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Contents

DISEASES

PESTICIDE CHEMISTRY

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 10 (1—2), pp. 1—6 (1975)

Effect of Heat Predisposition and Benzimidazole Treatment on Wheat Stem Rust Resistance

By

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Heating leaves of susceptible resistant and wheat cultivars for 20 sec at 49°C before inoculation with *Puccinia graminis* Pers. *tritici* increased the number of subsequent pustules as well as lesions. However, treatment with benzimidazole after inoculation decreased the enhanced susceptibility of heat-treated leaves significantly. These data support the view, that a slight damage to tissues has a favourable effect on the development of obligate parasites and this effect can be prevented by stabilizing the host metabolism with cytokinin-like materials.

It is a well known phenomenon that heat treatments can change susceptibility of plants to pathogens. Experiments dealing with the influence of heat on disease symptoms can be devided into two groups. In some experiments heat treatment was applied after inoculation while in others, before inoculation with pathogens. In the latter case heat predisposes plants to infections. On the other hand, heat treatment applied after inoculation usually results in heat therapy. Recently it has been shown by us that by means of heat therapy one can induce tissue necrosis and "hypersensitive reaction" in an originally compatible host/parasite complex (KIRÁLY *et al.*, 1972). Indeed, tissue necrosis and the development of the hypersensitive reaction characterizes incompatible host/parasite complexes and not the compatible ones.

While heat treatment applied after inoculation is usually connected to the development of symptoms characteristic to hypersensitive reaction, treatment prior to inoculation predisposes the host, in other words, in most cases susceptibility of the host increases (STRAIB and NOLL, 1944; YARWOOD, 1956; YARWOOD and HOOKER, 1965; CHAMBERLAIN and GERDEMANN, 1966; JHOOTY and YARWOOD, 1967; IKEGAMI, 1968).

The present study was initiated to determine the effect of heat predisposition on the wheat/stem rust complex in both compatible and incompatible combinations. ENGELBRECHT and MOTHES (1960) reported on the role of kinetin in heat-resistance of plants. Starting from this fact I tried to counteract the effect of heat treatment on different host/parasite relationships by cytokininlike actions.

Materials and Methods

Plants:

Little Club (*Triticum compactum* C. I. 4066), Vernal (*Triticum dicoccum* C. I. 3686), Khapli (*Triticum dicoccum* C. I. 4013), Reliance (*Triticum aestivum* C. I. 7370), and Arnautka (*Triticum durum* C. I. 1493) wheat cultivars were grown in the greenhouse at about 21°C. In every case 8–15-day-old plants were used in experiments.

Fungi:

Puccinia graminis (Pers.) f. sp. *tritici* race 11 was used throughout the experiments. The cultivars Little Club, Reliance and Arnautka are susceptible to this race (3–4 type reaction), while Vernal and Khapli are resistant (0; and 1 type reaction).

Heat treatment:

Eight-day-old plants were immersed in water at 49°C for 20 sec. This temperature and time period was the most effective without any visible damage on the treated leaves.

Treatment with benzimidazole:

Plants were sprayed with 200 ppm benzimidazole solution two times daily on 3 subsequent days. Treatments were started two days before or one day after the heat treatment.

Inoculation procedure:

The primary leaves of plants were washed with tap water and sprayed with a 50 mg urediospor/20 ml tap water suspension uniformly. At this concentration infection density was 60–80 pustules or lesions/untreated primary leaf. The inoculation was made one day after the heat treatment.

Estimation of the disease reaction:

Disease reaction was based both on the infection types of STAKMAN et al. (1962) and on pustule numbers. Each sample consisted of 4 pots and each pot contained 15–20 plants. The infection density was determined by the number of pustules/leaf and was expressed in percentage of untreated control.

Results

The heat treatment alone did not cause any visible damage on the plant. Sometimes the benzimidazole treatment alone caused withering on the top of the leaves.

Neither the heat treatment nor the benzimidazole treatment changed the infection type on the cul ivars applied in these experiments. However, the infection density was greatly affected by the different treatments. The pustule number was much higher on the heat-treated leaves compared with the untreated ones in each susceptible host. There was no significant difference after the benzimidazole treatment alone, but benzimidazole decreased the enhanced susceptibility of heat-treated leaves significantly (Fig. 1). Benzimidazole was effective only if it was

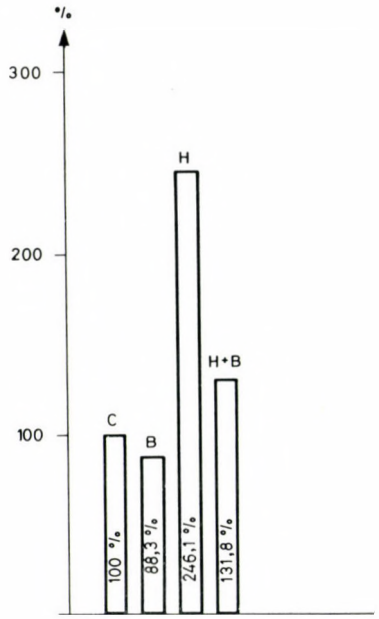

Fig. 1. Infection density of *Puccinia graminis tritici* race 11 on Little Club wheat cultivar after the different treatments. C = untreated control plants, B = plants sprayed with benzimidazole (200 ppm) two times daily on 3 subsequent days. The treatments started on the day of inoculation. H = heat treated plants (49°C for 20 sec). The heat treatment was applied one day before inoculation. H + B = heat treated + benzimidazole treated plants. The infection density was counted on pustule numbers per leaf and was expressed in percentage of untreated control. Each value is the average of ten replications. (P = 0.05)

applied after the heat treatment. Similar results were obtained in the case of hypersensitive (resistant) relationships (Fig. 2). There were significant differences between the heat-treated and untreated plants on the one hand, and between the heat-treated and heat-treated + benzimidazole-treated plants on the other.

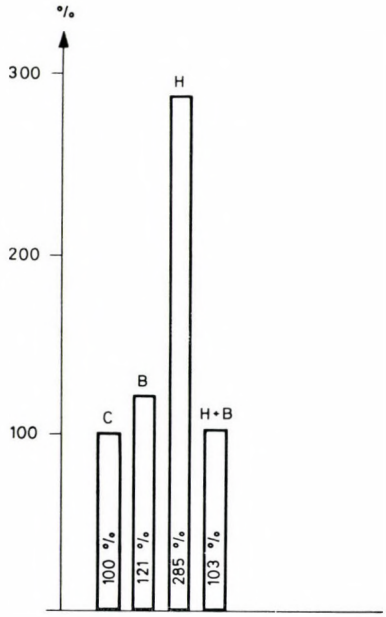

Fig. 2. Infection density of *Puccinia graminis tritici* race 11 on Vernal wheat cultivar after the different treatments. C = untreated control plants, B = plants sprayed with benzimidazole (200 ppm) two times daily on 3 subsequent days. The treatments started on the day of inoculation. H = heat treated plants (49°C for 20 sec). The heat treatment was applied one day before inoculation. H + B = heat treated + benzimidazole treated plants. The infection density was counted on lesion numbers per leaf and was expressed in percentage of untreated control. Each value is the average of ten replications. (P = 0.05)

Discussion

Different cases of decreased resistance of heat-treated plants have been reported in the literature. STRAIB and NOLL (1944) and IKEGAMI (1968) changed necrotic resistant host/parasite relationships to susceptible ones, JHOOTY and YARWOOD (1967) reported on changing "immune" type relationship (no visible symptom) to necrotic, or even to susceptible ones, while in other cases the number of pustules or lesions increased significantly (YARWOOD and HOOKER, 1965) after the treatment with heat. However, it is noteworthy that WAGENBRETH (1968) and YARWOOD and co-workers (1969) found increased resistance to fungi after heat predisposition in certain cases. The interpretation of the decreased resistance after heat predisposition is quite insufficient in spite of numerous data in the literature. The first possible interpretation of our results would be a damage to the stomata, in consequence of which more spore germ tubes could invade the leaf and more pustules would develop. However, the fact that the benzimidazole applied after

penetration of the pathogen was able to prevent the effect of the heat treatment precludes the possibility of the hypothesis on stomata damage.

JEROME and MÜLLER (1958), CHAMBERLAIN and GERDEMANN (1966) found positive correlation between the reduced resistance and the reduced phytoalexin content in heat-treated plants. But in the wheat stem-rust connection the heat treatment applied after inoculation caused typical hypersensitive symptoms with augmented peroxidase activity, so it is improbable that heat treatment decreases the phytoalexin capacity in wheat leaves (BARNA *et al.*, 1974).

Otherwise, the heat predisposition did not change the resistance type, only the number of pustules/lesions increased both in susceptible and hypersensitive connections. Presumably, another resistance mechanism works in this case which has been hardly studied until now.

Heat treatment has certainly a damaging effect on the host metabolism in spite of the lack of visible symptoms. Resistance of many wheat cultivars to stem rust is diminished after detaching the leaves (FORSYTH and SAMBORSKI, 1958). The detachment of leaves which exerts also a damaging effect on the host metabolism causes enzymatic changes in the detached leaves similar to those in tissues attacked by parasites (FARKAS *et al.*, 1964). These data suggest that the slight damage of tissues has a favourable effect on the development of certain parasites. Therefore by applying some cytokinin-like materials one can stabilize the metabolism of the host, thereby preventing from the favorable effect of heat treatment on the parasite. The results with benzimidazole support this hypothesis because, as was the case with detachment, benzimidazole prevented abolition of host resistance (PERSON *et al.*, 1958). However, to solve the problem other physiological and biochemical investigations are necessary which are in progress.

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Literature

BARNA, B., ÉRSEK, T. and MASHAAL, EL S. F. (1974): Hypersensitive reaction of rust-infected wheat in compatible host/parasite relationships. Acta Phytopath. Acad. Sci. Hung. *9*, 293–301.

CHAMBERLAIN, D. W. and GERDEMANN, J. W. (1966): Heat induced susceptibility of soybeans to *Phytophtora megasperma* var. *sojae*, *Phytophtora cactorum* and *Helminthosporium sativum*. Phytopathology *56*, 70–73.

ENGELBRECHT, L. and MOTHES, K. (1960): Kinetin als Faktor der Hitzeresistenz. Ber. Deutsch. Bot. Ges. *73*, 246–257.

FARKAS, G. L., DÉZSI, L., KISBÁN, K. and UDVARDY, J. (1964): Common pattern of enzymatic changes in detached leaves and tissues attacked by parasites. Phytopathol. Z. *49*, 343–354.

FORSYTH, F. R. and SAMBORSKI, D. J. (1958): The effect of various methods of breaking resistance on stem rust reaction and content of soluble carbohydrate and nitrogen in wheat leaves. Can. J. Bot. *36*, 717—723.

IKEGAMI, H. (1968): Heat-induced susceptibility of beans to rust. Phytopathology *58*, 773—775.

JEROME, S. M. R. and MÜLLER, K. O. (1958): Studies on phytoalexins II. Influence of temperature on resistance of *Phaseolus vulgaris* toward *Sclerotinia fructicola* with reference to phytoalexin output. Australian J. Biol. Sci. 11. 301—314.

JHOOTY, J. S. and YARWOOD, C. E. (1967): Heat-induced hypersensitivity in cowpea to *Erysiphe polygoni*. Phytopathology *57*, 148—150.

KIRÁLY, Z., BARNA, B. and ÉRSEK, T. (1972): Hypersensitivity as a consequence not the cause of plant resistance to infection. Nature *239*, 456—458.

PERSON, C., SAMBORSKI, D. J. and FORSYTH, F. R. (1958): Effect of benzimidazole on detached wheat leaves. Nature *180*, 1294—1295.

STAKMAN, E. C., STEWART, D.M. and LOEGERING W. Q. (1962): Identification of physiologic races of *Puccinia graminis* var *tritici* U. S, Eep- Agr. Res. Serv. Bull. E. 611.

STRAIB, W. and NOLL, A. (1944): Untersuchungen über dem Einfluss der Hitze auf dem Rostparasitismus. Zentr. Bakteriol. Parasitenk. Abt. II. *106*, 257—277.

WAGENBRETH, D. (1968): Die Wirkung von Hitzeschocks auf die Rostanfalligkeit von Pappelnblättern. Phytopath. Z. *61*, 87—97.

YARWOOD, C. E. (1956): Heat-induced susceptibility of beans to some viruses and fungi. Phytopathology *46*, 523—525.

YARWOOD, C. E. and HOOKER, A. L. (1965): Heat predisposition to corn rust. Phytopathology *55*, 510—511.

YARWOOD, C. E., IKEGAMI, H. and BATRA, K. K. (1969): Heat-induced anthocyanin, polysaccharide and transpiration. Phytopathology *59*, 596—598.

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Krankheitsresistenz von Pflanzen, ein komplexer Prozeß

Von

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Disease resistance of plants is discussed as the result of complex processes, that depends on how

the plant	satisfies the demand on nutrients of the pathogen
	exerts toxic effects on the pathogen
the pathogen	deprives the plant of important substances
	damages the plant with toxins.

The combinations of these factors and their results are presented. Combinations which lead to resistance prevail. There must be quantitatively and qualitatively different kinds of resistance.

In der Auseinandersetzung zwischen Pathogenen (Bakterien und Pilze) und ihren Wirtspflanzen spielen Resistenzerscheinungen eine wichtige Rolle, ohne daß indes Genaueres über ihre Physiologie und Biochemie bekannt wäre. Im Hinblick auf die große wirtschaftliche Bedeutung der pflanzlichen Resistenz ist das sehr zu bedauern. Bessere Kenntnis der Mechanismen und Vorgänge, die Kulturpflanzen resistent machen, wäre für Phytopathologie, Resistenzzüchtung und praktische Landwirtschaft von großem Nutzen.

Resistenz ist die Fähigkeit von Pflanzen, Infektionen durch Pathogene zu widerstehen oder so zu ertragen, daß keine wesentlichen Einbußen in Qualität oder Quantität des Ertrages entstehen, also nicht oder nur wenig zu erkranken. Zwichen Resistenz als dem einen Pol und voller Anfälligkeit als dem anderen Pol der möglichen Reaktionen einer Pflanze auf pathogenen Angriff gibt es zahlreiche Übergänge, wobei jahreszeitliche oder entwicklungsbedingte Schwankungen der Resistenz einer Pflanze noch nicht einmal berücksichtigt sind (GRAINGER, 1968). Obgleich absolute Resistenz sehr erwünscht ist, wäre in vielen Fällen schon viel geholfen, gelänge es, die Anfälligkeit einer Pflanze wesentlich zu verringern.

Resistenz hat viele Aspekte: wirtschaftliche, epidemiologische, genetische usw. Hier sollen vor allem die physiologischen und biochemischen Aspekte betrachtet werden mit dem Ziel, die Faktoren zu bestimmen, die entscheidenden Einfluß auf die Resistenz einer Pflanze gegen ein Pathogen haben oder haben können in der Hoffnung, dadurch bei der Züchtung besserer resistenter Formen von Kulturpflanzen zu helfen. Dabei soll von Resistenzerscheinungen, die anatomische

oder histologische Ursachen haben, abgesehen werden. Diese Abgrenzung ist selbstverständlich künstlich, da oft genug enge Zusammenhänge zwischen beiden Gruppen von Phänomenen bestehen. Sie ergibt sich aber aus den sehr verschiedenen Arbeitsmethoden. Außerdem wird von vornherein darauf verzichtet, Resistenzerscheinungen zu erklären oder zu verstehen, die auf andere Weise zustandekommen. Dazu gehören Resistenzen, verursacht durch

a) mechanische Barrieren auf und in der Pflanze;

b) Zeitunterschiede in Entwicklungsphasen von Wirt und Parasit, so daß der Wirt nicht empfänglich ist, wenn der Parasit infektionsbereit ist;

c) räumliche Trennung von Wirt und Parasit;

d) fehlende Vektoren;

e) von Parasiten benötigte, aber vom Wirt nicht erfüllte Ergänzung der genetischen Information oder des genetischen Apparates.

Es wird auch darauf verzichtet, physiologische Vorgänge in die Betrachtung hineinzuziehen, die die Erfüllung der oben genannten Bedingungen beeinflussen. Außerdem wird angenommen, daß die Temperaturbedingungen dem Pathogen zusagen.

Um zu erkennen, was die Resistenz (oder die Anfälligkeit) einer Pflanze bedingt, muß von den Faktoren ausgegangen werden, deren Zusammenwirken zur Krankheit der Pflanze führt. Wenn Wirt und Pathogen unter günstigen oder ausreichenden Infektionsbedingungen, als der zur Erkrankung unerläßlichen Voraussetzung, zusammenkommen, kann es zur Krankheit kommen, wenn

1. der Wirt für das Pathogen ein geeignetes Nährsubstrat darstellt;

2. der Wirt keine für das Pathogen toxischen oder hemmenden Substanzen oder solche Substanzen nur in unwirksamen Mengen oder Konzentrationen enthält;

3. der Verlust von Substanzen des Wirtes an das Pathogen ein bestimmtes Maß überschreitet;

4. der Wirt durch toxische oder hemmende Substanzen des Pathogens oder durch wirtseigene Toxine oder Inhibitoren, deren Synthese durch das Pathogen induziert oder durch seine Gegenwart verstärkt wurde, geschädigt wird (WOLFFGANG, 1972).

Die beiden ersten Bedingungen sind sozusagen Forderungen des Pathogens an den Wirt. Werden sie nicht erfüllt, kommt es nicht zur Infektion oder sie abortiert, so daß es wegen der mangelnden Eignung der Pflanze als Wirt, Axenie, nicht zur Krankheit kommt. Die beiden anderen Bedingungen betreffen die Empfindlichkeit des Wirtes für die Folgen der Infektion; sie entscheiden darüber, ob die Infektion zur Krankheit führt oder nicht. Werden sie nicht erfüllt – erträgt also der Wirt die Belastung seines Stoffwechsels durch die Infektion – so ist er infiziert, aber nicht krank. Diese Form von Resistenz soll als Toleranz bezeichnet werden. Erfüllt der Wirt die ersten beiden Bedingungen und die beiden anderen, so kann es entweder zur Erkrankung kommen – der Wirt ist also anfällig – oder aber der Wirt ist so empfindlich, daß es an den Infektionsorten zu hypersensiblen

Reaktionen kommt. Ob dazu die dritte und vierte Bedingung erfüllt sein müssen oder ob die vierte Bedingung dabei wichtiger ist, muß dahingestellt bleiben.

Jede der erwähnten Bedingungen ist komplex. Unter »Nahrungsbedarf des Pathogens« oder »Substanzverlust des Wirtes« sind wahrscheinlich je nach Wirtspflanze und Pathogen, aber auch vom Entwicklungszustand des Wirtes und des Pathogens abhängend, nach Art und Menge verschieden zusammengesetzte Gemische von Substanzen zu verstehen. Sinngemäß gilt das gleiche für Substanzen mit toxischer oder hemmender Wirkung auf Wirt und Pathogen. Außerdem ist damit zu rechnen, daß die Substrat- und die Toxinsituation sich gegenseitig beeinflussen, aber auch von der Umwelt und ihren Faktoren beeinflußt werden. Von diesen, wahrscheinlich sehr komplizierten und dynamischen Verhältnissen muß zunächst abgesehen werden.

Der Substanzbedarf des Pathogens. Es ist überraschend, wie wenig über Art und Menge der Substanzen bekannt ist, die Pathogene zu ihrer normalen Entwicklung brauchen, selbst wenn man die Schwierigkeiten berücksichtigt, die solche Untersuchungen bereiten. Die Schwierigkeiten, bestimmte phytopathogene Mikroorganismen auf künstlichen Medien zu kultivieren, zeigen jedoch, daß manche Pathogene offenbar sehr anspruchsvoll sind (MACLEAN, 1969; HENDRIX, 1970; TSAO, 1970; BUSHNELL, 1972). Es scheint auch wenig Neigung zu bestehen, dieses Problem zu untersuchen, oder aber man hält es für ein Scheinproblem oder für gelöst. So schreibt CRUICKSHANK (1963) »Recent work of Burrows (8) provides convincing evidence against a nutritional theory of resistance«. Nun sind die Beweise gegen eine Nahrungstheorie der Resistenz in dieser kurzen Mitteilung nicht eindeutig, es sind auch andere Deutungen möglich. Aber selbst wenn sie eindeutig wären, würde das doch nur bedeuten, daß sie für die Resistenz der untersuchten Wirts-Pathogen-Kombinationen gelten, nicht aber gegen eine »nutritional theory« der Resistenz überhaupt. CRUICKSHANK favorisiert die Rolle von Phytoalexinen in der Abwehr von pathogenen Angriffen auf die Pflanze. Von der Bedeutung der Ernährung des Pathogens für die Infektion und die dadurch ausgelöste Krankheit abzusehen, wäre aber ebenso einseitig, wie von der Bedeutung der vom Wirt erzeugten Toxine abzusehen. Gerade diese einseitigen Betrachtungsweisen haben wahrscheinlich dazu geführt, daß wichtige Faktoren in der Dynamik der Auseinandersetzung von Wirt und Pathogen übersehen wurden oder unbeachtet blieben. Sicherlich gibt es Wirt-Parasit-Kombinationen, bei denen über die Resistenz ganz oder fast ausschließlich durch Toxine entschieden wird, sicherlich aber auch solche, bei denen die Entscheidungen beim Nahrungsbedarf des Pathogens liegen. Weitaus häufiger sind aber wahrscheinlich die Fälle, in denen das Nahrungsangebot des Wirtes über die Toxinempfindlichkeit des Parasiten und die Toxinwirkung auf den Parasiten über seinen Nahrungsbedarf und damit über die Resistenz entscheiden.

Der Nahrungsbedarf eines Pathogens ist sicherlich komplex. Das bedeutet, daß verschiedene Substanzen in sehr verschiedenen Konzentrationen und Mengen zur Verfügung stehen müssen, wenn das Pathogen sich optimal entwickeln soll.

Suboptimales Nahrungsangebot kann qualitativ und quantitativ verursacht sein. Qualitativ insofern, daß etwa statt der optimalen Glukose Mannose oder statt der optimalen Glutaminsäure Asparaginsäure zur Verfügung steht, das Pathogen aber in der Lage ist, daraus die besser geeigneten Substanzen zu synthetisieren. Quantitativ suboptimal kann ein Nahrungsagebot in der Weise sein, daß etwa eine vom Pathogen benötigte Substanz, ein Kohlenhydrat, eine Phosphorverbindung, ein Vitamin in zu geringer Menge oder zu geringer Konzentration oder in schwer zugänglicher Bindung (chemisch oder an Zellstrukturen) vorliegt. Dabei ist es in diesem Zusammenhang unerheblich, ob das Pathogen seinen Nahrungsbedarf aus im Wirt fertig vorliegenden und leicht zugänglichen Substanzen decken kann, oder ob es mechanische Barrieren durchbrechen und fermentativ chemische Verbindungen aufspalten muß. Entscheidend ist nur, ob es dem Pathogen mit seinen Mitteln gelingt, seinen Nahrungsbedarf zu decken.

Die Toxinempfindlichkeit des Pathogens. Es ist zwar allgemein bekannt, daß Mikroorganismen durch zahlreiche Substanzen der verschiedensten Art und in weiten Konzentrationsbereichen gehemmt oder getötet werden. Solche Erkenntnisse werden in der Human- und Veterinärmedizin, aber auch im chemischen Pflanzenschutz verwertet. Von den Wirtspflanzen entweder vor oder nach der Infektion erzeugte Substanzen mit toxischer oder hemmender Wirkung auf die Pathogene spielen sicherlich auch bei der Pathogenese und bei der Abwehr pathogener Effekte eine wichtige Rolle. Einige solcher Substanzen, die nach K. O. MÜLLER Phytoalexine genannt werden, sind isoliert und ihre Struktur aufgeklärt worden (CRUICKSHANK, 1963; KUĆ, 1972; KOSUGE, 1969; ROHRINGER und SAMBORSKI, 1967). Ihre Rolle bei der Resistenz ist aber immer noch ziemlich unklar.

Um für die Resistenz eine Rolle zu spielen, müssen sie rechtzeitig in toxisch wirksamen oder hemmenden Konzentrationen dort verfügbar sein, wo sie auf das Pathogen einwirken sollen. Das alles hängt davon ab, ob präformiert vorliegende Substanzen in ausreichender Konzentration am Infektionsort vorliegen oder schnell genug dorthin transportiert werden, wenn die Infektion erfolgt oder ob ihre Synthese – bei Phytoalexinen im engeren Sinne – durch das Pathogen ausgelöst wird und dann schnell genug erfolgt. Die Tatsache allein, daß sie in wirksamen Konzentrationen nach Infektion in der Pflanze auftreten, beweist noch nicht, daß sie es sind, die zur Resistenz führen. Welche Mengen die Pflanze und wie schnell sie diese erzeugt, hängt sowohl von der Art des Pathogens, der Infektionsdosis, der Infektionsdichte als auch vom Entwicklungs- und Ernährungszustand der Pflanze ab, aber auch von den Umweltbedingungen wie z. B. der Temperatur. Beziehungen zwischen Toxinempfindlichkeit und Ernährungszustand des Pathogens waren bereits angedeutet worden. Es scheint, als ob die Toxin- und Hemmstoffsituation für das Resistenzgeschehen überbewertet wird, vielleicht auch deshalb, weil Fungizide eine so große Rolle in Pflanzenschutz spielen. Diese Bemerkung soll die Bedeutung von Toxinen und Hemmstoffen der Wirtspflanzen für ihre Resistenz nicht mindern. Es ist anzunehmen, daß sie für die Resistenz besonderes Gewicht haben.

Wie diese beiden Bedingungen erfüllt werden, ist für diese Überlegungen ohne Belang, ob also die Pflanze im infizierten Gewebe das Nahrungsangebot durch Erzeugung an Ort und Stelle, durch Neusynthese oder Hydrolyse vorhandener Substanzen oder durch Transport aus anderen Organen oder Geweben beschafft und ob toxische oder hemmende Substanzen nicht erzeugt werden oder in zu geringer Menge oder sofort unschädlich gemacht oder abtransportiert werden. Außerdem wird vorausgesetzt, daß das Pathogen sich immer in Geweben befindet, die diese Bedingungen befriedigen.

Die Substanzverluste des Wirtes. Sind Nahrungs- und Toxin- und Hemmstoffsituation im Wirt der Infektion eines Pathogens günstig, so entscheiden die Schäden, die das Pathogen dem Wirt zufügt, darüber, ob und wie der Wirt erkrankt. Dabei wollen wir hier von rein mechanischen Verletzungen absehen. Sie scheinen in vielen Fällen für den Schaden, den der Wirt durch die Infektion erleidet, unerheblich zu sein. So wenig über den Nahrungsbedarf der Pathogene bekannt zu sein scheint, so wenig scheint über die Substanzverluste der Wirte und ihre Bedeutung für diese bekannt zu sein. GRAINGER (1968) hat z. T. beträchtliche Substanzverluste in kranken Pflanzenorganen festgestellt. Es ist nicht zu erwarten, daß jede Pflanze starke, evtl. längere Zeit andauernde Substanzverluste unbeschadet erträgt, besonders dann nicht, wenn diese Verluste auch Substanzen mit speziellen, für die Pflanze wichtigen Wirkungen umfassen, etwa Vitamine u. ä.

Toxinwirkungen auf den Wirt. Zu dem dem Wirt von Pathogenen zugefügten Schaden durch Substanzverlust kommen in vielen Fällen noch Schäden durch Toxine oder Hemmstoffe des Parasiten oder Stoffwechselprodukte der Pflanze, die sich in und um die infizierten Gebiete anreichern. PRINGLE und SCHEFFER (1964) referieren Arbeiten über die spezifische Wirkung solcher Toxine und über enorme Unterschiede in der Empfindlichkeit anfälliger und resistenter Formen dafür. Auch BROWN (1965) führt zahlreiche toxische Wirkungen der Pathogene auf den Wirt an. In einer sehr schönen Arbeit haben VAN DIJKMAN und SIJPESTEIJN (1971) die Wirkung von Toxinen des Pathogens genauer untersucht. Wahrscheinlich wird die Toxinempfindlichkeit von vielerlei Faktoren beeinflußt, die Substanzverluste an das Pathogen dürften in vielen Fällen eine wichtige Rolle dabei spielen.

Für toxische oder hemmende Wirkungen ist es gleichgültig, wer das wirksame Prinzip erzeugt hat. So sind folgende Möglichkeiten vorstellbar:

1. Wirts-Toxin bzw. Hemmstoff schadet Pathogen
2. Pathogen-Toxin bzw. Hemmstoff schadet Wirt
3. Wirts-Toxin bzw. Hemmstoff schadet Wirt
4. Pathogen-Toxin bzw. Hemmstoff schadet Pathogen.

Dabei kann die Erzeugung eines Toxins oder Hemmstoffes durch den anderen Partner induziert werden (durch Fermentsubstrate, die bei Umsetzung durch Fermente des anderen Partners zu für diesen schädlichen Substanzen führen, durch eigene Fermente, die von anderen Partnern erzeugte Substrate zu schädlichen Substanzen für den anderen Partner machen usw.). Es können aber auch

normalerweise harmlose, weil nur in geringen Konzentrationen vorkommende Substanzen toxisch oder hemmend werden, wenn sie angereichert werden. Das kann dadurch geschehen, daß ihr Abtransport (oder ihre Umsetzung) verlangsamt oder aufgehoben wird oder wenn sie oder ihre Vorstufen aus anderen Geweben bevorzugt antransportiert und abgelagert werden.

Wichtig ist ferner der Zeitfaktor für toxische und hemmende Wirkungen. Dabei ist von Bedeutung, wann eine Substanz bestimmte, nämlich toxische Konzentrationen erreicht. Organismen bzw. ihre Gewebe und Organe sind nicht in allen Phasen ihrer Entwicklung gleichmäßig empfindlich. Es ist für die toxische Wirkung einer Substanz aber auch von Bedeutung, wie ihre Konzentration sich über die Zeit verhält, ob die Substanz einmalig oder dauernd und dann gleichmäßig zu- oder abnehmend oder oszillierend am Wirkungsort erscheint.

Auf Wechselwirkungen zwischen den vier über die Pathogenese entscheidenden Faktoren war schon mehrfach aufmerksam gemacht worden. Trotzdem soll hier noch einmal nachdrücklich darauf hingewiesen werden, daß diese Faktoren im Verlaufe der Auseinandersetzung zwischen einer Wirtspflanze und ihrem Pathogen keine festen Größen sein werden, sondern sich gegenseitig beeinflussen und verändern dürften. Diese Wechselwirkungen werden bei den folgenden Betrachtungen unbeachtet bleiben können. Sie werden beachtet und untersucht werden müssen, wenn etwas mehr über die einzelnen Faktoren und ihre Wirkung bekannt ist.

Kombination der Faktoren. Die für die Pathogenese und damit für die Resistenz einer Pflanze gegen ein Pathogen entscheidenden Faktoren

I Nahrungsangebot für das Pathogen
II Toxin- bzw. Hemmstoffgehalt des Wirtes
III Substanzverluste des Wirtes
IV pathogene Hemmstoff- oder Toxinwirkungen auf den Wirt

können in folgender Form auftreten:

Für das Wirt-Pathogen-Verhältnis wichtige Faktoren

I. 1. Nahrungsangebot des Wirtes für Pathogen ungenügend
2. Nahrungsangebot des Wirtes für Pathogen ausreichend
II. 3. Wirt enthält keine oder zu wenig für Pathogen toxische Substanzen
4. Wirt enthält wirksame Konzentrationen für Pathogen toxische Substanzen
III. 5. Wirt erträgt Substanzverlust durch Pathogen schadlos
6. Wirt erträgt Substanzverlust durch Pathogen nicht
IV. 7. pathogene Toxine schaden dem Wirt nicht
8. pathogene Toxine schaden dem Wirt.

Die Grenze zwischen wirksamer und unwirksamer Konzentration ist selbstverständlich keine scharfe Linie, sondern eine breite Zone, und sicher auch nicht unveränderlich. Umwelteinflüsse und epidemiologische Gegebenheiten werden sie verlagern können. Der Eindruck, der durch die Zweiteilung der mit römischen

Ziffern bezeichneten Gruppierung gegeben wird, als ob es für gegebene Konzentrationen nur eine von zwei Reaktionsmöglichkeiten gäbe, wie etwa Ein-Ausstellungen eines Lichtschalters, ist falsch. Es gibt sicherlich wirksame Konzentrationen mit schwacher, mäßiger und starker Wirkung, also mit quantitativen Wirkungsunterschieden. Von diesen muß zunächst abstrahiert werden, um die Diskussion überschaubar zu halten. Kombiniert man dann diese Faktoren so, daß aus jeder der mit römischen Ziffern bezeichneten Gruppen ein Glied in jeder Kombination erscheint, so ergibt sich das folgende *Grundschema:*

Tabelle 1

Grundschema von Kombinationen der für das Wirt-Pathogen-Verhältnis entscheidenden Faktoren

Nr.	Kombination	Wirkung auf Pathogen (I und II)	Wirkung auf Wirt (III und IV)	Reaktion des Wirtes
1	1 3 5 7	H–n	n–n	R
2	1 3 6 7	H–n	H–n	R
3	1 3 5 8	H–n	n–H	R
4	1 3 6 8	H–n	H–H	R
5	2 3 5 7	n–n	n–n	(T)
6	2 3 6 7	n–n	H–n	a
7	2 3 5 8	n–n	n–H	a (evtl. → Hy → R)
8	2 3 6 8	n–n	H–H	a → Hy → R
9	1 4 5 7	H–H	H–n	R
10	1 4 6 7	H–H	n–n	R
11	1 4 5 8	H–H	H–H	R
12	1 4 6 8	H–H	n–H	R
13	2 4 5 7	n–H	n–n	R (evtl. auch (T))
14	2 4 6 7	n–H	H–n	R (evtl. auch a oder (T))
15	2 4 5 8	n–H	n–H	R (evtl. auch a oder (T))
16	2 4 6 8	n–H	H–H	R (evtl. auch a oder a → Hy → R)

H = Hemmung
n = normal
R = resistent
a = anfällig
(T) = tolerant
Hy = Hypersensibilität

Dabei ist zu beachten, daß die Faktoren I und II über die Infektionsbereitschaft des Wirtes in biochemischer und physiologischer Beziehung entscheiden. Ist diese Entwicklung schon im negativen Sinne für das Pathogen gefallen, so ist die pflanze resistent, auch wenn sie durch Substanzverluste an das Pathogen oder durch Toxine des Pathogens Schaden erlitten hätte, wäre dem Pathogen die In-

fektion geglückt. Resistenz muß hierbei nicht bedeuten, daß dem Pathogen die Existenz und damit die pathogene Wirkung auf die Wirtspflanze vollkommen unmöglich, sondern nur, daß sie deutlich verringert sind.

Das Grundschema zeigt, daß von den möglichen Reaktionen der Pflanze die resistenten überwiegen. Bei einigen Kombinationen sind verschiedene Reaktionen des Wirtes möglich. Welche eintreten, hängt von der quantitativen Ausbildung der kombinierten Faktoren und den sich daraus ergebenden Auswirkungen ab. Bei der Kombination 7 z. B. sind die Verhältnisse im Wirt so, daß das Pathogen sich normal entwickeln kann. Der Wirt erträgt zwar den dadurch eintretenden Substanzverlust schadlos, kann aber, weil er gegen pathogene Toxine empfindlich ist, von diesen geschädigt werden. Bildet das Pathogen nur wenig oder schwach wirksame Toxine, so wird das zu pathologischen Erscheinungen führen. Ebenso wird es bei mittleren Konzentrationen oder Toxinen mittlerer Stärke sein, nur daß der Wirt dann stärker reagiert. Erzeugt das Pathogen aber sehr starke oder sehr viel Toxine, so kann ihre Wirkung umschlagen: Sie schaden den infizierten und den benachbarten Geweben so stark, daß sie schnell absterben, also hypersensibel reagieren. Bei der Kombination 14 hängt es von der Stärke der vom Wirt gebildeten Phytoalexine und ihrer Konzentration ab, wie stark das Pathogen gehemmt wird.

Bei schwacher Hemmung des Pathogens würde der Wirt durch seine Empfindlichkeit für Substanzverlust an das Pathogen gehemmt werden, also anfällig reagieren. Bei stärkerer Hemmung des Pathogens durch Phytoalexine wäre der Substanzverlust des Wirtes an das sich nur kümmerlich entwickelnde Pathogen dann gering, so daß der Wirt trotz seiner Empfindlichkeit nur schwach reagieren würde, die Pflanze würde mit Toleranz reagieren. Ist die Wirkung der Phytoalexine noch stärker, so daß das Pathogen abstirbt oder sich gerade noch an einigen Infektionsorten erhält, wäre – trotz der Empfindlichkeit des Wirtes für Substanzverlust – nur eine so geringe Reaktion zu erwarten, daß der Wirt als resistent eingestuft werden könnte. Die quantitative Wirkung der kombinierten Faktoren ist also von großer Bedeutung. Berücksichtigt man sie stärker, als es durch die Abstufung wirksam/unwirksam im Grundschema geschah, nimmt man also z. B. drei Stufen an, so bekommt man 81 Kombinationen, die sehr viel deutlicher als das Grundschema zeigen, wie vielfältig die Möglichkeiten zur Reaktion der Pflanze sein können. Ein solches erweitertes Kombinationsschema enthält eigentlich alle Reaktionen, die Pflanzen auf phytopathogene Angriffe ergeben können.

Bevor versucht wird, die Bilanz aus dem erweiterten Schema zu ziehen, sollen die Nachteile dieser Darstellung unterstrichen werden:

1. Die Beziehungen zwischen Wirtspflanze und Pathogen werden summarisch und statisch dargestellt. In Wirklichkeit handelt es sich aber sicherlich um dynamische Beziehungen, um Wechselwirkungen, die kybernetisch betrachtet werden sollten. Auch die wichtigen Umwelteinflüsse können in einer solchen vereinfachenden Darstellung nicht berücksichtigt werden.

2. Die quantitativen Aspekte der Faktorenkomplexe werden durch die

Tabelle 2

Erweitertes Schema von Kombinationen der für das Wirt-Pathogen-Verhältnis entscheidenden Faktoren

Nr.	Kombination	Wirkung auf Pathogen	Wirkung auf Wirt	Reaktion des Wirtes
1	1 4 7 10	H—n	(n—n)	R
2	1 4 7 11	H—n	(n—H)	R
3	1 4 7 12	H—n	(n—H)	R
4	1 4 8 10	H—n	(H—n)	R
5	1 4 8 11	H—n	(H—H)	R
6	1 4 8 12	H—n	(H—H)	R
7	1 4 9 10	H—n	(H—n)	R
8	1 4 9 11	H—n	(H—H)	R
9	1 4 9 12	H—n	(H—H)	R
10	1 5 7 10	H—H	(n—n)	R
11	1 5 7 11	H—H	(n—H)	R
12	1 5 7 12	H—H	(n—H)	R
13	1 5 8 10	H—H	(H—n)	R
14	1 5 8 11	H—H	(H—H)	R
15	1 5 8 12	H—H	(H—H)	R
16	1 5 9 10	H—H	(H—n)	R
17	1 5 9 11	H—H	(H—H)	R
18	1 5 9 12	H—H	(H—H)	R
19	1 6 7 10	H—H	(n—n)	R
20	1 6 7 11	H—H	(n—H)	R
21	1 6 7 12	H—H	(n—H)	R
22	1 6 8 10	H—H	(H—n)	R
23	1 6 8 11	H—H	(H—H)	R
24	1 6 8 12	H—H	(H—H)	R
25	1 6 9 10	H—H	(H—n)	R
26	1 6 9 11	H—H	(H—H)	R
27	1 6 9 12	H—H	(H—H)	R
28	2 4 7 10	n—n	n—n	R (T)
29	2 4 7 11	n—n	n—H	a
30	2 4 7 12	n—n	n—H	a
31	2 4 8 10	n—n	H—n	a
32	2 4 8 11	n—n	H—H	a → Hy → R
33	2 4 8 12	n—n	H—H	a → Hy → R
34	2 4 9 10	n—n	H—n	a
35	2 4 9 11	n—n	H—H	a → Hy → R
36	2 4 9 12	n—n	H—H	a → Hy → R
37	2 5 7 10	n—H	(n—n)	R
38	2 5 7 11	n—H	(n—H)	R
39	2 5 7 12	n—H	(n—H)	R
40	2 5 8 10	n—H	(H—n)	R
41	2 5 8 11	n—H	(H—H)	R
42	2 5 8 12	n—H	(H—H)	R

Tabelle 2 (Fortsetzung)

Nr.	Kombination	Wirkung auf Pathogen	Wirkung auf Wirt	Reaktion des Wirtes
43	2 5 9 10	n – H	(H – n)	R
44	2 5 9 11	n – H	(H – H)	R
45	2 5 9 12	n – H	(H – H)	R
46	2 6 7 10	n – H	(n – n)	R
47	2 6 7 11	n – H	(n – H)	R
48	2 6 7 12	n – H	(n – H)	R
49	2 6 8 10	n – H	(H – n)	R
50	2 6 8 11	n – H	(H – H)	R
51	2 6 8 12	n – H	(H – H)	R
52	2 6 9 10	n – H	(H – n)	R
53	2 6 9 11	n – H	(H – H)	R
54	2 6 9 12	n – H	(H – H)	R
55	3 4 7 10	n – n	n – n	R (T)
56	3 4 7 11	n – n	n – H	a
57	3 4 7 12	n – n	n – H	a
58	3 4 8 10	n – n	H – n	a
59	3 4 8 11	n – n	H – H	a → Hy → R
60	3 4 8 12	n – n	H – H	a → Hy → R
61	3 4 9 10	n – n	H – n	a
62	3 4 9 11	n – n	H – H	a → Hy → R
63	3 4 9 12	n – n	H – H	a → Hy → R
64	3 5 7 10	n – H	(n – n)	R
65	3 5 7 11	n – H	(n – H)	R
66	3 5 7 12	n – H	(n – H)	R
67	3 5 8 10	n – H	(H – n)	R
68	3 5 8 11	n – H	(H – H)	R
69	3 5 8 12	n – H	(H – H)	R
70	3 5 9 10	n – H	(H – n)	R
71	3 5 9 11	n – H	(H – H)	R
72	3 5 9 12	n – H	(H – H)	R
73	3 6 7 10	n – H	(n – n)	R
74	3 6 7 11	n – H	(n – H)	R
75	3 6 7 12	n – H	(n – H)	R
76	3 6 8 10	n – H	(H – n)	R
77	3 6 8 11	n – H	(H – H)	R
78	3 6 8 12	n – H	(H – H)	R
79	3 6 9 10	n – H	(H – n)	R
80	3 6 9 11	n – H	(H – H)	R
81	3 6 9 12	n – H	(H – H)	R

H = Hemmung n = normal

Eingeklammerte Reaktionen des Wirtes bedeuten, daß der Wirt sich normal verhalten dürfte, weil es durch magelnde Wirtlichkeit gar nicht erst (oder sehr vermindert) zu den ungünstigen Wirkungen des Pathogens auf den Wirt kommt.

R = resistent (T) = tolerant

a = anfällig Hy = Hypersensibilität

Berücksichtigung von nur drei Quantitäten recht ungenügend und etwas gewaltsam berücksichtigt.

Beide Nachteile wird man nur bei der Untersuchung konkreter Wirts-Pathogen-Beziehungen vermeiden können. Trotz dieser Schwächen erlaubt das erweiterte Schema folgende Schlüsse:

1. 73 von 81 Kombinationen ergeben Resistenz. Resistenz – in irgendeiner Form – ist also die weitaus häufigere Reaktion auf einen pathogenen Angriff. So häufig, daß man sie als den Normalfall ansehen kann.

2. Von den 73 zur Resistenz führenden Kombinationen ergeben 63 (1–27, 37–54, 64–81) deshalb Resistenz, weil das Pathogen ungünstige Verhältnisse vorfindet oder induziert. Der Angriff des Pathogens wird sozusagen in der ersten Verteidigungsstellung des Wirtes aufgefangen.

3. In 56 Kombinationen käme es zur Krankheit des Wirtes, bestünde diese »erste Verteidigungsstellung« nicht (2–9, 11–18, 19–27, 37–45, 47–54, 65–72, 74–81).

4. Hypersensible Reaktion ist ebenso häufig – oder selten – wie Anfälligkeit, je 8 Kombinationen.

5. In 7 Kombinationen ist die Resistenz doppelt gesichert: durch mangelnde Wirtlichkeit und mangelnde Empfindlichkeit der Pflanze.

6. Zur Toleranz führen die wenigsten – nur zwei – Kombinationen.

Es geht selbstverständlich nicht an, die Häufigkeit eines Reaktionstype in dieser formalen Faktorenkombination mit der Häufigkeit des Auftretens in den tatsächlich ablaufenden Auseinandersetzungen zwischen Wirtspflanzen und Pathogenen gleichzusetzen.

In der Praxis würde das bedeuten, daß verschiedene Formen von Anfälligkeit verschiedenen Formen von Resistenz gegenüberstehen. Dabei konnte natürlich die Dynamik der Auseinandersetzung von Wirt und Pathogen, ihre Interdependenz, positive und negative Rückkoppelungen usw. nicht berücksichtigt werden. Das ist für den Zweck dieser Studie nicht möglich und nicht nötig. Das erweiterte Schema zeigt aber, wie viele qualitativ und quantitativ verschiedene Formen von Resistenz möglich sind. In praxi dürften die Verhältnisse durch Wechselwirkungen zwischen Toxin- und Substratwirkungen und die verschiedenen Geschwindigkeiten der Reaktionen von Wirt und Pathogen noch komplizierter sein, als es die schematische Beurteilung des Resistenzverhaltens des Wirtes (die gelegentlich auch andere als die gewählten Ergebnisse zuläßt) vereinfachend zeigen. Keine der so »ermittelten« Resistenz oder Anfälligkeiten gleicht einer anderen vollkommen. Diese Fragen müssen durch die Untersuchung konkreter Wirt-Pathogen-Kombinationen beantwortet werden. Jedenfalls zeigen schon diese so sehr vereinfachenden Überlegungen, wie kompliziert die Vorgänge sind, die über Resistenz oder Anfälligkeit einer Pflanze entscheiden. Natürlich sind die Verhältnisse tatsächlich noch komplizierter, da die als »Merkmale« benutzten Eigenschaften selbst komplex und durch zahlreiche Faktoren (genetische, Umweltfaktoren) beeinflußt werden. Auch das Urteil darüber, ob Resistenz oder Anfälligkeit

die Folge der betreffenden Faktorenkombination ist, ist sehr summarisch. Das ergibt sich schon aus der groben quantitativen Abstufung der Faktoren in ungenügend, ausreichend und reichlich im Komplex I bzw. in diesen entsprechenden Angaben in den anderen Komplexen. Die Beziehungen zwischen der Hemmung des Pathogens durch für ihn ungünstige Nahrung- oder Toxinsituationen im Wirt und pathogenen Erscheinungen des Wirtes sind mit Sicherheit komplizierter, als es in dem erweiterten Schema dargestellt werden konnte. Das dürfte aber den heuristischen Wert dieser Überlegungen nicht mindern, solange man sich dieser Einschränkungen bewußt bleibt.

Dieses Modell berücksichtigt nicht bzw. nicht direkt Krankheiten, in deren Verlauf Transportwege geschlossen werden oder immunologische Reaktionen auftreten.

Toxische oder hemmende Wirkungen auf den Wirt oder seine Organe oder Gewebe, die durch das Pathogen induziert, vom Wirt aber selbst erzeugt werden, sind den von dem Pathogen ausgehenden gleichzusetzen. Sinngemäß gilt das auch für vom Pathogen unter dem Einfluß des Wirtes erzeugte, für das Pathogen toxische oder hemmende Substanzen.

Die hier dargestellte Konzeption widerspricht nicht dem Florschen Gene-for-Gene-Konzept, sondern könnte geeignet sein, die physiologische Basis dafür zu liefern: Dem Gen, das die Wirtsreaktion steuert, entspricht danach ein Gen im Parasiten, das seine Pathogenität regelt. Es liegt auf der Hand, daß in jeder der Faktorengruppen genetisch bestimmte Elemente darüber entscheiden, wie sich die Faktoren zusammensetzen. Benötigt das Pathogen z. B. eine bestimmte Substanz in einem bestimmten Entwicklungsstadium in bestimmten Mengen, so ist das genetisch bestimmt. Ob der Wirt diesen Bedarf erfüllt, hängt wiederum von seiner genetischen Konstitution ab.

Kein Platz wurde in diesen Überlegungen bis jetzt den »common antigens« eingeräumt. Falls sie tatsächlich bei der Entscheidung über Anfälligkeit oder Resistenz einer Pflanze gegen ein Pathogen von Bedeutung sind, dürfte ihre Rolle in den mit der Toxinsituation befaßten Faktorenkomplexen zu suchen sein. Dabei dürften sie direkt oder indirekt für toxische Wirkungen verantwortlich sein. Eine sehr wichtige Frage ist die nach der Vereinbarkeit verschiedener Zuchtziele. Falls die vorliegende Konzeption die physiologischen Ursachen für Anfälligkeit und Resistenz in der richtigen Richtung sucht, dürfte es naheliegen, anzunehmen, daß bestimmte Zuchtziele einander widersprechen. Die nach Menge und Qualität angestrebten hohen Ertragsleistungen unserer Kulturpflanzen führen wahrscheinlich dazu, daß ihre Eignung für phytopathogene Parasiten insofern zunimmt, daß das für diese wichtige Substanzangebot im Wirt nach Art und Menge immer besser wird. Außerdem dürfte die Empfindlichkeit für Substanzverluste und die Wirkung von Substanzverlusten auf Qualität und Quantität des Ertrages um so größer werden, je höher die Leistungsanforderungen an die Kulturpflanzen werden. Nach allen bisherigen Erfahrungen scheint es nicht möglich zu sein, Kulturpflanzen mit Resistenz gegen alle wichtigen Pathogene auszustatten. Man wird

also Kompromisse eingehen müssen und entweder auf bestimmte Resistenzen oder bestimmte Höchstleistungen verzichten müssen oder aber sich damit abfinden müssen, bestimmte Höchstleistungen durch Verzicht auf bestimmte Resistenzen zu erreichen und mit chemischen Abwehrmaßnahmen zu sichern.

Für das Wirt-Pathogen-Verhältnis wichtige Faktoren

(für erweitertes Schema)

I 1. Nahrungsangebot des Wirtes für Pathogen ungenügend
2. Nahrungsangebot des Wirtes für Pathogen ausreichend
3. Nahrungsangebot des Wirtes für Pathogen reichlich
II 4. Wirt enthält für Pathogen toxische Substanzen wenig oder gar nicht
5. Wirt enthält für Pathogen toxische Substanzen in wirksamen Mengen
6. Wirt enthält für Pathogen toxische Substanzen reichlich
III 7. Wirt erträgt Substanzverlust durch Pathogen schadlos
8. Wirt erträgt Substanzverlust durch Pathogen einigermaßen
9. Wirt erträgt Substanzverlust durch Pathogen sehr schlecht
IV 10. pathogene Toxine schaden dem Wirt nicht
11. pathogene Toxine schaden dem Wirt mäßig
12. pathogene Toxine schaden dem Wirt stark

Zusammenfassung

Die Krankheitsresistenz von Pflanzen wird als das Ergebnis eines komplexen Prozesses betrachtet, das davon abhängt, wie
die Pflanze den Substratbedarf des Pathogens befriedigt
toxische Wirkungen auf das Pathogen ausübt
das Pathogen der Pflanze wichtige Substanzen entzieht
der Pflanze durch Toxine schadet.

Die Kombinationen dieser Faktoren und ihr Ergebnis werden dargestellt. Es zeigt sich, daß zur Resistenz führende Kombinationen überwiegen und daß qualitativ und quantitativ unterschiedliche Resistenzen bestehen müssen.

Literatur

BROWN, M. (1965): Toxins and cell-wall dissolving enzymes in relation to plant disease. Ann. Rev. Phytopath. *3*, 1—18.

BURROWS, V. D. (1960): Nature of the resistance of oats varieties to races of oat stem rust. Nature *188*, 957—958.

BUSHNELL, W. R. (1972): Physiology of fungal haustoria. Ann. Rev. Phytopath. *10*, 151—176.

CRUICKSHANK, I. A. M. (1963): Phytoalexins. Ann. Rev. Phytopath. *1*, 351—374.

DIJKMAN, A. VAN und SIJPESTEIJN, A. K. (1971): A biochemical mechanism for the gene-for-gene resistance of tomato to Cladosporium fulvum. Neth. J. Pl. Path. *77*, 14—24.

GRAINGER, J. (1968): Cp/R_s and the disease potential of plant. Horticult. Res. *8*, 1—40.

HENDRIX, J. W. (1970): Sterols in growth and reproduction of fungi. Ann. Rev. Phytopath. *8*, 111–130.
KOSUGE, T. (1969): The role of phenolics in host response to infection. Ann. Rev. Phytopath. *7*, 195–227.
KUĆ, J. (1972): Phytoalexins. Ann. Rev. Phytopath. *10*, 207–232.
MACLEAN, M. J. (1969): Culturing of rust fungi. Ann. Rev. Phytopath. *7*, 123–146.
PRINGLE, P. B. und SCHEFFER, R. P. (1964): Host-specific plant toxins. Ann. Rev. Phytopath. *2*, 133–156.
ROHRINGER, R. und SAMBORSKI, D. J. (1967): Aromatic compounds in the host-parasite interaction. Ann. Rev. Phytopath. *5*, 77–86.
TSAO, P. H. (1970): Selective media for isolation of pathogenic fungi. Ann. Rev. Phytopath. *8*, 157–186.
WOLFFGANG, H. (1972): Resistenzphysiologie. Biol. Zbl. *91*, 625–637.

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The Role of Anthocyan and Phenol Compounds in the Resistance of Grapes against *Botrytis* Infection

By

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The grape varieties Ezerjó, Kecskemét 11, M-2 and M-4 were inoculated "in vitro" by the fungus species *Botrytis squamosa(?)882*, *Botrytis tulipae*, *Botrytis allii*, *Botrytis cinerea* and *Botrytis cinerea(?)9*. The experiments aimed to establish the virulence of the different *Botrytis* species, as well as the role of anthocyan and polyphenol compounds in the chemical axeny of grape berries.

From among the grape varieties studied, Kecskemét-11 was found the most resistant against *Botrytis* infection, followed by the variety M-4; both M-2 and Ezerjó were fairly susceptible against the parasite.

From the *Botrytis* species tested, the ones belonging to the morphological range of *B. cinerea* were found the most infectious: *Botrytis cinerea*, *Botrytis cinerea(?)9* and *Botrytis squamosa(?)882*, while *Botrytis tulipae* and *Botrytis allii* showed a less marked infectivity.

The quantitative and qualitative studies of the polyphenol and anthocyan compounds of different grape varieties indicated that the chemical axeny of the berries is related partly to these compounds. The role of anthocyans is in the first place a function of their qualitative composition, as shown by the most resistant grape variety. This variety contains only one anthocyan, the cyanidin, occurring in a quantity of 0.27 g/liter; this compound showed a marked inhibitory effect on the *Botrytis* species tested. In the axeny also the quantity of anthocyans may play a role as the variety M-4 (4.15 g/liter anthocyan) was found to be more resistant than M-2, containing only 1.89 g/liter anthocyan.

The participation of the polyphenols in the axeny depends also — similarly to the anthocyans — mostly on their qualitative properties and less on their quantity. This was indicated by the variety Kecskemét-11 (total phenol content 0.82 g/liter), which showed a higher resistance than Ezerjó, containing 1.10 g/liter. In course of the tests the following phenol components showed an inhibitory effect on the *Botrytis* species tested: tannic acid, chlorogenic acid, caffeic acid, gallic acid. The changes in the total polyphenol content depended more on the *Botrytis* species and less on the degree of infection, showing a specificity of the phenols participating in the axeny phenomenon, against the *Botrytis* fungi.

The *Botrytis* infection causes in some years considerable losses in the grape yield in spite of the large amount of fungicides applied to field plantations.

According to practical observations, the various grape varieties differ in their resistance against *Botrytis* attack. The actual damage caused on the grapes depends on the grade of virulence of the attacking *Botrytis* species (KUBICKAIA,

1969) and on the resistance or susceptibility of the grape variety in question. The resistance may be attributed to two main factors: to the axeny or preformed defense mechanism on one hand, and to the induced defense mechanism, on the other. The first factor exists in the plant already prior to the attack of the pathogen (HENKE, 1959, BREIDER, 1972) it may be considered, therefore, as a morphological or chemical property of the plant, independently of infection; the second factor appears following the infection, as an active defense mechanism (KIRÁLY, 1968).

In our experiments we aimed to study the chemical axeny of the grape berries, with special regard to the anthocyan and phenol compounds present (BOLCATO *et al.*, 1964; SINGLETON and ESAU, 1969; PLISANOVSKII and ZOTOV, 1971; RIBEREAU-GAYON, 1971).

The study intended to clear the following:

– to establish, whether there are connections between the resistance of grape varieties and the *Botrytis* species participating in the natural infection;

– to follow the changes occuring in the anthocyan- and phenol level of the berries following an attack by *Botrytis;*

– to orient ourselves on the inhibitory effect of some compounds in the berries, on *Botrytis* development.

The experiments – being of "*in vitro*" character–excluded the manifestation of induced defense reactions as resistance factors. On the other hand, the thickness of the skin, the phenolic acids of the wax coating covering the berries, can be regarded as morphological or chemical axenies (KIRÁLY, 1968; GREGORIEV and TODOROV, 1970). To exclude their disturbing effect, the berries were inoculated artificially, by injuring the skin with a needle. In carrying out the biological test, similarly to the experiments of GREGORIEV and TODOROV (1970) or KUBICKAIA (1969) the differences in activity of the *Botrytis* species were correlated to the number of infected berries. Also the speed of spreading on the berry surfaces was evaluated in function of time (percentage of the berry surface covered by the *Botrytis*).

Materials and Methods

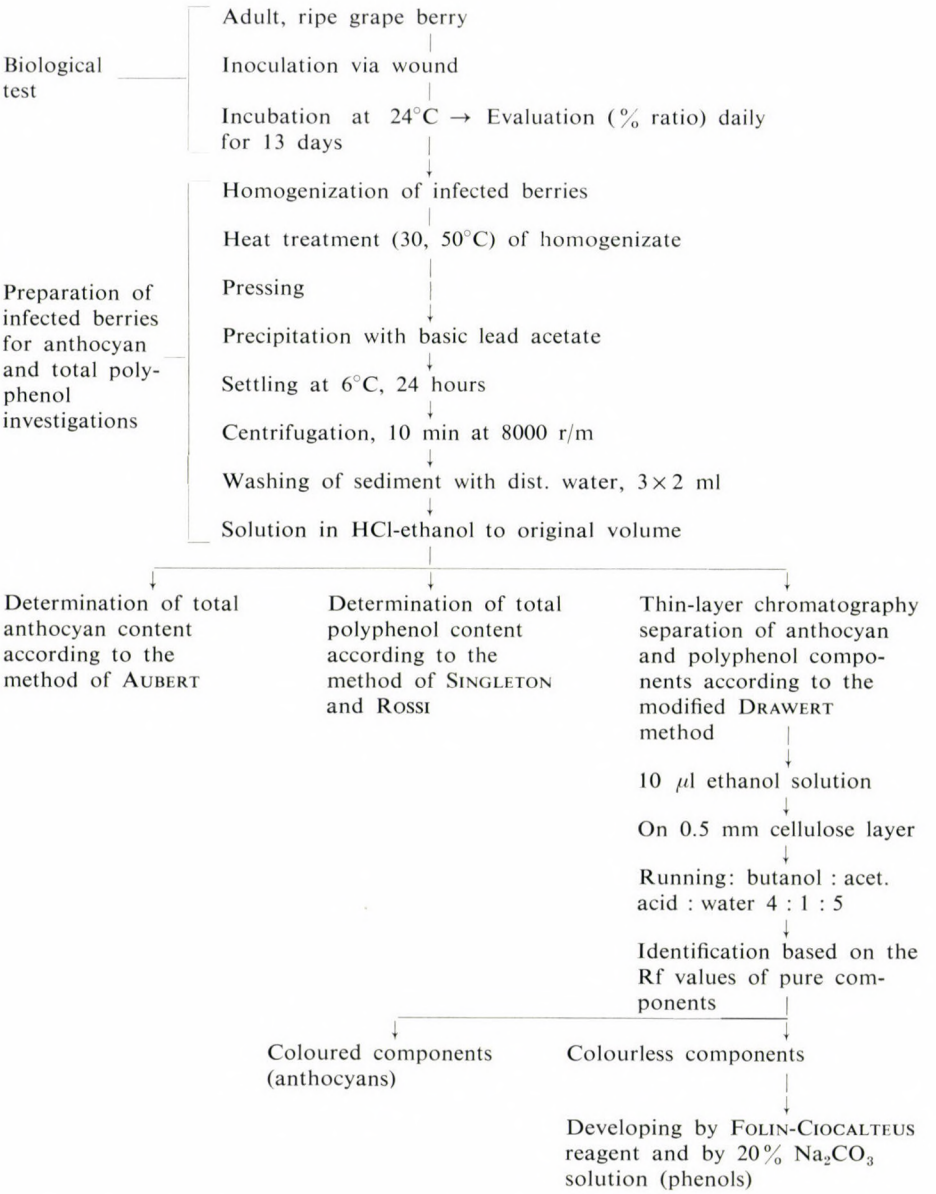
The experiments were carried out applying the grape varieties Ezerjó, Kecskemét-11, M-2, and M-4, harvested at the same date in the Experimental Vineyard of the Research Institute for Ampelology and Enology, Kecskemét-Miklóstelep.

The characteristics of the grape varieties are as follows:

– Ezerjó, a well-known, white wine-grape;
– Kecskemét-11, a wine-grape with flesh-colour (Ezerjó × Szürkebarát);
– M-2, a red wine-grape (Kadarka × Muscat Bouschet) with blue berries;
– M-4 a colouring grape (Kadarka × Petit Bouschet) with blue berries.

For inoculations the following *Botrytis* species were used: *Botrytis squa-*

The course of the investigation



Study of the phenol- and anthocyan compounds of grape berries on Botrytis species. Agar-diffusion method.

mosa(?)882, isolated from brown winestalks (collection of the Research Institute for Ampelology and Enology, Budapest), *Botrytis tulipae/A*, (collection of the University of Horticulture, Budapest), *Botrytis allii/F* isolated from onion (Station de Recherches de Viticulture, Pont-de-la Maye), *Botrytis cinerea(?)9* isolated from grape berries (collection of the Research Institute of Ampelology and Enology, Budapest), *Botrytis cinerea/F* isolated from grape berries (Station de Recherches de Viticulture, Pont-de-la Maye). In the following the origin of the different *Botrytis* strains will not be mentioned, only the species name *(Botrytis tulipae, Botrytis allii, Botrytis cinerea)*. All *Botrytis* cultures have been kept on malt-agar medium in test tubes, at 25°C under diffuse light. For the biological tests 10 day's old cultures were used.

Biological test

Intact, ripe, pruinous berries with short pedicels of the grape varieties Ezerjó, Kecskemét-11, M-2 and M-4 were inoculated via wounds made by needles, with conidia or mycelia, of the fungi *Botrytis squamosa(?)882*, *Botrytis tulipae*, *Botrytis allii*, *Botrytis cinerea* and *Botrytis cinerea(?)9;* the inocula were taken from the total surface of linear cultures. From each grape variety 10 – 10 berries were placed for incubation into sterile Petri dishes, in five repetitions for each fungus species. In course of continuous experiments for each variation at least 250 berries were investigated. During the 13 days of incubation the spread of infection on the berries was daily measured (% of berry surface covered by the fungus). In the evaluation also the per cent of infection on check berries (spontaneous infection) was considered.

Preparation of the infected berries for anthocyan- and total polyphenol content studies

After removing the short pedicels, the infected berries were homogenized, then heat-treated at 50°C for 30 minutes (GUETOV *et al.*, 1972). The sugar content of the homogenate disturbed the determination of the total phenol content, the anthocyan- and polyphenol components were therefore precipitated by basic lead acetate. DRAVERT (1961) recommended in case of red grapes the addition of lead acetate amounting to 0.5 of the sample to be treated; in case of white grapes one-tenth of the sample volume was sufficient according to the author. These amounts of lead acetate were found inadequate in our experiments, therefore, in the red M-2 and M-4 grapes 1.5 ml/2.5 ml, in the red Kecskemét-11 0.75 ml/2.5 ml, in the white Ezerjó 0.5 ml/2.5 ml lead acetate was used. A repeated washing of the precipitate was necessary for removing the sugar residues. As a deviation of DRAVERT's method, the precipitate was dissolved in ethanol containing 10% hydrochloric acid and completed to the original (2.5 ml) volume.

The total anthocyan content was measured according to the method of AUBERT (1970). The extinction values of the preparations dissolved in ethanol + + hydrochloric acid were measured at 550 nm wavelength, on a spectrophotometer (type Spektromom 202, MOM, Budapest).

The determination of the total phenol content was carried out with the method of SINGLETON and ROSSI (1965). The extinction of the blue colour developed with the FOLIN–CIOCALTEU reagent and with 20% Na_2CO_3 was measured at 765 nm in the spectrophotometer mentioned above.

For separating the anthocyan and polyphenol components, the original method of DRAVERT was modified by applying the samples on a cellulose layer (of 0.5 mm thickness) and also the composition of the running buffer was modified. The identification was carried out based on the R_f values of pure anthocyan and phenol fractions which were run parallelly.

The influence of phenol- and anthocyan compounds of grape berries on Botrytis species

The purified equivalents of polyphenol and anthocyan compounds, whose presence in the grape berries had been previously determined by chromatography, as well as other polyphenol compounds commonly occurring in grape berries, were tested on *Botrytis* species, by using the agar-diffusion method. In Petri dishes of 10 cm diameter, 0.1 ml quantities of thick *Botrytis* spore suspensions were spread on the surface of solidifying malt-agar gels. From the anthocyans or phenols to be tested, 0.1 ml quantities of stock-solutions were dropped into the circular holes cut into the agar layer. The radius of the inhibition zone was measured after a four-day incubation at 28°C. The compounds tested were the following: catechin, cumaric acid, coffeic acid, gallic acid, tannic acid, cinnamic acid, chlorogenic acid, phloroglucin, ellagic acid, malvidin and cyanidin. The analytically pure authentic compounds were used in aqueous solutions ten times more concentrated than their real concentrations in grape berries.

Results

Evaluation of the biological test

During the time of incubation the extension of the *Botrytis* colonies was measured daily on the infected berries. The rate of infection has been expressed in percentage value of the berry surface. As the inoculation was carried out artificially, the begin of infection could be accepted from the time of the treatment. In Fig. 1 the percentage values of the berries were shown which attained or surpassed the 50% infection on the 3., 5., and 13. day; the latter infection level was regarded as basic infection (Fig. 1).

The experiment supported the already known fact that the grape Ezerjó is one of the most susceptible varieties against *Botrytis;* the colonies of the fungus appeared the earliest and their spread was the fastest on this variety.

M-2 showed a similar susceptibility, although on the berries some *Botrytis* species *(Botrytis squamosa(?)882, Botrytis cinerea)* spread slower than on Ezerjó.

The variety Kecskemét-11 showed the highest grade of resistance against the five *Botrytis* species and the slow spread of the fungi on the berries was significant.

M-4 could be placed from the point of view of resistance between the varieties M-2 and Kecskemét-11 and the spread on the berries was generally faster than on Kecskemét-11.

The *Botrytis* species studied could be ranged for the grade of infectivity: the highest infection was caused by *Botrytis cinerea(?)9* and by *Botrytis squa-*

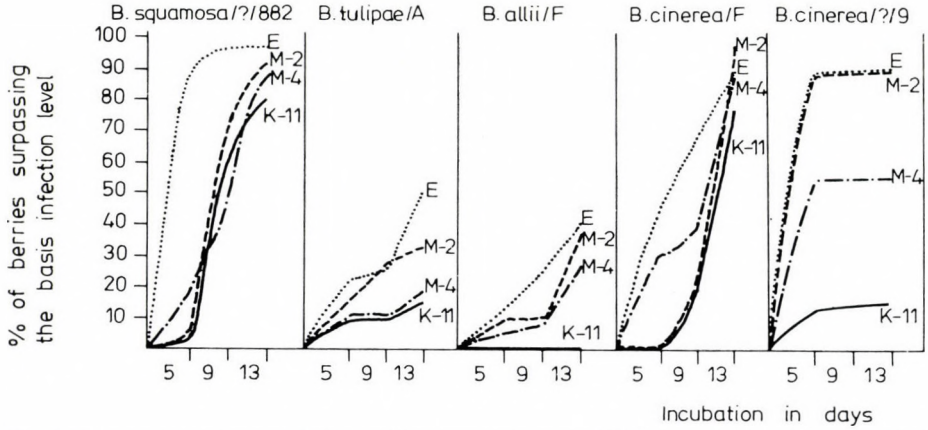


Fig. 1. Changes in *Botrytis* infection (%) of grape berries of different varieties. Kecskemét-11 variety was found to be the most resistant to *Botrytis* infection, Ezerjó the most susceptible. Those *Botrytis* species *(Botrytis allii, Botrytis tulipae)* for which the grapevine is no host plant, caused only medium damage on the varieties studied

mosa(?)882, followed by *Botrytis cinerea*, while *Botrytis allii* and *Botrytis tulipae* were markedly less infectious.

Although the different *Botrytis* species may cause different damage on the berries, the rate of infection depends by all means on the resistance factors of the grape present.

Changes in the anthocyan content as a result of Botrytis infection

The grape varieties studied showed different colour, contained therefore anthocyan in different quantities. The healthy grapes of M-4 contained 4.15 g/l, M-2 contained 1.89/l, the berries of Kecskemét-11 contained 0.27 g/l anthocyan. As a result of *Botrytis* infection, the anthocyan content decreased in each case; the rate of decrease was proportional to the extent of infection (Fig. 2).

In the Kecskemét-11 variety the juice of berries infected by *Botrytis squamosa(?)882* contained 13 days after incubation only 13.7% of the original anthocyan, the ones infected by *Botrytis cinerea* 14.6%, and the berries infected by *Botrytis cinerea(?)9* 11.9% of the original value. The remaining anthocyan con-

tent was higher in case of the less active *Botrytis tulipae* (35.5% of the original value) and in case of *Botrytis allii* the initial value decreased to 39.2%.

In the M-4 variety *Botrytis squamosa(?)882* decreased the initial anthocyan content to 36.2%, *Botrytis cinerea* to 32.2%, *Botrytis cinerea(?)9* to 27.8%. *Botrytis allii* and *Botrytis tulipae* showed also in this case a lower activity (decrease to 42.2 and 54.5% respectively).

In the M-2 variety the remaining anthocyan content was surprisingly low

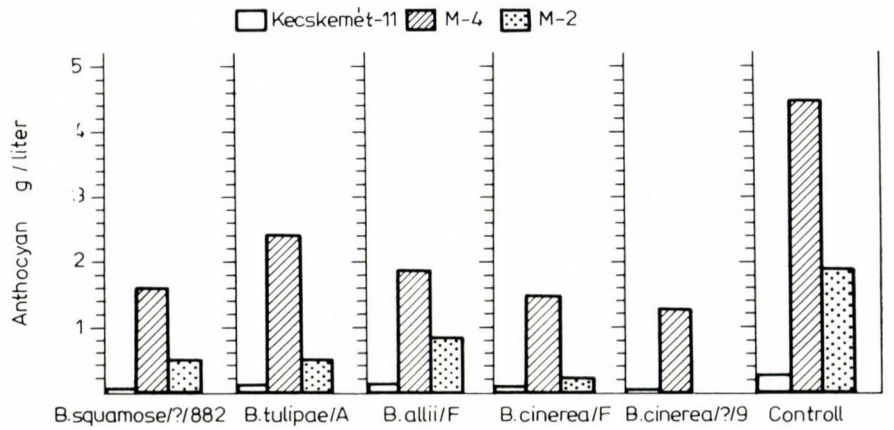


Fig. 2. Changes in the anthocyan content of grape berries as a result of *Botrytis* attack. The anthocyan content decreased in the grape varieties studied, after *Botrytis* infection. The decrease was consistently proportional to the grade of infection

following the infection by *Botrytis allii* and *Botrytis cinerea* (4.3 and 1.07% of the original content, respectively), while the fungi *Botrytis squamosa(?)882* and *Botrytis tulipae* decreased the initial level both to 29.0% (Table 1).

Table 1

Evaluation of remaining anthocyan and polyphenol contents in four grape varieties on the 13th day after *Botrytis* infection (compared to initial level)

Botrytis species	Ezerjó		Kecsk.-11		M-4		M-2	
	remaining							
	antho-cyan %	poly-phenol %	antho-cyan %	poly-phenol %	antho-cyan %	poly-phenol %	antho-cyan %	poly-phenol %
B. squamosa (?)882	—	26.6	13.7	16.7	36.2	51.2	29.0	63.8
B. tulipae	—	27.6	35.5	41.5	54.5	75.0	29.0	46.7
B. allii	—	53.2	39.2	75.1	42.2	47.0	4.3	49.8
B. cinerea	—	44.9	14.6	97.0	32.2	69.0	1.07	32.0
B. cinerea(?)9	—	70.5	11.9	64.5	27.8	29.5	—	—

The decrease in anthocyan- and polyphenol content was in correlation with the rate of infection of *Botrytis squamosa(?)882* and *Botrytis tulipae*. The *Botrytis* attack caused a moderate decrease in the polyphenol content.

Changes in polyphenol content as a result of Botrytis infection

The total phenol content of the grape varieties studied was the following as measured before inoculation: M-4: 2.91 g/l, M-2: 2.38 g/l, Ezerjó: 1.10 g/l, Kecskemét-11: 0.82 g/l. The content of polyphenols also decreased as a result of *Botrytis* infection. In case of *Botrytis squamosa(?)882* and *Botrytis tulipae* good correlations were observed with the grade of infection and anthocyan decrease. The same could be noticed with *Botrytis allii* on the varieties Ezerjó and M-4 (Fig. 3).

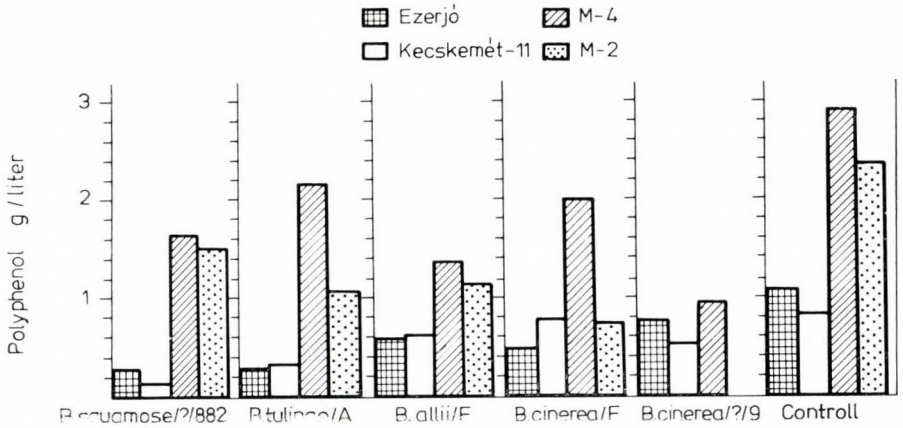


Fig. 3. Changes in the total polyphenol content of grape berries as a result of *Botrytis* attack. Depending on *Botrytis* species, the total polyphenol content of the berries decreased after *Botrytis* infection. In case of *Botrytis cinerea* and *Botritys cinerea(?)9* the polyphenol level decreased less than it was expected on the basis of the grade of infection

In berries infected with the fungi *Botrytis cinerea* and *Botrytis cinerea(?)9* the remaining polyphenol content was exceptionally high even in case of severe infection; the same was noticed with *Botrytis allii* on Kecskemét-11 and M-2 varieties. In the last mentioned varieties, however, the anthocyan content decreased in a good correlation with the grade of infection.

The resistance of our grape varieties studied could be attributed to one or more polyphenol compounds.

Evaluation of anthocyan and phenol components separated by thin-layer chromatography

The data of anthocyan and phenol components, separated in 4 : 1 : 5 BAW with thin-layer chromatography from the berry juices of Ezerjó, Kecskemét-11, M-4, M-2 varieties are shown in Table 2.

In our experiments the variety Kecskemét-11 was found the most resistant against *Botrytis* attack; its only characteristic anthocyan is cyanidine. In the varieties M-2 and M-4 components of identical R_f values were found. M-4 gave by

Table 2

Anthocyan and polyphenol reactions

Grape variety	Spot No.	R_f value	Colour/in phenols, after developing	Identified compound	Standard R_f value	Colour of standard /in phenols after developing
Ezerjó	1.	0.33 - 0.38	greyish blue	—	—	—
	2.	0.48 - 0.58	greyish blue	—	—	—
	3.	0.68 - 0.75	greyish blue	tannic acid	0.68 0.69	greyish blue
	4.	0.82 - 0.90	greyish blue	cumaric acid	0.90 0.94	greyish blue
Kecskemét-11	1.	0.47 - 0.58	crimson-red	cyanidin-monoglycoside	0.48 - 0.54	crimson red
	2.	0.57 - 0.63	greyish blue	—	—	—
	3.	0.63 - 0.68	greyish blue	—	—	—
	4.	0.64 - 0.76	greyish blue	tannic acid	0.68 - 0.69	greyish blue
	5.	0.88 - 0.93	greyish blue	cumaric acid	0.90 - 0.94	greyish blue
M-4, M-2	1.	0.20 - 0.30	deep purple	—	—	—
	2.	0.37 - 0.47	scarlet	—	—	—
	3.	0.46 - 0.53	scarlet	malvidin-monoglycoside	0.43 - 0.46	scarlet
	4.	0.48 - 0.58	crimson-red	cyanidin-monoglycoside	0.48 - 0.54	crimson red
	5.	0.53 - 0.60	purple-pink	malvin-aglycon	0.54 - 0.55	purple-pink
	6.	0.55 - 0.61	light crimson	cyanin aglycon	0.55 - 0.57	light crimson
	7.	0.60 - 0.66	purple red	delphinidin monoglycoside	0.57 - 0.61	purple red
	8.	0.71 - 0.75	light pink	—	—	—
	9.	0.77 - 0.79	light purple	—	—	—
	10.	0.83 - 0.86	purple pink	—	—	—

its higher anthocyan content more intensive spots than M-2. As the thin-layer chromatography studies showed, the number of anthocyan and polyphenol components did not decrease as a result of *Botrytis* infection, the intensities of the components (spots), however, decreased according to the grade of infection.

Effect of common polyphenol- and anthocyan compounds of grape on Botrytis species

By using the test method described in the chapter "Material and Methods" by the measurements of inhibited zones it was established that tannic acid

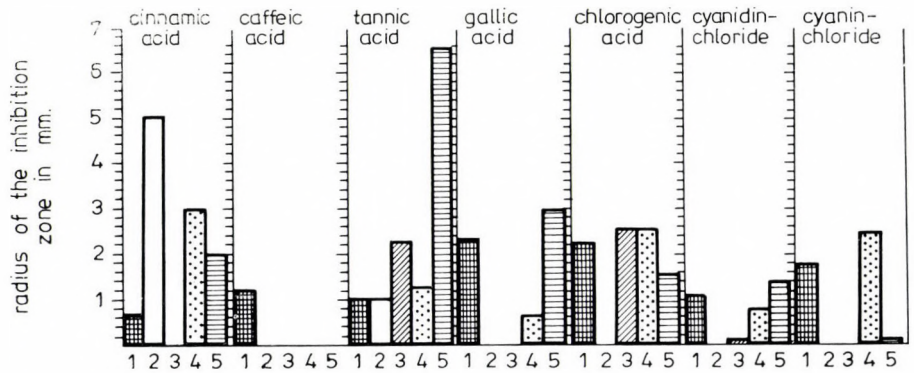


Fig. 4. Study of inhibitory effect of polyphenol and anthocyan compounds of grape berries on *Botrytis* species (agar-diffusion method). Legend: 1 = *Botrytis squamosa(?)882*, 2 = *Botrytis tulipae*, 3 = *Botrytis allii*, 4 = *Botrytis cinerea*, 5 = *Botrytis cinerea(?)9*. After four day incubation, according to *Botrytis* species and tested compound, differences were noted in *Botrytis* development. The phenol compounds exhibited more effective inhibition than anthocyan standards

(MUKHEREJE and KUNDU, 1973) inhibited the growth of all the five *Botrytis* species studied and was especially effective against *Botrytis cinerea(?)9*. Cinnamic acid proved to be ineffective against *Botrytis allii*, chlorogenic acid against *Botrytis tulipae*. Gallic acid did not inhibit the growth of *Botrytis tulipae* and *Botrytis allii*, caffeic acid, however, gave only against *Botrytis squamosa(?)882* a measurable inhibitory zone.

The following polyphenols did not inhibit any of the *Botrytis* species tested: catechin, cumaric acid, phloroglucin, ellagic acid.

From the anthocyan standards tested, cyanidin chloride and cyanin chloride inhibited *Botrytis* growth, malvidin, however, did not exhibit any activity against the *Botrytis* fungi (Fig. 4).

Conclusions

Our grape varieties showed resistance to a different degree against the *Botrytis* species included in the experiments. The highest resistance was observed with

K-11 (Kecskemét-11), followed by M-4, M-2 and Ezerjó. From the *Botrytis* fungi studied, the grape pathogens belonging to the morphological range "*cinerea*" showed the highest activity. As a result of infection – independently of grape variety – the anthocyan content of the berries decreased; in our experiments the decrease in the anthocyan level depended consistently on the rate of infection. From the grape varieties studied Ezerjó did not contain anthocyans; in M-2 and M-4 we succeeded to identify as main components malvidin, to a smaller extent cyanidin and delphidin, whereas in Kecskemét-11 only cyanidin was found. The results indicated that the resistance depended more on the quality of anthocyans present and less on their quantity. This was shown by the fact that the variety M-4, exhibiting the highest total anthocyan content, was less resistant than Kecskemét-11. The only anthocyan of the latter, cyanidin inhibited markedly the pathogenic *Botrytis* species for which grapevine is a regular host plant. From the two varieties M-4 and M-2, the variety M-4 showed a higher resistance.

In the "*in vitro*" experiment the polyphenol content decreased in all variations, independently of grape variety (AUBERT and POUX, 1969). The decrease of the polyphenol content did not show, however, in all cases a close correlation with the rate of infection. In all grape varieties the fungi *Botrytis cinerea* and *Botrytis cinerea(?)9*, and in Kecskemét-11 *Botrytis allii* did not lower the polyphenol level to any extent, as it could have been expected from the degree of infection. In the resistance against these fungi, therefore, the particular phenol components may play a significant role.

Similarly to the anthocyans, the resistance depends not on the absolute values of phenol level, but on the particular phenol components, as shown by the example of the least resistant grape variety Ezerjó, exhibiting a considerably higher total phenol level than the most resistant Kecskemét-11. In our experiments carried out with standard compounds, tannic acid was especially effective against the *Botrytis* species tested, as well as chlorogenic acid and cinnamic acid.

With the methods used in this study, we did not succeed yet to separate all the phenol components of the grape. We established the changes in the total phenol content resulting from *Botrytis* attack. However, there remains still the task to detect the ratio of the individual components in the healthy, intact berries and in the ones infected by *Botrytis*. Based on our results, it may be assumed that for the chemical axeny of the grape berries partly the anthocyan compounds, partly the polyphenols are responsible. The extent of axeny seems to depend in the first place on the type of the components and not on their total level.

Literature

AUBERT, S. and POUX, C. (1969): Extraction des composés phénoliques du raisin. Ann. Technol. Agric. *18*, 2, 111–127.

AUBERT, S. (1970): Dosages des composés phénoliques totaux. Ann. des Falsifications *63*, 108–117.

BOLCATO, V., LAMPARELLI, F. and LOSITO, F. (1964): Die Wirkung von Botrytis cinerea und Hefe auf die Farbstoffe von Traubenmosten. Rev. Enol. Viticolt. *17*, 415–421.

BREIDER, H. (1972): Über resistente Reben-Arthybriden und ihre Qualitätsleistungen. Mitteilungen *22*, 232–243.

DRAWERT, F. (1961): Über Anthocyane in Trauben, Mosten und Weinen. Vitis *2*, 288–304.

GREGORIEV, Z. and TODOROV, K. (1970): Vergleichende Schätzung der Resistenz von Trauben einiger Rebsorten gegenüber Botrytis cinerea. Losarstvo i viner (Sofia) *19*, 20–25.

GUETOV, G., NEDELTCHEV, N. and TZAKOV, D. (1972): Evolution des anthocyanes au cours de la vinification et de la conservation des vins. Bull. OIV. Paris *45*, 338–345.

HENKE, O. (1959): Untersuchungen über den Einfluss von Vitis cinerea Arnold auf einige Biochemische Eigenschaften der Kreuzungsnachkommen. Zeitschr. f. Pflanzenzüchtung, *41*, 253–270.

KIRÁLY, Z. (1968): Növényi betegségellenállóság élettana (Physiology of Plant Disease Resistance). In Hungarian. Akadémiai Kiadó, Budapest, pp. 1–138.

KUBICKAIA, M. (1969): Parasitische Aktivität der Botrytisstämme der Rebe. Selchozj. Biol., Moskva, pp. 709–712.

MUKHEREJE, M. and KUNDU, B. (1973): Antifungal activities of some phenoles and related compounds to three fungal plant pathogens. Phytopath. Z., *78*, 89–92.

PLISANOVSKII, V. A. and ZOTOV, V. V. (1971): Resistenzfaktoren der Traubenbeeren gegen Botrytis cinerea. Selchozj. Biol., *5*, 775–777.

POMPEI, C. and PERI, C. (1971): Einfluß der festen Teile der Traube auf die Konzentration der Polyphenole der Weine und auf die Empfindlichkeit gegen Alterung. Am. J. Enol. and Vitic. *22*, 55.

RIBEREAU-GAYON, P. (1971): Evolution des composés phénoliques au cours de la maturation du raisin. Conn. Vigne, Vins, pp. 247–261.

SINGLETON, V. L. and ROSSI, J. (1965): Colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. Am. J. Enol. Viticulture *16*, 144–158.

SINGLETON, V. and ESAU, L. (1969): Phenolic substances in Grapes and Wine and their significance. Acad. Press New York and London, pp. 131–133.

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Biochemistry of Resistance and Susceptibility in Cotton to *Alternaria macrospora*

By

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Total phenols, *ortho* dihydroxy (O. D.) phenols, total nitrogen, amino nitrogen, reducing sugars, non-reducing sugars, polyphenol oxidase (PPO) and peroxidase were estimated in healthy and *Alternaria macrospora* Zimm. inoculated *Gossypium hirsutum* L. variety MCU 5 (susceptible) and *G. arboreum* L. variety K. 7 (resistant) cotton leaves at periodic intervals. PPO and peroxidase activities were high in the resistant variety when compared to the susceptible variety. On inoculation, peroxidase activity increased in both the varieties, whereas PPO activity decreased. Total and amino nitrogen were high in the susceptible variety and inoculation decreased the nitrogen content. On inoculation, total nitrogen decreased in the resistant variety, and amino nitrogen slightly rose.

In recent years the leaf spot disease of cotton caused by *Alternaria macrospora* Zimm. has become a severe one in Tamil Nadu (India). Cotton varieties widely differ in their resistance to pathogenic attack by *A. macrospora*. Preliminary screening trials revealed that *Gossypium hirsutum* L. (variety MCU 5) is susceptible and *G. arboreum* L. (variety K.7) is resistant to *A. macrospora*. The information available on the nature of resistance and susceptibility exhibited by different cotton varieties to the leaf spot disease is scant. Certain physiological changes in these two cotton species as influenced by *A. macrospora* inoculation, from the point of view of host-parasite interrelationship, so as to interpret the biochemical nature of host resistance and susceptibility were studied and reported in this communication.

Materials and Methods

The two cotton species were grown in pots under field conditions. Twelve-day-old seedlings were sprayed with a heavy conidial suspension of *A. macrospora* and the pots were kept in a humid chamber. Leaves were used for all estimations. Samples were analysed at 0, 1, 3, 5 and 7-days after inoculation.

Enzyme extraction: Tissues were crushed in 5 ml of 0.1 M sodium phosphate buffer at pH 7.1 in a chilled porcelain mortar. The extract was filtered, centrifuged

at 2100 g for 20 min and the supernatant was used for enzyme assays. All the assays were performed in "Spectronic 20" colorimeter. Control cuvettes contained the same concentrations of the reaction mixture as that of the treatment except that the enzyme source was heated for 30 min at 100 °C (SRIDHAR *et al.*, 1969).

(i) Polyphenol oxidase (PPO): The reaction mixture contained 0.5 ml of 0.2 M sodium phosphate buffer at pH 7.0, 0.5 ml of 0.1 M catechol, 1.5 ml of distilled water and 0.5 ml of enzyme extract. Changes in absorbance at 495 mμ (MATTA and DIMOND, 1963) were measured at 30 sec intervals up to 3 min.

(ii) Peroxidase: The reaction mixture consisted of 0.1 ml of the enzyme extract, 1.0 ml of 0.001 M pyrogallol in 0.05 M sodium phosphate buffer at pH 6.5, 0.1 ml of 0.1 per cent hydrogen peroxide and 1.8 ml of distilled water. Absorbance changes at 470 mμ were recorded at 30 sec intervals up to 3 min (HAMPTON, 1963).

Ethanol extraction: Three grams of leaves were cut into pieces and extracted in 12 ml of boiling ethanol for 5 min. The extract was cooled, crushed in a mortar, filtered through cheese-cloth and the residue was re-extracted with 3 ml of ethanol. The filtrates were pooled, filtered through Whatman No. 42 filter-paper and the final volume was raised to 15 ml so as to represent 5 ml of extract for every g of tissue used (MAHADEVAN *et al.*, 1965; BHASKARAN and PRASAD, 1971). The extract was used for the estimation of total phenols, *ortho* dihydroxy (O.D.) phenols, amino nitrogen, reducing sugars and non-reducing sugars. In all the estimations 1 ml of extract was used and duplicate determinations were conducted. Simultaneously, the moisture content of a representative sample was determined by drying it in an oven at 105 °C for 25 hrs.

Estimation of phenols: Total phenols were estimated by employing Folin-Ciocalteau reagent (BRAY and THORPE, 1954) and O.D. phenols by using Arnow's reagent (JOHNSON and SCHAAL, 1957). Standard curves prepared from catechol were used to calculate the unknowns.

Estimation of nitrogen: Amino nitrogen was estimated by MOORE and STEIN's (1958) method using ninhydrin reagent. Total nitrogen was estimated by digesting 0.1 g oven dried (at 60 °C for 3 days) sample with a pinch of digestion mixture (10 parts of K_2SO_4, 1 part of $CuSO_4$ and 0.1 part of selenium metal powder), 4 ml of sulphuric-salicylic acid mixture and a few crystals of sodium thiosulphate. From the mixture ammonia was steam distilled in 0.1 N H_2SO_4 containing 1 or 2 drops of methyl red indicator. The excess of acid was back-titrated against 0.1 N KOH till the golden yellow colour appeared (BREMNER, 1960). From the volume of H_2SO_4 utilized, the quantity of total nitrogen was calculated.

Estimation of sugars: Reducing sugars were estimated by NELSON's (1944) method. Non-reducing sugars were hydrolyzed in 1 N H_2SO_4 at 50 °C for 30 min (INMAN, 1965) and estimated as reducing sugars.

Results

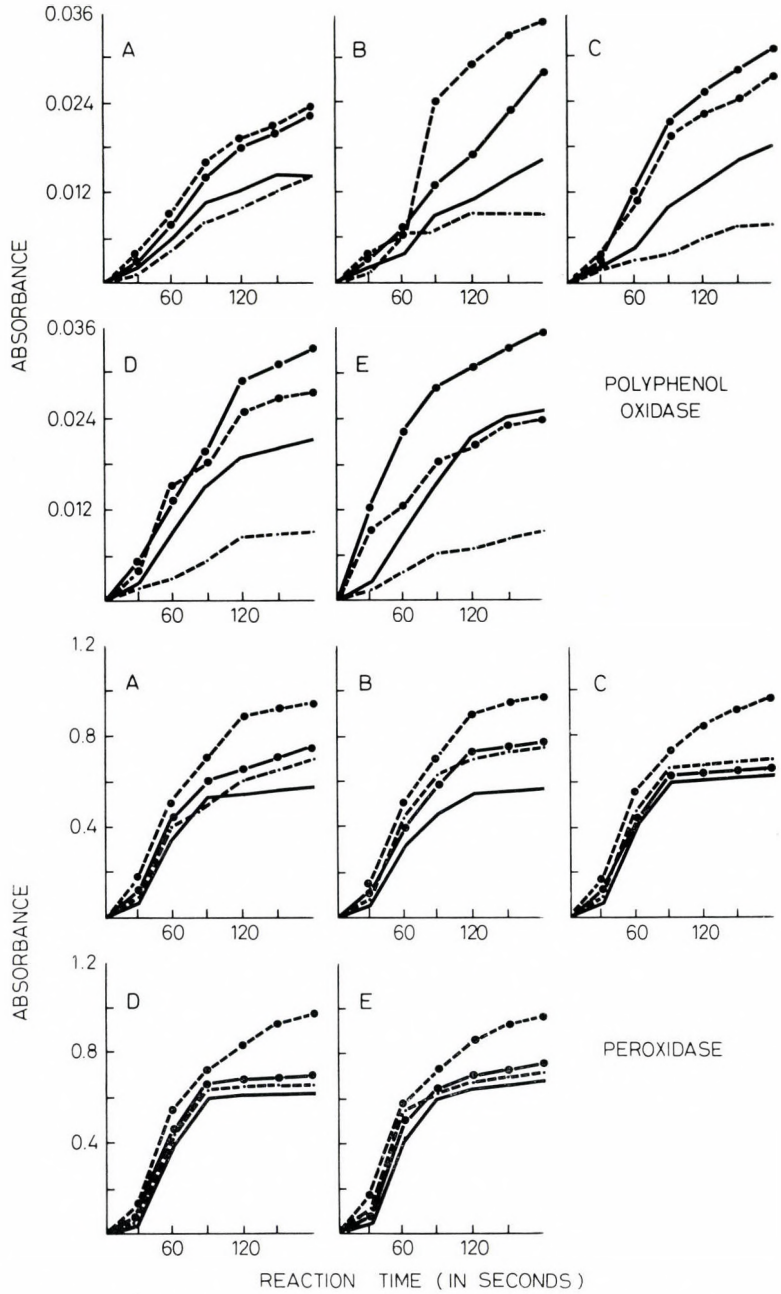
Oxidase activity: PPO and peroxidase activity was high in resistant leaves when compared to the susceptible one. PPO activity decreased on inoculation except in first day sample of the resistant variety (Fig. 1) whereas peroxidase activity increased (Fig. 2).

Table

Changes in total phenols, orthodihydroxy phenols, total nitrogen, amino nitrogen, reducing sugars and non-reducing sugars (as mg/g dry weight basis) in *Alternaria* inoculated cotton plants

	Sampling days	*Gossypium hirsutum*		*Gossypium arboreum*	
		healthy	inoculated	healthy	inoculated
Total phenols*	0	3.751	3.751	5.682	5.682
	1	3.830	4.942	5.750	7.160
	3	3.615	5.257	5.810	6.245
	5	3.730	5.570	5.397	5.757
	7	3.850	5.615	5.155	5.452
Orthodihydroxy phenols*	0	1.500	1.500	1.920	1.920
	1	1.541	1.420	1.910	2.113
	3	1.498	1.310	2.032	2.321
	5	1.581	1.275	2.001	2.355
	7	1.590	1.82	2.025	2.100
Total nitrogen	0	8.112	8.112	5.315	5.315
	1	8.210	8.012	5.315	5.320
	3	8.188	7.865	5.212	5.115
	5	7.988	7.812	5.272	5.015
	7	7.875	7.793	5.254	5.012
Amino nitrogen**	0	3.195	3.195	2.128	2.128
	1	3.174	3.235	2.224	2.212
	3	3.115	3.312	2.112	2.312
	5	3.012	3.082	2.142	2.315
	7	3.012	2.875	2.184	2.335
Reducing sugars***	0	3.213	3.213	2.532	2.532
	1	3.113	3.143	2.513	2.338
	3	3.082	2.974	2.448	2.332
	5	2.842	2.742	2.445	2.172
	7	2.838	2.532	2.325	2.028
Non-reducing sugars***	0	3.612	3.612	3.732	3.732
	1	3.612	3.582	3.735	3.614
	3	3.685	3.434	3.812	3.512
	5	3.732	3.417	3.942	3.514
	7	3.854	3.414	3.945	3.427

* Expressed as catechol equivalents
** Expressed as glutamic acid equivalents
*** Expressed as glucose equivalents



Figs 1 and 2.——— *G. hirsutum* (healthy); --------- *G. hirsutum* (inoculated); — .— .— *G. arboreum* (healthy); --- .--- .--- .--- *G. arboreum* (inoculated). Sampling: 0 hour (A), 1st day (B), 3rd day (C) 5th day (D) and 7th day (E)

Phenols: Total and O.D.phenols were more in resistant tissues than the susceptible one. On inoculation, total phenols increased in the latter. In the resistant variety total phenols increased initially and decreased gradually after three days (Table). In the susceptible variety, O.D.phenols decreased on inoculation, whereas in the resistant one, O.D.phenols accumulated (Table).

Nitrogen: Susceptible tissue was characterized by higher quantities of total and amino nitrogen when compared to the resistant one. On inoculation, even though there was an initial rise in amino nitrogen in the susceptible variety, the quantity decreased after the 3rd day. In the resistant tissue, there was slight increase in amino nitrogen on inoculation (Table). Total nitrogen decreased after inoculation in both the varieties (Table).

Sugars: While reducing sugars were more in the susceptible variety than the resistant one, non-reducing sugars were more in the latter. Both reducing and non-reducing sugars decreased after inoculation in both the varieties (Table).

Discussion

In host-parasite interactions, phenolic compounds are shown as possible defenders against pathogenic invasion (TOMIYAMA, 1963; MAHADEVAN, 1966; BHASKARAN and PRASAD, 1971; 1972). MAHADEVAN (1965) found inhibition of pectinolytic and cellulolytic enzymes of the pathogen by phenols and their oxidation products. *In vitro* studies conducted in this laboratory, also revealed that catechol, a constituent of cotton plant, inhibited the growth and pectinolytic enzyme synthesis of *A. macrospora* (Unpublished data). Hence the high concentration of total and O.D. phenols in the resistant variety can be attributed to its resistance to pathogenic invasion. Immediately after inoculation, total phenols increased in both the varieties. Phenols are stored in the vacuoles of plant cells in the form of phenolic glucosides (PRIDHAM, 1965) and the accumulation of phenolic compounds may be explained by their release from the glucosides by β-glucosidase of either the host or the pathogen. Another important mechanism of phenolic accumulation is the enhanced synthesis by the host through shikimic acid pathway (YOSHIDA, 1969) which is activated as a result of infection (GOODMAN *et al.*, 1967). Even though accumulation of phenolic compounds was observed after inoculation in both the varieties, in the resistant variety, the rate of accumulation was rapid which arrests the pathogenic development at the site of infection. The reduction in total phenols from the third day onwards in the inoculated resistant variety suggests the probable operation of the following reactions: i) incorporation of phenols into lignins or entering into the biochemical pathway after the rings are oxidatively split and ii) oxidative polymerization into melaninlike substances (THOMSON, 1964).

On inoculation *ortho* phenols decreased in susceptible leaves, whereas the quantity increased in resistant tissues. The reduction can be attributed to the

utilization by the pathogen as carbon source (BHASKARAN, 1971) or its polymerization to ligninlike compounds (THOMSON, 1964). GOODMAN *et al.* (1967) found that PPO system has a cresolase activity which catalyzes the synthesis of *ortho* diphenol from monophenol oxidatively. A similar reaction may probably take place in the inoculated resistant variety leading to the accumulation of O.D. phenols.

Ortho phenols upon oxidation become highly reactive and may result toxic to pathogen or inhibit enzymes (OKU, 1965; KUĆ, 1966; MAHADEVAN, 1966). The oxidation is mediated by PPO and peroxidase (TOMIYAMA, 1963) and the resulting quinones are effective inhibitors of sulfhydryl enzymes, which may inhibit the metabolic activities of the pathogen (RUBIN and ARTSIKHOVSKAYA, 1963; KUĆ, 1964). A similar phenomenon may exist in the resistant cotton variety. The higher activity of phenol oxidizing enzymes, PPO and peroxidase in the resistant variety than in the susceptible one, and activation of peroxidase immediately after inoculation, suggest a probable mechanism of disease resistance. The enhanced activity of phenol oxidizing enzymes may result from an increased synthesis by the host (GOODMAN *et al.*, 1967), activation of its latent enzymes (NAKAMURA and OKU, 1960) and also production by the pathogen.

Total and amino nitrogen was more in the susceptible leaves than the resistant one. High nitrogen content may reduce the toxicity of phenols (FLOOD and KIRKHAM, 1960; CHANDRAMOHAN *et al.*, 1967), thereby rendering the plant susceptible. Nitrogen content decreased on inoculation in the susceptible variety which can be attributed to i) pathogenic utilization (MCCOMBS and WINSTEAD, 1964; RAGHUNATHAN *et al.*, 1966) and ii) oxidative deamination of amino acids (HESS, 1958). The aromatic ring of the phenolic compounds is provided by the amino acids like phenylalanine and tyrosine (GOODMAN *et al.*, 1967) and since inoculation increased the total phenols it is suggested that aromatic amino acids be utilized for synthesis of phenolic compounds.

There was depletion of reducing and non-reducing sugars in the leaves of both the varieties due to inoculation. RAMAKRISHNAN (1966) observed a curtailment in reducing sugar synthesis in rice leaves due to *Piricularia oryzae* Cav. infection. Such changes in a susceptible variety can be attributed directly to pathogenic utilization or indirectly to altered metabolism of the host. Utilization of sugars by *Cercospora musae* Zimm. from banana leaves was reported by JAYAPAL and MAHADEVAN (1968). Reduction in sugar content in resistant leaves, on inoculation, can be attributed to a major part of them being shunted to polyphenol synthesis (NEISH, 1964). This evidence supports the rapid phenolic accumulation in resistant leaves after inoculation.

Considering all these factors, it can be suggested that the higher content of reducing sugars and nitrogen in *G. hirsutum* than in *G. arboreum* may lead to its susceptibility to *A. macrospora*. The toxicity of phenols is also reduced by high content of amino nitrogen. Regarding the factors leading to resistance, the resistant host has a higher quantity of phenols, besides the sugars and aromatic

amino acids being shunted to polyphenol synthesis, which arrest the development of the pathogen and inhibit the production of hydrolytic enzymes.

Literature

BHASKARAN, R. (1971): Studies on *Fusarium* wilt of musk melon. M. Sc. (Ag.) thesis, Annamalai Univ., India, p. 168.

BHASKARAN, R. and PRASAD, N. N. (1971): Certain biochemical changes in *Cucumis* species in response to *Fusarium* infection. Phytopathol. Medit. *10*, 238–243.

BHASKARAN, R. and PRASAD, N. N. (1972): Role of preformed inhibitors in *Cucumis* sp. in wilt resistance. Phytopathol. Z. *73*, 75–77.

BRAY, H. G. and THORPE, W. Y. (1954): Analysis of phenolic compounds of interests in metabolism. Meth. biochem. Analysis. *1*, 27–52.

BREMNER, J. M. (1960): Determination of nitrogen in soil by Kjeldahl method. J. agric. Sci. Camb. *55*, 11–33.

CHANDRAMOHAN, D., MAHADEVAN, A. and RANGASWAMI, G. (1967): Studies on some biochemical properties of leaf tissues of *Amaranthus tricolor* as related to resistance to infection by *Alternaria* sp. Indian Phytopathol. *20*, 109–113.

FLOOD, A. E. and KIRKHAM, D. S. (1960): The effect of phenolic compounds on the growth and sporulation of two *Venturia* spp. In: Phenolics in Plants in Health and Disease, Pergamon Press, London.

GOODMAN, R. N., KIRÁLY, Z. and ZAITLIN, M. (1967): The Biochemistry and Physiology of Infectious Plant Disease, D. Van Nostrand Company, Inc. Princeton, New Jersey, Toronto, London and Melbourne, pp. 354.

HAMPTON, R. E. (1963): Activity of some soluble oxidases in carrot slices infected with *Thielaviopsis basicola*. Phytopathology *53*, 497–499.

HESS, E. H. (1958): The polyphenolase of tobacco and its participation in amino acid metabolism: I. Manometric studies. Arch. Biochem. Biophys. *74*, 198–208.

INMAN, R. E. (1965): Qualitative sugar changes in barley infected with a facultative parasite. Phytopathology *55*, 341–345.

JAYAPAL, R. and MAHADEVAN, A. (1968): Biochemical changes in banana leaves in response to leaf spot pathogens. Indian Phytopathol. *21*, 43–48.

JOHNSON, G. and SCHAAL, L. A. (1957): Chlorogenic acid and other *ortho* dihydric phenols in scab-resistant Russet Burbank and scab-susceptible Triumph potato tubers of different maturity. Phytopathology *47*, 253–258.

KUĆ, J. (1964): Phenolic compounds and disease resistance in plants. In: Symposium: Phenolics in normal and diseased fruits and vegetables, Fourth Annual meeting plant phenolic group of North America, pp. 63–81.

KUĆ, J. (1966): Resistance of plants to infectious agents. Ann. Rev. Microbiol. *20*, 337–370.

MAHADEVAN, A. (1965): Biochemistry of resistance in cucumber against *Cladosporium cucumerinum*. II. The role of cucumber phenols on the growth and secretion of pectinolytic enzymes and on pectinolytic enzyme activity. Indian Phytopathol. *18*, 323–324.

MAHADEVAN, A. (1966): Biochemistry of infection and resistance. Phytopathol. Z. *57*, 96–99.

MAHADEVAN, A., KUĆ, J. and WILLIAMS, E. B. (1965): Biochemistry of resistance in cucumber against *Cladosporium cucumerinum*. I. Presence of a pectinase inhibitor in resistant plants. Phytopathology *55*, 1000–1003.

MATTA, A. and DIMOND, A. E. (1963): Symptoms of *Fusarium* wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology *53*, 574–575.

MCCOMBS, C. L. and WINSTEAD, N. N. (1964): Changes in sugars and amino acids on cucumber fruits infected with *Pythium aphanidermatum*. Phytopathology *54*, 233–234.

MOORE, S. and STEIN, W. H. (1958): A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. *211*, 907—913.

NAKAMURA, M. and OKU, H. (1960): Biochemical studies on *Cochliobolus miyabeanus*. IX. Detection of ophiobolin in the diseased rice laves and its toxicity against higher plants. Ann. Takamine Lab. *12*, 266—271.

NEISH, A. C. (1964): Major Pathways of Biosynthesis of Phenols. In: Biochemistry of Phenolic Compounds (E. B. HARBORNE, editor), Academic Press, New York, pp. 618.

NELSON, N. (1944): A photometric adaptation of the Somogyi method for the determination of glucose. J. biol. Chem. *153*, 375—380.

OKU, H. (1965): Host-parasite relationship in *Helminthosporium* leaf spot disease of rice plant. Ann. Sankyo. Res. Lab. *17*, 35—56.

PRIDHAM, J. B. (1965): Low molecular weight compounds in higher plants. Ann. Rev. Pl. Physiol. *16*, 13—36.

RAGHUNATHAN, R., MAHADEVAN, A. and RANGASWAMI, G. (1966): A comparative study of the resistant and susceptible banana varieties to fungal diseases. Indian Phytopathol. *19*, 141—149.

RAMAKRISHNAN, L. (1966): Studies in the host parasite relations of rice. II. Changes in the carbohydrates and phenolics. Phytopathol. Z. *56*, 279—287.

RUBIN, B. A. and ARTSIKHOVSKAYA, E. V. (1963): Biochemistry and Physiology of Plant Immunity. Pergamon Press, Oxford, pp. 358.

SRIDHAR, R., CHANDRAMOHAN, D. and MAHADEVAN, A. (1969): The role of parasite and its metabolites in triggering host physiology. Phytopathol. Z. *64*, 21—27.

THOMSON, R. H. (1964): Structure and reactivity of phenolic compounds. In: Biochemistry of Phenolic Compounds (E. B. HARBORNE, editor), Academic Press, New York, pp. 1—32.

TOMIYAMA, K. (1963): Physiology and biochemistry of disease resistance of plants. Ann. Rev. Phytopathol. *1*, 295—324.

YOSHIDA, S. (1969): Biosynthesis and conversion of aromatic amino acids in plants. Ann. Rev. Pl. Physiol. *20*, 41—62.

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Biochemical Nature of Root Exudates of *Abelmoschus esculentus* L. in Relation to Pathogenesis of Root and Collar Rot Caused by *Rhizoctonia bataticola*

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Root exudates of healthy and diseased 'okra' *(Abelmoschus esculentus)* plants with different ages have been investigated in relation to root and collar rot disease caused by *Rhizoctonia bataticola*. Qualitative and quantitative shifts brought about in the exudation pattern of amino acids and carbohydrates under the stress of root and collar rot infection have been correlated. An increasing trend in the exudation of amino acids and carbohydrates with the age of the plants was of interest to note in both diseased and healthy exudate samples. Qualitatively a greater number of amino acids was exuded by the healthy 'okra' plants, while the increased number of sugars was found to be present in the inoculated series. 'Fastglutamine' and citrulline were not detected in the exudates of inoculated series. Xylose and raffinose were not at all present in the healthy series. In inoculated series most of the amino acids of monoamino-monocarboxylic acid group, glutamine and arginine were exuded in greater amounts while glutamic acid, cystine and lysine in small amounts. Similarly, sucrose was abundantly high in inoculated series, while glucose and galactose were low. The possible explanation of these alterations in the amino acids and carbohydrate levels in response to the pathogenesis have been discussed.

Crude root exudates and sugar fractions were found to stimulate sclerotic germination and mycelial growth, while amino acid fractions were inhibitory in comparison with the control.

Introduction

The role of root exudates and redundant root tissues in governing the susceptibility of soil borne plant pathogens cannot be ignored as the root exudates and redundant tissues may stimulate a rhizospheric flora which may be conducive for the growth of the pathogens. Information on the role of root exudates in pathogenesis by root infecting fungi is limited to a few reports in the literature (JALALI and SURYANARAYANA, 1970; 1971; 1972; MAC RAE and CASTRO, 1967; CLAUDIUS and MEHROTRA, 1973). The review of SCHROTH and HILDEBRAND (1964) discussed root exudates in relation to root pathogens. The present investigation was carried out to demonstrate the significant shift in the amino acid and carbohydrate spectrum of 'okra' root exudates at different stages of plant growth with respect to pathogenesis and to see the effect of root exudate samples of 8 and 15 days growth on sclerotic germination and mycelial growth of the pathogen.

Materials and Methods

Rhizoctonia bataticola was isolated from the infected 'okra' roots. The fungus was tested for its pathogenicity, purified by the standard mycological techniques and subsequently maintained on PDA slants. 'Pusa sawani' variety of 'okra' was used throughout the experiment. Seeds were supplied by I.A.R.I., New Delhi.

Collection of root exudates

Surface sterilized 'okra' seeds were plated on PDA and contamination free healthy seedlings were transferred aseptically in culture cylinders (5 cm diameter, 25 cm long) containing 150 g coarse acid washed sand. Each glass cylinder had an exit tube at the base which was closed by a stop cock. The mouth of glass cylinders was plugged with cotton. Sufficient deionized water was added to moisten the sand uniformly before autoclaving. 15 seedlings were transferred to each cylinder and incubated at 28–30 °C. For collecting the root exudates from inoculated plants, 'okra' seeds were inoculated with 3 mm agar disc cut from the growing colony of *R. bataticola.* After 8 and 15 days of growth the water containing exudates was drained off via an exit tube at the base of culture cylinders, aseptically. The system was flushed 3–4 times with deionized water and the combined exudate samples were centrifuged for 30 minutes at 4000 rpm. The supernatant was decanted, and concentrated under vacuum at 40 °C to one ml for every 25 plants. Exudate samples from healthy uninoculated plants were obtained for comparison.

Fractionation of plant materials

Owing to the high salt contents which interfere in the separation of amino acids, the crude exudate samples were separated into cationic, anionic and neutral fractions by passing through IR-120(H^+) form and IRA-410(acetate form) ionic exchange resin column. The cationic fraction (amino acids) was retained on the IR-120(H^+) resin and recovered with 0.5 *N* ammonia. The eluates thus obtained were evaporated to dryness and then redissolved in 5 ml of 5% isopropanol. IRA-410(acetate form) resin retained the anionic fraction and the effluent from this column contained the neutral fraction (sugars). This neutral fraction was evaporated to dryness and then redissolved in 5 ml of 5% isopropanol.

Analysis of fractions

The various compounds present in cationic fractions (amino acids) and neutral fractions (sugars) were analyzed by paper chromatographic technique.

Amino acids

The amino acids were estimated qualitatively by one and two dimensional descending paper chromatography. The solvent systems used for one dimensional chromatography were butanol–water–acetic acid (40 : 4 : 15 v/v) and butanol–water–acetic acid (20 : 4.5 : 10 v/v). The chromatograms were developed in these two solvents, successively in the same direction for 15–16 hours. Two dimensional chromatograms were developed first in butanol–acetic acid–water (12 : 3 : 5 v/v) and in the second direction with phenol–water (160 : 20 v/v). The chromatograms were sprayed with ninhydrin solution to colour the major spots.

The quantitative analysis of amino acids was made by eluting the individual coloured spots in 5 ml of ethanolic solution of $CuSO_4 \cdot 5H_2O$. The eluates were then subjected to absorbance measurement by using Spectronic 20 (Bausch and Lomb, U.S.A.) at 540 nm. The concentration of each amino acid was calculated with reference to a standard curve.

Carbohydrates

Sugars were analysed qualitatively by one-dimensional chromatographic method. The solvent system was a mixture of n-butanol–acetic acid–water (4 : 1 : 5 v/v). The chromatograms were sprayed with aniline hydrogen phthalate reagent to colour the major spots.

For quantitative analysis individual coloured spots were cut out in 4 ml of colour eluting agent. (0.7 *N* HCl in 80% ethanol v/v). After complete elution their absorbance was determined by Spectronic 20 at 370 nm for hexoses and at 360 for pentoses. The concentration of each sugar was calculated with reference to a standard curve.

Effect of root-exudates, amino acid fractions, sugar fractions and various compounds on sclerotic germination and growth of the pathogen

Collected crude exudate samples, amino acid fractions, sugar fractions, and standards of all detected amino acids and sugars were used to see their effects upon sclerotic germination of pathogen so as to see which of them is stimulatory or inhibitory in the exudate. The experiment was repeated twice.

In order to study the effect of root exudates on growth rate of pathogen, 2% of the concentrated exudate samples, amino acid fractions or sugar fractions (1 ml for every 10 plants) were added to water agar medium. Dishes were plated and inoculated with 4 mm disc of the pathogen and incubated at 28° C. Duplicate plates were used for each sample. The growth rate was observed after every 12 hr of incubation period.

Results

Various amino acids present in the root exudates of healthy and inoculated 'okra' plants were detected, identified and their quantitative estimations were made. The results have been presented in Table I. Qualitatively 15 amino acids

Table 1

Amino acids in root exudates of healthy and diseased 'okra' plants

Amino acids µg/200 plants	8-day-old plants		15-day-old plants	
	healthy	diseased	healthy	diseased
(a) Monoaminomonocarboxylic acid group				
1. α-alanine	180	230	190.4	256
2. Glycine	90	130	96	140
3. Leucine	60	125	58	146
4. Serine	42	90	62.4	102.4
5. Threonine	110	130	120	180
6. Valine	—	—	130	196
7. Isoleucine	100	150	110	168
(b) Monoaminodicarboxylic acid group				
1. 'Fast glutamic'	122	—	143	—
2. Glutamine	106	162	140	180
3. Glutamic acid	140	94	145	112
(c) Sulphur containing amino acids				
1. Cystine	130	140	180	140
2. Methionine	80	96	120	120
(d) Basic amino acids				
1. Arginine	150	160	153	180
2. Lysine	185	148	222	190
(e) Heterocyclic amino acids				
1. Tryptophane	—	—	180	100
Other amino acids				
1. Citrulline	—	—	60	—
2. Unidentified	+	+	+	+

+ Present — Absent

were detected from 8 day healthy exudates, while from 15 days' exudate, in addition to these tryptophane, citrulline and valine were also found to be present. In the exudates of inoculated series, as in healthy plants, an increased number of amino acids was detected at 15 days of plant growth than at 8 days. 14 amino acids were detected from exudates of 8-day-old plants of inoculated series, while

from 15-day-old plants all the above 14 amino acids with an addition of valine and tryptophane were detected. Quantitatively, results indicate that there was a distinct abundance of amino acids of monoaminomonocarboxylic acid group, followed by monoaminodicarboxylic acid group. Next in order were basic amino acids, sulphur containing amino acids, aromatic amino acids, heterocyclic amino acids in all the exudate samples. One amino acid spot remained unidentified in both healthy and inoculated series.

Sugars were assayed from exudate samples of healthy and inoculated series and the results are presented in Table 2. Qualitatively in healthy series, glucose

Table 2

Sugars µg/200 plants	8 days		15 days	
	healthy	diseased	healthy	diseased
1. Galactose	—	—	80	70
2. Glucose	141	—	170	60
3. Raffinose	—	72	—	90
4. Sucrose	134	150	150	206
5. Xylose	—	184	—	190

and sucrose were detected from 8-days' exudates, while from 15-days' exudate in addition to these galactose was also detected. In the 8-day exudate samples of inoculated series raffinose, sucrose and xylose were detected while at 15 days in addition to these glucose and galactose were also found to be present. Raffinose and xylose which were detected from the inoculated series, were found to be absent in healthy exudates. Quantitatively, in healthy series, the relative abundance of sugars in the descending order was found to be as: glucose-sucrose-galactose. In the inoculated series, the concentration gradient was found to be in order of xylose-sucrose-raffinose-galactose-glucose.

Effect of root exudates, amino acid fractions, sugar fractions and individual compounds on sclerotic germination of the pathogen was investigated and the results are presented in Table 3 and 4. The time required for the germination of sclerotia of *R. bataticola* in all the exudate samples was 48 hours except in 8-day-old healthy exudate samples, where the time required for germination was 36 hours. There was no germination in α-alanine, leucine, isoleucine, glycine and tryptophane. In the sugar fractions of exudate samples, the germination time was 48 hours, while in amino acid fractions it was 72 hours. The germination time for control (distilled water) was found to be 60 hours.

Effect of root exudates, amino acids and sugar fractions was determined for the growth rate of pathogen on agar medium and the results are presented in Table 5. Exudate samples and sugar fractions of all samples were found to stimulate the growth of the pathogen, while the growth rate was considerably sup-

pressed by amino acid fractions. No difference was recorded in exudate samples, amino acid fractions and sugar fractions of healthy and inoculated series for growth response of the pathogen.

Table 3

Effect of crude exudates, individual amino acids and amino acid fractions on sclerotic germination of *R. bataticola*

S. No. Amino acids or Exudates	Sclerotial germination in hours					
	12	24	26	48	60	72
1. Distilled water	−	−	−	−	+	+
2. Healthy 8-day exudate	−	−	+	+	+	+
3. Healthy 15-day exudate	−	−	−	+	+	+
4. Diseased 8-day exudate	−	−	−	+	+	+
5. Diseased 15-day exudate	−	−	−	+	+	+
6. α-alanine	−	−	−	−	−	−
7. Glycine	−	−	−	−	−	−
8. Leucine	−	−	−	−	−	−
9. Serine	−	−	−	+	+	+
10. Threonine	−	−	−	+	+	+
11. Valine	−	−	−	−	+	+
12. Isoleucine	−	−	−	−	−	−
13. 'Fast glutamic'	−	−	−	−	+	+
14. Glutamine	−	−	+	+	+	+
15. Glutamic acid	−	−	+	+	+	+
16. Cystine	−	−	−	−	+	+
17. Methionine	−	−	+	+	+	+
18. Arginine	−	−	+	+	+	+
19. Lysine	−	−	+	+	+	+
20. Tyrosine	−	−	−	−	−	+
21. Tryptophane	−	−	−	−	−	−
22. Citrulline	−	−	−	−	+	+
23. Amino acid fraction (8-days Healthy)	−	−	−	−	−	+
24. Amino acid fraction (15 days Healthy)	−	−	−	−	−	+
25. Amino acid fraction (8 days Diseased)	−	−	−	−	−	+
26. Amino acid fraction (15 days Diseased)	−	−	−	−	−	+

+ = germination − = No germination

Discussion

The experimental findings showed an increasing trend in the exudation of amino acids with increase in age of plants. These findings are in conformity with JALALI and SURYANARAYANA (1972). Valine and tryptophane which were

Table 4

Effect of sugar fractions and individual sugars on the sclerotic germination of *R. bataticola*

S. NO. Sugar fractions on sugars	Sclerotic germination period in hours						
	12	24	36	48	60	72	84
1. Distilled water	−	−	−	−	+	+	+
2. Sucrose	−	−	−	+	+	+	+
3. Glucose	−	−	−	+	+	+	+
4. Raffinose	−	−	−	−	+	+	+
5. Galactose	−	−	−	−	+	+	+
6. Xylose	−	−	−	−	−	+	+
7. Sugar fractions:							
Healthy 8 days	−	−	−	+	+	+	+
Healthy 15 days	−	−	−	+	+	+	+
Diseased 8 days	−	−	−	+	+	+	+
Diseased 15 days	−	−	−	+	+	+	+

+ = Germination − = No germination

Table 5

Colony diameter in mm of the pathogen in the presence of root exudates, amino acid fractions and sugar fractions

S. No.	24	36	48	60	72	84
Control (water agar)	13	24	34	47	58	62
Healthy: 8-day exudate	14.5	25.5	37.5	51	63	67
15-day exudate	14	25.0	37.0	51	62.5	66
Diseased: 8-day exudate	14.0	25.0	36.0	50	61.0	64.5
15-day exudate	14.5	25.0	36.0	50	61.0	65.0
Amino acid fraction						
Healthy: 8-day exudate	12.0	22.0	31.5	43.5	54.5	58.0
15-day exudate	12.0	22.0	31.0	43.0	54.0	57.5
Diseased: 8-day exudate	12.0	22.5	31.5	43.0	54.0	57.5
15-day exudate	11.5	22.0	31.5	42.5	53.5	57.0
Neutral fraction (sugars)						
Healthy: 8-day exudate	13.5	25.0	35.5	49.0	60.0	65.0
15-day exudate	13.5	25.5	35.5	50.0	60.5	65.5
Diseased: 8-day exudate	13.5	25.0	36.0	48.0	59.5	64.0
15-day exudate	13.5	24.5	36.0	48.0	59.5	64.5

absent in 8-day exudates were shown to be present in 15-day exudate samples. The appearance of new substances with age does not necessarily mean that they have been excreted by the plants only after 8 days of growth. It is possible that traces of these substances might have been excreted continuously since germination

and only after the longer period did the concentration become sufficiently great to be detected. However, the findings of VIRTANEN *et al.* (1953) with germinating peas; ROVIRA (1956) with oats and peas indicated that there is a qualitative change in the free amino acid contents of the seed and plant during germination and early growth. It may, thus, be conceivable that the amino acid composition of the exudate may change with age. More amino acids were shown to be exuded by healthy 'okra' plants in comparison to inoculated exudate samples. This observation is similar to that of MAC RAE and CASTRO (1967), SULOCHANA (1962) who studied the root exudates of rice plants in relation to 'akagare' disease and cotton plants under the stress of wilt infection, respectively.

Quantitatively, most of the amino acids of monoaminomonocarboxylic acid group, glutamine and arginine were exuded in greater amounts in the root exudates of inoculated plants in comparison with healthy plants. It is supposed to be due to the significant shifts in the exudation of amino acids brought about by maceration activity, permeability change and cellular damage by the invading pathogen or by the selective nature of enzymes secreted by the pathogen causing disruption of the chain of cell proteins, in inoculated series, at points containing these amino acids. Protein degradation of cell walls has been observed to take place following infection by *Helminthosporium oryzae* by MAHADEVAN (1967) which may explain the alteration of amino acid contents of the exudates obtained from inoculated series. Glutamic acid, cystine, lysine were found to be considerably reduced in diseased exudates, while 'fast glutamic' and citrulline were not detected at all. The observed decrease in the number of amino acids may be the result of their utilization by the pathogen. The fungus utilizes these for its own protein synthesis.

Qualitatively and quantitatively an increased trend in the exudation of sugars with increase in the growth of plants was observed. Qualitatively, three sugars were detected in the root exudates of healthy plants at 15th day of growth, whereas in inoculated series, five sugars were shown to be present; thus revealing that more sugars are exuded under the stress of root rot infection. Sucrose was found to be abundantly exuded in inoculated series, while xylose and raffinose were not at all detected in healthy plants. This may be due to the cellular damage by the invading pathogen. The activity of carbohydrates in inoculated series may be affected by the fungal metabolites. The exudation of glucose and galactose was considerably reduced under diseased condition, thereby, indicating their preferential utilization by the pathogen for its own cellular synthesis. Findings of JALALI and SURYANARAYANA (1971) indicated that the changes brought about in the carbohydrate level of root exudates of wheat in response to its root rot infection may partly be attributed to the uncoupling of the oxidative phosphorylation and partly due to automatic augmentation in respiration caused by synthetic processes stimulated in diseased plants. This may be true also for the inoculated 'okra' plants.

The sclerotic germination of the pathogen indicated stimulatory effect of all the exudate samples. α-alanine, glycine, leucine, isoleucine and amino acid frac-

tions were found to be inhibitory, which may partly explain the fact that lesser disease incidence was noticed when plants of more than two weeks were inoculated with the pathogen. Sucrose, glucose, galactose and sugar fractions were stimulatory while raffinose and xylose were found to have inhibitory effects on the sclerotic germination of pathogens in comparison with the control.

Mycelial growth of the pathogen was stimulated with crude exudate samples and neutral fractions; while it was considerably reduced with amino acid fractions. This suppression in growth may be due to the inhibitory effect of α-alanine, glycine, leucine and isoleucine present in exudate samples. No appreciable difference in the exudate samples, amino acid fractions and sugar fractions of healthy and inoculated exudates in growth response of the pathogen was detected. Conclusively, it could be said that it is the balanced effect of all the compounds present in an exudate samples, which determines its stimulatory or inhibitory effect for the pathogen.

Literature

CLAUDIUS, G. R. and MEHROTRA, R. S. (1973): Root exudates from lentil (*Lens culinaris* Medic) seedlings in relation to wilt disease. Plant and Soil *38*, 315—320.

JALALI, B. L. and SURYANARAYANA, D. (1970): Biochemical nature of root exudates in relation to root rot of wheat. I. Amino acid shifts in relation to foliar treatments Z. Pflanzenkrank. Pflanzenschutz. *77*, 438—442.

JALALI, B. L. and SURYANARAYANA, D. (1971): Shifts in the carbohydrate spectrum of root exudates of wheat in relation to its root rot disease. Plant and Soil *34*, 261—268.

JALALI, B. L. and SURYANARAYANA, D. (1972): Biochemical nature of root exudates in relation to the pathogenesis of wheat. II. Amino acid shifts. Indian Phytopathology *25*, 195—199.

MAC RAE, I. C. and CASTRO, T. F. (1967): Root exudates of rice plant in relation to 'Akagare' — physiological disorder of rice. Plant and Soil *26*, 317—323.

MAHADEVAN, A. (1967): The role of proteolytic enzymes in disease development. Int. Symp. Plant Path. (Abstr.): I. A. R. I., New Delhi.

ROVIRA, A. D. (1956): Plant root excretions in relation to rhizosphere effect. I. The nature of root exudates from oats and peas. Plant and Soil *7*, 178—194.

SCHROTH, M. N. and HILDEBRAND, D. C. (1964): Influence of plant exudates on root infecting fungi. Ann. Rev. Phytopath. *2*, 101—132.

SULOCHANA, C. B. (1962): Amino acids in root exudates of cotton. Plant and Soil *16*, 312—316.

VIRTANEN, A. I., BERGE, A. M. and KARI, S. (1953): Formation of homoserine in germinating pea seeds. Acta Chem. Scand. *7*, 1423.

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Oxygen Release from Rice Seedlings: Effect of *Meloidogyne incognita* and *Helminthosporium oryzae* Infections

By

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Meloidogyne incognita infections caused a significant reduction in the amount of oxygen released from three-week-old seedlings of the rice varieties Bluebelle and Saturn. Increase of the nematode inoculum level from 1500 to 3000 larvae/pot produced increases in number of root galls. *Helinthosporium oryzae*, *M. incognita* or *H. oryzae* plus *M. incognita* root infection did not affect the root and shoot growth of three-week-old seedlings, however, each of these treatments significantly reduced oxygen release from seedlings when compared with nontreated controls.

Oxygen release from rice roots is a significant aspect of root oxidizing activity (ARMSTRONG, 1969; 1971). Recently quantitative measurements of oxygen production by the entire root system of intact rice seedlings were made with a polarographic oxygen monitor utilizing a Clark electrode (JOSHI *et al.*, 1973).

The purpose of this work was to determine the effects of *Meloidogyne incognita* and *H. oryzae* root infections on the growth of rice seedlings and oxygen release by the roots.

Materials and Methods

Experiments were conducted in the greenhouse in May 1973. In the first experiment, rice cultivars Bluebelle and Saturn and two inoculum levels of *M. incognita* were used. Sprouted seeds of both cultivars were planted separately in twelve 8-inch clay pots containing autoclaved clay loam soil. After emergence, rice seedlings were thinned to 3 seedlings/pot. One week after planting, groups of 4 pots of each cultivar were: a) inoculated with 1500 larvae/pot; b) inoculated with 3000 larvae/pot; and c) left uninoculated as a control. Holes were made in the soil around the plants with a glass rod and the nematode suspension was pipetted into the holes.

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Seedlings were washed from 4 pots of each treatment two weeks following inoculation. Oxygen release from seedling roots was measured by the method of JOSHI *et al.* (1973) and the number of galls and dry weights of roots and shoots were determined.

In the second experiment, only Saturn was used. Three sprouted seeds were planted in each of 16 pots which were then divided into four groups of 4 pots each. One week after planting groups were inoculated with: a) 6000 nematode larvae/pot; b) 98.5 mg (dry wt.) of fungus mycelium; c) 6000 nematodes/pot + 98.5 mg fungus mycelium; and d) left uninoculated as a control. Two weeks after inoculation, seedlings were removed for determination of oxygen release, number of galls and root and shoot dry weights.

Results and Discussion

M. incognita infection, at both inoculum levels resulted in a significant reduction in the amount of oxygen released from three-week-old seedlings of the rice varieties Bluebelle and Saturn (Table 1). Increase of nematode inoculum

Table 1

Effect of *Meloidogyne incognita* infection on oxygen release, root galling and root and shoot dry weights of three-week-old seedlings of two rice varieties[1,2]

Variety	No. of nematodes per pot	O_2 µl plant^{-1}min^{-1}	No. of gall per plant	Root dry wt. (g)	Shoot dry wt. (g)
Bluebelle	0 (control)	0.917 a	0 a	0.019 a	0.042 a
	1.500	0.776 b	41.7 b	0.017 a	0.025 a
	3.000	0.587 c	87.0 c	0.017 a	0.029 a
Saturn	0 (control)	0.987 a	0 a	0.014 a	0.044 a
	1.500	0.681 b	58. ob	0.021 a	0.035 a
	3.000	0.540 c	95. oc	0.019 a	0.036 a

[1] Inoculations were made one week after planting the seed. Data are averages of 4 replicates

[2] Separate analyses were done for both varieties. Means with unlike letters in a column differ significantly (P = 0.05)

level produced increases in the number of root galls of both varieties; however, root and shoot growth were not affected. Each of the three treatments significantly reduced oxygen release from seedlings when compared with nontreated control, although there were no significant differences between treatments (Table

2). Also there were no significant differences between the numbers of galls produced by nematode infection alone and by the nematode and fungus inoculum combined.

There was an absence of synergism between *H. oryzae* and *M. incognita* with respect to pathogenesis on rice variety Saturn, but it was clear that these pathogens decreased the oxidizing capacity of the seedling roots. Changes in respiratory patterns of plants due to pathogenic infections have been documented (GRIMM and WHEELER, 1963; URITANI and AKAZAWA, 1959). Root infections resulting in decreased oxygen release from rice seedlings are of interest since there is preliminary evidence that resistance of rice varieties to sulfide diseases may be related to their root oxidizing power (ARMSTRONG, 1969; 1971; JOSHI *et al.*, 1973).

Table 2

Effect of *H. oryzae*, *M. incognita*, and *H. oryzae*—*M. incognita* infections on oxygen release, root-gall formation and root and shoot dry weights of three-week-old Saturn seedlings[1,2]

Treatments	O_2 μl plant^{-1}min^{-1}	No. of galls per plant	Root dry wt. (g)	Shoot dry wt. (g)
UntreatedControl	1.011 a	0 a	0.021 a	0.056 a
H. oryzae	0.446 b	0 a	0.021 a	0.057 a
M. incognita	0.540 b	94 b	0.023 a	0.053 a
H. oryzae + *M. incognita*	0.446 b	102 b	0.021 a	0.055 a

[1] Inoculations were made one week after planting the seed

[2] Data are means of 4 replicates. Means with unlike letters in a column differ significantly ($P = 0.05$)

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Literature

ARMSTRONG, W. (1969): Rhizosphere oxidation in rice: an analysis of intervarietal differences in oxygen flux from the roots. Physiol. Plant. *22*, 296—303.

ARMSTRONG, W. (1971): Oxygen diffusion from the roots of rice grown under non-waterlogged conditions. Physiol. Plant. *24*, 242—247.

GRIMM, R. B. and WHEELER H. (1963): Respiratory and enzymatic changes in victoria blight oats. Phytopathology *53*, 436—440.

JOSHI, M. M., IBRAHIM, I. K. A. and HOLLIS, J. P. (1973): Oxygen release from rice seedlings. Physiol. Plant. *29*, 269—271.

URITANI, I. and AKAZAWA, T. (1959): Alteration of the Respiratory Pattern in Infected Plants. p. 349—390. In J. G. Horsfall and A. E. Diamond (ed.), Plant Pathology, the Diseased Plant. Vol. 1. Academic Press New York.

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Influence of Potassium Nutrition and Bacterial Blight Disease on Phenol, Soluble Carbohydrates and Amino Acid Contents in Rice Leaves

By

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Increased supply of potassium did not influence the blight disease caused by *Xanthomonas oryzae* in highly susceptible rice cultivar T(N) 1, but it markedly reduced the disease in less susceptible IR 8. Leaves of T(N) 1 contained higher concentrations of phenols, reducing and nonreducing sugars and amino acids than the cultivar IR 8. Inadequate supply of potassium favoured an accumulation of phenols, reducing and nonreducing sugars and amino acids in leaves of both varieties when compared to plants generously supplied with potassium. The levels of phenolic compounds and amino acids decreased in diseased leaves of cultivar T(N) 1, while that of IR 8 showed an increase and the increase being more in plants supplied with higher concentrations of potassium. In the infected leaves of the varieties, both the reducing and nonreducing sugars generally were low.

