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Academiae Scientiarum Hungaricae

Volume 1

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ACTA PHYTOPATHOLOGICA

Volume 1

Contents

To the Reader

By the decree of the Presidency of the Hungarian Academy of Sciences from January 1, 1966 a new periodical, Acta Phytopathologica will be published regularly.

The aim of the periodical is to inform scientists abroad of the recent achievements of plant pathological research in Hungary. It will include papers on general and experimental phytopathology, experimental mycology, phytobacteriology, plant virology and pathophysiology. Experimental results of studies on the ecology of harmful insects, insect pathology, insect physiology and on the mode of action of pesticides will also be reported.

Phytopathology has been known to exist as a science for not more than a hundred years. Its foundation is connectedwith the appearance of such standard works as JULIUS KÜHN'S "Die Krankheiten der Kulturgewächse" (1858), M. J. BERKELEY'S "Vegetable pathology" (in Gardeners Chronicle, 1854–1857), A. DE BARY'S "Morphologie und Physiologie der Pilze, Flechten und Myxomyceten" (1866) etc. which actually laid down the scientific principles of plant pathology. Since than phytopathology not only became independent of the sciences it originally evolved from, such as botany, microbiology, medical sciences, but also developed its own concepts, terminology and methodology. Thus, it became the exact scientific basis of present day practical plant protection. In our days instead of the earlier trends such as ethiology, symptomatology and mostly descriptive mycology, modern approaches characterized by microbiological, (virological, phytobacteriological), pathophysiological, genetical and even molecular biological aspects have gradually become predominant. These aspects have considerably contributed to the modernization of phytopathology and, by supplementing insect and plant ecological as well as entomopathological trends, have created a broad basis for the further development of up-to-date plant pathology and plant protection.

Acta Phytopathologica endeavours to follow and to promote this very significant change going on in our days and revolutionizing natural sciences. In the columns of this periodical we want to publish first of all results which reflect the above changes in phytopathology in Hungary, but papers from abroad

are also welcome, especially if they are connected with phytopathological research projects represented in Hungary.

Taking phytopathology in its broadest sense we want to open Acta Phytopathologica to all those who want to publicize the results of their thorough and detailed investigation at an international level and to promote thereby the solution of the problems involved in both theoretical and practical phytopathology.

To this we wish to all the present and future co-workers of our periodical a succesful cooperation.

The Editorial Board

Károly Schilberszky (1863—1935) the Founder of Horticultural Phytopathology in Hungary

Recently in a solemn session and special exhibition the 100 year anniversary of the great Hungarian phytopathologist KÁROLY SCHILBERSZKY was celebrated. It is justified to commemorate his person and life-work in the columns of Acta Phytopathologica about the foundation of which he so often dreamed.

Phytopathology and plant protection are sciences hardly 100 years old. In their formation and modernization several Hungarian scientists took part,

Fig. 1. Károly Schilberszky (1863—1935)

among others in the field of agricultural phythopathology LINHART, in the domain of viticultural protection ISTVÁNFFI, while in horticultural phytopathology Schilberszky. The activity of these three scientists from the very beginning determined the trends which plant protection research in Hungary and practical plant protection was to follow.

Schilberszky was born in 1863 in Budapest. He studied and graduated also in Budapest. Later on he completed his special knowledge with biological and chemical studies at the Faculty of Social Economy at the University of Technical Sciences. He obtained his diploma of doctor of philosophy in 1893 and as early

as 1894 he was appointed professor of the School (later College) of Horticulture. He became assistant professor at the Faculty of Philosophy of the University of Sciences in 1904, at the University of Technical Sciences in 1911 and at the Faculty of Soil Economy in 1923. Here he was appointed full professor in 1926. He was the first to obtain a chair of phytopathology at the University. He deceased in 1935 in Budapest.

Before he became professor at the School of Horticulture he worked for one year at the Experimental Station for Seed Testing and for 6 years in the Institute of Botany of the University. At the same time he lectured on botany and pharmacology from 1891 to 1899 for research students of pharmacology and participated in the edition of a textbook. From 1907 to 1910 he was professor of Botany at the Teacher's Training College of higher elementary schools. His pedagogical activity gained a high recognition when in 1900 at the world exhibition in Paris he was awerded a golden medal for his mycological and botanical preparations, drawings, tables and scientific publications. His whole life was spent in education, teaching and research work. He undertook the difficult task of pioneering and laying the foundations of the teaching of horticultural botany and phytopathology at the College of Horticulture and at the University which he systematically developed. As early as 1896 he urged the development of the Scool of Horticulture into a College. His work for many years as a professor and his relentless activity in the National Hungarian Horticultural Association attached his whole life's work to horticulture and horticultural phytopathology. For a period he was also co-editor of the journal "Kertészet" (Horticulture). In 1919 he was awarded a honorary state horticultural diploma. Although apparently pedagogical activity filled out his life, research work was nearest to his inclinations, to his heart. In him the ardent love for truth and the unappeasable desire to explore truth were coupled with great diligence and strong persistence. It is characteristic of his scientific activity that he was interested first of all in the scientific, theoretical aspects of the problems investigated, although he did not neglect the interests of practical production either.

His talent and gift, his unparalleled capacity of observation, his aim at exact knowledge became manifest very soon when he was still a university student. His first botanical study appeared in the 1884 year volume of the Österr. Botan. Zeitschrift on *Rosa dumetorum*. Not much later in the same journal he reports the new habitat in Buda of *Rosa sphaerocarpa*. His *Rubus* species originating from Pilis are mentioned by V. BORBÁS the great Hungarian botanist as new species. His hazel variant described from the same place is discussed in the great flora work of JÁVORKA under the name of f. *pilisiensis*. His longer study "Egy ázsiai steppenövény európai vándorútjáról (On the European wanderings of an Asian steppe plant) is an early valuable achievement of ecological and especially chorological phytogeography in Hungary. Namely in 1889 he found in Nagyhörcsögpuszta *Eurotia ceratoides* which originated from the Asian steppes, probably from the Mongolian desert, crossed the South Russian steppes and Hungary and advenced as far as the Spanish peninsula. On the basis of the structure of the fruit

of *Eurotia* he offered a plausible explanation of the wandering of this plant toward the West. In this "migration" the fauna of the steppes played a prominent part. CZAKÓ published a fern *(Nephrodium cristatum)* from his collections in the High Tátra. But even at that time he paid a great attention to mosses; his valuable collection of mosses (about 15 fascicles) came later into the possession of the Botanical Department of the Hungarian National Museum. In a work on the flora of Kecskemét by L. HOLLÓS he published in 1896 the first fossil moss species from Hun-

Fig. 2. A part of the exhibition from the life of Károly Schilberszky

gary. He wrote later on the same topic in his study "Pleistocénkorú mohafaj Kecskemétről *(Hypnum Hollosianum)*" (A moss species of the Pleistocene from Kecskemét). He found the new moss species in a peat layer which came to light in the court of a steam mill in Kecskemét in 1892 at the boring of a 203 m deep artesian well. In these years he published also a study on phototaxis and motional phenomena of diatoms.

His interest, however, turned soon toward plant teratology and subsequently phytopathology which was of decisive influence on his career. In this special field he wrote his important studies and popularizing papers which created for him a world-wide reputation. His first publication on teratology appeared in 1885 on a walnut with three cotyledons while the last of this kind of papers was published in 1934 on the mixed colour of vine-berres. During the period in between

he reported about 20—25 teratological cases. A longer study of his deals with the teratology of the *Gagea* flower, the development of the extra-fascicular vascular bundle, cabbage plants without plumula etc. The peak of his activities is marked, however, by his works in the field of mycology and phytopathology. One of his earliest studies type "A *Convolvulus arvensis* virág kétalakúságának helyes magyarázata" (The correct explanation of the two forms of the flower of *Convolvulus arvensis)* appeared in 1890. In this paper he described a new smut fungus *Thecaphora convolvuli* Schilb. Several studies deal with the systematics and spreading of slime fungi *(Myxophyta).*

As early as 1888 he had the great discovery of his life, the pathogen of the wart disease of potatoes at hand from the village of Hornyán in Trencsén county. He reports on this after thorough studies of many years for the first time in 1896 simultaneously in several foreign journals. (The most important communication appeared in "Berichte d. Deutsch. Bot. Gesellschaft" 1896, XV. p. 36—37.). According to his description this new pathogen *(Chrysophlyctis endobiotica* Schilb.) of the potato found by him causes the most dangerous fungus disease in potato, the wart disease of potatoes. The pathogen was later transferred by the British research worker PERCIVAL into another genus and is now generally known under the name of *Synchytrium endobioticum* (Schilb.) Perc. The author, however, never accepted this change in the denomination. In a monography that appeared in 1930 also in Munich ("Die Gesamtbiologie des Kartoffelkrebses") he summarized again the results of his detailed investigations on the wart disease of potatoes. His own system differs from the officially accepted system since he included into the family *Synchytriaceae* the genera *Woronina* and *Rosella* and kept on to insist on the legitimacy of the independent genus *Chrysophlyctis.* This he justified by the fact that the number of summer and winter sporangia of the pathogen of the wart disease of potatoes, the pattern of the formation of zoospores and the histopathology of the cancerous growths substantially differ from the corresponding characteristics of *Synchytria.*

In general was very thoroughly engaged in the study of potato diseases since another disease of the potatoes which occurred rather frequently in Hungary, potato blight *(Phytophthora infestans)* caused substantial damages to our potato crops. There was e. g. in 1924 an epidemic all over the country. As a scientist he was unable to accept the idea that in the practice this pathogen could not be sufficiently controlled. He attacked the difficult problem and in 1928 in a basic study which appeared with the support of the Ministry of Agriculture in 3 foreign languages and also in Hungarian under the title of "A burgonyavész gombájának ökologiája" (The ecology of the fungus causing Potato Blight) he reported his thorough observations in this field and his conclusions. In this study he discussed in a special chapter the control measures against the disease and the differences in the behaviour of potato varieties.

He showed a similar enthusiasm for the diseases and pathology of horticultural plants which were his special field at the College of Horticulture. A number of plant diseases such as those caused by *Monilia, Fusicladium,* some xylo-

Phagous fungi, and apoplexy of apricots etc. became widely known in Hungary owing to his literary activities. He studied with great thoroughness particularly the monilia disease of fruit trees and the apoplexy of apricots on which he reported in long articles. He was the first to report in 1908 the occurrence of the American gooseberry mildew which was spreading in Europe since 1900 and undertook the ask to work out the control measures. In the stone-fruit production of Hungary, particularly in apricot production the pathological phenomenon named apoplexy plays an important part. He observed this disease as early as 1899 but submitted his voluminous study only in 1932 to the St. Stephen Academy on the apoplexy of apricots, cherries and sour cherries stating that the immediate cause of the disease was the obstruction or blocking of the water transporting vessels due to pathological rubber production, gommosis ("A csonthéjas termésű gyümölcsfák gutaütésének okairól" The causes of the apoplexy of drupes, Kísérl. Közl. XXXVII. 1934. p. 1 – 16.). He devoted much of his time also to damages done by xylophagous fungi and published valuable data for the elucidation of the pathological role of *Daedalea unicolor* and *Schizophyllum commune*. He also dealt with the storage diseases of fruits, in the first place with the rot of the core and warty rot. In addition to *Puccinia pruni spinosae* causing the rust disease of prune trees he also reported the first occurrence in Hungary of *Puccinia cerasi* known as yellow rust of prune trees. Finally he wrote an extensive study on the new hosts of *Sclerotinia* and the better elucidation of its biology based on more exact data.

Besides horticultural phytopathology he did not overlook the most important phytopathological problem of Hungarian wheat, the question of wheat rust. As soon as 1918 he urged an intensive national campaign to control the alternate host of black rust, the common barberry *(Berberis vulgaris)*. Ten years later he demonstrated on the basis of exact observations that barberry makes the development of new black rust biotypes possible in the course of the formation of the diploid generation of rust. When in the Thirties the black rust of wheat caused an epidemic all over Europe he became again interested in wheat rusts and he urged to make the control of the barberry shrub compulsory (1930). At the end of his life in 1935 as a swansong of an uninterrupted scientific life he wrote a brilliant study on *Pseudomonas tumefaciens* occurring on fruit trees and on some agricultural crops ("Beiträge zur Biologie von *Pseudomonas tumefaciens*". Zeitschr. f. Pflanzenkrankh. XLV. 1935. p. 146 – 159).

His activity of unparalleled diligence, rich in initiatives and results did not take place only in the silent laboratory in the company of his beloved books and microscope. He always followed with great interest agricultural production and the practice of plant protection. He may be considered as one of the greatest educators of Hungarian farmers. With the discovery in Hungary of the gooseberry mildew referred to above and the initiation of its control, with the description of a great number of fruit and vegetable plant diseases, with his intensive participation in the construction of the law on plant protection and with more than 500 popularizing papers, innumerable lectures and education of a legion of students he gained an everlasting reputation.

When the several hundred workers of Hungarian plant protection commemorate Károly Schilberszky's 100th anniversary they not only render homage to the memory of the scientist of world fame but also to the great Hungarian educator in phytopathology, the excellent specialist who was always ready to help productive work with his special advices and to the great scientist who united science and practice with great competence and who indelibly inscribed his name into the heart of thousands of students, his colleagues in the domain of horticulture and on the glorius pages of Hungarian phytopathology. His name, his always initiating productive activity must be made well known and estimated by the youth of to-day as well as by the specialists of plant protection because his whole life-work remains a splendid monument and an example to be followed.

G. UBRIZSY

Suppression of Virus Multiplication and Local Lesion Production in Tobacco Following Inoculation with a Saprophytic Bacterium

By

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Half leaves of *Xanthi* tobacco (a local lesion host) infiltrated with living and heat-killed suspensions of *Pseudomonas fluorescens* prior to inoculation with TMV were less susceptible to the virus than the water-infiltrated half leaves. Especially the diameter of the viral lesions was reduced although the number of the lesions was also suppressed.

Samsun tobacco (a systemic host of TMV) became also resistant to TMV multiplication following infiltration with the living or the heat-killed bacterium.

There was no connection between suppression of local lesion production and the induced senescence of the tissues infiltrated with the bacterium.

This is the first report on the induction of virus inhibition in a plant following inoculation with a bacterium.

Introduction

The injection-infiltration method makes it possible to introduce a large number of pathogenic or saprophytic bacterial cells into the leaves of intact plants (KLEMENT, 1963). It has been shown previously by GOODMAN (1964, 1965) and LOVREKOVICH and FARKAS (1965) that plants injected with heavy suspensions of living non-pathogenic as well as heat-killed bacteria became "resistant" to a superinfection by the specific pathogens.

In our preliminary investigations a large number of *living* bacterial cells were injected into *Xanthi* tobacco and a similar "resistance" to tobacco mosaic virus (TMV) infectivity was experienced. This raised the question of the possible suppression of virus multiplication as well as local lesion production after the injection-infiltration of tobacco leaves with saprophytic bacteria.

Material and Methods

Plant material

Nicotiana tabacum var. *Samsun* and *N. tabacum* var. *Xanthi*-nc plants were used in the experiments as systemic and local-lesion hosts, respectively. Plants were grown in ordinary greenhouse conditions and had about 10–12 leaves at the time of the experiments.

Microorganisms

Half leaves of intact tobacco plants were infiltrated with sterile tap water to serve as controls. The other halves of the leaves were inoculated by injection of suspensions of 24 hr old cultures of *Pseudomonas fluorescens* (a saprophyte). Bacterial suspensions contained 10^8 cells/ml as a rule. These suspensions were injected by a fine hypodermic needle into the intercellular spaces of the tobacco leaf. The injected suspensions easily spread over a large surface of the leaf. After this injection-infiltration, the infiltrated water was evaporated from the intercellular spaces in 1 – 1.5 hr. Infiltrated tobacco leaves were infected with tobacco mosaic virus (TMV) at different time intervals to find out the most suitable interval for the inhibition of virus production. The U1 strain of TMV was kindly supplied by Professor M. ZAITLIN, Department of Agricultural Biochemistry, University of Arizona, Tucson, Arizona.

RNA and protein contents

For the extraction of RNA and DNA as well as proteins the procedure of SCHMIDT and THANNHAUSER (1945) modified by FLETCHER and OSBORNE (1965) was followed. Leaf disks were macerated and extracted in 80 per cent ethanol, then with 5 per cent TCA at 4°C and finally with absolute ethanol and ethanol: ether (3 : 1 v/v). For RNA determinations the residue remaining from the extraction with ethanol: ether was hydrolysed with 0.3 *N* KOH for 18 hr, the DNA precipitated by acidification to pH 2.0 with perchloric acid, and the RNA determined in the supernatant fraction by the orcinol reaction (cf. MARKHAM, 1955). For protein determinations the residue remaining from the final extraction was subjected to *N* NaOH at 100°C for 4.5 min. to solubilize the protein. An aliquot of the solution was assayed by the biuret method. To investigate the intensity of protein synthesis the incorporation of ^{14}C-glycine into the acid insoluble (protein) fraction was determined. Leaf disks were floated on ^{14}C-glycine (specific activity 26 mC/mM) solution containing 10 μC activity in 50 ml for 4 hours. Incorporation of nucleic acid bases (adenine and guanine) and a precursor of uracil (orotic acid) was also investigated. In each case a 10 μC/50 ml solution was applied to leaf disks by floating them on the solution. Specific activity for adenine, guanine and orotic acid was 11.4 mC/mM, 1.0 mC/mM and 16.0 mC/mM, respectively. The precipitate insoluble in 10 per cent TCA was dryed, making thereby an infinite thickness and counted using a gas-flow counter. The results are expressed in counts per minute (cpm).

Results

Inhibition of local lesion production

Bacterial suspensions of *Ps. fluorescens* did not effect the virus itself. TMV mixed with a suspension containing 10^8 cells/ml showed no loss of in-

fectivity. On the contrary, "infectivity" decreased if intact leaves of *Xanthi* tobacco were pretreated (infiltrated) with suspensions of living and heat-killed (100°C) bacteria. As a consequence of the pretreatments with bacteria the num-

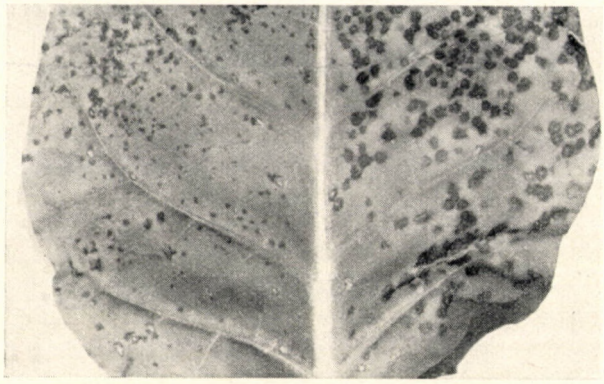

Fig. 1. Suppression of local lesion production in half leaf of *Xanthi* tobacco previously infiltrated with a suspension (10^8 cells/ml) of *Pseudomonas fluorescens*. Time interval between bacterial infiltration and TMV inoculation is 1 day. The control half leaf was infiltrated with water

Fig. 2. Inhibition of the diameter of the viral lesions in *Xanthi* tobacco previously infiltrated with *Pseudomonas fluorescens*. The control half leaf was infiltrated with water the opposite half leaf with the bacterial suspension

ber and the diameter of the lesions were significantly reduced. Especially the diameter of the lesions was influenced. In some cases where an apparent reduction in the number of lesions was experienced micro-lesions could be detected with a 10 × magnification. Heat-killed cells of *Ps. fluorescens* also caused a suppression

of local lesion production although to a lesser extent than the living ones. Results are shown in Table 1 and Figs 1 and 2. It is seen from Table 1 that the inhibition

Table 1

Inhibition of local lesion production in *Xanthi* tobacco following inoculation with *Pseudomonas fluorescens*

Infiltrated with	Time interval between infiltration and TMV infection	Relative number of lesions on leaf disks
Water (control)	—	100*
Living bacteria	1 hr	49
Living bacteria	1 day	14
Living bacteria	2 days	0
Heat-killed bacteria	1 day	77**

* Actual average number of lesions per 10 leaf disks (15 cm^2) for the control was 470.
** The diameter of the lesions was markedly reduced.

of the lesion production was greater when *Xanthi* leaves were infected with TMV one or two days after the infiltration of bacteria. Nevertheless, a considerable inhibition was also experienced if infection with TMV was carried out 1 hr after the infiltration of bacteria. In this case infiltrated water evaporated from the intercellular spaces in an hour or so and the leaves were immediately infected with the virus. Inhibition of the number and diameter of the viral lesions showed a considerable variation, depending on the light conditions during the year. During the winter months inhibition was usually less pronounced and 10^9 instead of 10^8 cells/ml were required to get a considerable suppression of local lesion production.

The experiments mentioned above indicate that first of all the diameter of the lesions is reduced. From this one would think that only tissue necrosis is inhibited and virus multiplication remains uninfluenced. However, experiments outlined in a further paragraph indicate that this is not the case.

Influence of Pseudomonas tabaci on the local lesion production

In some preliminary experiments *Ps. tabaci*, a pathogenic bacterium was used for injection-infiltration, however, without success. Infiltrated half leaves were killed as a consequence of the pathogenic effect. On the contrary, using the toxin-containing culture filtrate for injection leaves were not demaged and a considerable reduction in the number of local lesions was achieved if the leaves were infected with TMV 3 days after injection.

Inhibition of virus multiplication

In these experiments *Samsun* tobacco (a systemic host of TMV) served as a host for TMV multiplication. Disks were punched out 5 days after virus infection from half leaves which were pretreated with water (control), living and heat-killed bacteria *(Ps. fluorescens)*, respectively, homogenized in phosphate buffer (pH 6.5) and inoculated to a local lesion host to estimate the relative infectivity. Bacterial suspensions contained 10^9 cells/ml in these experiments. A slight yellow discolouration of half leaves infiltrated with these suspensions occurred 4–5 days after the injection. It is seen from Table 2 that multiplication of TMV was reduced to 20 per cent (80 per cent inhibition). In these experiments too, heat-killed bacteria induced inhibition of virus multiplication but to a lesser extent than the living cells of *Ps. fluorescens*.

Table 2

Relative infectivity of homogenates of leaf disks from TMV-infected *Samsun* tobacco pretreated with *Pseudomonas fluorescens* on the 5th day of multiplication

Infiltrated with	Time interval between infiltration and TMV infection	Relative number of lesions on leaf disks of *Xanthi* tobacco
Water (control)	1 day	100*
Living bacteria	1 day	20
Heat-killed bacteria	1 day	51

* Actual average number of lesions per 10 leaf disks (15 cm^2) for the control was 278.

Nucleic acid and protein synthesis

In a few experiments half leaves of *Xanthi* tobacco plants infected with *Ps. fluorescens* became yellowish in colour 2–3 days after the treatment. In these tissues an enhanced chlorophyll degradation was experienced. As seen from

Table 3

Incorporation of ^{14}C-glycine into the acid insoluble (protein) fraction of leaf disks of *Xanthi* tobacco infiltrated with *Pseudomonas fluorescens*

Infiltrated with	Mean cpm/100 mg fresh wt. 2 days after infiltration
Water (control)	2072
Ps. fluorescens 10^9 cells/ml	1417
Ps. fluorescens 10^8 cells/ml	2100

Table 3 in tissues pretreated with a large number of bacteria (10^9 cells/ml), incorporation of ^{14}C-glycine into the protein fraction was inhibited. This finding indicates that the synthesis of proteins may be suppressed in the bacterium-treated tissues and this results in tissue senescence. However, tissue senescence as well as suppressed syntheses were experienced only when the tissues were infiltrated with suspensions containing a larger cell number, i. e. 10^9 cells/ml. Using a suspension which contained 10^8 cells/ml there was no connection between senescence and inhibition of local lesion production. In this case a reduction of the number of virus lesions occurred even if TMV infection immediately followed bacterial inoculations. However, no chlorophyll degradation or inhibition of incorporation of ^{14}C-glycine into the protein fraction was detected in the tissues treated with a suspension of 10^8 cells/ml even after 3 days. Similarly the incorporation of adenine, guanine and orotic acid into the acid insoluble fraction and the level of RNA and protein remained unchanged. (Table 4 and 5). Therefore, one can rule out the possibility that senescence has a role in the

Table 4

Incorporation of ^{14}C-adenine, ^{14}C-guanine and ^{14}C-orotic acid into the acid insoluble (nucleic acid) fraction of leaf disks of *Xanthi* tobacco infiltrated with *Pseudomonas fluorescens*

Infiltrated with	Mean cpm/100 mg fresh weight 2 days after infiltration		
	adenine	guanine	orotic acid
Water (control)	285	219	426
Pseudomonas fluorescens 10^8 cells/ml	263	225	418

Table 5

Protein and RNA contents of leaves of *Xanthi* tobacco 3 days after infiltration with *Pseudomonas fluorescens*

Infiltration with	Protein mg/g fresh wt.	RNA expressed as mg ribose/g fresh wt.
Water (control)	9.1	3.4
Pseudomonas fluorescens 10^8 cells/ml	9.8	3.2

induction of reduced susceptibility to TMV following infiltration of bacteria. Further experiments are needed to decide whether or not tissue senescence after the inoculation with a larger number of cells (10^9 cells/ml) is in a cause and effect relation with the inhibition of virus production.

Discussion

As far as we know this is the first report on the induction of resistance in plants to a virus disease following inoculation with bacteria. However, a number of similar results were mentioned in the earlier literature. YARWOOD (1960), ROSS (1961a, 1961b) and LOEBENSTEIN (1963) demonstrated the role of induced resistance to viruses in uninfected parts of virus-diseased plants. LOEBENSTEIN and ROSS (1963) as well as SELA and APPELBAUM (1962) and SELA, HARPAZ and BIRK (1964) were able to show the production of antiviral factor(s) (interferon-like substances) in virus infected plants. The results of HECHT and BATEMAN (1964) interestingly pointed to the role of *Thielaviopsis basicola*, a fungal pathogen, in the induction of acquired resistance to viruses. Furthermore, LOVREKOVICH and FARKAS (1965) reported the induction of a delayed appearance of disease symptoms caused by *Ps. tabaci* in plants previously treated with heat-killed bacteria. GOODMAN (1964, 1965) got a similar effect with non pathogenic *E. amylovora*. All these results emphasized the non-specific role of the inducing agent in causing resistance to a second infection.

The data in the present report demonstrate that saprophytic bacteria are also able to induce resistance to a virus disease. It seems clearly established that the role of the inducing micro-organisms in all the cases mentioned above is non-specific, however, the nature of the influence on plant metabolism remains to be clarified. The work of LOEBENSTEIN (1964) and SELA, HARPAZ and BIRK (1965) show the possible importance of the altered protein metabolism in these reactions.

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Drifts in Enzyme Levels in Detached Senesceing Barley Leaves and their Relation to Enzyme Changes in Diseased Tissues

By

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The effect of detachment on some aspects of terminal oxidation systems in barley leaves was studied. It was found that in detached senesceing barley leaves the cytochrome oxidase activity increases markedly as compared to the control attached leaves. Digitonin-treatment increased the cytochrome oxidase activity in extracts from both attached and detached leaves. The response of cytochrome oxidase activity to digitonin treatment was greater with extracts from detached leaves. The O_2-uptake of both detached and control leaves increased upon treatment with 2,4-DNP. The increase was greater with detached leaves. No transhydrogenase activity was found in barley leaf extracts. NADPH-dependent cytochrome c-reductase was detected but its activity remained unchanged upon detachment. Isolation of the leaf tissues resulted in an increase in NADPH level. The results are discussed from the point of view of the role of senescence in the changes observed. It is pointed out that similar changes in the metabolic systems studied take place in diseased tissues as well.

Introduction

Senescence in leaves induced by detachment is associated with a characteristic rearrangement of enzyme levels (DÉZSI and FARKAS, 1964, KISBÁN et al., 1964, TODD and YOO, 1964, UDVARDY et al., 1964, HORVÁTH and UDVARDY, 1965, SAHAI-SRIVASTAVA, 1965). It has been pointed out by FARKAS et al. (1964) that the enzyme changes occurring in senesceing detached leaves are similar to those observed in a wide variety of diseased tissues. Apparently, senescence induced by parasitic attack is a major factor which contributes to the changed metabolic pattern of diseased leaf tissues. This conclusion is strongly supported by electron microscopic studies as well. It has been found that changes in the ultrastructure of senescent leaf cells are reminiscent of structural alterations observed in diseased tissues (SHAW and MANOCHA, 1965b).

The nature of enzyme changes associated with normal ageing or disease and the role of alterations of cell ultrastructure in these changes are poorly understood. On the one hand, evidence is available that at least part of the enzyme changes observed in virus-infected leaves and in detached senesceing leaf tissues is due to new enzyme synthesis triggered by the experimental induction of senescence (FARKAS and STAHMANN, 1966). On the other hand, the partial breakdown of cellular ultrastructure might lead to the activation, release or "solubilization" of latent enzymes from cell particles. Cytochrome oxidase being a typical particle bound enzyme the study of cytochrome oxidase level as affected by detachment

seemed to be especially desirable. In the present paper results pertaining to the effect of leaf excision on cytochrome oxidase activity and some other systems related to terminal oxidations are presented. The effect of rust-infection on cytochrome oxidase level has been described in a previous paper (FARKAS, 1962).

Materials and Methods

Barley seedlings (Hungarian variety "MFB") were grown under ordinary greenhouse conditions. Leaves of 10-day old plants were detached and placed with their petioles in water or 10^{-5} M kinetin. Samples were harvested and assayed at different times (2 to 7 days) after excision.

O_2 uptake of leaf pieces was measured by conventional Warburg technique. The effect of 2,4-dinitrophenol (2,4-DNP) on the respiratory rate was assayed by preincubating 3×3 mm tissue sections on 10^{-5} M 2,4-DNP dissolved in 0.05 *M* phosphate buffer at pH 5.0 (cf. RAU, 1962). Controls were simultaneously preincubated on 0.05 *M* phosphate buffer at pH 5.0.

The determination of cytochrome oxidase activity was carried out spectrophotometrically. The decrease in absorbance at 550 mμ was measured in a system containing chemically reduced cytochrome c and tissue extract (SMITH, 1955).

Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome c-reductase was assayed as described by EVANS (1955). The method is based on the measurement of the reduction of oxidized cytochrome c at 550 mμ in the presence of NADPH and plant extract.

The amount of total NADPH was estimated in tissue extracts prepared as described by FRITZ et al. (1963). The estimation of NADPH was carried out spectrophotometrically in a system containing plant extract, oxidized glutathione, and purified NADPH-dependent glutathione reductase to saturation. The amount of NADPH present was calculated from the decrease in absorbance at 340 mμ as described by BERGMEYER (1963). In control systems the oxidized glutathione was omitted.

Pyridine nucleotide transhydrogenase was assayed as described by KAPLAN (1955) in the presence of catalytic amounts of NADP, substrate amounts of nicotinamide adenine dinucleotide (NAD), substrate amounts of isocitric acid, and purified nicotinamide adenine dinucleotide phosphate (NADP)-specific isocitric dehydrogenase to saturation. The increase in absorbance at 340 mμ was followed.

NADP and glutathione reductase were purchased from Boehringer and Soehne GmbH Mannheim; cytochrome c, isocitric dehydrogenase, flavine mononucleotide (FMN) and kinetin from Sigma Chemical Company, St. Louis, Mo; NAD from Nutritional Biochemical Corporation, Cleveland, Ohio; NADPH from International Chemical Corporation, New York, N. Y. and adenosine triphosphate (ATP) from Schwartz Bioresearch Inc., Orangeburg, N. Y.

A Spectromom 201 ultraviolet spectrophotometer was used throughout the experiments.

Results

Cytochrome oxidase

As shown in Fig. 1 cytochrome oxidase activity increased considerably upon detachment of barley leaves. The increase in cytochrome oxidase level, as compared to the control, progressed continuously during detachment.

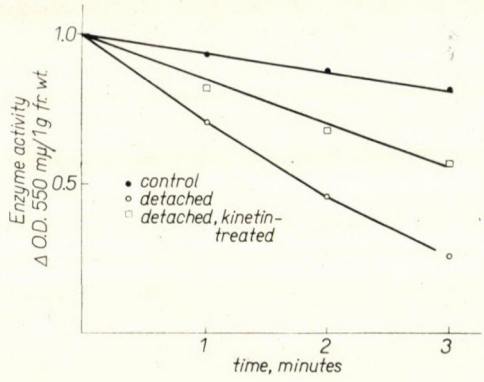

Fig. 1. Effect of detachment and treatment with 10^{-5} M kinetin on the cytochrome c oxidase activity of barley leaves

Kinetin is known to maintain normal cellular structure in isolated leaves (SHAW and MANOCHA, 1965a). Therefore, cytochrome oxidase activity was assayed in detached kinetin-treated leaves as well to find out whether or not the increase in cytochrome oxidase activity is lowered if the cellular ultrastructure (first of all that of the mitochondria) is kept more or less intact in detached leaves. The results presented in Table 1 indicate that kinetin treatment tends to decrease the level of cytochrome oxidase in detached leaves.

Table 1

Effect of 0.1 per cent digitonine on cytochrome oxidase activity in barley leaf extracts

Expt. No.	Enzyme activity expressed as ΔO. D. at 550 mμ/ 1g fresh weight/3 minutes								
	Without digitonin			With digitonin			Difference; digitonin-treated — untreated		
	C	D	D + K	C	D	D + K	C	D	D + K
1	0.28	0.78	0.75	0.55	1.80	1.71	0.27	1.02	0.97
2	0.15	0.73	0.48	0.35	1.22	0.92	0.20	0.48	0.45
3	0.25	0.77	0.65	0.78	1.67	1.28	0.53	0.90	0.63

C = control; D = detached for 5 days; D + K = detached treated with 10^{-5} M kinetin

These results lend support to the hypothesis that the increased cytochrome oxidase activity in detached leaves might be associated with the disorganisation of cell structure.

Digitonin treatment is routinely used to solubilize, or at least activate, latent enzymes in isolated cell particles by loosening their ultrastructure. It is tempting to suggest that the changes in cytochrome oxidase activity that occur upon detachment of leaves are due to a digitonin-like action, i.e. they are associated with a damage to the structure of cell particles. If the increase in cytochrome oxidase activity in detached leaves were due to a process similar to that induced by digitonin it could be expected that digitonin treatment has less effect on the cytochrome oxidase activity in extracts from detached leaves than in those from the controls. Results summarized in Table 1 indicate that this is not the case. Digitonin-treatment increased the cytochrome oxidase activity of extracts from detached leaves to a higher extent than that of the controls. The possible explanation of this apparent discrepancy will be discussed later.

Adenosine triphosphatase

To study further the eventual role of subcellular damage in the terminal phase of the respiratory process the effect of detachment on the ATP-ase activity was investigated. Damage to the mitochondria is accompanied by uncoupling and uncoupling is, as a rule, associated with an increase in ATP-ase activity.

As shown in Table 2 an increase in ATP-ase level was found in detached leaves, a phenomenon compatible with the assumption of damage to the mito-

Table 2

Effect of detachment and kinetin treatment on the adenosine triphosphatase activity of primary leaves of barley

Expt. No.	Enzyme activity expressed as mg inorganic P liberated/1 g protein/20 minutes				
	C	D	D + K	Ratios	
				D/C	D + K/C
1	17.3	23.9	17.7	1.38	1.02
2	18.4	27.2	17.7	1.48	0.96
3	14.6	23.2	12.3	1.59	0.84

C = control; D = detached for 5 days; D + K = detached treated with 10^{-5}M kinetin

chondria and resulting uncoupling. Therefore, the extent of eventual uncoupling due to leaf excision was studied by the use of 2,4-DNP. It is generally accepted that a positive response of tissues to treatment with 2,4-DNP is indicative of the operation of respiratory control; a phenomenon usually regarded as being dependent on the intactness of mitochondria. The results presented in Table 3 indicate

Table 3

Effect of 2,4-dinitrophenol on the oxygen uptake of detached barley leaves

Expt. No.	Oxygen uptake in μl O_2/lg fresh weight/h								
	C		D		D + K		Ratios: DNP-treatment/ buffer-treatment		
	Buffer	DNP	Buffer	DNP	Buffer	DNP	C	D	D + K
1	283	370	300	444	280	400	1.32	1.48	1.43
2	127	173	136	209	114	165	1.36	1.54	1.45
3	157	216	168	240	143	200	1.38	1.43	1.40

C = control; D = detached for 5 days; D + K = detached and treated with 10^{-5} M kinetin

that the O_2-uptake of both control and detached leaves was markedly increased upon treatment by 2,4-DNP. Surprisingly enough, the increase in O_2-uptake induced by dinitrophenol was greater with detached leaves than with the controls. This fairly unexpected finding is suggestive of the existence of a strong respiratory control in both control and detached leaves. Apparently the degeneration of mitochondria in detached leaves does not reach a level sufficient to abolish the respiratory control.

Transhydrogenase

The increased respiratory rate, the higher level of cytochrome oxidase activity, and the evidence for a more intense operation of the hexose monophosphate shunt in detached barley leaves (UDVARDY et al., 1964) made it desirable to study the most obvious link between the shunt and the mitochondrial cytochrome system: the NADPH + NAD $\rightleftarrows$ NADP + NADH transhydrogenation reaction. However, repeated attempts revealed no evidence for the presence of a transhydrogenase catalysing hydrogen transfer from NADPH to NAD either in control or in detached leaves.

NADPH-cytochrome c-reductase

Another, although less likely, link between the hexose monophosphate shunt and the terminal respiratory chain might be a NADPH-cytochrome c-reductase. The presence of a NADPH-dependent cytochrome c-reductase was, indeed, demonstrated in barley leaf extracts. Detachment, however, did not effect the activity of the enzyme appreciably (Fig. 2).

It follows from all these observations that if other NADPH-utilizing reactions are also relatively slow, in the absence of intensive transhydrogenation, NADPH might accumulate in detached leaf tissues. Further studies were carried out to study this problem.

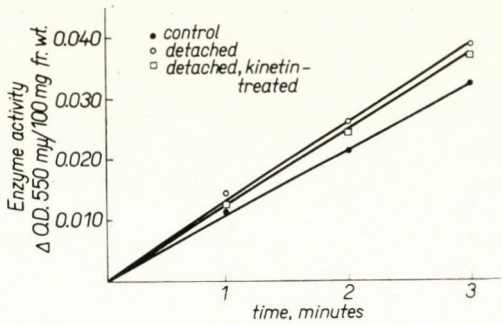

Fig. 2. Effect of detachment and treatment with 10^{-5} M kinetin on the NADPH-dependent cytochrome c-reductase activity of barley leaves

NADPH-level

Results summarized in Table 4 indicate that the isolation of barley leaves results in an increase in NADPH level by about 50 per cent. This observation is in line with the absence of substantial amounts of transhydrogenase and the un-

Table 4

Effect of detachment and treatment with kinetin on the NADPH level in barly leaves

Expt. No.	μmoles of NADPH/1 g protein				
	C	D	D + K	Ratios;	
				D + C	D + K/C
1	3.2	5.1	2.9	1.59	0.91
2	3.2	5.1	2.9	1.59	0.91
3	3.5	5.1	2.9	1.45	0.83

C = control; D = detached for 5 days; D + K = detached and treated with 10^{-5} M kinetin

changed level of NADPH-cytochrome c-reductase in barley leaves and also with our previous findings about the activation of the pentose phosphate shunt dehydrogenases in detached leaves (FARKAS et al., 1964, UDVARDY et al., 1964).

Discussion

The results presented indicate that the isolation of barley leaves results in an increase in cytochrome oxidase activity. As the excision of leaf tissues is associated with a damage to the cell ultrastructure (SHAW and MANOCHA, 1965a) one might postulate a causal correlation between these two phenomena. Indeed, when the

normal cellular structure is maintained in isolated leaves by the addition of kinetin (cf. SHAW and MANOCHA, 1965a) the increase in cytochrome oxidase activity due to excision is lowered. This seems to be in line with the idea that the increased cytochrome oxidase activity of detached leaves is due to the activation of latent cytochrome oxidase by breakdown processes loosening the structure of mitochondria. Such a mechanism is reminiscent of the mode of action of detergents in increasing enzyme activities in particulate preparations. Cytochrome oxidase activity is typically sensitive to such treatments, e.g. to treatment with digitonin. However, although the action of digitonin and the effect of detachment appear to be similar, there must be some basic difference between them as indicated by the essentially identical effect of digitonin on the cytochrome oxidase activity of extracts from control and detached leaves. In fact, digitonin treatment increased the cytochrome oxidase activity of extracts from detached leaves more than that of the controls. This indicates that the effect of digitonin and detachment is *additive* and most probably is not based on exactly the same mechanism. We might suppose that the structure of mitochondria and mitochondrial fragments is already loosened during detachment and this makes the access of digitonin to the site of action easier.

It is noteworthy that an increase in cytochrome oxidase level was found in rusted senesceing wheat leaf tissues as well (FARKAS, 1962) which are known to exhibit changes in cellular ultrastructure similar to those occurring in detached leaves (SHAW and MANOCHA, 1965b). It is likely that the increase in cytochrome oxidase level in rusted wheat leaves is to be explained, at least in part, by the accelerated senescence of the tissues and concomitant changes in mitochondrial structure.

Although the electron microscopic data of SHAW and MANOCHA (1965a) and the present results on cytochrome oxidase are in line and suggest that the increase in cytochrome oxidase activity in detached leaves is due to a damage to the mitochondrial structure, this damage is certainly not so excessive as to induce a marked uncoupling of respiration. This is indicated by the fact that 2,4-DNP induces a larger increase in O_2-uptake in detached leaves than in the controls. One explanation of this phenomenon could be an increased permeability of the cells of detached leaves to 2,4-DNP. However, this is unlikely as we have tested a range of concentrations of 2,4-DNP with both control and detached leaves and found that the optimal stimulatory concentration is the same in both cases.

The positive response of the tissues of both intact and detached leaves to 2,4-DNP is indicative of an effective respiratory control. The higher response of detached leaves to 2,4-DNP is puzzling. One possibility is that the synthetic processes are slowed down in the detached leaf, and ATP being utilized for synthetic processes to a lesser extent, the tissue respiration, when tested with 2,4-DNP, exhibits a stronger respiratory control.

The present investigations did not reveal any evidence for an effective link between the stimulated pentose phosphate shunt and the increased rate of terminal electron transport. Transhydrogenase activity was apparently absent from the

extracts of barley leaves. The NADPH-dependent cytochrome c-reductase, the in vivo role of which is anyhow highly questionable, was not markedly affected by detachment. At the same time NADPH accumulated in the barley leaf tissues upon detachment. All this is in line with the idea of the lack or insufficient capacity of hydrogen transfer systems between the hexose monophosphate shunt and terminal electron transport in mitochondria. Apparently, NADPH produced by the pentose phosphate shunt is not utilized at a rate high enough for synthetic processes either, although phenolics do accumulate in the detached barley leaves and phenol biosynthesis is supposed to be a NADPH requiring process (NEISH, 1960).

It is interesting that an increase in NADPH concentration (and also an increase in the NADPH/NADP ratio) was found by ROHRINGER (1964) in rusted wheat tissues as well. Although part of the NADPH accumulated in rusted tissues might be due to the presence of the fungus, the similarity of drifts in NADPH level in detached senesceing leaves and diseased tissues is worth of attention.

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Cytokinin Activity in Rust-Infected Plants: Juvenility and Senescence in Diseased Leaf Tissues

By

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As a result of rust infections cytokinin activity increases to a considerable extent in leaves of beans and broad beans. Cytokinin activity was measured in concentrated, partially purified extracts by two bioassays: (a) inhibition of root growth and (b) retention of chlorophyll on yellowing wheat leaves.

The higher intensity of protein synthesis and the ability to accumulate metabolites in the infected parts and in the "green islands" apparently results from the influence of cytokinins formed in rust infected leaves. Amino acids labelled with radioactive isotopes are incorporated to a higher extent into protein in the "green islands" than in healthy leaves. This may have a role in inducing juvenility. The activity of glycolic acid oxidase too, increases in the "green islands" and even reaches a higher level than in the healthy leaf tissues. However, the activity of this enzyme measured in extracts from the chlorotic leaf areas decreased to a very low level. One may conclude that the juvenile feature of rust infected leaf areas and of the "green islands" is directly connected with an increase in cytokinin content. The intensive senescence outside the "green islands" may be closely related to an increased export of nutrients from these parts of leaves induced by cytokinins.

The pattern of cytokinin-activity in extracts from uredospores was very similar to that of infected leaves. The greening effect of spore extracts on leaves (inhibition of senescence) can be explained by the relatively high cytokinin content of the spores. Compounds with cytokinin activity could also be isolated from healthy bean leaves, their activity, however, was much lower than that from rust-infected leaves. In healthy broad bean leaves no compound with cytokinin activity could be detected.

Introduction

In our previous paper (POZSÁR and KIRÁLY, 1966) we have shown that some physiological symptoms of rust-infected beans and wheat plants could be induced in healthy plants too by treating them with kinetin or benzyladenine. We suggested that the two cytokinins or some other similar compound are probably formed or accumulated in the diseased tissues in a higher concentration than normally. Our first attempts along these lines were unsuccessful, because we were unable to demonstrate directly the presence of cytokinins in the affected tissues. On the basis of indirect evidences, however, we suggested that the pathophysiological alterations described by us and others, such as accumulation of nutrients, pathological phloem-transport, inhibition of the growth of the apical shoots and secondary leaves and senescence were due to a relative accumulation of some cytokinin. Up to the

present no role has been attributed to this group of growth hormones in pathogenesis. The present paper will show that in rust-infected leaves of beans and broad beans cytokinins actually accumulate and that a kinin-like substance acting at the site of infection leads to the rejuvenation of the tissues and to the senescence of the uninfected tissues around the affected area.

Materials and Methods

Plant material

For the experiments rust-infected *(Uromyces phaseoli)* Pinto beans *(Phaseolus vulgaris)* and rust infected *(U. fabae)* broad beans *(Vicia faba)* were used. The leaves were always compared to healthy ones in different stages of infection. Investigations were usually carried out in the initial chlorotic phase of infection, during the sporulation period when the pustules had been already disrupted, and in the so-called green island phase, respectively. The green islands appear in a later stage of the sporulation period around the pustules, surrounding them in the form of a ring. In this advenced stage of infection the uninfected "healthy" parts of the infected leaves are already senescent and yellowing.

Demonstration of cytokinin-activity

a) *Root-growth test.* Cytokinins were extracted and isolated from infected and healthy leaves, respectively, by using the method of VAN DER KERK et al. (1964) and modified by VAN ONCKELEN et al. (1965). 10 g (fresh weight) of plant material were homogenized in 10 ml buffer, pH 5.5. The homogenate was extracted with 150 ml 96% ethanol, centrifuged, and the supernatant was evaporated to 10 ml. After adjusting the pH of the solution to 2.9, the residual chlorophyll, auxin, gibberellic acid etc. components were extracted from the water phase containing the cytokinin with 4 × 2 ml ethyl ether. The aqueous solution, after its pH had been adjusted to 7.8 extracted with 6 × 2 ml n-butanol and the butanol layer, containing the active ingredients was transferred to filter paper placed in Petri dishes. The filter paper was dried after the addition of 5 × 5 ml distilled water in order to remove traces of butanol. 50 barley seeds were germinated in 4 replications on the filter paper containing the active ingredients. The activity of cytokinins was expressed as the percentage of the inhibition of root growth after 3 and 4 days, respectively. Cytokinin activity in uredospore extracts was determined from 0.8 g spore material similarly to the method used with rust-infected leaves. For the biological testing of cytokinins a spring barley variety, H 1108, was used.

b) *The chlorophyll retention test.* Cytokinins were extracted and partially purified as described and the senescence-delaying activity was assayed. One-cm sections of first leaves of wheat were floated on test solutions for 72 hr and then

extracted in 80% ethanol. Chlorophyll retention was expressed by measuring the optical density of the extrects at 665 mμ. This method is based on the original bioassay of OSBORNE and MCCALLA (1961) with slight modifications.

Incorporation of labelled amino acids

The rate of incorporation of labelled amino acids (glycine-C^{14}, cysteine-S^{35}) into the protein fraction was determined in the precipitate insoluble in a 10 per cent trichloracetic acid solution at 4° C after floating discs, green islands or rings on a 50 μC/100 ml solution for 4 hours. The precipitate was washed with distilled water, dryed, making thereby an infinite thickness and counted using a gas-flow counter. The results are expressed as specific activities (μC/g fresh weigh).

Glycolic acid oxidase

Glycolic acid oxidase activity of healthy and infected bean leaves was compared. In infected leaves the green islands and the uninfected tissues which became yellow and senescent were always separated from each other. Glycolic oxidase activity was measured in a Warburg apparatus by a manometric method previously applied by us (KIRÁLY and FARKAS, 1957) with the difference that in the present experiments glycolic acid rather than lactic acid served as substrate.

Results

Demonstration of cytokinin activity in rust-infected leaves

Leaves from bean and broad bean plants, infected with *Uromyces phaseoli* and *U. fabae*, respectively, were harvested when the infection was about 10 to 12 days old and the uredo colonies were well developed i.e. when the fungus sporulated. Control leaves were taken from healthy plants. The cytokinin activity of purified leaf extracts was estimated by the bioassay described in the "Material and Methods". Cytokinin activity was measured by comparing activity of leaf extracts from both healthy and diseased leaves to that of different concentrations of kinetin and benzyladenine. The blanks were obtained by germinating barley seeds in distilled water instead of kinin solution or leaf extracts.

The results are shown in Table 1. It may be seen that the tissues of rust-infected leaves exhibit a higher cytokinin-activity than the healthy tissues. In the case of beans even in healthy leaves there was some activity present, a finding which is an additional contribution to data showing that kinin-like substances actually do occur in healthy plants. This has been shown recently by VAN DER KERK et al. (1964), VAN ONCKELEN et al. (1965), SETH and WAREING (1965). In healthy broad beans, however, no cytokinin activity could be detected. On the

Table 1

Cytokinin-activity in healthy and rust-infected leaves of bean and broad bean, respectively. The activity tested on the 3rd and 4th day of germination of barley by a bioassay based on the inhibition of growth of spring barley roots

Material	Day of testing	Length of barley roots in mm	Inhibition of root growth in per cent	Cytokinin activity in ppm*
Control (water)	4	25.0	—	—
Extract of healthy bean leaves	4	20.0	20.0	10
Extract of rust-infected bean leaves	4	13.6	54.0	50
Control (water)	3	17.0	—	—
Extract of healthy broad bean leaves	3	16.9	1.1	—
Extract of rust-infected broad bean leaves	3	8.5	50.0	48

* as related to kinetin content

other hand, the results indicate, that cytokinin activity in leaves of rust-infected bean and broad bean plants is considerably higher than in healthy leaves. This may well be the cause of the pathophysiological changes mentioned in the "Introduction".

Using the chlorophyll retention test, a higher cytokinin activity was also detected in the infected bean leaves. This bioassay is based on the capacity of cyto-

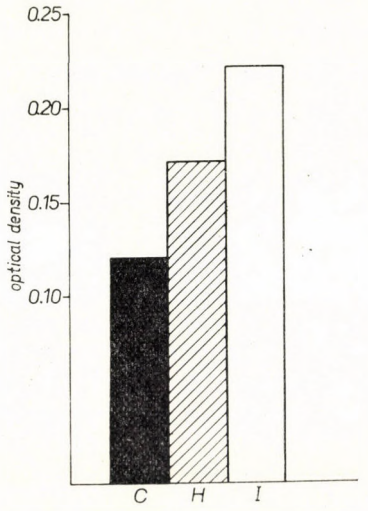

Fig. 1. Retention of chlorophyll by partially purified extracts from healthy and rust-infected bean leaves. Sections of first leaves of wheat were floated on water (C) on extract from healthy bean leaves (H) and on extract from rust-infected bean leaves (I), respectively. Optical densities of 80% ethanol extracts from wheat tissues determined at 665 mμ

kinins to delay chlorophyll degradation (Fig. 1.). It is remarkable that in this case too, even in healthy bean leaves there was possible to estimate some activity.

Cytokinin-activity in uredospores

In the extracts of the uredospores investigated cytokinin-activity is relatively high and similar in its effect to that of rust-infected leaves (Table 2). Extracts from uredospores of broad bean rust inhibit root growth by 30 per cent which is equivalent to the effect of a 20 ppm kinetin solution according to our bioassay. With wheat rust there is an inhibition of about 25 per cent.

Table 2

Cytokinin activity of uredospore extracts tested on the 4th day of germination by a bioassay based on the inhibition of growth of spring barley roots

Uredospore	Length of barley roots in mm	Inhibition of root growth in per cent
Control (water)	29	—
Spore extract of *Uromyces fabae*	20	31.0
Spore extract of *Puccinia graminis* var. *tritici*	22	24.8

Juvenility and senescence in infected leaves

It is a well known fact that so-called green islands develop around the infection site in different rust diseases. These green islands are physiologically very active. WANG (1961) for example has shown that there was an active starch synthesis in the green islands of rust-infected bean leaves. Our experiments to be described below will show that protein synthesis (incorporation of amino acids) is also activated in the green islands as compared to the surrounding tissues. First the green islands formed around the infection site were punched from the leaves and the rust colonies were removed from the middle of the discs. In this way green tissue-rings were obtained, and were then compared to samples which were cut from tissues of diseased leaves located near to the green islands. These somewhat senescent tissue zones were devoid of the fungus and were cut in such a way that tissue rings exactly alike the ones from the infected variants be obtained. The third variant consisted of rings prepared from healthy leaves. They were floated for 4 hours on a solution containing glycine-C^{14}. The activity of the incorporated radioactive carbon was determined in the TCA insoluble precipitate from all three kinds of leaf tissues. It may be seen in Table 3 that the highest rate of incorporation was found in the green islands. It was even higher than with intact healthy leaves. Thus, the green islands formed as a result of infection represent a very active

Table 3

Incorporation of glycine-C^{14} into the protein fraction of leaf tissue rings infected with *Uromyces phaseoli*

Material	Specific activity μC/g fresh weight
Healthy leaf (uninfected)	79.0×10^{-3}
Infected leaf	
1. Green island, pustule removed	129.7×10^{-3}
2. Senescent part near the green islands	32.1×10^{-3}

juvenile feature indeed probably due to the effect of some cytokinins. In the *senescent* leaf parts surrounding the sites of infection and green islands the rate of protein synthesis considerably decreased. The senescence in these tissue zones may have been brought about by the effect of the green islands near by. In order to prove the above hypothesis additional experiments were carried out in which half leaves of beans were infected with the rust fungus and the senescence of the other half was compared to that of intact, healthy half leaves. The results of the experiment are shown in Fig. 2 and Table 4. It may be seen that the uninfected half leaves

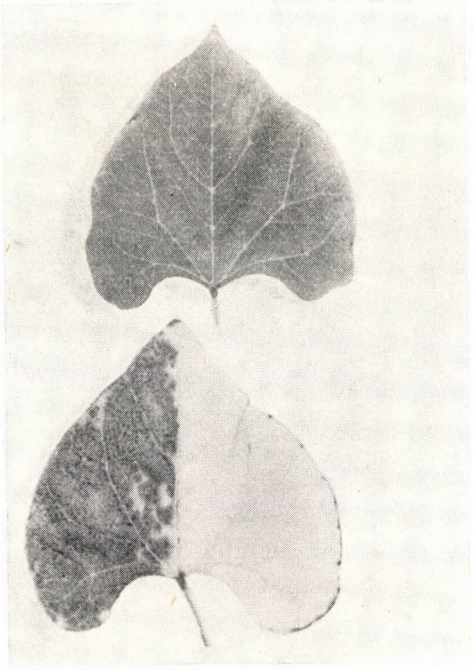

Fig. 2. Effect of *Uromyces phaseoli* in inducing juvenility and senescence, respectively, in a bean leaf. Leaf above: healthy control; leaf below: left half rust-infected. The uninfected right half shows senescence

Table 4

Incorporation of cysteine-S^{35} into the protein fraction of rust-infected bean leaves

Material	Specific activity μC/g fresh weight	
	Acid insoluble fraction (protein)	Acid soluble fraction
Uninfected leaf parts	19.0×10^{-3}	148.2×10^{-3}
Infected centers	36.1×10^{-3}	247.3×10^{-3}

displayed a considerable senescence. During winter months or under insufficient illumination the senescence of the tissues developed to such an extent that on the 15th day after infection the half leaves in the vicinity of the diseased tissues became yellow. The rate of incorporation of cysteine was also lower in the senescent leaf parts as compared to the infection sites containing the fungus.

In the three kinds of tissue samples mentioned above glycolic acid oxidase activity was also measured. This enzyme is always active in green tissues. Previously, we have obtained some data indicating that in the chlorotic stage of wheat rust disease i.e. when there is a loss of clorophyll in the infected parts, there is almost no enzyme activity at all (KIRÁLY and FARKAS, 1957). It may be seen in Table 5, that a similar situation occurs in the chlorotic stage of rust-infected beans.

Table 5

Effect of rust infection and kinetin on glycolic acid oxidase activity in bean leaves

Material	Glycolic oxidase activity Qo_2
Healthy	2.3
Infected with *U. phaseoli*, chlorotic stage	0.8
Infected with *U. phaseoli*, green islands	2.6
Chlorotic stage + kinetin treatment (50 ppm)	1.8

However, after the formation of the green islands the enzyme activity increases in the green centres exhibiting an intensive metabolism. As shown in Table 5 kinetin treatment applied in the cholorotic stage of disease led to a corresponding increase in enzyme activity.

The above results suggest that rust infections has a double effect on bean leaves: on one hand, there is a rejuvenation of the green islands surrounding the infection centre, and, on the other hand, the tissue zones between the green islands become senescent. By the artificial application of cytokinins both greening and senescing effects can be produced in plants (ENGELBRECHT and CONRAD, 1961; OSBORNE, 1962; LEOPOLD and KAWASE, 1964).

Discussion

According to the results reported in the present paper a number of the characteristic symptoms of rust diseases can be explained by cytokinin-like action. In some cases, by using different bioassays, healthy plants have been shown to contain kinin-like substances (MILLER, 1961; VAN DER KERK et al., 1964; BOTTOMLEY et al., 1963; ZWAR et al., 1963; VAN ONCKELEN et al., 1965; SETH and WAREING, 1965). In our previous experiments several indirect evidences have suggested that in rust-infected plants there was a cytokinin-like activity (POZSÁR and KIRÁLY, 1966). The results of the present paper provide direct evidence. Cytokinin activity can be demonstrated not only in rust-infected leaves but also in the uredospores, therefore, it appears that kinin-like substances are actually produced by the pathogens. In this respect it is interesting to note that BUSHNELL and ALLEN (1962) were able to produce green islands on detached barley leaves by applying unpurified extracts of uredospores i.e. reproduce typical cytokinin effect. The hypothesis outlined above is supported by the data shown in Fig. 1.

The bioassay suggested by VAN ONCKELEN et al. (1965) proved to be useful in our experiments too for the detection of kinin-like substances in plant organs. By this test we were able to show a linear correlation between cytokinin concentration and inhibition of root elongation.

According to the experimental data, in tissues where the fungus is acting directly, there is a juvenility-effect, whereas in tissues farther apart, from which an increased export of nutrients takes place, (POZSÁR and KIRÁLY, 1966) there is a senescence effect. Symptoms such as changes in phloem-transport and retardation of the secondary growth, can also be explained by the effect of cytokinin accumulated in the infected parts. A more detailed discussion of this phenomenon can be found in our paper cited above.

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Effect of Crown Gall Infection on the Respiration and on the Content of Reducing Sugars and Keto-acids in Maize

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In infected tissues (young galls) there is an increase in the level of reducing sugars. The opposite is true for the uninfected internodes located below the galls.

In infected tissues the respiratory rate increases. In uninfected tissues of diseased plants there is only a slight respiratory increase. In young galls the R. Q. is 1.05.

Pyruvic acid content in young galls decreases in the case of less susceptible varieties and increases in the case of highly susceptible varieties. The level of α-keto-glutarate and oxaloacetate is higher in infected stem tissues than in the control.

The rates of respiration obtained upon the infiltration of selective enzyme inhibitors suggest that in the early stage of infection there is no change in the respiratory pathway. Glycolytic inhibitors (NaF and iodoacetic acid) repress the oxygen uptake of infected tissues to a considerable extent. Respiration shows a higher sensitivity to the inhibitors of the enzymes of the tricarboxylic acid cycle (malonate, fluoroacetate) in infected tissues than in healthy ones. This suggests that the Krebs cycle plays a major part in the respiration of infected tissues.

The oxygen uptake is less sensitive to Na-azide, αα′-dipyridyl and salicylaldoxime in infected tissues than in healthy ones. It is very probable that in infected tissues flavoproteins play a role in terminal oxidation.

Introduction

In maize tissues infected with crown gall *(Ustilago maydis* (DC.) Cd.*)* the intensity of synthetic processes increases. The extent of this increase depends on the susceptibility of the variety concerned. In such cases the inorganic nitrogen content decreases in the tissues, whereas the content of free amino acids and proteins increases (PETHŐ, 1963; 1964a; 1964b; 1966). In the infected tissues metabolic processes leading to a higher protein content seem to be closely connected with respiration, with the concentration of respiratory substrates and with the amount of keto acids produced during respiration.

The rate of respiration, the content of carbohydrates and keto acids in maize plants infected with crown gall have been the subject of several investigations. E.g. HURD-KARRER (1926) reported reduced amounts of sap in infected internodes, HURD-KARRER and HASSELBRING (1927) found a decrease in the level of reducing sugars and sucrose. ESANU and NEGULESCU (1958; 1960) have demonstrated a lower level of total sugars and reducing sugars in non infected leaves of diseased

plants. DEVAY and ROWELL (1954), on the other hand, have established a higher fructose content in the galls.

According to GATINA (1960) the intensity of respiration increases in infected plants. ESANU and NEGULESCU (1958) have shown respiratory increase and an increase in peroxidase and catalase-activities in uninfected leaves of diseased plants. In contrast to the above findings MIHALEVSKAYA (1964) has reported a decrease in oxygen uptake by non-infected leaves of diseased plants, and a respiratory increase in leaf tissues surrounding the galls and in the galls themselves. FELDMAN (1948) has found a reduced rate of respiration, especially in the case of aerobic respiration, in the internodes below the infection sites. The respiratory quotient has been shown to be below unity in the uninfected internodes of diseased plants as well as in internodes of healthy plants, whereas in the galls it was higher than that. MIHALEVSKAYA (1964), on the other hand, has found the respiratory quotient to be lower than one in leaf tissues showing symptoms of crown gall.

TURIAN (1962) has pointed out that in stem tissues infected with *U. maydis* there is an increase in isocitritase activity as well as in the level of organic and α-keto-acids.

Most of the literary data cited above refer to uninfected organs and tissues of diseased plants. In addition, the contradictory results do not permit to get a uniform picture of the problems concerned.

The investigations to be presented were aimed to elucidate of the physiological and biochemical aspects of the host-parasite relation in question by studying the changes in reducing sugar content, respiratory rate, respiratory mechanism and keto acid content of maize tissues infected with *Ustilago maydis*.

Material and Methods

The experiments were carried out in 1964 and 1965 with maize plants grown on chernozem soil rich in calcium. For the analyses commercially produced varieties and hybrids as well as inbred lines of local varieties were used. The most important data on the inbred lines have been published in one of our previous papers (PETHŐ, 1966). The plants were artificially infected as usual (PETHŐ, 1963) by using a suspension of sporidia. In our previous experiments it has been found that the changes in the intensity of synthetic processes upon infection were most pronounced in young galls which did not contain any chlamydospores yet. Therefore the analyses to be presented in this paper were also performed in an early stage of infection, i.e. after the appearance of the galls.

Reducing sugar content was measured in a homogenate prepared in distilled water by SOMOGYI's method (1945) after removing proteins with lead acetate BRUGOVITZKY (1956). The results are given in mg/g fresh weight. Respiration was measured manometrically in a Warburg respirometer. Into the main compartment of the vessels 100 mg (fresh weight) of finely cut tissues and 2.0 ml phosphate buffer were placed. The central well contained 0.2 ml of a 20 per cent KOH solu-

tion. The respiratory inhibitors were dissolved in phosphate buffer of the desired pH and applied to the tissues by vacuum-infiltration (JAMES, 1953). The rate of respiration is expressed as O_2 uptake in mm^3 per hour by 1 mg tissue (dry weight) (Q_{s0}).

Keto-acid content of the tissues was determined in trichloroacetic acid extracts by the method of FRIEDEMANN and HAUGEN (1943). The results are represented as micromole/gr fresh weight.

Results

Changes in the reducing sugar content of leaves of the maize hybrid Mv. 5. upon infection are shown in Fig. 1. It can be seen that the reducing sugar content in leaves of healthy plants increases acropetally, whereas in leaves bearing galls

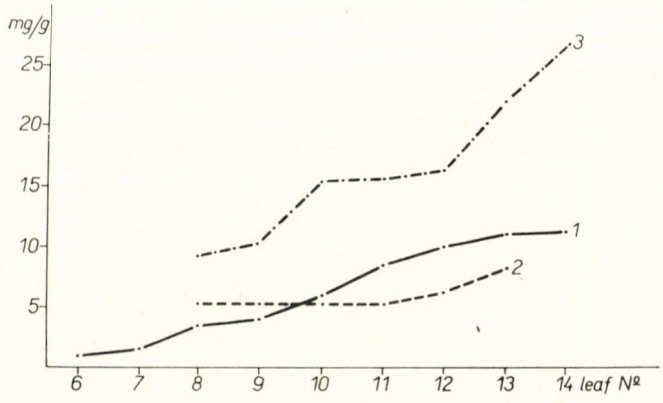

Fig. 1. Effect of crown gall infection on the reducing sugar content of leaves of the maize variety Mv. 5. 1: Leaves of healthy plants, 2: leaves bearing galls, 3: leaf-galls of infected plants

it remains at the same level, i.e. it does not follow the curve of the control leaves In the galls of infected leaves the reducing sugar content increases to a considerable extent.

A local increase in reducing sugar content takes place in the infected internodes as well (Fig. 2). In this case, however, the considerable decrease in reducing sugar content of the lower, non infected internodes seems to offer a basis for much more important conclusions. It is suggested that the accumulation of reducing sugars in the infected parts brings about a reduction in the level of reducing sugars in the uninfected internodes and may diminish thereby the energy supply of the root system as well.

The higher reducing sugar content on the infected tissues is one of the prerequisites for their higher respiratory rate. Therefore, changes in the respiratory rate

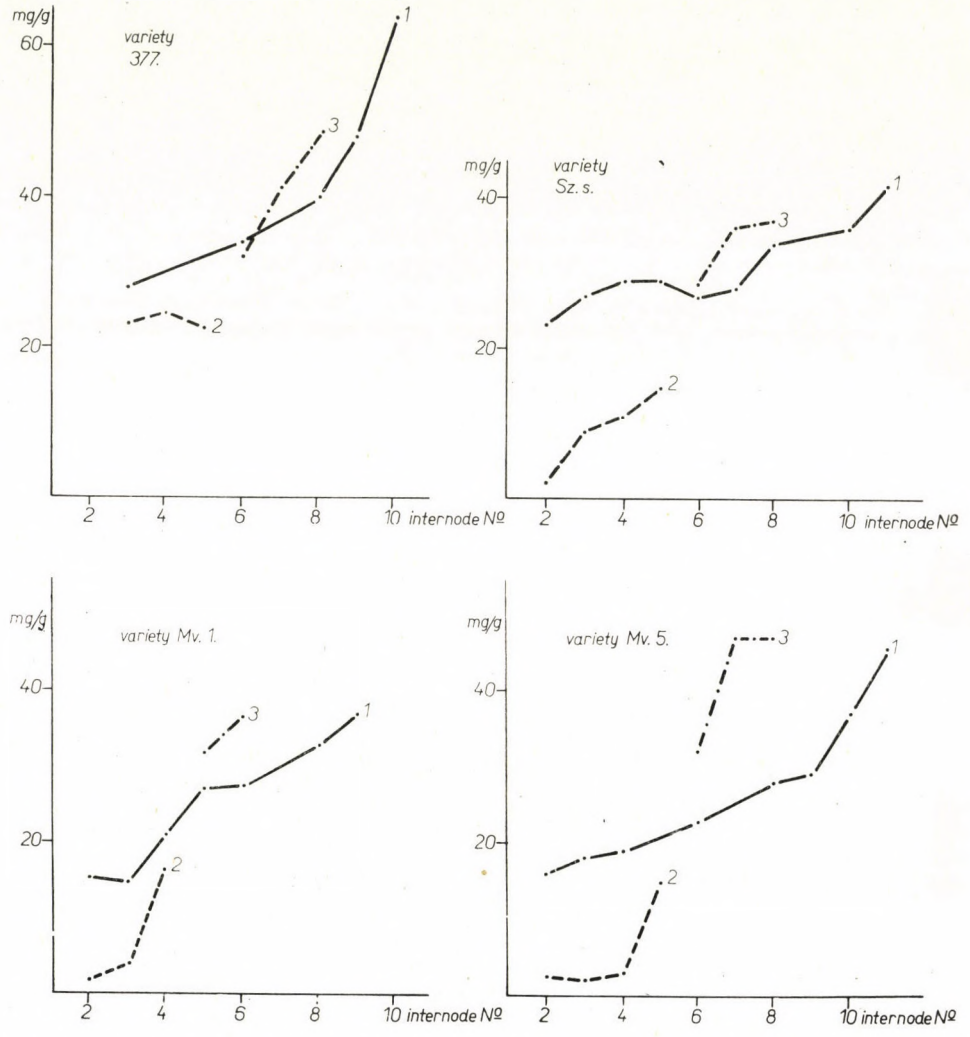

Fig. 2. Effect of crown gall infection on the reducing sugar content of internodes of maize plants belonging to four different varieties 1: Healthy plant, 2: lower, uninfected internodes of diseased plants, 3: infected internodes

(Q_{O_2}) of stem tissues of the highly susceptible local variety 62 infected with crown gall have been established at the infection site (gall) and in the uninfected internodia, respectively. To this end the respiratory rates of the galls located on the infected internodia and of the tissues surrounding the galls were compared to those of the corresponding internodia of healthy plants (Group II). The respiratory rate of internodes both below (Group I) and above (Group III) the internodes actually

infected with crown gall was also measured in order to establish the physiological effect of infection on non-infected internodes.

As seen in Fig. 3 the respiratory rate of stem tissues of infected plants was higher with all three groups than that of similarly located internodes of healthy plants. The greatest difference in respiration was found between the infected tissues (young galls) and the corresponding internodes of healthy plants. Thus, infection affects first of all the gall tissues themselves, but an increase in the respiratory rate can be experienced in non-infected tissues located further apart from the infection

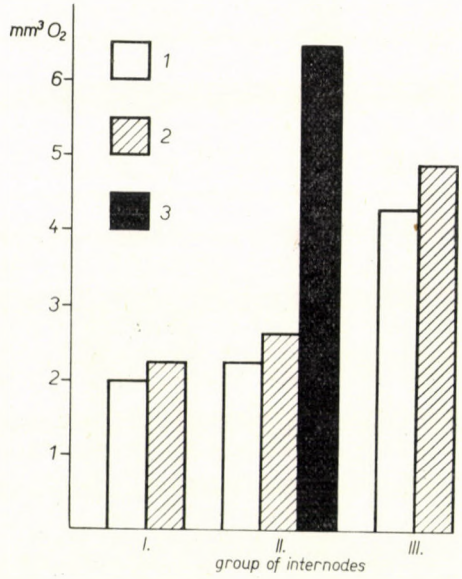

Fig. 3. Effect of crown gall infection on the respiration of internodes of maize plants belonging to the local variety No. 62. I: Internodes below the infection sites, II: internodes bearing galls, III: internodes above the infection sites. 1: healthy plants, 2: infected plants, 3: galls

site as well. The increase in the intensity of respiration exhibited by tissues further apart from the infection site can be induced by several factors. The results of the present investigations do not permit to decide which of these factors is involved.

Our previous experiments have shown unequivocally that in infected tissues of susceptible varieties there is an accumulation of amino acids (PETHŐ, 1962; 1963b; 1964b). In the experiments to be presented below we investigated whether or not the precondition of an increased rate of amino acid synthesis i.e. a higher content of keto acids exists in these tissues. TURIAN (1962) reported the accumulation of organic acids and α-keto acids in maize tissues infected with *Ustilago maydis*.

Fig. 4 shows that the content of pyruvic acid in infected tissues decreased in the case of less susceptible varieties whereas it increased with more suceptible

varieties. Alanine is formed from pyruvic acid. BEKMUHAMEDOVA's (1961) results suggest that alanine plays a special role in the nitrogen metabolism of maize. Previously we have established a parallelism between the susceptibility of different varieties and the alanine content in their infected tissues. It has been shown as well (PETHŐ, 1964a; 1964b) that in a later stage of infection it was the alanine content which decreased the most. When the fungus in its parasitic stage was grown on a synthetic medium alanine proved to be an especially suitable nitrogen source (PETHŐ, 1960).