Mineral nutrition greatly influences the physiology of the plant and consequently disease resistance (YARWOOD, 1959). The semi-dwarf, nitrogen responsive high yielding rice (*Oryza sativa* L.) varieties respond to large doses of potassium (KEMMLER, 1972). Most of these varieties grown under high nitrogen are extremely susceptible to blight caused by *Xanthomonas oryzae* (Uyeda *et* Ishiyama) Dowson. Potassium fertilization has been reported to reduce the intensity of blight (HASHIOKA, 1951; ONO, 1957; TAGAMI and MIZUKAMI, 1962). No detailed analytical study by which potassium increased the tissue resistance has been made. Therefore, this study was planned to investigate possible changes in plants grown in different concentrations of potassium and their relation to disease resistance.

Materials and Methods

T(N) 1 is highly susceptible to bacterial blight and IR 8 less susceptible were grown in nutrient medium. The nutrient solution adjusted to pH 5.0 contained 40 ppm nitrogen, 10 ppm phosphorous and potassium levels varying from 3, 25, 75, 125, and 175 ppm and balanced amounts of other mineral elements necessary for plant growth (YOSHIDA *et al.*, 1971). The level of nutrient solution was kept at more or less the same hight by addition of water. During the growth of plants,

pH values of the nutrient solution fluctuated and in order to eliminate the effect of different pH values on the growth of plants, the pH was checked and adjusted to 5.0. After 30 days of sowing, a nutrient solution but without potassium was added to the plants. They were inoculated by clipping the top portions of leaves with a pair of scissors previously dipped in a 48 hr old cell suspension (ca. 10^8 cells/ml) of *X. oryzae* (KAUFFMAN *et al.*, 1973) obtained from potato sucrose agar medium (TAGAMI and MIZUKAMI, 1962). The development of lesions was recorded from 3 days after inoculation until 10 days.

Leaf samples were collected from both healthy and inoculated plants, 4 and 8 days after inoculation in the morning for analyses. The leaves cut into small bits were extracted in boiling ethanol (SRIDHAR, 1972) and the extract was used to measure total phenols by employing Folin-Ciocalteau reagent (BRAY and THORPE, 1954). Standards prepared from chlorogenic acid were used to calculate the total phenols.

Reducing sugar content of the extract was estimated by Nelson's method (NELSON, 1944). For nonreducing sugars, the alcohol extract was hydrolyzed with 1 *N* H_2SO_4 on a hot water bath (INMAN, 1962) for 30 min at 49°C and after neutralizing it with 1 *N* NaOH using methyl red indicator, total soluble sugar content of the samples was measured by Nelson's method. Amounts of nonreducing sugars were calculated by subtracting the reducing sugars from total soluble sugars.

Soluble amino nitrogen in the extract was measured by the ninhydrin method (MOORE and STEIN, 1948). Standards prepared with glutamic acid were used to calculate the amino nitrogen in the extract.

Results

Disease development: Proneness of rice cultivar T(N) 1 to *X. oryzae* was not much influenced by the addition of potassium while it definitely increased the resistance of cultivar IR 8 (Fig. 1).

Changes in phenols: T(N) 1 contained higher concentrations of phenolic compounds than the less susceptible rice cultivar IR 8 (Table 1). Inadequate supply of potassium increased the phenols in leaves.

In the inoculated T(N) 1 leaves phenols decreased compared with the healthy leaves, while in IR 8, infection enhanced the phenols. Interestingly, leaves of IR 8 plants inoculated with the pathogen grown in high potassium level accumulated larger amounts of phenols than those grown at low potassium levels.

Soluble carbohydrates: Reducing and nonreducing sugars were generally more in the leaves of T(N) 1 than in the leaves of IR 8 grown at low potassium levels (Tables 2 and 3). Potash-deficient plants generally contained more reducing and nonreducing sugars than those receiving copious amounts of potash. Extremely high concentration of potassium favoured an increase in the quantity of

soluble sugars. Disease development caused varied effects in the amounts of reducing sugars in the leaves of T(N) 1, while it caused a general depletion in the leaves of IR 8. Disease development generally decreased the nonreducing sugar content in both the varieties.

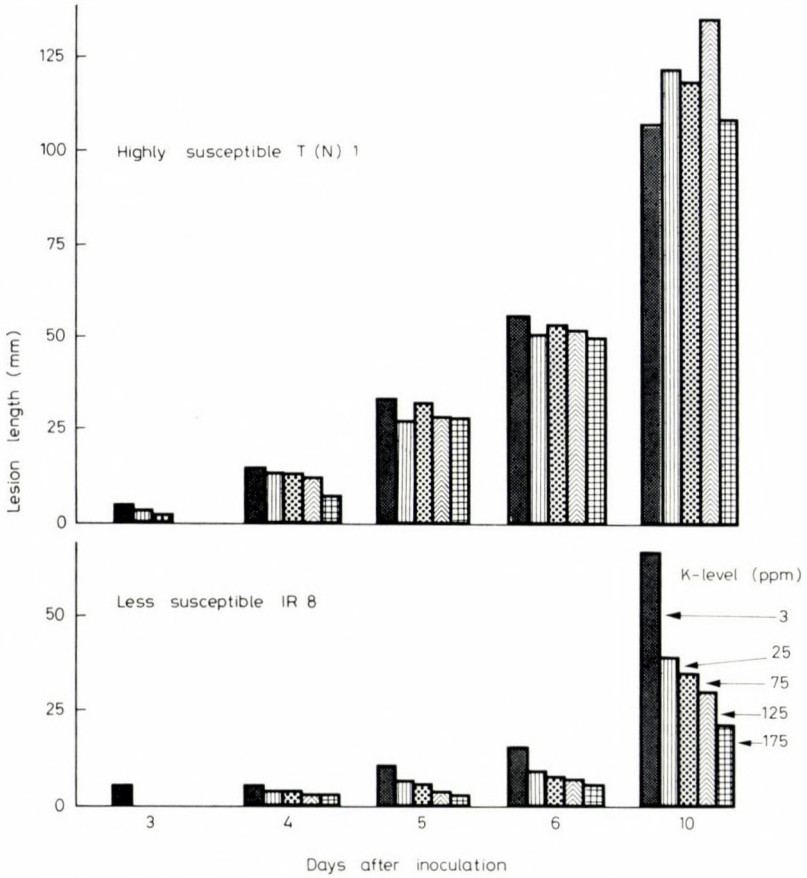

Fig. 1. Influence of different concentrations of potassium on bacterial blight development in two rice varieties

Soluble amino acids: Leaves of T(N) 1 plants contained more soluble amino acids than that of IR 8 (Table 4). Plants grown at lower potassium levels accumulated free amino acids. Inoculation of leaves with *X. oryzae* decreased amino acid content in the leaves of cultivar T(N) 1, while it increased in the leaves of rice cultivar IR 8, the rate of increase being more in plants grown at high potassium levels.

Table 1

Changes in total phenols of healthy and *X. oryzae* inoculated highly susceptible and less susceptible rice leaves. (Chlorogenic acid equivalent mg/100 g oven dry tissue)

K-level (ppm)	Highly susceptible — T(N) 1				Less susceptible — IR 8			
	Healthy		Inoculated		Healthy		Inoculated	
	Age of the plant (days)							
	34	38	34 (4)	38 (8)	34	38	34 (4)	38 (8)
3	488	502	451	425	362	344	411	402
25	421	459	311	311	239	307	313	430
75	320	359	292	234	287	289	358	335
125	525	449	273	320	301	309	412	414
175	359	425	292	336	335	320	488	449

( ) Represents days after inoculation.

Table 2

Changes in total soluble amino acid content of healthy and *X. oryzae* inoculated highly susceptible and less susceptible rice leaves. (Glutamic acid equivalent mg/100 g oven dry tissue)

K-level (ppm)	Highly susceptible — T(N) 1				Less susceptible — IR 8			
	Healthy		Inoculated		Healthy		Inoculated	
	Age of the plant (days)							
	34	38	34 (4)	38 (8)	34	38	34 (4)	(38) (8)
3	502	478	359	441	240	316	314	332
25	442	394	316	392	227	239	308	298
75	373	335	308	237	175	242	250	315
125	299	308	239	215	165	212	261	359
175	277	299	246	228	143	200	243	372

( ) Represents days after inoculation.

Discussion

A reduction of blight symptoms in IR 8 plants raised in large doses of potassium and the absence of this phenomenon in T(N) 1 leaves clearly indicate a differential response of cultivars to potassium. But analysis of leaf tissues of the two varieties for K did not show any significant deference.

Table 3

Changes in reducing sugars of healthy and *X. oryzae* inoculated highly susceptible and less susceptible rice leaves

(Glucose equivalent mg/100 g oven dry tissue)

K-level (ppm)	Highly susceptible — T(N) 1				Less susceptible — IR 8			
	Healthy		Inoculated		Healthy		Inoculated	
	Age of the plant (days)							
	34	38	34 (4)	38 (8)	34	38	34 (4)	38 (8)
3	693	717	588	621	502	841	454	679
25	550	660	660	588	430	645	406	531
75	530	645	454	578	392	602	382	516
125	249	406	306	421	253	430	235	391
175	308	548	359	502	320	501	311	454

( ) Represents days after inoculation.

Table 4

Changes in nonreducing sugars of healthy and *X. oryzae* inoculated highly susceptible and less susceptible rice leaves

(Glucose equivalent mg/100 g oven dry tissue)

K-level (ppm)	Highly susceptible — T(N) 1				Less susceptible — IR 8			
	Healthy		Inoculated		Healthy		Inoculated	
	Age of the plant (days)							
	34	38	34 (4)	38 (8)	34	38	34 (4)	38 (8)
3	1219	1558	799	1358	932	1147	932	1042
25	798	1491	487	1305	746	884	693	817
75	646	1081	550	1019	707	784	526	727
125	457	771	362	664	330	526	293	433
175	589	774	411	640	521	474	349	406

( ) Represents days after inoculation.

It is a well established fact that an inadequate supply of potassium leads to disturbed nitrogen and carbohydrate metabolism (HEWITT, 1963). In limiting concentrations of potassium, non-protein nitrogen increases greatly in plants (GRIFFITH *et al.*, 1964; SINCLAIR, 1969) possibly making the tissues more susceptible to infection. Higher amounts of soluble carbohydrates (LARSH and ANDER-

SON, 1948; BIRD, 1954), soluble nitrogen (BIRD, 1954; NAYUDU and WALKER, 1961; FANG *et al.*, 1963) and lower amounts of polyphenols (FANG *et al.*, 1963; EASWARAN, 1971) are reported to favour tissue susceptibility especially to *Xanthomonas* species.

Leaves of potassium-deficient plants which were susceptible to blight contained higher concentrations of total phenols, soluble sugars and free amino acids. However, no correlation between resistance or susceptibility to *X. oryzae* and differences in amino acid or sugar contents of rice plants was found (HSU, 1966).

Potassium-deficient plants contained large amounts of phenols (MULDER, 1949). Is it presumably due to high amount of soluble sugars in plants for the synthesis of phenols since aromatic synthesis is largely influenced by carbohydrate metabolism (NEISH, 1964)?

Phenols are rapidly broken down in presence of high amino nitrogen content by polyphenol oxidase (KIRKHAM, 1954; FLOOD and KIRKHAM, 1960) which may reduce their toxicity in potassium-deficient plants and thereby increase the susceptibility. Hence, the relationship between phenol and amino acid contents especially at low potassium levels becomes important.

Disease development in the highly susceptible cultivar T(N) 1 was faster and severe than in the less susceptible cultivar IR 8 at all the levels of potassium. Changes in soluble carbohydrates, amino acids and total phenols in T(N) 1 leaves due to different levels of potassium supply appeared not to influence tissue susceptibility. The reduction of soluble carbohydrates and amino acids in the infected leaves might be due to the utilization of these compounds by the pathogen and to the decreased synthetic ability of the severely infected leaves. The increase in amino acid content of inoculated IR 8 leaves could be attributed to hydrolysis of host protein during pathogenesis (FARKAS and KIRÁLY, 1961; RUDOLPH, 1963).

On the other hand, the phenolic content of inoculated leaves of cultivar IR 8 grown at high levels of potassium contained high concentration than the healthy. Moreover, the amino acid contents of these tissues were also considerably low. This would have provided a conductive situation for an efficient inhibition of the bacterium in the advancing portions of the lesions in IR 8 plants especially at high levels of potassium since, the bacterial population was always high at the advancing tip of the lesions (NWIGWE, 1973).

During host-parasite interaction permeability of the cells is altered (HANCHEY and WHEELER, 1969) and cellular compounds freely flow from one tissue to another. Potassium ion with its small charge and large hydration shell tend to increase permeability of the protoplasmic membrane (STOCKING, 1956). In leaves of plants generously supplied with potassium obviously, more phenolic compounds might have been mobilized towards the diseased portion to inhibit the multiplication and migration of the bacterium and thereby restrict the development of the disease.

Acknowledgements

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Literature

BIRD, L. S. (1954): Genetic-controlled carbohydrate and soluble nitrogen combinations in plant tissues causing resistance to the bacterial blight disease of cotton. Plant Dis. Reptr. *38*, 653–660.

BRAY, H. G. and THORPE, W. V. (1954): Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. *1*, 27–52.

EASWARAN, K. S. S. (1971): Physiology of resistance to bacterial disease in sorghum. I. Alterations in the phenols. Phytopath. Z. *71*, 141–146.

FANG, C. T., LIN, C. F., CHU, C. L. and SHU, T. K. (1963): Studies on the disease resistance of rice. II. Varietal resistance of rice to bacterial leaf blight and a preliminary analysis of its mechanism. Acta Phytopath. Sinica *6*, 107–112.

FARKAS, G. L. and KIRÁLY, Z. (1961): Amide metabolism in wheat leaves infected with stem rust. Physiol Plant. *14*, 344–353.

FLOOD, A. E. and KIRKHAM, D. S. (1960): The effect of some phenolic compounds on the growth and sporulation of two *Venturia* species. In: Phenolics in Plants in Health and Disease. Pergamon Press, Oxford. p. 81– 85.

GRIFFITH, W. K., TEEL, M. R. and PARKER, H. E. (1964): Influence of nitrogen and potassium on the yield and chemical composition of orchard grass. Agron. J. *56*, 473–475.

HANCHEY, P. and WHEELER, H. (1969): Pathological changes in ultrastructure: False plasmolysis. Can. J. Bot. *47*, 675–678.

HASHIOKA, Y. (1951): Bacterial leaf blight of rice and its control. Agri. and Hort. Tokyo *26*, 644–648.

HEWITT, E. J. (1963): The essential nutrient elements: Requirements and interactions in plants. In F. C. STEWARD (ed.) Plant Physiology – A Treatise Vol. III. Inorganic Nutrition of Plants. Academic Press, New York. pp. 137–360.

HSU, S. T. (1966): Nutritional requirements *in vitro* of *Xanthomonas oryzae* (Uyeda and Ishiyama) Dowson and its effect on host plant resistance. M. S. Thesis, College of Agri., Univ. Philippines, p. 58.

INMAN, R. E. (1962): Disease development, disease intensity and carbohydrate levels in rusted bean plants. Phytopathology *52*, 1207–1211.

KAUFFMAN, H. E., REDDY, A. P. K., HSIEH, S. P. Y. and MERCA, S. D. (1973): An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis. Reptr. *57*, 537–541.

KEMMLER, G. (1972): Under utilization of potash in developing countries. Fertilizer News *17*, 57–67.

KIRKHAM, D. S. (1954): Significance of the ratio between the water soluble aromatic and nitrogen constituents of apple and pear in the host-parasite relationship of *Venturia* sp. Nature *173*, 690–691.

LARSH, H. W. and ANDERSON, H. W. (1948): Bacterial spot of stone fruit with special reference to epiphytotics and dissemination of the causal organism. Bull. Ill. Agric. Expt. Sta. *530*, 31–55.

MOORE, S. and STEIN, W. H. (1948): Photometric method for use in the chromatography of amino acids. J. Biol. Chem. *176*, 367–388.

MULDER, E. G. (1949): Mineral nutrition in relation to the biochemistry and physiology of potatoes. I. Effect of nitrogen, phosphate, potassium, magnesium and copper nutrition on the tyrosine content and tyrosinase activity with particular reference to blackening of the tubers. Plant and Soil *2*, 59—121.

NAYUDU, M. V. and WALKER, J. C. (1961): Tomato leaf composition in relation to bacterial spot. Phytopathology *51*, 368—372.

NEISH, A. C. (1964): Biosynthetic pathways of aromatic compounds. Ann. Rev. Plant Physiol. *11*, 58—80.

NELSON, N. (1944): A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. *15*, 375—380.

NWIGWE, C. (1973): Persistance of *Xanthomonas oryzae* in the infected tissues of rice plants. Plant Dis. Reptr. *57*, 227—230.

ONO, K. (1957): The relation between rice diseases and potassium. p. 71—87. In Potassium Symp. (Japanese) International Potash Institute, Berne, Switzerland. pp. 137.

RUDOLPH, K. (1963): Weitere biochemische Untersuchungen Zum Wirt-Parasit-Verhaltnis am Beispiel von *Puccinia graminis tritici*. I. Der Einfluss der Infection auf den Sauerestoffwechsel. Phytopath. Z. *46*, 276—290.

SINCLAIR, C. (1969): The level and distribution of amines in barley as affected by potassium nutrition, arginine level, temperature fluctuation and mildew infection. Plant and Soil *30*, 423—438.

SRIDHAR, R. (1972): Influence of nitrogen fertilization and *Pyricularia oryzae* development on some oxidases, their substrates and respiration of rice plants. Acta Phytopath. Acad. Sci. Hung. *7*, 57—70.

STOCKING, C. R. (1956): Hydration and cell, physiology. I. Water content of cells and tissues. In: W. RUHLAND (ed.) Encyclopedia of Plant Physiology. Vol. II. General Physiology of the Plant Cell. Springer-Verlag, Berlin. pp. 22—37.

TAGAMI, Y. and MIZUKAMI, T. (1962): Historical review of rice caused by *Xanthomonas oryzae* (Uyeda *et* Ishiyama) Dowson. Special Rep. Plant Dis. and Insect Pests Forecasting Service, No. 10, pp. 112. Plant Protection Division, Ministry of Agri. and Forestry, Japan (Japanese—English Trans. by H. GUJII).

YARWOOD, C. E. (1959): Predisposition. In: J. G. HORSFALL and DIMOND, A. E. (eds.) Plant Pathology Vol. I. The Diseased Plant. Academic Press, New York. pp. 521—562.

YOSHIDA, S., FORNO, D. A. and COCK, J. H. (1971): Laboratory Manual for Physiologica Studies of Rice. The International Rice Research Institute, Los Banos, Philippines.

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Survival of *Xanthomonas oryzae* on Rice Leaves

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Xanthomonas oryzae was observed to survive for at least five days on the leaf surfaces of healthy susceptible rice varieties JC-70 and IR-8. Environmental conditions, especially temperature, appeared to determine the longevity of *X. oryzae* on rice leaves. It is concluded that *X. oryzae* maintains a temporary resident phase on healthy rice leaves which may provide part of the inoculum that incites blight epidemics when favourable conditions occur.

The bacterial leaf blight disease of rice incited by *Xanthomonas oryzae* (Uyeda *et* Ishiyama) Dowson, is the major bacterial diesase of rice and occurs in varying degrees at every stage in the life cycle of the crop. A common observation is the severe outbreak of the disease soon after rainstorms or typhoons, and the usual explanation is that strong winds and rainsplash disseminate the pathogen and transfer the inoculum from infected plants to otherwise healthy but injured rice leaves (GOTO *et al.*, 1955; TAGAMI and MIZUKAMI, 1958; TAGAMI, 1968). Another possible explanation of the infection would be that the organism could reside and possibly multiply on healthy rice leaves, but being a systemic pathogen incites blight epidemics only when it gains access into the leaf tissue especially through wounds such as those caused by strong winds and typhoons. It has been demonstrated (CROSSE, 1959; ENGLISH and DAVIS, 1960; CROSSE, 1963; LEBEN, 1963) that some pathogenic bacteria are capable of growing on the surface of healthy plants but induce disease development when favourable conditions occur. The aim of this experiment was to determine whether *X. oryzae* maintains a resident phase (epiphytic existence) on rice leaves as a first stage in the infection process.

Materials and Methods

Twelve pots each of 17-and 28-day-old plants of susceptible varieties JC-70 and IR-8 raised in the greenhouse were sprayed with a heavy inoculum suspension ($10^8 - 10^9$ cells/ml) of a streptomycin-resistant strain (S4000 B29-F38)

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of the pathogen, using a low pressure atomiser to avoid a forcible introduction of the pathogen into the leaf tissue. The sprayed plants were grouped into two lots; one lot was kept inside the greenhouse where the temperature varied between 26°C and 30°C, and the other lot was placed outside the greenhouse but shielded from rain with a polythene screen. The temperature outside the greenhouse varied between 27°C and 35°C. The plants were watered by moistening the soil in the pots thus avoiding washing off of the bacterial cells from the leaves by the usual sprinkler method. Leaf samples were taken from the plants daily for the first three days and later every other day for a total of nine days. The leaf samples were cut into three-cm pieces and vigorously shaken in test tubes containing sterile distilled water. Two transfer loops of the resulting suspensions were dispensed on streptomycin-treated Wakimoto's agar containing 4000 ppm streptomycin (NWIGWE, 1973). The plates were incubated at 28°C for four to five days and suspected colonies were picked out, multiplied on agar plants and finally inoculated into leaves of young JC-70 plants. The inoculated plants were kept in the greenhouse and observed for disease development.

Results and Discussion

It was easy to reisolate *X. oryzae* from leaf surfaces within two days of spraying the organism. After this period an increasing amount of contaminating bacteria were present on the isolation plates and *X. oryzae* could not very easily be distinguished from some of the yellow contaminants (NWIGWE, 1973) which also tolerate 4000 ppm of streptomycin. Inoculation tests with the bacteria reisolated from leaf surfaces showed that positive isolations were made up to five days from plants kept in the greenhouse and three days from those plants kept outside the greenhouse (Table 1). There was no noticeable difference on the longevity of *X. oryzae* on the 17-and 28-day-old plants. The results indicate that depending on the environmental conditions, especially temperature, *X. oryzae* can live on the surfaces of healthy rice leaves for up to five days. The viability of *X. oryzae* on various media, including infected leaf tissues has been shown to be adversely affected by high temperature of 34°C and above (EAMCHIT and OU, 1969). A recent report (GOTO, 1970) showed the pathogen could survive for a longer period between the leaf sheaths of rice plants where the micro-climatic conditions were more favourable than on exposed leaf blades. *X. oryzae* could survive for a long time in dried exudate at very high temperatures of 53–56°C but only five to six days when the dried exudate was soaked or kept in a moist chamber at a temperature of about 30°C (MIZUKAMI, 1961). There is the possibility that *X. oryzae* may survive on rice leaves for a longer period than five days in micro-colonies but in such numbers not detectable by the technique used in this experiment. However, the pathogenicity test of the reisolated organisms gave a reliable indication of the presence of the organism. Other methods of determin-

ing the longevity of *X. oryzae* on plant surfaces such as the phage and the fluorescin antibody techniques possess the disadvantage that these techniques could be applied very often with identical results on organisms other than *X. oryzae*. It is concluded from this experiment that *X. oryzae* lives for a short period on apparently healthy rice leaves. Under conditions favourable for infection it is probable that the pathogen residing temporarily on the healthy leaves of rice plants could be the source of inoculum for fresh leaf blight infections.

Table 1

Longevity of *X. oryzae* on rice leaves

Days after spray inoculation	Outside the greenhouse				Inside the greenhouse			
	Age of plants (days)							
	17		28		17		28	
	Rice varieties							
	JC—70	IR—8	JC—70	IR—8	JC—70	IR—8	JC—70	IR—8
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	—	—	+	+	—	—	+	—
5	—	—	—	—	—	+	—	+
7	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—

\+ Positive isolation of the pathogen
— Pathogen could not be detected

Literature

Crosse, J. E. (1959): Bacterial cankers of stone fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. Ann. Appl. Biol. *47*, 306—317.

Crosse, J. E. (1963): Bacterial cankers of stone fruits. V. A comparison of leaf surface populations of *Pseudomonas mors-prunorum* in autumn of two cherry varieties. Ann. Appl. Biol. *52*, 97—104.

Eamchit, S. and Ou, S. H. (1969): Effect of temperature on the viability of *Xanthomonas oryzae*. Phil. Phytopath. *5*, 2. (Abst.).

English, H. and Davis, J. R. (1960): The source of inoculum for bacterial cankers and blast of some fruit trees. Phytopathology *50*, 634 (Abst.).

Goto, K., Inoue, Y., Fukatsu, R. and Ohata, K. (1955): Field observation on the outbreak and fluctuation of severity of bacterial leaf blight of rice plant. Bull. Tokai-Kinki Agric. Expt. Sta. Div. Pl. Breed. Cultiv. *2*, 53—68.

Goto, M. (1970): Personal communication.

Leben, C. (1963): Multiplication of *Xanthomonas vesicatoria* on tomato seedlings. Phytopathology *53*, 778—781.

MIZUKAMI, T. (1961): Studies on the ecological properties of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, the causal organism of bacterial leaf blight of rice plant. Agric. Bull. Saga Univ. *13*, 1–85.

NWIGWE, C. (1973): Influence of epiphytic yellow bacteria on the pathogenicity of *Xanthomonas oryzae* on rice leaves. Il Riso *22*, 119–122.

TAGAMI, Y. (1968): Epidemiology of the bacterial leaf blight of rice. Rev. Plant. Prot. Res. *1*, 1–14.

TAGAMI, Y. and MIZUKAMI, T. (1958): Historical reviews of the researches on bacterial leaf blight of rice. Spec. Rept. Plant Dis. Inst. Forecast. *10*, 1–112.

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Reaction of *Physalis* Species to Plant Viruses. IV. *Physalis aequata* Jacq., *Physalis ixocarpa* Brot. and *Physalis viscosa* L. as less known indicators of plant viruses[1]

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In the course of studying the virus susceptibility of three less known *Physalis* species we found *Physalis aequata* Jacq. and *Ph. viscosa* L. to be locally susceptible to two further viruses (tobacco necrosis virus, R/* : */* : S/S : S/Fu; tobacco rattle virus, R/1 : 2.3/5 : E/E : S/Ne). New local host-virus relation was revealed between *Physalis ixocarpa* Brot. and tobacco necrosis virus. *Physalis aequata* proved to be locally and systemically susceptible to infection by alfalfa mosaic virus (R/1 : 1.3/18 : : U/U : S/Ap), potato aucuba mosaic virus (*/* : */* : E/E : S/Ap), potato virus Y (*/* : */* : E/E : S/Ap), tobacco mosaic virus (R/1 : 2/5 : E/E : S/*) and tobacco ring spot virus (R/1 : 1.8/42 : S/S : S/Ne). *Physalis ixocarpa* was found to be local and systemic host plant to four further viruses (cucumber mosaic virus, R/1 : 1/18 : : S/S : S/Ap; potato aucuba mosaic virus, tobacco mosaic virus and tobacco ring spot virus) while *Ph. viscosa* to three viruses (alfalfa mosaic virus, cucumber mosaic virus and potato virus X [R/1 : */6 : E/E : S/(Fu)]) not studied so far. All three *Physalis* species were resistant to infection by bean (common) mosaic virus (*/* : */* : : E/E : S/Ap), potato virus M (*/* : */* : E/E : S/Ap), potato virus S (*/* : : */* : E/E : S/Ap), radish mosaic virus (R/* : */* : S/S : S/Cl) and turnip yellow mosaic virus (R/1 : 1.9/37 : S/S : S/Cl).

According to our experiments *Physalis aequata* proved to be a new host plant to seven, while *Ph. ixocarpa* and *Ph. viscosa* to five viruses. The new host-virus relations provide further possibilities to identify the different viruses. And on the basis of the different reaction types of *Physalis* species included in our experiments (immunity, local susceptibility, local and systemic susceptibility) opportunity is given to separate the viruses.

Introduction

When reviewing the papers written on *Physalis* species as virus hosts it can be established that of the about 23 examined species *Physalis aequata* Jacq., *Ph. ixocarpa* Brot. (tomatillo, jamberberry) and *Ph. viscosa* L. show a less known reaction to viruses (reviewed by HORVÁTH 1970, 1974). According to the available data *Physalis aequata* is susceptible to five, *Ph. ixocarpa* to six and *Ph. viscosa* again to five viruses (see Table 1). In recent experiments the susceptibility of the above three *Physalis* species to other plant viruses not examined so far was studied.

[1] Earlier publications: I. Acta Phytopath. Acad. Sci. Hung. *5*, 65—72 (1970), II. Acta Phytopath. Acad. Sci. Hung. *9*, 1—9 (1974), III. Acta Phytopath. Acad. Sci. Hung. *9*, 11—15 (1974).

Material and Methods

In the course of our artificial infection experiments young *Physalis aequata*, *Ph. ixocarpa* and *Ph. viscosa* seedlings were inoculated with 14 viruses by means of the carborundum-spatula technique. The viruses used for inoculation were:

Table 1

Susceptibility of three *Physalis* species to plant viruses

Physalis species	Viruses	Literature
Ph. aequata Jacq.	Arabis mosaic virus	SCHMELZER (1963b)
	Cucumber mosaic virus	
	Potato virus X	LADEBURG *et al.* (1950)
	Prunus B virus	FULTON (1957), THORNBERRY (1966)
	Tomato ring spot virus	SCHMELZER (1963b), SCHMELZER and WOLF (1971)
Ph. ixocarpa Brot.	Alfalfa mosaic virus	SCHMELZER (1963a)
	Potato stem mottle virus	SCHMELZER (1957), SCHMELZER and WOLF (1971)
	Potato virus X	LADEBURG *et al.* (1950)
	Tobacco etch virus	GREENLEAF (1953)
	Potato virus Y	SCHMELZER and WOLF (1971)
	Beet pseudo-yellows virus	DUFFUS (1973)
	Physalis mosaic virus	PETERS and DERKS (1974)
Ph. viscosa L.	Potato aucuba mosaic virus	MACLEOD (1962), SCHMELZER and WOLF (1971)
	Potato virus Y	PONTIS and FELDMAN (1963), SCHMELZER and WOLF (1971)
	Tobacco mosaic virus	PRICE (1940), SCHMELZER and WOLF (1971)
	Tobacco ring spot virus	ANDERSON (1959)
	Tomato bunchy top virus	SMITH (1957, 1972), THORNBERRY (1966)

alfalfa mosaic virus (strain K2, R/1 : 1.3/18 : U/U : S/Ap; BECZNER, 1972), bean (common) mosaic virus (∗/∗ : ∗/∗ : E/E : S/Ap; HORVÁTH, 1973a), cucumber mosaic virus (R/1 : 1/18 : S/S : S/Ap; HORVÁTH, 1973b), potato aucuba mosaic virus (∗/∗ : ∗/∗ : E/E : S/Ap; HORVÁTH 1972b), potato virus M (∗/∗ : ∗/∗ : : E/E : S/Ap; HORVÁTH and DE BOKX, 1972), potato virus S (∗/∗ : ∗/∗ : E/E : : S/Ap; HORVÁTH, 1972a), potato virus X (R/1 : ∗/6 : E/E : S/[Fu]); HORVÁTH and BECZNER, 1968), potato virus Y (∗/∗ : ∗/∗ : E/E : S/Ap; HORVÁTH, 1967), radish mosaic virus (R/∗ : ∗/∗ : S/S : S/Cl; HORVÁTH *et al.*, 1973), tobacco mosaic virus (R/1 : 2/5 : E/E : S/∗; SIEGEL and WILDMAN, 1954), tobacco necrosis virus (strain *f*, R/∗ : ∗/∗ : S/S : S/Fu; SZIRMAI, 1964), tobacco rattle virus

(R/1 : 2.3/5 : E/E : S/Ne; HORVÁTH, 1973a), tobacco ring spot virus (R/1 : 1.8/42 : : S/S : S/Ne; HORVÁTH, 1973a) and turnip yellow mosaic virus (R/1 : 1.9/37 : S/S : : S/Cl; HORVÁTH *et al.*, 1973, JURETIĆ *et al.*, 1973). Considering that some *Physalis* host–virus relations had become known already in earlier investigations (e.g. *Physalis aequata* – cucumber mosaic virus, potato virus X ; *Ph. ixocarpa* – alfalfa mosaic virus, potato virus X, potato virus Y, tobacco rattle virus [syn.: potato stem mottle virus]; *Ph. viscosa* – potato aucuba mosaic virus, potato virus Y, tobacco mosaic virus, tobacco ring spot virus) (see HORVÁTH, 1970; 1974), in our present work only the new host-virus relations were studied. As for the maintenance of viruses, the methods of inoculation, the reisolation of viruses from the inoculated plants, the test plants as well as the details of serological identification of potato virus M and potato virus S precise descriptions are found in our earlier paper (HORVÁTH, 1974).

Results and Discussion

In the course of investigations into the virus susceptibility of *Physalis aequata* we found it both locally and systemically susceptible to alfalfa mosaic virus, potato aucuba mosaic virus, potato virus Y, tobacco mosaic virus and tobacco ring spot virus. On rubbed leaves of plants inoculated with alfalfa mosaic virus and potato virus Y – often only after the appearance of systemic symptoms – local chlorotic, irregular spots showed. On the non-inoculated or subsequently developed leaves vein clearing and intensive mosaic symptoms appeared five days after the inoculation. An especially powerful, bright yellow mosaic showed on *Physalis aequata* plants inoculated with alfalfa mosaic virus (Fig. 1A). Plants inoculated with potato aucuba mosaic virus did not show manifest local symptoms, but severe systemic interveinal mosaic and irregular scratchy necroses were observed on them. The latent local infection of *Physalis aequata* was unambigously pointed out in virus reisolation experiments with *Capsicum annuum* L. On rubbed leaves of plants inoculated with tobacco mosaic virus and tobacco ring spot virus 2–3 days after the inoculation numerous necrotic lesions appeared (in the case of tobacco ring spot virus the lesions were concentric surrounded by a light chlorotic ring; Fig. 1C). In the case of tobacco mosaic virus the necrotic lesions coalesced, and later the inoculated leaves withered and fell off. On the 6th day following the inoculation systemic symptoms (vein clearing, leaf blistering, leaf deformation and growth reduction; Fig. 1B) appeared. In plants inoculated with tobacco ring spot virus systemic interveinal necrotic spots showed too *Physalis aequata* only displayed a local susceptibility to tobacco necrosis virus (fierce green lesions on the yellowing leaves) and to tobacco rattle virus (greyish brown necrotic lesion surrounded by a chlorotic ring). The examined plant proved resistant to infection by bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic virus and turnip yellow mosaic virus. *Physalis ixocarpa* gave local and sys-

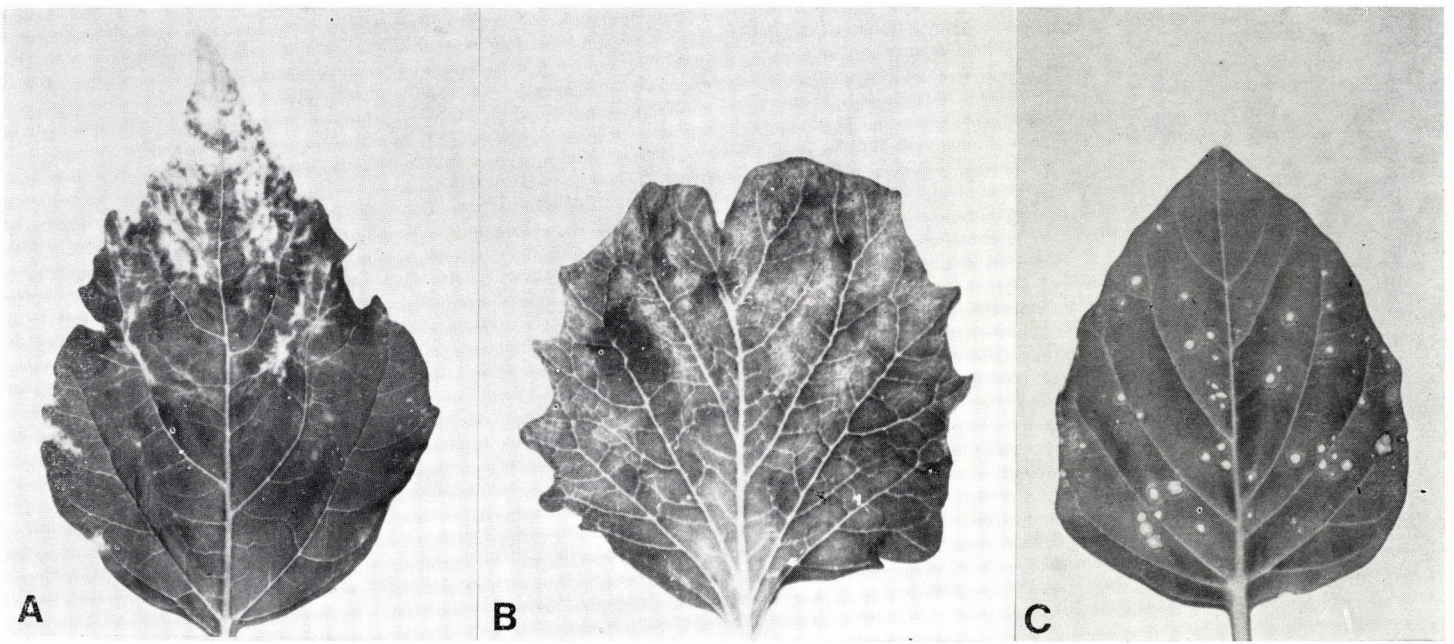

Fig. 1. Reaction of *Physalis aequata* Jacq. to plant viruses. A and B: systemic symptoms, C: local symptoms. A: alfalfa mosaic virus, B: tobacco mosaic virus, C: tobacco ring spot virus

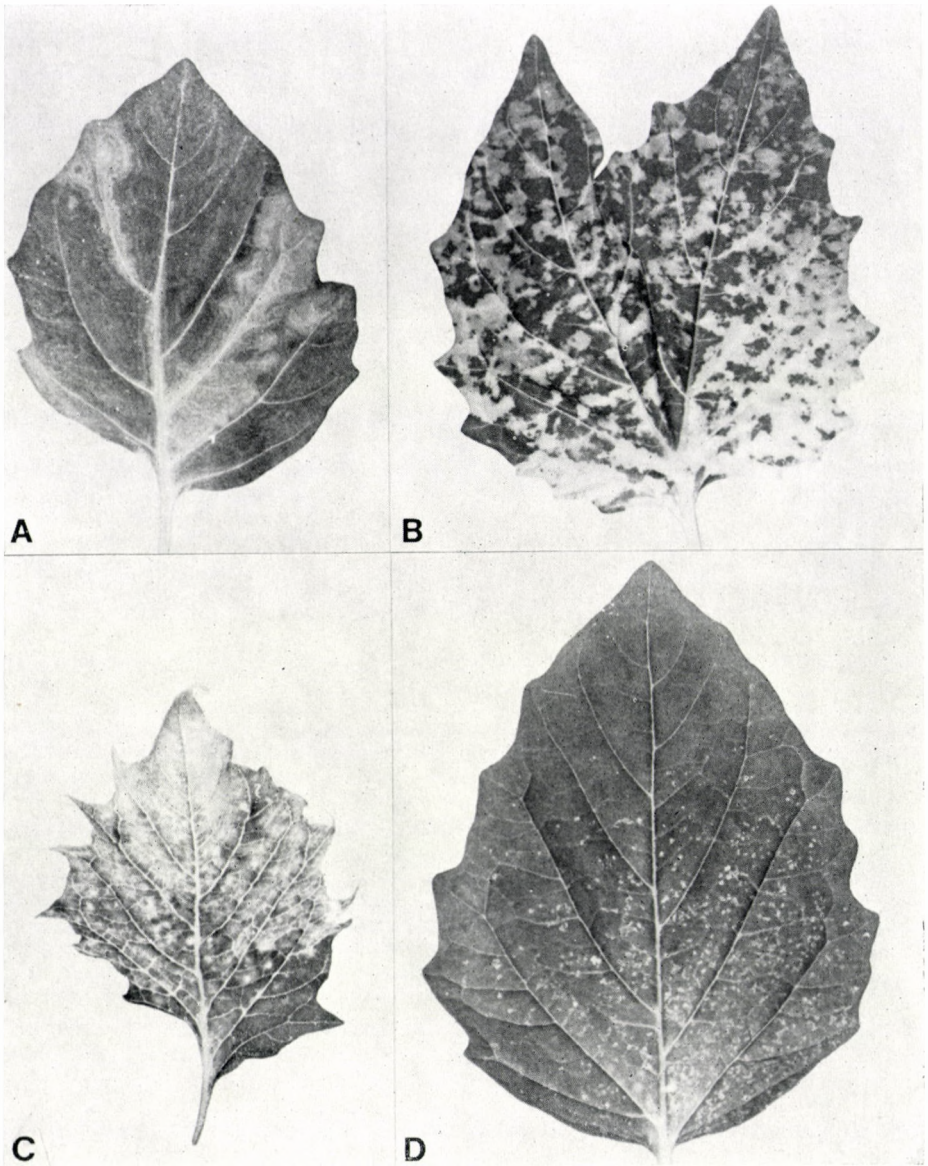

Fig. 2. Local (A) and systemic symptoms (B, C and D) of *Physalis ixocarpa* Brot. to three plant viruses. A and B: cucumber mosaic virus, C: tobacco mosaic virus, D: potato aucuba mosaic virus

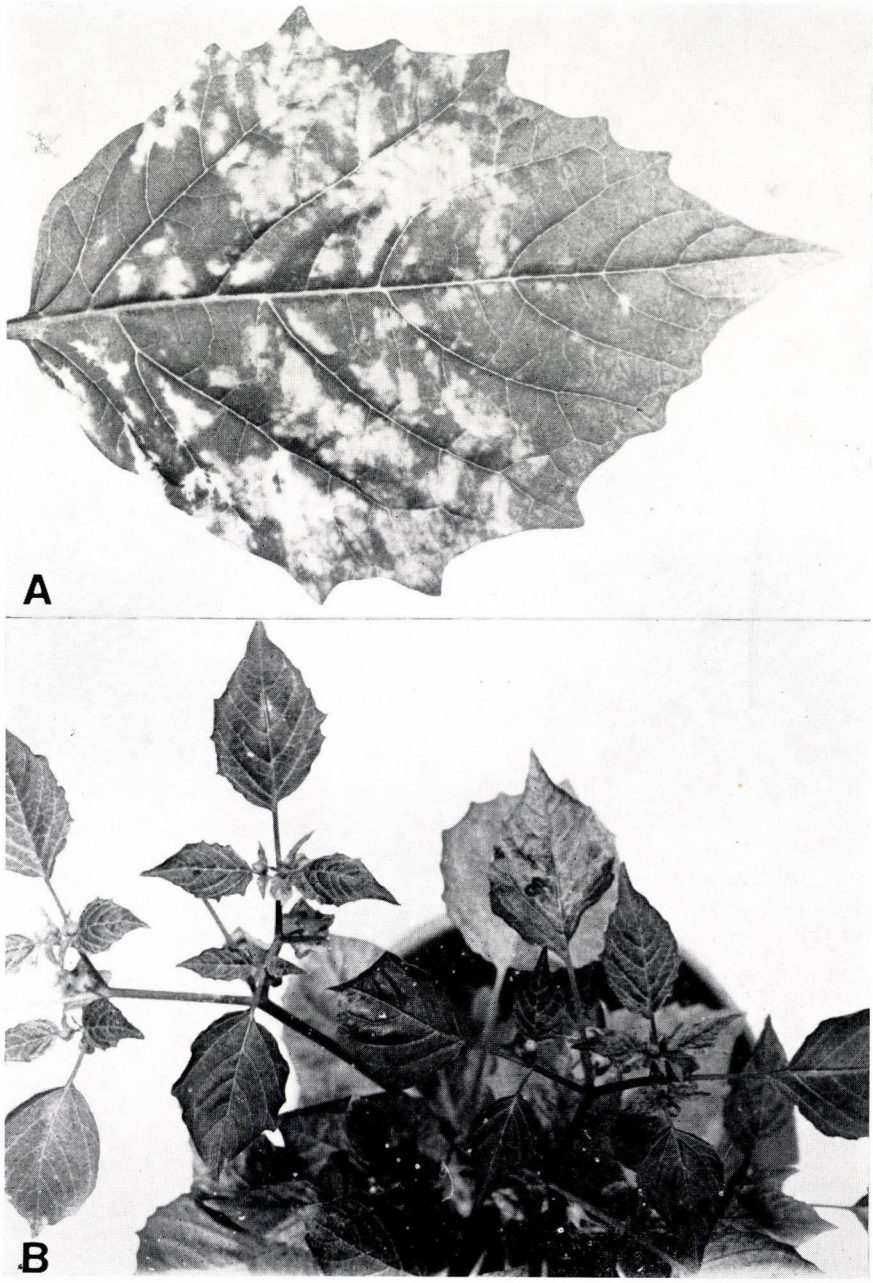

Fig. 3. *Physalis viscosa* L. systemically infected with alfalfa mosaic virus (A) and cucumber mosaic virus (B)

temic responses to infection by cucumber mosaic virus, potato aucuba mosaic virus, tobacco mosaic virus and tobacco ring spot virus. Local symptoms appearing on the 5th day after inoculation with cucumber mosaic virus were characterized by very intensive – though not necrotic – mosaic spots of about 0.5 cm diameter, and vein clearing (Fig. 2A). On the non-inoculated leaves vein clearing and very conspicuous ochre spots appeared which covered almost the entire leaf surface (Fig. 2B). Plants inoculated with potato aucuba mosaic virus did not show manifest local symptoms, but the inoculated plants proved latent susceptibility. The systemic symptoms were characterized by vein clearing and marked interveinal mosaic spottedness (Fig. 2D). Plants inoculated with tobacco mosaic virus and tobacco ring spot virus rubbed into the leaves developed necrotic lesions on the leaves 2–3 days after inoculation. In the case of tobacco mosaic virus the lesions were irregular, while the plants inoculated with tobacco ring spot virus displayed concentric lesions. In both cases the systemic symptoms appeared on the 6th day. The tobacco mosaic virus infection was characterized by intensive vein clearing, leaf blistering and mosaic spottedness (Fig. 2C). Some 40–50 per cent growth inhibition compared to the healthy control plants was also observed. In plants inoculated with tobacco ring spot virus systemic vein clearing, leaf deformation and tiny necrotic spots were found; the growth inhibition was substantially milder than in the plants inoculated with tobacco mosaic virus. *Physalis ixocarpa* only showed a local susceptibility to tobacco necrosis virus. The leaves of virus inoculated plants turned yellow and fierce green, mostly circular spots appeared on them. The examined plant proved to be resistant to five viruses: bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic virus and turnip yellow mosaic virus. In the course of investigations into the virus susceptibility of *Physalis viscosa* latter plant was found to be locally and systemically susceptible to three viruses (alfalfa mosaic virus, cucumber mosaic virus and potato virus X). The leaves of plants inoculated with alfalfa mosaic virus developed grey, irregular necrotic spots (mostly after systemic symptoms appearing on the 5th day). The systemic symptoms were: vein clearing, leaf blisters, and a particularly intensive and conspicuous bright yellow mosaic spottedness (Fig. 3A). Plants inoculated with cucumber mosaic virus rubbed into the leaves showed local, non-necrotic leaf spots of 0.5 cm diameter. The systemic symptoms appeared in the form of vein clearing and a conspicuous interveinal mosaic of ochre colour (Fig. 3B). The appearance of fierce green zones on the yellowing leaf surface was remarkable. Plants inoculated with potato virus X showed local and systemic susceptibility without displaying manifest symptoms. Symptoms appearing in the *Gomphrena globosa* L. test plants unambiguously proved the presence of the virus in both the inoculated and non-inoculated leaves. *Physalis viscosa* gave only a local response to infection by tobacco necrosis virus and tobacco rattle virus. The rubbed leaves of tobacco necrosis virus inoculated plants turned yellow, and fierce green spots appeared on them. Plants inoculated with tobacco rattle virus developed necrotic spots. The examined *Physalis viscosa* plant proved to be re-

sistant to infection by bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic virus and turnip yellow mosaic virus.

On the basis of our experiment results, of the examined *Physalis* species *Physalis aequata* is considered to be a new host plant of seven viruses, while *Ph. ixocarpa* and *Ph. viscosa* are hosts of five viruses. The examined species have a particularly important role in pointing out the polyphagous alfalfa mosaic virus, cucumber mosaic virus, potato virus Y, tobacco mosaic virus and tobacco ring spot virus. In addition to their virus diagnostical significance they have an important part in the separation of the different viruses (e.g. potato pathogen viruses) too.

Acknowledgements

I am indebted to Prof. Dr. J. HESLOP-HARRISON, Royal Botanic Gardens, Kew, Richmond, Surrey, England, for his valuable information about the nomenclature of *Physalis* species. Thanks are also due to the Botanischer Garten, Marburg, GFR; Giardino Botanico e Coloniale di Palermo, Palermo, Italy; Hortus Botanicus Bruxellensis, Bruxelles, Belgium; Institut für Phytopathologie der Friedrich-Schiller-Universität, Jena, GDR; Hortus Botanicus Universitatis, Nijmegen, The Netherlands; Hortus Plantarum Medicarum, Facultatis Pharmaceuticae Universitatis Comenianae, Bratislava, Czechoslovakia; Hortus Botanicus Universitatis, Košice, Czechoslovakia; Hortus Botanicus Universitatis, Budapest, Hungary for sending *Physalis* seeds. The author wishes to express his appreciation to Miss K. MOLNÁR and Miss M. BOLLÁN for their valuable technical assistance.

Note (added in proof)

In a recent paper PETERS and DERKS (Neth. J. Pl. Path. *80*, 124—132, 1974) described, that the *Physalis ixocarpa* Brot. is susceptible to *Physalis* mosaic virus newly isolated from *Physalis subglabrata* MacKenzie et Bush. in Illinois, USA. The three Physalis species (*Ph. floridana* Rydb., *Ph. peruviana* L. and *Ph. subglabrata*) are locally and systemically susceptible hosts of the *Physalis* mosaic virus.

Literature

ANDERSON, C. W. (1959): Tobacco ringspot virus on florist's hydrangea. Plant. Dis. Rep. *42*, 932—933.

BECZNER, L. (1972): A lucerna mozaik vírus új törzseinek magyarországi fellépése (Occurrence of new strains of alfalfa mosaic virus in Hungary). A Növényvéd. Korsz. *6*, 101—118.

DUFFUS, J. E. (1973): The yellowing vírus diseases of beet. Adv. Virus Res. *18*, 347—386.

FULTON, R. W. (1957): Comparative host ranges of certain mechanically transmitted viruses of *Prunus*. Phytopathology *47*, 215—220.

GREENLEAF, W. H. (1953): Effects of tobacco-etch virus on peppers (*Capsicum* sp.). Phytopathology *43*, 564—570.

HORVÁTH, J. (1967): Studies on strains of potato virus Y. 3. Strain causing browning of midribs in tobacco. Acta Phytopath. Acad. Sci. Hung. *2*, 95—108.

HORVÁTH, J. (1970): Reaction of *Physalis* species to plant viruses. 1. The cape gooseberry as a symptomless carrier of potato virus X and Y. Acta Phytopath. Acad. Sci. Hung. *5*, 65—72.

HORVÁTH, J. (1972a): Symptomless *Lycopersicon* host plants for potato virus S. Amer. Potato J. *40*, 339—341.

HORVÁTH, J. (1972b): *Lycium*-Arten als neue Testpflanzen für verschiedene Pflanzenviren Acta Phytopath. Acad. Sci. Hung. *7*, 465–468.

HORVÁTH, J. (1973a): *Paulownia fargesii* Franch. (Family: *Scrophulariaceae*) as a new host plant for several plant viruses. Acta Phytopath. Acad. Sci. Hung. *8*, 165–173.

HORVÁTH, J. (1973b): *Tetragonia echinata* Ait. als eine neue Testpflanze für das Gurkenmosaik-Virus (cucumber mosaic virus). Z. Pflanzenkrankheiten *80*, 129–132.

HORVÁTH, J. (1974): Reaction of *Physalis* species to plant viruses. II. Additional data on the virus susceptibility of *Physalis* spp. and new experimental results: *Physalis peruviana* L. var. macrocarpa and *Physalis pruinosa* L. Acta Phytopath. Acad. Sci. Hung. *9*, 1–9.

HORVÁTH, J. and BECZNER, L. (1968): A vírus gazdanövénykör kutatás újabb eredményei (Recent results of research on virus-host range). Növényvédelem *6*, 248–256.

HORVÁTH, J. and DE BOKX, J. A. (1972): Über die Anfälligkeit von *Chenopodium*-Arten und -Varietäten gegen das M-Virus (potato virus M). Phytopath. Z. *73*, 171–177.

HORVÁTH, J., JURETIĆ, N. and MILIČIĆ, D. (1973): *Crambe abyssinica* Hochst. ex. R. E. Frees as a new host plant for turnip yellow mosaic virus and radish mosaic virus. Phytopath. Z. *78*, 69–74.

JURETIĆ, N., HORVÁTH, J., MAMULA, D. and MILIČIĆ, D. (1973): Natural occurrence of turnip yellow mosaic virus in Hungary. Acta Phytopath. Acad. Sci. Hung. *8*, 175–183.

LADEBURG, R. C., LARSON, R. H. and WALKER, J. C. (1950): Origin, interrelation and properties of ringspot strains of virus X in American potato varieties. Wisc. Agr. Exp. Stat., Res. Bull. *165*, 1–47.

MACLEOD, D. J. (1962): Mosaic and streak viruses of the potato. Canad. Dept., Agr. Publ. *1150*, 1–80.

PETERS, D. and DERKS, A. F. L. M. (1974): Host range of some properties of *Physalis* mosaic virus, a new virus of the turnip yellow mosaic virus group. Neth. J. Pl. Path. *80*, 124–132.

PONTIS, R. E. and FELDMAN, J. M. (1963): A common weed, *Physalis viscosa*, new host for potato virus Y. Plant Dis. Rep. *47*, 22.

PRICE, W. C. (1940): Comparative host ranges of six plant viruses. Amer. J. Bot. *27*, 530–541.

SCHMELZER, K. (1957): Untersuchungen über den Wirtspflanzenkreis des Tabakmauche-Virus. Phytopath. Z. *30*, 281–314.

SCHMELZER, K. (1963a): Untersuchungen an Viren der Zier- und Wildgehölze. 1. Mitteilung. Virosen an *Viburnum* und *Ribes*. Phytopath. Z. *46*, 17–52.

SCHMELZER, K. (1963b): Untersuchungen an Viren der Zier- und Wildgehölze. 3. Mitteilung: Virosen an *Robinia*, *Caryopteris*, *Ptelea* und anderen Gattungen. Phytopath. Z. *46*, 235–268.

SCHMELZER, K. und WOLF, P. (1971): Wirtspflanzen der Viren und Virosen Europas. Nova Acta Leopoldina, Suppl. 2., Johann Ambrosius Barth., Leipzig 1971.

SIEGEL, A. and WILDMAN, S. G. (1954): Some natural relationships among strains of tobacco mosaic virus. Phytopathology *44*, 277–282.

SMITH, K. M. (1957): A Textbook of Plant Virus Diseases. J. et Churchill Ltd., London 1957.

SMITH, K. M. (1972): A Textbook of Plant Virus Diseases. Third edition., Longman Group Ltd., London 1972.

SZIRMAI, J. (1964): Durch Wirtspassage induzierte Varianten beim Tabaknekrosevirus. *In:* Z. KIRÁLY and G. UBRIZSY (eds.), Host-Parasite Relations in Plant Pathology. Symp. Hung. Acad. Sci., 19–22 October 1964, Budapest. pp. 97–104.

THORNBERRY, H. H. (1966): Index of Plant Virus Diseases. Agr. Handbook, U. S. Dept. Agr., Washington 1966.

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Natural Occurrence of Turnip Mosaic Virus in Hungary

By

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The present paper deals with the spontaneous occurrence of turnip mosaic virus (syn.: cabbage black ring virus, ∗/∗ : ∗/∗ : E/E : S/Ap) in cauliflower (*Brassica oleracea* L. var. botrytis [L.] Alef.) and garlic mustard (*Alliaria petiolata* [M. B.] Cavara et Grande [syn.: *A. officinalis* Andrz.]). The identification was based on test plant reactions, insect transmission (*Myzus persicae* Sulz.) and serology. In inoculation tests with cauliflower isolate (TuMV-*JN*) and garlic mustard isolate (TuMV-*All*) 8 of the 28 tested species proved to be local, and 9 to be systemic or local and systemic hosts of turnip mosaic virus. Five new host plant species of the *JN* and *All* isolates of turnip mosaic virus were: *Bunias orientalis* L., *Nicotiana chinensis* Fisch., *N. occidentalis* Wheeler, *Obione sibirica* (L.) Fisch., *Tetragonia echinata* Ait. The physical properties of the two isolates were found to be somewhat different, but the thermal inactivation point was generally between 54—56°C, the dilution end point in sap between 2×10^{-3} and 10^{-3}, and longevity *in vitro* was to 2 days. On the second day the activity of the *All* isolate of turnip mosaic virus was very low. In artificial infection experiments an antagonistic effect was demonstrated between turnip mosaic virus and cucumber mosaic virus in *Nicotiana tabacum* L. cv. Xanthi-nc plants. The cucumber mosaic virus inhibited multiplication of the turnip mosaic virus to about 60—70 per cent.

This is apparently the first report on the presence of turnip mosaic virus in Hungary.

Introduction

The turnip mosaic virus (syn.: cabbage black ring virus, ∗/∗ : ∗/∗ : : E/E : S/Ap) is spread all over the world, but generally occurs in the temperature zones of Africa, Asia, Europe and North-America (cf. YOSHII, 1963; TOMLINSON, 1970; SMITH, 1972). In spite of its wide distribution until recently only symptomatological observation and descriptions have been available concerning its occurrence in Hungary (cf. MILINKÓ, 1952). In the course of our investigations into the spread of virus diseases of plants belonging to the family *Cruciferae* in Hungary so far the occurrence of cucumber mosaic virus in rape (HORVÁTH, 1969a; HORVÁTH and HINFNER, 1969), radish mosaic virus (MAMULA *et al.*, 1972), and turnip yellow mosaic virus (HORVÁTH et al., 1973; JURETIĆ *et al.*, 1973) in turnip has been demonstrated. In experiments carried out in the recent past a further virus was isolated from cauliflower (*Brassica oleracea* L. var. botrytis [L.] Alef.) and garlic

mustard (*Alliaria petiolata* [M. B.] Cavara et Grande [syn.: *A. officinalis* Andrz.]). Considering that neither the newly isolated virus could be identified with those isolated and described in Hungary so far, nor were the above two plants known as natural Hungarian virus hosts, we carried out investigations with a view to the exact identification of the virus pathogen.

Material and Methods

In the summer of 1972 we found cauliflower plants with virus symptoms in the *Nezlov* garden at Keszthely (on the shores of Lake Balaton). The diseased plants showed vein clearing- and mosaic symptoms. Leaf deformations of the diseased plants were also remarkable (Fig. 1C). From the leaves collected on the plants with symptoms we prepared tissue extract in a porcelain mortar by adding an equal volume of phosphate buffer (0.1 *M*, pH 7.0), and with the extract we inoculated test plants (e.g. *Brassica rapa* L. var. rapa, *Chenopodium amaranticolor* Coste et Reyn., *Ch. quinoa* Willd., *Gomphrena globosa* L., *Nicotiana tabacum* L. cv. Samsun, *N. tabacum* L. cv. Xanthi-nc) using the carborundum spatula technique. On the third or fourth day following the inoculation local lesions appeared on the tobacco plants, and not much later on the *Chenopodium* plants too. The subsequent investigations concerned the identification of the isolate from cauliflower and determination of its properties. This isolate was designated by *JN*. Parallel with these investigations, during a virological-botanical collecting tour at Becehegy by Lake Balaton we found a garlic mustard plant whose leaves showed typical mosaic spottedness, dark green islands, deformation, leaf edge curling and intensive vein banding (Fig. 1A and B). The naturally infected leaves were ground with phosphate buffer added. The extract was mechanically applied to the following test plants in the greenhouse: *Ammi majus* L., *A. visnaga* L. (Lam.), *Brassica rapa* L. var. rapa, *Chenopodium amaranticolor* Coste et Reyn., *Ch. quinoa* Willd., *Cucumis sativus* L., *Datura stramonium* L., *Gomphrena globosa* L., *Nicotiana glutinosa* L., *N. tabacum* L. cv. Samsun, *N. tabacum* L. cv. Xanthi-nc, Samsun- and Xanthi-nc tobacco plants displayed local lesions 3–4 days after inoculation, and *Chenopodium* plants exhibited fully developed local lesions a couple of days later. These symptoms were similar to those induced by the *JN* virus isolate obtained from cauliflower. To identify the virus isolated from garlic mustard (designated by *All*), as well as isolate *JN*, we used various methods.

Host plant tests with the *JN*- and *All* isolates included 28 species from 8 families (see Table 1). The two isolates were examined serologically using microprecipitin method. In these tests was used an antiserum prepared against a Yugoslav strain of turnip mosaic virus *(cabbage isolate)*, as well as the turnip mosaic virus antiserum kindly sent us by Prof. Dr. R. BERCKS (Braunschweig). Physical properties and transmission by aphids were also studied. When studying physical properties we took into consideration the thermal inactivation point, longevity

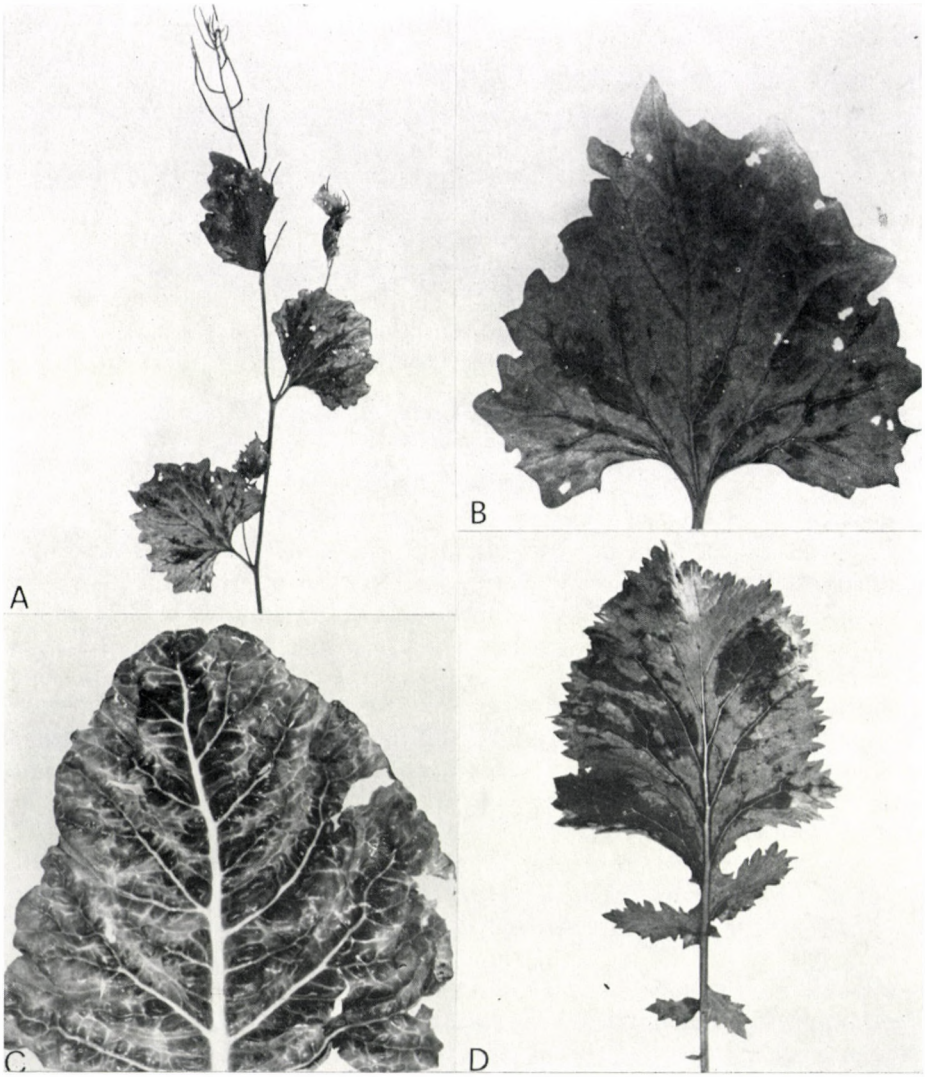


Fig. 1. Symptoms on the spontaneously infected *Alliaria petiolata* (M. B.) Cavara et Grande plant (A), leaf (B) and *Brassica oleracea* L. var. botrytis (L.) Alef. (C). D: Artificially inoculated *Brassica rapa* L. var. rapa with the isolate of *All* of turnip mosaic virus (TuMV-*All*)

in vitro and dilution end point. We used turnip as source plant, and *Chenopodium amaranticolor* Coste et Reyn. and Xanthi-nc as test plants.

Insect transmission tests were performed in case of both *All* and *JN* isolates by using *Myzus persicae* Sulz. as vector. The aphids reared on healthy turnip

plants were starved for three hours, then allowed to feed on diseased turnip leaves in Petri dishes for 8 – 10 minutes. Ten aphids were transferred to turnip test plants, kept there for one day, then killed by Phosdrin insecticide. The interaction between the *All* isolate and the *W* strain of cucumber mosaic virus (cf. SKIEBE and SCHMELZER, 1967) was studied in a separate experiment. Xanthi-nc tobacco plants were inoculated with identical quantities of tissue sap obtained from turnip and tobacco infected with *All* isolate and cucumber mosaic virus, respectively. The controls were Xanthi-nc tobacco plants infected only with the *All* isolate. The number of local lesions induced in tobacco by the complex inoculations (simultaneously performed with *All* isolate and the *W* strain of cucumber mosaic virus) was compared the number of those appearing on tobacco leaves inoculated only with *All* isolate. With respect to differences in susceptibility between leaves of different position on the stem the comparison was made with the 4th and 5th leaf.

Results and Discussion

In our artificial inoculation experiments the *JN* and *All* isolates were found to infect the same host plants (Table 1). On the basis of the type of symptoms shown by the test plants it seemed probable that both isolates were identical with the turnip mosaic virus. This supposition was supported by the results of an experiment in which *Nicotiana tabacum* L. cv. Samsun and *N. tabacum* L. cv. Xanthi-nc gave a local, while *N. glutinosa* L. a systemic response. This type of host reaction is characteristic of the turnip mosaic virus. Symptoms found on other test plants (see Table 1, Fig. 1D, Fig. 2A – C, and Fig. 3A – D) also proved identical with those caused by the turnip mosaic virus. It is to point out the fact that isolates *JN* and *All* differ fairly strong from one another with respect to reaction they caused in *Brassica oleracea* L. var. capitata and *Nicotiana glutinosa* L. plants. While *JN* isolate from cauliflower produced severe symptoms in both species, the infection with isolate *All* resulted in comparatively much milder symptoms in those plants. According to that property our isolate *JN* belongs to *cabbage strain* of turnip mosaic virus and *All* isolate to *ordinary strain*. This classification of turnip mosaic virus isolates in two strains was proposed by YOSHII (1963) who ordered a great number of turnip mosaic virus isolates into two named strains on the basis of the mentioned reaction of *Brassica oleracea* L. var. capitata and *Nicotiana glutinosa* L. It is interesting that *cabbage strain* apparently includes all turnip mosaic virus isolates from *Brassica oleracea* L. var. capitata known (cf. ULLRICH, 1955; LOVISOLO, 1960; MILIČIĆ *et al.*, 1963; ŠTEFANAC, 1964; 1967), while *ordinary strain* contains isolates from various cruciferous plants other than *Brassica oleracea* L. (cf. YOSHII, 1963) and non-cruciferous plant (*Tropaeolum majus* L., cf. MAMULA and LJUBEŠIĆ, 1975). In this respect several isolates from *Alliaria* sp. (BODE and BRANDES, 1958; SCHWARZ, 1959; WEIL, 1959; MILIČIĆ *et al.*, 1963; ŠTEFANAC, 1964; 1967), *Matthiola* sp. (POUND and WALKER, 1945; ULL-

Table 1

Reaction of several plants to two isolates of turnip mosaic virus from cauliflower *(JN)* and garlic mustard *(All)* in Hungary*

AIZOACEAE	
Tetragonia echinata Ait.**	I: Chlorotic spots II: Not infected
T. tetragonoides (Pall.) O. Ktze	I: Chlorotic spots II: Not infected
AMARANTHACEAE	
Gomphrena globosa L.	I: Gray local lesions with pink border II: Not infected
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	I: Chlorotic local lesions later turning into necrotic lesions II: Not infected
Ch. quinoa Willd.	I: Chlorotic local lesions II: Not infected
Obione sibirica (L.) Fisch.**	I: Chlorotic local lesions II: Not infected
CRUCIFERAE	
Brassica campestris L.	I: Not infected II: Systemic vein clearing, mosaic with light and dark green patches or blisters
Br. carinata A. Br.	I: Not infected II: Systemic vein clearing and mosaic
Br. oleracea L. var. capitata***	I: Local black necrotic rings (sometimes erratic reaction) II: Black ringspots (sometimes erratic reaction)
Br. rapa L. var. rapa	I: Chlorotic and sometimes necrotic local lesions II: Systemic vein clearing and veinal flecking, developing into severe mosaic with light and dark green islands. Severe distortion and stunting
Bunias orientalis L.**	I: Not infected II: Systemic mosaic spots
Cheiranthus cheiri L.	I: Not infected II: Systemic leaf distortion, mottling
CUCURBITACEAE	
Bryonia alba L.	I: Not infected II: Not infected
B. dioica Jacq.	I: Not infected II: Not infected

Cucumis sativus L.	I: Not infected
	II: Not infected
Cucurbita pepo L. var.	I: Not infected
patissonina Greb. f. radiata Nois.	II: Not infected
LEGUMINOSAE	
Phaseolus vulgaris L. cv.	I: Not infected
Red Kidney	II: Not infected
SOLANACEAE	
Capsicum annuum L.	I: Not infected
	II: Not infected
Datura stramonium L.	I: Not infected
	II: Not infected
Nicotiana chinensis	I: Local chlorotic spots
Fisch.**	II: Not infected
N. glutinosa L.***	I: Not infected
	II: Systemic mottling (sometimes erratic reaction)
N. occidentalis Wheeler**	I: Local necrotic lesions
	II: Systemic mosaic, sometimes necrotic spots
N. tabacum L. cv. Samsun,	I: Local necrotic lesions
Bel 61—10, and Xanthi-nc	II: Not infected
Petunia hybrida hort. ex Vilm	I: Black necrotic local lesions
	II: Systemic vein clearing, mottling and colour flower breaking
Solanum capsicastrum Link.	I: Not infected
	II: Not infected
S. ochroleucum Bast.	I: Not infected
	II: Not infected
UMBELLIFERAE	
Ammi majus L.	I: Not infected
	II: Not infected
A. visnaga (L) Lam.	I: Not infected
	II: Not infected

* I, denotes rubbed leaves (local symptoms); II, denotes leaves developed after inoculation (systemic symptoms).

** Plants with two asterisks are new experimental hosts of turnip mosaic virus

*** The two isolates of turnip mosaic virus *(JN* and *All)* reacted differently on both *Brassica oleracea* L. var. capitata and *Nicotiana glutinosa* L. test plants (text, page 80)

RICH, 1955; LOVISOLO, 1960; MILIČIĆ, 1962; YOSHII *et al.*, 1963; WEIL, 1964; USCHDRAWEIT and VALENTIN, 1957; TOCHIHARA, 1965) and other crucifers belong to *ordinary strain* (cf. YOSHII, 1963), because all of them produce weak or no symptoms in *Brassica oleracea* L. var. capitata and mild symptoms in *Nicotiana glutinosa* L., too.

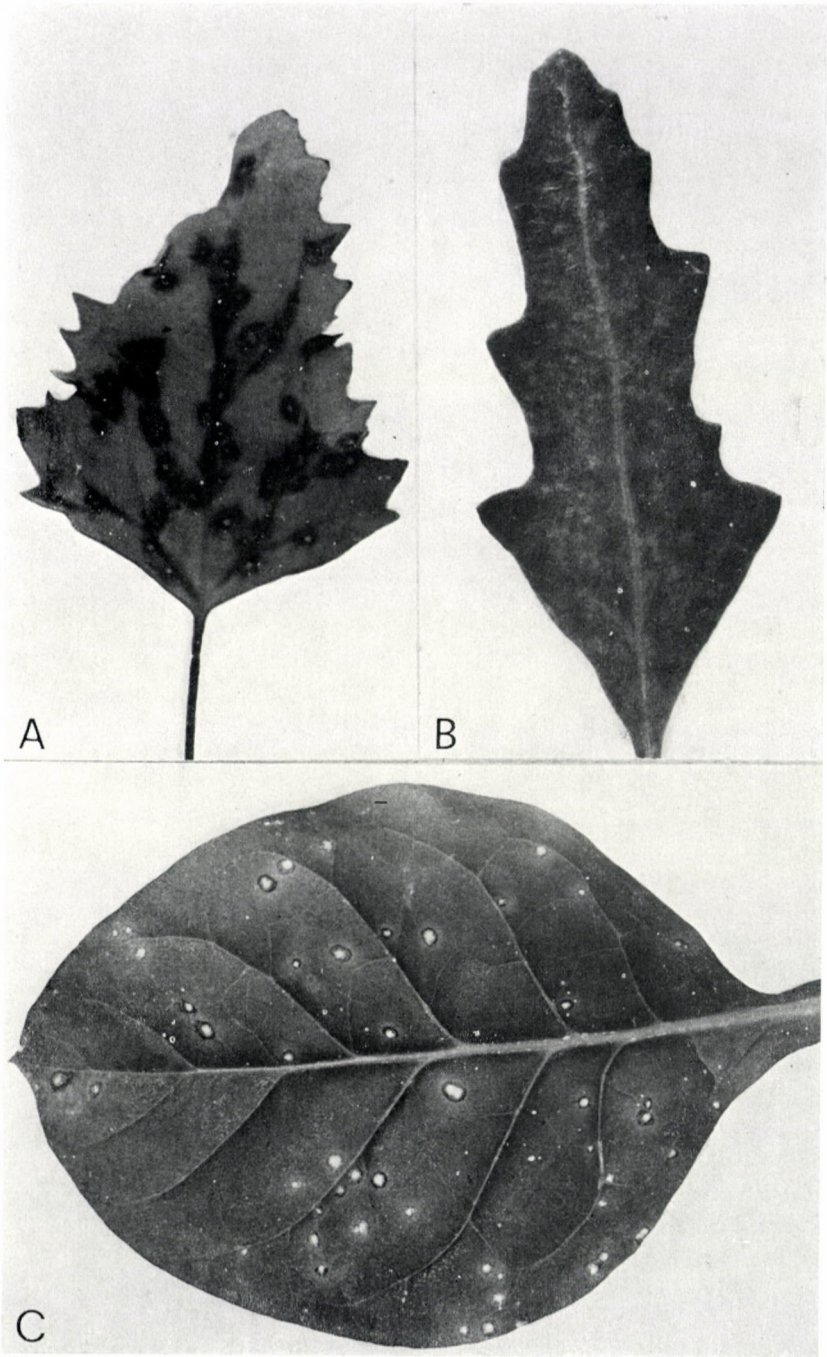
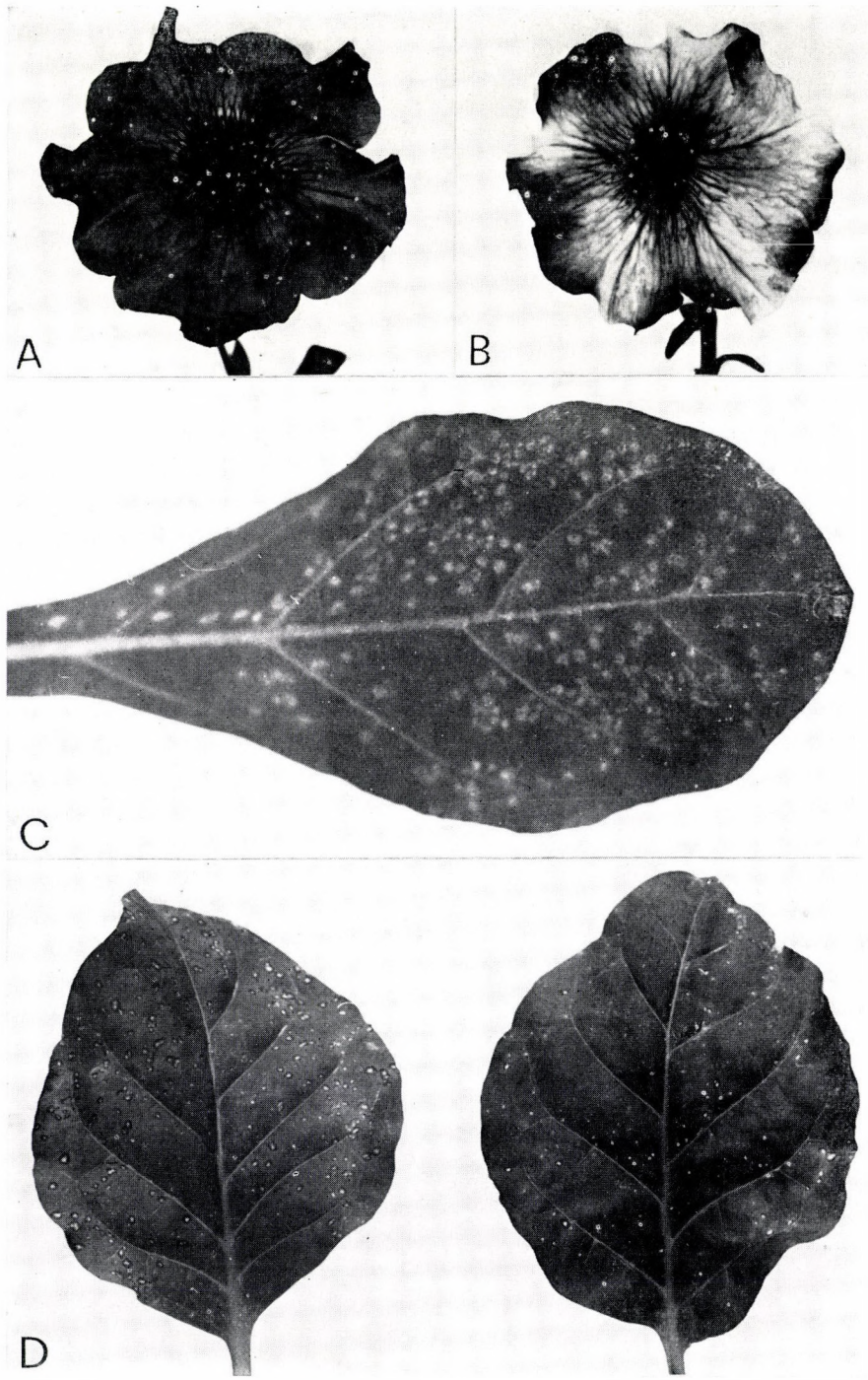


Fig. 2. Local symptoms on the various test plants inoculated with the *All* isolate of turnip mosaic virus (TuMV-*All*). A: *Chenopodium amaranticolor* Coste et Reyn., B: *Obione sibirica* (L.) Fisch., C: *Nicotiana tabacum* L. cv. Bel 61 – 10


A
B
C
D

We found a good specific reaction in the microprecipitin test with sap of infected turnip plants. None of the controls gave a positive reaction.

On the basis of test plant and serological examinations the identity of the *JN* isolate obtained from cauliflower and *All* isolate from garlic mustard with the turnip mosaic virus can be regarded as proved. As to the transmission of the *JN* and *All* isolates by aphids we found that in a stylet-borne manner they were easily transmitted to *Brassica rapa* L. var. rapa plants. The physical properties of the two virus isolates are very similar (Table 2). The values established by the experiments were, however, somewhat lower than those reported by TOMLINSON (1970) for turnip mosaic virus.

Table 2

Physical properties of the isolates of turnip mosaic virus isolated from cauliflower (*Brassica oleracea* L. var. botrytis [L.] Alef.) and garlic mustard (*Alliaria petiolata* [M. B.] Cavara et Grande) in Hungary

Isolates of turnip mosaic virus	Physical properties*		
	TIP (in °C)	DEP	Liv (in days)
JN	56	10^{-2}	1
All	54–56	2×10^{-2}	2**

* TIP, thermal inactivation point; DEP, dilution end point; Liv, longevity *in vitro*
** The activity of the virus was very low

Our investigations have confirmed the results of earlier researches carried on abroad concerning the turnip mosaic virus infection of *Brassica oleracea* L. var. botrytis (L.) Alef. and *Alliaria petiolata* (M. B.) Cavara et Grande (cf. MILIČIĆ, 1956; BROADBENT, 1957; USCHDRAWEIT and VALENTIN, 1957; MILIČIĆ *et al.*, 1958; BODE and BRANDES, 1958; ŠTEFANAC-UDJBINAC *et al.*, 1963; STEFANAC and MILIČIĆ, 1965; LAPIERRE and GRISON, 1969; SHUKLA and ŠCHMELZER, 1973). The complex infection of *Alliaria petiolata* (M. B.) Cavara et Grande by turnip mosaic virus and cucumber mosaic virus is also known in the literature (see HEROLD and BREMER, 1958; BRČÁK and POLÁK, 1963). According to our own experiments natural infection of *Alliaria* studied in this paper was caused by the turnip

Fig. 3. Healthy flower of *Petunia hybrida* Vilm. (A) and colour flower breaking (B) of the diseased plant inoculated with the *All* isolate of turnip mosaic virus (TuMV-*All*). C: Local symptoms on inoculated leaf of *Nicotiana occidentalis* Wheeler plant. D: Antagonistic interaction between the strains of turnip mosaic virus and cucumber mosaic virus. Left, local lesions on *Nicotiana tabacum* L. cv. Xanthi-nc leaf inoculated only with *All* isolate of turnip mosaic virus (TuMV-*All*); right, local lesions on *Nicotiana tabacum* L. cv. Xanthi-nc tobacco leaf inoculated with both *All* isolate of turnip mosaic virus (TuMV-*All*) and *W* strain of cucumber mosaic virus (CMV-*W*)

mosaic virus alone; cucumber mosaic virus could not be isolated from the diseased plant. This is proved by the immune *Cucumis sativus* L. and *Cucurbita pepo* L. var. patissonina Greb. f. radiata Nois., and by the only locally susceptible Samsun and Xanthi-nc tobacco plants.

When studying the interaction between the *All* isolate of the turnip mosaic virus and the *W* strain of the cucumber mosaic virus we found that in the case of a simultaneous inoculation the cucumber mosaic virus had an about 60 – 70 per cent inhibitory effect on the multiplication of the *All* isolate of turnip mosaic virus in Xanthi-nc tobacco leaves (Fig. 3D). In Xanthi-nc tobacco leaves inoculated either with the single *All* isolate or simultaneously with the *All* isolate and *W* strain of cucumber mosaic virus the number of local lesions was lower in the fourth than in the fifth leaf. These experimental results resemble those found earlier while studying some host-virus relations (HORVÁTH, 1969a, b, c, 1973). Considering that the cucumber mosaic virus and turnip mosaic virus often occur in a complex form in various plants (cf. SHUKLA and SCHMELZER, 1972), the antagonistic actions of the two viruses are of particular interest.

The results of investigations concerning a new virus pathogen of *Alliaria* (cf. PAPA and MICHELIN-LAUSAROT, 1973; PAPA *et al.*, 1973) suggest that besides the virus earlier called *Alliaria* mosaic virus but found to be identical with the turnip mosaic virus since, there exists an *Alliaria* mosaic virus which in some biological and biochemical properties differs from the turnip mosaic virus. According to our present knowledge both the turnip mosaic virus and the *Alliaria* mosaic virus belong to the potyvirus group.

Zusammenfassung

Natürliches Vorkommen des Kohlrübenmosaik-Virus *(turnip mosaic virus)* in Ungarn

Es wird im Rahmen der Untersuchungen zur Charakterisierung und Identifizierung der Crucifeeren-Viren über das dritte Virus, das sog. Kohlrübenmosaik-Virus (*turnip mosaic virus*, syn.: *cabbage black ring virus;* ∗/∗ : ∗/∗ : E/E : S/Ap) erst in Ungarn berichtet. Das Virus wurde aus Knoblauchsrauke (*Alliaria petiolata* [M. B.] Cavara et Grande, syn.: *A. officinalis* Andrz.) und aus Blumenkohl (*Brassica oleracea* L. cv. botrytis [L.] Alef.) isoliert. Auf Grund der Untersuchungen über Wirtspflanzenkreis, Virusübertragung, Serologie und physikalische Eigenschaften des Virus stellten wir fest, dass die Isolate *JN* aus Blumenkohl und Isolate *All* aus Knoblauchsrauke mit dem Kohlrübenmosaik-Virus identisch sind. Es wurden fünf Pflanzenarten (*Bunias orientalis* L., *Nicotiana chinensis* Fisch., *N. occidentalis* Wheeler, *Obione sibirica* [L.] Fisch. und *Tetragonia echinata* Ait.) als neue Wirte für die beiden Virusisolaten des Kohlrübenmosaik-Virus festgestellt. Das Virus ist gut durch *Myzus persicae* Sulz. zu übertragen. Die beiden Virusisolate reagierten positiv mit Kohlrübenmosaik-Virus-Antiserum. Zwei Isolate des Virus hatten einen thermalen Inaktivierungspunkt zwischen 54 – 56°C der Verdünnungsendpunkt lag zwischen 2×10^{-3} und 10^{-3}. Bei der Bestimmung der Beständigkeit *in vitro* im Preßsaft bei Zimmertemperatur wurden maximal 1 Tag (bei *JN* Isolaten) und 2 Tage (bei *All* Isolaten) ermittelt. Eine Mischinfektion der *Nicotiana tabacum* L. cv. Xanthi-nc-Tabakpflanzen mit dem Kohlrübenmosaik-Virus und Gurkenmosaik-Virus *(cucumber mosaic virus)* verminderte die Zahl der durch Kohlrübenmosaik-Virus hervorgerufenen Lokalläsionen um 60 – 70%. In Infektionsversuchen mit den zwei Iso-

laten des Kohlrübenmosaik-Virus stellten wir fest, dass die Anfälligkeit der Tabakblätter (Bel 61—10, Samsun, Xanthi-nc) gegen Virus von ihrer Sequenz am Sproß abhängt; die Virusanfälligkeit von unten nach oben graduell abnimmt.

Dies ist anscheinend der erste Bericht über das natürliches Vorkommen des Kohlrübenmosaik-Virus in Ungarn.

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Literature

BODE, O. and BRANDES, J. (1958): Elektronenmikroskopische Untersuchung des Kohlrübenmosaik-Virus (turnip mosaic virus). Phytopath. Z. *34*, 103-106.

BRČÁK, J. and POLÁK, Z. (1963): Identification of the viruses responsible for the mosaic disease of *Alliaria officinalis* Andr. in Central Bohemia. Preslia *35*, 110—117.

BROADBENT, L. (1957): Investigations of Virus Diseases of *Brassica* Crops. Cambridge Univ. Press, Cambridge 1957.

HEROLD, F. and BREMER, H. (1958): Untersuchungen zur Epidemiologie, Ökologie und Bekämpfung des Gurkenmosaikvirus. Gartenbauwissenschaft *23*, 254—274.

HORVÁTH, J. (1969a): Untersuchungen über eine Virose von *Brassica napus* L. Acta Phytopath. Acad. Sci. Hung. *4*, 29—44.

HORVÁTH, J. (1969b): Die Anfälligkeit von *Chenopodium amaranticolor* Coste et Reyn. gegenüber dem Kartoffel-Y-Virus im Hinblick auf die Blattsequenz. Acta Botanica Acad. Sci. Hung. *15*, 71—77.

HORVÁTH, J. (1969c): Die Anfälligkeit und Symptomausprägung der Blätter von *Nicotiana tabacum* L. cv. Hicks Fixed A2-426 nach Infektion mit dem tobacco mosaic virus in Abhängigkeit von ihrer Sequenz am Sproß. Acta Phytopath. Acad. Sci. Hung. *4*, 131—137.

HORVÁTH, J. (1973): On the susceptibility of tobacco and *Chenopodium* to virus infections in relation to the position of the leaves on the stem. Plant Virology. Proc. 7th Conf. Czechoslovak Plant Virologists, High Tatras 1971. pp. 305—310.

HORVÁTH, J. and HINFNER, K. (1969): Natural infection of rape with cucumber mosaic virus in Hungary. Acta Agr. Acad. Sci. Hung. *18*, 226—229.

HORVÁTH, J., JURETIĆ, N. and MILIČIĆ, D. (1973): *Crambe abyssinica* Hochst. ex R. E. Frees as a new host plant for turnip yellow mosaic virus and radish mosaic virus. Phytopath. Z. *78*, 69—74.

JURETIĆ, N., HORVÁTH, J., MAMULA, D. and MILIČIĆ, D. (1973): Natural occurrence of turnip yellow mosaic virus in Hungary. Acta Phytopath. Acad. Sci. Hung. *8*, 175—183.

LAPIERRE, H. and GRISON, C. (1969): Note sur une mosaique de l'alliaire. Ann. Phytopathol. *1*, 309—314.

LOVISOLO, O. (1960): Segnalazione di una nuova virosi della *Moricandia arvensis* ed osservazioni su altre virosi di piante ornamentali. Not. Malattie Piante *53—54*, 233—253.

MAMULA, D. and LJUBEŠIĆ, N. (1975): *Tropaeolum majus* L., natural host of turnip mosaic virus. Acta Botanica Croatica *34*, in press.

MAMULA, D., MILIČIĆ, D., ŠTEFANAC, Z. and HORVÁTH, J. (1972): Neue Angaben über Verbreitung und Wirtspflanzen des Rettichmosaik-Virus (radish mosaic virus). Acta Phytopath. Acad. Sci. Hung. *7*, 369—375.

MILIČIĆ, D. (1956): Virus-Zelleinschlüsse in *Alliaria officinalis*. Protoplasma *47*, 341—346.

MILIČIĆ, D. (1962): Viruskörper in den Zellen von *Matthiola incana* R. Br. Acta Botanica Croatica *20-21*, 21–34.

MILIČIĆ, D., PANJAN, M., BILANOVIĆ, D. and KATIĆ, B. (1958): Viruskrankheit von *Alliaria ofifcinalis* Andr. Acta Botanica Croatica *17*, 159–176.

MILIČIĆ, D., ŠTEFANAC-UDJBINAC, Z. and MAMULA, D. (1963): Rasprostranjenost nekih virusa krucifera u Jugoslaviji. Agronomski glasn. *1–2*, 92–100.

MILINKÓ, I. (1952): Két új virusféleség hazai előfordulása (Occurrence de deux nouvelles variétés de virus en Hongrie). Ann. Inst. Prot. Plant. 5, 241–250.

PAPA, G. and MICHELIN-LAUSAROT, P. (1973): In vitro aggregation of *Alliaria* mosaic virus protein. J. gen. Virol. *22*, 255–258.

PAPA, G., MICHELIN-LAUSAROT, P. and CASETTA, A. (1973): Purification and properties of *Alliaria* mosaic virus. Phytopath. Z. *78*, 344–356.

POUND, G. S. and WALKER, J. C. (1945): Differentiation of certain crucifer viruses by the use of temperature and host immunity reactions. J. Agr. Res. *71*, 255–278.

SCHWARZ, R. (1959): Epidemiologische Untersuchungen über einige Viren der Unkraut- und Ruderalflora Berlins. Phytopath. Z. *35*, 238–270.

SHUKLA, D. D. and SCHMELZER, K. (1972): Studies on viruses and virus diseases of cruciferous plants. VI. Effect of cabbage black ring and cucumber mosaic viruses in mixed infection on seed production of radish. Acta Phytopath. Acad. Sci. Hung. *7*, 315–324.

SHUKLA, D. D. and SCHMELZER, K. (1973): Studies on viruses and virus diseases of cruciferous plants. XIII. Cabbage black ring, nasturtium ringspot and alfalfa mosaic viruses in ornamental and wild species. Acta Phytopath. Acad. Sci. Hung. *8*, 139–148.

SKIEBE, K. and SCHMELZER, K. (1967): Zur Züchtung virusresistenter Hybridsorten beim Spinat (*Spinacia oleracea* L.). Z. Pflanzenzüchtung *58*, 323–342.

SMITH, K. M. (1972): A Textbook of Plant Virus Diseases. Third edition, Longman Group Ltd., London 1972.

ŠTEFANAC, Z. (1964): Prilog poznavanju virusa mozaika postrne repe (*Marmor brassicae* Holmes) (Contribution to the knowledge of turnip mosaic virus). Master's work, Faculty of Nat. Sci. and Mathematics, Zagreb 1964.

ŠTEFANAC, Z. (1967): Prilozi poznavanju svojstava virusa krucifera (Contribution to the knowledge of properties of crucifer viruses). Doctor's work, Faculty of Nat. Sci. and Mathematics, Zagreb 1967.

ŠTEFANAC, Z. and MILIČIĆ, D. (1965): Zelleinschlüsse des Kohlrübenmosaikvirus. Phytopath. Z. *52*, 349–362.

ŠTEFANAC-UDJBINAC, Z., MILIČIĆ, D. and ZELJKO, M. (1963): Wasserrüben- und Kohlrübenmosaikvirus (turnip mosaic virus) in Jugoslawien. Acta Botanica Croatica *22*, 107–117.

TOCHIHARA, H. (1965): Identification and serological comparisons of viruses causing mosaic diseases of Japanese radish. Bull. Nat. Inst. Agr. Sci. (Japan). Series C, *18*, 1–57.

TOMLINSON, J. A. (1970): Turnip mosaic virus. C. M. I./A. A. B. Description of Plant Viruses. No. 8., June 1970.

ULLRICH, J. (1955): Schwarzringfleckigkeit des Kohls in Deutschland. Nachrichtenblatt d. Deutsch. Pflanzenschutzd. *7*, 164–165.

USCHDRAWEIT, H. A. and VALENTIN, H. (1957): Untersuchungen über ein Kruziferen-Virus. Phytopath. Z. *31*, 139–148.

WEIL, B. (1959): Thermale Inaktivierung von zwei Pflanzenviren. Ein Beitrag zu Wärmetherapie pflanzlicher Viruskrankheiten. Phytopath. Z. *31*, 45–78.

WEIL, B. (1964): Beobachtungen zur Virusausbreitung im Freiland. Z. Pflanzenkrankheiten *71*, 302–310.

YOSHII, H. (1963): On the strain distribution of turnip mosaic virus. Ann. Phytopath. Soc. Japan *28*, 221–227.

YOSHII, H., SUGIURA, M. and IWATA, T. (1963): Studies of the daikon mosaic virus (DMV), the Japanese strain of turnip mosaic virus. Mem. Ass. Pl. Prot. Kyushi *1*, 1–26.

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Inhibition of Potato Virus X Infectivity by Bark Extract of *Prunus persica* (L.) Batsch

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The multiplication of potato virus X was completely inhibited by aqueous bark extract of *Prunus persica* (L.) Batsch. The inhibitor was found to be active at 1000 °C and also at 10^{-5} dilution. It withstands dialysis and activated charcoal treatments. It partially resisted aging *in vitro* for 287 days and desiccation 67 days at room temperature (23–25 °C).

Several plant extracts preventing potato virus X (PVX) infection have been reported in the literature (BAWDEN, 1954; BLASZCZAK *et al.*, 1959,; RAO and RAYCHAUDHURI, 1965; VERMA *et al.*, 1969; VERMA and RAYCHAUDHURI, 1970 and SINGH, 1971).

SINGH (1971) studied the effect of aqueous extract of thirty one higher plants on multiplication of PVX and found that bark extract of *Prunus persica* (L.) Batsch inhibited the multiplication of PVX completely. It is therefore considered necessary to study the properties of the virus inhibitor present in *P. persica* bark extract, which is reported in the present paper.

Materials and Methods

Chenopodium amaranticolor Coste and Reyn, PVX and aqueous bark extract of *P. persica*, were used as test plant, virus and inhibitor, respectively in the present experiment. Virus was maintained on *Nicotiana tabacum* L. var. Turkish. The usual preparation of PVX was made by grinding 5 g of young systemically infected tobacco leaves in a mortar with 6.0 ml distilled water. The extract obtained after squeezing the pulp through filter paper referred to have after as PVX.

100 g of fresh cleared bark obtained from stem of the apparently healthy *P. persica* plant was homogenized with 100 ml distilled water in a Waring Blendor. The extract was filtered through double layer of muslin cloth. The crude bark extract was used as inhibitor.

C. amaranticolor was used in local lesion assays for PVX. Seeds were sown in 22 cm clay pots in the green house, and after 20 days the seedlings were transplanted in the 10 cm clay pots at the rate of one plant per pot. The plants were

usually ready for inoculation after 40 – 50 transplantation by which time they had 4 – 6 nodes. Leaves of test plant were dusted with 600 mesh carborundum powder and 1.0 ml of inoculum was applied to small pads of muslin cloth which were then rubbed twice over the upper surface of each leaf in one direction from leaf base to the tip. In all experiments the amount of inoculum and the number of strokes per leaf were constant. The upper four fully expanded leaves on a plant were inoculated. Lesions were counted 4 – 6 days after inoculation. All the experiments were made in insect-proof chamber. The extract subjected to various treatments were mixed in 1 : 1 ratio with virus and assayed for infectivity using 1 : 1 dilution of the virus with distilled water as control. 10 plants were inoculated in each experiment. The virus inhibition was determined by comparing lesions produced by equal volumes of virus + distilled water and virus + extract.

Results

Properties of the inhibitor

Dilution: A dilution series of extract was prepared in distilled water and 1 ml of PVX was added to 1 ml of each dilution. The results (Table 1) indicate that the dilution affected the inhibitory property of the extract but it has been not completely removed at 10^{-5} dilution.

Table 1

Effect of dilution on the virus inhibitor

PVX mixed with	Number of lesion*
Distilled water (Control)	190.0
Diluted juice 10^{-1}	104.0
10^{-2}	119.5
10^{-3}	136.0
10^{-4}	147.6
10^{-5}	153.0

* Average lesions from 40 leaves

Resistance to heat: 2 ml of extract was placed in thin-walled glass tubes. The tubes containing extracts were immersed in constantly agitated water bath at temperature of 40, 60, 80, 90, 95 and 100 °C for 10 minutes. The tubes were immediately cooled in running tap water. Some of the inhibitory properties of the test juice was lost at 40 °C (Table 2) but the activity was not completely lost, however, when extract heated even up to 100 °C.

Resistance to ageing "in vitro": The inhibitive bark extract was stored in black painted stoppered glass vials at room temperature (23–25°C) for different periods of time. At different periods, stored extract was mixed in equal volume with PVX and used for inoculation. The extract was completely prohibitory when tested after 289 days.

Resistance to desiccation: Several samples of 10 ml of extract was desiccated at room temperature 23–25 °C in airtight desiccators containing anhydrous calcium chloride. Before use, the dried extracts were made to original volume with distilled water. The percentage of inhibition tested after 67 days of storage was found to be 77.7%.

Table 2

Effect of heat on inhibitor

PVX mixed with		Number of lesions*
Distilled water (Control)		226.8
Unheated bark extract		0.0
Bark extract heated at	40 °C	24.0
	60 °C	25.3
	80 °C	26.0
	90 °C	26.2
	100 °C	28.1

* Average lesions from 40 leaves

Dialyzability of the inhibitor: 20 ml of extract was placed in an inverted thistle funnel having its mouth tightly covered with cellophane and was dialyzed against 300 ml distilled and running water, separately. The water was changed three times during 24 hrs. The dialyzed extract in distilled water lost its inhibitory property up to 9.1% while dialyzed sap in running water up to 14.6%.

Adsorption on charcoal: 20 ml of extract was mixed well with 5 g of activated charcoal powder and centrifuged after 4 hrs at 3000 rpm for 10 min. The supernatant fluid was passed through Whatman filter paper No. 44 and then tested for its activity. The charcoal removed 35.6% inhibitory activity of the extract.

Application of extract before and after leaves were inoculated

Leaves of *C. amaranticolor* were gently rubbed with muslin cloth saturated with bark extract and the adjacent lateral leaves were rubbed with distilled water. These leaves were inoculated at 0, 2, 4, 8, 10, 12, 24 and 48 hrs with virus. The test extract was inhibitory up to 100, 60 and 20%, up to 0.2 and 4 hrs, respectively. No virus inhibition was noticed when leaves were inoculated with virus at first and then rubbed with extract.

Discussion

The results of the present study indicate that the *P. persica* contains a strong inhibitor of PVX in its bark. The inhibitor seems to be heat-resisting, effective in very high dilution, resistant to ageing *in vitro* more than 287 days and to desiccation more than 67 days at room temperature (23–25 °C) and is partially dialyzable. The exact nature of the inhibitor has not been known, but it appears to be very stable. Most of the properties of the inhibitor under discussion resembles to those described by SINGH and GUPTA (1970) and VERMA *et al.* (1969).

The bark extract was capable of inhibiting lesion formation on *C. amaranticolor*, when applied before the leaves were inoculated. This result indicates that the inhibitor forms a non-infectious complex with the virus (KASSANIS and KLECZKOWSKI, 1948) or the virus is aggregated by the inhibitor (FRANCKI, 1964) which prevents the virus multiplication.

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Literature

BAWDEN, F. C. (1954): Inhibitors and plant viruses. Adv. Virus Res. *2*, 31–57.

BLASZCZAK, W., ROSS, A. F. and LARSON, R. H. (1959): The inhibitory activity of plant juices on the infectivity of potato virus X. Phytopathology *37*, 781–791.

FRANCKI, R. I. B. (1964): Inhibition of cucumber mosaic virus infectivity by leaf extracts. Virology *24*, 193–199.

KASSANIS, B. and KLECZKOWSKI, A. (1948): The isolation and some properties of a virus inhibiting protein from *Phytolacca esculenta* J. Gen. Microbiol. *2*, 143–153.

RAO, D. G. and RAYCHAUDHURI, S. P. (1965): Further studies on the inhibition of a ring spot strain of potato virus X by plant extracts, culture filtrate of *Trichothecium roseum* and chemicals. Indian Gen. Microbiol. *5*, 9–12.

SINGH, R. (1971): Inactivation of potato virus X by plant extracts. Phytopath. Medit. *10*, 211–213.

SINGH, R. and GUPTA, S. N. (1970): Studies on a virus inhibitor from *Psidium guayava* L. Bark. Phytopathol. Z. *69*, 292–296.

VERMA, V. S. and RAYCHAUDHURI, S. P. (1970): Chemicals from *Emblica officinalis* Gaertn as inhibitors of potato virus X. Zentbl. Bakt. Parasitkde *124*, 393–398.

VERMA, V. S., RAYCHAUDHURI, S. P. and KHAN, A. M. (1969): Properties and nature of inhibitors of potato virus X in four medicinal plant extracts. Biologia Plantarum *11*, 384–387.

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Two Viruses Isolated from Patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.), a New Vegetable Natural Host in Hungary
I. Watermelon mosaic virus (general)

By

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The general strain (strain 2) of watermelon mosaic virus (WMV-G, ✳/✳ : : ✳/✳ : E/E : S/Ap) was isolated in Hungary from mosaic affected patisson plants (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.). The virus was identified on the basis of differential host range, aphid (*Myzus persicae* Sulz.) transmissibility, seed transmissibility by seeds of infected patisson, serology, electron microscopy, cytological observation of virus inclusion bodies in the infected plant tissue and physical properties. Out of 67 plant species 27 proved to be hosts of WMV-G. Eleven species (*Ammi visnaga* [L.] Lam., *Cucurbita pepo* var. *patissonina* f. *radiata*, *Lagenaria leucantha* Rusby, L. *siceraria* [Mol.] Standl., *Lavatera arborea* L., *Malva borealis* Wallm., *M. neglecta* Wallr., *M. pusilla* Sm. et Sow., *M. silvestris* L., *Tetragonia echinata* Ait., *T. crystallina* L'Hérit.) are recorded as new experimental hosts for WMV-G. Six species were locally, 12 species were systemically and 9 species were both locally and systemically susceptible. Forty species (out of which 30 were tested for the first time) proved to be resistant against infection with WMV-G.

The virus showed stylet-borne transmission by *Myzus persicae*. In our experiments WMV-G was not transmissible by seeds of infected patisson plants. The virus reacted positively with WMV-antiserum. Flexuous filamentous particles, 730—750 nm in length and cytoplasmic inclusion bodies (X-bodies) were found associated in infected tissue of patisson and cucumber (*Cucumis sativus* L.) plants. The thermal inactivation point was 60—62°C, and the dilution end-point 10^{-3}—2×10^{-4}, the longevity *in vitro* was 9—12 days. This is the first report of the occurrence of WMV-G in patisson.

Introduction

Patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois., BECKER-DILLINGEN 1956), an annual herbaceous vegetable plant belonging to the family of *Cucurbitaceae*, is known to have been used as a food by the North-American Indians even before the European settlers appeared. In the United States of America (California, Iowa, New York, New Jersey) as well as in the Soviet Union (Gruziya) it is a current vegetable crop even today. In Hungary the introduction and variety maintenance of patisson have been dealt with, and investigations into the possibilities of its storage and utilization made since 1968 (NAGY, 1970;

1973). According to observations made in Hungary besides two fungi (*Erysiphe cichoracearum* DC. ex Mérat, *Sphaerotheca fuliginea* [Schlecht. ex Fr.] Pollacci) the patisson is seriously damaged by the watermelon mosaic virus (WMV, ✳/✳ : : ✳/✳ : E/E : S/Ap) and cucumber mosaic virus (CMV, R/1 : 1/18 : S/S : S/Ap). We have already reported on the appearance of the latter two viruses (HORVÁTH et al., 1974). As it is known, yield losses caused in watermelon and summer squash by WMV easily transmitted and spread by a number of aphids (KARL and SCHMELZER, 1971; HORVÁTH, 1972; FRITZSCHE *et al.*, 1972; WEIDEMANN and MOSTAFAWY, 1972; ADLERZ, 1974a, b) may be in general of an extent of 9–43% (DEMSKI and CHALKLEY, 1972), 19–73% (DEMSKI and CHALKLEY, 1974), and 49–63% (THOMAS 1971a). In the case of early infection loss in marketability may even reach 100 per cent (DEMSKI and CHALKLEY, 1972).

Considering that – to our best knowledge – on the natural virus infection of patisson no paper has been published apart from our earlier short report, we give a detailed account of the isolation and properties of WMV in our present first report and of CMV in our second report.

WMV, first isolated by ANDERSON (1954a) in Florida (USA), has widely spread in various countries of the world for the last twenty years (VAN REGENMORTEL, 1971; Table 1). In Europe the virus was first isolated in Hungary, then in Bulgaria from *Citrullus lanatus* (Thunb.) Mansfeld (syn.: *C. vulgaris* Schrad.), *Cucurbita maxima* Duch. and *C. pepo* L. plants (MOLNÁR and SCHMELZER, 1963; 1964), though the X-bodies observed by GIGANTE (1935) suggest an about 40 years earlier appearance of WMV in Europe.

According to our present knowledge there are substantial differences in host plants, vector transmission and biological properties between the WMV strains isolated in various countries of the world. On this basis three strains (strain groups) of WMV can be distinguished: South African watermelon mosaic virus, watermelon mosaic virus 1, watermelon mosaic virus 2 (VAN REGENMORTEL *et al.*, 1962; MOLNÁR and SCHMELZER, 1963; 1964; WEBB and SCOTT, 1965; KARL and SCHMELZER, 1971). As regards the names of the individual virus strains or strain groups a new proposal was made by SCHMELZER (1969): "For WMV 2 we proposed general watermelon mosaic virus (GWMV), because it has a general distribution, i.e., it is to be found in the New World as well as in the Old World and because it has a more general host range, i.e., it is not confirmed to the *Cucurbitaceae*. WMV 1 we named specific watermelon mosaic virus (SWMV), because it seems to be specific for the New World and attacks specifically *Cucurbitaceae* (SCHMELZER, 1966a). The South African isolate investigated by VAN REGENMORTEL, BRANDES and BERCKS (1962) differs principally from SWMV, because it infects non-cucurbit hosts, too. Especially the pronounced different symptoms on pumpkin and squash and the striking velocity of symptom expression make it probable that it belongs to a third virus species which we preliminarily call South African watermelon mosaic virus (SAWMV)." Further on we apply the nomenclature suggested by SCHMELZER (loc. cit.) with certain modification. We note here that we think

Table 1

Observations on the occurrence of the strains of watermelon mosaic virus and their natural hosts

Virus strains[1,2]	Host plants	Locality	Literature
Type strain and yellow strain	*Cucurbita pepo* L. melopepo Alef. *C. pepo* L. medullosa Alef. *C. maxima* Duch. *C. moschata* Duch. *C. okeechobeensis* (Small) Bailey *Cucumis sativus* L. *C. melo* L. *Citrullus lanatus* (Thunb.) Mansfeld (syn.: *C. vulgaris* Schrad.) *C. vulgaris* Schrad. citroides Bailey *Melothria pendula* L. *Lagenaria siceraria* (Molina) Standl. *Luffa cylindrica* (L.) Roemer *L. aegyptiaca* Mill.	Florida	ANDERSON (1954a)
WMV–SA	*Cucurbita pepo* L. var. Caserta Bush	South Africa	VAN REGENMORTEL (1960) VAN REGENMORTEL *et al.* (1962)
WMV–G	*Citrullus lanatus* (Thunb.) Mansfeld *Cucumis sativus* L.	Japan	KOMURO (1962)
WMV–G	*Cicer arietinum* L. *Pisum sativum* L.	Japan	INOUYE (1964)
WMV–S and WMV–G	*Momordica charantia* L.	Hawaii	TOBA (1962), SHANMUGASUNDARAM *et al.* (1969)
WMV–G	*Citrullus lanatus* (Thunb.) Mansfeld *Cucurbita pepo* L. *C. maxima* Duch.	Hungary and Bulgaria	MOLNÁR and SCHMELZER (1963, 1964)
WMV–G	*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.	Hungary	HORVÁTH *et al.* (1974)
WMV–S	*Cucurbita pepo* L.	India	REDDY and NARIANI (1963)
WMV–S	*Trichosanthes dioica* L.	India	BHARGAWA and TEWARI (1970)

Virus strains[1,2]	Host plants	Locality	Literature
WMV—S	*Citrullus lanatus* (Thunb.) Mansfeld	India	JAGANATHAN and RAMAKRISHNAN (1971)
WMV—G	*Citrullus colocynthis* (L.) Schrad. *C. lanatus* (Thunb.) Mansfeld *Cucumis melo* L. *C. sativus* L. *Cucurbita pepo* L. *Luffa cylindrica* (L.) Roem. *Molucella laevis* L.	Israel	COHEN and NITZANY (1963), NITZANY (1970)
WMV—G	*Cucurbita pepo* L.	China	LIU *et al.* (1964)
WMV—G	*Cucurbita maxima* Duch.	Germany	SCHMELZER (1965)
WMV—S	*Citrullus lanatus* (Thunb.) Mansfeld	Cuba	SCHMELZER (1966a)
WMV—G	*Citrullus lanatus* (Thunb.) Mansfeld *Cucurbita maxima* Duch.	Roumania	SCHMELZER (1966b)
WMV—G	*Cucurbita maxima* Duch. *Cucurbita pepo* L.	Czechoslovakia, Moldavia	SCHMELZER and MILIČIĆ (1966)
WMV—G	*Citrullus lanatus* (Thunb.) Mansfeld	Yugoslavia	STAKIĆ and NICOLIĆ (1966, 1968)
WMV—S	*Melothria pendula* L.	Florida	ADLERZ (1969, 1972b)
WMV—G and WMV—S	*Citrullus lanatus* (Thunb.) Mansfeld	Venezuela	LASTRA (1968)
WMV—G	*Cucumis* spp. *Cucurbita* spp.	Argentina	ZABALA and RAMALLO (1969)
WMV—G	*Cucurbita maxima* Duch.	Egypt	BASILLIOUS *et al.* (1969)
WMV—G	*Malva parviflora* L.	Arizona	NELSON and TUTTLE (1969)
WMV—G	*Chenopodium ambrosioides* L.	Florida	MILNE and GROGAN (1969)
WMV—G and WMV—S	*Citrullus lanatus* (Thunb.) Mansfeld *Cucumis sativus* L. *Cucurbita maxima* Duch. *C. moschata* Duch. *C. pepo* L.	New York	PROVVIDENTI and SCHROEDER (1970)
WMV—G and WMV—S	*Cucurbita pepo* L.	Maryland	WEBB (1971)
WMV—G	*Phytolacca americana* L.	Maryland	WEBB (1971)
WMV—G	*Citrullus lanatus* (Thunb.) Mansfeld *Cucurbita pepo* L.	Georgia	DEMSKI and SOWELL (1970)

Virus strains[1,2]	Host plants	Locality	Literature
WMV—G	*Citrullus lanatus* (Thunb.) Mansfeld *Cucurbita moschata* Duch. *C. pepo* L.	Soviet-Union	MOSKOVETS *et al.* (1970)
WMV—S	*Citrullus lanatus* (Thunb.) Mansfeld *Cucumis sativus* L.	Kfnys	QUIOT *et al.* (1971)
WMV—G	*Cucurbita maxima* Duch.	New Zealand	THOMAS (1971b)
WMV—G and WMV—S	*Citrullus lanatus* (Thunb.) Mansfeld *Cucumis melo* L.	Iran	EBRAHIM-NESBAT (1974), WEIDEMANN and MOSTAFAWY (1972)
WMV—S	*Citrullus lanatus* (Thunb.) Mansfeld	El Salvador	DIAZ (1972)
WMV—S	*Momordica charantia* L.	Florida	ADLERZ (1972a)
WMV—G	*Cucumis melo* L. *Cucurbita maxima* Duch. *C. pepo* L.	Chile	AUGER *et al.* (1974)

[1] WMV—G: General strain (strain 2) of watermelon mosaic virus, WMV—S: Specific strain (strain 1) of watermelon mosaic virus, WMV—SA: South-African strain of watermelon mosaic virus

[2] The specific and/or general strain of watermelon mosaic virus were determined in many countries of USA, for example in California (GROGAN *et al.*, 1959), Texas (MCLEAN and MEYER, 1961), Arizona (NELSON *et al.*, 1962), Georgia (MORTON and WEBB, 1963), Washington (SKOTLAND *et al.*, 1963), California (MILNE *et al.*, 1969)

it more correct to write watermelon mosaic virus (general) instead of general watermelon mosaic virus, watermelon mosaic virus (specific) instead of specific watermelon mosaic virus, and replace the name of South African watermelon mosaic virus by watermelon mosaic virus (South African), therefore in our paper the following abbreviations are used: WMV-$G_{(eneral)}$, WMV-$S_{(pecific)}$, and WMV-$S_{(outh)}$ $A_{(frican)}$.

Material and Methods

At the end of August 1973 in the outdor patisson plots of the Horticultural Department of the Mosonmagyaróvár Faculty of Agronomy of the Keszthely University of Agricultural Sciences a disease characteristic of an almost total virus infection of plants was found. In the course of symptomatological observations two fundamental types of symptoms could be distinguished. Most of the diseased plants showed very severe mosaic symptoms, growth inhibition and leaf

deformation (Fig. 1A). A relatively low number of plants were found with severe mosaic and growth inhibition (Fig. 1B). The diseased plants displayed an equally severe shortening of internodes and proliferation of shortened laterals (Fig. 1C and D). Simultaneously with the symptomatological observations from the plants showing different disease symptoms (see Fig. 1A and B) leaf samples were collected in plastic bags. For mechanical inoculation young patisson leaves were ground

Fig. 1. Symptoms on the naturally infected patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.). A: Complex infection with cucumber mosaic virus and watermelon mosaic virus (deformed leaf), B: Watermelon mosaic virus infected leaf (not deformed), C and D: Shortened internodes and stem proliferations on naturally infected plants with watermelon mosaic virus (general strain, WMV-G)

(1 : 1 w/w) with 0.1 *M* phosphate buffer (pH 7.0). Inoculations were made with a glass rod using carborundum as abrasive to the following plants: *Ammi majus* L., *Chenopodium amaranticolor* Coste et Reyn., *Citrullus lanatus* (Thunb.) Mansfeld, *Cucumis sativus* L., *Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois,

Table 2

Reaction of various plants to the general strain of watermelon mosaic virus (WMV—G) isolated from patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.) in Hungary

Family and binominals[1]	Reactions
AIZOACEAE	
Tetragonia crystallina L'Hérit.*	IL: Chlorotic and sometimes necrotic lesions NIL: Not infected
T. echinata Ait.*	IL: Chlorotic and sometimes necrotic lesions NIL: Not infected
T. tetragonoides (Pall.) O. Ktze.	IL: Chlorotic and sometimes necrotic lesions NIL: Not infected
AMARANTHACEAE	
Amaranthus caudatus L.	IL: Local chlorotic lesions NIL: Not infected
Gomphrena decumbens Jacq.□	IL: Not infected NIL: Not infected
G. globosa L.□□	IL: Not infected NIL: Not infected
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	IL: Chlorotic local lesions NIL: Not infected
Beta macrocarpa Guss.□	IL: Not infected NIL: Not infected
B. trygina W. et K.□	IL: Not infected NIL: Not infected
B. vulgaris L.	IL: Not infected NIL: Not infected
COMMELINACEAE	
Commelina communis L.□	IL: Not infected NIL: Not infected
C. graminifolia H. B. et K.□	IL: Not infected NIL: Not infected
C. tuberosa L.□	IL: Not infected NIL: Not infected
CRUCIFERAE	
Brassica adpressa Boiss.□	IL: Not infected NIL: Not infected
Br. chinensis L.□	IL: Not infected NIL: Not infected
Cherianthus cheiri L.□	IL: Not infected NIL: Not infected

Family and binominals[1]	Reactions
CUCURBITACEAE	
Citrullus lanatus (Thunb.) Mansfeld (syn.: *C. vulgaris* Schrad.)	IL: Not infected NIL: Vein banding and mosaic
Cucumis melo L.	IL: Not infected NIL: Mosaic and leaf deformation
C. sativus L.	IL: No symptoms, sometimes chlorotic spots NIL: Vein clearing, mosaic and leaf deformation
C. sativus L. (P. I. 180280)	IL: Not infected NIL: Vein clearing, mosaic and leaf deformation
C. sativus L. (P. I. 124112)	IL: Not infected NIL: Vein clearing, mosaic and leaf deformation
Cucurbita ficifolia Bouche	IL: Not infected NIL: Mosaic spots and leaf deformation
C. moschata Duch.	IL: Not infected NIL: Mosaic spots and leaf deformation
C. pepo L. var. patissonina Greb. f. radiata Nois.*	IL: Not infected NIL: Mosaic, vein clearing
Ecballium elaterium (L.) A. Rich.	IL: Not infected NIL: Not infected
Echinocystis lobata (Michx.) Torr et Gray.	IL: Not infected NIL: Vein clearing, mosaic
Lagenaria leucantha Rusby*	IL: Not infected NIL: Vein clearing and severe mosaic
L. siceraria (Mol.) Standl.*	IL: Not infected NIL: Vein clearing, vein banding and mosaic
Luffa acutangula (L.) Roxb.	IL: Not infected NIL: Not infected
L. cylindrica (L.) Roem.□	IL: Not infected NIL: Not infected
ERICACEA	
Leiophyllum buxifolium (Berg.) Ell.□	IL: Not infected NIL: Not infected
GERANIACEAE	
Erodium ciconium Ait.□	IL: Not infected NIL: Not infected
E. cicutarium (L.) L'Herit.□	IL: Not infected NIL: Not infected
E. malacoides Willd.□	IL: Not infected NIL: Not infected
LABIATAE	
Ballota foetida Lamk.□	IL: Not infected NIL: Not infected

Family and binominals[1]	Reactions
Ocimum basilicum L.	IL: Not infected NIL: Not infected
O. canum Sims.□	IL: Not infected NIL: Not infected
LEGUMINOSAE	
Vicia faba L.	IL: Local, brown lesions NIL: Not infected
Vigna sinensis Savi. ex Hask.	IL: Not infected NIL: Not infected
MALVACEAE	
Lavatera arborea L.*	IL: Local chlorotic spots NIL: Chlorotic spots (Fig. 2B)
L. thuringiaca L.	IL: Not infected NIL: Not infected
L. trimestris L.	IL: Local necrotic lesions (Fig. 2C) NIL: Chlorotic lesions with central necrotic area (Fig. 2D)
Malva borealis Wallm.*	IL: Local chlorotic spots NIL: Vein clearing and mosaic spots
M. moschata L.	IL: Local chlorotic spots NIL: Vein clearing and mosaic spots
M. neglecta Wallr.*	IL: Local chlorotic spots NIL: Vein banding and mosaic spots
M. pusilla Sm. et Sow.*	IL: Local chlorotic spots NIL: Vein banding and mosaic spots
M. silvestris L.*	IL: Mild local chlorotic spots NIL: Vein clearing
M. verticillata L.	IL: Local chlorotic spots NIL: Vein clearing and mosaic spots
SOLANACEAE	
Atropa bella-donna L.□	IL: Not infected NIL: Not infected
Capsicum annuum L.□	IL: Not infected NIL: Not infected
Datura stramonium L.□	IL: Not infected NIL: Not infected
Nicotiana chinensis Fisch.□	IL: Not infected NIL: Not infected
N. glutinosa L.	IL: Not infected NIL: Not infected
N. knightiana Goodspeed.□	IL: Not infected NIL: Not infected
N. quadrivalvis Pursch.□	IL: Not infected NIL: Not infected
N. tabacum L. cv. Bel 61—10, Samsun and Xanthi-nc	IL: Not infected NIL: Not infected
Petunia atkinsiana Don.□	IL: Not infected NIL: Not infected

Family and binominals[1]	Reactions
P. axillaris (Lam.) Britt.□	IL: Not infected NIL: Not infected
P. hybrida Vilm.	IL: Not infected NIL: Not infected
P. parviflora Juss.□	IL: Not infected NIL: Not infected
P. violacea Lindl.□	IL: Not infected NIL: Not infected
TROPAEOLACEAE	
Tropaeolum majus L	IL: Not infected NIL: Not infected
T. minus L.□	IL: Not infected NIL: Not infected
T. peltophorum Benth.□	IL: Not infected NIL: Not infected
T. peregrinum L.□	IL: Not infected NIL: Not infected
UMBELLIFERAE	
Ammi majus L.	IL: Not infected NIL: Very severe mosaic, vein clearing, vein banding and leaf deformation (Fig. 2A)
A. visnaga (L.) Lam.*	IL: Not infected NIL: Very severe mosaic, vein clearing, vein banding and leaf deformation

[1] Plants designated with * are new hosts of the general strain of watermelon mosaic virus (WMV–G). Plants designated with □ are new resistant species against WMV–G. *Gomphrena globosa* L. (designated with□□) is susceptible against WMV (cf. MILNE and GROGAN, 1969)

Lavatera trimestris L., *Nicotiana glutinosa* L., *N. tabacum* L. cv. Bel 61–10, Samsun and Xanthi-nc, as well as *Ocimum basilicum* L. and *O. canum* Sims.

On the basis of differences between the symptoms induced in identical test plants – separately inoculated with tissue saps prepared from patisson plants showing the two different types of symptoms – it has become clear that the virus infection of leaves of the two original patisson plants can be traced back to infection at least by two viruses, one of the leaf samples (leaf *A* in Fig. 1) containing in all probability a complex of the two viruses (mixed infection). After the isolation and differentiation of the virus/es – in which *Citrullus lanatus*, *Datura stramonium* L., *Ecballium elaterium* (L.) A. Rich., *Nicotiana tabacum* cv. Bel 61–10, Samsun, Xanthi-nc, *Ocimum basilicum*, *O. canum* and *Lavatera trimestris* plants were of special importance – other test plants were also used (see Table 2).

The mechanically inoculated leaves were rinsed with tap water. Two isolates from the naturally infected patisson plants were designated by the symbols of

PCW (from leaf *A* in Fig. 1) and PW (from leaf *B* in Fig. 1). In the first paper only isolate PW will be dealt with.

In the insect transmission studies the green peach aphid (*Myzus persicae* Sulz.) was used. The aphids were starved for 3 hours, then permitted to feed on infected patisson and cucumber leaves for 8–10 minutes. Ten aphids were then transferred to healthy test plants (patisson, cucumber and Samsun- as well as Xanthi-nc tobacco) for 24 hours and afterwards killed with Phosdrin. The experiments were carried out in an insect-proof greenhouse.

To determine whether or not the virus was seed-borne 500 patisson plants were grown in greenhouse from seeds originating from infected fruit, and examined symptomatologically and serologically after 10 weeks. Serological reactions were performed by means of slide precipitin test using the procedure described previously (HORVÁTH, 1971). The examination of virus particles and infected tissues was carried out with a Siemens Elmiskop I electron microscope. The leaf samples were examined by the dip method (BRANDES and WETTER, 1959; BRANDES, 1964). Tissue sections for light microscope investigations were prepared from hair cells of patisson and cucumber plants, and immersed in tap water. Both infected and healthy plants were studied microscopically.

Physical properties of the virus were determined in extracted patisson sap, with *Chenopodium amaranticolor* used as assay host. The thermal inactivation point was determined in ultrathermostat (E. MOT., Typ. FF. 100/45) where 1 ml aliquots of infected sap were exposed to each degree of temperature from 48 to 60°C for 10 minutes. Other properties examined were the dilution end-point of infectious sap and longevity *in vitro* of the virus in sap at room temperature.

Results and Discussion

With tissue sap obtained from patisson plants showing mosaic growth inhibition and severe symptoms of shortened internodes as well as proliferation (Fig. 1B, C and D) successful experiments of transmission to *Ammi majus* (Fig. 2A), *Chenopodium amaranticolor*, *Citrullus lanatus*, *Cucumis sativus*, *Cucurbita pepo* var. *patissonina* f. *radiata* and *Lavatera trimestris* plants were carried out. On *Chenopodium* plants developed local symptoms, *Lavatera trimestris* local and systemic (Fig. 2C and D) while the other plants systemic symptoms. The *Nicotiana* and *Ocimum* species proved resistant to inoculation. On the basis of the symptoms induced by the PW isolate in the different test plants as well as the resistance of the individual plants it seemed probable that the infection had been caused by WMV. Of the viruses infecting cucurbitaceous plants in Europe CMV, tobacco necrosis virus (TNV, R/∗ : ∗/∗ : S/S : S/Fu), arabis mosaic virus (ArMV, R/1 : ∗/41 : S/S : S/Nc) and tobacco ring spot virus (TRSV, R/1 : : 1.8/42 : S/S : S/Nc) had to be excluded as possible pathogens because of the resistance showed first of all by the *Nicotiana*, *Ocimum*, *Gomphrena* and *Comme-*

lina species as well as by *Ecballium elaterium* and *Datura stramonium*. The presence of cucumber green mottle mosaic virus (CGMMV, R/✳ : ✳/✳ : E/E : S/✳) was made improbable by the fact that *Chenopodium* otherwise resistant to this virus was locally susceptible to the PW isolate. The systemic susceptibility of *Citrullus lanatus* also confirms the fact of WMV infection and excludes the possibility of squash mosaic virus (SqMV, R/1 : 2.4/35 : S/S : S/Cl) participating in the in-

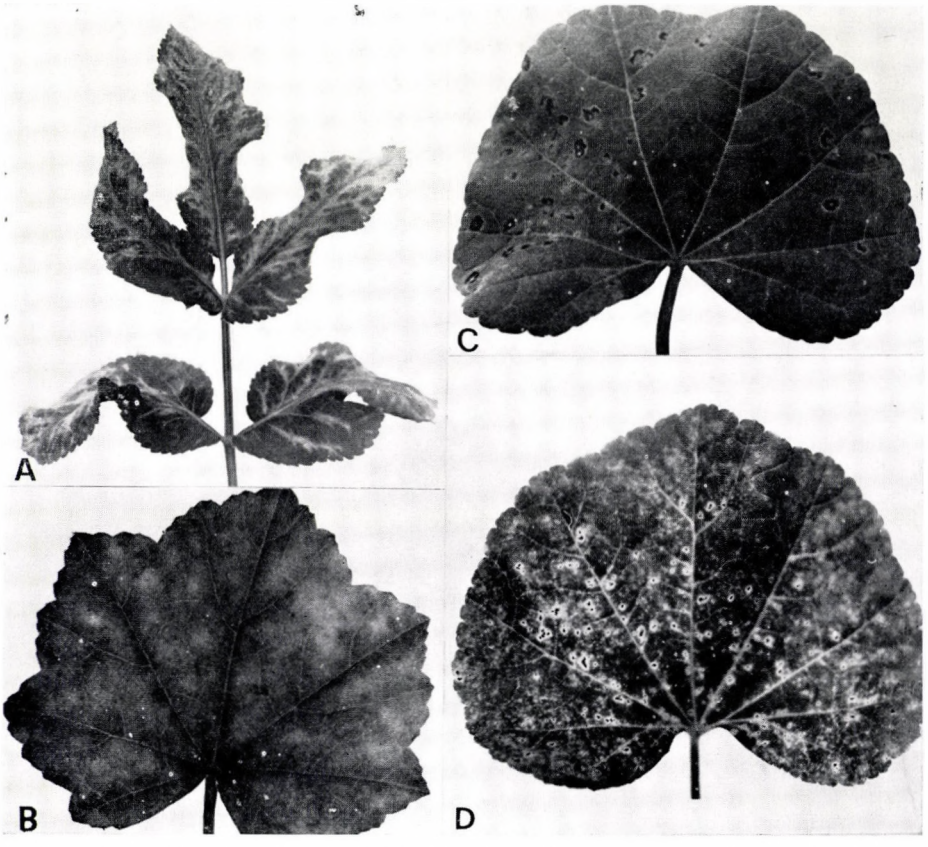

Fig. 2. Systemic (A, B and D) and local symptoms (C) on experimentally inoculated leaves of *Ammi majus* L. (A), *Lavatera arborea* L. (B), *Lavatera trimestris* L. (C and D) with the general strain of watermelon mosaic virus (WMV-G)

fection, as the latter is known to induce local symptoms only in the above plant (cf. THOMAS, 1974).

The results of test plant studies including some 67 species of 14 families (Table 2) – which with the exception of *Malva silvestris* L. agree with the investigation results of MOLNÁR and SCHMELZER (1964) – equally prove the occurrence

of WMV infection. On the basis of the results of test plant studies the PW strain isolated by us from patisson can be considered as identical with the general strain of WMV (WMV-G) occurring exclusively in Europe. As it is known, the WMV-G differs from the WMV-S first of all in its host plants not being restricted to the family *Cucurbitaceae* but infecting some 17 families including e.g. *Chenopodiaceae*, *Euphorbiaceae*, *Leguminosae*, *Malvaceae* (MOLNÁR and SCHMELZER, 1964; WEBB and SCOTT, 1965; PROVVIDENTI and SCHROEDER, 1970). As a further essential difference, the WMV-G causes local symptoms in *Chenopodium amaranticolor* (MOLNÁR and SCHMELZER, 1964; DEMSKI, 1968; MILNE and GROGAN, 1969), while the WMV-S is not pathogenic to *Chenopodium*. *Cucumis sativus* (P. I. 180280 and P. I. 124112) is only systemically susceptible to WMV-G, while both locally and systemically to infection by WMV-S (WEBB, 1963, WEBB and SCOTT, 1965; WEBB, 1974 personal communication). Another difference between the two virus strains is the resistance to WMV-G and systemic susceptibility to WMV-S of *Luffa acutangula* (L.) Roxb. (WEBB, 1965; PROVVIDENTI and SCHROEDER, 1970; DIAZ, 1972.)

Further on we use the symbol WMV-G/PW to designate the virus isolated from patisson. In the course of test plant studies we have found eleven new WMV-G/PW hosts: *Ammi visnaga* (L.) Lam., *Cucurbita pepo* var. *patissonina* f. *radiata*, *Lagenaria leucantha* Rusby, *L. siceraria* (Mol.) Standl., *Lavatera arborea* L., *Malva borealis* Wallm., *M. neglecta* Wallr., *M. pusilla* Sm. et Sow., *M. silvestris*, *Tetragonia echinata* Ait., and *T. crystallina* L'Hérit.

Thirty additional, so far not studied plants (*Atropa bella-donna* L., *Ballota foetida* Lamk., *Beta macrocarpa* Guss., *B. trygina* W. et K., *Brassica adpressa* Boiss., *Br. chinensis* L., *Capsicum annuum* L., *Cheiranthus cheiri* L., *Commelina communis* L., *C. graminifolia* H. B. et K., *C. tuberosa* L., *Datura stramonium* L., *Erodium ciconium* Ait., *E. cicutarium* [L.] L'Hérit., *E. malacoides* Willd., *Gomphrena decumbens* Jacq., *Leiophyllum buxifolium* [Berg.] Ell., *Luffa cylindrica* [L.] Roem., *Nicotiana chinensis* Fisch., *N. knightiana* Goodspeed, *N. quadrivalvis* Pursch., *Ocimum canum* Sims., *Petunia atkinsiana* Don., *P. axillaris* [Lam.] Britt., *P. parviflora* Juss., *P. violacea* Lindl.) have proved to be resistant to the virus.

In insect transmission studies (*Myzus persicae*) the WMV-G/PW was found to be readily transmitted in a non-persistent or stylet-borne manner from patisson and cucumber to patisson and cucumber, but – similarly to the mechanical transmission experiments – not to Samsun and Xanthi-nc tobacco plants. The aphid transmission of WMV-G/PW excludes again the possibility that the infection of patisson was caused by CGMMV or TRSV. As it is known, these two viruses cannot be transmitted by aphids. WMV-G can be distinguished and also separated from CGMMV and TRSV because it is transmitted by aphids.

In the course of WMV-G/PW transmission by patisson seed, plants grown from the seeds of diseased plants, were symptomatologically checked upon. Symptoms characteristic of WMV were not shown by any of the examined 500 plants. In the case of some plants suspected to have been infected by virus mechanical transmission was performed to *Ammi majus*, *Chenopodium amaranticolor*, *Cucumis*

sativus and *Cucurbita pepo* var. *patissonina* f. *radiata* plants, and the virus suspect patisson plants were also serologically tested with WMV antiserum. According to the results of symptomatological and serological examinations WMV-G/PW cannot be transmitted by patisson seed. These results agree with the unsuccessful virus transmission attempts made with the seeds of infected *Cucurbita maxima*, *C. pepo*, *Cucumis melo* and *Citrullus lanatus* plants (GROGAN *et al.*, 1959; MILNE and GROGAN, 1969; THOMAS, 1971b; EBRAHIM-NESBAT, 1974). WMV-G/PW was tested against WMV antisera kindly supplied by Prof. Dr. R. BERCKS (Braunschweig, Germany). Flocculent precipitates were observed in mixtures of virus preparations and WMV antiserum. No reaction was observed with normal serum.

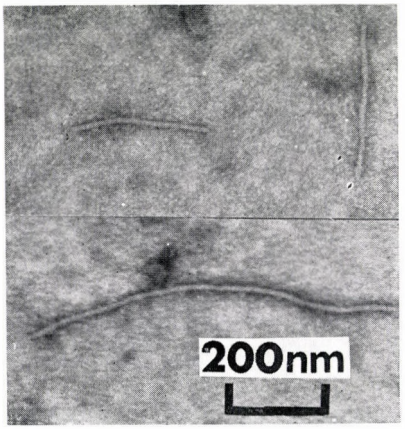

Fig. 3. Long flexuous filaments of the general strain of watermelon mosaic virus (WMV-G). Approx. ×37 500. Bar represents 200 nm

Preparations from systemically infected *Cucurbita pepo* var. *patissonina* f. *radiata* plants contained numerous flexuous filamentous, about 730–750 nm long particles (Fig. 3), while none were detected in similar preparations from uninfected, healthy plants. Our results are similar to those obtained in experiments where the normal length of WMV was found to range from 600–680 nm (WEBB, 1971), and 700–725 nm (VAN REGENMORTEL *et al.*, 1962; MOLNÁR and SCHMELZER, 1963; 1964; PROVVIDENTI and SCHROEDER, 1970; WEBB, 1971) to 746–765 nm (SCHMELZER, 1966a, PURCIFULL *et al.*, 1968; MILNE and GROGAN, 1969; THOMAS, 1971b; AUGER *et al.*, 1974). In hair cells of patisson and cucumber plants inoculated by WMV-G/PW numerous cytoplasmic inclusion bodies (X-bodies) characteristic of infection by viruses of the potyvirus group (cf. HARRISON *et al.*, 1971) including WMV (SCHMELZER and MILIČIĆ, 1966) were recognized (Fig. 4). They were mostly amorphous but sometimes consisted of accumulated needle-like structures. Inclusion bodies could not be seen in healthy patisson and cucumber plants.

Thermal inactivation point of WMV-G/PW in patisson sap occurred between 60 and 62 °C and the dilution end-point was at 10^{-3}–2×10^{-4}. In the *in vitro* inactivation test of crude sap from infected patisson the virus was found to be active after 9 days, but not after 12 days or more. These properties of WMV-G/PW agree with those found by VAN REGENMORTEL *et al.* (1962), and MOLNÁR and

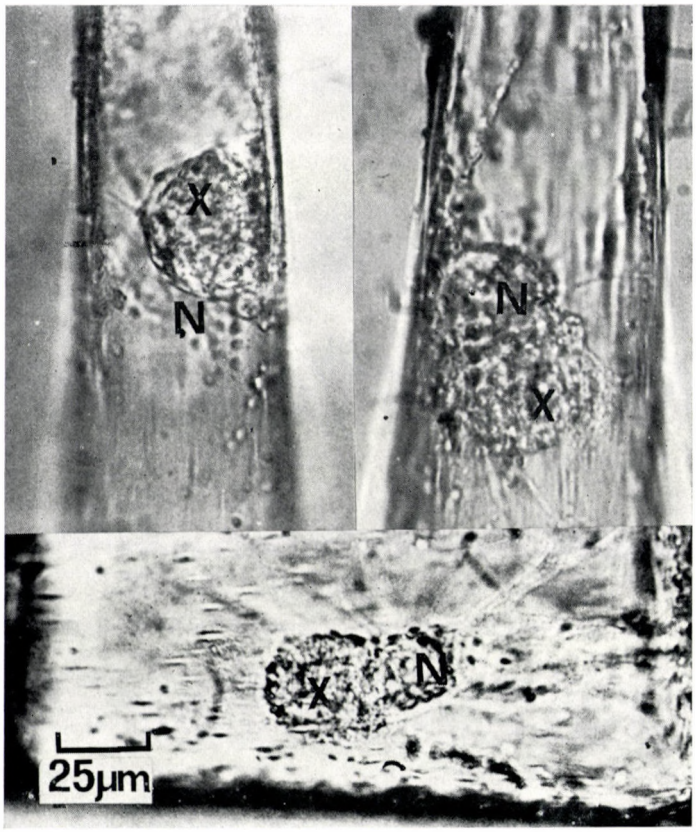

Fig. 4. Inclusion bodies (X-bodies) in hair cells of inoculated patisson leaves by the general strain of watermelon mosaic virus (WMV-G). X, inclusion bodies; N, nucleus

SCHMELZER (1964), but partly differ from the results of ANDERSON (1954a), LINDBERGH *et al.* (1956), MOLNÁR and SCHMELZER (1963), COHEN and NITZANY (1963) and THOMAS (1971b).

On the basis of the host range and symptomatology as well as aphid-stylet-borne transmission, serology, virus morphology and particle length, inclusion bodies and physical properties, the virus isolated from patisson and studied here

has been concluded to be a general strain (or strain 2) of WMV (WMV-G), and this is the first report on its occurrence in patisson.

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Literature

ADLERZ, W. C. (1969): Distribution of watermelon mosaic viruses 1 and 2 in Florida. Proc Fla. State Hortic. Soc. *82*, 161–165.

ADLERZ, W. C. (1972a): *Momordica charantia* as a source of watermelon mosaic virus 1 for cucurbit crops in Palm Beach county, Florida. Plant Dis. Rep. *56*, 563–564.

ADLERZ, W. C. (1972b): *Melothria pendula* plants infected with watermelon mosaic virus 1 as a source of inoculum for cucurbits in Collier county, Florida. J. Econ. Entomology *65*, 1303–1306.

ADLERZ, W. C. (1974a): Wind effects on spread of watermelon mosaic virus 1 from local virus sources to watermelon. J. Econ. Entomology *67*, 361–364.

ADLERZ, W. C. (1974b): Spring aphid flights and incidence of watermelon mosaic viruses 1 and 2 in Florida. Phytopathology *64*, 350–353.

ANDERSON, C. W. (1954a): Two watermelon mosaic virus strains from central Florida. Phytopathology *44*, 198–202.

ANDERSON, C. W. (1954b): Two muskmelon mosaic virus strains from central Florida. Phytopathology *44*, 371–374.

AUGER, J. C., ESCAFFI, O. and NOME, F. S. (1974): Occurrence of watermelon mosaic virus 2 on cucurbits in Chile. Plant Dis. Rep. *58*, 599–602.

BASILLIOUS, H. A., SOLIMAN, A. A. and NOUR-ELDIN, F. (1969): Studies of a squash mosaic virus diseases in U. A. R. 1. Properties of the causal virus. Agr. Res. Rev,. Cairo *47*, 91–104.

BECKER-DILLINGEN, J. (1956): Handbuch des gesamten Gemüsebaues. Paul Parey, Berlin und Hamburg 1956.

BHARGAVA, K. S. and TEWARI, J. P. (1970): *Trichosanthes dioica*, an additional natural host of watermelon mosaic virus. Plant Dis. Rep. *54*, 727–728.

BRANDES, J. (1964): Identifizierung von gestreckten pflanzenpathogenen Viren auf morphologischer Grundlage. Zugleich eine Zusammenstellung von grundlegenden Daten für die Klassifizierung dieser Viren. Mitt. Biol. Bundesanst. Berlin–Dahlem 1964., Heft *110*, 1–130.

BRANDES, J. and WETTER, C. (1959): Classification of elongated plant viruses on the basis of particle morphology. Virology *8*, 99–115.

COHEN, S. and NITZANY, F. F. (1963): Identity of viruses affecting cucurbits in Israel. Phytopathology *53*, 193–196.

DEMSKI, J. W. (1968): Local lesion reactions of *Chenopodium* species to watermelon mosaic virus 2. Phytopathology *58*, 1196–1197.

DEMSKI, J. W. and SOWELL, G., Jr. (1970): Susceptibility of *Cucurbita pepo* and *Citrullus lanatus* introductions to watermelon mosaic virus-2. Plant Dis. Rep. *54*, 880–881.

DEMSKI, J. W. and CHALKLEY, J. H. (1972): Effect of watermelon mosaic virus on yield and marketability of summer squash. Plant Dis. Rep. *56*, 147–150.

DEMSKI, J. W. and CHALKLEY, J. H. (1974): Influence of watermelon mosaic virus on watermelon. Plant Dis. Rep. *58*, 195–198.

DIAZ, F. F. (1972): Identification and distribution of watermelon mosaic virus 1 in El Salvador. Plant Dis. Rep. *56*, 437–440.

EBRAHIM-NESBAT, F. (1974): Distribution of watermelon mosaic viruses 1 and 2 in Iran. Phytopath. Z. *79*, 352–358.

FRITZSCHE, R., KARL, E., LEHMANN, W. and PROESELER, G. (1972): Tierische Vektoren pflanzenpathogener Viren. VEB Gustav Fischer Verlag, Jena 1972.

GIGANTE, R. (1935): Il mosaico della zucca. Bol. Staz. Patol. Veg., Roma *14*, 503–530.

GROGAN, R. G., HALL, D. H. and KIMBLE, K. A. (1959): Cucurbit mosaic viruses in California. Phytopathology *49*, 366–376.

HARRISON, B. D., FINCH, J. T., GIBBS, A. J., HOLLINGS, M., SHEPHERD, R. J., VALENTA, V. and WETTER, C. (1971): Sixteen groups of plant viruses. Virology *45*, 356–363.

HORVÁTH, J. (1971): *Lycopersicon*-Arten als neue Wirtspflanzen für das Kartoffel-M-Virus (potato virus M). Potato Res. *14*, 297–300.

HORVÁTH, J. (1972): Növényvírusok, vektorok, vírusátvitel (Plant viruses, vectors, virus transmission). Akadémiai Kiadó, Budapest 1972.

HORVÁTH, J., KUROLI, G. and NAGY, GY. (1974): A patisszon vírusbetegsége (Virus disease of patisson). Kertészet és Szőlészet *18*, 11.

INOUYE, T. (1964): A virus diseases of pea caused by watermelon mosaic virus. Ber. Ohara Inst. Landw. Biol. *12*, 133–143.

JAGANATHAN, T. and RAMAKRISHNAN, K. (1971): Studies on virus diseases of cucurbits. Madras Agr. J. *58*, 331–337.

KARL, E. and SCHMELZER, K. (1971): Untersuchungen zur Übertragbarkeit von Wassermelonmosaik-Virus durch Blattlausarten. Arch. Pflanzenschutz *7*, 3–11.

KOMURO, Y. (1962): Virus diseases of cucumber and watermelon caused by melon mosaic virus. Ann. Phytopath. Soc. Japan *27*, 31–36.

LASTRA, R. (1968): Occurrence of cucurbit viruses in Venezuela. Plant Dis. Rep. *52*, 171–174.

LINDBERGH, G. D., HALL, D. H. and WALKER, J. C. (1956): A study of melon and squash mosaic viruses. Phytopathology *46*, 489–495.

LIU, Y., JANG, R. Z., COU, Z. H. and CHIU, W. F. (1964): Studies on the mosaic disease of vegetable marrow (*Cucurbita pepo* L.). Acta Phytophylac. Sin. *3*, 15–24.

MCLEAN, D. M. and MEYER, H. M. (1961): A survey of cucurbit viruses in the lower Rio Grande Valley of Texas: Preliminary report. Plant Dis. Rep. *45*, 137–139.

MILNE, K. S. and GROGAN, R. G. (1969): Characterization of watermelon mosaic virus strains by serology and other properties. Phytopathology *59*, 809–818.

MILNE, K. S., GROGAN, R. G. and KIMBLE, K. A. (1969): Identification of viruses infecting cucurbits in California. Phytopathology *59*, 819–825.

MOLNÁR, A. and SCHMELZER, K. (1963): Nachweis des Wassermelonenmosaik-Virus in Europa. Naturwissenschaften *50*, 699.

MOLNÁR, A. and SCHMELZER, K. (1964): Beiträge zur Kenntnis des Wassermelonenmosaik-Virus. Phytopath. Z. *51*, 361–384.

MORTON, D. J. and WEBB, R. E. (1963): Symptoms associated with watermelon mosaic virus in South Georgia. Plant Dis. Rep. *47*, 772–773.

MOSKOVETS, S. M., FEGLA, G. I. and GLUSHAK, L. YU. (1970): Some virus diseases of cucurbits in the southern regions of the Ukraine. Mikrobiol. Zh. *32*, 735–738.

NAGY, GY. (1970): Új zöldségnövényünk – a patisszon (Patisson a new vegetable in Hungary). Kertgazdaság *1*, 47 - 52.

NAGY, GY. (1973): A patisszon (The patisson). Nemzetközi Mg. Szemle *3*, 111–112.

NELSON, M. R. and TUTTLE, D. M. (1969): The epidemiology of cucumber mosaic and watermelon mosaic 2 of cantaloups in an arid climate. Phytopathology *59*, 849–856.

NELSON, N. R., ALLEN, R. M. and TUTTLE, D. M. (1962): Distribution, prevalence and importance of some cantaloup virus diseases in Southwestern Arizona. Plant Dis. Rep. *46*, 667–671.

NITZANY, F. E. (1970): Virus diseases of annual crops in Israel. The Volcani Inst. Agr. Res. Virus Res. Unit., Bet Dagan 1970.

PROVVIDENTI, R. and SCHROEDER, W. T. (1970): Epiphytotic of watermelon mosaic among *Cucurbitaceae* in central New York in 1969. Plant Dis. Rep. *54*, 744–748.

PURCIFULL, D. E., EDWARDSON, J. R. and CHRISTIE, S. R. (1968): Aggregated filaments in extracts from *Lupinus* infected with watermelon mosaic virus. Virology *34*, 478–482.

QUIOT, J. B., KAAN, F. and BERAMIS, M. (1971): Identification of watermelon mosaic virus str. in the French West Indies. Ann. Phytopath. *3*, 125–130.

REDDY, K. R. and NARIANI, T. K. (1963): Studies on mosaic diseases of vegetable marrow (*Cucurbita pepo* L.). Indian Phytopath. *16*, 260–267.

SCHMELZER, K. (1965): Das Wassermelonenmosaik-Virus tritt auch in Deutschland auf. Nachrbl. dtsch. Pflschutzd. (Berlin) N. F. *19*, 69–71.

SCHMELZER, K. (1966a): Nachweis des spezifischen Wassermelonenmosaik-Virus in Kuba. Naturwissenschaften *53*, 619.

SCHMELZER, K. (1966b): Über den Virusgehalt von Cucurbitaceenfrüchten. Rev. Roum. Biol., Ser. Bot. *11*, 197–203.

SCHMELZER, K. (1969): Some more recent knowledge on cucurbit viruses. Proc. 6th Conf. Czechoslovak Plant Virologists, Olomouc 1967. Acad. Publ. House Czechoslovak Acad. Sci., Prague 1969. pp. 208–210.

SCHMELZER, K. and MILIČIĆ, D. (1966): Zur Kenntnis der Verbreitung des Wassermelonenmosaik-Virus in Europa und seiner Fähigkeit zur Bildung von Zelleinschlusskörpern. Phytopath. Z. *57*, 8–16.

SHANMUGASUNDARAM, S., ISHII, M., GILBERT, J. C. and NAGAI, H. (1969): Cucurbit virus studies in Hawaii. Plant Dis Rep. *53*, 70–74.

SKOTLAND, C. B., CLARK, R. L. and WEBB, R. E. (1963): Watermelon mosaic virus in Washington. Plant Dis. Rep. *47*, 772–773.

STAKIĆ, D. and NIKOLIĆ, V. (1966): Mozaik lubenice–novo oboljenje u Jugoslaviji (Watermelon mosaic a new virus disease in Yugoslavia). Savremena poljoprivreda *3*, 289–302.

STAKIĆ, D. and NIKOLIĆ, V. (1968): Uporedna ispitivanja raznih izolata virusa mozaika nice (Comparative studies of various isolates of watermelon mosaic virus). Savremena poljoprivreda *7–8*, 633–643.

THOMAS, W. (1971a): The incidence and economic importance of watermelon mosaic virus. New Zealand J. Agr. Res. *14*, 242–247.

THOMAS, W. (1971b): Watermelon mosaic virus, a disease of cucurbits in New Zealand. New Zealand J. Agr. Res. *14*, 235–241.

THOMAS, W. (1974): Seed-transimitted squash mosaic virus. New Zealand J. Agr. Res. *16*, 561–567.

TOBA, H. H. (1962): Studies on the host range of watermelon mosaic virus in Hawaii. Plant Dis. Rep. *46*, 409–410.

VAN REGENMORTEL, M. H. V. (1960): Zone electrophoresis and electron microscopy of a watermelon mosaic virus from South Africa. Virology *12*, 127–130.

VAN REGENMORTEL, M. H. V. (1971): Watermelon mosaic virus. C. M. I. /A. A. B. Description of plant viruses. No. 63, 1971.

VAN REGENMORTEL, M. H. V., BRANDES, J. and BERCKS, R. (1962): Investigations on the properties of watermelon mosaic virus. Phytopath. Z. *45*, 205–216.

WEBB, R. E. (1963): Local-lesion hosts for some isolates of watermelon mosaic virus. Plant Dis. Rep. *47*, 1036–1038.

WEBB, R. E. (1965): *Luffa acutangula* for separation and maintenance of watermelon mosaic virus 1 free from watermelon mosaic virus 2. Phytopathology *55*, 1379–1380.

WEBB, R. E. (1971): Watermelon mosaic viruses 1 and 2 in squash on the atlantic seaboard. Plant Dis. Rep. *55*, 132–135.

WEBB, R. E. (1974): Personal communication.

WEBB, R. E. and SCOTT, H. A. (1965): Isolation and identification of watermelon mosaic viruses 1 and 2. Phytopathology *55*, 895–900.

WEIDEMANN, H. L. and MOSTAFAWY, M. (1972): Watermelon mosaic virus type 2 in Iran and its transmission by different aphid species. Iranian J. Plant Path. *8*, 20–28.

ZABALA, S. and RAMALLO, J. C. (1969): The mosaic of *Cucurbitaceae*. Rev. Agr. N. E. Argentina *6*, 197–208.

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Transmission of Baculovirus by Mites. Study of Granulosis Virus of Codling Moth (*Laspeyresia pomonella* L.)

By

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Investigations were conducted on the possible role of mites in transmitting and disseminating insect pathogenic viruses. For this purpose *Tyrophagus putrescentiae* Schrank mites were studied which were observed to feed on cadavers of laboratory-reared codling moth larvae killed by granulosis virus. In experiments codling moth larvae were reared in the presence of mites which were allowed to move for 15 minutes on a dry glass surface contaminated by the virus. The resulting high mortality (78.9% on the 14th day) showed the fact of virus transmission, as compared to rearings with non-infected mites and no mites at all (2.8 and 2.1% mortality, respectively). Electron microscopy studies revealed the presence of inclusion bodies in the intestinal cavities of mites and in the folds of their integument, after having fed on larval cadavers; the virus units found in the intestine showed signs of digestion. Intact inclusion bodies with virus rods inside were found attached to the legs of mites. The significance of the observations is discussed from point of view of rearing hygiene and of the possible role played by mites in disseminating Baculoviruses under natural conditions.

The study of virus transmission represents a very important chapter both of medical, veterinary or agricultural science. Even if the transmission itself became established for the majority of virus diseases, the ways of virus transmission and dissemination are still only partly known. Good examples are given for this by the different pathways known for arboviruses in their dipterous intermediate hosts or by the cycles completed by plant pathogenic viruses in their homopterous vectors.

In the group of invertebrate viruses, especially in the Baculoviruses the transmission is facilitated by the fact that the virions are protected by very resistant proteinaceous inclusion bodies of different forms, like polyhedra or ovoid granules. These bodies are able to persist in the nature for a long period and can be transported by the water, wind or other means. It has been found also that biotic factors are also able to participate in the dissemination of viruses protected in their inclusion bodies and these factors are especially important in biotopes where the climatic factors seem to play a subordinate role. Basic work has been done in this field by BERGOIN and VAGO (1965), VAGO *et al.*, (1966), BERGOIN (1966a and b), who studied the ways of intestinal passage of different Baculoviruses through the intestinal tract of *Dictyoptera*, *Orthoptera* and other coprophagous or detri-

tiphagous invertebrates which ingested them and tested the persistence of virus in course of and following the passage in the vectors.

In present work observations are presented in connection with the possibility of Baculovirus transmission by coprophagous mites. This has been demonstrated on the granulosis virus of cooling moth, *Laspeyresia pomonella* L., by experiments and by electron microscopy.

Granulosis virus of codling moth and observations on its dissemination

The virus itself has been described in 1964 from a codling moth material of Mexican origin (TANADA, 1964). The histology of diseased larvae was studied by TANADA and LEUTENEGGER (1968), the morphology of the virus by STAIRS *et al.* (1966), the biochemical and cytological changes caused by the virus by BENZ and WÄGER (1971), WÄGER and BENZ (1971). The centrifugation of the virus and the study of infectivity of the centrifugate was studied by BAREFIELD and STAIRS (1970) whereas the field application was investigated by FALCON *et al.* (1968),

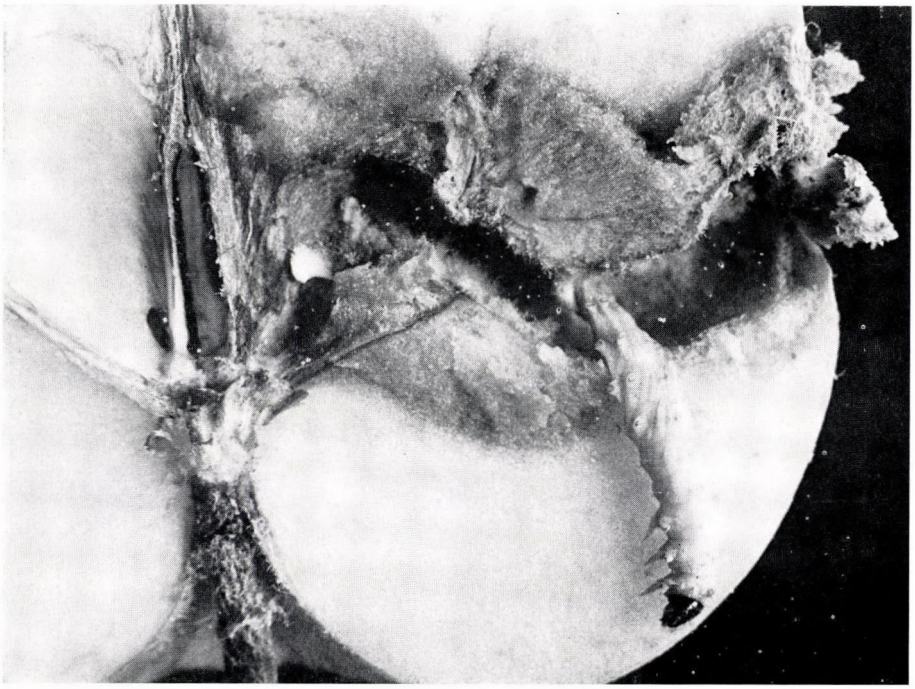

Fig. 1. Liquified cadaver of a codling moth larva killed by granulosis virus

FALCON (1971), KELLER (1973). The latter author gave also an excellent description on the pathophysiology and infectivity of the virus. Problems of codling moth rearing hygiene and action of alkaline substances on the virus was studied by SZALAY-MARZSÓ (1972a and b).

The infection hidden in laboratory-reared populations of codling moth may lead even under careful hygienic conditions to the destruction of the larval material, even when the laboratory accessories, rearing facilities, codling moth eggs are desinfected and the rearing is conducted on artificial media, with the larvae kept separately. The danger of an epidemic is especially imminent if the larvae are kept in groups, as the virus material contained in the larval faeces and the virus inclusion bodies liberated from the liquified cadavers (Fig. 1) present sources of infection (TANADA and LEUTENEGGER, 1968).

The possibility of virus transmission by mites presented itself in 1971 in the laboratories of the Station de Recherches Cytopathologiques (Sain-Christol-les-Alés) in codling moth rearings carried out on green apples. On the mouldy faecal pellets and gnawings numerous mites were observed; the mites have found also suitable humid hiding places in the cavities and mines resulting from codling moth

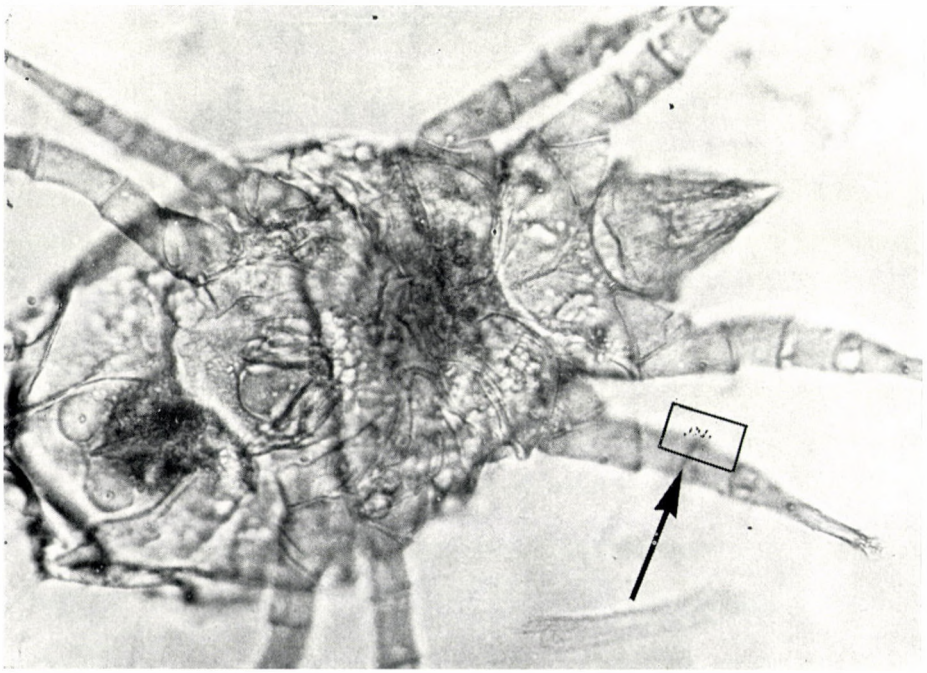

Fig. 2. Microphotograph of a *Tyrophagus putrescentiae* Schrank mite. The detail of the 2nd leg indicated also by an arrow is shown with a higher magnification in Fig. 7

larval feeding. The glassy white mites (Fig. 2) with a body length of 0.51 – 0.59 mm belonged to the species *Tyrophagus putrescentiae* Schrank which is well known from storehouses where the mites feed mostly on moulds (FLEURAT-LESSARD, 1974). The mites appeared in the rearings in high numbers and their relationship to granulosis virus became suspected when groups of mites were observed feeding on cadavers of codling moth larvae died with symptoms of granulosis (Fig. 3).

Fig. 3. *Tyrophagus putrescentiae* mites (arrows) feeding on the cadaver of a codling moth larva killed by granulosis virus

As the incidence of virus was found much higher in the rearing trays infested by mites, it was appropriate to study their possible role in virus transmission.

Experiments on virus transmission

For establishing the effectivity of *Acarina* in the virus transmission, the experiments were carried out in presence and absence of *Tyrophagus putrescentiae* mites. The mites were reared separately (in a neighbouring building) at 23°C, on artificial media containing alfalfa leaf powder, wheat germ, dried yeast, vitamine C and agar-agar.

The effectivity in virus transmission and dissemination was studied on one hand on mites fed on codling moth cadavers but mostly on mites which were allowed to move on a dry glass surface contaminated with the body contents of liquified larvae killed by virus. For this purpose the mites were swept by a very fine brush from the surface of their food into Petri dishes of 12 cm diameter, contaminated each with the material of 1 codling moth L_4 larva. The mites were allowed to move freely on the dry glass surface for 15 minutes at 23°C temperature, then were shaken into sterile Petri dishes by careful knocking. The mites were then transferred from there into codling moth single rearings, by using sterile brushes. The rearings consisted of small (2.5 × 5 cm), flat-bottomed glass vials with perforated plastic lids; each vial contained a quarter of a green apple which had been surface sterilized by a 3 per cent potassium hydrochloride solution (5 minutes) before cutting up, then washed in sterile distilled water. Into each vial 30 mites were transferred, then, after 15 minutes into each rearing a healthy L_3 codling moth larva was placed, by using again a sterile brush. The larvae originated from rearings kept on artificial media; only healthy larvae were used, showing no symptoms of virus disease. The larvae tunelled into the apple pieces which were in contact with the mites. The variations were as follows:

a) larvae developing in the presence of mites possibly contaminated by virus,

b) larvae developing in presence of mites transferred directly from their sterile artificial food,

c) larvae kept without the presence of mites.

In each variation 4 × 25 single rearings were made and kept on long photoperiod (17 hours photophase) at 23°C. The evaluations of the experiment by assessing larval mortality were made in the 4th, 8th and 14th day after the begin.

Results, based on larval mortality

Table 1

Variation	Per cent larval mortality		
	4th day	8th day	14th day
a) Possibly virus contaminated mites	18.2±1.2	77.5±4.6	78.9±5.5
b) Non-contaminated mites	—	2.6±0.1	2.8±0.3
c) Check (no mites)	—	1.9±0.2	2.1±0.3

The mortality values refer only to larvae which showed typical symptoms of virus disease (dark discoloration, liquified body contents). The high mortality of larvae reared in the presence of possibly virus-infected mites is conspicuous whereas the low mortality rate both in the rearings with non-infected mites and in the check indicate the presence of granulosis virus in the studied codling moth population.

Electron microscopy

For studying the mechanism of virus transmission in the mites, the tissues of mites which had been feeding on larval cadavers were examined by electron microscopy. The mites collected on cadavers and the ones exposed to contaminated glass surfaces were fixed in 8% glutaraldehyde solution and kept in the fixative for 24 hours at 5°C. The traces of the fixative were then washed out from the material by a repeated sodium cacodylate bath (pH 7.4). The mites were then transferred into 1% osmium tetroxyde (1 hour) then washed again in the buffer solution. The embedding was carried out into epon-resin (Epicote 812) by using the usual method (acetone dehydration, transfer of material into epon-aceton mixture, then into epon-accelerator mixture). The hardening of the embedding medium was done for 24 hours at 40°C, then for 48 hours at 60°C. The ultrathin sections were prepared by using an Ultratom III LKB; the preparations were contrasted with 3% lead citrate, stained with 5% uranyl acetate and examined under a Hitachi HU 11-C-S electron microscope.

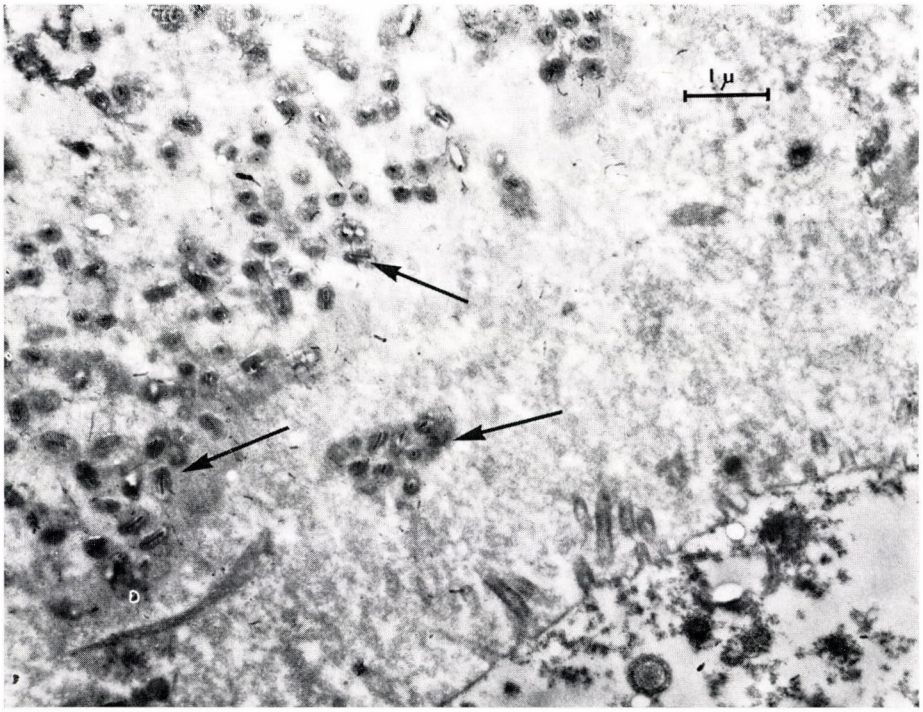

Fig. 4. Mass of granulosis virus inclusion bodies (arrows) in the intestinal cavity of a *Tyrophagus putrescentiae* mite. 16 000×

By examining the sections in many instances elements were found which were identified by shape and size as inclusion bodies of codling moth granulosis virus. So in the intestinal cavity of mites which had fed on cadavers, masses of the characteristic bodies were found (Fig. 4). In the areas surrounded by the microvilli of the intestine wall the inclusion bodies showed signs of digestion as seen by the clearing-up of the inclusion bodies whereas the virions inside them seemed still intact. Similar picture was seen in virus materials after a mild alcalic treatment (0.1% sodium carbonate solution, exposure of 3 minutes). In the mites which have fed on cadavers, masses of inclusion bodies were established deep

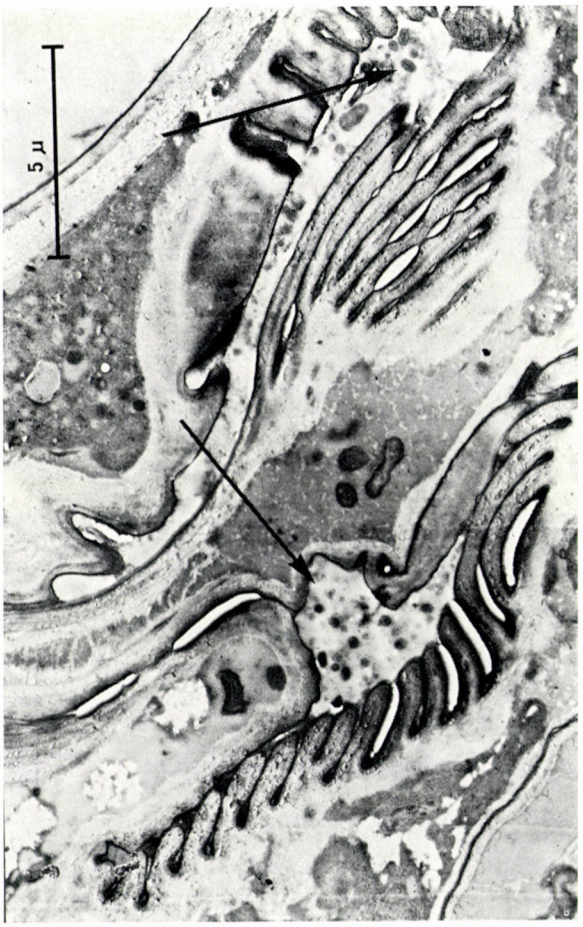

Fig. 5. Inclusion bodies of the virus (arrows) inside the folds of mite integumentum. Cross section, 7400×

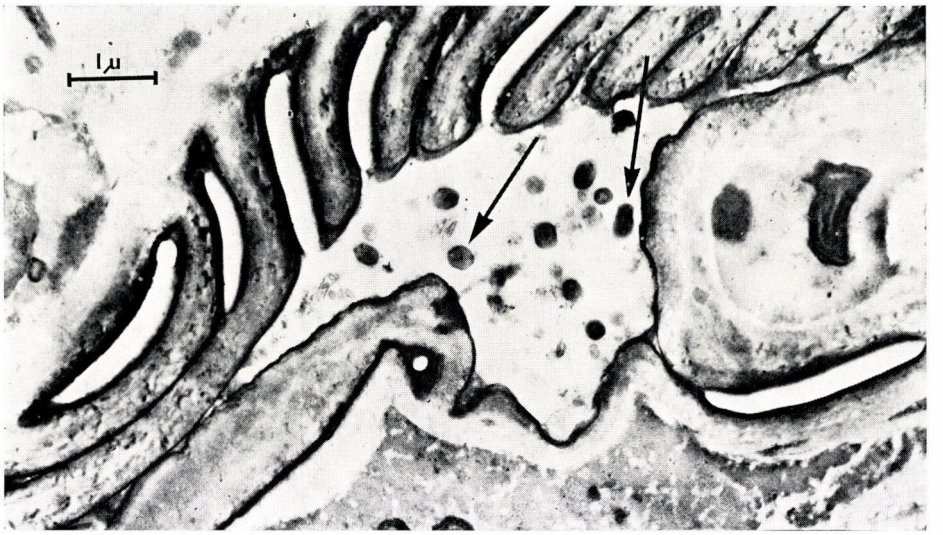

Fig. 6. Inclusion bodies of the virus (arrows) inside the folds of integumentum. Cross section, 16 200×

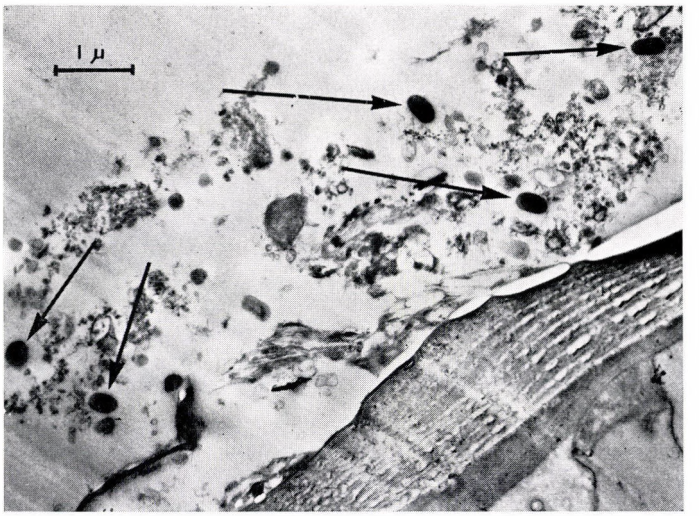

Fig. 7. Longitudinal section of the foreleg of a *Tyrophagus putrescentiae* mite (part indicated in Fig. 2), showing débris and virus inclusion bodies attached. 14 200×

in the folds of the integument (Figs 5 and 6), apparently as a result of a close contact with liquified cadavers. These groups of inclusion bodies and those found attached to the legs of mites exposed to contaminated glass surfaces (Fig. 7) did not show any alteration and were in the majority of cases electron dense.

Discussion and conclusions

For the study of ecology, biology and genetics of codling moth (*Laspeyresia pomonella* L.), but especially for the releases in sterile-male technique codling moth mass rearing are established in many laboratories (Yakima, Zürich, Avignon-Montfavet, Budapest). In these mass rearings the granulosis virus presents a continuous problem.

The experiments and observations described above indicate that coprophagous mites occurring in the rearings are able to participate in the transmission of codling moth granulosis virus.

The electron microscopy of mites having had contact with codling moth larval cadavers showed that inclusion bodies containing the intact virions of Baculovirus were in masses in the intestinal tract of the mites. These showed signs of digestion (clearing-up of the proteinaceous inclusion body), the ones, however, occurring in the folds of the integument were intact as well as those found attached to the legs of mites exposed to contaminated dry surfaces.

These findings are important from epidemiological point of view, as they show that copro- or mycophagous mites may act as vectors of insect pathogenic viruses. The observations call also attention to the problems of rearing hygiene as the *Tyrophagus* mites are quite common in any storeroom. The mites feeding on the faeces of diseased larvae or on the cadavers may carry the infective material to any parts of the rearing rooms. The danger of virus dissemination is further increased by the skin particles shed at the moulting, as the folds and crevices of the integumentum may contain many inclusion bodies. These are protected there not only from desinfectants but also may be carried away by currents of air (opening doors, ventillation etc.).

The laboratory transmission of viruses by mites is only one side of the question as the mites may be vectors of insect pathogenic viruses under field conditions as well.

The results obtained can probably be generalized also to other viruses which could be disseminated by coprophagous or necrophagous mites in the laboratory or in the nature, especially with regard to viruses protected by proteinaceous inclusion bodies. This possibility has to be taken into consideration not only in the epizootiology and ecopathology but also in the programmes of biological control.

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Literature

BAREFIELD, K. P. and STAIRS, K. P. (1970): Infectious components of granulosis virus of the codling moth, *Carpocapsa pomonella*. J. Inv. Path. *15*, 401–404.

BENZ, G. and WÄGER, R. (1971): Autoradiographic studies on nucleic acid metabolism in granulosis infected fat body of larvae of *Carpocapsa*. J. Inv. Path. *18*, 70–80.

BERGOIN, M. (1966a): Passage des corps d'inclusion de virus d'un poliédrie nucleaire dans la tube digestif d'un orthoptère détritivore *Acheta domesticus* L. *Entomophaga. 11*, 252–259.

BERGOIN, M. (1966b): Recherches sur la dissémination des germes entomopathogènes ingérés par des *Orthoptères* et des *Dictyoptères* prédateurs et detritivores. Thèse special, Marseilles, 136 pp.

BERGOIN, M. and VAGO, C. (1965): Passage de Rickettsiella grylli dans le tube digestif d'insectes prédateurs et détritivores. Arch. Inst. Pasteur, Tunis *1–2*, 66–75.

FALCON, L. A. (1971): Microbial control as a tool in integrated control programs. The granulosis virus of codling moth. In: HUFFAKER, C. B.: Biological Control, Plenum Press, New York–London, pp. 346–367.

FALCON, L. A., KANE, W. R. and BETHELL, R. S. (1968): Preliminary evaluation of a granulosis virus for control of the codling moth. J. Econ. Ent. *61*, 1208–1213.

FLEURAT-LESSARD, F. (1974): Contribution a l'étude des relations trophiques entre *Tyrophagus putrescentiae* Schrank (Acarina, Acaridae) et la mycoflore des graines de colza stockées. Mise au point d'une technique d'élevage de l'acarien sur moisissures. Ann. Zool. Écol. anim. *6*, 97–109.

KELLER, S. (1973): Mikrobiologische Bekämpfung des Apfelwicklers (*Laspeyresia pomonella* L.) (=*Carpocapsa pomonella*) mit spezifischem Granulosisvirus. Z. ang. Ent. *73*, 137–181.

STAIRS, G. R., PARRISH, W. B., BRIGGS, J. D. and ALLIETTA, M. (1966): Fine structure of a granulosis virus of the codling moth, *Carpocapsa pomonella* L. (*Lepidoptera, Tortricidae*). Virology *30*, 583–584.

SZALAY-MARZSÓ, L. (1972a): Az almamoly (*Laspeyresia pomonella* L.) granulózisvírusának kórszövettani és sejttani vizsgálata (in Hungarian with English summary) Növényvédelem *8*, 343–349.

SZALAY-MARZSÓ, L. (1972b): Az almamoly (*Laspeyresia pomonella* L.) granulózisvírusának lúgos inaktiválása és az almamoly laboratóriumi tenyésztésének egyes higiéniai kérdései (in Hungarian with English summary) Növényvédelem *8*, 402–407.

TANADA, Y. (1964): A granulosis virus of the codling moth, *Carpocapsa pomonella* (Linnaeus) (*Olethreutidae, Lepidoptera*). J. Ins. Path. *6*, 378–380.

TANADA, Y. and LEUTENEGGER, R. (1968): Histopathology of a granulosis virus disease of the codling moth, *Carpocapsa pomonella*. J. Inv. Path. *10*, 39–47.

VAGO, C., FOSSET, J. and BERGOIN, M. (1966): Dissémination des virus de polyédrie par les ephippigères, prédateurs d'insectes. Entomophaga *11*, 177–182.

WÄGER, R. and BENZ, G. (1971): Histochemical studies on nucleic acid metabolism in granulosis-infected *Carpocapsa pomonella*. J. Inv. Path. *17*, 107–115.

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Partial Recovery of Fertility in Irradiated Bean Weevil Males (*Acanthoscelides obtectus* Say, Col., *Bruchidae*)

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Different radiosensitivity and signs of repair processes were observed during the spermatogenesis of irradiated bean weevil males which successively were allowed to mate with normal virgin females on every 3rd day. An increase in fertility of the males was found with maximums on the 5th or 7th days (at the time of the second mating) after exposing to 4 or 6 krad of gamma radiation, treated either on the first or on the 7th day after emergence, respectively. There was no sign of recovery at 10 krad.

The fertilizing capacity (fertility) of untreated (control) males did not change significantly.

At the same time, the fecundity of the females mated with the untreated or irradiated males showed fluctuations. Females with significantly higher fecundity (t test, $P < 1\%$) on the 3rd and the 11th days (after the 1st and the 4th matings with the same male) were followed by females with low fecundity on the 8th and the 14th days (after the 3rd and 5th matings with the same male).

Somatic (YANG and DUCOFF, 1971; CAPROTTI et al., 1973) and reproductive (SOBELS, 1963, 1966; TRAUT, 1966; STRØMNAES, 1968; etc.) repair processes as the results of restoration mechanisms after mutagenic effects of ionizing radiation, if only a premutational stage is induced (SOBELS, 1963), are considered to be a common phenomenon.

The different radiosensitivity of developmental stages in spermatogenesis (VON BORSTEL, 1963; STRØMNAES, 1968; JAFRI and DAR, 1975) is thought to be the base of gradually increasing fertility of males when mated with virgin females after radiation treatment.

Recovery of fertility in males capable to mate several times is an important side effect of the sterile insect release method, especially after applying lower doses of radiation in order to produce more competitive males (HUTT and WHITE, 1973; ROBINSON, 1973). Doses resulting in complete sterility in the mature sperm, are not necessarily high enough to hinder spermatogenesis. Therefore, sperm-production can continue with relatively light chromosomal damage (RIEMANN and THORSON, 1969).

In a laboratory bean weevil strain, selected according to ovipositional responses, the males mated 12 to 15 times during an average of 16 days of their

lifetime (LABEYRIE, 1966) and their reproductive and fertilizing capacity showed variations, but changes in the rhythm of spermatogenesis were not found (HUIGN-ARD, 1971).

However, there are no data available on the natural process of spermatogenesis, the duration and radio-sensitivity of its stages in the bean weevil.

Taking into consideration the great number of matings, and supposing that the stages of spermatogenesis have different sensitivities, experiments were carried out to investigate, whether irradiated males regained their fertility or at least partly.

Material and Methods

Bean weevil males and females of the same age were taken from the laboratory rearing (SZENTESI, 1972). The method used to collect virgin adults was published elsewhere (SZENTESI et al., 1973).

In order to point out differences in radiosensitivity of stages during spermatogenesis the "brood pattern technique" (TRAUT, 1966; STRØMNAES, 1968) was used. The irradiated males were allowed to mate with virgin females periodically. As the spermatogenesis proceeded sperms of varying quality, originating from different irradiated stages of spermatogenesis, were used for insemination.

Two experiments were conducted at the same time:

Experiment I. One-day-old virgin males were irradiated, then allowed to mate with virgin females of the same age on every 3rd day for 14 days (the first mating was on the 2nd day).

Experiment II. Virgin males were mated on the 2nd and 5th days with virgin females of the same age, then irradiated on the 7th day after emergence. After irradiation they repeatedly were allowed to mate with females on every 3rd day for 7 days.

20 males were used in each variant: control, 4, 6 and 10 krad gamma radiation-treated males. Irradiation was performed in air. (^{137}Cs source; type: LMB-gamma-IM; dose rate: 229.5 krad/h.)

Males were kept in glass vials (2 cm diameter and 7 cm high) individually, in the absence of bean. One female for each male was put to them. After each mating the males were put away for next copulae, and the females were introduced into glass vials of the same size with 2 white bean seeds for oviposition. While matings were conducted at 28°C, males between matings and mated females for egg-laying were kept at 23°C. (R. h. was about 40–50%.)

At the end of the experiments all the viable and non-viable eggs laid were collected and counted.

Results and Discussion

Except of *Habrobracon* and *Drosophila* (VON BORSTEL, 1963) there are hardly any data available on the duration of stages of spermatogenesis in insect species.

Literature data about the relative sensitivity or resistance of stages of spermatogenesis to ionizing radiation vary greatly according to the mutation induced. First-day-sperm was usually found more sensitive than second-day-sperm (TELFER

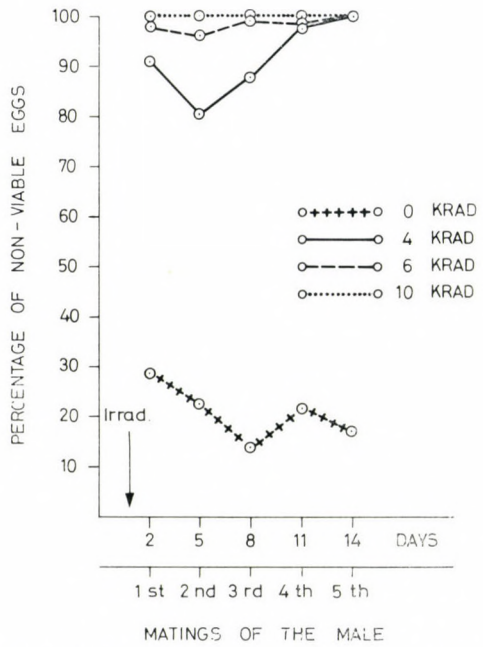

Fig. 1. Partial recovery of fertility of the male bean weevils treated with 4, 6 or 10 krad of gamma radiation on the first day after emergence. (Exp. I, open circles represent 4—20 replicates each)

and ABRAHAMSON, 1954; TRAUT, 1966; STRØMNAES, 1968). Similarly, the spermatids and spermatocytes were thought more sensitive comparing to the spermatogonia (SOBELS, 1966; TRAUT, 1966; STRØMNAES, 1968). Mature sperm were considered to be a phase of either less (STRØMNAES, 1968) or intermediate sensitivity (TRAUT, 1966).

When bean weevil males were irradiated on the first day after emergence (Exp. I), they showed relatively high radiosensitivity to the doses applied. Four krad of gamma radiation induced about 91 % of dominant lethality (Fig. 1). After 3 days (on the 7th day of the male's lifetime) there was an increase (recovery) in the fertilizing capacity, then the percentage of non-viable eggs rose slowly again,

reaching a maximum (100%) on the 14th day of the lifetime (after the 5th mating of the male).

Similar results were recorded after using 6 krad, although the amplitude of recovery was smaller. If the irradiation dose was too high (10 krad), all the stages were seriously damaged, and no sign of recovery appeared (Fig. 1).

Evidently all the stages of spermatogenesis were differently damaged at the time of irradiation, and the quality of sperm originating from these stages and

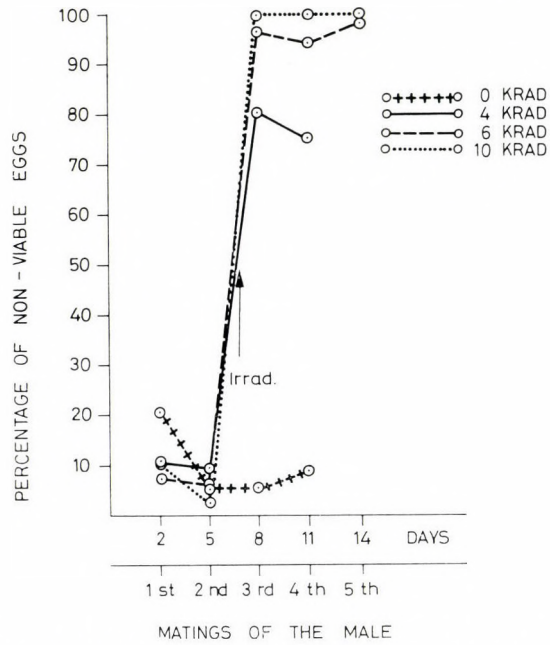

Fig. 2. Partial recovery of fertility of the male bean weevils treated with 4, 6 or 10 krad of gamma radiation on the 7th day after emergence. (Exp. II, open circles represent 3—20 replicates each. The data of control experiment on the 14th day are discarded)

utilized for fertilization showed the same differences. According to our results, those can be considered as the most radioresistant forms in the spermatogenesis that developed into sperm used for fertilization at about 7 days after irradiation at 23°C.

If irradiation was applied in the 7th day of the males' lifetime (Exp. II), almost the same results were gained. The percentage of induced dominant lethal mutations were little lower (about 80%) after using 4 krad of gamma radiation, and there was about 5% increase in fertility of males at the second mating. The maximum intensity of recovery process appeared 5 days after irradiation (Fig. 2).

HUIGNARD (1971) showed out individual fluctuations in the fertilizing

capacity *(fertility)* of untreated males in a selected bean weevil line, where males mated with virgin females daily. This fact was not in relation to changes in rhythms of the spermatogenesis during the lifespan of the insects, but it was caused by momentary exhaustion of amount of spermatozoa stored in the testes.

From the percentage of non-viable eggs found in the control groups (Exp. I and II) we could conclude that the factors affecting the mortality of eggs may vary considerably during the 14 days period (Figs 1 and 2). However, the ferti-

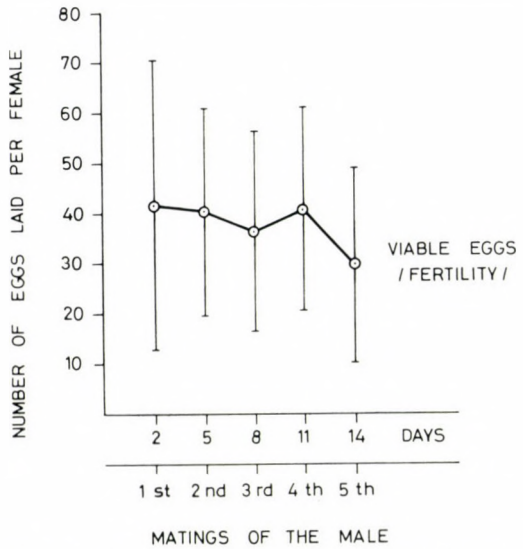

Fig. 3. Fertility of 5 bean weevil females successively mated with the same untreated male. (Summarization of $2 \times 3 - 20$ replicates of the control pairs' viable eggs in Exp. I and II)

lizing capacity (fertility) of untreated males, originating from our heterogeneous laboratory strain, did not show any significant alterations, especially with the first 11 days (Fig. 3, fertility). Thus the partial return of fertility could not be the results of such changes.

It has to be emphasized that the radiosensitivity of insects is known to be influenced by numerous factors (species, age of the insects, external and internal factors, method of irradiation etc.). Therefore, mutations induced can also differ greatly which makes still more difficult to determine the relative sensitivity of stages of spermatogenesis. We supposed that during a male's lifetime all the spermatogenic stages were simultaneously present in various quantities. Our experiments showed that at least one relative resistant stage existed, because recovery always appeared after 5 to 7 days postirradiation if treatment was applied either on the first or on the 7th day after the emergence of the male. However, there are not any significant differences among the percentage of non-viable eggs found

after each copulae, they only indicate a strong tendency of recovery. So we could state with certainty only the existence of different radiosensitive stages of spermatogenesis and the fact of partial recovery of fertility in irradiated bean weevil males.

The sterile insect release method is not applicable against the bean weevil in Hungary (SZENTESI, 1975). It is worth remarking, however, that in countries where there is possibility for using this technique, it is not necessary to take into account the recovery of male fertility, because higher doses (10–20 krad) can be

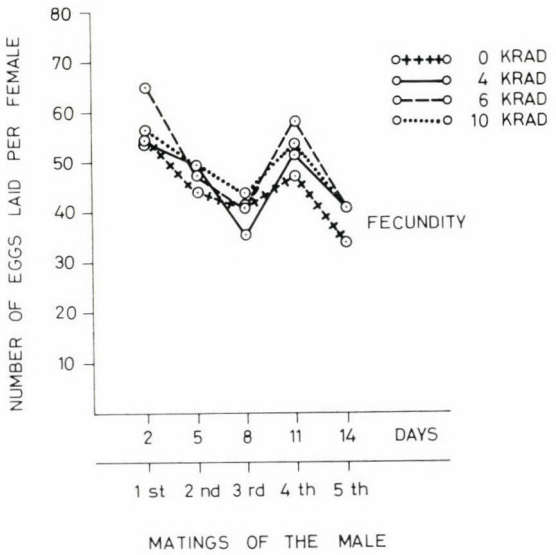

Fig. 4. Fecundity of 5 bean weevil females successively mated with the same untreated or treated male. (Summarization of all eggs of the control and the same radiation treatments in Exp. I and II. All the differences among the means of eggs of radiation treatments on the same day are not significant)

applied without any loss in sterile male competitiveness. Recovery does not take place at doses required for sterilization.

HUIGNARD (1971) experienced variations in the *fecundity* of females successively mated with the same male. It is because of the different quantities of sperm and accessory material secreted and transmitted from time to time. In our experiments (summarizing the data of the same variants of Exp. I and II) the number of eggs laid by females after successive matings of the same male also showed variations. Significant differences were found (Fig. 4, fecundity, t test, $P = 0.1$ and 1 %) between the average number of eggs laid per female on the 2nd and the 11th days (after the 1st and 4th matings of the same male) and those laid on the 8th and 14th days (after the 3rd and 5th matings of the male).

Acknowledgements

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Literature

VON BORSTEL, R. C. (1963): Effects of radiation on germ cells of insects: dominant lethals, gamete inactivation and gonial-cell killing. In: Radiation and Radioisotopes Applied to Insects of Agricultural Importance. Proc. Symp. Athens, 1963, IAEA, Vienna, 367—385.

CAPROTTI, M., SACCHI, L. and GRIGOLO, A. (1973): Effetto delle radiazioni ionizzanti su *Piophila casei* L.: possibilita' di riparazione dei danni radioindotti e influenza del "fattore tempo". Rivista Parassit. *34*, 161—166.

HUIGNARD, J. (1971): Variations de l'activité reproductrice des males d'*Acanthoscelides obtectus*. J. Insect Physiol. *17*, 1245—1255.

HUTT, R. B. and WHITE, L. D. (1973): Recovery of fertility in male codling moth (*Laspeyresia pomonella:* Lepidoptera, Olethreutidae) treated with 38 krad of gamma radiation. J. econ. Ent. *66*, 388—389.

JAFRI, R. H. and DAR, J. A. (1975): A cytological study of gamma irradiated testes of house fly, *Musca domestica* Linnaeus. In: Sterility Principle for Insect Control. Symp. Innsbruck, 1974, IAEA, Vienna (in press).

LABEYRIE, V. (1966): Évolution an cours de la vie individuelle de la capacité reproductrice des males d'*Acanthoscelides obtectus* Say (Coléoptère, Bruchidae). C. R. Acad. Sci. Paris, *263*, 774—777.

RIEMANN, J. G. and THORSON, B. J. (1969): Comparison of effects of irradiation on the primary spermatogonia and mature sperm of three species of Diptera. Ann. ent. Soc. Am. *62*, 613—617.

ROBINSON, A. S. (1973): Increase in fertility, with repeated mating, of gamma irradiated male codling moth, *Laspeyresia pomonella* (L.) (Lepidoptera: Olethreutidae). Can. J. Zool. *51*, 427—430.

SOBELS, F. H. (1963): Repair and differential radiosensitivity in developing germ cells of *Drosophila* males. In: Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells. Proc. Symp. (Sobels, F. H. ed.), Pergamon Press, London.

SOBELS, F. H. (1966): Processes underlying repair and radiosensitivity in spermatozoa and spermatids of *Drosophila*. In: Genetical Aspects of Radiosensitivity: Mechanisms of Repair. Proc. Panel Vienna, 1966, IAEA, Vienna, 49—65.

STRØMNAES, Ø. (1968): Radiation sensitivity and repair of chromosomal damage. In: Isotopes and Radiation in Entomology. Proc. Symp. Vienna, 1967, IAEA, Vienna, 341—354.

SZENTESI, Á. (1972): Studies on the mass rearing of *Acanthoscelides obtectus* Say (Col.; Bruchidae). Acta Phytopath. Hung. *7*, 453—463.

SZENTESI, Á. (1975): Field experiments using sterile adults for the suppression of bean weevil (*Acanthoscelides obtectus* Say, Col., Bruchidae) population. In: VIIIth Int. Plant Prot. Congr. Moscow, 1975, "Biol. Genet Control", pp. 171—181.

SZENTESI, Á., JERMY, T. and DOBROVOLSZKY, A. (1973): Mathematical method for the determination of sterile insect population competitiveness. Acta Phytopath. Hung. *8* (1—2), 185—191.

TELFER, J. D. and ABRAHAMSON, S. (1954): The higher egg mortality associated with insemination on the first then the second day after irradiation of *Drosophila* males. Genetics, *39*, 998—999.

TRAUT, H. (1966): Repair of genetic radiation damage — a possible cause of differential radiosensitivity in *Drosophila melanogaster*. In: Genetical Aspects of Radiosensitivity: Mechanisms of Repair. Proc. Panel Vienna, 1966, IAEA, Vienna, 67—78.

YANG, T. C. H. and DUCOFF, H. S. (1971): Recovery studies of X-irradiated *Tribolium castaneum* ("flour beetle") larvae. Radiat. Res. *46*, 290—300.

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Photoperiodic Regulation in the Population Dynamics of Certain Lepidopterous Species

By

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This paper gives a review about the effect of the short and long photoperiods and that of the diapause on the reproductive activity of *Laspeyresia pomonella* L., *Grapholitha funebrana* Tr. and *L. molesta* Busck. A hypothesis is offered on the basis of laboratory experiments for the explanation of the decreased reproduction in the field in autumn and spring.

Our previous papers (DESEŐ, 1967; 1971; 1973a, b; 1974, DESEŐ and SÁRINGER, 1970; 1973; 1975) dealt with multivoltine lepidopterous species (*Laspeyresia pomonella* L., *Grapholitha funebrana* Tr., and *L. molesta* Busck) having facultative, prepupal diapause induced during larval development by the shortening daylength in the autumn. The question arose on the one hand from our field observations, namely that the number of eggs laid in the orchard after diapause was usually very low resulting in a reduced infestation. On the other hand, references about the fecundity of lepidopterous species with similar life cycle have shown that the mean fecundity of females after diapause and even already in the autumn is lower than that of the ones of the summer generations (reviewed in DESEŐ, 1973b). The cause of lower fecundity in the autumn is usually explained "the preparation for diapause" induced by the shortening daylength in the autumn (HARCOURT and CASS, 1966; RAHN, 1970) and that after diapause by the loss in weight during diapause.

This paper gives a review on our results and intends to give an explanation for our observations.*

Results and Discussion

Laboratory experiments

The effect of short photophase during preadult development on the reproductive activity of *L. pomonella* was investigated with the Yakima (USA, Washington) laboratory strain. Small, green apples were used and diapause was inhibited by high temperature (28–30°C).

* We are indebted to Dr. T. JERMY for helpful criticism of the manuscript.

1. Short photophase (13/11 Light/Dark) reduced the usual ovipositing activity of mated females, extremely short photophase (8/16 L/D) even mating frequency (DESEŐ, 1972; 1973a, b).

2. The fecundity of mated females was decreased by the short photophase as well, while the long photophases increased or kept fecundity at normal level (DESEŐ, 1973a, b).

3. However, the question arose, whether the length of the photophases influences the fecundity during the whole preadult development, or only during a certain period. Therefore, eggs were exposed to short or long photoperiods for certain days and then transferred to the opposite illumination (Fig. 1). These ex-

Table 1

Fecundity of *Laspeyresia molesta* Busck developing under constant temperature regimes and photoperiods. (Egglaying at 23°C.) Two parental generations reared at temperatures altering between 18—28°C

Exp.	Developmental temperature (°C)	Photo/scotophase (hours)	No. of females	Eggs laid per female	
A	28	17/7	102	49.73±1.05	
B	28	11×13/11 → 17/7	81	49.90±2.04	
C	18	17/7	115	27.57±1.15	$p < 0.001$
D	18	11×13/11 → 17/7	92	42.39±0.08	$p < 0.001$

periments showed that when the embryo and the first instar larva had been exposed to eight short photophases, this information was enough to decrease the fecundity in the adults. As regards the long photophase, ten of them at the beginning of the embryonal/larval development were enough to induce not only normal, but even apparently increased fecundity. This sensitive period precedes the one, when diapause becomes induced. However, the developmental time of the population reared at short photoperiod after getting an information of ten long days prolonged with about 15 days. This observation was confirmed in *G. funebrana*, with the third generation in the laboratory. With *L. molesta* (about the 40th generation of a Hungarian population) we got similar results at alternating temperatures. However, the results with *L. molesta* differed from the ones with *L. pomonella* in three points: (a) Temperature and its changes during the preadult development have a strong influence on the fecundity. (b) The ecological experiences of two parent generations are enough to modify the response of the progeny to the ecological factors, expressed in the value of the fecundity. (c) Fecundity is influenced both by temperature and photoperiod. For example, 11 short photoperiods from the beginning of development at low temperature (Table 1. Exp. D.) resulted in reduced fecundity compared to the higher temperature regime (Exp. 3A, B)

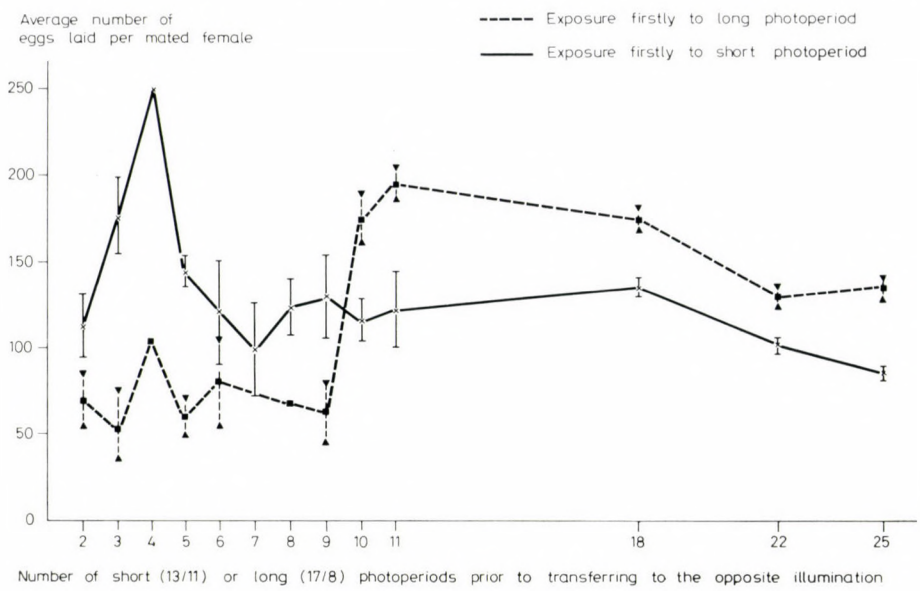

Fig. 1. The effect of short and long photoperiod during the beginning of the development on the fecundity of *L. pomonella* (DESEŐ and SÁRINGER, 1975, expanded with newer experiments)

but in the latter case fecundity was not so different as in the combination of long photoperiod with low temperature (Exp. C). The latter combination reduced fecundity definitively.

Fecundity of natural population

Observations on the fecundity of the field populations of *L. pomonella* are shown in Fig. 2. The decrease in fecundity after diapause (D, E) has its explanation partly in the shortening day in the autumn (C), partly in the loss in weight during diapause (D). The fecundity of the females which developed as larvae after summer solstice is lower (C) than that of females developed before it (B). However, the fecundity of females entering in diapause only in September (E) was somewhat higher than that of those entering in diapause in August (D).

In Fig. 3 data are presented concerning the changes of mating frequency and of the rate of ovipositing females during the season. The data show that shortening photophase in autumn reduces the rate of ovipositing females but does not affect mating frequency. However, mating frequency seems to become decreased after diapause.

As Fig. 2 shows, the mean weight of the females is the highest in autumn, whereas fecundity is the highest in summer. Thus, the relationship between female weight and fecundity is valid only for the same swarming period (DESEŐ, 1973b).

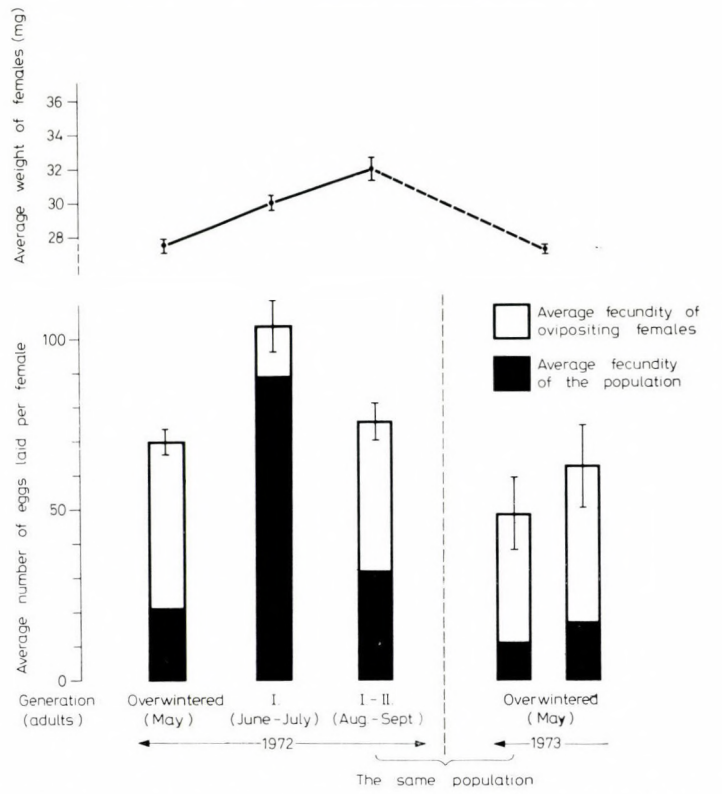

Fig. 2. Average fecundities and mean weights of native *L. pomonella* populations in the field during the season and after diapause (DESEŐ, 1973a, b)

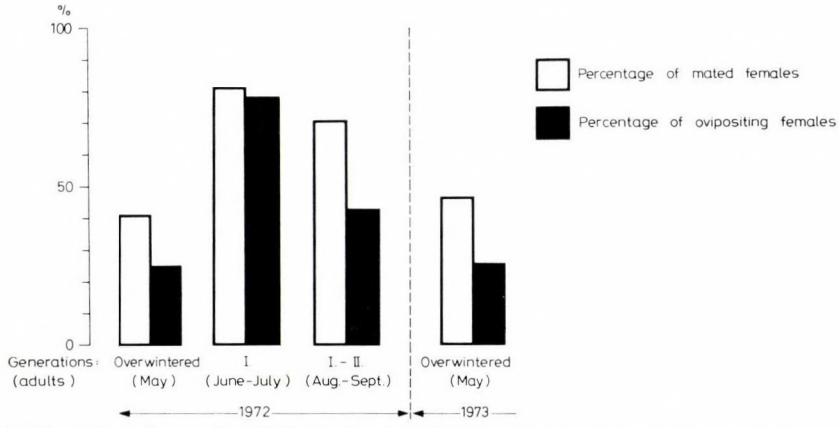

Fig. 3. The ratio of mated and that of ovipositing females in native field populations of L. *pomonella* during the season and after diapause (DESEŐ, 1973a)

The fact that high weight does not necessarily mean high fecundity shows that the population developing in May/June is not in the same physiological state than the one developing in July/August. Not only the number of ovipositing females is reduced then, but fecundity as well. Differences in the size of gonads are known (reviewed DESEŐ, 1973a). Differences in the endocrine system were observed as well (YIN and CHIPPENDALE, 1973), although it is not proved whether these changes develop also in the case when inspite of short photophase diapause does not occur. During diapause the changes concerning reproductive activity become intensified, so both receptivity of females and mating vigour are reduced in spring. Thus, from this standpoint one has to deal with reduced sexual and reproductive characteristics in the spring; consequently in case of the sterile male technique a lower number of sterile males would be required in spring than in summer, or in autumn. Furthermore, in case of pheromone traps this low reproductive activity is the reason that the number of males caught does not correlate with the number of eggs laid (with the degree of injury). According to the data of BRIOLINI *et al.* (1972) and MANI *et al.* (1972) not even the beginning of egg laying can be signal ised by the presence of males caught.

In the case of *G. funebrana* similar differences were found in the reproductive activities of the overwintering population and that of the summer generations. Furthermore, the fecundity of females entering diapause in August/September was lower than that of those diapausing already in August (DESEŐ and SÁRINGER 1970). In *L. molesta* the same pattern of the fecundity was found, females after diapause laid 51 eggs in average, those of the first generation 114, and the second generation 62 (REICHART and BODOR, 1972).

The possible role of photoperiodic selective pressure

Table 2 shows the differences in reproductive activity and mortality between field and laboratory populations after diapause. The laboratory strain seems to loose its ability to diapause: mortality rate is high and only 10% of the surviving

Table 2

Per cent of mortality during diapause and ratio of reproductive activity after diapause in field and laboratory populations

Population	Mortality (%) during diapause	No mating (%)	Mating (%)	Ovipositing females (%)
Gr. funebrana (field)	30	34	66	34*
L. pomonella (field)	25	50	50	24*
L. pomonella (laboratory)	75	90	10	5

* 50% of the females with reduced fecundity

females of the overwintered population mate. Thus, the mating vigour and female receptivity is stronger reduced than in the field population, however, the fecundity of all ovipositing females remains high. In both of the field populations only about 15% of females renews the population in the spring. This percentage can change slightly yearly and there are some differences between orchards as well (DESEŐ, 1973b).

However, considering the fact that only those species can live under our climatic conditions, which are adapted to these more or less, the question arises why is yet the percentage of the well-reproducing females so low. We can answer only with an assumption. In the case of species with more generations yearly and with short-photophase induced prepupal diapause, the change of daylength until and after summer-solstice influences the characteristics of a given population. The lengthening days in spring favour only those developing specimen which require long day conditions, or which have broad ecological plasticity as regards photoperiod. After summer solstice onward the shortening daylength is infavourable for a considerable part of the progeny of the parent generation developed under long day conditions. In spring a part of the population looses its ability to bear short day conditions; this process is supposingly similar to the laboratory strain which losts its ability to diapause. Thus, the photoperiodic selective pressure affects every year the population in more or less extent resulting in the survival of only a part of the population assumingly with broader photoperiodic plasticity. Whether this characteristic is genetically fixed or might be influenced in certain extent by the physiological conditions of the population must be investigated further.

Characteristics of reproduction affected by the length of photophase and diapause

Table 3 summarizes the effect of short and long photophases and that of the diapause on the reproductive activities of the codling moth. (a) Considering as normal the reproductive activity of females developing under longday conditions, or which seems to be adequate, in spring under gradually lengthening day, we can state that short photophase reduces the fecundity and the number of ovipositing females, however weight and mating frequency remain normal. (b) The weight of females exposed to short photoperiod does not change in the laboratory. However, in field conditions the weight increases in the autumn. There are two differences in comparison with laboratory conditions; the daylength becomes continuously shorter and the apples ripe normally. (c) The data in Table 3 suggest that short photophase does not damage the neuroendocrine control of the sexual behaviour only that of the oviposition. Where does the process become blocked, we do not know. Spermatozoa could be observed after mating even in the spermatheca of those females which do not begin oviposition after mating.

We may pose the question whether diapause has direct effect on mating frequency (mating vigour and receptivity of females) or it intensifies only the changes induced by short illumination in the autumn? On the one hand, we have to

refer to the numerous well known cases when diapause in short day species did not reduce reproductive activity. On the other hand, GEOFFRION (1959) with *L. pomonella* and LUM and FLAHERTY (1969, 1970) with *Plodia interpunctella* Hbn. showed that complete darkness in the first case and continuous illumination in the second case during preadult development reduced mating frequency and the beginning of oviposition. Our observations showed also that extremely short photoperiod influenced both processes. On the basis of all these data we assume that

Table 3

Influence of long and short photophases and that of diapause on the reproductive activity of *Laspeyresia pomonella* L.

	Long photophase → Development	Short photophase → Development	Short photophase → Diapause
Size of gonads*	normal	reduced	reduced
Weight of females	normal	increased / normal	*reduced*
Mating frequency	normal	normal	*reduced*
Begin of oviposition after mating	normal	reduced	reduced
Mean fecundity	normal	reduced	reduced
Mortality	normal	normal	*increased*

* see reviewed in DESEŐ, 1973a, b

the reduced reproductive activity, even the lower number of matings after diapause is the continuation of a process induced by the photophase in the autumn and not the consequence of the "preparation" for diapause or of the diapause stage *per se*. This assumption seems to be confirmed by histological observations; the same differences were observed in the ovarioles of these species developed under short day conditions as in those after diapause (DESEŐ, BENNETT, and LANDA unpublished data).

Among the changed characteristics showed in Table 3 the loss in weight and the higher mortality rate seem to be due to the diapause stage. However, the supposition is not excluded that even the higher mortality rate is the consequence of a not perfect diapause, as follows.

Like all biological processes in an insect also the development of the reproductive system seems to demand its optimal combination of illumination and temperature. In case of *L. molesta* we have seen that low temperature compensated the reducing effect of short photophase on the fecundity. Furthermore, in case of *G. funebrana* the rate of ovipositing overwintered females was higher when

entering diapause in August/Sept, then when in July/August. Moreover, as we showed in Fig. 1, the mean fecundity of codling moth was higher when entering diapause in September than in July/August.

Conclusion

A hypothesis is offered, namely that from the summer solstice on, the first token stimulus from the adversely changing environment is the gradually shortening length of day and the response of the insect is reduced reproductive activity. If the shortening photophase affects the developing insect in a combination with low temperature (that means usually later in the autumn), a perfect diapause will develop and reproductive activity will be about "normal". However, if then the temperature is high, the insect does not get the necessary information to swich over entirely to diapause induction: so the prolonged development or certain developmental processes will continue. In other words, the ability of the insect to protect itself perfectly against the adverse environment does not develop. As a result, the number of progeny will become reduced, or a part of the population cannot survive in winter. This photoperiodic regulation acting under our climate every year may play an important role in the population dynamics of certain multivoltine lepidopterous species with facultative diapause induced in the autumn by the shortening daylength.

Literature

BRIOLINI, G., CAPORALE, F. and CASTELLARI, P. L. (1972): Ricerche su *Cydia pomonella* L. (Lep. Tortricidae) e su di un methodo razionale per combatterla. Boll. Entom. Bologna *XXX*, 153–188.

DESEŐ, K. V. (1967): The role of olfactorial stimuli in the egg-laying behaviour of plum moth (*Grapholitha funebrana* Tr.). Acta Phytopath. Hung. *2*, 243–250.

DESEŐ, K. V. (1971): Study of factors influencing the fecundity and fertility of codling moth (*Laspeyresia pomonella* L., Lepid.; Tortr.). Acta Phytopath. Hung. *6*, 243–250.

DESEŐ, K. V. (1972): The role of Farnesylmethyl-ether applied on the male influencing the oviposition of the female codling moth (*Laspeyresia pomonella* L., *Lepidopt.*; *Tortricidae)*. Acta Phytopath. Hung. *7*, 257–266.

DESEŐ, K. V. (1973a): By-effect of diapause inducing factors on the reproductive activity of some Lepidopterous species. Nature New Biology. *242*, 126–127.

DESEŐ, K. V. (1973b): Reproductive activity of codling moth (*Laspeyresia pomonella* L. Lepidopt.; Tortr.) exposed to short photophase during preimaginal state. Acta Phytopath. Hung. *8*, 193–206.

DESEŐ, K. V. (1974): The regulation of fecundity by the length of photophase during preadult development in some *Lepidopterous* species. Int. Conf. on "Regulation of Insect Reproduction" Liblice 9–14, 1974. Only abstracts. Edited ČSAV. Prague.

DESEŐ, K. V. and SÁRINGER, GY. (1970): Influence of conditions prevailing during the ontogenesis on the number of follicles and fecundity of plum fruit moth (*Grapholitha funebrana* Tr.). Acta Phytopath. Hung. *5*, 355–365.

DESEŐ, K. V. and SÁRINGER, GY. (1973): Short photophase during preimaginal development influencing reproduction of some *Lepidoptera* spp. Seventh Conf. of European Comp. Endocrinol. Budapest. Only abstracts. Akadémiai Kiadó 275.

DESEŐ, K. V. and SÁRINGER, GY. (1975): Photoperiodic influence on fecundity of *Laspeyresia pomonella* L., *Grapholitha funebrana* Tr. and *L. molesta* Busck: The sensitive period. Entomologica experimentalis & applicata. *18*, *187—193*.

GEOFFRION, R. (1959): Étude biologique de la cochylis et de l'eudémis (*Clysia ambiguella* Hb. et *Polychrosis botrana* Schiff.) dans les vignobles du Val de Loire et recherches expérimentales sur la fecondité de la carpocapse (*Laspeyresia pomonella* L.) Thése Fac. Sci. Univ. Poitiers, Angers 204.

HARCOURT, D. G. and CASS, L. M. (1966): Photoperiodism and fecundity in *Plutella maculipennis* (Curt.). Nature 503, 317—218.

LUM, P. T. M. and FLAHERTY, B. M. (1969): Effect of mating with males reared in continuous light or in light-dark cycles on fecundity in *Plodia interpunctella* Hb. (*Lepidoptera: Phyticidae*). J. stored Prod. Res. *5*, 89—94.

LUM, P. T. M. and FLAHERTY, R. B. (1970): Effect of continuous light on the potency of *Plodia interpunctella* males (Lep.: *Phyticidae*). Ann. Ent. Soc. Amer. *63*, 1470—1471.

MANI, E., WILDBOLZ, TH. and RIGGENBACH, W. (1972): Die Männchenfalle, eine neue Prognosemethode für den Apfelwickler; Resultate 1969—1971. Schweiz. Z. Obst- u. Weinbau *108*, 337—344.

RAHN, R. (1970): Rôle de la photopériode sur l'activité de la reproduction de la dernière génération d'Acrolepia assectella Zeller (Lépidoptère, *Plutellidae*) dans l'ouest de la France. Comptes rendus Soc. Biol. *164*, 2630—2636.

REICHART, G. and BODOR, J. (1972): Biology of the oriental fruit moth (*Grapholitha molesta* Busck) in Hungary. Acta Phytopath. Hung. *7*, 279—295.

SÁRINGER, Gy. and DESEŐ, K. V. (1972): The annual development of the plum fruit moth (*Grapholitha funebrana* Tr. Lep. Tortr.) in Hungary. Acta Phytopath. Hung. *7*, 425—438.

YIN, C. M. and CHIPPENDALE, G. M. (1973): Endocrine system of mature diapause and non-diapause larvae of the southwestern corn borer, *Diatreae grandiosella*. Ann. Ent. Soc. Amer. *66*, 943—949.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 10 (1—2), pp. 141—151 (1975)

Observations on the Moulting of Aphids, Scales and Mealybugs

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The author carried out observations on the moulting of different *Homoptera* with special regard to the moulting of mouth parts. Different theories of the ways are discussed, how the new sucking bristles are led into feeding position and how the old ones are cast off. It is concluded that — especially in case of *Diaspididae* — the protractor and retractor muscles of the sucking apparatus play the most important role in moving the bristles forward and inserting them into the host plant.

In earlier observations the author studied the moulting of the pineapple mealybug (*Dysmicoccus brevipes* Cockerell) in Cuba and published his results in two papers (PATAKI, 1971; 1974).

The studies have been continued in Hungary and extended to aphids and scale insects. Based on literature data concerning the moulting of *Rhynchota*, some earlier conclusions of the author had to be modified or completed.

The moulting process — one of the most critical and significant phenomena in insect life — is basically similar in the different groups of *Rhynchota*, there are, however, differences in the different families, resulting from differences in anatomy and feeding behaviour. In insects with short haustellum, like bugs and most aphids, where the length of the bristles is nearly identical with the length of the labium, the moulting is comparatively simple. It is, however, more complicated in insects where the sucking bristles surpass many times the length of the labium, even of the body, like in scales, mealybugs and psyllids (WEBER, 1930; 1933; SNODGRASS, 1935). The moulting becomes modified also in the female larvae of the family *Diaspididae*, which settle down in the first larval instar and loose their organs of promotion. As these latter are unable to change their place, they have to insert their new bristles very near to the old ones and are able to modify only the direction and depth of penetration. This, however, may be even beneficial for the individual, enabling it to use more economically its salivary secretion, the histolytic and cecidogenic effects of which are well known.

In the earlier publication of the author (PATAKI, 1974) the moulting of the mandibular and maxillary bristles was supposed too, by assuming that the bristles become separated from the old skin and coil up in the head or thorax, as they had been in the embryonal stage.

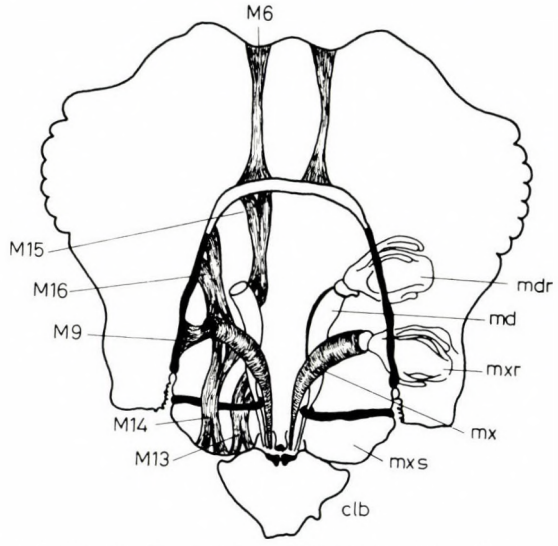

Fig. 1. Anatomy of the head of a one-day old *Myzus persicae* larva. After PONSEN (1972), based on nine transversal sections, studied by electron microscopy. clb = clypeolabrum, md = mandibular bristle, mdr = retort-shaped organ of mandible, mx = maxillary bristle, mxr = retort-shaped organ of maxilla, mxs = maxillary sclerit, M 9 = lateral muscle of maxillary bristle, M 13 = protractor muscle of mandibulary bristle, M 14 = protractor muscle of maxillary bristle, M 15 = retractor muscle of mandibular bristle, M 16 = retractor muscle of maxillary bristle

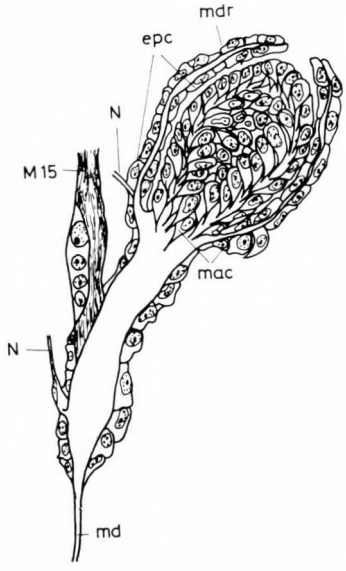

Fig. 2. Longitudinal section of the mandibular bristle and retort-shaped organ of a one-day-old *Myzus persicae* larva. After an electron microscopic photo of PONSEN (1972). epc = epidermal cells, mac = matrix cells, md = mandibular bristle, M 15 = retractor muscle of mandible

A detailed description of the moulting of homopterous insects was given by WEBER (1930). The stylets (bristles) consist of lifeless chitinous material and are produced by cells capable for proliferation. The latter are situated in the "retort-shaped" organ. Before moulting the new bristle is secreted by the matrix cells, becomes elongated backwards and coiles up like in the embryonal stage. PONSEN (1972) studied with electron microscopy in longitudinal and transversal ultrathin sections the internal anatomy of the green peach aphid, *Myzus persicae* Sulz., and presented detailed descriptions on the retort-shaped organs and inside those on the matrix cells (Figs 1 and 2). Each cell produces a very thin, long filament (fibril) which unite to form a new bristle. The observations made by the author of this paper have been carried out by considering above details of moulting.

Materials and Methods

For microscopic studies the insects were mounted in FAURE–BERLESE embedding medium, according to the classic method described by SZALAY-MARZSÓ (1969). The drawings and microphotos were prepared from these slides.

The species studied were the following:

Aphidoidea – Aphididae:

Aphis schneideri Kalt.
Dysaphis reaumuri Mordw.
Phorodon humuli Schrank
Aphis fabae Scop.
Macrosiphum avenae F.

Adelgidae:

Sacchiphantes viridis Ratz.

Pemphigidae:

Pemphigus spirothecae Pass.

Coccoidea – Ortheziidae (= Pseudococcidae):

Pseudococcus adonidum L.
Dysmicoccus brevipes Cock.

Diaspididae:

Quadraspidiotus perniciosus Comst.

Results and Discussion

SNODGRASS (1935) described in his comprehensive work the different types of piercing-sucking mouth parts of *Rhynchota*. In groups with short haustellum the bristle fascicle lies in the labial groove. The bristles can be inserted into the plant tissues by a simultaneous telescoping of the labial segments (*Aleurodid* adults, *Aphididae*) or the insertion of the bristles is accompanied by an elbowlike bend

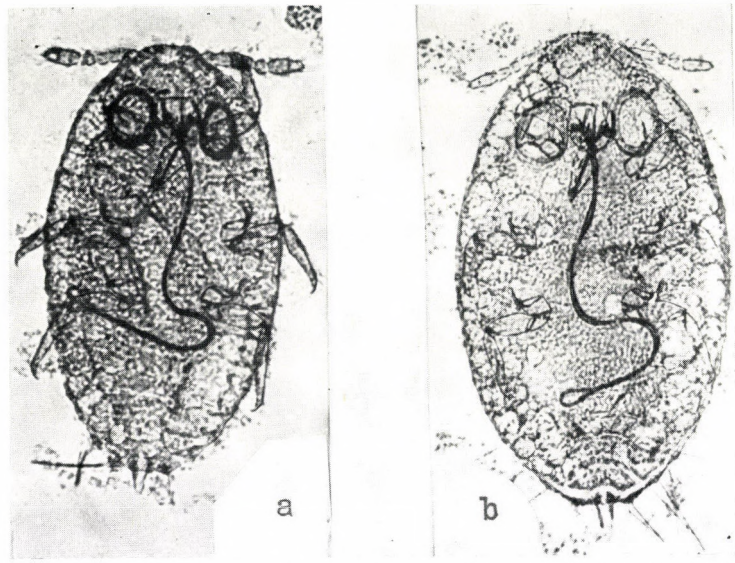

Fig. 3. *Dysmicoccus brevipes* larvae in moulting. a = first larval stage, b = second larval stage (females)

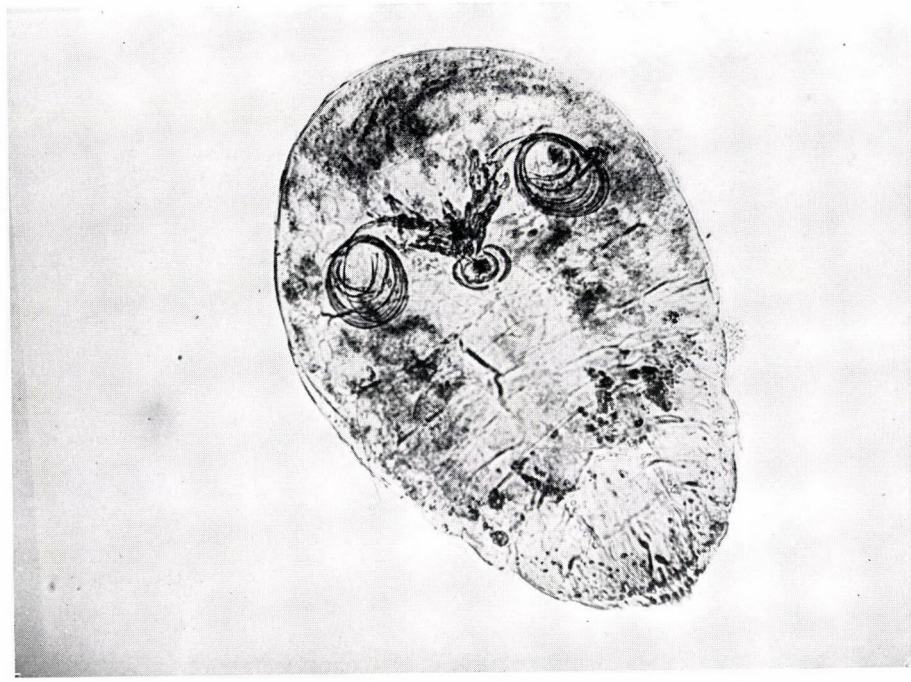

Fig. 4. Second larval instar of *Quadraspidiotus perniciosus* female in moulting

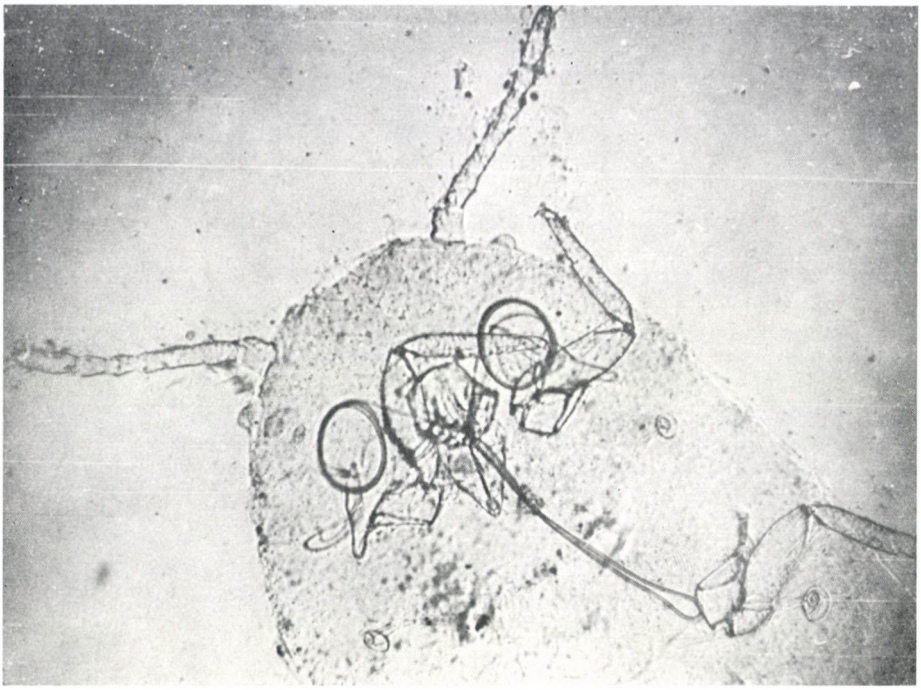

Fig. 5. Third larval instar of *Pseudococcus adonidum* female in moulting

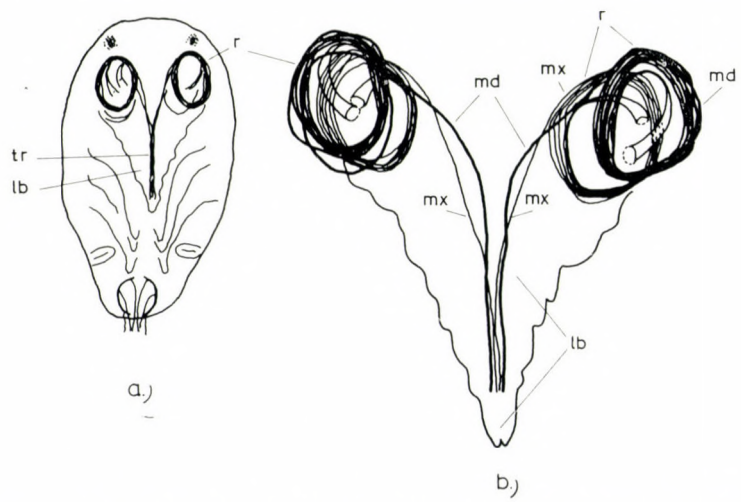

Fig. 6. *Dysmicoccus brevipes:* a = embryo, b = mouth parts of embryo, showing coils of mandibular and maxillary bristles, lb = labium, md = mandible, mx = maxilla, r = coils of bristles, tr = haustellum

in the labium between the first and the second segment, the stylet bundle becomes thus partly exposed and stays rigid. In many *Heteroptera* the first and second, in *Cimex* species (as the bedbug) the third and fourth labial segments are bent.

In those groups, where the bristles are longer than the labium, the problem of bristle storage has been solved in many ways. In larval *Psyllids* the bristle fascicle, when retracted, is projected forward from the base of the labium in a large

Fig. 7. Mandibular and maxillary coils of *Dysmicoccus brevipes* from Fig. 3a

free loop beneath the head. In *Coccids* and *Aleurodids* as well as in the larvae and females of *Ortheziidae* the retracted fascicle is received in a loop into an elongated internal pouch, the crumena, extending into the body. In the *Coptosomatidae* family *(Heteroptera)* the retracted bristle fascicle is looped posteriorly in a large membranous diverticulum in the second labial segment. In the *Aradidae*, finally, the retracted bristle fascicle is coiled in a large chamber of the preoral cavity anterior to the mouth. The retracted, stored stylets rest thus in the pharynx, where their bulblike basal-parts are well visible. The elongated middle portion of the bristle bundle forms a loop, either free in front of the head or extends into the thorax or even deeper into the body and rests in the membranaceous crumena or is coiled in the preoral cavity. The distal end of the bristle fascicle lies in the labium, inside the labial groove.

The above given descriptions may be completed by the data of SCHMUTTERER (1959), regarding the larvae and female adults of *Diaspididae*. These do not retract their bristle fascicle from the plant and it remains in the plant tissues even after moulting. The author's observations showed that the larvae can be easily lifted in course of their moulting from the plant surface, because their old bristle breaks off. If the larva removed was not in moulting, the bristle could be removed

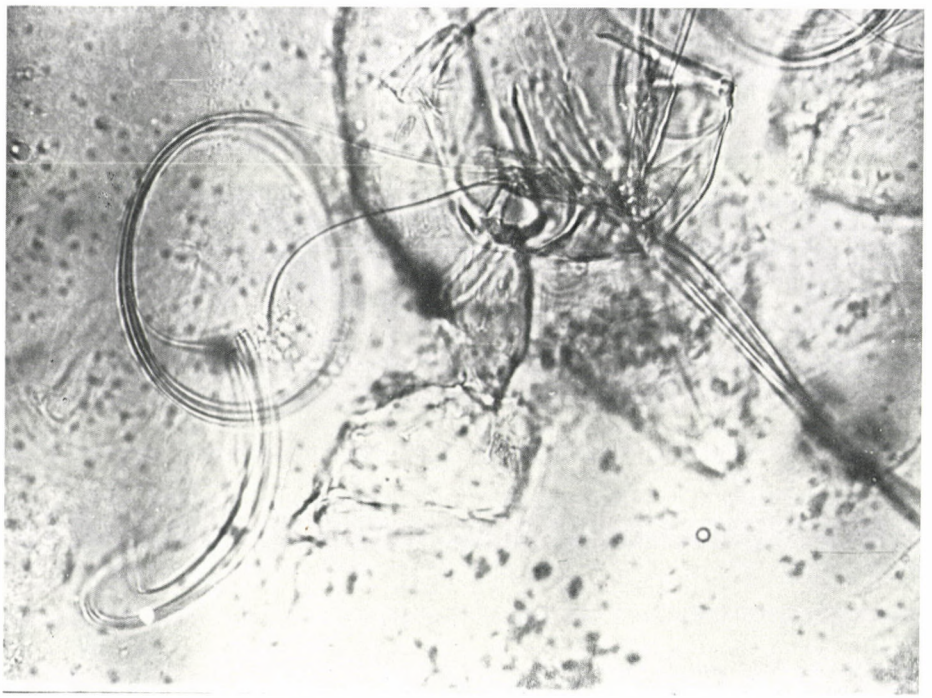

Fig. 8. Mandibular and maxillary coils of *Pseudococcus adonidum* from Fig. 5

too from the plant tissues and this became visible in the microscopic preparation. In moulting, the old bristles are cast off with the old skin, the latter becomes attached to the dorsal and ventral scale, the bristles, however, remain in the plant. Fig. 4 shows a San José scale larva fixed during the moulting, with the old bristles broken off at the labium.

The length of the bristles can represent also taxonomic characteristics inside the systematic group: in the two mealybug species studied by the author, the retracted bristle fascicle, resting ventrally in the crumena (forming a loop), extended to the second coxae in case of *Pseudococcus adonidum* (Fig. 5), in *Dysmicoccus brevipes*, however, over the third coxae, with other words into the abdomen (Figs 3 and 7).

WEBER (1930) emphasized the fact that after moulting the growth of the larval body parts is not proportional. The largest growth can be observed in the abdominal region, the least in the head. In young larvae of *Rhynchota* the bristles are nearly as long as in the adults, which is quite understandable, considering their identical feeding sites. As shown in Fig. 3 the first and second instar larvae of *Dysmicoccus brevipes* show nearly identical bristle lengths.

All developmental stages of *Dysmicoccus brevipes* have been studied in Cuba

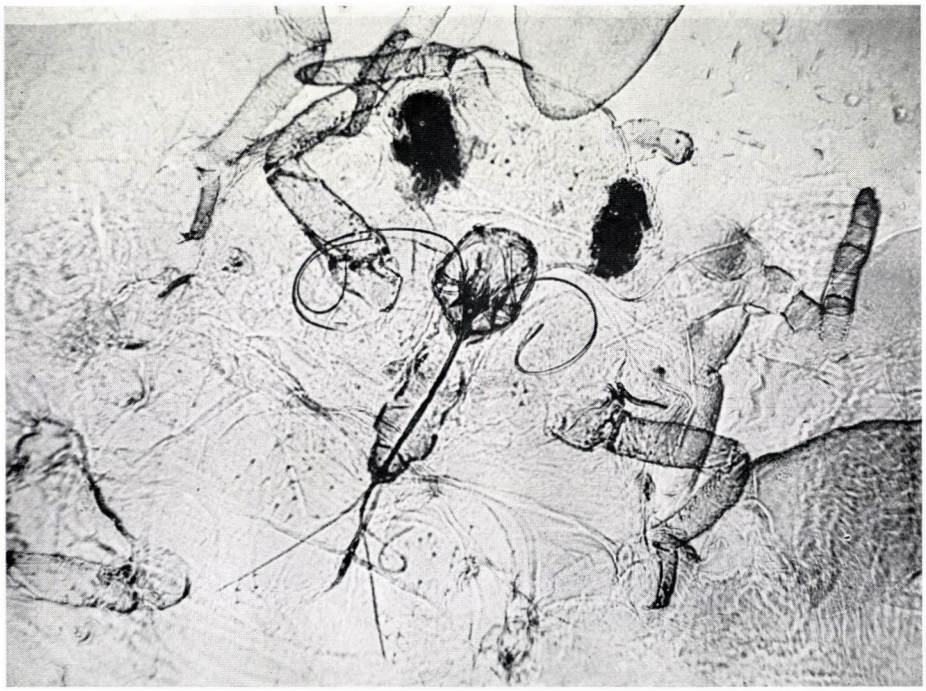

Fig. 9. Larva of winged *Sacchiphantes viridis* female in course of moulting

(PATAKI, 1971). As shown in Fig. 6 the maxillary and mandibular bristles form in the embryo coils of six windings; the same can be seen in larvae before moulting, where the new bristles formed in the retort-shaped organs constitute also six windings (Fig. 7). The shorter bristles of *Pseudococcus adonidum* form coils of four windings (Figs 5 and 8). The coils of *Quadraspidiotus perniciosus* are also six-fold (Fig. 4) whereas in the aphids studied, whose bristles are short *(Aphididae, Adelgidae, Pemphigidae)* both in embryonal and larval stages only simple coils can be observed (Fig. 9).

WEBER (1930) presents the following explanation to the question, how the bristles are formed from the coiled structures (Fig. 10): the new bristle – formed

in the retort-shaped organ – lies with its tip in the funnel-like basal end of the old bristle, visible also in Fig. 7. The new bristle is connected to the old one by a very fine chitinous filament. When in course of the moulting the head is withdrawn from the old skin, the departing old bristle pulls the new one into the proper position. At the same time, the inner chitinous membrane, which enveloped the base of the old bristle, folds back like the finger of a rubber glove (Fig. 10, e and f) and after hardening forms the base of the new bristle. When the new bristle ar-

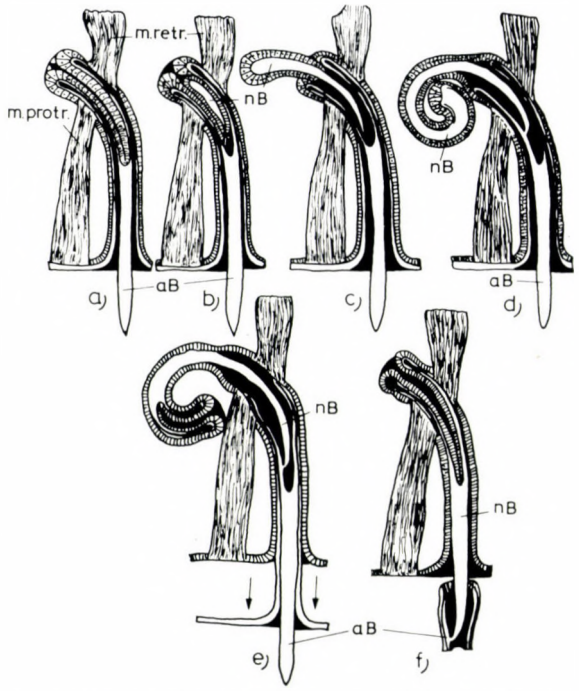

Fig. 10. Moulting of one sucking bristle of *Psylla mali* (simplified diagram, after WEBER, 1930) Chitinous parts white, the hollow parts black. a and b = begin of new formation of the retort-shaped organ, c and d = growth and coiling of the retort-shaped organ, e and f = begin of moulting, m. protr. = protractor muscle, m. retr. = retractor muscle, aB = old bristle, nB = new bristle

rived into its new position, the thin filament at its tip breaks off and the moulting is finished. The retractor and protractor muscles are thus able to move the bristles during the whole moulting process, as these are attached not immediately to the bristles but to the enveloping membrane.

This explains completely the moulting of the sucking insects with short bristles, which rest retracted in the labial groove. But how is formed the haustel-

lum in long-bristled insects, in which only the bristle tips rest in the labium? And how is the first bristle fascicle formed, to help the feeding of the newly hatched larva which possesses yet no old one to direct the new bristle fascicle into feeding position? How is the haustellum formed in the *Diaspididae*, in which the old, long bristle fascicle remains lost in the plant tissues and the larva is unable to change its place?

It can be observed that in the embryos the tips of the coiled-up bristles lie already in the labial groove (Fig. 6). In species with long bristles it is very likely that the old, cast-off bristle pulls down the new one only until the tip of the labium, setting thus only its distal end into the sucking position. In course of moulting the looped portion of the bristle fascicle is pulled out through the mouth opening and remains hanging on the cast-off skin with its base in the mouth, with its tip in the labium. In the *Diaspididae* the bristles, remaining in the plant, cannot pull the new ones further than the end of the labium, because the larva – having lost its legs after settling down – can only raise itself slightly but is incapable of other movement.

To find an explanation for these phenomena we have to refer again to the data of WEBER (1930), according to which the retractor and the protractor muscles are not attached directly to the bristles but to their developing membrane. It follows that the parting old bristles, by pulling after them the new ones, have to pull the latter through this sheat. The bristles are then led into the labial groove – where they become joined together to form a bristle fascicle – then through the tip of the labium where the muscles of the labial clamp get hold on them. It may be assumed that the latter muscles are responsible for breaking away from the old bristle as it would be a disadvantage for the insect if the tip of the new bristle fascicle was pulled out of the labium without penetrating into the plant although this could not be confirmed with light microscopy, it is possible that the bristles are arranged this way already in the embryo.

It can be concluded, therefore, that the muscles are able to move the new bristles already in this initial position. The process takes place essentially the same way when the insect inserts its bristle fascicle from the retracted, stored position (loop or coil), with the only difference that the bristles move forward in their basal membranaceous sheat. According to our assumption the process goes as follows: the protractor muscle contracts whereas the retractor and labium muscles slacken, the bristle moves forward. In this way are pushed the mandibular bristles one after the other, followed by the two maxillary bristles, the four bristles forming together the well-known double tube. In this phase the muscles of the labial clamp become active and the bristle fascicle is firmly held in place; the protractor muscle slackens and the retractor muscle contracts. As a result, the membranaceous sheats of the four bristles, with the muscles attached to them, slide upward. By repeating many times these movements the bristles become gradually uncoiled whereas the fascicle penetrates into the host plant tissues. When the funnel-like extended base of the bristles arrives in their membranaceous sheat, this does not permit a further move-

ment and the bristles are exerted at full length. The whole bristle fascicle can be retracted by the retractor muscles, this occurs, however, only in cases when the insect changes its place and inserts again its bristles into the host plant tissues.

Literature

PATAKI, E. (1971): Morfologia de la cochinilla harinosa de la piña (*Dysmicoccus brevipes* COCKERELL). Rev. Cenic, Habana, Cuba *3*, 1–2 127–144.

PATAKI, E. (1974): Néhány megfigyelés a vándorpajzstetvek (*Pseudicoccidae* család) vedlésével kapcsolatban. (Some observations in connection with the moulting of mealybugs, *Pseudococcidae*) Folia Entomologica Hungarica *27*, 1.

PONSEN, M. B. (1972): The site of potato leafroll virus multiplication in its vector, *Myzus persicae*. An anatomical study. Medelingen Landbouwhogeschool, Vageningen *16*, pp. 147.

SCHMUTTERER, H. (1959): Schildläuse oder *Coccoidea* I. Deckelschildläuse oder *Diaspididae*. Die Tierwelt Deutschlands. Fischer Verl., Jena pp. 260.

SNODGRASS, R. E. (1935): Principles of Insect Morphology, McGraw-Hill, New York pp. 665.

SZALAY-MARZSÓ, L. (1969): Levéltetvek a kertészetben (Aphids in Horticulture). Mezőg. Kiadó, Budapest pp. 187.

WEBER, H. (1930): Biologie der Hemipteren. Eine Naturgeschichte der Schnabelkerfe. Springer Verl. Berlin pp. 543.

WEBER, H. (1933): Lehrbuch der Entomologie. Fischer Verl. Jena, pp. 726.

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The Nematoda Species of Horse Manure Used for the Cultivation of the Field Mushroom and Possibilities of Control

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In 9 samples from fresh horse manure, 6 from compost after 2nd turning up and 8 from ready compost, 700 nematodes were determined. The nematological investigation data were analysed on the basis of PARAMONOV's (1952, 1962) ecological classification too. In the course of evaluations the abundance values of nematodes from 25 fresh horse manure, 36 after 2 turnings up and 105 ready compost samples were established.

The number of nematode species was in fresh horse manure 13, after the 2nd turning up 16, and in ready compost 9. Two *Seinura* sp. represent the mycopathogenic species. The ecological group number was three in fresh horse manure and in compost after 2nd turning up and in ready compost four.

In all three technological phases the ecological group of eusaprobiont dominates. The ratio of the mycopathogenic group at the most critical time that is before bedding was 5.26%. The abundance value of nematodes is highest in fresh horse manure which decreases parallel with composting but the ratio of infection-free samples does not reach 25% even before the completion of composting.

As a final conclusion to be drawn from the stack foil covering experiments it can be established:

1. On the effect of covering the stack surface temperature does not rise above 50°C.

2. Only the double foil covering after the third turning up is the best. The nematicidal and yield increasing effect is favourable.

3. The Nemafos (5 cm^3/m^2) results in total nematicidal effect but this does not necessarily entail maximal yield results.

In Hungary the field mushroom is cultivated in an area of about 300 thousand square metres. The average yield of 5–6 kg is related to the fertilizer ton. The crop amount is 1.5–1.8 million kg. The initial basic material for cultivation in 99% of cases is fresh manure. Other substituting materials instead of compost are only very rarely used in experiments. The compost is prepared from horse dung, by the so-called aerobic composition by three turnings up without pasteurization. Most cultivation takes place in old stone mines and cellars. Nematological analysis in Hungary referring to horse manure has not been done so far. As far as we know there have been few investigations of this kind on the international level.

VAN HAUT (1956) PAESLER (1957) and SUMENKOVA (1964) are the most sig-

nificant in this respect. According to CAYROL (1962) and SUMENKOVA (1964) saprobiont species exclusively occur in ready compost.

HESLING (1964) also considers that the nematodes of fresh manure should not cause concern. Nevertheless the different views of the role of *Rhabditids* (STEINER, 1931; 1933; REMPE and KUX, 1953; VAN HAUT, 1956; MORETON and JOHN, 1956a; 1956b; HESLING, 1966; MOGENS, 1966 etc.) led to the investigation of the Nematode species in fresh manure and under composting and to the possibilities of protection.

Material and Method

1. Nematological investigations

The fresh horse manure and compost samples were cut with scissors, mixed well and made to run according to BAERMANN (1917) and EGER (1959) modified method for 24 hours. The sampling was repeated four times and the measured material at each replication was 10 g. The counting of nematodes was carried out on the basis of GRISSE's description (1963) in plastic dishes of 3 cm in diameter and having side walls of 45°C slant with the aid of a square grid micrometer built into the ocular leus. After counting, the material was fixed with high temperature and with FAA. A few days later 25 – 50 individuals per samplewere transferred into a 9 : 1 mixture of alcohol and glycerin 9 : 1 mixture for 48 hours followed by a preservative preparation.

In the course of our investigations extending to species, nematodes were determined in 9 fresh horse manure samples, 6 samples from material under composting (II turns) and in 8 ready composted samples. In the study of nematode abundance, individual densities were determined related to 100 g material in 25 fresh horse manure samples, 36 under composting (II turns) and 105 ready composted.

2. Control experiments

In the course of our control experiments we wished to utilize the heat produced during composting to kill the nematodes existing on the surface of the stack.

In the first experimental series (from straw horse manure) five stacks were prepared of $1.8 \times 2.5 \times 1.5$ m size. The number I stack was composted according to the usual method with the aerobic procedure for 16 days. The number II stack was covered by a simple PVC foil for 24 hours following eack (I – III) turning up. No. III stack in contrast with the previous one was covered with a double foil. The number IV stack was covered with a simple foil for a 48 hour period 24 hours after every three turns. The number V stack treatment only differed from IV in that it was covered with a double foil. During the last covering the tempera-

ture and humidity content of the stack surface was registered with a thermo-hygrograph. The number of live nematodes on the surface of the stack was established before the last covering and after the removal of the cover in 3 treatments in 3 repetitions each.

In the second experimental series the size and material of the stack was the same as in the previous ones. In this series of experiments the following treatments were used:

I. The usual so-called 16 day aerobic composting with three turnings.
II. In the 24 hours after eack turning up of the stack, it was covered with a plastic (PVC) foil for a 24 hours period.
III. The stack was covered only 24 hours after the 3. turning up, for 24 hours with a double PVC foil.
IV. 24 hours following the third turning the surface of the stack was treated with a 5 ml/m^2 dose of Nemafos mixed with water (1 litre/m^2) sprinkled from a watering can. Immediately after this the stack was covered for a 24 hour period with a double PVC foil.

The numerical investigation of the nematodes was carried out in three repetitions per treatment before the last covering and after removal of the cover.

The effect of the treatments on the crop results with the usual and foil bag cultivation was closely observed. In the first case 2×6 q compost for each treatment was arranged randomly and was bedded on a concrete cellar floor. With sack cultivation the 5×10 sacks (15 kg compost per sack) were filled in each treatment. To cover the sacks and beds nematode-free pulverized limestone was used. The yield was registered at each cropping until the end for the phase No. III. Evaluation of the yield results was done on the basis of 31 croppings.

Results and Evaluation

1. Nematological investigations

In the fresh horse manure (Table 1) 14 species of Nematodes of 10 genus were found. The highest dominance values ($D = 22\%$) were reached by *Rhabditis* sp. Among all species the dominance value of *Pelodera cylindrica* (COBB) DOUGHERTY (18.56%) was the highest. The further order: *Monochoides striatus* (BUTSCHLI) GOODEY 17.16% *Rhabditis axei* (COBBALD) DOUGHERTY 12.52% *Panagrolaimus subelongatus* (COBB) THORNE 10.44% *Rhabditoides inermis* (SCHNEIDER) DOUGHERTY 9.74%. 2 *Seinura winchesi* (GOODEY). GOODEY individuals represented the species parasitic on mushrooms, whose dominance value (0.46%) was insigificant.

On the basis of PARAMONOV's (1952, 1962) ecological classification (Fig. 1) the ratio of the eusaprobiont nematode species in fresh horse manure (85.84%) is the highest. The participation of devisaprobionts was 3.68%, that of special fungal parasite species (0.46%).

Table 1

Individual dominance of nematoda species in fresh horse manure

Numbers	Species	Number of species	Individual dominance (D %)
1.	*Pelodera teres*	3	0.69
2.	*Pelodera cylindrica*	80	18.56
3.	*Pelodera icosiensis*	1	0.23
4.	*Rhabditis longicaudata*	1	0.23
5.	*Rhabditis axei*	54	12.52
6.	*Rhabditis sp.*	99	22.96
7.	*Rhabditoides inermis*	42	9.74
8.	*Mesorhabditis spiculigera*	6	1.39
9.	*Diplogastrellus gracilis*	7	1.62
10.	*Diplogasteroides ruehmi*	3	0.69
11.	*Mononchoides striatus*	74	17.16
12.	*Panagrolaimus rigidus*	13	3.01
13.	*Panagrolaimus subelongatus*	45	10.44
14.	*Trilabiatus lignicolus*	1	0.23
15.	*Seinura winchesi*	2	0.46
	Altogether	431	99.93

Table 2

Individual dominance of nematoda species in horse manure after II turning up

Numbers	Species	Number of species	Individual dominance (D %)
1.	*Pelodera teres*	9	4.20
2.	*Pelodera coarctata*	3	1.40
3.	*Pelodera cylindrica*	19	8.87
4.	*Pelodera strongyloides*	3	1.40
5.	*Rhabditis longicaudata*	4	1.86
6.	*Rhabditis intermedia*	2	0.93
7.	*Rhabditis axei*	32	14.95
8.	*Rhabditis sp.*	76	35.51
9.	*Mesorhabdiris sp.*	1	0.46
10.	*Diplogastrellus gracilis*	18	8.41
11.	*Diplogasteritus consobrinus*	1	0.46
12.	*Mononchoides leptospiculum*	2	0.93
13.	*Butlerius butleri*	1	0.46
14.	*Panagrolaimus rigidus*	24	11.21
15.	*Panagrolaimus subelongatus*	7	3.27
16.	*Cephalobus sp.*	4	1.86
17.	*Trilabiatus lignicolus*	3	1.40
18.	*Seinura winchesi*	3	1.40
19.	*Seinura oxurus*	2	0.93
	Altogether	214	99.91

During composting (after II turning up) the number of the genera increased to 11, the number of species to 16 (Table 2). The *Rhabditis* spp. further dominated ($D = 35.51\%$). Amongst the species *Rhabditis axei* (COBB) DOUGHERTY takes over ($D = 14.95\%$) which is followed by *Panagrolaimus rigidus* (SCHNEIDER) THORNE, with dominance value of 11.21%. The fungus parasite species are represented by *Seinura winchesi* (GOODEY) GOODEY and *Seinura oxorus* (PAESLER) GOODEY with a dominance value of 1.40% and 0.93% respectively.

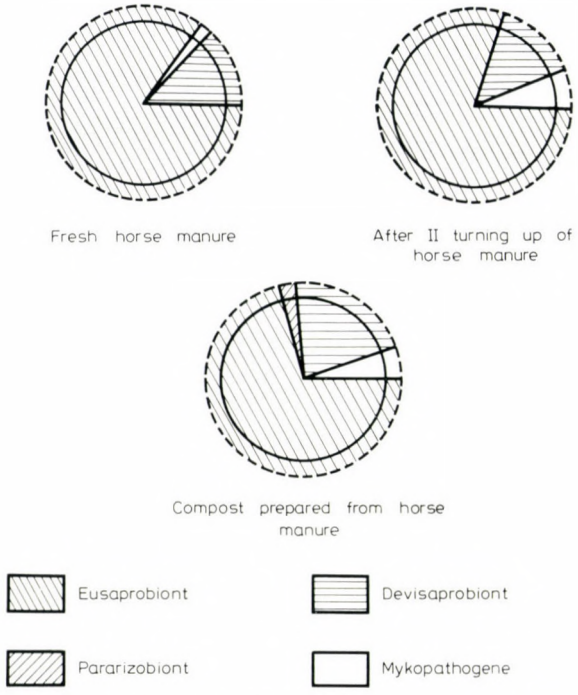

Fig. 1. Fresh horse manure and after the 2nd turning up and ecological characteristics and tendencies of nematodes from compost prepared from horse dung

On the basis of the ecological features during composting (Fig. 1) it can be concluded that inspite of the changes in the number of species the ecological characteristics do not change essentially. The ratio of eusaprobiont was 79.90%, the devisaprobionts was 14.01%, that of the fungal parasite species 6.09%. Nevertheless it is remarkable that the ratio of the latter species rose to the twelfth.

At the end of composting (before bedding doyn) the number of the genus dropped to 8, the species number to 9 (Table 3). Among the species the individual dominance value of *Rhabditis axei* (COBB) DOUGHERTY led by 40.35%. This was followed by *Pelodera teres* SCHNEIDER, 1866 dominance value of 12.28%. *Panagro-*

laimus rigidus (SCHNEIDER) THORNE was 10.52%. The only species representing the mycopathogenic species was *Seinura winchesi* (GOODEY) GOODEY dominance value of 5.26%.

With regard to ecological characteristics (Fig. 1) the most significant change was that a new ecological group (pararizobiont) appears too. MANKAU (1962) mentions a similar observation. In ready compost the ratio of eusaprobionts was 71.92% that of devisaprobionts 21.05% and mycopathogenic species 5.26%, of the pararizobionts 1.77%.

Table 3

Individual dominance of nematoda species from composted horse manure

Numbers	Species	Number of species	Individual dominance (D%)
1.	*Pelodera teres*	7	12.28
2.	*Pelodera cylindrica*	5	8.77
3.	*Rhabditis longicaudata*	1	1.75
4.	*Rhabditis axei*	23	40.35
5.	*Rhabditis sp.*	2	3.50
6.	*Mesorhabditis inarimensis*	1	1.75
7.	*Diplogastrellus gracilis*	2	3.50
8.	*Panagrolaimus rigidus*	6	10.52
9.	*Panagrolaimus subelongatus*	3	5.26
10.	*Cephalobus sp.*	3	5.26
11.	*Seinura winchesi*	3	5.26
12.	*Eudorylaimus sp.*	1	1.75
	Altogether	57	99.95

From the data relating to species investigations (Tables 1 – 3) it turned out and the abundance investigations of 166 samples results unanimously support this (Fig. 2) that the number of nematodes rapidly decreases parallel with composting but complete nematode-free states can be achieved in 1/4 of the cases.

A 25% nematode-free state developed after the II turning up and this does not change significantly after the last turning up either. The views on the role of *Rhabditis* which have developed cannot be debated (VAN HAUT, 1956; REMPE and KUX, 1953; HESLING, 1966b; MOGENS, 1966 etc.). However, contrary to SUMENKOVA (1964), CAYROL (1962) and HESLING (1964) investigations it must be stated that the compost prepared from the fresh horse manure is not free of fungal parasitic nematodes. It is true that the number of the species and ratio are not significant and the two species which have occurred do not belong to the most dangerous fungal parasites. According to HOOPER (1962) *Aphelenchus avenae* is frequent in fresh manure. We did not find it in a single sample. MANKAU (1962) states that parallel with composting the eusaprobionts are dominant but their ratio does not change significantly during composting.

According to our opinion the fungus compost prepared from horse manure is not the most dangerous infection source but the nematodes living in it are not desirable.

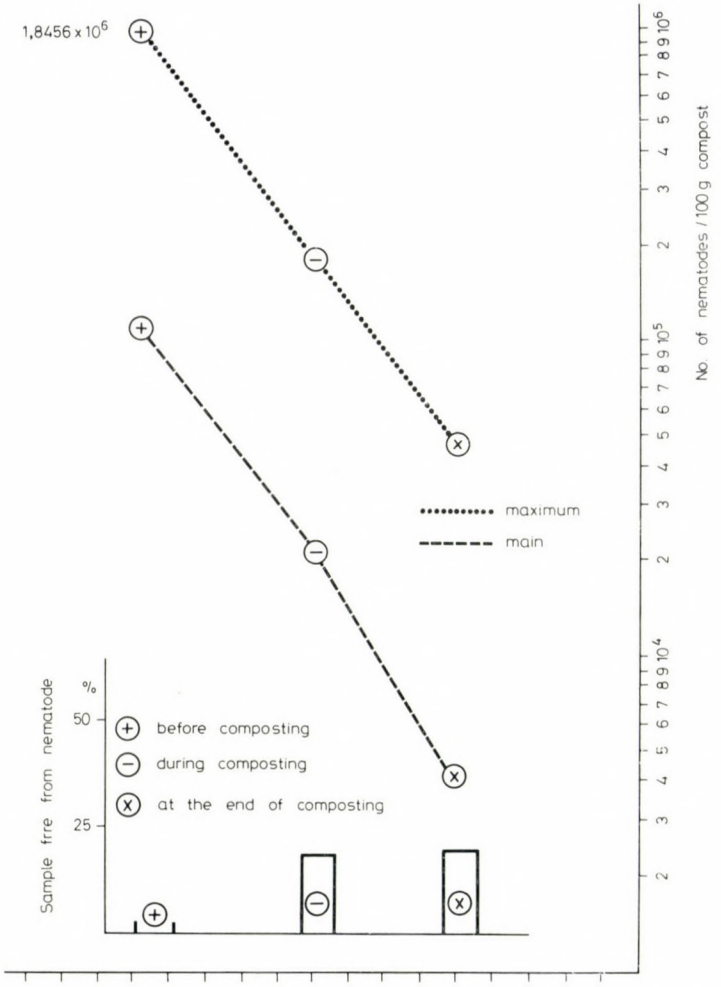

Fig. 2. Population dynamics of nematodes in horse manure during composting and the ratios of samples free from nematodes

2. *Control experiments*

The humidity content of the stack covered with foil was 100% in all treatments. Temperature (Fig. 3) did not reach 50°C in any treatment. The highest temperature was 49°C which occurred on the surface of the stack covered with

double foil for 49 hrs. The curve of the temperature of the stack covered with double foil for 24 hrs was closest to this value.

It is most remarkable that the maximum was reached in 6–8 hrs time

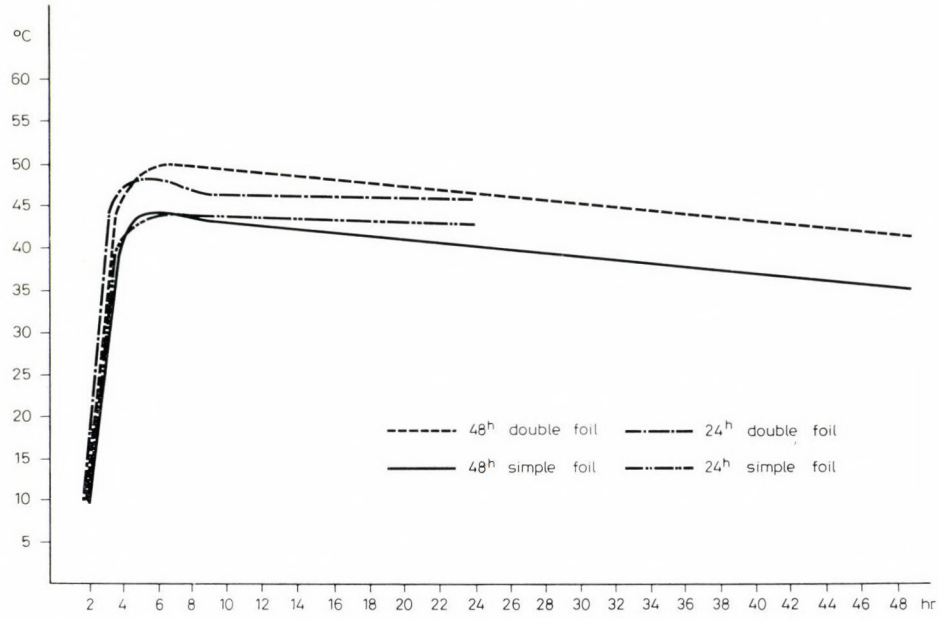

Fig. 3. The effect of foil covering during composting on the surface temperature

Table 4

Nematicidal effect of foil covering during composting

Treatment	Degree of effect
Control	—
3×24^h with simple foil	−28.90
3×48^h with simple foil	68.06
3×24^h with double foil	88.60
3×48^h with double foil	94.30

which from that time on, gradually decreased. The nematicidal effect reached by covering (calculated with ABBOT's formula) (Table 4) was in correlation with the formation of maximum temperatures. Total nematicidal effect was not achieved in any treatment. In spite of this the 94.30% effect obtained by double PVC foil covering of the stack for 48 hrs should be noted well. The same can be said for the 88.60% effect achieved with double covering for 24 hrs.

The experimental series repeated by using Nemafos (5 ml/1 m^2 in 1000 ml water) gave similar results (Table 5). Nemafos proved to be of total nematicidal effect (100%). The Nematode killing effect with double foil for 24 hrs in this case was 94.60%. The 3 × 24 hr double foil covering effect was 64.87%. It seems from these data that there is no need to cover after all turning up. The yield results of

Table 5

Nematicidal effect of foil covering during composting and Nemafos treatment

Treatment	Degree of effect %
Control	—
3×24^h with double foil	64.87
24^h with double foil	94.60
24^h with double foil + Nemafos	100

Table 6

Yield results of foil covering during composting of Nemafos treatment in the case of bedding of compost in the cellar floor

Treatment	Yield altogether kg
Control	56.25
3×24^h with double foil	47.65
24^h with double foil	81.55
24^h with double foil + Nemafos	76.96

S. D.5% = 2.64

the second foil covering experimental series (Tables 6–7) proved to be favourable. With the exception of the 3 × 24 hr double foil treatment each case was much higher than the control; according to our opinion in the last case as a consequence of lasting anaerobic conditions and built up of an ammonia atmosphere. In the sack cultivating experiment, the Nemafos treatment proved to be the most effective. In the usual cultivation conditions the double foil treatment yields (81.55 kg) were the best.

In other words the foil covering proved the suitability of such experiments carried out in Diskau.

As regards Nemafos, it was proved that it can replace the methyl bromide treatment (ARROLD, and BLACKE 1966), which is so difficult to carry out. The nematicidal effect (as OLIFF, 1965; HESLING, 1966a; LANGWEILER-REY, 1966; BURTON, 1969; HESLING and KEMPTON, 1969 etc. established) is most favourable and favourably influences the yield.

Table 7

Effect of foil covering during composting of Nemafos treatment on the yield results in foil sack cultivation

Treatment	Yield altogether
Control	26.55
3×24^h with double foil	19.30
24^h with double foil	32.75
24^h with double foil + Nemafos	34.15

S. D. = 1.04

Literature

ARROLD, N. P. and BLACKE, C. D. (1966): Some effects of *Ditylenchus mycelioplagus* and *Aphelenchoides composticola* on the growth on agar plates of the cultivated mushroom, *Agaricus bisporus* Nematologica. *12*, 501—510.

BAERMANN, G. (1917): Eine einfache Methode zur Affindung von Ankylostomum — (Nematoden) — Larven in Erdproben. Geneesk. T. Ned. Indie *57*, 131—137.

BURTON, A. U. (1969): Nemafos for pest control on mushroom. AMB/TAC. 1—8.

CAYROL, J. C. (1962): Importance des maladies vermiculaires dans les champignonnieres francaises. Mush. Sci. *V*, 480—496.

EGER, G. (1959): Grob-quantitativer Nematodennachweis im Champignonbetrieb. Die Deutsche Gartenbauwirtschaft *7*, 2—4.

GRISSE, A. (1963): A cauting dish for nematodes excluding border effect. Nematologica *9*, 162.

VAN HAUT (1956): Das Champignon-Myzel als Indicator für die Wirkung saprober Nematoden in Komposten. Nematologica *V*, 165—173.

HESLING, J. (1964): Alchen und Champignonzucht. Der Champignon *29*, 18—23.

HESLING, J. (1966a): Preliminary experiments on the control of mycophagus eelworms in mushrooms beds with a note on their swarming. Plant Pathology *15*, 163—166.

HESLING, J. (1966b): The effect of some microphagus saprobic nematodes on mushroom culture. Ann. Appl. Biol. *58*, 477—486.

HESLING, J. and KEMPTON, R. J. (1969): The control of nematodes in mushroom compost. Proc. 5th Br. Insectic. Fungic. Conf.

HOOPER, D. J. (1962): Effects of a nematode on the growth of mushroom mycelium. Nature *193*, 496—497.

LANGWEILLER-REY, V. (1966): Über die Bekämpfung von Nematoden in Champignon-Betrieben mit Kintensystem. Z. f. Pfl. Krankh. und Pfl. sch. *73*, 46—56.

MANKAU, R. (1962): The effect of some organic additives upon a soil nematode population and associated natural enemies. Nematologica *V*, 65—73.

MCLEOD, R. W. and CONROY, R. J. (1965): Occurrence of *Aphelenchoides compositicola* Franklin in mushroom compost from New South Wales. Nematologica *11*, 485—488.

MOGENS, J. (1966): Nematoden i champignonkulturer. Tidsskr. planteovl. N. *4*, 511—531.

MORETON, B. D. and JOHN, M. E. (1956a): *Aphelenchoides* sp. destruing mushroom mycelium. Nature *V*, 795.

MORETON, B. D. and JOHN, M. E. (1956b): A note on *Aphelenchoides* eelworms as mushroom pests. Mushroom Science *III*, 99—101.

OLIFF, K. E. (1965): Laboratory control of the nematode *Ditylenchus myceliophagus* living on *Agaricus campestris*. Hort. Res. *5*, 36—38.

PAESLER, F. (1957): Beitrag zur Kenntnis der Nematodenfauna in Champignon-Kulture. Nachrbl. für. den deutschen Pfl. N. *7*, 129—136.

PARAMONOV, A.A. (1952): Opyt ekologicheskoy classicafitsii fitonematod. Tr. Gelan. T. *VI*. 338 – 369.

PARAMONOV, AA. (1962): Osnovy fitogelmintologii. T.I. Akad. Nauk S. S. S. R. Moscow 351—405.

REMPE, H. and KUX, M. (1953): Experience with nematodes in sawdust compost. Mushroom Science *II*, 175—177.

STEINER, G. (1931): *Rhabditis lambdiensis* Maupas as a carrier of bacterial disease in mushrom beds. J. Parasit. *18*, 53.

STEINER, G. (1933): *Rhabditis lambdiensis* a nematode possibly acting as a disease agent in mushroom beds. Jurn. Agric. Res. *46*, 427.

SUMENKOVA, N. I. (1964): Fauna nematod kultiviruemüh sampinyonov v jijo biocenotichseskih svjazjah. Moskva Dissertacija pp. 273.

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Toxicity of Some Fungicides against the Greenhouse Whitefly (*Trialeurodes vaporariorum* West.), and its Parasite (*Encarsia formosa* Gah.)

I. Toxicity of Zineb (Perocin 75-B) against the greenhouse whitefly and its parasite

By

E. ELENKOV,[1] E. KHRISTOVA,[1] L. M. SHANAB[2] and P. SPASOVA[1]

In Bulgaria, experiments were carried out to study the effect of perocin (zineb 75-B) against the greenhouse whitefly *(Trialeurodes vaporariorum)* and its parasite (*Encarsia formosa* Gah.). Four concentrations were used: 0.15, 0.30, 0.60 and 1.2%. The effect of the fungicide against eggs was slight, only 1/3 of them was killed. Zineb at the high concentration applied directly before hatching, has a toxic effect on the hatched larvae. In treatments against larvae, zineb was effective only against the first instar. Against adults of the whitefly it was not effective, but against adults of *E. formosa*, the effect was slightly higher. Therefore, the application of zineb is recommended only if there is a fungal infection (e.g. *Pseudoperonospora cubensis* Berk.-Curt. Rostow), or if there is no *E. formosa* in the greenhouse.