The content of both α-ketoglutaric acid and oxaloacetic acid increases to a considerable extent in the infected tissues of the varieties studied (Fig. 5). These

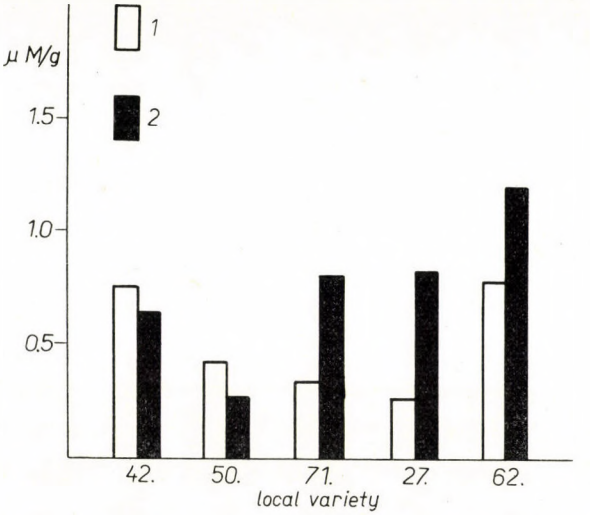

Fig. 4. Effect of crown gall infection on the pyruvic acid content of internodes of plants belonging to varieties with different degrees of susceptibility 1: Internodes of healthy plants 2: galls

keto-acids are the precursors of glutamic acid and aspartic acid, respectively. Previously it has been found (PETHŐ, 1964a; 1964b) that these two amino acids usually accumulate in the infected tissues of the varieties studied even with less susceptible varieties in which the amount of other amino acids considerably decreases. Glutamic acid is known to play a central role in the nitrogen metabolism of plants. This is also shown by the results presented above.

The lack of any connection between the amounts of α-ketoglutaric acid and oxaloacetic acid in the infected tissues, on the one hand, and the susceptibility of the varieties on the other, may be due to the fact that these keto-acids were estimated together and the changes in the overall concentrations observed do not reflect the behaviour of any of the two acids alone.

The connection between the enzymic reactions of the Krebs cycle and the biosynthesis of amino acids is well known (KRETOVICH and GALYAS, 1961). The

keto-acids mentioned above are members of the tricarboxylic acid cycle. Therefore, the hypothesis seems to be justified that the higher content of keto-acids in maize tissues infected with crown gall is due to an enhancement of the Krebs cycle in the overall respiration of such tissues.

The aim of further experiments was to investigate the qualitative changes caused by crown gall infection in the respiration of maize tissues. To this end

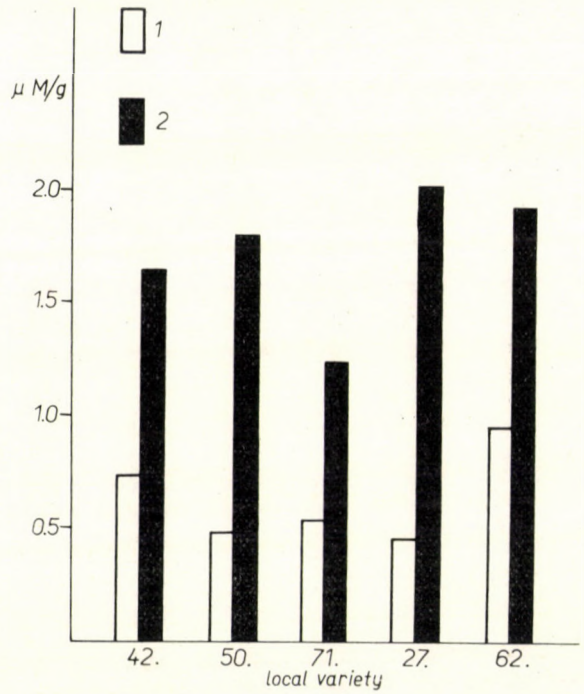

Fig. 5. Effect of crown gall infection on oxaloacetic acid and α-ketoglutaric acid contents of internodes of maize plants belonging to varieties with different degrees of susceptibility. 1: Internodes of healthy plants, 2: galls

conventional selective respiratory inhibitors were infiltrated into the tissues and the decrease in oxygen uptake of the treated tissues was measured. If a selective inhibitor inhibits oxygen uptake to a considerable extent the enzyme or enzyme system to which the inhibitor in question is specific is supposed to be functioning.

Respiration of plant tissues generally follows the glycolytic pathway. In cereals infected with obligate parasites, however, at least in later stages of infection glycolysis is being replaced by the pentose phosphate shunt (SHAW and SAMBORSKI, 1957). Our investigations were carried out with young galls being in an earlier stage of infection. Both the respiratory quotient of infected stem tissues and the effect of selective glycolytic inhibitors on oxygen uptake were studied. NaF has an

inhibitory effect on enolase which catalyzes the conversion of 2-phosphoglycerate into phosphoenolpyruvate (JAMES, 1953). Iodoacetate is one of the most efficient glycolytic inhibitors; it inhibits triosephosphate dehydrogenase.

It may be seen in Table 1 that in the early stage of infection when the galls are just appearing the respiratory quotient is practically one. This suggests that at this stage of the host-parasite relation the respiratory substrate is a carbohydrate.

Table 1

Effect of glycolytic inhibitors on the respiration of maize tissues infected with crown gall

Compound infiltrated	Q_{O_2}	Inhibition per cent	Q_{CO_2}	R. Q.
KH_2PO_4 $5 \cdot 10^{-2}$ M	4.13	0	4.34	1.05
NaF 10^{-2} M	0.10	97	—	—
Iodoacetate 10^{-3} M	0.34	91	—	—

The effect of the two inhibitors used indicates that at least in an early stage of infection the respiratory mechanism of the infected tissues follows the glycolytic pathway.

In order to study the fate of pyruvic acid, the end product of glycolysis, two selective inhibitors were used. Malonate exerts a competitive inhibitory effect on succinic dehydrogenase which catalyzes one of the steps of the tricarboxylic acid cycle. Inhibition of oxygen uptake upon addition of malonate suggests the presence of this enzyme as well as the operation of the tricarboxylic acid cycle. Inhibition by malonate can be reversed by the addition of excess succinic acid due to the mechanism of this type of inhibition. Fluoroacetate is also an inhibitor of the tricarboxylic acid cycle, and as such can be used in addition to malonate for the study of the Krebs cycle.

Table 2

Effect of inhibitors of the tricarboxylic acid cycle on the O_2 uptake by healthy and crown gall infected stem tissues of maize

Compound infiltrated	Healthy		Infected	
	Q_{O_2}	Inhibition per cent	Q_{O_2}	Inhibition per cent
KH_2PO_4 $5 \cdot 10^{-2}$ M	4.01	—	6.88	
Malonate 10^{-2} M	0.00	100.0	4.98	27.6
$2 \cdot 10^{-2}$ M	2.43	39.4	2.69	60.9
Malonate 10^{-2} M + Succinate 10^{-2} M			5.57	4.5
Fluoroacetate 10^{-2} M	0.00	100.0	4.19	39.0
$2 \cdot 10^{-2}$ M	2.91	27.4	—	—

The data presented in Table 2 indicate that in stem tissues of maize the tricarboxylic acid cycle is in operation. This pathway can be found in both healthy and crown gall infected tissues, with the latter, however, the oxygen uptake is more sensitive to the inhibitors applied. Thus, in the early stage of infection the enzyme systems which play a part in the biosynthesis of the keto acids accumulated are present and the respiration which gets activated upon infection follows the tricarboxylic acid cycle. Consequently, there is no qualitative difference in the respiratory mechanism between healthy and infected tissues. Infection, however, leads to an increased sensitivity of respiration to the inhibitors used. This suggests an enhanced role of the Krebs cycle in the early stage of infection.

To study the mechanism of terminal oxidation Na-azide, an inhibitor of metal containing oxidases is extensively used. αα'-dipyridyl is being applied as an inhibitor of iron containing enzymes though the nature of its inhibitory action is not well understood yet (JAMES, 1953). Under neutral or slightly acidic conditions it strongly reacts with the ferrous ions of oxidases and is therefore a suitable tool for the differentiation between iron and copper containing oxidases. Salicylic aldoxime, on the other hand, is specific to copper containing oxidases, especially polyphenoloxidase. By using these three inhibitors one can get a tentative picture about the changes in terminal oxidation upon infection.

Table 3

Effect of inhibitors of terminal oxidation on the O_2 uptake by healthy and crown gall infected stem tissues of maize

Compound infiltrated	Healthy		Infected	
	Q_{O_2}	Inhibition per cent	Q_{O_2}	Inhibition per cent
1/15 M phosphate buffer (pH 6.5)	4.47	—	6.41	—
Na-azide $2 \cdot 10^{-3}$ M	0.41	90.8	—	—
$5 \cdot 10^{-3}$ M	0.00	100.0	0.40	94.0
αα'-Dipyridyl $5 \cdot 10^{-3}$ M	2.78	37.8	4.26	33.6
Salicylaldoxime $5 \cdot 10^{-3}$ M	0.75	83.2	2.54	60.4

Table 3 shows that healthy stem tissues are more sensitive to azide than infected ones. A 5×10^{-3} M Na-azide solution inhibited O_2 uptake by 94 per cent in infected tissues, whereas with healthy tissues there was a 100 per cent inhibition. This suggests that metal containing oxidases play a more important role in the respiration of healthy tissues than in that of infected ones. This is supported also by the results obtained with the two other respiratory inhibitors. The fact that respiration was inhibited by salicylic aldoxime to a greater extent in both healthy and infected tissues than by αα'-dipyridyl suggests that copper containing oxidases play an especially important role in the terminal oxidation of maize tissues. Because inhibition with salicylic aldoxime was higher with healthy tissues than with

infected ones one may conclude that in the infected tissues flavoproteins play a more important role in terminal oxidation than the copper containing oxidases.

Discussion

In plants infected with obligate parasites there is generally an accumulation of substances around the infection sites (YARWOOD and JACOBSON, 1965) especially in the early stage of infection. In such cases part of the increase in dry matter content is due to an increased protein synthesis induced by the parasite in the host tissues. Dry matter content increases also in maize tissues infected by *Ustilago maydis*, as shown in our experiments. This finding does not contradict HURD-KARRER's (1926) observations according to which the amount of expressed sap per fresh weight decreases in non-infected stem tissues of diseased maize plants.

Carbohydrate content of the host is an important factor in host-parasite relationships. There is, however, no unequivocal connection between the sugar content of the host and the degree of its susceptibility to parasites (HORSFALL and DIMOND, 1957). In rust infected host tissues the sugar concentration decreases, whereas in mildew infected wheat leaves there is an accumulation of both sucrose and glucose (cf. FARKAS, 1965).

The experimental results presented show that in maize tissues infected with crown gall the level of reducing sugars increases at the site of infection and decreases in uninfected tissues of diseased plants, especially in the internodes located below the infection site. The higher sugar content in infected tissues (i. e. the galls) in the early stage of infection is a preprequisite for the enhanced respiration and synthetic processes both induced by infection. Increased fructose content in maize tissues infected by *Ustilago maydis* has been reported by DEVAY and ROWELL (1954) as well. Our finding on the decreased level of reducing sugars in the internodes below the gall tissues is substantiated by the observations of HURD-KARRER and HASSELBRING (1927) as well as by ESANU and NEGULESCU (1958, 1960). This phenomenon is due to the accumulation of substances at the infection site brought about by an enhanced translocation to tissues showing a high metabolic activity. The lower content of reducing sugars in tissues below the infection site probably involves a decrease in the nutrient supply of the root system. This, in turn, brings about a reduced physiological activity including nutrient uptake by the root system. This may be one of the causes of the altered metabolism and probably of the yield losses, as well as of the decreased growth rate of infected plants (PETHŐ, 1963a).

One of the very characteristic features of infected plant tissues is the activation of respiratory enzymes (cf. ALLEN, 1953; FARKAS and KIRÁLY, 1958; FARKAS, 1965; RUBIN, 1959). The significance of the increased respiration in the host-parasite relationship is not fully understood. In the present paper a marked respiratory increase in stem tissues of maize infected with crown gall has been demonstrated. MIHALEVSKAYA (1964) made similar observations with leaf tissues. She pointed out, however, that the respiratory increase can be observed only in

the early stage of infection. When the chlamidospores appear a pronounced decrease in the rate of respiration of infected tissues sets in. The respiratory quotient was in our case 1.05, whereas MIHALEVSKAYA reported a value of 0.89 to 0.98 and 1.20 for young and older galls, respectively. FELDMAN (1948) measured a respiratory quotient of 1.20 in the galls. The contradictory data may be due to the fact that in the experiments mentioned above maize varieties with different susceptibilities were used.

In the uninfected internodes of diseased plants of the highly susceptible local variety 62 we have found an increased respiratory rate. This is in contradiction with the observations of FELDMAN (1948) and MIHALEVSKAYA (1964) who reported a lowered intensity of respiration in uninfected internodes and leaves of diseased plants as compared to the control. This contradiction can be reconciled by referring to our previous observation (PETHŐ, 1963b) on the peculiar behaviour upon infection of this local variety. It has been established, namely, that with this peculiar variety even in the uninfected organs of diseased plants there was an accumulation of free amino acids in contrast to other less susceptible varieties. Therefore the slight respiratory increase seems to be justified.

In order to study the respiratory pathways in crown gall infected maize plants selective enzyme inhibitors were applied. Inhibitors of glycolysis (NaF and iodoacetate) markedly inhibited O_2 uptake by infected tissues. From this we conclude that respiration in the host-parasite complex follows, at least in the early stage of infection, the Embden-Meyerhof pathway, though FELDMAN (1948) has found NaF to be ineffective. In our experiments inhibitors of the citric acid cycle (malonate and fluoracetate) proved to have a pronounced inhibitory action on the O_2 uptake of infected tissues. This observation is in line with FELDMAN's (1948) data on the malonate sensitivity of infected internodes.

Accumulation of α-keto-acids in infected tissues, as reported by TURIAN (1962) as well, points to the increased significance of the tricarboxylic acid cycle in the respiration of the affected tissues. Increased respiratory rate, higher malonate sensitivity of the respiration and accumulation of keto acids in infected tissues, if taken together, suggest that the role of increased respiration in maize plants infected with crown gall is to ensure a higher level of keto acids which are necessary for an enhanced synthesis of amino acids and proteins. There seems to be a stimulation of the overall biosynthetic activity of infected cells as evidenced by a higher reducing sugar content, an increased respiration including activation of the tricarboxylic acid cycle, higher content of keto-acids and amino acids (PETHŐ, 1963b; PETHŐ, 1966; TURIAN, 1962). This leads to a higher protein content of the tissues involved (PETHŐ, 1966).

There is a definite connection between the level of amino acids and proteins in infected tissues and the susceptibility of the varieties concerned (PETHŐ, 1966). This suggests that the susceptibility of maize varieties to *Ustilago maydis* largely depends on the protein synthetizing ability of the individual varieties and on the sensitivity of their metabolic processes to the stimulatory effect brought about by the parasite.

Finally the experimental data presented suggest that upon infection with *Ustilago maydis* the terminal oxidation is also altered in maize tissues. At present, however, no final conclusion can be drawn about the nature of these alterations.

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Interaction of Phosphorus and *Fusarium* with the Major Element Nutrition of Cotton Plants

By

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Two cotton varieties "Ashmouni" and "Karnak" were grown in sand cultures to which extra amounts of phosphorus were added alone or in combination with either *F. oxysporum* or *F. moniliforme*.

Increasing the phosphorus concentration in the culture medium up to 100 p. p.m. resulted in an increase of the calcium content in the plants. Further increase of phosphorus concentration caused a decrease in the calcium content of both cotton varieties.

By varying the phosphorus level in the culture solution the sodium content of cotton plants belonging to the varieties "Ashmouni" and "Karnak" was affected in a way reciprocal to calcium content.

Phosphorus, potassium and magnesium contents in the tops of plants increased by increasing the phosphorus concentration in the culture medium.

Introduction of either *F. moniliforme* or *F. oxysporum* into the medium increased the magnesium and phosphorus contents of the tops of cotton plants much more than it was affected by the phosphorus levels alone.

At higher phosphorus levels viz. 200 to 500 p. p. m., the presence of *Fusarium* spp. in sand cultures increased the calcium content, and decreased the potassium content of the tops of plants as compared to the controls raised on non-inoculated sand cultures.

Introduction

Concerning the effect of phosphorus on the uptake of other essential elements by higher plants, it has been shown that lower concentrations of the former help the accumulation of total nitrogen in the different organs of the plant (MAC GILLIVARY, 1926, 1927; KRAYBILL, 1930; COLBY, 1933; CAROLUS, 1935; EMMERT, 1935; HAAS, 1936; RICHARDS and TEMPLEMAN, 1936; MC EVOY, 1951; and others). On the other hand, higher concentrations of phosphorus in the culture solution are usually accompained by a low nitrogen content of plants (CAROLUS, 1935). Phosphorus affects also the uptake of other elements, e. g. calcium, potassium, and magnesium (DANIEL, 1934, HAAS, 1936; and MCEVOY, 1951).

Another factor affecting plant nutrition is the effect of soil micro-organisms. As far as we know no work has been carried out on the effect of *Fusarium* spp. on the uptake of mineral elements by plants, except that carried out by SHAROUBEEM and NAIM (1962a). The results of these two authors indicate that *Fusarium oxysporum* produces two marked effects on the uptake of mineral elements by the cotton varieties 'Ashmouni' 'Mounoufi' and 'Karnak' namely: 1. a decrease

in the uptake of nitrogen, potassium and sodium, and 2. an increase in the utilization of phosphorus, calcium and magnesium by cotton plants.

The aim of the present work was to get some useful data of the nutrition of cotton plants, on one hand, and to use these data for choosing the proper levels of phosphorus applied in *Fusarium* – infested soils in order to ensure the best control of the vascular-wilt disease, on the other. Therefore, the effect of varying concentrations of phosphorus on the chemical composition of cotton plants belonging either to the wilt resistant 'Asmouni' or to the susceptible 'Karnak' variety was studied alone or in combination with either *Fusarium oxysporum f. vanifectum* or *Fusarium moniliforme*. These two *Fusarium* spp. were chosen because soils infested with *Fusarium oxysporum f. vasinfectum*, are liable to contain also *F. moniliforme*, which is pathogenic to corn. Many workers, however, have recorded the presence of *F. moniliforme* in the roots of different cotton varieties as well.

Materials and Methods

Seeds of both cotton varieties 'Ashmouni' and 'Karnak' were kindly provided by the Plant Breeding Department, Ministry of Agriculture, at Giza. Both *Fusarium oxysporum f. vasinfectum* and *Fusarium moniliforme* were isolated from 'Karnak' cotton seedlings and were kindly identified by Prof. W. C. SNYDER, Chairman of the Department of Plant Pathology, University of California. Single spore culture from the two *Fusarium* isolates were made. Suspensions of spores and mycelial fragments of each were prepared. Sterilized clay pots, the inside surfaces of which were coated with three layers of bitumen solution (HEWITT, 1952) were filled with sterilized washed sand. Each pot received 10 ml of washed fungal suspension in sterilized distilled water of either *F. oxysporum* or *F. moniliforme*. Five seedlings of equal height and vigour of either cotton variety, were transplanted to each pot. A basal nutrient solution (MONTASIR et al. 1960) which contained all the necessary macro-and micro-elements with phosphorus concentrations ranging from zero to 500 p. p. m., was used. All nutrient solutions were adjusted to pH 6.8. Each pot received a nutrient solution with the particular concentration, so as to reach 60% of the total water holding capacity of the sand. The decrease in weight of each pot was compensated every day by adding the same solution.

Control experiments were carried out without inoculation with either *Fusarium* sp. The pots were kept in a glass-house at a temperature of between 22° – 35° C.

After six weeks, the plants with the different treatments were harvested between 7 – 10 a. m. to avoid effects of diurnal variations in the leaf composition (CHAPMAN and GREY, 1949). Tops were quickly separated from roots, thoroughly washed with distilled water to remove all dust contamination (JACOBSEN, 1945) and oven dried. The dried shoots were finely ground and an aliquot was digested with sulphuric acid, clearified with hydrogen peroxide (LINDER, 1944 and WOLF, 1944) and made up to a certain volume with distilled water. Total nitrogen was

determined in aliquots of the peroxidase digested material using a micro-Kjeldahl distillation apparatus deviced by MARKHAM (1942). Magnesium was determined using titan yellow according to LINDER (1944). Determination of phosphorus was carried out by amino-naphtol sulphonic acid reagent (WOLF, 1944). Calcium, potassium and sodium were determined by flame-photometry.

Results

Chemical composition of plant tops, as affected by varying concentrations of phosphorus alone or in presence of either *F. moniliforme* or *F. oxysporum* in the culture medium, are given in Tables 1 to 3 for 'Ashmouni' and Tables 4 to 6 for cotton plants, variety 'Karnak'.

Table 1

Effect of varying concentrations of phosphorus on the chemical composition of 6-weeks old cotton plants (variety "Ashmouni") grown in non-inoculated sand cultures. Percentages of elements are calculated on a dry weight basis

Conc. of P in p. p. m.		% N	% PO_4	% K	% CaO	Na_2O	% MgO
	0	1.16	0.65	3.16	1.84	1.25	0.48
	20	2.96	1.04	3.55	2.10	1.18	0.74
	40	3.61	1.19	3.65	2.35	1.05	0.87
	60	4.12	1.35	3.89	3.43	0.89	1.02
	80	3.99	1.61	3.89	3.43	0.89	1.15
	100	3.87	2.13	4.13	3.29	0.89	1.34
	200	3.80	2.40	4.57	2.27	1.69	1.95
	300	3.61	2.79	4.92	2.02	1.81	2.30
	400	3.43	2.97	4.86	1.69	1.99	2.64
	500	2.99	3.17	5.10	1.45	1.99	3.02
Sig. diff.	P. 0.10	0.13	0.15	0.07	0.03	0.10	0.02
	P. 0.05	0.16	0.19	0.09	0.04	0.10	0.03

Discussion

1. Total nitrogen content in the tops of plants

Percentages of total nitrogen in in the tops of plants of both cotton varieties which were given different phosphorus and inoculation treatments are summarized in Fig. 1. The results show that nitrogen content increased with the increase of phosphorus concentration in the sand culture reaching its maximum at 60 p. p. m. for 'Ashmouni' and at 40 p. p. m. for 'Karnak'. These results are in accordance with the results obtained by MCEVAY (1951). Further increase in the phosphorus level up to 300 p. p. m. caused a decrease in the nitrogen content of the tops of plants. A similar effect caused by high phosphorus concentrations on the nitrogen

Table 2

Effect of varying concentrations of phosphorus on the chemical composition of 6-weeks old cotton plants (variety "Ashmouni") grown in sand cultures inoculated with *Fusarium moniliforme*. Percentages of elements are calculated on a dry weight basis

Conc. of P in p. p. m.		% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
	0	1.35	0.84	3.40	1.60	1.51	0.50
	20	2.38	1.62	3.64	1.86	1.38	0.81
	40	2.92	1.68	3.88	2.10	1.20	1.05
	60	3.65	1.85	4.13	3.29	1.02	1.17
	80	3.51	2.09	4.13	3.29	1.02	1.28
	100	3.38	2.62	4.19	3.29	1.02	1.48
	200	3.33	2.97	4.37	2.54	1.43	2.01
	300	3.25	3.15	4.50	2.26	1.65	2.39
	400	3.53	3.27	4.62	2.03	1.75	2.79
	500	3.60	3.40	4.62	2.03	1.75	3.19
Sig. diff.	P. 0.10	0.12	0.09	0.08	0.04	0.01	0.08
	P. 0.05	0.15	0.11	0.10	0.05	0.02	0.10

Table 3

Effect of varying concentrations of phosphorus on the chemical composition of 6-weeks old cotton plants (variety "Ashmouni") grown in sand cultures inoculated with *Fusarium oxysporum*. Percentages of elements are calculated on a dry weight basis

Conc. of P in p. p. m.		% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
	0	1.20	0.80	3.25	1.75	1.47	0.58
	20	2.14	1.86	3.45	2.05	1.08	1.00
	40	2.62	1.88	3.45	2.05	0.91	1.17
	60	3.24	1.96	3.98	3.64	0.68	1.34
	80	3.05	2.55	3.98	3.64	0.68	1.49
	100	3.00	3.02	3.98	3.44	0.88	1.60
	200	2.88	3.32	4.13	2.77	1.24	2.21
	300	2.76	3.64	4.37	2.27	1.42	2.62
	400	2.94	3.86	4.37	2.28	1.48	2.98
	500	3.16	4.04	4.37	2.28	1.48	3.39
Sig. diff.	P. 0.10	0.13	0.12	0.02	0.04	0.02	0.08
	P. 0.05	0.16	0.15	0.03	0.05	0.03	0.10

content of different plants was reported by CAROLUS (1935). However, higher phosphorus levels viz. 400 and 500 p. p. m. induced only a slight increase in the nitrogen content of the tops of plants raised on sand cultures which had been inoculated with either *Fusarium* sp. The presence of either *F. moniliforme* or *F. oxysporum* in the culture media depressed the nitrogen contents as compared to plants grown in non-inoculated sand cultures. The same finding has been re-

Table 4

Effect of varying concentrations of phosphorus on the chemical composition of 6-week old cotton plants (variety "Karnak") grown in non-inoculated sand cultures. Percentage of elements are calculated on a dry weight basis

Conc. of P in p. p. m.		% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
	0	1.08	0.92	3.40	1.65	1.95	0.42
	20	2.82	1.18	3.40	2.05	1.45	0.53
	40	3.58	1.22	3.76	2.25	0.99	0.73
	60	3.55	1.65	3.76	2.25	0.99	0.95
	80	3.41	2.19	4.13	2.90	0.47	1.10
	100	3.23	2.67	4.13	2.90	0.47	1.31
	200	2.90	3.11	5.10	1.83	0.73	1.89
	300	2.63	3.42	5.58	1.33	1.09	2.25
	400	2.58	3.62	5.62	0.94	1.54	2.60
	500	2.45	3.88	5.62	0.94	1.54	2.95
Sig. diff.	P. 0.10	0.15	0.20	0.11	0.05	0.08	0.17
	P. 0.05	0.19	0.25	0.14	0.06	0.10	0.21

Table 5

Effect of varying concentrations of phosphorus on the chemical composition of 6-weeks old cotton plants (variety "Karnak") grown in sand cultures inoculated with *Fusarium moniliforme*. Percentages of elements are calculated on a dry weight basis

Conc. of P in p. p. m.		% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
	0	1.32	0.68	3.89	1.16	1.75	0.45
	20	2.34	1.66	4.13	1.42	1.14	0.60
	40	3.15	1.65	4.37	1.62	0.80	0.85
	60	3.11	2.09	4.37	1.62	0.80	1.07
	80	2.91	2.49	4.86	2.17	0.41	1.27
	100	2.88	2.82	4.86	2.17	0.41	1.45
	200	2.63	3.37	4.86	2.07	0.58	2.10
	300	2.12	3.68	5.10	1.81	0.89	2.45
	400	2.62	3.88	5.58	0.98	1.35	2.74
	500	2.68	4.12	5.58	0.98	1.35	3.12
Sig. diff.	P. 0.10	0.10	0.13	0.10	0.08	0.03	0.09
	P. 0.05	0.12	0.16	0.12	0.10	0.04	0.11

ported by SHAROUBEEM and NAIM (1962a). In general it can be assumed that the change in total nitrogen content of plants due to the application of either *Fusarium* sp. at any given concentration of phosphorus is a result of variable plant growth. This is in turn a result of the effect of growth factors such as gibberellic acid or other related substances produced by *F. moniliforme* (SANDGREN and BELING, 1959; DAHLSTROM and SFAT 1961) or of vitamins, amino acids and auxins pro-

Table 6

Effect of varying concentrations of phosphorus on the chemical composition of 6-weeks old cotton plants (variety "Karnak") grown in sand cultures inoculated with *Fusarium oxysporum*. Percentages of elements are calculated on a dry weight basis

Conc. of P in p. p. m.		% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
	0	1.20	0.80	3.65	1.35	1.53	0.62
	20	2.11	1.80	4.05	1.86	0.99	0.91
	40	2.89	1.90	4.05	1.86	0.60	1.04
	60	2.82	2.38	4.25	2.78	0.35	1.18
	80	2.73	2.87	4.25	2.78	0.35	1.36
	100	2.69	3.31	4.25	2.78	0.35	1.59
	200	2.17	3.81	4.65	2.26	0.50	2.09
	300	1.65	4.15	4.65	2.26	0.69	2.65
	400	2.20	4.36	5.14	1.32	1.14	3.02
	500	2.31	4.69	5.14	1.32	1.14	3.35
Sig. diff.	P. 0.10	0.13	0.17	0.06	0.04	N.D.	0.04
	P. 0.05	0.16	0.21	0.07	0.05	N.D.	0.05

duced by *F. oxysporum* (MOUSTAFA and NAIM 1948: NAIM et al. 1957; and MONTASIR and YOUSSEF, 1960).

II. Phosphorus content in the tops of plants

Fig. 2 gives the phosphorus content in the tops of the experimental plants of different treatments. It is obvious that the phosphorus content in the tops of plants of both cotton varieties increased with the increase of phosphorus in the culture media, irrespective of inoculation. This result confirms the works of HAAS (1936) and Mc EVOY (1951). The presence of either *Fusarium* sp. especially of *F. oxysporum* in the culture solution further increased phosphorus content in the tops of plants. This can be attributed to the accumulation of fungal mycelia around the roots of cotton (MOUSTAFA and NAIM, 1955). The mycelia could in turn, accumulate large quantities of phosphate ions around the roots of the host (KRAMER and WILBUR, 1949) and absorb phosphate ions and transfer them to the roots (MELIN and NILSON, 1950).

III. Potassium content in the tops of plants

The potassium contents in the tops of plants which were given different inoculation and phosphorus treatments, are summarized in Fig. 3. As the phosphorus concentration in the culture media was increased, the potassium content of tops showed a slight increase. Similar results were obtained by MCEVOY (1951). This increase in the potassium content of the tops was greater with plants grown in presence of either *Fusarium* sp. than with the non-inoculated controls receiving

amounts of phosphorus ranging from zero to 100 p. p. m. Higher phosphorus levels, ranging from 200 to 500 p. p. m. induced a higher increase in the potassium content with plants raised on non-inoculated sand cultures than with those raised on sand cultures inoculated with either *Fusarium* sp. This is a result of a decrease in the potassium level of the culture solution due to the stimulatory effect of high

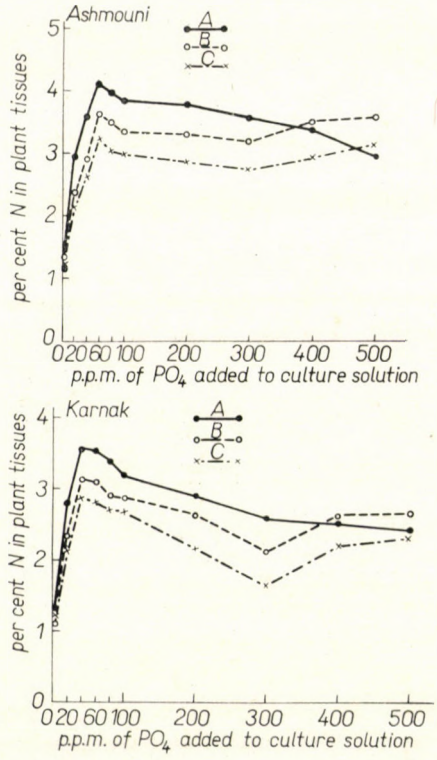

Fig. 1. Effect of varying concentrations of phosphorus in sand cultures inoculated or non-inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme* on the nitrogen content in the tops of plants of the cotton varieties "Karnak" (susceptible) and "Ashmouni" wilt resistant: A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated with *F. oxysporum* f. *vasinfectum*

phosphate concentrations on mycelial growth (SUBRAMANIAN, 1946 and SHAROUBEEM and NAIM 1962b) which is accompanied by an increase in the potassium content of the fungal mycelium (NAIM and SHAROUBEEM, 1963).

IV. Calcium content in the tops of plants

Concerning the effect of different phosphorus levels on the calcium content of the tops of plants – irrespective of inoculation – the results showed that the

calcium content of plants increased with the increase of phosphorus concentration in the culture media, reaching its maximum at 100 p. p. m. phosphorus. Further increase of the phosphorus level up to 500 p. p. m. in the nutrient solution was accompanied by a decrease in the calcium content of the tops of plants. (Fig. 4). These results are in accordance with the findings of DANIEL (1934). It is interesting

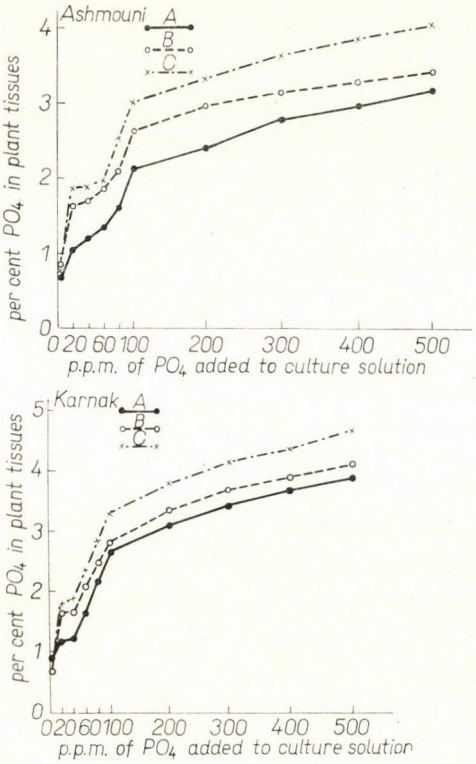

Fig. 2. Effect of varying concentrations of phosphorus in sand cultures inoculated or non-inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, on the phosphorus content in tops of plants of the cotton varieties "Karnak" (susceptible) and "Ashmouni" wilt resistant: A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated wilt *F. oxysporum* f. *vasinfectum*

to note that at any level of phosphorus in the culture media, the introduction of either *Fusarium* sp. affects the calcium content of the tops of cotton plant in a way reciprocal to its effect on potassium content especially at higher phosphorus levels ranging from 200 to 500 p. p. m. This result can be explained on the basis of an antagonistic effect between potassium and calcium in the culture medium. Utilization of a certain amount of potassium by *Fusarium* lowered the antagonism between potassium and calcium in the medium. This suggestion seems to be con-

firmed by comparing the potassium and calcium contents of the tops of plants. It is clear from these results that the increase in the calcium content in the tops of plants produced by the presence of either *Fusarium* sp. in the culture medium is associated with a decrease in the concentration of potassium.

V. Sodium content in the tops of plants

Sodium concentration in the plants decreased as the phosphorus concentraiton in the culture medium was increased up to 100 p. p. m. Addition of extra

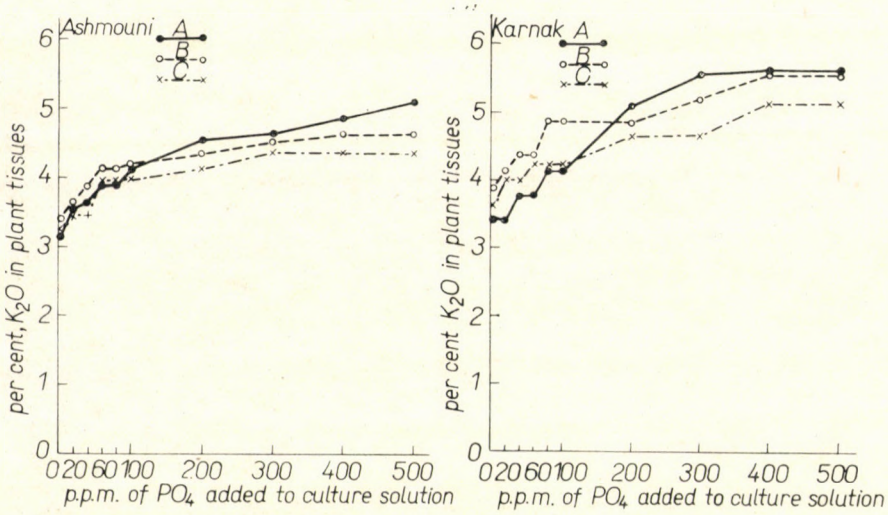

Fig. 3. Effect of varying concentrations of phosphorus in sand cultures inoculated or non-inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, on the potassium content in the tops of plants of the cotton varieties "Karnak" (susceptible) and "Ashmouni" wilt resistant: A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated with *F. oxysporum* f. *vasinfectum*

phosphorus ranging from 200 to 500 p. p. m. to the basic culture solution caused an increase in the concentration of sodium. These results are represented graphically in Fig. 5. It may be seen that the sodium content in the tops of cotton plants of both varieties, is also affected by the introduction of either *Fusarium* sp. to the sand culture, since the sodium content in the tops of plants which were given varying concentrations of phosphorus in presence of either *Fusarium* sp. was lower than when different phosphorus levels were used alone. This difference may be explained as a depressing effect of *Fusarium* on the uptake of sodium. Another explanation may be that an indirect effect due to the interaction of the mineral elements results in an increase or decrease of potassium or calcium contents of

the tops of plants. This, in turn, results in a decrease or increase in the sodium concentration of the plants. Results of COOPER and GERMAN (1943) confirm the latter conception.

VI. Magnesium content in the tops of plants

The magnesium content in the tops of plants belonging to both varieties 'Ashmouni' and 'Karnak' and given different inoculation and phosphorus treat-

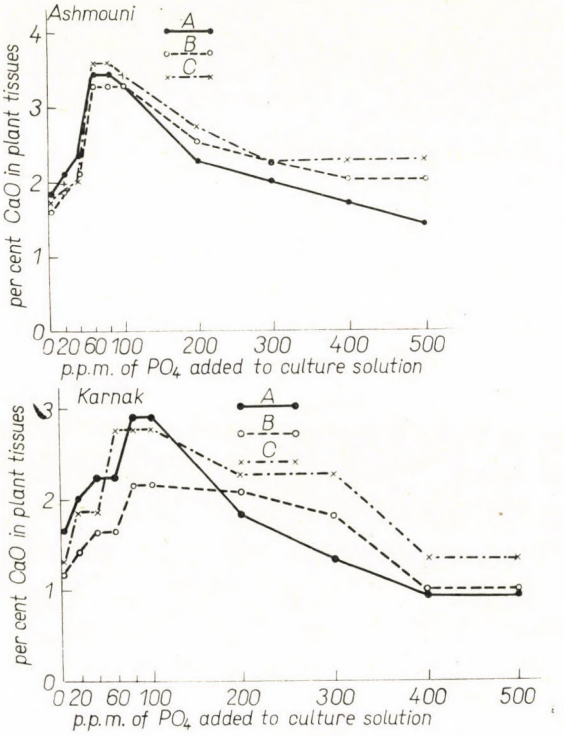

Fig. 4. Effect of varying concentrations of phosphorus in sand cultures inoculated or non-inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme* on the calcium content in the tops of plants of the cotton varieties "Karnak" (susceptible) and "Ashmouni" wilt resistant: A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated with *F. oxysporum* f. *vasinfectum*

ments are summarized in Fig. 6. There is a direct relationship between the increase of phosphorus concentration in the culture medium and the increase of magnesium content in the tops of plants of both cotton varieties. The increase of magnesium content in the tops was greater with plants grown with phosphorus and inoculated with either *F. moniliforme* or *F. oxysporum*, than with non-inocu-

lated plants grown with phosphorus. This difference in the increase of magnesium concentration in the plants must be attributed to the effect of the metabolites of *Fusarium* rather than to the fungus itself. This is based on the fact that *Fusarium* can penetrate only into cotton plants of the variety 'Karnak' but not into cotton

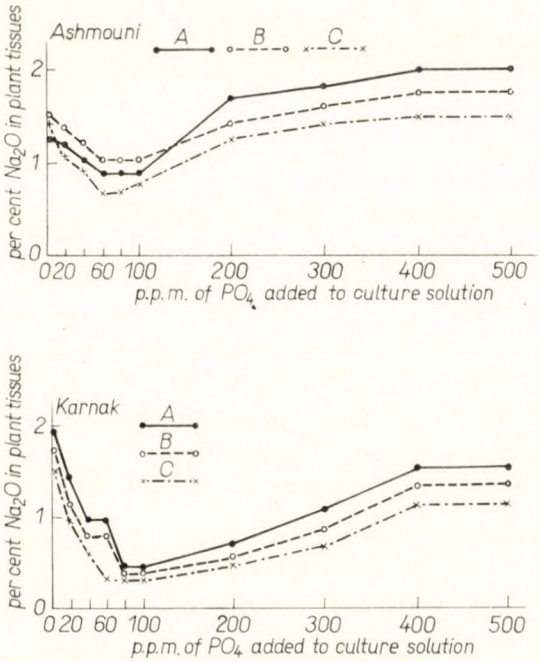

Fig. 5. Effected of varying concentrations of phosphorus in sand cultures inoculated or non-inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, on the sodium content in the tops of plants of the cotton varieties "Karnak" (susceptible) and "Ashmouni" wilt resistant: A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated with *F. oxysporum* f. *vasinfectum*

plants of the variety 'Ashmouni'. Assuming that these metabolites may form complex compounds with magnesium, will cause a depressing effect on chlorophyll formation. Therefore, the plants absorb much more magnesium to compensate for that loss. Chlorophyll is an organic complex compound containing a magnesium nucleus. Absence, lack, or inactivity of magnesium will induce chlorosis, and hence must be compensated by additional amounts of magnesium.

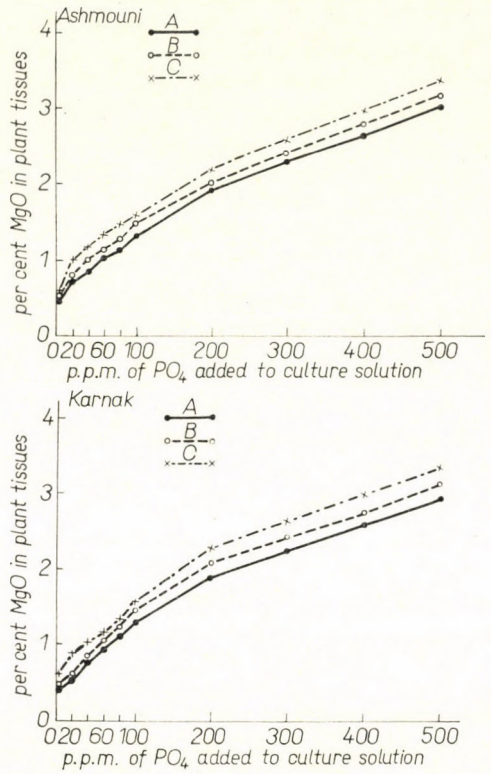

Fig. 6. Effect of varying concentrations of phosphorus in sand cultures inoculated or non-inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, on the magnesium content in the tops of plants of the cotton varieties "Karnak" (susceptible) and "Ashmouni" wilt resistant A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated with *F. oxysporum* f. *vasinfectum*

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Inducing Premunity in Plants by Attenuated Pathogens

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Results of the experiments described support the view that in the case of *Phytophthora infestans* premunity can be induced by zoospore suspension attenuated with the aid of heat treatment. Premunity developed on the cut surface of potato tubers is of local character.

Introduction

Human and animal organisms protect themselves against the pathogens of infectious diseases in different ways, premunity being perhaps the least widespread immune-reaction. In this case the organism or tissue infected by the primary pathogen remains immune from the superinfection by the same pathogen. This form of immunity, however, lasts only so long as the primary pathogen is viable in the organism attacked and has a pathogenic power. From this it follows that the premunity reaction is not able to destroy the primary pathogen in the organism. Thus, this reaction has only a practical significance if the primary infection is carried out by a strain of the pathogen causing a mild disease and the infection protects the host from infection of more virulent strain or strains belonging to the same species. Premunity being specific, may also afford aid in certain determination works.

Of the better known human diseases premunity plays a decisive role in the defence of the organism only in the cases of malaria, lues and tuberculosis. Premunity is considered also in the case of plant diseases as a potentiality of protection (SZEPESSY, 1963), which may obtain a significant role in the 5. phase of pathogeny, that is after the infection (SZEPESSY, 1964). This type of defence reaction was recognized in phytopathology for the first time by BERNARD (1909) in the case of mycorrhiza occurring in orchids. Later on, several workers succeeded in demonstrating the role of premunity in virus diseases (BERNARD, 1909; GÄUMANN, 1951; MÜLLER and BÖRGER, 1941; SALAMAN, 1933; YARWOOD, 1960) and subsequently with several diseases of fungal origin (ARNAUDI et al. 1964; MÜLLER and BÖRGER, 1941).

Material and Methods

The objective of the present work was to attempt to induce premunity with an artificially attenuated pathogen since we hope to get nearer this way to the

possibility of practical utilization of premunity reaction. As experimental object we too chose the fungus *Phytophthora infestans* (Mont) de By. which was already demonstrated by MÜLLER and BÖRGER (1941) to be able to induce local premunity on the cut surface of the potato tuber. As to the methods employed, however, we had to deviate from the experimental methodics suggested by the authors referred to. We proceeded by spraying the suspension of the pathogen weakened by various treatments on the whole surface of the tuber cut in two and after a certain time again with spraying we carried the pathogen on the whole surface. Intensity of the premunity obtained was measured by ranging the extent and intensity of the *Phytophthora* coating that appeared on the surface into classes marked by numbers (Fig. 1). This classification can be characterized as follows.

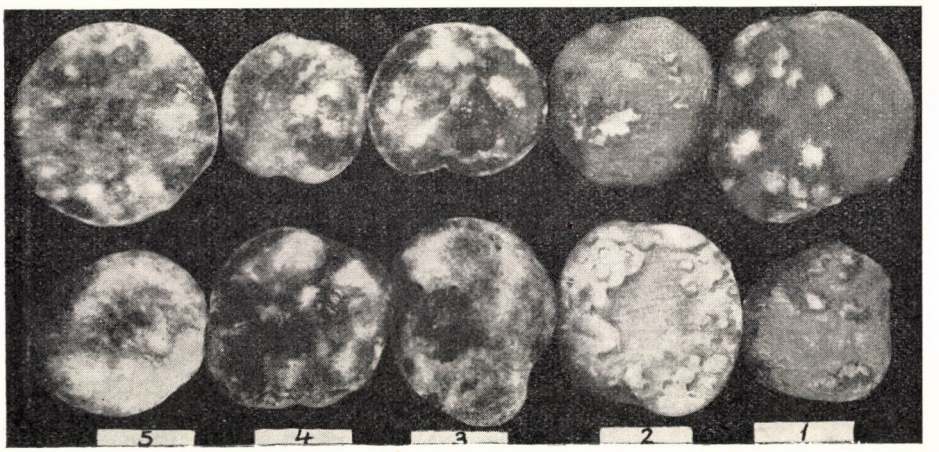

Fig. 1. Degree of mycelium coating of *Phytophthora* (5 = very pronounced, 1 = slight coating)

0 = this score is only given in the case when not even traces of the development of a fungus coating can be recognized with the naked eye on the surface of the cut and infected half tuber.
1 = mycelium is restricted to quite small spots or is remarkably poorly developed, hardly visible.
2 = development of mycelia is poor, the coverage highly defective.
3 = coverage is of the spotty type but the gaps are readily discernible, clear-cut.
4 = development of the mycelium is conspicuously poor although the mould is discernible on the overwhelming part of the surface.
5 = the intensively developed mycelial coating covers the whole surface of the cut half tuber.

It is clear from what has been said that this method of evaluation – although subjective – is highly suitable to measure the intensity of the premunity induced.

From the methodics of MÜLLER and BÖRGER (1941) we deviated also in the following details:

a) for superinfection the same strain was used – of course without any weakening – with which the primary treatment has also been carried out.

b) the superinfection was administered not 16 but 24 hours after the first treatment.

c) after superinfection the infected half tubers were kept at a temperature of 21° C.

Examinations were conducted in all cases in 4 series.

Results and Discussion

In the first experiments it was examined what degree of premunity the pathogen "weakened" at different temperatures can induce.

Therefore zoosporangia developed on the cut tuber surface were collected in a flask containing pure tap water. The flask was placed in a thermostat of 14°C temperature. After 4 to 5 hours swarming of the zoospores has set on. Subsequently 15 ml each of zoosporangium – zoospore suspension was measured into 5 flasks. The 5 flasks were then held separately on different temperatures according to the following pattern:

1. 5°C; 2. 15°C; 3. 22°C; 4. 30°C; 5. 45°C

The suspensions were held during 48 hours on these temperatures. It should be noted, however, that the given temperature values could not be precisely maintained: during 48 hours 4 readings were carried out the results of which are presented in Table 1.

Table 1

Change of the temperature values employed for the "weakening" of the pathogen

Measurements	Variants				
	1 5°C	2 15°C	3 22°C	4 30°C	5 45°C
1	6°C	15.5°C	25°C	30.5°C	44°C
2	5.5°C	15.0°C	20.0°C	31.0°C	43.0°C
3	3.5°C	13.5°C	20.0°C	30.5°C	44.0°C
4	4.0°C	13.0°C	25.0°C	31.0°C	45.0°C

After 48 hours the first "immunization" treatment of the cut half tubers was performed. The cut surfaces of 4 half tubers each per variant were sprayed with the suspensions containing the pathogen weakened in the way described. On the surface of the 4 half tubers marked out for control water was sprayed. Subsequently the half tubers were placed under separate bell-glasses but no moistening was performed as yet because the freshly cut half tubers were moist and did not imbibe the drops. The half tubers could not be arranged so that the cut surfaces should be entirely horizontal, therefore the suspensions ran off and even dripped

down. This is why two hours after the first spraying a second treatment was carried out. Between the two sprayings the suspensions stood in a thermostat of 14–15°C temperature.

One hour after the second treatment every half tuber separately was covered with a glass bell on the inner wall of which a band of filter paper that was kept continually wet ran along. 24 hours after the first "immunization" superinfection was carried out with fresh zoospore-suspension. The inoculum was carried on the surface also in this case with spraying. Subsequently the whole experimental material was placed in a room conditioned to 20–22°C temperature.

Evaluation was made after 7 days when the following results were obtained (Table 2).

Table 2

Results of evaluation of mycelial coating developed on the surface of the half tubers

Variants	Evaluation of half tubers per variants				Scores	
	1	2	3	4	Sum	Average
Control	5	5	5	4	19	4.75
1	5	3	5	4	17	4.25
2	4	3	3	3	13	3.25
3	3	4	4	4	15	3.75
4	2	3	2	3	10	2.50
5	2	4	1	1	8	2.00

Mycelial coating found on the half tubers of the variant No. 5 and the control is seen on Fig. 2. Since in the experiment described variant No. 5 figured as

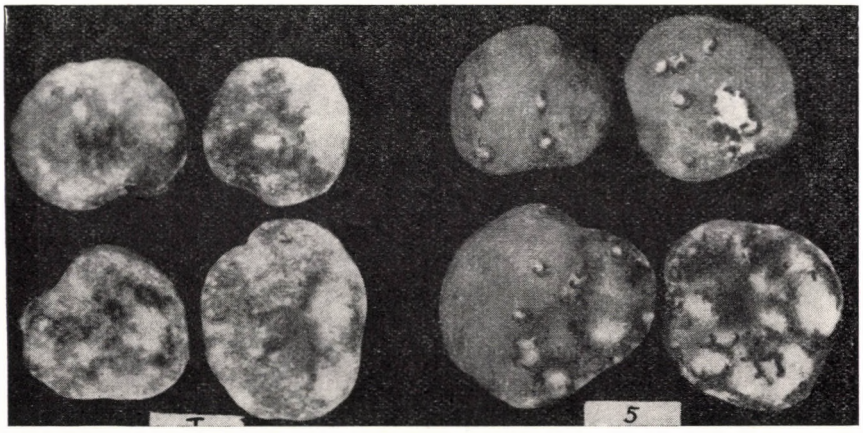

Fig. 2. Success of superinfection on the surface of control (T) and pretreated half tubers, respectively

best of all, that is the pathogen weakened at a temperature of 45°C induced the strongest premunity, in the following we wanted to know what time the pathogen used for primary infection must be kept at the temperature indicated to obtain best effect.

For comparability the suspensions were made at the same time in an identical way and subsequently kept for various periods at the desired 45°C temperature. In the variants where the suspension had to be stored for a certain time the flasks were placed in a thermostat of 5°C temperature, as previously we made the experience (Table 2.) that this temperature from the view-point of inducing the premunity is almost ineffective on the pathogen. Treatments can be read off Table 3.

Table 3

Data of heat treatment of suspensions containing the pathogen used for primary infection

Variants	Period of storage of suspensions in hours		
	at 5° C	at 45° C	total
A	34	12	46
B	22	24	46
C	10	36	46
D	0	46	46

At the end of the treatment superinfection was carried out with spraying and further on the same procedure was followed as in the previous experiment. At evaluation the following results were obtained (Table 4.)

Table 4

Results of evaluation of the mycelium coating developed on the surface of the half tubers

Variants	Evaluation of half tubers per variants				Scores	
	1	2	3	4	Sum	Average
Control	5	5	4	5	19	4.75
A	4	4	3	4	15	3.75
B	1	1	1	2	5	1.25
C	2	2	1	3	8	2.0
D	3	4	3	4	14	3.5

These results point to the fact that 45°C heat treatment for 24 hours is sufficient for the "weakening" of the pathogen. In the following we were guided by this statement.

Finally we examined the question whether the first, so-called "immunization" treatment had any long-distance effect since GÄUMANN (1951) took definitely the stand that premunity which can be observed in the case of plant diseases is always of a local character. To elucidate this question we proceeded as follows. The suspension for the primary infection was kept for 22 hours at a 45°C temperature. Then 4 half tubers were sprayed with water (control) and 8 half tubers with

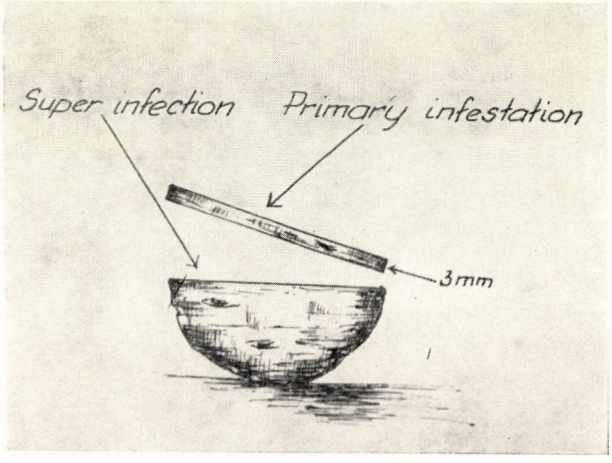

Fig. 3. Method for the demonstration of local premunity. (Explanation in the text)

the suspension of "weakened" pathogen. Spraying was repeated in 2 hour's time. In 24 hours from the first spraying, of the 8 half tubers sprayed with the suspension of the "weakened" pathogen on the cut surface of 4 half tubers (I) superinfection was carried out in the way described in the previous experiments.

From the 4 half tubers of the second group (II), however, according to the scheme on Fig. 3, disks of 3 mm thickness were cut off and superinfection carried out on the newly cut surface. Later, after reading of the results (Table 5) we came to the conclusion that premunity induced by the primary infection is as a matter of fact of local character, more exactly, in the given case it does no more make its effect felt at a distance of 3 mm.

Table 5

Results of the evaluation of mycelium coating developed on the surface of the half tubers

	Evaluation of the half tubers per variants				Scores	
Variants	1	2	3	4	Sum	Average
Control	5	5	4	3	17	4.25
I	1	2	1	1	5	1.25
II	5	5	4	5	19	4.75

Thus the experimental data support the assumptions of GÄUMANN (1951). Further details of the problem must be answered by further and more extended experiments.

In summary, in the present work premunity induced in potato tubers by *Phytophthora infestans* was studied. A method different from those used so far (MÜLLER and BÖRGER, 1941) were developed and an evaluation scale (Fig. 1) has been worked out which is suitable for the measurement of premunity. In the "immunization" treatments zoospore suspensions were used which have been previously attenuated by heat treatment. It has been established that 45°C is best suited for this purpose (Table 2). It has also been shown that 24 hours are needed at 45°C for the weakening of the pathogen (Table 4).

After "immunization" with a pathogen weakened by such treatment premunity develops on the surface of the potato half tubers and superinfection after the first infection can no more induce a disease of full value (Tables 2 and 4).

Experiments also indicated that in this case premunity is of local character (Table 5).

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Mycological Investigations in Some Hungarian Forest Types and Special Sites, I

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In the Hungarian Bükk-Mountains soil fungi produced the largest quantities of fruit bodies in the *Melica* type of the forest association *Querco petraeae-Carpinetum pannonicum*, but the forest types *Genisti tinctoriae-Quercetum petraeae subcarpaticum* and *Quercetum petraeae-cerris pannonicum* showed also a considerable mycopotential. Out of the forest types of the Noricum (Sopron) the associations *Luzulo-Querco-Carpinetum noricum*, *Piceetum excelsae-carpinoso-quercosum* and *Querco petraeae — Carpinetum transdanubicum* proved to be of highest mycopotential. Due to their abundance in characteristic species *Querco-Betuletum-Callunetosum*, *Abieti-Fagetum noricum* and *Aceri pseudoplatani-Alnetum glutinosae* are floristically the most interesting associations. From the mycofloristic aspect *Castaneo-Quercetum noricum* is a transitional community between *Luzulo-Quercetum* and *Piceetum*.

Introduction

In researches into the cryptogamic vegetation modern coenological methods have recently become more and more conspicuous (HUECK, 1953; W. B COOKE, 1955; UBRIZSY, 1956; BOHUS and BABOS, 1960). It follows as a matter of course that moss and lichen communities accessible for coenological analyses were investigated long before and more thoroughly than fungus populations of exceedingly ephemeral appearance and changing in their composition from time to time. However, since the pioneering and highly prolific researches by HAAS (1932) and HÖFLER (1938) the fungus coenoses living in the soil (geomycophyta) and occurring epiphytally on trees as epixylous aeromycophyta are world-wide examined with modern methods; some research workers achieved considerable results already so far. These investigations were recently extended to the dynamic structure of mycocoenoses and to interactions existing among coenostructure, aspect rhythm as well as the processes of metabolism and transformation of energy. In mineralization, humification, nitrification etc., attributable to conversion of energy in forest soils the mycotrophic and saprophytic higher fungi play also an important role. The ectoenzyme system of these macroscopic soil fungi decomposes a larger quantity of litter than needed for their metabolism. As to quantitative conditions they do generally not depend on the quality of forest soils, but all the more on the timber volume production of the forest type and, respectively, on the local biomass developing in the biotope. Therefore the quantity of macroscopic soil fungi and

their role in metabolic processes highly change according to the biocoenosis types. The activity of macroscopic geomycophyta and aeromycophyta in the metabolism of forest biocoenoses can be assessed on the strength of their fruit body production.

Mycocoenological investigations in Hungary are being carried on by the author since 1938 and the results have been published in several papers (1940, 1941, 1943, 1948, 1956, 1959). BOHUS performed excellent production-biological (1952, 1954) and recently mycocoenological researches of high level (BOHUS and BABOS, 1960) studying the fruit body production in different forest types, as sites, for many years and striving to detect the qualitative correlations between the various forest associations and the quantitative occurrence of fungus populations developing in them. Thus, he offered a scientific basis for the organization of fruit body collection on different sites. The investigations of KONECSNI (1960) in the black locust stands between the Danube and the Tisza are also worth mentioning.

The author continued his mycocoenological researches in forest associations and forest types determined in some characteristic tracts of the Hungarian Medium Mountains. Especially the years 1955 and 1965, rich in precipitation, were favourable for large-scale examinations and permitted to go ahead with the permanent observations begun in previous years on plots under study in the Buda-, Mátra- and Sátor-Mountains. In the intermediate years, much more adverse for fungus growth, chiefly the stationary quadrats of the Buda-Mountains (Nagyszénás) could be examined. The exceedingly rainy year 1965 promoted the development of an abundant fungus vegetation in the whole country. Beside the regions mentioned above in this year the investigations were also extended to the coniferous and mixed forests of the Noricum around Sopron. Finally, data were gained from rock swards, karst bush forests, from the region "Balatonfelvidék", the Buda-Mountains and the shifting sand steppes between the Danube and Tisza.

The systematic examination of macroscopic fungi of the forest types is chiefly justified by their areas most suitable for fruit body collection. Ninty-five per cent of the fruit body yield brought to inland markets and gathered in the open come from the forests, this explains why is the practice increasingly interested in forest sites suitable for the collection and growth of fungi. Indeed, from the aspect of a nation-widely organized fruit body collection it is important to know, in which regions of the country (macro-climate!) may the most advantageous conditions be found for fungus vegetation and within the different regions which of the forest associations can provide – due to their ecological and coenological prerequisites – the occurrence and increased turning up of the most valuable fungus species. The mass appearance of fruit bodies is a precisely definite, strictly seasonal phenomenon determined strongly always by ecological factors, especially by the R-factor (UBRIZSY, 1948, 1956), and depends thus chiefly on the weather conditions of the year, but within these the various forest communities – forming considerably different biotopes and biotope complexes for the fungus growth – provide fruit body yields of entirely dissimilar composition and changing also in their volume.

Prior to such production-biological establishments doubtlessly thorough floristic and site-coenological examinations are needed. Quantitative coenological surveys permit – if the researches are conducted for many years – to recognize accurately the composition of mycocoenoses, the annual rhythm of their aspects (dynamics) as well as the yearly total fruit body yield of the various aspects and of the whole forest type in certain forest associations and in their different, most characteristic sub-units (subassociation, sociation, facies etc.) (For fruit body collection it is very important to known the probable appearance and continuity of the aspects, i.e. their dynamics).

In their excellent methodological work BOHUS and BABOS (1960) presented a detailed analysis on the local and "combined" fruit body production of mycocoenoses. This production capacity (potential) of the various forest types, as biotopes, may be established with the combined or complex production values obtained as the product of the quantity and weight of fruit bodies. These values are simultaneously also suitable to analyze the elective influence exerted by the ecological factor collective of different forest types on various fungus groups. The summarized production values calculated on the basis of the minimum areas and expressed in hectares must be brought into correlation with the timber volume production data of forest types, because from this the relationship between the fruit body yield and the site quality of the biotope may also be deduced. On the strength of investigations performed in Hungary for nearly 10 years BOHUS and BABOS pointed out that the fruit body production, as biomass, is the highest in the typical oak forest *Genisti tinctoriae-Quercetum petraeae (= Luzulo-Quercetum petraeae)*. With higher timber yield the fruit body production of saprophytic geomycophyta generally increases, whereas that of mycorrhizal fungus species steps up only to the forest association mentioned above and diminishes in further forest types of the subsequent climax series. Accordingly the fruit body production the myco-biomass containing also the mycelia, do not directly depend on soil quality, because in climax forests and in those of most optimum site the yield is relatively lower and, on the other hand, a function of the special complex effect of ecological conditions determined by the forest types.

Hungarian investigations have also shown that forest types of acid soil and low site quality (e. g. the calcifugous *Vaccinium* oak forests) have a very notable mycorrhizal fungus vegetation, which plays a significant role in the nutrition processes of trees. With increasing humid conditions the importance of mycorrhizal fungus vegetation does not diminish in some forest types, while it falls behind the activity of saprophytic fungus vegetation in others. On slightly acid and neutral soils mineralization and humification become slower as a consequence of the thicker litter layer and in such soils the quantity of fungus mycelia is probably restricted by the increasing decomposition of animal residues.

The first investigation into the fruit body production of forest types, as biotopes, were performed by JURAVJEV in Karelia on 18 fungus species in 1933 and 1934. He found 3000 fruiting bodies, i. e. a quantity of about 60 kg per hectare annually. Birch forests proved to be the best sites. In Germany BÖTTICHER, PANN-

WITZ and NIER (1948) performed surveys in different forest types in 25 occasions and established 100 kg/ha total yield annually. The whole forest area of Germany amounts to 18 million hectares, the yearly fruit body production of fungi was assessed to 20 million tons. Similar analyses were conducted by FEHÉR and BESSENYEI (1933) in Hungary, near Sopron. LARSEN (1934) made analogous establishments in Denkmark, taking 39 fungus species into consideration. HÖFLER (1938) evidenced 62.5—120—180 kg fruit body yield per hectare in the various forest types of Austria. RAUTAVAARA (1947) obtained higher values in Finland having favourable precipitation conditions for macroscopic soil fungi. He published the following estimated data from 1944 and 1945: in dense humid woodlands the yield averaged to 302 kg, in light humid stands to 82 kg, in mossy lichenous forests to 107 and in productive pine moors to 93 kg per hectare. On Poland it was NESPIAK (1959) who carried out calculations on the production and the mycopotential of biotopes respectively, analysing the forest types with the method of W. B. COOKE.

On the results of Hungarian investigations pertaining to this matter a synoptic review was given by the present author in 1959. As to the complex fruit body production recently BOHUS and BABOS (1960) have made the following statemets: On the sample plots of the Buda- and Bükk-Mountains the yield was the highest in the *Melica* type of *Querco petraeae-Carpinetum pannonicum* (mixed oak-hornbeam forest), ranging in the rather unfavourable and arid Buda-Mountains from 7 to 132 kg, while in the favourable, humid Bükk-Mountains from 8 to 160 kg. As to the mycopotencial of the different Hungarian forest types the humid types of *Genisti tinctoriae-Quercetum petraeae* stand first and are followed by the different types of *Querco-Carpinetum pannonicum* and *Quercetum petraeae-cerris pannonicum (= Potentillo-Quercetum)*. On the basis of fruit body production precise data can also be obtained as to the proportion of edible and poisonous fungus species. In Hungary the proportion of edible fungi is 60 to 75 per cent of the whole fruit body production and is seldom less, while in Finland it touches 80 per cent according to RAUTAVAARA. In 75 per cent of the examined Hungarian forest types the proportion of toadstool species and their fruit body yield ranges only from 0.2 to 5.0 per cent in the different types of *Genisti tinctoriae-Quercetum*, while from 11 to 15 per cent in the others. Both total and edible fruit body yield is the highest in the oak forests *Genisti tinctoriae-Quercetum*, but the significance of these forest types is also increased by the fact that the best sites of *Boletus edulis*, the most important edible fungus species, are to be found in these woodlands providing also the highest production of *Cantharellus cibarius* and of the edible *Russula* species. Of course, these forests are also high-class sites for *Amanita phalloides* the most dangerous toadstool species.

Methods

In his mycocoenological surveys the author (UBRIZSY, 1956) applied already approved methods. As far as possible quadrats of 100 square metres were laid-out, the pieces of occurring fungus species counted, the data of abundance and domi-

nance (according to the scale of BRAUN-BLANQUET) as well as the sociability of the various species recorded and the values of frequency (local continuity) and constancy estimated HUECK, 1953; COOKE, 1955; BOHUS and BABOS, 1960). In the Tables also the substrata (Sb) and the life forms (F) were displayed. Beside the macroscopic soil fungi also the epixylous communities appearing in the herbaceous layer were valuated. To characterize the different forest types the locally faithful species and species combinations though still of disputable value were listed. Most useful and most expressive for both scientific investigations and practice is the clear and definite segregation of fungus aspects, termed usually according to the constant (frequent)-dominant species. However, we must not forget that these aspects, showing more or less regularity in their appearance from year to year, are not independent mycocoenoses but only "seasonal" parts of a mycocoenosis characteristic for a certain biotope or forest type. The higher soil fungi form a separate synusia in the herb layer, similarly also the xylophagous epixylous fungi appear as an independent synusia in the herb or tree layer of the site (UBRIZSY, 1941; 1943, 1955; PIRK, 1952).

It would led us too far if in this paper all the young and in many parts still undeveloped problems of mycocoenology would be tried to discuss. The standpoint of the author was already fixed in connection with some basic concepts and methodological procedures (UBRIZSY, 1956, 1959). Very valuable and suggestive reviews are presented on the already clarified as well as on the still disputable problems in the synecological studies of HUECK (1953), HÖFLER (1955), KREISEL, NESPIAK (1955) and, from overseas, in those of W. B. COOKE (1955), whereas an entirely new and greater prospect is opened by the synthetic work of BOHUS and BABOS (1960) containing methodological and coenological analyses. Further details are taken from these papers.

Mycocoenoses changing on the same site at the different periods are called aspects. The seasonal mass phenomena of fungus fruit bodies are expressed by the regular return and sequence of fungus aspects (FRIEDRICH, 1940, 1954; HÖFLER, 1954). Their annual dynamics, however, does not integrate itself with the phenological order of phanerogamic vegetation, but develops, in an exceedingly ephemeral manner, independently, according to the changes of the R-factor. Therefore, the precise and detailed surveys performed in a single year cannot give a satisfactory picture on the fungus vegetation and aspect conditions of a certain area, because the run of the R-factor may show smaller or greater changes from year to year due to its different components and their mutual correlative substitution. According to the investigations of the author following most important aspects can be established: vernal (in April and May), pre-aestival (in June), aestival (in July and August), pre-autumnal (in September), autumnal (in October), medio-autumnal (in November) and hibernal (from December to February).

In the fruit body formation the annual distribution of the stenothermic species (= depending on temperature demand) is closely correlated with the seasonal phenomenon. As it was pointed out by BÄSSLER (1944), in the course of the vegetation period several species of the same genus are succeeded by one another

in the aspects, e. g. *Tricholoma georgii* and *Tri. melaleucum* by *Tri. terreum; Boletus impolitus* by *B. radicans; Russula emetica* by *R. fragilis* and *R. densifolia* by *R. nigricans;* while *Lactarius piperatus* by *L. vellereus* etc. On the probable mycocoenosis of a certain site the most precise picture is today afforded by the examination of aspect conditions, requiring, therefore, inevitably thorough and permanent analysis. The establishment of many authors is of general validity that the so-called maximum aspect falls on the late summer or autumnal months. For summer months the mass appearance of most of the *Boletus*, *Lactarius* and *Russula* species is characteristic, whereas in autumn the *Cortinarius*, *Inocybe*, *Limacium* etc. and in late autumn the cold-resistant *Mycena* species dominate. This phenomenon is closely correlated with the heat and moisture demand of the above species, accordingly no mass fructification can be expected from them in an other season. Hungarian investigations revealed that in the summer aspects e.g. the *Russula* species participate with a proportion of 40 per cent, decreasing to 4 – 5 per cent in the autumnal aspects. BOHUS and BABOS have pointed out that due to fluctuations depending on temporal, specific and local conditions the different myco-aspects cannot be considered equivalent to those of the phanerogamic vegetation and may rather be looked upon as "pseudoaspects".

Relations among the examined plant communities and their mycovegetation

The most important study area of the author was the Mount Nagyszénás (near the village Nagykovácsi) in the Buda-Mountains; the aspect analyses were started as early as 1953 conducted in the oak-hornbeam and mixed forests growing on the NE-slope of this mount. Owing to excessively large quantities of precipitation and to warm humid weather conditions hihgly favourable for the mycovegetation in the years 1955 and 1965 extremely large masses of fruit bodies produced. And not only the yield of fungi but also the number of appearing species increased considerably in comparison to that of previous years. 1956 was already a dry, droughty year permitting to survey only the aestival maximum aspect fragmentarely, while in 1957, despite the simultaneously unfavourable weather, the mycoaspects of the mixed forest type and of the *Carex silvatica* facies could also be observed. 1958, with its summer precipitation of relatively more favourable distribution resulted in a fairly regular fungus fructification (in June, July and October), whereas in 1959 and 1960, again, merely the so-called "rest aspects" could be registered in August and September. During the excessively arid years 1961 – 1964 the mycorrhizal fungi did not produce fruit bodies, and even the saprophytes, specific for the humus and litter, occurred very sparsely, unsuited for regular surveying (Fig. 1.)