During the last years, the introduction of formosa (Gah) took place in many countries, with well developed greenhouse complexes for the control of the greenhouse whitefly (*Trialeurodes vaporariorum*/West.). This biological method of control greatly succeeded in Northern European countries, where the growing of vegetables is taking place from February till September (STENSETH, 1973). In the Southern European countries the whitefly has more favourable conditions for reproduction, as shown also on their field occurrence. Therefore, due to the high population density of the whitefly, the use of *E. formosa* alone is insufficient for obtaining good results. In this case the application of pesticides not toxic to *E. formosa* is considered to be of great importance.

Insecticides applied for the control of the whitefly are: Thiodan, Bi-58, Unden, Bromex, Actellic, Nogos and related preparations. But these insecticides kill all the adults of the whitefly, and to different degrees, also the larvae. It is therefore not recommendable to apply these insecticides in order to secure parasite survival in the greenhouses.

BOYCE (1961), found that wettable zineb is effective against the larvae and the pupae of the *Aleyrodid* on tomatoes. Later, MCMULLEN (1964), also found that maneb and zineb were toxic against the first-instar larvae and the adults of the

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Aleyrodid. Then MCCLANAHAN (1970), observed that morestan is toxic for the eggs and the adults of the whitefly, but not toxic for *E. formosa.*

Our task is to elaborate a combined programme for the control against the whitefly and some plant diseases.

In 1966 *Pseudoperonospora cubensis* (Berk—Curt./Rostow) was found in Pazardzhik district on greenhouse cucumber for the first time in Bulgaria. During the following years, this disease was observed in various regions on field cucumbers. Ever since, it developed in fields during summer, and in greenhouses, and under folia during winter and spring.

Perocin which contains 75% zineb was used in a concentration of 0.4% for controlling the disease. We have not determined time or period for applying the fungicide, but we start to spray after the disease had been occurred. In the same time it is important to know the effect of this fungicide against the greenhouse whitefly and its parasites which occured on the greenhouse vegetables. Concentrations used were: 0.15, 0.30, 0.60 and 1.2%.

Materials and Methods

Tomato plants (with 4—6 leaves) were transplanted to small pots under isolators. 100 adults of the *Aleyrodid* (males and females) were transferred to each plant for 24 hours to lay eggs. The plants were treated by spraying them with the fungicide after 1—2, 4—5, and 7—9 days from oviposition, depending on the development of the eggs. Experiments were made to determine the effect of the fungicide against the different larval instars and nymphs. In case of egg treatment, the number of unhatched eggs, living and dead larvae were counted after one month. In the treatments against larvae and nymphs, the number of living and dead individuals were also counted after 20—30 days from treatment.

To study the effect of the fungicide against the whitefly, the experiment was carried out with the following variations:

1. Adults of the whitefly were anaesthetized with ether, then were placed on filter paper to be sprayed with the fungicide, then placed on untreated tomato plants under isolators.

2. Tomato plants were treated with the fungicide, and after drying, untreated adults of the whitefly were placed on them.

3. Tomato plants were treated with the fungicide and after drying, treated adults were placed on them.

4. 8 tomato plants were treated with the fungicide, and untreated adults of the whitefly were placed on them 1, 2, 3, 4 and 5 days after the treatment, respectively.

The number of living and dead larvae produced by adults were counted one month after treatment. Tests were also made with each of the adults of *T. vaporariorum* and *E. formosa* in Petri dishes with three variations of the fungicide as the following:

1. Adults of *T. vaporariorum* or *E. formosa* were anaesthetized with ether, then treated with the fungicide and placed on untreated tomato leaves on damp filter-paper in Petri dishes.

2. Tomato leaves were treated with the fungicide, and untreated adults were placed on them.

3. Tomato leaves were treated and also adults were treated and put on the leaves.

After 24 hours, the effect of the fungicide was determined as percentage of mortality of the adults. The fungicide was applied only as spray, and all the variations of the experiment were done with 3 replications. Observations on adults were carried out with 10 pairs of whitefly (10 males and 10 females) and with 10 females of *E. formosa* for each replication. All adults used were of the same age freshly emerged. The effectivity of the fungicide was calculated by the abott formula.

Results

Zineb has an effect against the various stages of the greenhouse whitefly. But it has only slight effect on eggs (Fig. 1), as it has only one-third of them. The ovicidal effect of zineb showed changes during the different periods of egg development.

Egg treatments showed also that zineb had a toxic effect on the hatched larvae especially at the high concentration applied directly before hatching. Therefore, the number of living larvae was small (Fig. 1). The one-day-old eggs were 3.57 times more resistant than the seven-day-old eggs (Table 1).

Table 1

LC50 of zineb against the eggs of the greenhouse whitefly

Age of eggs (days)	LC50	Coefficient of resistance compared with the 7-day- old eggs
1	0.25	3.57
4	0.18	2.57
7	0.07	—

Treatments against larvae showed that fungicide was effective only against the first-instar larvae (Fig. 2). The effect of zineb at the high concentration was higher, but still not enough to reduce the population of the whitefly. The LC50 for the first-instar larvae was already high, but for the other instars was even 59.3 times higher than that of the first instar larvae, which is practically impossible to apply (Table 2).

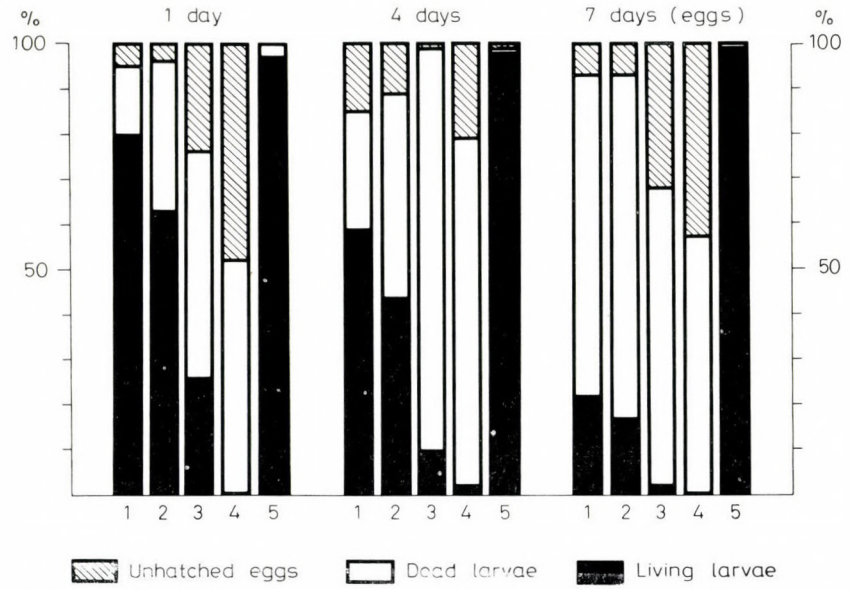

Fig. 1. Effect of perocin against eggs of the greenhouse whitefly in different periods of development. 1 = 0.15, 2 = 0.30, 3 = 0.60, 4 = 1.20%, 5 = untreated

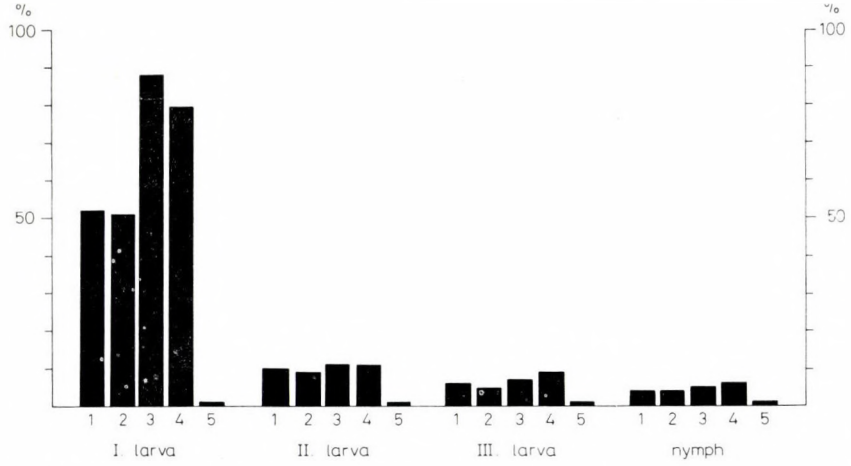

Fig. 2. The percentage of mortality of the larvae and the nymphs of the greenhouse whitefly treated with perocin. 1 = 0.15, 2 = 0.30, 3 = 0.60, 4 = 1.20%, 5 = untreated

Zineb was not effective against the adults of whitefly, and there were no differences between the various methods of application on the fecundity of the adults, or on the mortality rate of the hatched larvae.

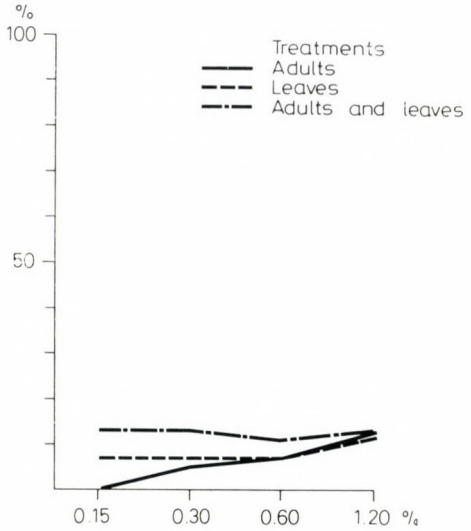

Fig. 3. The percentage of mortality of the adults of the greenhouse whitefly by different perocin applications

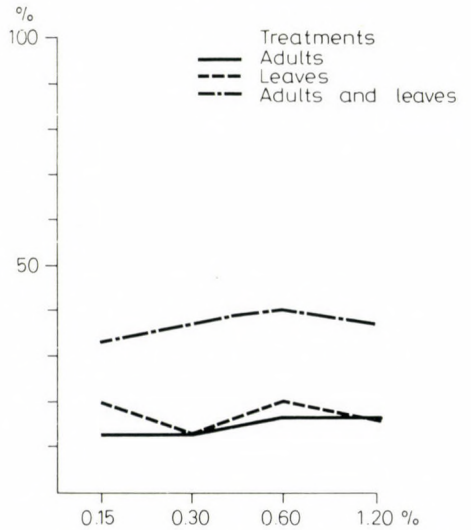

Fig. 4. The percentage of mortality of the adults of *E. formosa*, by different perocin applications

Direct spray on the adults of the whitefly, or indirect contact by treating tomato leaves, gave low mortality rate (Fig. 3). On the other hand, the mortality rate of the adults of *E. formosa* was slightly higher than that of the whitefly (Fig. 4),

especially in case of direct treatment. Zineb was not toxic to the larvae of *E. formosa*, parasiting the larvae of the whitefly.

Our results agree with those of McMULLEN (1964), as far as the first instar larvae are concerned. He reported also that the effectivity of zineb spray was one-half of that of ethyl-DDD against the adults of the whitefly. Our results, however, were not satisfactory against adults. We have also different results from those obtained by BOYCE (1961). His observations showed that zineb was effective against the larvae and the nymphs of the whitefly. Our experiments gave good results only against the first-instar larvae.

Table 2

LC50 of zineb against the first and the second instar larvae of the greenhouse whitefly

Larval instars	LC50	Coefficient of resis-t ance compared with the first instar larvae
L 1	0.17	—
L 2	10.08	59.3

Conclusion

Perocin (zuneb 75-B) has slight effect against the greenhouse whitefly (*Trialeurodes vaporariorum* West.), but shows a higher toxity against its parasite (*Encarsia formosa* Gah.). In case of applying perocin that damage will be more than the benefit when these two species are in the greenhouse. Therefore, the application of perocin is recommended only in case of great necessity for controlling a disease (e.g. Pseudoperonospora cubensis Berk.-Curt./Rostow), or if there are no *E. formosa* parasites introduced into the greenhouse.

Literature

BOYCE, H. R. (1961): Insecticidal activity of maneb formulations against the greenhouse whitefly, *Trialeurodes vaporariorum*, (West.) (Hemiptera: *Aleyrodidae*). Proc. ent. Soc. Ont. *92*, 197–200.

McCLANAHAN, R. J. (1970): Integrated control of greenhouse whitefly on cucumbers. J. econ. Entomol. *63*, 599–601.

McMULLEN, R. D. (1964): The toxity of malathion, perthane, maneb and zineb to *Trialeurodes vaporarioum* (Westwood). Canad. Entomol. *96*, 541–549.

STENSETH, CHR. (1973): Report about integrated control of pests under glass in Norway. Lutte integrée en cultures sous verre. Bulletin Srop WPRS bull. *4*, 4–7.

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Toxicity of Some Fungicides against the Greenhouse Whitefly (*Trialeurodes vaporariorum* West.) and its Parasite (*Encarsia formosa* Gah.)

II. Toxicity of Morestan against the greenhouse whitefly and its parasite

By

E. ELENKOV,[1] E. KHRISTOVA,[1] L. M. SHANAB[2] and P. SPASOVA[1]

In Bulgaria experiments were carried out to study the effect of morestan against the greenhouse whitefly and its parasite *E. formosa*. Morestan has an ovicidal effect against the whitefly, and increases gradually with the increasing concentrations and progressing egg development. Applying morestan before hatching resulted in high mortality, up to 100% of the hatched larvae. The LC90 was comparatively low (0.085), even against the two-days old eggs. On more developed eggs susceptibility to the fungicide increased. Against the first and the second instar larvae, morestan was highly effective, but not effective against nymphs. Spraying tomato plants with the fungicide caused reduction in the number of eggs laid by the adults if they were placed on the treated leaves one hour following the treatment. This non-direct treatment caused also a remarkable mortality of larvae. Spraying with morestan the adults of the whitefly and *E. formosa* less mortality resulted; a higher effect was observed if only the tomato leaves were sprayed and the highest effect was observed if both adults and leaves were treated. Applying morestan to control powdery mildew, two-spotted spider mites and greenhouse whiteflies are recommendable if *E. formosa* is present in the greenhouse, especially during the second half of the vegetation period.

The climatic conditions in Bulgaria are favourable for the development of: *Leveillula taurica* (Lev.), on tomato and pepper, *Sphaerotheca fuliginea* (Poll.), *Erysyphe cichoracearum* (D. C.), and *Leveillula cucurbitacearum* (Golov), on cucumber all over the year. It is therefore of great importance to control them not only in the field, but also in cultures under glass. Morestan is highly effective against the pathogens of these plant diseases, but shows also acaricidal and insecticidal effects against the two-spotted spider mite and the greenhouse whitefly, respectively.

Introduction as well as materials and methods were given in an earlier paper (ELENKOV *et al.*, 1975) dealing with the effect of Zineb (Perocin 75-B) on the greenhouse whitefly and *E. formosa*, in present paper the results of similar work with morestan are summarized.

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Results

Morestan fungicide applied in the following concentrations: 0.025, 0.05, 0.1 and 0.2% showed an ovicidal effect against the greenhouse whitefly (Fig. 1). This effect was slight at the low concentration against young eggs, but increased grad-

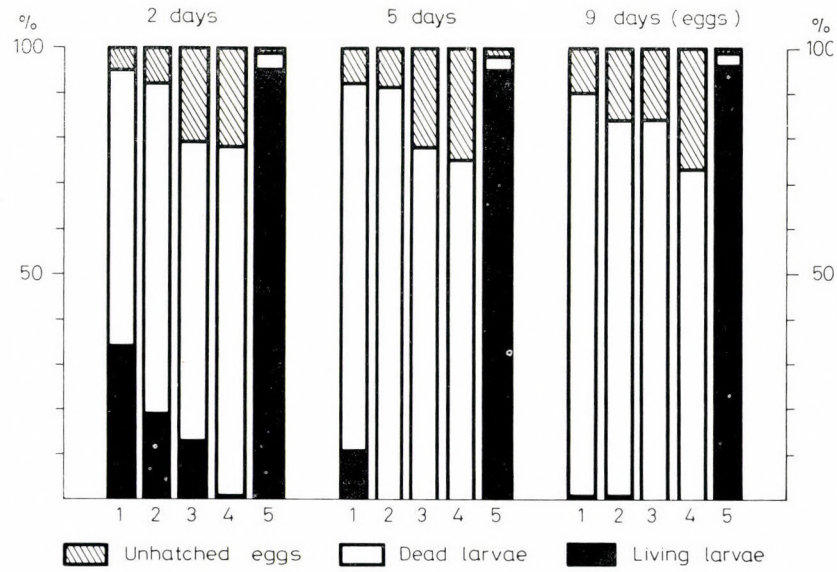

Fig. 1. Effect of morestan against eggs of the greenhouse whitefly in different periods of development. 1 = 0.025, 2 = 0.05, 3 = 0.10, 4 = 0.20%, 5 = untreated

Table 1

LC90 of morestan against the eggs of the whitefly

Age of eggs (days)	LC90%	Coefficient of resistance compared with the 9-days old eggs
2	0.085	5.99
5	0.028	3.81
9	0.014	—

ually at the higher concentrations, against older eggs. Using morestan against eggs gave good results for the hatched larvae also. The mortality rate of the hatched larvae increased gradually until 100% with the increase of concentrations

and egg development. LC90 was comparatively low (0.085) even against the two-days old eggs (Table 1), but in case of more developed eggs the susceptibility to the fungicide has increased. Spraying with low concentrations was highly effective against the first-instar larvae of the whitefly (Table 2), which were very susceptible compared to the third instar. Against the second instar larvae good results

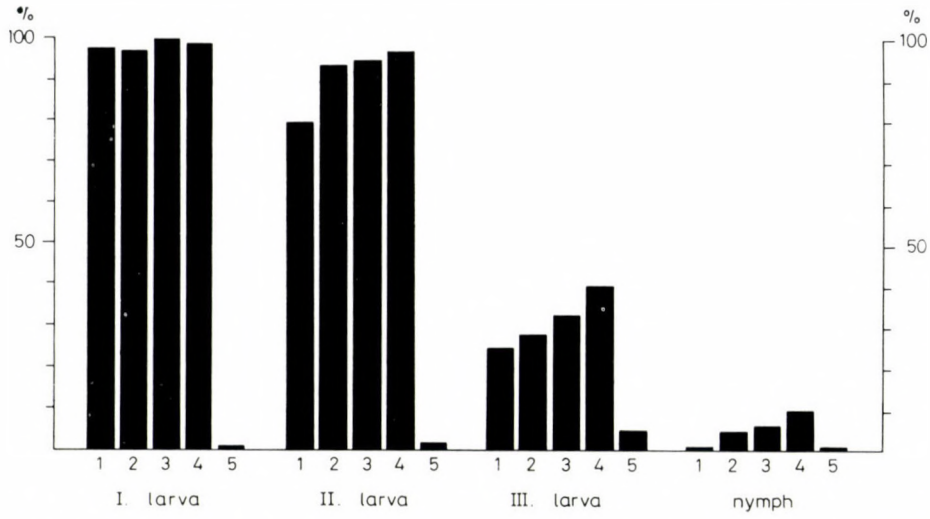

Fig. 2. The percentage of mortality of the larvae and nymphs of the greenhouse whitefly treated with morestan. 1 = 0.025, 2 = 0.05, 3 = 0.10, 4 = 0.20, 5 = untreated

Table 2

LC90 of morestan against the larvae and nymphs of the whitefly

Stadium	LC90%	Coefficient of resistance compared with the first instar larvae
L 1	0.005	—
L 2	0.01	4000
L 3	200.48	40000
Nymphs	10240.0	

were obtained only by using concentrations higher than 0.05%. Against nymphs, no effect was observed.

Treating the adults of the whitefly with direct spray of morestan affected their fecundity (Fig. 3). The reduction in their number of eggs laid was high when

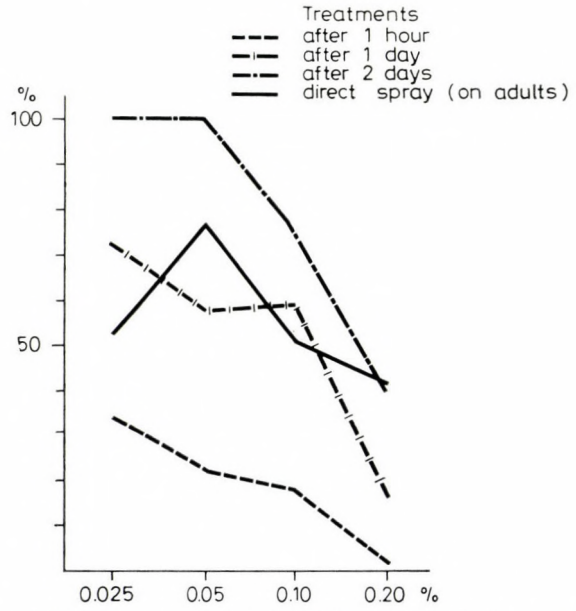

Fig. 3. The percentage of larva produced by the adults of the whitefly, transferred to treated tomato plants after different periods compared with 100% untreated

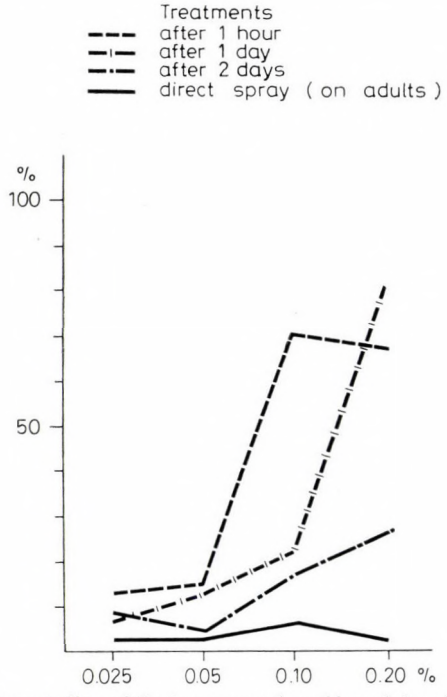

Fig. 4. The percentage of mortality of the larvae produced by adults of the whitefly transferred to treated tomato plants after different periods

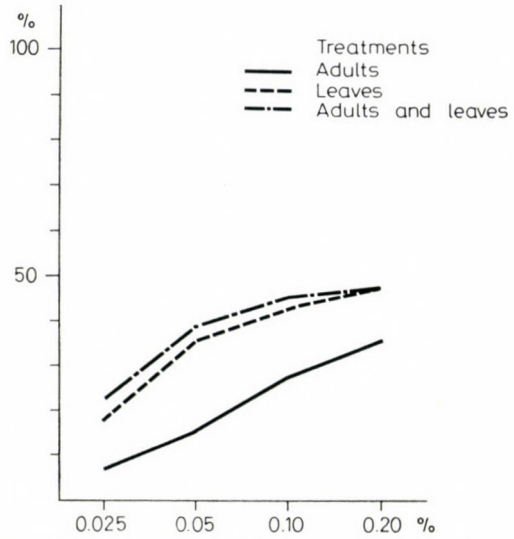

Fig. 5. The percentage of mortality of the adults of the greenhouse whitefly by different morestan applications

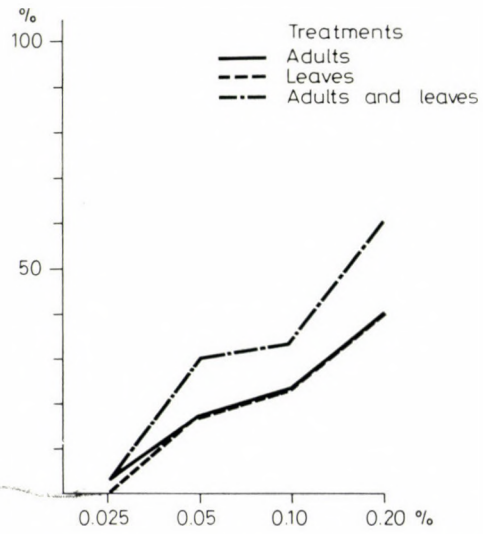

Fig. 6. The percentage of mortality of the adults of *E. formosa* by different morestan applications

the females had contacted the plants one hour after treatment; this was evident in three days. Otherwise, the effect of morestan on the vitality of the new generations originating from treated adults was different. The direct spray against adults

caused slight mortality in the larvae produced (Fig. 4). The highest percent of larvae mortality was noticed in larvae originating from adults placed on tomato plants during the first 24 hours after spraying.

Direct sprays of morestan on the adults of the whitefly and *E. formosa* gave lower mortality rate (Figs 5 and 6), but the effect was higher in case of treating leaves for the adults of the whitefly. The highest effect was in case of treating both of adults and leaves. Against *E. formosa*, the effect of morestan was higher when both of the adults and leaves were treated (Fig. 6). The LC50 was high, but it was higher against the whitefly than *E. formosa* (Table 3). Applying morestan at high concentrations kills more adults of *E. formosa* than adults of the whitefly.

Table 3

LC50 of morestan against the adults of the whitefly and *E. formosa*

Treatments	Whitefly		E. formosa	
	LC50	Coefficient of resistance compared with the 3rd treatment	LC50	Coefficient of resistance compared with the 3rd treatment
1 Adults	0.380	1.5	0.200	1.2
2 Leaves	0.240	—	0.200	1.2
3 Leaves and adults	0.240	—	0.160	—

Conclusion

Morestan is highly effective against eggs of the whitefly and against larvae in the first and second instars, and it has slight effect against *E. formosa*. Therefore, morestan is a preparation which is recommendable for controlling the mildew, the two-spotted spider mite and the greenhouse whitefly, especially in case of existence of *E. formosa* in the greenhouse and during the second half of vegetation period.

Literature

ELENKOV, E., KHRISTOVA, E., SHANAB, L. M. and SPASOVA, P. (1975): Toxity of Zineb (Perocin 75-B) against the greenhouse whitefly and its parasite. Acta Phytopath. Hung. (See the 165—170 pages of this issue).

MCCLANAHAN, R. J. (1970): Integrated control of the greenhouse whitefly on cucumbers. J. econ. Entomol. *63*, 599–601.

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Book Review

Aloysius KRIEG: Arthropodenviren. Georg Thieme Verlag, Stuttgart, 1973, pp. 328.

It may be stated without exaggeration that this comprehensive work, printed in German, is one of the most succesful popular publications in virology. The author — whose name is well known in insect pathology and virology — had chosen maybe a too modest title for this fine book, suggesting only viruses pathogenic for arthropods. It contains, however, much more summarizing all the most recent data on the relationships between the virus and its arthropod host, on virus biosynthesis and up-to-date virus systematics. It is first of all the clean, easy style and the logical construction of the chapters which catches the reader, besides the fine figures and synoptical tables. The clear-cut chapters of this book form closed units, each containing its own summary.

Following a short description of the main virus types the author describes the basic processes in a healthy, uninfected host cell, including the DNA and RNA synthesis, protein synthesis and assimilation. Then follow the chapters describing the relationships between the virus and host cell, host organism, even host population. The reader becomes thus familiar with the complicated and exciting interactions between host and parasite, with the details of virus transmission and the influence of viruses on the insect populations, before reading the detailed description of the viruses themselves. So the details of virus morphology and structure become significant even for untrained eyes and the reader becomes informed not only in the fields of insect pathogens but also in plant pathogenic viruses and in ones of medical or veterinary importance.

The chapters on the methods of virus isolation and determination and virus research give broad informations to readers who are interested in details of virus isolation, biological testing, titration, light- and electron microscopy, fractionation techniques, labeling and in analysis of nucleic acids, proteins and antigens.

In a final chapter on applied virology data of the most recent literature are summarized both on diseases of beneficial insects (like honeybee or silkworm) and on diseases of insect pests, on the possibilities of using viruses in controlling pest populations and on the problems of preventing virus transmission in plant pathology, medicine and veterinary science.

The book "Arthropodenviren" of A. KRIEG can be used successfully on the most variegated levels of scientific training: the text is equally fascinating and understandable for high school students, teachers, undergraduate and graduated students and professors or scientists working in different fields of theoretical or applied insect pathology, plant protection, plant pathology, medical and veterinary virology. Its translation to many languages can be expected and hoped for, to help the public to meet and understand a fascinating, rapidly developing scientific discipline.

L. SZALAY-MARZSÓ

PETER R. DAY: Genetics of Host-Parasite Interaction. W. H. Freeman and Co., San Francisco 1974. X + 238 p.

"Man prepared his crops in a way that seemed almost to invite their mass destruction." He must, therefore, control parasite populations by different means. One way is to develop and use his understanding of genetics to control pathogens. Day's book deals exclusively with the control of pathogens on the basis of genetical principles of the host-parasite interaction.

After giving some definitions the author treats the genetics of resistance, tolerance and cytoplasmic resistance, the genetics of pathogenicity, the gene-for-gene concept, the gene function in host–parasite interactions and the genetics of epidemics. Concerned with fungal parasites, the book also considers insects, nematodes, bacteria and viruses. The bibliography contains nearly 600 references. The mean feature of the book is that the author does not attempt a comprehensive treatment but uses examples to illustrate, excellently, general principles.

This book is highly recommended not only for research plant pathologists but researchers in plant breeding, genetics, epidemiology and entomology.

Z. K.

Contents

DISEASES

PESTS

PHYTOPATHOLOGISCHE ZEITSCHRIFT

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The Influence of the Grapevine Infectious Chlorosis Virus on Chloroplast Ultrastructure

By

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The infection with the grapevine infectious chlorosis virus results in an important destruction of chloroplasts, the growth of the number as well as the size of osmiophilic globulae and the accumulation of starch. The virus infection apparently results in an overstrain of the energetic resources of the cell and premature senescence of plastids.

The virus diseases of grapevine have been found in all countries where the viticulture is practized on a large scale. The diseases of fanleaf group, namely fanleaf, infectional chlorosis and vein banding are the most spreaded ones. The viruses of this group belong to NEPO-viruses, they are serologically related to the arabis mosaic virus and look like isometrical particles 28—30 nm in diameter.

The information about the influence of the fanleaf group viruses on the ultrastructure of affected plant's cell is scanty. It deals mainly with the fanleaf virus. For example, GEROLA *et al.* (1969) have shown, that the infection of different plants with the grapevine fanleaf virus results in destructive changes of chloroplasts, but these changes are not so clearly expressed as with other viral infections.

It is known, that virus infections in most cases damage cell chloroplasts first of all, producing different disorders in their organization. Thus, disorders expressed in chlorotic symptoms of different types. The absence of data dealing with chloroplast changes under the influence of the infectious chlorosis virus has prompted us to study these changes in the cells of the affected grapevine leaves.

Materials and Methods

Stock varieties Richtera 57 and Rupestris du Lot, infected with grapevine infectious chlorosis virus by grafting of cuttings, taken from affected vines, have been investigated.

Leaves were cut into small pieces 0.5×4 mm in a drop of fixing solution. Fixation lasted 2 hours in 6.5% glutaraldehyde and then 2 hours in 1% osmium tetroxide with phosphate buffer (MILLONIG, 1961). After dehydratation in growing concentration of ethanol the samples were embedded in metacrylate and epon Sectiones, obtained on LKB-8800 microtome had been further dyed with uranyl acetate and lead citrate and investigated under JEM-7 and Hitachi-11E electron microscopes.

Results and Discussion

The structure of chloroplasts in mesophyll cells of the leaves of healthy vine plants is common to that of the leaves of higher plants: the envelope consists of two membranes separated by electron transparent zone; a regular lamellae system and separate osmiophilic bodies are included in chloroplast stroma; grana consist of 15–16 tightly packed lamellae and are joined among themselves with intergrane lamellae (Fig. 1). There are electron transparent plots with DNA fibrillae in the stroma. It is established that such chloroplast structure is connected with high photosynthetic activity and an important accumulation of energy rich ATP and ADP compounds in leaves (NOBEL, 1970).

In the chloroplast stroma of the leaves of diseased plants numerous irregularly situated lammellae can be seen (Figs. 2, 3). Sometimes chloroplasts with clarified stroma and swollen lammellae and those with electron dense stroma and small number of lammellae can be seen in the same cell (Figs 3, 4). Often chloroplasts with numerous vesiculae and electron transparent plots with DNA fibrillae in their stroma can be seen. The latter can be found also in the case when the chloroplast stroma has undergone an important destruction (Fig. 4). Invaginations with mitochondria, ribosomes and other cytoplasmic elements in their cavity can be seen in the stroma (Figs 4, 5).

Under the influence of virus infection the quantity of pigments in chloroplasts decreases, while the content of lipid osmiophilic body increases. This shows, that the virus infection acts upon the whole chlorophyll–protein–lipid complex of chloroplasts. The infection of grapevine with the infectious chlorosis virus induces an increase of the number as well as the dimensions of osmiophilic body. The chloroplasts that have undergone strong destruction, show an important accumulation of osmiophilic body (Fig. 4). Such an increase of size as well as the number of osmiophilic body perhaps are being connected with the destruction of the internal structure of chloroplasts. The metabolites, that come as the results of this process, form a great number of body.

We investigated the accumulation of starch in the chloroplasts of mesophyll cells of diseased plant leaves (Fig. 6). This is accompanied by the growth of the number as well as the size of starch grains. The presence or absence of starch grains in plastids is an index of their definite functional state. The accumulation of starch in chloroplasts during day hours and the delay of carbohydrate deflux that results in the forming of starch, induce the suppression of photosynthesis in diseased plants even if the environmental conditions are favourable.

The study of the changes of cell structure under the influence of the virus infection is of great interest as it contributes to our understanding of how the virus uses the cell synthetical mechanism for its own reproduction. The disturbances of the internal structure of chloroplasts under the influence of the grapevine infectious chlorosis virus, that we have watched, are analogous to those, that are induced by other virus infections (GEROLA *et al.*, 1966; STEIN-MARGOLINA, 1971; CHALCROFT and MATTHEWS, 1966). Thus, a surplus accumulation of osmiophilic

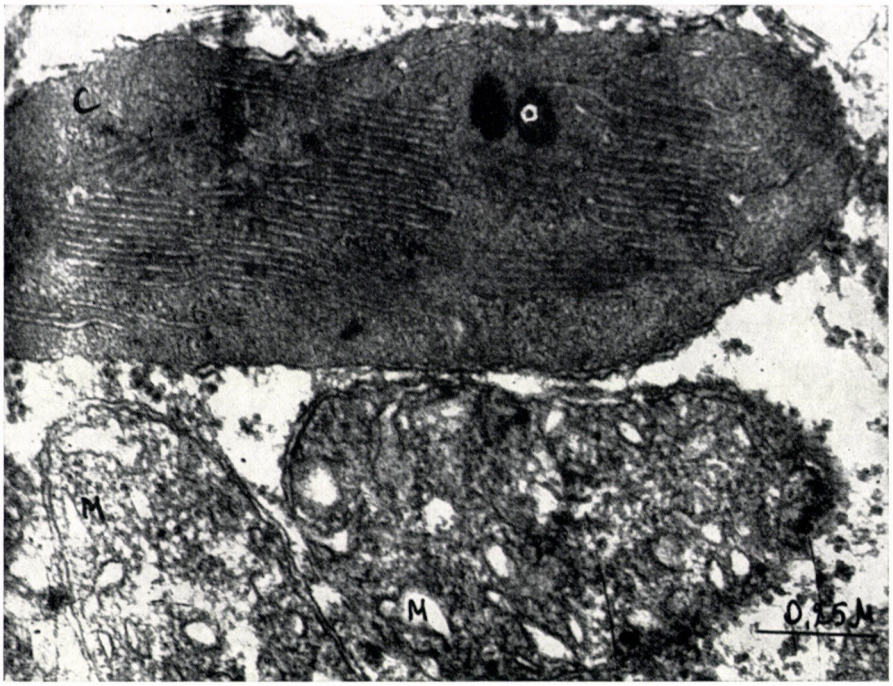

Fig. 1. A part of mesophyll cell of a healthy grapevine leaf (65,000×)

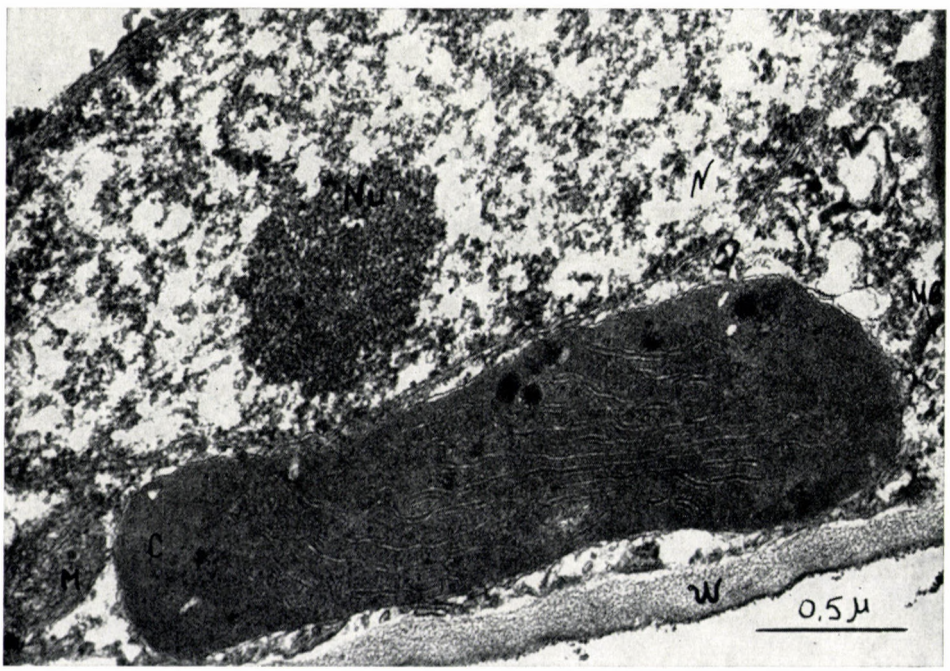

Fig. 2. A part of mesophyll cell of the leaf of grapevine, affected by infectional chlorosis virus (40,000×)

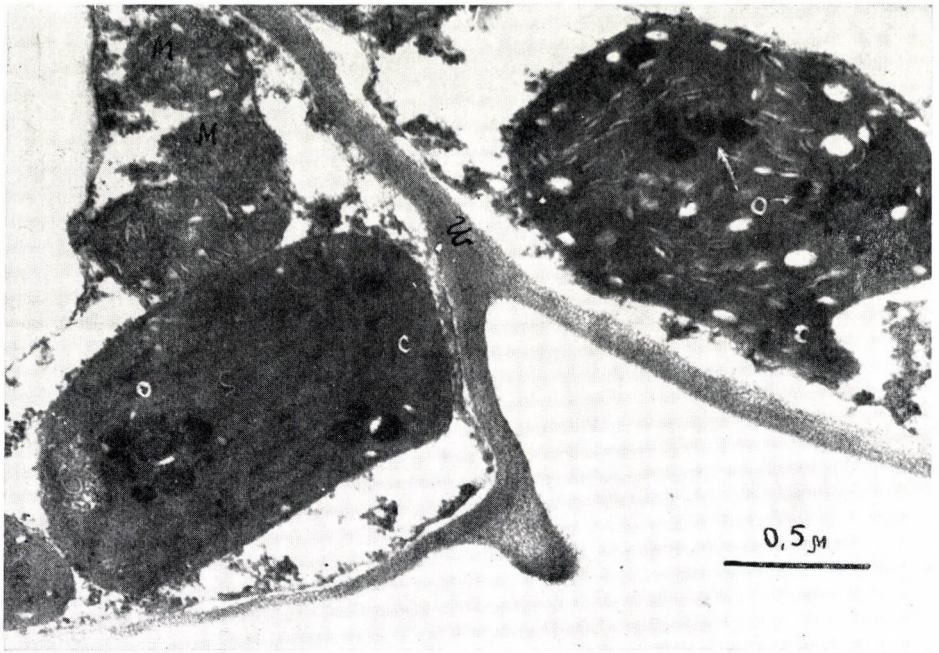

Fig. 3. The chloroplasts of mesophyll cell of the diseased grapevine leaf at different stages of destruction (40,000×)

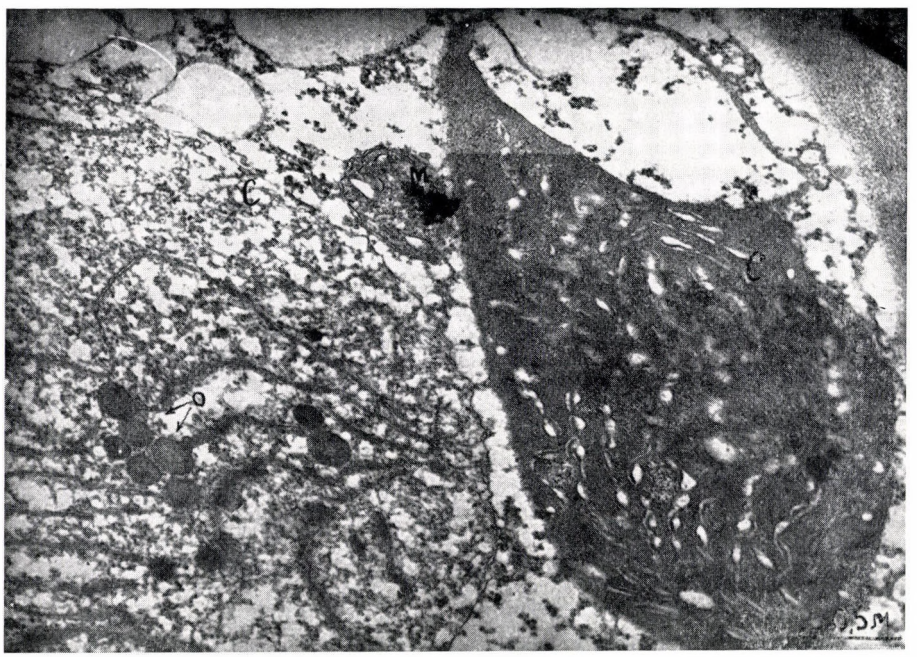

Fig. 4. A part of mesophyll cell of diseased grapevine leaf. A chloroplast with cleared stroma, destructed lamellae and a great numbers of osmiophilic body and a chloroplast with vesiculae and invagination cavity in which the ribosomae are disposed (38,000)

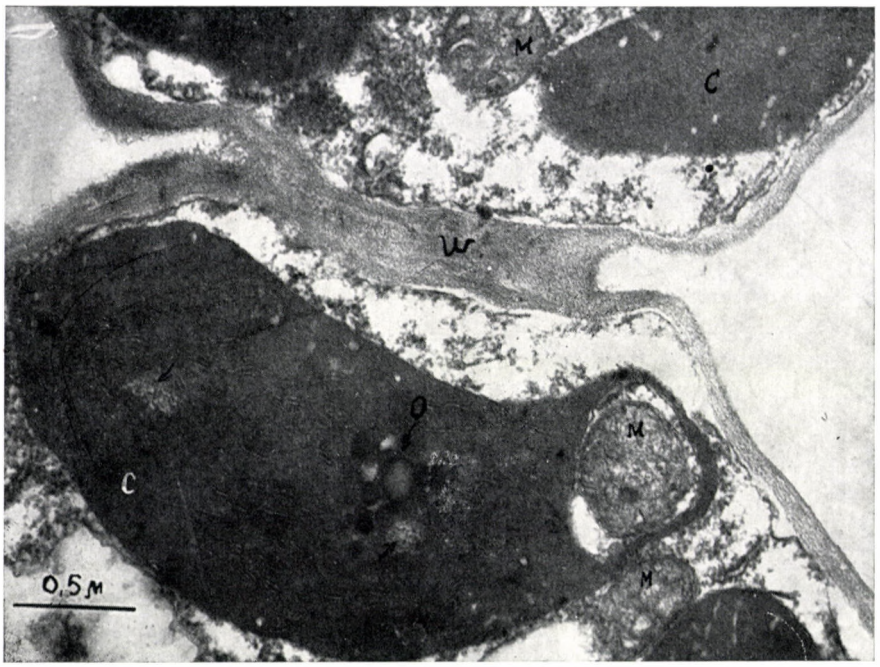

Fig. 5. A part of mesophyll cell of diseased grapevine leaf. In a cavity of invagination of chloroplast the mitochondria is disposed. (32,000×)

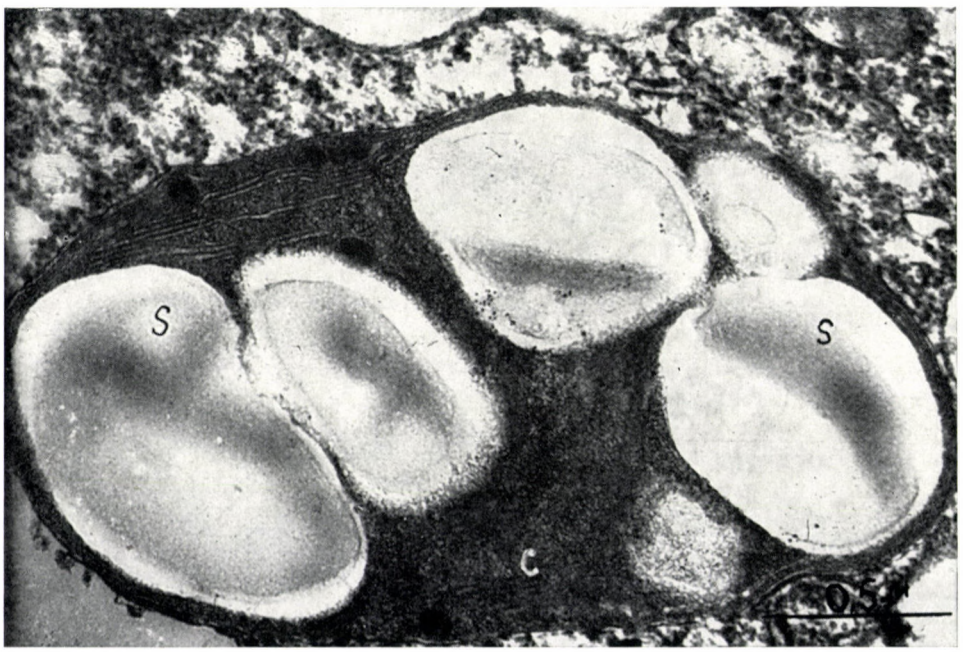

Fig. 6. A part of mesophyll cell of diseased grapevine leaf. A large quantity of starch is seen in the stroma of chloroplast. (50,000×)

Abbreviations used: O — osmiophilic body, W — cell wall, M — mitochondria, N — nucleus, Nu — nucleolus, St — starch, C — chloroplast, Mb — multivesicular bodies.

body in chloroplasts can be clearly seen in the leaves of sugar beet, infected with beet yellow mosaic virus. It is known that in this case the osmiophilic body grows to such an extent that it can induce the displacement of grana and even deform the plastids (ESAU, 1968). An increase in starch content of the leaves of plants infected by viruses had also been found by other researchers. With some diseases the accumulation of starch in chloroplasts is so important, that it may result in the rupture of membranes (WEINTRAUB and RAGETLI, 1964).

The changes in chloroplasts, similar to those described above, however, cannot be considered as the specific reaction to the virus infection. They may result from the senescence of leaves, soil chlorosis etc. (GREENWOOD *et al.*, 1963; LICHTENTHALER, 1966; STRUNK and WARTENBERG, 1960). The viral synthesis, apparently, induces an overstrain of the energetic resources of the cell and premature senescence of plastids.

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Literature

CHALCROFT, J. and MATTHEWS, R. E. F. (1966): Cytological changes induced by turnip yellow mosaic virus in chinese cabbage leaves. Virology, *28*, 555—562.

ESAU, K. (1968): Viruses in Plant Hosts. Form, distribution and pathologic effects. Univ. of Wisconsin Press. Madison, W. I.

GEROLA, F. M., BASSI, M. and GIUSSANI, G. (1966): Some observation on the shape and localization of different viruses in experimentally infected plants, and on the fine structure of the host cells. III. Turnip yellow mosaic virus in *Brassica chinensis* L. Caryologia *19*, 457—479.

GEROLA, F. M., BASSI, M. and BELLI, G. (1969): An electron microscope study of different plants infected with grapevine fanleaf virus. — Giorn. Bot. Ital *103*, 271—290.

GREENWOOD, A. D., LEECH, R. M. and WILLIAMS, J. P. (1963): The osmiophilic globules of chloroplasts. I.: Osmiophilic globules as normal component of chloroplasts and their isolation and composition in *Vicia faba* L. Biochem. Biophys. Acta *78*, 148—162.

LICHTENTHALER, H. K. (1966): Plastoglobuli und Plastidenstructur. Ber. Deut. Bot. Ges.2. *79*, 82—88.

MILLONIG, G. (1961): Advantages of a phosphate buffer for OsO_4. J. Appl. Phys. *32*, 1637.

NOBEL, P. S. (1970): Plant Cell Physiology (A physicochemical approach). Univ. Calif., San Francisco.

STEIN-MARGOLINA, V. A. (1971): Electrono-microscopicheskoe izuchenije virusov v rastenii hozjaine i klesche-perenoschike. — Doct. Thesis of Univ. of Moscow.

STRUNK, C. and WARTENBERG, H. (1960): Licht- und elektronenoptische Untersuchungen der Chloroplasten chlorotischer Maispflanzen. Phytopath. Z. *38*, 109—122.

WEINTRAUB, M. and RAGETLI, H. W. S. (1964): An electron microscope study of tobacco mosaic virus lesions in *Nicotiana glutinosa* L. J. Cell. Biol. *23*, 499—509.

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Serological Studies of Potato Viruses X, Y, A and Aucuba Mosaic

I. METHODS FOR THE ISOLATION OF ANTIGENS

By

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To obtain purified preparations of PVX, PVY, PVA and PAMV, pH were experimentally selected for buffer and temperature isolation. Making a denaturing influence on the admixtures of ballast substances (enzymes, structural cell components, etc.), they had only a slight effect on the virus isolated.

A primary extract was obtained by reducing vegetational material to fragments. Pigments were removed by the addition of chloroform of PVX, PVA and PAMV or ethyl alcohol of PVY. Relatively purified PVX, PXY, PVA and PAMV were isolated by differential centrifugation. The preparations obtained contained viruses in the following amounts: 8.25 mg/ml and 4.23 mg/ml of PVX isolated from *Datura stramonium* L. and potato var. Paul Wagner, respectively; 5.28 mg/ml and 2.14 mg/ml of PAMV obtained from *D. stramonium* and potato var. Epicurus; 2.14 mg/ml and 0.84 mg/ml of PVY from *Nicotiana tabacum* "*Samsun*" and potato var. Berlikhingen; 2.73 mg/ml and 1.16 mg/ml of PVA obtained from *Lycopersicon pimpinellifolium (Jusl.) Mill.* and potato var. Allerfrüheste Gelbe.

Introduction

Since the period of classic investigations of STANLEY, BAWDEN and PIRIE in the 1930's many efforts were directed to the development of methods for the isolation and purification of plant viruses. The first isolated and effectively studied viruses were stable enough and occurred in a host-plant in a relatively high concentration (TMV, potato virus X and bushy dwarf tomato virus). At present, viruses strongly differing in their concentrations in a host-plant and their resistance to various physical and chemical effects are given much consideration. There are no generally accepted rules in this case because methods effective with respect to one virus can be quite unapplicable to another. As it is known from literary sources, different strains of the same virus can require different methods for successful isolation (MATTHEWS, 1973).

The aim of our work is to study the properties and methods for purification of X, Y, A and F potato viruses. These viruses were not chosen by chance. X-virus is a classical object for investigations, for it shows high stability and concentration in the sap of a diseased plant. That is why it was used in our work as a control. Y-virus belongs to viruses which are isolated with difficulty because they

are labile and can aggregate during purification. However, the main problem with it is that it is present in the sap of a diseased plant in a very low concentration. F-virus is comparatively little studied but belongs to the X-group of potato viruses. A-virus, in spite of its wide distribution, is not completely studied as well but is known to belong to the group of potato virus Y. Consequently, it is a labile virus having a low concentration in a diseased plant.

Materials and Methods

Plants typically reacting to the viruses studied were taken as hosts. The plants were grown in a greenhouse in an autumn and winter period and in a vegetation house in a spring and summer period. Potatoes were grown in vegetation vessels in 5–6 kg of soil, indicator plants were grown in pots on 2–2.5 kg of soil. All test plants were regularly sprayed with test-killers for sucking insects. Checking of infected plants was performed in every 10 days by the drop analysis in accord with DUNIN and POPOVA (1937) with the use of antisera against X, Y, A, M, S, F potato viruses, CMV and TMV involving a control and by the method of indicator-plants in every 4 weeks.

Potato virus F or potato aucuba mosaic virus (PAMV) was kept on the following indicator-plants: *Capsicum annuum* L., *Nicotiana glutinosa* L., *N. tabacum* L. "Havana", *Physalis phyladelphyca* Lam., *Solanum tuberosum* var. Seedling 168, *Datura stramonium* L. and *S. miniatum* Bernh.

A test-plant of *C. annuum* had the most quick and obvious reaction for the F-virus infection. PAMV concentration was determined depending on the results of the indicator plants titration and on a serological analysis. The highest virus concentration happened to be in *D. stramonium* and *N. tabacum* var. "Havana". Leaf extracts of potato var. Epicurus and *D. stramonium* were used to obtain virus antigens.

Sap, containing potato virus Y (PVY), was obtained from leaves of potato var. Berlikhingen and tobacco var. "Samsun".

Tobacco and potato plants were infected with PVY after the appearance of 3–5 leaves. In 2–2.5 weeks tobacco leaves showed distinct lightening of the veins, after two more weeks mosaic spots characteristic of this virus appeared. Leaves with clearly pronounced symptoms served as sources for obtaining virus preparations. Potato leaves to be extracted for obtaining virus preparations were detached on the 21st day after the inoculation.

Potato virus X (PVX) was obtained from fresh necrosis on inoculated leaves of *Gomphrena globosa* L. after a series of passages through *N. tabacum* "Samsun." Pure virus was transferred to *D. stramonium*. Leaf extract of this plant and of potato var. Paul Wagner served as a source of obtaining virus preparations.

A potato virus A (PVA) preparation was obtained from diseased potato plants var. Allerfrüheste Gelbe and tomatoes *Lycopersicon pimpinellifolium* (Jusl.)

Mill. After inoculation of *Solanum demissum* A-6 leaves, in 3–5 days distinct star-like necroses characteristic of PVA were observed.

The purity of each virus preparation obtained after several cycles of differential centrifugation was determined in a spectrophotometer. The absorption value showed the amount of virus obtained. The absorption value ratio at 260 and 280 nm (E_{260}/E_{280}) along with the ratio E_{max}/E_{min} characterized the relative protein content in a sample, while the absence of changes in the spectrum served as additional confirmation of the purity of the preparation.

Results

All the procedures relating to the purification of A, Y, X and F potato viruses were performed in the cold at a temperature around 0°C. Common methods for protein isolation based on a relative virus stability in comparison with normal cell components were used.

PVX purification

Taking into account that PVX belongs to stable viruses, and that it is present in the sap of virus-infected plants in a rather high concentration, practically any method or a combination of methods applied for purification of phytopathogenic viruses were used. PVX concentration in the preparation obtained from *D. stramonium* and potato var. Paul Wagner were 8.25 mg/ml and 4.23 mg/ml, respectively.

PAMV purification

PAMV stability during homogenation of vegetational material gradually increased with selection of optimal molarity of this buffer. The usefulness of neutral phosphate buffer was compared with different molarity during test-plant inoculation with a homogenate obtained from infectious leaves of *Nicotiana tabacum* var. "Havana" (Table 1).

To obtain a pure PAMV preparation, the following methods were used: buthanol (TOMLINSON *et al.*, 1959), buthanol-chloroform (STEERE, 1956), chloroform (WETTER, 1960) and bentonite (DUNN and HITCHBORN, 1965).

An analysis of defects and advantages of all the methods tested resulted in a conclusion that the most reasonable method for purification and concentration of a virus-infected antigen is the following.

Leaves frosted at −14°C for 3–4 hr were triturated in a mortar where a 0.1*M* phosphate buffer (pH 7.0) containing 0.1% thioglycolic acid (1 : 3 w/v) was added. The resultant homogenate was wrung through a double cheese-cloth layer and centrifuged for 30 min at 6000 rev/min.

The sediment was discarded.

Table 1

Effect of phosphate buffer (pH 7.0) molarity on the PAMV infection of test plants

Buffer molarity	Test plants	
	Capsicum annuum	*Chenopodium amaranticolor*
0.5	1/5*	7
0.2	3/5	28
0.1	5/5	73
0.05	5/5	69
0.01	5/5	46
0.005	4/5	21

* — In denominator: number of plants tested which showed symptoms of top necroses
— In numerator: total number of plants tested

To the supernatant was added a 1/8 (v/w) portion of chloroform and thoroughly shaken for 10–15 min. The emulgated fluid was again subjected to a low-speed centrifugation for 20 min.

The sediment was discarded.

While stirring, to the supernatant a 1/4 part of $(NH_4)_2SO_4$ (volume/weight) was slowly added. In 60–90 minutes it was centrifuged for 15 min at 8000 rev/min.

The supernatant was discarded.

The sediment was diluted in a minimum amount of a 0.05 M phosphate buffer (pH 7.0); and the virus preparation was divided into two parts.

One part was centrifuged for 60 min at 40,000 rev/min.

The supernatant was discarded.

The sediment was resuspended in a 0.05*M* phosphate buffer and centrifuged for 10 min at 5000 rev/min.

The sediment was discarded.

The supernatant was centrifuged for 90 min at 40,000 rev/min.

The supenatant was discarded.

The other part was dialyzed for 20 hr against a 0.05 *M* phosphate buffer (pH 7.0) and centrifuged for 10 min at 5000 rev/min.

The supernatant was discarded.

The supernatant was additionally oxidized by a 10% acetic acid up to pH 5.0 and centrifuged for 20 min at 16,000 rev/min.

The supernatant was discarded.

The sediment was resuspended in a minimum amount of a 0.05 *M* phosphate buffer (pH 7.0) and centrifuged for 10 min at 5000 rev/min.

The sediment was discarded.

The sediment was resuspended in a minimum amount of a 0.05*M* phosphate buffer (pH 7.0) and centrifuged for 10 min at 5000 rev/min.

The sediment was discarded.

A pure virus preparation was obtained.

A partially purified virus preparation was obtained.

By means of the above methods rather pure PAMV preparations were obtained. The PAMV concentration obtained from virus-infected D. stramonium and from diseased potato var. Epicurus were 5.28 mg/ml and 2.14 mg/ml, respectively.

PVY purification

The method devised for PVY isolation is based on the modified procedures reported earlier by WETTER (1960), BARTELS (1957), DELGADO-SANCHEZ and GROGAN (1966) and DAMIRDAGH and SHEPHERD (1970). To obtain purified PVY preparations, young infected tobacco and potato plants were used because in older plant, more pigments are released which makes the virus isolation severely difficult. The following technique has been made a basis for obtaining a purified PVY preparation.

Leaves of diseased plants were frosted during 3–4 hours at −14°C then triturated in a mortar (during the trituration 5 grams of natrium diethyldithiocarbamate was added per 1000 grams of leaves) and centrifuged for 30 min at 6000 rev/min.

The sediment was discarded.

To the supernatant was added a 96% ethyl alcohol (20 ml of alcohol per 100 ml of sap), thoroughly shaken and centrifuged for 20 min at 6000 rev/min.

The sediment was discarded.

The supernatant was centrifuged for 120 min at 40,000 rev/min.

The supersedimental fluid was discarded.

The sediment was resuspended in a minimum amount of a 0.05 *M* phosphate buffer (pH 8.0) containing an 0.01 *M* trilon B (pH 7.8) and increased pH mixtures up to 8.0 .After 2-hours' standing the fluid was centrifuged for 15 min at 12,000 rev/min.

The supernatant was poured out in a specially prepared tube.

The sediment was again resuspended in the same buffer. After 1.5 hr of extracting, the mixture was centrifuged, the regime being the same.

The sediment was discarded.

The supernatant was combined with the first portion and the resultant mixture was centrifuged for 90 min at 40,000 rev/min.

The supernatant was discarded.

The sediment was extracted in a minimum amount of buffer and left to stand overnight. In the morning the fluid was subjected to centrifugation for 15 min at 12,000 rev/min.

The sediment was discarded.

The supernatant was again centrifuged for 60 min at 40,000 rev/min.

The supernatant was discarded.

The sediment was extracted in a minimum amount of a 0.05 *M* phosphate buffer (pH 7.0) and subjected to low-speed centrifugation at 5000 rev/min during 10 min.

The sediment was discarded.

A pure PVY preparation was obtained.

This method helped to isolate PVY from virose tobacco "Samsun" and potato var. Berlikhingen plants in concentrations of 2.17 mg/ml and 0.84 mg/ml, respectively, in purified virus preparations.

Partially purified PVY preparations were obtained by the method of fractional salting out. To this end, to the sap, preliminary freed from large cell fractions by low-speed centrifugation, sulphate ammonium was added to obtain a concentration which provides sedimentation of ballast components. The sediment was removed by a moderate centrifugation (8000 to 15,000 rev/min) and then discarded. The virus was separating and precipitating with further increase of sulphate ammonium concentration. After removing the salt by dialysis, the sediment was suspended in a buffered solution which finally enabled to obtain a partly purified PVY preparation.

PVA purification

The first attempts of many investigators to obtain a pure PVA failed (MATTHEWS, 1961). The properties of the virus itself and the absence of reliable test-plants wherein the virus could accumulate in high concentrations were primarily responsible. By the present time, the question dealing with PVA purification has been to some extent solved by the following investigators: ROLAND (1956), BRANDES and PAUL (1957), BARTELS (1963), SPIRE *et al.* (1969), FRIBOURG and DE ZOETEN (1970), STACE-SMITH and TREMAIN (1970), KRYLOV and GNUTOVA (1973).

Purified and partly purified PVA preparations were obtained by the method based on the following technique.

Fresh virus-infected plant leaves were quickly homogenized in a 0.5 *M* phosphate

buffer (pH 7.0) containing 0.01 *M* natrium diethyldithiocarbamate (1 : 1 w/v). The resultant homogenate was wrung through a double cheese cloth layer. The extract was added to a 1/8 (volume/weight) chlorophorm portion, shaken thoroughly for 2–3 min and centrifuged for 20 min at 5000 rev/min.

The sediment was then discarded.

The supernatant was subsequently recentrifuged for 90 min at 30,000 rev/min.

The resultant supernatant was discarded.

The sediment was resuspended in a minimum amount of an 0.02 *M* borate buffer (pH 7.4). The virus fluid was left to stand for 2 hours and then recentrifuged for 15 min at 8000 rev/min.

The sediment was discarded.

The supernatant was centrifuged for 45 min at 40,000 rev/min.

The resultant supernatant was discarded.

The sediment was resuspended in a minimum amount of a 0.02 *M* borate buffer (pH 7.0), and left to stand for 1.5 hours. It was then centrifuged for 10 min at 5000 rev/min.

The sediment was discarded.

A partially purified PVA preparation was obtained. To obtain a purified preparation, the differential centrifugation cycle was repeated once or twice, depending on the purity of the preparation obtained. This method for PVA purification allowed to isolate the virus from diseased tomato and potato var. Allerfrüheste Gelbe plants in concentration of 2.73 and 1.16 mg/ml, respectively. In our test the ratio E_{260}/E_{280} for PVX, PVY, PAMV and PVA was equal to 1.18, 1.46, 1.32 and 1.3, respectively.

During purification of PVA and PVY we came across difficulties which were not encountered in the work with PVX and PAMV. This is due to the nature of PVA and PVY, namely hightened ability to aggregation, lability, low concentration in virose plant tissues, ability to damage under the influence of pH extremes and even at small temperatures.

Before starting the purification or experimental application of a virus, stability of PVX, PAMV, PVY and PVA determined by the loss of infectivity in different conditions was primarily studied. As it appeared, frosting of these viruses present in the sap of virus-infected plants at -14 to -20°C did not damage them, while a part of normal cell proteins in such conditions became denaturated.

On carrying out purification of PVX, PAMV, PVY and PVA those values of pH and temperatures were chosen which, making a denaturing effect on the admixtures of various ballast substances (proteins, enzymes, cell structural components, etc.), would not affect the virus isolated.

PAMV inoculation on *Capsicum annuum* L. showed that the largest number of top necroses was obtained when sap pH of frosted tobacco leaves used for homogenation ranged from 6.0 to 8.0. Gradual reduce in the number of necroses was observed with the increase of pH with respect to its alkalinity or oxidation. That is why for leaf homogenation a buffer having pH 7.0 was used. In this connection, a definite role in virus purification belongs to the molar concentration of salts in the buffer. Our investigation results showed that PAMV stability during homogenation of plant material increased with the selection of optimal molarity of the buffer. On comparing of neutral buffer with different molarity at homogenate inoculation of test-plants with a homogenate obtained from infected *D. stramonium* leaves, it turned out that the largest quantity of necroses on *Chenopodium amaranticolor* Coste et Reyn and top necroses on *Capsicum annuum* appeared when a 0.05 *M* buffer was used.

We managed to considerably reduce the loss of PVA during its purification by means of a 0.05 *M* phosphorous buffer pH 7.0–7.1 usage during homogenation of diseased leaves involving a 0.01 *M* natrium diethyldithiocarbamate as a stabilizer.

As far as PVY is concerned, homogenation of infected leaves was performed without a buffer but with the use of an antioxidator due to its low concentration in plants.

All these precautions allowed to make further primary lightening of diseased plant sap infected with PVA and PVY by more strict means involving the application of chloroform and ethyl alcohol which helped to remove the most stable formations of normal cell components from virose sap.

Thus, studies of the possibilities of obtaining purified and partly purified PVX, PVY, PVA and PAMV preparations for their use as antigens in the preparation of diagnostic sera showed that the methods for virus purification used allowed to obtain relatively pure preparations for both stable PVX and PAMV present in the sap of a diseased plant in a high concentration and labile PVA and PVX with a low concentration in a virose plant.

Literature

Дунин, М. С., Попова Н. М. (1937): Капельный метод анализа вирусов в растениеводстве. М., Сельхозгиз: 45 с.

Крылов, А. В., Гнутова Р. В. (1973): Получение антисыворотки к А-вирусу картофеля. Тр. Биолого-почв. ин-та, новая серия т. 14 (117). Вирусные болезни растений Дальнего Востока, № 4:162—164.

Мэтьюз, Р. (1961): Серология растительных вирусов. М., ИЛ: 193 с.

Мэтьюз, Р. (1973): Вирусы растений. М., Изд. «Мир»: 600 с.

Bartels, R. (1957): Ein Beitrag zum serologischen Nachweis des X-virus in der Kartoffel. Phytopathol. Z., *30*, 1–16.

Bartels, R. (1963/1964): Untersuchungen über serologische Beziehungen zwischen Viren der Tobacco-etch-Gruppe. Phytopathol. Z., *49*, 395–406.

BRANDES, J. and PAUL, N. Z. (1957): Das Electronenmikroskop als Hilfsmittel bei der Diagnose pflanzeicher Virosen. Betrachtungen zur Vermessung faden- und stäbchenförmiger Virusteilchen. Arch. Microbiol., *26*, 358—368.

DAMIRDAGH, I. S. and SHEPHERD, R. I. (1970): Purification of the tobacco etch and other viruses of the potato Y group. Phytopathology, *60*, 132—142.

DELGADO-SANCHES, S. and GROGAN, R. G. (1966): Purification and properties of potato virus Y. Phytopathology, *56*, 1297—1404.

DUNN, D. B. and HITCHBORN, I. H. (1965): The use of bentonite in the purification of the plant viruses. Virology, *25*, 171—192.

FRIBOURG, C. B. and DE ZOETEN, G. A. (1970): Antiserum preparation and partial purification of the potato virus A. Phytopathology, *60*, 1415—1421.

ROLAND, G. (1956): Sur identification du virus A. Parasitica, *12*, 125—128.

SHEPHERD, R. J. and POUND, G. S. (1960): Purification of turnip mosaic virus. Phytopathology, *50*, 797—803.

SHEPHERD, I. F. (1970): The potential significance of potato hemagglutinins (lectins) in serodiagnosis. Phytopathology, *60*, 1621—1625.

SPIRE, D., FERAULT, A. C. and BERTRANDY, J. (1969): Extraction, purification et caracterisation du virus A de la pomme de terre. Ann. Phytopathol., *1*, 541—552.

STACE-SMITH, R. and TREMAIN, I. H. (1970): Purification and composition of potato virus Y. Phytopathology, *60*, 1857—1858.

STEERE, R. L. (1956): Purification and properties of tobacco ringspot virus. Phytopathology, *46*, 60—69.

TOMLINSON, I. A., SHEPHERD, I. and WALKER, I. C. (1959): Purification, properties and serology of cucumber mosaic virus. Phytopathology, *49*, 293—299.

WETTER, C. (1960): Partielle Reinigung einiger gestrechter Pflanzenviren und Verwendung als Antigens bei der Immunisierung mittles Freundschem Adjuvants. Arch. Microbiol., *37*, 278—292.

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Serological Studies of Potato Viruses X, Y, A and Aucuba Mosaic

II. PREPARATION OF DIAGNOSTIC ANTISERA

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Possibilities of preparation of antisera against viruses with different properties have been studied. Relatively pure virus preparations were used as antigens. The methods used to purify and concentrate PVX, PVY, PVA and PAMV allowed to obtain antisera with high antibody content (titre 1 : 512—1 : 4096). The combined scheme of animal immunization (intravenous and intramuscular injections involving an adjuvant, i.e. mixture of different volumes of 1% solutions of sodium alginate and gelatin) was considerably instrumental in the preparation of highly active diagnostic sera.

Diagnostic antisera play an important role in the study of plant virus diseases. At present antisera against 90 viruses have been prepared (MATTHEWS, 1973). Numerous methods which allow to obtain strong and specific antisera are described in the literature.

Sera involving different schemes of immunization were obtained in order to carry out serological studies of mosaic viruses.

Materials and Methods

Relatively pure and partially purified preparations of PVX, PVY, PVA and PAMV characteristics of which were given in the first part of the paper served as antigens.

The antibody formation was stimulated by the addition of non-specific stimulators (adjuvants) to the antigen (equal volumes of 1% solutions of gelatin and sodium alginate) prepared on a physiological solution.

Three immunization schemes were used.

The first immunization scheme (after DUNIN, 1959):

1st day — 1 ml	8th day — rest
2nd day — rest	9th day — 5.0—5.5 ml
3rd day — 2.0—2.5 ml	10th day — rest
4th day — rest	11th day — 5.0—5.5 ml

5th day – 3.0–3.5 ml
6th day – rest
7th day – 4.0–4.5 ml
12th day – rest
13th day – 5.0–5.5 ml
14–19th days – rest
20th day – blood sample was taken and titre tested. Antigen was injected intravenously without an adjuvant.

The second immunization scheme (after SCHELUDJKO, 1964): Antigen was injected intravenously with an adjuvant.

1st day – 1.5 ml
2nd day – 2.0 ml + adjuvant
3rd day – 3.0 ml
4th day – rest
5th day – rest
6th day – 4.0 ml + adjuvant
7th day – 5.0 ml
8th day – 5.0 ml
9th day – 5.0 ml + adjuvant
10th day – 5.0 ml
11, 12, 13, 14, 15, 16th days – rest
17th day – the first blood sample was taken and titre tested.

The third immunization scheme has been worked out by us:
1st day – 3.0 ml + adjuvant (intramuscular injection in hind paw muscules)
14th day – 4.0 ml + adjuvant (intramuscular injection in hind paw muscules)
28th day – 3.0 ml (intravenous injection)
30th day – 4.0 ml (intravenous injection)
32nd day – 5.0 ml (intravenous injection)
33, 34, 35, 36, 37, 38th days – rest
39th day – the first blood sample was taken and titre tested.

Results

Analysis of serum quality obtained by rabbit immunization with the virus antigens showed that it is more reasonable to use the third immunization scheme for preparation of diagnostic sera against PVX, PVY, PVA and PAMV (Table 1). Sera prepared by means of this scheme were highly active and specific with respect to homologous viruses and did not have antibodies against proteins of a host-plant. Higher titres of antibodies were obtained during reimmunization in 30–45 days after the first cycle of antigen injection.

Serum prepared against PVX was highly active. It had the highest antibody titre to a homologous virus in comparison with the sera obtained against PVA, PVY and PAMV. This can be explained by a high concentration of the given virus in the host-plant and its stability in the course of purification. Diagnostic serum obtained against PVX did not react with the plant sap in where other phytopathogenic viruses were present and also with sap of healthy plants. In detecting PVX in plants infected by this virus serum was highly sensitive and strictly specific as

Table 1

Influence of different animal immunization schemes on the titres of sera obtained

Viruses	Virus preparations	Schemes		
		first	second	third
X	I*	1 : 1024	1 : 2048	1 : 4096
	II**	1 : 1024	1 : 4096	1 : 4096
F	I	1 : 512	1 : 1024	1 : 2048
	II	1 : 1024	1 : 1024	1 : 2048
Y	I	1 : 512	1 : 1024	1 : 2048
	II	1 : 512	1 : 512	1 : 1024
A	I	1 : 256	1 : 512	1 : 512
	II	1 : 128	1 : 256	1 : 512

* — Partially purified virus preparation
** — Relatively pure virus preparation

evidenced by the results obtained on determinating specific antibodies in the serum studied with different control variants (Table 2).

Our earlier attempts to obtain immune serum against PAMV by means of injection of unpurified infected sap of *Nicotiana glutinosa* L. into rabbits resulted in preparing the antiserum with the titre to homologous antigen equal to 1 : 4096 (ZEMCHENKOVA, 1968) but this serum contained antibodies to normal components of test–plant cell. Application of this antiserum diluted to 1 : 256 secures obtaining only virus-specific antigen–antibody reaction. In further studies the presence of PAMV pure preparation favoured obtaining of a highly active and strictly specific antiserum. *Nicotiana tabacum* var. "Havana" and *Datura stramonium* L. have been used to accumulate the virus. The monoserum against F-virus prepared by us reacted distinctly with the sap of the plants inoculated by this virus but it reacted neither with the plant sap wherein other viruses were present nor with the sap of healthy plants (Table 2).

Thus, it was conclusively proved that diagnostic antiserum obtained against PAMV possesses a high titre (1 : 2048) and strict specificity.

An antiserum against PVA was prepared with the titre being 1 : 256–1 : 512. With dilutions 1 : 2–1 : 4 this serum reacted with the sap of the plants infected with PVY. This testified once again to the serological relationship between Y- and A-viruses. In the dilution of 1 : 5 the antiserum was strictly specific since it was reactive only towards the sap of the plants infected with PVA and did not react with the sap of the following affected plants: potato var. Paul Wagner and *D. stramonium* infected with PVX; *N. tabacum* var. "Havana" and potato var. Epicurus infected with PAMV; tomato infected with PVM; potato var. Lorkh infected with PVS; and the sap of healthy plants either.

Table 2

Titres of antisera obtained against

Viruses	Sap of plants tested	Control serum	Dilutions of homologous serum			
			1 : 2	1 : 4	1 : 8	1 : 16
F	*N. tabacum* "Havana"	−	++++	++++	++++	++++
	D. stramonium	−	++++	++++	++++	++++
	Potato var. Epicurus	−	++++	++++	++++	+++4
	N. glutinosa	−	++++	++++	++++	++++
X	*N. tabacum* "Samsun"	−	++++	++++	+++	+++
	Potato var. Berlikhingen	−	++++	+++	++	++
	Potato var. Paul Wagner	−	++++	++++	++++	++++
	D. stramonium	−	++++	++++	++++	++++
	Potato var. Negre	−	++++	++++	+++	+++
	Potato var. Aller-früheste Gelbe	−	++++	++++	+++	+++
	Potato var. Canzler	−	++++	++++	+++	++
A	Potato var. Arren Crest	−	+++	++	+	−
	N. tabacum "Samsun" infected by PVY	−	++	+	±	−

Application of the effective method for obtaining the antigen of PVY and increasing in the immunological reactivity of the rabbit organism due to the use of the third immunization scheme allowed to prepare a diagnostic serum against PVX of a relatively high titre − 1 : 512−1 : 2048. Specificity of the antiserum was determined by means of homologous reactions, by the absence of cross reactions with heterologous antigens and by the sap of healthy plants (Table 2).

Discussion

Our investigations showed that it was more reasonable to use two ways of antigen injections at the same time : intravenous and intramuscular. This conclusion was based on the experimental data which showed that in case of intravenous antigen injection into rabbits antibodies were detected only 5−6 days after single immunization. In some days the titre raised to its maximum and then sharply decreased. Antibodies appeared later during intramuscular antigen injection with an adjuvant but the titre was higher and decreased slower than after

X, Y, A and F-potato viruses

1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024	1 : 2048	1:4096	1:8192
++++	+++	+++	+++	++	++	+	±	−
++++	++++	+++	+++	+++	+++	++	++	−
+++	+++	++	±	−	−	−	−	−
++++	+++	+++	++	++	+	+	−	−
+++	+++	++	++	++	++	+±	±	−
+	+	−	−	−	−	−	−	−
++++	+++	+++	++	++	±	−	−	−
++++	++++	+++	+++	++	++	++	++	+
+++	++	+	±	−	−	−	−	−
++	++	++	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−

intravenous antigen injection. Considerably higher titres of antibodies were obtained after repeated injection of the same antigen some weeks or months after the first injection. Regularities of immunoreactivity of the animal organism served as the basis for designing of different immunization schemes.

A choice of immunization schemes for obtaining antisera against A, Y, X and F-viruses was defined first of all by the desire to prepare sera of the highest titre possible with strict specificity. As our results showed sera prepared by means of intravenous antigen injection had a lower titre than the antisera obtained by the application of the combined immunization scheme. Moreover, a relatively small number of injections which characterize the scheme and rather long intervals between them provided conditions of less fulltime work in comparison with the first and second schemes. However, the main advantage of the last scheme was that with the help of its diagnostic sera of higher titre could have been obtained (Table 1).

A serum titre was affected by the use of an adjuvant. On comparing results obtained in time of antigen injection according to the second and first schemes, it turned out that non-specific stimulators of immunogenesis increased the number of antibodies by one or two orders.

Soviet and foreign authors have often reported on the difficulties arising during the preparation of diagnostic sera against Y-virus. The properties of this virus (lability, low concentration in diseased plant tissues, ability to aggregation during its purification, etc.) are mainly responsible for these difficulties. Due to these reasons, a lot of difficulties arose which were connected with obtaining and testing the immune sera against the said virus.

Our experiments and results obtained testify to the possibility of the preparation of an antiserum against PVY of a relatively high titre (Table 2).

PAMV is relatively poor studied. Preparation of immune serum against F-virus was reported by KOLLMER and LARSON (1960), VAN SLOGTEREN (personal communication, cited after MATTHEWS, 1961), then later by JUO and RICH (1969), GNUTOVA (1971) and KRYLOV and GNUTOVA (1973a). As far as this virus is assigned to the serological group of PVX, its concentration in the host-plant can be expected to be relatively high. Identification of reliable test-plants (KRYLOV, 1966; 1971; BODE, 1966) and the absence of common antigens with other viruses (CHESTER, 1935) made it possible for us to obtain a monoserum against PAMV.

The first attempts to obtain a monoserum against PVA failed. Lack of reliable test-plants wherein the virus accumulated in a relatively high concentration, rapid aggregation under conditions in vitro and so on were primarily reason of it. Thus, working with labial viruses, we were able to prepare diagnostic serum not only against viruses which readily lose their antigenic activity in vitro but also against specific antigeno-active and stable products of metabolism of the diseased plant. Some problems connected with purification of viruses have to a certain extent been solved by us (KRYLOV and GNUTOVA, 1973b) and this allowed to prepare immune serum against PVA. The matter is that we succeeded in finding optimal conditions for obtaining concentrated preparations of PVA and in fitting certain stabilizers which prevented the virus from destruction in vitro and an appropriate buffer with molar concentration of salts in it.

Consequently, the data obtained allow to conclude that application of the combined immunization scheme favoured the preparation of strong and highly specific diagnostic sera. Methods developed for purification and concentration of PVA, PVY, PVX and PAMV made it possible to obtain high antibody content in immune sera.

Literature

Земченкова, Р. В. (1968): Получение антисыворотки к F вирусу картофеля. В кн.: «Некоторые вопросы биологии и медицины на Дальнем Востоке», Владивосток: 10—12.

Гнутова, Р. В. (1971): Получение антисыворотки к F вирусу картофеля. Тезисы докл. У Всесоюзн. совещ. по вирусным болезням растений, № 2, Киев: 26—27.

Крылов, А. В. (1966): Индикаторный метод диагностики мозаичных вирусов картофеля. Тр. 5 Всесоюзн. совещ. по вирусным болезням растений. «Вирусные болезни с.—х. растений и меры борьбы с ними», Киев; изд. «Наукова думка»: 228—233.

Крылов, А. В. (1971): Растения-индикаторы для диагностики мозаичных вирусов картофеля. Тр. Биол.-почв. ин-та № 4 «Вирусные болезни с.—х. растений ДВ», Владивосток, вып. 3:54—128.

Крылов, А. В., Гнутова, Р. В. (1973а): Диагностика F вируса картофеля. Тр. Биол.-почв. ин-та, новая серия, т. 115(118). «Выращивание семенного картофеля на безвирусной основе», Владивосток: 77—79.

Крылов, А. В., Гнутова, Р. В. (1973б): Получение антисыворотки к А-вирусу картофеля. Тр. Биол.-почв. ин-та, т. 114(117), «Вирусные болезни растений ДВ», Владивосток, № 4:162—164.

Мэтьюз, Р. (1961): Серология растительных вирусов. М., ИЛ:193с.

Мэтьюз, Р. (1973): Вирусы растений. М., изд. «Мир»:600 с.

Шелудько, Ю. М. (1964): О методике получения диагностической сыворотки к Х-вирусу картофеля. В кн: «Вирусные болезни растений», М., изд. «Колос»:102—109.

Bode, O. (1966): Untersuchungen über des Kartoffel-M-Virus. Proc. 3rd Trienn. conf. EAPR., Zürich, 226—227.

Chester, K. S. (1935): Serological evidence in plant-virus classification. Phytopathology, *25*, 686—701.

Dunin, M. S. (1959): On some methods of obtaining and applying antisera in phytopathological and entomological research. Proc. of the IVth Intern. Congr. of Grop. Protection, Hamburg, September 1957, Braunschweig 1959.

Juo, P. and Rich, A. E. (1969): Purification of potato virus aucuba mosaic. Phytopathology, *59*, 1816—1819.

Kollmer, G. F. and Larson, R. H. (1960): Potato virus F in relation to host range, properties, reaction of American potato varieties, and resistance. Wisconsin agr. expt. sta., Res. bull. *223*, 1—38.

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Serological Studies of Potato Viruses X, Y, A and Aucuba Mosaic

III. EXPANSION OF SENSITIVITY LIMITS OF SEROLOGICAL VIRUS DETERMINATION BY THE USE OF THE LATEX-TEST, BENTONITE FLOCCULATION, PASSIVE HAEMAGGLUTINATION AND COMPLEMENT FIXATION REACTION

By

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The following five immunological reactions have been studied and compared: the drop analysis in accordance with DUNIN and POPOVA (1937), latex-test and reactions of passive haemagglutination, bentonite flocculation and complement fixation. Relatively pure virus preparations and crude sap of virose plants preliminary subjected to low-speed centrifugation served as antigens. In obtaining potato viruses, the passive haemagglutination reaction showed the greatest sensitivity which helped to detect PVX, PVA, PVY and PAMV in the preparations studied in the following amounts: 0.002—0.003 mg/ml, 0.001—0.0015 mg/ml, 0.0015 mg/ml and 0.004 mg/ml, respectively. Latex-test was somewhat less sensitive than passive haemagglutination reaction. It fixed X, F, A and Y viruses in the amounts of 0.005—0.006 mg/ml, 0.005—0.007 mg/ml, 0.007 mg/ml and 0.015—0.001 mg/ml, respectively. Bentonite flocculation and complement fixation reactions often gave similar results and yielded to the latex-test and passive haemagglutination in their sensitivity. The minimum virus concentrations detectable by these reactions were 0.016—0.02 mg/ml, 0.015—0.02 mg/ml, 0.015 mg/ml and 0.05 mg/ml for X, Y, A and F viruses, respectively. The drop analysis on slides yielded to adsorptional serodiagnostic methods and complement fixation reaction in its sensitivity and accuracy which were less by 100—1000 times. It helped to detect 0.1—0.5 mg/ml of the virus in the material investigated.

In the complex of effective means in the struggle against plant virus diseases, serological method occupies an important place. Serology is currently a vast field of investigations and plays an important role in the present-day plant virology. In spite of practical results obtained by means of this method, the question on increasing sensitivity and accuracy of a serological test is of urgent importance.

In this connection, one of the main problems to be solved in serodiagnostics is the establishment of highly active diagnostic sera which would help to determine rather low virus concentrations in plants. Recently, to accomplish this, adjuvants are often used. Data on the practical application of the latex-test, bentonite flocculation reaction, passive haemagglutination and complement fixation in virology accumulate very rapidly. Unfortunately, the data on the reactions are not adequately known so far and the degree of their application in plant virology is still lower.

We used adsorptional serodiagnostic methods and the complement fixation reaction to increase the sensitivity of the method during the diagnostics and identification of X, Y, A and F potato viruses.

Materials and Methods

Two kinds of antigens were used: relatively pure virus preparations with the known virus concentration therein and crude sap of virose plants which was primarily subjected to low-speed centrifugation to remove large cellular components. Virus concentrations in the preparations studied were presented in the first communication.

Techniques of carrying out the drop reaction, passive haemagglutination, latex-test and reactions of bentonite flocculation and complement fixation have been reported in our previous papers (GNUTOVA and KRYLOV, 1973a, b; KRYLOV and GNUTOVA, 1974).

Results

On detecting Y, A, X and F viruses in the preparations investigated by means of the reaction on slides in accordance with DUNIN and POPOVA (1937) it was shown that in crude infectious sap of diseased plants X, F, Y and A could be detected in the following dilutions: 1 : 64–1 : 128, 1 : 32–1 : 64, 1 : 8–1 : 32, 1 : 16–1 : 32 respectively (Table 1).

In purified virus preparations we succeeded in detecting the viruses in the following amounts: X – 0.10–0.18 mg/ml; Y – 0.25–0.8 mg/ml; F – 0.18–0.27 mg/ml and A – 0.14–0.28 mg/ml.

Application of tanned red blood cells which can adsorb proteins on their surfaces for the quantitative virus determination suggested a conclusion that this immunological reaction is highly sensitive and specific.

Each test was followed by a control for spontaneous agglutination of sensibilized red blood cells, and for the absence of non-specific haemagglutinations (a reaction with normal red blood cells) in serum. A reaction of passive haemagglutination inhibition served as a specificity control.

For this purpose, to each virus preparation dilution heterologous antiserum (0.1 ml) and a drop of sensibilized red blood cells were added. Results were considered reliable when haemagglutination with normal red blood cells and heterologous antisera were absent.

With a positive reaction, red blood cells formed a uniform layer, covering the bottom of the tube, with a negative reaction, they formed a small compact sedimentation or a ring. The reaction was estimated by a 4-mark system.

The minimum virus concentrations detectable by the reaction of passive haemagglutination were 0.002–0.003 mg/ml for X; 0.001–0.0015 mg/ml for F;

Table 1

Comparative sensitivity of serological reactions in titrating X, Y, A and F potato viruses

Viruses	Antigen*	Serological tests				
		Drop analysis	Bentonite flocculation	Latex-test	Complement fixation	Passive haemagglutination reaction
X	I	1 : 128	1 : 512	1 : 2048	—	1 : 8192
	II	1 : 64	1 : 256	1 : 1024	—	1 : 2048
	III	1 : 64 (0.10)**	1 : 512 (0.10)	1 : 1024 (0.06)	1 : 256 (0.016)	1 : 2048 (0.002)
	IV	1 : 16 (0.18)	1 : 256 (0.012)	1 : 512 (0.005)	1 : 128 (0.020)	1 : 1024 (0.003)
F	I	1 : 64	1 : 512	1 : 1024	—	1 : 4096
	II	1 : 32	1 : 256	1 : 256	—	1 : 1024
	III	1 : 16 (0.18)	1 : 128 (0.015)	1 : 256 (0.005)	1 : 256 (0.010)	1 : 2048 (0.0010)
	IV	1 : 8 (0.27)	1 : 128 (0.020)	1 : 512 (0.007)	1 : 128 (0.015)	1 : 1024 (0.0015)
A	I	1 : 32	1 : 256	1 : 256	—	1 : 1024
	II	1 : 16	1 : 64	1 : 512	—	1 : 512
	III	1 : 4 (0.14)	1 : 64 (0.015)	1 : 128 (0.007)	1 : 64 (0.015)	1 : 512 (0.0015)
	IV	1 : 2 (0.28)	1 : 32 (0.0015)	1 : 64 (0.007)	1 : 32 (0.015)	1 : 256 (0.0015)
Y	I	1 : 32	1 : 128	1 : 256	—	1 : 512
	II	1 : 8	1 : 32	1 : 128	—	1 : 256
	III	1 : 2 (0.25)	1 : 32 (0.016)	1 : 256 (0.001)	1 : 64 (0.20)	1 : 512 (0.002)
	IV	— (0.80)	1 : 16 (0.050)	1 : 64 (0.015)	1 : 16 (0.25)	1 : 64 (0.004)

* I — crude sap of virose tobacco (in case of X-virus — D. stramonium; A-virus — tomatoes); II — crude sap of virose potato; III — purified virus preparations obtained from diseased tobacco plants (in case of X-virus — D. stramonium, A-virus — tomato); IV — purified virus preparations obtained from virose potato

** In parentheses — amount of the virus expressed in mg/ml

0.0015 mg/ml for A, and 0.002 — 0.004 mg/ml for Y. X-virus present in crude sap of *D. stramonium* and potato was able to provide observable haemagglutination with sap dilution of these plants not exceeding 1 : 2048 — 1 : 8192. F and Y viruses present in the sap of diseased tobacco and potato plants agglutinized, when sap dilutions of virose plants were not higher than 1 : 1024 and 1 : 256 — 1 : 512, respectively. A-virus present in tomato and potato sap infected with this virus was

detected by means of passive haemagglutination with the dilution of infectious sap not exceeding 1 : 512 – 1 : 1024.

Titration of virus antigens by means of the latex-test showed that with a positive reaction agglutinized latex particles sensibilized with γ-globulin looked like loose aggregations. With a negative reaction, the mixture resembled milk suspension.

Antibodies absorbed on latex helped to detect X, F, A and Y viruses in the following amounts: 0.005 – 0.006 mg/ml; 0.005 – 0.007 mg/ml; 0.007 mg/ml and 0.015 mg/ml, respectively. The maximum dilution of crude infectional sap of virose plants capable of providing visible agglutination with homologous sensibilized serum corresponded to 1 : 1024 – 1 : 2048; 1 : 512 – 1 : 1024 and 1 : 128 – 1 : 256 for X, F and Y and A viruses, respectively.

The tests were accompanied by the following controls: healthy plant sap + sensibilized latex suspension, healthy plant sap + latex emulsion and saline solution + "loaded" latex suspension. Specificity of the latex test was controlled by the following reactions: saline + immune serum, antigen + normal serum, and antigen + heterologous serum.

Titration of virus preparations by means of bentonite flocculation reaction showed (Table 1) that it can help to detect 0.01 – 0.012 mg/ml of X-virus, 0.015 – 0.02 mg/ml of F-virus, 0.015 mg/ml of A-virus and 0.016 – 0.05 mg/ml of Y-virus.

X-virus present in the sap of stramonium and potato can give visible agglutination with antibodies sensibilized with bentonite particles, with sap dilutions of these plants not lower than 1 : 256 – 1 : 512. F-virus and Y-virus present in sap of diseased tobacco and potato plants agglutinized when dilutions of virose sap were 1 : 256 – 1 : 512 and 1 : 32 – 1 : 128, respectively. In higher concentration virus was not detected by this reaction, A-virus present in tomato and potato sap was detected at dilutions not lower then 1 : 64 – 1 : 256.

The following reactions served as controls: healthy plant sap + sensibilized bentonite suspension healthy plant sap + bentonite suspension; and saline solution + sensibilized bentonite suspension.

To control the specificity of the results obtained, heterologous antisera were added to the dilutions of X, Y, A and F virus preparations and bentonite suspension was added therein.

The positive pattern of bentonite flocculation consisted in a blue layer covering the bottom of the tube. The negative pattern consisted in a blue uniform layer. The results were recorded in accord with the nature of the sediment.

When running the complement fixation, the following combination of sera-antigen units, providing the registration of specific and preventing the possibility of nonspecific reactions were taken into consideration: antigen + heterologous serum; antigen + normal serum; healthy plant sap + heterologous serum; and saline solution + normal serum.

The maximum virus concentrations essential for specific complement fixation in our experiments were 0.2 – 0.25 mg/ml; 0.01 – 0.015 mg/ml; 0.016 – 0.025 mg/ml and 0.015 mg/ml for Y, F, X and A viruses, respectively (Table 1).

Results were considered reliable provided that hemolysis in controls is faultless. Controls of serum, antigen, complement and heterologous system were imperative for this reaction.

Discussion

Drop reaction in accordance with DUNIN and POPOVA (1937) is one of the simplest immunological methods for investigations in plant virology. However, with all its advantages concerning high productivity and simplicity the drop method happens to be unadequately sensitive and accurate. We succeeded in detecting the virus in the preparations studied in a concentration corresponding to a tenth part of mg/ml, i.e. the given method allows to comparatively quickly diagnose those viruses which occur in the plant in a relatively high concentration, e.g. X and F viruses, while Y and A viruses cannot always be found by means of this reaction.

The reaction based on passive or indirect erythrocyte red blood cell haemagglutination conformably to plant viruses has been little studied so far while there are numerous examples of its wide usage as a quick and convenient method for the detection of viruses of man and animals.

Experimental investigations of the possibilities of the given reaction for the detection of X, Y, A and F potato viruses in the preparations studied allowed us to make a conclusion about its relatively high sensitivity and specificity. The passive haemagglutination reaction proved to be the most sensitive as compared to other serological reactions studied.

At the same time we have not noticed any influence of nonspecific inhibitors present in normal and immune sera on reaction results in our work with plant viruses, which sometimes is a reason of the appearance of nonspecific reactions with viruses of man and animals. Titration data of virus antigens by the passive haemagglutination reaction are indicative of this serological reaction as a rather promising method allowing to perform early diagnosing of viruses present in sap of a diseased plant in a low concentration.

It is known that sensibilized red blood cells cannot be stored for a long time. The difficulty owing to this falls away when polysterol latex particles are used. Antibodies adsorbed to latex helped us to obtain stable results of reactions conducted with the use of not only relatively pure preparations but with fresh sap of virus-infected plants as well. To carry out this reaction successfully, an antiserum with low content of antibodies to homologous virus is enough. This fact is of essential importance, since it permits to use them for identification and diagnoses of viruses to which immune sera of high titres is still obtained with difficulty.

Besides passive haemagglutination and latex-test, another method distinguished by its simplicity and sensitivity in which bentonite serves as adsorbent deserves attention. The first attempts of using inert bentonite particles to detect potato viruses showed that this reaction allows to reliably determine viruses present in sap of a diseased plant.

Along with adsorptional methods, the reaction of complement fixation can greatly extent the possibilities of serological analysis. The given reaction has not found wide application in the work with plant viruses probably because its technique is comparatively complex and labour-consuming. Nevertheless, as our investigations showed, complement fixation reaction can reasonably be used in some cases for diagnostics and identification of plant viruses. In conducting the reaction comparatively small volumes of serum and antigen were needed, 0.1 ml of the reagent studied was enough to obtain the result. Thus, the data obtained in virus antigen titration with adsorptional methods and the complement fixation reaction showed that the main advantage of these serological reactions is, first of all, their high sensitivity. Hence, specific interaction between Y-virus and loaded γ-globulin of homologous serum with latex, bentonite and red blood cells has been found in virus preparations diluted to 1 : 32 – 1 : 512, while with the drop reaction in accord with DUNIN and POPOVA the ratios were 1 : 2 – 1 : 16.

Dilutions of virose plant sap and purified preparations of this virus essential for A-virus detection by means of adsorptional diagnostic methods were 1 : 64 – 1 : 1024 and 1 : 2 – 1 : 32 for the drop reaction.

Passive haemagglutination between F-virus and sensibilized homologous serum was observed at virus preparation dilutions of 1 : 128 – 1 : 4096, while the drop analysis gave positive results at 1 : 4 – 1 : 64.

X-virus agglutinized with antibodies sensibilized with latex, bentonite and red blood cells when dilutions of virus preparation were 1 : 256 – 1 : 8192 and 1 : 16 – 1 : 128 with the drop reaction.

If the virus preparation dilutions were estimated quantitatively, then it would turn out that antibodies sensibilized with latex, bentonite and red blood cells could be detected in amounts of 0.001 – 0.007 mg/ml, 0.01 – 0.016 mg/ml and 0.0015 – 0.005 mg/ml, respectively. The drop reaction helped to detect only tenth mg parts of the virus.

Consequently, sensitivities of adsorptional serodiagnostic methods and complement fixation reaction were 100 – 1000 times higher than that of the drop reaction on slides. This great difference in sensitivity will not seem surprizing if one takes into consideration that fixation of one protein molecule on inert latex and bentonite particles or on red blood cells increases reagent system sizes by several orders, thus decreasing the amount of antibodies or antigen required for a visible reaction.

We came to the conclusion that passive haemagglutination reaction is the most universal method for the diagnostics and identification of plant viruses in laboratory investigation practice, as it possesses the greatest sensitivity. This reaction helped to fix the presence of the virus in the preparations studied in the amount of 0.0015 – 0.004 mg/ml. Besides, to obtain final results required only several hours, whereas the method of plant-indicators, resembling the given reaction in sensitivity, needed from one to several weeks. Contrary to passive haemagglutination reaction and complement fixation which were used only in laboratory conditions, latex-test and bentonite flocculation have successfully been

used in field conditions for the detection of potato affecting viruses. Antibodies of immune sera adsorbed on inert bentonite particles retained their sensitivity for up to 8 months, those adsorbed on latex for 3 years as in the case of BERCKS (1967), while red blood cells loaded with antibodies of sera remained sensitive for not more than 18 hr.

Consequently, reliable results in the diagnostics of X, Y, A and F potato viruses can be obtained by means of the latex-test, bentonite flocculation reaction, passive haemagglutination and complement fixation, which considerably extend possibilities of serodiagnostics.

Literature

Гнутова, Р. В., Крылов, А. В. (1973а): Применение адсорбционных методов серодиагностики и реакции связывания комплемента при изучении фитопатогенных вирусов. Труды Биол.-почв. ин-та, новая, Т. 114(117), Вирусные болезни растений Дальнего Востока, № 4: 99—107.

Гнутова, Р. В., Крылов, А. В. (1973б): Использование латекс-теста для диагностики X, У, Ф — вирусов картофеля. Труды Биол.-почв. ин-та, новая серия, Т. 114(117), Вирусные болезни растений Дальнего Востока, № 4: 108—111.

Дунин, М. С., Попова, Н. И. (1937): Капельный метод анализа в растениеводстве. М., Сельсхозгиз: 45 с.

BERCKS, R. (1967): Methodische Untersuchungen über den serologischen Nachweis pflanzenpathologener Viren mit dem Bentonit-Flockungstest, dem Latex-Test und Bariumsulfat-Test. Phytopathol, Z., *58*, 1—17.

KRYLOV, A. V. and GNUTOVA, R. V. (1974): On the possibility of using passive agglutination and complement fixation methods for potato X, F and Y virus diagnosis. Phytopathol. Z., *79*, 203—216.

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Studies on Viruses and Virus Diseases of Cruciferous Plants

XVIII. BEETLE TRANSMISSION AND SOME NEW NATURAL HOSTS OF ERYSIMUM LATENT VIRUS

By

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Erysimum latent virus (ELV) proved to be transmissible by the beetle species *Phyllotreta atra*, *P. nigripes*, and *P. undulata*. In tests with one beetle per plant the infection percentage was found by about five per cent. Trials with *Brevicoryne brassicae* and *Myzus persicae* in order to demonstrate the aphid transmissibility of the virus in persistent and nonpersistent manners, remained unsuccessful. Seven new natural host species of ELV, four belonging to the genus *Erysimum* and one each belonging to *Arabis*, *Barbarea*, and *Fibigia*, have been recognized. With the exception of the last mentioned, all these species were obviously infected without showing symptoms. The six ELV isolates tested in detail proved to possess the same antigenic property as none of them formed spurs in OUCHTERLONY tests.

SHUKLA and SCHMELZER (1972) described *Erysimum* latent virus (ELV) as a previously unknown crucifer virus. Its original host was symptomless *Erysimum helveticum* (Jacq.) DC. The host range of the virus was confined mainly to the species of *Cruciferae*. It had a dilution end point of 1 : 500,000, a thermal inactivation point between 76° and 78°C and was stable in crude sap at room temperature up to 21 days. The virus was easily transmissible by mechanical inoculation, but *Myzus persicae* (Sulz.) failed to transmit it in repeated trials when the aphids were allowed to feed on infected plants for a period of 15 minutes. In further studies (SHUKLA *et al.*, 1973), the virus was found to have polyhedral particles of 21 and 30 nm diameter in negatively stained and metal shadowed preparations, respectively. These properties were similar to those of other crucifer viruses known to be transmitted by chewing insects. However, no close relationship could be demonstrated between ELV and the earlier found beetle transmissible viruses of *Cruciferae* in serological and cross protection tests (SHUKLA and SCHMELZER, 1972; SHUKLA *et al.*, 1973).

In the present work, attempts have been made to transmit ELV by aphid (especially in persistent manner) and beetle species. Some new natural hosts of the virus, found during the investigation of viruses in cruciferous plants, are also mentioned herein.

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Material and Methods

The insect transmission tests were performed with the following aphid and beetle species: aphids – *Brevicoryne brassicae* (L.) and *Myzus persicae*: beetles – *Phyllotreta nemorum* (L.), *P. undulata* Kutsch., *P. nigripes* (F.), *P. atra* (F.), and *P. cruciferae* (Goeze). The aphids were bred on healthy cauliflower seedlings in greenhouse whereas the beetles were collected freshly from fields. All the animals tested were found to be not contaminated spontaneously with plant viruses. The acquisition and infection periods of the insects on plants were of different durations ranging from five minutes to 72 hours. The transmission tests with aphids were done with 5 or 10 insects per plant but in case of beetles only one insect per plant was employed. As soon as the virus symptoms appeared on test plants, serological tests were undertaken in order to confirm the results. *Brassica chinensis* L. and *B. juncea* (L.) Czern. et Coss. served as sources and test plants for insect transmission trials.

In order to find new hosts of ELV, samples of ornamental and wild crucifers were collected from various botanical gardens of the GDR and assayed for presence of the virus in greenhouse. Methods of preparing inoculum, inoculation and serology were the same as described in earlier papers (SHUKLA and SCHMELZER, 1970a; 1970b). The test plant exclusively used for isolation of ELV was *Sinapis alba* L. as it was found to be a very sensitive host for this virus (SHUKLA and SCHMELZER, 1972). Later on the isolates were compared on several host species of ELV.

Results

In general, *Brassica pekinensis* (Lour.) Rupr. proved to be an unsuitable plant species for insect transmission of ELV. In comparison to this, *Brassica chinensis* and *B. juncea* gave good results in such trials. All the experiments in which attempts were made to transmit ELV with the mentioned two aphid species using shorter and longer acquisition periods, characteristic for persistent and non-persistent viruses, remained unsuccessful. From the beetle species *Phyllotreta atra*, *P. nigripes*, and *P. undulata* proved to be vectors of ELV. The acquisition and infection periods in successful cases amounted to 16 and 24 hours, respectively. The infection percentage was found to be about five per cent on an average.

The new natural hosts of ELV found in the present investigation are listed in Table 1.

From Table 1 it can be seen that a majority of the plant species was symptomless when found spontaneously infected in the gardens. Also, the first two of the symptom-showing species contained cabbage black ring (CBRV) and other viruses additionally (SHUKLA and SCHMELZER, 1973). Only the mosaic symptom on *Fibigia clypeata* may be due to ELV infection. The presence of ELV in each of the species in Table 1 was confirmed by serological tests using *Sinapis alba* as a source. When six of the ELV isolates, obtained from different plant species,

Table 1

New natural hosts of Erysimum latent virus

Plant species	Places of collection	Observed symptoms
Arabis ludoviciana C. A. Mey.	Dresden	mosaic
Barbarea vulgaris R. Br.	Halle	no
Erysimum crepidifolium Rchb.	Dresden	flecks and mosaic
	Eberswalde	no
E. perovskianum Fisch. et Mey.	Halle	no
E. pulchellum (Willd.) Boiss.	Halle	no
E. silvestre (Cr.) Scop.	Dresden	no
Fibigia clypeata (L.) Med.	Dresden	mosaic

collected in different regions of the GDR, were tested side by side in spur tests, they reacted in an identical manner as none of them formed spur against any of the isolates (Fig. 1). Also no remarkable difference was found in test plant reactions.

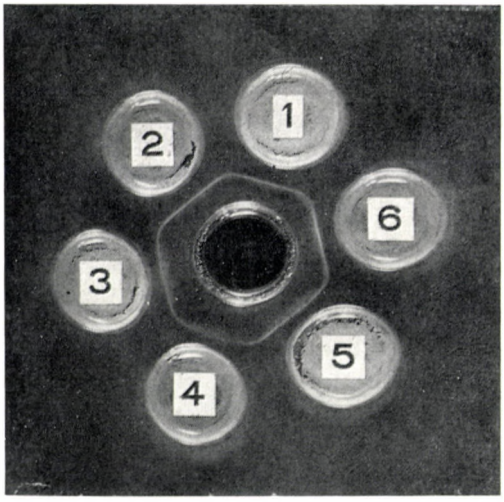

Fig. 1. Spur test with six isolates of *Erysimum* latent virus including the original one. The central well was filled with the antiserum against the original isolate. No spur formation occurred

Discussion

The results, presented here, showed that ELV is not transmissible by aphids neither in persistent nor in non-persistent manner. However, the virus could be transmitted by three species of *Phyllotreta*. The per cent infection, obtained through the beetle species, was rather low. This may be because only one insect per plant

was used in the transmission tests. It is possible that the two *Phyllotreta* species which could not transmit the virus nevertheless are also vectors. In spite of their low vector efficiency, the beetles can be of importance in the distribution of this virus in nature as they occur generally in rather large populations on cruciferous plants each year. Like other beetle-transmissible crucifer viruses, ELV also does not appear to depend on a particular vector species for its transmission (MARKHAM and SMITH, 1949; MARTINI, 1958; PROESELER, 1971; ŠTEFANAC and MAMULA, 1971). Although earlier investigated characters of ELV were found similar to those described for crucifer viruses having chewing insects as vectors and led to the incorporation of this virus into the group of beetle-transmissible viruses, the trials described here gave a solid basis for it.

The results proved that the name ELV given because of the latent presence in its first detected host *Erysimum helveticum* is justified even if other natural hosts are considered. With the exception of *Fibigia clypeata*, the plant species mentioned in Table 1 were found either latently infected or contained CBRV which may be responsible for symptom production. So far, ELV appears to be confined mainly to the species of *Erysimum*. Out of eight known natural hosts, five (including the original host) belong to this genus. The virus seems to be distributed throughout the GDR as it was demonstrated in samples from different regions of the country situated far from each other. Prehaps in future it may be shown to occur also in brassica crops as well as in other cruciferous species not only in the GDR but also in other countries. In contrast to turnip yellow mosaic virus (SHUKLA and SCHMELZER, 1974), no indication was found that ELV occurs in different strains.

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Literature

MARKHAM, R. and SMITH, K. M. (1949): Studies on the virus of turnip yellow mosaic. Parasitology *39*, 330—342.

MARTINI, C. (1958): The transmission of turnip viruses by biting insects and aphids. Proc. 3rd Conf. Potato Virus Diseases, Lisse Wageningen 1957, 106—113.

PROESELER, G. (1971): Übertragung des Wasserrübengelbmosaik -Virus (turnip yellow mosaic virus) durch Insekten mit beißenden Mundwerkzeugen. Arch. Pflanzenschutz *7*, 391—397.

SHUKLA, D. D. and SCHMELZER, K. (1970a): Studies on viruses and virus diseases of cruciferous plants. I. Viruses in some ornamentals. Acta phytopath. Acad. Sci. hung. *5*, 137—144.

SHUKLA, D. D. and SCHMELZER, K. (1970b): Studies on viruses and virus diseases of cruciferous plants. II. Tomato black ring virus in a weed and in some ornamentals. Acta phytopath. Acad. Sci. hung. *5*, 145—149.

SHUKLA, D. D. and SCHMELZER, K. (1972): Studies on viruses and virus diseases of cruciferous plants. IV. The previously undescribed *Erysimum* latent virus. Acta phytopath. Acad. Sci. hung. *7*, 157—167.

SHUKLA, D. D. and SCHMELZER, K. (1973): Studies on viruses and virus diseases of cruciferous plants. XIII. Cabbage black ring, nasturtium ringspot and alfalfa mosaic viruses in ornamental and wild species. Acta phytopath. Acad. Sci. hung. *8*, 139—148.

SHUKLA, D. D. and SCHMELZER, K. (1974): Studies on viruses and virus diseases of cruciferous plants. XVII. Serologically distinct strains of turnip yellow mosaic virus occurring in the GDR. Acta phytopath. Acad. Sci. hung. *9*, 237—246.

SHUKLA, D. D., PROLL, E., SCHMELZER, K. and SCHMIDT, H. B. (1973): Studies on viruses and virus diseases of cruciferous plants. VIII. Purification, properties and serology of *Erysimum* latent virus. Acta phytopath. Acad. Sci. hung. *8*, 91—103.

ŠTEFANAC, Z. and MAMULA, D. (1971): A strain of radish mosaic virus occurring in turnip in Yugoslavia. Ann. appl. Biol. *69*, 229—234.

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Studies on Viruses and Virus Diseases of Cruciferous Plants

XIX. ANALYSIS OF THE RESULTS OBTAINED WITH ORNAMENTAL AND WILD SPECIES

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During a period of two and a half years, 510 samples of ornamental and wild cruciferous plants were investigated for the presence of sap transmissible viruses. The material was collected in 12 botanical gardens or plantations distributed in the main regions of the GDR. Altogether 191 plant species or varieties, belonging to 58 genera, were tested. In 253 samples, taken from 129 species, viruses were found. In total 209 virus-host combinations could be distinguished, 185 of them were reported for the first time. The following 10 viruses were isolated which are transmitted in nature by aphids, nematodes and beetles, respectively: cucumber mosaic, cabbage black ring, tomato black ring, turnip yellow mosaic, *Erysimum* latent, broad bean wilt (syn. nasturtium ringspot), arabis mosaic, radish mosaic, alfalfa mosaic, and raspberry ringspot viruses. The sequence corresponds with the decreasing number of ornamental and wild crucifer species found to be spontaneously infected. Rather often several viruses were isolated together in one sample. Thirty-nine species contained more than one virus simultaneously. In all, 16 types of combined infections were found. Two viruses participated in 33, three in 13 and four in 2 combined or mixed infections. It was clearly demonstrated that in regions with light soils NEPO viruses (mostly tomato black ring virus) are dominating, whereas in regions with heavy soils and high aphid populations the aphid transmissible viruses are prevailing in ornamental and wild crucifer species. The significance of the results is discussed with special regard to epidemiology of the isolated viruses.

While working on viruses and virus diseases of cruciferous plants during a period of two and a half years starting from the autumn of 1969 to the early spring of 1972, special attentions were paid on the viruses occurring in ornamental and wild species in the German Democratic Republic. There were two important reasons for the intensive investigation of this plant group. Firstly, the previous investigators working on this problem studied only those species intensively which showed pronounced disease symptoms, for example, mosaic and flower breaking in *Matthiola incana* R. Br. Therefore, very little and often nothing is known about the occurrence and distribution of viruses in plant species which do not show any symptom under natural conditions. However, the work of USCHDRAWEIT and VALENTIN (1959) as well as our own findings clearly indicated that symptomless

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ornamental and wild crucifers contain various economically important viruses. Secondly, the majority of the biennial and perennial as well as vegetatively propagated members of this plant group can play a role in the epidemiology of certain viruses when serving as bridge-hosts or reservoirs.

During the investigation interesting results were obtained. Most of the investigated ornamental and wild cruciferous species was found to harbour various viruses at different places generally without showing symptoms. The names of the plant species and the viruses detected in them have already been reported in previous papers (SHUKLA and SCHMELZER, 1970a; 1970b; 1972a; 1973a; 1973b; 1973c 1974; and SHUKLA *et al.*, 1975). The major aim of the present article is to analyze these findings with regard to distribution and frequency of the isolated viruses in different regions of the country taking into consideration the soil and climate conditions prevailing there and the significance of the results in the epidemiology of the detected viruses. Various types of mixed or combined virus infections found during the studies and not discussed in the mentioned eight papers have also been included here. The first part of this paper deals with the plant species yielding no viruses in our investigation.

Sources of plant samples for virus isolation

The samples for virus assay were collected from the botanical gardens in Berlin-Baumschulenweg, Dresden, Eberswalde, Greifswald, Halle, Jena, Leipzig, and Rostock as well as from small plantations in the Zentralinstitut für Genetik und Kulturpflanzenforschung Gatersleben, Institut für Züchtungsforschung Quedlinburg, Institut für Phytopathologie Aschersleben and from a nursery for ornamental perennials near Potsdam. Two points were kept in mind while selecting these habitats for collection of the samples. Firstly, a large number of ornamental and wild crucifer species are concentrated there. Secondly, these places are rather well distributed over the GDR and represent the most important soil and climate conditions of that country.

Plant species yielding no viruses

Altogether 191 plant species or varieties, belonging to 58 genera of the *Cruciferae*, were investigated. From them 62 species, belonging to 36 genera and compiled in Table 1, did not yield virus, although 10 species displayed virus-like symptoms when collected. Seventeen of the species, yielding no virus, were tested from more than one place. Besides of real absence of viruses, unsuccessful trials may be due to the presence of strong inhibitors, low virus content or viruses which are not demonstrated by the used indicator plants. During the isolation experiments patches of dead tissues on leaves of test plants, like *Nicotiana megalosiphon* Heurck et Muell., *N. tabacum* L., and *Petunia hybrida* Vilm., were often observed one or

Table 1

Ornamental and wild crucifers yielding no viruses

Plant species	Places of collection
Aethionema iberideum (Boiss.) Boiss.	Jena
A. saxatile (L.) R. Br.	Leipzig
Alyssum arduinii Fritsch	Rostock
A. borzeanum Nyár.	Rostock
Anastatica hierochuntica L.	Greifswald
Arabis laxa Sibth. et Sm.	Dresden
A. pauciflora (Grimm) Garcke	Leipzig
A. pumila Jacq.	Eberswalde
A. x raetica Bruegg.	Dresden
A. turila L.	Jena*, Rostock
Biscutella didyma L.	Rostock
Calepina irregularis (Asso) Thell.	Halle*
Camelina sativa (L.) Crantz	Gatersleben, Halle*, Rostock
Cardamine trifolia L.	Potsdam
Cardaria draba (L.) Desv.	Jena*, Leipzig*
Cheiranthus alpinus Jacq.	Jena
Clipiola ionthlaspi L.	Rostock
Conringia orientalis (L.) Dumort.	Gatersleben, Halle, Rostock
Coronopus didymus (L.) Sm.	Rostock
Crambe cordifolia Stev.	Halle, Potsdam
C. grandiflora DC.	Leipzig
C. tataria Sebeok	Leipzig, Rostock
Draba argaea Kotschy	Rostock
D. armata Schott, Nym. et Kotschy	Leipzig
D. brunniifolia Stev.	Dresden, Halle
D. compacta Kotsch.	Jena, Rostock
D. dedeana Boiss. et Reut.	Leipzig
D. elongata Host	Rostock
D. haynaldii Stur.	Greifswald, Rostock
D. incana L.	Halle, Jena
D. magellanica Lam.	Greifswald*
D. oxycarpa Boiss. et Heldr.	Gatersleben, Halle
D. rigida Willd.	Gatersleben
D. tomentosa Wahlenb.	Halle, Rostock
Erucastrum gallicum (Willd.) O. E. Schulz	Jena
Erysimum aurantiacum Leyb.	Dresden*
E. linariifolium Tausch	Jena
Heliophila longifolia DC.	Rostock
Hesperis lutea Maxim.	Rostock
Hirschfeldia incana (L.) Lag.-Foss.	Greifswald
Iberis sempervriens var. *garrexiana* (All.) Cesati	Leipzig*
I. taurica DC.	Greifswald
Isatis glauca Gilib.	Leipzig
Lepidium graminifolium L.	Dresden
L. perfoliatum L.	Halle

Plant species	Places of collection
L. ruderale L.	Greifswald
L. sativum L.	Gatersleben, Jena
Lunaria rediviva L.	Berlin, Eberswalde, Jena, Leipzig
Malcolmia maritima (L.) R. Br.	Greifswald
Matthiola bicornis (Sibth. et Sm.) DC.	Rostock
Myagrum perfoliatum L.	Halle
Neslia paniculata Desv.	Halle
Ptilotrichum spinosum (L.) Boiss.	Greifswald
Raphanus caudatus	Jena*
Rapistrum perenne (L.) All.	Jena, Leipzig
Sisymbrium austriacum Jacq.	Leipzig*
S. strictissimum L.	Greifswald, Jena*, Leipzig, Rostock
Thlaspi alpestre L.	Jena, Rostock
T. densiflorum Boiss. et Kotsch.	Jena
T. stylosum (Ten.) Rchb.	Jena
Turritis glabra L.	Rostock
Vella spinosa Boiss.	Jena

* Plants on the marked places showed virus-like symptoms

two days after inoculation indicating cell damaging activities of the sap from certain ornamental and wild crucifers. The inoculum from few of the species, mentioned in Table 1, induced local lesions on *Chenopodium* spp., but in further trials viruses could not be recovered from them. Eleven species mentioned in Table 3, viz. *Camelina sativa*, *Conringia orientalis*, *Erucastrum gallicum*, *Lepidium ruderale*, *L. sativum*, *Malcolmia maritima*, *Matthiola bicornis*, *Neslia paniculata*, *Rapistrum perenne*, *Raphanus caudatus*, and *Sisymbrium austriacum* are known from literature to be susceptible to viruses (KLINKOWSKI *et al.*, 1968). Especially *Malcolmia maritima* is worth mentioning, because it has been shown to be a host of at least 11 different viruses.

Isolated viruses and the analysis of investigated samples

Ten different viruses, viz. cabbage black ring virus (CBRV), cucumber mosaic virus (CMV), broad bean wilt virus (BBWV, formerly named nasturtium ringspot virus), alfalfa mosaic virus (AMV), tomato black ring virus (TBRV), arabis mosaic virus (ArMV), raspberry ringspot virus (RRSV), radish mosaic virus (RMV), turnip yellow mosaic virus (TYMV), and *Erysimum* latent virus (ELV), were isolated and identified from ornamental and wild crucifers during the course of the studies. The first four are aphid transmissible, the following three NEPO and the last three are beetle transmissible viruses. From 129 (= 67.5 per cent) of the investigated species the mentioned viruses were obtained. From the botanical gardens of Berlin-Baumschulenweg, Dresden, Halle, Jena, and Leipzig the ma-

terials were collected two to three times. In this way, often the same species from one place was investigated repeatedly and mostly the same viruses were isolated from them. Additionally, often more than one sample from one species were collected in the same botanical garden because it was present at various locations. Therefore, the number of investigated samples was 510. Viruses were detected in 253 of them (about 50 per cent). The frequency of successful isolations did not differ very much between the habitats as it ranged from 37.9 to 60.7 per cent. Only Berlin-Baumschulenweg was an exception. About 80 per cent of the tested samples from there yielded virus isolates (Table 2).

Table 2

Frequency of virus-containing samples from ornamental and wild crucifers at different locations

Habitat	Number of investigated samples	Samples containing viruses	
		Number	Percentage
Rostock	58	22	37.9
Greifswald	35	16	45.7
Eberswalde	28	17	60.7
Berlin	31	25	80.6
Potsdam	37	18	48.6
Gatersleben	15	6	40.0
Aschersleben, Quedlinburg	25	10	40.0
Halle	57	32	56.1
Leipzig	80	37	46.2
Jena	83	34	40.9
Dresden	61	36	59.0
Total	510	253	49.2

It is interesting that in our investigations certain viruses were generally restricted to some particular genera of the *Cruciferae*, for instance ArMV and TYMV to *Arabis* and *Draba*, RMV and ELV to *Erysimum*. The only host of RRSV was *Iberis saxatilis*. BBWV and AMV have obviously rather limited natural host ranges among ornamental and wild crucifers, at least in the GDR.

Mixed or combined infections

Out of the 253 samples from ornamental and wild crucifers inducing virus infections, 49 (20 per cent) were found to contain more than one virus. They represent 47 different virus host combinations, two were found twice, varying only in the place of collection. The results are summarized in Table 3. Thirty-nine plant species were the sources giving rise to 16 types of virus combinations. Two viruses were involved in 32, three viruses in 13 and four viruses in two virus host com-

Table 3

Mixed or combined virus infections

	CBRV + CMV	CBRV + TBRV	CBRV + TYMV	CMV + BBWV	CMV + TBRV	CMV + TYMV
Alliaria petiolata (= A. officinalis)	H					
Alyssoides utriculatum	J*					
Arabis androsacea						
A. blepharophylla						J
A. caucasica		E				
A. jacquinii			H*		E	
A. ludoviciana						
A. muralis	J*					
A. scopoliana						D
Aubrieta olympica	E					
Barbarea intermedia					B	
B. vulgaris					G	
Berteroa incana	D*					
Biscutella lyrata						
Brassicella erucastrum	L*					
Cakile maritima						
Capsella grandiflora	H*					
Cardamine pratensis					B	
Cheiranthus cheiri						
Cochlearia officinalis	H*				B*	
Eruca sativa	L					
Erysimum crepidifolium						
E. hieraciifolium						
E. odoratum	L					
E. perovskianum						
E. silvestre						
Hesperis matronalis	H*, J*					
H. steveniana	D*					
H. tristis	J					
Hutchinsia alpina					E	
Iberis amara				H		
I. umbellata						
Lobularia maritima	J, L					
Lunaria annua						
Malcolmia bicolor						
Raphanus raphanistrum	L*					
Schivereckia podolica						D*
Thlaspi montanum					B	
Vesicaria utriculata	H*					

Explanations: B = Berlin D = Dresden E = Eberswalde G = Greifswald H = Halle J = Jena

in ornamental and wild crucifer species

CMV + RMV	CMV + ELV	RMV + ELV	CBRV + CMV + BBWV	CBRV + CMV + TBRV	CBRV + TBRV + ArMV	CBRV + TYMV + ELV	CBRV + RMV + ELV	CMV + BBWV + ELV	CBRV + CMV + BBWV + AMV
			B		H				
						D*			
	H		H*	B					
			L	B					
J							D*	H	
		D		R					
			H L	B					H* L

L = Leipzig R = Rostock * indicates that the plants showed symptoms

binations. CBRV was found in 32 types of mixed isolates, CMV in 41, BBWV in nine, AMV in two, TBRV in 13, ArMV in one, RMV in three, and TYMV and ELV in five types each.

The most frequent combinations were CBRV + CMV, CMV + TBRV, and CBRV + CMV + BBWV. They were detected in 16, 7 and 5 plant species. The other types were found one to four times. BBWV and AMV were isolated only in combined infections. In contrast to this, RRSV was the only virus detected in its host always singly. In one plant species three types of combined viruses were isolated, in six two types, and in the remaining 32 species only one type.

Mixed virus infections in ornamental and wild crucifers may be indeed very common, but our results given in Table 3 are not always the consequence of mixed infections. Leaves from one species at one place were often collected from several plants growing in groups and the whole material was treated as one sample. However, this cannot be true in all the cases. Rather often only one plant was present at one location and in spite of this more than one virus was isolated.

Relationships between virus types and their habitats

Due to combined isolation of viruses the number of virus isolates, summarized in Table 4, is higher than the number of virus-containing samples mentioned in Table 2. It can be seen that CMV was the most frequent virus followed by CBRV and TBRV. The other viruses were obtained in rather low percentages.

Of special interest is the demonstration of a close connection between habitat and prevailing occurrence of certain viruses in ornamental and wild crucifers. However, in this respect there can be considered only the viruses which are transmissible by aphids and nematodes, respectively. The beetle transmissible viruses were thoroughly recognized only in the last stage of the investigations. The want of their antisera was the main reason for this. After having obtained them the beetle transmissible viruses were isolated from samples collected in six botanical gardens within a rather short time. It is possible that they are present at other places, too.

Fig. 1 shows a map of the GDR in which the results of our investigation are recorded and, in a simplified form, the soil types after MATZ (1956) and the intensity of virus infections in potatoes after PFEFFER (1956). The latter property of a region depends from number and activity of aphids. On the other hand, the aphids depend from the climate.

It can be seen that in the north-eastern part of the GDR with light, alluvial or diluvial soils, nematode transmissible viruses with polyhedral particles (NEPO viruses) have a big share in the infestation of ornamental and wild crucifers in botanical gardens and plantations, whereas on loess and weathered mountain soils in the south-western part they are seldom. The reason for that seems to be the well-known fact that the nematode genus *Longidorus*, which is responsible for the trans-

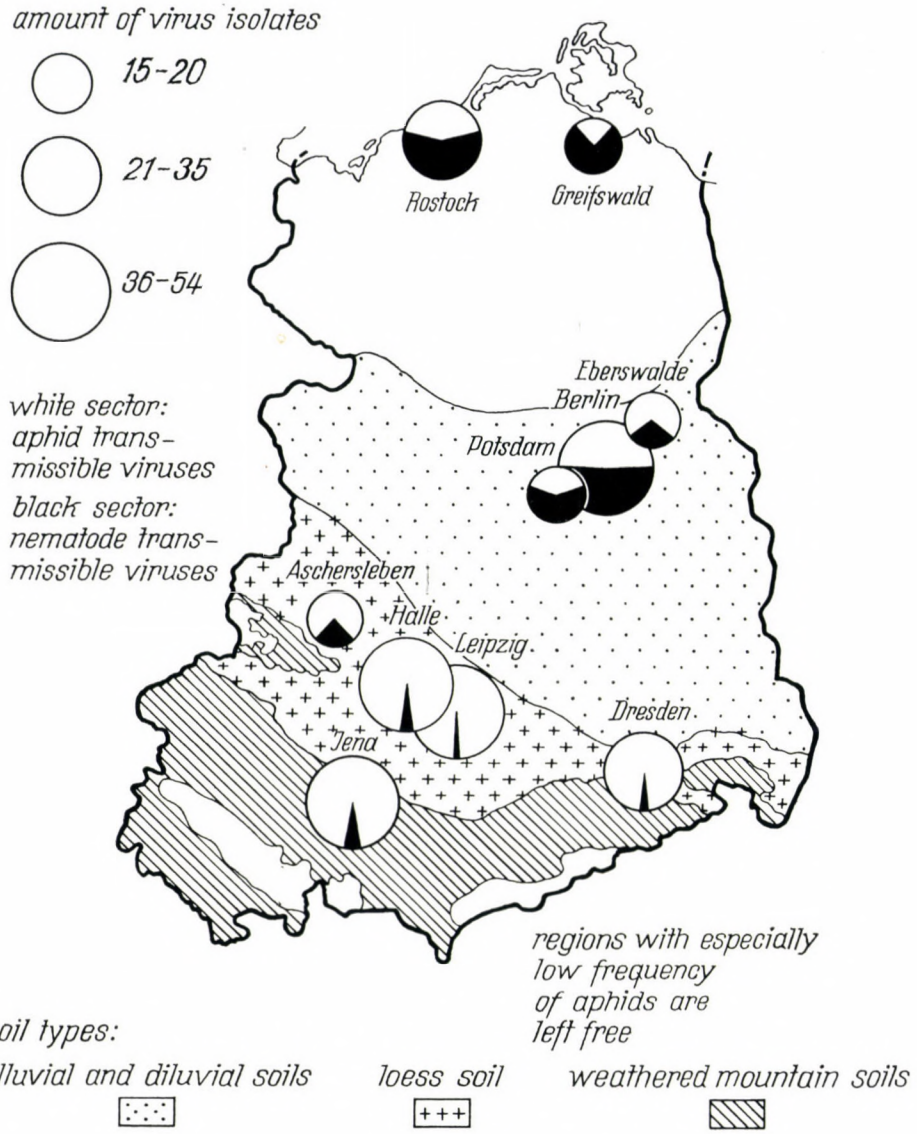

Fig. 1. A map of the GDR demonstrating the habitats of ornamental and wild crucifers investigated, types of viruses isolated, types of soil, and frequency of aphids

mission of TBRV and RRSV, occur only in light soils. In the south-western part of the GDR with heavy soils, the share of aphid transmissible viruses is absolutely predominating. In the middle and southern part of the country the climate enables the aphids to be very active and to increase strongly. Due to light soils and high

Table 4

Kind and number of virus isolates from ornamental and wild crucifers at different locations

Habitat	Aphid transmissible viruses				Nematode transmissible viruses			Beetle transmissible viruses		
	CBRV	CMV	BBWV	AMV	TBRV	ArMV	RRSV	TYMV	RMV	ELV
Rostock	4	5			12			1		
Greifswald		4			13					
Eberswalde	3	9			5			1		1
Berlin	5	13	1		19		1			
Potsdam		7			10		1			
Gatersleben	2	1			1	1	1			1
Aschersleben, Quedlinburg	7	2				1				
Halle	18	21	4	1	1	1		2		3
Leipzig	20	28	4	1		1				
Jena	13	21			1	1		4	1	
Dresden	11	18			1			6	3	5
Total	83	129	9	2	63	5	3	14	4	10

aphid populations there is a region in the middle part of the GDR where both NEPO and aphid transmissible viruses are rather frequent in ornamental and wild crucifers.

Among the investigated places only two yielded unexpected results, but these can be explained easily. The relatively high percentage of infections by NEPO viruses in the region of Aschersleben is in part due to plant import from light soil, a practice not met with other investigated habitats. Additionally, in Aschersleben as well as in other investigated places with heavy soils, ArMV was present. The vector genus of it, *Xiphinema*, is frequent in heavy soils. The other habitat with deviating results is Potsdam. Obviously the rather low number of tested samples in the first survey gave misleading results. The big share of NEPO virus infections was not found in trials performed two years later.

Discussion

It seems that our investigations on viruses in ornamental and wild cruciferous plants, reported in the parts XIII to XVIII of this series and in an additional paper (SHUKLA and SCHMELZER, 1973c), give the most extensive example of an exact virus identification within a well-defined plant group which is known until now. In all, we reported 209 different naturally occurring virus host combinations in 129 plant species or varieties, 185 combinations were reported for the first time. Ten viruses were involved. The total number of natural hosts including the new

hosts (written in brackets) for each virus is: CBRV – 45 (31), CMV – 81 (72), BBWV – 8 (8), AMV – 2 (1), TBRV – 44 (44), ArMV – 4 (4), RRSV – 1 (1), RMV – 3 (3), TYMV – 13 (13), ELV – 8 (8). Because a considerable amount of the investigated ornamental and wild crucifers are perennials and may be propagated vegetatively, the detection of such a large number of virus host combinations is not surprising. Nevertheless, besides the mentioned ones, many more viruses may occur in ornamental and wild crucifers in the GDR. In future not only ELV, but also other unknown viruses may be found in them.

The demonstrated connections between virus types on the one hand and soil types and frequency of aphids on the other in different regions of a country may not only be met with ornamental and wild crucifers but also with members of other plant families, if extended investigations will be undertaken. These connections seem to be both of academic and practical interest. They help to understand epidemiology and open possibilities for the control of viruses.

We were not able to isolate cauliflower mosaic (ClMV) and tobacco rattle (TRV) viruses which have been found to infect different ornamental and wild species of *Cruciferae* in earlier papers (USCHDRAWEIT and VALENTIN, 1956; BROADBENT, 1957). Also turnip crinkle and turnip rosette (TRoV) viruses could not be isolated in our investigations. Until now, however, these two viruses are reported to infect in nature only cruciferous crops and no ornamental or wild species (BROADBENT and HEATHCOTE, 1958). If test plants are used, which are suitable for each of them and if some measures are adopted against strong inhibitors in sap of crucifers, the chances of virus isolation may remarkably be increased. Except TRoV, all known beetle transmissible crucifer viruses can be isolated successfully using the easily available and quickly growing *Sinapis alba* L. This species shows pronounced symptoms also after infections with CBRV and ClMV. But for these two viruses cauliflower can also be used with satisfying results. *Nicotiana tabacum* cv. 'Samsun', which shows typical brown local lesions after infection with CBRV, is also a good test plant for TRV. For the isolation of CMV, BBWV, AMV and the mentioned NEPO viruses, *Nicotiana megalosiphon* and *Chenopodium quinoa* are very suitable as has already been proved by SCHMELZER (1970). Additionally, the two species are also adapted for cross protection and serological tests, respectively.

As already mentioned in our earlier papers, the majority of the naturally infected ornamental and wild crucifers was symptomless when collected in their habitats. The symptom-showing species contained mostly CBRV and/or CMV. From these findings it can be supposed that most virus infections cause little damage in this plant group. On the other hand, our results clearly indicate that the ornamental and wild crucifers are of importance for the epidemiology of certain diseases as they proved to be hosts of different viruses which induce serious diseases not only in economically important crucifers but also in plants of other families. Out of aphid transmissible viruses CBRV is especially significant for various brassica crops (SHUKLA and SCHMELZER, 1972b). CMV, BBWV, and AMV are known to cause severe diseases in representatives of many families. Perhaps

with the exception of horseradish, crucifers seem not to be damaged by the three NEPO viruses, but from epidemiological point of view the rather frequent infestation of ornamental and wild crucifers with TBRV in some regions should be considered as a danger for susceptible and heavily damaged plants belonging to other families.

On the whole it can be assumed that the virus work on ornamental and wild crucifer species gave new vistas especially in natural host ranges and geography of viruses. Further interesting results may come from more intensive research on epidemiology and geography of beetle transmissible viruses occurring in ornamental and wild crucifers.

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Literature

BROADBENT, L. (1957): Investigation of Virus Diseases of Brassica Crops. Cambridge University Press, 94 p.

BROADBENT, L. and HEATHCOTE, G. D. (1958): Properties and host range of turnip crinkle, rosette and yellow mosaic viruses. Ann. appl. Biol. *46*, 585—592.

KLINKOWSKI, M. and Co-workers (1968): Pflanzliche Virologie, Band II, second edition, Akademie-Verlag, Berlin.

MATZ, R. (1956): Agrar-Atlas über das Gebiet der Deutschen Demokratischen Republik Gotha, Haack.

PFEFFER, C. (1956): Untersuchungen über den Wert der in verschiedenen Gebieten erzeugten Pflanzkartoffeln. Züchter *26*, 257—269.

SCHMELZER, K. (1970): Untersuchungen an Viren der Zier- und Wildgehölze. VII. Mitt. Weitere Befunde an *Buddleja*, *Viburnum*, *Caryopteris* und *Philadelphus* sowie Viren an *Leycesteria*, *Chionanthus*, *Ribes*, *Hydrangea*, *Syringa*, *Spiraea* und *Catalpa*. Phytopath. Z. *67*, 285—326.

SHUKLA, D. D. and SCHMELZER, K. (1970a): Studies on viruses and virus diseases of cruciferous plants. I. Viruses in some ornamentals. Acta phytopath. Acad. Sci. hung. *5*, 137—144.

SHUKLA, D. D. and SCHMELZER, K. (1970b): Studies on viruses and virus diseases of cruciferous plants. II. Tomato black ring virus in a weed and in some ornamentals. Acta phytopath. Acad. Sci. hung. *5*, 145—149.

SHUKLA, D. D. and SCHMELZER, K. (1972a): Studies on viruses and virus diseases of cruciferous plants. IV. The previously undescribed *Erysimum* latent virus. Acta phytopath. Acad. Sci. hung. *7*, 157—167.

SHUKLA, D. D. and SCHMELZER, K. (1972b): Studies on viruses and virus diseases of cruciferous plants. VII. Occurrence and effects of cabbage black ring and cauliflower mosaic viruses on brassica crops. Acta phytopath. Acad. Sci. hung. *7*, 325—342.

SHUKLA, D. D. and SCHMELZER, K. (1973a): Studies on viruses and virus diseases of cruciferous plants. XIII. Cabbage black ring, nasturtium ringspot and alfalfa mosaic viruses in ornamental and wild species. Acta phytopath. Acad. Sci. hung. *8*, 139—148.

SHUKLA, D. D. and SCHMELZER, K. (1973b): Studies on viruses and virus diseases of cruciferous plants. XIV. Cucumber mosaic virus in ornamental and wild species. Acta phytopath. Acad. Sci. hung. *8*, 149—155.

SHUKLA, D. D. and SCHMELZER, K. (1973c): Occurrence of turnip yellow and radish mosaic viruses in the German Democratic Republic on previously unknown cruciferous hosts. Zbl. Bakt. Abt. II. *128*, 81—83.

SHUKLA, D. D. and SCHMELZER, K. (1974): Studies on viruses and virus diseases of cruciferous plants. XV. NEPO viruses in ornamental and wild species. Acta phytopath. Acad. Sci. Hung. *9*, 219—225.

SHUKLA, D. D., PROESELER, G. and SCHMELZER, K. (1975): Studies on viruses and virus diseases of cruciferous plants. XVIII. Beetle transmission and some new natural hosts of *Erysimum* latent virus. Acta phytopath. Acad. Sci. Hung. *10*, 211—215.

USCHDRAWEIT, H. A. and VALENTIN, H. (1956): Das Tabakmauchevirus an Zierpflanzen. Nachrbl. Dt. Pflanzenschutzdienst (Braunschweig) *8*, 132—133.

USCHDRAWEIT, H. A. and VALENTIN, H. (1959): Untersuchungen über Kultur- und Wildstauden als Zwischenwirte für wirtschaftlich wichtige Viren. Nachrbl. Dt. Pflanzenschutzdienst (Braunschweig) *11*, 89—92.

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Der Rauhhaarige Fuchsschwanz (*Amaranthus retroflexus* L.), ein wichtiges Reservoir des Gurkenmosaik-Virus (cucumber mosaic virus) in der Ungarischen Volksrepublik

Von

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By mechanical transmissions and serological investigations could be demonstrated that mosaic diseased *Amaranthus retroflexus* are infected with cucumber mosaic virus. The wide distribution of this weed, which is often colonized by aphids, and the frequency of the virus symptoms indicate the importance of *Amaranthus retroflexus* as a source of the mentioned virus in Hungary.

Der aus Nordamerika stammende Rauhhaarige Fuchsschwanz hat in weiten Teilen Europas Verbreitung erlangt. In Ungarn stellt er in vielen Gegenden eines der häufigsten Ackerunkräuter dar.

Auf der Suche nach Reservoiren der an Cucurbitaceen auftretenden Viren fanden wir Ende Juli 1973 in der Gegend von Hatvan in und am Rande mehrerer Kürbis-, Gurken-, Melonen- und Paprikafelder zahlreiche Pflanzen des Rauhhaarigen Fuchsschwanzes, die ein Mosaik aus dunkelgrünen sowie hellgrünen, zum Teil sogar graugelblichen oder grauweißlichen Blattbezirken und Verbeulungen sowie Umrißveränderungen der Blätter zeigten. Die betroffenen Pflanzen blieben deutlich kleiner, vor allem, wenn ihre Infektion im Jugendstadium erfolgt war. Die dunkelgrünen Gewebepartien zogen sich meist längs stärkerer Adern als unregelmäßig geformte Flecke und Streifen hin, sie waren auch bevorzugt an der Blattbasis vorhanden (Abb. 1, A—D). Am späteren Zuwachs konnte die Symptomausprägung zurückgehen, ohne jedoch völlig zu verschwinden (Abb. 1, E). Die kranken Pflanzen blühten und fruchteten normal, die Samenmenge war allerdings geringer als bei gesunden Pflanzen.

Von verschiedenen Feldern wurden insgesamt 11 Pflanzen mit typischen Symptomen ausgewählt und einzeln virologisch untersucht. Abreibungen ihrer zusammen mit Phosphatpuffer pH 8 und Aktivkohle hergestellten Blattbreie auf Testpflanzensortimente, bestehend aus *Celosia argentea* L., *Chenopodium amaranticolor* Coste et Reyn., *C. foetidum* Schrad., *C. murale* L., *Cucumis sativus* L., *Gomphrena globosa* L., *Nicotiana glutinosa* L. und *N. megalosiphon* Heurck et Muell., ergaben in jedem Fall hundertprozentige Infektionserfolge. Stets deuteten alle Reaktionen auf das Vorliegen des Gurkenmosaik-Virus (cucumber mosaic virus) hin. Serologische Prüfungen bestätigten diese Diagnose. In sämtlichen 11

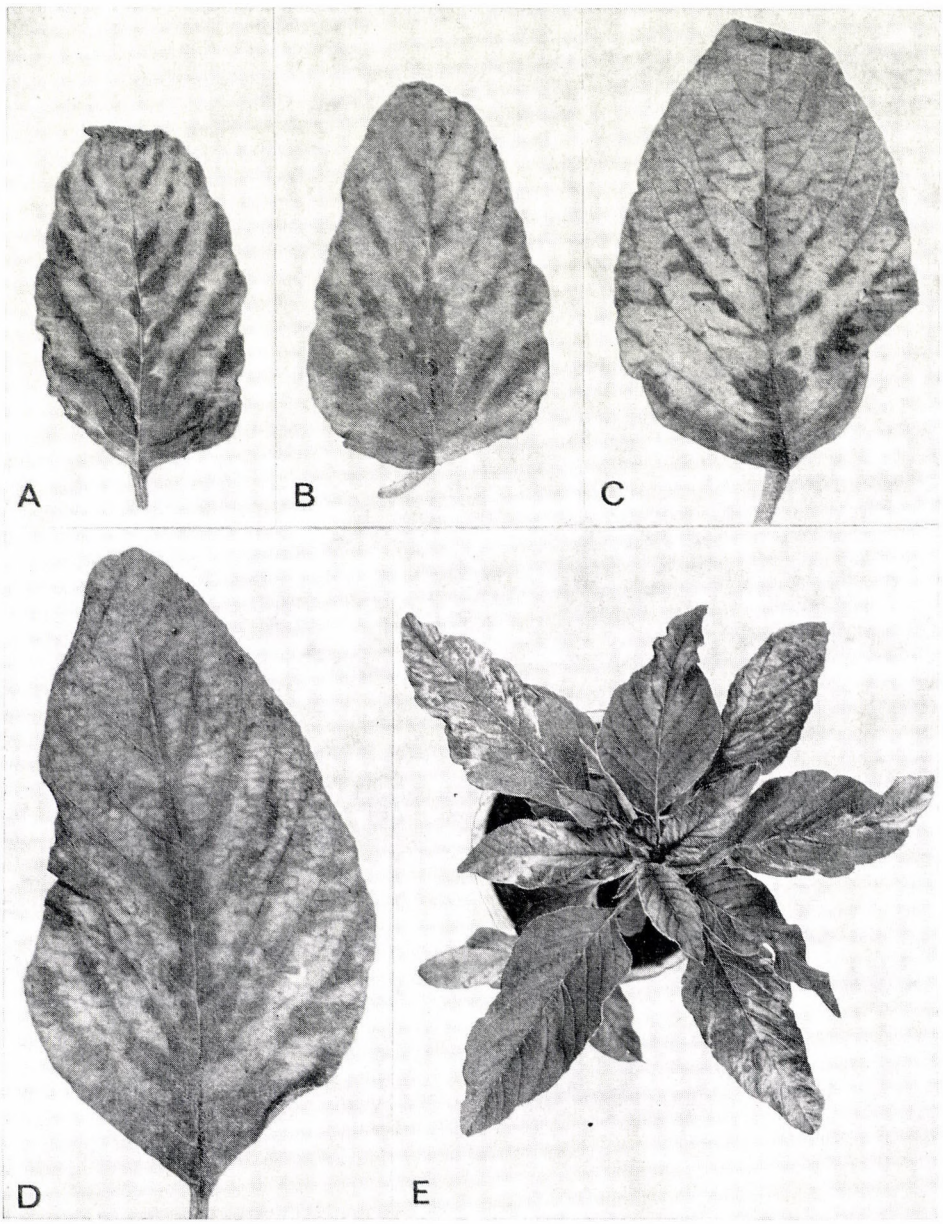

Abb. 1. Symptome des Gurkenmosaik-Virus an spontan im Freiland infizierten *Amaranthus retroflexus*. A—D: Blätter jung infizierter Pflanzen; E: vier Wochen im Gewächshaus weiterkultivierte Pflanze

Pflanzen lag das GMV in derjenigen Form vor, die insbesondere auf *Nicotiana*-Arten ein starkes Mosaik sowie ausgeprägte Umrißveränderungen der Blätter bewirkt und in Ungarn weit verbreitet ist. Bemühungen, zusätzlich andere Viren in den Isolaten zu ermitteln, verliefen ergebnislos. Durch Rückinfektionen auf junge *Amaranthus*-Sämlinge im Gewächshaus konnte das Symptombild mit einem der 11 Isolate reproduziert werden, das zufällig ausgewählt worden war.

Zusammen mit Feldbeobachtungen weisen die beschriebenen Versuche darauf hin, daß *Amaranthus retroflexus* in Ungarn sehr häufig vom GMV befallen ist und ein bedeutungsvolles Reservoir des Virus darstellt. Der Rauhhaarige Fuchsschwanz ist vielfach von Blattläusen besiedelt, die zweifellos bei der Ausbreitung des GMV eine große Rolle spielen. Aus diesen Tatsachen geht die Wichtigkeit der rechtzeitigen, gründlichen und wiederholten Unkrautvernichtung für die vorbeugende Virusbekämpfung in landwirtschaftlichen und gärtnerischen Kulturen sowie in deren unmittelbarer Nachbarschaft hervor.

Soweit uns bekannt ist, wurden in Europa an *Amaranthus*-Arten bisher folgende Viren in Spontaninfektion gefunden: das Rübenvergilbungs-Virus (beet yellows virus) in der UdSSR (GORJUŠIN, 1964), das GMV in der UdSSR und in der Schweiz (DAŠKEEVA und Mitarb., 1966; HÄNI, 1971), es handelte sich dabei stets um *Amaranthus retroflexus*. In Spanien fand man ein noch nicht näher bestimmtes Virus an *Amaranthus lividus* (RUBIO-HUERTOS und VELA-CORNEJO, 1966).

Herrn Dr. sc. J. RICHTER (Aschersleben) danken wir für die Überlassung des GMV-Antisefums. Die Abbildungen stellte Fräulein H. C. NORDMANN her.

Zusammenfassung

Durch mechanische Übertragungen und serologische Untersuchungen ließ sich nachweisen, daß mosaikkranke *Amaranthus retroflexus* vom Gurkenmosaik-Virus befallen sind. Die weite Verbreitung dieses viel von Blattläusen besiedelten Unkrautes und die Häufigkeit des Symptombildes weisen auf die Bedeutung von *Amaranthus retroflexus* als Reservoir des erwähnten Virus in Ungarn hin.

Literatur

DAŠKEEVA, K. N., SPATARENKO, S. S. und VETROVA, F. M. (1966): O roli sornjakov v sochranenii i rasprostranenii virusa ogurečnoj mozaiki. Infekcionnye zabolevanija kul'turnych rastenij Moldavii *6*, 22–27.

GORJUŠIN, V. A. (1964): Serologičeskoje izučenije želtuchi sacharnoi svekly i ejo vozbuditelja. Doklady TSChA *107*, 133–152.

HÄNI, A. (1971): Zur Epidemiologie des Gurkenmosaikvirus im Tessin. Phytopath. Z. *72*, 115–144.

RUBIO-HUERTOS, M. und VELA-CORNEJO, A. (1966): Light and electron microscopy of virus inclusions in *Amaranthus lividus* cells. Protoplasma *72*, 184–193.

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Mosaic Disease of *Peristrophe bicalyculata* Nees

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The symptoms, host range, physical properties and method of transmission of a virus causing mosaic disease of *Peristrophe bicalyculata* have been described. It constitutes the first record of a virus disease on *P. bicalyculata* in India.

Peristrophe bicalyculata Nees., an erect hispid herb in cultivated fields, has been found to be affected with a disease the symptoms of which suggested its possible viral nature. The symptoms of the disease as observed in nature comprise of characteristic mosaic of leaves (Fig. 1A, B) with reduction in size in severe instances. There are, however, no symptoms on flowers. This plant is a common weed distributed throughout India. It used as fodder for horses and as green manure in Southern India. Besides, the plant macerated in an infusion of rice is regarded as a remedy for snake bites.

Successful transmission of the disease was obtained with sap extracted from leaves in 0.1 *M* phosphate buffer of pH 7.0. To study the host range of the causal virus, plants belonging to eight families were inoculated with the standard extract

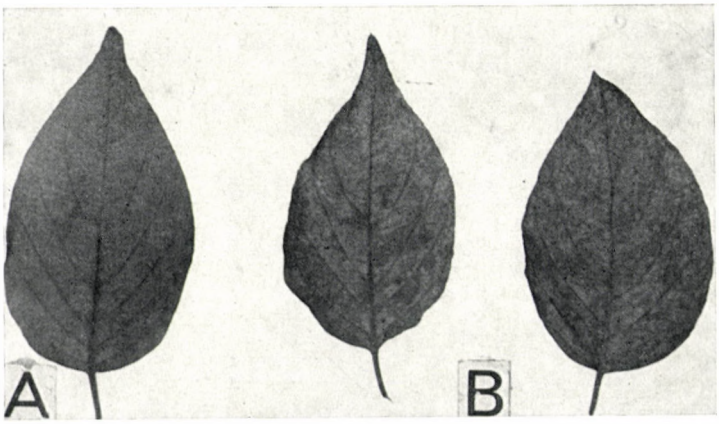

Fig. 1. a) Healthy leaf of *Peristrophe bicalyculata;* b) Diseased leaves of *Peristrophe bicalyculata*, showing mosaic symptoms

prepared from infected leaves of *P. bicalyculata*. The virus produced necrotic local lesions on the inoculated leaves of *Chenopodium* amaranticolor Coste & Reyn. The following plant species were not susceptible:

Acanthaceae:	*Barleria preonities* L.
	Justicia diffusa Willd.
Aizoaceae:	*Trianthema portulacastrum* L.
Compositae:	*Zinnia elegans* Jacq.
Cruciferae:	*Raphanus sativus* L.
Leguminosae:	*Crotalaria juncea* L.
	Vigna sinensis (Torner) Savi.
Malvaceae:	*Hibiscus esculentus* L.
Solanaceae:	*Datura stramonium* L.
	Lycopersicon esculentum Mill.
	Nicotiana glutinosa L.
	N. tabacum L. cv. Harrison's special, White Burley and Xanthi.
	Solanum melongena L.
	S. nigrum L.

The virus was transmitted by *Aphis gossypi* Glov. in a non-persistent manner but not by *A. craccivora* Koch. and *Rhopalosiphum maidis* Fitch.

The virus was non-infective when the infections sap was diluted more than 1 : 100. The virus was completely inactivated by heating the infectious sap for 10 minutes at 70°C but not at 65°C.

A perusal of literature reveals that there is no record of any virus disease on *P. bicalyculata*. This, therefore, constitutes the first record of a virus disease on *P. bicalyculata*. The present virus is designated as "Peristrophe Mosaic Virus". Cryptogram: ⁎/⁎ ⁎/⁎ ⁎/⁎ S/Ap.

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Effect of *Solanum khasianum* Mosaic Virus on Certain Growth Characters and Alkaloid Content of its Host*

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Solanum khasianum mosaic virus infection causes significant reduction in the rate of increase of number of leaves as well as branches of *S. khasianum*. There was a highly significant reduction in the height of main shoot of the plants from the 2nd month onwards and also a significant reduction in number of fruits due to the virus infection. It also causes a highly significant reduction in the fresh and dry weight of the fruits at both pale yellow and yellow stages. The percentage of moisture content of the fruits of infected plants was found to be more whereas the glyco-alkaloid (solasonine) content was considerably reduced at both the stages of the fruits.

Solanum khasianum Clarke is medicinally important because its fruits contain a good amount of solasonine (a glyco-alkaloid) which is used as a starting material for the synthesis of cortisone and other steroid hormones. THAKUR and SASTRY (1971) observed a mosaic disease on this plant species and established its viral nature. Detailed studies on host range, physical properties, purification, serology and electronmicroscopy of the virus were studied by RANGARAJU and CHENULU (1974). Based on the above studies they considered the virus causing mosaic disease of *S. khasianum* as a new one and designated it as *Solanum khasianum* mosaic virus (SKMV). Since viral infections are known to bring in drastic physiological and biochemical changes in the infected hosts, a study of the effect of SKMV on certain growth characters such as rate of increase of leaves and branches, height of main shoot, fresh weight, dry weight, moisture content and glyco-alkaloid content of fruits of infected plants was made and the results are reported in this communication.

Materials and Methods

Growth characters. Healthy seedlings were raised by sowing seeds of *S. khasianum* in the nursery bed during third week of February, 1970 and a month later, 25 seedlings were transplanted at 5 per row of 3' × 3' apart in well prepared plots in the experimental field of Division of Mycology and Plant Pathology.

* This paper forms a part of Ph. D. thesis of the senior author, accepted by the Post-Graduate School, Indian Agricultural Research Institute, New Delhi 110012.

A week after the transplantation, the plants were inoculated with the standard extract from the leaves of *S. khasianum* infected with SKMV. Similarly twenty five healthy plants were maintained at one corner of the field to serve as control. The plants were sprayed fortnightly with 0.1% Ekatox in order to avoid aphid infestation. Observations on the growth characters, viz., number of leaves and branches per plant at weekly intervals, and height of the main shoot and number of fruits per plant at monthly intervals were recorded from the date of inoculation.

Dry weight and moisture content of fruits. Ten uniform fruits were harvested randomly at two different stages, namely, pale yellow and yellow from healthy and diseased plants. Each fruit was immediately packed in a numbered paper envelope and weighed to determine the fresh weight. Later the material was dried at 65°C in an oven till constant weight. Dry weight was recorded for each fruit. From the difference between fresh and dry weight, moisture content per fruit was determined and expressed as percentage of fresh weight of the fruit.

Glyco-alkaloid (solasonine) content. Uniform fruits were harvested randomly at two different stages namely, pale yellow and yellow from healthy and diseased plants. Each sample consisted of 50 g of fruits from healthy plants and 40 g or 20 g from the diseased plants. The method described by BELL and BRIGGS (1942) for the extraction and estimation of solasonine was followed. The fresh material was minced and extracted with ethanol in a Soxhlet apparatus. The ethanol in the extract was removed by distillation, an excess of 2% acetic acid was added and the contents were warmed over a water-bath for the complete removal of ethanol. The aqueous solution was boiled after filtration and the crude solasonine was precipitated by the addition of liquor ammonia in excess. The precipitate was purified by redissolving in 2% acetic acid and reprecipitated with ammonia when hot. The process of purification of the precipitate was repeated twice. Later the precipitate was dried at 60°C in an oven till constant weight. The glyco-alkaloid content was estimated on dry weight basis.

Results

The effect of viral infection on certain of the growth characters such as rate of increase in the number of leaves, and branches, and height of the main shoot was studied according to the details given in the materials and methods and the results are presented in Tables 1–3.

Number of leaves. From the results in Table 1 it is evident that viral infection caused a highly significant increase in the rate of production of leaves in the first two weeks following infection. Thereafter there was a decrease in the rate of production of leaves in infected plants. However, the rate of decrease in leaves in infected plants was significant/highly significant only in 3rd, 5th, 9th and 10th weeks.

Number of branches. Results in Table 2 reveal that there is no branch development up to the 4th week in both infected and healthy plants. There was a

Table 1

Mean rate of increase in number of leaves of healthy and infected plants at weekly intervals

Treatments	Number of weeks												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Control	1.7	1.4	1.6*	2.6	51.2*	37.4	97.5	101.9	107.5**	101.3*	96.2	44.2	27.4
Infected	2.4**	2.4**	1.0	2.6	35.8	32.7	83.3	80.1	77.5	76.8	73.0	44.4	26.4
t-value	4.239	2.688	2.121	.000	2.434	1.614	1.346	1.962	2.669	2.140	1.793	0.039	0.812

t-value (48 df) at 5% = 2.000 and above
at 1% = 2.666 and above
* significant; ** highly significant

Table 2

Mean rate of increase in number of branches of healthy and infected plants at weekly intervals

Treatments	Number of weeks												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Control	—	—	—	—	3.5	3.6*	2.7	13.7	17.8**	55.2**	56.5**	51.8*	56.9**
Infected	—	—	—	—	5.8**	2.4	3.4	11.7	12.4	33.9	32.9	31.6	22.5
t-value	—	—	—	—	4.538	2.646	0.907	1.267	2.780	3.634	3.759	2.307	4.296

t-value (48 df) at 5% = 2.000 and above;
at 1% = 2.666 and above
— = no branch; * significant; ** highly significant

Table 3

Mean height (cm) of the main shoot of healthy and infected plants at monthly intervals

Treatments	Number of months				
	1	2	3	4	5
Control	10.3*	28.6**	29.0**	29.0**	29.0**
Infected	12.5*	23.7	23.8	23.8	23.8
t-value	—	5.781	6.285	6.300	6.300

t-value (48 df) at 5% = 2.000 and above; at 1% = 2.666 and above
* initial height; **highly significant

Table 4

Mean number of fruits of healthy and infected plants at monthly intervals

Treatments	Number of months				
	1	2	3	4	5
Control	—	—	67.2**	85.8**	97.3**
Infected	—	—	1.4	4.7	5.9
t-value	—	—	8.154	9.276	9.490

t-value (48 df) at 5% = 2.000 and above; at 1% = 2.666 and above
— = no fruit formation; ** highly significant

significant increase in the number of branches in the infected plants in the 5th week. In general, the rate of increase in branching was significantly/highly significantly less in infected plants compared to healthy.

Height of the main shoot. As can be seen from Table 3, the viral infection in general caused a significant reduction in the height of the main shoot in all the observations except the 1st observation. The slight increase in height observed in the first month was, however, not significant.

Effect of viral infection on fruiting. The mean number of fruits per plant in infected and healthy plants is presented in Table 4. The results significantly indicate the devasting effect of disease on fruit formation.

Effect of viral infection on the glyco-alkaloid (solasonine), moisture content, dry weight and fresh weight of fruits. The glyco-alkaloid content of the fruits at two stages of development namely, pale yellow and yellow collected from healthy and infected plants was estimated as per the method described under 'Materials and Methods'. The results presented in Table 5 reveal that the glyco-alkaloid content was reduced by more than 50 per cent in fruits collected from infected

Table 5

Effect of virus infection on fresh weight, dry weight, percentage of moisture content and glyco-alkaloid content of the fruits under different stages of development

Stages of the fruit	Fresh weight per fruit in g		t-value	Dry weight per fruit in g		t-value	Percentage of moisture		Glyco-alkaloid content as % of dry weight		Difference in alkaloid content between healthy and infected
	Healthy	Infected		Healthy	Infected		Healthy	Infected	Healthy	Infected	
Pale yellow	3.517**	1.836	14.366	0.70**	0.33	14.376	79.8	82.1	4.51+	2.64+	1.87
Yellow	3.522**	1.551	11.405	0.72**	0.26	17.240	79.5	83.2	4.15+	2.22+	1.93

t-value (48 df) at 5% = 2.000 and above
at 1% = 2.666 and above
+ Mean value of 2 samples analysed
** highly significant

plants. Further it is seen that viral infection brings about highly significant reduction in the fresh weight as well as dry weight of fruits at both pale yellow and yellow stages of development. However, the moisture content of fruits from infected plants was more when compared to that from healthy plants.

Discussion

Solanum khasianum mosaic virus infection caused a significant reduction in the rate of increase in number of leaves as well as branches of *S. khasianum*. Similarly, AKETSURA (1959) observed that the growth rate of the diseased leaves was half to one third of the normal. As emphasised by DIENER (1963), the depressed growth rate in virus-infected plants may be attributed to the disturbances in the metabolism of the growth-regulating substances. Another reason for the reduction in growth could be due to the increased respiration rate coupled with decreased photosynthetic activity in virus-infected plants.

The virus also caused a highly significant reduction in the height of the main shoot of *S. khasianum* resulting in severe stunting. Similarly, stunting of tomato plants infected with aucuba mosaic has been observed by CALDWELL (1934). Stunting due to virus infection may result from a reduced effective concentration of gibberellic acid in the plant as suggested by CHESSIN (1957).

The infected plants produced significantly lesser number of fruits compared to healthy plants. Heavy reduction in yield has been observed due to early infection of tomato plants with mosaic (HEUBERGER and MOYER, 1931). It is a common phenomenon that plants infected early in the season with virus diseases like mosaic suffer most and bear no marketable fruit whatever as stated by GARDNER and KENDRICK (1922).

The viral infection caused a highly significant reduction in the fresh as well as dry weight of the fruits. The reduction in dry matter content may be attributed to the initial reduction of chlorophyll content and the subsequent general starvation of the virus-infected plant.

SKMV infection caused significant reduction in glyco-alkaloid (solasonine) content of the fruits. Similar reduction in total alkaloid content of a number of tobacco varieties infected with potato virus X (PVX) (SCHUSTER, 1958) as well as in solasodine content of leaves infected with a virus inciting mosaic disease in *Solanum aviculare* (SASTRY, 1969) have been reported. As emphasized by GOODMAN et al. (1967), it is difficult to interpret the cause of changes in the alkaloid content of virus-infected plants, as indeed it is often with pathological metabolism in general.

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Literature

* AKETSURA, K. (1959): On the abnormal growth and the decrease of starch and tuber formation of potato plant affected with some viruses. Bull. Fac. Agric. Univ. Miyazak *4*, 222—230.

BELL, R. C. and BRIGGS, L. H. (1942): I. Solanum Alkaloids. Part I. The Alkaloid from the fruit of *Solanum aviculare*. J. Chem. Soc. 1—2.

CALDWELL, J. (1934): The physiology of virus diseases in plants: VI. Some effects of mosaic on the metabolism of the tomato. Ann. appl. Biol. *21*, 206—224.

CHESSIN, M. (1957): Growth substances and stunting in virus-infected plants. Proc. Third Conf. Potato Virus Diseases, Lisse-Wageningen, 24—28 June, 1957. pp. 80—84.

DIENER, T. O. (1963): Physiology of virus-infected plants. Ann. Rev. Phytopathology *1*, 197—218.

GARDNER, M. W. and KENDRICK, J. B. (1922): Tomato mosaic. Purdue Univ. (Ind.) Agr. Exp. Sta. Bull. 261.

GOODMAN, R. N., KIRÁLY, Z. and ZAITLIN, M. (1967): The Biochemistry and Physiology of Infectious Plant Disease. D. Van Nostrand Company, Inc., Princeton, New Jersey.

HEUBERGER, J. W. and MOYER, A. J. (1931): Influence of mosaic infection on tomato yields. Phytopathology *21*, 745—749.

*MOYCHO, W. and NIEMYSKI, K. (1962): Wplyw perazenia Wirusem mozaiki Tyloniowej na plon i sklad chemiczny Pomidorow. (The effect of tobacco mosaic virus infection on the yield and chemical composition of tomatoes). Acta agrobot. *12*, 261—273.

RANGARAJU, R. and CHENULU, V. V. (1974): Studies on a virus inciting mosaic disease in *Solanum khasianum* Clarke. Indian J. Exp. Biol., *12*, 356—359.

SASTRY, K. S. M. (1969): Investigation and control of some important diseases of medicinal plants. Indian Phytopath. *22*, 140—142.

SCHUSTER, G. (1958): Untersuchungen über die Auswirkungen von Virusbefall auf den Alkaloidgehalt verschiedener Arten und Sorten der Gattung Nicotiana L. Phytopathol. Z. *32*, 335—339.

THAKUR, R. N. and SASTRY, K. S. M. (1971): Studies on mosaic disease of *Solanum khasianum*. Indian Phytopath. *24*, 127—130.

* Original not seen.

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Gynandropsis pentaphylla DC — An Unrecorded Host of Tobacco Leaf-curl Virus

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G. pentaphylla showing leaf-curl symptoms was observed and reported to be an unrecorded host of tobacco leaf-curl virus. It might be acting as a natural host of this virus.

Gynandropsis pentaphylla DC is a medicinal weed growing wild during Kharif season. It was observed in the fields of Bajra crop at Delhi to be infected with leaf-curl disease. The diseased plants were stunted in growth and leaf size was somewhat reduced. The veins and veinlets were very prominent and thickened with enations on their undersurface. The leaves showed curling, puckering and occasionally rolling. In severe cases the margin of the leaves was curled upwards and the petioles twisted.

Scions were selected from severely affected plants and wedge-grafted on healthy stocks of *G. pentaphylla*, *Nicotiana tabacum* L. Cv. Harrison's special and *Lycopersicon esculentum* Mill. In addition, some healthy scions were also grafted as control on the stocks of above three plant species. All the test plants were raised and maintained under insectproof conditions.

The disease was readily transmitted within 15—30 days on all of them. Successful grafts in the control, however, did not show any symptoms anytime during four months under observation. The axillary shoots growing from the stock below the grafted portion showed severe leaf-curl. The disease could not, however, be transmitted when juice, extracted from the leaves of diseased plants, was rubbed with carborundum powder on healthy plants of *G. pentaphylla* or other two solanaceous hosts tried.

Attempts were made to transmit the disease with the help of whitefly (*Bemisia tabaci* Gen.). About 10—15 whiteflies given an aquisition feeding of 24 hours, were transferred to healthy plants of *G. pentaphylla* and *N. tabacum* for a transmission feeding of 24 hours. Typical leaf-curl symptoms appeared within 15—25 days on both the hosts. The symptoms produced on *N. tabacum were* comparable to tobacco leaf-curl (Nicotiana virus 10, SOTREY, 1932).

A perusal of literature indicates that this plant has been demonstrated experimentally as host of 'SK' strain of tobacco mosaic virus, chilli mosaic virus and mosaic of *Solanum khasianum* Clarke (VERMA *et al.*, 1972; VERMA and SINGH,

1974). No record of any virus naturally affecting *Gynandropsis* has come to the writers' notice. This report would, therefore, constitute the first record of a virus disease spontaneously occurring in *Gynandropsis*. Its importance also lies in the fact that this host of tobacco leaf-curl may act as a constant source of inoculum to nearby fields where various economically important crops such as tobacco, tomato, chilli etc. are grown.

Acknowledgements

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Zusammenfassung

Es wurde Symptomen von Blattkräuselkrankheit an *Gynandropsis pentaphylla* beobachtet. *Gynandropsis pentaphylla* ist eine neue, bisher nicht bekannte Wirtspflanze für Tabak-Blattkräusel-Virus, die als auch natürlicher Wirt gelten kann.

Literature

SOTREY, H. H. (1932): Leaf-curl of tobacco in Southern Rhodesia — Rhod. Agric. J. *29*, 186—192.

VERMA, V. S., SINGH, SHAMSHER, MISHRA M. D. and NAM PRAKASH (1972): Mosaic disease of a medicinal plant *Solanum khasianum* Clarke from India — Phytopath. *Z. 75*, 52—58.

VERMA, V. S. and SINGH, SHAMSHER (1974): Two medicinal plants *Gynandropsis pentaphylla* and *Trianthema portulacastrum*, as additional hosts of certain plant viruses. Phyton. Austria (in press).

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Reaction of *Physalis* Species to Plant Viruses

V. *PHYSALIS ALKEKENGI* L., *PHYSALIS ANGULATA* L., *PHYSALIS PHYLADELPHICA* LAM., AND *PHYSALIS PUBESCENS* L. AS NEW HOSTS OF SEVERAL PLANT VIRUSES

By

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Local and systemic host–virus relation has recently been pointed out between *Physalis alkekengi* L. (syn.: *Ph. franchetti* Mast.) and alfalfa mosaic virus (R/1 : 1.3/18 : S/S : S/Ap), potato aucuba mosaic virus (∗/∗ : ∗/∗ : E/E : S/Ap); *Ph. angulata* L. and alfalfa mosaic virus, tobacco ring spot virus (R/1 : 1.8/42 : S/S : S/Ne); *Ph. philadelphica* Lam. and alfalfa mosaic virus, *cucumber mosaic virus* (R/1 : 1/18 : S/S : S/Ap); potato aucuba mosaic virus, tobacco ring spot virus; *Ph. pubescens* L. and alfalfa mosaic virus, potato aucuba mosaic virus. *Physalis pubescens* proved only locally susceptible to tobacco necrosis virus (R/∗ : ∗/∗ : S/S : S/Ne) and tobacco rattle virus (R/1 : 2.3/5 : E/E : S/Ne). The above two viruses did not become systemic in the inoculated *Physalis pubescens* plants, they could only be pointed out in the inoculated leaves. *Physalis alkekengi* was found to be resistant to bean (common) mosaic virus (∗/∗ : ∗/∗ : E/E : S/Ap), potato virus M (∗/∗ : ∗/∗ : E/E : S/Ap), potato virus S (∗/∗ : ∗/∗ : E/E : S/Ap), radish mosaic virus (R/∗ : ∗/∗ : S/S : S/Cl) and turnip yellow mosaic virus (R/1 : 1.9/37 : S/S : S/Cl). In addition to the above viruses *Physalis angulata* showed resistance to tobacco necrosis virus too. *Physalis philadelphica* and *Ph. pubescens* were equally resistant to infection by bean (common) mosaic virus, radish mosaic virus and turnip yellow mosaic virus.

In the course of reviewing the literature of *Physalis* host–virus relations and studying various new host–virus relations in experiments the reaction of some 23 *Physalis* species to 76 viruses was found to have been investigated so far (reviewed by HORVÁTH, 1970, 1974a, 1975). Recent investigations revealed that *Physalis floridana* Rydb., *Ph. ixocarpa* Brot., *Ph. peruviana* L. as well as *Ph. subglabrata* MacKenzie et Bush., the original natural host plant were artificial hosts of the 27 nm diameter, isometric, 38 per cent RNA-content *Physalis* mosaic virus, a relative of potato (Andean) latent virus, belladonna mottle virus, dulcamara mottle virus, eggplant mosaic virus and the so called *Physalis* mottle strain (cf. MOLINE and FRIES, 1974) of the belladonna mottle virus, that had been isolated from *Physalis subglabrata* plants in Illinois, U. S. A. (PETERS and DERKS, 1974). Again, recent studies have thrown light upon the fact that *Physalis floridana* is not only a new host plant but also a good production host of satsuma dwarf virus (TANAKA and

[1] Earlier publications: I. Acta phytopath. Acad. Sci. hung. *5*, 65—72 (1970), II. Acta phytopath. Acad. Sci. hung. *9*, 1—9 (1974), III. Acta phytopath. Acad. Sci. hung. *9*, 11—15 (1974), IV. Acta phytopath. Acad. Sci. hung. *10*, 67—75 (1975).

IMADA, 1974). Taking into consideration results published during the few last years the number of viruses infectious for some 23 *Physalis* species has increased up to 78. Present paper gives account of new *Physalis* host–virus relations pointed out in the last several years.

Materials and Methods

In our experiments young *Physalis alkekengi* L. (bladder cherry, alkekengi, winter cherry, Chinese lantern plant; syn.: *Ph. franchetti* Mast.), *Ph. angulata* L. (tooth leaved winter-cherry), *Ph. philadelphica* Lam. and *Ph. pubescens* L. (husk tomato, Barbados gooseberry) plants were inoculated with 11 plant viruses to which the reactions of the above *Physalis* species had not been known so far. The following viruses or virus strains were inoculated in the experiments: alfalfa mosaic virus (strain K2, R/1 : 1.3/18 : S/S : S/Ap; BECZNER, 1972), bean (common) mosaic virus (∗/∗ : ∗/∗ : E/E : S/Ap, HORVÁTH, 1973a), cucumber mosaic virus (R/1 : 1/18 : S/S : S/Ap, HORVÁTH, 1973b), potato aucuba mosaic virus (∗/∗ : ∗/∗ : E/E : S/Ap, HORVÁTH, 1972), potato virus M (∗/∗ : ∗/∗ : E/E : S/Ap, HORVÁTH and DE BOKX, 1972), potato virus S (∗/∗ : ∗/∗ : E/E : S/Ap, HORVÁTH, 1972), radish mosaic virus (R/∗ : ∗/∗ : S'S : S/Cl, HORVÁTH *et al.*, 1973), tobacco necrosis virus (strain f = TNV_f, R/∗ : ∗/∗ : S/S : S/Fu; SZIRMAI, 1964), tobacco rattle virus (R/1 : 2.3/5 : E/E : S/Ne, HORVÁTH, 1973), tobacco ring spot virus (R/1 : 1.8/42 : S/S : S/Ne, HORVÁTH, 1973a), and turnip yellow mosaic virus (R/1 : 1.9/37 : S/S : S/Cl, HORVÁTH *et al.*, 1973; JURETIĆ *et al.*, 1973). As to the maintenance of the viruses, the methods of inoculation, the reisolation of viruses from the inoculated plants and the test plants, as well as concerning the serological demonstration of potato virus M and potato virus S detailed data can be found in our earlier publication (HORVÁTH, 1974a).

Results

In the course of artificial inoculation experiments *Physalis alkekengi* (syn.: *Ph. franchetti*) was found to react with latent local and manifest systemic symptoms (vein clearing, mosaic) to infections by alfalfa mosaic and potato aucuba mosaic viruses. To bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic virus and turnip yellow mosaic virus, on the other hand, it proved to be resistant. The latent infectivity of inoculated but symptomless leaves was pointed out by using *Phaseolus vulgaris* L. cv. Red Kidney and Pinto bean plants in the case of plants infected with alfalfa mosaic virus, and *Capsicum annuum* L. test plants for plants inoculated with potato aucuba mosaic virus in the following way: from inoculated leaves of *Physalis alkekengi* plants after a surface sterilization tissue sap was prepared and transferred to virus susceptible test plants by carborundum-spatula technique.

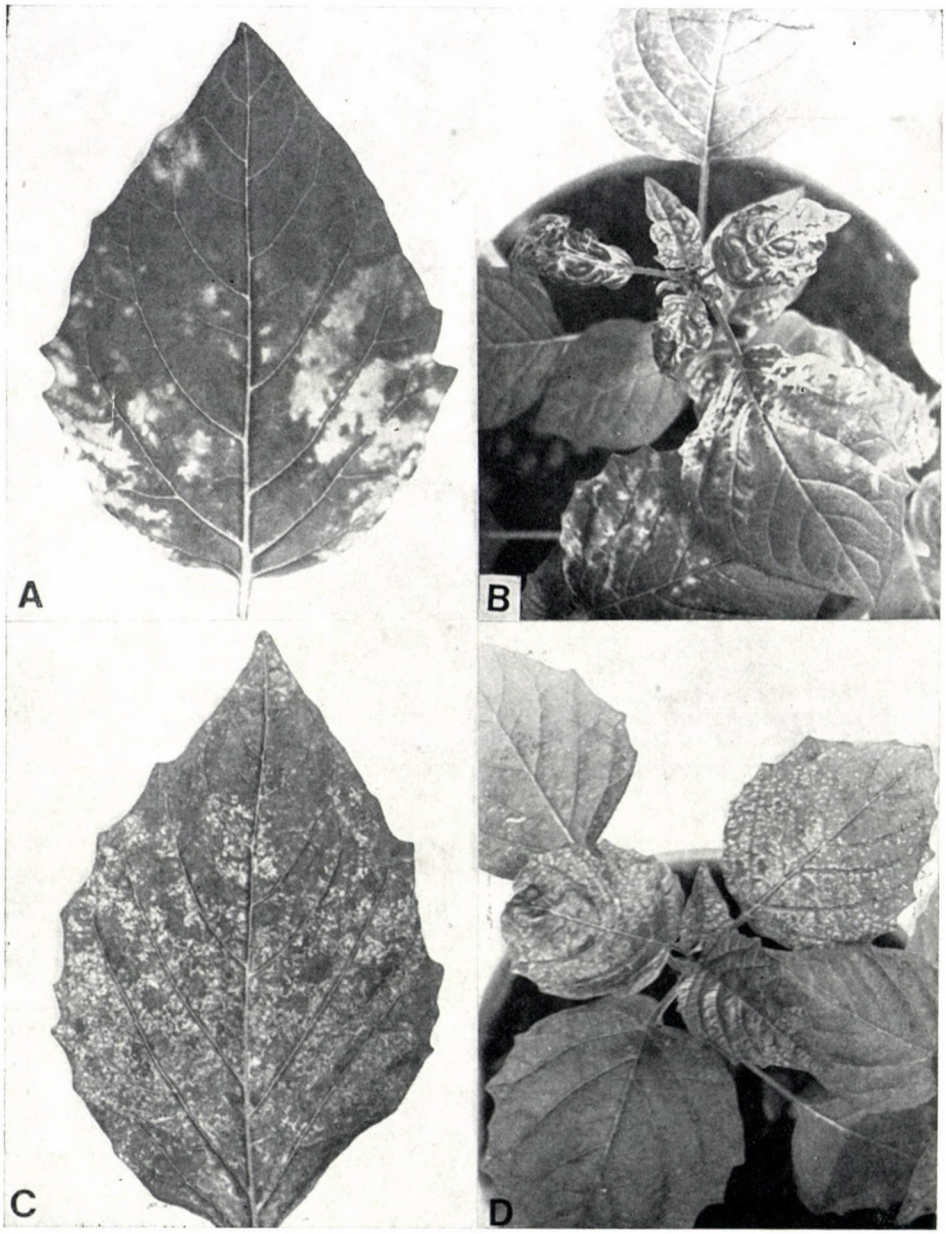

Fig. 1. Systemic symptoms of *Physalis philadelphica* Lam. to alfalfa mosaic virus (A and B), and potato aucuba mosaic virus (C and D)

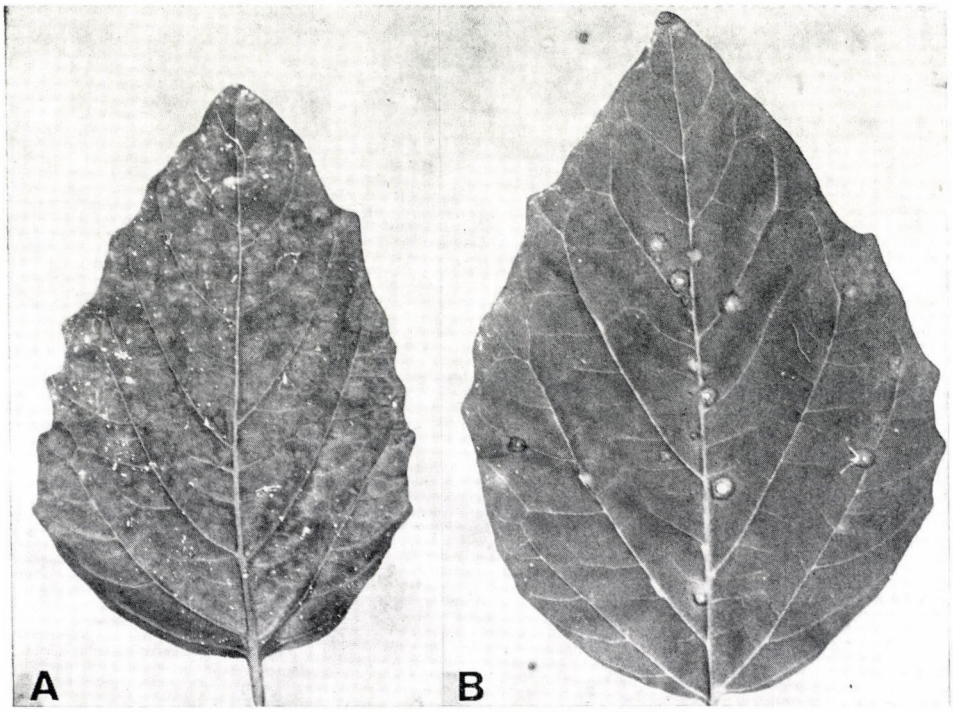

Fig. 2. Systemic (A) and local (B) reactions of *Physalis philadelphica* Lam. to two plant viruses A: Cucumber mosaic virus, B: Tobacco ring spot virus

Physalis angulata proved to be locally and systemically susceptible to two viruses. In the case of plants inoculated with alfalfa mosaic virus latent local infectivity as well as severe systemic mosaic symptoms and leaf deformation were found. Plants inoculated with tobacco ring spot virus by rubbing the virus into the leaves displayed chlorotic and later necrotic spots on the leaves 4–5 days after inoculation. On non-inoculated or subsequently developed leaves systemic mosaic was observed. The inoculated *Physalis angulata* plants proved to be resistant to infections by bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic virus, tobacco necrosis virus and turnip yellow mosaic virus both locally and systemically.

Physalis philadelphica was found to be locally and systemically susceptible to four viruses (alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic virus, and tobacco ring spot virus). Plants inoculated with alfalfa mosaic virus and potato aucuba mosaic virus showed, however, no visible local symptoms; the systemic symptoms manifested themselves in vein clearing, mosaic and leaf deformation (Fig. 1A–D). Manifest local and systemic symptoms were found in *Physalis philadelphica* plants inoculated with cucumber mosaic virus and tobacco

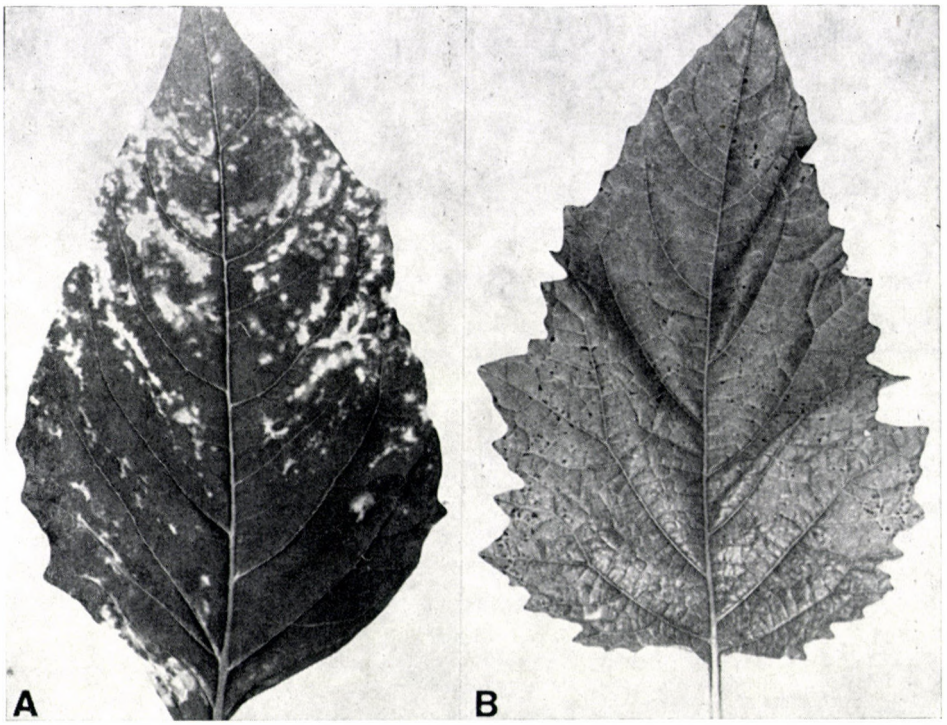

Fig. 3. Systemic symptoms of *Physalis pubescens* L. to alfalfa mosaic virus (A) and potato aucuba mosaic virus (B)

ring spot virus. Plants inoculated with cucumber mosaic virus by rubbing the leaves showed chlorotic local spots on the leaves, while on the non-inoculated leaves a very intensive vein clearing, ochre spots appeared (Fig. 2A). On rubbed leaves of plants inoculated with tobacco ring spot virus ring-shaped necrotic lesions with grey centre and dark edges were found 4–5 days after inoculation (Fig. 2B) followed later by systemic symptoms (ring-shaped chlorotic then necrotizing lesions) on the non-inoculated leaves. *Physalis philadelphica* plants inoculated with bean (common) mosaic virus, radish mosaic virus and turnip yellow mosaic virus proved to be equally resistant to virus infection.

Physalis pubescens gave only a local response to infection by tobacco necrosis virus and tobacco rattle virus. The rubbed leaves of plants inoculated with tobacco necrosis virus turned yellow several days later, but on the ochre leaves typical dark green local lesions were observed. The virus infectivity of inoculated leaves was pointed out in *Phaseolus vulgaris* cv. Red Kidney plants in the course of virus reisolation experiments. On the rubbed leaves of plants inoculated with tobacco rattle virus necrotic lesions surrounded by a characteristic chlorotic ring were found. *Physalis pubescens* proved to be locally and systemically susceptible

Table 1

New host–virus interrelations of some *Physalis* species

Physalis species	Viruses and the type of the host reaction*	Literature
Ph. aequata Jacq.	L: Tobacco necrosis virus Tobacco rattle virus L + S: Alfalfa mosaic virus Potato aucuba mosaic virus Potato virus Y Tobacco mosaic virus Tobacco ring spot virus R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Turnip yellow mosaic virus	HORVÁTH (1974c, 1975)
Ph. alkekengi L. (syn.: *Ph. franchetti* Mast.)	L + S: Alfalfa mosaic virus Potato aucuba mosaic virus R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Turnip yellow mosaic virus	HORVÁTH (1974c, and the present work)
Ph. angulata L.	L + S: Alfalfa mosaic virus Tobacco ring spot virus R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Tobacco necrosis virus Turnip yellow mosaic virus	HORVÁTH (present work)
Ph. floridana Rydb.	R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Turnip yellow mosaic virus	HORVÁTH (1974b)
Ph. ixocarpa Brot.	L: Tobacco necrosis virus L + S: Cucumber mosaic virus Potato aucuba mosaic virus Tobacco mosaic virus	HORVÁTH (1974c, 1975)

Physalis species	Viruses and the type of the host reaction*	Literature
	Tobacco ring spot virus R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Turnip yellow mosaic virus	
Ph. peruviana L.	L + S: Alfalfa mosaic virus Cucumber mosaic virus Potato aucuba mosaic virus Potato virus X Potato virus Y Tobacco mosaic virus Tobacco ring spot virus R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Tobacco necrosis virus Turnip yellow mosaic virus	HORVÁTH (1970, 1974b, c)
Ph. peruviana L. var. macrocarpa	L: Tobacco rattle virus S: Potato virus Y L + S: Alfalfa mosaic virus Cucumber mosaic virus Potato aucuba mosaic virus Potato virus X Tobacco mosaic virus Tobacco ring spot virus R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Tobacco necrosis virus Turnip yellow mosaic virus	HORVÁTH (1974a, c)
Ph. philadelphica Lam.	L + S: Alfalfa mosaic virus Cucumber mosaic virus Potato aucuba mosaic virus Tobacco ring spot virus R: Bean (common) mosaic virus	HORVÁTH (1974c, and the present work)

Physalis species	Viruses and the type of the host reaction*	Literature
	Radish mosaic virus Turnip yellow mosaic virus	
Ph. pruinosa L.	L + S: Alfalfa mosaic virus Cucumber mosaic virus Potato aucuba mosaic virus Potato virus X Potato virus Y Tobacco mosaic virus Tobacco ring spot virus R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Tobacco necrosis virus Turnip yellow mosaic virus	HORVÁTH (1974a, c)
Ph. pubescens L.	L: Tobacco necrosis virus Tobacco rattle virus L + S: Alfalfa mosaic virus Potato aucuba mosaic virus R: Bean (common) mosaic virus Radish mosaic virus Turnip yellow mosaic virus	HORVÁTH (1974c, and the present work)
Ph. viscosa L.	L: Tobacco necrosis virus Tobacco rattle virus L + S: Alfalfa mosaic virus Cucumber mosaic virus Potato virus X R: Bean (common) mosaic virus Potato virus M Potato virus S Radish mosaic virus Turnip yellow mosaic virus	HORVÁTH (1974c, 1975)

* L: local, S: systemic, L + S: both local and systemic, R: no reaction (immune)

to infections by alfalfa mosaic virus and potato aucuba mosaic virus. Plants inoculated with alfalfa mosaic virus showed a manifest local (tiny grey, necrotic spots) and manifest systemic (vein clearing, interveinal mosaic, leaf blistering 5 days after inoculation) disease (Fig. 3A). Plants inoculated with potato aucuba mosaic virus developed latent local and manifest systemic symptoms. The systemic symptoms

were vein clearing, interveinal mosaic, and in some plants tiny black, spot-like necrotic lesions (Fig. 3B). Plants inoculated with bean (common) mosaic virus, radish mosaic virus and turnip yellow mosaic virus were found to be resistant to virus infections.

Conclusions

In the course of studying the virus susceptibility of the *Physalis* species we have revealed numerous new host–virus relations as published earlier and in the present paper (see Table 1). The susceptibility of *Physalis peruviana* var. *macrocarpa* to some eight, and of *Ph. aequata*, *Ph. peruviana* and *Ph. pruinosa* to seven new viruses is particularly remarkable. *Physalis ixocarpa* and *Ph. viscosa* proved to be susceptible to five, *Ph. philadelphica* and *Ph. pubescens* to four while *Ph. alkekengi* and *Ph. angulata* to two new viruses. In the artificial inoculation experiments we found four *Physalis* species resistant to six viruses, further five *Physalis* species to five viruses, and two *Physalis* species to be resistant to three viruses (Table 1). Plants, locally or systemically, as well as both locally and systemically susceptible to various viruses, further, *Physalis* species perfectly resistant to the different viruses are of importance not only in identifying and separating the viruses but also from an epidemiological point of view, as perennial virus reservoirs (e.g. *Physalis heterophylla*, *Ph. peruviana*, *Ph. peruviana* var. *macrocarpa*) and virus production hosts (e.g. *Physalis floridana*).

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Literature

BECZNER, L. (1972): A lucerna mozaik vírus új törzseinek magyarországi fellépése (Occurrence of new strains of alfalfa mosaic virus in Hungary). A Növényvéd. Korsz. *6*, 101—118.

HORVÁTH, J. (1970): Reaction of *Physalis* species to plant viruses. 1. The cape gooseberry as a symptomless carrier of potato virus X and Y. Acta phytopath. Acad. Sci. hung. *5*, 65—72.

HORVÁTH, J. (1972): Symptomless *Lycopersicon* host plants for potato virus S. Amer. Potato J. *48*, 339—341.

HORVÁTH, J. (1973a): *Paulownia fargesii* Franch. (Family: *Scrophulariaceae*) as a new host plant for several plant viruses. Acta phytopath. Acad. Sci. hung. *8*, 165—173.

HORVÁTH, J. (1973b): *Tetragonia echinata* Ait. als eine neue Testpflanze für das Gurkenmosaik-Virus (cucumber mosaic virus). Z. Pflanzenkrankheiten *80*, 129—132.

HORVÁTH, J. (1974a): Reaction of *Physalis* species to plant viruses. II. Additional data on the virus susceptibility of *Physalis* spp. and new experimental results: *Physalis peruviana* L. var. *macrocarpa* and *Physalis pruinosa* L. Acta phytopath. Acad. Sci. hung. *9*, 1—9.

HORVÁTH, J. (1974b): Reaction of *Physalis* species to plant viruses. III. *Physalis floridana* Rydb. and *Physalis peruviana* L. as immune and screening plants to several plant viruses. Acta phytopath. Acad. Sci. hung. *9*, 11—15.

HORVÁTH, J. (1974c): Potato pathogenic plant viruses and their new host plants. Symp. Potato Virology, Section Virology Meeting E. A. P. R., Bonin 1974. pp. 5—6.

HORVÁTH, J. (1975): Reaction of *Physalis* species to plant viruses. IV. *Physalis aequata* Jacq., *Physalis ixocarpa* Brot., and *Physalis viscosa* L. as less known indicators of plant viruses. Acta phytopath. Acad. Sci. hung. *10*, 67—75.

HORVÁTH, J. and DE BOKX, J. A. (1972): Über die Anfälligkeit von *Chenopodium*-Arten und -Varietäten gegen das M-Virus (potato virus M). Phytopath. Z. *73*, 171—177.

HORVÁTH, J., JURETIĆ, N. and MILIČIĆ, D. (1973): *Crambe abyssinica* Hochst. ex R. E. Frees as a new host plant for turnip yellow mosaic virus and radish mosaic virus. Phytopath. Z. *78*, 69—74.

JURETIĆ, N., HORVÁTH, J., MAMULA, D. and MILIČIĆ, D. (1973): Natural occurrence of turnip yellow mosaic virus in Hungary. Acta phytopath. Acad. Sci. hung. *8*, 175—183.

MOLINE, H. E. and FRIES, R. E. (1974): A virus isolated from *Physalis heterophylla* in Iowa. Phytopathology *64*, 44—48.

PETERS, D. and DERKS, A. F. L. M. (1974): Host range of some properties of *Physalis* mosaic virus, a new virus of the turnip yellow mosaic virus group. Neth. J. Pl. Path. *80*, 124—132.

SZIRMAI, J. (1964): Durch Wirtspassage induzierte Varianten beim Tabaknekrosevirus. *In:* Z. KIRÁLY and G. UBRIZSY (eds.), Host–Parasite Relations in Plant Pathology. Symp. Hung. Acad. Sci., 19—22 October 1964, Budapest, pp. 97—104.

TANAKA, H. and IMADA, J. (1974): *Physalis floridana*, a good production host for satsuma dwarf virus. Plant Dis. Rep. *58*, 603—605.

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Two Viruses Isolated from Patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.), a New Vegetable Natural Host in Hungary

II. CUCUMBER MOSAIC VIRUS

By

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The present paper deals with the spontaneous occurrence of the green strain of cucumber mosaic virus (CMV—G/PC, R/1 : 1/18 : S/S : S/Ap) in patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.) in Hungary. The virus was identified on the basis of differential hosts, insect transmission, serology, physical properties and cross protection test. The PC isolate of CMV-G induced systemic symptoms on *Ecballium elaterium* (L.) A. Rich. and some *Nicotiana* species. These plant species are not susceptible to the general strain of watermelon mosaic virus (WMV-G, ∗/∗ : ∗/∗ : E/E : S/Ap), found associated with CMV in patisson (HORVÁTH *et al.*, 1975a). *Ecballium elaterium* and *Nicotiana* species are very important in the differential diagnosis and separation of the two viruses. Seventeen species, *Ammi visnaga* (L.) Lam., *Commelina graminifolia* H. B. et K., *C. tuberosa* L., *Cucurbita pepo* var. *patissonina* f. *radiata*, *Erodium ciconium* (L.) L'Hérit., *E. cicutarium* (L.) L'Hérit., *E. malacoides* Willd., *Gomphrena decumbens* Jacq., *Malva borealis* Wallm., *M. moschata* L., *M. neglecta* Wallr., *M. pusilla* Sm. et Sow., *M. verticillata*, L., *Nicotiana chinensis* Fisch., *N. knightiana* Goodspeed, *N. quadrivalvis* Pursch., and *Tetragonia crystallina* L'Hérit. are recorded as new experimental hosts for CMV-G/PC. The virus showed stylet-borne transmission by *Myzus persicae* Sulz. In our experiments CMV-G/PC was not transmissible by seeds of infected patisson plants. The virus reacted positively with CMV antiserum. The thermal inactivation point was between 62 and 64°C, and the dilution end-point was 2×10^{-4}. *In vitro* the virus kept active for 12 days, after 15 days it was inactive. On *Nicotiana tabacum* L. cv. Xanthi-nc the cross protection test between CMV-G/PC and white strain of CMV (CMV-W) resulted in protection against the multiplication of the CMV-W in the challenging inoculations. This is the first report of the CMV-G occurrence in patisson.

In an earlier paper (HORVÁTH *et al.*, 1974) we gave information about the virus disease of patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.), a new vegetable plant in Hungary, which — according to our previous investigations — was caused by the infection watermelon mosaic virus (WMV, ∗/∗ : ∗/∗ : E/E : S/Ap) and/or cucumber mosaic virus (CMV, R/1 : 1/18 : S/S : S/Ap). In the course of subsequent symptomatological examinations we found (HORVÁTH *et al.*, 1975a) the severe mosaic-, growth inhibition-, internode shortening- and proliferation symptoms displayed by the patisson to have been caused

by the general strain of WMV (WMV-G). Further, by differential host plants (e.g. *Ecballium elaterium* [L.] A. Rich, *Citrullus lanatus* [Thunb.] Mansfeld [syn.: *C. vulgaris* Schrad], *Lavatera trimestris* L., *Nicotiana glutinosa* L., *N. tabacum* L.) we pointed out that the very severe leaf deformation occurring in patisson was induced by CMV (HORVÁTH *et al.*, 1975a).

The simultaneous occurrence of CMV and WMV in various cucurbitaceous plants is known both in the United States of America (WEBB, 1961; PROVVIDENTI and SCHROEDER, 1970) and in Europe (SCHMELZER, 1965; MOSKOVETS *et al.*, 1970). However, as regards the CMV infection of patisson no data have been available so far. Present paper gives account of the isolation and properties of CMV obtained from patisson.

Materials and Methods

From patisson plants showing severe mosaic symptoms, leaf deformation, growth inhibition and proliferation, leaf samples were collected in plastic bags at the end of August 1973. The young diseased patisson leaves were ground (1 : 1 w/w) in a mortar with 0.1 *M* phosphate buffer (pH 7.0). With the tissue sap thus obtained we inoculated test plants (*Ammi majus* L., *Chenopodium amaranticolor* Coste et Reyn., *Citrullus lanatus* [Thunb.] Mansfeld, *Cucumis sativus* L., *Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois., *Datura stramonium* L., *Ecballium elaterium* [L.] A. Rich., *Gomphrena globosa* L., *Lavatera trimestris* L., *Nicotiana glutinosa* L., *N. tabacum* L. cv. Bel 61 – 10, Samsun, Xanthi-nc, as well as *Ocimum basilicum* L. and *O. canum* Sims) previously dusted with 500 mesh carborundum.

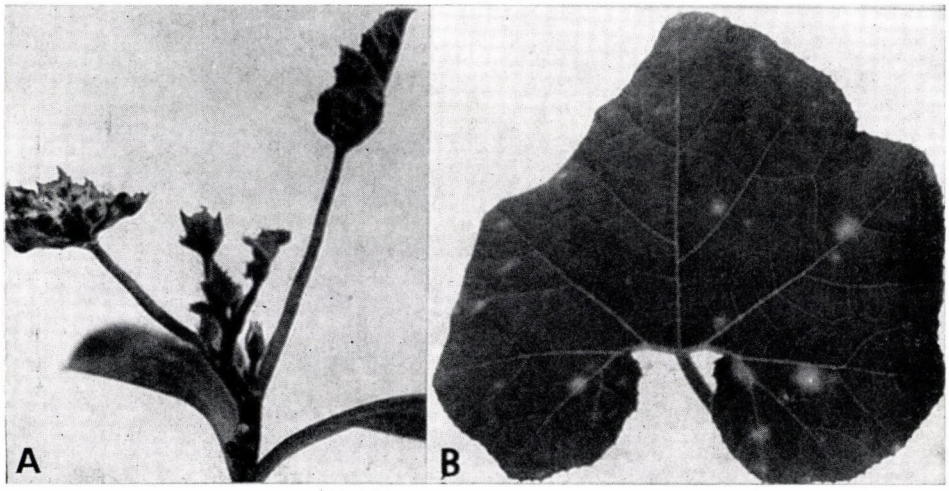

Fig. 1. Systemic symptoms on the experimentally inoculated patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.) plant (A) and on *Cucurbita ficifolia* Bouche (B)

Table 1

Reaction of various plants to PC isolate of the green strain of cucumber mosaic virus (CMV-G/PC) isolated from patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.) in Hungary

Family and binominals[1]	Reactions
AIZOACEAE	
Tetragonia crystallina L'Hérit.*	IL: Chlorotic lesions NIL: Not infected
T. echinata Ait.	IL: Chlorotic lesions NIL: Not infected
T. tetragonoides (Pall.) O. Ktze	IL: Chlorotic lesions NIL: Not infected
AMARANTHACEAE	
Amaranthus caudatus L.	IL: Chlorotic and sometimes brown necrotic lesions NIL: Not infected
Gomphrena decumbens Jacq.*, ⌀	IL: Brown necrotic lesions NIL: Leaf deformations, and dark-green spots
G. globosa L. ⌀	IL: Brown necrotic lesions NIL: Leaf deformations, and dark-green spots
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	IL: Chlorotic local lesions NIL: Not infected
COMMELINACEAE	
Commelina communis L. ⌀	IL: Not infected NIL: Severe stripe mosaic
C. graminifolia H. B. et K.*, ⌀	IL: Not infected NIL: Severe stripe mosaic
C. tuberosa L.*, ⌀	IL: Not infected NIL: Severe stripe mosaic (Fig. 2D)
CRUCIFERAE	
Brassica adpressa Boiss.	IL: Not infected NIL: Not infected
Br. chinensis L.	IL: Not infected NIL: Not infected
Cherianthus cheiri L. ⌀	IL: Not infected NIL: Latent, systemic susceptible
CUCURBITACEAE	
Citrullus lanatus (Thunb.) Mansfeld (syn.: *C. vulgaris* Schrad.) ⌀	IL: Not infected NIL: Not infected
Cucumis melo L.	IL: Not infected NIL: Mosaic spots (Fig. 2B)
C. sativus L.	IL: Chlorotic spots NIL: Vein clearing and mosaic (Fig. 2A)
Cucurbita andreana Naud.	IL: Not infected NIL: Mosaic spots
Cucurbita ficifolia Bouche	IL: Not infected NIL: Severe mosaic (Fig. 1B)

Table 1 (continued)

Family and binominals[1]	Reactions
C. moschata Duch.	IL: Not infected NIL: Severe mosaic, leaf deformation and growth reduction
C. pepo L. var. *patissonina* Greb. f. *radiata* Nois.*	IL: Not infected NIL: Severe mosaic and leaf deformation (Fig. 1A)
Ecballium elaterium (L.) A. Rich. ⧄	IL: Not infected NIL: Vein clearing, vein bending, mosaic spots (Fig. 2C)
Echinocystis lobata (Michx.) Torr. et Gray	IL: Not infected NIL: Vein clearing, vein banding, mosaic spots
Luffa acutangula (L.) Roxb. ⧄	IL: Not infected NIL: Mosaic spots
L. cylindrica (L.) Roem. ⧄	IL: Not infected NIL: Mosaic spots
ERICACEA	
Leiophyllum buxifolium (Berg.) Ell. □	IL: Not infected NIL: Not infected
GERANIACEAE	
Erodium ciconium (L.) L'Hérit.*	IL: Latent susceptible NIL: Mosaic
E. cicutarium (L.) L'Hérit.*	IL: Latent susceptible NIL: Mosaic
E. malacoides Willd.*	IL: Latent susceptible NIL: Mosaic
LABIATAE	
Ocimum basilicum L. ⧄	IL: Latent susceptible NIL: Vein clearing, mosaic
O. canum Sims. ⧄	IL: Latent susceptible NIL: Vein clearing, vein banding, leaf deformation, mosaic spots
LEGUMINOSAE	
Vicia faba L.	IL: Necrotic, brown lesions NIL: Not infected
Vigna sinensis (L.) Endl. ⧄	IL: Redish-brown necrotic lesions NIL: Not infected
MALVACEAE	
Lavatera trimestris L. ⧄	IL: Not infected NIL: Not infected
Malva borealis Wallm.*	IL: Not infected NIL: Vein clearing and mosaic
M. moschata L.*	IL: Not infected NIL: Vein clearing and mosaic
M. neglecta Wallr.*	IL: Not infected NIL: Vein clearing and mosaic
M. pusilla Sm. et Sow.*	IL: Not infected NIL: Vein clearing and mosaic
M. silvestris L.	IL: Not infected NIL: Vein clearing and mosaic

Table 1 (continued)

Family and binominals[1]	Reactions
M. verticillata L.*	IL: Not infected NIL: Vein clearing and mosaic
SOLANACEAE	
Atropa bella-donna L. ⧄	IL: Not infected NIL: Latent susceptible
Capsicum annuum L. ⧄	IL: Weak necrotic spots, leaf drop NIL: Vein clearing, mosaic
Datura stramonium L. ⧄	IL: Mosaic spots NIL: Severe mosaic
Nicotiana chinensis Fisch.*, ⧄	IL: Not infected NIL: Vein clearing, mosaic
N. glutinosa L. ⧄	IL: Not infected NIL: Vein clearing, leaf deformation, mosaic and severe growth reduction
N. knightiana Goodspeed*, ⧄	IL: Not infected NIL: Vein clearing, mosaic
N. quadrivalvis Pursch.*, ⧄	IL: Not infected NIL: Vein clearing, mosaic
N. tabacum L. cv. Bel 61—10, ⧄ Samsun⧄ and Xanthi-nc ⧄	IL: Not infected NIL: Vein clearing, mosaic
Petunia hybrida Vilm. ⧄	IL: Not infected NIL: Vein clearing, mosaic
UMBELLIFERAE	
Ammi majus L.	IL: Not infected NIL: Vein clearing, mosaic
Ammi visnaga (L.) Lam.*	IL: Not infected NIL: Vein clearing, mosaic

[1] Plants designated with * are new experimental hosts of cucumber mosaic virus (CMV). Plants designated with □ are new resistant, as well as plants designated with ⧄ are differential hosts of CMV and watermelon mosaic virus (WMV)

After inoculation the plants were sprayed with tap water. In a part of the test plants *(Ammi majus, Chenopodium amaranticolor, Citrullus lanatus, Cucumis sativus, Cucurbita pepo, Lavatera trimestris)* the same symptoms as earlier found in plants inoculated with WMV-G appeared (HORVÁTH *et al.*, 1975a). It was conspicuous, however, that on the leaves of the inoculated patisson plants a very severe deformation occurred (Fig. 1A), while no leaf deformation was observed on patisson plants inoculated with WMV-G alone. *Datura stramonium, Ecballium elaterium, Gomphrena globosa, Nicotiana tabacum* (cvs. Bel 61—10, Samsun, Xanthi-nc) as well as the two *Ocimum* species proved to be systemically susceptible. The responses of *Ammi majus, Chenopodium amaranticolor, Citrullus lanatus, Cucumis sativus, Cucurbita pepo, Lavatera trimestris* as well as of the above mentioned plants suggest that the isolate obtained from patisson and originally designated with the symbol PCW (cf. HORVÁTH *et al.*, 1975a) has two components:

WMV and – in all probability – CMV. With a tissue sap prepared from systemically susceptible tobacco plants – which, otherwise, are immune against WMV-G – we inoculated WMV–susceptible and CMV-resistant *(Citrullus lanatus, Lavatera trimestris)*, as well as WMV-resistant and CMV-susceptible *(Ecballium elaterium)* plants in order to conclude on the identity of the virus separated from WMV. Following the inoculation the CMV-resistant and WMV-susceptible plants *(Citrullus lanatus, Lavatera trimestris)* were found to be resistant, while the CMV-susceptible and WMV-resistant *Ecballium* plant proved susceptible, indicating that the virus inoculum obtained from tobacco plants only contained CMV. We use PC to designate the virus isolate separated from tobacco plants which is free of the $W_{(atermelon\ mosaic\ virus)}$-component of the original isolate (see PCW, HORVÁTH *et al.*, 1975a).

For the purpose of studying the host range of the PC isolate numerous plants were artificially inoculated (Table 1). In the course of studying the aphid transmission of the virus *Myzus persicae* Sulz. aphids were used. Before the experiments the aphids were starved for 3 hours, then placed for 8–10 minutes on patisson, cucumber and tobacco plants previously infected with PC isolate. After this the aphids were transferred to healthy patisson and cucumber plants of cotyledon- and Samsun and Xanthi-nc tobacco plants of 2–4 true-leaf stage. Twenty-four hours later the aphids were killed with Phosdrin and the plants placed in an insect-proof greenhouse.

Investigations were made to find out whether the PC isolate can be transmitted with the seeds of diseased patisson plants. For this purpose 500 seeds collected from the fruits of diseased patisson plants were planted in greenhouse. Plants grown from these seeds were symptomatologically checked upon, and those suspected to be diseased tested with *Chenopodium amaranticolor*, *Cucumis sativus* and *Cucurbita pepo* var. *patissonina* f. *radiata* plants.

In the serological examinations of the PC isolate the agar gel double diffusion test was used (VAN REGENMORTEL, 1966; 1967).

When determining the physical properties of the virus we took the thermal inactivation point, longevity *in vitro* and dilution-end point into consideration. The physical properties of PC isolate were determined in extracted patisson sap with *Chenopodium amaranticolor* used as assay host.

In the course of cross protection experiments young Xanthi-nc tobacco plants were inoculated with the PC isolate to prove that the PC isolate was identical with CMV. After the systemic symptoms had appeared the tobacco plants were inoculated with the white strain of CMV (CMV-W) as challenge strain.

Results and Discussion

Of the plants inoculated with tissue sap obtained from patisson plants showing mosaic, growth inhibition as well as severe internode shortening, proliferation and leaf deformation, and originally containing two viruses, the *Citrullus lanatus* and

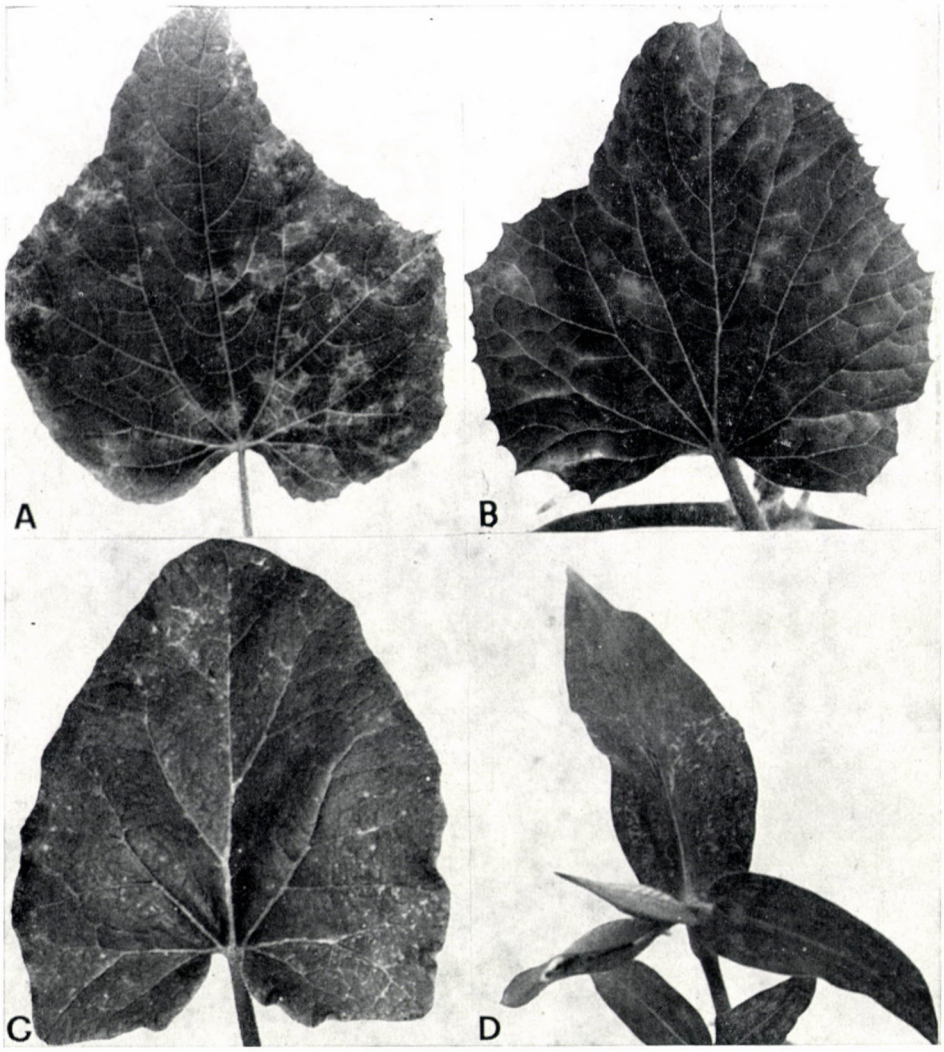

Fig. 2. Systemic symptoms on experimentally inoculated *Cucumis sativus* L. (A), *Cucumis melo* L. (B), *Ecballium elaterium* (L.) Rich. (C) and *Commelina tuberosa* L. (D)

Lavatera trimestris displayed symptoms characteristic of WMV. In another group of the inoculated plants – which had proved resistant to WMV *(Datura stramonium, Ecballium elaterium, Gomphrena globosa, Nicotiana glutinosa, N. tabacum, Ocimum basilicum, O. canum)* – symptoms characteristic of CMV appeared. In the course of studying the host range of pure PC isolate (containing one virus only) separated from systemically susceptible tobacco plants – known to be immune of WMV – seven plants showed local, 27 systemic and ten plants local and sys-

Table 2

Separation of watermelon mosaic virus (WMV) and cucumber mosaic virus (CMV) isolated from patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.) with differential hosts

Differential hosts	Separated/eliminated viruses
Atropa bella-donna L.	CMV/WMV
Capsicum annuum L.	CMV/WMV
Cheiranthus cheiri L.	CMV/WMV
Citrullus lanatus (Thunb.) Mansfeld	WMV/CMV
Commelina communis L.	CMV/WMV
C. graminifolia H. B. et K.	CMV/WMV
C. tuberosa L.	CMV/WMV
Datura stramonium L.	CMV/WMV
Ecballium elaterium (L.) A. Rich.	CMV/WMV
Erodium ciconium (L.) L'Hérit.	CMV/WMV
E. cicutarium (L.) L'Hérit.	CMV/WMV
E. malacoides Willd.	CMV/WMV
Gomphrena decumbens Jacq.	CMV/WMV
G. globosa L.	CMV/WMV
Lavatera trimestris L.	WMV/CMV
Luffa acutangula (L.) Roxb.	CMV/WMV*
L. cylindrica (L.) Roem.	CMV/WMV
Nicotiana chinensis Fisch.	CMV/WMV
N. glutinosa L.	CMV/WMV
N. knightiana Goodspeed	CMV/WMV
N. quadrivalvis Pursch.	CMV/WMV
N. tabacum L. cv. Bel 61—10	CMV/WMV
N. tabacum L. cv. Samsun	CMV/WMV
N. tabacum L. cv. Xanthi-nc	CMV/WMV
Ocimum basilicum L.	CMV/WMV
O. canum Sims.	CMV/WMV
Petunia hybrida Vilm.	CMV/WMV
Vigna sinensis (L.) Endl.	CMV/WMV

* *Luffa acutangula* (L.) Roxb. is locally susceptible to the specific (strain 1) strain of watermelon mosaic virus (EBRAHIM-NESBAT, 1974).

temic susceptibility (Table 1, Fig. 2). Five plants were found to be resistant to inoculation. On the basis of the examined host-virus relations the PC isolate is considered identical with the green strain of CMV (CMV-G) earlier isolated by us in Hungary (HORVÁTH, 1969; 1973, HORVÁTH and HINFNER, 1969, HORVÁTH and SZIRMAI, 1973; HORVÁTH and BECZNER, 1973; HORVÁTH *et al.*, 1975b). During the host plant studies 17 new experimental host plants of the PC isolate were found: *Ammi visnaga* (L.) Lam., *Commelina graminifolia* H. B. et K., *C. tuberosa* L., *Cucurbita pepo* var. *patissonina* f. *radiata*, *Erodium ciconium* (L.) L'Hérit., *E. cicutarium* (L.) L'Hérit., *E. malacoides* Willd., *Gomphrena decumbens* Jacq., *Malva borealis* Wallm., *M. moschata* L., *M. neglecta* Wallr., *M. pusilla* Sm. et Sow., *M. verticillata* L., *Nicotiana chinensis* Fisch., *N. knightiana* Goodpseed, *N. quadrivalvis* Pursch., and *Tetragonia crystallina* L'Hérit.

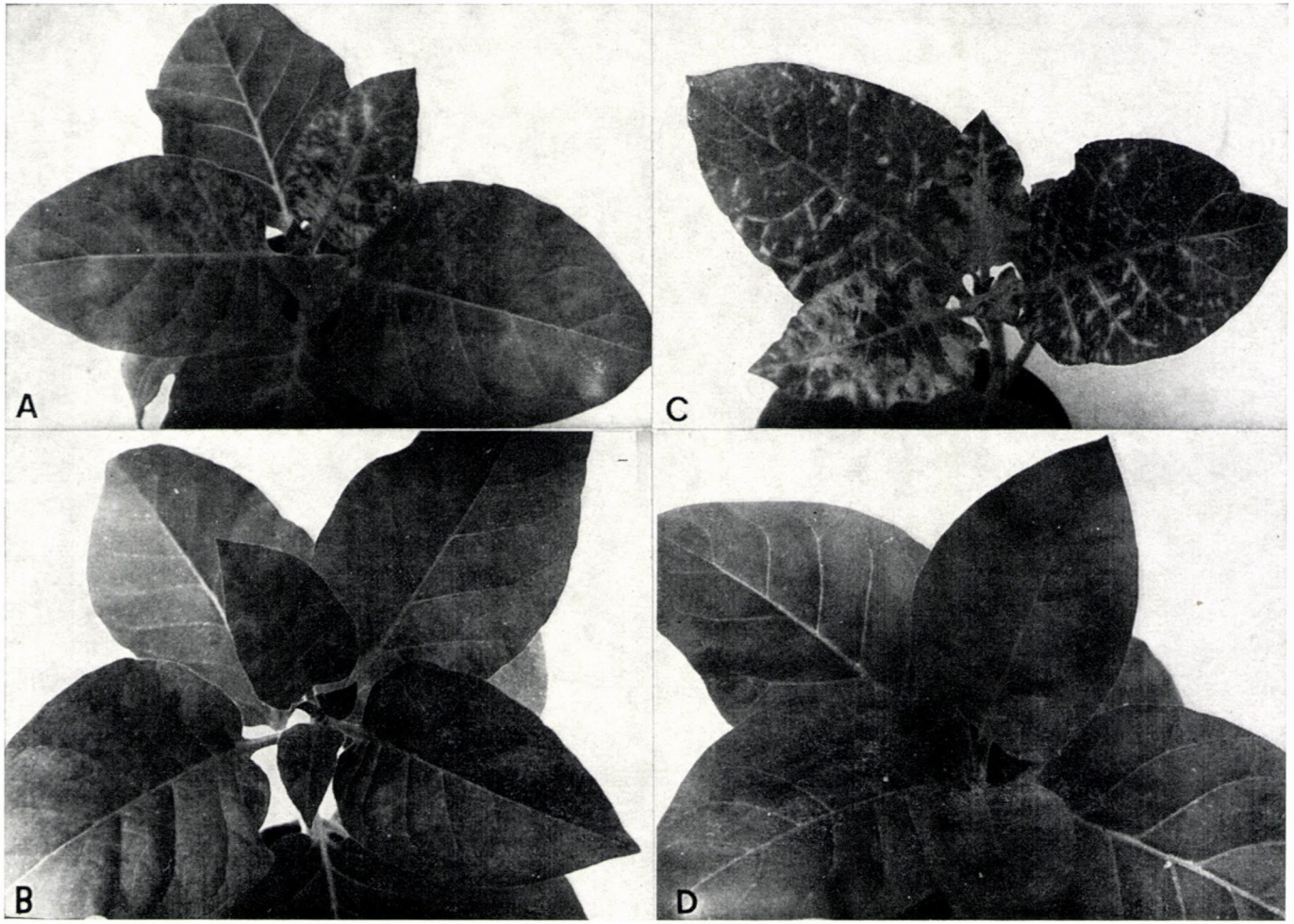

Fig. 3. *Nicotiana tabacum* L. cv. Xanthi-nc tobacco plants inoculated with CMV-W (A), CMV-G/PC (B). C and D: Cross protection test. CMV-W challenged with CMV-G/PC (C), CMV-G/PC challenged with CMV-W (D)

Leiophyllum buxifolium (Berg.) Ell. *(Ericaceae)* proved to be so far unknown new resistant plant. A comparison of the results of host plant studies performed with WMV-G (HORVÁTH *et al.*, 1975a) as well as in the present paper (see Table 1) with the PC isolate reveals that a number of plants are suitable to separate WMV–G and the PC isolate (CMV) from (Table 2). Of the two virus complexes, however, WMV is most easily separated from the CMV-resistant *Lavatera trimestris* and *Citrullus lanatus* plants, while in separating CMV the WMV-immune *Ecballium elaterium* and various *Nicotiana* species are considered to be of primary importance. We should like to point here to the fact that the transmission of the PC isolate from tobacco plants to cucurbitaceous plants (cucumber, patisson) involved difficulties, and later the virus could not in most cases be transmitted from tobacco, while its transmission from cucurbitaceous plants remained unchanged.

In the course of transmission experiments carried out with *Myzus persicae* aphids the PC isolate was readily transmitted in stylet-borne manner. We note, however, that the patisson is also a cucumber virus source, and in the case of patisson, and cucumber test plants the percentage of virus transmission was substantially higher (75 – 80 per cent) than with Samsun and Xanthi-nc as virus source and test plants (40 – 46 per cent). In studying the transmission of the PC isolate by patisson seeds we found that the virus could not be transmitted with the seeds of the diseased plants.

In the course of serological examinations the PC isolate gave a positive response to the CMV antiserum kindly placed at our disposal by Prof. Dr. Y. KOMURO (Aobacho, Chiba, Japan) and Prof. Dr. E. LUISONI (Torino, Italy). The results of the serological examinations proved then beyond any shadow of doubt that the identity with CMV had been reasonably assumed on the basis of the host range of the PC isolate and the characteristic symptoms of the different plants.

Further on we use the symbol CMV-G/PC to designate the virus isolated from patisson.

When studying the physical properties we found the thermal inactivation point of CMV-G/PC to be 62 – 64°C, its dilution end-point 2×10^{-4}, and longevity *in vitro* to be maximum 15 days.

In cross protection experiments the tobacco plants inoculated with the CMV-G/PC isolate were protected against a subsequent infection by the CMV-W strain (Fig. 3). The results of the cross protection test give evidence of a relationship between the CMV-G/PC and CMV-W strains – in the same way as between the strains isolated earlier in Hungary.

These investigation results confirm the ones obtained in the course of host plant-, aphid transmission- and serological examinations.

Our investigations into the natural occurrence of CMV called attention to numerous new host plants in Hungary in the last years. There are among them particularly remarkable cultivated and weed plants including herbaceous and woody species, e.g. *Brassica napus* L. (HORVÁTH 1969; HORVÁTH and HINFNER, 1969), *Echinocystis lobata* (Michx.) Torr. et Gray. (HORVÁTH and SZIRMAI, 1973), *Lycopersicon esculentum* Mill. (HORVÁTH and BECZNER, 1973), *Paulownia impe-*

rialis Sieb. et Zucc. (HORVÁTH, 1973), *Aristolochia clematitis* L., *A. durior* Hill. (syn.: *A. sipho* L'Hérit), and *A. elegans* Mast. (HORVÁTH *et al.*, 1975b).

The results presented here indicate that *Cucurbita pepo* var. *patissonina* f. *radiata* (patisson) plants are important reservoirs of CMV in the nature. This is apparently the first report on the presence of CMV in patisson.

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We express our thanks to Prof. Dr. Y. KOMURO (Aobacho, Chiba, Japan) and Prof. Dr. E. LUISONI (Torino, Italy) for kindly supplying the antiserum against cucumber mosaic virus. We are also grateful to Dr. GY. NAGY and Miss I. DOBROVSZKY (Mosonmagyaróvár, Hungary) for the facilities they offered concerning the field works. The technical assistance of Miss K. MOLNÁR and Miss M. BOLLÁN of the Laboratory of Virology (Keszthely, Hungary) is very much appreciated.

Literature

EBRAHIM-NESBAT, F. (1974): Distribution of watermelon mosaic viruses 1 and 2 in Iran. Phytopath. Z. *79*, 352—358.

HORVÁTH, J. (1969): Untersuchungen über eine Virose von *Brassica napus* L. Acta phytopath. Acad. Sci. hung. *4*, 29—44.

HORVÁTH, J. (1973): A mosaic, vein banding and chlorotic ring spot disease of *Paulownia* caused by cucumber mosaic virus. Phytopath. Z. *76*, 182—185.

HORVÁTH, J. and BECZNER, L. (1973): A paradicsom páfránylevelűség etiológiája. 1. Uborka mozaik vírus és dohány mozaik vírus izolálása és fertőzési kísérletek (Etiology of fern leaf disease of tomato. 1. Isolation of cucumber mosaic virus and tobacco mosaic virus and infection experiments). Növénytermelés *22*, 223—238.

HORVÁTH, J. and HINFNER, K. (1969): Natural infection of rape with cucumber mosaic virus in Hungary. Acta agr. Acad. Sci. hung. *18*, 226—229.

HORVÁTH, J. and SZIRMAI, J. (1973): Untersuchungen über eine Virose der Wildgurke (*Echinocystis lobata* [Michx.] Torr. et Gray.). Acta phytopath. Acad. Sci. hung. *8*, 329—346.

HORVÁTH, J., KUROLI, G. and NAGY, GY. (1974): A patisszon vírusbetegsége (Virus disease of patisson). Kertészet és Szőlészet *18*, 11.

HORVÁTH, J., JURETIĆ, N., BESADA, W. H. and KUROLI, G. (1975a): Two viruses isolated from patisson (*Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois.), a new vegetable natural host in Hungary. 1. Watermelon mosaic virus (general). Acta phytopath. Acad. Sci. hung. *10*, 93—111.

HORVÁTH, J., JURETIĆ, N. and MAMULA, D. (1975b): Some data on natural hosts of cucumber mosaic virus in Hungary and Yugoslavia. Acta Bot. Croatica *34*, 9—16.

MOSKOVETS, S. M., FEGLA, G. I. and GLUSHAK, L. YU. (1970): Some virus diseases of cucurbits in the southern regions of the Ukraine. Mikrobiol. Zh. *32*, 735—738.

PROVVIDENTI, R. and SCHROEDER, W. T. (1970): Epiphytotic of watermelon mosaic among *Cucurbitaceae* in central New York in 1969. Plant Dis. Rep. *54*, 744—748.

SCHMELZER, K. (1965): Das Wassermelonenmosaik-Virus tritt auch in Deutschland auf. Nachrbl. dtsch. Pflaschutzd. (Berlin) N. F., *3*, 69—71.

VAN REGENMORTEL, M. V. H. (1966): Plant virus serology. Adv. Virus Res. *12*, 207—271.

VAN REGENMORTEL, M. H. V. (1967): Serological studies an naturally occurring strains and chemically induced mutants of tobacco mosaic virus. Virology *31*, 467—480.

WEBB, R. E. (1961): Separation and identification of some viruses infectious to cucurbits. Phytopathology *51*, 802—803.

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The Production of Lubimin by Potato Tubers Inoculated with *Erwinia carotovora* var. *atroseptica*

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Tubers of potato cultivars Maris Peer, Desirée, King Edward and Pentland Dell were examined after inoculating with *Erwinia carotovora* var. *atroseptica* G120 for the presence of phytoalexins. In addition to rishitin and phytuberin, lubimin was found in all four cultivars at mean concentrations of 2.3 to 5.5 μg/g fresh weight of rotted tissue. Lubimin showed antibacterial activity towards *E. atroseptica* G120 in zone inhibition tests after thin-layer chromatography but only at concentrations approximately 10-fold greater than those found in the tubers.

Lubimin was first described as a phytoalexin produced in potato tubers infected with *Phytophthora infestans* (OZERETSKOVSKAYA, VASIUKOVA and METLITSKII, 1969; METLITSKII, OZERETSKOVSKAYA, VULFSON and CHALOVA, 1971a). A structure was originally proposed by METLITSKII *et al.* (1971b) and a revised structure has been suggested by STOESSL, STOTHERS and WARD (1974) and by KATSUI, MATSUNAGA and MASAMUNE (1974). Lubimin has also been isolated from potato leaves inoculated with *P. infestans* (METLITSKII, OZERETSKOVVSKAYA, VASIUKOVA, DAVIDOVA, SAVELYEVA and DYAKOV, 1974) and from the egg plant *(Solanum melongena)* and *Datura stramonium* inoculated with *Monilia fructicola* or other fungi (STOESSL *et al.*, 1974).

Other phytoalexins, rishitin and phytuberin, which were originally discovered in potato tubers inoculated with *P. infestans*, have subsequently been shown to occur in potatoes inoculated with *Erwinia carotovora* var. *atroseptica* (LYON, 1972; LYON, LUND, BAYLISS and WYATT, 1975). We wish to present evidence that lubimin is also formed as a consequence of infection with this bacterium.

Materials and Methods

Tubers

The potatoes were grown in Norfolk, England. Tubers of the cultivars Desirée and King Edward *(Solanum tuberosum)* and the *P. infestans*-resistant hybrids Maris Peer and Pentland Dell were harvested in August or September 1974

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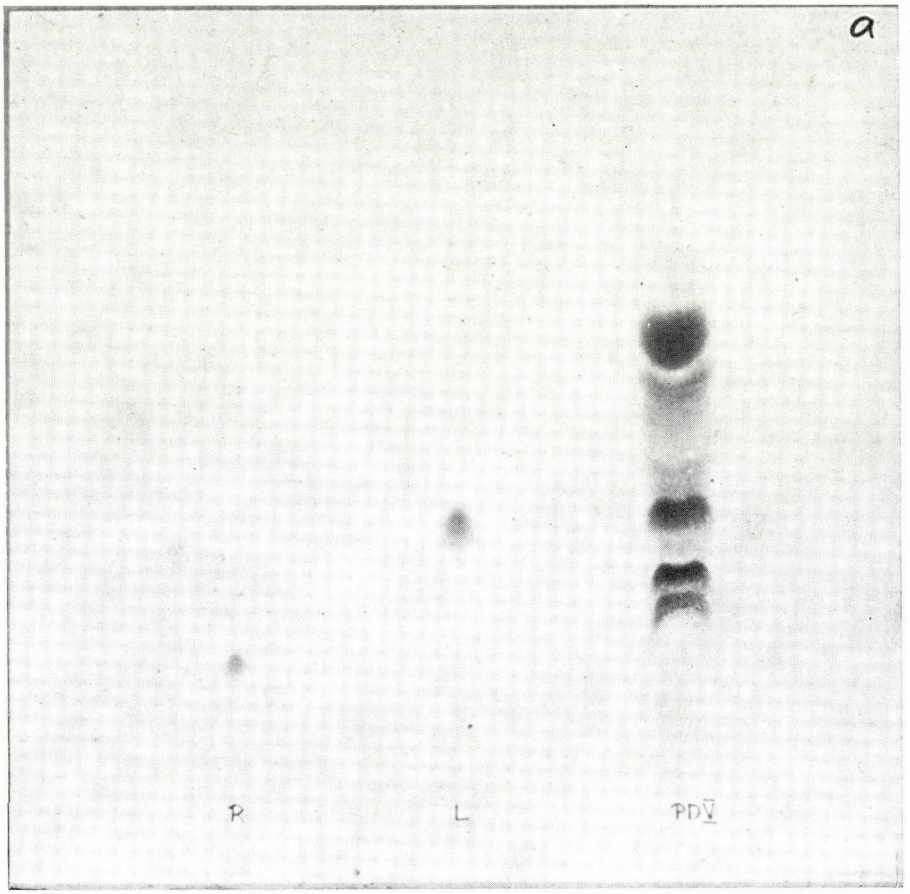

Fig. 1a. Thin-layer chromatogram of pure rishitin, lubimin and chloroform extract of Pentland Dell inoculated with *E. atroseptica* (Silica gel G_{uv} 254 running in chloroform acetone 9 : 1 v/v 2 times) Rishitin 50 μg Lubimin 50 μg. Tissue extract: 0.2 g dry wt a) developed with vanillin $-H_2SO_4$ reagent

and used for these experiments after storage for up to 5 weeks. Immediately after harvesting the potatoes were stored at 10°C for 2 weeks; the remainder of the storage was at 3°C.

Bacteria

Erwinia carotovora var. *atroseptica (E. atroseptica)* strain G120 (LUND and NICHOLLS, 1970; LYON, 1972) was used.

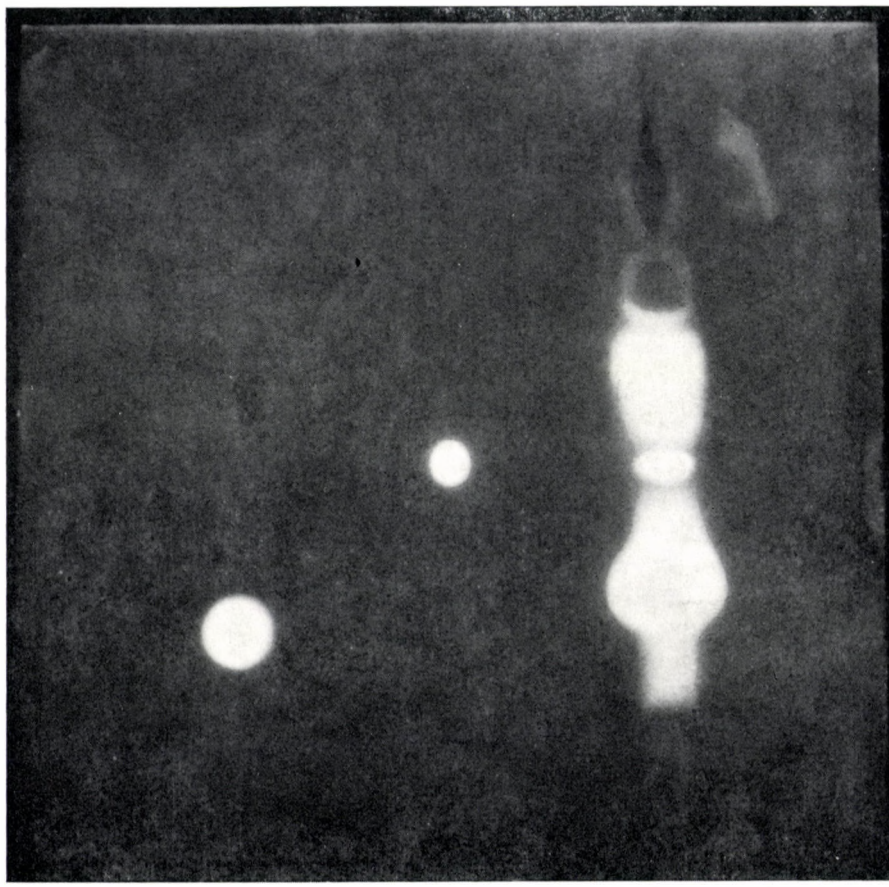

Fig. 1b. Antibacterial plate, sprayed with *E. atroseptica* G120, incubated overnight at 25°C, sprayed with aesculin reagent. Photograph taken under UV light

Inoculation of tubers

Bacteria were grown on slopes of "Difco" Heart Infusion Agar at 30° for 24 h. Growth was washed from the slopes with sterile distilled water and the suspension was adjusted to contain approximately 10^9 bacteria per ml. The method of inoculating the tubers was as previously described (LYON *et al.*, 1975). Ten or twenty tubers were inoculated per treatment, and control tubers were wounded and inoculated with sterile water.

Measurement of amount of rotting

The method of determining the fresh weight of rotted tissue was described by LYON *et al.* (1975).

Extraction of rishitin, phytuberin and lubimin

Immediately after removal from the tubers, the rotted tissue was placed in liquid nitrogen. It was then freeze-dried and stored at −20°C until extracted. Freeze-dried tissue (1 to 20 g dry weight) was macerated three times in a homogenizer (Measuring and Scientific Equipment Ltd.) at a speed of 7000 rpm for 2 min with 5 or 10 times its weight of 70% v/v methanol. The macerate was centrifuged at 8000 g for 15 min at +1°C. The material which sedimented was re-extracted twice in the same way, the combined supernatants were filtered through Whatman No. 1 filter paper and dried under reduced pressure at 40°C. The residue was dissolved in a mixture of water plus methanol (30 : 45) and extracted three times with 75 ml of chloroform, allowing one hour for the phases to separate. The combined chloroform phases were dried under reduced pressure at 40°. For further purification the residue was dissolved in ethyl acetate and applied onto thin-layer chromatography (t. l. c.) plates of non-activated silica gel G. 0.5 mm thick. The plates were developed twice in chloroform-acetone (9: 1 v/v). Compounds were detected by spraying markers with vanillin-sulphuric acid reagent (1 g vanillin, 30 ml methanol, 0.2 ml conc. sulphuric acid). Rishitin ($R_f = 0.22$) appeared turquoise, phytuberin ($R_f = 0.81$) purple and lubimin ($R_f = 0.42$) blue after heating the plates at 100°C for 5 min. In the case of the chloroform extracts of rotted potato, lubimin was obscured in this solvent system by desacetylphytuberin ($R_f = 0.43$) which gave a purple colour with the vanillin reagent. Bands adjacent to rishitin, phytuberin and lubimin markers were taken from the plates and eluted with ethyl acetate. Methyl stearate (1 mg) was added to each sample as internal standard, the samples were dried, re-dissolved in 0.2 ml cyclohexane and analyzed by gas-liquid chromatography (g. l. c). For the purpose of comparison, two authentic samples of lubimin were used. The first was purified by one of the authors (J. B.) in METLITSKII's laboratory in 1972; the second was obtained from Dr. S. F. OSMAN.

Estimation of rishitin, phytuberin and lubimin by g. l. c.

Conditions were those described by LYON (1972). The methyl stearate internal standard had a retention time of about 17 min. The retention times of rishitin, phytuberin and lubimin relative to methyl stearate were 0.59, 0.50 and 0.85 respectively. Quantitative estimations were made by relating peak areas (height of peak × width at half height) to the peak area of the internal standard. The average response ratios were for rishitin 0.69, for phytuberin 0.85 and for lubimin 0.72.

Results and Discussion

For the initial experiment extracts of rotted tissue from Desirée tubers were used. When fractions with R_f between 0.30 and 0.45 were eluted from t. l. c. plates and examined by g. l. c. an unknown peak was identified as lubimin by reference

to an authentic sample. The identity was confirmed by combined g. l. c. – mass spectrometry and comparison with authentic samples. The amounts of lubimin found in rotted tissue of four varieties of potato are shown in Table 1, with results

Table 1

Lubimin, rishitin and phytuberin in rotted tissue of four cultivars of potato inoculated with *E. carotovora* var. *atroseptica G120*

Tubers had been stored for less than five weeks after harvesting. After inoculation they were stored in air at 10°C and 90–100% relative humidity for 16 days

Cultivar	Concentration (μg per g. fresh wt. of rotted tissue) of:		
	Lubimin	Rishitin	Phytuberin
Maris Peer*	5.5	223	16
Desirée*	3.5	281	448
King Edward+	4.0	166	141
Pentland Dell+	2.3	120	189

* Results are the mean from two experiments each using 20 tubers
\+ Results are from a single experiment using 20 tubers

for rishitin and phytuberin for comparison. The concentrations of lubimin detected (2.3 to 5.5 μg per g wet weight of tissue) in the rotted tissue were low compared with those of rishitin and phytuberin. The concentrations of lubimin previously reported in potato tissue infected with *P. infestans* were 97 to 150 μg/g wet weight of tissue (METLITSKII *et al.*, 1974) and 0.2 μg per g wet weight of tissue (KATSUI *et al.*, 1974).

When tested for antibacterial activity on t. l. c. plates as described by LUND and LYON (1975), 50 μg of lubimin gave a zone of inhibition of *Erwinia atroseptica* G120, (Fig. 1) but it is likely that the concentration of lubimin required to significantly inhibit this bacterium is higher than that found so far in infected tubers.

Acknowledgements

We wish to thank Dr. S. F. OSMAN, U. S. D. A., Eastern Regional Research Center, Philadelphia, Pa, for supplying a sample of lubimin, Dr. G. D. LYON for valuable advice regarding the method of extraction and analysis by g. l. c., Dr. P. A. T. SWOBODA for advice on g. l. c., Dr. D. T. COXON for helpful discussions and Mr. R. SELF for mass spectral data. The work was financed by a Fellowship from the International Atomic Energy Agency to J. B.

Literature

KATSUI, N., MATSUNAGA, A. and MASAMUNE, T. (1974): The structure of lubimin and oxylubimin, antifungal metabolites from diseased potato tubers. Tetrahedron Lett. *51/52*, 4483–4486.

LUND, B. M. and LYON, G. D. (1975): Detection of inhibitors of *Erwinia carotovora* and *E. herbicola* on thin-layer chromatograms. J. Chromat. (In Press).

LUND, B. M. and NICHOLLS, J. C. (1970): Factors influencing the soft-rotting of potato tubers by bacteria. Potato Res. *13*, 210–215.

LYON, G. D. (1972): Occurrence of rishitin and phytuberin in potato tubers inoculated with *Erwinia carotovora* var. *atroseptica*. Physiol. Plant Pathol. *2*, 411–416.

LYON, G. D., LUND, B. M., BAYLISS, C. E. and WYATT, G. M. (1975): Resistance of potato tubers to *Erwinia carotovora* and formation of rishitin and phytuberin in infected tissue. Physiol. Plant Pathol. *6* (1), 43-50.

METLITSKII, L. B., OZERETSKOVSKAYA, O. L., VULFSON, N. S. and CHALOVA, L. I. (1971a): Effects of lubimin on potato resistance to *Phytophthora infestans* and its chemical identification. Mikol. Fitopatol. *5*, 438–443.

METLITSKII, L. V., OZERETSKOVSKAYA, O. L., VULFSON, N. S. and CHALOVA, L. I. (1971b): Chemical nature of lubimin, a new potato phytoalexin. Dokl. Akad. Nauk. SSSR (Biochemistry). *200*, 1470–2.

METLITSKII, L. V., OZERETSKOVSKAYA, O. L., VASIUKOVA, N. I., DAVIDOVA, M. A., SAVELYEVA, O. H. and DYAKOV, YU. T. (1974): Role of phytoalexins in vertical resistance of potato plants to *Phytophthora infestans*. Mikol. Fitopatol. *8*, 42–49.

OZERETSKOVSKAYA, O. L., VASIUKOVA, N. I. and METLITSKII, L. V. (1969): Izutsenie fitoaleksinov kartofelia (Study of the phytoalexins of potatoes). Dokl. Akad. Nauk. SSSR. *189*, 1146–1149.

STOESSL, A., STOTHERS, J. B. and WARD, E. W. B. (1974). Lubimin: a phytoalexin of several *Solanaceae*. Structure revision and biogenetic relationships. J. C. S. Chem. Comm. 709–710.

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Contributions to the Mechanism of Infection of *Erwinia uredovora*, a Parasite of Rust Fungi

By

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Strains of rust parasitic *Erwinia uredovora* were isolated from broad bean and wheat. It was established that the isolates could not be regarded specific for different rust species.

In the course of investigations on the mechanism of bacterial infection it was found that the bacterium itself does not damage the host tissue, thus, with respect to the plant *E. uredovora* may be considered as a saprophyte. The bacterium can multiply in the host tissue only if it has been infected with rust. The bacterium kills the rust in every phase of development. Heat-killed cells have no rust inhibiting effect. We found the same in the case of the saprophyte *Pseudomonas fluorescens*. It turned out that the bacterium is able to multiply merely in the living rust fungus. As a result of bacterial infection, a symptom similar to the hypersensitive necrosis is formed in the host tissues around the dead rust fungi.

Xanthomonas uredovorus a rust parasitic bacterium is well known from the earlier literature (cf. PON *et al.*, 1954). KLEMENT and KIRÁLY (1957) reported on the occurrence of the bacterium in Hungary. DYE (1963) suggested puting the species into the *Erwinia* genus, giving it the name of *Erwinia uredovora* Dye. Although PON *et al.* (1954) described the optimal conditions for infection as well as the host range, we know little about the host—parasite relationship.

Our primary aim was to investigate the mechanism of infection by *Erwinia uredovora*. We wanted to get an answer to the following: How specific is the bacterium regarding its host range? Does it damage the host tissue directly? Is it able to multiply in the plant tissue or is its multiplication restricted to the rust? In what stage of development is the fungus susceptible to bacterial infection? Are only living bacterial cells able to counteract rust infection? Can saprophytic bacteria *(Pseudomonas fluorescens)* exhibit a rust inhibiting effect? Can the bacterium multiply only on living rust fungi or also on plant tissues containing rust but killed by heat?

In our investigations we examined bacterium strains isolated by us from different rust infected plants (broad bean and wheat).

Materials and Methods

Three bacterial strains were isolated from broad bean and two from wheat. The biochemical tests necessary for their identification were carried out according to DOWSON (1957). *Pseudomonas fluorescens* Migula was used as the saprophytic control. The rust fungi in the investigations were the following: *Puccinia graminis* f. sp. *tritici* race 11, *Uromyces phaseoli*, *Uromyces fabae*. The test plants were grown in the greenhouse in sterile soil. Seven-day-old wheat seedlings (*Triticum aestivum* cv. Reliance C. I. 7370) twelve-day-old broad bean *(Vicia fabae)* and 14-day-old bean plants *(Phaseolus vulgaris* cv. *Pinto)* were inoculated with rust or with the bacterium. The suspension of the bacterium containing 10^7 cell/ml was introduced into the plant by spraying, by brushing or by direct injection. The change in the cell number of the bacteria could be followed on the basis of 10 leaf disc sample (12.5 mm^2 area) homogenates. The homogenate was serially diluted in physiological saline and spread onto nutrient broth agar medium. From the number of the developed colonies we could conclude as to the number of the bacteria in the original tissue. A bacterial suspension was held in a water bath at 100°C for 30 min to get the killed cells. To kill the rust fungi, the rusted leaves were dipped in 50°C water for 25 seconds (YARWOOD, 1963).

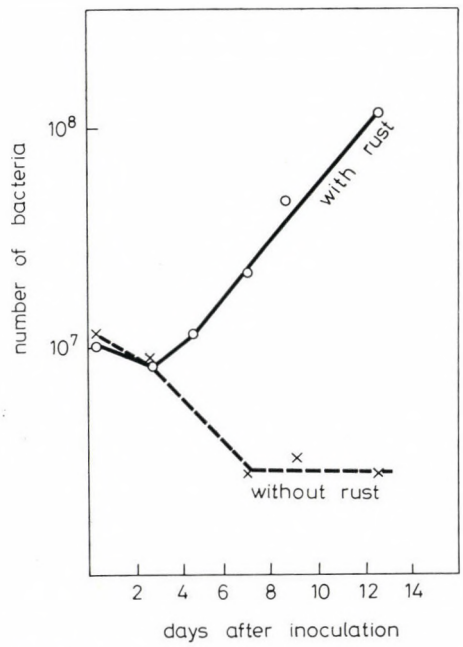

Fig. 1. Multiplication of populations of *Erwinia uredovora* in broad bean leaf tissue. ——— Simultaneously inoculated with *Uromyces fabae* and *Erwinia uredovora*. — — — — Healthy, non-rusted leaves inoculated only with *E. uredovora*

Fig. 2. Pinto bean leaf inoculated with *Uromyces phaseoli*, then the right half leaf inoculated after 1 day with *E. uredovora* 10^7 cell/ml

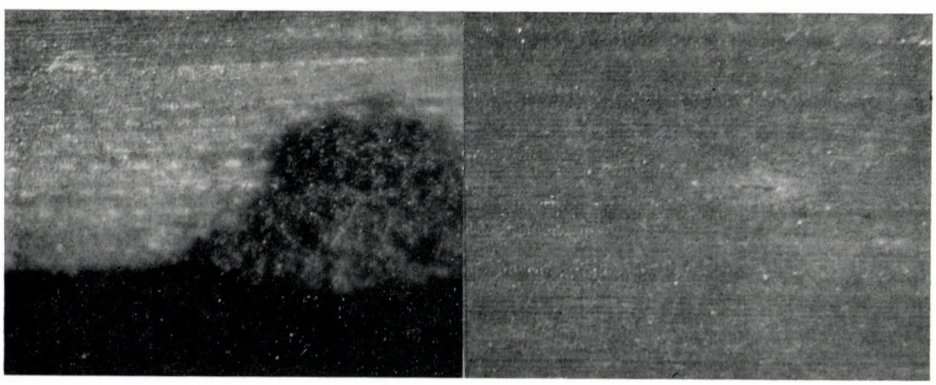

Fig. 3. Rust *Puccinia graminis* development on Reliance wheat leaves. A: simultaneously inoculated rust and bacterium; B: inoculated only with stem rust

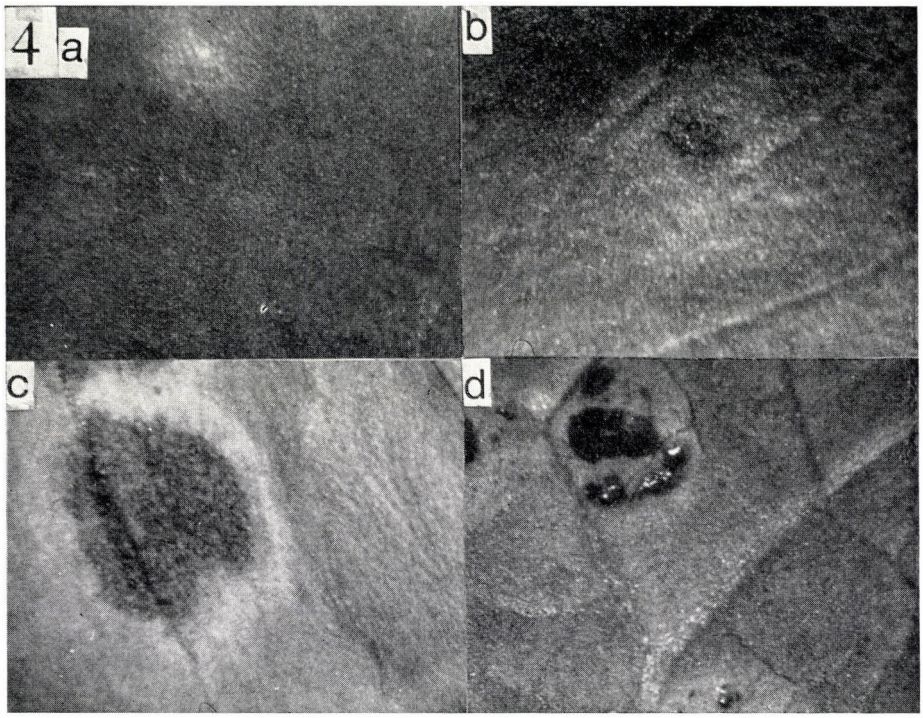

Fig. 4. Rust *(Uromyces fabae)* development on broad bean leaves. A: inoculated with rust and four days after rust infection with *E. uredovora;* B: seven days; C: ten days after rust infection with *E. uredovora;* D: inoculated only with rust

Results and Discussion

The bacterium studied by us was identical in morphological, physiological and biochemical properties to the characteristics of 0086 (the Hungarian strain) and to those of PON's strain.

If the suspension of bacteria was in contact with the rust colonies by spraying, brushing or if the suspension was injected directly into the intercellular tissue of rusted leaf we could always observe the rust-damaging effect of the bacterium. Bacterial treatment stopped the development of fungi and destroyed the developed colonies which discoloured to dark brown or black. It could be observed that the parasitized colonies were limited by a water saturated transparent tissue. The most effective result was achieved with the injection of the bacterial suspension into the host intercellulars. It was shown that the bacterial isolate originating from the broad bean infected the wheat rust and the isolate originating from the wheat rust infected the broadbean rust. Both isolates proved to be pathogenic on bean

rust too (Figs 2, 3a, 4a, b, c). Our further investigations were carried out using the R-1 strain originating from wheat rust.

To determine whether *E. uredovora* itself does not cause tissue destruction and whether the translucent spots around the rust colonies can be considered as a consequence of bacterial damage, the bacterial suspension was injected without the presence of rust into healthy bean, broad bean and wheat leaves. In two weeks after injection there was no sign of any symptom or other changes on the plants. *E. uredovora* injected into the tobacco leaf (KLEMENT *et al.*, 1963) in more than 10^7 cell/ml concentration did not induce the hypersensitive reaction.

We also studied that question whether the *E. uredovora* is able to increase in plant tissue or whether its multiplication is limited to rust infection? So we infected the leaves of broad bean plants with rust and bacteria. The change in the cell-number of the injected bacteria were detected every second day on the basis of cell count of the sample. The bacterial cell-number in the case of two kinds of treatments showed a slight drop on the third day following the injection while in the rust-infected tissue it began to rise on the 5th day and on the 11th day it rose by ten fold. During this time the bacterial number always remains lower than the cell-number of the initially injected cell-number (Fig. 1). Thus *E. uredovora* seems to be unable to multiply in the plant tissue without rust infection.

In order to determine the sensitive phase of the rust fungus the bacterial suspension was injected 2, 4, 6, 8 or 10 days after the stem rust infection into the leaf tissue until the appearance of the rust colonies. At simultaneous infection (bacteria and fungi) the colonies were not visible (Fig. 3a). On the seventh day after rust infection in the so called chlorotic phase host tissues turned to necrotic and the effect of the bacteria on the host tissue was very similar to a hypersensitive necrosis (Fig. 4b, c). Similar tissue necroses were observed when rust colonies were killed by heat or by chemical agents (BARNA *et al.*, 1974; ÉRSEK *et al.*, 1973; KIRÁLY *et al.*, 1972). When bacteria were killed with heat the development of the rust colonies was not influenced at any rate. Thus living bacterial cells are necessary for rust inhibition.

Another question arose namely whether rust inhibition merely occurred on the effect of *E. uredovora* or whether the same can be induced with saprophytes too. To clarify this question we applied *Pseudomonas fluorescens* suspension instead of *E. uredovora*. *P. fluorescens* did not inhibit rust development, and so necrotic spots were not observed in the leaves.

We wanted to find out whether the bacterium was able to multiply only on living rust fungi and so the following experiment was conducted. Starting on the second day after rust inoculation the broad bean were treated every second day with heat in water bath in order to kill the rust in the host tissue at 2, 4, 6, 8, 10, 12 and 14 day after inoculation. Then the leaves were infiltrated with *E. uredovora* suspension and the number of bacteria in broad bean tissue was determined. We found that the bacteria did not multiply in the tissue where the fungus was killed, just as in the healthy, not rusted plants. It turned out, therefore, that it is not the

host tissue which is parasitized by the bacterium, even if its metabolism might be changed by rust infection but the living fungus itself.

Literature

BARNA, B., ÉRSEK, T. and MASHAAL, S. F. (1974): Hypersensitive reaction of rust-infected wheat in compatible host-parasite relationships. Acta phytopath. Acad. Sci. hung., *9* 293 – 300.

DOWSON, W. J. (1957): Plant Disease due to Bacteria. Cambridge Univ. Press., England.

DYE, D. W. (1963): The taxonomic position of *Xanthomonas uredovorus* Pon et al. 1954. N. Z. J. Sci. *6*, 146 – 149.

ÉRSEK, T., BARNA, B. and KIRÁLY, Z. (1973): Hypersensitivity and the resistance of potato tuber tissue of *Phytophthora infestans*. Acta phytopath. Acad. Sci. hung. *8*, 3 – 12.

KIRÁLY, Z., BARNA, B. and ÉRSEK, T. (1972): Hypersensitivity as a consequence, not the cause, of plant resistance to infection. Nature *239*, 456 – 458.

KLEMENT, Z. and KIRÁLY, Z. (1957): Hyperparasitic chain of a fungus, bacterium and its phage on wheat. Nature, *179*, 157 – 158.

KLEMENT, Z., FARKAS, G. L. and LOVREKOVICH, L. (1964): Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology *54*, 474 – 477.

PON, D. S., TOWNSEND, C. E., WOSSMAN, G. E., SCHMIDT, C. C. and KINGSOLVER, C. H. (1954): A *Xanthomonas* parasitic on uredia of cereal rust. Phytopathology *44*, 707 – 710.

YARWOOD, C. R. (1963): Heat therapy of bean rust. Phytopathology, *53*, 1313 – 1316.

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An Unknown Symptom on Onion Plants Caused by *Pseudomonas alliicola* (Burkholder) Starr et Burkholder

By

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On the seedstalks and leaves of onion plants collected in the field in the summer of 1974 diesase symptoms of unknown origin were found. From the necrotic lesions the authors isolated the bacterium *Pseudomonas alliicola*, which had not been reported before from Hungary. The pathogenic character of the bacterium was demonstrated by successful inoculations on leaves, seedstalks and onion bulbs. The inoculation of onion bulbs yielded characteristic "mushy rot" symptoms, which were well distinguishable from those of the smelly "soft rot" well known to onion growers.

It has been established that both the dry necrosis on onion leaves and the mushy rot in onion bulbs are caused by the same pathogen, i.e. by *P. alliicola*. It has not been described so far in the literature that *P. alliicola* would cause symptoms under field conditions on the onion foliage as well.

In the summer of 1974 our attention was called to interesting symptoms which appeared in spots in the onion stands grown either from set or for seed. On the green parts of the onion plants white, scattered, longitudinally elongated spots appeared which were sunken and made the impression of tissue necrosis.

In his 1942 paper describing the bacterial diseases of onion, BURKHOLDER mentioned in a new pathogen under the name *Phytomonas alliicola* n. sp. which has been found in more than one state of the United States. This bacterium was later re-named as *Pseudomonas alliicola* (Burkholder) Starr et Burkholder, by STARR and BURKHOLDER (1942). The occurrence of this pathogen has been reported in Europe until now only in one case, from Bulgaria (VITANOV, 1967, 1970). Both BURKHOLDER and VITANOV isolated the bacterium from onion bulbs and regarded the organism as exclusively an onion-bulb pathogen.

Material and Methods

From leaves, seedstalks and bulbs of diseased plants collected in the field; many isolations were carried out by using as a medium beef-extract peptone agar, The pure cultures obtained in this way were transferred into healthy onion leaves and seedstalks by needle. The inoculations were extended also to the bulbs, by injecting 10^7 cell/ml density of bacteria suspension into the fleshy scales of the bulbs or, by an other method, by dropping the suspension onto the bulb neck.

For the identification of the bacterium the following cultivation techniques and biochemical tests were used: Gram staining, gelatine liquification, litmus milk test, nitrate reduction, indole production, hydrogen sulphide production, lipolytic activity, production of acids from different sugars, starch hydrolysis (DOWSON, 1957); production of pectinase on potato and carrot slices (HENNIGER, 1965); hypersensitive reaction on Xanthi and Burley tobacco (KLEMENT *et al.*, 1964). Both in the inoculations and in the biochemical tests mentioned, three *P. alliicola* strains were used for comparison, which we have received from the United States (strains Pa 6 and Pa 15) and from England (No. 2500).*

Results

Three–four days following the inoculation of onion leaves and seedstalks the same characteristic symptoms appeared which had been established earlier on the field-collected material. At the site of inoculation white, oblong, slightly sunken, tongue-shaped spots appeared with well marked contours; from these spots both up- and downward along the veins thin yellow-green stripes were visible, indicating the direction of bacterial invasion into the tissues. The progress of the pathogen was much faster towards the tip of the leaf than in the opposite direction (Fig. 1). The infected leaves later showed wilt symptoms, then soon became dry (Fig. 2). Similar symptoms were observed also on the seedstalks.

In case of onion bulb inoculations, the tissues around the inoculation points became first yellow and sunken then later the sunken parts changed colour to very pale brown. In the following stage the discoloured tissues disintegrated and became mushy (Figs 3 and 4). The authors suggested for the disease the name "mushy rot of onion" by considering the latter symptom. It occurs quite often that heavily attacked fleshy scales are surrounded by completely healthy, uninfected ones (Fig. 5).

After the successful reinfections it became obvious that the bacterium was pathogen. In the following cultivations it was noted that the isolates produced on beef-extract peptone agar first small, white colonies which changed later to dirty-white or grey and discoloured the medium to deep brown. The bacteria proved to be Gram-negative, they liquified gelatine, produced nitrites from nitrates, peptonized the litmus milk and reduced litmus. There was neither indole nor hydrogen sulphide production observed, at the same time a high lipolytic activity was noted. The bacterium produced acid from the sugars L-arabinose, rhamnose, D-galactose, fructose, D-lactose, and mannitol; no acid was produced, however, from saccharose. No starch hydrolysis and pectine decomposition occurred. The isolates studied caused distinct necrotic lesions, on the leaves of both tobacco varieties but no continuous necrotized areas were produced.

* Thanks are due to M. P. STARR, International Collection of Phytopathogenic Bacteria (ICPB) and R. A. LELLIOT, National Collection of Plant Pathogenic Bacteria (NCPPB), for sending us the strains of *Pseudomonas alliicola* (Burkholder) Starr et Burkholder.

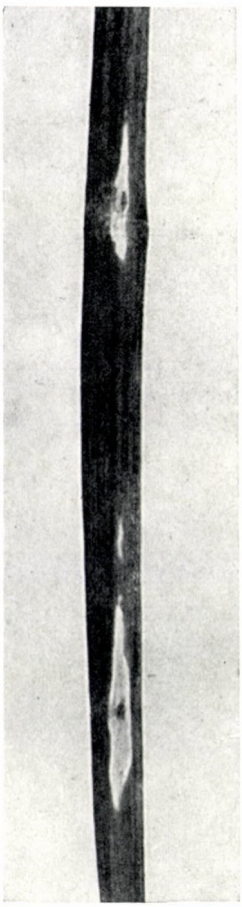

Fig. 1. Tissue necrosis on onion leaf inoculated with *P. alliicola*

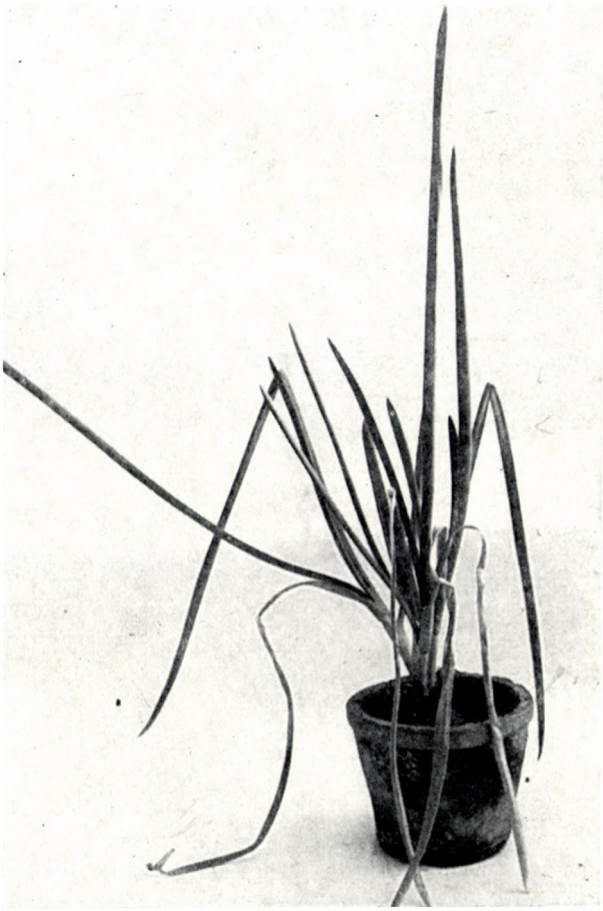

Fig. 2. Dried onion leaves 10 days after inoculation with *P. alliicola*

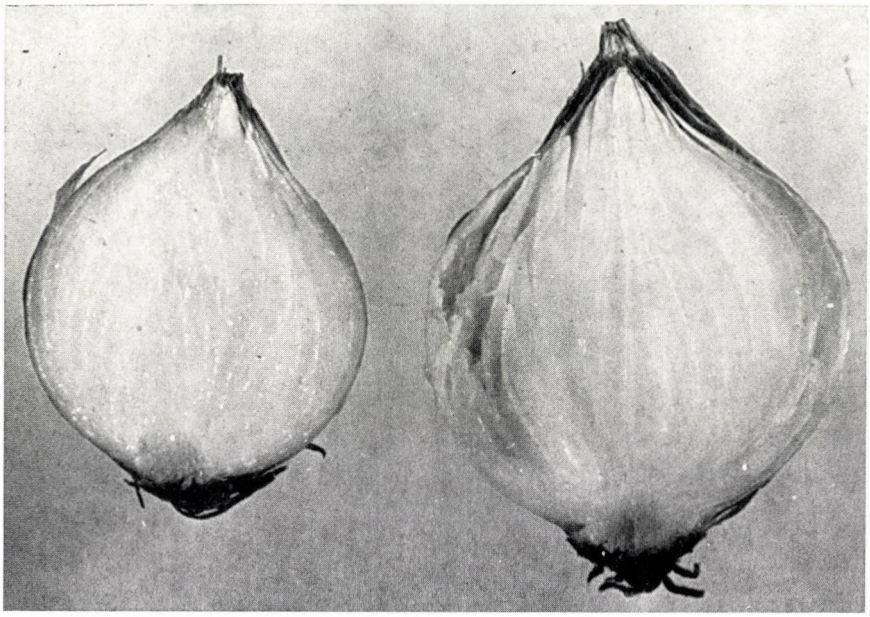

Fig. 3. Mushy rot of onion bulb 5 days after the inoculation with *P. alliicola*

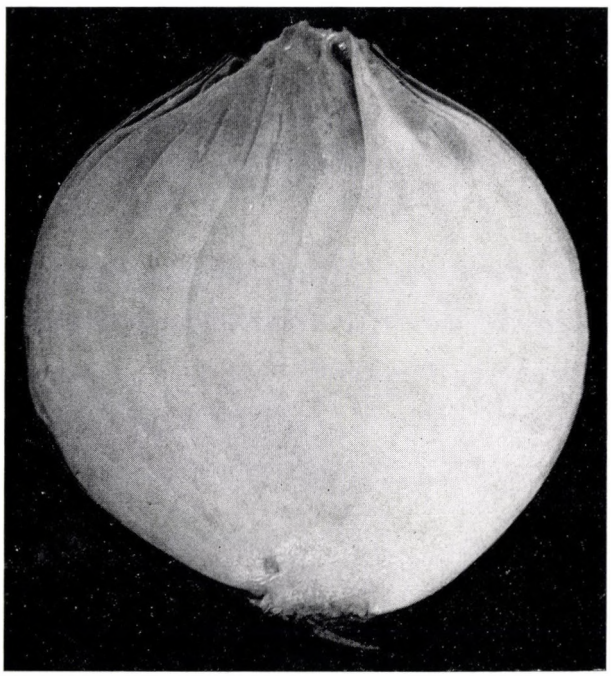

Fig. 4. Discoloration of the invaded tissues of an onion bulb inoculated with *P. alliicola*

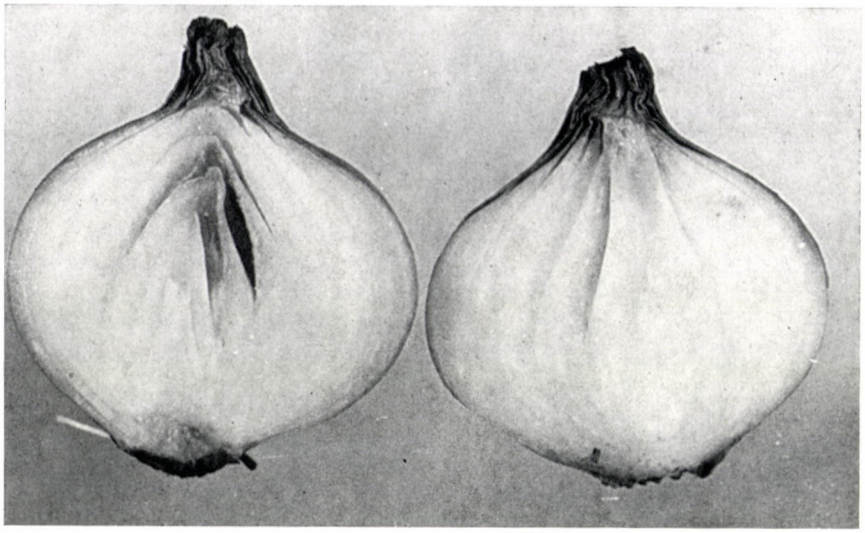

Fig. 5. Diseased fleshy scales next to healthy ones inside an onion bulb inoculated with *P. alliicola*

Discussion

The literature did not report any symptoms on the leaves and seedstalks of onion caused by *P. alliicola*. BURKHOLDER (1942) describing the disease the first time mentioned only the rotting on onion bulbs and VITANOV (1970) observed some leaf injuries only by inoculating the seedstalks.

In the biochemical and pathological investigation carried out by the authors, both the strains received from abroad and those isolated in Hungary from onion leaves, seedstalks and bulbs, behaved in an identical way; it could be stated therefore that the dry necrotizations on foliage and the mushy rot symptoms on bulbs are caused by the same pathogen *Pseudomonas alliicola*. The present paper gives the first description of symptoms on onion leaves and seedstalks observed under natural conditions.

Literature

BURKHOLDER, W. H. (1942): Three bacterial plant pathogens: *Phytomonas caryophylli* sp. n., *Phytomonas alliicola* sp. n., and *Phytomonas manihotis* (Arthaud-Berthet et Bondar) Viegas. Phytopathology *32*, 141—149.

DOWSON, W. J. (1957): Plant Diseases due to Bacteria. Cambridge at the University Press, London.

HENNIGER, H. (1965): Untersuchungen über Knollen-, und Lagerfäulen der Kartoffel. I. Zur Methodik der Resistenzprüfung mit dem Erreger der bakteriellen Knollennaßfäule (*Pectobacterium carotovorum* var. *atrosepticum* (van Hall) Dowson). Züchter *35*, 174—180.

KLEMENT, Z., FARKAS, G. L. and LOVREKOVICH, L. (1964): Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology *54*, 474—477.

STARR, M. P. and BURKHOLDER, W. H. (1942): Lipolytic activity of phytopathogenic bacteria determined by means of spirit blue agar and its taxonomic significance. Phytopathology *32*, 598—604.

VITANOV, M. (1967): Sipkavo meko gniene na luka. Gradinarstvo *5*.

VITANOV, M. (1970): Sipkavo meko gniene na luka, prichinyavano ot *Pseudomonas alliicola* Burkholder. Grad. i Loz. Nauka *7*, 83—89.

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The Effect of Grapevine Pollen on the Germination of Conidia of *Botrytis cinerea*

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The grapevine pollen markedly stimulated the germination of conidia of *Botrytis cinerea*. On its effect the germination period significantly decreased and after 2 hours 2.1—62.0% of the conidia was already germinated, whereas, there was no germination in the controls. On the effect of the pollen diffusate the germination per cent in the highest conidium concentration was 48 times, in the lowest concentration 3—3.5 times, while it was on the average 17 times higher than in distilled water. The stimulatory effect influenced the length of the germ tube, too. The germ tube was 4 times longer in the lowest conidium concentrations, while it was on the average 2 times longer than in the distilled water controls.

Many factors affect the germination of spores of pathogenic fungi on the surface of the host plant. The effect of atmospheric factors such as rainwater, air-humidity and air-temperature are well known, but many data prove that in addition to these, there are other important factors, too. So the germination of the conidia of *Botrytis cinerea* and other pathogenic fungi is significantly influenced by the exudates on the surface of the plant organs and either inhibit (PURKAYASTHA and DEVERALL, 1965; BLAKEMAN and SZTEJNBERG, 1973) or stimulate it (BROWN, 1922; KOVÁCS and SZEŐKE, 1956; HUNTER *et al.*, 1972).