In Hungarian deciduous forests (of both the Great Plain, the so-called Alföld, and the Medium Mountains) the aestival and pre-autumnal aspects (i.e. the communities developing in the months July to September) are the richest not only in species but also in the number of specimens, representing thus the "maximum"

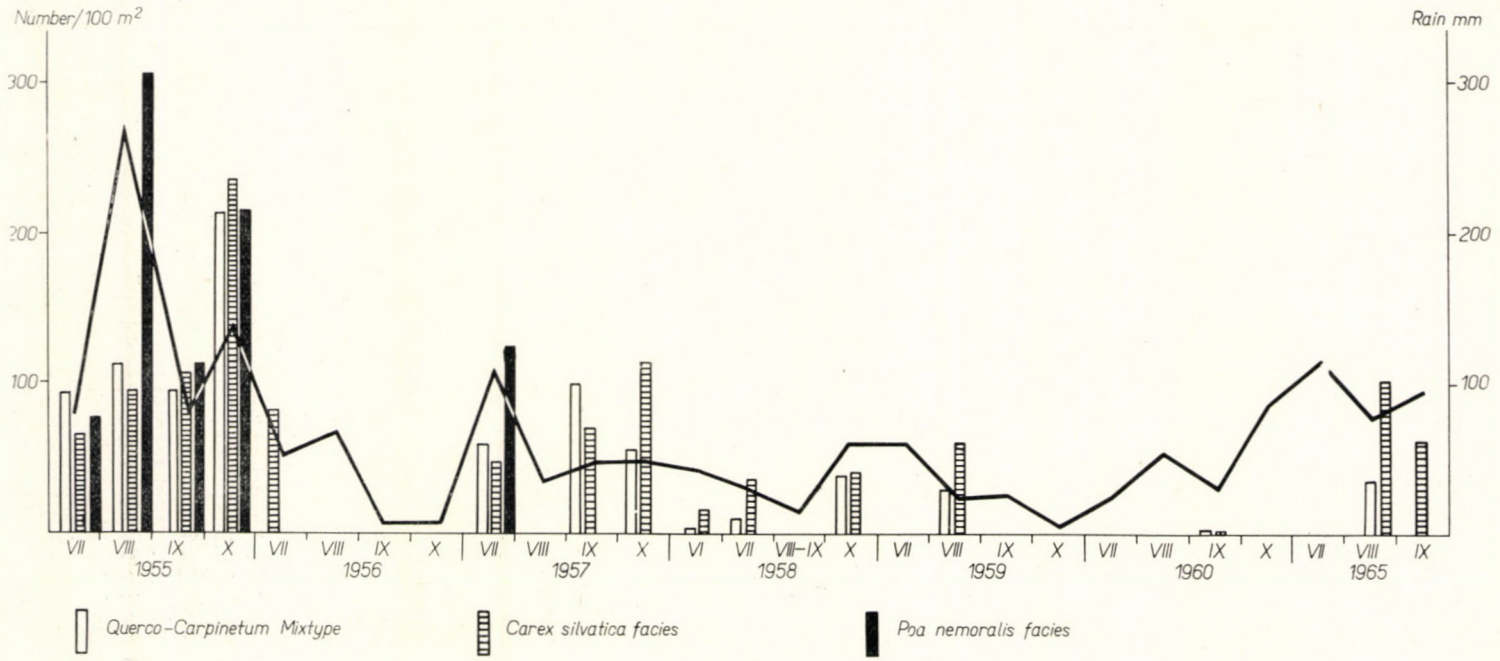
Number/100 m²
Rain mm
300
200
100
VII VIII IX X VII VIII IX X VII VIII IX X VI VII VIII–IX X VII VIII IX X VII VIII IX X VII VIII IX
1955 1956 1957 1958 1959 1960 1965
Querco-Carpinetum Mixtype
Carex silvatica facies
Poa nemoralis facies

Fig. 1

aspect according to FRIEDRICH's interpretation. Under the summer drought climate, e. g. in summer 1956, only 11 fungus species comprising 81 pieces could be collected in the relatively most hygrophilous *Carex silvatica* facies most favourable for fungus growth; this happened in June, and no fungus fructification was observed neither before nor afterwards. On the other hand, in mixed forest types as well as in the *Carex* and *Poa nemoralis* facies in July 88, 51, 73, in August 105, 84, 301, in September 83, 97, 110 and in October 207, 231, 208 fruit bodies were found. In November the fungus vegetation already stood still except the epixylous aeromycophyton synusia. The October aspect represented the secondary maximum of fungus appearance.

The author's investigations (UBRIZSY, 1948, 1956) on the R-factor (= soil temperature + the tenfold quantity of soil moisture) have revealed that its value characterized best the quantitative and qualitative composition of the fungus populations in all biotopes and in every case. It was the highest in the forests of the Great Plain, but culminated twice in those of the Medium Mountains: in summer (exactly in June or July, afterwards in August or September) and in autumn (in October or November, not very often in November or December). In summer the R-factor is rather characterized by data of high soil temperature and in autumn by a higher level of soil moisture. Unfortunately, this factor requiring complicated examinations on the spot could not be analysed in the last years, therefore, dealing with ecological relations of recent investigations, the author could merely rely on the meteorological data of the macro-climate (Diagram 1).

Areas investigated

1. *Nagyszénás. — a)* The *Carex silvatica* type of *Querco petraeae-Carpinetum pannonicum* Soó. The tree layer comprises: *Acer pseudoplatanus, Carpinus betulus, Fagus silvatica, Fraxinus excelsior, Prunus avium, Quercus petraea, Tilia cordata.* The degree of its cover ranges from 70 to 75 per cent. In the shrub layer: *Cornus mas, C. sanguinea, Crataegus oxyacantha, Carpinus, Sambucus nigra;* cover: 15 to 20 per cent. The herb layer is dominated by *Carex silvatica* (A – D: 4) and contains the following species: *Asarum europaeum, Campanula persicifolia, Hieracium murorum, Lilium martagon, Euphorbia amygdaloides, Lathyrus vernus, Viola silvestris, Pulmonaria officinalis, Lamium galeobdolon, Campanula trachelium, C. rapunculoides, Fragaria elatior, Sanicula europaea, Valeriana officinalis* etc.; cover: 80 to 90 per cent.

Due to the frequently occurring drought the summer maximum mycoaspect generally appears only in August or September. So a fragmentary aspect developed in September of 1953, in August and September of 1955, in July of 1956, 1957 and 1958, while 1959 in August and 1960 again, in September. In summer of the rainy year 1965 the maximum aspect manifested itself during August and September. Out of all (148) species found in the area 95 belonged to this mycocoenosis. The rendzina soil is covered with a dense rich herb layer, therefore the fungi appeared en mass chiefly on the fairly bare spots around the trees. After heavy rains and if

temperature conditions are also favourable, often very big or even gigantic fruit bodies of the different fungus species appear (e.g. *Boletus edulis*, *Lactarius piperatus*, *Russula cyanoxantha*, *R. virescens* etc.).

On the basis of permanent investigations conducted for 12 years the constant-dominant species of the mycocoenosis developing in the forest type or rather biotope are as follows: *Amanita rubescens*, *A. vaginata*, *Cantharellus cibarius*, *Collybia radiata*, *Lactarius piperatus*, *Limacium eburneum*, *Mycena pura*, *Russula lepida*, *R. vesca*, *Stereum hirsutum*, *Trametes versicolor*, *T. zonata* and *Vuilleminia comedens*. The perennial epixylous synusia *Schizophyllum commune — Stereum hirsutum — Trametes versicolor (— Lentinus cochleatus)* developing on stumps is also important. *Collybia dryophila*, *Lactarius pyrogalus* ssp. *circellatus*, *L. subdulcis*, *Russula cyanoxantha*, *R. ochroleuca* are further frequent, and *Strobilomyces strobilaceus*, *Lactarius pyrogalus* ssp. *circellatus*, *L. volemus*, *Lentinus cochleatus*, *Ramaria amethystina* characteristic species of the mycocoenosis (Table 1).

As fairly constant aestival and pre-autumnal maximum aspects — beside those published in previous papers (1956, 1959) — the following should be mentioned: the *Amanita rubescens — Lactarius piperatus* aspect; in July of dry years the xerothermophilic types: *Cantharellus cibarius — Lactarius piperatus*, and *Cantharellus — Lactarius subdulcis;* moreover the aspects *Russula lepida — Lactarius azonites* (in August 1957); *Amanita vaginata — Lactarius piperatus — Russula foetens* in August, and the epixylous aspect: *Pluteus cervinus — Panus stipticus*. For September and October the subsequent autumnal aspects are characteristic: *Armillaria mellea — Hebeloma crustuliniforme — Limacium eburneum* and *Hebeloma crustuliniforme — Limacium eburneum;* while *Collybia acervata — Mycena pura* appears only in September and the *Cortinarius brunneus — Mycena pura — Armillaria mellea* merely in October.

HÖFLER (1954), in his very noteworthy study of ecological view on myco-aspects, differentiated the following aspect types: dry aspects (Trockenaspekte), wet aspects (Nässeaspekte), warm aspects (Wärmeaspekte), cold aspects (Kälteaspekte) and rest aspects (Restaspekte), the latter is usually very poor in species. In the forests of the Hungarian Great Plain *Collybia dryophila — Scleroderma vulgare* appears frequently as rest aspect at Szarvas; it is a highly xerophilic myco-aspect consisting of xerothermophilic species. On the other hand, on the Mount Nagyszénás the aspect *Cantharellus cibarius—Lactarius piperatus* consists chiefly of xeromycophytes (in July and August). Extremely dry hot summers (e.g. 1960 to 1964) are meagre in precipitation and, consequently, due to the low moisture content of the soil, the fructification of fungi stops. Only the most xeromycophytes, living from dew and enduring the drought well, can grow, e. g. *Collybia*, *Marasmius* species and some *Gasteromycetes*. The aspect attains its full shape only in the ecological optimum.

The total number of species of the mycocoenoses developing in this forest type amounted to 148 during the 12 years of investigations but touched only 65 between 1958 and 1965; the number of fruit bodies was found ranging from 44 to 231 per 100 square metres (m^2) between 1953 and 1958, but only from 12 to

Table 1

Querco petraeae — Carpinetum pannonicum Soó	Sb.	S.	F.	Nagy-			
				19.6.1958	19.6.1958	28.7.1958	28.7.1958
Agaricus bisporus	T.	1	M	—	—	2, +	—
Aleuria vesiculosa	T.	2	S	—	—	—	—
Amanita pantherina	T.	1	M	—	—	—	—
Amanita rubescens	T.	1	M	—	—	—	—
Boletus chrysenteron	T.	1	M	—	—	—	—
Boletus edulis	T.	1	M	—	—	—	—
Boletus granulatus	T.	1	M	—	—	—	—
Boletus luridus	T.	1	M	—	—	—	—
Boletus pseudoscaber	T.	1	M	—	—	—	—
Boletus scaber	T.	1	M	—	—	—	—
Bovista plumbea	T.	1—2	S	—	—	—	—
Cantharellus cibarius	T.	1	M	—	—	—	—
Chlitris quercina	S.	3	P	—	—	—	—
Clitocybe geotropa	T.	1	S	—	—	—	—
Clitocybe infundibuliformis	T.	1	S	—	—	—	—
Collybia butyracia	T.	1	S	—	8, +	—	—
Collybia dryophila	T.	1—2	S	2, +	3, +	—	—
Collybia longipes	T.	1	S	—	—	—	—
Collybia platyphylla	T.	1	S	—	—	—	—
Collybia radicata	T.	1	S	—	1, +	1, +	—
Collybia tenacella	T.	1	S	—	—	—	—
Coprinus plicatilis	T.	1	S	—	—	—	—
Cortinarius anomalus	T.	1	M	—	—	—	—
Disciotis venosa	T.	1	S	—	—	—	—
Geastrum hygrometricum	T.	1	S	—	—	—	—
Hygrophorus obrosseus	T.	1	S	—	—	—	—
Hypholoma fasciculare	Tr.	3	S—P	—	—	12, +	—
Inocybe fastigiata	T.	1	S	—	—	—	—
Inocybe geophylla	T.	1	S	—	—	—	—
Lactarius piperatus	T.	1	M	—	—	13, + — 1	2, +
Lactarius pyrogalus ssp. *circellatus*	T.	1	M	—	—	—	—
Lactarius subdulcis	T.	1	M	—	—	—	—
Lactarius vellereus	T.	1	M	—	—	2, +	—
Lactarius volemus	T.	1	M	—	—	—	—
Lepiota erminea	T.	1	S	—	—	—	—
Lepiota gracilenta	T.	1	S	—	—	—	—
Lycoperdon gemmatum	T.	1—2	S	—	—	—	—
Marasmius rotula	T.	1—2	S	—	—	—	—
Mycena galericulata	Tr.	1—2	S	—	—	—	—
Mycena pura	T.	1	S	—	—	1, +	—
Nectria cinnabarina	Su.	3	S	—	—	—	—
Panus stipticus	Tr.	1—2	S	—	—	—	—
Phellinus pomaceus	P	2	P	—	—	—	—
Peniophora quercina	Su.	3	S	—	—	2, +	—
Polyporus adustus	Tr.	3	P—S	—	—	—	—
Psathyrella candolleana	T.	2	S	1, +	—	—	—
Rhodophyllus lividus	T.	1	M	—	—	—	—

szénás									Fr.
12.10.1958	12.10.1958	2.8.1959	2.8.1959	11.9.1960	11.9.1960	13.8.1965	13.8.1965	15.9.1965	
–	–	1, +	–	–	1, +	8, +	–	–	2
–	–	–	–	–	–	4, +	–	–	1
–	–	–	–	–	–	–	–	5, +	1
–	–	2, +	2, +	2, +	–	1, +	–	1, +	2
–	–	–	–	–	–	–	–	3, +	1
–	–	1, +	6, +	1, +	–	–	2, +	–	2
–	–	2, +	–	–	–	–	–	–	1
–	–	3, +	–	–	–	–	–	3, +	1
–	–	1, +	–	–	–	–	–	–	1
–	–	–	1, +	–	–	1, +	2, +	1, +	2
4, +	–	–	–	–	–	–	–	–	1
–	–	–	3, +	–	–	4, +	–	1, +	2
–	M, +	–	–	–	–	–	–	–	1
–	8, + – 1	–	–	–	–	–	–	–	1
–	2, +	–	–	–	–	2, +	3, +	5, +	2
1, +	1, +	–	–	–	–	–	–	–	2
2, +	–	–	–	–	–	–	–	–	2
–	–	–	–	–	–	5, +	2, +	–	2
–	–	–	–	–	–	1, +	–	–	1
–	–	1, +	2, +	–	–	–	–	2, +	3
–	–	–	–	–	–	–	5, +	–	1
–	2, +	–	–	1, +	–	–	–	–	1
3, +	–	–	–	–	–	–	–	–	1
–	–	–	–	–	–	2, +	–	–	1
–	–	–	–	–	–	–	1, +	–	1
–	–	–	–	–	–	3, +	–	–	1
–	15, +	–	–	–	–	–	–	–	2
–	–	1, +	–	–	–	–	–	–	1
–	–	–	–	–	–	–	–	2, +	1
–	–	1, +	18, 1	–	2, +	–	–	–	3
–	–	8, +	–	–	–	5, +	–	3, +	2
–	–	–	–	–	–	22, + – 1	–	1, +	2
–	–	–	–	–	–	–	2, +	8, + – 1	2
–	–	–	–	–	–	–	3, +	–	1
–	1, +	–	–	–	–	–	–	–	1
–	3, +	–	–	–	–	–	–	–	1
3, +	–	–	–	–	–	–	–	–	1
–	–	–	10, +	–	–	–	–	–	1
2, +	–	–	–	–	–	–	–	–	1
6, +	4, +	–	–	–	–	1, +	2, +	–	3
M, +	–	–	–	–	–	–	–	–	1
–	–	–	–	–	M, +	–	–	–	1
4, +	–	–	–	–	–	–	–	–	1
–	–	–	–	–	–	–	–	–	1
–	–	–	M, +	–	M, +	–	M, +	–	2
–	–	–	3, +	–	–	–	–	–	1
–	–	–	2, +	–	–	–	–	–	1

Querco petraeae — Carpinetum pannonicum Soó	Sb.	S.	F.	Nagy-			
				19.6.1958	19.6.1958	28.7.1958	28.7.1958
Rhodophyllus nidorosus	T.	1	M	—	—	—	—
Russula cyanoxantha	T.	1	M	—	2, +	1, +	—
Russula foetens	T.	1	M	—	—	—	5, +
Russula lepida	T.	1	M	—	—	—	3, +
Russula lutea	T.	1	M	1, +	—	—	—
Russula luteotacta	T.	1	M	—	—	—	—
Russula ochroleuca	T.	1	M	—	—	—	—
Russula vesca	T.	1	M	—	—	2, +	—
Russula virescens	T.	1	M	—	—	—	—
Schizophyllum commune	Tr.	3	S—P	M, +	M, +	—	—
Stereum hirsutum	Tr.	3	S	M, +	M, +	—	—
Trametes unicolor	Tr.	2—3	S	—	—	—	—
Trametes versicolor	Tr.	3	S	—	M, +	—	M, +
Trametes zonatus	Tr.	3	S	—	—	—	—
Tricholoma conglobatum	T.	1—2	S	—	—	—	—
Tricholoma melaleucum	T.	1	S	—	—	—	—
Tricholoma nudum	T.	1	S	—	—	—	—
Vuilleminia comedens	Su.	3	S	M, +	M, +	M, +	—
Total number of species: 66							
Number of species/Number of fruit bodies				6 : 7	8 : 18	11 : 36	5 : 12

79/100 m^2 between 1958 and 1965. The fairy-ring formation of *Lactarius piperatus* and the mass occurrence of *Amanita vaginata* f. *alba* are characteristic for this forest type, being of importance as the site of *Lactarius volemus*, *Cantharellus cibarius* and *Boletus edulis*.

b) *Poa nemoralis* facies of *Querco petraeae-Carpinetum pannonicum* Soó. The tree layer consists of the same species as that of the previous association, its cover amounts to 70 per cent, that of the shrub layer ranges from 20 to 30 per cent. In the herb layer (with a cover of 60 to 65 per cent) the following species are present: *Poa nemoralis* (A—D: 2—3), the plants mentioned in the tree layer, furthermore: *Satureia vulgaris*, *Chrysanthemum corymbosum*, *Veronica officinalis*, *Neottia nidus-avis*, *Dactylis aschersoniana*, *Dentaria bulbifera*, *Aconitum vulparia*, *Genista elata* etc. The mycocoenosis developing here and containing usually greater quantities of fruit bodies (i. e. the pieces per species occur in larger numbers) may only be examined in favourable years, because in dry summers (as happenned to be e.g. from 1956 to 1964) it does not evolve at all. This is due the fact that this facies occupies the higher, drier section of the slope. On the other hand, the minor cover of the ground vegetation promotes the mass appearance of fruit bodies in the optimum period (LEISCHNER—SISKA, 1939). Constant-dominant period species: *Lactarius piperatus*, *L. pyrogalus* ssp. *circellatus*, *Collybia radicata*, *Cortinarius multiformis*, *Russula luteotacta*, furthermore the members of the epixylous aeromycophyton synusia: *Stereum hirsutum*, *Trametes versicolor*, *Vuilleminia comedens*.

szénás									Fr.
12.10.1958	12.10.1958	2.8.1959	2.8.1959	11.9.1960	11.9.1960	13.8.1965	13.8.1965	15.9.1965	
–	–	–	–	–	–	1, +	1, +	–	1
–	–	–	2, +	–	–	–	–	–	2
–	–	–	–	–	–	–	–	–	1
		3, +	1, +	–	–	5, +	5, +	–	3
–	–	–	–	–	–	2, +	–	–	1
–	–	–	1, +	–	–	–	–	–	1
–	–	–	1, +	–	–	–	–	–	1
–	–	–	7, +	1, +	–	3, +	–	2, +	3
–	–	–	–	–	–	–	1, +	–	1
M, +	–	–	–	–	–	3, +	M, +	–	3
–	M, +	–	M, +	–	–	M, +	M, +	M, +	4
–	M, +	–	–	–	–	–	–	–	1
–	–	–	M, +	M, +	–	M, +	–	M, +	3
–	–	–	–	–	–	M, +	–	–	1
5, +	–	–	–	–	–	–	–	–	1
2, +	–	–	–	–	–	–	–	–	1
8, +	–	–	–	–	–	–	–	–	1
–	M, +	–	M, +	–	–	–	M, +	–	4
14 : 44	11 : 38	12 : 28	18 : 63	6 : 7	4 : 5	21 : 79	17 : 36	16 : 39	

Frequently still *Russula vesca*, *R. fragilis*, *Amanita vaginata*, *A. rubescens* are encountered. Differential species: *Boletus edulis*, *Craterellus cornucopioides*, *Lepiota granulosa*.

Aspect conditions: The aestival maximum aspect develops in July or August as the *Russula vesca – Lactarius piperatus* or *Collybia radicata – Lactarius pyrogalus* ssp. *circellatus* aspect (1955, 1964); in September *Craterellus cornucopioides – Marasmius peronatus – Hypholoma fasciculare*, in October *Limacium leucophaeum – Clitopilus prunulus*, and in October or November *Cortinarius multiformis – Tricholoma conglobatum* are the prevalent aspects. The number of species in the coenosis amounted to 68, that of the specimens per 100 m^2 ranged from 35 to 301 between 1953 and 1958, while from 7 to 63 in the more adverse period 1958 to 1965. The site has a podsolizing soil (Table 1).

c) Mixed type of *Querco – Carpinetum*. In the tree and shrub layer also planted specimens of *Pinus nigra* are to be found. Cover of the tree layer 80, in the shrub layer 30 and in the herb layer 40 per cent. This association stands on the higher part of the slope on a calcareous-marly soil. Constant-dominant species of the coenosis: *Agaricus bisporus*, *Russula alutacea;* frequently occur still: *Hebeloma crustuliniforme*, *Lactarius insulsus*, *Limacium leucophaeum*, *Marasmius wynnei*, *Russula nauseosa*. Differential species: *Agaricus bisporus*, *Cortinarius fulgens*. Number of species 18, with 16 to 207 pieces per 100 m^2 in 1954 to 1958 and 12 to 63 between 1958 and 1963. Valuable as the site of *Agaricus bisporus* (Table 1).

Table 2

Pinetum nigrae mixtum auct.	Sb.	S.	F.	Árpád-	
				15.9.1955	23.10.1957
Armillaria mellea	Tr.	2	P—S	—	—
Boletus granulatus	T.	1	M	4, +	2, +
Clitocybe aurantiaca	T.	1	S	—	—
Clitocybe cerussata	T.	1	S	2, +	—
Clitocybe dealbata	T.	1	S	5, +	2, +
Clitocybe geotropa	T.	1	S	—	—
Clitocybe infundibuliformis		1	S	—	—
Clitocybe inversa	T.	1	S	6, +	3, +
Clitocybe metachroa	T.	1	S	—	—
Clitocybe nebularis	T.	1	S	—	—
Clitocybe phyllophila	T.	1	S	—	2, +
Collybia esculenta	T.	1	S	2, +	—
Flammula spumosa	T.	1	S	—	—
Geastrum fimbriatum	T.	1	S	2, +	—
Geastrum rufescens	T.	1	S	4, +	—
Gomphidius viscidus	T.	1	M	3, +	—
Hypholoma fasciculare	Tr.	2	S	—	—
Inocybe friesii	T	1	S	—	12, +
Inocybe lucifuga	T.	1	S	5, +	—
Lactarius deliciosus	T.	1	M	2, +	—
Lepiota carcharias	T.	1	S	—	—
Lepiota cristata	T.	1	S	1, +	2, +
Lepiota erminea	T.	1	S	2, +	—
Limacium arbustivum	T.	1	M	—	3, +
Limacium eburneum	T.	1	M	—	—
Limacium lucorum	T.	1	M	—	—
Lycoperdon gemmatum	T.	1	S	1, +	1, +
Lycoperdon saccatum	T.	1	S	—	—
Marasmius androsaceus	T—Su	2	S	—	—
Marasmius epiphyllus	T—Su	2	S	—	—
Marasmius peronatus	T.	1	S	3, +	5, +
Marasmius wynnei	T.	1	S	2, +	—
Mycena gracilis	T.	1	S	—	—
Mycena pura	T.	1	S	—	—
Naucoria furfuracea	T.	1	S	—	—
Naucoria temulenta	T.	1	S	—	—
Paxillus atromentosus	Tr—T	1	M	2, +	—
Peniophora quercina	Su	2	S	—	M, +
Pholiota marginata	T.	1—2	S	—	—
Polyporus adustus	Tr.	2	P—S	—	—
Ramaria abietina	I.	3	S	1, +	—
Ramaria stricta	I.	3	S	3, +	—
Russula fragilis	I.	1	M	1, +	—
Schizophyllum commune	Tr.	2	S—P	—	—
Stereum hirsutum	Tr.	2	S—P	—	—
Stropharia merdaria	I.	1	S	—	—
Trametes unicolor	Tr.	2	S—P	—	—

hegy				Hármashatár-hegy 9.11.1965	Fenyőgyöngye 9.11.1965	K
2.9.1960	2.9.1960	31.10.1965	7.11.1965			
–	–	5, +	–	–	–	1
7, +	5, +	1, +	–	–	–	4
2, +	–	5, +	–	–	–	1
–	2, +	2, +	–	–	–	2
2, +	–	–	–	–	–	2
–	–	2, +	–	–	–	1
–	–	4, +	–	–	–	1
5, +	8, +	2, +	9, + – 1	–	2, +	5
–	–	–	–	6, +	2, +	1
–	–	–	–	8, + – 1	–	1
11, +	2, +	–	3, +	–	–	3
2, +	–	–	–	–	–	1
–	–	6, +	–	–	–	1
–	–	3, +	1, +	–	–	2
–	–	–	–	–	–	1
1, +	–	–	2, +	–	2, +	3
–	–	–	–	4, +	3, +	1
–	–	–	–	–	–	1
–	25, + – 1	–	–	–	–	2
–	–	–	–	–	–	1
–	–	–	–	5, +	–	1
3, +	2, +	1, +	–	–	–	3
1, +	1, +	–	–	–	–	2
5, +	–	4, +	–	–	–	2
2, +	–	–	–	–	–	1
–	–	–	–	–	15, +	1
2, +	–	–	–	–	2, +	3
–	1, +	–	–	2, +	–	1
–	–	–	–	3, +	–	1
–	–	–	3, +	–	–	1
–	–	–	–	–	–	1
–	–	–	–	–	–	1
–	–	–	1, +	–	–	1
3, +	–	–	–	–	–	1
–	1, +	–	–	5, +	–	1
–	–	–	1, +	12, +	–	2
–	–	–	–	1, +	1, +	2
–	–	M, +	–	–	–	1
–	–	–	–	3, +	2, +	1
–	–	M, +	–	–	M, +	1
–	–	–	–	–	–	1
–	–	–	–	–	–	1
–	–	–	–	–	–	1
–	–	–	M, +	M, +	M, +	2
–	–	M, +	M, +	M, +	–	2
–	–	–	–	1, +	–	1
–	–	M, +	M, +	–	–	1

Pinetum nigrae mixtum auct.	Sb.	S.	F.	Árpád-	
				15.9.1955	23.10.1957
Trametes versicolor	Tr.	2	S—P	—	M, +
Tricholoma albobrunneum	T.	1	S	2, +	—
Tricholoma melaleucum	T.	1	S	—	—
Tricholoma portentosum	T.	1	S	—	—
Tricholoma sejunctum	T.	1	S	—	—
Tricholoma terreum	T.	1	S	8, +	15, +
Volvaria loveiana	P.	1	P	—	—
Xylaria hypoxylon	Tr.	2	S	—	—
Total number of species: 55					
Number of species/Number of fruit bodies				21 : 61	12 : 49

Aspect conditions: *Agaricus bisporus — Boletus granulatus* in July and August; *Amanita rubescens — Lactarius insulsus, Cortinarius glaucopus — Lactarius pyrogalus* ssp. *circellatus, Agaricus bisporus — Hebeloma crustuliniforme* in September; *Clitocybe geotropa — Marasmius wynnei, Limacium olivaceo-album — Cortinarius fulgens — Hebeloma crustuliniforme* in October and *Cortinarius brunneus — Entoloma lividum* in October —November.

2. *Árpádhegy* (Budapest). — a) Planted *Pinetum nigrae mixtum* auct. (highly mixed with the original rocky stand of *Fraxinetum orni).* In the tree and shrub layer (merging fairly into one another): *Pinus nigra, Fraxinus ornus, F. excelsior, Tilia cordata, Viburnum lantana, Quercus petraea, Crataegus monogyna* etc. Cover: 50 per cent. In the herb layer: *Brachypodium silvaticum, Festuca sulcata, Filipendula hexapetala, Teucrium chamaedrys, Melica ciliata, Silene venosa, Anthericum ramosum, Polygonum dumetorum, Geum urbanum, Mycelis muralis, Stachys recta, Reseda lutea, Viola odorata* etc. Cover: 70 to 80 per cent. Constant-dominant species: *Boletus granulatus, Clitocybe inversa, C. cerussata, Geastrum fimbriatum, Inocybe lucifuga, Lepiota cristata, Tricholoma terreum.* Frequently still occur: *Russula fragilis, Lycoperdon gemmatum, Lepiota erminea, Clitocybe phyllophila.* Characteristic species: *Geastrum rufescens, Gomphidius viscidus, Lactarius deliciosus, Lepiota erminea, Inocybe lucifuga, Paxillus atrotomentosus, Tricholoma albobrunneum* (Table 2).

Aspects: *Boletus granulatus — Ramaria stricta, Inocybe lucifuga — Clitocybe dealbata — Lepiota erminea, Geastrum fimbriatum — Ramaria abietina* (July and August); *Inocybe lucifuga — Gomphidius viscidus — Clitocybe inversa, Marasmius peronatus — Gomphidius viscidus — Paxillus atrotomentosus* (August and September); *Marasmius peronatus — Tricholoma terreum, Lepiota cristata — Tricholoma terreum, Inocybe Friesii — Tricholoma terreum* (October and November). Epixylous synusia: *Schizophyllum commune — Stereum hirsutum — Trametes unicolor.*

b) Highly mixed stand of *Mercuriali — Tilietum* Zólyomi et Jakucs *(Tilio-Fraxinetum),* to which the *Prunus mahaleb — Fraxinus ornus* stand and the arti-

h e g y				Hármashatár-hegy 9.11.1965	Fenyőgyöngye 9.11.1965	K
2.9.1960	2.9.1960	31.10.1965	7.11.1965			
—	—	M, +	—	M, +	M, +	3
—	—	—	—	—	—	1
2, +	—	—	—	—	—	1
—	—	2, +	—	—	—	1
—	—	—	—	2, +	—	1
16, +	12, +	—	—	—	—	3
—	—	—	—	1, +	—	1
—	—	—	—	M, +	—	1
15 : 64	10 : 59	17 : 42	10 : 23	17 : 57	11 : 32	

ficially established *Pinetum nigrae* association are admixed to a considerable degree. The tree layer of 80 per cent cover contains *Tilia platyphyllos*, *T. cordata*, *Fraxinus excelsior*, *Acer platanoides*, *A. campestre*, *Quercus petraea*. In the shrub layer of 50 to 60 per cent cover *Fraxinus ornus*, *Acer*, *Tilia*, *Quercus*, *Viburnum lantana*, *Rosa canina* etc. are to be found. The herb layer is formed by *Poa nemoralis*, *Melica uniflora*, *Hypericum perforatum* etc. and its cover touches scarcely 10 to 20 per cent. Constant-dominant species: *Clitocybe dealbata*, *C. inversa*, *Lactarius subdulcis*, *Marasmius epiphyllus*, *Russula fragilis*. Characteristic species: *Fomes torulosus*, *Limacium pudorinum*, *Tricholoma grammopodium*.

Most important aspects: *Lactarius subdulcis* – *Clitocybe cyathiformis* (in August); *Limacium pudorinum* – *Scleroderma vulgare* (in October); *Limacium pudorinum* – *Collybia dryophila* (in September); *Clitocybe inversa* – *Omphalia maura*, *Clitocybe nebularis* – *Rhodophyllus sericeus* – *Tricholoma scalpturatum* (in November). In this forest stand rock swards also occur and their characteristic fungus species *(Clitocybe, Omphala, Rhodophyllus)* appear in the coenosis as well (see later).

Both sites are fairly dry, with karstic rocky parts. The mycocoenoses described above develop only in very favourable rainy years.

* * *

Part II to be published in the next issue.

Life Cycle of Blumeriella jaapii (Rehm) v. Arx Infecting Stone-Fruits

By

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The pathogen *Blumeriella jaapii* (Rehm) v. Arx (previously named as *Cylindrosporium padi*) has been known in Hungary since 1939. Today it may be found in all parts of the country. It causes early defoliation on seedlings and bearers of the sweet and sour cherry. Beside the leaves of *Prunus avium* and *Prunus cerasus* those of *P. mahaleb*, *P. amygdalus*, *P. armeniaca* and *P. domestica* become also considerably infected by this fungus.

In April and May 1963 the apothecia of the pathogen were detected on overwintered leaves of sweet cherry infected in 1962.

This was the first evidence for the existence of the perfect stage of the fungus on cultivated stone-fruit trees in Europe.

The paper describes briefly the perfect form and the entire developmental cycle of the fungus. The plectenchymatous stroma overwintered on infected leaves develops either in to an apothecium or in to an acervulus. Conidium formation in empty apothecia or development of apothecia in the acervuli could never be observed. But from stromata appearing on the same leaf either apothecia or acervuli may evolve. The primary infection is accordingly caused by ascospores or "spring conidia". The summer conidia have absolutely no role in the overwintering of the fungus, they perish in winter.

For the destruction of the ascospores or conidia of the pathogen organic fungicides (thiocarbamates) in a 0.2 to 0.4 per cent solution can be applied. Sprayings should start in the first half of April, in the uni- or bifoliate stage of trees (immediately after the drop of petals) and must be carried out at least four times, every 8 to 10 days.

Introduction

The first appearance of the pathogen in Hungary was observed only on cherry grafts in a nursery near Cegléd (HUSZ, 1939). Investigations carried out in the years 1950 to 1955, however, revealed damages done by this fungus to leaves of seedlings and grafts of sour cherry *(P. cerasus)*, mahaleb (or perfumed) cherry *(P. mahaleb)*, almond *(P. amygdalus)*, apricot *(P. armeniaca)* and plum *(P. domestica)* growing in four nurseries (Alsótekeres, Fertőszentmiklós, Mezőhegyes, Nyírtelek) located far from one another (KASZONYI, 1955a, 1955b).

In the last years – as it could be expected – the pathogen spread out from the nurseries and caused considerable defoliation also on the bearers of sweet and sour cherry.

Today the pathogen can already be found in every part of the country. Its appearance on the bearers indicates that in a short time this fungus will become a serious problem of the large-scale orchards in Hungary just as abroad (BLUMER, 1958). This justified the further investigations into the still unknown details of the pathogen's habit in Hungary and Europe.

Symptoms of the Disease on Cultivated Stone-fruits

The symptoms of the disease on different host plants were already described by the author (KASZONYI, 1955a, 1955b), therefore they are dealt with only in brief here.

In Hungary the pathogen attacks merely the leaves of grafts and bearers of cultivated stone-fruits. The symptoms become visible as soon as the beginning of May in the form of 1 to 3 mm large purple-lilac circular or angular spots. Primary spots in spring are few in number, but if the first infection is followed by a fairly warm, rainy weather, on the reverse of the spots the yellowish-white "summer" conidium colonies appear. As a consequence the leaves become covered with densely confluent secondary lilac spots. The green leaf parts are getting yellow and the tree sheds most of its leaves already in the second half of July.

The leaf spots of the sour and sweet cherry as well as of the mahaleb cherry do not drop, but from the leaves of the almond, apricot and plum the spots fall out, together with the pathogen, in every case.

In contradiction to the finding of VIENNOT-BOURGIN (1949) on shoots or branches no infection was ever found.

The Pathogen

The first fairly thorough description of the pathogen was given by KARSTEN who described it as *Cylindrosporium padi* (Lib.) Karst. in 1884 and classified it taxonomically in the *Melanconiales* series of *Fungi imperfecti.*

The fungus thus was examined and described found on living leaves of *Prunus padus.* JAAP detected the perfect stage of the fungus on rotting leaves of *Prunus padus* about 60 years ago, and REHM (1907) described it as *Pseudopeziza jaapii Rehm* nova species.

However, the perfect stage of the pathogen infecting cultivated stone fruits in Europe has not been discovered before 1963. This explains that the name, taxonomical position of the fungus as well as the way of its overwintering were recurrent problems in the phytopathological literature for many decades (DARPOUX, 1945; HOCHAPFEL, 1952; KASZONYI, 1955a, 1955b; BLUMER, 1958). Recently the fungus was identified with *Pseudopeziza jaapii Rehm* by v. ARX (1961), who did not found its perfect stage either, but investigated thoroughly the imperfect form and suggested, therefore, *Blumeriella jaapii (Rehm) v. Arx* as the new name of the pathogen.

Overwintering and developmental cycle of the fungus

In the autumn of 1962 from bearers and nursery grafts of cherries leaves infected by "*C. padi*" were collected and examined after overwintering in the laboratory. From the stromatic plectenchymae of diseased fallen leaves either acervuli or apothecia developed in April and May 1963. From the acervuli and apothecia, developed on the stromata, conidia and ascospores, respectively, were taken and used to infect potted seedlings of sweet and mahaleb cherry. In mid-May, on the 10th to 12th day after infection, the purple spots appeared on the leaves. The acervuli developed on the reverse side of the leaves and large masses of typical conidia were discharged from under the disrupted epidermis.

The results of the investigations, one specimen of all microphotographs made from the perfect form, and some of the leaves bearing apothecia were first sent to Professor Dr. J. A. VON ARX (Baarn, Holland), who gave the following answer (July 29, 1963): "Für die Fotos von *Blumeriella jaapii* danke ich Ihnen vielmals. Es freut mich sehr, dass Sie diesen Diskomyceten in Europa wiedergefunden haben. – Und dies ist auch der erst europäische Fund auf der Kirsche. JAAP bzw. REHM hatten den Pilz seinerzeit nur auf der wildwachsenden *Prunus padus* gefunden und beschrieben."

Thus in Europe, it was proved for the first time by the author's investigations that the pathogenic stage on stone fruits is merely part of the whole life cycle of "*Cylindrosporium padi*".

The first proof that VON ARX (1961) was right in his conclusions, was also presented by the author's investigation. Thus the confusion prevailing for 80 years in the whole life cycle and the taxonomy of the "not fully known" fungus has finally found its solution.

The results of the author's studies are also confirmed by the observations of ROOSJE[1] who was the first to report from Holland that he had also detected the perfect (apothecium-bearing) form of the pathogen.

Description of the perfect form

Beginning with September, on the diseased leaves in the place of summer acervuli stromata develop, which fill up the space between the lower and upper epidermis; after defoliation they become dark coloured, protrunde slightly and hemispherically on the reverse side of the leaves (Fig. 1).

In April or May on the stromata acervuli (spring conidia, marked with *a* on Fig. 2) or sessile, stellately dehiscing apothecia) marked with *b* on Fig. 2) develop, having a diameter of 126 to 306 μ (see also Fig. 3).

The asci are roughly club-sharped, slightly stalked, 53.40 to 106.0 × 17.57 to 14.24 μ in size and contain on their upper end 8 hyaline ascospores (Fig. 4).

[1] Letter from Ir. G. S. ROOSJE, Proefstation voor De Fonitt. In de Volle Grand, Willhelminadorp, Nederland, Mai 22, 1964.

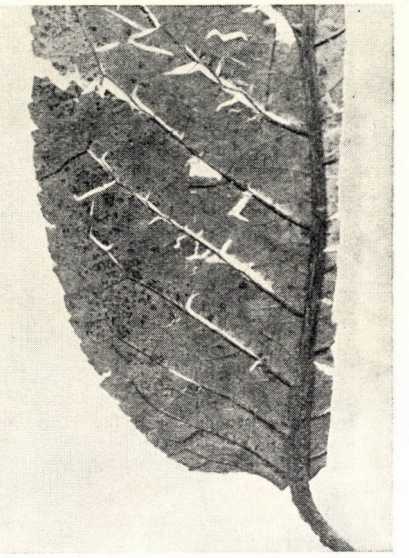


Fig. 1. Overwintered sweet cherry leaf with black dot-like stromata of the pathogen (Phot.: KASZONYI 1963)

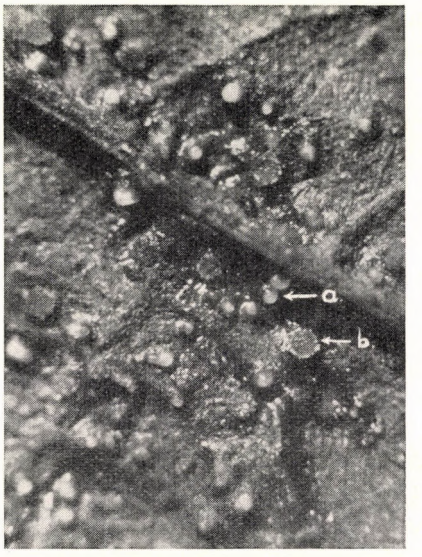


Fig. 2. Acervuli (a) and apothecia (b) developed on the reverse side of a sweet cherry leaf in spring (Phot.: KASZONYI 1963)

Among the asci many filiform or ramifying paraphyses may be seen, which are usually hook-like incurved or thickened on their end. The ascospores are short, filiform and 30.60 to 51.40 × 1.78 to 2.67 μ in size (Figs 5 and 6).

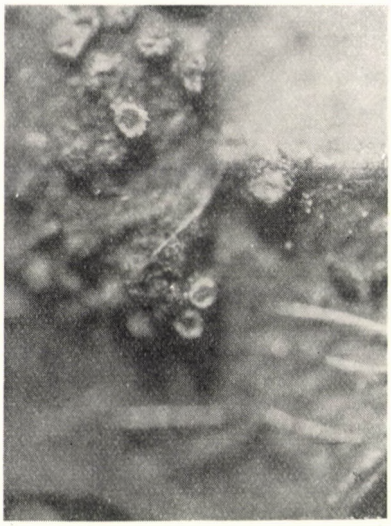


Fig. 3. Mature apothecia on the lower surface of a sweet cherry leaf (Phot.: KASZONYI 1963)

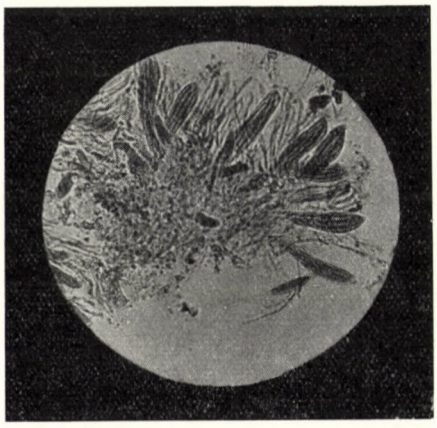


Fig. 4. Detail of an apothecium of *Blumeriella jaapii* (Phot.: KASZONYI 1963)

Prerequisites for the further development of stromata

The further development of stromatic plectenchymae in spring depends on temperature and precipitation.

It was established by replicated experiments and observations that from the stromata occurring on the same leaf either acervuli or apothecia develop.

During heavy rains the leaves lying on the ground and overwintered with plectenchymatous stromata become abundantly soaked with water. If after the rain the leaves retain their moisture for 1 or 2 days and the temperature rises to

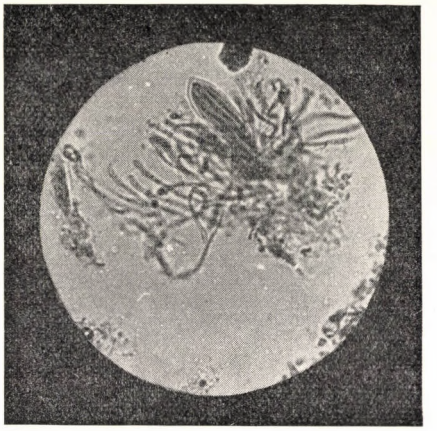


Fig. 5. An ascus of *Blumeriella jaapii* with paraphyses (Phot.: KASZONYI 1963)

Fig. 6. An ascus of *Blumeriella jaapii* with ascospores (Phot.: KASZONYI 1963)

18.0 (±3)°C, the stromata begin to develop rapidly and produce within 24 to 48 hours as mentioned above – always either acervuli or apothecia.

On the other hand, if during this time the leaves dry out, neither acervuli nor apothecia develop.

We never observed that in empty apothecia later asexual conidia would develop (in acervuli). Similarly, no observation was made either that stromata producing once acervuli would have later, after sporulation, developed apothecia.

This fact must be emphasized, because according to the well-known paper of HOCHAPFEL (1952) in the apothecia conidia may be formed.

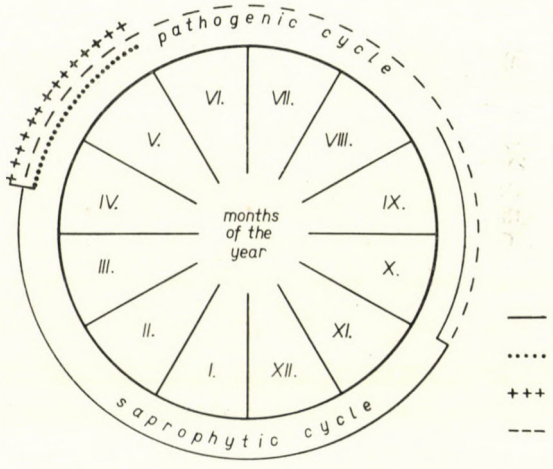
Knowing the above facts and relying on observations in Hungary it can be stated that – in contradiction to DARPOUX (1945) and others – the saprophytic life cycle of the pathogen may have two trends: 1. stroma-acervulus, 2. stroma-apothecium; accordingly the primary infection can be induced either by "spring" conidia or by ascospores. DARPOUX's (1945) opinion divergent from the author's view may be tracted back to the fact that neither he nor others observed the real phenomenon as yet that from two stromata developing on the same leaf next to each other, one produces an acervulus while the other an apothecium.

As to the reason why the stromata are able to continue their development in two directions, there is no satisfactory explanation available as yet. Investigations pertaining to this matter will be published later.

The pathogenic and saprophytic life cycle of the fungus is demonstrated in Table 1.

Table 1

Life cycle of *Blumeriella jaapii (Rehm) v. Arx*



Conidia developed in the vegetation period have absolutely no role in the overwintering, and in the inducing infection in spring.

Methods of Control

To control the pathogen 0.2 to 0.4 per cent solutions of organic fungicides can successfully be used, if sprayings are carried out by taking into consideration the development of fungus and of the host plant. Accordingly, the first treatment has to be performed about the 9th of April, in the uni- or bifoliate stage of the plants (immediately after the drop of petals). Depending on the incubation period of the fungus until mid-July sprayings must be performed every 8 to 10 day, and repeated 4 to 8 times as required by the degree of infection and weather conditions. The first four treatments are of decisive importance. At the end of August and in September no further sprayings are needed, because the stromata developing in leaf tissues cannot be destroyed by the fungicides.

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Production of Actidione (Cycloheximide) and its Use for Plant Protection in Hungary

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Examining the fungistatic effect of *Streptomyces* strains isolated from different soil samples actidione production was evidenced in 6 isolates. For the determination of actidione a very sensitive, bio-assay method based on diffusion and suitable also for routine work was elaborated. Shaken cultures of the strains B.64, B.81 and B.128 produced 250 ppm actidione in 72 hours.

For the extraction of actidione the adsorption on 0.5 per cent activated carbon and for its eluation acetone of 80 per cent concentration and applied three times in 10 per cent quantities proved to be most suitable.

The inhibitory effect of the antibiotic was examined in vitro on 37 fungus species. It has been found that both the examined *Phytophthora* species, some yeasts and *Colletotrichum lindemuthianum* causing the anthracnose of beans were most sensitive to actidione.

In the course of glass-house experiments carried on to control the anthracnose of beans it turned out that plants were not damaged by using the antibiotic in concentrations below 10 ppm. Into new plant parts grown after the treatment the antibiotic was not translocated to such an extent that could have been prevent later infection.

Introduction

The cycloheximide (otherwise known as actidione), produced by some strains of *Streptomyces griseus* and *Streptomyces noursei* is one of the most minutely investigated fungistatic antibiotics. After its first description (FORD and LEACH, 1948; WHIFFEN, 1948) a great number of papers has dealt especially with the possibilities of its agricultural use; a fine review of these works and results was given by FORD, KLOMPARENS and HAMNER (1958). The application of actidione against plant diseases gained ground not only due to its high efficiency, but also because its systemic action proved to be very advantageous. Experimental quantities of actidione were already produced in Hungary in 1954 by the Pharmaceutical Research Institute, subsequently – between 1955 and 1958 – this antibiotic was obtained as the by-product of streptomycin manufacturing of the Research Institute for Plant Protection (SZIRMAI and VÖRÖS, 1955). On the basis of field experiments conducted for many years it was found out that seed dressing with actidione provides a protective effect, equivalent to that of the usual mercury-containing disinfectants, against wheat bunt *Tilletia foetida* (Wallr.) Liro (VÖRÖS and SZIRMAI, 1955). An other field of detailed investigations was the control of apple mil-

dew. It turned out that due to its phytotoxicity and despite the satisfactory fungicidal effect the raw antibiotic can not practically be applied against this disease. The protective effect of actidione against apple scab was also of a very high degree (SZIRMAI and VÖRÖS, 1959). These Hungarian results are in conformity with similar data achieved abroad (HENRY et al. 1951, 1952; SZKOLNIK and HAMILTON, 1959).

In the last years exceeding results were gained with actidione and its derivates in controlling rust diseases (MOSS, 1961; MOSS et al., 1960; HACKER, 1959). the powdery mildew of roses (JONES and SWARTWOUT, 1961), grass diseases, cherry leaf spot, the powdery mildew of different ornamental plants (THOMPSON, 1961; VALÁSKOVÁ, 1962), the monilia disease of fruit trees (GROVER, 1960), and the *Sorosporium* smut of maize (KRÜGER, 1959).

Experiments against the powdery mildew of barley carried on recently already with actidione produced in the Institute should separately be mentioned. Out of the 5 preparations tested in field experiments, actidione proved most effective: sprayings with a solution of 20 ppm concentration diminished the mildew infection by more than 60 per cent (PODHRADSZKY J., results not yet published).

By the concern UPJOHN Co. (Kalamazoo, Mich., USA) large quantities of actidione are produced and put on the market for plant protection purposes. From the different actidione-containing plant protectives "Actispray" is used against cherry leaf spot caused by the fungus *Cylindrosporium*, "Actidione BR" against the *Cronartium*-damages of conifers, the preparations "Actidione RZ", "Actidione-Thiram" and "Actidione-Ferrated" are applied against the diseases of grasses (rust, mildew, snow-mould etc.), "Actidion PM" is sprayed against the powdery mildew of roses and other ornamentals, whereas the combination "Actidione-Captan" chiefly against the black leaf spot of roses.

The action mechanism of actidione (LATUASAN and BERENDS, 1958; WESCOTT and SISLER, 1964) as well as the conditions of its uptake and translocation (LEMIN et al., 1960; LEMIN and THOMAS, 1961) were also thoroughly studied in the last years.

Earlier favourable domestic and foreign results justified the continuation of experiments with actidione, but due to difficulties in inland production this intention could not be realized until 1954. As a consequence of changes in Hungarian streptomycin fabrication it was not possible to manufacture this antibiotic as a by-product, and *Streptomyces* strains yielding actidione were not available until 1964.

Examining the antibiotic production capacity of about 800 isolates of *Streptomyces* isolated from Hungarian soil samples during recent years, in many cases a fungistatic effect was observed. Out of the active strains 6 isolates showed actidione production according to paper-chromatographic comparison. The experiments to gain the antibiotic in Hungary were started with these strains.

Material and Methods

For the maintenance and propagation of strains, oblique agar cultures containing 1 per cent crude glucose, 1 per cent peptone and 0.5 per cent common salt were used, and strains of high performance stored in lyophilized state.

The cultivation was carried out on a horizontal shaking table of 320 revolutions per minute at 27 to 28°C. The Erlenmeyer flasks of 500 ml volume fixed at the table contained 100 ml medium each. Inoculation was generally performed with spore suspensions. The medium proving best for actidione production contained: 1 per cent glucose, 1 per cent soy flour, 0.5 per cent $CaCO_3$, its starting pH-value ranged from 6.8 to 7.0.

Actidione was extracted from culture filtrates – acidified with HCl to the pH-value 3 and filtered to mycelium-less state – by adsorption on activated carbon and by eluation with acetone. For plant protection experiments the reddish-brown watery remnant obtained after vacuum-evaporation of eluates was used.

For bio-assay the agar cup method with the test organism *Saccharomyces carlsbergensis* was applied. The agar layers were put into Petri dishes of 10 cm diameter and consisted of a ground layer (10 ml) and an inoculated layer (5 ml). To the latter 10 per cent suspension was mixed at 50°C so that the inoculated agar contained 5.10^7 of yeast cells per ml. The medium used for bio-assay comprised following substances per litre: 3 g peptone, 30 g saccharose, 2 g glucose, 2 g $NaNO_3$, 1 g $MgSO_4$, 0.5 g KCl, 0.01 g $FeSO_4$, 20 g agar, 2 g K_2HPO_4 and 200 ml yeast extract. Its hydrogen ion concentration was fixed at 8.0. Into the Petri dishes 6 holes of 9 mm mm diameter were bored with a sterile plug drill and into two opposite holes 0.1 ml standard actidione solution of 2.5 ppm concentration was measured each. The diameter of the inhibition zone of the four materials to be examined was corrected on the basis of this procedure.

In evaluation, paper-chromatography etc. the crystalline actidine of 90 per cent activity obtained from the UPJOHN Co. served as comparative material.

The spectrum and the minimal inhibiting concentrations were established by halving serial dilutions prepared with the Czapek medium containing 2 per cent malt extract. These dilutions were inoculated with cell- or spore-suspensions of identical concentration, whereas in case of fungi not sporulating in artificial cultures, the inoculation was performed with blocks of identical size excised from the culture.

For ultra-violet irradiation a Hungarian device, the "Germicid" tube was used from 1 m distance. The *Streptomyces* strain suitable for UV-treatment was dispersed on the surface of the medium in the Petri dishes and at the termination of the treatment the strain was immediately incubated in darkness to avoid photoreactivation.

In glass-house experiments against the anthracnose of beans 2 to 4 three-leaved seedlings of the variety "Kompolti Gyöngybab" were used. Artificial infection was carried out with a suspension of *Colletotrichum lindemuthianum* containing 5.10^5 conidia per ml, and subsequently the plants were kept in a wet chamber

for 20 to 24 hours. The *Colletotrichum lindemuthianum* strain serving for the experiments sporulated best on agar containing 1 per cent oat flour, consequently for maintaining and propagation of the fungus this medium was used.

Results

Elaboration of a bio-assay method

The first precondition of the experimental work pertaining to the production of actidione was the elaboration of a reliable bio-assay method suitable also for routine investigations. On the strength of literature data and earlier domestic ex-

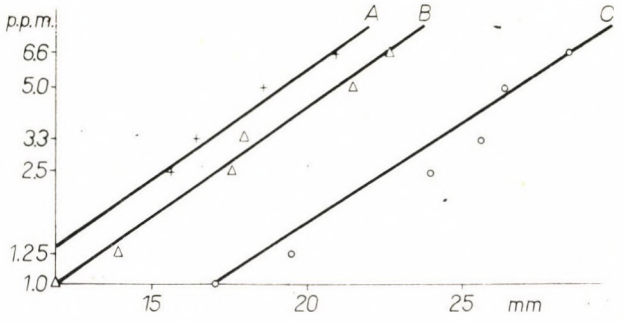

Fig. 1. Bio-assay of actidione with the test organism *Saccharomyces carlsbergensis* on different media
Ordinate: Logarithm of the actidione concentration. Abscissa: Diameter of the inhibition zone in mm. *A* = yeast extract-glucose agar; *B* = supplemented yeast agar at pH 6; *C* = supplemented yeast agar at pH 8

perience the yeast fungus *Saccharomyces carlsbergensis* was chosen as test organism. Applying the agar cup method described above, on the simple yeast extract-glucose agar (containing 10 g glucose, 1 g KH_2PO_4, 20 g agar, and 200 ml yeast extract per litre on the pH-level 6.0) the development of small sized, indistinct inhibition zones hardly to be measured were observed, therefore this method had to be amended. To do so, the evaluation was repeated with the same test organism on 32 media containing different substances and showing diverse pH-values; a detailed description of these materials is disregarded.

For bio-assay a medium proved to be most suitable comprising following substances per litre: 30 g saccharose, 2 g glucose, 3 g peptone, 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 0.01 g $FeSO_4$, 20 g agar and 200 ml yeast extract. The inhibition zones induced by actidione were largest and best measurable on this medium. It turned out that the diameter of inhibition zones depends also on the acidity of the medium. On a medium of pH 8.0 the same actidione concentration caused a larger inhibition zone than on a 6.0 pH-medium. The difference is clearly demonstrated by Fig. 1 in which the results obtained on the above de-

scribed medium are compared with the values achieved on the simple yeast extract-glucose agar.

Comparison of the actidione production by different Streptomyces isolates

Out of the fungistatic *Streptomyces* strains isolated from different soil samples the actidione production of 6 strains was demonstrated by the aid of paper-chromatography; these were marked with the symbols B.11, B.64, B.81, B.128, B.147 and B.161. The actidione production of the strains was compared in shaken cultures, kept on a medium containing – as described above – 1 per cent soy flour. The cultivation took 6 days and the actidione contents of samples

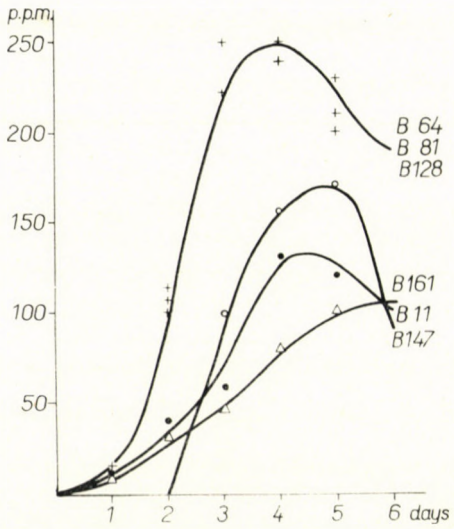

Fig. 2. Actidione production of different *Streptomyces* strains in shaken cultures on the 1st to 6th day of the cultivation

Ordinate: Actidione content in ppm. Abscissa: Cultivation time in days

drawn every 24 hours were compared. The results thus obtained are illustrated in Fig. 2; each of them represents the mean of three replications.

It may be seen that the actidione production of the strains B.64, B.81 and B.128 was essentially of identical trend. The agent production reached its maximum – about 250 ppm – on the 3rd or 4th day of cultivation and showed afterwards a slight decline. The antibiotic production of the strains B.11, B.147 and B.161 was lower, the peak of the agent content (100 to 150 ppm) could be observed on the 4th or 5th day of cultivation.

The strain B.64 was treated with ultra-violet irradiation in order to gain isolates of higher actidione production. After an irradiation of 3 hours performed with the described method on the surface of the treated layers 4 to 6 isolated thalli

appeared, the productiveness of which was examined in shaken cultures. The examination of 80 isolates treated with UV-irradiation revealed that none of them produced more actidione than the initial culture. By about half of the isolates actidione was not produced at all or but sporadically. These cultures differed macroscopically even in thallus formation from those yielding actidione. On the agar used for cultivation their rugose, slightly violescent and weakly sporulating thalli differed sharply from the smooth white colonies of well-producing strains.

Effect of the medium composition on actidione production

From many media of different types that containing – as mentioned above – glucose and soy flour proved to be most suitable for actidione production. However, it had to be clarified, to what extent the degree of agent production might be influenced by the concentration of carbon and nitrogen source. To find out the optimum composition of the medium, experiments were performed with the strains B.81 and B.128 in shaken cultures. All medium variants contained uniformly 0.5 per cent sodium chloride and 0.1 per cent calcium carbonate, only the quantities of soy flour and crude glucose were changed from 0.25 to 4.0 per cent. In soy flour variants the medium contained 1 per cent glucose and in sugar concentration variants 1 per cent soy flour. Cultures were shaken for 6 days and from the 2nd day onward the actidione content was established from samples taken every 24 hours. The results are summarized in Table 1, showing the agent contents in ppm as the means of three replications.

Table 1 reveals that for the actidione production of both examined strains the medium containing 1 per cent glucose and 1 per cent soy flour was most favourable. For all variants 72 hours have been the optimum fermentation period followed by a slow decline of agent production. In the medium of best composition this decrease was of a higher degree than in that containing only 0.5 per cent soy flour and being less suitable for production.

In the presence of 2.0 or 4.0 per cent glucose both *Streptomyces* strains did not grow at all or only to a very limited degree, therefore, in these variants, naturally, no actidione production was observed. Both strains grow well even on media containing more than 1 per cent soy flour, but their agent production was very low.

Experiments on actidione extraction

The extraction of actidione from culture filtrates was carried out by adsorption on activated carbon and by eluation with acetone (FORD and LEACH, 1948). In order to obtain the maximum quantity with minimum material consumption the amount of carbon necessary for extraction and that of acetone required for eluation had to be established.

For fixing of the agent the activated carbon "Carbo C extra" was used. From the culture filtrate gained with the medium of already described composi-

Table 1

Actidione production of the strains B.81 and B.128 on media containing different quantities of glucose and soy flour respectively, in cultures incubated for 48 to 144 hours

Strains	Content of the medium	Actidione production (ppm)				
		48	72	96	120	144
		hours				
B.81	*Soy flour*					
	0.25	0	5	5	2	3
	0.50	12	37	29	26	21
	1.00	35	74	71	49	16
	2.00	28	17	6	0	0
	4.00	9	0	0	0	0
	Glucose					
	0.25	11	17	10	0	0
	0.50	21	43	53	48	15
	2.00	0	0	0	0	0
	4.00	0	0	0	0	0
B.128	*Soy flour*					
	0.25	5	13	28	15	10
	0.50	31	84	84	62	35
	1.00	75	161	75	40	11
	2.00	67	57	23	7	0
	4.00	23	17	5	3	0
	Glucose					
	0.25	21	17	8	0	0
	0.50	26	59	36	33	8
	2.00	0	0	0	0	0
	4.00	0	0	0	0	0

tion actidione was fixed (in a maximum concentration of 250 to 300 ppm) likewise perfectly by 0.5 and 1 per cent carbon. However, by the 0.1 per cent carbon related to the quantity of the culture filtrate only 75 per cent of the agent present in the filtrate was fixed, therefore, under the given conditions for actidione extraction 0.5 per cent activated carbon proved to be most suitable.

Actidione was eluated from the air-dried carbon by an organic solvent in several replications. For each eluation from the solvent a 10 per cent quantity related to the volume of the culture filtrate was used. Comparing acetone and methyl-alcohol (both of 80 per cent concentration) it turned out that the latter eluated 70 per cent less actidione from the carbon than acetone under identical conditions. Between the eluation capacities of cold and warm (60°C) acetone no difference was found. It had to be clarified, however, how often the eluation with 80 per cent acetone in 10 per cent quantities should be replicated to eluate the agent most perfectly. For this purpose the same amount of carbon was five times eluated in succession with identical quantities (10 per cent) of acetone of 80 per cent concentration. The eluates thus obtained comprised 50, 25, 12, 6 and 3 per cent of the

original agent content. Accordingly in actidione extraction an acetone quantity of 10 per cent – if applied three times – suffices for the eluation of the agent from the carbon, because the fourth eluate includes only about 6 per cent of the original agent content.

The investigations showed that in the mycelium filtered from the acidified culture fluid no considerable quantities of actidione occurred.

Investigations on the in vitro inhibiting effect of actidione

The inhibition effect of actidione was examined on 37 fungus species in the laboratory by the aid of the serial dilution method between the concentrations 0.25 to 100 ppm. Results were recorded after 48 hours of incubation at 27°C. The minimum inhibiting concentrations for the different fungi are presented in the following enumeration. (see page 109.)

The actidione sensitiveness of the examined fungi is very different and from the obtained results hardly conclusions can be drawn as to the higher sensitiveness or resistance of certain fungus groups or genera. The examined two *Phytophthora* species were obviously not able to grow in the presence of 0.25 ppm actidione either, but the same sensitiveness was shown by a yeast fungus *(Cryptococcus diffluens)* and this was the minimum inhibiting concentration also for *Colletotrichum lindemuthianum*. Some yeast fungi, especially *Geotrichum candidum* and *Candida parapsilosis* proved to be extremely actidione-resistant, but so was *Aspergillus flavus*, too. The growth of the examined *Mucorales*, *Rhodotorula* and of some *Aspergillus* species was inhibited by actidione only in 100 ppm concentration. In general it may be stated, that the decidedly phytopathogenic fungi are far more sensitive than parasites attacking weakened plants or saprophytic organisms.

Glass-house experiments to control the anthracnose of beans

Examining in vitro the inhibiting effect of actidione it turned out that out of the 37 tested fungus species *Colletotrichum lindemuthianum* causing the anthracnose of beans was one of the most sensitive organism. In the laboratory its growth was already inhibited by 0.25 ppm concentration of actidione. Therefore experiments were conducted in the glass house to control this disease.

In the first experiment series bean plants were sprayed with actidione solutions of different concentrations and 24 hours after the treatment artificial infection was carried out. The anthracnosic spots, developed on the shoots 5 days after infection, served as basis for the evaluation of the experiment. The average number of spots found on the shoots of control plants was 49; the treatment with actidione of 2.5 ppm concentration resulted in 6, that of 5 ppm in 2.5 and that of 10 ppm in 0.5 spots, while 20 ppm concentration damaged severely the bean plants grown in the glass-house, therefore they could not be evaluated. On the specimens treated with 10 ppm concentration appeared necrotic spots as well due to the phytotoxic effect of actidione. For this reason in subsequent experiments the 5 ppm con-

Test organisms	Minimum inhibiting concentration in ppm
Phytophthora cactorum	below 0.25
Phytophthora cinnamomi	below 0.25
Candida parapsilosis	above 100.00
Candida pulcherrima	5.00
Candida guillermondii	25.00
Torulopsis famata	1.00
Torulopsis inconspicua	0.50
Trichosporon cutaneum	50.00
Geotrichum candidum	above 100.00
Cryptococcus diffluens	below 0.25
Saccharomyces carlsbergensis	5.00
Saccharomyces cerevisiae	5.00
Saccharomyces bajanus	5.00
Saccharomyces exiguus	5.00
Rhodotorula glutinis	1.00
Rhodotorula rubra	100.00
Rhodotorula mucilaginosa	100.00
Actinomucor repens	100.00
Cunninghamella echinulata	100.00
Sclerotinia sclerotiorum	5.00
Phoma betae	2.50
Colletotrichum lindemuthianum	0.25
Colletotrichum lagenarium	2.50
Monilia sitophila	5.00
Botrytis cinerea	25.00
Trichothecium roseum	10.00
Aspergillus niger	50.00
Aspergillus tamarii	100.00
Aspergillus fumigatus	50.00
Aspergillus wentii	100.00
Aspergillus flavus	above 100.00
Helminthosporium sativum	10.00
Helminthosporium sp.	50.00
Fusarium moniliforme	25.00
Fusarium graminearum	10.00
Myrothecium verrucaria	50.00
Myrothecium roridum	50.00

centration of actidione, providing satisfactory protective effect without damaging the plants, was applied. The results of spraying carried out with actidione of 5 ppm concentration against the anthracnose of beans may be seen in Fig. 3.

Because actidione is a systemic fungistatic compound, it was assumed that if applied after infection a therapeutic effect may be achieved. Therefore after artificial infection 10—10 plants were sprayed with an actidione solution of 5 ppm concentration every 24 hours. This experiment was evaluated by the above described method, on the basis of spots appearing on the shoots. In untreated specimens the average number of spots amounted to 33. On plants sprayed 24 hours after artificial infection 13 and on those treated 48 hours later 9 spots were counted

in the average. Individuals sprayed with actidione solution of 5 ppm concentration 96 hours after infection showed 28 spots in the average on the shoots, indicating that actidione is not able to inhibit the development of symptoms if applied three days after infection. However, according to the results obtained it is obvious that an earlier treatment with actidione exerts a decisive therapeutic effect.

Investigations being presently in progress have to clarify, how long the plants sprayed with actidione remain protected after the treatment against the infection

Fig. 3. Seedlings of the bean variety "Kompolti Gyöngybab" one week after the infection with *Colletotrichum lindemuthianum*. Left: specimens treated with 5 ppm actidione; right: untreated plants

by *Colletotrichum lindemuthianum*. Informative experiments showed that full protection is provided by actidione even 3 days after treatment: on sprayed leaves and shoots no anthracnosic spots developed, but on plant parts growing after spraying the infection by the pathogen was not inhibited. This, accordingly, revealed that the antibiotic was not translocated into new plant parts developing after spraying or its translocation was of such a low degree unable to provide protection against the attacking pathogen.

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Untersuchungen über die Infektion durch *Alternaria porri* f. sp. *solani* (E. & M.) Neerg. und *Rhizoctonia solani* Kühn an Tomatensamen

Von

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Die Infektion der Tomatensamen durch die pathogenen Pilze *Alternaria porri* f. sp. *solani* (E. & M.) Neerg. und *Rhizoctonia solani* Kühn wurde bisher aus Ungarn nicht mitgeteilt. In der vorliegenden Mitteilung werden die Ergebnisse der diesbezüglichen Untersuchungen des Verfassers erörtert.

Einleitung

Unsere Gemüsepflanzen werden durch die verschiedenen bakteriellen und Pilzkrankheiten von Jahr zu Jahr schwer geschädigt. Die meisten Krankheitserreger, die diese Erkrankungen verursachen, werden durch die Samen verbreitet. Vom Standpunkt der Bekämpfung dieser sich auch mit dem Samen verbreitenden Krankheitserreger ist die Analyse der Umstände der Sameninfektion bzw. die Ausarbeitung der auf die Bekämpfung der Infektion gerichteten Schutzmassnahmen von grosser Bedeutung.

Die auf die Infektion des Saatguts der Halmfrüchte, ferner auf die Ausarbeitung der als Schutz dienenden Beizverfahren bezüglichen Forschungen blicken nunmehr auf eine Vergangenheit von 300 Jahren zurück und sind – mit der Ausnahme kleinerer Einzelheiten – heute schon als abgeschlossen anzusehen. Die Aufklärung der Möglichkeiten und Umstände der Infektion der verschiedenen Gemüsesamen sowie deren systematische Prüfung ist mannigfaltiger und umständlicher als im Falle der Getreidesamen. Die Ausarbeitung der Schutzmassnahmen wird durch die erhöhte Empfindlichkeit der Samen und Keimpflanzen gegen Chemikalien erschwert. Es lässt sich unter anderem hiermit erklären, dass die Untersuchung der samenbedingten Verbreitung der wichtigsten Krankheitserreger der Gemüsepflanzen nur durch die Forschungen nach der Jahrhundertwende auf die Tagesordnung gesetzt wurde. Die anfänglichen, in erster Reihe auf die Hülsenfrüchtler beschränkten Untersuchungen haben in den letzten drei Jahrzehnten einen neuen Aufschwung genommen und erstreckten sich sozusagen auf alle wichtigeren Gemüsearten.

Diese Forschungsarbeit nahm in Ungarn mit den Untersuchungen von SCHILBERSZKY (1922) ihren Anfang.

Die durch die pathogenen Pilze *Alternaria porri* f. sp. *solani* (E. & M.) Neerg. und *Rhizoctonia solani* Kühn verursachte Infektion der Tomatensamen wurde aus Ungarn bis jetzt nicht mitgeteilt. In der vorliegenden Mitteilung werden die Ergebnisse unserer diesbezüglichen Untersuchungen erörtert.

Übersicht der Literatur

Die durch *Alternaria solani* und *Rhizoctonia solani* verursachte Infektion der Tomatenfrüchte und Samen wurde bisher geringer Aufmerksamkeit gewürdigt.

Die systematische und schwere Schädigung durch Krankheitserreger ist auch in Ungarn bekannt, aber *Alternaria solani* wurde als laubinfizierender, *Rhizoctonia solani* als sämlinginfizierender Pilz angenommen (KIRÁLY et al., 1960).

Die bezüglichen einheimischen Literaturquellen erwähnen die Infektion der Früchte und der Samen nur auf Grund ausländischer Forschungen (KIRÁLY et al., 1960; UBRIZSY, 1965) oder überhaupt nicht (UBRIZSY, 1960).

Auf Grund der Forschungsarbeit in den über einen entwickelten Tomatenbau verfügenden westlichen Ländern betonen ausser den bedeutenderen Handbüchern (WALKER, 1950; NEERGAARD, 1945; KOTTE, 1952; DOOLITTLE et al., 1961) hinaus zahlreiche Mitteilungen die Wichtigkeit der Früchten- und Sameninfektion durch *Alternaria* und *Rhizoctonia*. (MASSEE [cit. in NEERGAARD, 1945]; RAMSEY et al. [cit. in BARKER, 1947]; SAMSON [cit. in NEERGAARD, 1945]; BAKER [1947]; CICCARONE et al., [1957]; CICCARONE, [1956]; JOSHI et al., [1957]; CHROSSAN et al., [1960]; GONZALES et al., [1963]; SMITH, [1964]).

Material und Methode

Zu Beginn unserer Arbeit haben wir uns die Untersuchung der Alternaria-Infektion der Tomatenfrüchte und Samen zum Ziel gesetzt.

An der Zentralanlage des Versuchsinstituts in Kecskemét sowie in den Betriebsanpflanzungen in der Umgebung von Kecskemét haben wir im Verlaufe des Monats August 1964. die reifen Früchte der auf Grund der Symptome mit *A. solani* mässig infizierten Sorte Kecskeméti Determinált San Marzano und der stark infizierten Sorte K – 42 eingesammelt. Die Samen wurden nach Auswaschung auf Samenwaschleinwand und nach der Trocknung bis zur Verwendung in Papiertüten aufbewahrt.

Das Ausmass der Sameninfektion wurde durch Keimung auf sterilem Filterpapier in Petri-Schalen sowie durch Aussaat in desinfizierte irdene Schüssel von der Dimension 15 × 25 cm in mit Dampf sterilisierten Flusssand am 5. bzw. 25. Tage festgestellt, obwohl über die sich entwickelnde Infektion auch am 14. und 20. Tage Aufzeichnungen verfertigt wurden.

Je Wiederholung wurden 100 Samen gekeimt und ausgesät. Die Temperatur des Keimthermostats war während der Dauer der Versuche 28° C, die Temperatue

des Gewächshauses schwankte aber in den Monaten Oktober – Februar zwischen 20 ± 4° C, in den Monaten März–Mai zwischen 24 ± 4° C.

Die Rückisolierung des Krankheitserregers aus den infizierten Pflanzen wurde insgesamt zweihundert Male vorgenommen.

Die zu den histologischen Untersuchungen verfertigten Schnitte wurden mit Baumwollenblau und Safraninrot gefärbt.

Ergebnisse

1. Die Infektion der Früchte

Die massenhafte Alternaria-Infektion der Tomatenfrüchte am Ende der Vegetationsperiode zog im Herbst 1963 unsere Aufmerksamkeit auf sich. Im Jahre 1964 setzten wir unsere Beobachtungen sowohl im Zentral-Versuchsgebiet wie auch an anderen Anbaugebieten des Landes fort.