The effect of other exogenous factors is also noticeable. Among these it should be mentioned that certain saprophytic bacteria and fungi living on the surface of leaves inhibit the germination of spores (BLAKEMAN, 1972; FOKKEMA and LORBEER, 1974), while the pollen grains sticking to the surface of the organs stimulate it. The stimulation helps the infection and generally, the surface colonization by fungi (OGAWA and ENGLISH, 1960; BACHELDER and ORTON, 1963; CHOU and PREECE, 1968; FOKKEMA, 1968; 1971). The aim of these investigations was to get an answer as to the influence of the grapevine pollen on the germination of grey mould conidia.

Materials and Methods

In our investigations a Hungarian hybrid of *Vitis vinifera* L. the cultivar "Ezerjó" and an American cultivar, "Othello" were used. The pollen was gathered during blooming, by shaking. These were cleaned with a fine sieve from the flower parts and used according to the following procedure.

A 6 ml basic suspension was prepared from the pollen grains with bidistilled water (suspension "A") the concentration of which was set 60 000 pollen grains/ml with a haemocytometer. The suspension was soaked for 12 hours while slowly revolving (50 turns/min) and finally the pollen grains were eliminated with filtration from the diffusate.

The conidial basic suspension was prepared with an isolate of *Botrytis cinerea* Pers. (No. BoCi.-Tar. 972–2), originating from the Tokaj grape–growing–district. Conidia of the two-week-old culture grown on potato-dextrose-agar medium were used firstly for inoculation of some new grapevine shoots in a hygrostat. After 15–20 days, the abundantly developed conidiophores were washed with a wet brush to collect conidia in order to prepare 6 ml basic suspension of 200,000 conidia/ml and it was used as suspension "B".

The following dilutions were prepared from the above-mentioned two basic suspensions for the concentrations used in the experiment:

concentration a: 1 ml suspension "A" + 1 ml suspension "B" = 30 pollen grains + 100 conidia/μl.
concentration b: 1 ml suspension "A" + 1 ml 10× diluted suspension "B" = = 30 pollen grains + 10 conidia/μl.
concentration c: 1 ml suspension "A" + 1 ml 100× diluted suspension "B" = = 30 pollen grains + 1 conidium/μl.

Of each concentration 0.05 ml was dropped onto a slide and this was placed on a 10 cm ø Petri dish lined with wet filter paper on 2 glass sticks and covered. Three series were prepared from each of the concentrations while only one was used each from the sterile bidistilled water, and tap water controls for the three different concentrations.

The drop cultures were kept at 22°C room temperature in diffused light and evaluated after 2, 6, 10 and 22 hours. The investigation was carried out with a microscope having 300× magnification. From every series the total and the germinating conidia were counted in 20 fields, and then the germination per cents were calculated. In the course of the experiment 20,000 conidia were investigated.

Results

The effect of pollen grains on the germination of *B. cinerea* conidia is shown in Tables 1 and 2. The results prove that the grape pollen stimulates the germination of the conidia. The stimulating effect is well proved by the fact that the period of germination is decreased and after two hours conidia already germinated in the presence of pollen in every concentration, while they did not without pollen in the distilled and tap water controls. This quick germination was especially significant on the effect of the Othello pollen, (15.3–62.0%), while of the Ezerjó it was markedly less (2.1–8.73%).

Table 1

The effect of Ezerjó pollen on the germination of *Botrytis cinerea* conidia

Cultivar	Concentrations		Series	Germination of the conidia and the lenght of the germ tube after							
	pollen grains μl	conidia μl		2 hours		6 hours		10 hours		22 hours	
				%	μ	%	μ	%	μ	%	μ
Ezerjó	30	100	1	1.9	3	26.4	45	32.0	72	42.9	90
			2	1.5	3	23.9	36	32.6	54	36.8	118
			3	2.8	3	21.8	40	32.1	72	42.2	109
			Ø	2.1	3.0	24.0	40.3	32.2	66.0	40.6	105.7
	0	100	control dest. water	0.0	0	0.5	22	2.2	36	6.0	90
	0	100	control tap water	0.0	0	0.0	0	0.0	0	0.3	27
	30	10	1	4.4	5	44.4	51	55.0	85	64.8	242
			2	3.8	5	45.8	85	50.4	130	70.9	170
			3	9.5	5	65.1	68	67.0	187	87.1	208
			Ø	5.9	5.0	51.8	68.0	57.5	134.0	74.3	206.7
	0	10	control dest. water	0.0	0	2.5	25	5.3	68	15.2	123
	0	10	control tap water	0.0	0	0.0	0	0.0	0	0.4	21
	30	1	1	12.7	5	72.3	68	85.6	280	85.6	404
			2	5.6	5	63.8	51	83.6	210	91.2	510
			3	7.7	5	60.0	75	89.1	245	94.9	510
			Ø	8.7	5.0	65.4	64.7	86.1	245.0	90.6	475.0
	0	1	control dest. water	0.0	0	3.1	55	9.3	65	26.6	102
	0	1	control tap water	0.0	0	0.0	0	0.7	3	1.5	34

Comparing the two cultivars, on the effect of the Othello pollen after 2 hours, in the same conidium concentrations, the germination was $7-10\times$ higher than in the case of Ezerjó. However, the pace of the latter later increased and after 10 hrs the Ezerjó pollen was stimulating more markedly.

The stimulating effect was convincing later, too, but its degree changed dependent on the conidium concentration. The highest percentage of germination

Table 2

The effect of Othello pollen on the germination of *Botrytis cinerea* conidia

Cultivar	Concentrations		Series	Germination of the conidia and the lenght of the germ tube after							
	pollen grains µl	conidia µl		2 hours		6 hours		10 hours		22 hours	
				%	µ	%	µ	%	µ	%	µ
Othello	30	100	1	15.2	3	20.8	34	27.3	42	31.9	85
			2	10.2	3	16.6	42	23.7	42	24.5	85
			3	20.6	3	22.9	42	25.0	51	29.7	62
			Ø	15.3	3.0	20.1	39.4	25.3	45.0	28.7	77.3
	0	100	control dest. water	0.0	0	1.4	25	3.4	34	7.1	34
	0	100	control tap water	0.0	0	0.0	0	0.0	0	0.0	0
	30	10	1	57.6	5	76.4	51	77.9	68	78.7	104
			2	59.6	5	77.4	51	77.4	51	81.7	100
			3	68.7	5	70.4	68	75.8	68	77.3	102
			Ø	62.0	5.0	74.7	56.7	77.0	62.3	79.2	102
	0	10	control dest. water	0.0	0	3.7	25	8.3	42	18.3	42
	0	10	control tap water	0.0	0	0.0	0	0.0	0	0.6	20
	30	1	1	51.2	5	72.5	51	81.3	85	84.3	115
			2	54.5	5	75.5	51	83.5	51	86.5	129
			3	62.9	5	80.8	59	87.5	81	95.0	140
			Ø	56.2	5.0	76.3	53.7	84.1	72.3	88.6	128
	0	1	control dest. water	0.0	0	4.8	21	10.2	34	30.7	68
	0	1	control tap water	0.0	0	0.0	0	0.0	0	0.9	16

(88.6% and 90.6%) was found in the lowest conidium concentration, but the highest stimulatory effect was noted of Ezerjó pollen in the highest conidium concentration, where after 6 hrs the germination percentage was 48 × more than the germination found in distilled water. At both cultivars the germination dependent on the conidium concentration was generally 17 × more than in distilled water controls. The least difference, that is the least stimulatory effect in both cultivars

was found in the lowest conidium concentration, here the germination was merely 3–3.5× higher than in the distilled water. In tap water, germination after 22 hrs was insignificant at both cultivars, it was not higher than 1.5%.

The pollen stimulation influenced the germ tube length, too. The pollen of both cultivars stimulated the development of the germ tube and the absolute values of their length varied dependent on conidium concentration. At both cultivars the shortest tubes occurred in the highest concentration, while the longest were found in the lowest conidium concentration. The stimulatory effect, irrespectively of cultivars, resulted in almost double length in every conidium concentration, but the greatest effect opposed to the germination per cent, found in the lowest concentration. On the effect of the Ezerjó pollen the tube length was 4× more than in the distilled water control. The tube growth in tap water after 22 hrs was insignificant.

Discussion and Conclusions

The results prove that the grapevine pollen also stimulates the germination of conidia of *B. cinerea* as it has been observed on the effect of *American ilex*, the almond, strawberry, black currant, raspberry, cherry, sour-cherry, apricot, peach, apple and several ornamental plants such as hyacinth, iris and narcissus pollen (OGAWA and ENGLISH, 1960; BACHELDER and ORTON, 1963; CHOU and PREECE, 1968; BORECKA and MILLIKAN, 1973).

So far it has not been clarified, which substance(s) of the pollen stimulate. CHOU and PREECE (1968) found that the stimulative substance of the strawberry pollen was water soluble, dializable and heat-stable. They investigated the role of reducing sugars in the formation of the stimulatory effect, since the germinability of several week old conidia were restored by the effect of pollen diffusate. The reduction of the germinability of the old conidia is related to the exhaustion of the energy sources which can be restored if the C-source is repleted from outside. In connection with the C-supply the sugars have an important role, as it has also been supported by other investigations (KOSUGE *et al.*, 1962; KOSUGE and HEWITT, 1964; SHIRAISHI *et al.*, 1970). Besides the reducing sugars saccharose, maltose and mannose also stimulate the germination. The biological analysis of the dilutions prepared from the pollen diffusate, however, showed that in such a great dilution as 10,000×, in which the level of reducing sugars had already decreased to 0.43 μg/ml, the conidium germination was 70.0% whereas on the effect of 0.50 μg/ml reducing sugar solution, the germination did not surpass the 4.0%. This proves without doubt that in the pollen apart from the reducing sugars, there are other substances which have further marked role in the formation of a stimulatory effect (CHOU and PREECE, 1968). Concenrning this BORECKA and PIENIAZEK (1968: loc. cit.: BORECKA and MILLIKAN, 1973) consider that the abscisin acid which occurs in the pollen, has a role, too.

The pollen diffusate stimulates not only germination, but the growth of the germ tube, too. Investigations have revealed that an increased aggressiveness also associated with the stimulated hyphal growth which, according to the inoculation experiments, do not originated similarly only from the effect of the reducing sugars. The aggressiveness of *B. cinerea* on bean leaves is increased by the effect of the strawberry pollen, likewise that of *Helminthosporium sativum* and *Septoria nodorum* on rye leaves as influenced by the effect of rye pollen (CHOU and PREECE, 1968; FOKKEMA, 1971).

The pathological consequences of the pollen stimulation, taking into consideration CHOU and PREECE's (1968), FOKKEMA's (1971) statements and our results, can be summarized as follows.

1. It reduces the inoculum threshold needed for infection.
2. It shortens the period needed for germination which increases the possibility of infection in direct way, through epidermal tissue even under changing weather conditions, too.
3. It restores the germinability and infectivity of senescent conidia on the surface of plant organs and increases the probability of infection.
4. It increases the pathogenicity and agressiveness of *B. cinerea.*
5. It enriches the antagonistic microflora of the plant phyllosphere (*Aureobasidium pullulans*, *Sporobolomyces roseus* etc.) which, however, moderates the possibility of infection.

According to our results, it can be taken for certain that the stimulation of grapevine pollen has an important role in aiding the early infection of *B. cinerea* on clusters during blooming, furthermore, that in the last phase of blooming the fungus can easily colonize on the anthers covered by pollen grains. This latter increases the possibility of contact infection on berries after blooming (BALDACCI *et al.*, 1962; LEHOCZKY, 1970; 1972; MCCLELLAN and HEWITT, 1973).

Literature

BACHELDER, S. and ORTON, E. R. (1963): *Botrytis* inflorescence blight on American holly in New Yersey. Plant Dis. Reptr., *46*, 320.

BALDACCI, E., BELLI, B., FOGLIANI, G. (1962): Osservazioni sul ciclo vitale della *Botrytis cinerea* Pers. nella vite. Notiz. sulle Malattie delle Piante, N. *62* (N. S. 41) 1—15.

BLAKEMAN, J. P. (1972): Effect of plant age on inhibition of *Bortrytis cinerea* spores by bacteria on beetroot leaves. Physiol. Plant Pathol., *2*, 143—152.

BLAKEMAN, J. P. and SZTEJNBERG, A. (1973): Effect of surface wax on inhibition of germination of *Botrytis cinerea* spores on beetroot leaves. Physiol. Plant Pathol., *3*, (2) 269—278.

BORECKA, H. and MILLIKAN, D. F. (1973): Stimulatory effect of pollen and pistillate parts of some horticultural species upon the germination of *Botrytis cinerea* spores. Phytopathology, *63*, (11) 1431—1432.

BORECKA, H. and PIENIAZEK, J. (1968): Stimulatory effect of abscisic acid on spore germination of *Gloeosporium album* Osterw. and *Botrytis cinerea* Pers. Bull. Acad. Pol. Sci. Biol., *61*, 657—661.

BROWN, W. (1922): On the exosmosis of nutrient substances from the host tissue into the infection drop. Ann. Bot., *36*, 101—119.

CHU CHOU, MYRA and PREECE, T. F. (1968): The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. Ann. appl. Biol., *62*, 11—22.

FOKKEMA, N. J. (1968): The influence of pollen on the development of *Cladosporium herbarum* in the phyllosphere of rye. Neth. J. Pl. Path., *74*, 159—165.

FOKKEMA, N. J. (1971): The effect of pollen in the phyllosphere of rye on colonization by saprophytic fungi and on infection by *Helminthosporium sativum* and other leaf pathogens. Neth. J. Pl. Path., *77* (Supplement No 1.) 1—60.

FOKKEMA, N. J. and LORBEER, J. W. (1974): Interactions between *Altenaria porri* and the saprophytic mycoflora of onion leaves. Phytopathology *64*, (8) 1128—1133.

HUNTER, J. E., ROHRBACH, K. G. and KUNIMOTO, R. K. (1972): Epidemiology of *Botrytis* blight of *Macadamia* racemes. Phytopathology *62* (3) 316—319.

KOSUGE, T. and HEWITT, WM. B. (1964): Exudates of grape berries and their effect on germination of conidia of *Botrytis cinerea*. Phytopathology *54*, 167—172.

KOSUGE, T., DUTRA, J. C. and HEWITT, WM. B. (1962): The effect of grape washings on the germination of spores of fungi associated with bunch rot of grapes. Phytopathology *52*, 739 (Abstr.)

KOVÁCS, A. and SZEŐKE, É. (1956): Die phytopathologische Bedeutung der kutikularen Exkretion. Phytopath. Z., *27*, 335—349.

LEHOCZKY, J. (1970): A szürkepenészes megbetegedés egy ritkább, korai esete a szőlő fürtvirágzatán és a fiatal fürtökön. Növényvédelem, *5* (5) 207—211.

LEHOCZKY, J. (1972): A szürkepenész *(Botryotinia fuckeliana)* biológiája, kórfolyamata a szőlőn és a hatásos fürtvédelem alapkövetelményei. Szőlő- és Gyümölcstermesztés, *7*, 217—251.

MCCLELLAN, W. D. and HEWITT, WM. B. (1973): Early *Botrytis* rot of grapes: time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. Phytopathology *63* (9) 1151—1157.

OGAWA, J. M. and ENGLISH, H. (1960): Blossom blight and green fruit rot of almond, apricot and plum caused by *Botrytis cinerea* Plant Dis. Reptr., *44*, 265—268.

PURKAYASTHA, R. P. and DEVERALL, B. J. (1965): The growth of *Botrytis fabae* and *Botrytis cinerea* into leaves of bean *Vicia faba* L.). Ann. appl. Biol., *56*, 139—147. (ref.: R. A. M., 45. 52. 1966.)

SHIRAISHI, M., FUKUTOMI, M. and AKAI, S. (1970): Germinability of aged conidia of *Botrytis cinerea* Pers. by several saccharides. Ann. Phytopath. Soc. Japan, *36* (5) 297—303.

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Further Studies on the Interaction between Wheat Mosaic Streak Virus and *Helminthosporium sativum**

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When wheat plants were inoculated first with virus and then by *Helminthosporium sativum*, there was marked reduction in growth as manifested by fewer tillers, fewer fertile flowers, and stunting of plants. Wheat variety HD-1927 was affected more than the other varieties tested. There was a considerable decrease in yield per spike, yield per plant and thousand kernel weight.

In nature plants are often exposed to more than one pathogen at a time, which may result in effects showing synergism, antagonism or independent associations. CHADHA and RAYCHAUDHURI (1965) have shown antagonistic effect, while RAJU *et al.* (1969) have shown synergistic effects between virus and fungus pathogens. This study presents observations with regard to effects on plant growth and yield.

Material and Methods

The plants of four wheat varieties namely, Kalyansona, N. P. 824, HD-1927 and Ridley used in the study were raised in 25 cm pots. For each treatment four pots having five plants each were used for each variety. When the plants were 20 days old, these were divided into four sets. Two sets were inoculated with wheat mosaic streak virus (WMSV) by the usual leaf rubbing method using fine carborundum powder as an abrasive. Fifteen days after virus inoculation, one of these sets was further inoculated with spore suspension of *H. sativum*,while the other set was kept as such. Third set of plants was inoculated with *H. sativum* alone. All these plants were kept in a humid chamber for 48 hours for providing humid conditions for establishment of *H. sativum* infection, while the fourth set was kept uninoculated as control. Observations on important plant characters were taken at different times during plant growth and on yield after harvesting the crop.

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Results

The data in Table 1 illustrate that there was a reduction in growth of plants as manifested by fewer tillers, fewer fertile flowers and stunting of plants due to infection with WMSV or *H. sativum*. This effect was increased when the two pathogens were present together (Fig. 1). Wheat variety HD-1927 is resistant to *H. sativum* but when the plants were inoculated with both pathogens, there was significant decrease in growth of plants. The number of tillers per plant in this variety was lessened by 27% when inoculated with WMSV plus *H. sativum*, but only 4% when inoculated with *H. sativum* alone. The length of stalk with spike in the same variety was lowered by 24% when inoculated with both the pathogens, but only 15% when inoculated with *H. sativum* alone. Again the number of fertile flowers in this variety was lessened by 46% when inoculated with WMSV plus *H. sativum* but only 12% when *H. sativum* alone was the infecting agent.

The data in Table 2 show that the combined damaging effect of WMSV and *H. sativum* was evident in all the four wheat varieties, particularly regarding production of grains per spike and plant, and thousand kernel weight. The damaging effect is more apparent and significant on wheat variety HD-1927 than on other three varieties. The number of kernels per spike was reduced in this variety by 40% when inoculated with WMSV plus *H. sativum*, but 14% when inoculated with *H. sativum* alone. Similarly production per spike was lessened by 45% when inoculated with WMSV plus *H. sativum* but only 9% when inoculated with *H. sativum* alone.

Discussion

The present studies show that there was decrease in growth and yield of wheat in plants doubly infected with WMSV and *H. sativum*. Among the four varieties studied, a difference exists in combined damaging effect of WMSV and *H. sativum* which may be due to the differences in varietal response to WMSV. The most damaging effect of WMSV plus *H. sativum* was shown by wheat variety HD-1927, although the harmful effects on other varieties were also considerable. Altered metabolism of the plant due to virus infection might have favoured an enhanced damaging effect due to *H. sativum*.

RAJU *et al.* (1969) observed that due to the combined effect of wheat streak mosaic and leaf rust a synergistic effect was seen on all but one of the wheat cultivars studied and concluded that the synergism found with WMSV was not universal but confined to specific wheat cultivars, and apparently no synergism occurred in wheat when brome grass mosaic virus was used instead of WMSV. Dry wheat of foliage in the Wichita cultivar grown in the greenhouse was decreased significantly by WMSV and rust combined when compared with WMSV infection alone.

Fig. 1. Response of variety HD-1927 to interaction of pathogens. Plants in the right pot show marked reduction of height due to combined inoculations of WMSV and *H. sativum*. Plants in the left pot are inoculated only with *H. sativum*

Table 1

Effect of wheat mosaic streak virus and *Helminthosporium sativum* singly and in combination on the growth of 4 wheat varieties

Properties studied	Plant	Kalyansona	N.P. 824	HD-1927	Ridley
No. of tillers plant	Uninoculated	4.3 (100.0)	5.8 (100.0)	4.8 (100.0)	7.5 (100.0)
	Infected with WMSV	3.9 (90.7)	5.0 (86.2)	4.4 (91.6)	7.4 (98.6)
	Infected with *H. sativum*	3.3 (76.7)	4.0 (68.9)	4.6 (95.8)	5.7 (76.0)
	Infected with WMSV & *H. sativum*	3.0 (69.8)	4.0 (68.9)	3.5 (72.9)	6.4 (85.3)
No. of fertile tillers per plant	Uninoculated	3.7 (100.0)	5.5 (100.0)	4.8 (100.0)	7.3 (100.0)
	Infected with WMSV	3.5 (94.6)	4.6 (83.6)	4.0 (83.3)	6.8 (93.1)
	Infected with *H. sativum*	2.9 (78.3)	3.8 (69.1)	4.6 (95.8)	6.6 (90.4)
	Infected with WMSV & *H. sativum*	2.8 (75.7)	3.7 (67.3)	3.3 (68.7)	6.7 (91.8)
Length of stalk with spike (cm)	Uninoculated	73.0 (100.0)	95.6 (100.0)	83.5 (100.0)	95.0 (100.0)
	Infected with WMSV	71.4 (97.8)	93.5 (97.8)	74.9 (89.7)	80.4 (84.6)
	Infected with *H. sativum*	71.0 (97.2)	70.4 (73.6)	71.5 (85.6)	76.2 (80.2)
	Infected with WMSV & *H. sativum*	69.5 (95.2)	81.6 (85.4)	63.9 (76.5)	79.8 (84.0)
Length of spike	Uninoculated	11.4 (100.0)	11.6 (100.0)	12.9 (100.0)	12.8 (100.0)
	Infected with WMSV	11.4 (100.0)	11.6 (100.0)	12.2 (94.6)	11.4 (89.1)
	Infected with *H. sativum*	10.8 (94.8)	10.7 (92.2)	12.7 (98.4)	10.2 (79.7)
	Infected with WMSV & *H. sativum*	10.7 (93.8)	11.3 (97.4)	10.6 (82.2)	9.6 (75.0)
No. of fertile flowers	Uninoculated	56.8 (100.0)	49.3 (100.0)	48.7 (100.0)	53.5 (100.0)
	Infected with WMSV	56.6 (99.6)	45.5 (92.3)	47.5 (97.5)	51.8 (96.9)
	Infected with *H. sativum*	48.0 (84.5)	41.0 (83.2)	42.7 (87.7)	51.5 (96.5)
	Infected with WMSV & *H. sativum*	44.4 (78.2)	40.9 (82.9)	25.3 (54.0)	51.8 (96.9)

Numbers in parentheses are percentages	*Kalyan-sona*	*N. P. 824*	*HD-1927*	*Ridley*
C. D. at 5% level (No. of tillers/plant)	1.13	0.70	0.70	0.70
C. D. at 5% level (No. of fertile tillers/plant)	0.37	0.25	1.40	1.69
C. D. at 5% level (Length of stalk with spike)	2.41	2.41	4.67	2.93
C. D. at 5% level (Length of spike)	1.35	1.13	4.74	1.08
C. D. at 5% level (No. of fertile flowers)	2.53	3.88	2.03	2.66

Table 2

Effect of wheat mosaic streak virus and *Helminthosporium sativum* singly and in combination on yield of four wheat varieties

Properties studied	Plant	Kalyansona	N.P. 824	HD-1927	Ridley
No. of kernels per spike	Uninoculated	50.7 (100.0)	34.0 (100.0)	36.7 (100.0)	40.6 (100.0)
	Infected with WMSV	50.2 (99.0)	30.5 (89.7)	34.8 (94.8)	40.5 (99.8)
	Infected with *H. sativum*	33.5 (66.1)	27.2 (80.0)	31.7 (86.3)	35.5 (87.4)
	Infected with WMSV & *H. sativum*	33.9 (66.8)	27.1 (79.9)	22.1 (60.2)	36.2 (89.1)
Yield per spike (g)	Uninoculated	1.30 (100.0)	1.40 (100.0)	1.10 (100.0)	1.20 (100.0)
	Infected with WMSV	1.20 (92.3)	1.15 (82.1)	1.00 (90.9)	1.20 (100.0)
	Infected with *H. sativum*	0.75 (57.7)	0.75 (53.6)	1.00 (90.9)	0.85 (70.8)
	Infected with WMSV & *H. sativum*	0.65 (50.0)	0.55 (39.3)	0.60 (54.5)	0.80 (66.6)
Yield per plant (g)	Uninoculated	4.55 (100.0)	7.30 (100.0)	5.30 (100.0)	7.40 (100.0)
	Infected with WMSV	4.20 (92.3)	5.40 (73.9)	4.80 (90.5)	6.70 (90.5)
	Infected with *H. sativum*	2.50 (54.9)	3.70 (50.7)	5.00 (94.3)	5.30 (71.6)
	Infected with WMSV & *H. sativum*	2.60 (56.0)	2.30 (31.5)	3.30 (62.2)	5.10 (68.9)
Weight of 1000 kernels (g)	Uninoculated	24.50 (100.0)	38.50 (100.0)	25.20 (100.0)	21.50 (100.0)
	Infected with WMSV	23.00 (93.9)	34.50 (89.6)	23.10 (91.6)	20.80 (96.7)
	Infected with *H. sativum*	15.50 (61.4)	24.50 (63.7)	24.40 (96.8)	15.50 (72.1)
	Infected with WMSV & *H. sativum*	13.50 (55.1)	16.50 (42.8)	15.30 (60.3)	14.80 (68.8)

Numbers in parentheses are percentages	*Kalyan-sona*	*NP. 824*	*HD-127*	*Ridley*
C.D. at 5% level (No. of kernels/spike)	2.39	2.44	2.12	2.93
C.D. at 5% level (Yield/spike)	0.09	0.06	0.04	0.06
C.D. at 5% level (Yield/plant)	0.09	0.49	0.49	0.70
C.D. at 5% level (Weight of 1000 kernels)	3.17	2.74	2.58	3.67

CRANE and CALPOUZOS (1969) showed that *Cercospora beticola* infection produced more lesions when sugar beet leaves were inoculated with beet yellows virus and damage to the plant in terms of dry leaves is greatly increased when both pathogens occur together.

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Literature

ADLAKHA, K. L. and RAYCHAUDHURI, S. P. (1975): Interaction between *Helminthosporium sativum* and wheat mosaic streak virus. Z. Pflkrankh. Pflschutz, *82*, 201–206.

CHADHA, K. C. and RAYCHAUDHURI, S. P. (1965): Interaction between sterility virus and *Fusarium udum* Butl. in pigeon pea. Indian J. Agric. Sci. *36*, 133—139.

CRANE, G. L. and CALPOUZOS, L. (1969): Synergism of *Cercospora beticola* and beet yellow virus in killing sugarbeet leaves. Phytopathology *59*, 1338—1339.

RAJU, D. G., SILL, W. H. and BROWDER, L. E. (1969): The combined effects of two viral diseases and leaf rust on wheat. Phytopathology *59*, 1488—1492.

RAYCHAUDHURI, S. P. and GANGULY, B. (1968): A mosaic, streak disease of wheat. Phytopath. Z. *62*, 61—65.

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Certain Morphological and Pathological Characters of *Alternaria cucumerina*, the Cause of Watermelon Leaf Spot Disease in Egypt

By

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Alternaria cucumerina (Ellis & Everh.) Elliott was isolated from diseased leaves of watermelon (*Citrullus vulgaris* Schrad.) plants, for the first time in Egypt. Four isolates of the fungus varying in their morphological and spore measurements were obtained.

Spraying of spore or mycelial fragment suspensions of the fungus realized leaf spot on both cotyledons and leaves, however the formers were less susceptible than the latters.

Watermelon (*Citrullus vulgaris* Schrad.) is the most economically important cucurbitaceous crop in Egypt. It occupies nearly two thirds of the total area cultivated with the different cucurbits, and is considered a main vegetable crop in the newly reclaimed sandy soils. Its cultivated area reached 87,291 feddan during 1971 (ANON., 1971).

Watermelon plants are subjected to the infection with several diseases, of which leaf spot is one of the most destructive in reducing the growth and yield. No serious work, however, has been carried out on this disease in Egypt (EL-HELALY *et al.*, 1966). Symptoms, morphological and certain pathological characters of the pathogen will be presented here.

Materials and Methods

Spotted watermelon leaves collected from different localities were used for the isolation trials. Isolation was carried out as usual on the potato dextrose agar medium, and by the use of the filter paper technique (KILPATRICK, 1966). The surface sterilized spotted tissues were placed on wet sterile filter papers in petri dishes. The growing fungus was examined, after 2 days incubation at 28°C, under the microscope and single spored by transferring off a single conidium to slants of PDA medium.

Pathogenicity test was carried out by spraying the plants with spore suspension of the fungus (about 250,000 spores/ml) in moist chambers at 25°C for 2 days, where the severity of infection was estimated after one week.

Results and Discussion

Symptoms of the disease

A survey of the leaf spot disease of watermelon was carried out in different governorates during four successive seasons (1969 – 1972). Disease symptoms could be described as follows.

Minute, circular, tan coloured spots less than 0.5 mm in diameter, and surrounded by a pale green to yellow halos were the first to appear on both cotyledons and leaves. They were gradually enlarged and became dark brown to black in colour, with definite concentric rings and margins on the upper leaf surface, while indefinite on the lower one.

A single spot may vary in its size to reach about one cm in diameter, but in the case of numerous spots, they coalesce to involve a large part of the leaf surface. Occasionally, in warm and highly moistened weather, the disease may cause considerable defoliation. Fig. 1 shows naturally infected watermelon leaves of the cultivar Giza 1.

Isolation of the pathogen

Cultivation of the old or well developed lesions of the diseased leaves on FDA medium, yielded several fungi either solely or in combinations. The most frequent fungi were a species of *Alternaria* which produced abundant, small, nonbeaked and chain forming spores and a species of *Helminthosporium* which was characterized by its small, ellipsoid-shape, and three septated spores. Other fungi were also obtained but in less frequency.

Cultivation of the young and newly formed spots (0.5 – 1.0 mm in diameter) on the same medium yielded a nonsporulated fungal growth or produced very slight spores.

Using the filter paper technique, however, the newly formed spots produced abundant spores which were very large and having very long, hyaline and septate beaks. Nine single spored cultures were identified by the writers according to the spore characteristics and measurements (NEERGAARD, 1945; JACKSON and WEBER, 1959; ALEXOPOULOS, 1964) as follows:

Nos 1 – 4: *Alternaria cucumerina* (Ellis & Everh.) Elliott.
No. 5: *Alternaria tenuis* Nees.
Nos 6, 7: *Helminthosporium* spp.
No. 8: *Curvularia* sp.
No. 9: *Stemphylium* sp.

The same nine cultures were also kindly identified by The Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, England. Identifications that carried out by the writers were found to be in complete accordance with those of the Commonwealth Mycological Institute. However, isolate 3 was identified by

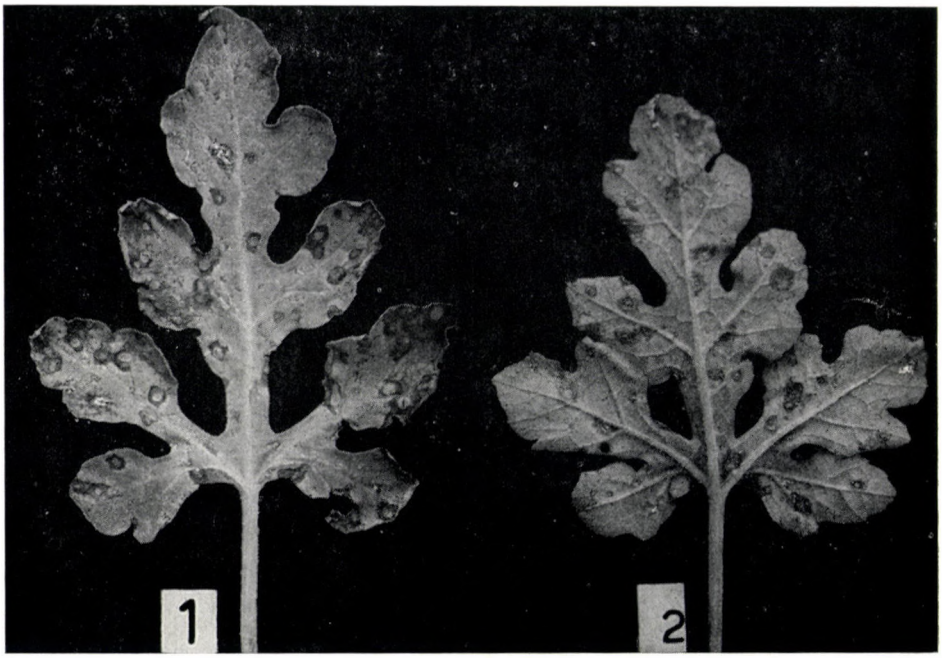

Fig. 1. Naturally spotted watermelon leaves of the cultivar Giza 1; (1) upper and (2) lower leaf surface

Fig 2. Artificial infection on watermelon leaves and cotyledons (Giza 1) induced by inoculation with *Alternaria cucumerina* (isolate 2)

the genus only as *Alternaria* sp. They have the following numbers IMI 173647, IMI 173648, IMI 174649 and IMI 173650.

Pathogenicity tests proved *A. cucumerina* to be the sole causal organism responsible for watermelon leaf spot disease in Egypt. The four isolates could be arranged in the following sequence based on their virulence 2, 1, 3 and 4 and they were readily reisolated from the artificially inoculated plants.

Typical symptoms, similar to those found in nature were developed on the inoculated leaves. Symptoms were developed on the true leaves within 2–4 days, while more longer incubation period was needed in the case of cotyledons (Fig. 2). Stems and leaf petioles, however, were always free of the disease. In addition, cotyledons were less susceptible than the true leaves. This could be attributed to the difference in their anatomical structures, in addition to the thickened nature of the former.

In this respect, it must be stated that injecting the spore suspension of *A. cucumerina* inside the stems and leaf petioles showed no infection on any part of the plant even after one month of injection. The points of injection, however, became somewhat elevated after about 5 days without any further symptoms. This indicated that the fungus could not transmit internally through the plant. Similar results were previously reported by BRISLEY (1923) and JACKSON (1959).

Morphological characters of A. cucumerina

Colour, rate of growth, zonation, spore production ability, and spore measurements of the four isolates of *A. cucumerina* were studied on vegetable 8 juice agar medium after 14 days incubation at 28°C. Results are presented in Table 1 and Fig. 3.

Mean conidial body length and width, however, were not excessively variable among the four isolates. Generally, the spore body width was less variable than the body length. Beak length was more variable than both body length and width.

Hyphae in culture is hyaline to olivaceous, branched, irregularly septate, white to black in mass. Conidiospores light to dark brown and septate.

Conidia apical on conidiospores, yellow to pale brown, ovoid to obclavate, with few longitudinal septations when young, becoming dark brown to black, obclavate, with more transverse and longitudinal septations when mature; conidial body smooth to papillate. Beaks straight, slender, quite variable in length, hyaline, smooth, septated.

Conidia rarely formed in two-spore chains (specially isolate 2). The basal one was older than the terminal which was somewhat different from the basal one. It was semicoloured, mostly malformed, with or without beak, small in size in comparison with the basal, having a limited number of transverse and longitudinal septations and seemed to be resulted from the swelling and enlargement of the terminal cell of the beak of the basal spore. In very few cases, conidia were formed in three spore chains as shown in Fig. 4.

Table 1

Morphological characters of the four isolates of *A. cucumerina* on vegetable 8 juice agar medium

Character	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Colour of growth	olive	black	white to pale olive	dark olive
Rate of growth	moderate	rapid	slow	moderate
Sporulation	slight	moderate	rare	slight
Zonation	apparent	apparent	rare	apparent
Range of spore body length, μ	36—75	46—108	35—80	36—81
Average of spore body length (100 spores), μ	51	68	56	58
Range of beak length, μ	30—222	31—310	33—177	29—291
Average of beak length (100 spores), μ	101	147	103	128
Range of spore body width, μ	14—24	14—33	14—23	14—31
Average of spore body width (100 spores), μ	18	19	17	21

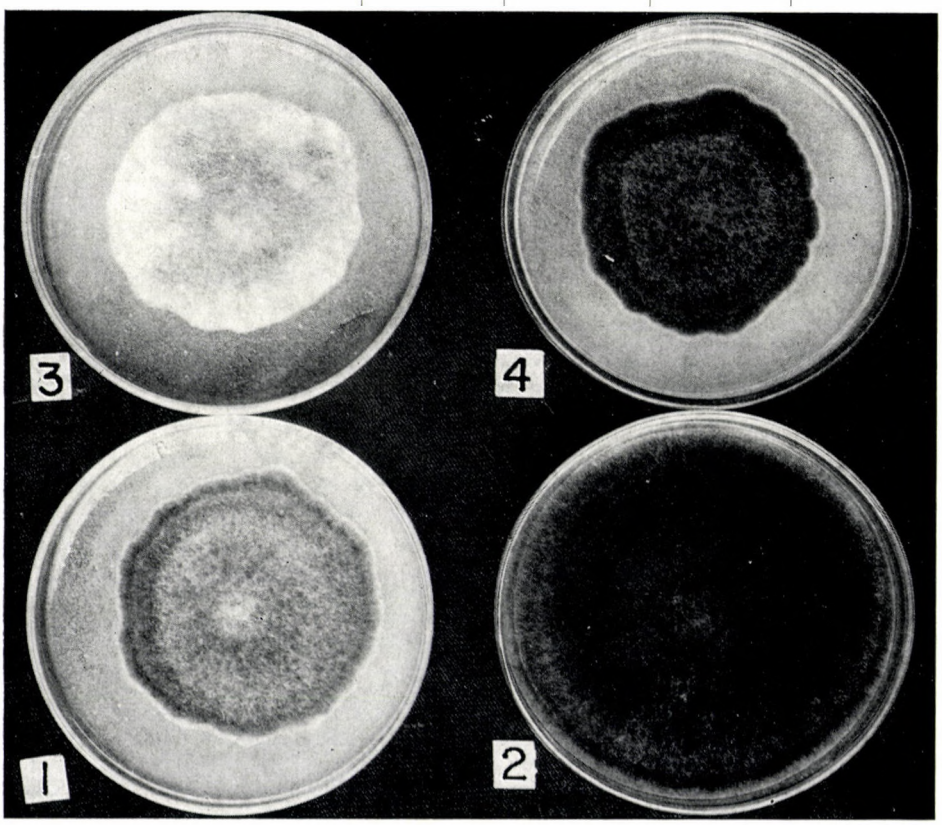

Fig. 3. Fungal growth of the four isolates of *Alternaria cucumerina* grown on vegetable 8 juice agar medium for 14 days at 28°C

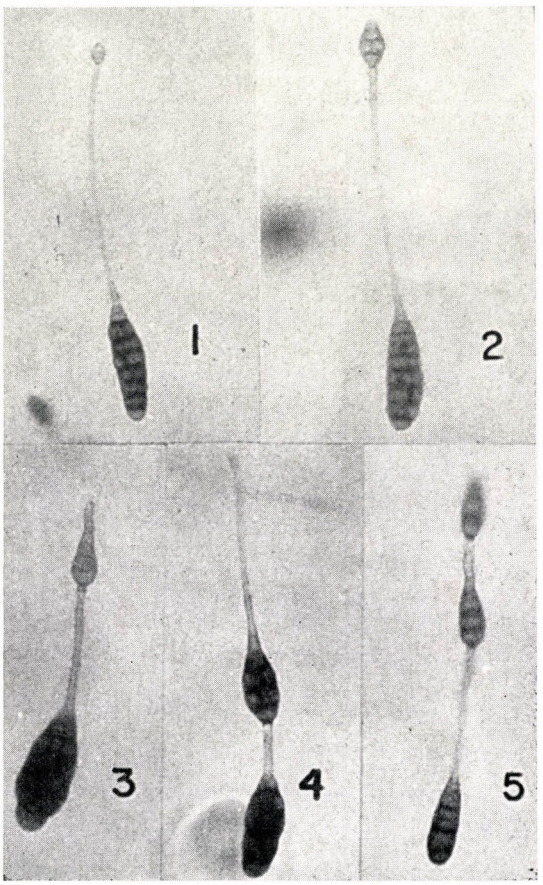

Fig. 4. Different stages of spore chain formation of *Alternaria cucumerina* (isolate 2)

Regarding spore germination, conidia mostly produced more than one germ tube, while in some cases, conidia produced conidiophores bearing conidia directly.

Literature

ANONYMOUS, (1971): Report of the Agric. Economics Dept. of 1971, Min. of Agriculture, ARE.

ALEXOPOULOS, C. J. (1964): Introductory Mycology. John Wiley & Sons, Inc. N. Y. 613 pp.

BRISLEY, H. R. (1923): Studies on the blight of cucurbits caused by *Macrosporium cucumerinum* E. & E. Phytopathology, *13*, 199—204.

EL-HELALY, A. F., IBRAHIM, I. A., ASSAWAH, M. W., ELAROSI, H. M. and ABDEL-REHIM, M. A. *et al.* (1966): General survey of plant diseases and pathogenic organisms in the U. A. R. (Egypt) until 1965. Alex. J. Agric. Res. Bull. 15.

JACKSON, C. R. (1959): Symptoms and host-parasite relations of the *Alternaria* leaf spot disease of cucurbits. Phytopathology, *49*, 731—733.

JACKSON, C. R. and WEBER, C. F. (1959): Morphology and taxonomy of *Alternaria cucumerina*. Mycologia, *51*, 401—408.

KILPATRICK, R. A. (1966): Induced sporulation of fungi on filter paper. Plant Dis. Reptr. *50*, 789—790.

NEERGAARD, P. (1945): Danish species of *Altenearia* and *Stemphylium* Einar Munksgaard. Copenhagen. Oxford Univ. Press. London, 560 pp.

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Survival of *Alternaria cucumerina*, the Causal Organism of Leaf Spot Disease of Cucurbits

By

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Conidia of *Alternaria cucumerina* were quite unable to germinate after four months storage at room temperature, while mycelial threads in watermelon leaf tissues kept perfectly dry, retained their viability after two years. Mycelial fragments of the fungus proved to be pathogenic on watermelon leaves, and the resulted spots were similar to those formed by using conidial suspensions. Mycelium in plant debris supplied the inoculum for the primary infection, while conidia were responsible for disseminating the disease. The fungus is not an internal seed borne one.

A. cucumerina was newly recorded on watermelon and other cucurbits in Egypt (IBRAHIM *et al.*, 1975). An apparent confliction, however, had been cited in the literature among the results of BRISLEY (1923) and those of JACKSON (1958) concerning the viability of conidiospores and the source of inoculum and also between the results of the previous authors and those of MANNS and ADAMS (1927).

Hence, it was found of great interest to study the viability of both mycelium and conidiospores, the capability of mycelium for inciting infection, and seed transmission of *A. cucumerina* to clarify this confliction and to define the responsibility for the new infection.

Materials and Methods

Two-week-old cultures of A. *cucumerina* (IMI 173648) grown on glucose peptone agar medium were evaporated to dryness at 33°C for three days. Percentage of spore germination was estimated after 1, 10, 30, 60, 90, 120 and 150 days of storage at room temperature using the same dried cultures in each case.

Naturally spotted watermelon leaves collected from different localities in Egypt during 1971 and 1972 were dried between folds of filter papers and kept at room temperature. Isolation of the fungus was carried out once every six months using the filter paper technique (KILPATRICK, 1966; MAHMOUD, 1971). Hundred spots were used for each trial, and the resulted fungus was examined for pathogenicity in the greenhouse using healthy watermelon plants.

The mycelium-free spores were obtained by culturing the fungus on doubled Richard's medium for 10 days at 28°C. Mycelial suspension was sprayed on watermelon plants, incubated for 2 days in moist chambers, and infection was recorded 5 days later under greenhouse conditions.

Local and imported watermelon seeds were kindly supplied by the Egyptian Agricultural Organization and the Egyptian Agricultural Quarantine. One hundred seeds of each local variety and 10 seeds of each imported one, were surface-sterilized and placed on potato dextrose agar medium. The resulted fungi were recorded after 7 days incubation at 28°C.

Later, watermelon seeds collected from severely diseased plants were surface-sterilized and divided to three groups. The first group was left without treatment, the second was dusted with conidia of *A. cucumerina* and planted immediately. The third group, however, was dusted with the pathogen and kept for four months before planting. Plantation was carried out in sterilized sandy soil, and 200 seeds were used for each treatment.

Results and Discussion

Survival of conidiospores

Percentage of spore germination showed a gradual decrease by increasing the period of storage (Table 1). Viability was over 70% after one month of storage, while it reached 3% after 3 months. This was in agreement with BRISLEY (1923), who reported that spores of *Macrosporium cucumerinum* became nonviable after storage in a dry culture for three months, while disagreed with JACKSON (1958, 1959) who mentioned that the viability of conidiospores of *A. cucumerina* was over 90% after eight months storage *in vitro* at constant temperatures of 8, 28 and 36°C. However, he mentioned that conidia lost rapidly their viability when placed in soil.

Table 1

Effect of different periods of storage on the viability of conidia of *A. cucumerina*

Periods of storage in days	Per cent of spore germination
1	99
10	93
30	71
60	18
90	3
120	0
150	0

Survival of mycelium

Mycelial fragments that were kept perfectly dry in leaf tissues of watermelon plants, retained their viability after two years. Viability of mycelial threads in leaf tissues was 100, 91, 82 and 36% after 6, 12, 18 and 24 months of storage respectively (Table 2). This confirmed the results of BRISLEY (1923) who reported that the dried mycelial fragments readily retained their viability after nine months storage at room temperature. No samples, however, were kept for a longer period than nine months, so it was impossible to determine for how long the mycelium would keep its viability. Similarly, ANON. (1926) revealed that the organism retained its viability possibly for a year in the soil.

Table 2

Survival of mycelium of *A. cucumerina* in naturally infected watermelon leaves that were dried and preserved for different periods

Locality	Date of collection	Date of isolation	Number of isolates obtained from 100 spots
Tahrir Region	August, 1971	Feb., 1972	100
		Aug., 1972	91
		Feb., 1973	82
		Aug., 1973	36
Haush Eisa	July, 1972	Jan., 1973	97
		July, 1973	92
Abo-El-Matamer	August, 1972	Feb., 1973	100
		Aug., 1973	83

Artificial infection with mycelial fragments

Mycelial fragments of *A. cucumerina* proved to be pathogenic on both cotyledons and leaves of watermelon plants. The resulted spots were quite similar to those obtained by using the spore suspension for infestation. In addition, conidiospores bearing conidia were developed on the surface of these spots as they were placed in moist chambers. This result led the writers to believe that probably spores had no role in transmitting the fungus from one season to another. That is to say that, mycelium in plant debris supplied the inoculum for the primary infection in the beginning of the season, while the spores were responsible for disseminating the disease from diseased leaves to another healthy ones during the same season. Similar results were previously reported by BRISLEY (1923) and VAN HALTERN (1933). They found that living mycelium in the dead vines supplied the spring inoculum. On the other hand, JACKSON (1959) found that conidia were unable to survive in the soil, and probably have no important function in overwintering the fungus in the field. However, he found conidial inoculum to be effective in

inciting the disease, while the use of mycelial fragments suspensions never resulted in infection. Hence, he suggested that dormant mycelium in recovered leaf fragments, resumed growth under favourable conditions and produced conidia which served as inoculum for the new infection. Accordingly, rotation and removing of plant debris at the end of the season are of great importance in reducing the disease.

Seed transmission

The writers failed to isolate *A. cucumerina* from several hundreds of surface-sterilized local and imported watermelon seeds; even from those which were collected from severely diseased plants. This assured that the fungus was not an internal seed borne one.

Artificially contaminated seeds planted just after contamination, gave about 7% diseased seedlings with small brown spots on their cotyledons. This may be due to the mechanical transmission of spores from seed surface to cotyledons during seed germination. In this respect, JACKSON (1959) observed small brown lesions on the cotyledons of 4% of seedlings resulted from seeds previously surface-sterilized, artificially inoculated with conidia of *A. cucumerina* and stored for four months before planting. Seedlings from surface-sterilized and uninfested seeds, however, showed no signs of infection. On the other hand, MANNS and ADAMS (1927) mentioned that the disease appeared to be seed-borne. VAN HALTERN (1933) using untreated seeds artificially inoculated with conidia of the pathogen, found an increase in disease incidence related to seed-borne conidia. Similarly MIDDLETON and WHITAKER (1946) suggested that seed-borne conidia were responsible for the early infection of crown leaves.

Plating of the artificially contaminated seeds, in the present investigation, after four months from contamination, gave healthy plants. This indicated that the disease was probably unable to transmit on the seed surface from one season to another. Seeds are usually stored for a period longer than four months before planting.

Literature

ANONYMOUS, (1926): The melon blights. New Yersey Agric. Exp. Stat. Circ. 194, 2 (cf. Rev. Appl. Mycol., *5*, 679, 1926).

BRISLEY, H. R. (1923): Studies on the blight of cucurbits caused by *Macrosporium cucumerinum*. Phytopathology, *XIII*, 199–204.

IBRAHIM, A. N., ABDEL-HAK, T. M., FADL, F. A. and MAHROUS, M. M. (1975): Certain morphological and pathological characters of *Alternaria cucumerina* the cause of watermelon leaf spot disease in Egypt. Acta phytopath. Acad. Sci. hung. *10*, 301–307.

JACKSON, C. R. (1958): Taxonomy and host range of *Alternaria cucumerina*. Phytopathology, *48*, 343–344.

JACKSON, C. R. (1959): Symptoms and host parasite relations of the *Alternaria* leaf spot disease of cucurbits. Phytopathology, *49*, 731–733.

KILPATRICK, A. R. (1966): Induced sporulation of fungi on filter paper. Plant Dis. Reptr. *50*, 789—790.

MAHMOUD, B. K. (1971): Survey and studies on organisms causing leaf spot of cotton in UAR. M. Sc. Thesis, Al-Azhar Univ. Egypt.

MANNS, T. F. and ADAMS, J. F. (1927): Dept. of Plant Pathology. Rept. Delaware Agric. Expt. Stat. for the fiscal year ending June 1927, Bull. *152*, 40—51.

MIDDLETON, J. T. and WHITAKER, T. W. (1946): Some diseases of cantaloupes and honey dew melons observed in Southern Caifornia. Plant Dis. Reptr. *30*, 373—375.

VAN HALTERN, F. (1933): Spraying cantaloupes for the control of downy mildew and other diseases. Georgia Expt. Stat. Bull. *175*, 53.

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The Role of *Sclerotinia laxa* (Ehrenb.) Aderh. et Ruhl. in the Sexual Expression of Apricot, *Armeniaca vulgaris* Mill.

By

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In infected apricot trees the author found a correlation between late-blossoming flowers of abnormal structure and the fact of infection. The major changes are as follows: a reduction in number and phyllody of the stamina capable of function, empty anthers and enlargement of the sepals — which jointly result in full-flowers. Even more important is the decrease in vitality of the pistils and, in the majority of cases, their phyllody. Abnormal flowers did not produce fruits, i.e. fungus infection also indirectly decreased the yield.

Changes in normal sexual expression may be induced by genetical and environmental factors. In the handbook edited by RUHLAND (1967) a most detailed summary is presented about these: soil, soil- and air humidity, light, temperature, geographic location, mechanical intervention, injury, the position of the flower and the age of the plant individual, hormone treatment, pathogens and pests are all capable of changing the sexual conditions.

According to UBRIZSY (1965) and RUHLAND (1967), the following abnormalities of the gynoeceum and androeceum occur in plants.

The *strengthening of the gynoeceum* is induced, as a rule, by infections with fungi of the *Ustilago* species, e.g. *Ustilago vaillantii* Tul. brings about a multiplication of carpels or an elongation of the pistils in *Muscari comosum*, *Ustilago longissima* (Schlechtend.) Meyen exerts the same effect in *Glyceria maxima*, *Ustilago oxalidis* Ellis et Tracy on *Oxalis stricta* and *Ustilago caricis* Hazsl. on *Carex praecox*.

Masculinization is much less frequent and rather met with in dioecious plants. *Ustilago violacea* (Pers.) Roussel induced the formation of male flowers on female individuals of *Melandrium album* and *Uromyces scutellatum* (Scrank.) Lév. promoted the abortion of female flowers in *Euphorbia cyparissias*.

A change *in both directions* may ensue following infection with *Peronospora violae* De Bary. Slighter infections may induce staminodia; as a result of intense infection even a reduction of the gynoeceum can ensue in the species *Knautia arvensis*, *Scabiosa columbaria* and *Scabiosa succisa*. *Ustilago maydis* (Dc.) CDA may call forth the formation of female flowers in the tassels and male flowers in the ears of maize.

In the present paper the author reports on the effect of the fungus *Sclerotinia laxa* (Ehrenb.) Aderh. et Ruhl. strengthening the gynoeceum of apricots.

Material and Methods

At the Újhartyán farm the C. 256 *Magyar kajszi* (Hungarian apricot) trees have been affected by infection with *Sclerotinia* for several years. On April 19, 1967, the author could observe that on the bottom part of the branches of the infected trees the flowers of certain female formations were blossoming much later than those of the unaffected trees.

40 characteristically teratogenic flowers were collected and examined according to the author's own method (SURÁNYI, 1972). The morphological data of the abnormal flowers were compared with those of the healthy ones. — Over and above this, the author presents in this paper (founding himself on mean values of four years) how sexual correlation developed in five varieties of *Armeniaca vulgaris*, also including C. 256 *Magyar kajszi* (Hungarian apricot).

Results

On the bottom parts of infected branches one rather often finds late-blossoming flowers which have suffered 12 to 15 days' delay regarding the blossoming

Fig. 1. Blossoming delay in flowers at a distance from the infection spot, compared with healthy ones (as fruit)

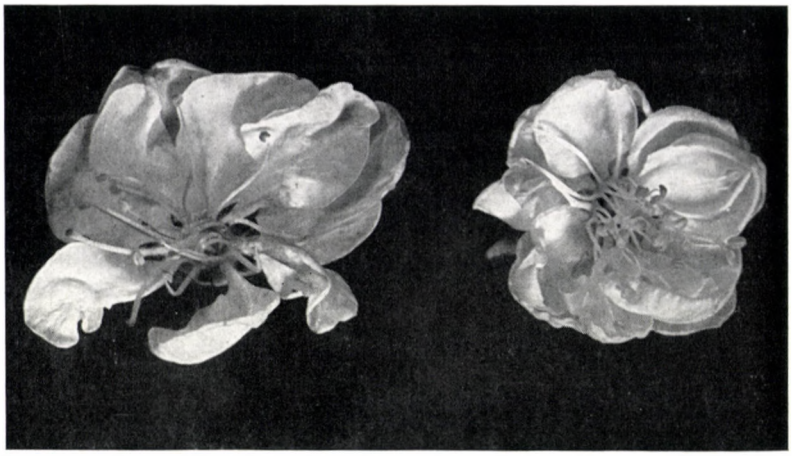

Fig. 2. The number of petals increases (9.8), at the same time also the petals are thickening

Fig. 3. The number of stamina decreases (20.7) staminodia bring about full-flowers

phenophase as compared with fruits from healthy flowers. Conspicuous differences in morphology appear in such flowers: the number of petals is increased to nearly a twofold of the normal ones, the petals are thickened and almost fleshy in touch, the outer petal circle assumes a greenish hue (Fig. 2).

In each teratogenic flower the author could find the total number of approximately 30 pistils characteristic of C. 256 *Magyar kajszi* (Hungarian apricot), however, their function reduced by about 50 per cent. In many instances the stamina which still had anthers did not produce pollen. In this way full flowers were produced (Fig. 3).

Fig. 4. The flower has five separate pistils, sepals are considerably enlarged (Photo: I. TÓTH)

Although not to be distinguished in the photographs, there were 5 carpels in each normal flower, and in correlation with this also an enlargement of the sepals could be observed (Fig. 4). This is a definite proof of the existence of the "vegetative" order of flower organs as set up by RESENDE (1967).

Numerically, the results of the author's surveys are demonstrated in Table 1. It appears from these data that the number of sepals did not change upon the effect of the infection; the more significant, however, was the multiplication of the petals and carpels, as well as the decrease of stamina capable of function.

Table 1

Data of flowers from healthy and infected trees, C. 256 *Magyar kajszi* (Hungarian apricot) (n = 40)

Flower-organs pc.	Normal	Teratogenic	L. S. D. 0.1%
	flowers		
Sepals	5.0	5.0	—
Petals	5.0	9.8	1.32
Stamina	30.2	20.7	3.40
Pistils	1.1	5.2	0.29

Discussion

In some places and in certain years, the fungus *Sclerotinia laxa* (Ehrenb.) Aderh. et Ruhl. may cause considerable damages among others also in apricot plantations. Its effect decreasing the yield is partly direct: withering of flowers and shoots in the white-bud stage – and partly indirect: inhibitions of fertilization-biological character ensue. The present paper deals with this very problem, since levelling up the calyx size, the number of the petals and stamina would not mean problems in themselves, on the other hand, the phyllody of the carpels, as well as morphologically normal but empty anthers mean serious obstacles to fertilization. Not one fruit was produced by free pollination from such flowers.

Sexual changes caused by fungus infection can be well explained by the correlation between gynoeceum and androeceum (Fig. 5). The marked weakening of the androeceum (which need not necessarily inhibit the setting of fruits) and the excessive strengthening of the gynoeceum are consequences of this antagonistic relationship. In the strengthening of the gynoeceum the primitive *Prunus* flower habit – namely five carpels – reappears. This is undesirable since such carpels are unable to function, i.e. fruit-setting in a sexual way becomes quite impossible. The optimum sexual relation has effects on fertilization, all of which can be demonstrated also statistically (SURÁNYI, 1973).

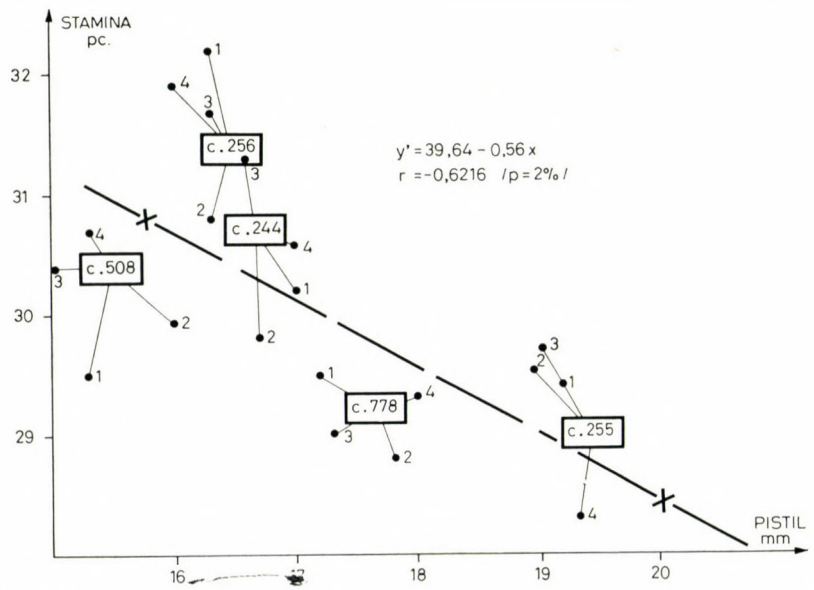

Fig. 5. Correlation between androeceum and gynoeceum in five apricot varieties (Years: 1 – 1968, 2 – 1969, 3 – 1970, 4 – 1971)

By way of summary it can be stated that flower abnormalities induced by fungi in C. 256 *Magyar kajszi* (Hungarian apricot) can develop in a way that the relative hormone level necessary for normal sexual organization is being changed. A further chemical analysis of and physiological studies into the said hormone level need to be conducted. According to RUHLAND (1967) the gynoeceum strengthens in consequence of a rise in the level of the endogenous stimulants; presumably also the fungus acts in this way: either it directly affects the auxin level or may inhibit the catabolism of the stimulants and / or prompt the decomposition of the inhibitors, thus rising the relative level of the stimulants.

Literature

RESENDE, F. (1967): Flowering and sex expression. In: Handbuch der Pflanzenphysiologie. Band XVIII. p. 268—75.

RUHLAND, W. (Edit.) (1967): Handbuch der Pflanzenphysiologie. Band XVIII. Sexualität. Fortpflanzung. Generationswechsel. Springer, Berlin—Heidelberg—New York. 874 p.

SURÁNYI, D. (1972): Teratological changes in *Prunus* varieties and their interpretation by sex correlation between pistil and stamina. Bot. Közl. *59*, 119—23.

SURÁNYI, D. (1973): Sexual correlation by self-compatible and self-incompatible varieties of some *Prunus*. Acta Bot. Acad. Sci. Hung. *18*, 179—85.

UBRIZSY, G. (Edit.) (1965): Növénykórtan (Phytopathology) Vol. II. Akadémiai Kiadó, Budapest. 942 p.

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Studies on the Biology and Ecology of Onion Downy Mildew *(Peronospora destructor)* Berk. (Fries) in Hungary

III. EPIDEMIOLOGY OF THE DISEASE

By

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A close correlation between the prevailing wind and the direction of the spread of the disease was many times observed. The effect of local air convection, however, proved to be also of great importance in the dissemination of *P. destructor* conidia, at least up to 0.50 m above ground.

The epidemiological investigations carried out indicate that dynamics of infection mainly depends on the frequency and length of time of humid conditions in the field. In addition, these investigations have shown that other factors as free water on the plant surface, as well as air temperature are also responsible for the fungus development. Based on the data obtained, a continuous registration of leaf wetness is proposed to determine the exact time of infection. Under field conditions two humid nights are usually needed for taking place the whole infection cycle including sporulation, conidium dissemination and germination, and at last penetration.

Incubation period lasts about 11 to 14 days. The disease symptoms can appear of two different kinds, namely either by direct fungus sporulation or by the discoloration of the host tissue, both depending on the weather situation out of which air humidity is of decisive importance.

Similarly to other plant diseases, the coefficiency of several factors including pathogen, host plant and the environmental conditions proved to be essential for the multiplying of onion downy mildew, caused by *Peronospora destructor*. Therefore, the epidemiological investigations carried out by the author in 1971, 1972 and 1973, were divided into two parts: 1. the overwintering of the pathogen and the primary source of inoculum, 2. the infection cycle connected with the environmental conditions. The investigations about the overwintering of *P. destructor* showed, that the principal way of fungus transmitting from one growing season to the other, at least in Hungary, was by means of mycelium surviving inside the onion bulbs (VIRÁNYI, 1974a).

In a further paper the most important factors influencing sporulation and conidium germination under laboratory and glasshouse conditions were described (VIRÁNYI, 1974b). The results obtained indicate that high air humidity (up to 100 per cent) and free water on the leaves are of great importance in the beginning of the reproduction phase of the fungus.

Studying the dissemination of onion downy mildew, both NEWHALL (1938) and YARWOOD (1943) underlined the wind-borne nature of this disease. VAN

DOORN (1959) was the first, who made exact field experiments to recognize the number of periods with weather conditions favourable to infection. Later on, RONDOMANSKI (1967) also improved the knowledge of epidemiology of *P. destructor* by having some ideas about the preconditions of epidemic.

The aim of the present work was to study the infection cycle of the pathogen, *P. destructor*, as related to the environmental conditions existing in the field.

Material and Methods

The epidemiological investigations were carried out in the Experimental Farm of the Research Institute for Plant Protection, in "Júlia major", not far from Budapest. The experimental field of specific arrangement was divided by paths into eight sectors, all of which contained plants of three different ages. The scheme of this experimental field is shown in Fig. 1.

Onion sets, variety Makói, used for planting originated from the Experimental Station for Onion Breeding in Makó (Southern Hungary). Onion bulbs, artificially inoculated with a conidium suspension described previously (YARWOOD, 1943), were planted in the centre of the experimental field in order to ensure the source of inoculum (Fig. 1). Meteorological data were obtained from a thermo-hygrograph placed closely to the crop at a height of 0.20 m above ground. When the first sporulation on the systemically infected plants was noticed, the

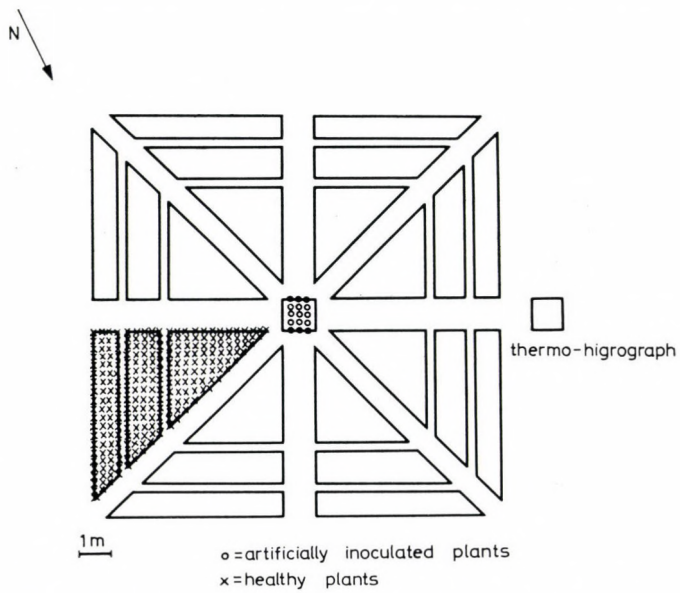

Fig. 1. Scheme of the experimental field in "Júlia major", 1973

investigations began and were carried out from May 28 to August 1 every day. The diseased leaves per sector were counted and removed to keep the centre as the only source of infection.

To determine the directive influence of wind on the spread of the disease, further investigations were made in onion fields grown both from set and for seed situated in several parts of Hungary.

Results

Spread of the disease

The fungus, *Peronospora destructor* may be characterized as an anemochor organism, which means, that the conidia disseminate from one plant to the other mainly by wind. The direct influence of the prevailing wind on the spread of onion downy mildew could be many times observed. Starting from an infection centre, including one or more systemically infected onion plants, the extent of area covered with diseased plants increased and assumed an elongate shape, according to the wind direction. Nevertheless, the investigations on the experimental field gave unlike results. There was no correlation between the position of plots with severe infection and the direction of the prevailing wind (Fig. 2). In spite of the main wind of northwest existing in the experimental field, the greatest number of plants with disease symptoms were found in the plots of northwest and west.

The role of environmental factors in the infection process

In an experiment lasted about two months a positive correlation was found between the relative air humidity and the dynamics of infection (Fig. 3). The diagram below shows the length of time with a relative air humidity at least or more than 80 per cent in hours per day. The upper graph represents the number of leaves with new symptoms during the same period. The diagram indicates that three peaks of infection have been formed, all of which were preceded by a period with high air humidity. During the examination period, there were 17 days with a weather situation favourable for the development of the pathogen.

(Favourable weather situation means either a 12–14-hour period with a relative air humidity more than 95 per cent or a 6-hour period with more than 95 per cent RH – first night – and an 8-hour period with more than 95 per cent RH– second night –).

On the other hand, there were only 7 days from the 17 mentioned, on which infection could take place as a result of sporulation in the infection centre. This contradiction proves that other factors, as free water existing on the plant surface as well as air temperature must also be considered among the determining factors of infection process.

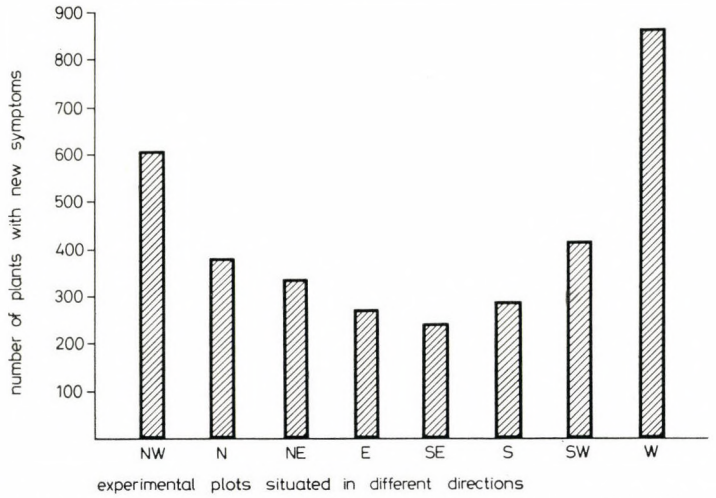

Fig. 2. Frequency of plants with new disease symptoms on plots located in different directions, "Júlia major" 1973,

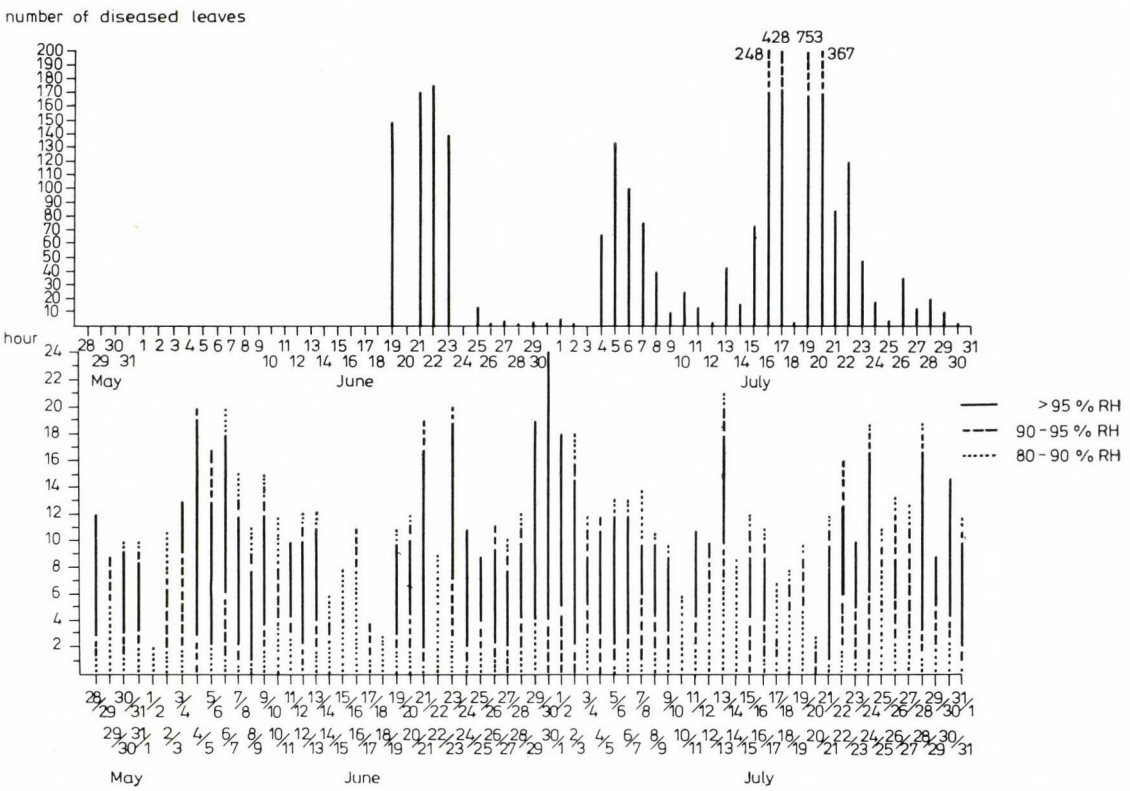

Fig. 3. Connection between air humidity and the dynamics of infection of *P. destructor* in "Júlia major" 1973,

Incubation period

According to the data obtained, during the summer of 1973, the length of time between the infection and the first disease symptoms, i.e. the incubation period has lasted about 11 to 14 days with a maximum of 20 days (Fig. 4). Except the first infection cycle, the exact time of incubation was difficult to determine, due to the continuous appearing of secondary symptoms. The diagram (Fig. 4) also indicates that during the interval of two months examined, four days without

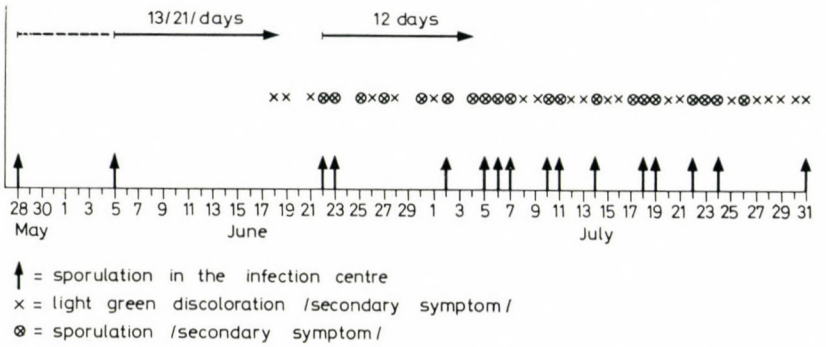

Fig. 4. Incubation period and the mode of symptom-appearance on onion leaves, "Júlia major", 1973

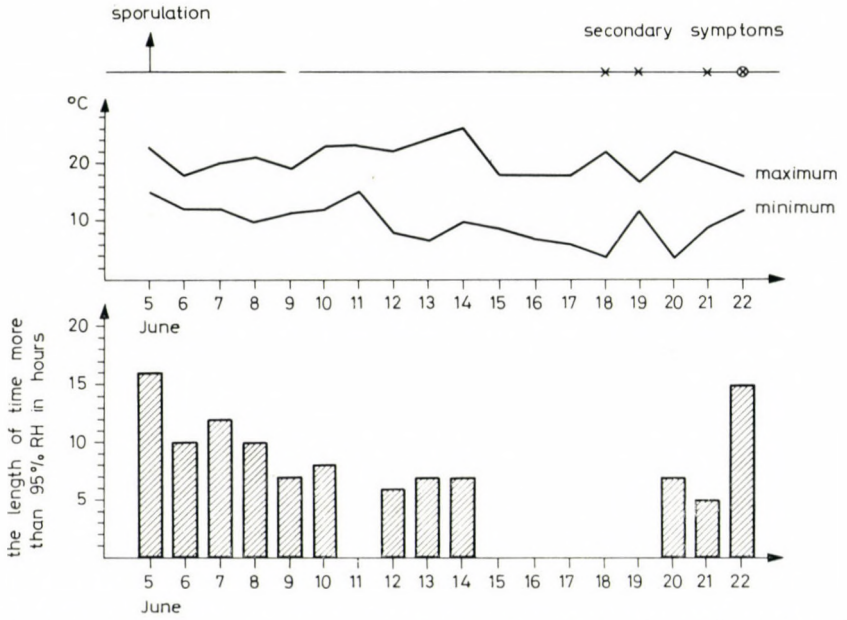

Fig. 5. One infection-cycle connected with the main meteorological factors in "Júlia major", 1973

any symptoms occurred only. It is worth to mention that disease symptoms were very often manifested by developing conidiophores and conidia on the plants without any previous discoloration of the host tissue.

The mode of appearance of the disease symptoms by discoloration or sporulation mainly depends on the weather situation during the incubation period. This opinion seems to be supported by the meteorological conditions showed in Fig. 5. On June 5 there was a period of 16 hours with more than 95 per cent RH followed by a humid night on June 6 during which more than 95 per cent air humidity lasted for 10 hours.

Under these circumstances infection could certainly take place. The next days were a little drier with a temperature ranged between 7 and 26°C. The first light-green spots appeared on June 18, i.e. 13 days after infection. Because of the dry weather, the fungus could not sporulate. Four days later, however, heavy sporulation occurred due to the high air humidity, which lasted for 15 hours.

Discussion

The wind-borne nature of onion downy mildew has already been known a long time before (YARWOOD, 1943). Many investigations made in the United States indicated that conidia of *P. destructor* might be caught in the air even at a a height of 500 m above the onion fields (NEWHALL, 1938). Similarly to YARWOOD's (1943) and RONDOMANSKI's (1967) results, the author has found that the main direction of disease spread in the field is determined by the prevailing wind, at least in the beginning of the epidemic. Later on, however, after infection became severe, differences between various parts of the field could be no longer apparent.

According to RONDOMANSKI (1967), the highest number of infection spots occur in Poland from west, north-west and north direction. In Hungary, even in our experimental field, the wind usually blows from north-west. For this reason, the results obtained were unexpected, and allow to suppose that the local air convection is of great importance in the dissemination, at least at a height of 0.40 – 0.50 m above ground.

A close correlation between high air humidity and conidium formation was previously observed (YARWOOD, 1943; RONDOMANSKI, 1967; VIRÁNYI, 1974b). Studying on the effect of environment in epidemiological respect, VAN DOORN (1959) has found that the favourable weather situation begins at 5 p.m., when the air humidity increases up to 100 per cent. He also found that infection does not take place on all days which are favourable for it. The author obtained the same results.

Many experiments, carried out under laboratory conditions, confirm VAN DOORN's (1959) and others' opinion according to which conidium germination occurs only in free water (VIRÁNYI, 1974b). This finding can explain the lack of infection observed in cases when high air humidity existed. According to the

author's opinion for a better understanding of fungus development it is not enough to consider the air humidity alone, but the exact determination of the length of periods, during which water in liquid form exists on the plant surface, is also important.

From an epidemiological point of view, besides the rain, dew formation is also of great importance (McKAY, 1939). This experience supports the author's opinion about the need for a continuous registration of leaf wetness.

YARWOOD (1943) and others have found that two humid nights are usually necessary for the whole infection process including sporulation, dissemination of conidia and infection. Looking for the cause of this phenomenon, RONDOMANSKI (1967) has supposed that the night-time is too short for the completion of both sporulation and infection, especially when the air temperature achieves or exceeds 20°C resulting in a great delay of germination.

On the basis of the investigations of both fungus development and weather situation, the following conclusion could be drawn: one night with a humid period of at least 6 hours is needed for sporulation, and a second night almost similar to the former one, completed with free water on the plants for 6 hours or longer for conidium germination and penetration (Fig. 3). Both the dew formation and the length of time during which the plants are covered with water, are closely correlated with air humidity and temperature, as well as with the temperature of the onion leaves (YARWOOD, 1943).

On the basis of these results it can be concluded that the length of time and intensity of plant wetness seem to be one of the most important factors influencing onion downy mildew epidemic.

In agreement with RONDOMANSKI (1967) and others, the author has found that incubation period usually lasts 11 – 14 days, sometimes a little longer. According to RONDOMANSKI (1967), the secondary infection appears firstly with fungus sporulation and the symptoms of light-green discoloration of the infected tissues develop later. A great number of investigations carried out by the author indicate that RONDOMANSKI's statement is an extreme generalization of the problem. The mode of appearance of the secondary symptoms is considerably determined by the environmental conditions, among which air humidity is the most important.

Acknowledgements

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Literature

McKay, R. (1939): Observation on onion mildew caused by the fungus *Peronospora schleideniana* W. G. Sm. J. roy. hort. Soc. *64*, 272—285.

Newhall, A. G. (1938): The spread of onion mildew by wind-borne conidia of *Peronospora destructor*. Phytopathology *28*, 257—269.

Rondomanski, W. (1967): Studies on the epidemiology of onion downy mildew, *Peronospora destructor* (Berk.) Fries. Final Techn. Rep. 1962—1967. Inst. Veg. Crops, Skierniewice.

Van Doorn, A. M. (1959): Onderzoekingen over het optreden en de bestrijding van valse meeldauw *(Peronospora destructor)* bij uien. T. Pl. Ziekten, Meded. No. 210.

Virányi, F. (1974a): Studies on the biology and ecology of onion downy mildew (*Peronospora destructor* (Berk.) Fries) in Hungary I. Overwintering of the pathogen in onion bulbs. Acta phytopath. Acad. Sci. hung. *9*, 311—314.

Virányi, F. (1974b): Studies on the biology and ecology of onion downy mildew (*Peronospora destructor* (Berk.) Fries) in Hungary II. Factors influencing sporulation and conidium germination. Acta phytopath. Acad. Sci. hung. *9*, 315—318.

Yarwood, C. E. (1943): Onion downy mildew. Hilgardia *14*, 595—691.

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Sulfide Diseases of Rice on Iron-excess Soils

By

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A synthesis of information involving a new concept is given in this paper, which is primarily a review, also presenting original data.

Hydrogen sulfide toxicity to rice has been discovered on iron-excess soils. Etiological and ecological boundary conditions for H_2S on iron-excess soils have been developed and characterized with respect to rice cultivars, soil series, soil profiles and type of clay, insoluble sulfides—FeS and FeS_2, and H_2S detoxification factors. The symptomatology and etiology of two sulfide diseases—straighthead sulfide disease (SSD) and mild sulfide disease (MSD)—has been discovered and determined sufficiently to indicate that they may occur on iron-excess soils throughout the world. Two simple and rapid laboratory procedures involving seedling oxygen release and nutrient uptake *in vitro* have been developed for testing sulfide reactions of rice breeding lines.

Hydrogen sulfide (H_2S) induced physiological or toxicant diseases of paddy (flooded) rice on iron-deficient soils (soils not containing sufficient iron to convert all soluble sulfide to iron sulfides) have been adequately reviewed (BABA *et al.*, 1965; HOLLIS, 1967a; TANAKA and YOSHIDA, 1970), but recent evidence shows that H_2S diseases causing reductions in rice yields occur also on iron-excess soils (soils containing more than enough iron to convert all soluble sulfides to iron sulfides). For reasons, which will be outlined here, the extensive formation of insoluble iron sulfides does not prevent the build-up of rice-toxic concentrations of H_2S in the soil solution. A condensed but comprehensive summary of this information is presented for everyone interested in wetlands ecosystems (anaerobic environments) consisting of rice paddies, marshes, swamps, deltas and estuaries. Two compelling arguments for this presentation are: (a) rice and native plants with their root systems growing in soils submerged under water are distributed throughout the world primarily on iron-excess soils, (b) evidence now available makes it necessary for us to challenge the basic assumption that excess iron protects these aquatic plants against damage by soluble sulfides.

Materials and Methods

A sulfide electrode was used for measurements of soil sulfide levels in Louisiana and Texas rice fields. The observed potential obeyed the Nernst equation as a function of sulfide ion activity ($^aS^=$) or concentration [$S^=$] and the calibration curve could be extended to [$S^=$] = 10^{-20} M by direct calculation from this equation, on the single assumption that complexing of $S^=$ with protons produced a sum of concentrations [$S^=$], [HS^-], and [H_2S] > 10^{-7} M. Thus, free [$S^=$] was determined by its simultaneous measurement with pH. Methods of calibration and measurement of [$S^=$] and pH in A^1 soil horizons have been summarized (ALLAM *et al.*, 1972).

Results

Sulfide diseases on iron-deficient soils. — Diseases of rice stemming from inhibition of nutrient uptake by H_2S produced by sulfate-reducing bacteria, *Desulfovibrio* spp. have been confined to soils deficient in iron and other elements, found mainly in Japan (MITSUI, 1964; MITSUI, 1965). One disorder which exhibits several manifestations is called akiochi (autumn decline). At one time after World War II, akiochi disease occupied 20–25 per cent of the Japanese rice acreage. Plants in later stages of development exhibit deficiency symptoms for silica, bases, and in final stages for nitrogen and phosphorus. *Helminthosporium* leaf spot caused by the fungus *H. oryzae* v. Breda de Haan is commonly associated with the disease. Akiochi has been controlled by the development of resistant varieties and by applications of basic slag, iron rich soil and elimination of fertilizers containing sulfate. The average yield of rice per acre in Japan for the 5 year period, 1965–69, was 4609. lbs and akiochi is no longer considered a major problem (ADAIR *et al.*, 1973).

A recent compilation of physiological disorders or diseases of rice (SUBRAMONEY, 1965; TANAKA and YOSHIDA, 1970; Table 1) shows various degrees of implication for H_2S as a causal agent in 12 of 27 cases. Diffusion of knowledge about such disorders up to the late nineteen sixties had been confined within separate regions; however, establishment in the Philippines of the International Rice Research Institute in 1960 and activation of research in 1962 has promoted coordination of rice research findings and the major cause of akiochi is now accepted as H_2S toxicity. Sulfide is known to inhibit respiration and oxidizing power of rice roots, which in turn retards the uptake of various nutrient elements. Initially, the oxidizing power of rice roots causes sulfide to be oxidized (detoxified) but when the soil is low in iron, sulfide stays in the soil solution without being precipitated as ferrous sulfide (detoxified) and eventually (in the latter part of the growing season) exerts initial effects as an inhibitor of oxidative enzyme systems (ALLAM and HOLLIS, 1972; BABA *et al.*, 1965; MITSUI, 1964; MITSUI, 1965; MITSUI *et al.*, 1951; TANAKA and YOSHIDA, 1970).

Table 1

Reported disorders of the rice plant with hydrogen sulfide listed as one of the possible causes*

Country	Name of disorder
Ceylon	Bronzing
Colombia	Espiga Erecta (straighthead)
Hungary	Brusone
India	Bronzing (Dahal land)
India	Kuttipachal in Kerala
Japan	Akiochi
Japan	Akagare Type II
Japan	Straighthead
Korea	Akiochi
Portugal	Branca
U.S.A.	Straighthead
Vietnam	Acid sulfate soil induced toxocity

* Summary from SUBRAMONEY (1965) and TANAKA and YOSHIDA (1970)

Apart from rice, citrus is the only economic crop extensively affected by H_2S toxicity. Water damage of Florida citrus occurs on 78 per cent of the growing area, on sandy flatwoods soils deficient in iron and other nutrients. The damage which manifests both chronic and acute phases is considered by KNORR (1973) as, "the most prevalent cause of citrus decline and loss of production in Florida". Water damage was conceived originally as a drainage problem meriting engineering approaches but recent studies on its acute manifestations have demonstrated that the principal factor in the lowered yields, decline and death of citrus trees is H_2S (FORD, 1973).

Sulfide diseases on iron-excess soils. – Knowledge of soils in the Southwest Louisiana rice area is now based on recent intensive surveys of Acadia and Evangeline parishes (CLARK *et al.*, 1962; TOUCHET *et al.*, 1974), supplemented by spot determinations in the other major rice-producing parishes – Calcasieu, Vermillion and Jefferson Davis (S. A. LYTLE, personal communication). There are approximately 37 soil series in Acadia and Evangeline parishes alone, ranging in texture from fine sandy loams to heavy clays. The most commonly-occurring soil series are Crowley and Midland and their surface layers are mainly silt loams in texture. In Louisiana, sulfide samplings were confined mainly to central and northern portions of Acadia and Jefferson Davis parishes with silt and sandy loam soil associations predominating in montmorillonite clay minerals.

One viewpoint which has permeated agronomic thought in recent years is that the tendency of H_2S to form insoluble sulfides with metals, principally iron; solubility product constant 3.7×10^{-19} at 18°C, (HODGMAN, 1948), makes it impossible for rice-toxic levels of H_2S (0.1 to 1.0 μg/ml) to occur in soils containing excess iron because, "in normal soils, the presence of Fe^{++} keep the concentration

of H_2S below 10^{-8} moles per liter and makes it undetectable . . ." (PONNAMPERUMA, 1965). STURGIS (1936) determined acid-extractable Fe_2O_3 levels ranging from 1.7 to 4.17 per cent in a normal Louisiana rice soil (Crowley silt loam); essentially, these levels of iron define an iron-excess soil in the U. S., Gulf Coast region. Subsequent determinations of iron oxides have indicated a wider range of values may exist, viz., 1 to 6 per cent acid-extractable iron (W. H. PATRICK, Jr., Personal communication).

Our approach in the Department of Plant Pathology, Louisiana State University was made against informed opinion which placed strong emphasis upon the importance of iron oxides in preventing free, soluble sulfide formation by the observed processes of reduction of these iron oxides to soluble ferrous iron (Fe^{++}) (BABA *et al.*, 1965; MITSUI, 1964; MITSUI, 1965; PONNAMPERUMA, 1965). We examined possibilities for occurrence of rice-toxic levels of H_2S in normal Louisiana and Texas rice soils containing excess-iron oxides by focusing attention on Fe^{++}. Initially, theoretical predictions of rice-toxic H_2S levels were derived from experimental determinations of $[Fe^{++}]$ and $[H^+]$ in the soil solution of biochemically-reduced rice field soils flooded for periods exceeding 40 days, and the use of equations for the solubility product constant of FeS and dissociation constants of H_2S. The predictions were verified by extraction of H_2S from flooded soils in inert gas (nitrogen) streams and its determination by chemical methods (HOLLIS, 1967b, c, d, e).

Later, a comprehensive pattern of rice sulfide disease etiology on Gulf Coast rice soils was revealed by theoretical and experimental: (a) identification of the insoluble sulfides, FeS and FeS_2 and determinations of H_2S levels (ALLAM, 1971; ALLAM *et al.*, 1972; PITTS, 1971; PITTS *et al.*, 1970; 1972a); (b) quantitative inhibition by H_2S of oxidative enzymes, including cytochrome oxidase, and of oxygen release and nutrient uptake of rice seedlings (ALLAM and HOLLIS, 1972, JOSHI, 1974; JOSHI and HOLLIS, 1974a, b; JOSHI *et al.*, 1973; 1975); and (c) parallel reactions of rice cultivars to these physiological parameters and to sulfide diseases (ADAIR *et al.*, 1973; ATKINS *et al.*, 1956a, b; JOSHI, 1974; TISDALE and JENKINS, 1921).

Straighthead and mild sulfide diseases. — Straighthead disease of rice has been a recognized physiological disorder for more than 60 years, and was first reported by Collier (TISDALE and JENKINS, 1921) from Arkansas. Cause of the disorder or disease has been unknown, except in special cases where its occurrence has been linked with soil arsenic levels built up by repeated applications of arsenical insecticides on cotton land subsequently planted to rice (ADAIR *et al.*, 1973). The particular arsenic compounds involved are unknown but it is likely that arsenic acids are formed in the soil (HODGMAN, 1948). Straighthead is named from the upright heads which lack sufficient weight to bend over and is characterized by glume abnormalities. The disease has been found in the United States where it is of major importance, and in Japan, Colombia, Portugal and several other countries (OU, 1972; TANAKA and YOSHIDA, 1970; Table 1); it occurs commonly on sandy soils predominating in kaolinite and related clay minerals under con-

tinuous flooding (CLARK *et al.*, 1962, MOWERY *et al.*, 1960). Drainage of fields prior to panicle development 50 to 60 days after flooding and the use of resistant cultivars have been common practices for straighthead control (ADAIR *et al.*, 1973, ATKINS *et al.*, 1956a, b).

Field testing for straighthead reaction in the U. S. A. Gulf Coast region has been conducted by the U. S. D. A. and Texas Agricultural Experiment Station since 1952 on the Dave Winterman farm containing Crowley and Edna fine sandy loam soils near Eagle Lake, Texas. These soils, formerly classified in the Hockley series (MOWERY *et al.*, 1960), have recently been renamed (J. W. STANSEL, personal communication). They are lower in acid-extractable iron than Crowley silt loam and other related rice soils of higher clay content (CONNELL and PATRICK, 1969). Katy, Crowley and Edna fine sandy loams have A horizons, respectively, of more than 51 cm, 25 to 50 cm and less than 25 cm and are common throughout the Eagle Lake, Texas (Western Division rice area, J. W. STANSEL, personal communication). They are normal soils containing excess iron and "concretions containing iron and manganese are scattered throughout their profiles in most cases" (MOWERY *et al.*, 1960, p. 9).

H_2S levels determined in 1971 with the sulfide electrode were higher in Eagle Lake area soils (Table 2) than in comparable determinations made across a spectrum of soil series in Louisiana (ALLAM, 1971; ALLAM *et al.*, 1972; HOLLIS *et al.*, 1972). The hundreds of cultivars and breeding lines tested at Eagle Lake over the years have, collectively, exhibited a continuous range of variability in straighthead reaction (J. G. ATKINS, personal communication in HOLLIS, 1967b), but practical separations of reaction levels have been based on 5 ratings: highly susceptible, moderately susceptible, moderately resistant, resistant (ATKINS *et al.*, 1956a, b). Test results in 1973 and 1974 were disappointing because of the general occurrence of a low level of straighthead disease in the experimental area (J. W. STANSEL, personal communication).

A summary of evidence that straighthead is caused by H_2S would include those points cited under sulfide diseases on iron-excess soils along with a selection of data from recent publications (JOSHI, 1974; JOSHI and HOLLIS, 1974a; JOSHI *et al.*, 1973; 1975): (a) straighthead resistant cultivars include Bluebelle, Belle Patna and Saturn; susceptible cultivars include Dawn, Zenith, Blue Rose and Arkrose, (b) cultivars with higher oxygen release are resistant; those with lower oxygen release are susceptible, (c) also, after 12 hr pretreatments with 0.2 μg/ml H_2S, Bluebelle and Saturn released more O_2 (were more resistant to effects of H_2S) than Dawn and Zenith, (d) likewise, with respect to nutrient uptake, Bluebelle and Saturn were unaffected by H_2S pretreatments at both 0.1 and 0.2 μg/ml, whereas 58 and 50 per cent reduction, respectively, occurred in the nutrient uptake of Dawn and Zenith after pretreatment with 0.1 μg/ml H_2S, (e) specific inhibition of P^{32} uptake after pretreatment with 1.0 μg/ml H_2S was highest in Zenith, followed by Dawn, Saturn and Bluebelle in decreasing order of inhibition.

It has been correct in the recent past, before accumulation of the data re-

Table 2

H_2S concentrations (toxic levels > 0.01 μg/ml) in Louisiana and Texas rice fields during the 1971 growing season. (Figures in μg/ml)

Sample number	Louisiana					Texas
	May 17	May 31	June 17	July 14	July 28	Aug. 5, 1971
1	.4011	.4011	.7943	.1585	.5012	.2581
2	.3162	.0501	.5012	.1991	.3162	.0125
3	.0199	.0794	.3162	.0501	.1258	.7943
4	.1585	.3162	.0794	.1258	.0362	.1258
5	.7943	.1991	.0501	.3165	.3162	.2581
6	.1991	.7943	.0316	.0199	.2581	.2581
7	.7943	.1991	.0200	.1258	.3162	.1991
8		.3162	.1258	.1991	.2583	.1258
9		.5012	.3163	.0316	.3162	.1623
10		.1991	.1585	.1258	.7943	.1991
11				.5012		.0125
12				.1258		.5012
13				.1991		.5012
14				.1991		.2581
15				.1991		.5012
16						.5012
17						.7943
18						.3162
19						.3981
20						.3162
21						.7943
22						.1258
23						.7943
Average	.3976	.3155	.2393	.1584	.3233	.3611
Total number of samples	20*	14	21	30	22	26
Per cent of fields showing >.01 μg/ml	35	70	50	50	47	92

* Fields sampled at each date. These data may be compared with those published previously, ALLAM *et al.* (1972)

viewed in this paper, to consider straighthead of rice as a recognizable disease without a known cause. Likewise mild sulfide disease (MSD) could by regarded as a disease with a defined cause, but without recognizable symptoms (ALLAM, 1971; ALLAM *et al.*, 1972; HOLLIS, 1967a; HOLLIS *et al.*, 1972).

Although symptomless toxicant diseases of rice had been suspected to occur

since the mid-nineteen sixties (HOLLIS, 1967a), it was the determination of rice-toxic concentrations of H_2S (ALLAM and HOLLIS, 1972; MITSUI, 1964; MITSUI *et al.*, 1951) and their actual demonstration in flooded Gulf Coast rice fields during booting, heading-flowering and ripening stages of rice plant development (ALLAM *et al.*, 1972; HOLLIS, 1967c, d) which made it necessary to invent MSD as a hypothetical disease that reveals a general underlying susceptibility of rice cultivars to H_2S, for which straighthead, hereafter called straighthead sulfide disease (SSD), is the acute symptomatic manifestation. Straighthead caused by applications of arsenical insecticides on cotton land subsequently planted to rice is renamed straighthead arsenic disease.

The existence of MSD is strongly supported by the etiology and widespread occurrence of SSD and the correlations outlined above respecting cultivar reactions to oxygen release, nutrient uptake and SSD symptoms, following exposure to rice-toxic concentrations of H_2S (JOSHI, 1974; JOSHI and HOLLIS, 1974a, b; JOSHI *et al.*, 1973, 1975). Additional lines of evidence which posit the existence of MSD are: (a) involvement of H_2S in many physiological diseases of rice around the world (Table 1), (b) a possible absence of root detoxifying mechanisms *per se* (for elimination of H_2S) once the plow layer is biochemically reduced and the rice root system in this soil layer (A_1 horizon) has been blackened by deposition of iron sulfides, (c) the reactions to varied nitrogen levels of the Louisiana rice cultivars Saturn and Zenith. These cultivars are comparable in most respects but Saturn is superior on the basis of responsiveness to higher levels of nitrogen and the resulting increase in grain yield; its introduction in Louisiana some 10 years ago brought about yield increases which ranged commonly up to 800 pounds of rough (unhulled) rice per acre (N. E. JODON, personal communication).

Symptoms of MSD in Louisiana resemble those of a mild akiochi disease in Japan (BABA *et al.*, 1965; HOLLIS *et al.*, 1972; MITSUI, 1964): these are; late season expression, premature death of lower leaves, nitrogen deficiency yellowing of lower leaves, occurrence of *Helminthosporium* leaf spot (HLS) and lowered grain yields. It is of interest to recall that rice yield is a more sensitive indicator of the occurrence of SSD than the straighthead symptom (J. G. ATKINS, personal communication in HOLLIS, 1967a). HLS (brown spot) is found generally on poor soil (OU, 1972); it appears to occur also on rice plants weakened by other factors, including akiochi disease (BABA *et al.*, 1965) and ring nematode. Brown spot has been observed by the author in both Louisiana and Texas on 30 to 50 day-old rice plants during the first half of the growing season in conjunction with high populations of *Criconemoides onoensis* Luc 1959 (HOLLIS, 1973).

Sulfide diseases and plant breeding. — The combined laboratory and field data outline an etiologic role for H_2S in physiological or toxicant diseases of rice; physiological responses of rice seedlings make possible the rapid differentiation of sulfide resistant and susceptible breeding lines. Since failure of a rice plant selection tested in the field to respond to higher levels of nitrogen and/or other nutrients may be caused by H_2S inhibition of nutrient uptake on both iron-deficient and iron-excess soils, rice breeders in all parts of the world should routinely

direct attention toward laboratory tests of H_2S effects on their breeding lines. Such tests would be consistent with past and present practices since the principal objective of rice breeders has been to obtain higher yields with the use of nitrogenous fertilizers (LAMBERS, 1970). This objective has been linked to lodging resistance in both temperate and tropical regions; in the U. S. A. to other correlated variables including high seedling density, low tillering rate, and in tropical regions to plant type for maximum light absorption, early maturity and insensitivity of floral initiation to photoperiod and temperature.

Straighthead resistant breeding lines of rice in the United States have been originated from the varieties Pa Chiam from Taiwan and Sinapagh from the Philippines-countries where straighthead has not been reported as a disease (ADAIR *et al.*, 1973).

The higher yields of Saturn variety in Louisiana may have resulted in part from its selection for H_2S resistance; thus the plant breeder, Dr. N. E. JODON could have unconsciously derived a yield increase effect from sulfide resistance without the means for its identification as a separate factor. The intrinsic disadvantage of an approach made without benefit of this distinction is that genes for higher yield per se may have been discarded in favor of combinations of genes for lower yield and for sulfide resistance favoring higher yield, or the reverse combination. There is evidence for apparent support of this view in the extreme susceptibility of certain high-yielding varieties such as Dawn to SSD, and in the fact that the high-yielding Saturn variety is only moderately resistant to SSD.

Ecology of sulfide diseases of rice. Although measurements of ferrous iron in the soil solution and theoretical considerations (ALLAM, 1971; ALLAM *et al.*, 1972; HOLLIS, 1967a, b, c, d; PITTS, 1971; PITTS *et al.*, 1970, 1972a), have provided chemical and thermodynamic explanations for equilibria between H_2S and iron sulfides (FeS, FeS_2), the actual mechanisms of occurrence of rice-toxic levels of H_2S in the soil solution of submerged iron-excess soils have not been elucidated experimentally. Hydrogen sulfide has been observed to evolve from anaerobic cultures, even though the cultures contained enough (soluble) ferrous iron to precipitate sulfide as insoluble ferrous sulfide (BLOOMFIELD, 1969). Bloomfield found that very different FeS: H_2S ratios were produced by the organic matter—rice blade or lucerne—added to the cultures, although dissolved Fe, $[Fe^{++}]$ and pH were equivalent. One explanation for the failure of ferrous iron to precipitate sulfide is the formation of iron chelates with certain fractions of organic matter (W. H. PATRICK, Jr., personal communication).

The two most important factors now known to regulate accumulation of H_2S in submerged rice soils are soil reaction (pH) and oxidizable carbon (ALLAM *et al.*, 1972); however, it is of greater interest to consider actual detoxifying mechanism for H_2S. In addition to soluble (ferrous) iron content, two additional factors for detoxification of H_2S have been identified in rice soils. It has long been evident that a lack of H_2S odor in most Gulf Coast rice fields may be related to sorption of H_2S from the soil solution by the soil clay fraction (HOLLIS, 1967a, b, d). We have been able to show that bentonite (montmorillonite clay) sorbs H_2S

under laboratory conditions, whereas kaolinite clay does not sorb H_2S (ALLAM *et al.*, 1972). This is in agreement with the fact that SSD occurs on sandy soils predominating in kaolinite, halloysite and illite rather than montmorillonite clays (CLARK *et al.*, 1962; MOWERY *et al.*, 1960).

It was found that *Beggiatoa*, a filamentous bacterium found in the rice rhizosphere may be an important factor influencing the distribution and severity of sulfide diseases in rice (PITTS, 1971; PITTS *et al.*, 1972b). *Beggiatoa* is autointoxicated by hydrogen peroxide (H_2O_2); the rice root catalase decomposes H_2O_2. In return, *Beggiatoa* removes (detoxifies) H_2S from the rice rhizosphere by oxidizing it to elemental sulfur. Thus, rice root and *Beggiatoa* comprise a mutually protective system. The possible role of *Beggiatoa* in rice culture is currently being explored.

Sulfide diseases of rice have their origin in toxic concentrations of H_2S in the biochemically- and microbiologically-reduced plow layer (PL) or A_1 horizon, and in the undisturbed plow sole (PS) or accumulation zone for reduced products just below the PL. The PL contains the bulk of organic matter and is the only layer saturated with water. Beneath the PS lies oxidized layers (OL) containing hydroxides and oxides of iron and manganese, high clay and a very low content of organic matter.

The profile of the "average soil" embodying the principal soil types in flooded Louisiana rice fields has been characterized previously from the phytopathological standpoint (HOLLIS, 1967c; HOLLIS and RODRIGUEZ-KABANA, 1967). The most important feature is the water-impervious layer (WIL) which extends from the region of the PS beneath the A_1 horizon. This layer commonly ranges in thickness from 10 to 24 cm and comprises the lower A and upper B soil horizons. The top of the WIL is generally found from 12 to 20 cm below the soil surface; these dimensions represent therefore the range of thickness of the PL. The WIL intergrades with the OL below and contains a minimum of organic matter, a moderate to high content of clay, and reduced products leached from the PL-including organic acids, iron sulfides, hydrogen sulfide and ferrous iron detected, respectively, by odor (HOLLIS and RODRIGUEZ-KABANA, 1967), color and physical criteria (HOLLIS, 1967d; PITTS, 1971; PITTS *et al.*, 1970, 1972a) and by chemical analyses (ALLAM, 1971; ALLAM *et al.*, 1972; CONNELL and PATRICK, 1969; HOLLIS, 1967a, b, c, d).

The three soil layers of interest in the submerged rice soil profile are: (a) the reduced PL (A_1 horizon), (b) the WIL, and (c) the OL. Oxidation is indicated by the presence of mottles and other configurations of iron and manganese hydroxides and oxides and by water unsaturation. The OL appearance, texture and physical similarity to the WIL under water suggest that the OL must function to support the surface water table, and thus, it can be regarded as an extension of the WIL in Louisiana rice fields. Absence of a WIL in river bottom areas of Louisiana where rice is also grown suggests that partial retention of surface water here may depend solely on the OL.

Prior to 1968, it was common knowledge that the rice root system in Louisiana fields was confined to the PL because the PS was considered impenetrable.

Observations of the soil profile by the author with the use of a shovel indicated, however, that rice roots penetrated into the OL (HOLLIS, 1967e). Consequently, a study of soil layer distribution of the rice root system was made during the 1967 growing season (HOLLIS, 1968). Penetration depth was studied with reference to average soil profile dimensions: PL (0–15 cm), WIL (15–25 cm), and OL (25–45 cm). Cylindrical cores (4.7 cm in diam × 45.7 cm long) were removed at weekly intervals from four flooded rice field sites on four different soil series and divided into 0 to 15, 15 to 30 and 30 to 45 cm segments. The soil was leached away under a fine spray of water to free the roots, which were collected on screens and then preserved in formal-acet-alcohol. Root surface area was based on length and diameter measurements of sample aliquots with an ocular micrometer. Root penetration (surface area) in the lower core segments increased progressively from the fourth through the seventh sampling (42–70 days). Percentages of root surface at 15.2 to 45.7 cm depth, at 42, 48, 63 and 70 days after flooding and planting, were 16.5, 34, 46.1 and 39.3, respectively; and at 30.5–45.7 cm, the percentages were 1.1, 6.6, 15.1 and 19.6. At midseason (63 days) approximately 40 per cent of the rice root system (surface area) had developed below the plow layer. Although age of the plant had also increased, with consequent shifts in the requirements for specific nutrients (BABA *et al.*, 1965; MITSUI, 1964; MITSUI, 1965), it is apparent that such an increase in surface area should facilitate nutrient absorption from the WIL and OL. This portion of the root system taps a compensatory nutrient reservoir and provides anchorage simply because it becomes functional during the booting, heading-flowering and ripening stages of plant development when toxic concentrations of H_2S are produced in the PL. Soil profiles with A horizons thicker than 55 cm, such as exhibited by Katy fine sandy loam (J. W. STANSEL, personal communication), have deeper and less effective OL. These characteristics frequently contribute to very high rice yields in SSD resistant cultivars, and to SSD in susceptible cultivars. Where soils such as Edna fine sandy loam have a relatively thin A horizon (25 cm) the WIL may become important in SSD and MSD initiation because of leachings from the A (A_1) horizon (PL) resulting in higher concentrations of ferrous iron, FeS, FeS_2 and H_2S in the WIL than in the PL (HOLLIS, 1967a, b, c; PITTS *et al.*, 1970, 1972a).

Sethi, according to ALBERDA (1953), was the first to note that the rice plant produces still another root system spread on the soil surface; and this system, occupying a thin oxidized surface zone of soil characterized by PATRICK and DELAUNE (1972), has been observed commonly in Louisiana by the author during the latter part of the growing season, particularly in rice where aerial applications of nitrogen (top dressings) have been made during the panicle initation stages of plant development. This surface root system also exerts a compensating effect on loss of nutrient absorptive function by sulfide inhibition of roots in the PL and WIL.

Discussion

Although sulfide diseases of rice as now known are endemic (confined to certain regions), their occurrence and severity is seen to represent the interplay of physical, chemical and microbiological factors.

The extension of sulfide diseases of rice to iron-excess soils in the U. S. Gulf Coast region provides a basis for their potential discovery, recognition and quantification in vast areas of the globe where rice production levels are still limited by undefined soil factors. Specifically, rice researchers working in areas of iron-excess soils now possess tools for the independent evaluation of sulfide resistance and of fertilizer uptake capacity, utilization and response of rice breeding lines.

Stabilization and rejuvenation of ecologically degraded or geographically reduced areas of wetlands ecosystems involving aquatic plant species other than rice are an economic and political necessity; success will depend in part upon increased knowledge of aquatic plant sulfide diseases on organic soils containing excessive quantities of both iron and organic carbon.

Vast areas of acid sulfate soils in Southeast Asia have been considered free of the sulfide diseases of both rice and other aquatic plant species by most authors because sulfate-reducing organisms do not operate under very acid conditions – at least in laboratory tests. However, BLOOMFIELD and COULTER (1973) admit the possibility of H_2S toxicity to plants where the acidity decreases after prolonged waterlogging. The demonstrated occurrence of high rice-toxic H_2S concentrations in Louisiana rice fields at the relatively low pH levels of 5 to 6 (ALLAM, 1971; ALLAM *et al.*, 1972; HOLLIS, 1967a, b, c, d) indicates there can be a slow accumulation of H_2S under conditions adverse to its rapid production by sulfate-reducing bacteria. Such data are consistent with the detection and occurrence of H_2S in physiological disorders of plants on acid sulfate soils (Table 1).

An interesting consequence of established sulfide disease etiology on iron-excess soils is the interpretation of nitrogen deficiency and other nutrient deficiencies in wetlands ecosystems, in an inverse sense – as simply sulfide inhibition of nutrient uptake. Experiments have demonstrated favorable responses of *Spartina* spp. (cord grasses) to nitrogen supplements in Louisiana coastal marshes; and the relevant question of sulfide-resistance-enhanced-nitrogen-utilization efficiency arises because high H_2S levels have been measured in coastal marsh soils (W. H. PATRICK, Jr., personal communication).

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Literature

ADAIR, C. R., BOLLICH, C. N. BOWMAN, D. H. JODON, N. W. JOHNSTON, T. H. WEBB B. D. and J. G. ATKINS (1973): U. S. D. A. Agr. Hdbk. No. 289 (Rev.) Washington, pp. 1, 47, 142, 145.

ALBERDA, TH. (1953): Growth and root development of lowland rice and its relation to oxygen supply. Plant and Soil *5*, 1—28.

ALLAM, A. I. (1971): Soluble sulfides in rice fields and their in vitro effects on rice seedlings. Ph. D. dissertation, Louisiana State University, Baton Rouge, 66 p.

ALLAM, A. I. and HOLLIS J. P. (1972): Sulfide inhibition of oxidases in rice roots. Phytopathology *62*, 634—639.

ALLAM, A. I., PITTS G. and HOLLIS J. P. (1972): Sulfide determination in submerged soils with an ion-selective electrode. Soil Sci. *114*, 457—467.

ATKINS, J. G., BEACHELL, H. M. and CRANE L. E. (1956a): Reaction of rice varieties to straighthead. Texas Agr. Exp. Sta. Progress Report 1865, 1—2.

ATKINS, J. G., BEACHELL H. M. and CRANE L. E. (1956b): Testing and breeding rice varieties for resistance to the straighthead disease. Rice Journal *59* (6), 36—38.

BABA, I., INADA K. and TAJIMA K. (1965): Mineral nutrition and the occurrence of physiological diseases, pp. 173—195. In: The Mineral Nutrition of the Rice Plant, A. Tanaka, Ed. John Hopkins, Baltimore.

BLOOMFIELD, C. (1969): Sulfate reduction in waterlogged soils. The Jour. of Soil Sci. *20*, 207—221.

BLOOMFIELD, C. and COULTER J. K. (1973): Genesis and management of acid sulfate soils. Adv. in Agron. *25*, 265—326.

CLARK, H. L., HALEY, G. L. HEBERT, E. J. HOLLIER, R. M. Jr. and ROY A. J. (1962): U. S. D. A. Soil Conserv. Serv., Soil Survey. Acadia Parish, Louisiana, Ser. 1959, No. 15.

CONNELL, W. E. and PATRICK, W. H. Jr. (1969): Reduction of sulfate to sulfide in waterlogged soil. Proc. Soil Sci. Soc. Amer. *33*, 711—715.

FORD, H. W. (1973): Levels of hydrogen sulfide toxic to citrus roots. J. Amer. Soc. Hort. Sci. *98*, 66—68.

HODGMAN, C. D., Ed. (1948): Handbook of Chemistry and Physics. (31st Ed.) Chem. Rubber Publ. Co., Cleveland, p. 1448.

HOLLIS, J. P. (1967a): Toxicant diseases of rice. Louisiana Agr. Exp. Sta. Bull. *614*, 24 p.

HOLLIS, J. P. (1967b): Some new facts about soil diseases of rice. Louisiana Agriculture *10*, 8, 9.

HOLLIS, J. P. (1967c): Concentration of toxicants in the rice soil profile. Phytopathology *57*, 460 (Abstr.).

HOLLIS, J. P. (1967d): Soluble sulfide and iron in rice soils. Phytopathology *57*, 461 (Abstr.).

HOLLIS, J. P. (1967e): Nature of the nematode problem in Louisiana rice fields. Plant Dis. Rep. *57*, 167—169.

HOLLIS, J. P. (1968): Soil layer distribution of the rice root system. Phytopathology *78*, 1053—1054 (Abstr.).

HOLLIS, J. P. and RODRIGUEZ-KABANA R. (1967): Fatty acids in Louisiana rice fields. Phytopathology *57*, 841—847.

HOLLIS, J. P., ALLAM A. I. and PITTS G. (1972): Sulfide diseases of rice. Phytopathology *62*, 764—765 (Abstr.).

HOLLIS, J. P. (1973): A summary of ring nematode studies. 65th Ann. Rept. Rice Exp. Sta., Crowley, Louisiana, pp. 153—174.

JOSHI, M. M. (1974): Physiological responses of rice to hydrogen sulfide. Ph. D. dissertation, Louisiana State University, Baton Rouge, 50 p.

JOSHI, M. M. and HOLLIS J. P. (1974a): Effects of hydrogen sulfide on oxygen uptake and nutrients uptake of four rice varieties. Proc. Rice Tech. Working Grp. Meet. Fayetteville, Arkansas, March 12—14, p. 69 (Abstr.).

JOSHI, M. M. and HOLLIS J. P. (1974b): Physiological tests for sulfide diseases of rice. Proc. 66th Ann. Meet. Amer. Phytopath. Soc., Vancouver, B. C., Canada, Aug. 11—15, p. 144—145 (Abstr.).

JOSHI, M. M., I. IBRAHIM K. A. and HOLLIS J. P. (1973): Oxygen release from rice seedlings. Physiol. Plantarum *29*, 269—271.

JOSHI, M. M., IBRAHIM I. K. A. and HOLLIS J. P. (1975): Hydrogen sulfide: Effects on the physiology of rice plants and relation to straighthead disease. Phytopathology *65*, 1165–1170.

KNORR, C. (1973): Citrus Diseases and Disorders. The Univ. Presses of Florida, Gainesville, pp. 101—103.

LAMBERS, HILLE RIS D. (1970): Varietal Improvement in Rice, In: Aspects of Rice Growing in Asia and the Americas, R. Best, Ed., H. Veenman & Zonen N. V., Wageningen, The Netherlands, p. 97.

MITSUI, S. (1964): Inorganic Nutrition, Fertilization and Soil Amelioration for Lowland Rice. Yokanda Publ. Co., Ltd., Tokyo, 107 p.

MITSUI, S. (1965): Dynamic aspects of nutrient uptake, pp. 53—62. In: The Mineral Nutrition of the Rice Plant, A. Tanaka, Ed. Johns Hopkins, Baltimore. p. 318.

MITSUI, S., ASO S. and KUMAZAWA K. (1951): Dynamic studies on the nutrient uptake by crop plants. I. The nutrient uptake of rice roots as influenced by hydrogen sulphide. J. Sci. Soil Manure, Japan *22*, 46—52.

MOWERY, I. C., MCFEE, G. S. MATANZO F. and FRANCIS E. (1960): U. S. D. A. Soil Conserv. Serv., Soil Survey, Fort Bend County, Texas, Ser. *1955*, No. 5.

OU, S. H. (1972): Rice diseases. Commonwealth Mycol. Inst., Kew, England, 368 p., pp. 358, 359.

PATRICK, W. H. Jr. and DELAUNE R. D. (1972): Characterization of the oxidized and reduced zones in flooded soil. Proc. Soil Sci. Soc. Amer. *36*, 573—576.

PITTS, G. (1971): Explorations in the chemistry and microbiology of Louisiana State University, Baton Rouge, 60 p.

PITTS, G., ALLAM A. I. and HOLLIS J. P. (1970): Identity of iron sulfides on rice roots. Phytopathology *60*, 687 (Abstr.).

PITTS, G., ALLAM A. I. and HOLLIS J. P. (1972a): Aqueous iron-sulfur systems in rice field soils of Louisiana. Plant and Soil *36*, 251—260.

PITTS, G., ALLAM A. I. and HOLLIS J. P. (1972b): *Beggiatoa:* Occurrence in the rice rhizosphere. Science *178*, 990—992.

PONNAMPERUMA, F. N. (1965): Dynamic aspects of flooded soils and the nutrition of the rice plant, pp. 295—328. In: The Mineral Nutrition of the Rice Plant, A. Tanaka, Ed., Johns Hopkins, Baltimore, p. 318.

STURGIS, M. B. (1936): Changes in the oxidation-reduction equilibrium in soils as related to the physical properties of the soil and the growth of rice. Louisiana Agr. Exp. Sta. Bull. *271*, 37 p.

SUBRAMONEY, N. (1965): Injury to paddy seedlings by production of H_2S under field conditions. Jour. of Ind. Soc. of Soil Sci. *13*, 95—98.

TANAKA, A. and YOSHIDA S. (1970): Nutritional disorders of the rice plant in Asia. Int. Rice Res. Inst. Tech. Bull. *10*, 51 p.

TISDALE, W. H. and JENKINS J. M. (1921): Straighthead of rice and its control. Plant Dis. Rep. Farmer's Bull. *1212*, 1—16.

TOUCHET, B. A., CLARK, H. L. REDLICH, C. M. ROY, A. J. GRIFFIS, B. J. ZAUNBRECHER S. J. and HOLLIER R. M. (1974): Soil survey of Evangeline Parish, Louisiana, U. S. D. A. Soil Conserv. Serv. in cooperation with Louisiana Agr. Exp. Sta.

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Indole Hydroxylation in Various Plant Species

By

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Following the infiltration of indole and incubation, the formation of hydroxy derivatives was detected in the organs of plant species. A larger quantity of hydroxy derivatives was formed in the plastid concentrate than in the organs. In the plant species examined the hydroxylation of the indole occurred on the carbon atom at position 5, while in the case of the chloroplasts of the bean and the leaf and chloroplast of *Tradescantia* it also proceeded at position 6. There are differences in the extent of hydroxylation between the individual plant species, and this provides the starting basis for further experiments.

Indole derivatives are known to exert resistance-increasing effects in the plant kingdom. In our experiments indole hydroxylation has been studied in the organs of plants of various species. We were unable to find literature references to the indole hydroxylation of the three plant species studied, or to the quantitative development of the hydroxy derivatives of indole. Endogenous indole has been detected in some plant species, e.g. maize (REVIN and SMOLYANINOV, 1971). Different products are formed in hydroxylation systems; some systems are substrate-specific, but the quantitative and qualitative distributions of the products are not reported in a number of publications (MARIÁN, 1968, 1969; MATKOVICS *et al.*, 1972a, b; NEMCSÓK, 1972). Accordingly, in the present work we have attempted the isolation and quantitative determination of the products formed.

Material and Method

The experiments were carried out with MFB barley (Martonvásár variety), pearl bean (Baranya County variety) and one species from the *Commelinaceae* family, *Tradescantia albiflora* Brückn. (syn. *T. viridis* hort.).

A study was made of the hydroxylating abilities of the organs and tissues of the plants, and of the plastids and plastid-free fractions. The hydroxy derivatives formed were detected by thin-layer chromatography on 10×20 cm plates. A 90 : 10 mixture of benzene + acetone was employed as solvent. The derivatives were identified *via* chemically-prepared compounds, on the basis of the colours and shapes of the spots, their R_f values and their amounts. The compounds were

prepared in the Fenton-Cier system (EICH and ROCHELMEYER, 1966). By this means 6-membered rings can be monohydroxylated; in this case indole gives rise to the 4-, 5-, 6- and 7-hydroxyindoles.

The indole was added to the plant parts by infiltration, and then incubated for 5 hours at 20°C. The indole was employed in a concentration of 100 γ/ml, in phosphate buffer. The amounts of the hydroxy derivatives formed were determined spectrophotometrically *via* a calibration curve recorded for the indole substrate.

Results and Discussion

The averages of the values from the 64 experiments carried out are listed in Table 1.

Table 1

Indole hydroxy derivatives calculated in γ/g fresh weight

		Indole residue		5-OH-indole	6-OH-indole
5-day bean	Shoot	R_f	0.65	0.17	*
			102	2.90	
Complete plant	Stem	R_f	0.83	0.28	*
			292	5.40	
part	Root	R_f	0.75	0.15	*
			210	2.95	
	Plastid concentrate	R_f	0.72	0.23	0.24
			265	5.80	4.60
Cell fraction	0.25 M saccharose	R_f	0.70	0.25	0.19
from 20-day	Plastid-poor fraction		397	1.60	1.65
bean leaf	0.35 M saccharose	R_f	0.71	0.28	0.21
	Plastid-poor fraction		465	1.70	1.65
	Shoot	R_f	0.70	0.26	*
4-day barley			635	3.65	
	Root	R_f	0.72	0.24	*
			247	3.40	
	Leaf	R_f	0.73	0.30	0.25
Tradescantia			165	1.15	1.25
	Plastid concentrate	R_f	0.76	0.28	0.21
			310	2.45	2.65

* 6-OH-indole not formed

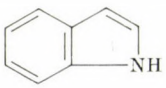
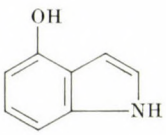
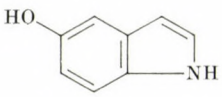
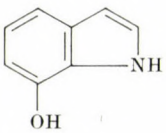
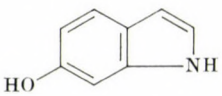
It can be seen from the Table that the formation of 5- and 6-OH-indoles was detected in the plants under examination. On development with the van Urk reagent, 5-OH-indole appeared as a purple spot, and 6-OH-indole as a Paris-blue one. In the shoot, stem and root of the bean only 5-OH-indole was formed. A sim-

ilar result was obtained for the barley (Table). Both 5- and 6-OH-indoles were found in the leaf and plastid concentrate of *Tradescantia*, and in the plastid-free fractions at the two saccharose concentrations. The *Tradescantia* differed from the other two plant species with regard to the 6-OH derivative; the difference may be produced by the very good regenerating ability, and in connection with this by the intensive synthesis processes.

6-OH-indole was produced in the chloroplast and the chloroplast-free fractions in both the bean and *Tradescantia*. This indicates that in both cases the enzyme or enzymes hydroxylating in the 6-position could be enriched.

In the evaluation of the amounts of the derivatives it is necessary to compare the quantities of the same derivatives of the 3 plant species. In the bean shoot 2.9 γ, in the barley shoot 3.65 γ, and in the leaf of *Tradescantia* 1.15 γ 5-OH-indole was formed per gram of fresh plant weight. The hydroxylating ability of the barley was thus the strongest. Comparison of the roots of bean and barley leads to a similar finding.

FENTON-CIER SYSTEM

No.	Colour		Formula	R_f value
1	Purplish-red	Indole		0.81
2	Blue	4-OH-indole		0.53
3	Purple	5-OH-indole		0.46
4	Pink	7-OH-indole		0.38
5	Paris blue	6-OH-indole		0.37

The chloroplast concentrate of the bean leaves produced both derivatives to a greater extent (5-OH-indole 3.8 γ, 6-OH-indole 4.6 γ) than did the plastids of *Tradescantia* (2.45 and 2.65 γ, respectively).

The quantity of the derivative was highest in the bean stem. This may be related with the synthetizing processes of the stem and with the presence of materials being transported in both directions. In the stem, the shoot and the root, 5.4 γ, 2.90 γ and 2.95 γ 5-OH-indole, respectively, were measured.

Both 5- and 6-OH-indoles exhibit high values in the plastid concentrate of the bean. The values obtained for the plastid concentrate may indicate that if the entire cell is considered, then the hydroxylation is more intensive in the plastid. As regards the hydroxylating ability, no difference was found between the shoot and root of barley. In the case of *Tradescantia* it can be observed that the amount of derivatives formed is greater in the plastic concentrate than in the leaf. It is possible that a number of enzymes involved in the final oxidation processes may participate in the formation of the hydroxy derivatives of indole.

Literature

EICH, E. and ROCHELMEYER, H. (1966): Über die präparative Anwendung der radikalischen Hydroxylierung von Indol. Pharm. Acta Helvetiae *41*, 109—123.

MARIÁN, M. (1968): Indolvázas vegyületek ferroaszkorbátos hidroxilálása (Ferrous ascorbate hydroxylation of indole-skeleton compounds). Course work, József Attila University, Szeged.

MARIÁN, M. (1969): *In vitro* nem specifikus hidroxilázok vizsgálata különböző modell vegyületeken (Study of *in vitro* non-specific hydroxylases on various model compounds). Diploma work, Attila József University, Szeged.

MATKOVICS, B., MARIÁN, M. and SIMON, MÁRIA (1972a): Some data on the *in vitro* hydroxylation of anthranilic acid and its biological transformation products. Acta Biologica, Szeged *18*, 135—144.

MATKOVICS, B., SIMON, M., SZŐNYI, D. G. and MÉSZÁROS, H. M. (1972b): *In vitro* hydroxylation, Part 18. changes of the anthranilic acid hydroxylation of bean plants during development. Biochem.-Physiol. Pflanzen *163*, 324—327.

NEMCSÓK, J. (1972): A peroxidáz enzim és hidroxiláló képességének változása bab csíranövényben (Peroxidase and the variation of its hydroxylating ability in bean seedlings). Diploma work, Attila József University, Szeged.

REVIN, A. F. and SMOLYANINOV, V. V. (1971): Isolation, purification and quantitative determination of indole derivatives. Fiziol. Rast. *18*, 642—645.

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Occurrence of *Fusarium* Species in Hungary

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Formal descriptions of the *Fusarium* species found in Hungary are given. Identification was carried out according to the Booth's system. Among the species listed *F. solani*, *F. poae*, *F. sporotrichioides*, *F. fusarioides*, *F. avenaceum*, *F. semitectum*, *F. semitectum* var. *majus*, *F. moniliforme*, *F. moniliforme* var. *subglutinans*, *F. oxysporum*, *F. concolor*, *F. equiseti*, *F. acuminatum*, *F. sambucinum*, *F. culmorum*, *F. heterosporum*, *F. sulphureum* and *F. graminearum* are included. Many of these fungi are recorded for the first time from the territory of Hungary.

During the last five years numerous reports on the economic importance of *Fusaria* have been presented in Hungary. Many *Fusarium* epidemics in different crops have been observed and occurrence of several species was reported. Studying the stalk and the cob rot of maize BÉKÉSI and HINFNER (1970) stated that the main pathogens are *F. semitectum*, *F. culmorum*, *F. acuminatum*, *F. graminearum* and *F. oxysporum*. During the investigation of the *Fusarium* flora of the ear and the grain of wheat BÉKÉSI and HINFNER (1971) isolated additional species, too. These were identified as *F. solani*, *F. avenaceum*, *F. equiseti* and *F. moniliforme*. According to MESTERHÁZY (1974) 14 *Fusarium* species are associated with the wheat and the triticale: two of them, *F. culmorum* and *F. graminearum* are predominant. Studies on the etiology of alfalfa wilt in Hungary led to a conclusion that the pathogens are *F. oxysporum* f. sp. *medicaginis* and *F.* "*roseum*" (MESTERHÁZY and MANNINGER, 1972).

As one can see there are observations on the occurrence of *Fusaria* nevertheless the real presence of these species in Hungary remains quite uncertain, because the workers cited did not confirm their statements by giving a formal mycological description or the descriptions are either incorrect or obsolete. In a recent review VÖRÖS and LÉRÁNTH (1974) listed the Hungarian *Fusarium* species. From their paper it becomes evident that only a few valid records on *Fusaria* are available and for the most part these date back to more than thirty years. Such old informations always rise to criticism especially in this genus where different taxonomic conceptions have coexisted.

For this reason the aim of this article is to give the formal description of *Fusarium* species recently found in Hungary. Former records without correct characterization will not be cited here.

Materials and Methods

Isolations were made in part at this laboratory and cultures were also received from different institutions of Hungary. Identification was carried out according to BOOTH (1971) using potato-dextrose agar and soil extract agar as culture media. Growth rate is given as the average diameter of the colony attained by a series of some mycelia settled on a small piece of water agar. Measurement was made after four days' growth on potato-dextrose agar at room temperature and at normal laboratory light. Strains are maintained under sterilized paraffine oil.

Identified species

Fusarium solani (Martius) Saccardo

Growth rate 3.0–3.5 cm. Aerial mycelium greyish-white or cream-coloured, striate and in some cases sparse. Agar develops a blue, bluish-green or light-brown discoloration. Pionnotial-like sporodochia blue or beige. Conidiophores elongated, branched, phialides measure $40-60 \times 2.5-3.0\ \mu$. Microconidia generally abundant. They are oval measuring $10-14 \times 3-5\ \mu$. Macroconidia have a somewhat rounded foot cell, 3–5 septate and measure $30-55 \times 4-6\ \mu$. Chlamydospores both smooth- and rough-walled are generally abundant and are both terminal and intercalary, $10\ \mu$ diam. (Fig. 1A).

Isolated from soil, from wheat (rhizosphere, stem and leaf), from alfalfa (rhizosphere and root), from onion (bulb), from hop (root).

Former record from Hungary (cf. VÖRÖS and LÉRÁNTH, 1974): UBRIZSY G. (1941).

Fusarium poae (Peck) Wollenweber

Growth rate 4.5 cm. Cultures white to carmine red, a carmine pigment diffuses into the agar. Microconidia are formed from doliiform phialides, $8-12 \times 3-5\ \mu$ with an apical collar. These conidia are lemon-shaped, $8-10\ \mu$ diam. Some strains produce a pionnotial-like sporodochia with orange-coloured masses of macroconidia wich are uniform, 3 septate, $30-40 \times 4\ \mu$. Chlamydospore production is sparse. They are, if present, smooth walled, globose, $10-15\ \mu$ diam. (Fig. 1B).

Isolated from wheat (stem and leaf), from alfalfa (root), from onion (bulb). No former record.

Fusarium sporotrichioides Sherbakoff

Growth rate 4.0 cm. Superficial mycelium honey-coloured from below red to reddish-brown. In most cases the agar remains colourless but sometimes a red pigment diffuses into the media. Conidia are formed from polyphialides which

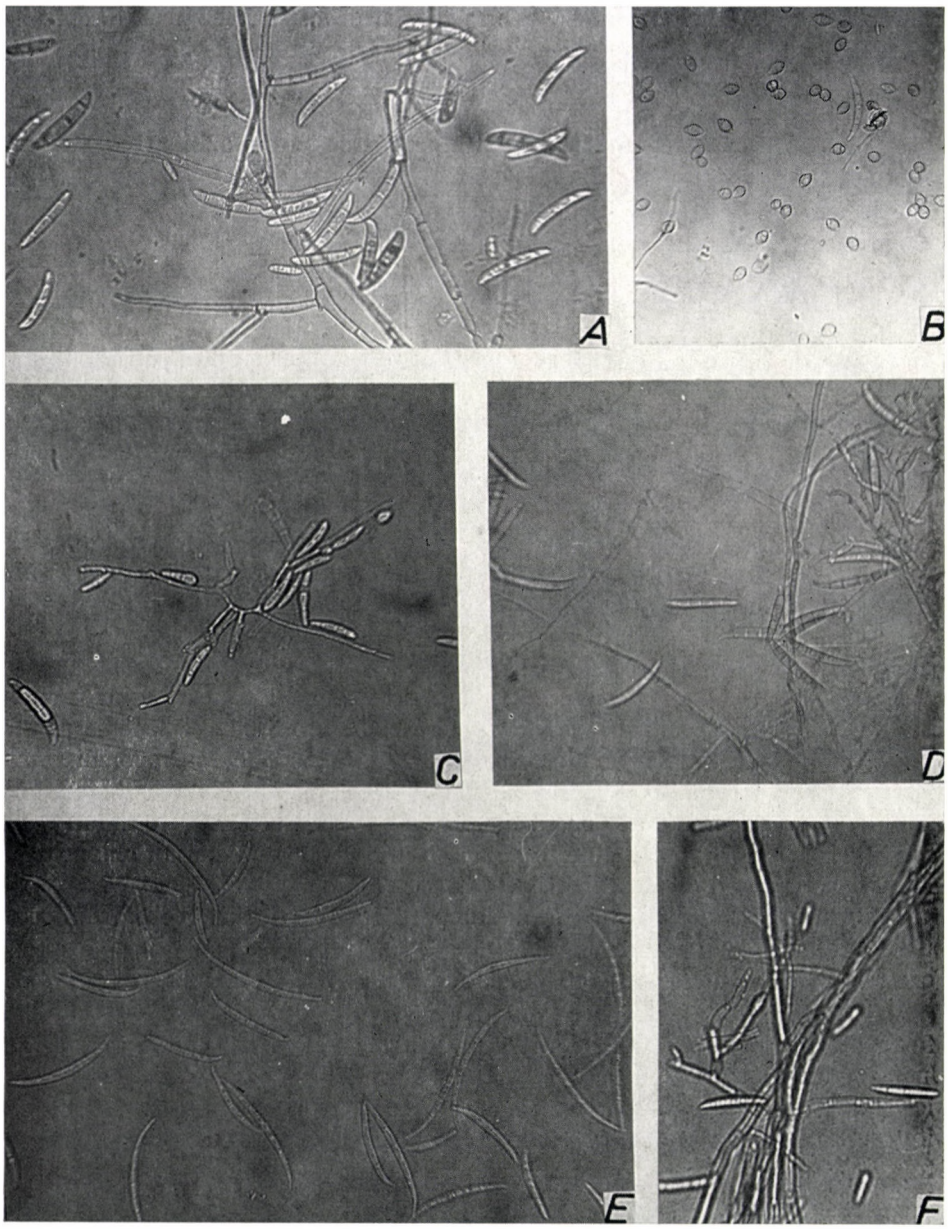

Fig 1. A, *F. solani*, macroconidia, microconidia, phialides; B, *F. poae*, macroconidium, microconidia; C, *F. sporotrichioides*, macroconidia, microconidium, polyphialides; D, *F. fusarioides*, macroconidia, polyphialides; E, *F. avenaceum*, macroconidia; F, *F. semitectum*, macroconidia, polyphialide. (×400)

measure 15×3 μ. Microconidia pyriform to clavate often becoming 1 septate, $8-10 \times 3-5$ μ. Macroconidia are fusoid, with an apedicellate foot cell, 3–5 septate, $25-45 \times 3.5-5$ μ. Globose chlamydospores 10–15 μ diam. with smooth walls are formed singly or in short chains (Fig. 1C).

Isolated from wheat (rhizosphere, leaf and grain), from alfalfa (root), from fodder mixture.

No former record.

Fusarium fusarioides (Fragoso et Ciferri) Booth

Growth rate 4.5 cm. Cultures typically rose to carmine red, aerial mycelium reddish-brown with a powdery appearance due to the formation of conidia. Agar develops a dark red discoloration. Microconidia are narrowly clavate to fusiform, relatively sparse, 0–1 septate, $10-15 \times 4$ μ. They are formed from polyphialides, which measure 20×3 μ. Macroconidia are curved, fusoid with a pointed apex and well-developed foot cell, 5 septate, $40 \times 5-6$ μ. Chlamydospores are abundant, globose, smooth-walled 20 μ diam. (Fig. 1D)

Isolated from wheat.

No former record.