Die Ergebnisse der Aufnahmen über das Ausmass der Fruchtinfektion sind in der Tab. 1. dargestellt.

Die Angaben der Tabelle wurden auf so vielen zufallmässig ausgewählten Punkten des in Rede stehenden Anbaufeldes aufgenommen, auf wie viel Hunderte von Früchten sich die Infektions-Durchschnittswerte beziehen. Von den Angaben verdient die in dem der Aufarbeitung harrenden Rohmaterial der Konservenfabrik von Békéscsaba festgestellte 34%ige Durchschnittsinfektion besondere Aufmerksamkeit – mit Rücksicht darauf, dass in den früheren Jahren als Nebenprodukt der Konservenfabriksaufarbeitung auch Saatgut zurückbehalten wurde, welches ohne weitere Behandlung den Erzeugern zur Verfügung gestellt worden ist.

Das Ausmass der Fruchtinfektion zeigte eine enge Korrelation mit der Infektion des Stengels und das Laubes. Auf dem stark infizierten teilweise schon abgetrockneten Laube der Pflanzen war die Sporulation von Anfang September an jede Woche ausserordentlich reichlich. An den teilweise schon unbelaubten, auf dem Boden liegenden Stengeln zeigten auf den grünen Früchten verschiedenen Entwicklungsgrades, meistens von der Gegend des Blütenstiels ausgehende, etwas eingesunkene braune Flecke von verschiedener Grösse und glatter Oberfläche die Infektion an. An den älteren Flecken von 10–20 mm Durchmesser entwickelte sich ein dichter, bräunlich-schwarzer Konidiumrasen (Abb. 1–2).

An den in Reife begriffenen oder bereits gereiften Früchten hat sich die Infektion intensiver gestaltet. Grösse und Erscheinung der Flecke ist so wie bei den grünen Früchten, ihre Farbe ist jedoch schwarz.

Am Rande der älteren, langsam eintrocknenden Flecke zieht die elliptisch verlaufende Runzelung der Schalenoberfläche charakteristische Ringe um das nnere Gebiet der Flecke (Abb. 2). Auf den Flecken entwickelt sich an den Tagen nach reichlichem Tau oder Regen ein dichter samtig schwarzer Konidiumrasen.

Tabelle 1

Das Ausmass der *Alternaria solani* Infektion der Tomatenfrüchte in verschiedenen Anbaugebieten in 1964

Ort	Zeitpunkt der Aufnahme	Sorte	Untersuchte Fruchtzahl St.	Durchschnittliche Infektionsprozente	Charakterisierung
Kecskemét	25. VIII.	K—42	500	26	Reife, aufplatzende Früchte
Kecskemét	1. IX.	K—42	400	30	Grüne und in Reife begriffene Früchte
		Det. San Marzano	500	37	Reife Früchte
Borbáspuszta	4. IX.	K—merevszárú	200	40	Reife Früchte
		K—törpe	300	31	Reife Früchte
		Manitóba	300	21,6	Reife Früchte
Városföld	7. IX.	Budai Korai	500	43,8	Reife Früchte
		K—Fh—70 Det.	500	44	Reife Früchte
		K—507	500	38,2	Reife Früchte
Cegléd	9. IX.	K-konzerv	500	3	Reife und grüne Früchte
		Det. San Marzano	500	25	Reife und grüne Früchte
Mohács	10. IX.	No 10 × Bizon	Schätzung	6—8	Reife Früchte
		Budai Korai	,,	4—6	Reife und grüne Früchte
Pécs	11. IX.	Budai Korai	Schätzung	8—10	Reife und grüne Früchte
		K—524	,,	3—5	,,
		K—512	,,	3—5	,,
Kiskunfélegyháza	14. IX.	Scarlet Dawn	500	8	,,
		K—42 × K—törpe	500	45	
Békéscsaba (Konservenfabrik)	17. IX.	Unbekannt	500	34	Der Aufarbeitung harrendes Produkt
		K—konzerv	Schätzung	2—5	Reife und grüne Früchte
Szabadkígyós	17. IX.	K—42	,,	15—20	,,

Bei den grünen oder am Anfang der Reife stehenden Früchte beschränkt sich die Infektion mit wenigen Ausnahmen auf die Gewebe in unmittelbarer Nähe der Flecke. Mit dem Fortschreiten der Reife oder im Falle der Infektion der in Reife befindlichen Früchte verbreitet sich der Pilz rasch in den fleischigen Geweben und jenen des Stiels der Früchte. Die Symptome werden von schwärzlicher Färbung begleitet. Im Zeitpunkt der Marktreife erstreckt sich diese schwarze Gewebezerstörung meistens auf ein Drittel des Fruchtfleisches, aber auch innere Gewebezerstörungen von viel höherem Prozentsatz sind häufig (Abb. 3).

Die erwähnte innere Zerstörung der Früchte trägt den Charakter der Trokkenfäule. Häufig ist die Erscheinung von Maden einer Diptera-Art in der erkrank-

ten Frucht zu beobachten. Diese auch sekundär infizierten Früchte übergehen infolge der Tätigkeit der Maden in nasse Fäule. Manchmal wird der Krankheitsprozess auch durch saprophytische Bakterien und Pilze ähnlich beeinflusst.

Die durch *Rhizoctonia solani* hervorgerufene Infektion der Tomatenfrüchte ist weniger augenfällig und charakteristisch. Auf die *Rhizoctonia*-Infektion eines

Abb. 1. Schwere *Alternaria solani* Infektion auf K. Det. San Marzano Früchten

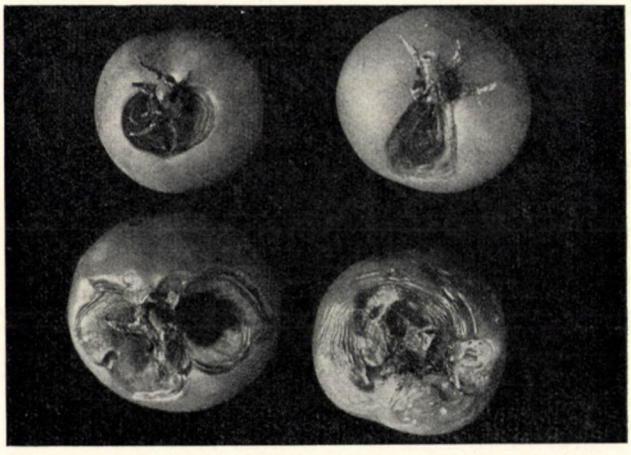

Abb. 2. *A. solani* Infektionen verschiedenen Alters auf grünen und reifenden Früchten

kleineren Anteils des mit der von uns eingesammelten *Alternaria* infizierten Untersuchungsmaterials wurde unsere Aufmerksamkeit nur durch die während der Frucht- und Samenuntersuchung getätigten Kulturen gelenkt.

RAMSEY et al. (cit. in BAKER, 1947) haben schon in 1926 die *Rhizoctonia*-Infektion der Tomatenfrüchte angezeigt. In der weiteren Folge berichteten auch

BAKER (1947), JOSHI et al., (1957), CHROSSAN et al., (1960), DOOLITTLE et al., (1961), GONZALES et al., (1963) und SMITH, (1964) über diese Schädigung von *Rhizoctonia solani.*

Zweifellos ist auf jedem Anbaugebiet, wo der Boden mit *Rhizoctonia* infiziert ist, und die in der Reife begriffenen Früchte auf dem Boden liegen, die Infektion durch den Pilz in Abhängigkeit von den Feuchtigkeitsverhältnissen der Bodenoberfläche auch in Ungarn beachtlich.

Die Ergebnisse der Untersuchung der von uns eingesammelten Früchte zeigen, dass auch gemeinsame Infektion durch die Pilze *A. solani* und *Rh. solani* vorkommt.

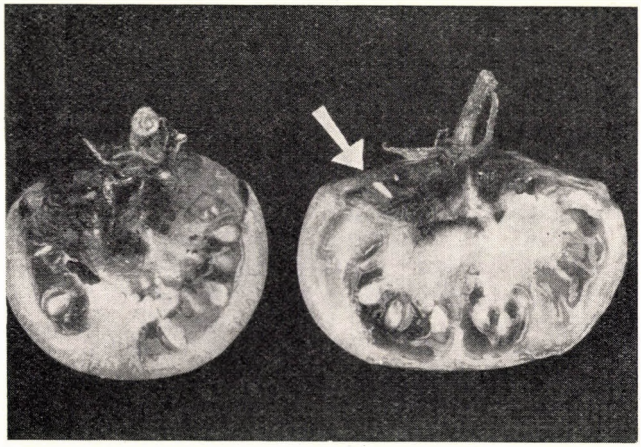

Abb. 3. *Alternaria* Infektion der inneren Gewebe der Früchte (in der Richtung des Pfeiles ist das Ei der sekundär infizierenden Diptera Art zu sehen)

2. Die Infektion der Samen

Beim Durchschneiden der eine vorgerücktere Infektion zeigenden reifen Früchte ist die intensive Infektion der inneren Gewebe des Fruchtknotens schon makroskopisch auffallend. Vom Standpunkt der Infektionsmöglichkeit der Samen haben zwei Faktoren die grösste Bedeutung im Zeitpunkt der Entwicklung der inneren Infektion:

a) Der Zustand der äusseren und inneren Zellschichten der Samenschale in Abhängigkeit vom Reifezustand der Samen.

b) Der Umstand, ob sich der Keimträger (Nabelstrang) in engem Zusammenhang mit dem Endosperm befindet, oder die Absperrung der Nabelöffnung mit der Ausbildung der Endosperm-Kutikula beendet wurde.

Die Vorbedingung der Ausbildung der inneren *Alternaria-* und *Rizoctonia-*Infektion ist die Reife der Früchte und parallel damit die der Samen oder zumindest der Beginn des Reifeprozesses.

Tabelle 2

Das Ausmass der Infektion von Tomatensamen durch die Pilze *Alternaria solani* und *Rhizotonia solani*

Sorte	Ursprung der Sameninfektion	Fungizide Behandlung vor der Aussat	Keimungsprozente auf Filterpapier				Aufgangsprozente in sterilem Sand 25. Tag	
			3. Tag		5. Tag			
			insges.	krank	insges.	krank	insges.	krank und umgefallen
K—42	*A. solani*	0	86.3	16.6	90.3	22.6	86.0 ± 4.3	25.6 ± 1.8
	natürliche Infektion	0.05 %iges Formalin	88.0	10.0	91.0	21.0	92.0 ± 3.3	15.3 ± 1.0
Det. San Marzano	*A. solani*	0	86.6	6.3	89.6	9.3	98.3 ± 1.1	13.0 ± 4.0
	natürliche Infektion	0.05 %iges Formalin	88.3	0.6	89.6	3.0	97.3 ± 2.3	1.0 ± 0.0
Nr. 1080	*A. solani* künstliche Infektion	0	93.6	21.6	95.0	45.0	88.6 ± 3.2	56.0 ± 9.0
Nr. 1080	*Rh. solani*	0	97.3	35.6	98.0	78.0	96.6 ± 1.5	57.6 ± 4.4
	künstliche Infektion	0.05 %iges Formalin	98.0	4.0	99.0	9.6	98.0 ± 1.2	6.0 ± 2.4
K—42	Kontrolle	0	90.0	0.0	92.0	0.0	91.0 ± 3.1	0.0
Det. San Marzano	(gesund)	0	89.0	0.0	91.0	0.0	96.0 ± 2.7	0.0
Nr. 1080		0	97.6	0.0	98.6	0.0	97.3 ± 1.6	0.0

Zu Beginn der physiologischen und biochemischen Prozesse der Reife setzt eine Konkurrenz zwischen dem sich im Inneren des Fruchtknotens entwickelnden Krankheitserreger und dem Wachstumsrhythmus der Samen ein.

Falls die Fruchtinfektion nicht früh genug einsetzte und ihre Ausdehung in den oberen Zellschichten des Fruchtknotens nicht allzu gross ist, so erreicht die Reife der Samen und die Kutinalisation der Nabelöffnung ihr Ende, noch bevor der Pilz die Plazenta, den Funikulus sowie die ganze Oberfläche der Fruchtschale erreichen würde.

Vom Gesichtspunkt der Vermeidung der inneren Infektion der Samen ist auch der Umstand günstig, dass die Nabelöffnung bei den Tomatensamen kleiner ist als z. B. bei den Samen des Paprikas oder mehrerer anderer Gemüsearten.

Die obigen Faktoren erteilen gemeinsam eine Erklärung für die verhältnismässig geringfügige innere Infektion der Tomatensamen (Tab. 2).

Bei der Prüfung mikroskopischer Schnitte wurde das ruhende Myzelium des Pathogens *A. solani* in den folgenden Teilen der Samen beobachtet:

a) An der Oberfläche der Fruchtschale.

b) Bei der Nabelöffnung, an den Resten des Nabelstranges, an der äusseren Oberfläche der Endosperm-Kutikula.

c) In den Geweben um die Nabelöffnung des Endosperms (Abb. 4).

Vom Standpunkt der chemischen Bekämpfung der Infektion bildet die schwerste Aufgabe die Zerstörung des unter Punkt *c)* erwähnten tiefliegenden Myzels. Dies bildet zugleich die Gewähr für den vollständigen Erfolg.

3. *Ergebnisse der Untersuchung des Ausmasses der Sameninfektion*

Die aus den eingesammelten infizierten Früchten gewonnenen Samen wurden in drei Versuchsserien mit 6 Wiederholungen auf Filterpapier ohne Vorbehandlung und nach einer 0,05%igen Oberflächen-Formalindesinfektion von 10 Minuten gekeimt.

Im Keimbett der Samen der stärker infizierten Sorte K – 42 schwankte die Erkrankung zwischen 14,0 und 32,4%, mit einem Durchschnittswert von 22,6%. Die Samen der schwächer infizierten Sorte San Marzano zeigten im Filterpapier-Keimbett einen durchschnittlichen Infektionswert von 9,3%. Die Wirksamkeit der oberflächlichen Desinfektion wurde durch die dichte Verteilung der Samen bzw. Keimpflanzen sowie durch die für die rasche Verbreitung des Pilzes optimale Umgebung gestört oder verhindert.

Aus den kranken Keimpflanzen wurden auf Kartoffeldextrose und Czapek-Dox Agar zweihundert Impfungen vorgenommen, deren 78,4% *A. solani*, 8,3% *Rh. solani* Infektionen zur Folge hatte. Aus 13,3% der Isolate entwickelten sich *Mucor racemosus* und *Alternaria tenuis* Pilze.

Weiterhin wurden je 20 Stück unversehrte, in Reife befindliche Früchte der zur Verfügung stehenden Hybride Nr 1080 mit je einem von den früher infizierten Keimpflanzen isolierten Stamm von *A. solani* und *Rh. solani* künstlich infiziert.

Nach 15 Tagen zeigten alle Früchte eine intensive Infektion. Dann wurden die Samen auf die übliche Weise ausgewaschen und getrocknet.

Im Verlaufe unserer weiteren Untersuchungen waren wir bestrebt, unter Ausschluss der im Filterpapier-Keimbett erschienenen sekundären Organismen nur das Ausmass der durch die pathogenen Pilze *A. solani* und *Rhizoctonia* verursachten Infektion festzustellen, auf Grund der Virulenz und Pathogenität der an den Samen gehafteten pilzlichen Gebilde. Darüber hinaus wollten wir auch die Möglichkeit der gegenseitigen Infektion vermindern.

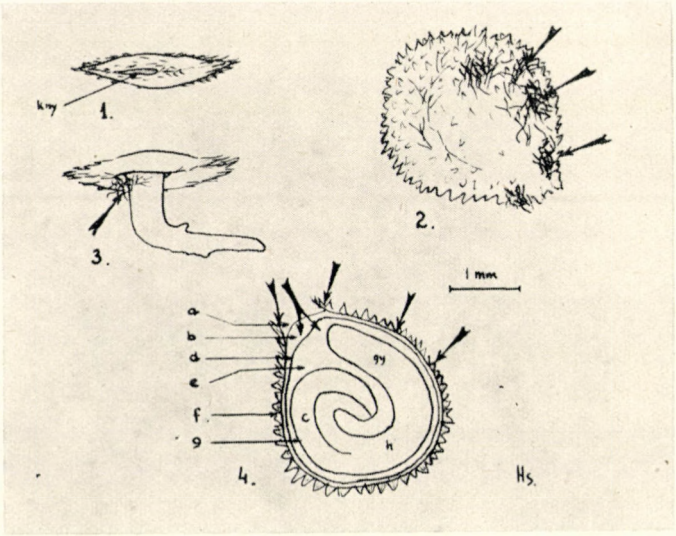

Abb. 4. Die Stellen der *Alternaria* Infektion der Samen: 1. Nabelöffnung. 2. Myzeliumherde an der Oberfläche der Samenschale. 3. Die Infektion des Hypocotyls bei der Nabelöffnung. 4. Querschnitt eines Tomatensamens mit Pfeilen, die die bestimmten Infektionspunkte anzeigen. a = Nabelöffnung, b = Reste des Nabelstranges, c = Cotyledon, d = Endosperm — Cuticula, e = Endosperm, f = Samenschale, g = Embryo, gy = Radicula, h = Hypocotyl

Auf Grund der von WILHELM (1956) zur Isolierung von Wurzelfäule verursachenden Pilzen ausgearbeiteten Methode haben wir die infizierten Samen verschiedenen Ursprungs in 6 Wiederholungen auf die bereits erörterte Weise in mit Dampf sterilisierten Flusssand ausgesät u. zw. unbehandelt und mit Formalin-Vorbehandlung. Auf dem Saatbett betrug die Distanz der Samen untereinander 2—2,5 cm.

Das Verfahren hat sich als zweckdienlich erwiesen. Die Infektion erschien in den einzelnen Wiederholungen mit ausgeglichenen Werten. Zugleich hat sich auch die Einwirkung der oberflächlichen Desinfektion geltend gemacht, den prozentuellen Wert der äusserlich und innerlich infizierten Samen anzeigend.

An den von mit *A. solani* infizierten Samen erhaltenen Sämlingen erschienen die Krankheitssymptome am 6—10. Tage nach dem Auflaufen. Am Wurzelhals

hat sich eine schwarze Gewebenekrose von 5—10 mm Ausdehnung ausgebildet. Die kranken Pflanzen sind langsam vertrocknet, aber nur selten umgefallen.

Ein Teil der aufgegangenen Pflanzen zeigte auf charakteristische Weise die auf die innere Infektion des Samens verweisende, oft noch vor Abwurf der Samenschale in Erscheinung tretende Zerstörung des Keimblattes und Stengelvegetationspunktes, die von oben nach unten fortschritt (Abb. 5).

Aus den kranken Pflanzen konnten wir in jedem Falle nur den Krankheitserreger *A. solani* isolieren.

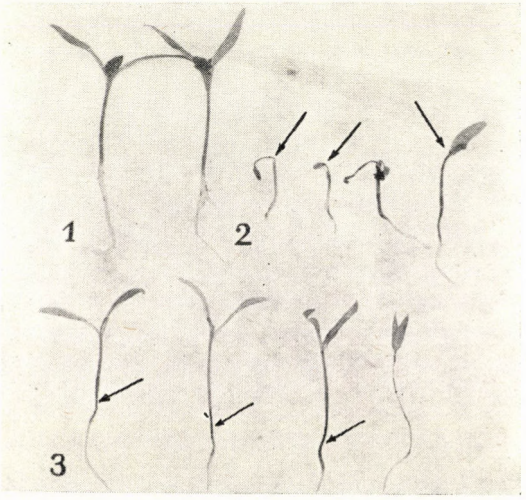

Abb. 5. *Alternaria* Infektion infizierten Samen entstammender Keimpflanzen. 1. Gesund, 2. Zerstörung des Stengelvegetationspunktes und des Keimblattes, 3. Wurzelhalsnekrose

Die Samen der mit *Rhizoctonia* künstlich infizierten Früchte zeigten eine durchschnittliche Infektion von 57,6%. Die Krankheitssymptome erschienen jedoch nur in der Form einer auf den Wurzelhals und den Wurzelstamm begrenzten milden Nekrose von 3—5 mm Ausdehnung.

Der angewendete Sand hat offenbar zur Entfaltung der Infektion des Pilzes ungünstige Bedingungen geschaffen.

Die Ergebnisse der auf die Feststellung des Ausmasses der Infektion gerichteten Untersuchungen sind in der Tab. 2. zusammengefasst.

Die in der vorliegenden Mitteilung erörterten Infektionsuntersuchungen wurden im Verlaufe unserer weiteren Arbeit mit Versuchen zur Bekämpfung der Infektion fortgesetzt. Über die Ergebnisse dieser Versuche werden wir in einem späteren Zeitpunkt berichten.

Diskussion

In der vorliegenden Mitteilung wird zum ersten Male aus Ungarn über die *Alternaria-* und *Rhizoctonia*-Infektion der Tomatenfrüchte und Samen berichtet.

Im Verlaufe unserer Untersuchungen haben wir uns in erster Reihe mit dem Krankheitserreger *Alternaria solani* befasst, aber auch gemeinsame Infektion durch die Pilze *A. solani* und *Rhizoctonia solani* festgestellt.

Die *Alternaria*-Infektion der Früchte ist im Herbst der Jahre 1963 und 1964 im ganzen Lande massenhaft aufgetreten (Abb. 1). In 1965 erschien sie schon im Monat Juli in mehreren Tomatenanbaugebieten in beachtlichem Ausmasse. Das Ausmass der Fruchtinfektion zeigt eine enge Korrelation mit der Infektion des Stengels und des Laubes. Die anfängliche, sich auf die äusseren Gewebeteile des Perikarps beschränkende Infektion dringt mit dem Fortschreiten der Reife in die inneren Gewebeteile des Fruchtknotens ein, wo sie ein Krankheitsbild vom Typ einer schwarzen Trockenfäule hervorruft (Abb. 3.). Infolge der ausgedehnten inneren Infektion greift der Pilz die Schalenoberfläche der Samen und den Nabelstrang an, durch welchen er fallweise auch in das Endosperm eindringt (Abb. 4).

Zur genauen Bestimmung des Infektionsgrades der Samen erwies sich die Aussaat in sterilen Flusssand am geeignetesten.

An den Sämlingen, die aus mit *A. solani* infizierten Samen hervorgingen, erschienen die Krankheitssymptome am 6—10. Tage nach dem Auflaufen in der Form einer beim Wurzelhals auftretenden schwarzen Gewebenekrose von 5 bis 10 mm Ausdehnung. An den auch innerlich infizierten Samen trat eine charakteristische Zerstörung des Stengelvegetationspunktes auf, die sich von oben nach unten verbreitete (Abb. 5).

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Studies on Strains of Potato Virus Y[1)]

1. Strain C

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Results of transmission experiments showed that *Gomphrena globosa* L. *Capsicum annuum* L. var. *Markgärtner*, *Datura stramonium* L. var. *tatula* and *Lycopersicon pimpinellifolium* (Jusl.) Mill. are not susceptible to infection with an isolate (EP) of strain C (PVY^C) of potato virus Y. Many other plant species, however, exhibited characteristic symptoms, both systemic and local. *Nicotiana tabacum L. "V 20"* proved to be a symptomless carrier of the virus. The isolate EP has a thermal inactivation point of 58 to 60° C, dilution end point of 2×10^{-4} to 1×10^{-4}, and retains its infectivity in vitro for 15 to 18 days. It can be kept in dried tobacco tissues without loosing its infectivity for a period of between 365 to 426 days. The virulence based on results of biological tests and serological reactions proved to be fairly small with isolate EP.

Introduction

It has been known for a long time that the rugose mosaic disease of potato (SCHULTZ and FOLSOM, 1923) is caused by a complex infection of both potato virus X (PVX) and potato virus Y (PVY) (SMITH, 1931; KOCH, 1931). Later on it turned out that these viruses after being separated from each other still consisted of several components.

It was SALAMAN (1930) who first reported that a virus, isolated from the potato variety *Di Vernon* caused top necrosis on the variety *President*, whereas no such symptoms were produced on the variety *Arran Victory*. Not too much later KOCH and JOHNSON (1935) as well as DYKSTRA (1936) came to the conclusion that as far as symptom production on potato is concerned there were considerable differences between PVY originating from England and a virus which caused vein clearing and was of American origin. In the same year BAWDEN (1936) made similar observations and reported a virus, to be called by him in 1943 virus C, which induced top necrosis on the potato variety *Monocraat*. COCKERHAM (1943) was the first to suggest a possible relationship between PVY and PVY^C and to show that PVY^C in contrast to PVY was not transmissible by aphids. This has

[1] The experiments were carried out in the Department of Plant Pathology, Institute of Plant Breeding, Gross Lüsewitz in the framework of a scientific exchange program among the Research Institute for Plant Protection, Hungary, the Hungarian Academy of Sciences and the German Agricultural Academy.

been later confirmed (BAWDEN and SHEFFIELD, 1944) by experiments in which PVY^C could not be transmitted by *Myzus persicae* Sulz. One year later, however, BALD and NORRIS (1945) were successful in transmitting PVY^C by aphids. Two years later BAWDEN and KASSANIS (1947) reported on repeated failures to transmit PVY^C by aphids and suggested that BALD and NORRIS (1945) had obtained their positive results by working with an unknown variety of PVY rather than PVY^C. In her experiments WATSON (1956) also demonstrated that PVY^C could not be transmitted by *M. persicae* from potato. Some *Nicotiana* species, however, served as efficient sources of the virus for aphid transmission tests. Positive transmissions were also obtained from plants infected with both PVY and PVY^C (WATSON, 1960). In the mean time it has been demonstrated that PVY and PVY^C are serologically related (BAWDEN, 1943; BAWDEN and SHEFFIELD, 1944), having a common antigenic group (BAWDEN and KASSANIS, 1951) and similar physical properties (BALD and NORRIS, 1945). Furthermore, it has been shown that PVY^C gave premunity against PVY (BAWDEN and SHEFFIELD, 1944) and that PVY and PVY^C exhibited different symptoms on the same host plant species (HUTTON and PEAK, 1952). *Datura stramonium* L. (JOHNSON, 1930; DARBY et al., 1951) is an exception to this, as well as *Emilia sagittata* L. which recently proved to be resistant to both PVY^N (normal strains) and PVY^C (HOLLINGS and STONE, 1963).

Material and Methods

Virus culture

Strain PVY^C was kindly supplied by Dr. G. COCKERHAM. It was isolated from the potato variety *Edgecote Purple* and designated by us as "EP"

For studies on the virus-host relationship two strains of potato virus X(PVX) were also included. The ringspot strain (PVX^{RS}) was kindly supplied by Dr. N. S. WRIGHT. Originally it had been isolated by Dr. R. H. LARSON (WRIGHT, 1964). The so-called normal strain of PVX (PVX^N) was isolated from the potato variety *Sirtema*, a gift by Dr. H. WENZL.[2]

Using our method for the separation of potato viruses (HORVÁTH, 1966a) we checked whether there was any virus contamination in our cultures. The pure virus strains were kept in *Nicotiana glutinosa* L., *Nicotiana tabacum* L. var. *Samsun* and *Nicotiana tabacum* L. var. *White Burley*. In addition, the strains were kept also in *Nicotiana* species[3] resistant to *Peronospora tabacina* Adam. and in dried

[2] Thanks are due to Dr. G. Cockerham (Scottish Plant Breeding Station, Edinburgh, Scotland), Dr. N. S. Wright (Canada Department of Agriculture, Vancouver, Canada) and Dr. H. Wenzl (Institute for Plant Protection, Wien, Austria) for providing me with their virus strains.

[3] The *Nicotiana* species resistant to *Peronospora tabacina* Adam (*N. exigua* Wheeler *N. goodspeedii* Wheeler, *N. megalosiphon* Heurck et Muell, *N. debneyii* Domin, *N. tabacum* L. Resistant Hicks) proved to be susceptible to different strains of PVY and PVX.

tobacco leaf tissues *(Nicotiana tabacum* L. var. *Samsun)* over $CaCl_2$ in an exsiccator according to McKINNEY's method (McKINNEY, 1947).

Growing of plants and experimental conditions in the greenhouse

The following plant species were used in our experiments:

Family and binomal	Source[4]
Amaranthaceae	
Gomphrena globosa L.	b
Solanaceae	
Capsicum annuum L.	a
C. annuum L. var. *Markgärtner*	b
Datura metel L.	a
D. stramonium L. var. *tatula*	c
Lycium halimifolium Mill.	a
Lycopersicon esculentum Mill.	a
L. pimpinellifolium (Jusl.) Mill.	a
Nicotiana glutinosa L.	e
N. repanda Willd.	f
N. tabacum L. var. *"V 20"*	i
N. tabacum L. var. *Havana* 38	g+h
N. tabacum L. var. *Havana* 425	g+h
N. tabacum L. var. *Samsun*	d
N. tabacum L. var. *Sanderae*	a
N. tabacum L. var. *White Burley*	d
Petunia hybrida Vilm.	c
Physalis floridana Rydb.	b
Solanum nigrum L.	c
Solanum tuberosum L. (Seedling No. 59/558.)	j

Growing and inoculation of test plants were carried out in the greenhouse. During our experiments from spring to autumn the light conditions were favourable. The temperature, however, varied between 20 to 25°C during the day and often decreased to a value as low as 15°C during the night. Because of technical difficulties we had no means of controlling these fluctuations in the temperature. The test plants to be infected were planted in pots of a diameter of 9 cm and were kept at the same temperature throughout the experiment. The virus cultures were

[4] The seed samples of the test plants were obtained from a) Dr. Chr. Lehmann (Gatersleben, Germany), b) Institute for Plant Breeding, Gross Lüsewitz, Germany; c) Botanical Garden, Leipzig, Germany; d) Dr. P. Berger, Dresden, Germany; e) Dr. W. Blaszczak, Poznan, Poland; f) Dr. F. E. Nitzany, Rehovot, Israel; g) Dr. W. B. Raymer, Beltsville, Maryland and Dr. J. C. Walker, Madison, USA; h) Dr. R. Roland, Gembloux, Belgium; i) Dr. W. Endemann, Dresden, Germany; j) Dr. K. H. Möller, Gross Lüsewitz, Germany and are highly appreciated.

maintained under controlled conditions, in vector-proof isolators and were subjected to passages in order to prevent them from loosing their activity. Against the vectors sprayings with the insecticide Tinox in an 0.05 per cent solution were carried out once a week. According to recent data of ACKERMAN et al. (1964) Tinox, being a systemic insecticide keeps its activity even for longer periods.

Inoculation methods

Mechanical transmission was carried out by grinding the plant material in a mortar and applying the undiluted sap obtained with a glass spatula to the leaves of test plants previously dusted with carborundum (500 mesh). The infections were done in several replications, consisting of 5 plants each. After inoculation the plants were rinsed with water.

Aphid transmissions were performed by using adults of the aphid *Myzus persicae* Sulz. as vectors[5] and *Nicotiana* species and *Physalis floridana* Rydb. seedlings as test plants. Usually 20 to 25 aphids were subjected to a starvation period of four hours and a feeding period of eight minutes and put on the test plants under glass covers for infection feeding. After 24 hours the aphids were killed by spraying the plants with a 0.05 per cent Tinox solution.

Symptoms on test plants and recovery of the isolates

The susceptible (+) test plants infected showed either systemic (□) or local (0) symptoms, or both (□0). Some of them remained symptomless (−). In many cases the virus could be recovered by subinoculations (× +), in other cases not (× −). For the subinoculations the sap from *Capsicum annuum* was diluted 1 : 3 with distilled water, in case of the other test plants, however, undiluted sap was employed. For the designation of symptoms abbrevations are used, as indicated in the pertaining chapters. For the description of symptoms local symptoms are marked with (I), systemic ones with (II). Since PVY and its strains induce similar symptoms on *Nicotiana* species the determination of the incubation period (first arabic number), the pathogenecity (second arabic number), the infectivity (low = = 50, medium = 75, high = 100) and the percentage of infection (third arabic number) also seemed to be necessary. The incubation period is expressed in days and the infectivity in percentages.

The pathogenicity is considered to be high (10) if the time period of the morbid state is less than 45 days, medium (20) if longer than 45 days but shorter than 65 days and low (30) if longer than 65 days.

The degree of the severity of symptoms is expressed as 10 = weak, 20 = medium, 30 = severe, and 40=very severe (E. g. *Capsicum annuum* L.: +(□) × +;

[5] Thanks are due to Dr. K. Neitzel, Institute for Plant Breeding, Gross Lüsewitz, Germany for providing the aphids.

(II)Vc, Vb, Mo; 32/30/80/10.) The above designation means that in the experiment *C. annuum* L. was susceptible to PVY^C (+) there were systemic symptoms (□) and the virus could be recovered (× +); the systemic symptoms (II) consisted of vein clearing (Vc), vein banding (Vb) and mosaic (Mo), the incubation period took 32 days, the pathogenicity was 30 (low) the infectivity was 80 (medium) whereas the severity of symptoms was 10 (weak).

Determination of the virulence

Interactions between virus species: 5 tobacco plants were infected with sap diluted 1 : 10 and containing PVX^{RS} or PVX^N. After 24 hours the tobacco plants were superinoculated with undiluted sap containing strain EP.

Physical properties

Thermal inactivation point: 1 ml freshly pressed sap from virus-infected *N. tabacum* L. var. *Samsun* was diluted 1:1 with distilled water and heated in cotton plugged test tubes at different temperatures for 10 minutes in an ultrathermostate. After the treatments the sap was cooled with running tap water and immediately inoculated to 5 *N. tabacum* L. var. *Samsun* plants.

Dilution end point: Infected leaves of *N. tabacum* L. var. *Samsun* were homogenized in a mortar, the brei obtained filtered through sterile cheese cloth and the filtrate diluted to the desired extent with distilled water. The individual dilutions, starting with the highest one, were applied to 5 *N. tabacum* L. var. *Samsun* plants each.

Ageing in vitro: 10 ml of unfiltered sap was stored at room temperature in rubber stoppered test tubes and inoculated every third day to 5 *N. tabacum* L. var. *Samsun* plants.

Storage over calcium chloride

According to the method developed by McKinney (1947) infected leaves of *N. tabacum* L. var. *Samsun* were finely cut and stored in a dried state over $CaCl_2$ in an exsiccator at 0° C. At different time intervals samples taken from the dried leaf tissues were homogenized in a mortar in the presence of distilled water and phosphate buffer, pH 7. The homogenate was applied to 5 tobacco plants.

Serology

5 weeks after inoculation some top leaves of the plants serving as stock cultures of the virus were expressed into test tubes of a volume of 0.8 ml. With PVX, PVY and PAMV the sap was taken from tobacco plants, whereas PVS and PVM was obtained from potatoes. The sap was centrifuged for 30 minutes at 6000 r.p.m.

in a Model "Eispiruette" centrifuge and the supernatant was mixed on slides with the antiserum[6] in a 1 : 1 ratio. The slides were placed in a thermostate of 25° C for 40 minutes with PVY, 20 minutes with PVX and 15 minutes with PVS, PVM and PAMV, and evaluated in a light microscope with a 80 fold magnification. The degree of the reaction was designated as weak = +; medium = ++; strong = +++; very strong = ++++. The evaluations were based on relative values obtained by comparing serological reactions with 21 PVY strains.

Cross protection tests

Interaction among virus strains: 5 tobacco plants were inoculated first with 1 ml of undiluted sap containing the virus to be tested. After the appearence of the symptoms the plants were superinoculated with 1 ml of undiluted sap which contained the other virus strain. Since the plants reacted with systemic symptoms to all the strains involved cross protection tests were carried out in both directions.

Results

Susceptible and non-susceptible hosts[7]

Mechanical transmission:

Amaranthaceae

Gomphrena globosa L.: −/× −

Solanaceae

Capsicum annuum L.: +(□)× +; (II)Vc, Vb, Mo; 32/30/80/10
Datura metel L.: +(□)× +; (II)Vc, Mo, Ld, Cl; 14/30/100/10
D. stramonium L. var. *tatula:* −/× −
Lycium halimifolium Mill.: +(0)× +; (I)BlNRi Le Ab; 8/10/90/20
Lycopersicon esculentum Mill.: +(□)× +; (II)Vc, Mo; 17/30/80/10
L. pimpinellifolium (Jusl.) Mill.: −/× −
Nicotiana glutinosa L.: +(□)× +; (II)Vc, Lc, Cl, Ld, Rg; 12/30/100/20; (Fig. 1)
N. repanda Willd.: +(□)× +; (II)Vc, Vb, Rg, 15/30/100/10
N. tabacum L. var. "V 20": −/× +;
N. tabacum L. var. *Havana* 38: +(□)× +; (II)Vc, Vb, Mo; 13/30/100/10

[6] The serological reactions were carried out with antisera prepared against PVY (a), potato aucuba mosaic (PAMV) (b), potato virus X (PVX) (c), potato virus S (PVS) (d), and potato virus M (PVM) (e). Thanks are due to Dr. R. BARTELS (a), Braunschweig, Germany; Dr. D. H. M. VAN SLOGTEREN (b), Lisse, Netherland; Dr. J. NOHEJL (c, d, e) Havlickuv Brod, Czechoslovakia for providing me with the antisera mentioned.

[7] The abbreviations used below are as follows: BlNRi = black necrotic ringlike spot; Cl = crinkling of leaves; Dif = diffuse; Lc = leaf-curl; Ld = leaf distortion; LeAb = leaf abscission; Mo = mosaic; NSp = necrotic spots; Rg = retardation of plant growth; Vb = vein banding; Vc = vein clearing; Vn = vein necrosis; YM = yellow mosaic; YRi = yellow ring-like spots.

N. tabacum L. var. *Havana* 425.: +(□)× +; (II)Vc, Vb, Mo; 13/30/100/10
N. tabacum L. var. *Samsun:* +(□)× +; (II)Vc, Vb, Mo; 11/30/100/10
N. tabacum L. var. *Sanderae:* +(□)× +; (II)Vc, Vb; 14/30/100/10
N. tabacum L. var. *White Burley:* +(□)× +; (II)Vc, Vb; 12/30/100/20
Petunia hybrida Vilm.: +(□)× +; Vc, DifMo, YM; 23/30/60/20
Physalis floridana Rydb.: +(□0)× +; (I)NSp, LeAb; (II)Mo, Lc, YRi, LeAb; 18/20/100/20
Solanum nigrum L.: +(□)× +; (II)Vc; 32/30/80/10

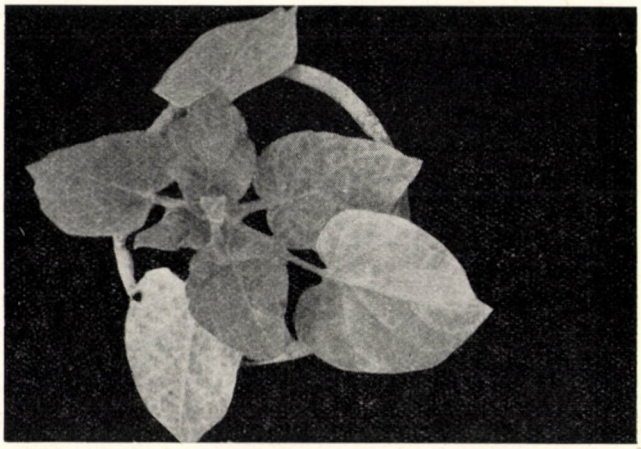

Fig. 1. *Nicotiana glutinosa.* Showing systemic veinclearing, leaf distortion and suppression of plant growth induced by potato virus Y (strain C) 26 days after inoculation (7/11—EP)

Solanum tuberosum L. (Seedling No. 59/558): +(□0)× +; (I)BlRi, Vn, LeAb; (II)Mo; 21/20/100/40 (Fig. 2)
Edgecote Purple (variety): +(0)× +; (II)Vc, Mo.

Four of the test plants listed above did not become infected by mechanical inoculation and the virus could not be recovered from them either. *Nicotiana tabacum* L. "V 20" did not show symptoms, but contained the virus as evidenced by positive subinoculation tests.

Aphid transmission:

Solanaceae

Nicotiana tabacum L. var. *Havana* 38: −/× −
Nicotiana tabacum L. var. *Havana* 425: −/× −
Nicotiana tabacum L. var. *Samsun:* −/× −
Nicotiana tabacum L. var. *White Burley:* −/× −
Physalis floridana Rydb.: −/× −

Experiments to transmit the virus by vectors and to recover it were unsuccessful.

Determination of the virulence of strain EP

It is a known fact that the characteristics of PVY strains can be distinguished on *Nicotiana glutinosa* L. plants simultaneously infected with PVX and PVY strains (HUTTON, 1948, LADEBURG et al., 1950; DARBY et al., 1951; HUTTON and PEAK, 1952). Among the test plants reacting with local lesions *Lycium halimifolium* Mill. and *Physalis floridana* Rydb. proved to be suitable host plants for the differentiation of PVY strains (ROSS, 1948, 1953; DARBY et al., 1951; HUTTON and PEAK, 1952).

Fig. 2. Local lesions on *Solanum tuberosum*. (Seedling No. 59/558) induced by potato virus Y (strain C) 26 days after inoculation (11/40—EP)

As a consequence of interaction between strain EP and PVX^{RS} spot necroses on tobacco became considerably more severe. The interaction between strains EP and PVX^{N} was quite weak on tobacco whereas on potato it was strong. It is worth mentioning that some of the strains studied later (HORVÁTH, 1966b) did not exhibit any additive effect if inoculated together with PVX^{N}, the symptoms even became less severe as compared to the control. Among the host plants reacting with local lesions *Lycium halimifolium* Mill. was not suitable for quantitative studies, since if inoculated with the strains under investigation, including strain EP, it reacted with leaf drop as soon as the lesions started to appear. As far as the severity of the symptoms is concerned *Physalis floridana* Rydb. also reacted in a quite irreproducible manner to the individual strains.

Summing up the results of our observations strain EP proved to be slightly virulent as evidenced by biological tests. The serological reactions of strain EP were relatively weak compared to other PVY strains.

Determination of the physical properties

As shown by heat inactivation experiments (Table 1) carried out in two replications, strain EP becomes inactivated at 58 to 60°C. Dilution end point was between 2×10^{-4} and 10^{-4} in both experiments (Table 2). The in vitro stability was 15 days in the first, 18 days in the second, 18 days in the third and again 15 days in the fourth experiment (Table 3). Thus, stability in vitro varies between 15 to 18 days. In dried tobacco leaf tissues strain EP was active even after 365 days though its virulence decreased considerably. In 426 days, however, its activity was completely lost as evidenced by negative transmission experiments (Table 4).

Table 1

Thermal Inactivation of strain EP[a)]

Temperature (°C for 10 min.)	Expt. 1 August 11, 1964	Expt. 2 August 18, 1964
	b)	b)
Unheated	5/5	5/5
54	5/5	5/5
56	5/5	4/5
58	3/5	1/5
60	0/5	0/5
62	0/5	0/5
64	0/5	0/5
66	0/5	0/5

a) Diluted 1 : 1 with distilled water
b) Infected/tested

Table 2

Dilution end point of strain EP

Dilution	Expt. 1 June 15, 1964	Expt. 2 July 13, 1964
	a)	a)
Undiluted	2/2	2/2
10^{-1}	2/2	2/2
10^{-2}	4/4	3/4
2×10^{-3}	4/4	3/4
10^{-3}	6/6	3/6
2×10^{-4}	4/8	4/8
10^{-4}	0/10	0/10
2×10^{-5}	0/12	0/12

a) Infected/tested

Table 3

Ageing in vitro of strain EP [a]

Days	Expt. 1 June 9, 1964	Expt. 2 June 9, 1964	Expt. 3 July 9, 1964	Expt. 4 July 9, 1964
	b)	b)	b)	b)
0	5/5	5/5	5/5	5/5
3	5/5	5/5	5/5	5/5
6	5/5	5/5	5/5	5/5
9	4/5	3/5	4/5	3/5
12	1/5	2/5	2/5	1/5
15	0/5	1/5	2/5	0/5
18	0/5	0/5	0/5	0/5
21	0/5	0/5	0/5	0/5
24	—	0/5	0/5	0/5

a) Undiluted extracts of virus infected plants
b) Infected/tested

Table 4

Storage over Calcium chloride of tobacco leaves infected with strain EP

Storage at 0° C for days	Expt. 1 September 28, 1964	Expt. 2 November 11, 1964	Expt. 3 July 25, 1965	Expt. 4 July 25, 1965
	a)	a)	a)	a)
120	5/5	5/5	—	—
192	—	3/5	4/5	—
365	—	—	2/5	3/5
426	—	—	—	—

a) Infected/tested

Serological relationship

During serological tests (Table 5) strain EP reacted with antisera prepared against PVY alike the normal strain (PVY^N), the "Rippenbräune" strain (PVY^R) and the so-called "anomalous" strain (PVY^{An}), i.e. the reaction was weak. This is in agreement with the low intesity of the reactions obtained in our biological tests. No positive reactions were obtained with antisera prepared against other virus species.

Cross protection between PVY strains on tobacco

One of the important problems of present day plant virology is to investigate the biological factors which affect the host-virus relationship in vivo i.e. to study the interaction among different virus species and among different strains of the

Table 5

Serological relationship of strain EP

Antiserum	Serological extracts of tobacco August 4—5, 1964	Reaction extracts of potato (Edgecote Purple) August 4—5, 1964
PVY	+	+
PVX	—	—
PVS	—	—
PVM	—	—
PAMV	—	—

same virus. In many cases experiments along these lines did not yield conclusive results yet (BAWDEN, 1950; BENNETT, 1953; KLINKOWSKI and SCHMELZER, 1957; KÖHLER, 1954, 1961, 1964 a, b, c; PRICE, 1964; ROCHOW and ROSS, 1955, ROSS 1950; 1959; SCHMELZER et al., 1960; SILBERSCHMIDT, 1959; SILBERSCHMIDT and RIMPAU, 1962; WATSON, 1960; WITTMANN, 1961).

Premunity tests with different PVY strains have shown that strain EP premunized against some PVY^N strains (HORVÁTH, 1966 b) whereas no such effect was obtained against strain PVY^R and PVY^{An}, respectively.

Conclusions

In this paper, the first one from a series of publications on strains of PVY, we have been dealing with strain PVY^C (isolate EP). The susceptibility of some mechanically infected plants has been established. It has been found that 4 *(Gomphrena globosa* L., *Capsicum annuum* L. var. *Markgärtner*, *Datura stramonium* L. var. tatula and *Lycopersicon pimpinellifolium* (Jusl.) Mill.) out of the 20 species studied were not susceptible. From these 4 plant species the virus could not be recovered either. 12 plant species showed systemic symptoms *(Capsicum annuum* L., *Datura metel* L., *Lycopersicon esculentum* Mill., *Nicotiana glutinosa* L., *N. repanda* Willd., *N. tabacum* L. var. *Havana* 38, *N. tabacum* L. var. *Havana* 425., *N. tabacum* L. var. *Samsun*, *N. tabacum* L. var. *Sanderae*, *N. tabacum* L. var. *White Burley*, *Petunia hybrida* Vilm. and *Solanum nigrum* L.) and 1 species *(Lycium halimifolium* Mill.*)* reacted with local lesions. Both systemic and local symptoms were exhibited by *Physalis floridana* Rydb. and a seedling (No. 59/558.) *of Solanum tuberosum*. *N. tabacum* "V 20" proved to be a symptomless carrier of strain EP. Vector transmissions both to *Nicotiana* species and *Physalis floridana* Rydb. were unsuccessful. The termal inactivation point was found to be between 58 and 60°C, the dilution end point between 2×10^{-4} and 10^{-4}. The virus remained active in expressed sap for 15 to 18 days, whereas in dried tobacco leaf tissue it had lost its activity between the 365th and the 426th day. Isolate EP gave positive serological reactions with antisera prepared against PVY but did not react with antisera

prepared against other virus species. Isolate EP had a premunizing effect against some PVY^N strains, but no premunity was observed with strains PVY^R and PVY^{An}.

Experiments to establish the virulence of isolate EP have shown that interaction with strain PVX^{RS} led to an intensification of spot necroses as compared to the control. This additive effect could be observed, however, with other isolates as well and was not in agreement with the symptoms caused by the individual strains on the host plants involved. DARBY et al. (1951) have given detailed descriptions about the use of spot necroses for quantitative evaluations. In our own experiments no quantitative evaluations could be carried out because the symptoms were indefinite and inconsistent. This can be explained by the effect of fluctuations in the temperature. DARBY et al. (1951) have namely pointed out that in order to get reliable results with the spot necroses the plants had to be kept at constant temperatures. At the optimum temperature of 24°C great differences in the appearance of the spot necroses were obtained with the individual isolates. At 16°C the symptoms were too weak or even absent, whereas at 28°C the spot necroses were so severe as to fail to reveal any difference among the isolates. Although the interaction between the isolate EP and PVX^N proved to be more useful to determine the virulence of our isolate, than the synergism between EP and PVX^{RS}, the reactions obtained were not always in agreement with the symptoms induced on other host plants. On the other hand, in case of a synergism with some other isolates, instead of an additive effect reduction in the severity of the symptoms was observed.

Among the host plants showing local symptoms *Lycium halimifolium* Mill. could not be used for quantitative assays, since it reacted with rapid leaf drop even with isolate EP, which proved to be little virulent in comparison with other strains. ROSS (1948), DARBY et al. (1951), HUTTON and PEAK (1952) and SCHMELZER and KLINKOWSKI (1959) have pointed out that *Physalis floridana* Rydb. is very suitable for the demonstration of differences between individual PVY strains. According to the data of SCHMELZER and KLINKOWSKI (1959) the strains PVY^R and SILBERSCHMIDT's necrotic fleck strain (YS) do not produce local lesions on *Physalis floridina* Rydb. Our own later experiments (HORVÁTH and SOLYMOSY, 1962; HORVÁTH 1964) have shown that PVY^R strains are also able to induce local symptoms on *Physalis floridana* Rydb. It must be stressed, however, that there must have been some difference between the two PVY^R strains studied by SCHMELZER and KLINKOWSKI (1959) and that investigated by us, as evidenced by differences in symptom production on *Petunia hybrida* Vilm. ROSS (1953) has shown that with *Physalis floridana* Rydb. temperature does not play an important role. Our own observations as well as the experiments of NIENHAUS (1957, 1961), however, have pointed out that temperature does have a definite influence on symptom production in *Physalis floridana* Rydb. In our hands *Physalis floridana* Rydb. was not suitable for the differentiation of either individual strains or isolates of the same strain. It would be worthwhile, however, to investigate the reaction of *Physalis floridana* Rydb. to PVY strains under constant environmental conditions.

By comparing the behaviour of isolate EP with that of 22 isolates belonging

to 4 PVY strains we have demonstrated by biological tests and serological reactions that isolate EP of strain PVY^C is only moderately virulent.

*

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Effect of Photoperiod and Temperature on the Diapause of Athalia Glabricollis Thomson (Tenthred. Hym.)

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The present paper reports on experimental results obtained by examining the joint effect of temperature (of 18, 23 and 28°C) and photoperiods of different length. According to the data the diapause is induced by the photoperiod at 18 and 23°C, and by the temperature at 28°C. The critical light period for the diapause lies between 14 and 15 hours. The prepupa in the cocoon is the diapausing developmental form.

Introduction

Until the middle of the fiftieth out of the species of the *Athalia* genus only *A. rosea* L. (= colibri Christ.) was kept in evidence as a harmful insect. Detailed ecological studies (SÁRINGER, 1957) on this species, however, revealed in a little while, that considerable damages in mustard and rape stands are done, besides the larval population of *A. rosae*, also by the larvae of *A. glabricollis*. With the new pest – which was called, on the basis of examinations on host plant selection "mustard saw-fly" – detailed ecological investigations were conducted (SÁRINGER, 1958). It turned out that *A. glabricollis* is, like its associate species *A. rosae*, a multivoltin saw-fly easy to cultivate in the laboratory. The study of its diapause conditions seemed, therefore, advisable.

Of the ecological factors inducing the diapause the joint effect of different temperatures (18, 23 and 28°C) and of different lengths of the photoperiod are dealt with in this paper. (The latter are expressed by the fractions 4/20, 12/12, 13/11, 14/10, 15/9 and 16/8, where the numerator indicates the duration of light and the denominator that of darkness in hours). The establishment of the developmental stage sensitive to light from the point of view of the diapause will be discussed in this paper as well.

On the role of the photoperiod and temperature in influencing the diapause of different insect species, detailed and systematized reports may be found in the reviews of BONNEMAISON (1945), ANDREWARTHA (1952), LEES (1955), MÜLLER (1960), FUZEAU-BRAESCH (1961), DANILEVSKIJ (1961), BECK–CLOUTIER–MCLEOD (1962), DE WILDE (1962), JOURDHEUIL–MISSONNIER (1964) and SÁRINGER (1964).

Material and Methods

The imagines used in the experiments were caught with sweeping nets in stands of *Sinapis alba* L. near Keszthely in mid-May 1962. The females of the collected imagines laid fertile eggs into the leaf blades of young, 5 to 10 cm high, potted seedlings of *Sinapis alba* precultivated in the glass-house laboratory. All cultures were kept during the period of embryonic development at the temperature prevailing in the laboratory. The young larvae hatched from the eggs were put – just on the same day – with a fine brush onto the leaves of *S. alba* raised in water

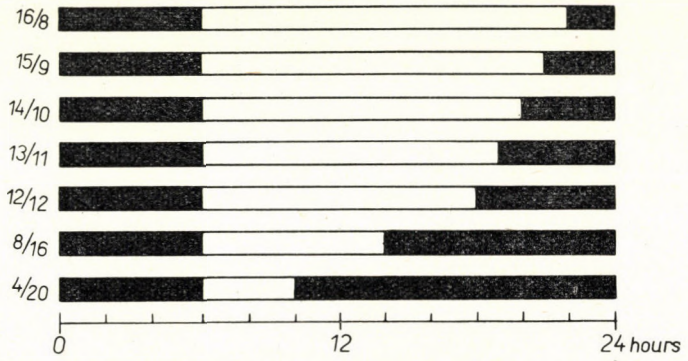

Fig. 1. The proportion of daily light and dark periodes in hours. □ light; ■ darkness

hygrostats. Each hygrostat contained 5 larvae. In every experimental series 6 × 5 larvae were used and all experiments were repeated twice, so that the data represent records on the behaviour of 60 individuals.

The larvae of stage L_1 were taken from the hygrostats and placed in thermostat chambers of 18 (±1)° C, 23 (±0.8)°C and 28 (±0.5) °C, respectively where 3 parallel white Tungsram strip-lamps of 40 W (with a total candle-power of about 3000 lux) served as light surces; the lamps were fixed in the middle of the chambers 1 m below the ceiling. The light periods of different lengths were regulated by putting the hygrostats into trays half-filled with dry sand and covered with light-tight clay-pots at the desired moment; the pots were removed every morning.

At the beginning the food of the larvae was changed daily and later, when the intensive feeding of the larvae had started (from stage on L_3), twice or three times every day, as needed. Larvae which finished their feeding were transferred to flowerpots filled with sieved earth and covered with cellophane to provide light for the larvae until their burrowing into the soil. Subsequently the pots were left on their original place.

The diapause percentages shown in Fig. 2 were calculated on the 60th day after the burrowing of the larvae into the soil. Examining the ontogeny it turned out namely that even the larvae bred at the lowest (18°C) temperature finished their development in 20 to 25 days and came to the surface as imagines if in the

larval stage long-day treatment (light for 15 hours and darkness for 9 hours) was applied. Therefore fixing the number of diapausing individuals on the 60th day after the last larva had burrowed into the soil seemed to be justified.

The proportions of the daily light and dark periods are presented in Fig. 1.

The light-sensitive developmental stage was established so that larvae hatched from eggs laid by a female within 24 hours at 23° C were bred partly under 13-hour and partly under 16-hour illumination, and after the third moult the cultures kept for 16 hours in light received a 13-hour while those of 13-hour photoperiod a 16-hour light treatment.

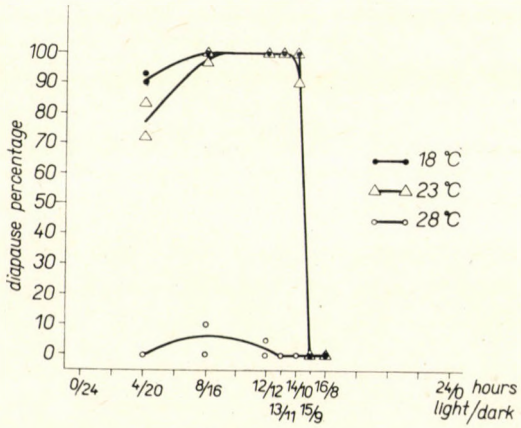

Fig. 2. Correlation between the photoperiod and the percentage of diapausing individuals at three temperatures

Results and Discussion

At 18°C

From the run of the curve showing the diapause percentages of larvae bred under photoperiods of different lengths (Fig. 2) it may be seen that the number of prepupae which remained in *diapause* was diminished by a short (4-hour) photoperiod. When a daily 8-hour illumination was applied 100 per cent of the population remained in diapause. A daily photoperiod of 14/10 hours resulted still in 100 per cent diapause, while with photoperiods of 15/9 and 16/8 hours the diapause per cent was zero. Accordingly at 18°C the critical photoperiod for the diapause lies between 14 and 15 hours, i. e. it depends on one hour in the photoperiod length whether the prepupa in the cocoon will continue its development with or without diapausing.

At 23°C

The trend of the curve demonstrating the diapause of larvae bred at this temperature (Fig. 2) corresponds almost entirely to that illustrating the diapause

at 18°C. Differences of some per cent may be observed with the photoperiods 4/20, 8/16 and 14/10. At 23°C already an effect of the higher temperature prevails, diminishing slightly the diapause induced by the photoperiod. The critical photoperiod for the diapause falls between 14 and 15 hours, so even at this temperature a difference of one hour in the length of the photoperiod is decisive, whether a diapause comes into being in the prepupa stage or the development continues to proceed without interruption.

At 28°C

The curve showing the diapause at this temperature (Fig. 2) differs considerably from those obtained at 18 and 23°C. Applying the same photoperiods

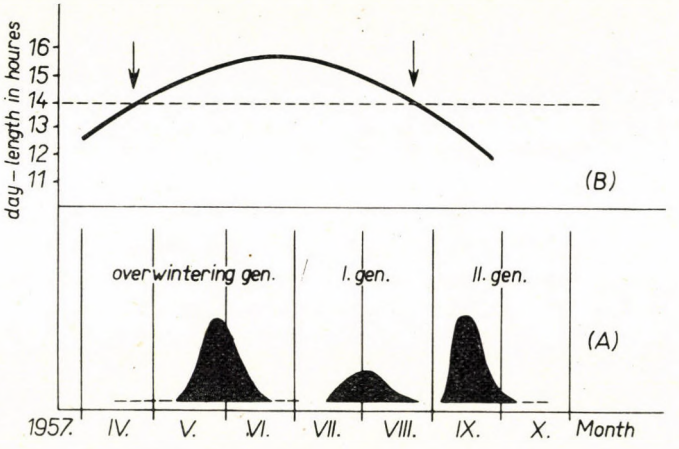

Fig. 3. Flight of the imagines of different generations of *Athalia glabricollis* Thomson in the region of Keszthely in 1957 (after SÁRINGER, 1958). A = relative frequency of the populations of flying imagines. B = day-lengths in hours in the region of Keszthely

only a very low percentage of prepupae remained in diapause. Out of all experimental conditions only the temperature was higher than in the previous treatments, therefore the data obtained reveal that the diapause is governed by the photoperiod only up to a certain temperature limit. At 28°C the temperature has already the decisive role. Accordingly, it can be stated — as it was also proved with *Athalia rosae* (SÁRINGER, 1964) — that beyond a certain temperature limit in *A. glabricollis* the diapause-inducing role of the photoperiod falls behind the effect of temperature.

The above results made it evident that within a certain temperature range the diapause of *A. glabricollis* in the prepupal stage is regulated by the photoperiod prevailing during the larval development. The investigations have also proved that the critical daily photoperiod for the diapause lies between 14 and 15 hours.

On the basis of the results presented above the yearly course of development of this species under field conditions will now be analysed and an attempt will be made to give a satisfactory explanation as to the number of generations per year.

The number of generations per year of *A. glabricollis* has been discussed in a previous paper (SÁRINGER, 1958). It has been shown that near Keszthely this insect flew from the beginning of May to the end of June, mostly in the second half of May. The first summer generation flew in July and August (by far the greater part in the first half of August), whereas the second summer generation from the beginning of September to mid-October.

Accordingly to laboratory investigations a diurnal photoperiod exceeding 14 hours did not induce diapause. From Fig. 3 it can be seen that daily photoperiods longer than 14 hours occur in the region of Keszthely from April 22 to August 21. The maximum photoperiod of nearly 16 hours, is that of June 21. Fig. 3 also reveals that the developmental period on the larval offspring of the overwintered generation falls on June, i. e. on the month of the longest days, and, therefore, the development proceeds without diapause. The larval progeny of the first summer generation live on the plants from the second half of July to the second half of August. These larvae can also complete their development if the light period exceeds 14 hours. But the larval offspring of the second summer generation develop from the beginning of September to mid-October only on days of a photoperiod below the critical 14 hours, and remain, therefore, in diapause.

From the average temperatures prevailing in the region of Keszthely during the development of the generations the conclusion can be drawn that the larvae of the overwintered generation develop at 16 to 20°C, those of the first summer generation at 20 to 22°C and those of the second summer generation at 12 to 17°C (averages of 50 years, BACSÓ, 1959). In some years during the development of the first summer generation besides the photoperiod a mean temperature higher than the average of many years may occur, which is able to bring the diapause to an end independently of the photoperiod. But for the overwintered and for the second summer generation only the photoperiod must be regarded as a diapause-forming factor, because in these periods temperature, due to its low level, cannot be supposed to affect the diapause.

Experimental results have shown that under the influence of a long photoperiod (exceeding 15 hours) no diapause develops in *A. glabricollis*. Therefore it should be considered as a long-day insect which has a facultative diapause according to the nomenclature of ANDREWARTHA (1952), and the prepupa in the cocoon is the diapausing developmental form.

Experiments to establish the light-sensitive developmental stage revealed that larvae from the last (L_4) stage on do perceive the light conditions decisive for the diapause.

Comparing the results of experiments conducted on *A. glabricollis* and the data obtained by investigations on *A. rosae* (SÁRINGER, 1964) under similar experimental conditions, it becomes evident, that both species are sensitive to the effect of photoperiod and temperature. From this fact it may be concluded that populations of different species living in the same biotope and belonging to the same genus of *Tenthredinidae* show ecologically similar behaviour.

Conclusion

Experiments on the correlation between the diapause of *A. glabricollis* on the one hand, and photoperiod and temperature on the other, indicated that the diapause is governed by phothoperiod at 18 and 23°C and by temperature at 28°C.

The diurnal light period critical for the diapause lies between 14 and 15 hours.

Light conditions decisive for the diapause are perceived by the larvae from their fourth developmental stage on; earlier no sensitivity to light is displayed.

Because under the influence of long-day light period (exceeding 15 hours) no diapause develops, *A. glabricollis* is to be considered as a long-day insect having facultative diapause. The prepupa in the cocoon is the diapausing developmental form.

Comparing the above results with those obtained by investigations on *A. rosae* it can be established that as to the diapause populations of different species living in the same biotope and belonging to the same genus of *Tenthredinidae* behave similarly toward external factors.

The author is very indebted to Mrs. Flóra T.-Péczely, laboratory technician, for her valuable help in tending the ample experimental series.

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Phytopathogenic and Saprophytic Fungi from Hungary, I.

Contributions to the Peronosporales, Erysiphales and Deuteromycetes Flora of Hungary

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Peronosporales and Erysiphales

During the course of the collection of microscopic fungi in Hungary between 1946 and 1965, 38 species of Peronosporales were found and identified. 12 of them are new records from Hungary, while 2 additional species are new. They are described here as *Bremia xanthii* n. sp. and *Peronospora lobulariae* n. sp. Two of the 64 Erysiphales species listed here were not known earlier from Hungary. They are *Phyllactinia syringae* and *Trichocladia robiniae*. The collection presented herewith contains 93 new data concerning host plants of different mildews. The nomenclature and systematic arrangement of fungi applied in this paper is identical with the conception of G. Moesz (in Fungi Hungariae III. Ascomycetes, Pars 1. – Ann. Mus. Nat. Hung., 1939, 32: 1–61. and Die Pilze von Budapest und seiner Umgebung. Bp. Term. Tud. Társ., 1942. pp. 1–320.). The collection is deposited in the herbarium of the Research Institute for Plant Protection, Budapest. New records are signed with *.

1. *Phycomycetes*

Chytridiales

Cladochytriaceae

Physoderma maculare WALLR. (= *Cladochytrium alismatis* BÜRGEN in COHN, Beitr. Biol. II. 280.) – On the leaves of **Alisma lanceolata*, Szarvas-Bikazug, 8. 23. 1948. (Sporangia: 26–35 × 17–30 micron, det. by J. Podhradszky). (Moesz, G., Ann. Mus. Nat. Hung., 1937–1938, 31: 75.; This fungus was mentioned in Hungary from *Alisma plantago aquatica* by L. Hollós).

Peronosporales

a. *Peronosporaceae*

Bremia lactucae REGEL. – On *Lactuca sativa*, Szarvas 6. 6. 1949; Debrecen 6. 16. 1965 (leg. O. Tóth), on **Lactuca serriola*, Debrecen, 6. 16. 1965 (leg. O. Tóth).

Bremia sonchi K. SAWADA. – On the leaves of *Sonchus oleraceus*, Nyíregyháza 7. 3. 1947.

Bremia lapsanae Gm. – On *Lapsana communis*, Szarvas, 7. 23. 1948.

Bremia xanthii n. sp. (Figures 1 and 2)

Conidiophoris e stomatibus exeuntibus, 350–550 micr. altis, trunco 9–11 micr. crasso, basi oblongati. Ramis 2–6ies dichotome ramosis, a terminibus

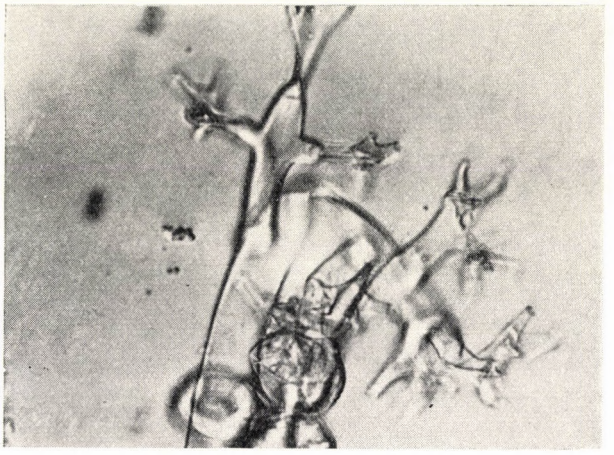

Fig. 1. Conidiophore of *Bremia xanthii* n. sp. (1000×)

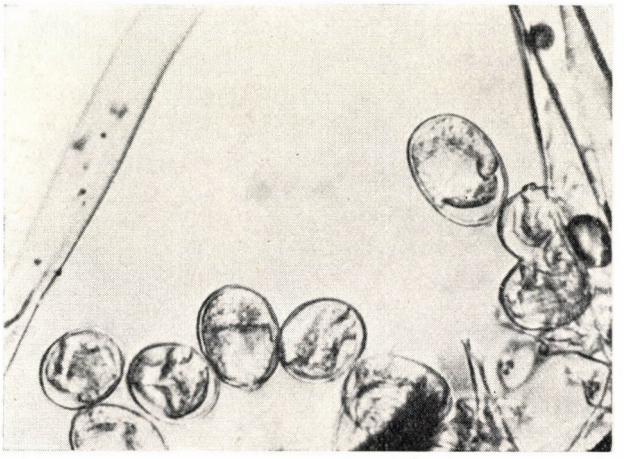

Fig. 2. Conidia of *Bremia xanthii* n. sp. (1000×)

latis, cum 3–5 sterigmatis, 4.5–9 micr. longis. Conidiis breviter ellipsoideis 16–29.5–(33.6) micr. longis, 13–16.8–(25.2) micr. latis. – Habitat in foliis *Xanthii strumarii* L. in Hungaria.

The collective species *Bremia lactucae* was divided first by K. SAWADA. Later J. SCHWEIZER (1919, 1920) has demonstrated the heterogeneity of *Bremia*

lactucae by means of artificial infections. *Bremia* strains from certain host plants could not infect others. *Bremia centaureae* and *Bremia sonchi* were segregated by SYDOW in 1923 (Ann. Mycol. 21: 169) on the very same basis. During the past decades ITO and TOKUNAGA (1935), MILOTZOVA (1937), JAGGER and CHANDLER (1933), SCHULTZE and RÖDER (1938), as well as LEE LING and TAI (1945) were dealing with the collective species *Bremia lactucae*. Finally the fungus which infects *Lapsana communis* was segregated as *Bremia lapsanae* Gm. The *Bremia* species found in Hungary on *Xanthium strumarium* L. clearly differs from the *Bremia* spp.

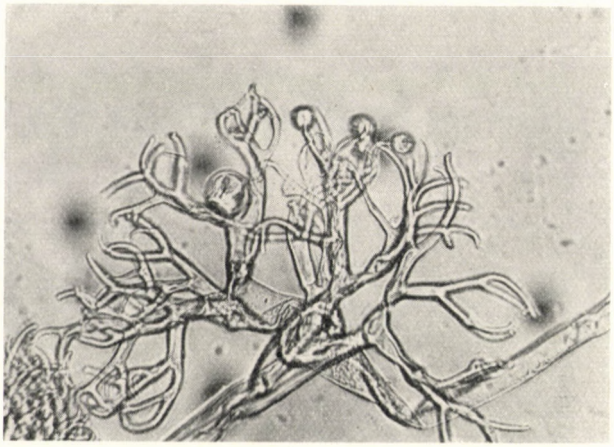

Fig. 3. Conidiophore of *Peronospora lobulariae* n. sp. (600×)

living on other *Compositae* host plants on the basis of the size of the conidia. This was the main reason to segregate the new species. The material was collected by O. Tóth at Gyulatanya (Nyírség), 6. 8. 1965.

Bremia sp. div. sine nominis – On *Arctium lappa*, Gyulatanya (Nyírség) 6. 8. 1965 (leg.: O. Tóth). Conidia 16.8–25.2 × 15–16.8 micron. On *Arctium tomentosum*, Bak. 6. 23. 1950 (leg.: B. Husz).

Peronospora lobulariae n. sp. (Figure 3.)

Conidiophoris singulis vel plurimis e stromatibus exeuntibus, 200–250 micr. altis, 13–18 micr. crassis, basi leviter oblongati. Ramis 3–6ies dichotome ramosis, fere leviter curvatis, furcis terminalibus 9–23 × 1.8–2.2 micr. magnis. Conidiis breviter ellipsoideis vel ellipsoideis rotundatis, 20–25 micr. longis, 16–22 micr. latis. Longitudine media 22.5 micr., latitudine media 18.8 micr. Existat conidiis globosis 19–21 micr. diametris. Oosporis ignotis. – Habitat in foliis *Lobulariae maritimae* (L.) Desv. in Hungaria.

A number of small *Peronospora* species from *Cruciferae* host plants have been segregated since the monographic work of Gäumann (1923). The *Peronospora* species from *Lobularia maritima*, described here, however, differs from these on

the basis of the size of the conidia. This new species appeared many times in large number all over the country during the last years. The type material was collected by O. Tóth in Debrecen, 1964.

Peronospora destructor (BERK.) CASP. This pathogen is common all over the country on cultivated *Allium* spp. *(Allium cepa, Allium fistulosum*, etc.). It was epidemic during the last years, especially until the rainy vegetation period of 1965.

**Peronospora polygoni* (HALS.) FISCHER. — On *Polygonum convolvulus*, Gyulatanya (leg.: O. Tóth), 6. 14. 1964.

Peronospora chenopodii SCHLECHT. — On *Chenopodium hybridum*, Debrecen (leg.: O. Tóth) 6. 4. 1964.

Peronospora schachtii FUCK. — Common on cultivated *Beta vulgaris*. During the last years it was sometimes epidemic almost all over the country. This fungus was not registered by G. Moesz from the present territory of Hungary. — Szarvas, 7. 3. 1949.; Előszállás, 6. 4. 1959.

**Peronospora kochiae* GÄUM. — On *Kochia scoparia*, Debrecen, (leg.: O. Tóth), 6. 24. 1965.

Peronospora variabilis GÄUM. — On *Chenopodium album*, very common all over the country. — Szarvas, 5. 10. 1947.; Badacsony, 6. 18. 1953.; Csepelsziget, 7. 3. 1953, etc.

Peronospora hiemalis GÄUM. — On *Ranunculus acer*, Sátorhegység (Upponyi forrás) (leg.: O. Tóth), 5. 16. 1965.

Peronospora ranunculi GÄUM. — On *Ranunculus polyanthemos* and on *Ranunculus repens*, Sátorhegység (Upponyi forrás) (leg.: O. Tóth), 5. 16. 1965.

Peronospora arborescens (BERK.) DEBARY. — Under wet climatic conditions it is common on cultivated poppies. — Seregélyes (leg.: J. Podhradszky), 6. 15. 1949.

Peronospora brassicae GÄUM. — On **Brassica oleracea*, Debrecen (leg.: O. Tóth), 5. 15. 1964.; Fertőd, 11. 5. 1953.; Keszthely, 7. 27. 1962. and on *Sinapis alba*, Debrecen (leg.: O. Tóth), 7. 9. 1965.