Fusarium avenaceum (Corda ex Fries) Saccardo

Growth rate 5.0 cm. Aerial mycelium red to white. The agar becomes reddish-brown. In young cultures very few polyphialides are produced together with a great number of simple phialides, measuring $20-25 \times 3$ μ. Macroconidia are sparse and heterogenous in the aerial mycelium. They are more abundantly formed in orange-coloured pionnotes. These conidia are very characteristic to the *F. avenaceum*, range 3–7 septate, $40-70 \times 3.5$ μ. Neither microconidia nor chlamydospores have been observed (Fig. 1E).

Isolated from wheat (leaf), from alfalfa (root), from maize (cob), from pea (stem), from fodder mixture.

Former records from Hungary (cf. VÖRÖS and LÉRÁNTH, 1974): UBRIZSY G. (1941)

MOESZ G. (1916) as: *Fusarium corallinum* Corda
UBRIZSY G. (1941) as: *Fusarium herbarum* (Corda) Fries
MOESZ G. (1942) as: *Fusarium viticolum* Thümen
MOESZ G. (manuscript) as: *Fusarium aecidii tussilaginis* Allescher
MOESZ G. (manuscript) as: *Fusarium graminum* Corda
MOESZ G. (manuscript) as: *Fusarium zeae* (Westergreen) Saccardo

Fusarium semitectum Berkeley et Ravaz

Growth rate 6.0 cm. Superficial mycelium peach-coloured and assumes a powdery appearance with the formation of conidia. Agar remains colourless.

Conidiogenous cells are polyphialides, $20 \times 3.5\ \mu$. Macroconidia are curved with an apedicellated foot cell and pointed apex. They vary from 3 to 5 septate and measure $25-40 \times 3-4\ \mu$. Chlamydospores formed singly or in chains. They are globose, intercalary, $10\ \mu$ diam. (Fig. 1F).

Isolated from wheat (rhizosphere and grain), from alfalfa (rhizosphere), from peppermint (rhizosphere).

No former record.

Fusarium semitectum var. majus Wollenweber

Cultures are similar in their appearance to those of *F. semitectum* except macroconidia are larger. They measure $35-55 \times 3-6\ \mu$.

Isolated from wheat (rhizosphere).

No former record.

Fusarium moniliforme Sheldon

Growth rate 4.5 cm. Aerial mycelium dark violet or cream-coloured. Microconidia formed in chains are oval, $10 \times 3\ \mu$. Although occasionally some conidiogenous cells resemble polyphialides most of them are characteristic simple phialides, measuring $20-30 \times 2.5\ \mu$. Macroconidia are inequilaterally fusiform with 3–5 septa, $40 \times 3.5\ \mu$. Chlamydospores were not observed (Figs 2A, 2B).

Isolated from wheat (leaf and ear).

Former record from Hungary (cf. VÖRÖS and LÉRÁNTH, 1974):

KASZONYI S. (1964) as: *Fusarium moniliforme* Sheldon var. *minus* Wollenweber.

Fusarium moniliforme var. subglutinans Wollenweber et Reinking

Cultural characteristics of this variety are similar to those of *F. moniliforme* except simple phialides are replaced by polyphialides which measure $15-20 \times 3-4\ \mu$. Microconidia examined in situ appear to form heads.

Isolated from maize (cob), from wheat (leaf and grain).

No former record.

Fusarium oxysporum Schlechtendahl

Growth rate 4.5 cm. Mycelium peach-coloured to white, from below usually tinged with a violaceous colour. This pigment diffuses into the agar. Microconidia borne on short phialides are oval, $6-15 \times 2.5-3.5\ \mu$. Macroconidia are fusoid with a pedicellated foot cell, 3–5 septate, $30-40 \times 3.5-4\ \mu$. Chlamydospores are globose, mainly rough-walled, $8-10\ \mu$ diam. They are formed terminally on short lateral branches or are intercalary (Fig. 2C).

Isolated from wheat (rhizosphere, stem and leaf), from alfalfa (root), from onion (bulb), from maize (grain), from potato (tuber).

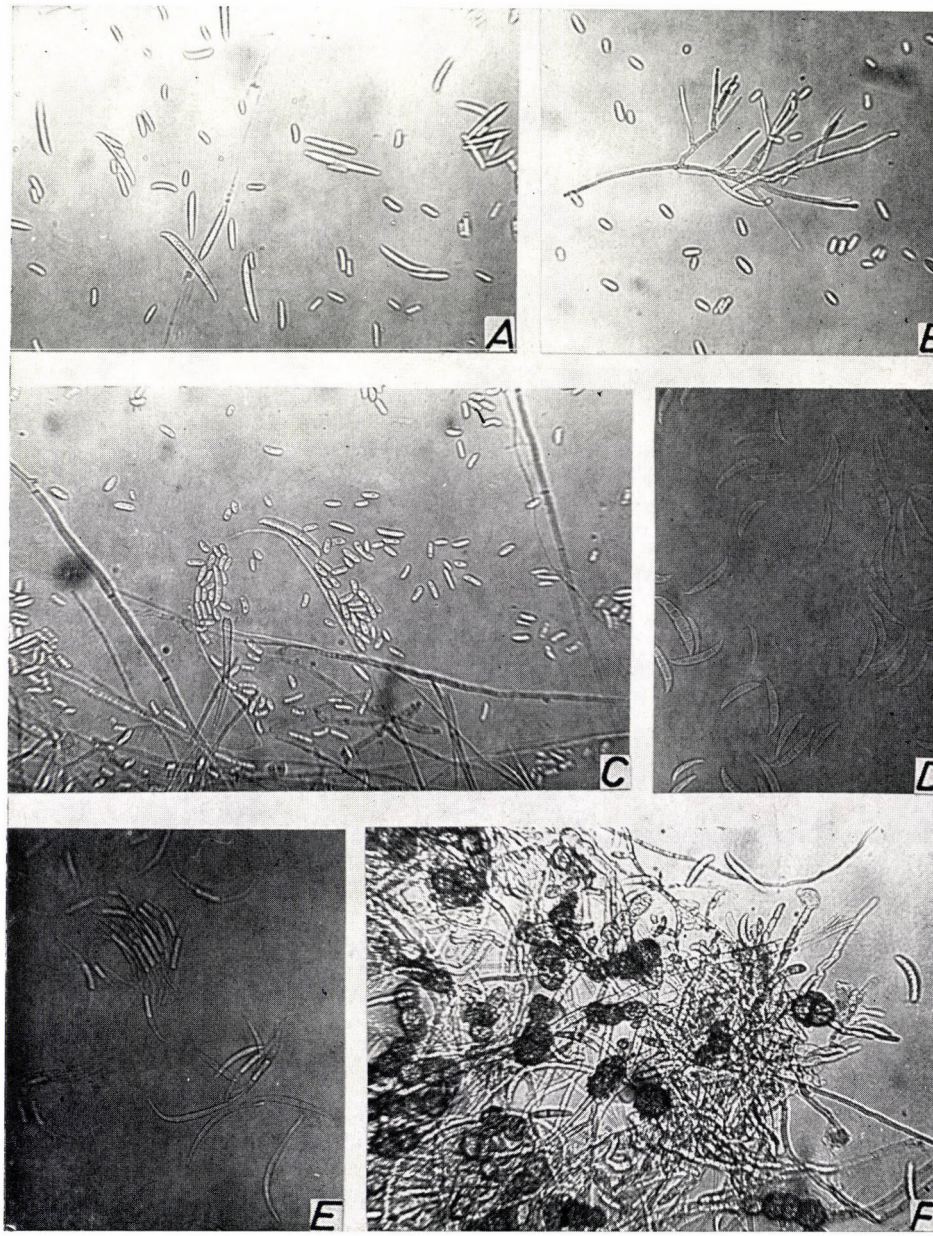

Fig. 2. A, *F. moniliforme*, macroconidia, microconidia; B, *F. moniliforme*, phialides, microconidia; C, *F. oxysporum*, macroconidium, microconidia, phialides; D, *F. concolor*, macroconidia, phialides; E, *F. equiseti*, macroconidia from pionnotes; F, *F. equiseti*, macroconidia, phialides, chlamydospores. (×400)

Former records from Hungary (cf. VÖRÖS and LÉRÁNTH, 1974):*
HOLLÓS L. (1913) HOLLÓS L. (1933) as: *Fusarium aurantiacum* (Link) Saccardo.

Fusarium concolor Reinking

Growth rate 5.0 cm. Culture peach to light brown. In our strains a tendency towards pionnote formation was repeatedly observed. Phialides measure $10-12\times 3-3.5\ \mu$. Conidia are fusoid with a pointed apical cell and well-developed foot cell, 3–5 septate, $25-35\times 4.5-5.5\ \mu$. Chlamydospores are smooth-walled, intercalary with an average diameter of $10\ \mu$ (Fig. 2D).

Isolated from potato (tuber).
No former record.

Fusarium equiseti (Corda) Saccardo

Growth rate 5.5 cm. Cultures are white to beige, from below become brown. Agar turns light brown with age. Phialides are simple and measure $10-15\times 3\ \mu$. Macroconidia formed in the aerial mycelium are heterogenous, without an elongated apical cell, 3–5 septate, $30-50\times 3.5-5\ \mu$. Macroconidia from pionnote or effuse sporodochia are falcate with a well-marked pedicellated basal cell and a curved elongation of the apical cell, 3–6 setate, $40-60\times 5\ \mu$. Chlamydospores dark-coloured, rough-walled, globose, $10\ \mu$ in diameter, abudant, in chains or in knots (Figs 2E, 2F).

Isolated form wheat (rhizosphere, stem, leaf and grain), from alfalfa (rhizosphere), form barley (rhizosphere).
No former record.

Fusarium acuminatum Ellis et Everhardt

Growth rate 4.5 cm, cultures carmine red, aerial mycelium white with a reddish-brown tinge. A dark red pigment diffuses into the media. Conidiophores show reach proliferation, phialides measure $10-15\times 3-4\ \mu$. Macroconidia are falcate, strongly dorsi-ventral, 3–6 septate, $35-50\times 4\ \mu$. Chlamydospores sparse, intercalary, smooth-walled, oval, $15-20\ \mu$ diam. (Figs 3A, 3B).

Isolated from wheat (rhizosphere, stem, leaf and grain), from alfalfa (rhizosphere).
No former record.

Fusarium sambucinum Fuckel

Growth rate 5.0 cm. Aerial mycelium white to rose. A light red pigmentation develops on the surface of the agar and diffuses into the medium. In the aerial

* Since 1913 many formae speciales of *F. oxysporum* have been described from the territory of Hungary. As the purpose of this article is to summarize only species and varieties of the genus *Fusarium*, formae speciales are not listed here.

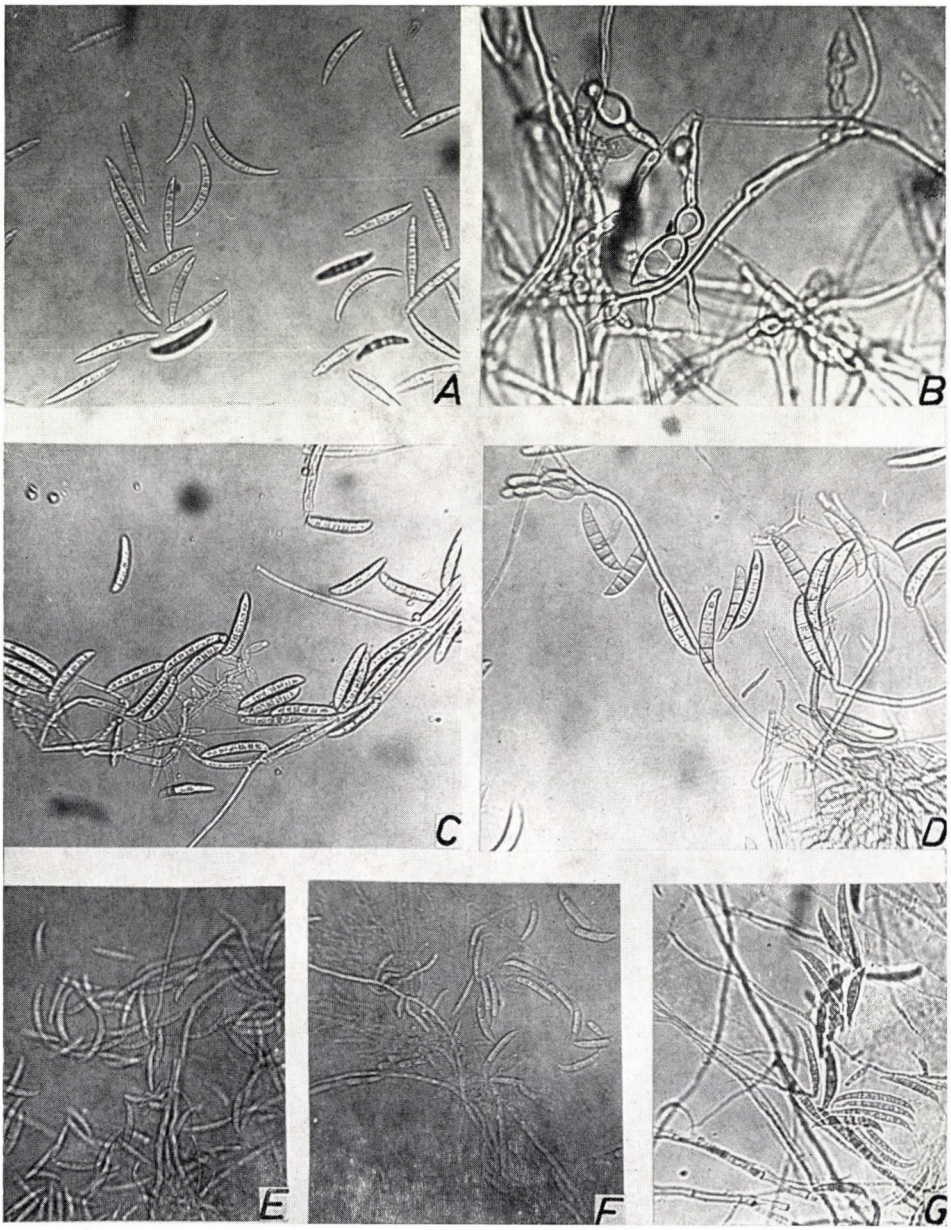

Fig. 3. A, *F. acuminatum*, macroconidia; B, *F. acuminatum*, chlamydospores; C, *F. sambucinum*, macroconidia, phialides; D, *F. culmorum*, macroconidia, phialides; E, *F. heterosporum*, macroconidia, phialides; F, *F. sulphureum*, macroconidia, phialides; G, *F. graminearum*, macroconidia, phialides. (×400)

mycelium macroconidia are formed sparsely from cylindrical phialides which measure $10-18 \times 3-5\ \mu$. These conidia are variable in their form and size. Conidia produced in sporodochia are typical, fusoid, 3–5 septate, $40-45 \times 5\ \mu$. Chlamydospores are formed sparsely as single cells, in chains or in clumps. They are globose, smooth-walled and measure $10-15\ \mu$ diam. (Fig. 3C).

Isolated from wheat (leaves).

Former records from Hungary (cf. VÖRÖS and LÉRÁNTH, 1974):

MOESZ G. (1909)
HOLLÓS L. (1913)
HOLLÓS L. (1933)
MOESZ G. (1942)
HOLLÓS L. (1913) as: *Fusarium sclerodermatis* Oudemans
HOLLÓS L. (1933) as: *Fusarium sarcochroum* (Dezmaziéres) Saccardo
MOESZ G. (manuscript) as: *Fusarium sclerodematis* Oudemans

Fusarium culmorum (W. G. Smith) Saccardo

Growth rate is greater than 8.0 cm. Cultures are carmine red to rose with a yellow discoloration around the point of inoculation. Agar becomes reddish-brown with the age. Within a few days brown pionnotial-like sporodochia begin to develop on the surface of the cultures. Phialides are cylindrical, $10-20 \times 4-5\ \mu$. Macroconidia are uniform, not strongly curved, with a beaked apical cell, 3–5 septate, $30-50 \times 5.5-6\ \mu$. Chlamydospores are both terminal and intercalary, solitary or in chains, smooth- to rough-walled, $10-15\ \mu$ diam. (Fig. 3D).

Isolated from wheat (rhizosphere and leaf), from alfalfa (rhizosphere), from potato (tuber).

Former records from Hungary (cf. VÖRÖS and LÉRÁNTH, 1974):
KRENNER J. A. (1941)
MOESZ G. (1942)

Fusarium heterosporum Nees ex Fries

Growth rate 4.5 cm. Cultures pale-rose to white. Agar develops a yellowish discoloration. After a week orange-coloured pionnotes begin to form. In the aerial mycelium conidia form sparsely and show a great variation in form and size. In the pionnotes macroconidia are produced abundantly from short ellipsoidal phialides, $10 \times 3\ \mu$. These conidia are 3 septate, $25-35 \times 3.0-3.5\ \mu$. Instead of chlamydospores only swollen, thick walled hyphal cells were observed. (Fig. 3E).

Isolated from wheat (leaves).

Former records from Hungary (cf. VÖRÖS and LÉRÁNTH, 1974):
MOESZ G. (1942) UBRIZSY G. (1941) as: *Fusarium heterosporum* Nees ex Fries var. *lolii* (W. G. Smith) Wollenweber

MOESZ G. (manuscript) as: *Fusarium heterosporum* Nees ex Fries var. *lolii* (W. G. Smith) Wollenweber.

Fusarium sulphureum Schlechtendahl

Growth rate 5.0 cm. Cultures pale rose to cream. Aerial mycelium sparse, pionnote sporodochia with orange-coloured masses of macroconidia are present. Phialides with an apical collar measure 12×3.5 μ. Macroconidia are curved, with pointed tips, 3–4 septate, $35 \times 3.5-4.0$ μ. Chlamydospores with smooth wall are solitary and measure 8 μ diam. (Fig. 3F).

Isolated from potato tuber.
No former record.

Fusarium graminearum Schwabe

Growth rate is greater than 8.0 cm. Cultures carmine red to rose with a brownish tinge. A reddish-brown pigment diffuses into the medium. Only macroconidia are formed. These arise from simple, globose phialides, which measure 10×4.5 μ. Macroconidia are variable in their form, but each have an apical cell narrowing gradually, 3–5 septate, $40-70 \times 3.5-5$ μ. Mycelial chlamydospores are rare, they are more frequently found in the conidia and measure 6–8 μ in diameter. (Fig. 3G).

Isolated from wheat (grain), from alfalfa (rhizosphere).
Former record from Hungary (cf. VÖRÖS and LÉRÁNTH, 1974):
HOLLÓS L. (1913) as *Fusarium roseum* Link var. *maydis* Saccardo

Acknowledgements

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Literature

BÉKÉSI, P. és HINFNER, K. (1970): Adatok a kukorica fuzáriumos eredetű megbetegedéseinek ismeretéhez. Növényvédelem *6*, 13–18. (In Hungarian with English summary.)

BÉKÉSI, P. és HINFNER, K. (1971): *Fusarium*-fajok előfordulása őszibúza-kalászokon és az egyes fajták szemtermésén. Növényvédelem *7*, 353–357. (In Hungarian with English summary.)

BOOTH, C. (1971): The genus *Fusarium*. Commonwealth Mycological Institute, Kew.

HOLLÓS, L. (1913): Kecskemét vidékének gombái. Math. és Termtud. Közl. *33*, 149–235.

HOLLÓS, L. (1933): Szekszárd vidékének gombái. Math. és Termtud. Közl. *37*, 1–215.

KASZONYI, S. (1964): Újabb növénykórtani adatok hazánkból. II. Kertészeti és Szőlészeti Főiskola Évkönyve *28*, 145–162.

KRENNER, J. A. (1941): Néhány mikológiai és növénykórtani adat hazánkból. Bot. Közl. *38*, 62–67.

MESTERHÁZY, Á. (1974): *Fusarium* diseases of wheat and triticale in South-East Hungary. Cereal Research Communication *2*, 167—173.
MESTERHÁZY, Á. és MANNINGER, S. (1972): A lucerna fuzáriumos hervadása. Növényvédelem *8*, 260—265. (In Hungarian with English summary.)
MOESZ, G. (1909): Gombák Budapestről és környékéről. Bot. Közl. *8*, 217—237.
MOESZ, G. (1916): Gombák a Száva partjáról. Bot. Közl. *15*, 81—92.
MOESZ, G. (1942): Budapest és környékének gombái. Term. Tud. Társ. Kiadása, Budapest.
MOESZ, G. (manuscript): Meghatározott gombák jegyzéke. — Kézirat nyomán készült cédulakatalógus. Term. Tud. Múzeum Növénytára, Budapest.
UBRIZSY, G. (1941): A Nyírség gombavegetációja. Tisia *4*, 44—91.
VÖRÖS, J. and LÉRÁNTH, J. (1974): Review of the mycoflora of Hungary. XII. *Deuteromycetes: Moniliales* and *Myceliales*. Acta phytopath. Acad. Sci. hung. *9*, 333—361.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 10 (3—4), pp. 359—376 (1975)

Powdery Mildews on Ornamentals in Hungary

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Informations about powdery mildew diseases of 25 ornamental plant species are presented. Of these 19 host–parasite relationships give new data as to Hungary.

Besides some references – in mycological papers – the powdery mildews of ornamental plants were published the first time by UBRIZSY (1970, 1972) in Hungary.

It is since 1969 that we keep collecting and observing ornamental plants infected by powdery mildews. This activity embraced first of all the perennial plants, but powdery mildews were collected from potted plants, trees or shrubs as well. Systematic collection was started in 1974, in the largest parks of Budapest. The collected material can be seen in the herbarium of the Research Institute for Plant Protection.

The determination of plants was performed on the basis of the work of SOÓ and JÁVORKA (1959), as well as JÁVORKA and CSAPODY (1962); the identification of pathogens by the work of BLUMER (1967). The occurrence up to now of the pathogens on host plants are displayed on home relation by the work of UBRIZSY (1968), as to world relation on the basis of the data of HIRATA (1966).

Data up till now worked up from the collected material are presented in an alphabethic order of host plants. The host–parasite relationships marked by * mean new data for Hungary.

* A c h i l l e a p t a r m i c a L. (Compositae)
Erysiphe cichoracearum DC. ex MÉRAT emend. SALMON

HIRATA (1966) mentions pathogens of several European countries, among others from the neighbouring Rumania, telling as pathogen *Erysiphe cichoracearum*. From the USA an *Oidium* species was informed about; while from France, Rumania and USSR *Léveillula taurica* ARN. is known.

On the garden variety of A. p t a r m i c a (cv. 'Plena') the powdery mildew causes a slight cover on the leaves (Budapest, Rózsadomb, 2. 7. 1973; Tata, 1973, 1974 summer). On the basis of the born, form, and germination of conidia the pathogen was determined as *E. cichoracearum*. Cleistothecia were not found.

The perfect form of *E. cichoracearum* was found by UBRIZSY and VÖRÖS (1966) on some other wild Achillea spp. (A. collina, A. millefolium).

Aquilegia vulgaris L. (Ranunculaceae)
Erysiphe aquilegiae DC. ex MÉRAT

Aquilegia vulgaris – as other Aquilegia spp. and hybrids – gives a preferred ornamental plant of perennial beds. It is attacked by powdery mildew in the form of a slight covering and it was published on the name *Erysiphe aquilegiae* by MOESZ (1939) from several parts of the Carpathian basin, in general from areas outward of our present borders, while by UBRIZSY (1941) from Debrecen. UBRIZSY (in litt.) found even its perfect form in Budapest, Mechwart tér (29. 10. 1971) on Aquilegia sp. cult. BLUMER (1967) and HIRATA (1966) reported *E. aquilegiae* (syn. *E. communis* GREV.) from different parts of the world.

Conidial form of *E. aquilegiae* was found on the Gellérthegy in Budapest (7. 8. 1974; conidia: $36.74 \times 15.06\ \mu$).

Aster spp. (Compositae)
Erysiphe cichoracearum DC. ex MÉRAT emend. SALMON

Many spp. and varieties are known, which are often placed to parks, perennial beds or rockeries. They are characteristic ornamental plants of the autumn, with blue or violet flowers. There is a strong powdery mildew covering (Euoidium) found on different varieties, the sexual state of which is *Erysiphe cichoracearum* (BLUMER, 1967).

HIRATA (1966) published practically from every part of the world *E. cichoracearum*, in some cases *Oidium* sp.; *Sphaerotheca fuliginea* and *Uncinula asteris* from Japan on Aster ageratoides TURCZ. ssp. ovatus NAKAI, again *S. fuliginea* from Canada on A. puniceus L.

In Hungary UBRIZSY and VÖRÖS (1966) described conidial and perithecial state of *E. cichoracearum* on A. novi-angliae L. and A. novi-belgii L. (Szarvas, Balatonakarattya).

Own observations prove powdery mildew a rather widespread disease of most Asters. Almost all over the year one or other form of the fungus can be found on the green parts of the plant. The form of highest occurrence is conidial state (from springtime to autumn) on leaves, stems, sometimes on calices as well (Fig. 1). In the autumn on the white powdery mildew covering some black pin-prick forms will hint to cleistothecia. On the rosette forming in the autumn a characteristic mycelium coating is developing, a so-called secondary mycelium (Fig. 2). This one has a thicker wall than the summer one. Protected by the foliage and snow cover, the leaf rosette – and the mycelium cover developed on it – will survive the cold of the winter (Fig. 3). With the spring coming, the hyphae will start growing and producing conidium chains on the edges of colonies. The earliest that was found sporulating colonies in open field, in a rather protected spot, was on 6 March,

Fig. 1. *Aster novi-belgii* infected by powdery mildew

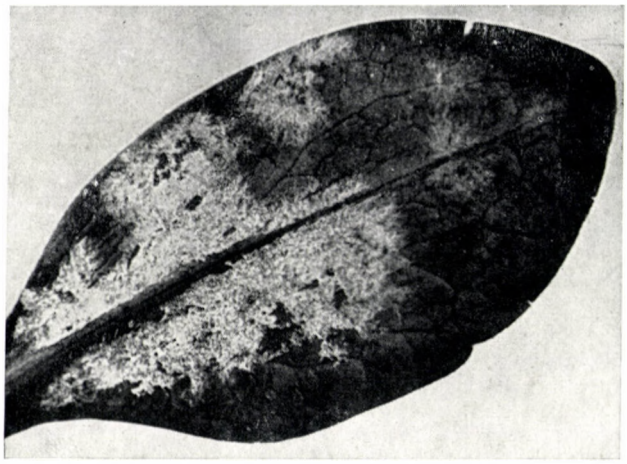

Fig. 2. Overwintering secondary mycelium on the leaf of *Aster* sp.

1972. If, in wintertime such leaves with mycelia get in laboratory, the higher temperature will start a growth of hyphae and a formation of conidia on the edges of colonies. This mycelial state plays the main role in overwintering of pathogen, so in the start of primary infection respectively.

Fig. 3. Overwintered leaf under snow and foliage of *Aster* sp. infected by powdery mildew

Conidium-form of the pathogen could be found on several Aster spp. The conidia develop in long chains. There are 4–5 conidia born per day: the air motion makes them separated and getting on plants so causing new infections. In places without any wind some chains of 16–20 conidia may be found. But the conidia on the end of chain, that is the oldest ones are shrivelled, unable of germination. Conidia are cylindrical, with sizes 33.24×15.24; L/W = 2.25; by BLUMER (1967) it is proved 37×18 μ. They have simple germ tubes, starting from one corner of the conidia. Appressorium club-shaped.

In cases the host plants could not be properly identified; these are presented with the name *Aster* sp. herewith (Tata, 30. 5. 1971; Budapest, Rózsadomb 26. 7. 1972; Városliget, 10. 9. 1974; Gellérthegy, 29. 9. 1974; conidia 41.46×15.24 μ; L/W = 2.22).

* A s t e r d u m o s u s L. was found at Tata (9. 7. 1972) and in Budapest, in the Tabán (14. 8. 1974) (conid 31.42×14.19 μ; L/W = 2.21).

A s t e r n o v i- b e l g i i L. with glossy leaves, coming form America is a very good host to powdery mildew (Tata, 5. 6. 1971; Budapest, Rózsadomb, 16. 6. 1971; Tata, 10. 6. 1973); on the contrary, on A. n o v a e - a n g l i a e L., coming also from America, with very hairy leaves, powdery mildew could not be found. Though its cleistothecia were found by UBRIZSY (1966). SZ. NAGY (1972) inoculation attempts seemed to prove the species' resistance to *E. cichoracearum*. Inoculation tests with leaf disc afforded the result that on the leaf discs of A. n o v a e - a n g l i a e the developing of conidia was slower to start, there was only a colonia of 1–2 mm of diameters to form on the discs with 1–2 conidium chains, while the leaf-discs of A. d u m o s u s and A. n o v i- b e l g i i were

densely covered (in 8–10 days) by a colony of powdery mildew consisting of conidium chains and mycelia.

Both sexual and asexual states of the fungus was found on every green part of *A. rotundus (Garden of the University of Horticulture, Budapest, 26. 8. 1974). Cleistothecia were plenty, with 5–15 (6–8) ascospores in each. As a rule, there are 2, seldom 1 or 3 ascospores in a single ascus. It is worth mentioning as something interesting, that in this very year, in the perennial bed next to above ones, no cleistothecia formed on A. dumosus.

Perfect form was discovered as well on the slope of Gellérthegy (29. 9. 1974) on a stock of Aster sp., but developed rather scattered, with cleistothecia of 120 μ in diameter as an average. In the mycelia and conidia of *E. cichoracearum* hyperparasitic pycnidial fungus *Cicinnobolus cesatii* DE BARY could be found.

This is the first paper about the powdery mildew of *Aster dumosus* and A. rotundus in Hungary. HIRATA (1966) does not give any mention of A. rotundus either.

Begonia rex PUTZ. (Begoniaceae)
Oidium begoniae PUTT.

Ornamental plant of leaves, rather preferred; often attacked – together with other Begonia varieties – by powdery mildew. Its conidial state has been known in Europe for about 50 years (BLUMER, 1967), denominated *Oidium begoniae* PUTT. Size of conidia: $20-36 \times 13-17$ μ. HAMMARLUND (1945) ranged this powdery mildew to *Erysiphe polyphaga*, but this name is not valid (SZ. NAGY, 1975). Perfect form was found by SIVANESAN (1971) and ELIADE (1972) describing it as *Microsphaera begoniae* sp. nov. and *M. tarnavschii* sp. nov., respectively.

UBRIZSY and VÖRÖS (1966) published on the basis of the PODHRADSZKY collection (Budapest, 20. 11. 1960) as a new observation by the name of *Oidium begoniae* from Begonia rex.

At the working of diploma for dissertation, there were found rather considerable damages on plants Begonia rex in the Agricultural Cooperative "Március 15.", Budapest (Fig. 4).

Conidia on the leaves of Begonia rex Pesthidegkút (17. 5. 1972) are born in short chains (2–3 conidia each). Sizes 50.60×16.63 μ; L/W = 3.04. This may afford a basis to range to *Oidium begoniae* var. *macrosporum* (conidia $38-64 \times 14-22$ μ) described by DE MENDOÇA and DE SEQUEIRA (1963 ap. BLUMER, 1967). Conidia are without fibrosine bodies. Germ tube starting from a corner of conidium. Appressoria lobated, branching.

Campanula rapunculoides L. (Campanulaceae)
Oidium sp.

A perennial plant, indigenous in Hungary, blooming in summertime: it is rather preferred to plant in gardens. Often attacked by powdery mildew, like other Campanula spp. In our country only its form having conidium is known

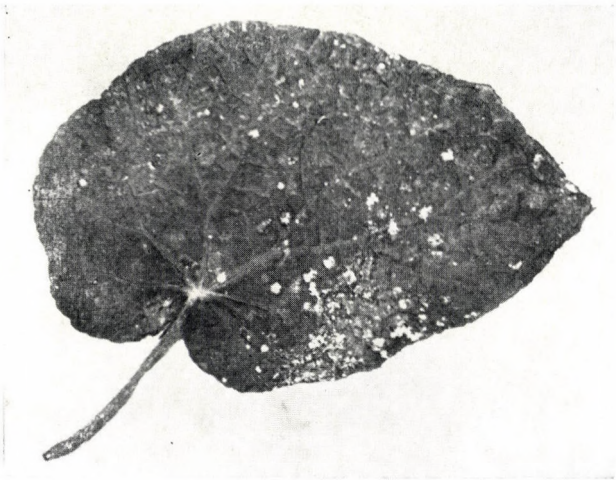

Fig. 4. Oidium colonies on leaf of *Begonia rex*

(Euoidium), by BLUMER (1967) likely to belong to *E. cichoracearum*. HIRATA (1966) published it under the name *E. cichoracearum* from Rumania and Lithuania; from Denmark, Germany, Norway and Armenia as *E. communis;* from Finland, Poland, Switzerland and Hungary as *Oidium* sp. He gives information about other Campanula spp., having the same pathogens.

UBRIZSY (in litt.) found *Oidium* on C. glomerata, in Budapest (Gugger-hegy, 27. 8. 1966.)

There were two collections made in Budapest (Rózsadomb, 18. 6. 1974., Gellérthegy, 7. 8. 1974.) for C. rapunculoides plants infected with powdery mildew. The coating of powdery mildew on the leaves or stems of the plant seems rather scarce. Conidia form in chains (2–4 conidia), are elliptical, having a size of $27.14 \times 13.88\ \mu$; L/W = 1.98.

*Chrysanthemum × hortorum BAILEY,
*Ch. carinatum L. (Compositae)
Oidium chrysanthemi RABENH.

Many species of Chrysanthemi are growing wild, a large number of species and varieties of it are grown in gardens or glasshouses. One the most commonly known species of it may be Chrysanthemum × hortorum BAILEY (syn.: Ch. indicum L.) produced by the cross-breeding of several spp. Its powdery mildew was described by RABENHORST in 1853 (BLUMER, 1967) as *Oidium chrysanthemi.* HIRATA (1966) reported it from different Chrysanthemum spp. most often as *Oidium* sp., *Oidium chrysanthemi*, *Erysiphe cichoracearum*, *E. polyphaga* from very different parts of the world; once he mentions *Sphaerotheca fuliginea* (on Ch. carinatum L., Rumania) and *Léveillula taurica* (on Ch. cineriaefolium (TREV.) VIS., Greece and Italy).

From Chrysanthemum vulgare (L.) BERNH. was reported by MOESZ (1939), later by UBRIZSY and VÖRÖS (1966) from Szarvas, and from Budapest (Jánoshegy) respectively, under the name *E. cichoracearum* DC. emend. SALMON; its perfect form was found both in Budapest and at Pomáz.

Its conidial state was found on different varieties of Ch. × hortorum BAILEY (syn.: Ch. indicum L.) at Tata (24. 10. 1974., 29. 7. 1973., 23. 9. 1973., 1. 9. 1974), at Szomód (7. 10. 1973). The powdery mildew appears in late summer, but even more in the autumn on the leaves. Development of conidia is generally scarce, they are longish (37.93 × 15.89 μ; L/W = 2.38), without fibrosine bodies, developing in short chains; they have simple germ tubes, longish, to start from one corner of the conidia. On Ch. carinatum L. (Tata, 29. 7. 1973) there could be observed beside this some unripe cleistothecia in yellow; but these ones, in lack of ripe asci, were unfit for identification. *Cicinnobolus cesatii* DE BARY was found in the hyphae.

This is the very first home report about the powdery mildew of Chrysanthemum × hortorum and Ch. carinatum. Its conidial form may be classified to Pseudoidium; germ tube of Erysiphe type; in spite of it, on the basis of way of born and the size of conidia, it cannot be ranged to *E. cichoracearum*. Denomination *Oidium chrysanthemi* RABENH. seems much more fit. A developing of perfect state may promise a help for a correct identification.

*Cucurbita spp.,
*Lagenaria siceraria (MOLINA) STANDLEY (Cucurbitaceae)
Erysiphe cichoracearum DC. ex MÉRAT emend. SALMON
Sphaerotheca fuliginea (SCHLECHT. ex FR.) POLLACCI

In the recent years quite a fashion developed as to different forms of calabash gourds and pumpkins; their fruit serves to decorate flats in wintertime. The most known of them are Lagenaria siceraria and varieties of C. pepo and Cucurbita maxima. The fruits of C. maxima DUTCH convar. turbaniformis (ROEM.) ALEF. may reach a diameter of 20–25 cm, its hat remembering a turban is cinnober red and covers the ventral part of yellowish white. Of the varieties over a hundred in number of C. pepo L. the most preferred are convar. verrucosa ALEF., the pearform, convar. pyriformis (ROEM.) ALEF. and the orangeform one, convar. pomiformis ALEF.

The green parts of them are often covered by powdery mildew, causing – at serious cases – a full defoliation (Figs 5, 6.). In Hungary, there are two pathogens of them known, like with other cucurbits (cucumber, melon, watermelon, vegetable marrow): *Erysiphe cichoracearum*, *Sphaerotheca fuliginea* (SZ. NAGY, 1970).

The symptoms caused by both of the pathogens are identical: morphological differences can be found only in the form of conidia, in the presence of fibrosine bodies, in the way of germination as well as in the number of asci developing in one cleistothecium.

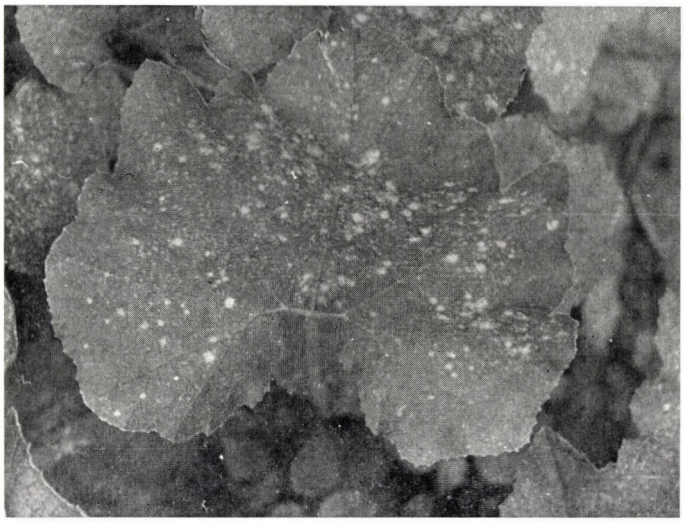

Fig. 5. Powdery mildew on leaf of *Cucurbita maxima* var. *turbaniformis*

Fig. 6. Powdery mildew on leaf of *Cucurbita pepo* var. *pyriformis*

E. cichoracearum has conidia cylindrical, with sides almost parallel, sized $29.98 \times 15.05\ \mu$; L/W = 2.00. There are no fibrosine bodies inside, have simple germ tubes, starting from one corner of the conidia.

Conidia of *S. fuliginea* are almost regularly elliptical, more "pouched" compared to the other species, with a size of $30.66 \times 18.63\ \mu$; L/W = 1.65; they

have stick or disc-like fibrosine bodies found inside. The germ tubes start from the ventral part of conidia, mostly simple, some crooked but rather often forked. There is a single 8 spores ascus developing in the cleistothecium.

There was a continuous possibility to observe occurrence of both the pathogens since 1969 in Julia Major, Budapest, at Tata, and at Tiszavárkony.

*Dahlia pinnata CAV. (Compositae)
Erysiphe cichoracearum DC. ex MÉRAT emend. SALMON

Much liked flower of parks and gardens is dahlia: cut it is much favoured in vases. By BLUMER (1967) its powdery mildew may be either *Erysiphe cichoracearum* or *E. polyphaga*. HIRATA (1966) reported powdery mildew of Dahlia spp. as *E. cichoracearum*, *E. polyphaga*, *E. communis*, *Sphaerotheca fuliginea*, *Oidium ambrosiae* and *Oidium* sp. from very different parts of the world. There is no information known about occurrence in Hungary.

Conidium form was found at Szomód (22. 9. 1974) on the bottom and middle leaves as well as on the sprouts of a cactus-dahlia of flowers in dark scarlet colour, in forms of separate young colony. The conidia formed in chains, elliptical, sometimes barrel form, as to size $31.22 \times 17.03\ \mu$; L/W = 1.83, without fibrosine bodies. Supposedly the pathogen belongs to *E. cichoracearum*. Neighbouring stocks belonging to other vars. were not infected by powdery mildew.

Delphinium cultorum VOSS. (Ranunculaceae)
Erysiphe ranunculi GREVILLE

It is one of the most showy, most beautiful perennial plants of summer. Its disease caused by powdery mildew is generally known, it greatly spoils the esthetical value of the plant, sometimes causing total loss of foliation. The cause of disease is *Erysiphe ranunculi* (syn.: *E. nitida* (WALLR.) RABENH., *E. polygoni* DC. emend. SALMON) or *Sphaerotheca delphinii* (KARSTEN) BLUMER (syn.: *S. humuli* (DC.) BURR. var. *fuliginea* SALM.) (BLUMER, 1967). *Léveillula taurica* ARN. is also mentioned by HIRATA (1966) from Armenia.

MOESZ (1939) published under name *E. nitida* on Delphinium elatum L., D. intermedium SOL. var. alpinum W. et K. and Delphinium sp. cult.

Erysiphe ranunculi is rather spread in our country both in gardens and parks (Fig. 7). Its cleistothecial and conidial states were collected in Budapest in the Cooperative "Március 15" (1959), on the Gellérthegy (Garden of the University of Horticulture, 6. 7. 1973); in the Tabán (14. 8. 1974) and in the Horváth-kert (10. 9. 1974). The conidia are born by the single or in short chains (Pseudoidium type), are elliptical, $34.31 \times 14.81\ \mu$; L/W = 2.31. Cleistothecia appear in big masses in autumn (Fig. 8), their diameter is $90-110\ \mu$, they have brown appendages, rather scarce. In a cleistothecium there are 5–6 asci, with 5–6 ascospores in each. – Someway *E. ranunculi* may be a rather specialised sp., as the neighbouring *Paeonia* stocks remained quite free of infection in the Horváth-kert.

Fig. 7. Leaf of *Delphinium cultorum* with powdery mildew

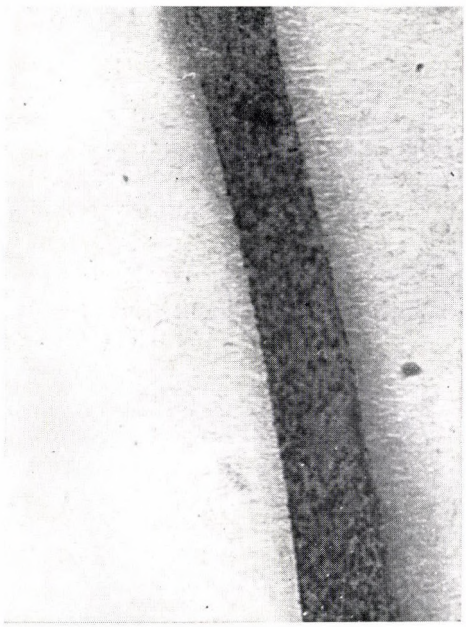

Fig. 8. Cleistothecia thickly covering the petiole of *Delphinium cultorum*

Fig. 9. Ruined inflorescence of *Filipendula ulmaria* (see to the left) and a healthy inflorescence (see to the right)

*Filipendula vulgaris MÖNCH. (syn.: F. hexapetala GILB.),
*F. ulmaria (L.) MAXIM. (Rosaceae)
Sphaerotheca macularis (WALLR. ex FR.) MAGNUS

They are our indigenous plants, the varieties of which are often used as ornamental plants. By BLUMER (1967) there are two powdery mildews on Filipendula spp., the *Sphaerotheca macularis* (WALLROTH ex FR.) MAGNUS causing deformations and the hardly noticeable *Erysiphe ulmariae* DESMAZIÈRES.

In Hungarian literature MOESZ (1939) speaks about *S. macularis* from F. ulmaria on the basis of the Lőcse collection of GRESCHIK, and *E. ulmariae* from F. hexapetala, from his own collection, in Budapest, Farkasrét.

It was the first time in 1971, on the 27 July, that were noticed in a Tata garden, that almost all the flowers of Filipendula ulmaria cv. 'Plena' were deformed (Fig. 9). The inflorescence axis (Fig. 10) and the buds were fully covered with thick, fluorlike white coating, on the leaves powdery mildew spots could be found. Since this these symptoms have been recurring every year, and have been getting always more serious (Tata, 27. 6. 1971, 25. 6. 1972, 10. 6. 1973, 23. 6. 1974). Since 1973 were observed the same but less serious symptoms, on F. vulgaris (Fig. 11), (Budapest, Garden of the University of Horticulture, 2. 6.

Fig. 10. Thick powdery mildew cover on the stem of *Filipendula ulmaria*

Fig. 11. Crooked inflorescence axis of *Filipendula vulgaris (F. hexapetala)*

1973, 23. 4. 1974, Budapest, Gellérthegy, 7. 8. 1974, Garden of the University of Horticulture, 19. 9. 1974, in the company of hyperparasite fungus *Cicinnobolus ceastii* DB.)

On the attacked parts of the plants tiny, but always growing spots in reddish-brown colour started developing to get cracked with time in their length. On the spots white, powdery coats get formed, caused by the mycelia and the mass of conidia. Both the axis of inflorescence and rather often even the main vein of the leaves may get a serious distortion. The way of developing of the deformation is, that practically the spots develop only on one side of the petiole, main vein, or inflorescence axis, here they stop, while on the other side the division of cells goes on.

F. v u l g a r i s displayed only a distortion of green parts: contrary to this at F. u l m a r i a even the inflorescence may get so vigorous infection what will make the flowers so seriously harmed, to prevent them even from opening. The difference above can find an explanation in the different blooming times of the two plant species. That is, F. v u l g a r i s blooms about the end of May or beginning of June, when a possible infection is little. But by the blossoming time of F. u l m a r i a – end of June – the pathogen's propagation may have got to a level to prevent perfectly any development of inflorescence; in case of slighter infection it is only the axis of inflorescence that gets distorted.

Both symptoms observed and microscopic tests allowed the consequence that the powdery mildew collected from both host plants i.e. *Sphaerotheca macularis*, (no information about yet as to coming from F. v u l g a r i s in Hungary and the same coming from *F. ulmaria* with an occurrence – as known – beyond our frontiers).

By the author's tests the inflorescence stem has a rather thick cover of mycelia, so have the peduncle and the buds; besides it, a plentiful development of it can be found on the leaves. Mycelia produce conidium-chains. Conidia are elliptical with some fibrosine bodies in them; conidia are of a size of $31 \times 17.2\ \mu$. Germ tube simple, starting from one side of conidium.

*G e r a n i u m s a n g u i n e u m L. (Geraniaceae)
Sphaerotheca fugax PENZIG et SACCARDO

It is a well-known plant of the forests of oaksand bushes, well supporting both blazing sun and half shadow and is rather liked to plant in perennial beds of parks. Several powdery mildew species are known as its pathogens (BLUMER, 1967): *Sphaerotheca fugax* PENZIG et SACCARDO and *Erysiphe communis* (WALLROTH.) LINK; on other Geranium spp. *S. macularis* (WALLROTH ex FRIES) MAGNUS and *Léveillula taurica* ARN. were found.

HIRATA (1966) reported *E. communis* from G. s a n g u i n e u m (France and Norway), and *S. fugax* (France, Germany, USA and the European part of USSR).

MOESZ (1939) – on the basis of the Lőcse collection of GRESCHIK – reported

S. fugax living on the leaves, stems and fruits (few of perithecia) of Geranium dissectum. Beside this there has not been any report about it from Hungary.

In Budapest parks everywhere the conidial state of *S. fugax* can be met on Geranium sanguineum L. (earliest about end May), and also the cleistothecia and the dirty whitish secondary mycelia characteristic of the species offer the feature to differ it from *S. macularis*. The fungus can be found on leaves, stems, calices and fruit. Conidia develop in chain forms with disc and stick like fibrosine bodies in them, what are of size $29.74 \times 15.90\ \mu$; L/W = 1.86. The secondary mycelium is white with thick walls but without partitioning; in masses they are dirty whitish, to develop only about the end of growth season. Cleistothecia are of a diameter of $100\ \mu$, appendages brown with a length identical to the same of the diameter of cleistothecia. One cleistothecium has one ascus, with 8 ascospores developing in.

Data collected (of this paper) – except one – come from Budapest: Garden of the University of Horticulture (11. 6. 1973), Júlia Major, Nagykovácsi (30. 8. 1973), University of Horticulture (29. 5. 1974), Rózsadomb, Garden of Institute (18. 6. 1974), Gellérthegy (7. 8. 1974), Vérmező (26. 8. 1974) (cleistothecia and secondary mycelia), Horváth-kert (10. 9. 1974) (cleistothecia and secondary mycelia), University of Horticulture (19. 9. 1974), (on the peduncle pycnidia and conidia of hyperparasite fungus *Cicinnobolus cesatii* De Bary were found; pycnidia: $50.31 \times 34.18\ \mu$; conid.: $2 \times 0.67\ \mu$).

*Kalanchoë blossfeldiana Poelln. (Crassulaceae)
Oidium kalanchoeae Lüstn.

Ornamental plant rather liked, grown in pots. In glasshouses it is often attacked by powdery mildew *(Oidium kalanchoeae)*, by Hammarlund (1945) belonging to *Erysiphe polyphaga.*

By Hirata (1966) the powdery mildews of different Kalanchoë species in Australia, Canada, the Netherlands, Norway and the USA by the name *O. kalanchoeae;* from Great Britain, France, Sweden and Switzerland known as *E. polyphaga; S. fuliginea* was found on K. laciniata Dc. in the USA.

On the potted plants coming from Óbuda Cooperative, Budapest, on the leaves and stems conidial state of the pathogen was found (*Oidium kalanchoeae* Lüstn.), what may cause, in case of vigorous infection, a full defoliation of the plant. Conidia are born by single, are cylindrical, $36.58 \times 16.37\ \mu$ of size; L/W = = 2.18, without fibrosine bodies. Their germ tubes start from one corner of the conidia, they are straight, very short in general and often immediately produce the lobated appressoria with multifold branching. The terminology *E. polyphaga* is not valid (Sz. Nagy, 1975), this is why the name *O. kalanchoeae* is correct.

About indigenous occurence there has not been any information as yet.

*M o n a r d a d i d y m a L. (Labiatae)
Oidium sp.

Perennial plant, coming from North America, with glossy leaves and characteristic fragrance. Together with its relative of hairy leaves (M. f i s t u l o s a L.) they give attractive decorations of perennial beds. HIRATA (1966) reports *Erysiphe galeopsidis (E. labiatarum)* from M o n a r d a d i d y m a L. (Armenria, SU); *E. galeopsidis* and *E. cichoracearum* from M. f i s t u l o s a L. and *Oidium sp.* from M. p u n c t a t a (USA); *E. galeopsidis* (Germany), *E. cichoracearum* (USA and Turkmenia) and *Oidium sp.* (USA) from M o n a r d a sp.

BLUMER (1967) does not enumerate the M o n a r d a spp. when giving the list of host plants of powdery mildews. He mentions three *Erysiphe* spp., on other plants belonging to Labiatae family, but their separation is not quite perfect:

1. *Erysiphe biocellata* EHRENBERG
   syn.: *E. galeopsidis* DC. emend. SALMON,
   E. labiatarum (WALLR. ex FR.) CHEV. Germ tube of conidia is sometimes lobated; ascospores are developed only as early as autumn.
2. *E. galeopsidis* DC. ex MÉRAT
   syn.: *E. labiatarum* (WALLR. ex FR.) CHEV. Germ tube of conidia of a decisive lobated form; ascospores forming only in the spring.
3. *E. salviae* (JACZ.) BLUMER
   syn.: *E. cichoracearum* DC. ex MÉRAT emend. SALMON,
   E. galeopsidis DC.,
   E. labiatarum CHEV. f. salviae JACZ. (Only on Salvia spp.) Germ tube of conidia not lobated.

On stem, as well as on adaxial surface and abaxial one of leaves there was found the conidium state of a fungus of powdery mildew (Budapest, Garden of the University of Horticulture, 6. 7. 1973., 26. 8. 1974.). Conidia are forming in chains (3–4 conidia), they are cylindrical without fibrosine bodies; their size is $28.95 \times 16.72\ \mu$; L/W = 1.73. Germ tube starts from a corner of the conidium, mostly simple, longish, with a club-shaped appressorium on the end; but the germ tube is often winting cork-screw like with several club-shaped appressoria on the parts connected with the substratum. Its perfect state has not been met yet.

It belongs to Euoidium type on the basis of conidium formation: considering the sizes of conidia it may be ranged to *E. galeopsidis* (by BLUMER, 1967, $26.40 \times 16.24\ \mu$). At the other two spp. BLUMER presents bigger sizes. Appressoria taken, it can be ranged only to *E. salviae*. Neither branching germ tube, nor lobated appressoria respective have ever been seen, what makes *Oidium* sp. denomination most usable. A finding of perfect state may give some further support for its identification.

This is the first report in Hungary about the Monarda powdery mildew.

Even HIRATA (1966) mentions it from a single European country (Germany), coming from M o n a r d a sp.

*P a e o n i a o f f i c i n a l i s L. (Ranunculaceae)
Erysiphe ranunculi GREVILLE

It is the most showy perennial of garden flowers blooming in spring. Its powdery mildew is caused by *Erysiphe ranunculi* GREV. (syn.: *E. nitida* (WALLR.) RABENH., *E. polygoni* DC. emend. SALMON). MOESZ (1939) informs about it coming P a e o n i a c o r a l l i n a RETZ on the name *E. nitida* (from Nemespodhrágy).

The powdery mildew creates a scarce, hardly noticeable sheathing, grey, powderlike, on the leaves. Cleistothecia rather early of forming (Alsóörs, 9. 6. 1973). Some further localities Budapest, Garden of the University of Horticulture, 30. 9. 1973, Tata, 7. 10. 1973, 1. 9. 1974, Budapest, Gellérthegy, 29. 9. 1974. Formation of conidia is in the single or in short chain; 28.28×14.03 μ as to size L/W = 2,01. There is scarce, mycelium-like appendages on the cleistothecia; 1–3 asci develop in one cleistothecium with 4–5 ascospores forming in each.

P h l o x p a n i c u l a t a L. (Polemoniaceae)
Erysiphe cichoracearum DC. ex MÉRAT emend. SALMON
**Sphaerotheca fuliginea* (SCHLECHT. ex FR.) POLLACCI

It is one of the generally favoured flowers of summer. Some varieties of it are covered by thick layer of powdery mildew. As pathogens *Oidium drummondii* VON THÜM. and *Léveillula polemoniacearum* GOLOV. are mentioned by BLUMER (1967). As to other P h l o x spp., he mentions *Sphaerotheca fuliginea* (SCHLECHT. ex FR.) POLLACCI. HIRATA (1966) reports *E. cichoracearum* from P h. p a n i c u l a t a L. (Yugoslavia, Germany, Canada, USA), *S. macularis* (USA) and *Oidium* sp. (Switzerland).

In Hungary, it was UBRIZSY (1970) to report about powdery mildew of P h l o x. *E. cichoracearum* perithecia were collected in a rather large number, in Budapest, Keszthely, Szentendre on P h l o x p a n i c u l a t a.

In the years of 1973 and 1974 we also made an abundant collection of P h l o x p a n i c u l a t a plants with powdery mildew. The susceptibility of the varieties proves rather different. There are stocks quite healthy, while others are perfectly white of powdery mildew covering them: it attacks leaves, stem, and inflorescences, so the leaves perish and fall before time. It could be found – on the basis of conidia, beside *E. cichoracearum* (Tata, 23. 9. 1973) *S. fuliginea* (Fertőszentmiklós, 15. 8. 1974, Tata, 1. 9., 22. 9. 1974, Budapest, University of Horticulture, 19. 9. 1974 infected with; *Cicinnobolus cesatii* hyperparasite fungus, Szomód, 22. 9. 1974). Conidia of *S. fuliginea* contain so-colled fibrosine bodies of strong refraction, but in the same of *E. cichoracearum* similar formulae never can be found. Perfect state of *E. cichoracearum* was found in Budapest on the area of János Hospital (11. 9. 1974): 8–27 (10–18) asci are developing in a single

cleistothecium with regularly 2 ascospores in each, but seldom 1, 3, or 4 ones as well.

Perfect state can be met only together with the conidial state not containing fibrosine bodies: never any cleistothecium could be found at imperfect states having fibrosine bodies. Conidium state may prove that there are two pathogens in Hungary as to Phlox paniculata: *E. cichoracearum* DC. ex MÉRAT emend. SALMON and *S. fuliginea* (SCHLECHT. ex FR.) POLLACCI. This last one is a new report from our country.

*Phlox drummondii HOOK (Polemoniaceae)
Erysiphe cichoracearum DC. ex MÉRAT emend. SALMON

Annual ornamental plant. No powdery mildew of it has been reported in Hungary. By HIRATA (1966), powdery mildews as follows occurred on it: *Oidium drummondii*, *Erysiphe cichoracearum*, *Sphaerotheca fuliginea*, *Léveillula taurica*.

Phlox drummondii plants with powdery mildew found by Dr. János BODOR in a balcony box, Budapest, Rózsadomb. By the end of the summer cleistothecia got formed as well. I was asked for determination of the pathogen. Conidia form in chains, not containing fibrosine bodies, size of 30.26×14.68 μ; L/W = 2.06. The cleistothecia with myceliumlike appendages. In a cleistothecium there are 8–19 (12–15) asci forming. The asci are regularly of 2 spores, but seldom asci of 3, 4 spores may be met. The pathogen proved *Erysiphe cichoracearum* on the basis of conidium and ascus states.

Solidago canadensis L. (Compositae)
Erysiphe cichoracearum DC. ex MÉRAT emend. SALMON

Perennial ornamental plant, bringing its yellow blooms in summer and autumn, rather liked. Its powdery mildew is generally known, pathogen: *Erysiphe cichoracearum* DC. ex MÉRAT emend. SALMON. HIRATA (1966) reports (from Poland) *S. fuliginea* (SCHLECHT. ex FR.) POLLACCI.

E. cichoracearum is reported from S. serotina AIT. (Budapest) by UBRIZSY and VÖRÖS (1966); while by TÓTH (1957) from Solidago canadensis (Gödöllő).

Author's collection data: Budapest, Gellérthegy (24. 9. 1969), Rózsadomb (5. 8. 1971), Tata (5. 6. 1971, 23. 9. 1973), Budapest, János Hospital (7. 8. 1974 – only mycelium –), Budapest, Tabán (14. 8. 1974). Coating is greyish, dusty-coloured, few conidia are forming of sizes 33.32×18.17 μ; L/W = 1.83; without fibrosine bodies, their simple germ tube starts from one of the corners of conidium. Perfect state has not been met yet.

*Veronica incana L. (Scrophulariaceae)
Sphaerotheca fuliginea (SCHLECHT. ex FR.) POLLACCI

Much liked perennial ornamental plant with blue flowers and tormentose leaves. *Sphaerotheca fuliginea* (SCHLECHT. ex FR.) POLLACCI and *Erysiphe polyphaga*

HAMM. may cause the powdery mildew of Veronica spp.; the latter was found by HAMMARLUND (1945) on V. speciosa R. CUNN.; its cleistothecia contain 8–12 asci each with 2–4 ascospores. Beside these, HIRATA (1966) reports *E. cichoracearum* DC. ex MÉRAT emend. SALMON from Veronica spp. and *S. fuliginea* from Veronica incana L. (Germany, Norway and USA).

As to Hungary, *S. fuliginea* was found on V. chamaedrys L. and V. crassifolia WIERZB. by MOESZ (1939, 1942), while the same was met by UBRIZSY and VÖRÖS (1966) on V. hederaefolia L.

Author found conidial state of *S. fuliginea* at Tata (9. 7. 1972, 29. 7. 1973, 23. 6. 1974), at Szomód (9. 7. 1972, 30. 6. 1973), and in Budapest, in the Tabán (14. 8. 1974). Conidia are developing in chains, containing fibrosine bodies, with a size of $29.36 \times 15.52\ \mu$; L/W = 1.89. Perfect state has not been met. This is the first report of powdery mildew of Veronica incana L. in Hungary.

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Literature

BLUMER, S. (1967): Echte Mehltaupilze *(Erysiphaceae)*. G. Fischer Jena.

ELIADE, E. (1972): *Microsphaera tarnavschii* sp. nov. sur les bégonias cultivés en Roumanie. Lucrarile Gradini Botanice din Bucuresti (1970/71) 391–399.

HAMMARLUND, C. (1945): Beiträge zur Revision einiger imperfekten Mehltau-Arten. *Erysiphe polyphaga* nov. sp. Bot. Not. *1*, 101–108.

HIRATA, K. (1966): Host range and geographical distribution of the powdery mildews. Faculty of Agric., Niigata University, Niigata, Japan.

JÁVORKA, S. and CSAPODY, V. (1962): Kerti virágaink. Mezőgazdasági Kiadó, Budapest.

MOESZ, G. (1939): Fungi Hungariae 3. Ascomycetes 1. Annales Musei Nationalis Hungarici. Pars Botanica *32*, 1–61.

MOESZ, G. (1942): Budapest és környékének gombái. K. Magyar Termtud. Társ., Budapest.

SIVANESAN, A. (1971): A new *Microsphaera* species on Begonia. Trans. Br. mycol. Soc. 56. *2*, 304–306.

SOÓ, R. and JÁVORKA, S. (1959): A magyar növényvilág kézikönyve I–II. Budapest.

SZ. NAGY, GY. (1970): Die Identifizierung des Mehltaus der Kürbisgewächse auf Grund der Konidienmerkmale. Acta phytopathol. Acad. Sci. hung. 5. *2–4*, 231–248.

SZ. NAGY, GY. (1972): Studies on powdery mildews of cucurbits I. Host and maintenance of *Sphaerotheca fuliginea* and *Erysiphe* sp. under laboratory and glasshouse conditions. Acta phytopathol. Acad. Sci. hung. 7. *4*, 415–420.

Sz. Nagy, Gy. (1974): Lisztharmat a Filipendulán. Növényvédelem *10*, 451—454.
Sz. Nagy, Gy. (1975): Revision of the powdery mildew species *Erysiphe polyphaga* Hamm. Acta phytopath. Acad. Sci. hung. *10*, 377—388.
Tóth, S. (1957): Adatok Magyarország mikroszkópikus gombáinak ismeretéhez II. Bot. Közl. *47*, 55—61.
Ubrizsy, G. (1941): Újabb adatok a Nyírség gombaflórájának ismeretéhez. Borbásia *3*, 151—155.
Ubrizsy, G. (1968): Review of the mycoflora of Hungary, V. Acta phytopath. Acad. Sci. hung. *3*, 241—260.
Ubrizsy, G. (1970): Két hazai dísznövény érdekes lisztharmatgombája. Növényvédelem *6*, 167.
Ubrizsy, G. (1972): Further notes on the powdery mildews of Hungary I. Acta phytopath. Acad. Sci. hung. *7*, 77—84.
Ubrizsy, G. and Vörös, J. (1966): Phytopathogenic and saprophytic fungi from Hungary I. Contributions to the *Peronosporales*, *Erysiphales* and *Deuteromycetes* flora of Hungary. Acta phytopath. Acad. Sci. hung. *1*, 145—164.

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Revision of the Powdery Mildew Species *Erysiphe polyphaga* Hamm.*

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Denomination *Erysiphe polyphaga* described by HAMMARLUND (1945) does not valid, as the paper does not present description in Latin;
and does not prove right, as

1. the origin of inoculum applied at tests is unknown;
2. the inoculation experiments could not be reproduced;
3. asci with more than two spores may occur even at perfect states of *E. cichoracearum;*
4. the born and way of germination of conidia of powdery mildews belonging to *E. polyphaga* are heterogenous;
5. perfect form of powdery mildew of *Begonia* was discovered and is proved to be a *Microsphaera* species.

Powdery mildews belonging to *E. polyphaga* developing their conidia in chains, not containing fibrosine bodies, having simple germ tube forming longish, club-shaped appressoria may be ranged to *Erysiphe cichoracearum* As to the other powdery mildews known in imperfect forms, the denomination *Oidium* seems correct, up till finding the perfect forms of each.

It was in 1945 when HAMMARLUND described in Germany *Erysiphe polyphaga* as a new species. In Hungary there are two pathogens to cause mildew disease of cucurbits: *Sphaerotheca fuliginea* (SCHLECHT. ex FR.) POLLACCI and a species of *Erysiphe* (SZ. NAGY, 1970, 1972, 1973). A more exact definition of this *Erysiphe* species made the author face a rather serious problem, as the proper place of it did not come up to this time to identical standpoints: formerly *E. cichoracearum* DC. ex MÉRAT emend. SALMON took the general opinion, while recently the denomination *E. polyphaga* HAMM. has been met rather often. Even BLUMER (1967) cannot represent a definite standpoint. With the aim of clearing up the situation, author decided to go through the literature concerned for trying to work up in a research work the fungi of powdery mildew classed in this group.

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Literature Review

HAMMARLUND (1945) found the conidial form of a powdery mildew on *Veronica speciosa* in the year 1934. The perfect form of it was discovered next year and described by him as *Erysiphe polyphaga* nov. sp. Some later he found 6 small perithecia on *Begonia Gloire de Lorraine;* similarly he could meet cleistothecia on *Cucumis sativus* in Sweden in field cultivation two times and on *Dahlia variabilis* grown in South America. He defined the same as *E. polyphaga* as well.

He published this new species in a "preliminary report" in German as "preliminary diagnosis" ("Vorläufige Diagnose"): "Mycelia congested, conidiophores dense and simple. Conidium form elliptical, or cylindrical, born in long chainforms. Perithecia are scattered. Appendages are basale, mostly with a length. over the diameter of cleistothecium, in dark brown. Asci may be 8–12 (f.m. *veronicae spinosae*) or 10–20 (f.m. *begoniae Gloire de Lorraine*): with 2–4 spores (f.m. *veronicae speciosae*)". He holds out an opinion that this species "seems to be rather near to *E. cichoracearum*. There is a single morphological difference to exist: at the asci of general occurrence with 2 spores . . . there can be found some with 3 or 4 spores." Whether this fact can be ascribed to genetical causes, he does not offer any argument.

In glasshouse cultivation he could observe spontaneous infections: *Chrysanthemum→Begonia Gloire de Lorraine→ Kalanchoë blossfeldiana.* He came to supposing that the fungi mildew known up to this time in an imperfect form and ranged to the different species of powdery mildew (e.g. *Oidium chrasanthemi*, *O. begoniae*, *O. kalanchoeae*) all belong to *E. polyphaga.* To get evidence to this, he carried out artificial inoculations. 62 species of 100 belonging to 11 families of plants got infected. The origin of inoculum is not presented in his paper, but as he mentioned to use only conidia for inoculations, he may have taken this as single biotype. But this biotype morphologically (e.g. size and form conidia) seems rather variable. This fact he admits as matrical modification, proposing a denomination "forma matricalis" (f.m.). So the name of powdery mildew found on *Veronica speciosa* is: *Erysiphe polyphaga* f.m. *veronicae speciosae.*

Following HAMMARLUND there were some in Europe to take over the denomination *E. polyphaga.* E.g. VIENNOT-BOURGIN (1956, 1971), CIFERRI and CAMERA (1962) take it without any condition. BLUMER (1967) treats it as a different species, but with the remark, it hardly can be told from *E. cichoracearum;* differentiate them is problematical; *E. polyphaga* can be taken as a hardly specialised physiological race of *E. cichoracearum.* The last he ranged as the one for the members of the family *Compositae;* though the powdery mildews of the species belonging to the families *Cucurbitaceae*, *Solanaceae* etc. was ranged to the *E. polyphaga.* It is the description in German language that keeps JUNELL (1967) from accepting this denomination valid: he presents it in quotation marks ("Erysiphe polyphaga" HAMMARL.) as "nomen nudum", and says: "but until the species is better known will use this name". BOEREMA and VERHOEVEN as well consider this denomination

invalid. Some others (e.g. SANDU-VILLE, 1967) do not even give a mention to this species in their works.

HAMMARLUND (1945) reports the most important host plants as follows (synonyms of powdery mildews given by HAMMARLUND in brackets): *Kalanchoë blossfeldiana* (*Oidium kalanchoëae* LÜST.), *Begonia Gloire de Lorraine* (*Oidium begoniae* PUTT.), *Veronica speciosa*, *Cyclamen persicum* (*Oidium cyclaminis* WENZL.), *Lycopersicum esculentum* (*Oidium lycopersici* COOKE et MASSEE), *Nicotiana tabacum* (*Oidium tabaci* THEUM.), *Solanum tuberosum* (*Oidium solani* auct.), *Cucumis melo* and *C. sativus* (*Erysiphe cichoracearum* (DC.) SALMON p.p.), *Linum usitatissimum* (*Oidium lini* ŠKORIĆ), *Chrysanthemum indicum* and *C. morifolium* (*Oidium chrysanthemi* RAB.), *Dahlia variabilis* (*E. cichoracearum* p.p.), *Verbena hybrida* (*Oidium verbenae* THÜM. et BOLLE), *Ricinus communis* (*Oidium ricini* JACZ.) and other species of *Kalanchoë*, *Sedum* and *Sempervivum* etc.

BLUMER (1967) published about some of these plants (e.g. *Chrysanthemum*, *Cucurbitaceae*, *Veronica)* various powdery mildew species.

He even managed to extend this host range when performing inoculation experiments with a form of powdery mildew of cucumber producing asci of 2–3 spores *(Helianthus annuus)*. But repetition of HAMMARLUND's inoculation tests could not meet at all time a recurrent success. VIENNOT–BOURGIN (1951 ap. BLUMER, 1967) could not transfer powdery mildew of *Begonia* onto *Kalanchoë*. VON ARX (1951 ap. BLUMER, 1967) is of the opinion that the powdery mildew of *Begonia (Oidium begoniae)* is a specific form, not transferable to *Cyclamen*, *Kalanchoë*, *Chrysanthemum* and *Primula*. Powdery mildew coming from *Cyclamen*, *Chrysanthemum* and *Kalanchoë* did not infect *Begonia* either. Neither STONE (1962 ap. BLUMER, 1967) could manage to transfer powdery mildew of cucumber onto *Begonia* and vice versa. By BLUMER (1967), HAMMARLUND, in this case, seems to have fallen victim of an error brought about by an alien infection.

The perceft state of powdery mildew of *Begonia (Oidium begoniae)* was found in Great Britain and Rumania and was described by SIVANESAN (1971) as *Microsphaera begoniae* sp. nov. and by ELIADE (1972) as *Microsphaera tarnavschii* sp. nov.

An erlier paper of author (SZ. NAGY, 1972) gives account of the result of some tests of infection. These seem to prove, the transfer was a success in infecting *Aster dumosus* and *Cichorium intybus* with *Erysiphe* conidia coming from *Cucumis sativus*. On the other hand, cucumber could be infected in transferring *E. cichoracearum* conidia coming from *Aster dumosus*, *C. intybus* and *Lactuca serriola*. An infection coming from cucumber developed very slight traces on the leaves of *Kalanchoë blossfeldiana*, even not after each inoculation; the reciprocal tests brought the same results.

Material and Methods

The powdery mildews found on host plants classified by BLUMER (1967) and HAMMARLUND (1945) in the host range of *E. cichoracearum* and *E. polyphaga* were collected. Their morphological characteristics – born, form, size, way of germination (both on dry slides and water agar), form of germ tubes and appressoria, number of asci and ascospores developing in the cleistothecia – were observed under a microscope.

Artificial inoculations were carried out in the laboratory, with leaf disc method (SZ. NAGY, 1972) and glasshouse, on potted plants. Germinative conidia of 2–3 days were used for inoculations.

Results

1. *Morphological tests*

a) Characteristics of powdery mildews occurring on species belonging to the host range of *Erysiphe poliphaga*

Begonia rex PUTZ.

Conidia born by single, seldom in chains of 2 or 3 conidia; exceedingly long: 50.60×16.63 μ (length/width = 3.04). Germ tube starting from the corner of conidium. Appressoria lobated, branching.

Chrysanthemum spp.

Chrysanthemum × *hortorum* BAILEY (syn.: *Ch. indicum* L.) and *Ch. carinatum* L. have the mycelia of powdery mildew forming a slight coating on the leaves. There is very little conidium forming. These are developing in very short chains in sizes 37.93×15.89 μ (L/W = 2.38), without fibrosine bodies; they have a long germ tube starting from one of the corners of the conidium. Appressoria are club-shaped. There could be some cleistothecia found on *Ch. carinatum*, immature, unfit for identification.

Cucumis and *Cucurbita* spp.

These plants have two species of powdery mildews: one of the *Erysiphe* sp. and the *Sphaerotheca fuliginea* (SCHLECHT. ex FR.) POLLACCI. The latter one is very distinguishable on the basis of the fibrosine bodies, the germination of conidia and the single ascus developing in the cleistothecium. Conidia of *Erysiphe* sp. form in long chains, without fibrosine bodies, they are cylindrical, with nearly parallel sides, their size is 29.98×15.05 μ (L/W = 2.00). The germ tube is simple, starting from one of the corners of the conidium (Fig. 1), appressorium club-shaped. 8–12 asci can be found in each of the cleistothecia, with 2, seldom 3 ascospores in each (Fig. 2).

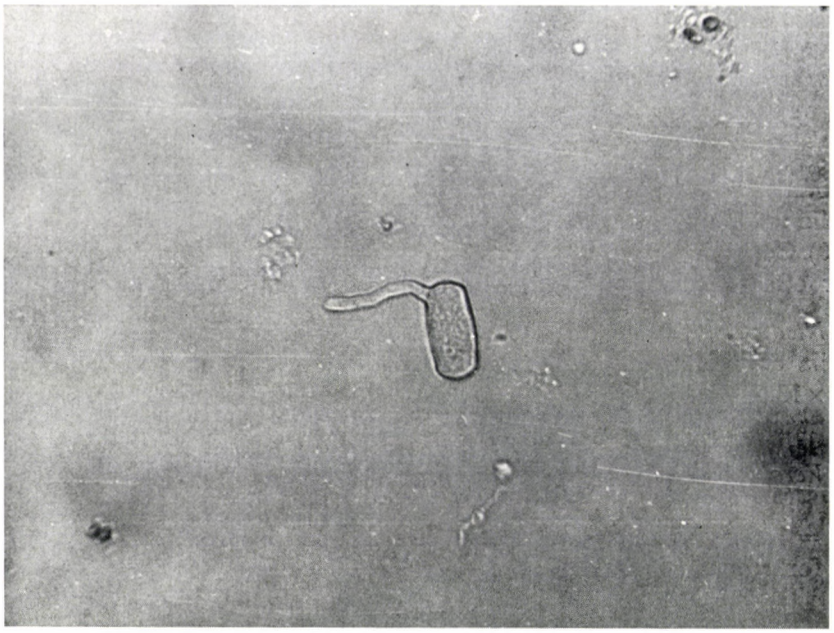

Fig. 1. Germinating conidium of. *Erysiphe* sp. from *Cucumis sativus*

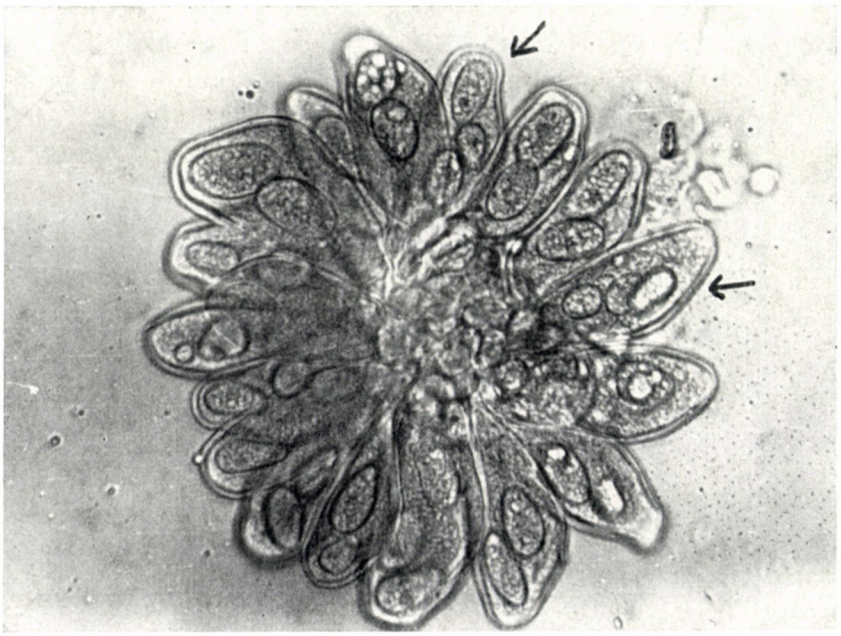

Fig. 2. Asci of *Erysiphe* sp. with 2- and 3-spores from *Cucumis sativus*

Helianthus annuus L.

It is exposed to the attacks of two species like Cucurbitaceae: *Erysiphe* sp. and *Sphaerotheca fuliginea*. Conidia of *Erysiphe* sp. are of size $29.04 \times 15.17\ \mu$ (L/W = 1.91). Their way of formation, germination is in total accord with the characteristics of *Erysiphe* sp. forming on Cucurbitaceae.

Kalanchoë blossfeldiana POELLN.

Conidia are born by single, they are cylindrical, $36.58 \times 16.37\ \mu$ in size. (L/W = = 2.18). Germ tube starts from one corner of conidium, it is straight, very short and rather often creates lobated appressorium with multifold branching (Fig. 3).

Nicotiana tabacum L.

Conidia develop in long chains, they are cylindrical without fibrosine bodies, $27.50 \times 13.52\ \mu$ of size (L/W = 2.07). Germ tube starts from one corner of conidium, it is simple, straight, its appressorium is club-shaped.

Veronica incana L.

Conidia developing in long chains, with fibrosine bodies. Size $29.36 \times 15.52\ \mu$ (L/W = 1.89). Germ tube starts from the ventral side of conidium, it is short, bent, often forked.

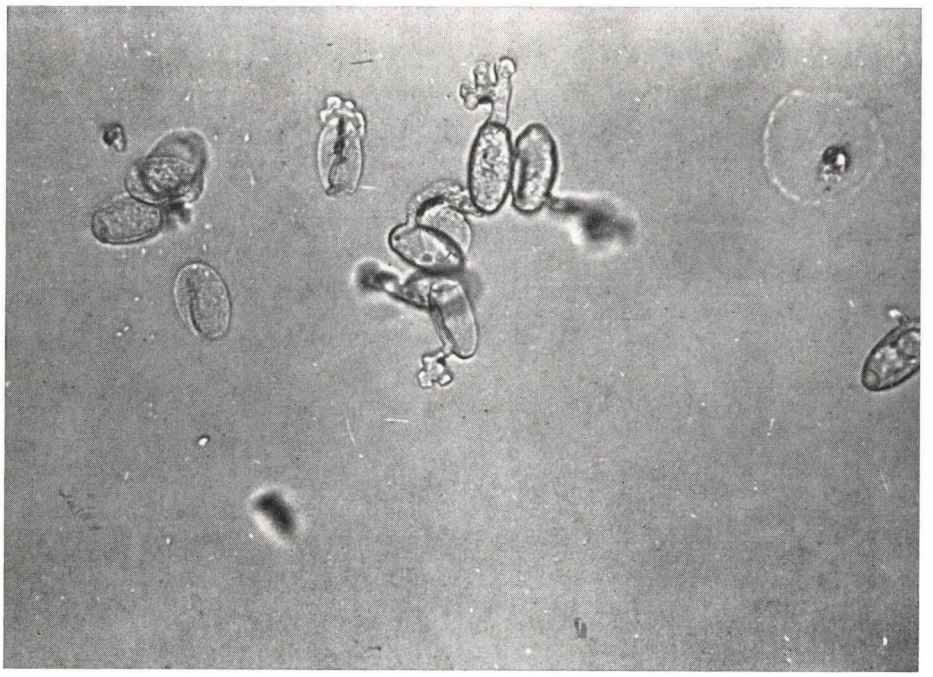

Fig. 3. Germinating conidia of *Oidium kalanchoeae*

b) Characteristics of powdery mildews occurring on species belonging to the host range of *Erysiphe cichoracearum*

Conidia are born in long chains (4–6 members), that are cylindrical with almost parallel sides, without fibrosine bodies; their size shows some difference in relation of host plant, the lenght: width ratio is about 2 (1.83–2.08); some bigger on Aster spp. (Table 1). Germ tubes start from one of the corners of conidia, straight, without branching; appressoria are club-shaped.

Table 1

Characteristic sizes of conidia of *Erysiphe cichoracearum* on hosts

Hosts	Length μ	Width μ	Length/width
Aster spp.	33.24	15.24	2.25
Carduus acanthoides	34.14	18.16	1.88
Cichorium intybus	29.83	15.39	1.94
C. intybus var. *foliosum*	28.09	14.28	1.96
Lactuca serriola	31.57	15.20	2.08
Phlox paniculata	29.41	14.75	2.01
Solidago canadensis	33.32	18.17	1.83
Sonchus spp.	30.33	15.20	2.00
Mean	31.24	15.80	1.98

Cleistothecia are of diameters of about 100–150 μ. Appendages mycelium-like. These are developing many (5–18) asci, most often 8–10, in a single cleistothecium. Normally there are 2 ascospores in an ascus, but in cases 1, 3 or 4 spores can be found as well. 3 spore asci were found on Aster spp. (Fig. 4) and *Cichorium intybus* var. *foliosum*, 3 and 4 spore ones on *Phlox paniculata* (Fig. 5).

2. *Inoculation tests*

Oidium begoniae conidia collected from leaves of *Begonia rex* were not apt to infect the leaf of either *Cucumis sativus* or *Aster dumosus* or *Kalanchoë blossfeldiana*. The conidia germinated, but died in a couple of days; they were living the longest on *K. blossfeldiana*, even to developed some hyphae, but on the fifth day following inoculation they perished as well.

Erysiphe conidia from *Helianthus annuus* were a success to infect cucumber and vice versa, both in laboratory and glasshouse.

No result at all could be reached as to cross-inoculations either in laboratories or in glasshouses between *Phlox paniculata* and *Cucumis sativus*. In the same way no infection could be reached from cucumber to *Chrysanthemum* × *hortorum* and *Nicotiana tabacum*, and neither reciprocally either.

Contrary to this, *E. depressa* from *Arcticum lappa* and *E. artemisiae* from *Artemisia vulgaris* formed colonies on the leaves of cucumber.

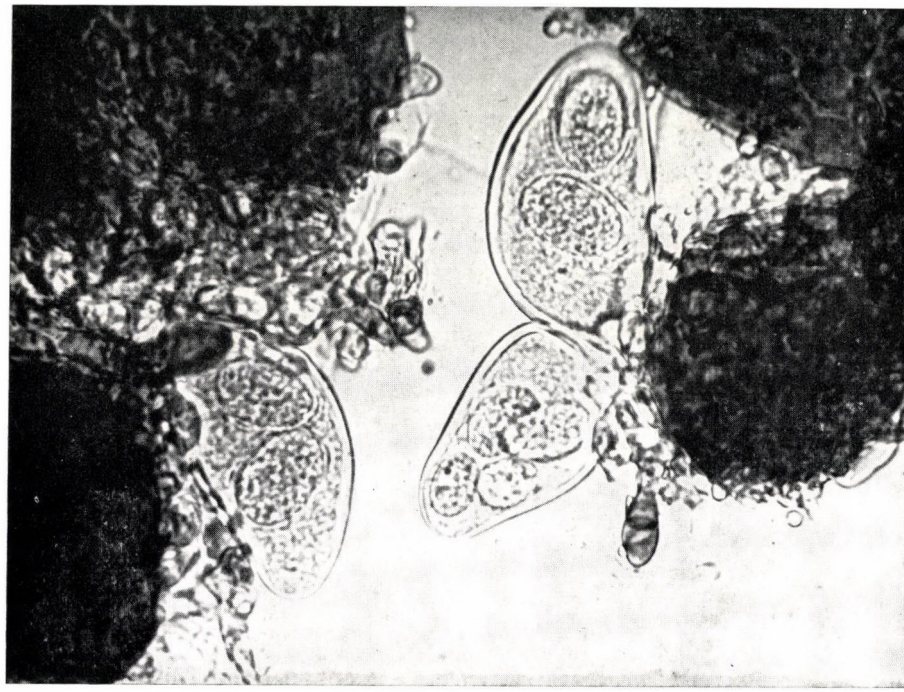

Fig. 4. Asci of *Erysiphe cichoracearum* with 2- and 4-spores from *Phlox paniculata*

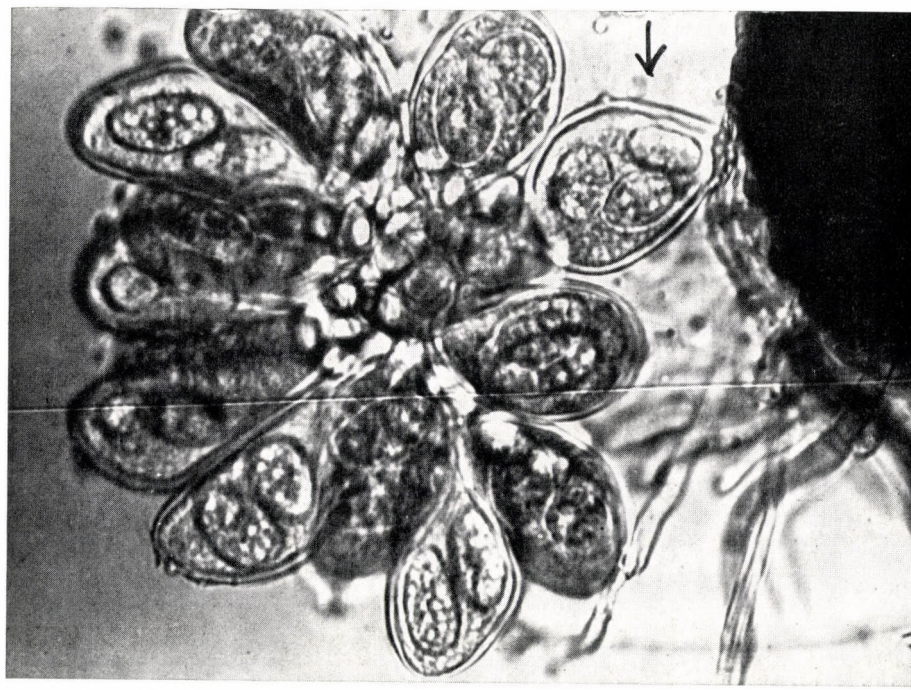

Fig. 5. Asci of *Erysiphe cichoracearum* with 2- and 3-spores from *Aster* sp.

Discussion

Denomination *Erysiphe polyphaga* – first of all – cannot be accepted as valid, as the description was reported in German language. Especially as the fungus names published since 1935 – as ruled by the International Code of Botanical Nomenclature – may be valid only if the publication contains descriptions in Latin too. In this case, the "preliminary diagnose" of HAMMARLUND was not followed by any other.

The host range of new species was determined by inoculation tests, but there is no information given, where the primary inoculum came from. Neither is information given as to the experimental circumstances: so there is the possibility that he evaluated spontaneous infections as results of his own inoculation tests. This supposition may be supported by the inoculation tests of VON ARX (1952) and the same of author, according to which the *Chrysanthemum – Begonia – Kalanchoë*-chain of inoculation could not be proved. There is the probability that *Begonia* and *Kalanchoë* plants happened in the glasshouse beside *Chrysanthemum* infected by powdery mildew, and the infection of the first ones could not be seen with naked eye. Powdery mildews from *Chrysanthemum*, *Begonia* or *Kalanchoë* could successfully inoculate neither cucumber nor *Aster*.

As to ranging powdery mildews occurring on different host plants, HAMMARLUND seems to be rather biased. Beside the inoculation tests of rather doubtful value – he was interested only in the number of ascospores forming within a single ascus, though there are rather many factors to be observed at systhematization of powdery mildews. The systhematisation of *Erysiphe* is based on the morphology of perfect state (number of asci developing in a single ascus, the form of appendages). The first and most striking characteristic at the examination of perfect state is the form of appendages, and it is just this what HAMMARLUND failed to observe at *Begonia* powdery mildew. Even before SIVANESAN and ELIADE he found the cleistothecia of *Oidium begoniae*, but failed to notice the dichotomical branching of appendages, characteristic to genus *Microsphaera*. It is this, on the basis of which SIVANESAN (1971) and ELIADE (1972) described *Begonia* powdery mildew as *Microsphaera begoniae* sp. nov. and *Microsphaera tarnavschii* sp. nov., respectively.

The only morphological difference between *E. cichoracearum* and *E. polyphaga* – by HAMMARLUND – is the occasional appearance of asci with 3 and 4 spores among the ones of 2 spores. In the course of cleistothecium test author met asci of 3 or 4 spores not only on the species of the family *Cucurbitaceae* in case mildew fungi, but also on *Aster* and *Phlox* powdery mildews taken as *E. cichoracearum*. This cannot be of genetical origin, much more can it be ascribed to an irregularity taking place in the course of cell-division.

Some features of conidial state may give systematical values as well (BLUMER, 1967). Information of HAMMARLUND is only about the way of development of conidia: "conidia develop in long chains", leaving without any examination the other features of conidia. HIRATA (1942, 1955, 1956) gave a detailed work about the

germ tubes characteristic of individual species. ZARACOVITIS (1965), proved a close connection existing between the ways of formation and germination of conidia.

The conidium formation and germination of powdery mildews tested by author considered, a different development can be noticed as to the powdery mildews ranged by HAMMARLUND to *E. polyphaga*. On the basis of conidium formation, powdery mildews of *Cucumis* and *Cucurbita* species, *Helianthus annuus*, *Nicotiana tabacum*, *Veronica incana* as well as *E. cichoracearum* (on *Aster* spp., *Carduus acanthoides*, *Cichorium intybus* and *Cichorium intybus* var. *foliosum*, *Lactuca serriola*, *Phlox paniculata*, *Solidago canadensis* and *Sonchus* spp.) form their conidia in long chains, thus they belong to the type *E. cichoracearum* (*Euoidium*). To the type *E. polygoni* (*Pseudoidium*) producing its conidia by the single or in short chains there may be ranged the powdery mildews of *Begonia rex*, *Chrysanthemum* × *hortorum* and *Kalanchoë blossfeldiana*. By ZARACOVITIS (1965) the *Microsphaera* and *Uncinula* spp., *Oidium begoniae* and some *Erysiphe* spp. belong to this group. HIRATA (1968) informs about a powdery mildew producing *E. polygoni* type appearing on some species belonging to family *Cucurbitaceae*; the occurrence of the mildew is in Japan and China.

Species belonging to the group *Euoidium* at germination test carried out on slides grow long germ tubes with club-shaped appressoria on them, on conidia of *Pseudoidium* there are very short germ tubes, appressoria are lobated.

Club-shaped appressoria were met on the powdery mildews of *Cucurbitaceae*, *Chrysanthemum* × *hortorum*, *Helianthus annuus*, *Nicotiana tabacum*, *Aster* spp., *Cichorium intybus*, *Lactuca serriola*, *Phlox paniculata*, *Solidago canadensis* and *Sonchus* spp.; lobated appressoria were found on the germing conidia of the powdery mildews of *Begonia rex* and *Kalanchoë blossfeldiana*. Different from any others, conidia of the powdery mildew of *Veronica incana* contain so-called fibrosine bodies; they grow characteristic, sometimes forked germ tubes, coming from the ventral part of the conidia: this is the characteristic conidial form of *Sphaerotheca fuliginea*.

Thus the conidial state of powdery mildews of *Cucurbitaceae*, *Helianthus annuus* and *Nicotiana tabacum* displays characteristics conform to those of *E. cichoracearum*. The conidia of mildew found on *Chrysanthemum* spp. form in short chains, are considerably longer than those of *E. cichoracearum*, but have identical ways of germination. On *Kalanchoë blossfeldiana* conidia form mostly by single, they have short germ tubes and lobated appressoria. The biggest deviation from *E. cichoracearum* is displayed by the powdery mildew of *Begonia:* conidia of extraordinary length, mostly by the single, lobated appressoria.

HAMMARLUND greatly simplified the identification of powdery mildew fungi known in imperfect form. He considered only two factors (number of ascospores and inoculation), and, at one of them, he seems to have fallen victim to an alien infection ("Hier scheint HAMMARLUND einer Fremdinfektion zum Opfer gefallen zu sein." – BLUMER, 1967, p. 83.). He classified powdery mildews with different characteristics into one species; some of these show quite a close relationship with *E. cichoracearum*. Originally they did belong there, so neither HAMMARLUND nor

BLUMER denies this connection. The stand taken by BLUMER seems interesting: he takes *E. polyphaga* as a separate species, expresses his doubt as to some results of some inoculation tests made by HAMMARLUND, in spite of all these, he extends on the host range. But he absolutely rejects the determination "forma matricalis".

Author is of the opinion, – on the basis of characteristics of perfect and imperfect states – that the powdery mildew of *Cucurbitaceae*, sunflower and tobacco may be ranged to *E. cichoracearum*, representing different specialised forms of this "collective species" dividing in innumerable physiological races.

The powdery mildew of *Begonia* belongs to the *Microsphaera* genus. As to the other powdery mildews known only in imperfect forms, keeping the denomination *Oidium* seems more justified.

It is due to give a special mention to the powdery mildew of *Veronica* spp. It is true, that it was the first, that HAMMARLUND found the species of powdery mildew, the one with three ascospores to describe the new species from. Author found *S. fuliginea* on this plant: its perfect state (the cleistothecium has always one ascus) is considerably different from the cleistothecia of *Erysiphe* species, with many asci. This is no contradiction, as both the species are known from *Veronica* spp. (BLUMER, 1967). *S. fuliginea* may occur beside *Erysiphe* on other plants as well, e.g. on cucumber, vegetable marrow, melon, watermelon and sunflower.

Literature

BLUMER, S. (1967): Echte Mehltaupilze *(Erysiphaceae)*, Gustav Fischer Verlag, Jena.

BOEREMA, G. H. and VERHOEVEN, A. A. (1972): Check-list for scientific names of common parasitic fungi – Series 1a: Fungi on trees and shrubs Neth. J. of Plant Path. *78*, 1–63.

CIFERRI, R. and CAMERA, C. (1962): Tentativo di elencazione dei funghi italiani I. *Erisifali*. Quad. Ist. Bot. Univ. Pavia, No. 21.

ELIADE, E. (1972): *Microsphaera tarnavschii* sp. nov. sur les bégonias cultivées en Roumanie. Lucrarile Gradini Botanice din Bucuresti (1970/1971) p. 391–399.

HAMMARLUND, C. (1945): Beiträge zur Revision einiger imperfekten Mehltau-Arten. *Erysiphe polyphaga* nov. sp. Bot. Not. *1*, 101–108.

HIRATA, K. (1942): On the shape of the germ tubes of *Erysipheae* I. Bull. Chiba Coll. Hort. *5*, 34–49.

HIRATA, K. (1955): On the shape of the germ tube of *Erysipheae* II. Bull. Fac. Agric. Niigata Univ. *7*, 24–36.

HIRATA, K. (1956): On the shape of the conidial germ tubes of *Erysipheae* III. Germ tubes formed on host plant leaves – Bull. Fac. Agric. Niigata Univ. *8*, 1–4.

HIRATA, K. (1968): Notes on host range and geographic distribution of powdery mildew fungi 5. Powdery mildew fungi of cucurbits – Transactions of the Mycological Society of Japan *9*, 80–83.

JUNELL, L. (1967): *Erysiphaceae* of Sweden – Symbolae Botanicae Upsaliensis *19*, 1–117.

SANDU-VILLE, C. (1967): Ciupercile *Erysiphaceae* din Romania. Studiu Monografic. Ed. Acad. Rep. Soc. Rom., Bucuresti.

SIVANESAN, A. (1971): A new *Microsphaera* species on *Begonia*. Trans. Br. mycol. Soc. *56*, 304–306.

STONE, O. M. (1962): Alternate hosts of cucumber powdery mildew — Ann. App. Biol. *50*, 203—210.

SZ. NAGY, GY. (1970): Die Identifizierung des Mehltaus der Kürbisgewächse auf Grund der Konidienmerkmale — Acta phytopathologica Acad. Sci. hung. *5*, 231—248.

SZ. NAGY, GY. (1972): Studies on powdery mildews of cucurbits I. Host range and maintenance of *Sphaerotheca fuliginea* and *Erysiphe* sp. under laboratory and glasshouse conditions. Acta phytopathologica Acad. Sci. hung. *7*, 415—420.

SZ. NAGY, GY. (1973): A kabakosok liszharmatbetegsége Magyarországon. Doctoral Dissertation, University for Horticulture, Budapest.

VIENNOT-BOURGIN, G. (1956): Encyclopédia mycologique. XXVI—XXVII. Mildious, oidiums, caries, charbons, rouilles des plantes de France. Paul Lechevalier, Paris.

VIENNOT-BOURGIN, G. (1971): *Erysiphacées* nouvelles ou peu connues an France. Annls Phytopath. *3*, 337—352.

VON ARX, J. A. (1952): Meeldauw op *Cinearia* en andere sierplanten. Tijdschr. Plantenziekten *58*, 10—13.

ZARACOVITIS, C. (1965): Attempts to identify powdery mildew fungi by conidial characters. Trans. Brit. Mycol. Soc. *48*, 553—558.

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Armillaria Root Rot: Distribution and Severity in Softwood Plantations in Newfoundland

By

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Armillaria root rot is the most important disease of softwood plantations in Newfoundland and is Island-wide in distribution. It has been observed on most species and in most localities examined. The incidence of the disease, however, varied between species, between planting locations and sites, and between various methods of establishment of plantations. A few recommendations are given to minimize the loss caused by this disease in Newfoundland.

Armillaria root rot or the shoe-string root rot is caused by an agaricaceous fungus, *Armillaria mellea* (Vahl ex Fr.) Kummer. It is an important disease of a wide variety of tree and shrub species in both temperate and tropical regions of the world, and is one of the most important diseases of commercial forests in North America (PATTON and VASQUEZ BRAVO, 1967).

The fungus is a non-specific facultative parasite of underground plant parts, such as roots, root collar and base of the stem. It lives as a soil-borne saprophyte but can become a virulent pathogen whenever suitable host material and conditions are available. The spread of the disease from an infected tree or an infected stump takes place by means of rhizomorphs or by contact or grafting between the diseased and healthy roots (BOYCE, 1966). Although the role of *A. mellea* in the decadence of forest trees is not well understood, it is known that the disease causes abrupt or gradual reduction in growth, decline in vigour, thinning of the crown, and may ultimately result in tree mortality. The pathogen is particularly destructive in plantations and in natural stands already weakened by other factors such as drought, water logging, and damage by an insect or other pathogenic fungi (TWAROWSKI and TWAROWSKA, 1959; PATTON and VASQUEZ BRAVO, 1967). PEACE (1962) reported that this pathogen is responsible for considerable tree mortality in young as well as older plantations and considers *Armillaria* root rot as one of the important problems in the establishment of plantations in several countries in Europe and North America.

In Newfoundland (latitude 46° 37' N to 52° 01' N, longitude 52° 37' W to 59° 25' W; Fig. 1) the root rot has been recorded on both softwood and hardwood species in forests[1]. The first record of the disease in natural stands dates back to

[1] The forests of Newfoundland are situated in the Boreal Forest Region of Canada (ROWE, 1972) and consist mainly of balsam fir, *Abies balsamea* (L.) Mill.; black spruce, *Picea mariana* (Mill.) B.S.P., and white birch, *Betula papyrifera* Marsh.

1958 when it was observed in young black spruce regeneration on old burns at several places in western Newfoundland (CARROLL and PARROTT, 1958, DAVIDSON and NEWELL, 1958). Since then the disease has been recorded in regeneration, semi-mature and mature stands of black spruce and balsam fir and scattered tamarack, *Larix laricina* (Du Roi) K. Koch, trees at several locations in western and central Newfoundland. The disease has also caused the death of balsam fir trees previously damaged by the balsam woolly aphid, *Adelges piceae* (Ratz.). In such cases the incidence of the root rot varies directly with the level of aphid damage (HUDAK and SINGH, 1970). Recent surveys have shown that it causes extensive tree mortality of native and exotic species in softwood plantations, and is considered as the most important cause of the deterioration, decay and death of such trees (WARREN and SINGH, 1968; 1969).

This paper presents the results of comprehensive surveys conducted during the years 1968 to 1971 to investigate the distribution and severity of *Armillaria* root rot and the influence of root weevils, *Hylobius* spp., on the incidence of the disease in softwood plantations on the Island. The paper is designed not only to assess the status of this disease, but also to provide some recommendations for minimizing damage in areas designed for artificial regeneration.

Materials and Methods

Although *Armillaria* root rot has been observed in forests throughout the Island of Newfoundland, detailed investigations on the distribution and severity of the disease have only been conducted in 17, 6- to 18-year-old, softwood plantations established in a variety of site conditions. These plantations were located at Bauline Line on the Avalon Peninsula; at North Pond in central Newfoundland; and at Birchy Lake, Cormack, Serpentine Lake, Stephenville, Bottom Brook, Middle Brook and Highlands River in western Newfoundland (Locality Nos 1 to 17 in Fig. 1, Table 1). They contained 16 native and introduced softwood species, represented by 65 provenances. Data on the plot location, history, moisture regime of the site, planted tree species, mode of establishment, soil characteristics, numbers of *A. mellea* infected stumps per acre and average percent *A. mellea* infection in these plantations are summarized in Table 1.

Sampling for Armillaria root rot

Preliminary surveys had shown that most dead and chlorotic trees had *A. mellea* infection. Therefore emphasis was placed on examining dead and chlorotic trees, but some apparently healthy trees were also examined in all plantations.

The intensity of sampling in plantations was as follows: every chlorotic and dead tree, and approximately 10% of the apparently healthy trees at Bauline Line, Cormack, Stephenville, Bottom Brook, Middle Brook and Highlands River; every tree encountered at North Pond; and 20 trees in each of the 18, 1/5 acre

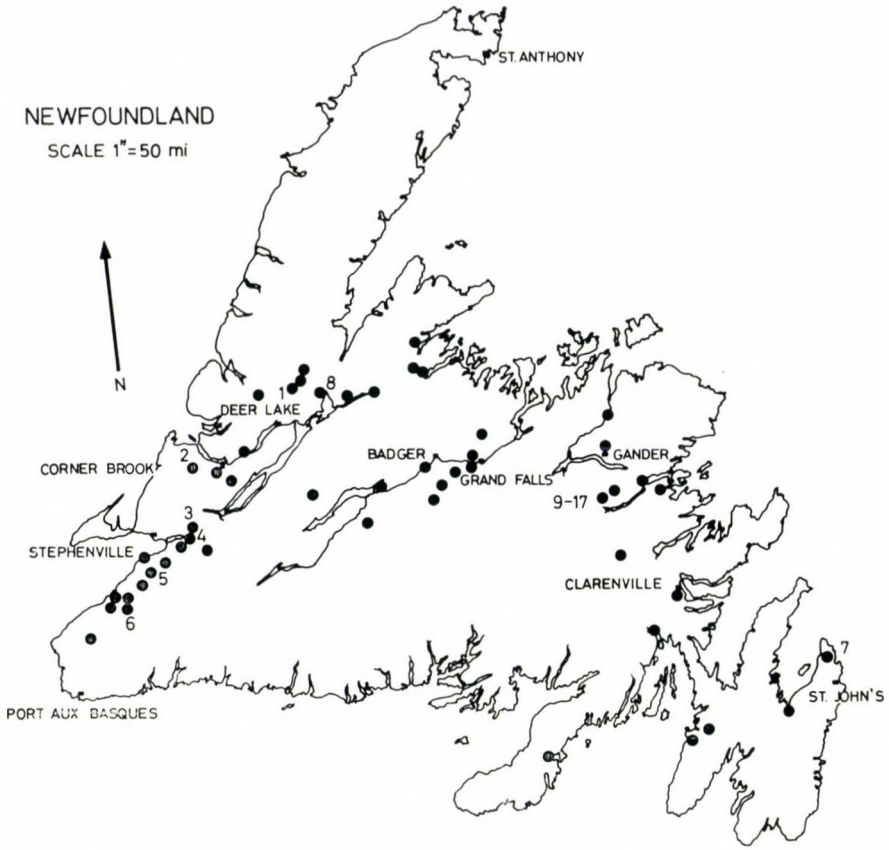

Fig. 1. Map of the Island of Newfoundland showing distribution of *Armillaria* root rot. Nos 1 to 17 indicate the location of 17 plantations where comprehensive surveys were conducted

subplots in the plantation at Serpentine Lake. On areas where seed was sown on prepared seed spots, every tree on 863 randomly selected spots was examined as were all trees in 341 equally spaced milacre quadrats established in areas where seeds were broadcast. A total of 9095 trees were examined in the 17 plantations; 5640 were established by bare root stock, 159 by container stock, 1175 by seed spotting, and 2121 by broadcast seeding.

Examination of trees/seedlings consisted of an inspection of the primary and secondary roots, and the root collar for symptoms of the disease and signs of the pathogen. Evidence of the disease was established by chlorosis or browning and thinning of the foliage, formation of a canker and resin exudation at the point of infection on the root/root collar, white or cream colored mycelial fans under the bark of an infected root/root collar, dark rhizomorphs, and characteristic honey-colored mushrooms. Isolations from mycelial fans and rhizomorphs were made on 2% malt agar to confirm the identity of the fungus.

Table 1

Locality Nos, plot location, history, moisture regime of site, planted tree species, mode of infection in the

Locality Nos (also on the map, Fig. 1)	Plot location	History	Moisture regime**	Planted tree species
1	Cormack (1)*	Burned-cutover	Fresh	Norway spruce, *Picea abies* L.; black spruce, *Picea mariana* (Mill.) B.S.P.; white spruce, *Picea glauca* (Moench) Voss; Sitka spruce, *Picea sitchensis* (Bong.) Carr.; Japanese larch, *Larix leptolepis* (Sieb. & Zucc.) Gourd.; Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco; and a cross between Sitka and white spruce.
2	Serpentine Lake (1)	Cutover	Fresh to moist, with patches of wet poorly drained soil	Red spruce, *Picea rubens* Sarg.; and Sitka spruce.
3	Stephenville (1)	Farmland	Fresh, with a few patches of wet poorly drained soil.	White spruce — 28 provenances.
4	Bottom Brook (1)	Cutover	Fresh	Six species of Japanese fir and one balsam fir, including needle fir, *Abies holophylla* Maxim.; Nikko fir, *A. homolepis* Sieb. & Zucc.; Maries fir, *A. mariesii* Mast.; *A. mayriana* Myiabe & Kudo; Saghalin fir, *A. sachalinénsis* Mast.; Veitch fir, *A. Veitchii* Lindl.; and balsam fir.
5	Middle Brook (1)	Cutover	Moist to very moist	— Same as in Bottom Brook —
6	Highlands River (1)	Cutover	Dry	— Same as in Bottom Brook —

establishment, soil characteristics, number of *A. mellea* infected stumps per acre and per cent 17 plantations

Mode of establishment	Soil characteristics					No. of infected stumps per acre	Average per cent infection
	Texture	pH	Organic matter (%)	Available nitrogen (lbs/acre)	Available phosphorus (lbs/acre)		
Bare root planting	Loam, deep, stone-free, overlain with a very thin layer of raw humus	4.8	12	41	10	529	29
Bare root planting	Loam, with variable amounts of sand and clay, overlain with a thick (6"—9") layer of raw humus	4.8	15	36	6	4	9
Seedspotting		4.8	15	36	6	4	0
Bare root planting	Loam, deep, stone-free, overlain with a very thin layer of raw humus	4.7	11	34	2	24	3
Bare root planting	Sandy loam, well drained, stone-free, overlain with a thin layer of raw humus	4.5	15	41	8	1106	18
Bare root planting	Loam, stone-free, well drained, overlain with a thin layer of raw humus	4.6	13	42	4	1084	35
Bare root planting	Loam, mostly stone-free, well drained, overlain with a thin layer of raw humus	4.4	15	32	8	695	28

Table 1 (*contd*).

Locality Nos (also on the map, Fig. 1)	Plot location	History	Moisture regime**	Planted tree species
7	Bauline Line (1)	Pastureland	Moist to wet heathland with a few dry patches	Red pine, *Pinus resinosa* Ait.; Norway spruce, and Sitka spruce.
8	Birchy Lake (1)	Cutover-burned	Fresh to wet	Norway spruce, black spruce, white spruce, red spruce and a cross between a Sitka spruce and a white spruce
9—17 (a)	North Pond (2)	Burned-cutover	Fresh to moist	Sitka spruce
(b)	North Pond (3)	Burned-cutover	Dry to wet (mostly fresh to moist)	Black spruce
(c)	North Pond (4)	Burned-cutover	Fresh to wet (mostly fresh to moist)	Black spruce; Sitka spruce; Jack pine, *Pinus banksiana* Lamb.

The term distribution in the present text refers to geographic location of the disease. However, the term severity refers to the amount of infection present in an area, which is expressed as a percentage of the total number of trees examined.

Sampling for Hylobius weevils

The survey for estimating the combined damage by Hylobius weevils, *H. pinicola* (Couper) and *H. warreni* Wood, was conducted only in the plantation located at Bauline Line; no weevil damage was encountered in any of the other plantations examined. The method of sampling was the same as for *Armillaria* root rot; primary roots and root collars of every chlorotic (119) and dead (103) tree, and approximately 10% of the apparently healthy trees (452) were examined. The percent weevil-infested trees and the per cent trees infected by *A. mellea* were calculated from the total trees examined.

Trees infested by the weevils exhibit characteristic symptoms of injury. Weevil larvae feed on the inner bark and cambium of the roots and root collars producing tunnels of resin and frass, frequently girdling and killing infested trees. The foliage of severely damaged trees becomes chlorotic and ultimately turns red. Trees under 1-inch stump diameter are seldom attacked and those over 8 inches, although attacked, tend to survive complete girdling, but become weak and susceptible to windthrow.

Mode of establishment	Soil characteristic					No. of infected stumps per acre	Average per cent infection
	Texture	pH	Organic matter (%)	Available nitrogen (lsb/acre)	Available phosphorus (lsb/acre)		
Bare root planting	Clay loam, thin, extremely stony, overlain with a thin layer of raw humus	4.9	12	35	6	6	12
Seedspotting	Sandy loam	4.3	12	32	4	0.7	2
Container planting***	Sandy loam	4.3	13	34	6	154	0
Seedspotting	Stony clay	4.3	14	33	6	904	0
Broadcast seeding	Sandy loam	4.3	13	31	4	759	0.2

* The numbers in patentheses indicate the number of plantations examined in that area
** After DAMMAN (1964)
*** Two types of containers were used in the two plantations: 9/16" diam. high mpact polystyrene tubes and 3" long plastic bullets

Sampling and analysis of soil

Representative soil samples were collected with a soil auger from all plantations. Each sample was composed of a mixture of the top 9-inch layer of soil[2] collected from four widely separated locations in each plantation. The composite samples were air dried at 40°C and sieved through a 2 mm mesh screen. A sample of each was also sieved through a 100 mm mesh screen to obtain a finer aggregate required for the analysis of organic matter.

The pH of the soil samples was determined, before drying, in 1 : 1 soil–water suspension using a Beckman pH meter Model Zeromatic II (JACKSON, 1958). Mechanical analysis of the dried soil samples was conducted by the hydrometer method (BOUYOUCOS, 1934; 1951) to determine the soil texture. The percent organic matter was determined by estimating the organic carbon, using the wet

[2] The surface humus and litter, along with living mosses and small herbs, were removed before collecting the soil samples.

digestion method of WALKLEY and BLACK (1934), and multiplying the results with van Bemmelen's factor of 1724. Available nitrogen (NH_4-N plus NO_3-N) was determined by the steam distillation method (BREMNER and KEENEY, 1965) and available phosphorus was determined by TRUOG's method (1930).

Results and Discussion

Results of the surveys have shown that *Armillaria* root rot is Island-wide in its distribution, and it has been observed in most plantations and on most of the 16 species examined. The distribution and severity of the disease, however, varied considerably between species and between plantations.

Tree species and provenances

Data in Table 2 show that the highest average infection of *Armillaria* root rot occurred in the genus *Pseudotsuga*, 31%; followed by 29% in the genus *Abies;* 11% in the genus *Picea;* 5% in the genus *Larix;* and 3% in the genus *Pinus*. The genera *Larix*, *Pseudotsuga* and *Pinus* had only one species each and were present in not more than one plantation.

The highest percentage of infection among the *Abies* species occurred in *A. holophylla* from Korea with an average of 79 and a range from 68 to 100; the lowest occurred in *A. balsamea* from New Brunswick, averaging 5 and ranging from 0 to 9. Two Japanese provenances of *A. Veitchii* showed the least infection (8% (b) and 13% (c)) of the *Abies* species from Asia. Infection in all the Asiatic (exotic) *Abies* species averaged 32%, but it averaged 29% in all the *Abies* species.

Picea sitchensis, from several locations in western North America, was the most susceptible among the *Picea* species. The percentage of infected trees for *P. sitchensis* averaged 35 and ranged from 0 to 67. However, per cent infected trees for all *Picea* species averaged 11. No infection was observed on a few provenances of *Picea abies*, *P. glauca*, *P. mariana*, *P. rubens* and *P. sitchensis* × *glauca*.

The root rot infection in *Larix* species, represented only by *L. leptolepis* at Cormack, averaged only 5%. Infection in *Pinus* species, represented only by *P. resinosa* at Bauline Line, averaged only 3%; and in *Pseudotsuga* species, represented only by *P. menziesii* at Cormack, it averaged 31%.

The results also indicated that in most cases the exotic species are more susceptible to the disease than are native species (Table 2). For example, infection in exotic firs averaged 32% as compared to 5% for the native fir (all bare root planting). In exotic spruces it averaged 11%[3] for native spruces in bare root plant-

[3] In native spruces (only *P. glauca* and *P. mariana*) the per cent infection averaged 20% for bare root planting. However, this data was obtained from a mixture of these two species and only from one plantation.

Table 2

Average per cent infection in provenances of sixteen conifer species by different methods of the establishment of plantations. (E = exotic or introduced species, N = native species, N* = These species are native, but their planting stock was introduced from Acadia Forest Experiment Station, New Brunswick; SS = seedspotting, BC = broadcast seeding; the figures have been rounded to the nearest whole number)

Species	Origin	Average per cent infection		
		Bare root planting	Container planting	Seedling (seedspotting & broadcast)
ABIES				
Abies balsamea (N*)	New Brunswick, Acadia For. Exp. Sta.	5		
Abies holophýlla (E)	Korea	79		
Abies homólepis (E)	Japan	33		
Abies marièsii (a) (E)	Japan, Aomori Prefecture, Arakawa Nat. Forest	33		
Abies marièsii (b) (E)	Japan, Aomori, Mt. Hakkoda	25		
Abies mayriàna (E)	Japan, Hokkaido, Ishikari, Atsuta	33		
Abies sachalinènsis (E)	Japan, Hokkaido, Sorachi, Yamabe, Tokyo Univ. Forest	35		
Abies Veitchii (a) (E)	Japan, Nagano, Kamiina, Hishiminowa, Mt. Kyogatake	30		
Abies Veitchii (b) (E)	Japan, Nagano Prefecture, Usuda	8		
Abies Veitchii (c) (E)	Japan	13		
Average for native Abies species			5	
Average for all Asiatic (exotic) Abies species			32	
Average for all Abies species			29	
LARIX				
Larix leptolepis (E)	Holland	5		
Average for Larix species			5	
PICEA				
Picea abies (E)	Bindal, Norway			0 (SS)
Picea abies (E)	Nord, Norway			4 (SS)
Picea abies (E)	Nord, Norway			0 (SS)

Table 2 (contd).

Species	Origin	Average per cent infection		
		Bar root planting	Container planting	Seeding (seedspotting & broadcast)
Picea abies (E)	—	12		
Picea abies (E)	—	13		1.4 (SS)
Average for Picea abies		13		1
Picea glauca —				
S-2438 (E)	Peterborough, Ont.	3		
S-2444 (E)	Beachburg, Ont.	6		
S-2445 (E)	Cushing, P. Q.	2		
S-2446 (E)	Beloeil, P. Q.	2		
S-2447 (E)	Grandes Piles, P. Q.	4		
S-2449 (E)	St Raymond, P. Q.	7		
S-2450 (E)	Casey, P. Q.	6		
S-2452 (E)	Lake Mattawin, P. Q.	8		
S-2453 (E)	Franchere Township, P. Q.	3		
S-2454 (E)	N. Baskatong Lake, P. Q.	2		
S-2455 (E)	Lac Dumoine, P. Q.	5		
S-2462 (E)	McNally Lake, P. Q.	3		
Picea glauca —				
S-2463 (E)	Notre Dames du Laus, P. Q.	0		
S-2464 (E)	Chalk River, Ont.	2		
S-2467 (E)	Miller Lake, Ont.	0		
S-2469 (E)	Aylmer Lake, Ont.	4		
S-2470 (E)	St. Sylvestre, P. Q.	3		
S-2471 (E)	Monk, P. Q.	3		
S-2472 (E)	Price, P. Q.	3		
S-2473 (E)	Edmundston, N. B.	5		
S-2475 (E)	Upper Green River, N. B.	3		
S-2480 (E)	Kakabeka Falls, Ont.	1		
S-2484 (E)	Mitchinamekus Lake, Ont.	1		
S-2485 (E)	Lac Simard, P. Q.	3		
S-2486 (E)	Swastika, Ont.	7		
S-2491 (E)	Valcartier, P. Q.	3		
S-2603 (E)	Marquette County, Mich.	4		
Picea glauca —				
S-2604 (E)	Shipshaw River, P. Q.	1		
Picea glauca (E)	Jutland, Denmark			0 (SS)
Picea glauca (N)	Labrador, Newfoundland			0 (SS)

Table 2 (contd.)

Species	Origin	Average per cent infection		
		Bare root planting	Container planting	Seeding (seedspotting & broadcast)
Picea glauca (E)	Sewert, Alaska			0 (SS)
Picea glauca (E)	Sewert, Alaska	14		
Average for native provenances of Picea glauca				0
Average for exotic provenances of Picea glauca		4		0
Average for all provenances of Picea glauca (N & E)		4		0
Picea mariana (E)	Ontario			0 (SS)
Average for Picea mariana				0
Picea glauca and P. mariana (N)	Newfoundland	20		0 (SS) 0.1 (BC)
Average for Picea glauca and P. mariana		20		0 0.1
Picea rubens (E)	Digby, Nova Scotia			14
Picea rubens (E)	Halifax, Nova Scotia			0
Picea rubens (E)	Unknown	2		0
Average for Picea rubens		2		5
Picea sitchensis (E)	Fisk Bay, Alaska	20		
Picea sitchensis (E)	Krozow, Alaska	55		
Picea sitchensis (E)	Lillisnoo, Alaska	34		
Picea sitchensis (E)	Old Sitka, Alaska	43		
Picea sitchensis (E)	Petersburg, Alaska	38		
Picea sitchensis (E)	Queen Charlotte Islands, B. C.	67		
Picea sitchensis (E)	Unknown	19	0	0 (SS) 0.3 (BC)
Picea sitchensis (E)	Terrace, B. C.	5		
Average for Picea sitchensis		35	0	0 0.3
Picea sitchensis × *glauca* (E)	Denmark	15		
Picea sitchensis + *glauca* (E)	Denmark			0 (SS)
Average for Picea sitchensis × *glauca*		15		0
Average for all native Picea species				0 0.1

Table 2 *(contd.)*

Species	Origin	Average per cent infection		
		Bare root planting	Container planting	Seeding (seedspotting & broadcast)
Average for all exotic Picea species		11		1.4
Average for all Picea species		11	0	1.1 0.2
PINUS				
Pinus resinosa (N*)	Newfoundland	3		
Average for Pinus resinosa		3		
PSEUDOTSUGA				
Pseudotsuga menziesii (E)	Vancouver Island, B. C.	31		
Average for Pseudotsuga species			31	
Average for all exotic species			15	1.4 0.1
Average for all native species			9	0

ing, and 1.4% as compared to 0% in seedspotting. Average infection for all exotics was 15% while that for all native species was 9% in bare root planting, and it was 1.4% for the exotic species and 0% for the native species in seedspotting.

Although no quantitative data were collected, it was observed that the root rot was progressing and its severity was increasing over the years 1968 to 1971. Many trees, which were designated healthy in 1968, were found to be chlorotic and infected in the later years. Similarly, infected trees which were noted as chlorotic in 1968, were found dead in the later years. Thus the incidence of the disease and the mortality caused by the disease has increased during recent years.

Site conditions

It is apparent that there are several factors which have influenced the severity of the root rot in these plantations. It was not possible to isolate one factor and compare its influence with the other, but moisture regime, nutrient status of the soil, history of the site, and presence of old infected stumps from previous stands appeared to have the greatest effect on the severity and distribution of the disease (Table 1).

There were marked differences in moisture conditions, ranging from dry to wet, between and sometimes within the plantations. The percentage of infection was higher (18 to 35) in fresh, moist and dry sites than in very moist to wet areas

(3 to 9) (Table 1). Similar results were also obtained by HUNTLY *et al.* (1961), TWAROWSKI and TWAROWSKA (1959), and ONO (1965 and 1970) who reported that the disease was influenced by the moisture conditions of the soils; it was least active or even absent in extremely dry or extremely wet conditions in Ontario (Canada), Poland and in northern Japan. HUNTLY *et al.* also remarked that under extreme soil moisture conditions the tree may be reduced to a low state of vigour in which it is unable to resist subsequent attack by *A. mellea.*

Furthermore, there is no direct evidence (Table 1) that variations in organic matter, pH, and available nutrients (N and P) affected the incidence of the disease. However, the high percentage of organic matter, ranging from 11 to 15 and the low pH ranging from 4.4 to 4.9 occurring on these sites apparently favored the growth of the fungus. TWAROWSKI and TWAROWSKA (1959) and SOKOLOV (1964) while working in Poland and Russia, found that a pH range of 4 to 6.6 and a high humus content were highly favorable to the growth of *A. mellea.* Available nitrogen (30 lb. to 42 lb. per acre) and phosphorus (2 lb. to 10 lb. per acre) were also below the optimum requirement of 45 lb. of nitrogen and 13 lb. of phosphorus per acre, for the growth of coniferous seedlings (WILDE, 1958). This presumably increased the susceptibility of seedlings to infection. DAY (1929), THOMAS (1934) and HUNTLY *et al.* (1961) had also remarked that trees growing in nutrient-deficient soils were more susceptible to attack by *A. mellea.*

The history of a site in the present text refers to an area having been cutover, burned, burned-cutover, farmland or pastureland. These conditions are indirectly related to the occurrence of old infected stumps. Stumps are usually present in cutovers; they are rare or almost absent in farmland or pastureland. It was observed that the disease was more abundant in cutover areas with a large number of old infected, hardwood and softwood stumps than in farmland or pastureland which had fewer such stumps (Table 1). In cutovers where the number of infected stumps per acre varied from 529 to 1106, the percentage of infection was high, ranging from 18 to 35. However, the infection was comparatively low in plantations established on old pastureland, 12%, and on abandoned farmland, 3%, where the number of infected stumps averaged 6 and 24 per acre, respectively. Infection was lowest, 2%, at Birchy Lake where infected stumps numbered less than 1 per acre. Stumps were numerous in this area but they had been severely burned by a slash fire and showed no evidence of *A. mellea;* most of them were heavily burned and therefore were probably too nutrient-poor to support the growth of *Armillaria.* HUNTLY *et al.* (1961) from Ontario, BARANYAY and STEVENSON (1964) from Alberta (Canada), and GREIG (1967) from Britain also found that the occurrence of widespread infection by *A. mellea* in plantations is always associated with the presence of abundant infection centres in the form of infected stumps; the larger and more numerous the stumps, the worse the attack.

Mode of establishment of plantations

SINGH and RICHARDSON (1973) reported that the distribution and severity of the root rot varied considerably with the mode of establishment of these plantations. Present data (Tables 1 and 2) also show that the disease was present in all plantations established with bare root stock; it was also present on some seedlings in some seeded areas but was absent on seedlings established by the container method. In areas planted with bare root seedlings the infection averaged 17%, in seedspotted areas 1%, and in broadcast seeded areas only 0.2% (Table 1).

A total of 16 species were examined during the surveys but only four species *(Picea glauca, P. mariana, P. abies* and *P. sitchensis)* had been established by more than one method. Data for these four species show that percent infection ranged from 4 to 35 for bare root planting, 0 for container planting, 0 to 4 for seedspotting, and 0.1 to 0.3 for broadcast seeding (Table 2). These results suggest that regardless of species and the number of infected stumps, trees in plantations established with bare root stock are much more susceptible to attack by *A. mellea* than those established by container planting or by direct seeding.

BUCKLAND (1953) and ONO (1970) reported that planting methods, including the skill and depth of planting, were related to the occurrence of *Armillaria* root rot. One found that the incidence of the disease was greater in unskilful plantings and in seedlings planted at depths of 5 cm and 15 cm than in those planted at a depth of 10 cm. However, he did not study the effect of various methods of establishing plantations, such as direct seedling, bare root planting and container planting. In the present investigations with these methods, records of plantation establishments indicate that seedlings in the bare root stock were planted with all the possible skill and at a depth reasonably optimum and constant in all plantations.

Damage by Armillaria root rot and Hylobius weevils

Results of the survey in the plantation infested by both the *Hylobius* weevils, *H. pinicola* (Couper) and *H. warreni* Wood, and *A. mellea* have shown that the weevil infestation in all trees was higher than infection by *A. melley*, particularly in living trees (Table 3). Damage by the weevils was highest in Sitka spruce (58%) and lowest in red pine (33%). However, *A. mellea* infection was highest in Norway spruce (5%), followed by 4% in Sitka spruce, and 3% in red pine. The per cent infection by *A. mellea* increased in trees injured by the weevils, particularly Sitka spruce, where infection was 15%, followed by 7% in Norway spruce (WARREN and SINGH, 1970). WHITNEY (1961) also found that wounds caused by these weevils provide more infection courts for the entry of root-rotting and staining fungi than any other wound type in white spruce. SMERLIS (1961) recorded a similar role for *H. pinicola* in balsam fir. HUDAK and SINGH (1970) reported a similar increase in the damage by *A. mellea* in balsam fir trees already damaged by the balsam woolly aphid.

Table 3

Per cent trees damaged by *Hylobius* weevils and *Armillaria* root rot in the Bauline Line plantation

Damaged trees	Tree species (number of trees examined are shown in brackets)			
	Pinus resino (337)	*Picea abies* (222)	*Picea sitchensis* (115)	*Pinus sylvestris** (38)
Weevil only				
Apparently healthy	31	17	17	53
Chlorotic	1	12	23	3
Dead	1	8	19	11
Total	33	37	58	66
Root rot only				
Apparently healthy	1	0	0	0
Chlorotic	0	1	1	0
Dead	1	5	3	0
Total	3	5	4	0
Weevils and root rot in the same tree				
Apparently healthy	<1	0	0	0
Chlorotic	0	<1	2	0
Dead	<1	6	13	0
Total	1	7	15	0
Total trees damaged				
Apparently healthy	32	17	17	53
Chlorotic	1	14	25	3
Dead	3	18	36	11
Total	36	49	77	66

* *Pinus sylvestris* examined are not included in the total number of trees examined in the plantation (4.620) because these were not within the limits of the 64 plots

The impact of the damage in the plantation can only be fully evaluated after considering the combined effect of the weevils and the fungus. Damage to Sitka spruce approached 77 %, it was 49% in Norway spruce, and less than 36% in red pine. Obviously Sitka spruce was the most vulnerable of the species examined when the weevils and the root rot occur simultaneously.

Conclusions and Recommendations

These surveys have shown that *Armillaria* root rot is a potentially serious disease in young softwood plantations in Newfoundland, causing up to 79% infection and up to 35% tree mortality in some plantations. The severity of the disease, however, varied between species, between planting locations, and between different modes of establishment of the plantations.

The disease was more severe on the exotic species than on the native species, particularly on firs and spruces. It was also more severe in plantations established by bare root stock than those established by container planting or direct seeding, and the incidence was highest in plantations established on cutovers which contained numerous infected hardwood and softwood stumps. The root rot was also more abundant in fresh, moist and dry sites than in very moist or wet sites.

The results of these investigations support many of the findings of previous research workers elsewhere, and indicate that the losses caused by this disease in Newfoundland can possibly be minimized by taking some precautionary measures when establishing new plantations. The least susceptible species, such as native black and white spruces, should be selected for planting. The problem may also be considerably reduced or almost eliminated by seeding rather than planting; seeding has been a practical and successful method of reforesting upland sites on the Island. All forest soils in Newfoundland have a low pH and are nutrient poor. Consequently, it may be practical to improve soil conditions by the addition of lime and fertilizer as part of the standard treatment in reforestation practices. When selecting areas for reforestation, preference should be given to those having a low number of infected stumps. Alternatively, stumps should be removed or thoroughly burned to reduce their potential as a source of infection.

The paper describes the severity and distribution of *Armillaria* root rot in softwood plantations in Newfoundland. Surveys have revealed that the root rot is Island-wide in distribution and is the most important disease in plantations. It was present on most species and in most localities examined. However, its incidence varied between species and provenances, between planting locations, and between various methods of establishment of plantations. The root rot was more abundant on exotic species than on native species. The disease was more severe in fresh, moist and dry sites than in very moist or wet areas; and in cutovers than in farmlands or pasturelands, particularly the cutovers with a larger number of old, infected stumps of hardwood and softwood trees. The root rot was also more severe in plantations established by bare root stock planting than in those established by container planting or by direct seeding. Damage by the disease increased in trees previously damaged by *Hylobius* weevils.

Literature

BARANYAY, J. A., and STEVENSON, G. R. (1964): Mortality caused by *Armillaria* root rot, *Peridermium* rusts and other destructive agents in Lodgepole pine regeneration. Forest Chron., *40*, 350–361.

BOUYOUCOS, G. J. (1934): A comparison between pipette method and the hydrometer method for making mechanical analysis of soils. Soil Sci., *38*, 335–345.

BOUYOUCOS, G. J. (1951): A recalibration of the hydrometer method for making mechanical analysis of soils. Agron. J., *43*, 434–438.

BOYCE, J. S. (1966): Forest Pathology. McGraw-Hill Book Company, New York. 572 p.

BREMNER, J. M., KEENEY, D. R. (1965): Steam distillation methods for determination of ammonium, nitrite and nitrate. Anal. Chim. Acta. *32*, 485—495.

BUCKLAND, D. C. (1953): Observations on *Armillaria mellea* in immature Douglas fir. Forest. Chron., *29*, 344—347.

CARROLL, W. J., PARROTT, W. C. (1958): Summary of forest disease conditions. In: Annual report of Nfld. Forest Protection Assoc., St. John's, Nfld. ,Pp. 81—84.

DAMMAN, A. W. H. (1964): Some forest types of central Newfoundland and their relation to the environmental factors. Soc. Amer. Foresters, For. Sci. Monograph *8*, 62 p.

DAVIDSON, A. G. and NEWELL, W. R. (1958): Forest Disease Survey. Atlantic Provinces. In: Annual Report of Forest Insect and Disease Survey. Forest Biol. Div., Sci. Serv., Can. Dept. Agric., Ottawa, pp. 25—29.

DAY, W. R. (1929): Environment and disease. A discussion on the parasitism of *Armillaria mellea*. Forestry, *3*, 94—103.

GREIG, B. J. W. (1967): Honey Fungus. Leaflet No. 6. Forestry Commission, London, England. 10 p.

HUDAK, JANOS and SINGH, PRITAM (1970): Incidence of *Armillaria* root rot in balsam fir infested by balsam woolly aphid. Can. Plant Dis. Surv., *50*, 99—101.

HUNTLY, J. H., CAFLEY, J. D. and JORGENSEN, E. (1961): *Armillaria* root rot in Ontario. Forest. Chron., *37*, 228—236.

JACKSON, M. L. (1958): Soil Chemical Analysis. Prentice Hall, Inc., Englewood Cliffs, New Jersey. 498 p.

ONO, KAORU (1965): *Armillaria* root rot in plantations of Hokkaido. — Effects of topography and soil conditions on its occurrence. Govt. Forest Expt. Sta., Tokyo, Japan. Bull. No. *179*. 62 p.

ONO, KAORU (1970): Effect of soil conditions on the occurrence of *Armillaria* root rot of Japanese larch. Govt. Forest Expt. Sta., Tokyo, Japan. Bull. No. *229*, 219 p.

PATTON, R. F. and VASQUEZ BRAVO, R. (1967): *Armillaria* root rot, *Armillaria mellea* (Vahl ex Fr.) Kummer. In: Important Forest Insects and Diseases of Mutual Concern to Canada, the United States and Mexico . . . Edited by A. G. Davidson & R. M. Prentice. Dept. of Forestry and Rural Development, Canada, Pp. 36—38.

PEACE, T. R. (1962): Pathology of Trees and Shrubs with Special Reference to Britain. Clarendon Press, Oxford. 753 p.

ROWE, J. S. (1972): Forest Regions of Canada. Dept. of the Environment, Can. For. Serv. Publ. No. *1300*, 172 p.

SINGH, PRITAM and RICHARDSON, J. (1973): *Armillaria* root rot in seeded and planted areas in Newfoundland. Forest. Chron., *49*, 180—182.

SMERLIS, E. (1961): Pathological condition of immature balsam fir stands of *Hylocomium-oxalis* type in the Laurentide Park, Quebec. Forest. Chron., *37*, 109—115.

SOKOLOV, D. V. (1964): Kornevaya Gnil' ot Openki i ber'ba S nei. (Root rot caused by *Armillaria mellea* and its control.) Izadatel' "Lesnaya Promyshlennost". Moscostvo. pp. 1—183. Russian—English translation by Canada Dept. of Forestry, Ottawa, pp. 1—235, 1966.

THOMAS, H. E. (1934): Studies on *Armillaria mellea* (Vahl) Quél., infection, parasitism and host resistance. J. Agric. Res., *48*, 187—218.

TRUOG, E. (1930): The determination of the readily available phosphorus in soil. J. Amer. Soc. Agron., *22*, 874—882.

TWAROWSKI, Z. and TWAROWSKA, I. (1959): Studia i obserwacje nad opien'kg miodowq *Armillaria mellea* (Vahl) Quél. jako przyczynq mqsowego zamierania drzewostanów (Studies and Observations on *Armillaria mellea* (Vahl) Quél. as the cause of mass dying-off of forest stands). Prace Instytutu Badawczego Lesnictwa. No. 192. Pp. 3—63. Polish—English translation by U. S. Dept. of Agric. & Nat. Sci. Found., Washington, D. C. Pp. 1—60, 1967.

WALKLEY, A. and BLACK, I. A. (1934): An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., *37*, 29—38.

WARREN, G. L. and SINGH, PRITAM (1968): Important Forest Diseases. In: Annual Report of the Forest Insect and Disease Survey, Newfoundland Region. Can. Dep. Fisheries & Forestry, Can. For. Service, Ottawa, pp. 10—17.

WARREN, G. L. and SINGH, PRITAM (1969): Important Forest Diseases. In: Annual Report of the Forest Insect and Disease Survey, Newfoundland Region. Can. Dep. Fisheries & Forestry, Can. For. Serv., Ottawa, pp. 10—19.

WARREN, G. L. and SINGH, PRITAM (1970): *Hylobius* weevils and *Armillaria* root rot in a coniferous plantation in Newfoundland Bimonthly Res. Notes, *26*, 55.

WHITNEY, R. D. (1961): Root wounds and associated root rots of white spruce. Forest. Chron., *37*, 401—411.

WILDE, S. A. (1958): Forest Soils. Their Properties and Relation to Sylviculture. The Ronald Press Company, New York. 537 p.