**Peronospora diplotaxidis* GÄUM. — On *Diplotaxis muralis*, Esztergom, 7. 28. 1950.

Peronospora lepidii-sativi GÄUM. — Fairly frequent on *Lepidium draba*, Martonvásár, 6. 8. 1953.; Budakalász, 6. 8. 1955.; Nagykovácsi 6. 21. 1963, etc. and on *Lepidium ruderale*, Gyulatanya (leg.: O. Tóth), 6. 8. 1965.

Peronospora niessleana BERL. — Common on *Alliaria officinalis*, Szarvas, 6. 23. 1949.; Debrecen (leg.: O. Tóth), 6. 19. 1965., etc.

Peronospora sisymbrii-orientalis GÄUM. — On *Sisymbrium orientale*, Nadab, 4. 8. 1959.

Peronospora thlaspeos-arvensis GÄUM. — On *Thlaspi arvense*, Kismacs (leg.: O. Tóth), 4. 19. 1964.

**Peronospora astragalina* SYDOW. — On *Astragalus cicer*, Nyírtelek (leg.: O. Tóth), 7. 8. 1965.

Peronospora pisi SYDOW. — On *Pisum sativum*, during wet periods, Martonvásár, 5. 23. 1950.

**Peronospora trifolii-hybridi* GÄUM. — On *Trifolium hybridum*, Mezőtúr, 6. 1. 1948. The size of the conidia: 20—25 × 19—23 micron.

Peronospora trifolii-repentis SYDOW. — *On *Trifolium repens*, Halásztelek (Békés), 4. 16. 1948.

Peronospora conglomerata FUCKEL. — On *Geranium pusillum*, Debrecen, Sátorhegység (Upponyi pincék) (leg.: O. Tóth), 5. 16. 1965.; and on **Geranium phaeum*, Debrecen, 6. 3. 1948.

Peronospora hyoscyami DEBARY. — On *Hyoscyamus niger*, Nyírtelek (leg.: O. Tóth), 5. 23. 1964.

Peronospora alta FUCK. — On *Plantago major*, Debrecen (leg.: O. Tóth), 6. 24.1965.

**Peronospora borealis* GÄUM. — On *Galium boreale*, Halásztelek (Békés), 4. 17.1948.

Plasmopara aegopodii (CASP.) TROTT; — On *Aegopodium podagraria*, Kőkapu (Sátorhegység), 6. 3. 1948.; l. c. (leg.: O. Tóth), 7. 4. 1965.

**Plasmopara geranii-pratensis* TR. et O. SAVUL. — On *Geranium pratense*, Nagykovácsi, 6. 18. 1950.

Plasmopara nivea (UNGER) SCHROETER. — On **Bupleurum falcatum*, Lillafüred (leg.: O. Tóth), 5. 3. 1964.

Pseudoperonospora cubensis (B. et C.) ROSTOW. — Fairly common on muskmelon and on watermelon, during wet periods.

Pseudoperonospora humuli (MIY. et TAK.) WILS. — Common on wild and cultivated *Humulus lupulus* in humid years.

b. *Albuginaceae*

Albugo bliti (BIV.) O. KUNTZE. — On *Amaranthus retroflexus*, frequent, Nyíregyháza, 7. 5. 1947.; Szarvas, 8. 23. 1947.; Szarvas, Pepikert, 6. 29. 1948., etc.

Albugo candida (PERS.) O. KUNTZE. — On *Camelina microcarpa*, Nagykovácsi, 6. 9. 1953. On *Coronopus procumbens*, Szarvas, 6. 8. 1947,; Tiszasüly, 6. 16. 1966. On *Erysimum repandum*, Budapest, Vérmező, 6. 6. 1949. On *Sisymbrium strictissimum*, Visegrád, 6. 22. 1950. On *Sisymbrium altissimum* (= *S. sinapistrum*), Visegrád, 6. 22. 1950.

Albugo candida (PERS.) O. KUNTZE var. *globulosus* f. *microspora* O. SAVUL. On *Arabis turrita*, Nagyszénás (Budapest), 6. 11. 1957.

Albugo portulacae (DC) O. KUNTZE. — Frequently on *Portulaca oleracea*, Szarvas, 8. 18. 1948.; Tápiószele, 7. 17. 1953., etc.

Albugo tragopogonis (PERS.) S. F. GRAY. — On *Scorzonera laciniata*, Vizesfás (Békés), 7. 17. 1950.

Albugo tragopogonis (PERS.) S. F. GRAY f. *tragopogi* O. SAVUL. — On *Tragopogon dubius*, Szarvas 7. 28. 1948.

2. *Ascomycetes. Erysiphales*

a. *Erysiphaceae*

Erysiphe aquilegiae DC. — On **Clematis integrifolia*. Perithecia: 74.5—102.3 × 74—83.7 micron; perithecium appendages: 102—204.6 micron; asci: 50.2 × 28—32.5 micron; ascospores: 18.6 × 11 micron; conidia: 37.2 × 17.5—18.6 micron. Halásztelek (Békés), 8. 18. 1947; Szarvas, 8. 23. 1948.

Erysiphe artemisiae (WALLR.) GREV. – On **Artemisia absinthium*, Börzsöny, Királyrét (with many perithecia), 11. 15. 1951. On *Artemisia vulgaris*, common, Szarvas, 9. 23. 1947.; Budapest, Zugliget, Jánoshegy (perithecia in large numbers), 9. 27. 1949. etc.

Erysiphe cichoracearum DC. emend. SALMON. – On **Achillea collina*, Szarvas (with perithecia), 10. 27. 1948.; Pomáz (with perithecia), 10. 19. 1954. On **Achillea millefolium*, Szarvas, Köröspart (with perithecia), 10. 3. 1948. On **Centaurea micranthos*, Budapest, Rózsadomb, 10. 18. 1952. On *Centaurea pannonica*, Szarvas, Pepikert, 11. 16. 1946. On *Chrysanthemum vulgare*, Szarvas, 9. 24. 1948.; Budapest, Jánoshegy (with perithecia, associated with the teleuto form of *Puccinia tanaceti* FUCK.), 9. 23. 1950.; Pomáz (with perithecia), 11. 18. 1950.; Frequent on *Cichorium intybus*, Szarvas, 9. 3. 1947.; Galambos (Békés), 9. 3. 1947.; Szarvas, Pepikert, 9. 15. 1948., etc. On **Echinops multiflorus*, Halásztelek (Békés), 9. 3. 1947. On **Hieracium murorum*, Nagykovácsi (Nagyszénás), 8. 5. 1953. On **Hieracium sabaudum* (sometimes with perithecia), Budapest, Kecskehegy, Vadaskert, etc. 11. 7. 1965. On **Inula britannica*, Szarvas, Mezőtúr, 9. 8. 1947., Hortobágy, 9. 3. 1948. On **Inula oculus christi*, Pomáz, 9. 6. 1953. On *Inula salicina*, Budapest, Jánoshegy, 9. 27. 1949. On **Aster novi-angliae* and on *Aster novi-belgii*, common. Szarvas, Pepikert (perithecia: 67.5–110 × 47.5–70 micron, perithecium appendages 120–240 micron, asci with 1–2 ascospores: 35 × 15–17.5 micron; ascospores: 20.5 × 10.5 micron), 9. 24. 1947.; Balatonakarattya (perithecia: 84–111 × 83–102 micron; perithecium appendages: 65–102–130 micron; asci: 57 × 37.2 micron; ascospores: 18–22 × 11–12 micron; conidia: 27.5–36 × 9.4–13.5 micron), 9. 12. 1965. On **Lactuca serriola* and *Lactuca dichotoma*, Szarvas, 8. 2. 1947. On **Eupatorium cannabinum*, Ócsa (Pest), 8. 31. 1950. On *Lapsana communis*, Szarvas, 8. 28. 1947. On *Prenanthes purpurea* (with perithecia, common), Sopron, Károlymagaslat, 9. 27. 1965. On **Scorzonera hispanica*, Szarvas, Pepikert (perithecia: 130–158 × 121–130 micron, perithecium appendages: 65–121 micron, asci: 74.4 × 38.2 micron, ascospores: 28 × 19.5 micron, conidia: 45.2 × 14.8 micron), 8. 5. 1948. On **Scorzonera cana* (in company with *Albugo tragopogonis* (PERS.) S. F. GRAY), Vizesfás (Békés), 4. 11. 1950. On **Senecio vulgaris*, Szarvas, 11. 3. 1947.; Gyöngyös, 9. 6. 1965. On **Senecio jakobaea* and *Senecio barbareifolius*, Halásztelek (Békés), 9. 3. 1947. On **Solidago serotina*, Budapest, Margitsziget, 9. 23. 1950. On *Sonchus oleraceus*, *Sonchus arvensis* and *Sonchus asper*, Szarvas, 11. 15. 1949, 8. 27. 1948. On *Taraxacum officinale*, Szarvas, Pepikert, 11. 15. 1949.

**Erysiphe cichoracearum* DC. emend. SALMON f. *nicotianae* JACZ. – On *Nicotiana tabacum*, Budapest, Rózsadomb, 9. 12. 1953.

Erysiphe communis (WALLR.) LINK. – Frequently on *Armoracia lapathifolia*, Nyíregyháza, 8. 7. 1947.; Gyoma. 8. 25. 1948.; Budapest (leg.: B. Husz), 1949. On **Alliaria officinalis*, Szarvas, 11. 2. 1948., Pusztavacs, 8. 27. 1950.

On **Brassica oleracea* (in greenhouse), Budapest, Rózsadomb, 9. 17. 1953. On *Capsella bursa-pastoris*, Mezőgyán (Békés), 9. 17. 1948. On *Circaea lutetiana*, Pusztavacs (Pest), 8. 27. 1950. On **Diplotaxis tenuifolia* and *Diplotaxis muralis*, Gyoma (perithecia: 87.4 × 95 micron, perithecium appendages: 171–304 micron, ascospores: 26.6 × 15.2 micron), 11. 8. 1947. On **Gypsophila muralis* and *Gypsophila acutifolia*, Debrecen (perithecia: 93–120–139.5 micron, perithecium appendages are short: 46.5–65.2 micron, asci: 67 × 30.6–37.2 micron, ascospores: 16.7–21.4 × 11.2 micron), 8. 9. 1948. On **Knautia drymeia*, Mátraháza, 11. 23. 1950, Sopron, 11. 25. 1965. On *Knautia arvensis*, Budapest, Zugliget, 9. 27. 1949. On **Lepidium draba*, Budapest, Rózsadomb (with perithecia), 8. 19. 1950. On **Lepidium ruderale*, common, Hortobágy, Tiszafüred, Sarkad, Mezőgyán (Békés), 9. 7. 1948., Szarvas, Mezőtúr, 9. 11. and 15. 1948. and 10. 6. and 17. 1948. (perithecia: 83.5–102.7–120.9 × 102.5–120.9 micron, perithecium appendages: 55.8–83.7 micron, asci: 67 × 30.5 micron, ascospores: 39.3 × 9.3 micron, conidia: 36–43.7 × 13.2–14.5 micron, in company with the hyperparasitic fungus *Cicinnobolus cesatii*, which has 93–111.6 micron diam. piriform pycnidia). On **Lunaria rediviva*, Kékestető, 10. 1. 1950. On *Papaver somniferum*, Tordas, (perithecia: 108.3–119.3–136.1 micron), 7. 3. 1950. On **Scabiosa ochroleuca*, Halásztelek (Békés), 9. 23. 1948. On *Sinapis arvensis*, Mezőtúr, 10. 4. 1947. On *Sisymbrium strictissimum*, Budapest, Nagyhárshegy, 9. 14. 1952. (with perithecia). On **Sisymbrium officinalis*, Gyula, 8. 12. 1948. On **Sisymbrium sophia*, Szarvas, Kondoros, 7. 12. 1948. On **Sisymbrium loeselii*, Nyíregyháza, Debrecen (perithecia: 102–129 × 129–138.3 micron, perithecium appendages: 167.4–251.2 micron, asci: 74.4 × 46.5 micron, ascospores: 24.2–30.6 × 14 micron), 8. 8. 1948.; On *Statice gmelini*, Gyula, 8. 7. 1944., Halásztelek (Békés), 9. 3. 1947. (perithecia: 83.7–111.5 × 81.2–110 micron, perithecium appendages: 55.8–74.4–112.6 micron, asci: 52 × 27.9 micron, ascospores: 34.5 × 10.2 micron). On *Thesium intermedium*, Budapest, Széchenyi hegy (with perithecia), 10. 23. 1953.

Erysiphe convolvuli DC. – Frequently, on *Calystegia sepium*, Szarvas, 9. 23. 1947.; Ócsa (Pest), 8. 27. 1950., Tiszazug (Csongrád), 8. 31. 1953. (with perithecia). On *Convolvulus arvensis*, very common all over the country, always with perithecia at fall.

Erysiphe cruchetiana BLUMER. – Common, on *Ononis spinosa*, Szarvas (with perithecia), 9. 10. 1947.; Budapest, Jánoshegy (with perithecia), 9. 27. 1949.; Budakalász (with perithecia), 8. 8. 1955.; Ágasegyháza (with perithecia), 10. 2. 1958. On *Ononis columnae*, Nagykovácsi, Nagyszénás, 8. 11. 1953.; 8. 2. 1959.; Budapest, Zugliget, 9. 21. 1951. (with perithecia).

Erysiphe depressa (WALLR.) SCHLECHT. – On *Arctium lappa*, Szarvas (with perithecia), 8. 22. 1948.; Budapest, Jánoshegy (with perithecia), 10. 8. 1949. On *Arctium minus*, Szarvas, 10. 3. 1947. On *Arctium tomentosum*, Budapest, Óbuda (with perithecia), 9. 12. 1947.

Erysiphe fischeri BLUMER. — On *Senecio vulgaris*, Szarvas, 9. 23. 1947.; Szentendre (with perithecia), 10. 3. 1965.

Erysiphe galeopsidis DC. — Frequent on *Ballota nigra*, Szarvas, 9. 23. 1947.; Budapest, Rózsadomb, between 1948 and 1965 many times. On *Galeopsis pubescens*, Budapest, Hűvösvölgy (with perithecia), 8. 21. 1951. On *Galeopsis tetrahit*, Ócsa (Pest), (with perithecia), 8. 27. 1950. On *Lamium purpureum*, Nyíregyháza, 5. 17. 1948.; Szarvas-Pepikert, 5. 2. 1948. On *Lamium amplexicaule*, Balatonakarattya (with perithecia), 10. 13. 1965. On *Leonurus cardiaca*, Szarvas, Kondoros (with perithecia), 9. 29. 1951. On *Phlomis tuberosa*, Pomáz, Kőhegy, 5. 28. 1954. On **Stachys recta*, frequent, Budapest, Széchenyi hegy (with perithecia), 10. 23. 1953.; Budapest, Árpádhegy (with perithecia), 9. 17. 1955. and 11. 1. 1965.; Hármashatárhegy (with perithecia), 10. 31. 1965. On **Stachys officinalis*, Budapest, Széchenyi hegy (with perithecia), 10. 23. 1953. On *Stachys silvatica*, Mátraháza (with perithecia), 10. 1. 1952. On *Teucrium chamaedrys*, Budapest, Széchenyi hegy (with perithecia), 10. 23. 1953.

Erysiphe galii FUCKEL. — On **Galium mollugo*, Budapest, Nagyhárshegy (with perithecia), 9. 28. 1950.

Erysiphe graminis DC. — On *Aegilops cylindrica* (with perithecia), Budapest, Óbuda, 5. 27. 1951.; Budapest, Rózsadomb, 6. 3. 1953. (perithecia: 159.8—204.8 micron, conidia: 45.9 × 13.8 micron). On **Agropyron caninum*, Nyíregyháza, 7. 5. 1947. On *Agropyron repens*, common, Szarvas, 7. 11. 1948. On *Dactylis glomerata*, Szarvas, 9. 18. 1947. On **Bromus sterilis*, Budapest, Hármashatárhegy, 6. 10. 1951.; Budapest, Óbuda, 7. 18. 1951. (in company with *Ustilago bromivora* (TUL.) FISCH. v. WALDH., perithecia: 144—206.4 micron, asci: 41.7—69.7—88.15 × 30.75—33.21 micron). On **Setaria verticillata*, Siófok, 7. 20. 1952.

Erysiphe heraclei DC. — On *Conium maculatum*, Szarvas (with perithecia), 9. 23. 1947. On **Daucus carota*, Maklár (leg.: B. Husz), 7. 2. 1948. On *Falcaria vulgaris*,fr equent, Szarvas, Pepikert (perithecia: 108—120.5 × 120—125 micron, perithecium appendages: 74.4—102.3 micron, asci: 55.8—65.1 × 27.9—37.2 micron, ascospores: 19.5 × 10.2 micron); Vizesfás (Békés), (perithecia: 107.5—112.5 × 120.9—125.2 micron). On *Heracleum sphondylium*, Nyíregyháza (with perithecia), 8. 18. 1948.; On *Pastinaca sativa*, Vizesfás, 8. 16. 1950. On *Petroselinum hortense* (in large numbers), Csabacsüd (Békés), 7. 3. 1948. and Szarvas, 9. 13. 1948. (perithecia: 93—112 × 102.3—120.9 micron, ascospores: 37.2—41.8 × 9.3—11.2 micron). On *Pimpinella major*, Szarvas, Pepikert, 9. 24. 1948. On **Torilis arvensis*, frequent Szarvas, 9. 23. 1947.; Budapest, Hűvösvölgy, 7. 8. 1954. (perithecia: 83.7—111.6 × 112.0—125.5 micron, perithecium appendages: 65.1—74.4 micron, asci: 55.8 × 23.2 micron, ascospores: 27.9 × 13.9 micron).

Erysiphe horridula (WALLR.) LÉV. — On *Anchusa officinalis*, Budapest, Árpádhegy, 5. 30. 1954.; Nagykovácsi, Nagyszénás (with perithecia), 6. 3. 1953. and 8. 2. 1959. On *Cerinthe minor*, Nagyvázsony (Veszprém), 6. 26. 1953.

Nagykovácsi, 10. 12 1955. On *Cynoglossum officinale*, Debrecen, Pallag, 9. 19. 1949. On *Lithospermum arvense*, Szarvas, 5. 15. 1948. On **Myosotis collina* and *Myosotis arvensis*, Szarvas, Pepikert, 7. 11. 1947. On **Myosotis micrantha*, Szarvas, 8. 30. 1948. On *Pulmonaria mollissima*, Budapest, Jánoshegy, 7. 6. 1953.; Mátraháza (with perithecia), 10. 13. 1954. On *Symphytum officinale*, frequent, Ócsa (Pest), 8. 31. 1950.

Erysiphe hyperici (WALLR.) FRIES. — On *Hypericum maculatum*, Budapest, Hűvösvölgy, 9. 29. 1951.; Kékestető (with perithecia), 10. 1. 1952. On *Hypericum perforatum*, Nagykovácsi, Nagyszénás, 9. 17. 1959.

Erysiphe labiatarum (WALLR.) CHEV. — On *Lycopus exaltatus*, Halásztelek (Békés), (perithecia: 83.7—111.6 × 83.5—102.3 micron, perithecium appendages: 83.7—130.0 micron, asci: 65 × 35 micron, conidia, 37.2 × 18.6 micron), 9. 3. 1947.; On **Mentha aquatica*, Halásztelek, 9. 3. 1947. On **Prunella vulgaris*, Halásztelek, 9. 3. 1947.

Erysiphe lamprocarpa (WALLR.) DUBY. — On **Plantago intermedia*, Szarvas, 10. 9. 1948. (with perithecia). On **Plantago lanceolata*, ib. On *Plantago major*, frequent, Szarvas, 10. 8. 1946., 9. 18. 1947.; Hortobágy, 9. 3. 1948. etc.

Erysiphe martii LÉV. — On **Robinia pseudacacia* (this species is more frequent than *Trichocladia robiniae* TSCHERMIENKA), Szarvas, 10. 17. 1946.; Nyíregyháza, 9. 18. 1948. (perithecia: 107.5 × 95.76 micron, conidia: 25.2 × 18.5 micron). On *Coronilla varia*, frequent, Szarvas, 8. 20. 1947, Halásztelek, 10. 25. 1947. (with perithecia); Békésszentandrás, 10. 24. 1947. On **Glycyrrhiza echinata*, Szarvas, Halásztelek, 10. 25. 1947.; Békésszentandrás, 10. 9. 1948. (perithecia: 105.8—118.8 micron, ascospores: 41.8—55.8 × 11—12 micron, conidia, 26—65.1 × 15.2 × 18.6 micron); On **Lathyrus tuberosus*, Szarvas, 9. 25. 1947. On **Lotus corniculatus*, Szarvas, 9. 24. 1947. On **Lupinus polyphyllus*, Börzsöny, Királyrét (perithecia: 105.8—118.4 micron), 9. 21. 1951). On *Melilotus officinalis*, frequently all over the country, Szarvas, Mezőtúr, 8. 25. 1948.; Halásztelek, 8. 25. 1948.; Budapest, Árpádhegy (with perithecia), 10. 31. 1965. On *Melilotus albus*, Szarvas, 10. 17. 1946. On *Onobrychis viciaefolia*, Szarvas, 8. 30. 1948. On *Trifolium hybridum*, Mezőtúr, 9. 3. 1948. On *Trifolium pratense*, common. On **Trifolium repens*, Szarvas, 9. 23. 1947. On **Trifolium campestre*, Nagykovácsi, Nagyszénás, 8. 2. 1959. On **Trifolium incarnatum*, fairly frequent, Szarvas, 8. 30. 1948. On **Trifolium minus*, Zebegény (with perithecia), 8. 31. 1950. On **Trifolium rubens*, Zebegény (with perithecia), 8. 31. 1950.

Erysiphe mayorii BLUMER. — On *Cirsium arvense*, frequent, Szarvas, 10. 9. 1948.; Vizesfás, (in company with a *Helminthosporium* sp., and with the teleuto sori of *Puccinia suaveolens* (PERS.) ROSTR.) (perithecia: 93—116 × 93 micron, perithecium appendages: 111.6—140 micron, asci: 93—136 × 37.2 micron, ascospores: 63—65 × 18.5 micron), 8. 17. 1950.

Erysiphe nitida (WALLR.) RABH. — On **Ranunculus polyanthemos*, Szarvas and Vizesfás, 9. 23. 1947. (perithecia: 76—102.3 × 74.4—83.7 micron, perithecium appendages: 74.4—102.3 micron, asci: 57.6 × 35—37.2 micron,

ascospores: 20.2 × 10.2 micron, conidia: 30.8 × 13.5 micron). On *Ranunculus repens*, Szarvas, Halásztelek, Mezőtúr, 8. 21. 1947. On *Ranunculus sardous*, Szarvas, Mezőtúr, 9. 9. 1948, Körtvélyes (Csongrád), (leg.: L. Timár), 8.24. 1951.

Erysiphe pisi DC. – On **Medicago lupulina* and on *Medicago sativa*, Szarvas, 9. 23. 1947. On *Pisum sativum*, Tiszafüred (with perithecia), 9. 4. 1948. On *Vicia cracca*, Halásztelek, Mezőtúr, 9. 28. 1947. On **Vicia villosa*, Szarvas (with perithecia), 9. 27. 1947. On *Phaseolus vulgaris*, Szarvas (only conidia), 8. 18. 1947.

Erysiphe polygoni DC. – On *Polygonum aviculare*, common, all over the country. On **Polygonum lapathifolium*, Öcsöd (Békés), (with perithecia), 9. 22. 1948. On **Polygonum persicaria* and on *Polygonum lapathifolium*, Szarvas, Mezőtúr, 9. 23. 1948.; Csongrád, 8. 31. 1954. (perithecia: 74.4–120.9 × 74.4–102.3 micron, perithecium appendages: 65–176.7 micron, asci: 46.5–55.8 × 20–37.3 micron, ascospores: 23–28 × 11.2 micron. – in company with large numbers of *Cicinnobolus cesatii)*. On *Rumex limosus*, Nyíregyháza, 8. 8. 1948. (in company with *Uromyces rumicis* (SCHUM.) WINT.). On **Rumex acetosa* (in large numbers), Szarvas, 10. 25. 1947. (perithecia: 83.7–111.6 × 74.4–102.3 micron, perithecium appendages: 46.5–102.3 micron, conidia: 36.5 × 12.2 micron – in company with the uredo sori of *Uromyces rumicis)*. On **Rumex acetosella*, Debrecen (perithecia: 83–111.6 × 74.4–93 micron, perithecium appendages: 46.5–158–214 micron, asci: 55.8 × 32.2 micron, ascospores: 28 × 11.2 micron, conidia: 37.2–44.6 × 10.5–21.2 micron); 8. 9. 1948.; Szarvas (with perithecia, in company with the uredo sori of *Uromyces rumicis)*, 9.17. 1948. On **Rumex crispus*, Szarvas (perithecia: 78–102.3 × 74.4–83.7 micron, perithecium appendages: 65–156 micron, asci: 60.3 × 37.2 micron, ascospores: 20.4 × 10.2 micron, conidia: 37.2–44.6 × 11–18.6 micron), 10. 25. 1947. On **Rumex paluster*, Szarvas (perithecia: 93–111.6 × 83.7–102.3 micron, perithecium appendages: 46.5–65.1 micron, ascospores: 23.2 × 9.3 micron), 9. 23. 1947.; On **Rumex patientia*, Szarvas, 9. 23. 1947. On **Rumex stenophyllus*, Szarvas, Mezőtúr, (perithecia: 93–111.6 × 83.7–102.3 micron, perithecium appendages: 83.7–111.6 micron, asci: 55.8–60.5 × 28–37.2 micron, ascospores: 18.6–28 × 9.3–11 micron), 10. 25. 1947.

Erysiphe salviae (JACZ.) BLUMER. – On *Salvia verticillata*, Sarkad (Békés), 8. 31. 1948. On **Salvia nemorosa*, Halásztelek (only conidia), 5. 19. 1948.

Erysiphe urticae (WALLR.) KLOTZSCH. – On *Urtica dioica*, Szarvas, 9. 25. 1947.

Erysiphe verbasci (JACZ.) BLUMER. – On **Verbascum blattaria* var. *blattariforme*, Szarvas, 9. 17. 1947.

Léveillula taurica (LÉV.) ARN. – On *Chondrilla juncea*, Halásztelek, 9. 18. 1947.; Nagykovácsi, 10. 3. 1953.

Microsphaera quercina (SCHW.) BURR. – (The imperfect form is very common, all over the country, nevertheless perithecia are also frequent). On *Quercus robur*, Szarvas, Pepikert, Sopron, Budapest, etc. On *Quercus petraea*, Budapest,

Nagyhárshegy, 9. 14. 1952.; Szarvas (perithecia: 106.4–110.2 × 102.8–114 micron, perithecium appendages: 114–122 micron, ascospores: 53.2–54 × 62.2 micron, conidia: 28.2 × 17.2 micron), 10. 14. 1947.; Árpádhegy (perithecia: 83.7–121 × 79–102.3 micron, perithecium appendages: 93–121 micron, ascospores: 50–52 × 28–32.5 micron) 11. 1. 1965.

Microsphaera betae VANHA. – The imperfect form is frequent but perithecia are rare on *Beta vulgaris* var. *esculenta* and on the var. *saccharifera*, Sopronhorpács, 10. 1. 1950.; Martonvásár, 9. 12. 1952.; Szarvas, 10. 9. 1948.; Hatvan, 8. 25. 1959.; Nagygombos, 8. 21. 1961.; Mezőhegyes, 9. 22. 1963. (perithecia: 34. 85–70.5 micron, conidia: 41. 8–55.8 × 11–12 micron, in company with *Cicinnobolus cesatii*-pycnidia: 139.2 × 18.6–35.3 micron.).

Microsphaera berberidis (DC.)LÉV. – On *Berberis vulgaris*, frequent, Debrecen, 9. 19. 1947.; Szarvas, 9. 24. 1947.; Nagykovácsi, Nagyszénás, Budapest-Árpádhegy, 10. 31. 1965., etc. On **Mahonia aquifolium*, Szarvas, 10. 2. 1946. On **Mahoberberis ilicifolia*, Szarvas, Pepikert, (perithecia: 93–111.6 × 93–102.3 micron, perithecium appendages: 148.8–186.0 micron), 9. 24. 1947.

Microsphaera friesii LÉV. – On *Rhamnus cathartica*, Szarvas-Pepikert, 10. 2. 1947, Budapest, Hűvösvölgy, (with perithecia), 9. 27. 1953.

Microsphaera divaricata (WALLR.) LÉV. – On *Frangula alnus*, Ócsa (Pest), 8. 27. 1950. (This is the first occurrence of the species in Hungary. It was already announced in G. UBRIZSY: Növénykórtan, Vol. II. p. 195. – Budapest, 1965.).

Microsphaera grossulariae (WALLR.) LÉV. – On *Ribes grossularia*, Mátraháza (with perithecia), 9. 23. 1958.

Microsphaera lonicerae (DC.) WINTER. – On *Lonicera tatarica*, frequent, Nyíregyháza, 8. 8. 1947.; Szarvas, Pepikert, 9. 20. 1947. (perithecia: 65–97.4 × 63.2–74.4 micron, perithecium appendages: 93–111.6 micron, asci: 28–37 micron.).

Microsphaera mougeotii LÉV. – On *Lycium halimifolium*, frequent, Szarvas, 8. 31. 1948., 10. 9. 1948. (with perithecia); Budapest, Rózsadomb, 9. 22. 1952.; Pomáz, 9. 6. 1953., etc.

**Microsphaera syringae* JACZ. nec. MAGN. – This species occurred only in a single case on *Syringa vulgaris* in Hungary. The common, and widespread powdery mildew of lilac is caused by the fungus *Phyllactinia suffulta* (REBENT.) SACC., as it was already demonstrated by G. Moesz (1939). – Nyíregyháza, 9. 29. 1946.

Phyllactinia mespili (CAST.) BLUMER. – On *Crataegus monogyna* and on *Crataegus oxyacantha*, Szarvas-Pepikert, 9. 29. 1947., 10. 2. 1947., 10. 12. 1947. (perithecia: 186–204.5 × 167–186 micron, perithecium appendages: 111.6–158.2 micron, bulbous base of the appendages: 23.2 micron).

Phyllactinia roboris (GACHET) BLUMER. – On *Quercus robur*, Szarvas, Pepikert, (with perithecia), 10. 2. 1947.

Phyllactinia suffulta (REBENT.) SACC. – On *Carpinus betulus*, Szarvas (with peri-

thecia in large numbers), 9. 12. 1947., 10. 24. 1947. On *Cornus sanguinea*, Szarvas, (perithecia: (158.1-unripe) – 167 – 186 micron, perithecium appendages: 260 micron, bulbous base: 27.9 micron), 10. 15. 1947. On **Cornus mas*, Szarvas, Pepikert (perithecia: 176.7 – 214 × 172 – 204.6 micron, appendages: 139.5 – 269.7 micron, bulbous base: 27.9 – 32.5 micron, asci: 55.8 × 41.5 micron), 10. 15. 1947. On *Corylus avellana*, and on *Corylus americana*, frequent, Szarvas, Pepikert, 10. 2. 1947. On **Corylus americana* var. *laciniata*, Szarvas, Pepikert, 9. 24. 1948.; Pusztavacs (Pest), 8. 22. 1950.; Bükkhegység, Szinvaforrás (perithecia in large numbers), 10. 12. 1953. On *Fagus silvatica*, common, Szarvas, Pepikert, 10. 2. 1947.; Kékestető (Mátra hegység) (with perithecia), 10. 5. 1952. On **Fraxinus pannonica*, frequent, Szarvas, Pepikert, and Erzsébetliget, 10. 20. 1947.; Bükk, Szinvaforrás, 10. 2. 1953.; Bükk, Vesszősvölgy, 10. 15. 1953. (perithecia in large numbers: 167. 4 – 204.6 × 232.5 micron, appendages: 250.2 – 325.5 micron). On **Fraxinus ornus*, Szarvas, Pepikert, 10. 20. 1947.; Budapest, Árpádhegy, 10. 15. 1965. (perithecia in large numbers). On *Fraxinus excelsior* var. *monophylla*, Szarvas, Pepikert, (perithecia: 167.4 – 204.6 × 232.5 micron, appendages: 250.2 – 325.5 micron, bulbous base: 37.2 micron), 10. 15. 1947. On *Syringa vulgaris*, common, Nyíregyháza, 9. 18. 1946.; Szarvas, Pepikert, (perithecia: 232.2 – 251.5 micron), 9. 29. 1946.

Podosphaera leucotricha (ELL. et EV.) SALM. – On *Malus pumila*, especially on the varieties "Jonathan", "Török Bálint", etc. It is a serious parasite everywhere in apple growing areas. Some years perithecia occur in large numbers.

Podosphaera oxyacanthae (DC.) DE BARY. – On **Cydonia vulgaris*, Szarvas, (perithecia: 62.5 – 67.8 × 73.6 micron), 11. 2. 1946.

Podosphaera tridactyla (WALLR.) DE BARY. – On *Prunus domestica*, frequent, Szarvas, 9. 17. 1947.; Mezőtúr, 10. 2. 1948.; (perithecia: 74 – 93 × 72.2 – 91.5 micron, appendages: 251 – 361.2 micron). On *Prunus spinosa*, frequent, Nyíregyháza (in company with *Puccinia pruni spinosae*), 9.29. 1946.; Szarvas, 10. 2. 1947.; Budapest, Jánoshegy, 10. 5. 1958.; Pomáz (with perithecia), 10. 18. 1952.

Sphaerotheca balsaminae WALLR. – On *Impatiens noli tangere*, Sopron, Várvölgy, 9. 27. 1965.

Sphaerotheca euphorbiae (CAST.) SALMON. – On *Euphorbia palustris*, Halásztelek (Békés), 9. 3. 1947. On **Euphorbia virgata*, Halásztelek, 8. 27. 1947.; Szarvas, 8. 27. 1948. On **Euphorbia cyparissias*, Szarvas, 8. 18. 1948. On **Euphorbia esula*, Pomáz, 10. 18. 1952. On **E. exigua* Nagykovácsi, 10. 12. 1955.

Sphaerotheca fuliginea (SCHLECHT.) SALMON. – On *Bidens tripartitus*, Szarvas, and Halásztelek, 9. 23. 1947. On **Dipsacus laciniatus*, Halásztelek (perithecia: 111.2 – 130 × 111.2 – 128.2 micron, appendages: 93.5 – 214.3 micron, asci: 104.2 × 66.2 micron, with the hyperparasitic fungi *Cicinnobolus cesatii*, in large numbers.). On **Dipsacus silvester*, Szarvas (with perithecia), 8.21. 1947. On *Erigeron canadensis*, Martonvásár (with perithecia), 8. 12. 1965. On **Euphrasia stricta*, Mátraháza (with perithecia), 9. 29. 1958. On *Odonti-*

tes rubra, Mátraháza, 9. 29. 1958.; Nagykovácsi, Nagyszénás, (with perithecia), 8. 2. 1959. On *Physalis alkekengi*, Zebegény (with perithecia), 8. 31. 1950.; Bükk, Szinvaforrás (with perithecia), 10. 12. 1953. On **Veronica hederaefolia*, Gyöngyös (in large numbers), 9. 3. 1965. On *Xanthium spinosum* and on *Xanthium italicum*, Halásztelek, Hortobágy, (perithecia: 115.2–145.2 × 110–130 micron, appendages: 65–83.7 micron, asci: 65–93–(120) × 65–83.5–(111.6) micron), 10. 3. 1948. On *Xanthium strumarium*, frequent, Szarvas, Halásztelek, 9. 3. 1947.; Mezőtúr, 9. 25. 1948.; Budapest, Margitsziget, (with perithecia), 9. 12. 1952.

Sphaerotheca fusca (FRIES) BLUMER. – On *Senecio nemorensis*, Magastátra, Ótátrafüred (Stari Smokovec) (with perithecia), 8. 21. 1961.

Sphaerotheca humuli (DC.) BURR. – Frequently on cultivated and wild *Humulus lupulus*, Szarvas, 9. 23. 1947.; Ócsa (Pest), 8. 27. 1950.; Budapest, Zugliget, 10. 3. 1952.; Iregszemcse (Tolna), 9. 7. 1960. (with perithecia), etc.

Sphaerotheca macularis (WALLR.) JACZ. – On *Alchemilla silvestris*, Lillafüred, 10. 4. 1953. On **Alchemilla palmata*, Ótátrafüred (Stari Smokovec), 8. 28. 1962. (with perithecia); On **Potentilla argentea*, Halásztelek, 8. 21. 1947. On **Rubus idaeus* (not cultivated), Mátraháza, (perithecia: 82.4–103.6 micron, conidia: 22.3 × 14.2 micron), 9. 26. 1951.

Sphaerotheca mors-uvae (SCHWEINITZ) BERK. – This species is very frequent on wild and on cultivated *Ribes grossularia*, sometimes causing heavy losses.

Sphaerotheca pannosa (WALLR.) LÉV. – var. *rosae* WORON. – Common on "Crimsom rambler" and on other varieties of cultivated roses, in most cases in company with *Phragmidium disciforme*.

Sphaerotheca pannosa (WALLR.) LÉV. var. *porsicae* WORON. – On the leaves and fruits of *Prunus persica*, sometimes epidemic.

Sphaerotheca sanguisorbae (DC.) BLUMER. – On **Sanguisorba minor* and on *Sanguisorba minor* var. *muricata*, Budapest, Jánoshegy and Zugliget, 9. 27. 1949.; Budapest, Jánoshegy, 9. 24. 1950. (with perithecia).

Trichocladia astragali (DC.) NEGER. – On *Astragalus glycyphyllus*, frequent, Nyíregyháza, Debrecen, 8. 8. 1948.; Pusztavacs (Pest) (in company with *Uromyces euphorbiae-astragali* (OPIZ) E. JORDI.; (perithecia: 108–115.2–120 micron). 8. 27. 1950.; Putnok, 10. 13. 1948;. Leányfalu, 7. 26. 1950. On **Astragalus cicer*, Mátraháza (with perithecia), 10. 13. 1954.

Trichocladia evonymi (DC.) NEGER. – On *Evonymus vulgaris*, frequent, Szarvas, 9. 17. 1947.; Szentgotthárd (leg.: B. Husz), 6. 24. 1949.; Debrecen, 8. 12. 1953.; Budapest, Jánoshegy (with perithecia), 10. 3. 1958. On **Evonymus verrucosa*, Budapest, Hármashatárhegy, 11. 1. 1965.

**Trichocladia robiniae* TSCHERMIENKA. – On *Robinia pseudacacia*, Szarvas (perithecia: 65.2–83.7–93 × 65.2–88.8 micron, perithecium appendages: 195.3–465–585.8 micron, asci: 57.5–63.5 × 31.3–36.5 micron, ascospores: 23–25.5 × 13.2–15.2 micron, conidia: 32.5 × 10.2 micron), 10. 17. 1946.

Trichocladia tortilis (WALLR.) NEGER. – On *Cornus sanguinea*, Nyíregyháza, 9. 29.

1946.; Budapest, Hűvösvölgy, 9. 27. 1953.; Budapest, Jánoshegy, 10. 3. 1958.; Budapest, Árpádhegy, 10. 25. 1965. (with perithecia). On **Cornus mas*, Budapest, Árpádhegy-Hármashatárhegy (with perithecia), 10. 25. 1965.

Uncinula aceris (Dc.) Sacc. – On *Acer campestre*, Szarvas, (perithecia: 158–186 × 130.5 micron, appendages: 93–121 micron), 9. 23. 1948. On *Acer platanoides* (see in A. Krenner: Bot. Közl. 38: 63, 1936.; this contribution was left out from the monograph of G. Moesz).

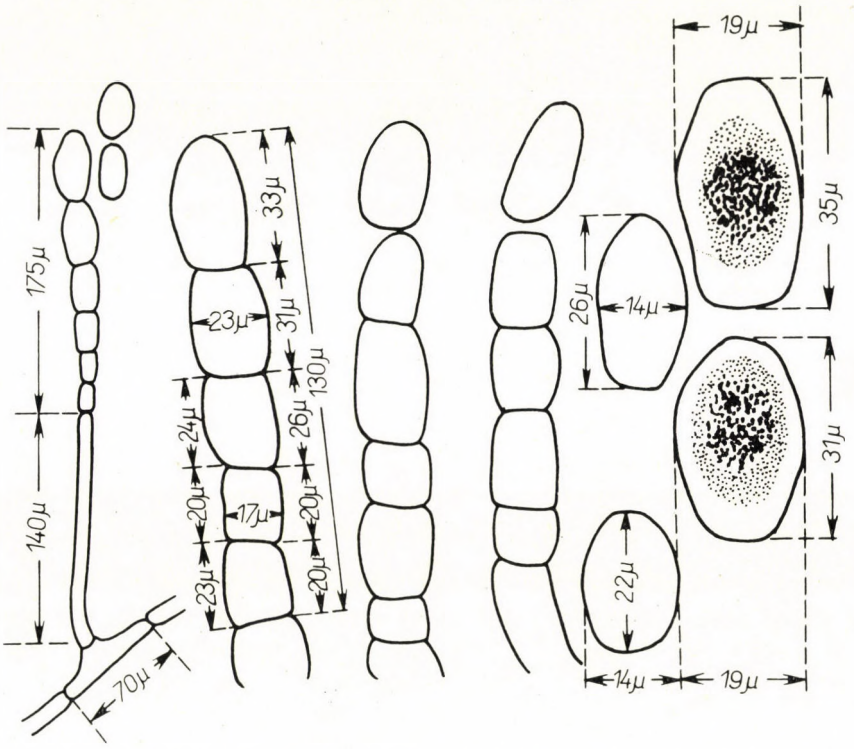

Fig. 4. Conidium formation of *Oidium solani*. Chains of conidia and individual conidia

Uncinula prunastri (Dc.) Sacc. – Frequently on *Prunus spinosa*, Mátraháza, 9. 3. 1958.; Budapest, Jánoshegy, (with perithecia), 8. 25. 1957.

Uncinula salicis (Dc.) Winter. – On *Salix caprea*, Mátra, Kékestető, (with perithecia), 10. 1. 1952. On *Populus pyramidalis*, Szarvas, 9. 25. 1947. On *Populus nigra*, Budapest, Jánoshegy, 9. 27. 1949.; Galyatető (with perithecia), 10. 3. 1952. On *Populus tremula*, Mátraháza, 10. 14. 1954. On **Populus canadensis* and on *Populus virginica*, Szarvas (perithecia: 139.5–176.7 × 139.5–167.4 micron, appendages: 120.8–195.3 micron, ascospores: 18.6 × 17.5 micron), 9. 25. 1947.

**Oidium begoniae* Puttemans. – On *Begonia rex*, Budapest (leg.: J. Podhradszky), 9. 20. 1960.

Oidium evonymi-japonici (ARC.) SACC. – On *Evonymus japonica*, common.

Oidium lini SKORIC. – On *Linum usitatissimum*, frequent, Martonvásár, (conidia: 37.2–50.2 × 16.7–18.6 micron), 6. 25. 1954.

Oidium solani auct. – Frequently on *Solanum tuberosum.*; Kisvárda 11. 9. 1950. (on 11 different varieties of cultivated potato, e. g. "Aranyalma", "Ackersegen", "Bintje", "Ella", "Gülbaba", "Korai sárga", "Merkur", "Sickingen", "Májuskirály", "Lovászpatonai", etc. – conidia: 22–26 – 31–36 × 14–19 micron) (Figure 4). Recently this fungus is considered to be the imperfect stage of *Léveillula taurica* f. *solani* GOLOVIN. On **Lycopersicon esculentum*, Keszthely, 8. 28. 1964. – It is quite possible, that this conidial form belongs to *Léveillula taurica* f. *lycopersici* GOLOVIN.

Oidium verbenae THÜM. et BOLLE. – On *Verbena chamaedryfolia*, Budapest, Rózsadomb, 10. 10. 1954.; Mátraháza, 11. 13. 1954.

Oidium vincae (SAVUL. et SANDU VILLE) UBRIZSY. – On *Vinca minor*, *Vinca major* and on *Vinca herbacea*, (conidia: 28.8–46.5 × 13.2–16.8 micron).

**Oidium helianthi* auct. – On *Helianthus annuus*, common, all over the country, and on *Helianthus tuberosus*. The question, whether this imperfect form belongs to *Léveillula taurica* f. *helianthi*, or to *Erysiphe cichoracearum*, is not solved yet.

Oidium spec. div. – On *Cucumis sativus*, on *Cucurbita pepo*, on *Colocynthis vulgaris* and on *Phaseolus vulgaris*, all over the country, e.g. Pusztaecseg, Kelemenzug (Békés), 7. 27. 1950.; Szarvas in 1947–1949, Keszthely, 1957–1965.

Deuteromycetes

This report contains data about imperfect fungi collected in Hungary, between 1959–1964. The indentified 42 species presented here represent 28 genera. During the course of the identification of the material a new *Phomopsis* species was found on the host plant *Amaranthus retroflexus*. This fungus is described here as *Phomopsis amaranthi* n. sp. Some of the listed species were not known from Hungary earlier. Their names are signed with *. *Hyalostachybotrys bisbyi* SRINIVASAN, a *Hyphomycetes*, which occurred in Europe for the first time, is described in detail on the basis of the material we have found on *Sorghum* roots.

Measurements or/and characters of some fungi estimated by us were divergent from those were described by other authors. These data are in bracelets after the names of the fungi. In this respect it is worth to mention that the size of the conidia of *Septoria rubi* WEST., a fungus causing leaf spot on cultivated *Rubus idaeus*, were consequently larger than the data of any earlier description.

The letters and numbers in parentheses after the names of fungi are the marks of the herbarium exemplares. The collection is deposited in the herbarium of the Research Institute for Plant Protection, Budapest.

Phyllosticta aegopodii ALL. (K. 222.) – On the leaves of *Aegopodium podagraria* Bükk hegység, Szinvaforrás (leg.: G. Ubrizsy).

Phyllostictina cruenta (FR.) PETR. et SYDOW (K. 223., K. 224.) – On *Polygonatum*

odoratum and on *Polygonatum latifolium*, Budapest, Hármashatárhegy, and Szarvas, Pepikert.

**Phoma acervalis* SACC. (K. 220.) – On dry branches of *Salix* sp., Zebegény.

Phoma acuta FUCK. (K. 184.) – On dry stalk of *Urtica dioica*, Nagymaros.

Phoma amaranthi BRUN. (K. 187.) – On dry stalk of *Amaranthus retroflexus*, Nagymaros.

Phoma ruborum WEST. (K. 110.) – On the twigs of *Rubus idaeus* (cult.), Sopron.

Phoma urticae SCH. et SACC. (K. 184.) – On the dry stalks of *Urtica dioica*, Nagymaros.

Phomopsis amaranthi n. sp. (K. 187. and K. 194.) – Pycnidia sparsa vel seriata, depresso-ellipsoidea, immersa dein papilla nigra emergentia, contextu dilute brunneo circa porum obscuriore, 300–600 × 200–300 micra. Conidia 1. ovali-fusoidea, hyalina, biguttulata, attenuata, 6.7–9 × 2.7–3.6 micr., 2. filiformes, curvata 18–27 × 0.5–1.5 micr. magna. Conodiophora 18–20 × 0.5–0.8 micr. – Hab. in stipitibus emortuis *Amaranthi retroflexi.*

Pycnidia on the faded spots of the dead, gray stalks densely scattered, or arranged in longitudinal rows, depressed-ellipsoidal, or conical, at first covered, then emerging by a papilla, which is much darker, than the lower part of the pycnidia. The texture of the pycnidia is prosenchymatic, one- or multiloculated. The inner surface densely covered with conidiophores. Pycnidia 300–600 × 200–300 × 50–150 micron. Conidia 1. oblong-fusoid, attenuated, biguttulate, 6.7–9 × 2.7–3.6 micron, 2. filiform, hooked or curved, 18–27 × 0.5–1.5 micron. Conodiophores subulate, 18–20 × 0.5–0.8 micron. – On the dry stalks of *Amaranthus retroflexus*, Nagymaros.

According to the informations available for us it is no *Phomopsis* species described from *Amaranthus* host plant. The size of the conidia and other characters of *Phoma amaranthi* BRUN. and *Phoma amaranthicola* BRUN. are quite different from the data described here. – One of the two exemplars we have examined contains only type 1. conidia.

Phomopsis arctii TRAV. (K. 202.) – On the dry stalk of *Arctium* sp., Rétság.

**Phomopsis dipsaci* GROVE (K. 182.) – On the dry stalk of *Dipsacus* sp., Pálmajor.

**Phomopsis hysteriola* GROVE (K. 185.) – On the dry stalk of *Heracleum sphondylium*, Nagymaros.

Phomopsis juglandina (FUCK). HÖHN. (K. 170.) – On dead branches of *Juglans regia*, Nagymaros.

Phomopsis oncostoma HÖHN. (K. 201.) – On dry branches of *Robinia pseudacacia*, Rétság.

**Phomopsis polygonorum* GROVE (K. 203.) – On dry stalk of *Polygonum* sp., Nagymaros. (The size of the conidia in our exemplar is identical with the data of GROVE – 7.2–9 × 2.7 micron, but the conidiophores are longer. Instead of the 10–15 × 1.5–2 micron measurement, we have found that the conidiophores in our material are 11–18 × 1.8 micron).

Phacidiopycnis pseudotsugae (M. WILS.) HAHN. (K. 175. and K. 177.) – On *Pseudotsuga taxifolia* saplings, Ózd.

Cytospora decipiens SACC. (K. 174.) – On dead branches of *Carpinus betulus*, Budapest.

Cytospora prunorum SACC. et SYD. (K. 193.) – On dry branches of *Prunus armeniaca*, Ecséd.

Ascochyta allii HOLLÓS (K. 208.) – On dry leaves of *Allium cepa*, Selyp. (We have found that the size of the conidia is 10–12.5 × 3.5–4.5 micron instead of the data of the original description, which is 12–15 × 2.5–3 micron)

Septoria convolvuli DESM. (K. 171. and K. 172.) – On the leaves of *Convolvulus arvensis*, Nagymaros and Budapest. (As distinguished from the data of DIEDICKE – 30–40 × 1–1.5 micron, – as well as from the data of GROVE – 35–55 × 1–1.5 micron –, the sizes of the conidia in our materials were 30–43 × 1.4–1.8 micron and 45–60 × 1.8–2.5 micron).

**Septoria plantaginea* PASS. var. *plantaginis majoris* SACC. (K. 212.) – On the leaves of *Plantago major*, Sopron.

Septoria rubi WEST. (K. 169., K. 211., K. 216. and K. 217.) – On the leaves and young shoots of *Rubus idaeus* (cult.), Nagymaros, Sopron, Szombathely and Magyarnándor. (The size of the conidia according to DIEDICKE is 40–55 × 1.5 micron, and according to GROVE it is 35–60 × 1.5–2 micron. However, this characteristic was variable in our collection depending on the locality of the material, the size of the conidia was always larger: 45–60 × 2–2.7 micron, 36–81 × 2–2.7 micron, and 70–80 × 3–3.5 micron respectively.)

Septoria tritici ROB. et DESM. (K. 214.) – On the leaves of *Triticum vulgare*, Bánkút.

Coniothyrium fuckelii SACC. (K. 205). – On the twigs of *Rubus idaeus* (cult.), Magyarnándor. – (K. 199.) On shoots of *Rosa* (cult.), Kunhegyes. (The size of the conidia on *Rosa* host plant: 3.6 × 2.7 micron, or 3.2 micron diam.).

**Coniothyrium vagabundum* SACC. (K. 178.) – On dead branches of *Cornus sanguinea* Budapest. (According to the original description the size of the conidia is 4 × 1.5 micron, however, in our material this was 4.5–7 × 1.2–1.5 micron).

**Diplodia elaeagni* PASS. (K. 215.) – On dry branches of *Elaeagnus angustifolia*, Iregszemcse. (The size of the conidia in our material was: 18–25 × 9–10.2 micron).

Diplodia juglandis FR. (K. 195.) – On dry branches of *Juglans regia*, Ecséd.

**Hendersonia vitis* DIEDICKE (K. 200.) – On dead shoots of *Vitis vinifera*, Várvölgy. (The size of the conidia in our material was: 16–18 × 6–7 micron).

Woinowicia hirta (SCHROET.) SACC. (K. 192.) – On the haulms of *Triticum vulgare*, Budapest.

Camarosporium robiniae SACC. (K. 201. and K. 218.) – On dry branches of *Robinia pseudacacia*, Rétság and Tura.

**Myxosporium carpini* GROVE (K. 174.) – On dry branches of *Carpinus betulus*, Budapest.

Colletotrichum ficus KOORDERS (K. 191.) – On the leaves of *Ficus elastica*, Budapest.

Colletotrichum trifolii BAIN. et ESSARY (K. 207.) – On the living stems of *Medicago sativa*, Kunszentmárton.

Vermicularia circinans BERK. (K. 206.) – On dry leaves of *Allium cepa*, Selyp.

Vermicularia dematium FR. (K. 185., K. 186. and K. 204.) – On dry stalks of *Heracleum sphondylim*, *Trifolium pratense* and on the dead twigs of *Rubus idaeus*, Nagymaros, Csepreg and Magyarnándor.

Actinonema rosae (LIB.) FR. (K. 173.) – On the leaves of *Rosa* (cult.), Győr.

Septogloeum mori (LÉV.) BRI. et CAV. (K. 179. and K. 181.) – On the leaves of *Morus* sp., Kecskemét and Eger.

Cylindrosporium castaneae (LÉV.) KRENNER (K. 183. and K. 209.) – On the leaves of *Castanea sativa*, Nagymaros and Sopron. (The measurements of the conidia in our exemplars were: 36–47 × 2.7–3.6 micron, and 40–47 × 3–3.5 micron).

Cylindrosporium padi KARST. (K. 176.) – On the leaves of *Prunus avium*, Szeged.

Melanconium juglandinum KUNZE (K. 170. and K. 198.) – On dry branches of *Juglans regia*, Nagymaros.

Coryneopsis rubi GROVE (K. 205. and K. 210.) – On the twigs of *Rubus idaeus*, Magyarnándor and Sopron.

Oedocephalum glomerulosum (BULL.) SACC. (K. 189.) – On the seeds of *Cornus mas*, Budapest.

**Botryosporium pyramidale* (BONORD.) COST. (K. 180.) – On the dead leaves and stalks of *Lycopersicon esculentum.*

**Spicaria elegans* (CORDA) HARZ (K. 190.) – On the seeds of *Carpinus betulus*, Budapest.

Botrytis cinerea PERS. (K. 221.) – On the dry stalk of *Helianthus annuus*, Iregszemcse.

Arthrobotrys superba CORDA (K. 188.) – On the seeds of *Carpinus betulus* and *Celtis occidentalis*, Budapest.

**Hyalostachybotrys bisbyi* SRINIVASAN (= *Stachybotrys aurantia* BARRON) (K. 219.) – On the roots and stalks of *Sorghum bicolor*, Martonvásár. (This fungus was isolated – from the rhizosphaere of *Erianthus munja* and *E. arundinaceus* – and described for the first time by SRINIVASAN (J. Indian bot. Soc., 1958, 37 : 334–342). Since it was not known from Europe till now, we present a short description of the species on the basis of our exemplar from the roots and stalks of *Sorghum bicolor*. The colonies are pale orange coloured. The conidial heads are entirely confluented, forming a continuous, mucoid layer on the surface. Conidiophores and conidia are Stachybotrys-like, but they remain hyalin or pale orange. Sterigmata in 3–6 clusters are hyalin, pitted. Conidia smooth, with somewhat pointed ends, ellipsoidal, with 1- or sometimes 2, very large oil dropps. Conidiophores: 50–115 × 3.5–5.4 micron, streigmata: 13.5–18 × 3.5–5.4 micron, conidia: 10.8–12.5 × 7.2–8.5 micron).

Ramularia ajugae (NIESSL) SACC. (K. 225.) – On the leaves of *Ajuga genevensis*, Szarvas, Pepikert.

Ramularia macularis (SCHROET.) SACC. (K. 226.) – On the leaves of *Chenopodium urticum*, Szarvas, Erzsébetliget.

Heterosporium echinulatum (BERK.) COOKE (K. 213.) – On the sepala of *Dianthus barbatus*, Szeged.

Cylindrocolla urticae (PERS.) BON. (K. 184.) – On the dry stalks of *Urtica dioica*.

Phytopathogenic and Saprophytic Fungi from Hungary, II.

Contributions to the Ustilaginales Flora of Hungary

By

J. PODHRADSZKY

Research Institute for Plant Protection, Budapest

The monographic work of G. MOESZ concerning the *Ustilaginales* flora of the Carpathian basin was revised and edited by UBRIZSY in 1950. The smut fungi listed in this paper have been collected and identified since the closing date (1949)

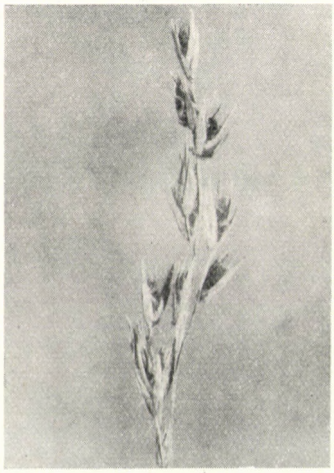

Fig. 1. *Ustilago bullata* in the bloom of *Festuca arundinacea*

of the monographic book, mentioned above. Common and widespread species, like the frequent cereal and maize smuts, are omitted. Therefore this enumeration contains the following data:

Fungi, which are signed with

* = occurred on new host plant in Hungary,

** = occurred in Hungary for the first time, and

*** = occurred on completely new host plant, which was not observed earlier.

The collection is deposited in the herbarium of the Research Institute for Plant Protection, Budapest.

1. ****Ustilago bullata* BERK. (= *Ustilago bromivora* (TUL.) DE WALDH.) – In the ovary of *Festuca arundinacea*, Vácrátót (Pest), June, 1953. (This fungus was

very frequent on a number of *Festuca* spp. artificially infected with the ergot fungus (Á. BOROS and A. GARAY, 1955; Fig. 1)

**Ustilago bullata* BERK. – In the ovary of *Bromus inermis*, Kecskemét, 1954. (Figure. 2.).

Fig. 2. *Ustilago bullata* in the bloom of *Bromus inermis*

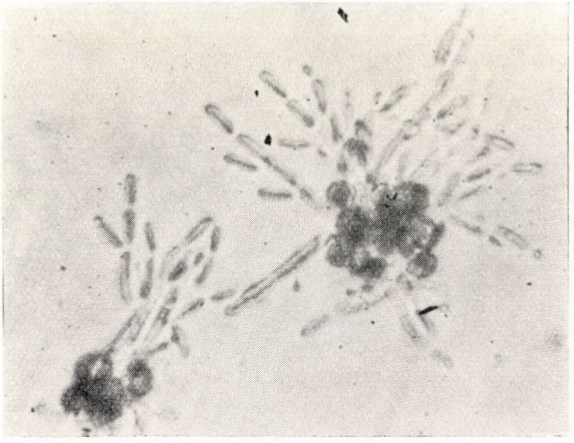

Fig. 3. Germinated spores of *Ustilago nigra*

Ustilago bullata BERK. – In the ovary of *Bromus sterilis*, Pomáz (Pest), June, 1956.; Martonvásár (Fejér), June, 1956.

2. **Ustilago crameri* KOERNICKE – In the ovary of *Setaria italica* var. *maxima* (csumiz), Martonvásár (Fejér), September, 1956. (leg. et det. Dr. G. Ubrizsy). *Ustilago crameri* KOERNICKE – In the ovary of *Setaria italica*, Vácrátót (Pest), Aug. 1954.

3. *Ustilago longissima* (SCHLECHT.) MEYEN – On the leaves of *Glyceria aquatica*, Keszthely (Veszprém), June, 1962.; Kalocsa (Bács-Kiskun), June, 1963.
4. *Ustilago neglecta* NIESSL (= *Ustilago panici-glauci* (WALLR.) WINT.) – In the ovary of *Setaria glauca*, Budapest, 1954.; Martonvásár (Fejér), 1956.; Hatvan-Nagytelek (Heves), 1963.
5. ***Ustilago nigra* TAPKE – In the ears of cultivated *Hordeum* spp., (Podhradszky–Király, 1954). In consequence of the general use of barley seed treatments, this fungus occurs less and less frequently in Hungary. (Fig. 3)

Fig. 4. *Ustilago nuda tritici* in the ear of *Secale cereale*

6. **Ustilago nuda tritici* SCHAFFN. (= *Ustilago vavilovii* JACZ.) – In the ear of *Secale cereale* grown in a wheat field, Végardó (near Sárospatak, Borsod-Abaúj-Zemplén), June, 1956. (Fig. 4)
7. *Ustilago perennans* ROSTRUP (= *Ustilago decipiens* (WALLR.) LIRO) (HILLE, 1958). – In the ovary of *Arrhenatherum elatius*, Keszthely (Veszprém), June, 1954.; and May, 1959.; Martonvásár (Fejér), July, 1956.
8. *Ustilago spegazzinii* HIRSCHH. var. *agrestis* (SYD.) G. W. FISCHER et HIRSCHH. (= *Ustilago agrestis* SYDOW, *Ustilago hypodites* FRIES). – On the haulm of *Agropyron repens*, Szarvas (Békés), 1958–1960.; Martonvásár (Fejér), 1960.; Karcagtilalmas (Szolnok) (in an irrigation canal of a rice plantation), July, 1961.; Tajó (Bács-Kiskun) (leg.: Mrs. Szomolenszky), 1962.; Sopronhorpács (Győr-Sopron), 1963.; Budapest (leg.: Dr. B. Nagy), June, 1963.; Budakalász (Pest), June, 1964.; Érd-Elviramajor (Pest), June, 1964.; Hatvan-Nagytelek (Heves), June, 1964.

**Ustilago spegazzinii* HIRSCHH. var. *agrestis* (SYD.) G. W. FISCHER et HIRSHH. – On the haulm of *Agropyron intermedium*, Budapest, Gellérthegy (leg.: Dr. A. Pénzes), June, 1964.; Martonvásár (Fejér), June, 1965.; Budapest, Rózsadomb (leg.: Dr. G. Ubrizsy), June, 1965.

9. ***Ustilago striiformis* (WESTEND.) NIESSL – On the leaves of *Dactylis aschersonia*, Mátraháza (Heves), June, 1964.
10. *Ustilago utriculosa* (NEES) UNGER (= *Ustilago reticulata* LIRO) – In the flowers

Fig. 5. *Sphacelotheca cruenta* in the bloom of *Sorghum vulgare*

of *Polygonum lapathifolium*, Sellye (Baranya) (leg.: Dr. Ö. Szatala), September, 1959.; Balatonboglár (Somogy) (leg.: Dr. G. Ubrizsy), 1959.

11. *Sphacelotheca andropogonis* (OPIZ) BUBÁK (= *Ustilago ischaemi* FUCKEL, *Sphacelotheca ischaemi* (FUCKEL) CLINTON) – In the flowers of *Andropogon ischaemum*, Pomáz-Kőhegy (Pest), June, 1956.; Tordas (Fejér), July, 1962.
12. ***Sphacelotheca cruenta* (KÜHN) Potter – In the ovary of *Sorghum vulgare*, Magyaróvár (Győr-Sopron), 1960. (Fig. 5.).
13. *Sphacelotheca hydropiperis* (SCHUM.) DE BARY – In the ovary of *Polygonum hydropiper*, Bakonybél (Veszprém), (leg.: P. Tallós), 1954.
14. **Sphacelotheca sorghi* (LINK) CLINTON – In the ovary of *Sorghum vulgare* (brown and white sorghum), Martonvásár (Fejér), 1959.
15. **Sorosporium holci-sorghi* (RIV.) MOESZ (= *Sphacelotheca reiliana* (KÜHN) CLINTON) – In the inflorescence of *Sorghum halepense* var. *sudanense* (f. *sorghi* (GESCHELE) SAVUL.), and in the inflorescences (♂ and ♀) of *Zea mays* (f. *zeae*

Fig. 6. *Sorosporium holci-sorghi.* Infected and healthy grains in a sorghum bloom

Fig. 7. *Sorosporium holci-sorghi* in the bloom of *Sorghum vulgare*

(PASS.) SAVUL.), from many localities all over the country, e.g. Martonvásár (Fejér), August, 1956, 1959, etc. (Figs 6–8)

16. **Tilletia contraversa* KÜHN – In the grains of *Agropyron cristatum*, Budapest-Sashegy, August, 1959.; In the grains of *Triticum aestivum*, Szentjakabfa (Veszprém), 1953. This fungus occurs sporadically all over the country, on plains and on hills as well (PODHRADSZKY, 1962.). Dwarf bunt was epidemic in Baranya county in 1964, causing sometimes infections as high as 15–20%. *Tilletia contraversa* KÜHN – In the grains of *Agropyron intermedium*, Budapest–Sashegy, August, 1959.

Fig. 8. *Sorghum halepense* var. *sudanense* plants infected with *Sorosporium holci-sorghi*

Tilletia contraversa KÜHN – In the grains of *Agropyron repens* plants infected artificially with spore material obtained from *Agropyron intermedium* and from *Triticum aestivum* hosts. 1957.

17. ***Tilletia intermedia* GASSNER (= *Tilletia caries* (DC.) TUL. × *Tilletia foetida* (WALLR.) LIRO) – In the grains of *Triticum aestivum*, in company with *Tilletia caries* and *Tilletia foetida*, Szentistván and Kisbocsva (Borsod-Abaúj-Zemplén) 1949.; Bocfölde (Zala), 1949.; (PODHRADSZKY, 1962.).

18. ***Tilletia lepturi* SIGRIANSKY – In the ovary of *Lepturus pannonicus*, Ecsegfalva-Templomzug (Szolnok) (leg.: Dr. G. Ubrizsy and Dr. J. Podhradszky), 1949. (Fig. 9.).

19. ***Tilletia separata* KUNZE – In the ovary of *Apera spica venti*, Végardó (near Sárospatak) (Borsod-Abaúj-Zemplén), July, 1959.

20. **Urocystis agropyri* (PREUSS) SCHROET. – In the leaf on the ear axle of *Arrhenatherum elatius*, Magyaróvár (Győr-Sopron), 1956 and 1959.
Urocystis agropyri (PREUSS) SCHROET. – In the leaves of *Agropyron repens*, Szarvas (Békés), May, 1959.; Kalocsa (Bács-Kiskun), June, 1963.; Sásd (Baranya), September 30, 1964. (Fig. 10.)

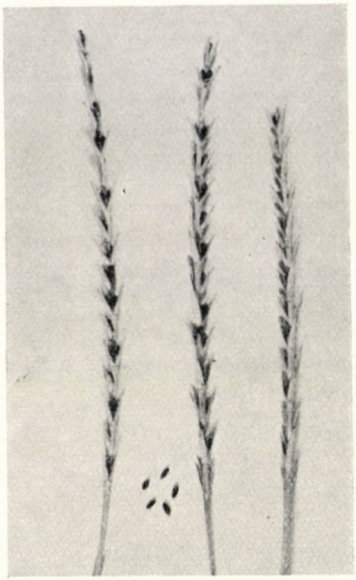

Fig. 9. *Tilletia lepturi* in the ear of *Lepturus pannonicus*

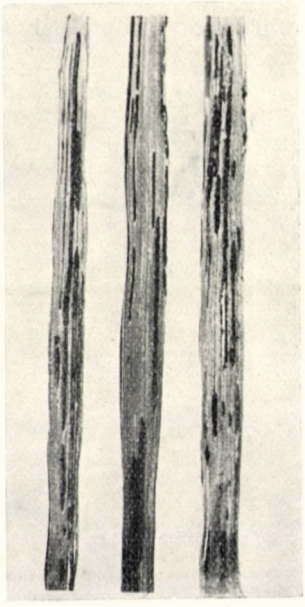

Fig. 10. *Urocystis agropyri* in the leaf of *Agropyron repens*

21. *Urocystis colchici* (SCHLECHT.) FUCKEL – In the upper part of the leaves of *Colchicum arenarium*, Szany (Győr-Sopron) (leg.: Dr. J. Lehoczky), June, 1959.
22. ***Ginanniella primulae* (ROSTRUP) CIFERRI – In the ovary of *Primula veris*. This fungus was found in the experimental garden of the Research Institute for Medical Plants, Budapest, on stocks originated from the Bakony mountain and from the hills of Buda. September, 1952.

During the course of the identification of smut fungi collected in Hungary between 1949–1965 a number of new data and informations were revealed:

1. In the territory of Hungary 8 smut species occurred on 10 new host plants (these are signed with*):

Ustilago crameri on *Setaria italica* var. *maxima*
Ustilago spegazzinii on *Agropyron intermedium*
Ustilago bullata on *Bromus inermis*
Ustilago nuda tritici on *Secale cereale*
Sphacelotheca sorghi on *Sorghum vulgare*
Sorosporium holci-sorghi on *Sorghum halepense* var. *sudanense* and in the ears of *Zea mays*
Tilletia contraversa on *Agropyron cristatum* and on *Triticum aestivum*
Urocystis agropyri on *Arrhenatherum elatius*.

2. The occurrences of 7 smut species are new records in Hungary (these are signed with**):

Ustilago nigra, *Ustilago striiformis*, *Sphacelotheca cruenta*, *Tilletia intermedia*, *Tilletia lepturi*, *Tilletia separata* and *Ginanniella primulae*.

3. One smut species occurred on a completely new host plant (this is signed with***):

Ustilago bullata on *Festuca arundinacea* (it was frequent on different *Festuca* spp. artificially infected with ergot).

Book Review

G. UBRIZSY (editor): *Növénykórtan* (Phytopathology) Vol. I—II. Second revised and amplified edition. Publishing House of the Hungarian Academy of Sciences, Budapest 1965.

The totally revised, second enlarged edition of 1519 pages of the book entitled Phytopathology is edited by UBRIZSY. All chapters were written by research workers of the Research Institute for Plant Protection who have been working for a long time in their special field and thus the material presented in each chapter is based on own research works and on recent literary data. The Research Institute for Plant Protection is the centre of phytopathological research in Hungary and therefore the two big volumes truly reflect the extent and level of this research.

Selection and elaboration of the material is most up-to date. The problems of phytopathology are discussed with a special emphasis on conditions in Hungary but the most important foreign literary data are also included. Therefore phytopathologists of the neighbouring countries can benefit by using the work and even research workers of countries farther away may draw upon it. A great merit of the book is the numerous references. Hungarian literature referred to may be considered as complete but also foreign literature is very detailed, containing 2475 citations.

The two volumes are rather independent of each other. The work falls into the following main chapters:

Volume I:

1. General Phytopathology
2. Physiological Plant Diseases
3. Plant Viruses and Virus Diseases
4. Bacterial Plant Diseases

Volume II:

5. Phytopathogenic Fungi
6. Phanerogamous Parasitic Plants

Chapter 1 offers a completely newly written general phytopathology. Its parts are: history of phytopathology, fundamental aspects, scope and auxiliary sciences of phytopathology, concepts of health and disease, recognition and identification of symptoms, etiology of diseases, classification of plant diseases and plant disease control (by G. UBRIZSY); sources of infection, infection chains, mechanism of infection, invasion, transmission, aggressiveness of pathogens, genetics of plant pathogens, disposition and resistance of the host, epidemiology of infectious plant diseases (by Z. KIRÁLY). Valuable chapters are the physiology and biochemistry of plant diseases and the perspectives in the study of host-parasite relationships with regard to practical plant protection, written by G. FARKAS. The next chapter discusses the possibilities of biological control. The role of plant breeding and agricultural practices in the control of plant diseases is reviewed by Z. KIRÁLY, while the utilization of

hyperparasitic and antagonistic micro-organisms and of antibiotics is compiled by G. UBRIZSY and J. VÖRÖS. All these parts give a brief survey of the results obtained in Hungary and abroad, they stress the essential aspects and point to possibilities in the future. G. UBRIZSY deals also with the importance of phytoncides in phytopathology. The part of General Phytopathology is concluded by the chapters on the therapy of plants (G. UBRIZSY and J. VÖRÖS) and on the prognosis of plant diseases (G. UBRIZSY and L. SÁNTHA).

In Chapter 2 Ö. SZATALA discusses the physiological diseases. Its parts are diseases induced by unfavorable climate and weather conditions, diseases caused by anomalies of nutrition. A particularly useful part is that on deficiency diseases with many beautiful coloured plates. Then follow damages by gases and sewage waters and those caused by mechanical injuries.

Chapter 3 is a summary of plant viruses and virus diseases. The general part has been written by F. SOLYMOSY, the descriptive part by J. SZIRMAI. In the general part morphology, structure, serology, variability, systematics, transmission of plant viruses, host—virus relationship and the control of plant virus diseases are reviewed. The descriptive part surveys virus diseases arranged according to host plants. Thus separate chapters are devoted to the virus diseases of cereals, tuber crops, industrial plants, forage crops, fruit trees, vegetables, ornamental plants and medicinal herbs. Many excellent original photos facilitate the recognition of virus diseases.

In Chapter 4 Z. KLEMENT discusses bacterial plant diseases. In the general part the characteristic properties of the phytopathogenic bacteria are excellently surveyed. In the descriptive part the phytopathogenic bacteria and the diseases caused by them are discussed in the systematic order of bacteria. Not only species found in Hungary but also those widespread in Europe and expected to turn up in Hungary are reviewed.

Volume I is concluded by ample references and the indices. The latter consists of a taxonomic index and a subject index with a separate enumeration of the vectors. All these facilitate the rapid finding of the parts sought for.

The overwhelming part of Volume II, more than 800 pages, deals with the pathogenic fungi. This is easy to understand because fungi are represented with the highest number of species among the pathogenic organisms and their damage is the highest. The general part is short and reviews only the system followed in the book. From the foreword we learn that the elementary concepts of general mycology had to be left out owing to limited space. This is regrettable because it would be necessary for a great variety of fungi.

In the first edition the system of GÄUMANN was followed while in the present second edition a modified form of MARTIN's system is used which appeared in 1964 in Volume IX of the Yearbook of the Research Institute for Plant Protection by UBRIZSY and VÖRÖS. This system is beyond doubt complicated and in some of its parts — as every system — contestable, but was compiled on the basis of latest research work.

In the descriptive part the diseases of fungal origin are dealt with in more or less detail depending on their importance. The descriptions follow the systematic order of fungi. *Myxomycotina* and *Phycomycetes* were elaborated by L. SÁNTHA and G. UBRIZSY, *Ascomycetes* by Z. CSORBA and I. BEREND, of *Basidiomycetes: Exobasidiales*, *Polyporales*, *Agaricales*, *Gasteromycetes* and *Tremellales* by G. UBRIZSY, *Uredinales* by J. PODHRADSZKY, B. HUSZ, Z. KIRÁLY and J. DOBY, *Ustilaginales* by J. PODHRADSZKY, Z. KIRÁLY and I. BEREND. The chapter on *Deuteromycetes* is the work of J. VÖRÖS and B. HUSZ. All these chapters are worked out on the basis of latest mycological and phytopathological knowledge and the results include a good deal of own research work conducted for several decades. Morphology, physiology and occurrence of the practically significant species, the disease symptoms and the control measures are discussed in full detail while the less important species are dealt with very briefly and in many cases only the spore dimensions and the host is given. This is also a consequence of the limitation of space and does not diminish the value of the work because the material has been worked up in a way that the phytopathological viewpoints are fully realized and only the general mycological parts reduced.

In the last Chapter 6 G. UBRIZSY deals with the phanerogamous pharasitic plants giving a short but relevant survey of the parasitic and semiparasitic phanerogamous plants.

Subsequently follow the special literature on fungi and the enumeration of the pathogens according to their hosts dealt with in the two volumes. Finally in an Appendix a register and an index of the fungi concludes the volume.

Summing up it may be established that the big work in two volumes is a significant achievement of the Hungarian agricultural and biological literature and it may reckon also upon the interest of phytopathologists of foreign countries. It is to be regretted that the work written in Hungarian can be used by foreign specialists only to a limited extent. Recognition of the pathogenic organisms is highly promoted by the 412 figures, the major part of which are original photos.

The work was published in a careful presentation which is the merit of the Publishing House of the Hungarian Academy of Sciences.

J. BÁNHEGYI

L. SZEMERE: *Die unterirdischen Pilze des Karpatenbeckens. Fungi Hypogaei Territorii Carpato-Pannonici.* — Akadémiai Kiadó, Budapest, 1965.

Das vorliegende Buch ist das zweite als Monographie zu bewertende Werk, das die in Ungarn bzw. im Karpatenbecken vorkommenden unterirdischen Pilze *(Fungi Hypogaei)* behandelt. Die erste Arbeit dieser Art stammt von László HOLLÓS aus dem Jahre 1911, in der 68 Arten beschrieben wurden. Einige dieser sind auf Grund der neueren und strengeren taxonomischen Beurteilungen dem Formenkreis anderer Arten einverleibt worden oder erwiesen sich als Synonyme, so dass das obenerwähnte Material nach der auch derzeit gültigen Nomenklatur 56 »gute« Arten umfasst. Nach dem Erscheinen seiner Monographie entdeckte L. HOLLÓS noch zwei Arten.

L. SZEMERE, der den grössten Teil seines mykologischen Wirkens der Auffindung und zeitgemässen Beschreibung der unterirdischen Pilze Ungarns widmete, führt in seinem schön ausgestatteten, auch inhaltlich reichen Werk vom Gebiet des Karpatenbeckens bereits 83 *Fungi Hypogaei* an. Von diesen sind taxonomisch 4 Arten zu *Phycomycetes*, 47 zu *Ascomycetes* und 32 zu *Basidiomycetes* zu zählen (unter den letzteren fand auch *Paxillus involutus* status subterraneus, ein besonderer unterirdischer Pilz Platz). Mit der früheren Arbeit von HOLLÓS verglichen wurde also das Vorkommen von 27 neu entdeckten Arten verzeichnet, die zum grossen Teil der Verfasser selbst gesammelt hatte.

Die auf Kunstdruckpapier herausgebrachte, auch äusserlich elegante Monographie umfasst 314 Seiten und enthält auf 8 schwarz-weissen sowie 10 Farbtafeln etwa 41 Abbildungen. Die Zeichnungen und die farbigen Abbildungen wurden vom Verfasser hergestellt. Besondere Beachtung verdient die praktische Bestimmungstafel mit den Abbildungen von Sporen usw., die für die Identifizierung und Erkennung der Pilze nötig sind.

Nach dem als Einleitung dienenden Abschnitt über die allgemeine Charakterisierung und Phylogenie der unterirdischen Pilze werden in den folgenden Kapiteln ihre geographische Verbreitung, Autökologie, ferner die Technik ihrer Auffindung bzw. Sammlung behandelt. Nachher wird ein kurzer geschichtlicher Rückblick über die diesbezüglichen Forschungen in Ungarn geboten. Der spezielle Abschnitt enthält die taxonomische Übersicht, die eingehende Beschreibung und Charakterisierung der behandelten Pilze, bei den einzelnen Arten sind auch konographische und bibliographische Angaben zu finden.

Die Bestimmungsschlüssel sind darauf ausgerichtet, nicht nur den Fachleuten, sondern auch anderen interessierten Kreisen eine entsprechende Methode zur Erkennung der Arten zu übermitteln. Ein musterhaft zusammengestelltes Autoren- und Sachverzeichnis bilden als gut brauchbare Behelfe den Abschluß.

Das mit anerkennungswürdiger Sorgfalt bearbeitete Buch wird dank seines mächtigen Beobachtungs- und Erfahrungsmaterials ein Standardwerk des internationalen mykologischen Fachschrifttums darstellen.

G. UBRIZSY

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Hypersensitive Reaction Induced in Apple Shoots by an Avirulent Form of Erwinia amylovora

By

Z. KLEMENT and R. N. GOODMAN

Research Institute for Plant Protection, Budapest and
Department of Horticulture, University of Missouri, Columbia, Mo. U. S. A.

The role of an avirulent bacterium in a normally sensitive host plant was investigated.

Apple shoots were inoculated with virulent and avirulent strains of *Erwinia amylovora* and *Erwinia*-like (saprophytic) bacterium. Virulent strains produced the typical "fire blight" symptom while the avirulent form induced a small brown discoloration at the site of injection. The *Erwinia*-like organism caused neither blight symptom nor brown discoloration.

Both of the virulent and avirulent *E. amylovora* strains induced hypersensitive necrosis in tobacco leaves (pathogenicity test). *Erwinia*-like organism was unable to induce this type of defence reaction.

Both the virulent and avirulent bacteria started to multiply in apple shoots, the multiplication of the avirulent bacterium, however, stopped or decreased 24 hr after infection.

The results have shown that the avirulent bacteria induced a hypersensitive reaction in the normally sensitive host similar to that caused by virulent bacteria in an incompatible host.

The ability of an avirulent bacterium to induce hypersensitive reaction makes it necessary to differentiate between pathogenicity and virulence which are discussed in this paper.

Introduction

It has been shown that plant pathogenic bacteria are able to induce hypersensitive reaction in an incompatible host-pathogen combination [6, 7, 13]. All of the investigated pathogenic pseudomonads except the homologous plant pathogens have been reported to multiply in tobacco leaves immediately after inoculation by injection-infiltration [6] and to induce a rapid necrotic response which is considered to be a defence reaction of the infiltrated leaf tissue. On the other hand, saprophytic pseudomonads do not multiply in plant tissues and hence do not produce visible reactions.

A similar (hypersensitive) defence reaction appears in resistant hosts infected with homologous virulent pathogenic bacteria [3, 4, 10, 11, 15].

AVERRE and KELMAN [1] supposed that the usual localization of the avirulent *Pseudomonas solanacearum* at the inoculation court and the marked morphological reaction of the host tissue may be considered as a type of hypersensitive

reaction. The aim of the present study was to investigate the fate of an avirulent form of a homologous bacterium in a normally sensitive host plant. The question to which we sought answer was, is the nonvirulent bacterium able to multiply in such plant tissues and if so is there a defence reaction which prevents the bacteria from multiplying at an increased rate.

Material and Methods

Fifty-two strains of *Erwinia amylovora* (Burill) Winslow et al. and a yellow *Erwinia*-like organism [2] were investigated for pathogenicity and virulence. These strains were isolated from apple and pear orchards in the U. S. A. and England, preserved in a lyophilized state until used. Bacteria were grown on nutrient yeast (0.5%) glucose (1%) agar (1.5%) at 28 °C. Suspensions of bacteria washed from nutrient agar slants with sterile tap water and incubated for 24 hr were used for inoculations.

The bacterial strains were investigated for pathogenicity in leaves of *Nicotiana tabacum* L. *White Burley* and *Vigna sinensis* L. A suspension of 10^7 bacterial cells/ml was injected (infiltrated) into the intercellular spaces of tobacco and cow pea leaf panels [5]. To establish pathogenicity the hypersensitive reaction as previously described [5] was used as an indicator.

In order to establish virulence young shoots of potted *Jonathan* apple trees 10 to 15 cm long were infected with the strains of bacteria under investigation. Bacterial suspensions containing 10^8 cells/ml of these strains were injected into the apple shoots with a hypodermic needle at a point midway between the apex and the first node. The treated plants were grown under greenhouse conditions at approximately 28 °C to 30 °C.

The population trends of bacteria in the infected apple shoots were determined as follows: 15 apple shoots were infected with the same bacterial strain. Then 1.5 cm long pieces of three shoots were cut out with a corkborer at the site of injection at five intervals after infection and homogenized in a mortar in 0.5 ml sterilized distilled water. The number of bacteria present in the shoot tissues was estimated by agar-plate counting.

Results

As far as pathogenicity is concerned all *E. amylovora* strains induced hypersensitive reaction in tobacco and cow pea leaves except the yellow *Erwinia*-like organisms. A rapid necrosis appeared 7 to 9 hours after the injection of 10^7 cells/ml. The yellow *Erwinia*-like organisms did not evoke necrosis even when a highly concentrated inoculum of 10^9 cells/ml was applied.

Regarding virulence, all of the investigated *E. amylovora* strains produced the typical symptoms of "fire blight" disease in apple shoots, except one of the bacterial strains, No 21a, which did not cause any blight symptom. No blight symptoms were produced on apple shoots injected with the yellow *Erwinia*-like organism.

The typical symptom started to appear on the 3rd or 4th day with inocula containing 10^6 bacterial cells/ml (Fig. 1). With the avirulent strain No 21a no blight symptom was produced on the shoots injected with as many as 10^{10} cells/ml. This avirulent form was repeatedly reinvestigated for virulence but in no case were blight symptoms obtained. Instead grown necrosis of a diameter of 1 to 2 mm appeared on the shoots (Fig. 2). Neither control shoots injected with water nor

Fig. 1. Typical "fire blight" symptoms on an apple shoot infected with a virulent strain of *Erwinia amylovora*

shoots treated with yellow *Erwinia*-like organisms showed this brown discoloration at the site of injection.

The avirulent form was compared with other virulent strains of *Erwinia amylovora* for cultural and biochemical characters, phage sensitivity and antigenic structure. Cultural and biochemical characteristics and phage sensitivities of the virulent, avirulent and yellow type bacteria were studied in detail by SCHAFFER and GOODMAN [14]. In these experiments the virulent and avirulent isolates of *E. amylovora* gave similar reactions (nutrient gelatine, litmus milk, nitrate reduction, methyl red, Voges-Proskauer tests, pectate gel, thermal death points, pH tolerance and carbohydrate utilization). The yellow *Erwinia*-like organisms, however, gave different results in these tests. The phage sensitivity of the investigated bacterial strains varied independently of pathogenicity or virulence.

For the further identification of the virulent and avirulent forms immune sera were prepared for a virulent strain (No S-1), the avirulent form (No 21a) and a yellow *Erwinia*-like organism (No 35A). The preparation of sera, the agglutination and gel diffusion tests were made as described previously [9]. The sera for No S-1 and No 21a gave cross-reactions with virulent and avirulent strains, therefore, no serological differences even between the virulent and avirulent forms of *E. amylovora* could be detected. By contrast, the yellow *Erwinia*-like organism

Fig. 2. Brown discoloration at the site of inoculation on apple shoot treated with avirulent *Erwinia amylovora*

gave only a weak non-specific reaction with *E. amylovora* No S-1 and No 21a in the agglutination test. However, the yellow *Erwinia*-like organisms could clearly be distinguished from the virulent and avirulent *E. amylovora* strains if tested by the gel diffusion method (Table 1).

The results presented above clearly show that the avirulent form kept all its original properties characteristic for the virulent strains of E. amylovora with the exception of its virulence which was lost.

Studies on population trends of virulent and avirulent forms in apple shoots. To gain a further insight into the host-parasite interaction between an avirulent *E. amylovora* and the "homologous" apple shoot, population trends of bacteria injected into the host tissue were investigated.

Two different types of host-parasite relation were studied in detail: 1. infection of susceptible apple shoots with the virulent *E. amylovora* strain No S-1, and 2. injection of apple shoots with the nonvirulent *E. amylovora* strain No 21a. The results obtained are shown in Fig. 3. The curves indicate bacterial population increases as a function of time.

In the first case, cells of virulent *E. amylovora* started to multiply immediately. A logarithmic growth curve was obtained. The symptoms typical for the "fire

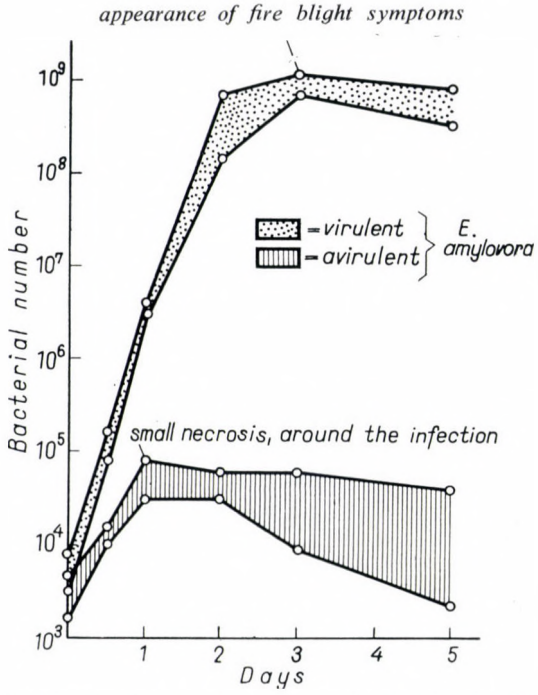

Fig. 3. The population trends of a virulent and an avirulent strain of *E. amylovora* in apple shoots of a length of 7.5 cm

Table 1

Pathological and serological differences among virulent and avirulent *Erwinia amylovora* and yellow *Erwinia*-like bacteria

	In apple shoots		In tobacco	Gel diffusion test with sera for		
	blight symptom	hypersensitive reaction	hypersensitive reaction	S−1	21a	35
Virulent *E. amylovora* No S−1	+	−	+	+	+	−
Avirulent *E. amylovora* No 21a	−	+	+	+	+	−
Yellow *Erwinia*-like No 35A	−	−	−	−	−	+

blight" disease appeared only when the maximum cell number was reached. This host parasite system is a typically compatible one. Avirulent cells of *E. amylovora* injected into apple shoots also started to multiply. In about 24 hr, however, multiplication stopped or decreased. A local necrosis of host cells around the infection site could be observed simultaneously with the inhibition of bacterial growth. Therefore, the avirulent form of *E. amylovora* induced a defence reaction (similar to the hypersensitive reaction) in the potentially susceptible apple shoot. This resulted in the rapid death of the host cells around the infection site and in the inhibition of bacterial multiplication.

Discussion

The results of our investigations on the avirulent form of *E. amylovora* make it necessary to discuss the phenomena of pathogenicity and virulence. This is important because several authors use the terms pathogenicity and virulence synonymously. However, an increasing number of them regard the two phenomena as being separate and make a precise distinction between the two. The results mentioned above provide definitive criteria by which pathogenicity may be differentiated from virulence.

According to MILES [8] "pathogenicity is best regarded as an attribute of a species, . . ." We can then describe *e. g. E. amylovora* as pathogenic for apple and pear trees "without necessary implying that all strains of this species produce disease in its respective hosts". "*Virulence*, on the other hand, is conveniently reserved for pathogenicity of a given stable homogenous strain of microbe, as determined by observation of its action on the host in relation to which the statement about virulence is made".

All of the virulent and avirulent *E. amylovora* strains investigated were able to establish themselves in the host and induce a defence reaction, in the form of hypersensitive necrosis in the non-host plant. These common properties, multiplication in the host and induction of the hypersensitive reaction are the characteristic pathogenic features of *E. amylovora*. All strains, except one, were virulent because they were able to produce typical blight symptoms on apple shoots. However, the one which was unable to do so could induce hypersensitive reaction on some other plants. Therefore, a potential pathogenic property is always carried in all the *E. amylovora* strains both the avirulent and virulent. Further proof for the potential pathogenic property is that the avirulent form is able to induce hypersensitive reaction in the homologous apple shoots as well. In the absence of virulence, however, the infection does not become manifest, the bacteria do not multiply at an increased rate and so typical symptoms of the disease fail to appear. Therefore, it seems that the avirulent strain lost its virulence but kept its potential pathogenicity.

Yellow *Erwinia*-like organisms did not induce either blight symptom or hypersensitive necrosis on apple shoots and in tobacco and cow pea leaves, there-

fore, they may be regarded as saprophytes. From a plant pathological point of view, saprophytic bacteria have to be distinguished from avirulent bacteria. Whereas avirulent bacteria have kept their fundamental pathogenic property which is indicated by their capacity to induce hypersensitive reaction, saprophytic bacteria do not pass this capacity.

The investigations on host-parasite relationships are summarized in Table 2. It seems that hypersensitivity is a general defence reaction of plants against plant pathogenic bacteria in incompatible combinations.

Table 2

Host-parasite relationships

Combinations	Hyper-sensitive reaction	Typical disease symptom
Virulent bacteria — Sensitive host plant	−	+
Avirulent bacteria — Normally sensitive host plant	+	−
Normally virulent bacteria — Resistant host plant	+	−
Pathogenic bacteria — Non-host plant	+	−
Saprophytic bacteria — Plant (all)	−	−

The studies on avirulent forms would be more satisfying if avirulent mutants from a virulent strain could be produced by mutagenesis.

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Studies on the Nitrogen Metabolism of Vine Infected with Yellow Mosaic Virus

By

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Analyses carried out twice during the vegetation period in 1965 on vine (var. *Pirosveltelini)* leaves infected with yellow mosaic virus have shown that in the infected leaves there are significantly more alcohol soluble tryptophan, methionine + valine, arginine + histidine and alcohol soluble nitrogen than in healthy leaves.

According to the results of the first sampling there is no significant difference in the protein-nitrogen content between infected and healthy leaves. However, there is significantly less tryptophan contained in protein and significantly more histidine + arginine in the infected leaves than in the healthy ones.

Introduction

BOSC and BERLAN (1961), SEHGAL and BOONE (1964), LALORAYA and JEE (1955) as well as REINDEL and BIENENFELD (1965) have shown that in virus-infected plants some of the free amino acids accumulate. According to the data of HENKE (1956), WYND (1943), WILDMAN et al. (1949), RAJARAO et al. (1956), SEHGAL and BOONE (1954) and ORLOB and ARNY (1961) virus infection of plants results in a decrease in the amounts of total nitrogen, protein-nitrogen and amino acids contained in proteins. ELBERTZHAGEN (1958), MICZYNSKI (1961) and PORTER and WEINSTEIN (1960) have pointed out that there is a parallelism between the change in the nitrogen content of virus-infected plants as compared to that of healthy ones and the process of disease development. In their review papers KIRÁLY and FARKAS (1959) as well as DIENER (1963) emphasize the fact that virus infection leads to serious disturbances in nitrogen metabolism.

Very little is known about the pathophysiology of virus-infected vines and no data are available on the nitrogen metabolism of vine plants infected with yellow mosaic virus. Vine plants infected with the "Hungarian Yellow Mosaic Virus" show severe symptoms (MARTELLI et al.,1966) which consist of chrome yellow bands along the veins (Fig. 1) followed after some years by a reduction in size of the leaves with quite yellow of yellowish white discoloration (Fig. 2) and often with necrotic margins (Fig. 3). There is a considerable reduction of growth (Fig. 4) and in 5 to 6 years the entire plant may die. In our biochemical studies leaves of yellow mosaic infected (middle stage) and healthy vines were compared with respect to their dry matter content, alcohol soluble nitrogen, protein-nitrogen and amino acid contents.

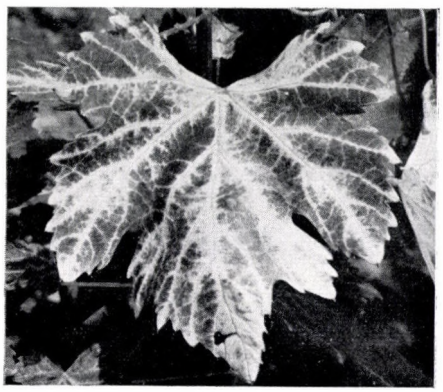

Fig. 1. Symptoms caused by yellow mosaic virus on the leaves of vine var. *Pirosveltelini*

Fig. 2. Small yellow leaves

Material and Methods

In the Badacsony vineyard of the Ampelological Research Institute 4 healthy and 4 yellow mosaic infected vine plants of the variety *Pirosveltelini* were selected to serve as sources of experimental material. In July and in September, 1965, leaves were collected from the above plants and analyzed. The fresh leaves were extracted in 70 per cent alcohol. The extracts and the residues were both hydrolyzed at 105 °C in sealed bomb tubes in 6 N HCl for 48 hours and in 14 per cent barium-hydroxide for 24 hours, respectively. The amino acid content of the hydrolysates was analyzed by BLACK's (1956) paper chromatographic method. The chroma-

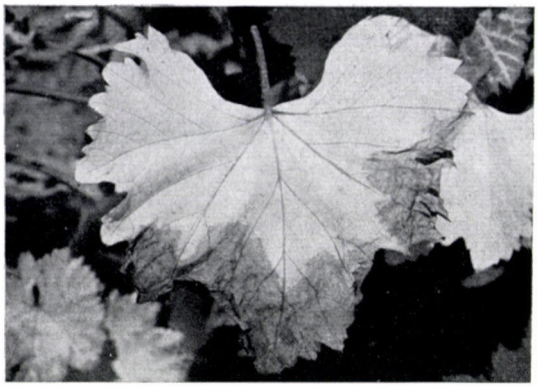

Fig. 3. Necrosis of the margins of yellow leaves

Fig. 4. Growth reduction of infected plants. 1. Height of a healthy plant. 2. Height of a diseased plant

tograms were run at 27 °C in three replications. N-butanol–glacial acetic acid–water (125 : 30 : 125) and with tryptophan isopropanol–ammoniumhydroxide–water (170 : 10 : 30) were used as solvents. The chromatograms were developed with ninhydrine and p-dimethylamino-benzaldehyde, respectively. In Fig. 5 chromatograms prepared from the acid hydrolysates of alcoholic extracts of healthy and virus-infected leaves, respectively, are compared. The quantitative differences in the amino acid contents of the extracts are indicated by the different sizes and colour intensities of the spots. The quantitative evaluations were carried out in a Type ERI 10 (Carl Zeiss, Jena) automatic extinctiometer by reading the

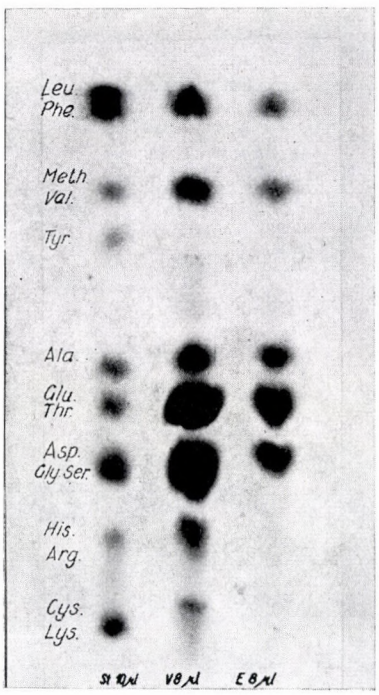

Fig. 5. Chromatograms of 48 hour acid hydrolysates of alcoholic extracts of yellow mosaic virus infected and healthy leaves. St.: Chromatogram of a standard amino acid mixture. V: Chromatogram of an extract of infected leaves. E: Chromatogram of an extract of healthy leaves

absorptions directly on the chromatographic paper and comparing the values obtained to those given by standard amino acid solutions. The nitrogen contents of the alcoholic extracts and of the residues were determined by the Kjeldahl method. The average results of four measurements are expressed as mg per cent and per cent by dry weight. Significance values are also reported.

Results

In the case of both samplings there was more alcohol soluble nitrogen in yellow mosaic virus-infected leaves than in healthy ones (Table 1). The difference was significant at a probability level of P. 0.1%. As far as dry matter content is concerned no significant difference was found between infected and healthy leaves.

Table 1

Dry matter and alcohol soluble nitrogen contents of yellow mosaic virus-infected and healthy vine leaves

Time of sampling and denomination of the sample	Dry matter content	Alcohol soluble nitrogen content in hydrolysates of alcoholic extracts	
		mg/100 g dry weight	
	g/100 g fresh weight	48h acid hydrolysis	24 h alkaline hydrolysis
July, 1965			
Virus-infected leaf	24.13	259.52	54.49
Healthy leaf	24.34	71.20	19.82
September, 1965			
Virus-infected leaf	27.92	76.44	27.18
Healthy leaf	27.65	42.66	12.06
Significant difference	—	35.03	6.28

The concentration of both free amino acids and amino acids contained in alcohol soluble peptides was higher in virus-infected leaves than in healthy ones (Table 2). With the sample taken in July the quantitative differences in tryptophan, methionine + valine, arginine + histidine, alanine, aspartic acid + glycine + + serine and cystine + lysine contents and with those of September the differences in the amounts of tryptophan, methionine + valine, arginine + histidine were significant. The differences are as a rule more pronounced in the case of the samples taken in July than with those collected in September.

According to the data of the analyses carried out in July (Table 3) there are no significant differences in the total nitrogen contents and in the nitrogen contents of the acid hydrolysates of proteins between virus-infected and healthy vine leaves. On the other hand the nitrogen content of the alkaline hydrolysates of proteins is significantly lower in the infected leaves than in healthy ones. Of the amino acids contained in proteins there is a significant reduction in tryptophan and a significant increase in arginine + histidine in virus-infected leaves as compared to the control.

Table 2

Alcohol soluble amino acid content of yellow mosaic virus-infected and healthy vine leaves

Time of sampling and denomination of the sample	Amino acid content of hydrolysates of alcoholic extracts								
	Trypto-phan	Leucine + Ph. alanine	Methi-onine + valine	Tyro-sine	Alanine	Glutam-ic acid + thre-onine	Aspart-ic acid + gly-cine, serine	Argi-nine + histi-dine	Cystine + ly-sine
	mg/100 g dry weight								
July, 1965									
Virus-infected leaf	1.75	11.93	11.16	9.60	14.40	25.56	21.93	20.37	14.04
Healthy leaf	1.09	9.88	8.65	7.22	9.49	27.04	16.77	7.53	8.79
September, 1965									
Virus-infected leaf	0.97	7.53	8.47	9.51	15.14	22.98	12.71	19.23	9.32
Healthy leaf	0.53	6.34	5.29	8.66	15.09	17.42	10.15	9.29	7.88
Significant difference	0.12	—	2.28	—	3.29	—	4.61	4.56	2.49

Table 3

Protein nitrogen and amino acid contents of yellow mosaic virus-infected and healthy vine leaves

Time of sampling and denomination of the sample	Alco-hol inso-luble nitro-gen	Protein nitrogen in		Content in amino acids contained in proteins								
		alka-line	acid	Tryptophan	Leucin + Ph. alanine	Methionine + valine	Tyrosine	Alanine	Glutamic acid + threonine	Aspartic acid + glycine	Arginine + histidine	Cystine + lysine
		hydrolysate		g/100 g dry weight								
July, 1965												
Virus-infected leaf	6.22	1.81	3.39	0.14	1.19	0.78	0.90	1.29	1.56	1.53	1.59	0.86
Healthy leaf	5.94	2.13	3.55	0.19	1.29	1.18	1.53	1.17	1.95	1.37	1.28	0.88
Significant difference	—	0.26	—	0.03	—	0.43	0.81	—	—	—	0.22	—

Discussion

Papers dealing with the nitrogen metabolism of virus-infected plants report altered levels of free amino acids, amino acids contained in proteins, total nitrogen and protein nitrogen. SEHGAL and BOONE (1964) have shown that in virus-infected

strawberry plants asparagine as well as free aspartic acid and glutamic acid accumulate whereas the amount of amino acids contained in proteins decreases. In tobacco leaves infected with tobacco mosaic virus or leaf curl virus there are more aspartic acid, histidine and lysine (LALORAYA and JEE, 1955) and in potato leaves infected with potato leaf roll virus there are more free glutamic acid and valine (REINDEL and BIENENFELD, 1956) than in the controls. RAJARAO et al. (1956) have found that virus infection results in a decrease in the aspartic acid, methionine and valine contents of leaf proteins. HENKE (1956), WYND (1943), WILDMAN et al. (1949) and ORLOB and ARNY (1961) reported a decrease in the total nitrogen and protein nitrogen contents of leaves upon virus infection. In the authors' view this decrease is due to the inhibition of protein synthesis and to an increased rate of degradation of leaf proteins in infected plants. ELBERTZHAGEN (1958) and MICZYNSKI (1961) have demonstrated that in plants infected with tobacco mosaic virus and potato mosaic virus respectively, total and alcohol soluble nitrogen contents decrease at an early stage of infection and increase at a later stage, as compared to the control. PORTER and WEINSTEIN (1960) have found a variation in the amounts of nitrogen compounds depending on how long the tobacco plants had been infected with cucumber mosaic virus.

The literary data mentioned above unequivocally prove that virus-infected leaves exhibit a higher content in alcohol soluble free amino acids and lower levels of amino acids contained in proteins, total nitrogen and protein nitrogen than the healthy ones. These quantitative differences may be subject to alterations as the infection process goes on. The results presented in this paper are in line with the above findings. We have demonstrated that amino acids contained in alcohol soluble peptides as well as free amino acids occur in higher concentrations in yellow mosaic infected leaves than in healthy ones. In the infected leaves we found in the case of both samplings significantly higher quantities of alcohol soluble tryptophan, methionine + valine, arginine + histidine and alcohol soluble nitrogen than in the control leaves. As far as amino acids contained in proteins are concerned more arginine + histidine and less tryptophan was found in infected leaves than in healthy ones. The rise in alcohol soluble nitrogen and amino acid contents as well as the decrease in the amounts of leaf protein and some amino acids contained in proteins suggest that upon virus infection nitrogen metabolism of leaves is stimulated and leaf proteins mobilized. It has to be noted, however, that as the disease develops, even in terms of the relatively short period investigated, the increments found in the alcohol soluble nitrogen and amino acid contents show a decreasing trend.

The change in the tryptophan content of infected leaves is of special interest. Accumulation of tryptophan in leaves leads to a deficiency in B-indole-acetic acid, which in turn results in stunting as reported by BOSC and BERLAN (1961) in the case of "court noué" of vine. It may be hypothesized that the increase in alcohol soluble tryptophan at a probability level of P. 0.1 % as shown to occur in this paper in yellow mosaic virus-infected vine leaves is actually responsible for the reduction of growth of vine shoots (Fig. 4).

Acknowledgement

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Nitrogen Metabolism and Susceptibility to Smut in Different Maize Varieties

By

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In the infected tissues there is an accumulation of proteins. The protein content of the galls shows a concomitant increase with the increasing susceptibility of the varieties tested.

Leaf galls of plants of less susceptible varieties have less amino-nitrogen than control leaves. With moderately and highly susceptible varieties there is an accumulation of amino acids in the leaf galls. Amino nitrogen content of infected tissues increases parallel with increasing susceptibility. Amino acid accumulation can be demonstrated in infected stem tissues as well.

In young stalk galls and in internodes bearing galls there is a change in the relative amounts of free amino acids with aspartic acid and glutamic acid predominating. By the time the chlamydospores appear the free amino acid content of the infected tissues and of the internodes bearing galls decreases to a considerable extent. Thus, accumulation of amino acids occurs only at an early stage of the host-parasite relation.

In the infected internodes there is a concomitant decrease in the inorganic nitrogen level.

The total nitrogen content of the primordial leaves above the galls considerably decreases. The reduced growth of infected plants may be partly explained by the influence of the infected tissues on non-infected organs by reducing their N-content.

The results presented in this paper further support the hypothesis that the nitrogen metabolism of the host-parasite complex is a deciding factor in determining susceptibility.

Introduction

The concentration in host tissues of compounds serving as nutrients for the parasite and the sensitivity of the systems synthetizing these compounds to the toxic effects of the parasite are important factors in the formation of the host-parasite relation, in the appearance of symptoms and finally in the degree of susceptibility of the host. Therefore, for a successful control of parasites it is absolutely necessary to study host-parasite relations from a nutritional-physiological point of view.

The significance of the nitrogen-nutrition of the host plant in the infection process has been studied in a number of host-parasite relations. Most studies have been carried out in this respect with rust diseases of cereals. In general infection results in the accumulation of soluble N-compounds, primarily amino acids (SHAW and COLOTELO, 1961).

Changes in the amino acid content of maize tissues infected with *Ustilago maydis* have been reported by DEVAY and ROWELL (1954) as well as by TURIAN (1962). Our own experimental results (PETHŐ, 1960, 1962, 1964a, 1964b, 1964c) have shown free amino acid content of maize tissues infected with *U. maydis* to decrease with less susceptible varieties and to increase with highly susceptible ones. Among the free amino acids which occur in higher quantities in maize tissues aspartic acid and glutamic acid usually accumulate depending on the degree of susceptibility of the variety whereas the amount of alanine decreases (PETHŐ, 1964b, 1964c).

Maize varieties considerably differ in their free amino acid content even without infection (PETHŐ, 1964a, 1964c). No definite correlation was found, however, between susceptibility and free amino acid content without infection when different varieties were compared.

Protein content of host plant tissues usually increases upon infection. This is true for maize tissues infected with *U. maydis* as well (PETHŐ, 1960). Accumulation of N-compounds in the infected tissues greatly affects the non-infected tissues of the host plant (PETHŐ, 1964a, 1964b, 1964c).

The above data show that in some cases, e. g. with the crown gall and maize complex N-metabolism is an important factor of susceptibility. The present paper reports further data to this problem.

Material and Methods

For the experiments in addition to the maize variety "*Mindszentpusztai Sárga Lófogú*" (MPS) some inbred lines of local varieties were also used. The varieties have been chosen as to represent a whole range of slightly, moderately and highly susceptible varieties (Table 1).

The degrees of susceptibility of the varieties shown in Table 1 were confirmed in our own experiments. The variety MPS is slightly susceptible.

Table 1

Some important characteristics of the inbred lines of the local varieties used in the experiment

Local variety	Convar.*	Origin*	Ripening*	Inbred for years	Susceptibility to *U. maydis*
50.	dentiformis	Magyaróvár	medium	5	slight*
70.	dentiformis	Medgyesháza	medium	4	medium*
71.	dentiformis	Középhídvég	medium	4	medium
27.	dentiformis	Félegyháza	medium	4	high
62.	dentiformis	Újszentiván	medium-late	5	high*

* Data by G. Székács

Sowing was carried out on April 21, 1964 in a chernosem soil, infection by the end of June at certain intervals andsamp ling in the first half of July continuously. Colonies of germinated chlamydospores were shaken horizontally for 36 hours at room temperature. The inoculum thus prepared was applied near the apical shoot with a hypodermic syringe. Tissues of organs in a similar position from healthy plants served as a control.

The organ to be investigated was always freshly prepared by excising it from the plants. Amino-N content was estimated photometrically by the ninhydrine test in alcoholic extracts (PETHŐ, 1964a). Amino acids were separated by paper chromatography) one dimensional, descending, Whatman paper 4, solvent system:

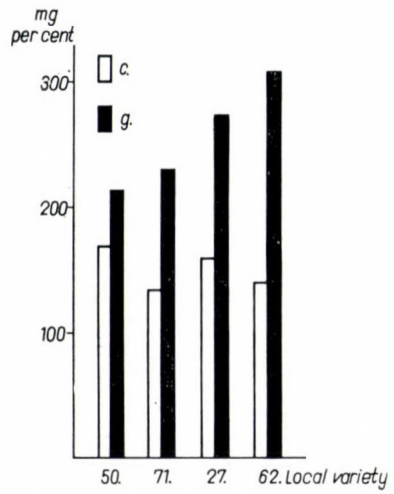

Fig. 1. Effect of crown gall infection on the protein nitrogen content of internodes of maize plants belonging to local varieties differing in susceptibility. g.: gall; c.: comparable internodes of healthy plants

88% phenol: water = 100 : 20) after desalting of the alcoholic extracts (PETHŐ, 1964b) and their quantity was determined by comparing the size and color intensity of the spots developed with ninhydrine with those of standard amino acids of known quantities (HAIS and MACEK, 1961). Protein and total N-contents were determined by the Kjeldahl method. The amount of inorganic and amide-N was estimated by the method of VARNER et al. (1953) following deproteinisation of the water extracts with wolframic acid. The results have been expressed in mg per cent of fresh weight.

Results

In maize tissues infected with *Ustilago maydis* dry matter content increases. E. g. in young and old stalk galls of plants of the local variety 62 the dry matter content was 10.12% and 11.06%, respectively. In the internodes of the same loca-

tion and age of healthy plants it was as low as 7.88 %. Results of earlier experiments suggest that this increase in dry matter content is partly due to an increased protein level.

Figure 1 shows the protein-N content in galls formed on the internodes of infected plants and in similarly located internodes of healthy plants with five different local varieties of maize differing in susceptibility. On the abscissa the varieties figure in the order of increasing susceptibility. It may be seen that the

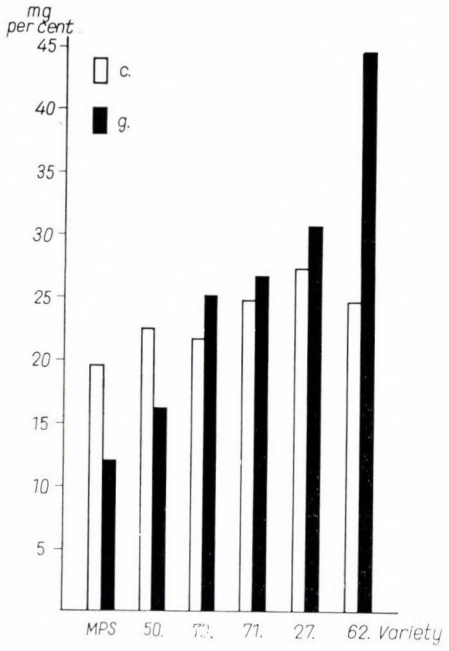

Fig. 2. Effect of crown gall infection on the amino nitrogen content of leaf tissues in six different maize varieties. g.: Leaf galls of infected plants. c.: Comparable leaves of healthy plants

protein-N content is higher in infected tissues (galls) than in healthy ones, and that it increases with increasing susceptibility. The difference between the protein-N contents of healthy and infected tissues is greater with highly susceptible varieties than with moderately susceptible ones.

In infected tissues protein accumulation may be due to an increased synthetic activity of the cells. The prerequisite for an intensive protein synthesis is a higher amino acid content (enhanced amino acid synthesis).

Our earlier investigations (PETHŐ, 1964a, 1964c) have shown that in infected tissues of maize varieties differing in susceptibility the free amino acid content changes with changing susceptibilities of the varieties. Because of the limited number of varieties included in these experiments, however, no definite correlation

could be established between the degree of susceptibility and amino-N content of infected tissues.

In the present experiments six varieties were included in order to be able to draw more reliable conclusions about the correlation mentioned above. As seen in Fig. 2, in the varieties which are represented in the order of increasing susceptibility the amino-N content of the infected tissues shows a parallel increase. With the varieties MPS and N°-50, both slightly susceptible to *U. maydis*, free amino acid content is lower in the infected tissues than in the healthy ones. In the case of

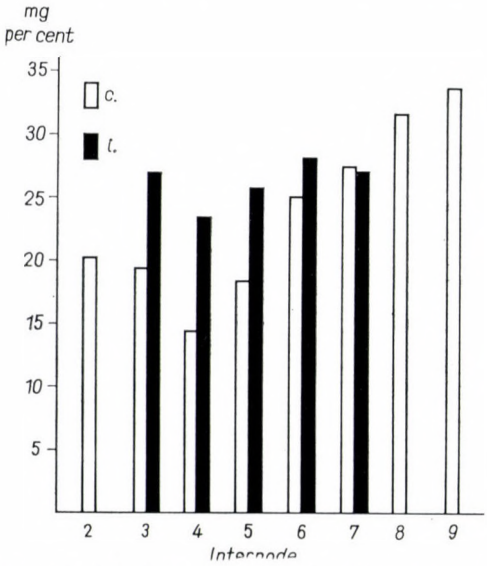

Fig. 3. Effect of crown gall infection on the amino nitrogen content of internodes of maize plants belonging to the highly susceptible local variety 27. i.: Infected internodes c.: Comparable internodes of healthy plants

moderately and highly susceptible varieties there is an accumulation of amino acids in the infected tissues. The difference in amino-N content between infected and control tissues is greatest with the highly susceptible local variety 62.

These results are in agreement with those reported earlier (PETHŐ, 1964a, 1964c). It may be supposed, therefore, that one of the deciding factors in the susceptibility of maize varieties to *U. maydis* is the accumulation of amino acids in the infected tissues. In highly susceptible varieties the conditions prevailing in the infected tissues make a preferential accumulation of free amino acids possible. This is a prerequisite for the intensive protein synthesis necessary for the multiplication of the fungus, for the formation of the hypertrophic tissues and of the chlamydospores. According to this theory the reason for the differences in the susceptibility of different varieties may be sought for in the varying activities of biosynthetic processes leading to the accumulation of amino acids.

Fig. 3 shows the amino-N contents of healthy and infected internodes of plants of the highly susceptible local variety 27. The internodes are numbered acropetally. Amino acids accumulate in infected internodes too. It is surprising that the amino-N content is about the same in all the infected internodes; it does not follow the curve showing the changes in the amino-N levels of the internodes of control plants. This phenomenon explains the observation that in the internodes which are located near the top of the plants the differences in amino-N-content between healthy and infected internodes decrease.

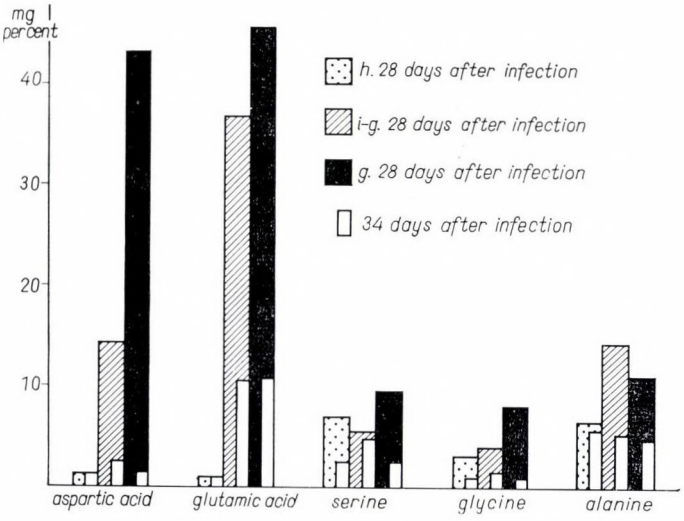

Fig. 4. Effect of crown gall infection on the amino acid content of different tissues of maize plants belonging to the local variety 42, 28 and 34 days after infection. h.: Non-infected internodes. i—g.: Internodes bearing galls. g.: Galls

As far as the amino acid composition is concerned there are remarkable differences between healthy and infected leaf tissues (PETHŐ, 1964b, 1964c). In the infected tissues there is more aspartic acid and glutamic acid, whereas the amount of some other amino acids decreases. Similar changes are to be found in infected internodes as well (Fig. 4). Among the five amino acids which occur in the highest quantities in maize tissues aspartic acid and glutamic acid accumulate both in the infected internodes bearing galls and in the young galls. The amount of the other three amino acids, however, does not increase considerably. This preferential increase in the aspartic acid and glutamic acid levels in infected tissues points to a considerable change in the metabolism of maize plants upon infection. Six days later, when the chlamydospores appear, the amount of the amino acids investigated decreases to a considerable extent both in the internodes bearing galls and in the galls themselves. Thus, amino acids accumulate only at an early stage of infection and later with the appearance of the chlamydospores the amino acids accumulated are used up for protein synthesis.

Figure 5 shows the differences in the inorganic nitrogen contents of healthy and infected tissues. The lower level of inorganic nitrogen in infected tissues is presumably due to an enhanced amino acid synthesis. The above indicate that

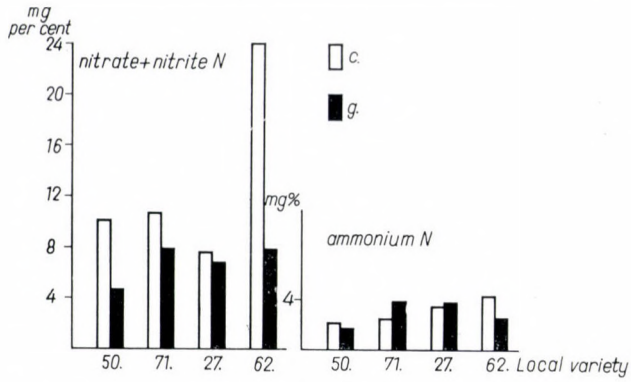

Fig. 5. Effect of crown gall infection on the inorganic nitrogen content of maize plants. g.: Galls. c.: Comparable internodes of healthy plants

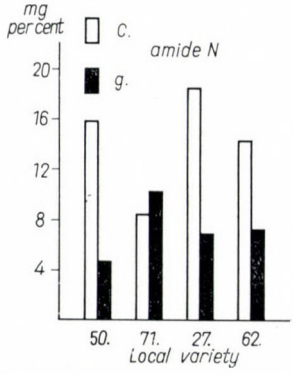

Fig. 6. Effect of crown gall infection on the amide nitrogen content of maize plants. g.: Galls. c.: Comparable internodes of heal thy plants

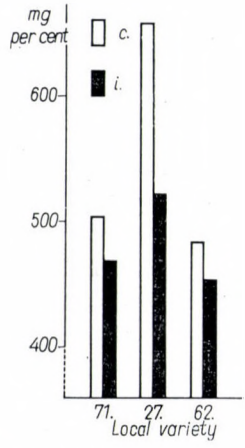

Fig. 7. Total nitrogen content of top leaves of healthy (c.) and crown gall infected (i.) maize plants

the higher amino acid content upon infection is due to a de novo synthesis of amino acids rather than to the hydrolysis of proteins.

The reduced amide content (Fig. 6), as observed with most of the varieties tested, also points to an enhanced amino acid synthesis in the infected tissues. It has to be mentioned, however, that the literary data bearing on this problem are contradictory. DEVAY and ROWELL (1954) have reported a lower glutamic acid

content in the galls as compared to the control, whereas TURIAN (1962) has found the opposite.

It may be suggested that the enhanced protein synthesis in the infected tissues, leading to protein accumulation in the galls, has an unfavourable effect on the nitrogen metabolism of the non-infected organs, inhibiting their nitrogen supply and thereby their protein synthetizing activity and their growth. This hypothesis is supported by the data in Fig. 7. The young top leaves of infected maize plants have a lower total nitrogen content than those of healthy plants. In these leaves there is a nitrogen deficiency which may explain the reduction of growth in infected plants, as observed earlier (PETHŐ, 1963).

Discussion

Ustilago maydis is able to infect any growing organ of the maize plant. There is no maize variety which would be absolutely resistant to this particular pathogen, there are, however, differences in the susceptibility of individual varieties. The pathogen remains localized to the infection site. Therefore, a special attention has to be given to the study of the physiology of this particular host-parasite complex.

In order to be able to develop proper control measures against plant pathogens the study of the physiological aspects of resistance seems to be indispensable. Pathophysiological studies during the last few years have considerably contributed to our knowledge about the physiology of infected tissues. In plant tissues infected by obligate parasites dry matter content increases and an enhanced synthetic activity can be observed. These physiological phenomena accompanying symptom development are mainly characteristic for the eraly stage of the infection process (FARKAS, 1965).

In maize tissues infected with *Ustilago maydis* dry matter content increases. Part of this increase is due to protein accumulation. Shortly after infection a very intensive tissue proliferation sets in. The formation of these hypertrophic tissues involves a very intensive accumulation of substances and an enhanced physiological activity of the cells. This affects the nutrition of the entire plant (PETHŐ, 1964a, 1964c).

The data presented in this paper show that there is a parallelism between the degree of susceptibility and the protein content of the infected tissues in the maize varieties tested. Apparently the metabolism of plants varying in susceptibility is affected by the parasite to a different extent.

The accumulation of proteins in the tissues of plants of highly susceptible varieties is connected with an increased free amino acid content. This correlation between the degree of susceptibility and free amino acid content shows that in the infected tissues of plants belonging to highly susceptible varieties conditions are suitable for an increased rate of amino acid synthesis. A higher keto-acid content which is the prerequisite for an enhanced amino acid synthesis has been established in the infected tissues (PETHŐ, 1964c, TURIAN, 1962). This problem will be dealt with in more detail elsewhere.

The growth of infected plants is inhibited (PETHŐ, 1963). Reduction in growth is directly proportional to the degree of infection. The inhibition of growth can be explained by the fact that the intensive protein synthesis at the site of infection results in a depletion of the nitrogen supply of the organs located above the infection site. The reduced nitrogen content of the top leaves involves a decrease in the protein synthetizing activity of these organs, and this, in turn, leads to the inhibition of growth.

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Rust Resistance Induced by Amino Acids: A Decrease of the Enhanced Protein Synthesis in Rust-Infected Bean Leaves

By

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Half leaves of "Pinto" bean infected with the rust fungus *Uromyces phaseoli* incorporated labeled amino acids both into the acid insoluble and acid soluble fractions more intensively than the uninfected halves of the same leaves. This stimulation of incorporation was more pronounced in the acid insoluble (protein) than in the acid soluble fraction. In a resistant host (variety "Fürj") the rates of incorporations were suppressed upon infection. Similarly, protein synthesis was suppressed in "Pinto" bean leaves where a resistance to rust was induced by treatment with an excess of methionine or serine. Fungal growth was also inhibited, and this may give an explanation for the decreased protein synthesis in the whole host-pathogen complex.

Introduction

There are many indications on the induction of host resistance to pathogens by natural amino acids (VAN ANDEL, 1958; KUĆ, et al., 1959; OORT and VAN ANDEL, 1960; 1961; PAPAVIZAS and DAVEY, 1963). SAMBORSKI and FORSYTH (1960) and SAMBORSKI, ROHRINGER and PERSON (1961) have shown that excessively high levels of histidine, isoleucine, methionine, and serine inhibited leaf rust *(Puccinia recondita)* development on detached leaf sections of wheat. Rust-infected plants are characterized by an increase in the total N-content and in the protein level (SHAW and COLOTELO, 1961; STAPLES, 1964; POZSÁR and KIRÁLY, 1966). In this paper increased incorporation of labeled amino acids into the protein fraction of rust-infected bean leaves and suppression of stimulated incorporations into rust-infected leaves as a consequence of treatment with serine and methionine will be reported.

Materials and Methods

Plant material

Primary leaves of Pinto bean *(Phaseolus vulgaris)*, a variety susceptible to rust *(Uromyces phaseoli)* infection, were used in the experiments. One half of each leaf was infected and the other half served as a control. In some experiments opposite leaves were compared. Incorporations were determined at three stages of the disease: (a) at the chlorotic stage, (b) at the beginning of the sporulation, and (c) at the end of the sporulation stage.

Incorporation of the labeled amino acids

Discs punched out from leaves were floated for 4 hr. on solutions of C^{14}--glycine, C^{14}-serine, S^{35}-methionine or S^{35}-cysteine. Each solution had an activity of 50 μC/100 ml. The specific activity for cysteine, methionine, glycine and serine was 17 mC/mM, 47 mC/mM, 26 mC/mM and 9 mC/mM, respectively. Following floating leaf discs were homogenized in 10 per cent TCA at +4 °C and centrifuged. This was repeated twice. The pellet was then washed with distilled water, centrifuged and used for measuring radioactivity. 0.8 ml of the precipitate suspended in water was put in small planchettes, carefully dried, making thereby an infinite thickness and counted using a gas flow counter (in the case of C^{14}) or a GM thin-endwindow tube (S^{35}) and a proportional scaler. Radioactivity was expressed in counts per minute (c. p. m.) per 200 mg fresh weight.

In experiments where the rust-inhibitory action of methionine and serine was investigated, discs on which "chlorotic" symptoms just started to develop were floated for 4 days on a solution containing 2000 p. p. m. of these amino acids. By the end of this treatment control leaf discs, floated on water, were in the sporulation stage, whereas on leaf discs which were treated with methionine and serine sporulation was inhibited. After 4 days the discs were transferred to a solution of C^{14}-glycine for 4 hr. and incorporation of this amino acid into the acid insoluble (protein) fraction was determined as described before.

Results

The stimulation of amino acid incorporation into rust-infected tissues

As seen in Table 1, S^{35}-cysteine was incorporated at the sporulation stage into the protein fraction of rust-infected leaves more intensively than into that of healthy ones. As is shown, incorporation was stimulated both in the acid soluble

Table 1

Incorporation of S^{35}-cysteine into the acid insoluble fraction of rust-infected and uninfected half leaves of Pinto bean at the sporulation stage

Half leaf	C. p. m./200 mg fresh wt.		Ratio of $\frac{\text{soluble}}{\text{insoluble}}$
	Acid insoluble fraction	Acid soluble fraction	
Healthy	241	2.430	10.0
Infected	458	3.588	7.8
Ratio of radioactivities infected/uninfected	1.9	1.4	—

and insoluble fractions. However, stimulation was more pronounced in the acid insoluble fraction. Therefore, the ratio of incorporation into acid soluble: insoluble fractions was diminished in rust-infected leaves. This indicates that stimulated incorporation into infected tissues cannot be explained solely by a stimulated absorption from the labeled solution but, in part at least, by enhanced protein synthesis.

Incorporations were also stimulated at the chlorotic stage of disease. This is shown in Table 2 where results with four amino acids are represented in both the chlorotic and the sporulation stages. Interestingly, the rates of incorporation were different with different amino acids. At the chlorotic stage the incorporation of C^{14}-glycine, S^{35}-cysteine and C^{14}-serine was stimulated by 25, 39 and 65 per cent, respectively. However, no significant stimulation was observed in the case of incorporation of S^{35}-methionine.

Table 2

Incorporation of amino acids into the acid insoluble fraction of rust-infected and uninfected Pinto bean leaves at two stages of the disease

Compounds	C. p. m./200 mg fresh wt.		
	Healthy	Infected	
		Chlorotic stage	Sporulationstage
S^{35}-Methionine	241	258	349
S^{35}-Cysteine	267	371	608
C^{14}-Glycine	683	856	3.975
C^{14}-Serine	397	655	1.814

At the sporulation stage, incorporations were enhanced to a higher extent. Twice as much S^{35}-cysteine, 4.5 times as much C^{14}-serine, and 5 times as much C^{14}-glycine were incorporated into rust-infected half leaves as into the healthy halves of the same leaves. At this stage of the disease the stimulation of incorporation of S^{35}-methionine showed a comparatively low rate (44 per cent). Generally speaking, the S^{35}-containing amino acids were incorporated to a lesser extent in this experiment than were C^{14}-glycine or C^{14}-serine.

Incorporation of amino acids in a resistant host

By using the variety "Fürj" as a host we were able to follow the changes of protein synthesis in an incompatible host-pathogen complex. As shown in Table 3 there is no stimulation in the incorporation of C^{14}-glycine or S^{35}-cysteine at the chlorotic stage of the disease. Even in a later stage, namely in the necrotic phase, the stimulation is rather low (64 per cent with C^{14}-glycine and 32 per cent with S^{35}-cysteine) as compared to that in the variety "Pinto" (a compatible host-pathogen

Table 3

Incorporation of C^{14}-glycine and S^{35}-cysteine into the acid insoluble fraction of rust-infected and uninfected half leaves of Fürj bean (resistant)

Compound	Healthy	C. p. m./200 mg fresh wt.	
		Infected	
		Chlorotic stage	Necrotic stage
C^{14}-Glycine	151	168	248
S^{35}-Cysteine	287	262	379

combination). In this case the contribution of the fungal body to the incorporation of amino acids into the protein fraction must be very low.

Suppression of protein synthesis by treatment with methionine or serine

According to the data shown in Table 4 treatment of leaf discs with methionine or serine greatly suppresses the enhanced incorporation of C^{14}-glycine into rust-infected tissues. Incorporation into the protein fraction increased only by 36 per cent in infected leaf discs pretreated with methionine or serine for 4 days before transferring the discs to a solution containing C^{14}-glycine. On the contrary, incorporation of this amino acid increased by 100 per cent or more when the infected discs were floated on water before the labeled glycine was added. In this experiment stimulation of incorporation in the control (floated on water) as a consequence of rust infection did not attain the value indicated in Table 2. This is because the leaf discs were floated for 4 days before the incorporation experiment was started. During this period the leaves began to senesce and this lowered the rate of protein synthesis. Still, the tendency to increased protein synthesis is similar in both experiments.

Table 4

Incorporation of C^{14}-glycine into the acid insoluble fraction of rust-infected and uninfected leaf discs of Pinto bean at the beginning of the sporulation stage following treatment with methionine or serine for 4 days

Pretreatment	C. p. m./200 mg fresh wt.	
	Healthy	Infected
Water	490	1.126
Methionine (2000 p. p. m.)	368	373
Serine (2000 p. p. m.)	580	790

As a consequence of treatment with methionine or serine rust development was fully inhibited (sporulation was suppressed). In these experiments the fungal body did not contribute to a considerable extent to the incorporation of amino acids.

Discussion

It is known from earlier results of SHAW and COLOTELO (1961) and STAPLES (1964) that the protein level of rust-infected susceptible hosts is higher than that of healthy ones. The results presented in this paper indicate that an *enhanced incorporation* of amino acids into the protein fraction also occurs as a consequence of rust infection. Most probably this is so because the relatively large mass of fungal body contributes to the stimulation of incorporations. For example, STAPLES and LEDBETTER (1958) have shown by microradioautography that tritium-labeled glycine fed to infected leaves was incorporated mainly into the fungal mycelium and uredospores. Similarly, SYDOW and DURBIN (1962) claim that at least in the later stages of rust infection chemical changes in diseased wheat tissues may be ascribed mainly to the fungus. Consequently it is suggested that protein increase is due first of all to the protein synthesis of the pathogen also in our experiments.

In the resistant host both fungal development and rates of incorporations were suppressed. The action of treatment with methionine or serine on fungal development as well as on amino acid incorporations was quite similar. Here too, both fungal growth and the stimulation of protein synthesis were suppressed. Therefore, an excess of the above-mentioned amino acids may induce not only resistance but also a decrease in the enhanced protein synthesis in rust-infected beans. One can explain this decrease by the inhibited growth of the pathogen which mainly contributes to the enhanced incorporation of amino acids.

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Effects of Different Levels of Potassium, Alone or in Combination with *Fusaria* spp., on the Nutritional Status of Cotton Plants

By

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1) Sand culture experiments were conducted to investigate the effects of different levels of potassium, alone or in combination with either *Fusarium oxysporum f. vasinfectum* or *Fusarium moniliforme*, on the chemical composition of wilt-resistant "Ashmouni" and susceptible "Karnak" cotton variety plants.

2) Increasing the potassium level in the culture solution up to 500 p. p. m. is accompanied by an increase in the nitrogen content in both varieties, while at higher potassium levels the content of nitrogen was decreased.

3) The increase in potassium supply of the sand culture up to 500 p. p. m. decreased the phosphorus content of plant tops while 750 and 1000 p. p. m. potassium caused an increase in the phosphorus content of tops.

4) There is a parallelism between the potassium content of plant tops and its concentration in the culture medium.

5) The calcium, magnesium and sodium contents of plant tops decreased with the increase of potassium concentration in the nutrient solution.

6) Phosphorus contents of plant tops were reciprocal to their nitrogen contents as affected by varying levels of potassium in culture medium.

7) Inoculation with *Fusarium oxysporum* or *Fusarium moniliforme* induced a further increase or decrease of the chemical constituents of cotton plants, as compared to the controls.

Introduction

It has been shown in the previous works that potassium affects plant growth. However, the specific role of potassium in the uptake of other elements by higher plants is not well understood. An inverse correlation exists between potassium and calcium contents in alfalfa (FONDER, 1929). A low supply of potassium in the soil might cause an increased absorption of other elements such as magnesium and phosphorus in tomato (JONSTON and HOAGLAND, 1929). The effect of ommitting potassium from the fertilizer might also result in an increased absorption of either nitrogen or phosphorus by plants in general (THOMAS, 1930). Other workers also support these results (COLBY, 1933, PHILLIPS et al., 1934; MCCALLA and WOOFORD, 1935, 1938; OPTIZ, 1943; LAFON and CAUILLARD, 1953; SHEAR et al., 1953; and others) based on work on various plants such as prune, tomato, wheat, grape, etc.

On the other hand, potassium in the form of a fertilizer might also affect its own uptake by the higher plant, as exemplified by the work of SKINNER et al. (1944) on the uptake of potassium by the cotton plant. Increased amounts of potassium in the fertilizer had a two-fold effect, i.e. they increased the percentage of potassium, and at the same time, reduced the percentage of magnesium, absorbed by the plant. Other elements were similarly affected, i.e. nitrogen, calcium and phosphorus. Other workers obtained similar results on studying the effect of potassium on either its own uptake or on that of other elements (GILBERT et al., 1951; and MCEVOY, 1951).

Microorganisms or their metabolic products can also exert an influence on the growth of higher plants (WENT 1945, 1949; SKOOG, 1947; ZIMMERMAN and HITCHCOCK, 1948 and STEINBERG, 1947), among others through the formation or destruction of plant growth substances.

Therefore, the present work was aimed to investigate the effect of different levels of potassium alone or in combination with growth promoting factors such as gibberellic acid and other related substances produced by *Fusarium moniliforme* (SANDEGREN and BELING, 1959; DAHLSTROM and SFAT, 1961) or other growth factors of vitamin, amino acid and auxin nature produced by *Fusarium oxysporum f. vasinfectum* (MOSTAFA and NAIM, 1948; NAIM et al. 1957 and MONTASIR and YOUSSEF, 1960), on the chemical composition of cotton plants.

Materials and Methods

Two cotton varieties, "Ashmouni" and "Karnak", were chosen for the present study. The former is known to be highly resistant against vascular-wilt disease, while the latter is susceptible to vascular-wilt disease caused by *F. oxysporum*. Sand cultures with varying concentrations of potassium were prepared (SHAROUBEEM et al. 1965). The experiments carried out can be grouped into three main series to all of which potassium sulphate was added to supply extra potassium to the basic culture solution in increasing concentrations of 50, 100, 150, 200, 250, 300, 500, 750 and 1000 p.p.m. The first and second series were inoculated with *Fusarium moniliforme* and *Fusarium oxysporum* respectively, while the third series was kept without inoculation for controls.

Seedlings of cotton plants, equal in height and vigour, were transplanted, three per container. In all the three series, treatments were carried out in triplicate for each of the two cotton varieties.

All nutrient solutions were adjusted to pH 6.8. Decrease in weight of each pot was compensated every day by additional amounts of the same solution. All plants were kept in a glasshouse at a temperature ranging from 25 to 35 °C.

Strict adherence to sampling at the proper stage of development is of fundamental importance, because after the stem and leaves are fully grown, variations in some index values of phosphorus and potassium become uncontrollable, since as the leaves age gradually, they become depleted of their mobile nutrients (LUN-

DEGARDH, 1943). For this reason, it has become apparent that the analysis, to be of significance, should be carried out on plants before flowering.

Certain investigators have restricted sampling for analysis to a particular time of the day, to avoid effects of the diurnal variation in the leaf composition, as suggested by CHAPMAN (1941), NIGHTINGALE (1942), and CHAPMAN and GREY (1949). During the present work, samples were taken between 7 and 10.30 a. m.

Plants, six weeks old, were uprooted, the tops were separated from the roots. The shoots were quickly, but thoroughly, washed with distilled water (JACOBSEN, 1945), and then transferred to a ventilated electric drying oven, at 105 °C., for 24 hours, cooled in a desiccator and weighed. The dried shoots were finely ground using a hard glass-mortar, then, a known weight was digested using conc. sulphuric acid and clarified with hydrogen peroxide (LINDNER, 1944 and WOLF, 1944). Total nitrogen was determined in aliquots of the peroxide digested material using the micro-Kjeldahl distillation apparatus developed by MARKHAM (1942). Magnesium was determined by the method of LINDNER (1944) using titan yellow. The determination of phosphorus was carried out by amino naphthol sulphonic acid reagent (WOLF, 1944) as the blue colour produced by this compound does not fade quickly. For the determination of calcium, potassium and sodium a Flame-photometer was used.

Results

(1) Total Nitrogen content of tops:

Fig. 1 summarizes the effect of varying concentrations of potassium as well as different inoculation treatments on the total nitrogen content of the tops of plants of both cotton varieties. It is interesting to note that these results show resemblance

Table 1

Effect of varying concentrations of potassium on the chemical composition of 6-weeks old "Karnak" cotton variety plants, grown in non-inoculated sand cultures. Percentages of elements are calculated on a dry weight basis

Conc. of K in p. p. m.		% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
0		1.42	3.63	0.85	4.00	2.15	2.96
50		1.85	3.22	2.92	3.49	1.09	1.90
100		2.25	2.75	3.28	3.29	0.93	1.75
150		2.65	2.37	3.88	2.79	0.83	1.65
200		3.15	1.84	4.25	2.54	0.71	1.52
250		3.56	1.45	4.45	2.30	0.61	1.43
300		3.91	1.10	4.86	2.09	0.55	1.36
500		4.25	0.83	5.30	1.95	0.25	0.96
750		3.13	1.87	6.09	1.85	0.25	0.66
1000		1.75	3.26	6.65	1.73	0.25	0.40
Sig. Diff.	P. 0.10	0.08	0.11	0.19	0.12	0.04	0.05
	P. 0.05	0.10	0.14	0.26	0.15	0.05	0.06

to the data of dry weights of tops as affected by different potassium levels and inoculation treatments. These results show a relationship between the nitrogen content of the plant and its dry weight. In other words, the nitrogen content of

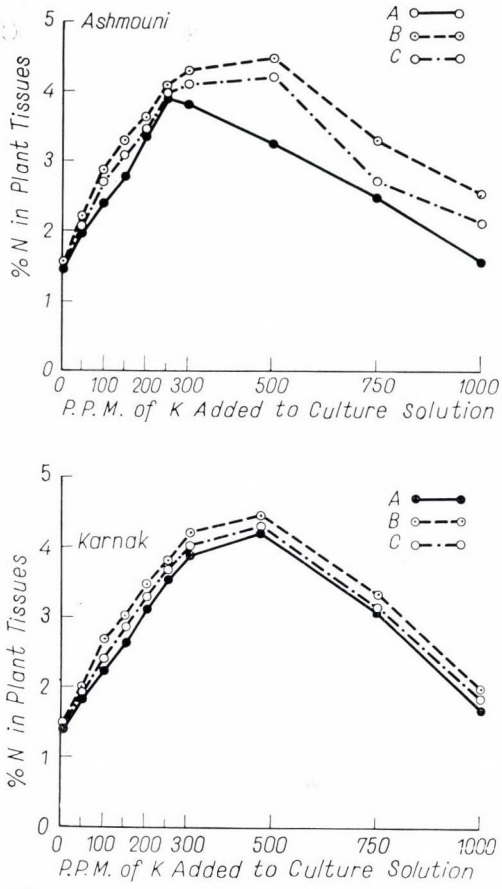

Fig. 1. Effect of varying concentrations of potassium in sand culture inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, or non-inoculated culture, on which are raised susceptible "Karnak" and wilt-resistant "Ashmouni" cotton plants on the nitrogen content of plant tops: A = non-inoculated sand culture; B = inoculated with *F. moniliforme;* C = inoculated with *F. oxysporum* f. *vasinfectum*

"Ashmouni" and "Karnak" plant tops increased with the increase of potassium supply in the nutrient medium up to 500 p. p. m., while at higher concentrations viz. 750 and 1000 p. p. m. potassium, the nitrogen content decreased.

Concerning inoculation treatment, it was found that the nitrogen contents of plant tops raised on inoculated sand cultures with either *F. moniliforme* or *F. oxysporum* were higher than their criteria of plant tops raised on non-inoculated sand cultures when compared under similar potassium levels.

(II) Phosphorus content of tops:

It can be seen from Fig. 2 that increasing the application of potassium up to 500 p. p. m. in either non-inoculated or inoculated sand cultures with *F. moniliforme* or *F. oxysporum*, causes a decrease in the phosphorus content of both "Ashmouni" and "Karnak" cotton variety plant tops to a minimum. Further increase of potassium concentration up to 1000 p. p. m. was accompanied by an increase of the phosphorus content of plant tissues.

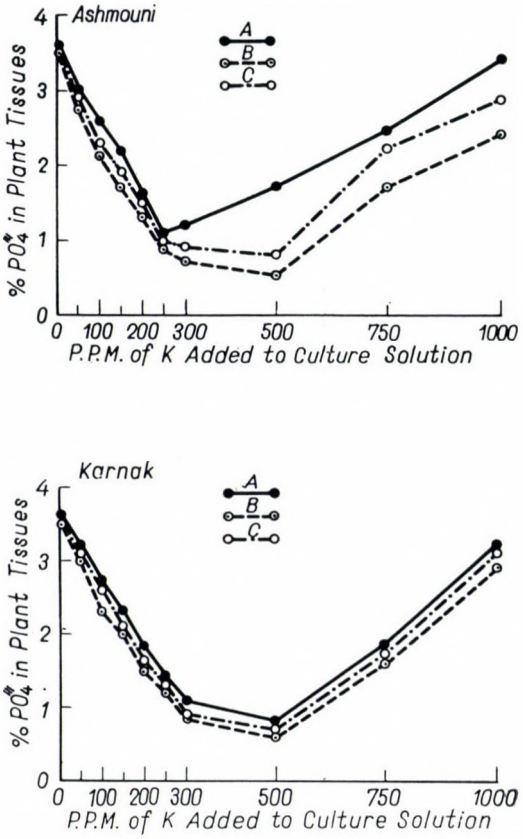

Fig. 2. Effect of varying concentrations of potassium in sand culture inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, or non-inoculated culture, on which are raised susceptible "Karnak" and wilt-resistant "Ashmouni" cotton plants on the phosphorus content of plant tops: A = non-inoculated sand culture; B = inoculated with *F. moniliforme;* C = inoculated with *F. oxysporum* f. *vasinfectum*

The addition of either *Fusaria* spp. to the sand culture containing different levels of potassium, depressed the phosphorus content of plant tops as compared with those of the controls raised in non-inoculated sand cultures.