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Factors Regulating Diapause in Alfalfa Ladybird, *Subcoccinella 24-punctata* L. *(Col., Coccinellidae)*

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Study of the effect of photoperiod and temperature on the induction of diapause in alfalfa ladybird, *Subcoccinella 24-punctata* L. revealed that it is a long-day species and the young adult is the sensitive stage that responses to photoperiod and temperature effects. Long photoperiod (more than 15/9 hr L/D) prevented the induction of diapause to a great extent, while short one (shorter than 15/9 hr L/D) produced the greatest percentages of adult diapause. High temperature (28°C) inhibited diapause induction even under short photoperiods.

The critical photophase of alfalfa ladybird lies between 14 and 15 hr daily light. It was found that a temperature difference of 10°C caused a change of one hour in the critical photophase. Therefore, it is suggested that diapause in alfalfa ladybird is induced by the combined effect of photoperiod and temperature and that the photoperiod reaction in this species is practically temperature-dependent.

Diapause in *S. 24-punctata* L. is not only a reflect of photoperiod and temperature effects, but also a result of food quality. Adults fed on *Medicago sativa* L. developed with lower percentage of adult diapause, while those fed on red clover (*Trifolium pratense* L.) developed with higher percentage of diapause.

Alfalfa ladybird, *Subcoccinella vigintiquatuorpunctata* L. *(Col., Coccinellidae)* is one of the most common pests feeding on alfalfa (*Medicago sativa* L.) in the southwestern part of Hungary (Keszthely). There are 2—3 generations a year depending on the enviromental conditions of summer and autumn. The beetles overwinter as adults.

HAGEN (1962) described three main types of dormancy in predaceous coccinellids based upon the seasons in which the adults are reproductively inactive. LEES (1955) indicated that while diapause may be influenced by immediate environmental factors, such as temperature, food supply, and humidity, the length of day is more consistent indicator of the seasons. Photoperiod, therefore, is probably the primary factor in the initiation of diapause. The stage of the insect sensitive to the photoperiodic induction of diapause and the photoperiod regimen which induces diapause vary from species to species. The photoperiod which induces or inhibits diapause may vary with the latitude.

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HODEK and CERKASOV (1961) showed that photoperiod, temperature, and food prevent or induce diapause in *Coccinella septempunctata* L. MCMULLEN (1967) determined that these factors affect diapause in *Coccinella novemnotata*, and that the adult from emergence to the seventh day of age is sensitive to diapause induction and inhibition has also been studied in *Chilocorus bipustulatus* (PANTYUKHOV, 1968), *Hippodamia tredecimpunctata* (STORCH and VAUNDELL, 1972) and *Coccinella transversoguttata* (STORCH, 1973).

The purpose of the present investigation was to determine the effects of photoperiod, temperature, and food quality on diapause induction and inhibition of one of polyphagous coccinellids, particularly, all these factors were tested for most of predeacous coccinellids.

Materials and Methods

Experiments were performed during the years: 1970, 1971, and 1972. Adult beetles were collected in alfalfa fields at Felsőpáhok (Southwest Hungary). Stock cultures of 10 females and 10 males were placed on alfalfa culture to oviposite.

Experiments were performed in three thermostate chambers with constant temperatures of 18 ± 1 °C, 23 ± 0.8 °C, and 28 ± 0.5 °C respectively where three parallel white Tungsram florescent lamps of 40 W (with total candle-power of about 300 lux) served as light sources; the lamps were fixed in the middle of the chambers 0.7 m below ceiling. The duration of light was 17/7 hr L/D, at the same time each thermostate chamber was supplied with four photoperiod boxes of 16/8, 15/9, 14/10 and 13/11 hr L/D.

In each experimental series all the immature stages (egg, larva and pupa) and adult were exposed to each of the photoperiod regimens. Experimental series were tested for each photoperiod regimen and replicated three times.

Eggs were collected each morning from the stock cultures and placed under experimental conditions. About 16 hygrostate cultures, each containing 30–40 eggs, were kept in each five photoperiods. When the eggs hatched, the larvae were reared together and fed daily on the leaves of the host plant. In addition, the influence of host plant was included; the larvae of the dishes from 1 to 4 were fed on the leaves of *Medicago sativa* L., 5–8 on leaves of *Trifolium pratense* L., 9–12 on leaves of *Saponaria officinalis* L., and from 13–16 on *Chenopodium album* L.

Newly emerged adults were reared on the shoots of the previously mentioned food plants. Adults were exposed to each of the five photoperiod regimens. The same experiments were carried out in outdoor insectarium using the same photoperiods.

The criteria used to diagnose diapause were: (a) stop of feeding, hiding and aggregation inside the corrugated paper strips; (b) failure of a female to oviposite within a certain period (20 days) after emergence; (c) the condition of female and male reproductive gonads.

Results

Influence of photoperiod on diapause induction

The effect of different photoperiods on the incidence of diapause at rearing constant temperatures which is depicted graphically in Figure 1 show that the curve is not unique for *Subcoccinella 24-punctata* L., but it is typical for long-day insects — those enter diapause in response to short photoperiods.

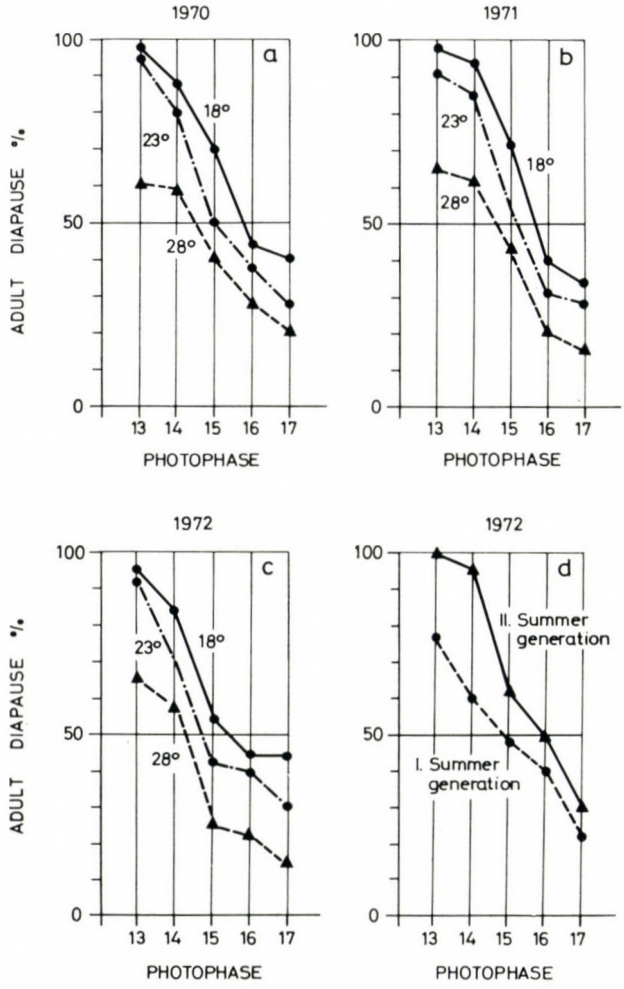

Fig. 1. Photoperiod and temperature effects on the induction of diapause in alfalfa ladybird, *S. 24-punctata* L. during three successive years (1970—1972). a, b, c, under laboratory conditions; d: incidence of diapause under natural temperature

Under the conditions tested, it was found that the critical photophase lies between 14 and 15 hours. In other words, exposing of young adults to photoperiods shorter than 14 hr daily light may induce 100% adult diapause, while photoperiods exceeding 15 hr daily light may prevent the induction of adult diapause. No significant differences were found between the proportions of adults entering diapause at one photophase and the same temperature conditions during three subsequent years (Table 1). Long photoperiod (LD 17/7 hr) appeared to prevent diapause, however, exposing of newly emerged adults to long photoperiod caused a part of the adult population to enter diapause. About 20–30% of adults went into diapause under long photoperiod even at high temperature (28°C), while 53% and more than 90% adult diapause were obtained by exposing the young adults to intermediate (LD 15/9 hr) and to short (LD 13/11 hr) photoperiods, respectively.

The influence of photoperiod on the induction of diapause in adults kept under insectarium conditions and exposed to different photophases revealed that they responded similarly to those tested at constant temperatures (Fig. 1–D). Critical photophase lies also between 14 and 15 hr daily light. Long photoperiods induced 28.1% adult diapause in the population of the first summer generation, while 29.7% was obtained in the adult population of the second summer generation. On the other hand, exposing of adults to short photoperiods resulted in 76 and 100% adult diapause for each of the first and second summer generations re-

Table 1

Percentages of adult diapause of alfalfa ladybird exposed to different photoperiods and temperatures during three successive years (1970–1972)

Temp. °C	Photoperiod hrs/day L/D	Number of individuals			% Adult diapause		
		1970	1971	1972	1970	1971	1972
18°	13/11	24	75	163	98.0±1.5	97.4±2.7	95.0±2.7
	14/10	42	60	178	97.4±2.8	98.5±2.8	83.9±2.3
	15/ 9	20	75	196	70.4±4.7	73.1±6.7	53.7±8.2
	16/ 8	40	75	169	44.3±5.5	40.6±4.5	44.2±7.3
	17/ 7	24	80	167	40.3±2.8	34.3±2.9	44.0±7.6
23°	13/11	20	95	135	95.0±3.0	91.5±2.5	92.5±4.2
	14/10	16	75	151	70.0±2.1	85.2±4.1	70.0±0.5
	15/ 9	15	75	131	61.4±4.2	55.4±3.7	42.1±4.9
	16/ 8	35	70	122	42.0±5.0	31.4±4.7	39.7±4.7
	17/ 7	40	95	143	28.2±2.2	27.7±3.3	30.2±1.9
28°	13/11	14	60	88	69.2±6.6	65.9±7.3	65.0±9.2
	14/10	15	55	71	59.4±4.6	62.7±5.1	57.0±3.8
	15/ 9	15	85	77	49.6±7.0	42.8±2.2	24.8±7.2
	16/ 8	27	85	50	38.8±3.8	22.0±5.0	21.1±2.4
	17/ 7	37	75	61	20.4±3.3	15.1±3.7	14.2±3.9

spectively. Adults exposed to intermediate photoperiod (LD 15/9 hr) showed an intermediate response to photoperiod and resulted in intermediate percentages of adult diapause.

The entering of the whole population of the last summer generation adults into diapause under insectarium conditions (Fig. 1 – D) demonstrate the great importance of photoperiod in diapause induction. During September, the daily average of temperature (15°C) was still favourable for adult development, however, the occurrence of natural day-length of about 13 hr daily light prevented adults from further development and obliged all of them to enter diapause. These results indicate that photoperiod may be the primary factor inducing diapause in alfalfa ladybird adults.

Influence of temperature on diapause induction

The results showed that the percentages of adults entered diapause under the same photoperiod diminish by the increase of temperature. Adults exposed to long photophases induced only 20 – 30% diapause, while 60 – 66% adult diapause was obtained by short photophases when they held at 28°C, comparing with 34 – 50% and 95 – 100% adult diapause at 18°C when they exposed to long and short photophases respectively. These observations demonstrate that the high temperature may avert the effect of photoperiod and diapause induction in *S. 24-punctata* L. adult is regulated by the combined effect of photoperiod and temperature.

The points of intersection between the 50% diapause line and the curves representing the three different temperatures (Fig. 1 – A, B, and C) lay within a distance corresponding to about one hour, i.e. a temperature difference of 10° centigrades causes a clear change in the critical photoperiod. This means that the photoperiod reaction of alfalfa ladybird is practically temperature-dependent.

Influence of food plants on diapause induction

The induction of diapause have been studied under laboratory as well as under insectarium conditions with young adults which had developed from eggs and larvae fed on the food plants: *Chenopodium album* L., *Medicago sativa* L., *Saponaria officinalis* L., and on *Trifolium pratense* L. Table 2 gives the percentages of adults that entered diapause at different constant temperatures and under insectarium conditions when they were exposed to various photoperiods.

It was found that the percentages of diapause induced at one temperature and photoperiod were higher in case of the adults fed on the leaves of *T. pratense* L., while the lowest were obtained by adults fed on the leaves of *M. sativa* L. There were no significant differences between the percentages of diapausing adults fed on *C. album* and those fed on *S. officinalis* L. (Table 2).

With regarding to the percentages of diapause induced under insectarium conditions, similar results were obtained; only the rate of diapause was slightly

Table 2

The percentage of adult *Subcoccinella 24-punctata* L. entering diapause under different treatment levels of photoperiod, temperature and food plant

Adult diapause %				
Photoperiod (hr/day)	0 (Total darkness)			
Food plant	*C. album*	*M. sativa*	*S. officinalis*	*T. pratense*
18°	55.5±0.2 (30)	41.2±0.2 (36)	58.7±05. (29)	66.1±1.0 (27)
23°	56.9±0.4 (30)	32.3±0.3 (30)	74.3±0.2 (30)	64.4±0.8 (20)
28°	36.0±0.3 (34)	27.1±0.2 (44)	56.6±0.2 (15)	55.2±0.5 (23)
Insectarium (1)	81.4±0.2 (30)	51.4±0.4 (30)	70.9±0.6 (30)	57.4±0.3 (30)
Insectarium (2)*	± (−)	± (−)	± (−)	± (−)
	13			
18°	94.4±5.8 (206)	95.2±3.2 (163)	98.0±4.9 (148)	100.0±0.0 (98)
23°	95.5±7.0 (145)	92.7±4.2 (135)	95.6±4.9 (169)	99.3±1.0 (133)
28°	67.2±2.0 (69)	65.5±9.2 (88)	62.5±3.9 (58)	68.4±6.9 (61)
Insectarium (1)	78.8±1.8 (83)	76.0±2.3 (122)	82.0±4.2 (82)	79.5±6.3 (76)
Insectarium (2)	100.0±5.0 (37)	100.0±0.0 (85)	100.0±7.2 (40)	100.0±1.5 (25)
	14			
18°	92.5±4.1 (163)	83.9±2.3 (178)	94.2±5.5 (73)	93.3±5.6 (116)
23°	82.0±2.0 (77)	70.0±0.5 (181)	80.8±3.3 (84)	78.3±2.3 (97)
28°	52.5±6.6 (69)	57.0±3.8 (71)	53.9±1.6 (58)	57.3±5.6 (75)
Insectarium (1)	72.2±3.5 (71)	59.1±2.1 (110)	74.0±4.8 (67)	64.9±6.5 (60)
Insectarium (2)	97.4±1.5 (45)	95.4±1.9 (60)	92.0±3.0 (40)	94.2±3.2 (25)

Adult diapause %				
Photoperiod (hr/day)	15			
Food plant	*C. album*	*M. sativa*	*S. officinalis*	*T. pratense*
18°	54.5±5.4 (158)	53.7±8.2 (186)	63.3±6.6 (96)	70.0±5.8 (147)
23°	52.2±6.7 (133)	42.1±5.0 (131)	66.2±2.5 (119)	63.9±1.7 (83)
28°	49.4±7.6 (53)	24.8±7.2 (85)	53.8±3.5 (46)	55.1±5.7 (76)
Insectarium (1)	54.0±5.8 (75)	44.5±2.7 (132)	57.0±5.1 (100)	57.0±2.4 (80)
Insectarium (2)	64.1±4.8 (53)	61.8±2.9 (71)	66.2±3.2 (40)	68.3±3.8 (25)
	16			
18°	52.7±5.1 (133)	44.2±7.3 (169)	55.1±3.5 (82)	55.7±4.0 (133)
23°	46.0±5.0 (91)	39.7±7.4 (122)	54.5±4.9 (80)	68.2±11.6 (115)
28°	49.8±3.6 (20)	22.0±5.0 (49)	43.0±4.6 (60)	48.0±3.9 (31)
Insectarium (1)	44.0±2.4 (90)	36.5±0.9 (105)	41.7±1.8 (77)	46.0±4.0 (105)
Insectarium (2)	52.9±3.0 (39)	49.1±1.3 (60)	55.1±2.5 (33)	57.5±3.6 (25)
	17			
18°	48.9±6.7 (134)	44.0±7.6 (167)	45.0±8.2 (90)	48.2±5.4 (134)
23°	39.0±4.9 (131)	30.2±2.0 (143)	43.4±3.6 (119)*u*	44.4±5.0 (99)
28°	24.8±6.8 (45)	14.2±4.0 (61)	42.2±5.2 (62)	47.9±3.9 (76)
Insectarium (1)	34.5±3.5 (110)	22.1±1.5 (113)	37.5±2.4 (95)	38.0±3.6 (104)
Insectarium (2)	36.9±2.8 (54)	29.7±2.0 (76)	43.4±2.4 (40)	47.0±3.2 (22)

* Not determined
The figures in parentheses indicate the number of newly emerged adults
(1) First summer generation
(2) Second summer generation

higher. The noticeable increase of diapause ratio can be ascribed to the fluctuation of the prevailing temperature during the experimental period. Data also revealed that the percentages of adults entering diapause under complete darkness both at constant and variable temperatures were similar to those recorded under long photoperiod (LD 17/7 hr), addition the highest percentage of diapause was ascertained also by adults fed on the leaves of *T. pratense* L.

However, the food plant influenced the induction of diapause, it was noticed also that the highest rate of adult diapause always occurred under short photoperiods. These results show that among the factors governing diapause in alfalfa ladybird, photoperiod was the prime factor and that of temperature and the type of food plant were only secondary factors that must be taken into consideration and cannot be excluded.

Discussion

Diapause in *Subcoccinella 24-punctata* L. was induced by the combined effects of photoperiod, temperature and food quality. The highest the temperature and the longer the daylength, the smaller the percentage of adults entering diapause. Beetles showed an intermediate response to photoperiod when they exposed to photoperiod of 15/9 hr LD. These results are in agreement with that obtained by STORCH and VAUNDELL (1972), LYNCH *et al.* (1972) and STORCH (1973).

The present work proved that alfalfa ladybird looks like other coccinellids, it is a long-day insect and the young adult is only the sensitive stage to photoperiod and temperature reactions. Exposing of preadult stages to different durations of light did not control the induction of diapause. Similar observations were recorded for each of *Leptinotarsa decemlineata*, (DE WILDE, 1958), *Coccinella septempunctata* (HODEK and CERKASOV, 1961), *Coccinella novemnotata* (MCMULLEN, 1967), and *Chilocorus renipustulatus* (PANTYUKHOV, 1968).

The role of photoperiod inducing diapause is much evident among long-day insects. Photophases exceed than 15 hr inhibit the onset of diapause, while shorter one induce a 100% diapause. The most interest observations on diapause phenomenon in *S. 24-punctata* L. is that there was a certain percentage of adult population entered diapause even under long photoperiod; whereas under such conditions diapause may be completely prevented. Diapause induced under long photoperiod can be ascribed to genetical variations among the individuals of the species as a result of which certain percentage is obliged to enter diapause.

This suggestion coincides with the interpretation given by HODEK and CERKASOV (1961) for the presence of a similar behaviour in the population of *C. septempunctata.* They suggested that the population of *C. septempunctata* is a mixed population, consisting of monovoltine insects with obligatory diapause and of polyvoltine ones with facultative diapause, seems to live in their country (Czechoslovakia) and probably in Central Europe generally.

Temperature plays also a great role in regulating diapause. Temperature could change the critical photophases of *Acronycta rumicis* (GORYSHIN, 1955; 1958), *Chloridea obsoleta* (DANILEVSKII, 1965), and *Athalia rose* (SÁRINGER, 1967). With alfalfa ladybird, a temperature difference of 10°C could change the critical photophase one hour, so photoperiod reaction is temperature dependent. JERMY (1967) got contradicting results with *Cydia pomonella* and concluded that the photoperiod reaction in this species is temperature-independent.

DE WILDE (1959), and JERMY (1967), found that the quality of food is one of the most decisive factors, regulating diapause. Those observations support the conclusion obtained in alfalfa ladybird, when beetles fed on alfalfa developed with small percentage of diapause, while those fed on red clover produced the highest diapause. So one can conclude that diapause in alfalfa ladybird is controlled by the combined effects of photoperiod, temperature and food quality.

Literature

DANILEVSKII, A. S. (1965): "Photoperiodism and Seasonal Development of Insects". English translation, 1965. Oliver and Boyd, Edinburgh and London.

GORYSHIN, N. I. (1955): The relation between light and temperature factors in the photoperiodic reaction in insects. Entomol. Obozr., 34, 9—14.

GORYSHIN, N. I. (1958): The effect of day length on the onset of diapause in the Colorado beetle (*L. decemlineata* Say). In the book, the Colorado beetle. Acad. Sci. USSR, M. 2, 136—149.

HAGEN, K. S. (1962): Biology and ecology of predaceous Coccinellidae. Ann. Rev. Entomol., *7*, 289—326.

HODEK, I. and CERKASOV J. (1961): Experimental influencing the imaginal diapause in *Coccinella septempunctata* L. Acta Soc. Zool. Bohemslov., *24*, 70—90.

JERMY, T. (1967): Experiments on the factors governing diapause in the codling moth, *Cydia pomonella* (Lep., Tortricidae). Acta phytopath. Acad. Sci. hung., *2*, 45—60.

LEES, A. D. (1955): The physiology of diapause in arthropods. Cambridge Univ. Press, *1*, 151 pp.

LYNCH, R. E., BRINDLEY, T. A. and LEWIS, L. C. (1972): Influence of photophase and temperature on survival and oxygen consumption of diapausing European corn borers. Ann. Entomol. Soc. Amer., *65*, 433—436.

MCMULLEN, R. D. (1967): The effect of photoperiod, temperature and food supply on rate of development and diapause in *Coccinella novemnotata*. Can. Entomol., *99*, 279—286.

PANTYUKHOV, G. A. (1968): On the photoperiodic reaction of *Chilocorus renipustulatus* Scriba. (Col., Coccinellidae). Ent. Rev. Wash., *47*, 26—29.

SÁRINGER, GY. (1967): Investigations on the light-sensitive larval instar determining the diapause of *Athalia rosae* L. Acta phytopath. Acad. Sci. hung., *2*, 119—126.

STORCH, R. H. (1973): The effect of photoperiod on *Coccinella transvensoguttata* (Col., Coccinellidae). Ent. Exp. et Appl., *16*, 77—82.

STORCH, R. H. and VAUNDELL, W. L. (1972): The effect of photoperiod on diapause induction and inhibition in *Hipodamia tredecimpunctata* (Col., Coccinellidae). Can Entomol., *104*, 285—288.

WILDE, J. DE (1958): Perception of the photoperiod by the Colorado potato beetle (*Leptinotarsa decemlineata* Say). Proc. 10th Intern. Congr. Entomol. Montreal, 1956, *2*, 213—218.

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Effect of Oviposition Stimuli and Subsequent Matings on the Viability of Eggs of *Acanthoscelides obtectus* Say *(Coleoptera, Bruchidae)*

By

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Virgin bean weevil females were mated either with normal or sterile males subsequently, and were kept in the absence of dry beans for 0, 4, 8, 12, 16 and 20 days. Their fertility and fecundity were significantly affected. The percentage of non-viable eggs proportionally increased in the absence of oviposition stimuli. The maximum increase in the number of non-viable eggs was 50% in 20 days. At the same time there was a significant decrease in the number of eggs laid. If oviposition stimuli were provided only the 20th day, the number of eggs laid was one-fifth, one-third of those laid from the first day. Females mated with normal males, and afterwards repeatedly with either sterile or normal males show high level of non-viable eggs if prevented from egg-laying for at least 12 to 16 days by the absence of oviposition stimuli.

Examining the possibilities of using the sterile insect release method (SIRM) against the bean weevil (*Acanthoscelides obtectus* Say) it has been found that, on the one hand mating competitiveness of irradiated males was equal to that of the normal ones (SZENTESI *et al.*, 1973), and on the other hand, although bean weevil females were able to mate twice and sterile sperms were even slightly higher competitive (sperm mixing), the second mating had little influence on the viability of eggs laid (HUIGNARD, 1971; SZENTESI and JERMY, 1973).

Among many factors influencing the viability of eggs and the oviposition, one with rather great importance is the availability of a suitable oviposition site within a certain period of time. Unfavourable conditions, e.g. the absence of oviposition stimuli can induce both the absorption of eggs (WIGGLESWORTH, 1965; BITSCH, 1968), and significant losses in sperm-quantity in the female during sperm storage (CUNNINGHAM *et al.*, 1971). In *Acanthoscelides obtectus* ovosorption was observed in the ovarioles (LABEYRIE, 1960).

Our observations have shown (SZENTESI, unpublished), that the majority of females mated already in the storage room before migrating to the bean fields and before seeking for suitable oviposition sites. Therefore we supposed, that the SIRM might succeed in the case only if there was a delay — caused by the absence of bean — in the oviposition of females which mated in the fields with sterile males. During this time a sterility could increase due to sperm mixing. [Sperm mixing is reported by authors in females of some species mated with sterile and fertile males, e.g. *Ceratitis capitata* (HOOPER, 1972), *Dysdercus koenigii* (HARWALKAR and RAHALKAR, 1973), *Anthonomus grandis* (LINDQUIST and HOUSE, 1967).]

Regarding these problems, experiments were carried out to determine the relative importance of factors affecting ovogenesis and oviposition and the viability of eggs laid by females alternately mated with normal and irradiated males, as well as inhibited in egg-laying by the absence of bean during sperm storage.

Material and Methods

Bean weevil adults were gained from the laboratory mass culture (SZENTESI, 1972) by applying a hatching device (SZENTESI *et al.*, 1973). In the experiments one to 7 days old virgin bean weevil females and males were used. Till the beginning of the experiments they were stored at 15°C. Males were irradiated with a dose of 10 krad of gamma radiation (^{137}Cs source, type: LMB-gamma-IM).

In order to determine the basic egg-production of the bean weevil females used, 40 virgin females of the same origin were kept at 15°C for one to seven days and then dissected and the number of mature eggs in both lateral oviducts was counted.

Virgin females were mated with normal males of the same age. (Females and males were placed into an arena and illuminated by a 250 W "Infrasec" lamp, under which the temperature varied between 25–30°C. Pairs "in copula" were separated from the others. Having finished mating the males were removed immediately in order to prevent repeated mating. Only those females of pairs were considered as "mated" in which copulation lasted at least 7 minutes, a time required for normal matings. The matings were conducted in the absence of bean.)

One female (once mated with either normal or sterile male and for the second time with a normal or a sterile male) was put into glass vials of which upper parts were dipped into talcum in order to prevent escaping of the animals. Surface provided for the movement was cca. 7.5 cm^2/animal. Three white dry beans served as ovipositional stimuli, provided either on the 1st or on the 4th, 8th, 12th, 16th, and 20th days, respectively. Each variant was repeated 20 times. The number of eggs laid in absence and in presence of oviposition stimuli was counted. The lifespan of the females and males was observed as well. Temperature of the experimental chamber was 23°C; Rh. was about 50–60%.

Results and Discussion

Factors influencing oviposition and fecundity

According to HUIGNARD (1975) no external stimuli are needed for the bean weevil females to produce about 30 mature eggs (basic egg-production) during the first days of their lifetime. There were considerable differences between selected laboratory strains in regard to response to oviposition stimuli represented by dry beans (LABEYRIE, 1961; HUIGNARD, 1975). Our experiments were carried out with

Table 1

Number of egg-laying females from 20 specimens in the absence of oviposition stimuli, and the average number of eggs deposited

Days without ovipos. stimuli	N_v♀		N♀N♂		N♀N♂ / N♀N♂		N♀N♂ / N♀S♂		N♀S♂ / N♀N♂		N♀S♂ / N♀S♂	
	No. of ♀	No. eggs	No. of ♀	No. eggs	No. of ♀	No. eggs	No. of ♀	No. eggs	No. of ♀	No. eggs	No. of ♀	No. eggs
4	—	—	3	2.3	0	0	4	9.5	0	0	3	4.5
8	—	—	11	4.3	3	9.0	5	5.6	2	15.5	5	14.6
12	—	—	11	5.9	10	8.7	13	8.0	15	10.7	11	16.0
16	—	—	9	5.3	10	11.2	9	12.4	7	16.7	11	16.7
20	13	11.1	13	14.1	10	7.1	13	11.7	11	12.6	15	14.6

No. of ♀ = number of responding females from 20 specimens
N_v♀ = normal virgin female
N♀ = normal female
N♂ = normal male
S♂ = sterile male

a natural strain which is supposedly heterogeneous in respect of oviposition behaviour. Mating is further external stimulus for ovogenesis and oviposition.

Basic egg-production. At 15°C the number of mature eggs in the lateral oviducts was 1.4 ± 2.0 on the first day, and 10.0 ± 8.6 on the 7th day. None to 15 of 20 females laid an average of 0 – 16 eggs without any stimulating effect of the host plant (Table 1). The eggs were deposited on the surface of the glass vials randomly. Thus we also found, that there was a spontaneous ovarian and ovipositional activity in virgin females without any stimulating effect.

Effect of oviposition stimuli. Fig. 1 shows that in the presence of oviposition stimuli even virgin females laid similar number of eggs compared to those of mated females. We found significant differences in the fecundity of females if bean seeds were present on the first or on the 20th days. The number of eggs laid on the 20th day was below 10 in each variant. LABEYRIE (1960) also found significant differences between the number of eggs laid during 8 days in the presence or absence of dry beans. However, the reduction in egg-number is not due to the absorption processes, because mature eggs are not resorbed in the bean weevil (LABEYRIE, 1960). From this we can conclude that not only oviposition is induced mainly by the presence of the host plant but also ovogenesis is affected.

Effects of mating(s). Examining the effect of mating HUIGNARD (1969) transplanted another bursa copulatrix (containing only one spermatophore with accessory material of a male) into bean weevil females mated earlier with normal males. There was no significant difference found, during the first 6 days between the number of eggs laid in the presence of dry bean by females having one or two spermatophores, however, significant difference appeared after 12 days. MERLE (1970) also showed increase of fecundity as a result of mating in *Drosophila.*

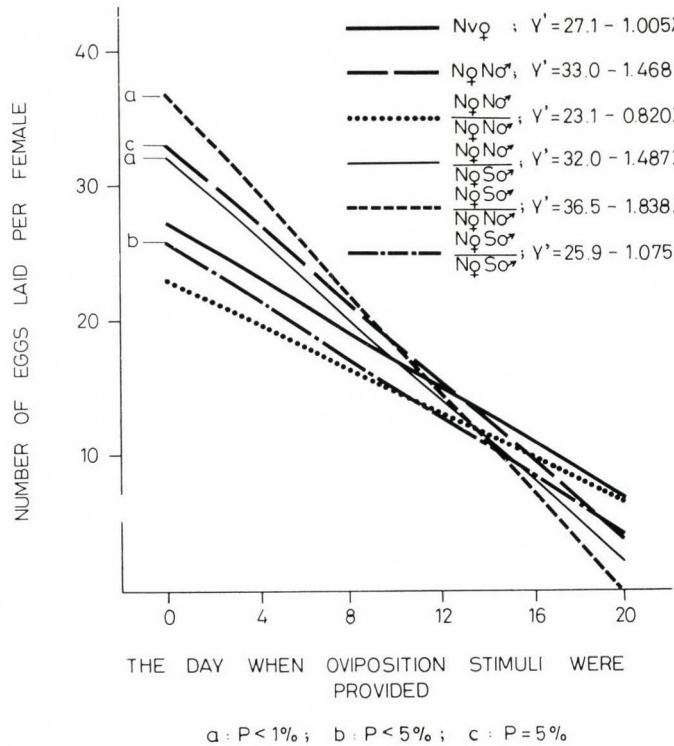

Fig. 1. Effect of time on the number of eggs laid by virgin, and mated bean weevil females, respectively, if oviposition stimuli were provided on the first, 4th, 8th, 12th, 16th, and 20th days. $N_v♀$ = normal virgin females; $N♀N♂$ = normal females mated with normal males; $\frac{N♀N♂}{N♀N♂}$ = normal females, both matings with normal males; $\frac{N♀N♂}{N♀S♂}$ = normal females, first mating with normal males, second mating with sterile males; $\frac{N♀S♂}{N♀N♂}$ = normal females, first mating with sterile males, second mating with normal males; $\frac{N♀S♂}{N♀S♂}$ = normal females, both matings with sterile males. (The values of significance refer to the number of eggs laid from the first and the 20th days, not to the relations among the curves)

Contrary to the above findings the number of eggs laid at any time in our experiments did not differ significantly after one or two matings, and from the 20th day it was equal to that of the basic egg-production. ($1.4 \pm 2.0 - 10.0 \pm 8.6$ eggs) at 15°C (Fig. 1). Females after the 20th day were not dissected, thus the exact number of mature eggs retained in the lateral oviducts is not known.

It was experienced that mating itself did not stimulate oviposition, because there were no significant differences among the number of eggs laid on any day either by virgin or twice mated females. This fact allows the conclusion that mating did not affect ovogenesis either (Fig. 1).

"Effect of duration of time" on the fertility

Virgin females either in the presence or the absence of bean lay non-viable eggs (Fig. 2, A). Females mated with only one normal male laid eggs with gradually increasing non-viability (Fig. 2, A). The rate of increase, however, is not sharp.

Females subsequently mated with normal males, laid only few non-viable eggs till the 12th day, however, there was a strong increase in the percentage of non-viable eggs from that time if oviposition stimuli were not present. There is significant difference between egg-mortality on the first and on the 20th days (Fig. 2, B).

In respect of fertility the value of one or two matings are different in certain extent. There were more non-viable eggs laid in case of one mating as compared to two matings (Fig. 2, A – B). This can be the result not only of the quantity of sperms transferred, but also of the amount of accessory gland material received by the females.

The values of non-viable eggs got, if the second mating was with a sterile male (Fig. 2, C) are the results of three factors: (1) basic egg-mortality existing even if the first mating is with a normal male, (2) the egg-non-viability increased by the sterile sperms from the second mating and (3) both are affected by the "time factor", i.e. by the duration of absence of oviposition stimuli.

If the first mating is with a sterile male, the majority of the eggs are non-viable, though the second mating with a normal male also has some effect (Fig. 2, D). Of course there is no measurable change in the percentage of non-viable eggs using totally sterile males in both matings.

Thus our experiments have strengthened the earlier findings (HUIGNARD, 1971; SZENTESI and JERMY, 1973), i.e. the first mating has a decisive role in the viability of eggs laid, however, there is sperm mixing in the bean weevil females at least for some extent.

The ratio of non-viable eggs can only be modified by the second mating, if oviposition stimuli are present immediately. This ratio increased in time with each variant in certain extent due to the absence of oviposition stimuli.

There can be several physiological reasons of the increasing ratio of non-viable eggs. The absence of oviposition stimuli either seriously affects the viability of normal sperms or favours sperm mixing during sperm storage, by which the probability of insemination by sterile sperms increases. It can be also the result of increasing genetic damage of treated sperm during storage (cited by NORTH, 1967) or similar degradation process during sperm storage in the spermatheca as reported by CUNNINGHAM *et al.* (1971) in *Ceratitis capitata*, and/or perhaps changes in the viability of mature eggs during storage in the lateral oviducts due to the inhibition in egg-laying.

The longevity of females having no chance to lay eggs on beans, was always longer than those of males and usually longer than those of females having the possibility to lay eggs just after introducing the bean seeds. (It has to be mentioned that egg-laying often began 20 – 30 minutes after providing the oviposition stimuli.)

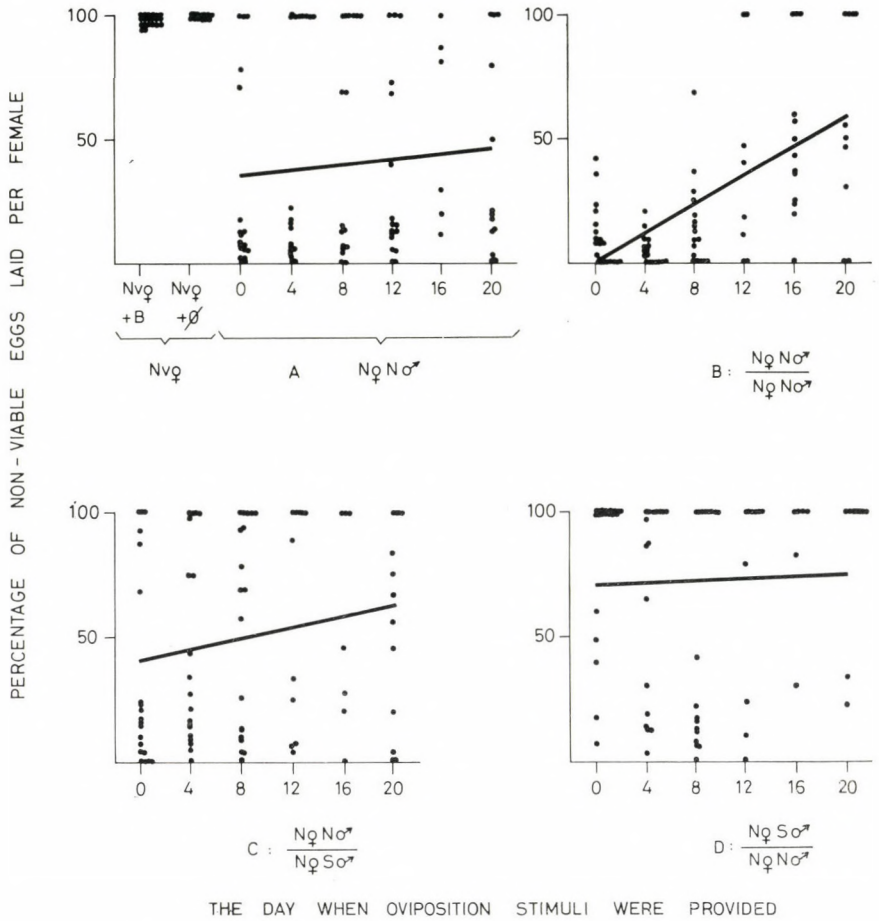

Fig. 2. Percentage of non-viable eggs laid by virgin and mated females, if oviposition stimuli were provided on the first, 4th, 8th, 12th, 16th, and 20th days. A: normal virgin females + bean ($N_v♀ + B$); normal virgin females without bean for 20 days ($N_v♀ + Ø$); normal females mated with normal males ($N♀N♂$); B: normal females, both matings with normal males $\frac{N♀N♂}{N♀N♂}$; C: normal females, first mating with normal males, second mating wi h sterile males $\frac{N♀N♂}{N♀S♂}$; D: normal females, first mating with sterile males, second mating with fertile males $\frac{N♀S♂}{N♀N♂}$. (Each point means the percentage of non-viability of eggs deposited by one female. The values of significance refer to the percentage of non-viable eggs laid from the first and the 20th days, not to the relations among the curves)

Conclusions

1. In the bean weevil, the most stimulating effect for fecundity is the presence of host plant. Oviposition depends upon the character of the bean weevil strain selected, i.e. it takes place with or without external stimuli. In case of a heterogeneous breeding line the most stimulating effect for egg-laying is the presence of host plant.

2. It is repeatedly pointed out that a second mating has no significant effect on egg-viability. Therefore, from the point of view of practical application of SIRM this species can be regarded a pseudomonogamic one.

3. On the basis of biology of bean weevil and the results gained earlier and now we can conclude that females emerging in May (approximately under field conditions), mated with normal males and migrating to the bean fields, would not find any bean plants suitable for egg-laying. In the absence of adequate oviposition stimuli they would not lay eggs till the end of their lifetime (which in this case can be even 40 days). Some females got to the fields later (e.g. from the end of July) would have the chance to lay eggs. However, if there is no available bean field nearby, it would take quite a long time for them to get there. During this period the viability of eggs would decrease and considerable sterility could occur. If there would be another possibility to mate with released sterile males, there would appear a somewhat higher level of nonviable eggs. At the same time the number of eggs laid would also show significant decrease.

On the contrary, females hatching from stored beans, mating in the store and migrating to the bean field later in the season when ripening bean pods are already present, would lay a great number of viable eggs even if mated in the field with released sterile males. Thus, releasing sterile populations in bean fields would hardly prevent infestation as it has been pointed out by HUIGNARD (1971).

Acknowledgements

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Note added in proof: Findings reported by LABEYRIE (1974) are in good accordance with the above results.

Literature

BITSCH, J. (1968): Sur les phénoménes de résorption d'oocytes chez les *Machilis* (Insectes, Thysanoures). Bull. Soc. Zool. France, *93*(3), 385—395.

CUNNINGHAM, R. T., FARIAS, G. J., NAKAGAWA, S. and CHAMBERS, D. L. (1971): Reproduction in the Mediterranean fruit fly: depletion of stored sperms in females. Ann. ent. Soc. Am. *64*, 312—313.

HARWALKAR, M. R. and RAHALKAR, G. W. (1973): Sperm utilization in the female red cotton bug. J. econ. Ent. *66*(3), 805—806.

HUIGNARD, J. (1969): Action stimulatrice du spermatophore sur l'ovogenèse chez *Acanthoscelides obtectus* Say (Insecte, Coléoptère). C. R. Acad. Sci. Paris, *268*, 2938—2940.

HUIGNARD, J. (1971): Étude a l'aide d'une technique d'irradiation aux rayons gamma de la migration et de l'utilisation des spermatozoides chez la bruche du haricot, Acanthoscelides obtectus (Say). In: Sterility principle for insect control or eradication. Symp. Athens, IAEA, 1970, Vienna, 203—216.

HUIGNARD, J. (1975): Internactions between the host-plant and mating upon the reproductive activity of *Acanthoscelides obtectus* females (Coleoptera: Bruchidae). Symp. Biol. Hung. *16*, 101—108.

HOOPER, G. H. S. (1972): Sterilization of the Mediterranean fruit fly with gamma radiation: effect of male competitiveness and change in fertility of females alternatively mated with irradiated and untreated males. J. econ. Ent. *65*(1), 1—6.

LABEYRIE, V. (1960): Action de la présence des grains de haricot sur l'ovogénèse d'*Acanthoscelides obtectus* Say (Coléoptère, Bruchidae). C. R. Acad. Sci. Paris, *250*, 2626—2628.

LABEYRIE, V. (1961): Longevité et capacité reproductrice de lignées d'*Acanthoscelides obtectus* selectionnées en fonction de la résponse aux stimuli de ponte. C. R. Soc. Biol. *155*(6), 1366—1369.

LABEYRIE, V. (1974): Actions combinées de l'age et de la date d'introduction des grains de *Phaseolus* sur la fecondité d'*Acanthoscelides obtectus*. (Abstract only). In: Regulation of insect reproduction. Int. Conf. Liblice, 9—14 Sept. 1974, Inst. Ent. Czech. Acad. Sci. Praha.

LINDQUIST, D. A. and HOUSE, V. S. (1967): Mating studies with apholate-sterilized boll weevils. J. econ. Ent. *60*(2), 468—473.

MERLE, J. (1970): Role des sécrétions génitales males dans la physiologie de la *Drosophile* femelle. In: L'influence des stimuli externes sur la gamétogenese des insectes. Coll. Int. C. N. R. S. *189*, 311—330.

NORTH, D. T. (1967): Sperm storage: modification of recovered dominant lethal mutations induced by tretamine and analogs. Mutat. Res. *4*, 225—228.

SZENTESI, Á. (1972): Studies on the mass rearing of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Acta phytopath. Acad. Sci. hung. *7*, 453—463.

SZENTESI, Á. and JERMY, T. (1973): A babzsizsik (*Acanthoscelides obtectus* Say) petéinek életképessége steril és fertilis hímekkel való párzás sorrendjétől függően. (Biability of eggs of the bean weevil depending on the succession of copulation with sterile and fertile males). Növényvéd. Korsz. *7*, 87—93.

SZENTESI, Á., JERMY, T. and DOBROVOLSZKY, A. (1973): Mathematical method for the determination of sterile insect population competitiveness. Acta phytopath. Acad. Sci. hung. *8* (1—2), 185—191.

WIGGLESWORTH, V. B. (1965): The Principles of Insect Physiology. Methuen et Co. Ltd. London

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Evidence and Preliminary Investigations on a Male Aphrodisiac and a Female Sex Pheromone in *Mamestra brassicae* (L.)

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The presence of a male aphrodisiac and a female sex pheromone in *Mamestra brassicae* (L.) has been proved by behavioural and physicochemical investigations.

The possibility of interfering with the chemical communication of insects influencing their reproductive behaviour is a potent tool to control them.

The chemical communication in mating has been investigated in the cabbage armyworm, *Mamestra brassicae* (L.), a widely polyphagous and bivoltine species, and a frequent and occasionally serious pest of cabbage in Hungary.

There are a few data concerning the male scent apparatus and aphrodisiac as well as their functions in some Noctuid species (ELTRINGHAM, 1925; APLIN and BIRCH, 1968, 1970; BIRCH, 1970, 1974; GRANT, 1971). The scent brushes described firstly by ELTRINGHAM (1925) and later by APLIN and BIRCH (1968) in *Leucania* species can be found in a pair of pockets on the ventral abdominal part of the male. The brushes only store the scent material which is secreted by a pair of glands in the second abdominal segment (Stobbe's glands). The males of *M. brassicae* possess a pair of scent brushes, arising from the second abdominal sternite and having similar morphology and function described in *Leucania*.

As for the female sex pheromone gland, SASS (unpublished) found a glandlike structure under the dorsal part of the intersegmental membrane, between the 8th and 9th abdominal segments of the female. It consists of cylindrical epithelium cells which contain many unstainable vacuoles during the imaginal life from the 3rd to 4th day on. The gland has no exit tube.

Experiments were carried out in order to prove the existence of the male aphrodisiac and the female sex pheromone in *M. brassicae*.

A laboratory strain of *M. brassicae* reared for 10 generations on a semiartificial diet (NAGY, 1970) was used. Rearing conditions: 28°C, about 50% rel. hum., 18/6 light/dark photoregime. There was a relatively high level (10—50%) of diapause in spite of the above conditions. Only diapausing specimens were used for the experiments. After storing the diapusing pupae at +5°C for a month they were sexed and kept at +25°C and about 60% rel. hum. for hatching. The investigations were carried out always during a reversed photo-regime. In order to make observations on calling, mating, etc. behaviour the dark period was provided

during working hours (9.30 a.m. to 3.30 p.m. dark, 3.30 p.m. to 9.30 a.m. light). For making a crude solution of the male scent material and the sex pheromone, the scent brushes of the males and the tips of the calling females' abdomen were cut off and extracted in n-hexane. Both extracts were stored at −20°C and then analysed by a Packard 7400 series gas chromatograph under the following conditions: *detector:* ECD; *column:* glass, length: 60 cm, inner diameter: 6 mm, filled with Anakron ABS 90−100 mesh prepared with 10% SE-30 silicone; *carrier gas:* nitrogen: 50 ml/min; *temperatures:* injection port: 150°C, column: from 50 to 140°C, programme rate: 18°C/min, detector: 200°C; *electrometer range:* 3.10^{-10} ampere.

The male aphrodisiac

According to the preliminary investigations the gaschromatogram of 3 μl of the male scent brush crude extract showed 3 peaks (Fig. 1). It can be supposed therefore, that the male scent material has 3 different components. This is in accordance with the data of BIRCH (1974) and APLIN and BIRCH (1970) who found the aphrodisiac of a closely related species, *Mamestra persicariae* (L.) to be a mixture of benzaldehyde, phenylmethyl alcohol and phenylethyl alcohol, in a rate of 10 : 2 : 85, respectively. Benzaldehyde, alone or with other minor components has been identified from five more species of *Noctuidae* (BIRCH, 1974). However, our investigations have not yet revealed benzaldehyde to be a component of the *M. brassicae* aphrodisiac. APLIN and BIRCH (1970) supposed, that the aphrodisiac of *M. brassicae* was consisted in 85% of a compound (undefined by them) with a molecular weight of 166.

In order to prove the biological activity of the extract, several experiments were carried out during the scotophase. In a series of preliminary experiments 5 days old females were placed into a petri-dish and 10 μl of the crude scentbrush extract was dropped into it. After some minutes a low percentage of the females assumed calling position (wings quivered, ovipositors extruded and the tip of the abdomen bent slightly downwards). When under the same conditions the opening of a glass vial, coated inside with the crude extract, was held close to the antennae of the females, all of them responded. Thus, it seemed to be very probable that the aphrodisiac acted only from a short distance and in a high concentration. The calling position of the females could be elicited even at daylight by the above-mentioned method. There has not been any similar result described in the literature so far. However, we could not yet prove experimentally any other role of the male scent material (i.e. arresting and receptivity stimulating effects on the female) otherwise known from the literature (BIRCH, 1970).

The responses of females of different ages to male brush crude extracts were examined in another experiment. The females to be tested were placed in a 4 litre glass jar into which air was pumped through a tube containing the crude brush extract. Twenty females were used in each trial. Some 15% of the 1 day old

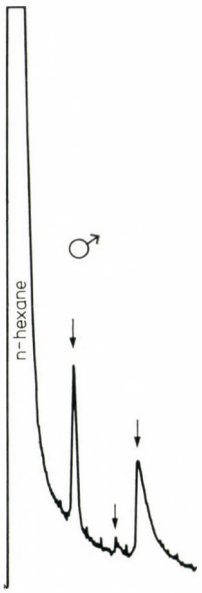

Fig. 1. Gas chromatogram of the male aphrodisiac of *Mamestra brassicae* (L.) using 3 μl of a crude extract of scent apparatuses. (The arrows indicate the supposed 3 components)

females exhibited calling position and another 15% were quivering their wings after 5 minutes of pumping. When the air flow ceased they stopped calling. Using 2 days old females 35% of them were calling after the first 5 minutes, 85% after the first hour and 100% in two hours. A 100% calling could be induced in 3 to 8 days old females after 5 minutes. When the air flow ceased the females of such ages continued calling. Each experiment was repeated 3 times.

The female sex pheromone

Under experimental conditions intensive flight and moving activity in virgin *M. brassicae* females were observed some minutes after the setting in of darkness. Mature (3 to 4 days old) females usually began to show typical calling behaviour 1 to 1.5 hours after initiating the dark period and it was maintained during the whole scotophase. However, the 1 day old females produced a surprisingly low level of calling activity, and it was in good accordance with the observations of SASS (unpublished) concerning the size of the sex pheromone gland. Simultaneously these females showed a high degree of flight activity which may serve the dispersion of the species during early adult life.

The gas chromatogram of the crude abdomen tip extract showed only one peak (Fig 2), most probably of a highly volatile substance, the composition of

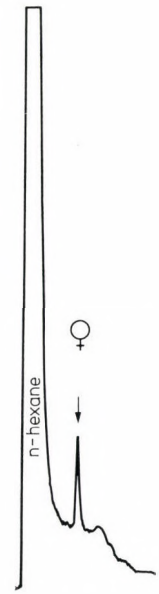

Fig. 2. Gas chromatogram of 3 μl of the crude extract made of the distal end of *Mamestra brassicae* (L.) female abdomens. (The arrow shows the supposed sex pheromone)

which was not investigated yet. Data of the female sex pheromone and its chemical characters could not be found in the literature.

The biological activity of the supposed sex pheromone has been investigated in behavioural experiments. A small cage containing 3–4 females of different ages was placed into the cage of the males. The males showed a strong flight activity, localized the cage in no time, but did not exhibit any clusper extending (which could be regarded as an adequate response to the female pheromone). It is very likely that perhaps crowding or other disturbing effects (lack of tactile stimuli, low pheromone concentration) caused the failure. However, the existence of an attractant could be assumed.

In another experiment males were placed in a glass container of 4 litres and air was pumped through it from another container holding calling females. Twenty males were used and ten 2 to 8 days old females in each case. Using 1 day old males only a slight increase in flight activity was registered after 1 hour of air flow, however, clusper extrusion could not be observed. Two days old males exhibited 3 clusper extrusions (15%) after 10 minutes of air flow and an increased flight activity could be observed. After a half an hour of ventilation with cleaned air the experiment was repeated and 50% of the males extruded their cluspers during 10 minutes of air flow. This strong response could be detected in the following 3 repetitions, too. Using 3 days old or older males, 50 to 60% of them were

extruding their cluspers during 10 minutes of air flow and all of them were highly active. Some of them even tried to mate with other males.

The experiments were repeated by pumping air into the container through a tube containing crude abdomen tip extract taken from 16 females. The same responses could be detected, thus the suspected sex pheromone was present in the extract, too. The relatively low response of the 1 day old males to the sex pheromone is very similar to that of young females to the aphrodisiac and may also serve the dispersion of the moth.

Thus, the above experiments proved the existence of both the aphrodisiac and the female sex pheromone in *Mamestra brassicae* (L.). There are experiments designed to produce a quantity needed for identification by means of gas chromatography and other methods, and to carry out laboratory behavioural investigations using olfactometers in order to gain statistically reliable data.

Literature

APLIN, R. T. and BIRCH, M. C. (1968): Pheromones from the abdominal brushes of male Noctuid Lepidoptera. Nature, Lond. *217*, 1167—1168.

APLIN, R. T. and BIRCH, M. C. (1970): Identification of odorous compounds from male Lepidoptera. Experientia, *26*, 1193.

BIRCH, M. C. (1970): Pre-courtship use of abdominal brushes by the nocturnal moth *Phlogophora meticulosa* (L.) (Lepidoptera: Noctuidae). Anim. Behav. *18*, 310—315.

BIRCH, M. C. (1974): Aphrodisiac pheromones in insects. 116—134. In: (Birch, M. C., ed.): Pheromones. Frontiers of Biology 32. North. Holland, Amsterdam. 495

ELTRINGHAM, H. (1925): Trans. Entomol. Soc. London. *73*, 1. [Cited by Aplin, R. T. and Birch, M. C. (1968)].

GRANT, G. G. (1971): Scent apparatus of the male cabbage looper, *Trichoplusiani*. Ann. ent. Soc. Amer. *64*, 347—352.

NAGY, B. (1970): Rearing of the European corn borer (*Ostrinia nubilalis* Hbn) on a simplified artificial diet. Acta phytopath. Acad. Sci. hung. *5*, 73—79.

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Beitrag zur Kenntnis der Krankheiten der Mehlmotte, *Ephestia kühniella* Zell. und der Getreidemotte, *Sitotroga cerealella* Ol. im Gebiet von Kosova

Von

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Extensive sutdies have been carried out on the diseases of two pests of stored products: flour moth (*Ephestia kühniella* Zell.) and cereal moth (*Sitotroga cerealella* Ol.) in the area of Kosova (South-Yugoslavia). The most numerous were the diseases caused by protozoa: *Gregarina polimorpha* Hammerschmidt *(Eugregarina)* and *Mattesia dispora* Naville *(Schizogregarina)*. Also combined infections by *Nosema* spp. and *Thelohania* spp. were observed.

The incidence of diseases showed considerable differences in the storerooms surveyed; in the average the larvae of flour moth were diseased to 72%, the ones of cereal moth to 32%.

Die Mehlmotte, *Ephestia kühniella* Zell. und die Getreidemotte *Sitotroga cerealella* Ol. sind häufige Schädlinge gelagerter Nahrungsmittel im Gebiet von Kosova. Wenn wir die Zahl der Generationen (bis 4 jährlich) und auch die Zahl der abgelegten Eier in Betracht nehmen, ist es leicht verständlich dass bei den klimatischen Bedingungen des Gebietes beide Schädlinge in kurzer Zeit in Mühlen und Speichern grossen Schaden verursachen können.

Mit Rücksicht auf dieser wichtigen Rolle, welche die zwei Arten spielen, war es notwendig festzustellen, welche natürliche Begrenzungsfaktoren bei diesen im Gebiet Kosova vorkommen, da in dieser Gegend viele alte Mühlen, Speicher und Lagerräume existieren, wo sich die Beziehungen zwischen den Schädlingen und deren Krankheiten und Parasiten schon seit längerer Zeit einspielen konnten und wo die zeitgemässe Bekämpfungsverfahren noch nicht eingegriffen haben.

Material and Methode

Lebendige und tote Exemplare verschiedener Stadien des *Ephestia kühniella* Zell. und *Sitotroga cerealella* Ol. wurden auf Resten alter gespeicherter Vorräte in verscheidenen Fundorten des Gebietes Kosova gesammelt und untersucht. Die Proben stammten aus den Ortschaften Pec, Radavci, Novosela, Istogu, Vitina und Liplani. Diese Dörfer liegen in einer Entfernung von 12—120 km von einander und die genannten Speicher und Mühlen liegen meist in einem Umkreis von 5—24 km um die Ortschaften. Diese Entfernungen hatten sicher einen isolie-

renden Effekt nicht nur auf die Krankheitserreger, sondern beschränkten auch die Verbreitung der Wirte.

Die Ermittlung der Infektion wurde in zwei Phasen durchgeführt, zuerst an Ort und Stelle auf Grund äusserer Symptome wie milchige Trübung, rötliche Farbe und Verkürzung des Körpers oder beschränkte Beweglichkeit der Tiere (WEISER, 1966). Als zweite Phase galt die Untersuchung der gesammelten Schädlinge unter dem Lichtmikroskop, lebendig oder in totem Zustand. Ausstriche aus den Organen der Raupen, Puppen oder Adulten wurden getrocknet, mit Methylalkohol fixiert und nach Abtrocknen mit Giemsa gefärbt. Für resistente Stadien, wie die Sporen wurden lange Färbungen über Nacht durchgeführt. Von dem ganzen gesammelten Material wurden 250 Larven der Mehlmotte und 150 Larven der Getreidemotte untersucht, das übrige Material wurde in Laborzuchten weitergeführt, um eventuelle inapparente Infektion auszulösen.

Resultate und Diskussion

In der Literatur finden wir Angaben verschiedener Authoren (STEINHAUS, WEISER u.a.) über Symptomen und Pathologie der einzelnen Krankheiten des *E. kühniella* und *S. cerealella*. Diese wurden bei Gelegenheit verschiedener auffallenden Epizootien gesammelt und untersucht. Unser Material stammt aus Lokalitäten wo die Pathogene keine auffallende Epizootien hervorgerufen haben. Dieser Umstand kann auch auf die geringe Individuenzahl zurückgeführt werden. Diesen Infektionen könnten aber bei einer höheren Dichte der Wirtspopulation in Epizootien aufflammen.

Die häufigsten Erkrankungen wurden durch *Eugregarina* (Abb. 1. a, b, c, d, e), ferner durch *Mattesia dispora* NAVILLE (Abb. 2. b, c, d) und durch gemischte Infektionen von *Nosema* spp. und *Thelohania* spp. (Abb. 2. a) verursacht. Die Teilnahme einzelner Pathogene im Gesamtbild der Erkrankungen ist aus Tabelle 1 ersichtlich.

Tabelle 1

Infektion in den Raupenpopulationen (absolute Zahlen und Prozentsatz)

Mikroorganismus	*Ephestia kühniella*		*Sitotroga cerealella*	
	Zahl	%	Zahl	%
Eugregarina spp.	95	38	16	11
Gregarina polimorpha	37	16	—	—
Mattesia dispora	28	11	—	—
Nosema und *Thelohania* Mischinfektion	18	7	41	27
Insgesamt	178	72	57	38

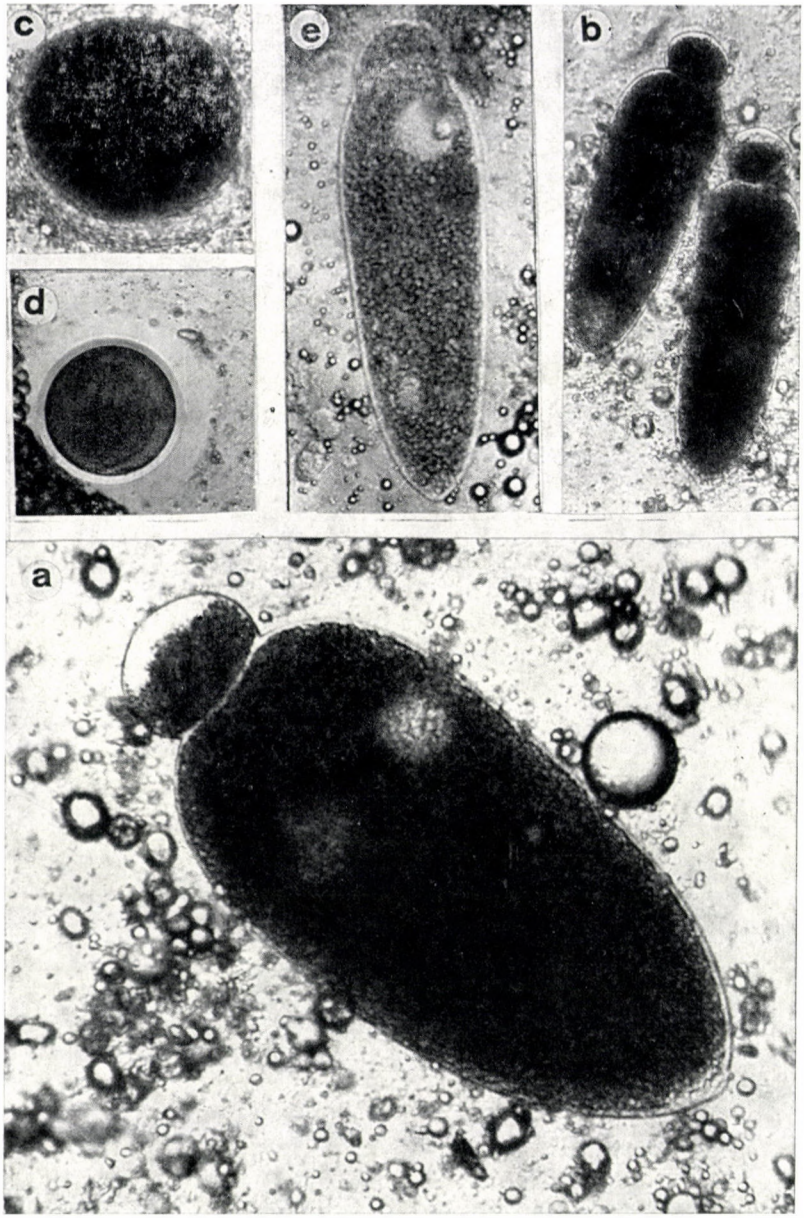

Abb. 1. a, b, c, d — *Gregarina polimorpha*, a, b — Gamonten, nativ, a — 103×, b — 400×, c, d — Cysten, nativ, c — 400×, d — 103×, e — *Gregarina* spp., Gamont, nativ, 400×

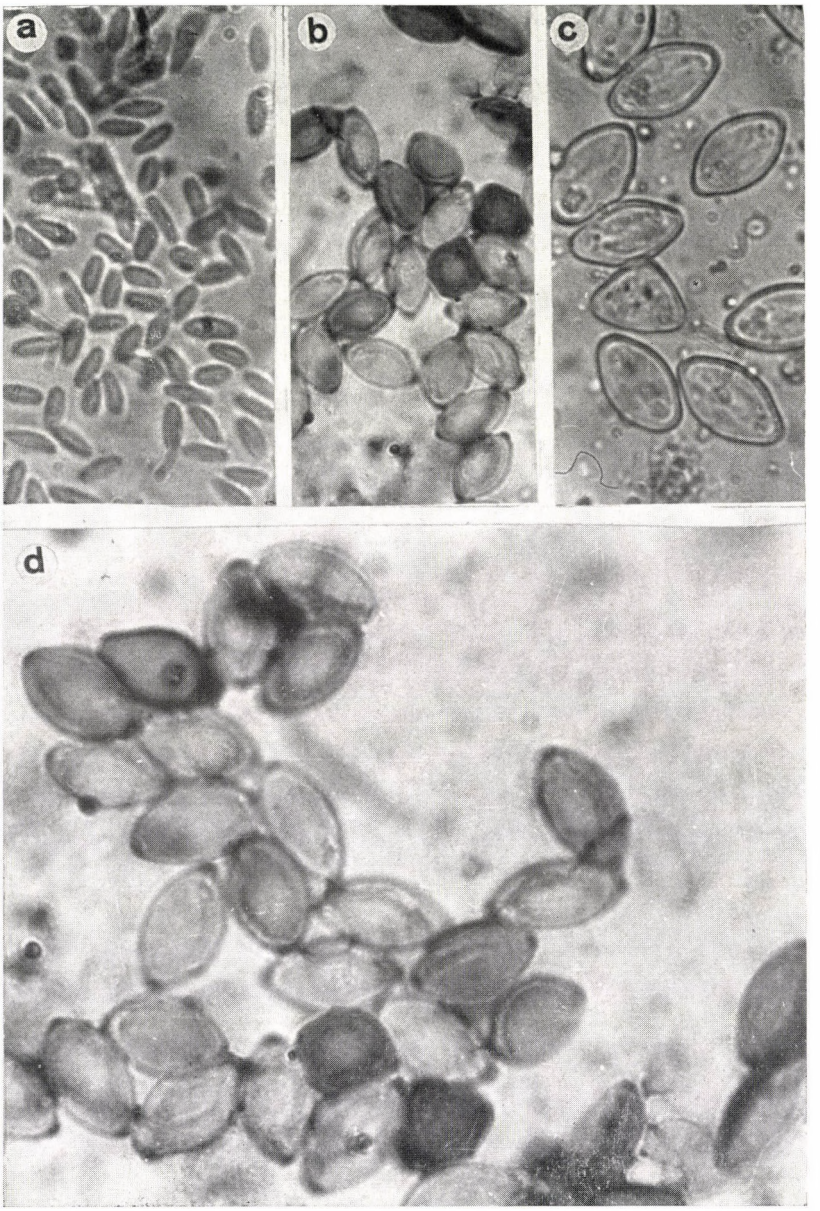

Abb. 2. a—*Nosema* spp., reife Sporen, nativ, 1000×, b, c, d — Mattesia dispora, reife Sporen, b, d — Giemsafärbung, 630×, c — nativ, 100×

Aus der Tabelle ist ersichtlich, dass die Infektion der Mehlmotte bis 72% erreicht hat, bei der Getreidemotte dagegen nur 38%. Der Unterschied kann durch das Fehlen der Mattesia und durch die niedrigere Teilnahme der Gregarinen im Infektionsbild erklärt werden.

Einzelne Erkrankungen bzw. Pathogene sind in den Abbildungen dargestellt, eine Beschreibung der neu aufgefundenen Infektionen wird später erscheinen. Im allgemeinen ist es interessant, dass die Zusammensetzung der Pathogene die in den Proben vorkommen, eine bestimmte Eigenart aufweist. Bei *Ephestia* wurden in der ČSR vor allem Erkrankungen durch *Mattesia dispora* festgestellt, dagegen waren die Mikrosporidien selten. In den Vereinigten Staaten fand KELLEN und LINDEGREN (1969) in Lepidopteren in Vorratsräumen neben *Mattesia* auch zwei Mikrosporidien, *Nosema plodiae* und *N. heterosporum*, neben einiger Virus-Infektionen. Ihr Material war jedoch reichlicher als unsere Sammlungen.

Es ist auch interessant, dass *Mattesia dispora* nicht auf *Sitotroga cerealella* überging, obzwar die Mikrosporidien auf diesem Wirt sogar reichlicher waren als bei *Ephestia*. Diese Tatsache ist auch deswegen interessant, da ein Auftreten von Mikrosporidien auf *Sitotroga* noch nicht beschrieben wurde, obzwar diese Art so häufig zur Massenzucht von *Trichogramma* benutzt wurde. Eine Mikrosporidien-Erkrankung könnte in diesen Massenzuchten einen grossen Schaden anrichten.

Es fehlen noch Angaben über die Verbreitung der Infektionen in den verschiedenen Punkten eines grösseren Gebietes, wo die abiotischen Faktoren eigentlich überall ziemlich gleich genommen werden können und nur die biotischen Faktoren eine gewisse Variabilität aufweisen. Einen Auskunft über diese Frage bringt die Tabelle 2.

Ursachen der Unterschiede zwischen den einzelnen Fundorte im Gebiet Kosova sind sicher komplex, doch die wichtigsten sind aller Wahrscheinlichkeits nach die geographischen Isolationen. Die letzteren sind durch die Gewohnheiten der Bevölkerung zustande gekommen, die von einem gewissen Kreis ihr Getreide in die Mühle oder Lagerhäuser gebracht hat. Zur Zeit unserer Untersuchungen

Tabelle 2

Verbreitung einzelner Pathogene in den verschiedenen Lokalitäten

Fundort	*Ephestia kühniella*						*Sitotroga cerealella*			
	Eugreg.		*Mattesia*		*Nosema + Thelohania*		*Eugreg.*		*Nosema + Thelohania*	
	Zahl	%	Zahl	%	Zahl	%	Zahl	%	Zahl	%
Pec (Peja)	29	22	8	28	7	39	6	38	21	51
Radavci	58	44	16	57	8	45	10	62	16	39
Novosella	23	17	—	—	—	—	—	—	4	10
Istogu	22	17	4	15	3	16	—	—	—	—
Vitina	—	—	—	—	—	—	—	—	—	—
Liplani	—	—	—	—	—	—	—	—	—	—

war die Lokalität Radavci ein wirklicher Infektionsherd für *Ephestia* sowie für *Sitotroga*. Andere, wie z. B. Vitina oder Liplani waren von den Infektionen verschont. In Novosela wurden Mikrosporidien nur bei *Sitotroga*, dagegen in Istogu nur bei *Ephestia* gefunden.

Wenn es sich noch zeigen wird, dass verschiedene Jahreszeiten auch bei Erkrankungen der Vorratsschädlinge eine wichtige Rolle spielen können und dass die beschriebenen Pathogene weit nicht die einzigen seien, brachte diese Untersuchung doch den Beweis, dass natürliche Begrenzungsfaktoren der zwei Schädlingsarten auf den Untersuchungsgebiet reichlich vorhanden sind. Sie lassen auch eine Möglichkeit zu, sie in einer biologischen Schädlingsbekämpfung zu benutzen.

Für die freundliche Hilfe bei der Bestimmung und Bearbeitung dieses Materials wie auch für alle Hinweise bei der Vorbereitung der Manuskripte danke ich herzlichst Herrn Dr. Jaroslav Weiser Dr. Sc. aus dem Entomologischen Institut der Akademie, Prag, Tschechoslowakei.

Literature

Doflein, F. (1953): Lehrbuch der Protozoenkunde, G. Fischer, Jena.

Franz, M. J. (1966): Praktische Aspekte der mikrobiologischen Bekämpfung von Schadinsekten, Med. Rijk. Landbouw. Weten, Gent, n. *3*, XXXI.

Grassé, P. (1952): Traité de Zoologie, I—II tome, Masson, Paris.

Jirovec, O. (1953): Protozoologie, Academie, Praha.

Kellen, W. R. and Lindengren, J. E. (1968): Biology of *Nosema plodiae* spp. n., a microsporidian pathogen of the Indian-meal moth, *Plodia interpunctella* Hübner. (Lep. *Phycitidae*), J. Invertebrate Pathology *11*, 104—111.

Kellen, W. R. and Lindengren, J. E. (1969): Host pathogen relationships of two previously undescribed microsporidia from the Indian-meal moth, *Plodia interpunctella* Hübner (Lep., *Phycitidae*), J. Invertebrate Pathology *14*, 328—335.

Kovaččvić, Z. (1962): Primijenjena Entomologija II, G. Zavod, Zagreb.

Krieg, A. (1972): Mikrobiologische Schädlingsbekämpfung, Biologie in unseren Zeit *2*, 178—184.

Murraj, Gj. (1970): Manual i dëmtuesve kryesore të kulturave bujqësore, Instituti i lartë shtetëror, Tiranë.

Roecki, K. (1941): Das Leben der Einzeller, Franckh'sche Verlagshandlung Stuttgart.

Steinhaus, E. A. (1947): Insect Microbiology, Comstock, Ithaca, N. Y.

Steinhaus, E. A. (1949): Principles of Insect Pathology, McGraw Hill, New York.

Weiser, J. (1961): Die Mikrosporidien als Parasiten der Insekten, Parey, Hamburg und Berlin.

Weiser, J. (1966): Nemoci hmyzu, Academie, Praha.

Weiser, J. (1969): An Atlas of Insect Diseases. Academie, Praha.

Weidner, H. (1953): Bestimmungstabellen der Vorratsschädlinge und des Hausungeziefers Mitteleuropas, G. Fischer, Jena.

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Biochemical and Chemical Factors of the Selective Antifungal Effect of Triforine

I. THE CAUSES OF SELECTIVITY OF THE CONTACT FUNGICIDAL ACTION

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The biochemical basis of selectivity of the systemic fungicide, triforine, belonging to the group of chloral-amides, was studied on seven fungus species. A positive correlation was established between the sensitivity of the fungi against triforine and the quantities of unmetabolized triforine in the mycelia or spores, respectively, following the incubation. In some of the triforine-resistant fungi the resistance is based on the lack of uptake of the active ingredient, whereas in other resistant species the fungicide becomes metabolized shortly after the uptake.

The control of plant pathogenic fungi has been considerably promoted in the last two decades by the development of systemic fungicides. Besides their advantages, however, it became soon apparent that in some microorganisms the resistance against these fungicides with specific action can be formed much faster than against the more general, contact fungicides. The recognition of this disadvantage stimulated the research for new types of active materials, which could help to enlarge the assortment of fungicides to prevent the formation of resistance in the field. One group of these new active materials is represented by the chloral-amide derivatives, which are effective mostly against powdery mildews and whose contact fungicidal activity was known earlier; a practical application was achieved, however, only with the systemic derivatives, e.g. triforine, and chloraniformethan (WOODCOCK, 1974). The most known representant of the group, triforine (N,N'-bis(1-formamido-2,2,2-trichloro-ethyl)-piperazine) appeared first under the name CELA W-524 in 1967; following some years of experimentation (FUCHS *et al.*, 1971a) it has been used since against the powdery mildew of cereals, cucumber powdery mildew, bean rust, powdery mildew and scab of fruit trees and some other fungal diseases. It may be very perspectivic, together with the chemically related chloraniformethan, which is effective against the same pathogens. Studies on this group of active materials may yield further active derivatives (CARTER *et al.*, 1972).

The inhibition of ergosterin biosynthesis is regarded as the fundamental element of the mechanism of action of triforine (SHERALD *et al.*, 1973; SISLER, 1974). Unknown was, however, the cause of selectivity of fungicidal action, which aroused the interest of many researchers. FUCHS *et al.* (1971b) have found triforine active besides against powdery mildews and rusts also against many facultative

parasites. The under "*in vitro*" conditions, (i.e. for contact action) sensitive fungi belonged in most cases to the order *Moniliales*, considerable differences were found, however, in the susceptibility of systematically closely related species (DRANDAREVSKI and FUCHS, 1973). No correlation has been established between activities observed "*in vitro*" and "*in vivo*" (on diseased plants). The data published so far referred to the spectrum of effectivity and tried to conclude on the similarity in the mechanism of action of different compounds from the identities of this spectrum (JENKIN and PREW, 1973; SHERALD *et al.*, 1973).

The lack of informations on the causes of selectivity induced the authors of the present paper to study the biochemical basis of high selectivity in fungicidal action of triforine. We hoped that at least one part of the results could be generalized to other compounds of the group as well. As the systemic character postulates a more specific (therefore also more selective) fungicidal effectivity, the discovery of the cause of selectivity may present a basis for the further research work on systemic fungicides. An organism, which is susceptible against an active material, has to be able to take up the ingredient in sufficient quantities and has to possess receptor sites which are attacked by the material in question; these receptor sites have to be essential for the organism from biochemical point of view and it is also very important that the organism should not display (or possess only very slow) biochemical processes which could metabolize and inactivate the active material before it had exerted its full effectivity. In case of precursors also the ability has to be present in the organism to transform the compound into its active form. The resistance of a given organism may follow from the absence of any of these conditions mentioned above. Therefore, from the beginning all experiments are foredoomed to failure which try to find correlations between the selectivity of action and one single biochemical factor. In our experiments we tried therefore to exclude some of the possible causes of tolerance and, in turn, to establish the presence of other causes differentiated to fungus species.

Material and Methods

The triforine used in our experiments was provided as technical grade active ingredient by the firm Celamerk. The necessary dilutions were made from the 0.5% ethanol solution of the compound.

The experiments were carried out with the following fungi:

Cladosporium cucumerinum
Stemphylium radicinum
Trichothecium roseum
Botrytis fabae
Colletotrichum atramentarium
Rhizoctonia solani
Aspergillus niger

The fungus species were selected on the basis of the agar disc test (VÖDRÖS, 1973) and their triforine susceptibility was also tested in shaken liquid cultures, in darkness, at 25–27°C, in 100 ml Erlenmeyer flasks, which contained 20 ml 2 and 5% malt medium, respectively. Into these flasks different triforine doses were added, by completing the concentration of ethanol in each case to 2%. The susceptibility tests were conducted in general with triforine concentrations of 500, 100 and 50 ppm; in case of *Cladosporium* fungi this series was completed by doses of 15 and 5 ppm. The triforine culture media were inoculated by the different fungi after a shaking of 24 hours. The inocula were taken from cultures maintained on 5% malt agar; the fungicidal effect was evaluated on the 4th and 7th day following the inoculation.

The study of uptake and metabolism was carried out with the lowest toxic triforine concentrations observed with the most susceptible fungi. Here, similarly to the conditions of activity studies, 20 ml quantities of liquid containing 2 and 5% malt, respectively, and 2% alcohol, were pre-incubated for 24 hours with equivalents of 100 and 50 ppm triforine; into the media prepared thus, were placed the mycelium units of 7–10 days (live weight of each was about 1 gram) which had been cultivated on triforine-free medium. The surface water was sucked off on G-2 glass filter. Simultaneously, identical amounts of the same mycelium were sampled and their dry weight determined. All possible measures were taken during the incubation to keep the sterile conditions. After the incubation the fungus colonies were separated of the liquid medium on porous G-2 glass filter and washed with low quantities of distilled water. The mycelia were then dried at room temperature and ground with quarz sand in 10 ml acetone; the acetone extracts were then filtered through G-5 filters, evaporated to dryness and taken up again in 2 ml ethanol. The filtrate of the incubation medium was extracted by shaking with 10 ml, then with 5 ml chloroform, the united chloroform phases evaporated to dryness and re-dissolved in 2 ml ethanol.

The triforine content of the ethanol solutions prepared from the mycelia and media, respectively, was determined by polarography. The polarographed solutions contained in 25 ml quantities 5 ml ethanol (with the aliquot part of the extract to be measured), and 10 ml Britten-Robinson buffer (pH 9). The polarography was carried out with dropping mercury electrode, measuring the direct current (between potentials of 0 and −2V) in nitrogen atmosphere. The height of the wave of triforine between −0.5 and −0.8 V was evaluated. The detection limit of triforine found 0.01 mg. (There was no possibility of spectophotometry because the absorption peak of triforine coincided with that of fungal materials present in the extracts.)

The different experimental variations were set up with three repetitions; in those series where the possibilities of a statistical analysis were given, the SD values are indicated in the Tables.

Results and Discussion

In the first phase of the experiment the order of susceptibility against triforine was studied on the fungus species included in the experiment; this was carried out both with the agar-disc method well proved also in other experiments (VÖDRÖS, 1973) and in the liquid cultures, which corresponded to the conditions of the subsequent uptake and metabolism studies. The orders of susceptibilities established with the two methods are presented in Table 1.

Table 1

Effect of different triforine concentrations on selected fungus species a) in agar disc, b) in liquid culture tests

Fungus species	a) Impregnating concentration in agar-disc test			b) Nominal concentration in liquid medium culture			
	0.5%	0.1%	0.01%	0.05%	0.01%	0.005%	0.0015%
Cladosporium	+	+−	−	+	+	+	−
Botrytis	+			+	(+)	+−	
Aspergillus	+	−	−	+	+	+	
Colletotrichum	−	−	−	+−	−	−	
Trichothecium	−	−	−	+−	−	−	
Rhizoctonia	−	−	−	(−)	−	−	
Stemphylium	−	−	−	−	−	−	

Abbreviations: + = total inhibition; (+) = nearly total inhibition;
+− = partial inhibition; (−) nearly no inhibition;
− = no inhibition

According to these studies the nominal concentrations of 50 and 100 ppm triforine, respectively, were found suitable for differentiating between susceptible and resistant species, the following experiments were then conducted with these concentrations. It was considered, that these dosages surpass the water solubility of triforine, but partly because of the mycelial uptake and as a result of decomposition, no solid-phase active material remained at the end of the incubation period. This was confirmed in distinct experiments. The low water solubility of triforine necessitated on the other hand a pre-incubation to obtain an equilibrium before adding the mycelia to the medium; the adequate dose of triforine was, therefore, shaken in the medium for 24 hours. This was followed by an incubation with the mycelia for 24 or 48 hours, then the amount of undecomposed triforine was measured both in the medium and in the mycelium, according to the method described above. Connections between the amount of triforine found in the mycelium and the susceptibility of the fungus are shown in Table 2. In case of *Cladosporium cucumerinum* and *Stemphylium radicinum* a concentration of 50 ppm was used, with an incubation period of 48 hours; with the other fungi the experimental conditions were 100 ppm triforine and 24 hours incubation.

Table 2

Relationship between the sensitivity of seven fungus species against triforine and the amounts of triforine measured in the mycelium

Fungus species	Concentration limits of toxic effect %	Data of incubation experiment:		
		treatment concentr. (ppm)	incub. time (hours)	triforin found in mycelia mg/g dry weight
Cladosporium	0.005–0.0015%	50	48	1.7 ±0.8
Botrytis	0.01 –0.005	100	24	0.65±0.1
Aspergillus	<0.005	100	24	<0.1
Colletotrichum	0.05	100	24	<0.1
Trichothecium	0.05	100	24	<0.1
Rhizoctonia	≧0.05	100	24	<0.1
Stemphylium	>0.05	50	48	<0.1

In the mycelia of fungi which were resistant against triforine in the concentrations used (Table 2) no active material could be observed, whereas the most susceptible *Cladosporium* showed nearly three times as much as the less susceptible *Botrytis*. The triforine content and susceptibility showed no correlation only in case of *Aspergillus niger*. For the proper interpretation of the latter we have to remind that the incubation experiments were carried out with pre-incubated (7–10 days old) mycelia, whereas the study of susceptibility was made with spore suspensions (naturally with the exception of *Rhizoctonia*). We were forced to do this from methodological reasons, but also theoretically it seemed admissible as literature data indicated that in case of many fungi triforine inhibits not the spore germination but the mycelial growth (DRANDAREVSKI and FUCHS, 1973; SHERALD *et al.*, 1973). So it is probable that in establishing the sensitivity, the effect exerted on the mycelial growth was measured. To interpret the abnormal reaction of *Aspergillus*, we have still to assume in this single case a relatively higher sensitivity of the spores. For this assumption the evidences were studied from two directions: first, the effectivity was studied besides on spore suspension also on pre-incubated mycelia, second, an incubation experiment was carried out with a bigger quantity of *Aspergillus* spores, to establish their triforine content. The results of both experiments are shown in Table 3.

Table 3

Comparison of sensitivity of *Aspergillus niger* spores and mycelia against 100 ppm triforine and of the amount of triforine found in them

Test object	Amount of triforine established	Inhibiting effect
Spores	0.32 mg/culture	100%
Developed mycelium	0.1 mg/g dry weight	40%

The inhibition of mycelial growth was measured on the dry weight, compared to untreated parallel incubated for the same period, as the visual evaluation of mycelium development would have contained too many subjective elements. The quantity of triforine found in the spores was expressed as the amount found in one culture unit, because the different dry weights of spores and mycelia made it difficult to find the acceptable basis of reference in both experiments. The amount of spores used in the experiment did not attain even as live weight 1 gram, so the triforine content of the *Aspergillus* spores is not less than the values presented for *Cladosporium* and *Botrytis* mycelia, shown in Table 2, regarding the correlation of sensitivity and the amount of triforine established in the fungi.

Before drawing the other conclusions, we had to get answer to the following question: is the higher triforine content found in the sensitive fungi the cause or the consequence of death. To settle this question, an incubation was made with the sublethal dose of the most sensitive fungus, *Cladosporium cucumerinum* (15 ppm triforine). Even in this case a detectable dose of triforine was present in the mycelia. The ratio of the inner concentration to the external concentration at the end of experiment was not lower in case of the little dose, than one founded in the high dose (Table 4), which gave also a quantitative evidence. The experiment proved unambigously that the triforine content of the mycelium is correlated not with the damage caused in the fungus but with the sensitivity present in advance.

The fact that for determining the triforine content, a specific chemical method could be used instead of microbiological methods (DRANDAREVSKI and FUCHS, 1973) or isotope labelling (FUCHS *et al.*, 1972) used by other authors, and also that the values established could be correlated to sensitivity, rendered very possible that triforine is the active material itself and not a mere precursor of an active ingredient. This latter statement, however, had to be proven also by biological tests on mycelium extracts. The mycelial extracts of both extremes, the sensitive *Cladosporium* and tolerant *Stemphylium* made from mycelia incubated with 50 ppm triforine, were tested against both fungi, together with extracts of untreated mycelia (Table 5). The extract of *Stemphylium* treated with triforine was not active against any of the fungi, (according to expectations), which showed that the metabolism chemically

Table 4

Amount of triforine found in *Cladosporium cucumerinum* mycelia and the ratio of concentrations formed between the fungus and medium after an incubation with lethal (50 ppm) and sublethal (15 ppm) triforine doses

Treatment	Triforine found in mycelia (mg)	Triforine concentration ratio
15 ppm	0.02(7)±0.01	22
50 ppm	0.23 ±0.04	14

Table 5

Toxic effect of extracts of *Stemphylium radicinum* and *Cladosporium cucumerinum* incubated with triforine and without triforine on the fungi tested

Variants \ Test fungi	*Stemphylium*	*Cladosporium*
Stemphylium check	−	+−
Stemphylium + triforine	−	−
Cladosporium check	−	−
Cladosporium + triforine	−	+−

Abbreviations: +− = partial inhibition, − = no inhibition

demonstrated is identical with an inactivation. It has to be mentioned only that the exudate of *Stemphylium* not treated with triforine exerted an inhibition on the development of *Cladosporium cucumerinum*, this inhibitive factor, however, was not observed in the triforine variant. This inhibition phenomenon is dealt with by one of the authors (Maya GASZTONYI) in a separate paper. The extract of triforine-treated *Cladosporium* proved to be toxic for the same fungus in the agar-disc test, but was ineffective against *Stemphylium radicinum*. This latter observation may be interpreted as follows: in the sensitive *Cladosporium* the triforine became not activated to any material which would be then effective also against the resistant *Stemphylium*, only triforine itself is present in a relatively high quantity. This in itself would not be a sufficient proof for the specific activation, as examples are known when an activation was necessary for the effectivity, the fungus could take up, however, only the precursor; we believe, however, that together with the ones mentioned above, it was sufficiently proven that triforine itself has to be regarded as the active fungicide, in accordance with the opinion expressed in the literature but not supported by experimental evidence.

There is no reason now why we could not draw the conclusion that – at least in case of the fungi studied – the cause of triforine tolerance was the lack of unmetabolized active material in the organism; so the tolerance has to be explained by no other causes than by a low uptake rate or by a strong inactivating process.

The next step was to establish, which of the two factors is present in the different species. This could be found out by determining the amount of active material in the incubation medium. In the first experiments, however, it became evident that triforine showed a slow decomposition in the aqueous phase even if no biological factors were present. This chemical decomposition had to be considered, and, therefore, we determined the rate of decomposition both in the incubation medium and in the basic solution (2% ethanol), the latter without malt content. The rate of decomposition is shown in Fig. 1.

The chemical decomposition is considerable in the aqueous solution, as shown in Fig 1 and even twice as fast in the incubation medium. In course of the experiment we maintained the sterile conditions and the incubation was made in total darkness, excluding thus the possibilities of both microbiological degra-

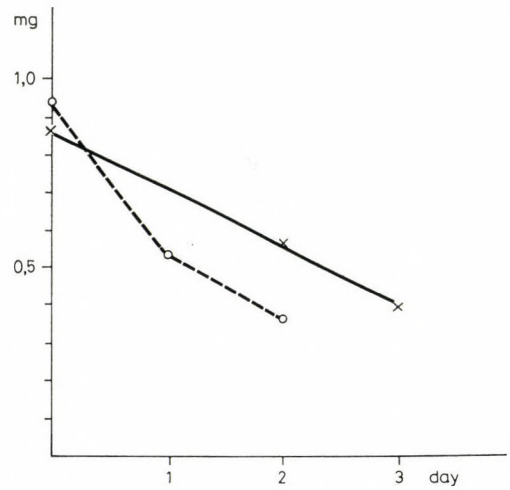

Fig. 1. Chemical decomposition of triforine — × — in 2% alcohol and — ○ — in 2% alcoho containing 2% malt. Ordinate: mg triforine per 20 ml; abscissa: No. of days

dation or photocemical decomposition, the latter mentioned by BUCHENAUER (1974). Most authors do not consider the role of mere chemical decomposition in the decrease of activity and the ones who consider it (DRANDAREVSKI and FUCHS, 1973) underestimate the speed of decomposition, based only on biological tests. The cause of this may be also the fact that these authors worked with suspensions of higher concentrations and the results were expressed in "half-life periods" that is irrealistic under these conditions. We do not know any exact data on the chemical decomposition in solutions in the literature.

It seemed the most suitable to correct the chemical decomposition (which was found not only considerable, but also depending on the experimental conditions), by carry out control experiments without fungi, parallel to each incubation and to relate the results to these. Data evaluated thus are presented in Table 6.

Significant metabolism was established in three species *(Stemphylium radicinum, Aspergillus niger* mycelium, *Colletotrichum atramentarium)*, in this order of sequence. Regarding *Aspergillus*, the different behaviour of spores and mycelia were treated already above; this manifested itself also in the fact that metabolism was observed only in incubating the active material with well developed mycelia, whereas in spore suspensions no metabolism occurred.

It was remarkable that in susceptible fungi the triforine content measured in the medium was hardly less than in the control, besides the considerable amount found inside the mycelia. So the total triforine content proved to be also higher than that of the check. This may be explained by the fact that the active material taken up (and not metabolised) in the mycelia is not exposed, or to a smaller extent, to chemical decomposition, compared to the amount of triforine staying in the outer medium. It is therefore admissible to compare only the latter amount

Table 6

Measure of triforine uptake and metabolism in seven fungus species and the conclusions drawn from the experiments

Fungus species	Triforine found in		Evaluation		
	fungi	medium			
	expressed in % of check		uptake	metabolism	result
Cladosporium	60	84	+	−	sensitive
Botrytis	5	96	+	−	sensitive
Aspergillus mycelia	2	71	+	+	resistant
Aspergillus spores	45	79	+	−	sensitive
Colletotrichum	1	79	+	+	resistant
Trichothecium	1	95	−	−	resistant
Rhizoctonia	2	93	−	−	resistant
Stemphylium	2	66	+	+	resistant
$SD_{95\%}$	3%	17%			

Abbreviations: + = positive (uptake, metabolism)
− = negative (no uptake, metabolism)

with that of the control. In fungi which metabolize triforine, the uptake cannot be shown directly but we may assume it because the metabolism occurs probably inside the organism and not by enzymes released into the medium. There is no question, however, about the absence of uptake in fungi where the presence of triforine in the mycelium could not be demonstrated and, in addition the outer medium did not exhibit a significant decrease in active material. These were: *Trichothecium roseum*, *Rhizoctonia solani*.

In the studied cases, therefore, in two species from the triforine-resistant fungi *(Stemphylium* and *Colletotrichum)* the metabolism of the active ingredient, in the other two *(Trichothecium* and *Rhizoctonia)* the low level of uptake presents the basis for their tolerance. It may be mentioned that the differences in uptake were not correlated to the pH of the incubation medium, which changed by the different fungi between 4.7 and 5.1. *Cladosporium* and *Botrytis* showed a relative sensitivity corresponded to the level of uptake; in *Aspergillus* only the spores proved to be sensitive, the developed mycelia metabolize the active material, it is, therefore, relatively more resistant.

It is worthwhile to make a difference between the two types of tolerance, from practical point of view too. The resistance based on triforine metabolism has to manifest itself in systemic effect similarly as in contact activity, while the rate of uptake may show considerable differences if it happens from the cells of the host plant or from the aqueous solution of an artificial culture. Here we have to refer again to the studies of DRANDAREVSKI and FUCHS (1973) which did not show correlation between the spectrum of effectivity measured *in vivo* and *in vitro*.

On the other hand, a resistance based on metabolism can be counteracted theoretically by using enzyme inhibiting synergists, to which many examples can be found with other pesticides.

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The authors express their sincere thanks to Mrs. M. Vödrös for the valuable technical assistance in course of the biological experiments.

Literature

Buchenauer, H. (1974): Transformation systemischer Fungizide durch Bestrahlung mit UV- und Sonnenlicht auf Oberflächen von Glas und Pflanzen. IV. Reinhardsbrunner Symposium "System-Fungizide".

Carter, C. A., Summers, Lu. A. and Wain R. L. (1972): Fungitoxicity of N-(2,2,2-trichloro-1-methoxyethyl)-formamide and related compounds. Ann. Appl. Biol., *70*, 233—243.

Drandarevski, Ch. A. and Fuchs A. (1973): The in vitro and in vivo antifungal activity of triforine. Meded. Fac. Land.-wet. Rijksuniv. Gent, *38*, 1525—1536.

Fuchs, A., Viets-Verweij, M., Vörös, J., and de Vries F. W. (1971a): Some observations on activity and metabolism of a new systemic fungicide N,N'-bis(1-formamido-2,2,2-trichloroethyl)-piperazine (CELA W 524). Acta phytopath. Acad. Sci. hung., *6*, 347—354.

Fuchs, A., Doma, S. and Vörös J. (1971b): Laboratory and greenhouse evaluation of a new systemic fungicide N,N'-bis(1-formamido-2,2,2-trichloroethyl)-piperazine (CELA W 524). Neth. J. Pl. Path., *77*, 42—54.

Fuchs, A., Viets-Verweij, M., and de Vries F. W. (1972): Metabolic conversion in plants of the systemic fungicide triforine [N, N'-bis (1-formamido-2,2,2-trichloroethyl)-piperazine, CELA W 524]. Phytopathol. Z., *75*, 111—123.

Jenkin, J. F. and Prew R. D. (1973): The effect of fungicides on incidence of Sporbolomyces spp. and Cladosporium spp. on flag leaves of winter wheat. Ann. Appl. Biol., *75*, 253—56.

Sherald, J. L., Ragsdale, N. and N. Sisler H. D. (1973): Similarities between the systemic fungicides triforine and triarimol. Pestic. Sci., *4*, 719—727.

Sisler, H. D. (1974): Fungicides affecting sterol metabolism. IV. Reinhadrsbrunner Symposium "System-Fungizide".

Vödrös M. (1973): Új módszer fungicid hatóanyagok gyors szűrővizsgálatára. A Növényvédelem Korszerűsítése, *7*, 157—162. (In Hungarian with English summary)

Woodcock, D. (1974): Factors affecting the fungitoxicity of triforine, chloraniformethan and related compounds. IV. Reinhardsbrunner Symposium "System-Fungizide".

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Effect of Copper and Chelating Agents on the Toxicity of Chelating Fungicides

By

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The effect of copper sulfate as well as the non-toxic chelator sodium dibutyldithiocarbamate on fungal growth inhibition with chelating fungicides was studied. The two powerful chelating fungicides oxine (8-hydroxyquinoline) and sodium dimethyldithiocarbamate were included. Three fungus species namely *Ascochyta pisi*, *Botrytis cinerea* and *Helminthosporium turcicum* were used as test organisms.

Results showed that the presence of copper increased the toxicity of oxine while, in most cases, did not affect that of sodium dimethyldithiocarbamate. The enhancing effect caused by copper was reduced in the presence of the dibutyldithiocarbamate salt. A sequence of zones of fungal growth and growth inhibition was obtained against *A. pisi* in the presence of sodium dimethyldithiocarbamate together with both copper sulfate and dibutyldithiocarbamate salt.

It is well known that fungicides which exert their fungitoxicity through the chelation process are greatly affected by metals. The role of metal chelation in the mechanism of the toxic action of oxine and sodium dimethyldithiocarbamate has been reviewed by several authors (HOLLINGSHEAD, 1956; HORSFALL, 1956; OWENS, 1969; LUKENS, 1969, 1971 and ALBERT, 1973).

The present work concerns the effect of some chelating fungicides on the growth of fungi and the modification of their fungitoxicity by the addition of metals and of non-toxic chelating agents.

An experimental design was applied to allow the formation of different complex compounds in the medium on which the fungus was grown.

Materials and Methods

Two highly active chelating fungicides namely oxine and sodium dimethyldithiocarbamate (Na-DMDT) were tested. Cupric sulfate pentahydrate ($CuSO_4 . 5H_2O$) and sodium di-n-butyldithiocarbamate (Na-DBDT) were selected to show the influence of metals and non-toxic chelating agents, respectively, on the toxicity of the compounds tested.

Oxine was commercially available (Reanal, Budapest) and the dithiocarbamate salts were prepared as described in literature (KLOPPING and VAN DER KERK, 1951).

Three species of fungi namely, *Ascochyta pisi*, *Botrytis cinerea* and *Helminthosporium turcicum* were used as test organisms.

The diffusion technique applied in this investigation has been used earlier to study the mode of action of dithiocarbamates against unicellular green algae (LINDAHL, 1966).

The effect of different compounds on fungal growth was investigated on Czapek's agar in 10 cm diameter petri dishes. Fifteen ml of nutrient agar was poured into each plate and covered with a 5 ml of the same nutrient medium containing a two weeks spore suspension. Filter paper strips (Whatman No. 1.4 × 40 mm), soaked in 10^{-3} mole/L water or alcoholic solution of the substance to be investigated and dried, were placed on the agar surface after the agar medium had solidified. The strips were placed to form the letter (V) in the case of the interaction between two components. In the case of studying the interaction of three components, the strips were arranged as a triangle.

Tests were carried out using glass-distilled water and precautions were taken to minimize the possibility of contamination with metals. Care was taken to maintain sterile conditions during all manipulations.

Results were recorded photographically 5 days after incubation at 25°C.

Results and Discussion

Copper sulfate was found to increase the area of growth inhibition caused by oxine agains all fungi tested (Figs 1a, 2a and 3c). These findings agreed with the earlier results of RUBBO *et al.* (1950) and ALBERT *et al.* (1953), who suggested that toxicity of oxine to bacteria is due to its chelate with metals. Chelate enters the cell as the 2 to 1 (oxine to divalent metal) complex. Within the cell, the 2 : 1 complex is in equilibrium with the 1 : 1 complex which is said to be the true toxic agent.

Both oxine and copper seem to contribute to the high fungitoxicity of the oxine : copper complexes. This was quite clear as seen from the effect of the powerful chelator Na-DBDT, which is known to be of stronger chelating capacity than oxine (SIJPESTEIJN *et al.*, 1957b). This compound was able to decrease toxicity by capturing copper ions rendering them unavailable to chelate with oxine. SIJPESTEIN and JANSSEN (1959) found that the antagonistic activity of some chelating agents, including Na-DBDT, never surpass the barrier of 5 ppm oxine. In our results, however, antagonistic effect was still observed by Na-DBDT at the applied oxine concentration which was much more higher (145 ppm).

The inhibition zones resulted with Na-DMDT were not affected by the presence of copper sulfate (Figs 1a and 2b). The only exception was the case of *H. turcicum* (Fig. 3b), where Na-DMDT alone was less toxic and copper, when added, increased the area of growth inhibition.

The growth inhibition zones observed with Na-DMDT alone is suggested to be due to that the amount soluble of the 2 : 1 (dithiocarbamate : copper) complex,

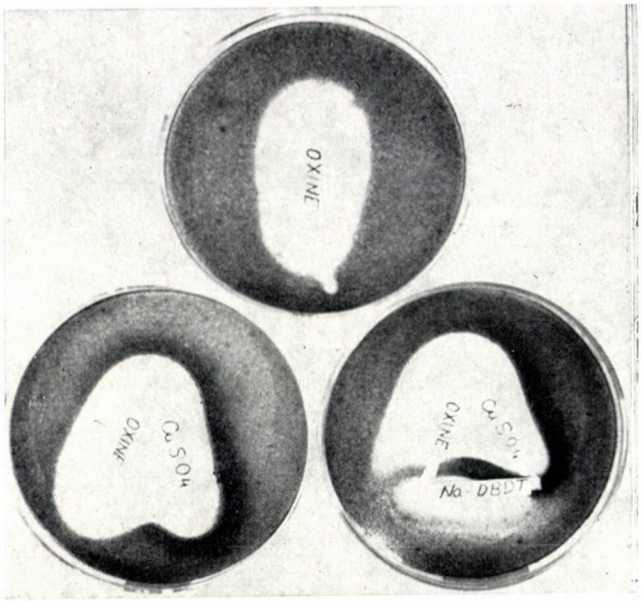

Fig. 1a. *Ascochyta pisi*

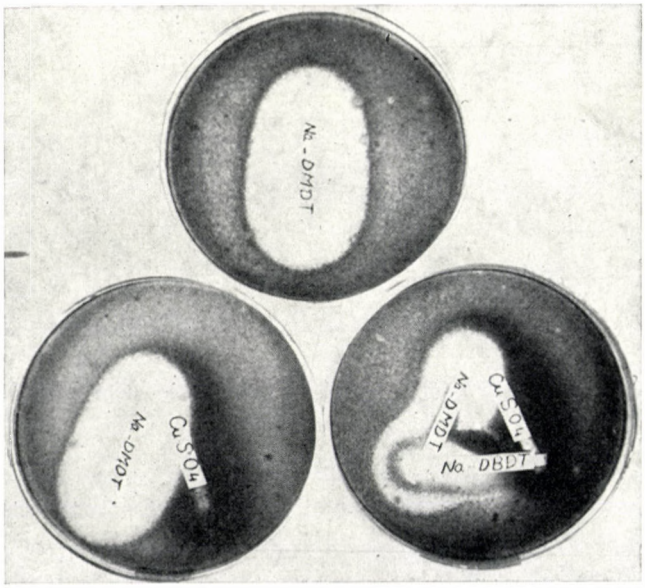

Fig. 1b. *Ascochyta pisi*

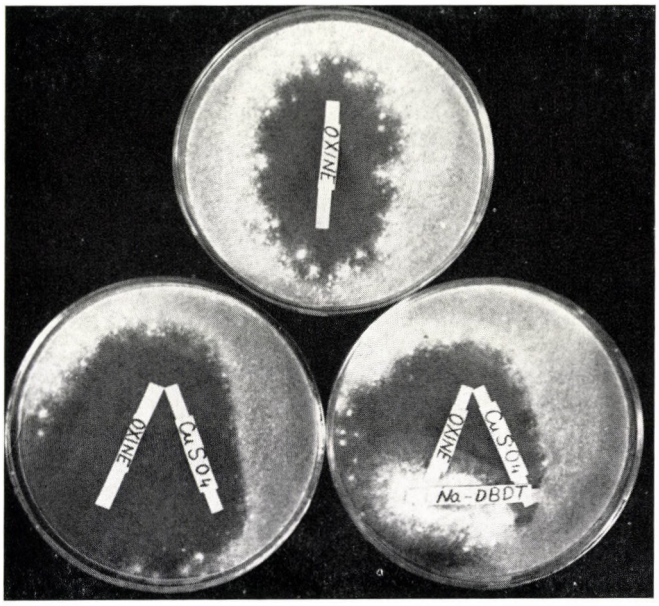

Fig. 2a. *Botrytis cinerea*

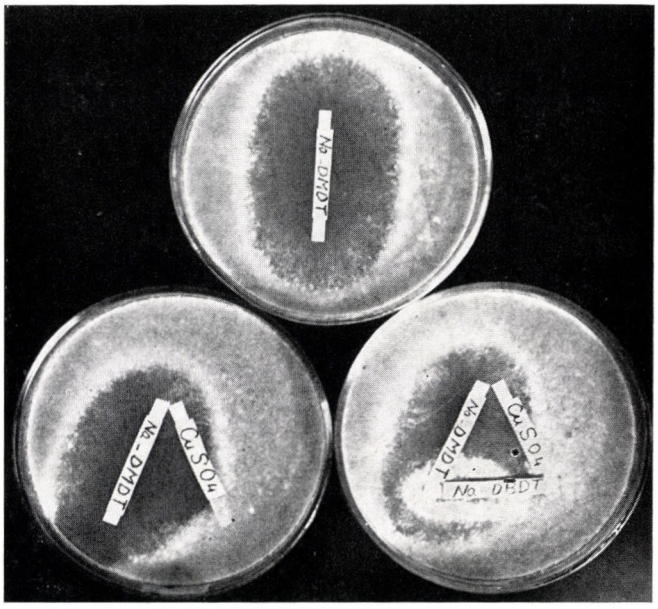

Fig. 2b. *Botrytis cinerea*

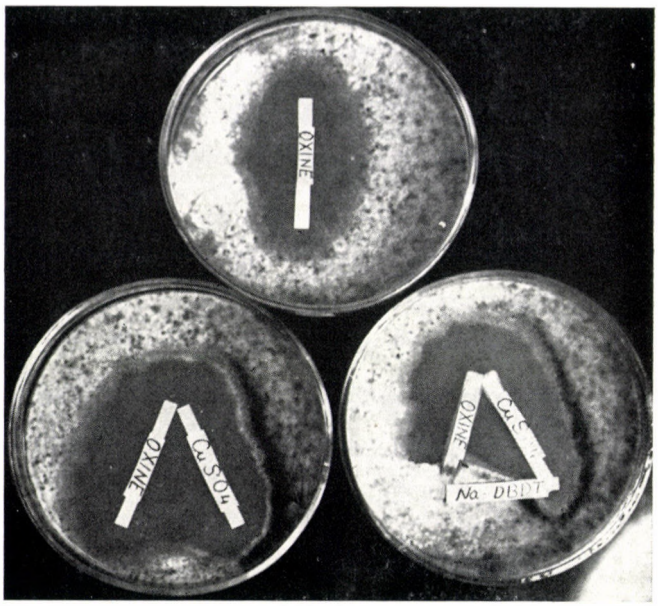

Fig. 3a. *Helminthosporium turcicum*

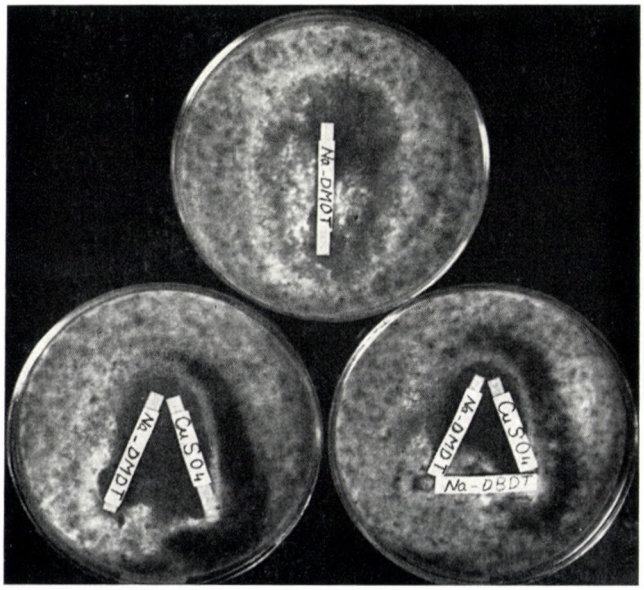

Fig. 3b. *Helminthosporium turcicum*

with copper inevitably present in the medium, is enough to inhibit the growth of *A. pisi* and *B. cinerea*. This soluble amount was not enough, however, to be inhibitory for the growth of the insensitive *H. turcicum*. The growth of the latter fungus is only inhibited by the 1 : 1 complex formed by the addition of cupric sulfate. In an investigation carried out by SIJPESTEIJN and JANSSEN (1959); they found that *Aspergillus niger* was not inhibited by the 2 : 1 ($DMDT^-$: Cu^{2+}) complex but only by the 1 : 1 complex. For *Glomerella cingulata*, however, the 2 : 1 complex had proved to be toxic in their tests.

Na-DBDT, a much more stronger chelating agent (JANSSEN, 1957) and much more less toxic than the dimethyl derivative, has a general reduction of toxicity with all fungi. Since the activity of Na-DMDT was suggested to be attributable to the complexing with copper, whether naturally present in – or experimentally added to – the medium, the antagonistic action of Na-DBDT is due to a chelate formation with those cupric ions. This complexing of Na-DBDT with copper makes the medium looks like a metal-depleted medium.

The most interesting phenomenon was observed in the effect of Na-DMDT on *A. pisi* (Fig. 1b). A sequence of zones of fungal growth and growth inhibition resulted by the addition of both cupric sulfate and Na-DBDT to the medium. This zonation was not observed in the case of Na-DMDT alone or with copper only. This so-called inversion phenomenon has been observed with Na-DMDT against *A. niger* (SIJPESTEIJN *et al.*, 1957a) and also against yeast (GOKSØYR, 1955), and was attributed to the presence of cupric ions in the medium. On the same grounds the present results may be explained. When Na-DMDT diffuses from the strip, its concentration will gradually fall because of its dilution. Thus, at the outer zone, where the dithiocarbamate concentration is less, the inhibition is due to the toxic 1 : 1 complex with copper. The zone of inversion growth occurred when the concentration of Na-DMDT was more, and at the same time, the free copper concentration was less due to the presence of the powerful chelator, Na-DBDT. Thus, the presence of the 2 : 1 complex was responsible for nontoxicity at this zone. At the inner zone, where the dimethyldithiocarbamate ions were at the maximum concentration, and the free cupric ions were at minimum, the zone of inhibition was due to the free DMDT ions. Thus, the presence of Na-DBDT resulted in a regulation of cupric ion concentration to make this phenomenon apparent.

This inversion phenomenon was observed to a limited extent with oxine against the same fungus. Differences in sensitivity of several fungi to different complexes are suggested to be the reason that it was not observed with the other fungi tested.

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Thanks are due to Dr. J. VÖRÖS for stimulating discussions and for his interest in this work.

Literature

ALBERT, A. (1973): "Selective Toxicity", 5th ed. Methuen & Co. Ltd., London.

ALBERT, A., GIBSON, M. I. and RUBBO, S. D. (1953): The influence of chemical constitution on antibacterial activity. VI. The bacterial action of 8-hydroxyquinoline (oxine). Brit. J. Exptl. Path. *34*, 119—130.

GOKSØYR, J. (1955): The effect of some dithiocarbamyl compounds on the metabolism of fungi. Physiol. Plant. *8*, 719—835.

HOLLINGSHEAD, R. G. W. (1956): Oxine and its Derivatives. Chap. 46. Butterworth, London.

HORSFALL, J. G. (1956): Principles of Fungicidal Action. Waltham, Mass., Chronica Botanica Co.

JANSSEN, M. J. (1957): The stability constants of metal complexes of some N-dialkyldithiocarbamic acids. Part II. Copper complexes in ethanol—water mixtures of various compositions. Rec. Trav. Chim. *76*, 827—835.

KLOPPING, H. L. and VAN DER KERK, G. J. M. (1951): Investigations on Organic Fungicides. IV. Chemical constitution and fungistatic activity of dithocarbamates, thiuram sulfides and structurally related compounds Rec. Trav. Chim. *70*, 917—939.

LINDAHL, P. E. B. (1966): On the mechanism of the growth inhibition in unicellular green algae by some N-disubstituted dithiocarbamate derivatives. Lantbrukshögsk. Ann. *32*, 65—86.

LUKENS, R. J. (1969): Heterocyclic Nitrogen Compounds. In: Torgeson, D. C. (ed.), Fungicides. Vol. II. pp. 395—445. Academic Press. New York and London.

LUKENS, R. J (1971): Chemistry of Fungicidal Action. In: Molecular Biology, Biochemistry and Biophysics. Springer-Verlag. Berlin, Heidelberg and New York.

OWENS, R. G. (1969): Organic Sulfur Compounds. In: Torgeson, D. C. (ed.), Fungicides Vol. II. pp. 147—302. Academic Press. New York and London.

RUBBO, S. D., ALBERT, A. and GIBSON, M. I. (1950): The influence of chemical constitution and antibacterial activity. X. The antibacterial action of 8-hydroxyquinoline (oxine). Brit. J. Exptl. Pathol. *31*, 425—441.

SIJPESTEIJN, A. KAARS and JANSSEN, M. J. (1959): On the mode of action of dialkyldithiocarbamates on moulds and bacteria. Antonie Van Leeu-Wenhoek, J. Microbiol. Ser. *25*, 422—438.

SIJPESTEIJN, A. KAARS, JANSSEN, M. J. and VAN DER KERK, G. J. M. (1957a): Investigations on Organic Fungicides. XI. The role of metals and chelating agents in the fungitoxic action of sodium dimethyl-dithiocarbamate (Na DDC). Biochim. Biophys. Acta *23*, 550—557.

SIJPESTEIJN, A. KAARS, JANSSEN, M. J. and DEKHUIJZEN, H. M. (1957b): Effect of copper and chelating agents on growth inhibition of Aspergillus niger by 8-hydroxyquinoline and pyridine-N-oxide-2-thiol. Nature *180*, 505—506.

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Inhibitors of Steroid Biosynthesis as Potential Insect Antihormones*

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Several compounds known or supposed to act as inhibitors of steroid-biosynthesis in different organisms were tested as potential inhibitors both of insect juvenile hormones and of ecdysones. Based on the analogy between early steps of human cholesterol biosynthesis and the formation of insect juvenile hormones, some mevalonic acid-analogues, known to interfere with cholesterol formation in the terpenoid stage, were tested as potential inhibitors of juvenile hormones. From the other hand, hypocholesterolaemic agents used in human medicine, as well as some systemic fungicides acting by the inhibition of ergosterol-biosynthesis were investigated for antiecdysone activity.

While the synthetic analogues of the natural mevalonic and homomevalonic acid proved to be inactive as anti-juvenoides, some inhibitors of steroid biosynthesis retarded insect pupariation, probably by interfering with ecdysone formation. Highest activity was effected by the fungicide triarimol, 2,4-dichloro-α-pyrimidin-5-ylbenzhydrol. Its effect could be reversed by simultaneous application of ecdysterone.

Although considerable interest has been devoted to insect juvenile-hormones (JH) and moulting hormones (ecdysones), as well as to synthetic JH-mimics, there is relatively little information about inhibitors of hormones regulating metamorphosis in insects. SLÁMA (1971) reported that none of the synthetic juvenile hormone mimics exerted an anti-JH effect, though many of them can be regarded as potential competitive antagonists of the natural hormones. SVOBODA *et al.* have shown, that triparanol, 1-[*p*-(2-diethylaminoethoxy)-phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl-ethanol, azasteroids, 3β-hydroxy-24-norchol-6-en-23-oic acid, as well as several new branched and straight chain secondary and tertiary amines inhibit metamorphosis and the Δ^{24}-sterol reductase in insects (SVOBODA and ROBBINS 1967; 1968; SVOBODA *et al.*, 1968; 1972; ROBBINS *et al.*, 1975).

The search for inhibitors of insect hormone-biosynthesis ("insect antihormones") represents a hopeful approach of finding selective anti-insect agents. A rational research on this field is impeded by the inadequate understanding of the mechanism of action and biogenesis of insect hormones. Yet pharmacological and human-biochemical analogies may serve as a theoretical basis for a semi-rational approach to this problem.

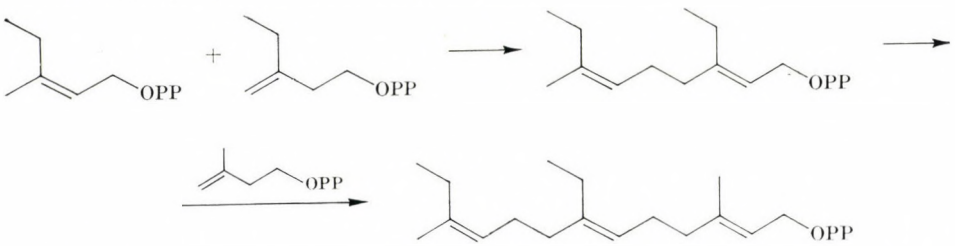
* Lecture presented at the Third International Congress of Pesticide Chemistry (IUPAC), Helsinki, 1974.

Potential inhibitors both of insect juvenile-hormones and of ecdysones were included in our experiments. This was motivated by integrating the formation of these two types of insect hormones with the general biogenesis pattern of terpenes and steroles in different living organisms. Based on similarities between the biosynthesis of early intermediates in human steroid biosynthesis and of insect juvenile-hormones, as well as on the steroidal character of ecdysones, an attempt was made to investigate the insect anti-hormone activity of compounds known or supposed to act as inhibitors of analogous processes in other organisms.

Materials and Methods

Potential inhibitors of juvenile hormone-biosynthesis

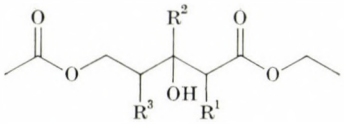
Due to insufficient knowledge on insect JH-biogenesis at time of initiating our experiments, a rational design of potential anti-juvenoides could be based on analogies rather than on a sound biochemical basis. KOYAMA *et al.* (KOYAMA *et al.*, 1972; OGURA *et al.*, 1972) proposed a biosynthesis route for JH involving a head-to-tail condensation of 3-ethyl-allyl-pyrophosphate and of 3-ethyl-but-3-enyl-pyrophosphate into bis-homo-geranyl-pyrophosphate, followed by bis-homo-farnesyl-pyrophosphate as biointermediates.

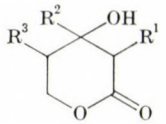


Based on a close analogy with the formation of cholesterol via mevalonate and farnesyl-pyrophosphate in vertebrates, the intermediacy of 3-ethyl-3,5-dihydroxy-valerolactone (homomevalolactone *(7)*) could be postulated. Later on, SIDDALL *et al.* (1974) gave experimental evidence of the involvement of homo-nevalonic acid in JH-biogenesis, thus confirming our assumption.

Based on the close analogy between the biogenesis of juvenile hormones and early steps in human cholesterol biosynthesis from the one hand and by successful inhibition of cholesterol formation in test organisms by mevalonate-analogues (STEWART and WOOLLEY, 1959; WRIGHT, 1957) from the other, several synthetic mevalonate-analogues were tested by us for anti-JH activity. Ethyl 2-methyl-3-ethyl-3-hydroxy-5-acetoxy-valerate *(2)*, the esterified 2-methyl-analogue and potential synthetic antimetabolite of homomevalonic acid, as well as the corresponding lactone *(8)* were not known in the literature and were prepared on the analogy of the method described by HOFFMAN *et al.* (1957). Ethyl 5-acetoxy-

homomevalonate *(1)*, ethyl 5-acetoxy-mevalonate *(3)*, its methyl- and dimethyl-homologues *(4, 5, 6)*, as well as the corresponding lactones *(7, 9, 10, 11, 12)* were re-synthesized according to known methods (STEWART and WOOLLEY, 1959; WRIGHT, 1957; HOFFMAN *et al.*, 1957).





	R^3	R^2	R^1		R^3	R^2	R^1
1	H	C_2H_5	H	**7**	H	C_2H_5	H
2	H	C_2H_5	CH_3	**8**	H	C_2H_5	CH_3
3	H	CH_3	H	**9**	H	CH_3	H
4	H	CH_3	CH_3	**10**	H	CH_3	CH_3
5	CH_3	CH_3	H	**11**	CH_3	CH_3	H
6	CH_3	CH_3	CH_3	**12**	CH_3	CH_3	CH_3

To test compounds for anti-juvenile hormone activity penultimate instar larvae of the bug, *Pyrrhocoris apterus* were used, as recommended by SLÁMA (1974). The freshly moulted animals were narcotized by submerging under water for 10–15 min. 1 μl of the acetonic solution of the chemical was topically applied to the larvae. The insects were then reared in petri-dishes, supplied with food and water. The evaluation was performed after the next ecdysis. A dosage of 50 μg/insect was used in the first and 30 μg/insect in a second series of experiments.

Potential anti-ecdysones

While a proposed analogy between JH biogenesis and the early steps of cholesterol formation served for us as theoretical basis in testing mevalonate analogues for anti-juvenile effect, the steroidal character of the moulting hormones (ecdysones) inspired us to assay for anti-ecdysone activity some hypocholesterolaemic agents interfering with late steps of steroid biosynthesis. Compounds acting on that way are widely used for the prevention of atheroschlerosis. Additional support for our investigations was provided by the encouraging results of SVOBODA et al. obtained with 20,25-diazacholesterol and triparanol. These compounds inhibit Δ^{24}-sterol reductase, thus affecting larval development regulated by the steroidal ecdysone hormones.

The following known hypocholesterolaemic agents were tested for anti-ecdysone activity: butyl 2-phenyl-butyrate; 2-(4-chlorophenyl)-valeric acid; 2-(4-chlorophenyl)-valeronitrile; β-diethylaminoethyl 2,2-diphenyl-valerate hydrochloride (SKF 525); 2-(4-chlorophenoxy)-2-methylpropionic acid; bis-(4-chlorophenoxy)-acetic acid; β-diethylaminoethyl bis-(4-chlorophenoxy)-acetate; 1-cyano-1-phenylamino-cyclohexane; ethyl N-benzyl-N-benzyloxy-carbamate *(13)*; trans-

1,4-bis-(2-chlorobenzyl-aminomethyl)-cyclohexane dihydrochloride *(14)*; 2-(β-diethylaminoethyl-mercapto)-benzimidazole.

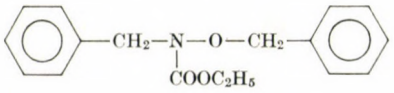
The systemic fungicides triforine, 1,4-di(2,2,2-trichloro-1-formamidoethyl)-piperazine *(15)* and triarimol, 2,4-dichloro-α-pyrimidin-5-ylbenzhydrol *(16)* are effective inhibitors of ergosterol biosynthesis (SHERALD *et al.*, 1973; SISLER, 1974), justifying their inclusion into our tests. Although not demonstrated experimentally, structural similarities suggest the same mechanism for chlotrimazol, 1-(o-chlorotrityl)-imidazol used in human therapy against dermatomycoses and trichomoniasis

Two DDT-analogues devoid of insecticidal activity, such as 2-o-chlorophenyl-2-p'-chlorophenyl-1,1-dichloroethane (o,p'-DDD) and bis-p-hydroxyphenyl-1,1,1-trichloroethane were also tested within this series. The test of these two compounds was motivated mainly by the inhibited hydroxylation of cholesterol in animals following administration of o,p'-DDD (BERGENSTAL *et al.*, 1960).

The hydroxylation reaction leading to the conversion of plant sterols required in the diet into ecdysones is probably governed by a yet unidentified metal dependent oxydative enzyme (FUNATSU *et al.*, 1972). As these enzymes are highly sensitive to chelating agents, 8-oxyquinoline and some of its lipophylic derivatives were tested by us for anti-ecdysone activity. 8-Butylcarbamoyloxy-quinoline and 8-phenylcarbamoyloxy-quinoline are described in the literature as antimicrobial and antimalarial agents, respectively. 2-(2'-Hydroxy-2',2'-diphenyl-ethyl)-8-hydroxyquinoline *(17)*, one of the compounds synthesized in the course of our studies on specific inhibitors of ecdysone biosynthesis (MAEKAWA and MATOLCSY, 1975) was also tested within this series.

Several polyene macrolide antibiotics and saponins exert their antifungal action by physicochemical adsorption to steroles serving as vital constituents of the protoplasmic membrane. It seemed us indicated to test whether these compounds can result an anti-ecdysone effect based on physicochemical adsorption to the steroidal insect hormones. The compounds tested for anti-ecdysone activity within this type were: nystatin, a polyene macrolide antibiotic; cyclamin, an antifungal saponin present in Cyclamen sp., digitonin known to yield insoluble complexes with β-steroles and methanolic extracts of mycelia obtained from cultures of Streptomyces strains producing polyene macrolides.

Full-grown larvae of the fleshfly *Sarcophaga bullata* were used as test organism for testing anti-moulting hormone activity. After the larvae had left their food (pork liver), they were kept under dry conditions for 24 hours, allowing them to empty the gut. In some experimental series these animals were then used for injections (non-water treated larvae). Owing to some inhomogeneity of insect material as to the age before pupation, a "synchronization" of their physiological state seemed necessary for more exact test purposes. Therefore the old larvae were kept in contact with wet filterpaper in a refrigerator at 5–8 centigrades for 5 days before use (water-treated larvae). As described by OHTAKI (1966) and by ZDAREK and FRAENKEL (1970) for other *Sarcophaga* species, this pretreatment prevents pupation by an inhibition of ecdysone-release in connection with a



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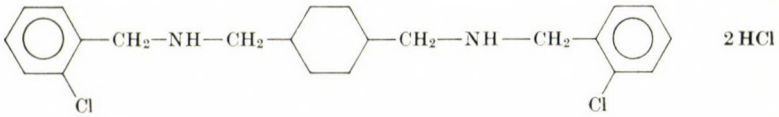

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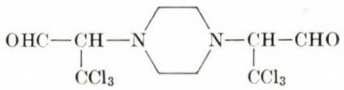

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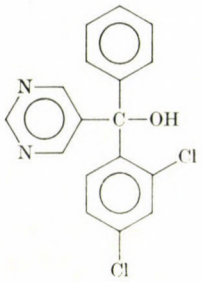

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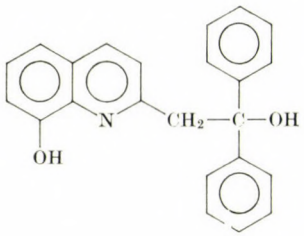

17

possible metabolic breakdown of ecdysone in the haemolymph. 20–30 larvae were used in each treatment.

The larvae were immobilized by chilling on melting ice for a short period. Evaluation of activity was carried out with suspensions prepared by pouring the appropriate quantities of an ethanolic solution into water, with stirring. This method yields sufficiently stable suspensions of very small particles. 2 μl of this suspension has been injected into the body-cavity by means of a micro-injector and a No. 18 record needle. 20 μg of the compound has been applied to each larvae. After 3–5 hours the dead larvae were selected and discarded. The remainders were preserved in petri-dishes at 18–20 centigrades. In 6–8 hours intervals the pupae an dead larvae were counted. Untreated and solvent-treated (injected) larvae were used as control.

The application of high dosages was limited by the solubility of the compounds and by the tolerance of the larvae to ethanol. In some instances (e.g.

cyclamin and digitonin) the larvae survived only lower (5 – 10 μg) dosages of the compound which seemed toxic in itself.

A preliminary screening test of the above listed compounds has been carried out by using a 3 – 5 μg/specimen dosages. The compounds exerting a certain degree of activity were selected for further detailed assay. Results of these tests are presented in Table 1.

Table 1

Effect of inhibitors of steroid biosynthesis on duration of larval pupation

Compound	Dosis μg/spec.	Treatment[a]	Retarded larval mortality %	Delay in pupariation[b]
N-benzyl-N-benzyloxy-carbamate	30	NWT	0.0	1.3
(13)	20	WT	11.5	1.1
2-(β-diethylaminoethyl-mercapto)-benzimidazole	30	NWT	0.0	1.3
2,4-dichloro-α-pyrimidin-2-ylbenz-hydrol (triarimol) *(16)*	30	NWZ	10.0	1.2
	20	WT	20.0	2.3
8-oxiquinoline	20	NWT	8.0	1.3
8-butylcarbamoyloxy-quinoline	30	NWT	0.0	1.3
Bis-p-hydroxyphenyl-1,1,1-trichloroethane	30	NWT	10.0	1.9
Untreated control	—	NWT	3.3	1.0
Injected control	—	NWT	4.0	1.0
	—	WT	0.0	1.0

[a] NWT = non-water-treated larvae: larvae kept under dry conditions; WT = water-treated larvae: larvae kept wet for 5 days

[b] Ratio of the interval between treatment and 50% pupariation of the treated larvae to the adequate period needed for the control larvae. Dead larvae have not been taken into consideration

Larvae kept under wet conditions (water treated), as well as larvae not subjected to pre-treatment were used in these tests. Activity was assessed by the increase of a retarded larval mortality and by delay of pupation expressed as the ratio of the interval between treatment and 50% pupariation of the treated larvae to the adequate period required by the injected control larvae. Deviations from value 1.0 ranging to 0.3 can be derived also from a possible inhomogeneity of the larval material, especially in case of the non-pretreated (non-water-treated) larvae. Only deviations higher than 0.3 can be regarded therefore as reliable.

Results and Discussion

None of the compounds tested for anti-juvenile hormone activity *(1 – 12)* gave rise to symptomps characteristic of a disturbance in juvenile hormone function. Some of the compounds revealed toxic effects, others were completely inactive.

The inactivity of these mevalonic acid analogues does not necessarily mean that an interference into juvenile hormone biosynthesis by antimetabolite analogues of its biointermediates is beyond possibilities. Reversibility of action might serve as one of the possible explanations for the lack of activity of these compounds. Much importance can be attributed therefore to Baker's active site directed irreversible inhibition concept in designing new derivatives of this type.

From the compounds, which were tested for anti-ecdysone effect, the systemic fungicide triarimol, 2,4-dichloro-α-pyrimidin-5-ylbenzhydrol yielded the most promising result.

Much less unequivocal results were obtained with other compounds listed in Table 1.

A high activity for cyclamin had been observed in our former experiments (MATOLCSY *et al.*, 1974). Since that time, however, due to a more detailed investigation of the symptoms and to the refinement of our anti-ecdysone test, it has become evident that the effect observed by us was the manifestation of a sublethal toxic action rather than an anti-ecdysone effect.

If the observed anti-ecdysone activity of triarimol is based on the inhibition of ecdysone synthesis, it was to be expected that its action can be reversed by simultaneous application of ecdysterone. Triarimol have been applied in doses of 20 μg/larvae and ecdysterone in doses of 2 μg/larvae, by using the same method as described above.

The data presented in Table 2 indicate that the activity of triarimol could be reserved by administration of ecdysterone. Due to the application of exogenous ecdysterone the pupariation has been accelerated by about 10 hours as compared with the control. The joint action of triarimol and ecdysterone have resulted not only in the recovery of normal rate of pupariation but also in the accelerated pupation due to exogenous ecdysterone. The results of these reversal studies give

Table 2

Reversal of action of triarimol by ecdysone

Dosage μg/larva		Hours required for pupation of	
		50%	100%
triarimol	ecdysone	of the test animals	
0	0	23	44
20	0	48	75
0	0.02	25	38
20	0.02	38	60
0	0.2	12	38
20	0.2	14	47
0	2.0	11	15
20	2.0	12	34

strong support to the idea that the action of triarimol is a result of an interference into ecdysone biosynthesis.

Further studies on this field are in progress.

Literature

BERGENSTAL, D. M., LIPSETT, M. B., MOY, R. H. and HERTZ, R. (1960): Biological Activities of Steroids in Relation to Cancer. (G. Pincus and E. P. Vollmer, eds.), 463. Academic Press, New York.

FUNATSU, M., HAYASHI, K., INABA, T., ISHIGURO, M., NAMIKIRA, G., KARIYA, M., YAMAURA, J., TSUKAMOTO, T. and SUYAMA, K. (1972): Nature of latent phenoloxidase complex in the prepupae of housefly and its physiological significance. Sci. Bull. Fac. Agr. Kyushu Univ. *26*, 85.

HOFFMAN, C. H., WAGNER, A. F., WILSON, A. N., WALTON, E., SCHENK, C. H., WOLFF, F. D. E., HOLLY, F. W. and FOLKERS, K. (1957): Synthesis of DL-3,5-dihydroxy-3-methylpentanoic acid (mevalonic acid). J. Am. Chem. Soc. *79*, 2316.

KOYAMA, T., OGURA, K. and SETO, S. (1972): Enzymatic reaction of isopentenyl pyrophosphate homologs — an approach to the biosynthesis of insect juvenile hormone. 16th Symp. Chem. Natl. Prod., Osaka.

MAEKAWA, K. and MATOLCSY, G. (1975): Synthesis of 2-(2'-hydroxy-2',2'-diphenyl-ethyl)-8-hydroxy-quinoline. Agr. Biol. Chem. (Japan) *39*, 663.

MATOLCSY, G., VARJAS, L. and BORDÁS, B. (1974): Assay of steroid inhibitors for insect antihormone activity, Acta phytopathol. Acad. Sci. hung. *9*, 161.

OGURA, K., KOYAMA, T. and SETO, S. (1972): Enzymatic synthesis of new farnesyl pyrophosphate homologues. J. Chem. Soc. Chem. Comm. *1972*, 881.

OHTAKI, T. (1966): On the delayed pupation of the fleshfly, Sarcophaga peregrina Robineau-Desvoidy, Jap. J. Med. Sci. Biol. *19*, 97.

ROBBINS, W. E., THOMPSON, M. J., SWOBODA, J. A., SHORTINO, T. J., COHEN, C. F., DUTKY, S. R. and DUNCAN, O. J. (1975): Nonsteroidal secondary and tertiary amines: inhibitors of insect development and metamorphosis and Δ^{24}-sterol reductase system of tobacco hornworm. Lipids *10*, 353.

SHERALD, J. L. RAGSDALE, N. N. and SISLER, H. D. (1973): Similarities between the systemic fungicides triforine and triarimol. Pestic. Sci. *4*, 719.

SIDDALL, J. B., JUDY, K. J., SCHOOLEY, D. A., JENNINGS, R. C., BERGOT, B. J. and SHARON HALL, M. (1974): Insect juvenile hormones and insect growth regulators. Third Intern. Cong. Pestic. Chem. (IUPAC), Helsinki.

SISLER, H. D. (1974): Fungicides affecting sterol metabolism. Kurzfass. IV. Reinhardsbrunner Symp. System Fungizide. Schloss Reinhardsbrunn.

SLÁMA, K. (1971): Insect juvenile hormone analogues. Ann. Rev. Biochem. *40*, 1079.

SLÁMA, K. (1974): Physiological and Biochemical Effects of Juvenoids. In: K. Sláma, M. Romanuk, F. Sorm: Insect Hormones and Bioanalogues. p. 217—302 Springer Verlag, Wien—New York.

STEWART, J. M. and WOOLEY, D. W. (1959): Antimetabolites of mevalonic acid. J. Am. Chem. Soc. *81*, 4951.

SVOBODA, J. A. and ROBBINS, W. E. (1967): Conversion of β-sitosterol to cholesterol blocked in an insect by hypocholesterolaemic agents. Science *156*, 1637.

SVOBODA, J. A. and ROBBINS, W. E. (1968): Desmosterol as a common intermediate in the conversion of a number of C_{28} and C_{29} plant sterols to cholesterol by the tobacco hornworm. Experimentia *24*, 1131.

SVOBODA, J. A., THOMPSON, M. J. and ROBBINS, W. E. (1968): 3β-Hydroxy-24-norchol-5-en-23-oic Acid, a new inhibitor of the Δ^{24}-sterol reductase enzyme system(s) in the tobacco hornworm, *Manduca sexta* (Johanson). Steroids *12*, 559.

SVOBODA, J. A., HUTCHINGS, R. F. N., THOMPSON, M. J. and ROBBINS, W. E. (1969): 22-trans-cholesta-5,22,24-trien-3β-ol- an intermediate in the conversion of stigmasterol to cholesterol in the tobaco hornworm, Manduca sexta (Johansson). Steroids *14*, 469.

SVOBODA, J. A., THOMPSON, M. J. and ROBBINS, W. E. (1972): Azasteroids: potent inhibitors of insect moulting and metamorphosis. Lipids *7*, 553.

WRIGHT, L. D. (1957): Antimetabolites of mevalonic acid utilization on lactobacillus acidophilus. Proc. Soc. Exptl. Biol. Med. *96*, 364.

ZDAREK, J. and FRAENKEL, G. (1970): Overt and covert effects of endogenous and exogenous ecdysone in puparium formation (pupariation) of flies. Proc. Nat. Acad. Sci. U. S. A. *67*, 331.

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