Table 2

Effect of varying concentrations of potassium on the chemical composition of 6-weeks old "Karnak" cotton variety plants, grown in sand cultures inoculated with *Fusarium moniliforme*. Percentages of elements are calculated on a dry weight basis

Conc. of K in p. p. m.	% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
0	1.51	3.52	1.22	4.50	2.28	3.09
50	2.03	3.01	2.42	3.84	1.24	2.15
100	2.71	2.32	2.88	3.49	1.13	2.04
150	3.05	2.04	3.32	3.14	1.04	1.95
200	3.48	1.53	3.71	2.79	0.95	1.90
250	3.85	1.20	4.13	2.54	0.83	1.74
300	4.22	0.84	4.27	2.44	0.79	1.70
500	4.48	0.60	4.92	2.19	0.39	1.30
750	3.39	1.62	5.59	2.09	0.39	0.87
1000	2.04	2.96	6.27	1.89	0.39	0.55
Sig. Diff. P. 0.10	0.10	0.21	0.52	0.06	0.01	0.05
Sig. Diff. P. 0.05	0.20	0.34	0.71	0.07	0.01	0.06

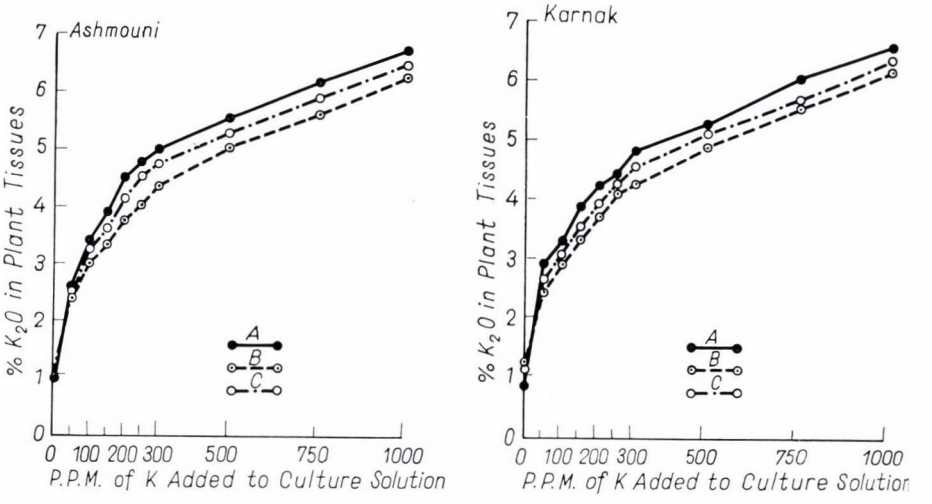

Fig. 3. Effect of varying concentrations of potassium in sand culture inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, or non-inoculated culture, on which are raised susceptible "Karnak" and wilt-resistant "Ashmouni" cotton plants, on the potassium content of plant tops: A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated with *F. oxysporum* f. *vasinfectum*

(III) Potassium content of tops:

The potassium content of plant tops increased by increasing the concentration of potassium in the culture solution. This result (Fig. 3) is shown by all experimental plants in both non-inoculated and inoculated sand cultures with either

Table 3

Effect of varying concentrations of potassium on the chemical composition of 6-weeks old "Karnak" cotton variety plants, grown in sand cultures inoculated with *Fusarium oxysporum*. Percentages of elements are calculated on a dry weight basis

Conc. of K in p. p. m.	% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
0	1.48	3.53	1.15	4.15	2.20	3.01
50	1.93	3.10	2.65	3.66	1.17	2.05
100	2.42	2.61	3.06	3.39	1.05	1.90
150	2.88	2.13	3.57	2.98	0.95	1.83
200	3.31	1.65	3.95	2.66	0.84	1.74
250	3.74	1.32	4.28	2.45	0.72	1.63
300	4.04	0.90	4.59	2.25	0.66	1.57
500	4.35	0.75	5.17	2.05	0.30	1.21
750	3.18	1.75	5.76	1.97	0.30	0.72
1000	1.93	3.10	6.43	1.80	0.30	0.46
Sig. Diff. P. 0.10	0.07	0.28	0.33	0.07	0.01	0.06
Sig. Diff. P. 0.05	0.09	0.35	0.41	0.09	0.01	0.07

Table 4

Effect of varying concentrations of potassium on the chemical composition of 6-weeks old "Ashmouni" cotton variety plants, grown in non-inoculated sand cultures. Percentages of elements are calculated on a dry weight basis

Conc. of K in p. p. m.	% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
0	1.49	3.60	0.97	4.44	2.09	2.90
50	1.99	3.02	2.59	3.64	1.09	1.93
100	2.40	2.64	3.40	3.01	0.81	1.69
150	2.81	2.19	3.90	2.65	0.71	1.55
200	3.39	1.64	4.50	2.34	0.60	1.47
250	3.93	1.10	4.78	2.24	0.52	1.36
300	3.84	1.21	5.00	2.04	0.42	1.24
500	3.28	1.74	5.59	1.89	0.32	1.11
750	2.51	2.49	6.20	1.79	0.32	0.83
1000	1.58	3.44	6.78	1.64	0.32	0.55
Sig. Diff. P. 0.10	0.10	0.19	0.29	0.08	0.03	0.02
Sig. Diff. P. 0.05	0.12	0.24	0.38	0.10	0.04	0.03

Fusaria spp. However, it is interesting to notice that increase in the potassium content of the plants, due to increasing potassium concentration in the culture medium, is greater when raised on non-inoculated cultures than on the inoculated treatments.

(IV) Calcium content of tops:

Results of the determination of the calcium content of plants are shown in Fig. 4. Increasing the potassium concentration of either non-inoculated or inoculated sand cultures with *F. moniliforme* or *F. oxysporum*, causes a decrease in the calcium content of "Ashmouni" and "Karnak" cotton plants. The presence of either

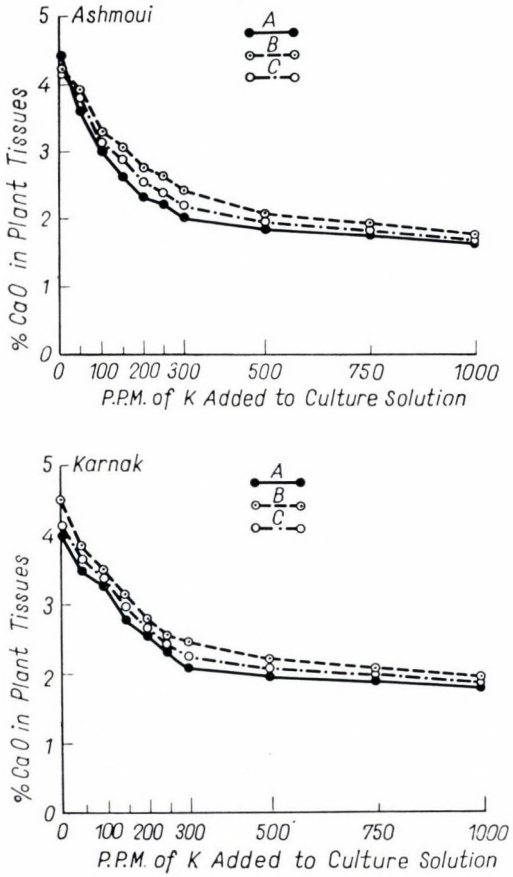

Fig. 4. Effect of varying concentrations of potassium in sand culture inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, or non-inoculated culture, on which are raised susceptible "Karnak" and wilt-resistant "Ashmouni" cotton plants, on the calcium content of plant tops: A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated with *F. oxysporum* f. *vasinfectum*

Fusarium sp. in the sand cultures with different levels of potassium, raises the calcium content of tops to higher levels than those of the controls raised on non-inoculated sand cultures.

(V) Magnesium content of tops:

Figure 5 summarizes the effect of applying different levels of potassium to non-inoculated or inoculated sand cultures with *F. moniliforme* or *F. oxysporum* on the magnesium content of both cotton varieties. It can be seen from the figure that increasing the application of potassium up to 500 p. p. m. in the nutrient medium, irrespective of inoculation, causes a decrease in the magnesium content of all the experimental plants, while higher potassium levels, ranging from 500 to 1000 p. p. m., do not affect the magnesium content of the plant tops. The magnesium content

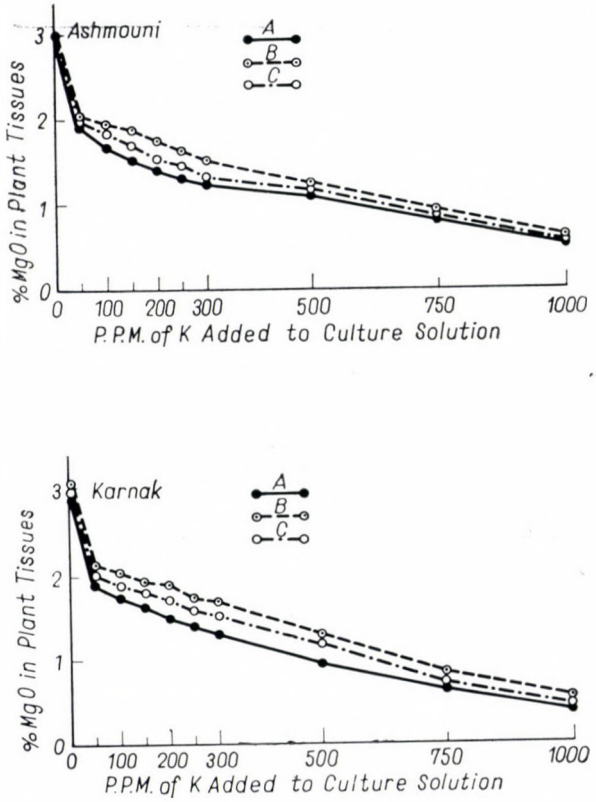

Fig. 5. Effect of varying concentrations of potassium in sand culture inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, or non-inoculated culture, on which are raised susceptible "Karnak" and wilt-resistant "Ashmouni" cotton plants, on the magnesium content of plant tops: A = non-inoculated sand culture; B = inoculated with *F. moniliforme*; C = inoculated with *F. oxysporum* f. *vasinfectum*

Table 5

Effect of varying concentrations of potassium on the chemical composition of 6-weeks old "Ashmouni" cotton variety plants, grown in sand cultures inoculated with *Fusarium moniliforme*. Percentages of elements are calculated on a dry weight basis

Conc. of K in p. p. m.		% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
0		1.53	3.52	1.09	4.19	2.22	3.03
50		2.23	2.77	2.43	3.94	1.23	2.04
100		2.88	2.13	3.02	3.29	1.15	1.96
150		3.30	1.72	3.32	3.09	1.06	1.89
200		3.65	1.30	3.76	2.79	0.95	1.76
250		4.11	0.89	4.03	2.64	0.85	1.64
300		4.32	0.73	4.37	2.42	0.71	1.52
500		4.50	0.55	5.04	2.09	0.52	1.25
750		3.32	1.72	5.62	1.95	0.52	0.94
1000		2.56	2.44	6.31	1.78	0.52	0.62
Sig. Diff.	P. 0.10	0.17	0.07	0.20	0.05	0.02	0.13
	P. 0.05	0.21	0.09	0.25	0.06	0.03	0.16

of the plant tops raised in inoculated sand culture with either *Fusaria spp.* was higher than that of the control plants raised in non-inoculated sand cultures when compared under similar potassium levels.

Table 6

Effect of varying concentrations of potassium on the chemical composition of 6-weeks old "Ashmouni" cotton variety plants, grown in sand cultures inoculated with *Fusarium oxysporum*. Percentages of elements are calculated on a dry weight basis

Conc. of K in p. p. m.		% N	% PO_4	% K_2O	% CaO	% Na_2O	% MgO
0		1.50	3.50	1.02	4.23	2.15	2.96
50		2.08	2.94	2.50	3.80	1.20	2.01
100		2.75	2.35	3.28	3.14	1.01	1.85
150		3.10	1.93	3.61	2.91	0.90	1.70
200		3.46	1.51	4.15	2.59	0.76	1.57
250		3.98	1.02	4.56	2.40	0.68	1.48
300		4.13	0.93	4.75	2.21	0.52	1.33
500		4.25	0.83	5.30	1.99	0.42	1.19
750		2.75	2.26	5.92	1.85	0.42	0.90
1000		2.15	2.90	6.52	1.74	0.42	0.59
Sig. Diff.	P. 0.10	0.13	0.13	0.29	0.04	0.01	0.05
	P. 0.05	0.16	0.16	0.36	0.06	0.01	0.06

(VI) Sodium content of tops:

The sodium content of plants of both cotton varieties grown under different potassium levels, as well as under different inoculation treatments, are summarized in Fig. 6. The increasing supply of potassium in the culture medium caused a decrease in the sodium content of both cotton varieties. The decrease was greater in plants raised in inoculated sand cultures with either *F. moniliforme* or *F. oxysporum*.

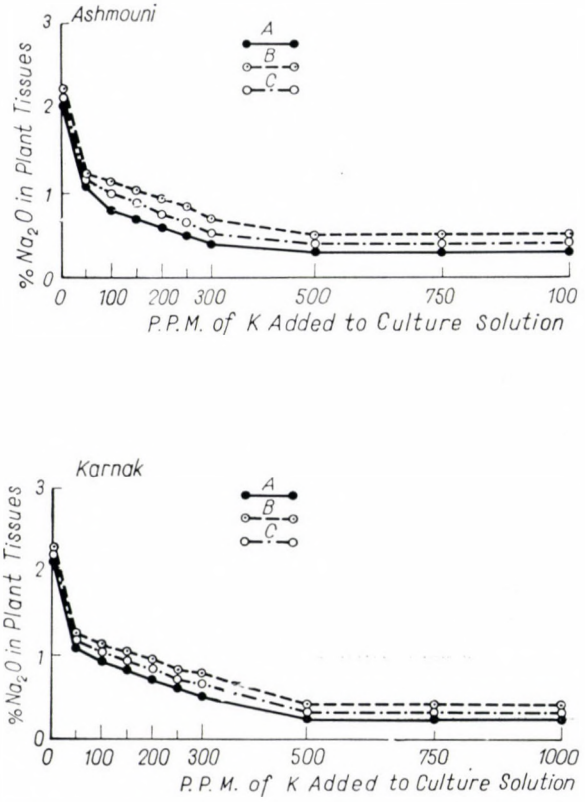

Fig. 6. Effect of varying concentrations of potassium in sand culture inoculated with *F. oxysporum* f. *vasinfectum*, *F. moniliforme*, or non-inoculated culture, on which are raised susceptible "Karnak" and wilt-resistant "Ashmouni" cotton plants, on the sodium content of plant tops: A = non-inoculated sand culture; B = inoculated with *F. moniliforme;* C = inoculated with *F. oxysporum* f. *vasinfectum*

Discussion

Introduction of different levels of potassium to the sand culture, irrespective of inoculation with *Fusaria* produced a marked effect on the nutritional status of cotton plants. As a general rule, the plant nitrogen showed a gradual increase,

while the phosphorus content resulted in a marked decrease, with increase of potassium in the culture medium. The former result is in accordance with the results obtained by McEvoy (1951) and Lafon and Cauillard (1953), while the latter confirms the work of Emmert (1935), Skinner et al. (1944), and Shear et al. (1953). However, high levels of potassium were accompanied with a decrease in nitrogen content and an increase in phosphorus content of plants. Similar results were obtained by Shear et al. (1953) and Lafon and Cauillard (1953) respectively. Thus, it can be assumed that varying levels of potassium affect the nitrogen content in a way reciprocal to its effect on the phosphorus content of cotton plant tops.

The difference of total nitrogen and phosphorus contents of cotton plants due to infection with *Fusarium spp.* at any given concentration of potassium is a result of an effect on plant growth.

It has also been observed that the potassium content of plant tops was affected in a way reciprocal to that of calcium and magnesium on varying the potassium levels in the culture medium. Increasing the amount of potassium in the culture medium causes a continual increase of potassium contents as well as a continual decrease in the calcium and magnesium contents of both cotton varieties. Similar results were obtained by Skinner et al. (1944) and McEvoy (1951), Gilbert (1951) and Shearetal (1963) respectively. This result can be explained on the basis of an antagonistic effect between potassium, on the one hand, and calcium and magnesium, on the other hand, in the culture medium.

It is of great interest to note that the introduction of either *F. moniliforme* or *F. oxysporum* in the culture media receiving varying levels of potassium caused an increase in calcium and magnesium in both cotton varieties above the controls. This difference in increase of both magnesium and calcium may be attributed to the utilization of certain amount of potassium by *Fusarium* which lowered the antagonistic effect between these three elements in the medium (Naim and Sharoubeem, 1963 and Sharoubeem and Naim, 1962).

It is hard to draw a decisive conclusion as to the effect of varying concentrations of potassium in the sand culture on the sodium content of plant tops, as sodium was present in the basic culture solution in an infinitesimal amount, viz. 0.0047 p. p. m. in the form of sodium molybdate. Absorption of sodium by cotton plants is depressed by varying levels of potassium. This may be an indirect effect of the experimental element, results in an increase of potassium content of the plants which in turn results in a decrease in the plant sodium (Cooper et al. 1953).

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Antifungal Activity of Dithiocarbamate Derivatives

By

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The effect of substituents bound to the N or S atoms of dithiocarbamate on the antifungal activity of the compounds obtained was investigated. The 20 different derivatives obtained in our laboratory were tested on the test-organisms *Alternaria tenuis* and *Botrytis allii* in the form of dried spray and wet spray. The activities were evaluated by estimating the degree of inhibition of spore germination and mycelial growth, respectively. N-methyl-N-cyanoethyl derivatives, in contrast to S-cyanoalkyl compounds described earlier, proved to have a considerable antifungal activity on their own, and are not considered to be the precursors of fungicides. This activity may be due to the ability of the cyano-group to form complexes.

Introduction

Derivatives of dithiocarbamate not only represent an important group of fungicides used in plant protection but are the subject of thorough investigations on their application as systemic fungicides (van der KERK, 1956). That is the reason for the ever increasing interest in this group of compounds. As far as their spectrum, mode of action and metabolic effects are concerned reference is made only to some review papers (LUDWIG and THORN, 1960; RICH and HORSFALL, 1961; THORN and LUDWIG, 1962).

In our previous publication (MATOLCSY and JOSEPOVITS, 1966) the methods of synthesis and the physical and chemical properties of some dithiocarbamate derivatives have been reported. In the present paper investigations on the antifungal activity of the compounds synthesized will be described and an attempt will be made to draw some conclusions as to the connections between the formulae and the biological effects of the compounds in question.

Materials and Methods

The compounds used in the experiments are listed in Table 1. In the following the compounds will mostly be referred to by the decimal numbering indicated in Table 1. The data always refer to results obtained with a 50 per cent suspension of the compound concerned in powder form.

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Table 1

Dithiocarbamic acid derivatives with the general formula $R_1-N(CH_3)-C(=S)-S-R$ included in the experiments

R_1 = / R_2 =	CH_3-	$NC-C_2H_4-$	$HOOC-C_2H_4-$	$HOOC-CH_2-$
Na	1.1 Na-dimethyl-dithiocarbamate	2.11 Na salt of N-methyl-N-cyano-ethyl-dithiocarbamic acid		4.1 Na salt of N-methyl-N-carboxymethyl-dithio-carbamic acid
Zn/2		2.12 Zn salt of N-methyl-N-cyano-ethyl-dithiocarbamic acid		
$-S-C(=S)-N(CH_3)-R_1$	1.2 tetramethyl-thiurame-disulfide	2.2 bis (methyl-cyano-ethyl-thiocarbamyl) disulfide		
$-CH_2-COOH$	1.3 N,N-dimethyl-S-carboxy-methyl-dithiocarbamate	2.3 N-methyl-N-cyano-ethyl-S-carboxymethyl-dithio-carbamate	3.3 N-methyl-N-carboxyethyl-S-carboxymethyl-dithio-carbamate	
$-C_2H_4-COOH$	1.4 N,N-dimethyl-S-carboxy-ethyl-dithiocarbamate	2.4 N-methyl-N-cyanoethyl-S-carboxyethyl-dithiocarbamate	3.4 N-methyl-N-carboxyethyl-S-carboxyethyl-dithio-carbamate	
$-N(CH_3)_2$	1.5 dimethylamide-dimethyl-dithiocarbamic acid	2.5 dimethylamide-N-methyl-N-cyanoethyl-dithiocarbamic acid		
$-CH(CCl_3)-CH(CH_3)-NO_2$	1.6 1,1,1-trichloro-2-(N,N-dimethyl-thiocarbamyl-mercapto)-3-nitrobutane	2.6 1,1,1-trichloro-2-(N-methyl-N-cyanoethyl-thiocarbamyl-mercapto)-3-nitro butane		
$-CH_3$	1.7 N,N-dimethyl-S-methyl-dithiocarbamate	2.7 N-methyl-N-cyanoethyl-S-methyl-dithiocarbamate	3.7 N-methyl-N-carboxyethyl-S-methyl-dithiocarbamate	4.7 N-methyl-N-carboxy-methyl-S-methyl-dithio-carbamate

For biological tests conidia taken from 8 to 10 and 10 to 12 days old colonies of *Alternaria tenuis* and *Botrytis allii*, respectively, were used. Earlier observations (BÁNKI, 1959; ANDRISKA et al., 1963; HAMRÁN, 1964) have shown that *Botrytis allii*, due to the size of its conidia and to some physiological characters is about ten times as sensitive to fungicides as *Alternaria tenuis*. Therefore it is exceptionally well suited for testing substances with low activity. This proved to be true in the present experiments as well: ED_{50} values given in Tables 4 to 7 are

in 44 cases higher with *Alternaria*
in one case equal
and in 5 cases higher with *Botrytis*.

It should be kept in mind, however, that out of these 5 cases 3 values refer to compounds with 2 maxima (2.12 and 2.2) and 2 values to a substance (3.7) with undefined effects where ED_{50} was estimated in a deliberate fashion.

The difference in sensitivity between the two molds is clearly shown in Figs 1 to 3 and Tables 7 and 8.

Conidia were brought into contact with the chemicals by either of two methods:

1. Dried spray method (marked as *bp*): Slides were sprayed first with the chemical to be investigated and the suspension of conidia was later placed on the surface of the layer of the dried spray (to imitate preventive spraying).

2. Wet spray method (marked as *f*): The conidia were directly suspended in the spraying solution and placed as such on the slide (to imitate post-infectional spraying, though this method is even more drastic).

With these two methods the chemicals were applied at a rate which was found satisfactory in our previous work (BÁNKI, 1959; ANDRISKA et al., 1963). In method *bp* the chemicals were applied to the slides of unit area in a concentration of about 100 ml per m^2 which is an amount ten times less than that which would sediment during the time of the experiment from the suspension used in method *f*. Of course, in addition to this quantitative difference there was also a qualitative difference between the two forms of application.

The results have shown that with the compounds and fungi employed the inhibitory effect with method *f* was 100 to 1000 times higher than with method *bp*.

The slides were made hydrophobic by using vapour of chloro silane "*Silonite V. 1.*". The plastic layer thus formed was distroyed with chromic acid at 3 places with areas 15 mm in diameter each (PÁRKÁNY, 1961). In this way wells with regular shapes were obtained. The suspensions of conidia placed in these wells had flat drop surfaces very suitable for microscopic observations.

The conidia were suspended in distilled water to which glucose was added to a final concentration of 0.2 per cent.

As activity indices (JUVANCZ, 1965) inhibition of germination and inhibition of growth were chosen. Since the non-germinated conidia were not re-inoculated to the culture medium because of technical difficulties, "inhibition of germination" is defined as the sporostatic effect which developed during 48 to 72 hours. "Inhibi-

tion of growth" as measured by the length of the mycelia also applies to the above time period. This effect was expressed by measuring the actual lengths of the mycelia in a microscope equipped with a net micrometer and not on the basis of size ranges as suggested by McCallan (1929).

The rate of inhibition of germination was expressed in percentage values on the basis of the proportions of non-germinated to germinated conidia. Corrections with the values obtained with untreated controls were made according to Abbot. Inhibition of growth was expressed in percentage values, the length of the untreated controls being taken as 100 per cent. No deformations of mycelia were observed in the samples investigated.

Between the two inhibitory effects there are considerable differences depending on the chemical and physico-chemical properties of the substances. This has been shown in one of our earlier publications (Andriska et al., 1963). In general, with the same dose the percentage value of the inhibition of growth is higher than that of the inhibition of germination. This is easily understandable since with non-germinated conidia there is no growth whatsoever, whereas in the determination of the total length of mycelia the total number of conidia in involved. Thus, if the percentage of non-germinated conidia is X and the growth of the rest of the conidia is not inhibited then the rate of inhibition of growth as compared to the control is $Y = X$. If, however, the growth of the germinated conidia is inhibited, $Y > X$. If the inhibitor of germination enhances the growth of the mycelia, which is a rare phenomenon, Y can be smaller than X. If there is inhibition of growth without germination being affected, only Y is to be considered, of course.

Experimental

From what has been said above it is apparent that for each chemical no more than 8 variables of effectivity can be considered: 2 fungal species × 2 indices × 2 types of spraying. Not always were all the variants used, however. The 2 species (except 1.3) and the two indices with *bp* were always employed, *f*, however, was used only when necessary (see Tables 2 to 5).

The tests were always started by preparing logarithmic dilutions, as usual in biometric experiments (Finney, 1964; Cavalli-Sforza, 1965). If necessary, serial dilutions according to ^{5}log and ^{2}log values were prepared for further tests.

As a standard the simplest member of the group of compounds was used: Na-dimethyl-dithiocarbamate (1.), TMTD (1.2) and Zineb, respectively, according to the formula of the preparation under investigation. To get a clear cut picture of the qualitative characters all chemicals were used in an unstabilized form. This, of course, decreased the accuracy of the quantitative measurements. A more detailed analysis of this phenomenon will be given in the "Discussion".

In about 100 experimental series, with 5 to 6 different doses and with 2 controls in each experiment the germination and growth of about 300,000 conidia were observed and measured, respectively. The results which were not reliable from a qualitative point of view were not taken into consideration. Reliable re-

sults were plotted in the form of log dosage-probit. Points located on a line were connected. Approximations were done by graphical regressions. Special care was taken to follow the laws of weighting (JUVANCZ, 1965; FINNEY, 1964; CAVALLI-SFORZA, 1965). Since the work was done with unstabilized dithiocarbamic acid derivatives, in addition to processes characterized by two maxima such characterized by a "blunt angle" were often observed (e. g. Fig. 2 : 3.4 T; Fig. 3: Alternaria 2.2) similarly to our previous experiments which had been carried out with unstabilized or contaminated Zineb or Maneb preparations, respectively. Sometimes processes characterized by "serrated" curves with 2 maxima of low deviation were also observed. When the amplitude of the deviation was not too large an attempt was made to evaluate the results by linear regression. If the deviation was higher than $\pm$ 0.3 probit, no regression was made use of.

Naturally, with such a heterogeneous material (see Figs 1 to 3) from the point of view of activity not only probit analysis but also some other flexible methods of calculation (e. g. SPEARMAN—KÄRBER) were avoided, because in the present work no attempt was made to carry out quantitative analyses on the relative activities of pairs of chemicals which are characterized by unquestionably straight and parallel processes.

Much rather it was our aim to give a coherent picture which can be developed by using graphical methods and by analysing the parameters obtained by these graphs.

In Tables 2 to 5 the following indices are given in accordance with the system outlined in Table 1.

"log" Dilution used in the experimental series indicated; the number shows the base of logarithm.

"test" An explanation for this symbol has been given above. Other marks: *A* = *Alternaria tenuis; B* = *Botrytis allii*, *bp* = dried spray; *f* = wet spray.

"ED_{50}" and "ED_{95}" Values taken from the graphs in the percentage of the spray and wet spray, respectively. The dose *bp*/surface is about ten times smaller than the dose *f*/surface. These two values give a direct indication as to the effectivity, the zone of the effective doses and the slope of the curve representing the process (DIMOND et al., 1941; JANKE, 1953). In order to give a visual representation of the slope, which is very characteristic of the mode of action, the "angles" with respect to lg and to scales of *x* and *y* as shown in Figs 1 to 3 are also given.

"Angles" By using the data of the 3 latter columns and the well-known equation $Y = a + bx$ (and by substituting ED_{50} for *x* and probit 5 for *y*) the equation of any of our linear curves can be calculated.

"St" refers to the standard used for the individual measurements. 1.1 = Na-dimethyl-dithiocarbamate, 1.2 = T = TMTD; Z = Zineb, Z_M = Zineb (Montecatini).

"Ratio of effectivity". The effect of the individual chemicals can be quantitatively compared to the standard only if it is well centered and parallel to it. Very often, however, these requirements were not fulfilled. In such cases the ratio of effectivity was roughly determined by using the values of both ED_{50} and ED_{95}.

Table 2

Indices of effectivity of dimethyl-dithiocarbamic acid

Compound No. 1	log	Test and assay method	Inhibition of germination				
			ED_{50} per cent	ED_{95} per cent	Angle of inclination in degrees	St	Factor of effectivity as related to St
1.1	10	A bp	0.084	0.470	38	—	—
	2		0.035	0.070	62	—	—
	10	A f	0.001	0.003	49	—	—
	10		0.00003	0.00006	62	—	—
	2	B bp	0.02	0.075	45	—	—
	2		0.0036	0.007	63	—	—
	2	B f	0.0005	0.0013	54	—	—
	10		0.000003	0.000015	39	—	—
1.2	10	A bp	0.006	0.035	37	—	—
	10	f	0.0003	0.013	19	—	—
	10	B bp	0.00063	0.003	40	—	—
	10	f	0.0000004	0.00001	22	—	—
1.3	10	A bp	0.3	—	19	T	1/5
1.4	10	A bp	—	—	—	—	—
	10	B bp	(1.0)	—	29	—	—
1.5	10	A bp	0.1	0.35	46	T	1/4
	10	B bp	0.007	0.030	42	T	3
1.6	10	A bp	0.020	0.044	59	T	1/3
	10	B bp	0.0005	0.0025	39	T	±
1.7	10	A bp	(10.0)	—	11	Z_M	1/50
	10	B bp	2.0	—	13	Z_M	1/50

The numbers express how many times as effective is the chemical in question as the standard.

"Coded effectivity". With chemicals so different in qualitative properties the quantitative characteristics can be determined fairly inaccurately. In the case of non-parallel process differences between chemicals three times more and three times less effective than the standard are not significant. The difference is significant only if the values of ED_{50} or ED_{95} differ by one order of magnitude. Therefore for the classification of the chemicals a decimal code (ANDRISKA et al., 1963) has been used which is based on the value of ED_{95}. ED_{95}, known, is of a major importance for both total effectivity and practice. The numbers in the Figures represent the negative logarithms raised to make a round figure of ED_{95} characteristic for the

derivatives of type 1 (For further explanation see the text)

Coded effectivity	Remarks	Inhibition of growth: ED_{50} per cent	ED_{95} per cent	Angle of inclination, in degrees	St	Factor of effectivity as related to St	Coded effectivity	Remarks
1	degrading	0.008	0.050	36	—	—	2	degrading
1—2	fresh	0.009	0.036	37	—	—	2	fresh
3	degrading	0.003	0.0023	33	—	—	3	degrading
4—5	fresh	0.00003	0.00006	62	—	—	4—5	fresh
1—2	degrading	0.01	0.025	55	—	—	2	degrading
2—3	fresh	0.003	0.0065	59	—	—	2—3	fresh
3	degrading	0.0002	0.0010	39	—	—	3	degrading
5	fresh	0.000001	0.00001	30	—	—	5	fresh
2		0.002	0.010	39	—	—	2	
2		0.000003	0.0013	13	—	—	3	
3		0.00036	0.002	37	—	—	3	
5		0.0000005	0.000013	23	—	—	5	
0		0.01	0.07	27	T	1/10	1—2	
Ø		—	—	—	—	—	Ø	
Ø	25% effect	1.0	—	—	—	—	0	60% effect
1	(T-break)	0.03	0.20	34	T	1/3	1	(T-break)
2	break	0.0043	0.0250	37	T	10	2	
2		0.01	0.04	43	T	1/5	2	
3		0.0007	0.0023	47	T	≈	3	
Ø		(2.0)	—	19	Z_M	1/100	Ø—0	
Ø—0		0.8	(2.7)	47	Z_M	1/100	0	

chemical in question. Chemicals which are practically ineffective were marked by the sign "∅".

Naturally in the case of such rough coding, raising to make round figures from fractions cannot be done according to the ordinary arithmetic rules since if this were done for example chemicals with ED_{95} values of 0.06 and 0.45, respectively, would equally have code number 1. Therefore in raising to make round figures from the code numbers the factor for st, ED_{50}, slope etc. have been taken into consideration. In some cases it seemed necessary to use non-logarithmic fractions as code numbers too, e.g. 3—4 = 3.5; 2—3 = 2.5.

In the columns "Remarks" in the Tables apparent abnormalities are indicated.

Table 3

Indices of effectivity of cyanoethyl-dithiocarbamic acid

Compound No. 2	log	Test and assay method	Inhibition of				
			ED_{50} per cent	ED_{95} per cent	Angle of inclination, in degrees	St	Factor of effectivity as related to St
2.11	2	A bp	0.012	0.018	73	1.1	3
	2	B bp	0.004	0.030	33	1.1	$\approx$
	2	f	0.0006	0.0009	72	1.1	1/4
2.12	5	A bp	0.0045	0.0170	44	Z	$\approx$
	5	f	0.00001	0.00002	61	Z	2
	5	B bp	0.0009	0.0080	31	Z	1/2
	5	f	0.00067 0.000008	0.0023 —	59 21	Z	1/10 $\approx$
2.2	10	A bp	0.02	1.00	19	Z	$\approx$
	10	f	0.00013	0.0014	28	Z	1/10
	10	B bp	0.0004	0.034	28/8/42	Z	$\approx$
	5	f	0.0013	0.0028	59	Z	1/100
2.3	10	A bp	—	—	0	Z	—
	10	B bp	0.8	—	25	Z	1/200
2.4	10	A bp	1.4	—	21	T	1/100
	10	B bp	0.047	0.370	32	T	1/50
2.5	10	A bp	2.000 — 0.002	1.500 0.020 0.010	39/11 34 64	Z_M	$\approx$
	10	A f	0.0031 0.00075 0.000004	— 0.000017 0.000009	5 41 58	Z_M	$\approx$
	5	B bp	0.0044	0.016	45	Z_M	$\approx$
	10	B f	0.0001	0.00032	48	Z_M	3
2.6	10	A bp	0.010	0.025	54	Z	1/2
	10	B bp	0.0022	0.016	32	Z	1/5
2.7	10	A bp	(10.0)	—	22	Z_M	1/50
	10	B bp	0.34	(2.3)	35	Z_M	1/10

derivatives of type 2 (For further explanation see the text)

germination		Inhibition of growth						
Coded effectivity	Remarks	ED_{50} per cent	ED_{95} per cent	Angle of inclination, in degrees	St	Factor of effectivity as related to St	Coded effectivity	Remarks
2		0.009	0.016	66	1.1	$\approx+$	2	
2		0.002	0.017	32	1.1	$\approx$	2	
3		0.0003	0.0006	62	1.1	1/2	3—4	
2		0.0004	0.0060	25	Z	$\approx$	2—3	
5	(break in Z)	0.0000065	0.000015	57	Z	2	5	(break in Z)
2	2-maxima	0.002	0.012	36	Z	1/3	2	
3	(Z is a straight line)	0.00003 0.0002	(0.000002) 0.0013	26 34	Z	$\approx$	3—4	2 maxima (break in Z, too)
0	tendency	0.018	0.40	23	Z	$\approx$	1	1 upward
3	for break	(0.000001)	0.0016	8/36	Z	1/10	3	shift, 3 tendency for
2	intersection, breaks	(0.000001)	0.01	8	Z	$\approx+$	2	break intersection
3	tendency for break	(0.0000003)	0.0001	13	Z	1/5	4	breaks
Ø		—	—	0	Z	—	Ø	
0		0.54	—	18	Z	1/1000	0	
Ø		0.23	1.00	41	T	1/80	0	
1		0.025	0.160	35	T	1/100	1	
2	2-maxima see Fig. 1	3.0000 — 0.0005	1.700 0.01 0.005	39/11 30 70	Z_M	$\approx+$	2—3	2 maxima
5	2 maxima	— 0.0012 0.0000033	(0.1) 0.00003 0.000008	16 21 56	Z_M	$\approx+$	5	2 maxima
2		0.0053	0.014	54	Z_M	$\approx+\approx+$	2	
4		0.00019 (0.00001)	0.0005 —	32 14/52	Z_M		3—4	2 maxima (ED_{35})
2		0.008	0.022	51	Z	1/4	2	
2	break	0.001	0.013	27	Z	1/10	2	break
Ø		1.0	—	35	Z_M	1/20	0	
0		0.13	0.60	40	Z_M	1/10	0—1	

Tables 4 and 5

Indices of effectivity of dithiocarbamic-acid derivatives of types 3-(carboxymethyl-)

Compound No. 3	log	Test and assay method	Inhibition of germination				
			ED_{50} per cent	ED_{95} per cent	Angle of inclination in degrees	St	Factor of effectivity as related to St
3.3	10	A bp	—	—	—	T	—
	10	A bp	—	—	—	Z	—
	10	B bp	1.5	—	21	T	1/1000
	10	B bp	0.047	(~10.0)	13	Z	1/20
3.4	10	A bp	—	—	—	T	—
	10	A bp	—	—	—	Z	—
	10	B bp	0.03 —	0.22 —	33 3	T	1/10
	10	B bp	0.6	—	24	Z	1/200
3.7	10	A bp	0.3	1.0	47	Z_M	1/3
	5	B bp	(1.0)	—	5	Z	1/1000
No 4.							
4.1	10	A bp	0.007	(10.0)	10	1.1	1/10
	10	A bp	(8.0) —	— —	27 —	1.1	1/100 —
	10	B bp	0.004 —	0.02 —	40 —	1.1 1,1	10 —
4.7	10	A bp	(10.0)	—	15	1.1	1/500
	10	B bp	(10.0)	—	11	1.1	1/250

In Table 6 the code numbers and factors for st as shown in Tables 2 to 5 are represented in the average of the values obtained for the inhibition of germination and inhibition of growth. The averages of the values of *f* and *bp* as well as of *A* and *B* cannot be taken because of the great differences in sensitivity. With chemicals showing two maxima (and having more than one, considerably different ED_{95} values) coding was carried out with precaution taking usually the lower values. The effect as related to that of the standard was expressed as a fraction which indicates the corresponding value of the given standard and that of the factor. With chemicals which were easily degraded (e.g. 1.1, 4.1) the averages taken were based on the indices of the fresh substance.

and 4-(carboxymethyl-) (For further explanations see the text)

		Inhibition of growth						
Coded effectivity	Remarks	ED_{50} per cent	ED_{95} per cent	Angle of inclination, in degrees	St	Factor of effectivity as related to St	Coded effectivity	Remarks
Ø		—	—	—	T	—	Ø	
Ø		—	—	—	Z	—	Ø	
Ø		0.3	1.9	37	T	1/1000	0	
0	break	0.2200 0.0370 0.0047	— — —	24/14 19 4	Z	1/10	1—2	2 maxima
Ø	slightly "ser-	—	—	—	T	—	Ø	
Ø	rated" blunt angle	—	—	—	Z	—	Ø	
1	lower part "serrated"	0.074 —	0.32 —	47 7	T	1/25	1	"serrated", break in a blunt angle
Ø—0	degrading	0.3	—	28	Z	1/500	Ø—0	
0		0.2	0.8	48	Z_M	1/5	0	
Ø	(on a wide range up to ED_{16})	—	—	0	Z	—	Ø	
Ø—1	fresh, intersection, break	0.00001	1.0	10	1.1	$\pm$ $\approx$	0—2	fresh
Ø	rapidly degrading	1.5 —	— —	44 —	1.1 1.1	1/500 —	Ø Ø	rapidly degrading
2	fresh	0.00003	0.0008	26	1.1	50	3	fresh
Ø	degrading	—	—	—	1.1	—	Ø	degrading
Ø		1.0	—	19	1.1	1/50	Ø—0	"serrated"
Ø	degrading	0.01	(20.0)	12	1.1	—	Ø—1	intersection, degrading

The most characteristic dosage-responses were also recorded. In Figs 1 to 3 out of the 8 effectivity variants 4 are represented: Inhibition of germination *A bp*, inhibition of germination *B bp*, inhibition of growth *A* and *B f*. The two latter variants are represented together due to the limited number of the variants involved. In this way evaluation is also easier (see next Chapter).

Comparative measurements made at different times and in different combinations necessarily involve some contradictions if the results are represented together. Such contradictions are evident in Tables 2 to 5 and Figs 1 to 3. We think, however, that by a very cautious evaluation the real situation can be interpreted with a fairly high degree of accuracy.

Table 6

Average activity of the compounds synthesized. Averages were taken on the basis of code numbers and of the factors related to the standard, as shown in Tables 2 to 5. *A, Alternaria tenuis; B, Botrytis allii.* Decimal numbering of the columns and lines refers to the compound in question

	1.–		2.–		3.–		4.–	
	Code	Factor of effectivity	Code	Factor of effectivity	Code	Factor of effectivity	Code	Factor of effectivity
–.11 A bp	1.8		2	2			0.5	1/3
A f	4.5							
B bp	2.5		2	1			2.5	20
B f	5		3.3	1/3				
–.12 A bp			2.3	1				
A f			5	2				
B bp			2	1/3				
B f			3.3	1/3				
–.2 A bp	2		0.5	1				
A f	2.5		3	1/10				
B bp	3		2	1				
B f	5		3.5	1/25				
–.3 A bp	0.8	1/7	Ø		Ø			
B bp			0	1/500	0	1/100		
–.4 A bp	Ø		Ø	1/90	Ø			
B bp	Ø		1	1/70	1	1/20		
–.5 A bp	1	1/4	2.3	1				
A f			5	1				
B bp	2	6	2	1				
B f			3.8	2				
–.6 A bp	2	1/4	2	1/3				
B bp	3	1	2	1/7				
–.7 A bp	Ø	1/70	Ø	1/30	0	1/4	Ø	1/150
B bp	0	1/70	0.3	1/10	Ø	1/1000	0	1/250

Evaluation of the Results

In the evaluation which follows the known or supposed chemical, biochemical and physico-chemical properties of the dithiocarbamic acid derivatives will not be taken into consideration. Classification will be based only on the degrees of effectivity of the individual compounds. In the next chapter an attempt will be

Table 7

Distribution of dosage responses to the compounds tested according to the slope of the curves. Curves with 2 maxima are not included in this Table

Angle of inclination 0 to 19°		20 to 39°		over 40°	
Alternaria tenuis	*Botrytis allii*	*Alternaria tenuis*	*Botrytis allii*	*Alternaria tenuis*	*Botrytis allii*
1.4	1.4	1.2	1.2	1.1	1.1
1.7	3.7	1.3	1.7	1.5	1.5
2.3	4.1	2.2	2.12	1.6	1.6
3.3	4.7	2.4	2.3	2.11	2.11
3.4		2.7	2.4	2.12	
4.1			2.6	2.6	
4.7			2.7	3.7	
			3.3		
			3.4		

made to explain our conclusions arrived at in the above manner in chemical and biochemical terms. The agreement between these two kinds of interpretation will show how reliable our results actually are.

The data in Tables 2 to 5 and Figs 1 to 3 show that as far as effectivity is concerned there is a fairly high variability among many dithiocarbamic acid derivatives. Part of them completely lose their activity sooner or later (i.e. get decomposed into fractions which are inactive or which neutralize each other's effect). Such are e.g. 1.1, 3.3 and 4.1.

1.1, which served in many cases as a standard lost completely its high initial activity during 3 months when kept as a 1 per cent solution in water at 5 °C (see e.g. 1.1. in Table 2 and 4.1 in Table 5). This fact, as mentioned earlier, made its use as a standard somewhat equivocal. Of course, for the final evaluations its activity exerted in a fresh state was considered.

4.1 lost its activity even more rapidly. It was always kept at 5 °C in the form of a 1 per cent solution and was allowed to sit only for 1 hour at room temperature before use.

Serial dilutions prepared from 2.2 were kept at about 24 °C. In 48 hours there was a considerable reduction of activity as shown with both fungal species. The dosage-response curves with these two materials were "serrated" and broken, respectively, even if applied in a fresh state. This is an indication for substances which are easily degraded. "Serrated" curves and curves with a blunt angle are shown by 1.5, 2.12 and 3.4 (Table 1 to 3).

2.12 (in its *Bf* form), 2.5 and 3.3 have curves with definitely 2 maxima which can be seen also in Tables 3 and 4. Between chemicals which induce processes characterized by "serrated", broken or 2 maximum-curves, respectively, there is no

Table 8

Order of effectivity of the compounds synthesized according to three different principles

	a) with *Alternaria tenuis*	b) with *Botrytis allii*
According to code numbers	2.12 = 2.5 1.1 = 1.2 = 1.6 = 2.11 = 2.6 1.3 = 1.5 = 2.2 = 4.1 3.7 1.4 = 1.7 = 2.3 = 2.4 = 2.7 = 3.3 = 3.4 = 4.7	1.1 = 1.2 = 1.6 2.2 = 2.5 = (4.1) 1.5 = 2.11 = 2.12 = 2.6 2.4 = 3.4 1.7 = 2.3 = 2.7 = 3.3 = 4.7 1.4 = 3.7
According to factors of effectivity related to the standard	2.11 = 2.12 1.1 = 1.2 = 2.5 1.3 = 1.5 = 1.6 = 2.2 = 2.6 = (3.7) = 4.1 1.7 = 2.4 = 2.7 4.7 1.4 = 2.3 = 3.3 = 3.4	1.1 = 1.2 = 1.5 = 1.6 = 2.11 = 2.5 = (4.1) 2.12 = 2.2 = 2.6 1.7 = 2.4 = 2.7 = (3.3) = (3.4) 2.3 = (3.3) = (3.4) = 3.7 = 4.7 1.4
According to zones as shown in the Figures (*bp*, inhibition of germination)	1.1 = 1.2 = 1.6 = 2.11 = 2.12 = 2.5 first maximum = 2.6 *strong* 1.5 = 2.2 = 2.5 second maximum = 3.7 = 4.1 *medium* 1.3 = 1.7 = 2.4 = 2.7 = 4.7 *slight to very slight* 1.4 = 2.3 = 3.3 = 3.4 *no effect*	1.1 = 1.2 = 1.6 = 2.12 = 2.5 *strong* 1.5 = 2.11 = 2.2 = 2.6 = 4.1 *strong to medium* 2.4 = 2.7 = 3.3 = 3.4 *slight* 1.4 = 1.7 = 2.3 = 3.7 = 4.7 *very slight*

sharp borderline. E.g. 2.2 with one of the 8 variants (inhibition of germination *B bp*, Fig. 2) has already 2 slight maxima 4.1 behaves similarly.

With all these chemicals one has to keep in mind the formation of components with varying effectivities by degradation or rearrangement during dilution (LUDWIG and THORN, 1960; RICH and HORSFALL, 1961).

With unstabilized Zineb serving as a standard "serrated" curves or curves with a blunt angle were observed many times whereas with TMTD this was the case only twice, and even then only to a limited extent. This fact has to be emphasized because TMTD is regarded as a classical example of fungicides with 2 maxima (LUDWIG and THORN, 1960; DIMOND et al., 1941).

This reduced variability of the standards certainly contributed to the fact that the results obtained at different times and using different standards were contradictory (see the two marked substances related to Z and T in Fig. 2) with compounds with reduced and erratic activities (e. g. 3.3 and 3.4 in Table 4).

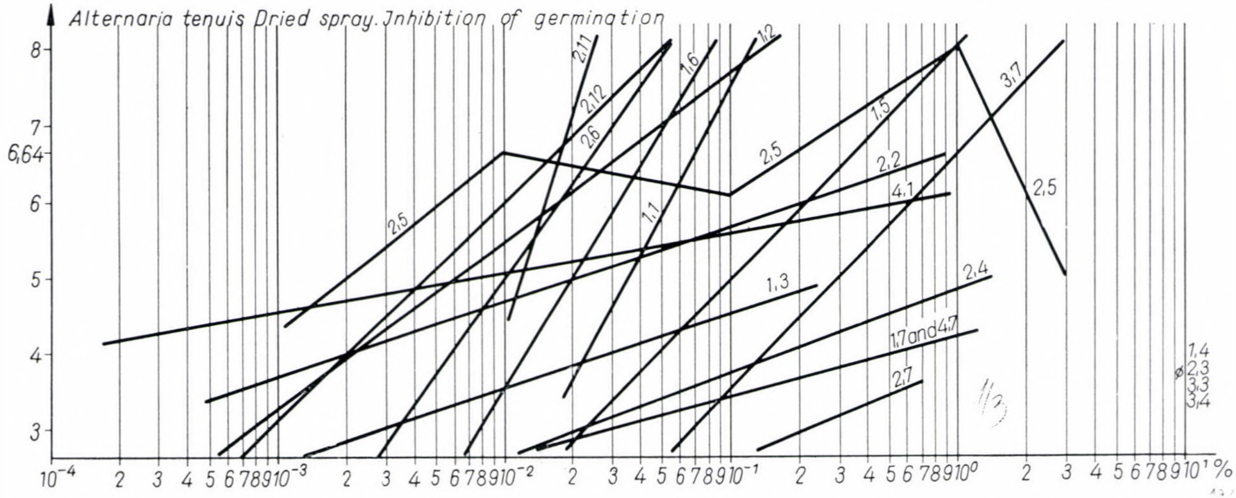

Alternaria tenuis Dried spray. Jnhibition of germination
8
7
6,64
6
5
4
3
10⁻⁴ 2 3 4 5 6 7 8 9 10⁻³ 2 3 4 5 6 7 8 9 10⁻² 2 3 4 5 6 7 8 9 10⁻¹ 2 3 4 5 6 7 8 9 10⁰ 2 3 4 5 6 7 8 9 10¹ %
2,11
2,12
2,6
1,6
1,2
2,5
1,1
1,5
3,7
2,2
4,1
2,5
1,3
2,4
1,7 and 4,7
2,7
1,4
2,3
3,3
3,4

Fig. 1

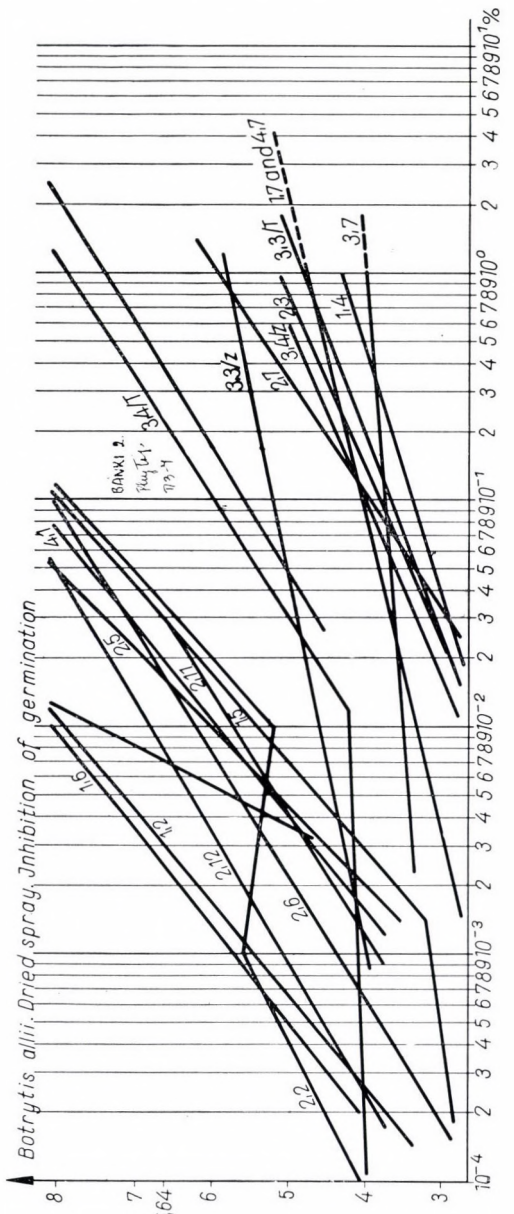

Botrytis allii. Dried spray, Inhibition of germination
8
7
6,64
6
5
4
3
10⁻⁴ 2 3 4 5 6 7 8 9 10⁻³ 2 3 4 5 6 7 8 9 10⁻² 2 3 4 5 6 7 8 9 10⁻¹ 2 3 4 5 6 7 8 9 10⁰ 2 3 4 5 6 7 8 9 10¹ %
BÁNKI 2.
1,6
2,5
2,11
1,5
1,2
2,12
2,6
2,2
3,4Γ
3,3Γ
2,1
2,3
1,4
3,7
1,7 and 4,7

Fig. 2

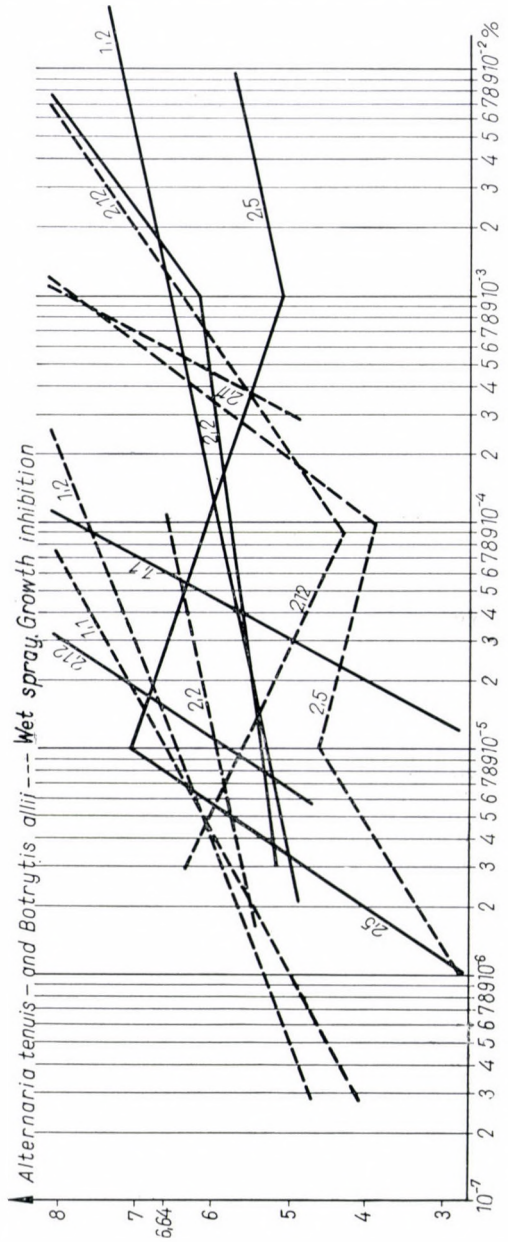

Alternaria tenuis – and Botrytis allii – – – Wet spray. Growth inhibition
1,2
2,2
2,5
1,1
2,1
2,3
8
7
6,64
6
5
4
3
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2} %

Fig. 3

One of the important qualitative characteristics of dosage-response processes expressed by a regression line (FINNEY, 1964; CAVALLI-SFORZA, 1965; DIMOND et al., 1941) is the tangent of the line (the regression coefficient of x related to y, which is equal with b in the equation $Y = a+bx$). If this is expressed in angle-degrees, the size of the angle will show directly, provided that x and y have the same scale, the change of the activity of the chemical upon dilution.

The small angle of inclination may have several causes, e.g. the small number; a too high degree of heterogeneity of the test organisms used or a relatively high degree of susceptibility of the species concerned. Regression lines taken with *Botrytis allii* are for the above reasons usually more flat than those with *Alternaria tenuis* (Figs 1 to 3). If, however, these causes are disregarded and the same experimental conditions are supposed to exist, the small angle of inclination suggests that the effectivity of the chemical, irrespective of the absolute activity, does not change considerably upon dilution. The reason for this, just with the derivatives tested by us, may be that upon dilution degradation products with highly differing activities are formed, which even on a wider dose-range give a fairly uniform total effect.

With larger angles of inclination we have just the reversed situation. In the case of a very steep angle of inclination (if the chemical is otherwise active, i.e. it has a favourable ED_{50} and ED_{95}) one has to think of a very drastic but unequivocal change of the active ingredient upon dilution or of an effect on an "all or nothing" physiological reaction leading to a sharp effect threshold.

Between the two extreme cases there may exist, of course, several transitory types.

In order to establish the effectivity of a chemical in addition to the angle of inclination the point of intersection between the curve and axis y (a in the equation) has to be determined. With equal angles of inclination the higher the value of a the higher the effect. The magnitude of a can be calculated from the values of ED_{50} and the angles of inclination reported.

Disregarding chemicals with 2 maxima the compounds listed in Table 7 can be divided into 3 typical categories according to the angle of inclination. The classification was carried out on the basis of the average values of the angles obtained with inhibition of germination and inhibition of growth as listed in Tables 2 to 5. Most of the chemicals were placed in the same categories with respect to both fungal species. With the more sensitive *Botrytis* species the chemicals close to the borderlines of the categories were shifted by one class in the direction of the smaller angles of inclination.

As repeatedly mentioned above, the activity of chemicals which are qualitatively so different cannot be defined exactly by a single index-number. The significance as well as the fiducial limits of the differences in activity cannot be given either. Therefore, a quantitative evaluation and the classification of the chemicals can be made only approximately by considering different toxicological parameters. Results of such efforts are shown for both test-species in Table 8.

The classification of the chemicals was based on three principles:

1. Code number; 2. effectivity as related to the standard; 3. zones of location as shown in Figs 1 to 3. The data for 1. and 2. were taken from Table 6. The categories established are, of course, not absolute: there may be and certainly are overlappings and transitions among them. The numbers of chemicals exhibiting abnormalities figure in parentheses.

The data for 3. were taken from Figs 1 to 3 on the basis of the principle that the shifts of the dosage response curves from the right bottom to the left top sides indicate an increasing tendency of effectivity. For this compilation the data of 1. and 2. were also taken into consideration.

Because of the striking correspondence of the three compilations in the case of classification 3. the individual categories were provided with denominations. (Between *Botrytis* and *Alternaria* there is in this respect also an overlapping of a half category.)

The final conclusion of the analysis of the activities of the chemicals tested is that disregarding the compounds which belong to the 1st group and which are known also from the literature (not to mention their lability) 6 new dithiocarbamate fungicides have been produced,

3 of which are as effective as Zineb and TMTD (2.11, 2.12, 2.5) and

3 of which have a medium activity (2.2, 2.6, 4.1).

The practical usefulness of these substances depends, of course, on the ease they can be stabilized, on their economic production and on their effectivity under field conditions. These problems are not the subject of the present paper.

Discussion

Though the compounds tested do not make a whole series there are still some conclusions which can be drawn on the correlation between structure and activity.

As a starting point to the experiments it was anticipated that in fungi N-cyanoethyl derivatives are hydrolysed to N-carboxyethyl compounds, just as it is the case with other some compounds, e.g. S-cyanoalkyl-dithiocarbamates (PLUIJGERS and van der KERK, 1961) in different organisms. It was further supposed that N-carboxyethyl derivatives undergo some additional modifications (decarboxylation, oxidation). The experiments have shown that the hydrolysis of N-cyanoethyl derivatives does not contribute very much to fungicidal activity but rather, at least with N-cyanoethyl-N-methyl-dithiocarbamic acid, the salts themselves have a considerable fungicidal effect on their own. The following experimental results support this conclusion:

a) The activity of N-cyanoethyl derivatives is not lower but sometimes even higher than that of the corresponding N-carboxyalkyl derivatives (This argument alone could be explained by their higher permeability).

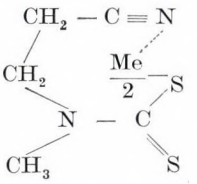
b) The angle of inclination of the dosage-response curve of some N-cyanoethyl derivatives significantly differs from that of the corresponding N-carboxyalkyl derivatives. This points to a different mode of action.

c) Inhibition of growth of mycelia was more pronounced than inhibition of

germination with three chemicals: S-carboxymethyl-dimethyl-dithiocarbamate, the Na salt of N-methyl-N-carboxymethyl-dithiocarbamic acid and to a lesser extent with N-methyl-N-carboxyl-methyl-S-methyl-dithiocarbamate. It has been observed with some fungi that herbicides with auxin-activity in concentrations which did not inhibit the germination of conidia did inhibit the growth of mycelia (WOODFORD and SAGAR, 1960). The auxin-activity of S-carboxymethyl derivatives has been known for a long time (van der KERK et al., 1955) and N-methyl-N-carboxymethyl-S-methyl-dithiocarbamate has recently been found to have auxin-activity, too (ROTHWELL and WAIN, 1963). In our own experiments (SÜDI et al., 1963) with cucumber seedlings the Na salt of N-methyl-N-carboxymethyl-dithiocarbamic acid also exhibited a strong auxin effect. Therefore it seems very likely that the striking inhibition of mycelial growth experienced with the above three derivatives is due to the same effect. Cyanoethyl compounds do not display this specific activity. Consequently, at least N-carboxymethyl and N,N-dimethyl derivatives are not formed in considerable amounts from cyanoethyl compounds.

The facts mentioned above suggest that the derivatives of N-methyl-N-cyanoethyl-dithiocarbamate are active in their original form of N-substitution. According to literary data increasing the number of substituents on N leads to a rapid activity decrease (CARTER et al., 1963). There are, however, very few data available on derivatives which have been susbstituted asymmetrically. By analogy it should be mentioned that among S-carboxymethyl-dithiocarbamates N-methyl-N-ethyl derivatives were still be found active (PLUIJGERS and van der KERK, 1961). At any rate, the high fungicide activity of cyanoethyl-dithiocarbamate is to be due to the specific influence of the cyano group.

In most authors' view the activity of dialkyl-dithiocarbamates is due to the formation of metal complexes. Since cyano groups are known to form complexes it may be supposed that in the case of N-cyanoethyl-dithiocarbamates the cyano group also takes part in complex formation which is an important factor in the activity of these compounds. The steric structure of the molecule allows for that. The metal complex may have the following structure:



One of our observations seemingly contradicts the finding that cyanoethyl derivatives have a fungicidal activity on their own. We have found several cyanoethyl compounds with dosage-response curves showing "breaks" or 2 maxima. Such are a disulfide derivative and a dimethylamide derivative. The broken curves characteristic for these compounds cannot be explained by two modes of action as it was the case with dialkyl-dithiocarbamate and TMTD (SIJPESTEIJN and JANSSEN, 1959) because in our experiments there were just these

latter which exhibited no "break" in their dosage-response curves. It may be supposed, however, that the enzymatic degradation products of the compounds mentioned above are more active than the original substances but, because degradation is limited by some biological factors, this enhanced activity will be manifest only at lower concentrations. Instead of the hydrolysis of the cyano group, however, here too, rather the reductive degradation of the disulfide group or the hydrolysis of the S-amide bond is to be considered. This is supported by the slope of the first ascending part of the dosage-response curve (at least with *Alternaria*) which resembles that of the Na salt of N-methyl-N-cyanoethyl-dithiocarbamate, the actual degradation product. The hydrolysis of the amide group seems to be facilitated by the presence of the cyano group, because with the corresponding but less active N,N-dimethyl derivatives no such change was observed.

It has to be noted that as far as sensitivity to cyanoethyl derivatives is concerned there is less difference between *Botrytis* and *Alternaria* than with the other substances investigated. With *Alternaria* the activities of the Na-salts, the Zn-salts, the S-dimethyl-amide and the S-carboxyethyl ester of the N-methyl-N-cyanoethyl derivatives are higher than those of the corresponding forms of dimethyl-dithiocarbamates. The S-derivatives of the two groups are equally active, only the activity of the disulfide derivative and that of the S-carboxymethyl derivative lag behind those of the cyanoethyl compound and the dimethyl analogue. With the latter this may be attributed to the growth-inhibiting effect of the N,N-dimethyl derivative.

Acknowledgements

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Aufnahme von Uracyl, Azauracyl und Maleinhydrazid durch Pflanzen in Abhängigkeit von der Konzentration und ihre gegenseitige Beeinflussung[1]

Von

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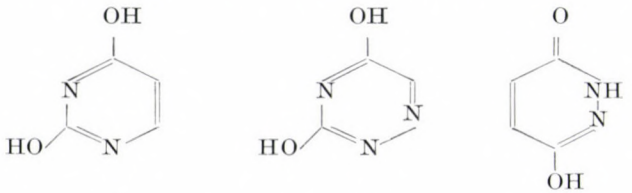
Mit Verwendung von Isotopenmethodik wurde untersucht, in welchem Masse Uracyl und zwei seiner Antimetabolite, das systemfungizid wirksame Azauracyl und der Pflanzenwuchsstoff Maleinhydrazid durch die Wurzeln einerseits in Abhängigkeit von der Konzentration, anderseits bei paarweise gleichzeitiger Verwendung aufgenommen werden. Die durch die Pflanzen aufgenommene Mengen von Uracyl und Maleinhydrazid erreichen bei Erhöhung der Konzentration der Nährlösung schnell ein relativ niedriges Maximum, dagegen steigt die Aufnahme von Azauracyl mit steigender Konzentration. Die aufnehmbare Mengen von Uracyl und Maleinhydrazid werden durch die Anwesenheit der anderen zwei Verbindungen nicht beeinflusst. Dagegen wird die Aufnahme von Maleinhydrazid in Anwesenheit von Uracyl verdoppelt und in Anwesenheit von Azauracyl auf das Vierfache erhöht.

Aus Arbeiten von DEKKER und OORT (1962, 1964) ist bekannt, dass Azauracyl (II) bei Pflanzen eine systemfungizide Wirkung besitzt, d. h. durch die Pflanzen aufgenommen wird und eine innertherapeutische Wirkung gegenüber pathogenen Pilzen ausübt. Da diese Verbindung als Antimetabolit des Uracyls (I) bekannt ist, kann man vermuten, dass die systemfungizide Wirkung durch einen ähnlichen Mechanismus ausgeübt wird: auf Grund seiner strukturellen Ähnlichkeit nimmt das Azauracyl die Stelle des Uracyls in der Nucleinsäure-Biosynthese ein (»Lethal Synthesis«).

Anderseits hat BASKAKOV (1958) die Hypothese entwickelt, wonach Maleinhydrazid (laut genauer Strukturbestimmung 3-Oxypiridazinon-6) (III), bekannt als Pflanzenwachstumregulator und Herbizid, seine biologische Wirkung gleichfalls als Antagonist des Uracyls ausübt. Dafür spricht die Tatsache, dass die pflanzenwachstumshemmende Wirkung des Maleinhydrazids bei gleichzeitiger Verwendung von Uracyl aufgehoben werden kann (BASKAKOV, 1958).



I II III

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Es stellt sich deshalb die Frage, wieweit diese strukturell ähnlichen und einander vermutlich antagonisierenden Verbindungen fähig sind, ihre Aufnahme durch die Pflanzen gegenseitig zu beeinflussen. Dabei können diese drei Verbindungen als Modellverbindungen angesehen werden, um festzustellen, ob ein Antagonismus bzw. Synergismus strukturell verwandter Verbindungen schon bei der ersten Phase der biologischen Wirkung, d. h. bei der Aufnahme und Translokation auftreten kann. Die gegenseitige Beeinflussbarkeit der Aufnahme dieser Verbindungen, vor allem des Systemfungizids Azauracyl, ist aber auch in praktischer Hinsicht nicht ohne Interesse.

Die Aufnahme biologisch aktiver Substanzen, zum Beispiel von 2-Heptadecyl-2-imidazolin (MILLER, et al., 1953), Sulphonamide (CROWDY und RUDD JONES, 1956), Antibiotika (CROWDY, 1957), Aryloxyalkylcarbonsäuren (CROWDY and WAIN, 1951), substituierte α-Methoxyphenylessigsäuren (MITCHELL, 1963), Maleinhydrazid (CRAFTS und YAMAGUCHI, 1965) wurde von verschiedenen Authoren ausführlich studiert. Über die gegenseitige Beeinflussbarkeit der Aufnahme dieser Verbindungen enthält die Literatur kaum einige Angaben; diese beschränken sich auf den Einfluss von Atmungsinhibitoren auf die Aufnahme (CRAFTS und YAMAGUCHI, 1965).

Vorbedingung für Untersuchungen über die gegenseitige Beeinflussbarkeit der Aufnahme war eine genaue Kenntnis der Aufnahme der einzelnen Verbindungen in Abhängigkeit ihrer Konzentration.

Material und Methode

Bei unseren Versuchen haben wir folgende Substanzen benützt: Uracyl (Merck), Azauracyl (Merck), Maleinhydrazid (Merck), Uracyl-C^{14} (The Radiochemical Center, 0,1 mc, 2,5 mg), Azauracyl-C^{14} (hergestellt nach dem Verfahren von GUT, 0.02 mc, 5.6 mg) und Maleinhydrazid-C^{14} (The Radiochemical Center, 0.1 mc, 4 mg).

Zur Bestimmung der Aufnahme der einzelnen Substanzen in Abhängigkeit von ihrer Konzentration wurden folgende Versuche durchgeführt: 10 Tage alte Buschbohnenpflanzen wurden in Erlenmeyer-Kolben in je 50 ml Lösung bis zum Wurzelhals eingetaucht (je 3 Pflanzen in einer Kolbe). Die einzelnen Lösungen enthielten gleiche Mengen aktiver und steigende Mengen inaktiver Substanz. Die Aktivität, bzw. die Menge der aktiven Substanz in den Lösungen war bei Uracyl 1 $\mu c = 25\ \mu g$, bei Azauracyl 0.8 $\mu c = 220\ \mu g$ und bei Maleinhydrazid 1 $\mu c = 40\ \mu g$. Die Menge der inaktiven Substanz war bei allen bei Verbindungen 1, 2, 3, 4, 5 und 7.5 mg, entsprechend einer Konzentration von 20, 40, 60, 80, 100 bzw. 150 ppm. Die Pflanzen wurden 48 Stunden bei natürlichem Licht in den Lösungen stehen gelassen, bei einer Temperatur von 22–23 °C. Danach wurde die in der Lösung verbliebene Aktivität gemessen und die gefundenen Werte mit denen der Kontrollversuche (gleiche Lösungen ohne Pflanzen) verglichen.

Bei den Versuchen zum Studium der gegenseitigen Beeinflussung der Aufnahme dieser Verbindungen haben wir die gleiche Methodik befolgt, jedoch mit dem Unterschied, dass die aktiven und die inaktiven Substanzen verschieden waren. Diese Versuche wurden mit allen sechs möglichen Verbindungs-Paaren (Uracyl-C^{14} + Azauracyl, Uracyl-C^{14} + Maleinhydrazid, Azauracyl-C^{14} + Uracyl, Azauracyl-C^{14} + Maleinhydrazid, Maleinhydrazid-C^{14} + Uracyl, Maleinhydrazid-C^{14} + Azauracyl) durchgeführt.

Resultate

Die Ergebnisse der Versuche, bei denen die Aufnahme der einzelnen Substanzen in Abhängigkeit von ihrer Konzentration untersucht wurde, sind aus der Tabelle 1 ersichtlich. Aus den Messwerten geht hervor, dass Uracyl und Maleinhydrazid durch die Wurzeln nur bis zu einer begrenzten, relativ niedrigen Menge aufgenommen werden. Diese Menge wird bereits bei niedriger Konzentration relativ schnell erreicht und bleibt auch bei Erhöhung der Konzentration praktisch

Tabelle 1

Aufnahme von Uracyl, Azauracyl und Maleinhydrazid durch die Wurzeln in Abhängigkeit von ihrer Konzentration in der Lösung

Substanz	Menge in der Lösung mg	Konzentration der Lösung ppm	Aufnahme		
			Menge mg	%	Relative Menge in den Pflanzen ppm
Uracyl	0.025	0.5	0.0235	94	6.4
	1	20	0.75	75	191
	2	40	1.08	54	264
	3	60	1.08	36	277
	4	80	1.00	25	246
	5	100	1.05	21	264
	7.5	150	1.05	14	258
Azauracyl	0.22	4.4	0.1078	49	24.1
	1	20	0.43	43	107
	2	40	—	—	—
	3	60	1.41	47	353
	4	80	1.44	36	360
	5	100	1.90	38	477
	7.5	150	2.85	38	710
Maleinhydrazid	0.04	0.8	0.0036	9	0.92
	1	20	0.45	45	107
	2	40	0.46	23	109
	3	60	0.42	14	98
	4	80	0.40	10	101
	5	100	0.45	9	115
	7.5	150	0.45	6	112

konstant. Diese aufnehmbare Menge beträgt bei Uracyl etwa 260, bei Maleinhydrazid etwa 110 ppm (auf Pflanzen-Rohrgewicht umgerechnet). Dagegen steigt beim Azauracyl die aufnehmbare Menge mit steigender Konzentration der Lösung und erreicht einen Höchstwert von 710 ppm.

Die Ergebnisse der Versuche über die gegenseitige Beeinflussung der Aufnahme sind in den Tabellen 2, 3 und 4 zusammengestellt. Aus den erhaltenen Messwerten geht hervor, dass die aufgenommene Menge von Uracyl weder durch Azauracyl, noch durch Maleinhydrazid, sowie die aufgenommene Menge von Azauracyl weder durch Uracyl, noch durch Maleinhydrazid beeinflusst wird. Dagegen wird die aufgenommene Menge von Maleinhydrazid in Anwesenheit von Uracyl verdoppelt, in Anwesenheit von Azauracyl auf das Vierfache erhöht.

Als Erklärung dafür kann entweder angenommen werden, dass Azauracyl und Maleinhydrazid eine synergistische Wirkung bei der Ersetzung des Uracyls ausüben, was eine erhöhte Aufnahme bewirkt, oder dass Azauracyl und auch Uracyl die Permeabilität der Zellenwände für Maleinhydrazid erhöhen. Die relativ niedrige Erhöhung der Aufnahme deutet darauf hin, dass die Aufnahme von Maleinhydrazid nicht nach dem Mechanismus von aktiver Aufnahme organischer Substanzen verläuft. Im Laufe weiterer Untersuchungen soll festgestellt werden, ob in der Aufnahme dieser Verbindungen die Zellenatmung eine unmittelbare Rolle spielt, d. h. ob die Aufnahme mit spezifischen Atmungs-Inhibitoren beeinflusst werden kann. Es muss ferner geklärt werden, ob die gegenseitige Beeinflussung dieser und verwandter Verbindungen auch bei der biochemischen Umwandlung durch die Pflanze in Erscheinung tritt.

Tabelle 2

Aufnahme von Uracyl durch die Wurzeln in Anwesenheit von Azauracyl bzw. Maleinhydrazid

Substanz neben 0,025 mg (0.5 ppm) Uracyl	Menge in der Lösung mg	Konzentration der Lösung ppm	Uracyl-Aufnahme		
			Menge mg	%	Relative Menge in den Pflanzen ppm
—	—	—	0.0235	94	6.4
Azauracyl	1	20	0.0190	95	6.8
	2	40	0.0182	91	6.4
	3	60	0.0192	96	6.6
	4	80	0.0178	89	6.3
	5	100	0.0162	76	6.1
	7.5	150	0.0170	85	6.3
Maleinhydrazid	1	20	0.0178	89	6.2
	2	40	0.0190	95	6.4
	3	60	0.0182	91	6.4
	4	80	0.0188	94	6.8
	5	100	0.0190	95	6.9
	7.5	150	0.0194	92	6.7

Tabelle 3

Aufnahme von Azauracyl durch die Wurzeln in Anwesenheit von Uracyl bzw. Maleinhydrazid

Substanz neben 0.22 mg (4.4 ppm) Azauracyl	Menge in der Lösung mg	Konzentration der Lösung ppm	Azauracyl-Aufnahme		
			Menge mg	%	Relative Menge in den Pflanzen ppm
—	—	—	0.1078	49	24.1
Uracyl	1	20	0.0924	42	23.1
	2	40	0.0924	42	21.7
	3	60	0.0858	39	21.2
	4	80	0.0858	39	22.7
	5	100	0.0924	42	23.4
	7.5	150	0.0823	38	20.9
Maleinhydrazid	1	20	0.1078	49	25.2
	2	40	—	—	—
	3	60	0.0858	39	22.3
	4	80	0.1056	48	23.4
	5	100	0.0823	38	23.1
	7.5	150	0.0792	36	22.9

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Tabelle 4

Aufnahme von Maleinhydrazid durch die Wurzeln in Anwesenheit von Uracyl bzw. Azauracyl

Substanz neben 0.04 mg (0.8 ppm) Maleinhydrazid	Menge in der Lösung mg	Konzentration der Lösung ppm	Maleinhydrazid-Aufnahme		
			Menge mg	%	Relative Menge in den Pflanzen, ppm
—	—	—	0.0036	9	0.92
Uracyl	1	20	0.0092	23	1.9
	2	40	0.0076	19	2.1
	3	60	0.0072	18	1.8
	4	80	0.0088	22	2.0
	5	100	0.0080	20	1.9
	7.5	150	0.0084	21	2.0
Azauracyl	1	20	0.0136	34	3.6
	2	40	0.0172	43	4.1
	3	60	0.0176	44	4.2
	4	80	0.0156	39	4.0
	5	100	0.0184	46	4.5
	7.5	150	0.0168	42	4.3

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Untersuchungen über die durch den Pilz *Fusarium oxysporum* Schl. f. l. Wr. verursachte pathologische Keimung der Kartoffel

Von

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Der pathogene Pilz kann die pathologische Keimung der Knollen in beträchtlichem Ausmasse auslösen. In dieser Beziehung bestehen unter den einzelnen Sorten wesentliche Unterschiede. Die ungünstige Wirkung äussert sich im Prozentsatz der pathologisch keimenden Knollen und noch mehr in den Prozenten der kranken Keime.

Die kranken Knollen treiben früher aus als die gesunden, und können daher zu Beginn des Keimens der gesunden Knollenmasse aus dem Pflanzgut ausgeschieden werden.

An den kranken Knollen entwickelt sich eine grössere Anzahl von Keimen als an den gesunden.

Die Anwesenheit des Pilzes in der Knolle verschiebt die Keimung entschieden in pathologischer Richtung, aber die Pilzlosigkeit der Knollen bedeutet nicht unbedingt gesunde Keimung. Die scheinbare Gesundheit der Keime bringt nicht unbedingt die Gesundheit der Knollen zum Ausdruck. Dickkeimige Knollen können in beträchtlichem Ausmasse von dem Pilz (oder dem Blattrollvirus) befallen sein. Die negative Staudenselektion hat auch in dieser Hinsicht Bekräftigung erfahren.

Das Blattrollvirus *(Corium solani Holmes)* spielt nicht einmal annähernd die gleiche Rolle in der Auslösung des pathologischen Keimens als die infektiöse Fusarienwelke.

In der Bekämpfung ist das Hauptgewicht auf die Prävention, namentlich auf die richtige Agrotechnik zu legen.

Einführung

Begriff und Bedeutung der pathologischen Keimung

Der Begriff der pathologischen Keimung umfasst solche schwere Sprossdefekte, die auf irgendeine, die Knospen der Knolle betreffende Erkrankung zurückzuführen sind. Der junge Spross ist daher schon ursprünglich krank, da die den Defekt auslösende Ursache bereits im embryonalen Zustand wirkte. Die pathologische Wirkung äussert sich am häufigsten in der Bildung von sog. »Fadenkeimen« oder »Dünnkeimen«, aber in gewissen Fällen kann auch die Bildung von sog. »Keimknöllchen« (»äussere Sprossknöllchen«, »Knöllchensucht«) oder »einwärts gerichteter Keimung« (Bildung von sog. »inneren Sprossknöllchen«) beobachtet werden.

Unter »*Fadenkeimen*« verstehen wir ausserordentlich dünne junge Sprosse, meistens mit einem Durchmesser von nicht über 1 mm, unter »*Dünnkeimen*« solche

von mehr als 1 mm Durchmesser, aber noch immer von pathologischer Dünnheit (viel dünner als ein Bleistift). Es ist wichtig, diese nicht mit dem Begriff des sog. »*Dunkelkeimes*« zu verwechseln, worunter wir nur infolge des Lichtmangels verlängerte, etiolierte, aber ursprünglich gesunde Keime verstehen. Die Knolle mit solchem Keim wird am Licht getrieben starke, gedrungene, farbige Keime, sog. »*Lichtkeime*« entwickeln, während die kranke Knolle auch im Lichte fadenartig oder dünn austreibt. Die Bildung von »*Keimknöllchen*«, »*Knöllchensucht*« erfolgt beim Austreiben der Knolle entweder aus einer Knospe ausgehend und verzweigend oder aus mehreren Knospen stammend, auf kürzeren oder längeren, dickeren oder dünneren Sprossen, sehr oft auf Fadenkeimen, mit der Entwicklung von nicht lebensfähigen, wertlosen, winzigen Knollen. Die »*einwärts gerichtete Keimung*« ist mit der Bildung von inneren Sprossknöllchen verbunden. Die wachsenden Knöllchen zerreissen die Knolle und stehen daraus in kleinerem oder grösserem Masse hervor. Schliesslich ist als folgenschwerste Veränderung der *vollkommene Mangel des Austriebvermögens* zu erwähnen, welcher in der Praxis als »Erblindung der Augen (Knospen)« der Knolle bezeichnet wird.

Da die Sprosse der pathologisch keimenden Knollen in den meisten Fällen nicht fähig sind, die Oberfläche des Bodens zu erreichen, oder wenn sie auch mit Mühe und Not aufgehen, ein verkümmertes, wertloses Kraut entwickeln, ist oft mit einem Ausfall der Anbaufläche bis 10% oder darüber hinaus zu rechnen. Dies ist aber aus volkswirtschaftlichem Standpunkt bei weitem nicht gleichgültig, da es sich unter ungarischen Verhältnissen um den Verlust eines Kartoffelareals von 35—40 000 Kat.-Joch handelt.

Die Ursachen der pathologischen Keimung und eine kurze Übersicht der einschlägigen Fachliteratur

Die das pathologische Keimen auslösenden Ursachen können *abiotische* und *biotische* Faktoren sein.

Von den *abiotischen* Faktoren ist als erster die zur Zeit der Knollenbildung vorherrschende *hohe Bodentemperatur* zu nennen. Nach LYSSENKO geht unter der Einwirkung der hohen Temperatur in den Knospen der Knolle ein gewisser »Altwerdensprozess« vor sich, welcher zum Sprossdefekt führt. Die in den wärmeren Zonen der Sowjetunion schädigende sog. »südliche Krankheit« ist ihrem Wesen nach ein durch die Wärme verursachter ökologischer Abbau, dessen wesentlichstes und kennzeichnendstes Symptom die Faden- oder Dünnkeimigkeit bildet. SZIRMAI (1951) hat bei der experimentellen Erforschung des durch die Wärme verursachten Abbaus festgestellt, dass Temperaturen von über 29 °C die Sprossentwicklung der Knollen schädlich beeinflussen, während bei 35 °C und darüber die Sprossbildung zum Stillstand kommt. Die Beobachtungen von WENZL und DEMEL (1952), STEINECK (1955) und SOKOLENKO (cit. SCHICK und KLINKOWSKI, 1962) deuten auf eine Erhöhung der Fadenkeimigkeit und der dadurch verursachten wirtschaftlichen Schäden in den Gebieten von hoher Temperatur hin.

Hohe Temperatur während der Lagerung (am häufigsten Erwärmen der Prismen oder der Mieten) kann insbesondere im Falle der sog. »heftigen Sorten« häufig pathologische Keimung auslösen (HINFNER und CSÁK, 1958).

Hohe Bodentemperatur in Verbindung mit Trockenheit führt zu sehr schweren Sprossdefekten. Dies ist die Erklärung für das nach Dürrejahren beobachtete höhere Ausmass von Fadenkeimigkeit und das Unterbleiben des Austriebes der Knollen (SZIRMAI, 1951; HINFNER und CSÁK, 1958). PETRÓCZI (1964) erbrachte in sehr beachtenswerten Versuchen den Beweis dafür, dass von den ungünstigen ökologischen Faktoren die hohe Temperatur im Verein mit dem bei der Entwicklung der Knollen auftretenden akuten Wassermangel pathologische Sprossbildung verursacht. Die sich während der niederschlagsreichen Periode nach langdauernder Dürre entwickelnden sekundären (Töchter-) Knollen haben stets gesunde Keime gebildet, die während der Dürreperiode entstandenen primären Knollen jedoch in ansehnlicher Zahl Faden- oder Dünnkeime (in Abhängigkeit von den gegebenen Bedingungen zu 25–30 % Faden-, und zu 50–60 % Dünnkeime).

Auch die *Trockenheit an und für sich* kann sich ungünstig auswirken. Nach GRAEBNER (1924) vollzieht sich in solchen Fällen – besonders auf Sandböden – in den in Entwicklung begriffenen Knollen ein »Notreife«-Prozess; die Knospen bilden sich unvollständig aus, sie treiben fadenartig oder sind zum Austreiben unfähig. In den Knospen der in trockenen Böden gepflanzten Knollen tritt ein gewisser Grad von »Welkeprozess« ein, der pathologische Keimung induziert.

Auf die schädliche Wirkung der *ungünstigen Bodentemperatur- und Feuchtigkeitsbedingungen nach dem Pflanzen* wurde von KÖHLER (1927) und BRANDL (1930) hingewiesen. Niedrige Temperaturen – meist im Verein mit Wassermangel – lösen die Bildung von Keimknöllchen aus. Das Übel steigert sich, wenn das Pflanzgut aus einer warmen Lagerstätte (erwärmten Prisma oder Miete) in kalten Boden gelangt (DOHY, 1951, HINFNER und CSÁK, 1958). Manchmal ist auch die nach einwärts erfolgte Keimung auf ähnliche Ursache zurückzuführen (HINFNER und CSÁK, 1958).

Langdauernder *Sauerstoffmangel* im Boden (schwere, nasse Böden) ist als eine Ursache oder zumindest als prädisponierender Faktor der pathologischen Keimung anzusehen (HINFNER und CSÁK, 1958).

Der *Sauerstoffmangel während der Lagerung* wirkt besonders schädlich bei Einmieten von unreifen Knollen, bei allzu dicker winterlicher Deckung des Prismas oder der Miete, infolge des durch das intensivere Atmen verursachten rascheren Sauerstoffverbrauchs und der CO_2 Anhäufung, vor allem auf das am Boden des Prismas oder der Miete befindliche Knollenmaterial (APPEL, 1948). Die pathologische Wirkung (Fadenkeim- und Keimknöllchenbildung) erhöht sich bei längerer Lagerung. Frühe und hauptsächlich die sog. »heftigen« Sorten erleiden grösseren Schaden (HINFNER und CSÁK, 1958). Bei kleinen Knollen ist der Schaden schwerer und häufiger (MADEC, 1956). PETRÓCZI (1964) hat experimentell nachgewiesen, dass der partielle oder totale Sauerstoffmangel zu wesentlichem bzw. totalem Sprossschaden führt.

Lagerung von grossen Mengen kann – *infolge des höheren Druckes* – in den am Boden des Prismas oder der Miete befindlichen Knollen gelegentlich einwärtsgerichtete Keimung verursachen (APPEL, 1948).

Mit der eine pathologische Keimung (Keimknöllchen-Bildung) auslösenden Wirkung des *wiederholten Abkeimens* hat sich KÖHLER (1927) eingehend befasst. Zur Bildung der primären Sprosse wird eine grössere Menge von Eiweiss verbraucht, so dass mit deren Entfernen eine unverhältnismässig grössere Kohlenhydratmenge für die sekundären oder sogar tertiären Sprosse zur Verfügung steht. Dieser in den Knospen und Sprossspitzen sich ansammelnde Kohlenhydratüberschuss kann besonders bei kaltem Boden nicht in der Form von regelmässiger Sprossbildung zur Geltung kommen, sondern veranlasst die Pflanze zur Entwicklung eines Speicherorgans.

Schliesslich sind hier gewisse *chemische Effekte* zu erwähnen, die besonders in der letzteren Zeit eingehend untersucht worden sind. Die Kenntnis dieser Wirkungen hat ihren Ursprung im wesentlichen in der Wirkungsprüfung der biotischen Faktoren. Es handelt sich um toxische oder Hormonsubstanzen, die von den Erregern gebildet werden und in reinem Zustand hergestellt, in der Form einer Lösung den Knospen zugeführt imstande sind, unmittelbar pathologische Sprossbildung herbeizuführen. Solche sind z. B. die als Stoffwechselprodukt der Fusarium-Pilze entstehende *Gibberellinsäure* und andere Gibberellinderivate (YABUTA, 1939; BRIAN, 1954; CURTIS, 1947; TAGAWA, 1959; UBRIZSY, VÖRÖS und KIRÁLY, 1960; SZABÓ-SZŰCS, 1961; PETRÓCZI, 1954, 1965). TANO und KOBAYASHI (1955) konnten mit dem K-Salz der β-Indolessigsäure Keimknöllchenbildung auslösen.

Von den *biotischen* Faktoren stehen die Viruskrankheiten seit längerer Zeit im Mittelpunkt des Interesses. Wie einheitlich die Auffassung der Forscher in bezug auf die pathologische Keimung verursachende Wirkung des Stolbur-Virus ist, ebenso gegensätzlich ist sie betreffs der Frage der Rolle des Blattrollvirus. APPEL (1914, 1948), HILTNER (1919), GILBERT (1923), MÜTTERLEIN (1923), SCHLUMBERGER (1924), BESSELER (1926), KOLTERMANN (1927), KROHN (1929), HUSZ (1941), SZIRMAI (s. UBRIZSY und Mitarbeiter, 1952), HINFNER und CSÁK (1958) betrachten das Blattrollvirus als die Ursache der pathologischen Keimung. Diese Ansicht wird nicht geteilt von SCHULTZ und FOLSOM (1921), APPLEMAN (1924), MURPHY und MCKAY (1925), BINSWANGER (1926), MCINTOSH (cit. ESMARCH, 1932), DYKSTRA (1948), ZOGG, HOBBER und SALZMANN (1949), VERHOVEN (1949), WENZL (1950a), SALZMANN (1950), STEINECK (1952), DOHY (1955), WENZL und GLAESER (1959), SÁRVÁRI I. (1963), SÁRVÁRI B. (mündl. Mitteilung: 1964). Zwischen den beiden gegensätzlichen Auffassungen nimmt GULYÁS (1938) eine Mittelstelle ein, der dem Blattrollvirus nicht ausgesprochen pathologische Keimung auslösende Wirkung zuschreibt, sondern nur geringere »Keimenergie« und schwächere Keimfähigkeit. Es ist interessant zu bemerken, dass WENZL (1965) in einer neueren Arbeit speziell bei der Sorte »Allerfrüheste Gelbe« die Fadenkeimigkeit als eine Begleiterscheinung des Blattrollvirus erwähnt.

Zu der pathologische Keimung auslösenden Wirkung des Stolbur-Virus bekennen sich einmütig mehrere Autoren: SZIRMAI (1956, 1958), WENZL (1956b),

VALENTA (1958), BLATTNY (1958), BOJNANSKY (1958), HINFNER und CSÁK (1958), SÁRVÁRI I. (1963), PETRÓCZI (1964).

SALZMANN (1950) fand einen Zusammenhang zwischen der Netznekrose und der Fadenkeimigkeit.

Von den *bakteriellen Krankheiten* mag die Ringfäule *(Corynebacterium sepedonicum)* sowohl an der Fadenkeim-, wie auch an der Keimknöllchenbildung teilnehmen (HINFNER und CSÁK, 1958).

Von den pathogenen Pilzen wurde die Rolle von *Colletotrichum atramentarium* durch WENZL (1950b, 1951) nachgewiesen. Um die durch den Pilz verursachte Fadenkeimigkeit zu bekämpfen wurde mit früher Ernte (Unreifrodung) experimentiert (WENZL, 1955). Neuestens hält dieser Autor auch eine gemischte (Stolbur-Virus + *Colletotrichum)* Infektion für möglich, wobei wahrscheinlich das Virus das primäre ist, welches die gummiartige Erweichung der Knollen verursacht und die Ansiedlung des Pilzes in den Geweben nur von sekundärem Charakter ist (WENZL, 1964).

Auf die pathologische Keimung auslösende Wirkung der infektiösen Fusariumwelke *(Fusarium oxysporum* Schl. f. 1. Wr.*)* haben meine eigenen Untersuchungen (DOHY, 1955), und später die weiter reichenden Forschungen von PETRÓCZI (1964, 1965) hingewiesen.

Eigene Untersuchungen

Den Ausgangspunkt dieser Untersuchungen bildete die praktische Beobachtung, dass nach den Jahren mit infektiöser Welkekrankheit ein wesentlicher Teil der Knollen unregelmässig, dünn oder fadenartig austreibt. Im Frühjahr 1948 war dies im ganzen Nyírség-Gebiet als Folgeerscheinung des sehr starken Welkekrankheitsbefalls des Jahres 1947 zu beobachten. Ähnlich war die Lage – obwohl in milderer Form – im Frühling 1951 nach dem Fusariumbefall des Jahres 1950. Die in Rede stehende Erfahrung war aber noch nicht von konkreten Untersuchungen bzw. zahlenmässigen Angaben unterstützt. Es war nicht genau aufgeklärt, ob die Pilzinfektion selbst oder der warme Jahrgang die pathologische Keimung auslöst, welch letzterer im Übrigen auch die Fusariumerkrankung begünstigt. Wir haben bisher keinen Vergleich zwischen der Keimung der von Fusarium befallenen und nicht befallenen Pflanzen stammenden Knollen gemacht. Es war uns nicht bekannt, in welchem Ausmasse der Fusariumbefall unregelmässiges Keimen verursacht.

Das niederschlagreiche und nicht allzu warme Sommerwetter des Jahres 1951 hat weder die Fusariumwelke noch die von der Wärme verursachten Abbauerscheinungen begünstigt. Die Wirkung der trotzdem sporadisch erscheinenden infektiösen Welke konnte daher unabhängig von der schädlichen Wärmewirkung untersucht werden.

Die Arbeit wurde in der Versuchswirtschaft Kisvárda mit dem zum Keimen-Bringen der Knollen der 1951 infizierten Kartoffelstauden im Jahre 1952 begonnen.

Tabelle 1

Keimungsangaben im Frühjahr 1952 der Knollen der von

Sorte	Knollen insgesamt	Gesunde Knollen	Abnormal (dünn oder fadenartig) keimende Knollen		Keime Insgesamt	Gesunde Keime	
	St	St	St	%	St	St	%
Korai sárga	37	24	13	35	130	72	55
Bintje	4	4	—	—	9	9	100
Patonai rózsa	26	22	4	15	50	27	54
Patonai 158	37	34	3	8	41	32	78
Eigenheimer	26	8	18	69	128	18	14
Aranyalma	38	35	3	8	35	29	83
Aranyalma (Kisvárdai)	8	8	—	—	10	10	100
Merkur	26	22	4	15	65	41	63
Merkur (Poln. imp.)	39	32	7	18	83	67	81
Wohltmann	5	1	4	80	20	3	15
Krüger	10	9	1	10	31	26	84
Lorch	140	68	72	51	359	98	27
Berlichingen	29	22	7	24	102	75	74
Moschestik	10	3	7	70	43	3	7
Korenovski	185	94	91	49	597	206	35
Koschelinskia	11	11	—	—	19	19	100
Pionir (Voran)	42	39	3	7	54	38	70
Parnassia	42	38	4	9	70	59	84
Insgesamt	715	474	241	—	1846	832	—
Durchschnitt	—	—	—	33.7	—	—	45.1

Von den in den Landes-Kartoffelsortenversuch 1951 einbezogenen Sorten zeigte sich in 18 ein Fusariumbefall. Die kranken Pflanzen wurden genau bezeichnet und im Herbst separat geerntet. Das Knollenmaterial wurde je nach Sorten sorgfältig getrennt, in Kartoffelkisten gelegt und in einem kühlen, trockenen Keller (4—7 °C) gelagert. Das zum Keimen-Bringen erfolgte vom 1. Februar 1952 an im Gewächshaus, bei einer Temperatur von 15—20 °C in diffusem Licht in einem Luftraum von über 70% relativer Feuchtigkeit. Als Kontrolle wurden je 100 gesunde Knollen derselben Sorten auf ähnliche Weise zum Keimen gebracht. Die Ergebnisse der Keimung sind in Tab. 1 dargestellt.

Es kann festgestellt werden, dass die Knollen der mit Fusarium befallenen Pflanzen in der überwiegenden Mehrzahl der Sorten auf die Infektion mit pathologischem Keimen reagierten. Hinsichtlich der Reaktion bestanden unter den Sorten augenfällige Unterschiede. Summiert und die Durchschnitte gezogen, kommt die schädliche Wirkung des Fusariumpilzes schon im Zahlenverhältnis der pathologisch keimenden Knollen (33.7%) entsprechend zum Ausdruck, aber noch mehr in der Prozentzahl der pathologischen Keime (54.9%). Sehr wesentlich ist die Gesetzmässigkeit, dass *die durchschnittliche Keimzahl der pathologisch keimenden Knollen höher ist* als die der Gesunden (im Durchschnitt 2.2 mal). Dies erklärt sich dadurch,

Welkekrankheit (Fusarium) im Jahre 1951 befallenen Kartoffelpflanzen

Dünnkeime		Fadenkeime		Abnormale Keime insgesamt		Durchschn. Keimzahl der gesunden Knollen	Durchschn. Keimzahl der kranken Knollen	Die Keimzahl der kranken Knollen grösser × (mal)
St	%	St	%	St	%	St	St	
36	28	22	17	58	45	3.0	4.5	1.5
—	—	—	—	—	—	2.2	—	—
21	42	2	4	23	46	1.2	5.7	4.8
9	22	—	—	9	22	0.9	3.0	3.3
72	56	38	30	110	86	2.3	6.1	1.6
6	17	—	—	6	17	0.8	2.0	2.5
—	—	—	—	—	—	1.3	—	—
21	33	3	4	24	37	1.9	6.0	3.2
16	19	—	—	16	19	2.1	2.3	1.1
17	85	—	—	17	85	3.0	4.2	1.4
5	16	—	—	5	16	2.9	5.0	1.7
209	58	52	15	261	73	1.4	3.6	2.6
25	24	2	2	27	26	3.4	3.8	1.1
13	30	27	63	40	93	1.0	5.7	5.7
284	48	107	17	391	65	2.2	4.3	2.0
—	—	—	—	—	—	1.7	—	—
3	6	13	24	16	30	0.9	5.3	5.9
11	16	—	—	11	16	1.6	2.7	1.6
748	—	266	—	1014	—	—	—	—
—	40.5	—	14.4	—	54.9	1.9	4.3	2.2

dass an den kranken Knollen auch die Knospen des Nabelteiles auskeimen. Unsere diesbezügliche Erfahrung steht im Einklang mit der Behauptung von APPLEMANN (1924a), SCHLUMBERGER (1924, 1926) und VASTERS (1926), derzufolge die niedrigere Keimzahl die grössere Treibkraft bedeutet und die aus vielen Knospen, zerstreut erfolgte Keimung für verminderte Treibkraft und geringeren Saatwert zeugt.

Die kranken Knollen treiben viel früher aus und die Länge ihrer Keime ist in einem gewissen Zeitpunkt viel grösser als bei den gesunden Knollen. Nach unseren Beobachtungen und Messungen kann festgestellt werden, dass die gesunden Knollen bloss über ein Sprossende von einigen mm verfügen, während sich die mit *Fusarium* befallenen Knollen mit 2–3 cm langen Fadenkeimen auszeichnen. Eine interessante Parallele lässt sich beim rascheren Austrieb der an virösem Abbau leidenden Knollen einiger Sorten erkennen (HILTNER, 1922). Nach BIRKNER (1955) treiben die mit Virus infizierten Knollen nach Rinditebehandlung rascher aus als die gesunden.

Die Knollen der nicht befallenen Kontrollpflanzen haben zu 100% gesund gekeimt, was zweifellos beweist, dass 1951 von einer pathologische *Keimung auslösenden Wärmewirkung nicht die Rede sein konnte.*

In der weiteren Folge waren wir bestrebt, Antwort auf die Frage zu erhalten, ob und inwiefern ein *Zusammenhang zwischen der Ringbräune* (Braunfäule des

Gefässbündelrings) der Knollen und *der pathologischen Keimung* besteht. Das auf die erörterte Weise eingesammelte und eingekeimte Knollenmaterial wurde durch das mehrfache feine Aufschneiden des Nabelteiles (unter Verfertigung einer Querschnitt-Serie) sowie durch das Durchschneiden der ganzen Knolle der Länge nach zum Gegenstand sorgfältiger Untersuchung gemacht. Inzwischen wurden aus der kranken Gefässbündelzone stichprobenartig zahlreiche Isolate verfertigt, wobei der Pilz teils in der Richardsschen Nährlösung, teils auf festem Nährboden mit Reisbrei gezüchtet wurde. Die Kulturen erwiesen sich als der Pilz *Fusarium oxysporum* Schl. f. 1. Wr. Sehr sporadisch und nur beim Nabelende erschienen auch andere Fusarien, aber auch hier dominierte der Pilz *F. oxysporum*.

Die Ergebnisse der beschriebenen inneren Knollenuntersuchung sind unter Berücksichtigung der Beschaffenheit der Keime in Tab. 2 zusammengefasst.

Obwohl die als Ausgangspunkt dienende Knollenzahl der einzelnen Sorten ziemlich niedrig war (einerseits weil bei mehreren Sorten schon ursprünglich wenig mit Fusarium behaftetes Material zur Verfügung stand, andererseits weil im Verlaufe des Keimprozesses mehrere Knollen zugrunde gingen) und daher unser Versuch nicht geeignet war, um das Benehmen der einzelnen Sorten festzustellen, können aus den Angaben für eine Gesamtzahl von 424 Knollen immerhin massgebliche Feststellungen abgeleitet werden. Demnach waren

1. Von insgesamt 424 Knollen

mit Ringbräune befallen	166 =	39.2%
nicht befallen	258 =	60.8%

2. Von 166 Knollen mit Ringbräune

dickkeimig	81 = 48.8%	
dünnkeimig	56 = 33.7%	51.2%
fadenkeimig	29 = 17.5%	

3. Von 258 nicht mit Ringbräune befallenen Knollen

dickkeimig	197 =	76.4%
dünnkeimig	44 = 17.0%	23.6%
fadenkeimig	17 = 6.6%	

4. Von insgesamt *278* Knollen mit dicken Keimen ringbräunekrank

	81 =	*29.1%*
von *100* Knollen mit dünnen Keimen ringbräunekrank	*56* =	*56.0%*
46 Knollen mit Fadenkeimen ringbräunekrank	*29* =	*63.0%*
146 Knollen mit kranken Keimen ringbräunekrank	*85* =	*58.2%*

Es kann ohne Zweifel festgestellt werden, dass

1. Die Anwesenheit des *Fusarium*-Pilzes in der Knolle die Keimung in eine pathologische Richtung verschiebt,

Tabelle 2

Befall der Knollen im Frühjahr 1952 der Kartoffelpflanzen, die im Jahre 1951 von Welkenkrankheit (Fusarium) befallen worden sind

Sorte	Ringbräune gegenwärtig						Keine Ringbräune					
	mit dicken	dünnen	Faden	Gesamtzahl		Insgesamt	mit dicken	dünnen	Faden	Gesamtzahl		Insgesamt
				gesunde	kranke					gesunde	kranke	
	Keimen in wieviel Knollen						Keimen in wieviel Knollen					
Korai sárga	9	3	1	9	4	13	15	4	1	15	5	20
Bintje	—	—	—	—	—	—	4	—	—	4	—	4
Patonai rózsa	3	4	—	3	4	7	14	—	—	14	—	14
Patonai 158	11	2	—	11	2	13	11	—	7	11	7	18
Eigenheimer	1	—	—	1	—	1	5	—	—	5	—	5
Aranyalma	1	1	—	1	1	2	4	2	—	4	2	6
Aranyalma (Kisvárdai)	3	—	—	3	—	3	2	—	—	2	—	2
Merkur	5	1	—	5	1	6	10	3	—	10	3	13
Merkur (poln.)	4	2	1	4	3	7	27	4	—	27	4	31
Wohltmann	—	2	—	—	2	2	—	3	—	—	3	3
Krüger	3	2	1	3	3	6	2	1	—	2	1	3
Lorch	2	1	7	2	8	10	11	9	2	11	11	22
Berlichingen	4	2	—	4	2	6	14	3	—	14	3	17
Majestic	—	1	4	—	5	5	3	2	—	3	2	5
Korenovski	9	26	14	9	40	49	20	13	5	20	18	38
Koschelinskia	2	—	—	2	—	2	9	—	—	9	—	9
Pionir (Voran)	7	—	1	7	1	8	31	—	2	31	2	33
Parnassia	17	9	—	17	9	26	15	—	—	15	—	15
Insgesamt:	81	56	29	81	85	166	197	44	17	197	61	258

2. die Tatsache, dass aus den Knollen kein Pilz isoliert werden kann, nicht unbedingt gesunde Keimung bedeutet. In diesem Falle ist darauf zu denken, dass der Krankheitserreger durch seine toxischen Substanzen imstande ist, pathologisches Keimen hervorzurufen,

3. die Gesundheit der Keime noch nicht bedeutet, dass auch die Knollen unbedingt gesund sind. Knollen mit dicken Keimen können in einem beträchtlichen Ausmasse (29.1 %) vom Pilze befallen werden.

I. J. 1952 wurden 15 Sorten in unsere einschlägigen phytopathologischen Versuchen einbezogen. Nach dem auf natürlichem Wege erfolgten Fusariumbefall des Krautes wurde die reife Knollenernte — je nach dem Gesundheitszustand der Kartoffelpflanzen — separat aufgenommen, auf die erörterte Weise gelagert und im Frühjahr 1953 zum Keimen gebracht.

Die Ergebnisse des Keimungsversuches werden in den Tabellen 3 und 4 dargestellt.

Aus den Tabellen ist es ersichtlich, dass die Knollen der mit *Fusarium* befallenen Pflanzen zu 21.1 % pathologische Keime entwickelten (darunter waren 14.1 % Knollen mit Fadenkeimen bzw. nicht keimend), die Knollen der gesunden Pflanzen insgesamt zu 4.7 % (darunter 1.8 % Knollen mit Fadenkeimen bzw. nicht keimend).

Tabelle 3

Ergebnisse der Keimung im Frühjahr 1953 der Knollen der im Jahre 1952 von Fusarium *(Fusarium oxysporum)* befallenen Pflanzen

Sorte	Gesamtzahl der Knollen	Knollen mit dicken Keimen	Knollen mit Fadenkeimen	Nicht keimende Knollen	Fadenkeimige und nicht keimende Knollen insges.		Knollen mit Dünnkeimen	Knollen mit pathologischen Keimen und nicht keimende insges.	
	Stück				St.	%	St.	St.	%
Gülbaba	101	66	29	—	29	28.7	6	35	34.6
Aranyalma	91	84	—	1	1	1.1	6	7	7.7
Margit	100	97	—	—	—	—	8	3	3.0
Mittelfrühe	99	52	43	—	43	43.4	4	47	47.4
Sabina	89	61	16	1	17	19.1	11	28	31.5
Ostbote	68	50	15	2	17	25.0	1	18	26.5
Lorch	106	75	18	—	18	17.0	13	31	29.2
Korenovski	101	78	15	—	15	14.9	8	23	22.8
Berlichingen	26	10	—	—	—	—	16	16	61.5
Moschestik	80	65	11	—	11	13.8	4	15	18.8
Merkur	50	35	—	1	1	2.0	14	15	30.0
Krüger	103	99	1	—	1	1.0	3	4	3.9
Parnassia	29	23	6	—	6	20.7	—	6	20.7
Ackersegen	11	9	—	1	1	9.1	1	2	18.2
Wohltmann	90	87	1	—	1	1.1	2	3	3.3
Insgesamt	1144	891	155	6	161	*14.1*	92	253	*22.1*

Tabelle 4

Ergebnisse der Keimung im Frühjahr 1953 der Knollen der im Jahre 1952 von Fusarium *(Fusarium oxysporum)* nicht befallenen Pflanzen

Sorte	Gesamtzahl der Knollen	Knollen mit dicken Keimen	Knollen mit Fadenkeimen	Nicht keimende Knollen	Fadenkeimige und nicht keimende Knollen insgesamt		Knollen mit Dünnkeimen	Knollen mit pathologischen Keimen und nicht keimende insges.	
	Stück				St.	%	St.	St.	%
Gülbaba	96	92	2	—	2	2.1	2	4	4.2
Aranyalma	100	98	—	1	1	1.0	1	2	2.0
Margit	99	98	—	—	—	—	1	1	1.0
Mittelfrühe	102	102	—	—	—	—	—	—	—
Sabina	92	89	—	—	—	—	3	3	3.3
Ostbote	102	98	2	—	2	2.0	2	4	3.9
Lorch	100	76	14	—	14	14.0	10	24	24.0
Korenovski	96	82	5	—	5	5.2	9	14	14.6
Berlichingen	100	100	—	—	—	—	—	—	—
Moschestik	103	97	2	—	2	1.9	4	6	5.8
Merkur	98	89	—	—	—	—	9	9	9.2
Krüger	100	98	—	1	1	1.0	1	2	2.0
Parnassia	98	98	—	—	—	—	—	—	—
Ackersegen	92	91	—	—	—	—	1	1	1.1
Wohltmann	106	106	—	—	—	—	—	—	—
Insgesamt	1434	1414	25	2	27	*1.8*	43	70	*4.7*

Es ist zu betonen, dass der Sommer des Jahres 1952, besonders der Monat August, ausserordentlich warm war, z. B. erreichte am 12. und 17. August das Maximum der Lufttemperatur in Kisvárda im Durchschnitt 35.6 °C, das der Bodentemperatur in 10 cm Tiefe 34.3 °C. Sehr ungünstig gestalteten sich auch die auf 1 mm Niederschlag entfallenden Wärmesummen im Juli und August, da sie auf das Dreifache des für die Kartoffel günstigen Wertes 10 anstiegen (35.5 bzw. 30.6). Diese schädliche Wärmewirkung hat sich auch in einem gewissen Prozentsatz von pathologischer Keimung der Knollen der gesunden Pflanzen geäussert. Aber gerade dieses Jahr erbrachte den Beweis dafür, dass *dem Fusariumbefall* in der Auslösung der pathologischen Keimung *eine viel bedeutendere Rolle* zufiel als der hohen Temperatur. Es soll aber betont werden, dass in diesem Falle *die kranken Pflanzen einen doppelten pathologischen Effekt* (*Fusarium* + hohe Wärme) *erlitten.*

Hinsichtlich der Reaktion sowohl gegenüber *Fusarium* wie auch gegenüber hoher Temperatur zeigten sich sehr wesentliche Sortenunterschiede, z. B. reagierten die Sorten *Sabina, Gülbaba, Mittelfrühe, Berlichingen* auf den Fusariumbefall mit hoher pathologischer Keimzahl (31.5–61.5%), während *Margit, Wohltmann, Krüger, Aranyalma* nur in geringem Ausmasse (zu 3.0–7.7%) pathologische Keime entwickelten. Aus dem mit *Fusarium* nicht befallenen Material zeichneten sich *Korenowski* und *Lorch* mit hoher pathologischer Keimzahl aus (14.6–24.0%),

während z. B. *Mittelfrühe*, *Berlichingen*, *Parnassia* und *Wohltmann* vollständig normal keimten.

Auch das Blattrollvirus (*Corium solani* Holmes) wurde vom Standpunkt der pathologischen Keimung geprüft. Der Kürze halber wollen wir von der Anführung der diesbezüglichen Tabellen absehen (ausführlich s. DOHY, 1955) und nur die Endergebnisse mitteilen. Beim Keimen der Knollen der 1951 mit Blattrollvirus befallen gewesenen Klone »A« bzw. »B« kamen wir im Frühjahr 1952 zum Ergebnis, dass in der Auslösung der pathologischen Keimung das Blattrollvirus bei weitem nicht in dem Masse in Betracht kommt als die infektiöse Fusariumwelke. Nur 1.4% der Knollen der von Blattrollvirus befallenen Pflanzen hat pathologische Keime entwickelt (aus 1981 Knollen 28). Wenn wir auch die nicht keimenden Knollen (von 1981 34 = 1.7%) hierher zählen, so können wir 3.1% schwere Veränderungen zu Lasten des Blattrollvirus nachweisen.

Aus den dickkeimigen Knollen der von Blattrollvirus befallenen Pflanzen entwickelte sich insgesamt ein zu 42.3% kranker Bestand (396 von 924 Pflanzen).

Bei dem Keimen im Frühjahr 1953 in diffusem Licht der Knollen der vom Blattrollvirus befallenen Klone des Jahres 1952 fanden sich 3% dünnkeimige, 1.2% fadenkeimige bzw. nicht keimende, insgesamt 4.2% schweren Defekt erlittene Knollen. Besonders hervorzuheben ist der Befund, dass im Falle des Klons 856, dessen jede einzelne Pflanze mit Blattrollvirus infiziert war, nur zu 0.5% pathologisch keimende Knollen erhalten wurden.

Wir nehmen an, dass die obige, zu Vergleichszwecken vorgenommene Untersuchung mit einem bescheidenen Schritt zur Klärung der Auffassungen und Ansichten betreffs des Blattrollvirus beitrug.

Die für die Praxis abzuleitenden Folgerungen und Erwägungen

1. Da die kranken Knollen früher austreiben als die gesunden, muss bei der frühjahrlichen Knollenselektion *nicht zugewartet werden, bis die Knollen vollständig auskeimen* (was besonders bei in Prismen oder Mieten gelagertem Material mit vielen Keimabbrüchen und einer Verminderung des Saatwertes einhergehen würde), sondern es genügt, wenn sie zu Keimen beginnen. Die kranken Knollen treten nämlich in der Anfangsperiode des Keimens der gesunden Hauptmasse entsprechend in Erscheinung. Von nachträglichem Sprossdefekt kann in dieser Hinsicht keine Rede sein.

2. Da die dickkeimigen Knollen in beträchtlichem Ausmasse mit *Fusarium* oder Virus infiziert sein können, gewinnt die Unerlässlichkeit der negativen Staudenselektion auf dem Kartoffelfeld einen erhöhten Nachdruck. Dies wird noch unterstützt durch die praktische Erfahrung, dass auch die von spät mit *Fusarium* infizierten Pflanzen stammenden, nicht mit Ringbräune befallenen Knollen oft — ohne auffälligere Veränderung des Keimens — verkümmerte Sprosse entwickeln. Diese Knollen erscheinen also auf Grund ihrer Keime als gesund und können daher

nicht ausgeschieden werden. Daraus folgt dann die wichtige Regel, dass die *negative Staudenselektion auch unmittelbar vor der Ernte vorgenommen werden muss.*

3. Da es sich um einen die Pflanze ständig bedrohenden bodenbewohnenden Pilz handelt, muss alles unternommen werden, um einerseits *die Möglichkeit der Infektion präventiv* bestmöglichst *einzuschränken* (richtige Fruchtfolge, Auswahl der Felder, Meiden der bekanntlich stark infizierten Areale), anderseits um *die Entwicklung der Pflanze in die Richtung* der möglichst *geringsten Prädisposition zu lenken:* mit einer rasche Entwicklung gewährleistenden Agrotechnik den Kampf mit Wärme und Trockenheit auf das Minimum zu vermindern (Vortreiben, entsprechende Nährstoffversorgung, Sicherung eines guten Saatbettes, zur richtigen Zeit vorgenommenes Legen, Pflanzenpflege und Ernte). Besonders gilt dies für *Gülbaba*, eine unserer wertvollsten Frühkartoffelsorten, die sich gegen die fusariöse bzw. doppelte (*Fusarium* + Wärme) pathologische Wirkung als ziemlich empfindlich erwies.

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Die Rolle der Gibberellinsäure und Gibberellinderivate bei der Fadensprossbildung der Kartoffelknollen

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Kartoffelknollen wurden mit dem Kulturfiltrat solcher *Fusarium*-Arten behandelt, die an der Stengelbasis Fäule erregen, vor allem mit dem des Pilzes *F. moniliforme*. Bei ihrem Antrieb wurde festgestellt, dass von ihren Stoffwechselprodukten, die in die Nährlösung übergegangen sind, die Gibberellinsäure und die Gibberellinderivate die physiologischen Vorgänge der Pflanze tiefgreifend beeinflussen. Die Ruheperiode wird unterbrochen, die schnell wachsenden Sprosse bleiben dünn, ihre Anthocyanbildung ist mangelhaft. In den auf natürlichem Wege entstandenen Fadensprossen ist in messbarer Menge Gibberellin vorhanden.

Einleitung

Eine sehr wertvolle Entdeckung der letzten Jahrzehnte auf dem Gebiet der Pflanzenpathologie und Pflanzenphysiologie bezieht sich auf die *Fusarium*-Pilze. Der Phytopathologe KUROSAWA, ein Formosa-Japaner, prüfte 1926 die Bakanaëkrankheit des Reises, die er als ansteckend erkannte und aus der er den Pilz *Gibberella fujikuroi* (Saw.) Wr. isolierte. In Pilzkulturen kommt dieser stets in der Konidialform *Fusarium moniliforme* Sheld. vor. Es wurde festgestellt, dass das 2- bis 3wöchige Kulturfiltrat des Pilzes auf die Reispflanzen ebenso wirkt wie der Krankheitserreger selbst; die behandelten Pflanzen wuchsen schnell, streckten sich, wurden schwach und gingen schliesslich ein. YABUTA und HAYASHI (1939) isolierten aus dem Filtrat den Wuchsstoff, das Gibberellin, das jedoch nur nach der Entwicklung der Fermentationsindustrie eine weite Verbreitung und umfangreiche praktische Anwendung erfuhr. Im Filtrat der *Fusarium*-Kultur wurden auch andere Stoffwechselprodukte gefunden, von denen die Fusarinsäure sowie die Malonsäure, ein sehr aktiver Enzymhemmstoff die wichtigsten sind (GÄUMANN, 1957). Auch die Verfasser befassten sich seit einigen Jahren mit den toxischen Stoffen der *Fusarium*-Pilze; es ist ihnen gelungen, mit den Filtraten einiger *Fusarium*-Kulturen unter Laborverhältnissen auf provokativem Wege eine pathologische Sprossbildung hervorzurufen. Die Untersuchungen der Verfasser wurden wesentlich gefördert durch die heimischen Forschungen bzw. durch den Beginn der Gibberellinerzeugung, die durch die Anstalt für Impfstofferzeugung »Phylaxia« in Zusammenarbeit mit dem Forschungsinstitut für Pflanzenschutz in Europa sozusagen zuerst gelöst wurde (UBRIZSY, VÖRÖS und KIRÁLY, 1960, SZABÓ und SZABÓ-SZŰCS, 1961).

Untersuchungsmethoden

Zur Durchführung der Versuche wurden in verschiedenen Teilen des Landes (Nyirség, Donau – Theiss-Zwischenstromland, Nordungarn) ringfäulekranke Knollen und vermorschende unterirdische Stengelteile solcher Kartoffelfelder gesammelt, auf denen die infektiöse Welkekrankheit massenhaft auftrat. Das Aussortieren der Knollen erfolgte im Frühjahr, als auf den Nabelpartien die Schädigung intensiver war und eine leichte Infiltration die ringfaulen Knollen auch ohne eine Halbierung erkennen liess.

Der Pilz wurde auf die übliche Weise isoliert. Nach einer entsprechenden Vorbereitung der Knollen wurde aus dem infizierten Teil unter sterilen Bedingungen ein Stückchen herausgehoben und auf Kartoffelagar übertragen. Die aus den Knollen und der Stengelbasis isolierten *Fusarien* wurden nach den Methoden von WOLLENWEBER und REINKING (1935) und RAJLLO (1950) nicht bestimmt, die Isolaten wurden mit einer laufenden Nummer bezeichnet.

Die in den Versuchen verwendeten, bestimmten Kulturen wurden dem Stammaterial des Instituts für Pflanzenschutz, des Lehrstuhls für Pflanzenpathologie der Hochschule für Garten- und Weinbau bzw. des Lehrstuhls Pflanzenschutz der Universität für Agrarwissenschaften entnommen. Die Massenvermehrung des Pilzes erfolgte in Kulturen in Roux-Flaschen, auf flüssigem Nährboden. Dazu wurden zwei Nährlösungen angewandt:

Lösung Nr. 1.	40.0	g Glukose
	9.5	g Ammoniumtartrat
	2.0	g Kaliumhydrogenphosphat
	0.2	g $MgSO_4$
	0.1	g Spurenelemente (Mn, Cu, Fe, Zn)
Lösung Nr. 2.	10.0	g Saccharose
	50.0	g Malzextrakt
	2.0	g Ammoniumtartrat
	0.3	g Bierhefe
	0.1	g Maismarmelade
	0.5	g Kaliumdihydrogenphosphat
	0.5	g $MgSO_4$
	0.1	g Spurenelemente (Fe, Zn, Mn, Cu, B)

Die Lösung Nr. 1 wurde auf pH 6.2, Nr. 2 auf pH 4.2 eingestellt. In die Roux-Flaschen wurden je 200 ml Nährlösung gefüllt und nachher an 2 aufeinanderfolgenden Tagen bei 1.5 Atm-Druck 1/4 Stunde lang sterilisiert. In der Standkultur wurde der Pilz bei 28 °C 8 Tage lang gezüchtet, worauf das Mycelium durch Filtrieren entfernt wurde. Danach wurden die Knollen 3 Minuten lang mit dem Filtrat behandelt und in schwachem diffusem Licht angetrieben. Die Stärke und die Zahl der Sprosse sowie ihre Wachstumsintensität wurden am Ende der 1. bzw. 2. Woche mittels einer mm-Einteilung gemessen.

Bei beiden Behandlungen wurden Knollen verwendet, die sich im Ruhestadium befanden, bzw. die Sprosse besassen. Versuchssorten waren *Gülbaba*, *Merkur* und *Goldapfel*.

Den Laborversuchen folgten Kleinparzellenversuche im Freiland, um die Wirkung des Gibberellins auch im Stadium des Knollenansatzes messen zu können. Bei der Ertragsanalyse wurde das Erntegewicht reihenweise ermittelt und summiert. Die ausgehobenen Knollen wurden im Frühjahr des folgenden Jahres nach dem Antrieb mit dem Kontrollmaterial verglichen, um feststellen zu können, ob die künstlich induzierte Fadensprossenbildung auch bei der Nachkommenschaft auftritt.

Der Gibberellingehalt der verschiedenen Fusariumkulturen wurde auf papierchromatographischem Wege mittels eines durch die Verfasser modifizierten Verfahrens bestimmt. Die Anzucht der Pilze erfolgte mit der Nährlösung Nr. 2, die sich als Nährboden am besten bewährte. Das Gibberellinvorkommen wurde nach 8 und nach 16 Tagen geprüft. Die papierchromatographische Untersuchung des Filtrats der Standkultur erfolgte durch einfaches Auftropfen, die der Fadenkeime durch Äthylacetatextraktion. Als Laufmittel wurde n-Butanol – 1.5-n-Ammoniak im Verhältnis 3 : 1 verwendet, die Laufzeit betrug 4 Stunden. Als Entwickler wurde Äthylalkohol und cc. Schwefelsäure im Verhältnis 3 : 2 benützt, durch dieses wurde das getrocknete Papier durchgezogen und mittels einer analytischen UV-Lampe durchleuchtet. Dabei traten die grünlichblau fluoreszierenden Gibberellinflecken in Erscheinung. Aus ihrem R_f-Wert, sowie aus ihrer Farbenintensität und Fleckengrösse wurde auf die Menge der Gibberellinsäure geschlossen. Als Standard diente eine Gibberellinsäurelösung bekannter Konzentration.

Im Laufe der Untersuchungen wurden nicht nur Fadenkeime mittels Gibberellin induziert, auch natürlich entstandene Fadenkeime wurden versuchsweise auf Gibberellin geprüft. Zu diesen Versuchen wurden etiolierte Fadensprosse bzw. gesunde, normal anthocyanierte Starksprosse verwendet.

Versuchsergebnisse

Nach den Untersuchungen der Verfasser entstanden die mittels der pilzfreien Kulturfiltrate verschiedener *Fusarium*-Arten ausgelösten tiefdringenden Veränderungen (Fadensprossbildung, vorzeitiges Austreiben der Knospen, Zunahme der Sprosszahl, Fehlen der Anthocyaniertheit) infolge des longitudinalen Wuchsbeschleunigungseffekts der Gibberellinsäure.

Die Gibberellinerzeugung der im Versuch verwendeten *Fusarium*-Arten wird in Tabelle 1, die an den behandelten Knollen erscheinende Wirkung in Abb. 1 angeführt. Von den Pilzen, die vom untersten Teil des Stengels der Kartoffelstaude, aus den Knollen und aus dem Boden der Kartoffelfelder isoliert wurde, verursacht *F. moniliforme* (Stamm 24) die grösste Veränderung; dieser Stamm wird auch zur heimischen Gibberellinerzeugung verwendet. Die Anthocyaniertheit der Sprosse der behandelten Knollen sowie ihr Fehlen steht im Zusammenhang mit der

Gibberellinsäure bzw. mit den Gibberellinderivaten (Tabelle 2). Dies beweisen auch die sehr starken provokativen Behandlungen mit der 0.01 %-igen Lösung der kristallinen Gibberellinsäure heimischer Erzeugung (Tabelle 3).

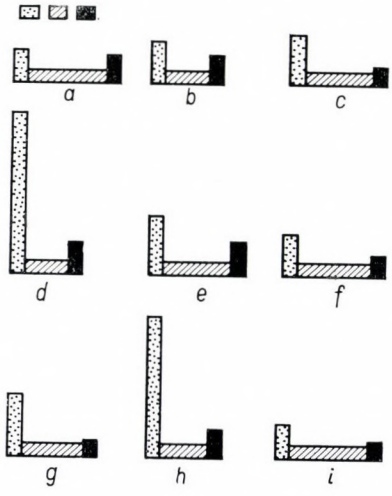

Abb. 1. Die Wirkung des Kulturfiltrats verschiedener Fusarium-Arten auf die Sprosse behandelter Knollen. Punktiert = Sprosszahl, schattiert = Sprossdurchmesser, gefüllt = Sprosshöhe, a—h: *Fusarium*-Arten nach Tabelle 1, i = Daten der Kontrollknollen

Die mit den Kulturfiltraten behandelten Knollen wurden in Freilandversuchen 2 Jahre hindurch geprüft. Es wurden im Vergleich zu den Kontrollreihen sehr bedeutende Unterschiede in der Zahl der Sprosse und im Wachstumsgang festgestellt (Abb. 2 und 3). Die Unterschiede verminderten sich mit der Vegetationszeit und mit dem Zunehmen der Nährstoffaufnahme aus dem Boden und

Tabelle 1

Die Untersuchung der Gibberellinerzeugung verschiedener *Fusarium*-Arten (Nährlösung Nr. 3)

Angewandte Fusarien	In der Nährlösung:	
	Gibberellinsäure mg/ml	Gibberellinderivate
F. moniliforme v. *minus* (a)	0	einerlei Gibb. Deriv.
F. oxysporum f. *niveum* (b)	0	einerlei Gibb. Deriv.
F. solani (e)	0	einerlei Gibb. Deriv.
F. moniliforme (d)	55	zweierlei Gibb. Deriv.
F. sp. von Stengelbasis der Kartoffel (e)	0	einerlei Gibb. Deriv.
F. sp. von Stengelbasis der Kartoffel (f)	0	einerlei Gibb. Deriv.
F. moniliforme (g)	0	einerlei Gibb. Deriv.
F. moniliforme (h)	10	zweierlei Gibb. Deriv.

Tabelle 2

Mittelwerte der Knollen bei Behandlung mit dem Filtrat der pilzfreien Nährlösung Nr. 1 verschiedener *Fusarium*-Arten
Sorte: Gülbaba

Benennung des Fusariums	Mittelwerte der Sprosse einer Knolle			
	Zahl St.	Länge mm	Durchmesser mm	Anthocyaniertheit
F. moniliforme v. *minus* (20)	3.2	7.8	3.7	normal
F. oxysporum f. *niveum* (21)	3.6	8.4	3.4	normal
F. solani (23)	5.7	7.8	3.4	normal
F. moniliforme (24)	12.6	16.4	1.8	keine
F. species (29)	5.1	8.4	3.6	normal
F. species (32)	5.2	8.0	2.9	schwach
F. moniliforme (33) (?)	6.0	9.8	3.8	schwach
F. moniliforme (34) (?)	3.9	10.0	2.6	schwach
Kontrolle	3.6	6.7	4.2	normal

verschwammen schliesslich gänzlich. Bei der quantitativen Bewertung des Ertrags ergaben sich dennoch wesentliche Differenzen in der Knollengrösse, Knollenzahl und im mittleren Gewicht des Ertrags einer Staude (Tabelle 4).

Die Nachkommenschaften der auf provokativem Wege gewonnenen fadensprossigen Knollen bzw. der Kontrollknollen wurden nach der Ernteanalyse getrennt eingelagert. Ihre Sprossbildung wurde im folgenden Frühjahr unter Laborbedingungen verglichen.

Tabelle 3

Die Wirkung des Gibberellins auf die Sprossbildung der Kartoffel
Sorte: Gülbaba

Serie	Sprosszahl St.			Sprossdurchmesser mm			Anthocyaniertheit	
	behandelt		Kontrolle	behandelt		Kontrolle	behandelt	Kontrolle
	0.01%	0.05%		0.01%	0.05%			
1.	11.8	11.4	3.8	1.0	1.0	2.7	keine	normal
2.	11.6	11.2	4.4	1.1	0.9	3.8	keine	normal
3.	12.1	12.0	4.3	1.0	1.2	3.6	keine	normal
4.	11.7	11.6	4.1	1.0	1.2	3.5	keine	normal
5.	10.2	10.9	3.9	0.9	1.0	3.5	keine	normal
6.	10.1	10.5	3.6	1.3	1.5	3.4	keine	normal
7.	10.8	9.4	3.0	1.4	1.6	3.8	keine	normal
8.	11.5	10.6	3.3	1.2	1.4	3.0	keine	normal

Abb. 2. Fadendünne Sprosse einer mit Gibberellin behandelten Knolle

Abb. 3. Freilandversuch zum Vergleich behandelter Reihen mit Kontrollreihen

Beim Antrieb konnte zwischen den Partien verschiedener Herkunft kein Unterschied festgestellt werden. Die Serien beider Gruppen begannen gleichzeitig zu treiben, sogar das Prozent der pathologischen Sprossbildung war im wesentlichen das gleiche.

In ihren Versuchen induzierten die Verfasser nicht nur eine Fadensprossbildung mittels Gibberellin, sondern wiesen auch in natürlich gebildeten Fadensprossen

Gibberellin nach (Abb. 4). Es wurde festgestellt, dass in den Fadensprossen Gibberellin bzw. Gibberellinderivate in messbarer Menge vorkommen, in den starken Sprossen können sie jedoch nur in Spuren nachgewiesen werden. Die Versuche der Verfasser bestätigen jene Versuche, nach denen sich in der Kartoffel Gibberellin bildet. Die Untersuchungen in bezug auf die Fadensprossbildung infolge abiotischer Faktoren (hohe Bodentemperatur und akuter Feuchtigkeitsmangel zur Zeit der Knollenbildung) und der Schadwirkung des Stolbur-Virus sowie in bezug auf die Anhäufung endogenen Gibberellins sollen ausgebreitet werden. Nach OKAZAWA (1959, 1960) nimmt der Gibberellingehalt des Laubes bei Temperaturen,

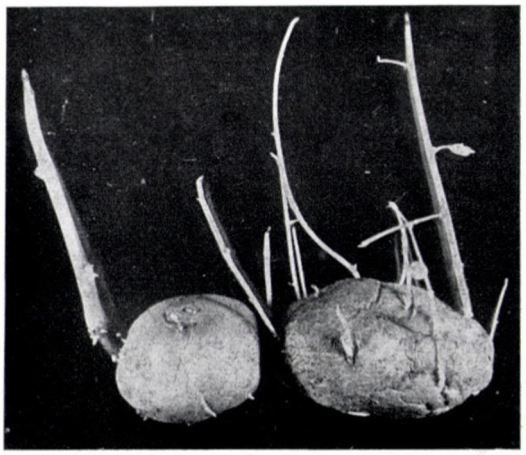

Abb. 4. Das Treiben einer doppelwüchsigen Knolle. Die Knollenpartie, die sich zur Dürrezeit bildete, treibt Fadensprosse. Die nach Niederschlägen entstandene Partie treibt starke, anthocyanierte Sprosse

die der Knollenbildung zusagen sowie an Kurztagen bedeutend ab. Nach der Meinung der Verfasser ermöglichen diese Ergebnisse eine Erklärung mehrerer umstrittener Fragen der Sommerpflanzung.

Tabelle 4

Der Ernteertrag Gibberellin-behandelter Pflanzknollen bei Frühjahrpflanzung

Sorte	Mittelgewicht in dkg der Knollen einer Staude		Vergleich der Erträge Kontrolle = 100
	behandelt	Kontrolle	
Gülbaba	40	81	49.4
Merkur	39	62	62.9
K. Rose	42	89	47.2
Goldapfel	46	95	48.4

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Mycological Investigations in some Hungarian Forest Types and Special Sites, II.

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3. *Sopron and its environment* provides ecological and climatic conditions quite different from those in the Hungarian Medium Mountains. Owing to the subalpine and montan climate the fungus vegetation is generally rich and it does not fluctuate to such a high degree either than in other regions. In the area studied (comprising the floristic districts Noricum: Ceticum; Pannonicum: Laitaicum; Castriferreicum and Arrabonicum) about 568 fungus species have been detected (LENKY in lit., CSAPODY 1963), this is, accordingly, floristically the richest territory of Hungary. Even in arid years the mycocoenosis is more or less rich in species, while in humid ones the fruit body production exceeds both in the minimum areas and per hectare that of the Bükk- and Sátor Mountains subjected to Subcarpathian influences.

The investigations were begun in seven characteristic plant associations of greater importance in September 1965. Each minimum area had an extent of 500 m^2, though due to the relative abundance of fruit body production quadrates of 100 m^2 would have sufficed. Number of surveys: 56. The features of the main forest associations and fungus vegetations are as follows:

a) In a mixed stand of *Piceetum excelsae-carpinoso-quercosum* Soó most widespread in the environment of Sopron, especially in the areas of the Noricum, the tree layer is composed of *Picea excelsa*, *Pinus silvestris*, *Abies alba*, *Quercus petraea*, *Castanea sativa*, *Carpinus betulus*, *Tilia cordata* etc. and shows a cover of 70 to 80 per cent, while the shrub layer – containing *Tilia*, *Corylus avellana*, *Castanea* – of 10 per cent. The herb layer including the types subnudum and nudum and having a cover of 15 to 20 per cent contains the following species: *Vaccinium myrtillus*, *Calluna vulgaris*, *Luzula nemorosa*, *Deschampsia flexuosa*, *Melampyrum cristatum*, *Convallaria majalis* etc.

The species of the mycocoenoses developed in this association totalled up to 98 representing that this forest community is the richest in species. The number of pieces ranged from 53 to 301 in the minimum areas of 500 m^2 extent. Constant-dominant species: *Amanita citrina*, *Boletus chrysenteron*, *B. subtomentosus*, *Armillaria mellea*, *Boletus granulatus*, *B. luteus*, *Cortinarius anomalus*, *C. multiformis*, *Craterellus cornucopioides*, *Laccaria laccata*, *Lactarius subdulcis*, *Russula cyanoxantha*, *R. ochroleuca*, *R. vesca* etc. (Tab. 3).

Table 3

Piceetum excelsae carpinoso-quercosum Soó	Sb.	S.	F.	Deák-kút 27. 9. 1965	Deák-kút 24. 9. 1965
Agaricus arvensis silvicola	TT.	1	S	—	—
Amanita citrina	T.	1	M	4, +	—
Amanita rubescens	T.	1	M	—	—
Amanita spissa	T.	1	M	1, +	—
Amanita vaginata	T.	1	M	—	—
Armillaria mellea	Tr.	2	P—S	45, 1	120, 1
Boletus chrysenteron	T.	1	M	32, 1	4, +
Boletus edulis	T.	1	M	—	2, +
Boletus flavus	T.	1	M	—	10, +
Boletus granulatus	T.	1	M	—	1, +
Boletus luteus	T.	1	M	—	—
Boletus placidus	T.	1	M	—	—
Boletus sanguineus	T.	1	M	—	—
Boletus subtomentosus	T.	1	M	6, +	6, +
Boletus viscidus	T.	1	M	—	2, +
Bulgaria polymorpha	Tr.	2	S	—	—
Calocera viscosa	Tr.	2	S	—	—
Cantharellus cibarius	T.	1	M	—	—
Clavaria flava	T.	3	S	—	—
Clitocybe drussata	T.	1	S	—	—
Clitocybe infundibuliformis	T.	1	S	—	—
Clitocybe inversa	T.	1	S	—	—
Clitocybe odora	T.	1	S	—	3, +
Clitocybe pythiophila	T.	1	S	—	—
Collybia acervata	T.	2	S	—	—
Collybia dryophila	T.	1	S	—	—
Collybia fusipes	T.	1—2	S—P	—	—
Collybia ionides	T.	1—2	S	—	—
Collybia radicata	T.	1	S	—	—
Coprinus micaceus	Tr.	2	S	—	—
Cortinarius anomalus	T.	1	M	—	3, +
Cortinarius cinnamomeus	T.	1	M	4, +	—
Cortinarius cinnabarius	T.	1	M	—	—
Cortinarius largus	T.	1	M	—	1, +
Cortinarius multiformis	T.	1	M	—	2, +
Cortinarius triumphans	T.	1	M	—	1, +
Craterellus cornucopioides	T.	1—2	M	28, + —1	—
Fomes annosus	P.	2	P	—	—
Geastrum hygrometricum	T.	1	S	—	—
Gomphidius viscidus	T.	1	M	—	1, +
Hebeloma crustuliniforme	T.	1	S	—	—
Hypholoma fasciculare	Tr.	2	S	—	—
Hypholoma sublateritium	Tr.	2	S	35, + —1	—
Inocybe fastigiata	T.	1	S	—	—
Laccaria amethystina	T.	1—2	S	—	3, +

Sopron							K
Deák-kút 28. 9. 1965	Deák-kút Fáberrét 24. 9. 1965	Károly-magaslat 17. 9. 1965	Károly-magaslat 22. 9. 1965	Károly-magaslat 27. 9. 1965	Várhely 22. 9. 1965	Fáberrét 27. 9. 1965	
2, +	–	–	–	–	2, +	–	1
2, +	3, +	4, +	1, +	2, +	–	3, +	5
3, +	–	–	–	3, +	–	–	1
4, +	–	–	–	–	–	1, +	2
1, +	1, +	–	–	–	–	–	1
15, + – 1	–	–	27, + – 1	–	–	22, + – 1	3
17, + – 1	23, + – 1	8, +	16, + – 1	6, +	–	15, + – 1	5
–	2, +	–	–	–	–	–	1
6, +	–	–	–	4, +	–	1, +	2
3, +	2, +	5, +	–	–	–	–	3
2, +	–	–	–	5, +	–	–	1
–	–	–	–	2, +	–	–	1
1, +	–	–	–	–	–	2, +	1
8, +	15, + – 1	3, +	6, +	–	–	2, +	1
–	3, +	–	–	–	–	–	1
–	–	–	–	–	M, +	–	1
–	–	–	–	2, +	–	–	1
–	–	2, +	–	3, +	–	–	1
–	–	–	–	–	2, +	–	1
8, +	–	–	–	–	–	–	1
4, +	–	–	–	–	–	–	1
12, + – 1	–	–	–	–	–	–	1
–	–	1, +	–	–	–	–	1
–	–	3, +	–	–	–	–	1
25, + – 1	–	5, +	–	–	–	–	2
9, +	–	–	–	–	–	–	1
2, +	–	3, +	–	2, +	–	–	2
–	–	–	–	–	3, +	–	1
–	–	–	–	–	–	3, +	1
–	–	–	–	–	36, 1	–	1
2, +	–	–	9, +	1, +	–	3, +	3
–	–	–	–	–	–	–	1
–	–	5, +	–	–	–	–	1
–	–	23, + – 1	–	–	–	–	2
3, +	–	5, +	2, +	–	–	–	3
–	–	–	–	–	–	–	1
–	5, +	–	35, + – 1	–	–	–	3
–	–	–	–	–	M, + – 1	–	1
1, +	–	–	–	–	–	–	1
–	–	–	–	–	–	–	1
3, +	–	–	–	–	–	–	1
17, + – 1	26, + – 1	–	–	–	–	–	2
45, 1	–	–	–	–	72, 1	–	3
–	–	–	–	–	–	1, +	1
–	–	3, +	–	–	–	–	1

Table 3 continued

Piceetum excelsae carpinoso-quercosum Soó	Sb.	S.	F.	Deák-kút 24. 9. 1965	Deák-kút 27. 9. 1965
Laccaria laccata	T.	1—2	S	6, +	—
Lactarius deliciosus	T.	1	M	2, +	—
Lactarius piperatus	T.	1	M	—	—
Lactarius quietus	T.	1	M	3, +	—
Lactarius scrobiculatus	T.	1	M	—	—
Lactarius subdulcis	T.	1	M	—	3, +
Lactarius vellereus	T.	1	M	2, +	—
Lenzites abietina	Tr.	2	S	—	—
Lepiota clypeolaria	T.	1	S	1, +	—
Lepiota erminea	T.	1	S	—	—
Lepiota procera	T.	1	S	—	—
Lepiota rhacodes	T.	1	S	—	1, +
Limacium russula	T.	1	S	—	—
Lycoperdon gemmatum	T.	1	S	3, +	2, +
Lycoperdon pyriforme	T.	1—2	S	—	—
Lycoperdon saccatum	T.	1	S	—	—
Marasmius peronatus	T.	1	S	½	½
Mycena pura	T.	1	S	—	—
Naucoria escharoides	T.	1	S	—	—
Otidea grandis	T.	1—2	S	—	—
Panus sripticus	Tr.	1—2	S	—	—
Paxillus atrotomentosus	Tr.	1—2	M	—	—
Paxillus involutus	T.	1	M	—	—
Phallus impudicus	T.	1	M	—	—
Pleurotus dryinus	Tr.	1—2	P	—	—
Pholiota terrigena	T.	1	S	3, +	—
Pluteus cervinus	Tr.	1	S	—	—
Polyporus cristatus	T.	1—2	S	—	—
Polyporus schweinitzii	Tr.	1—2	P—S	—	—
Psathyrella candolleana	Tr.	1—2	S	—	—
Psathyrella hydrophila	Tr.	2	S	—	—
Rozites caperata	T.	1	M	—	—
Russula atropurpurea	T.	1	M	—	2, +
Russula cyanoxantha	T.	1	M	2, +	—
Russula densifolia	T.	1	M	—	—
Russula foetens	T.	1	M	—	—
Russula fragilis	T.	1	M	—	—
Russula heterophylla	T.	1	M	—	—
Russula lepida	T.	1	M	—	—
Russula nigricans	T.	1	M	—	—
Russula ochroleuca	T.	1	M	2, +	—
Russula vesca	T.	1	M	3, +	—
Scleroderma vulgare	T.	1—2	M	—	—
Stereum hirsutum	Tr.	2	S—P	—	—
Trametes serialis	Tr.	2	P	—	—

Sopron							K
Deák-kút 28. 9. 1965	Deák-kút Fáberrét 24. 9. 1965	Károly magaslat 17. 9. 1965	Károly magaslat 22. 9. 1965	Károly-magaslat 27. 9. 1965	Várhely 22. 9. 1965	Fáberrét 27. 9. 1965	
22, + – 1	–	8, +	4, +	–	–	–	3
–	–	–	–	–	–	–	1
–	–	–	–	–	–	1, +	1
1, +	–	8, +	1, +	–	–	–	3
–	–	–	3, +	–	–	–	1
7, +	2, +	2, +	2, +	–	–	–	3
–	–	–	–	–	–	–	1
M, +	–	–	–	–	1, +	–	1
–	–	–	–	–	–	–	1
–	–	2, +	–	–	–	–	1
–	–	1, +	–	1, +	–	–	1
6, +	–	–	–	–	2, +	–	2
1, +	–	–	–	–	–	–	1
2, +	–	–	1, +	–	–	–	3
–	12, +	–	–	–	–	–	1
1, +	–	–	–	–	–	–	1
5, +	–	3, +	–	–	–	–	2
5, +	–		–	–	–	–	2
4, +	–	–	–	–	–	2, +	1
2, +	–	–	–	–	–	–	1
12, +	–	–	–	–	–	–	1
1, +	–	–	–	–	–	–	1
2, +	–	–	–	–	2, +	–	1
–	6, +	–	–	–	–	–	1
–	–	–	–	–	1, +	–	1
–	–	–	–	–	–	–	1
1, +	–	–	–	–	–	–	1
–	–	–	–	–	–	3, +	1
–	–	–	–	–	1, +	–	1
3, +	–	–	–	–	–	–	1
–	–	–	–	–	58, 1	–	1
4, +	–	–	–	–	–	–	1
2, +	–	2, +	–	–	–	4, +	3
2, +	10, +	–	–	4, +	–	–	3
–	3, +	–	2, +	–	–	–	1
–	–	–	2, +	–	–	–	1
2, +	–	1, +	–	1, +	–	–	2
–	–	–	–	–	–	2, +	1
1, +	–	–	–	–	–	1, +	1
–	4, +	–	5, +	12, + – 1	–	3, +	3
6, +	5, +	–	2, +	–	–	2, +	3
2, +	–	–	2, +	2, +	–	3, +	3
–	–	2, +	–	–	–	3, +	1
M, +	–	–	–	2, +	–	–	2
–	M, +	–	–	–	M, +	–	2

Table 3 continued

Piceetum excelsae carpinoso-quercosum Soó	Sb.	S	F.	Deák-kút 24.8.1965	Deák-kút 27.9.1965
Tricholoma acervum	T.	1	S	—	3, +
Tricholoma album	T.	1	S	2, +	—
Tricholoma columbetta	T.	1	S	—	—
Tricholoma inamoenum	T.	1	S	—	—
Tricholoma rutilans	Tr.	1—2	S	—	3, +
Tricholoma sapronacaeum	T.	1	S	—	—
Thelephora laciniata	T.	1—2	S	—	—
Xanthochrous obliquus	Tr.	2	P	—	—
Total number of species: 98 Number of species/Number of fruit bodies:				19 : 184	20 : 173

The maximum aspect develops in late summer or in autumn, culminating thus in September. In mild autumns hibernal-cryophilic aspects may be found even in October and November. This association is the site of *Boletus edulis*, *Cantharellus cibarius*, *Lactarius deliciosus* and of other fine species collected in great quantities.

Most important aspects (in autumn): *Amanita citrina — Boletus chrysenteron — Craterellus cornucopioides; Armillaria mellea — Boletus granulatus; Amanita spissa — Lepiota rhacodes — Hypholoma sublateritium; Boletus granulatus — Cortinarius multiformis; Amanita citrina — Boletus chrysenteron — Russula ochroleuca* (Figs 1 and 2).

Panus stipticus — Tricholoma rutilans is an epixylous aspect, while the coenoses *Fomes annosus — Trametes hirsuta — Tr. gibbosa* and *Trametes serialis — Anisomyces odoratus — Leptoporus caesius* are epixylous synusiae.

Fig. 1. *Amanita spissa* and *Amanita citrina* in *Piceetum* (Sopron, September 27, 1965)

Sopron							K
Deák-kút 28. 9. 1965	Deák-kút Fáberrét 24. 9. 1965	Károly magaslat 17. 9. 1965	Károly magaslat 22. 9. 1965	Károly magaslat 27. 9. 1965	Várhely 22. 9. 1965	Fáberrét 27. 9. 1965	
2, +	–	–	–	–	–	–	1
–	–	–	–	–	–	–	1
4, +	–	2, +	–	–	–	–	1
–	–	4, +	–	–	–	–	1
3, +	–	–	–	3, +	3, +	–	2
2, +	–	–	–	–	–	4, +	1
–	–	–	–	3, +	–	–	1
–	–	–	–	–	2, +	–	1
53 : 301	17 : 103	25 : 109	18 : 121	16 : 53	16 : 178	21 : 81	

b) Abieti-Fagetum Knapp. *noricum typ. Oxalis et nudum* Soó. – In the tree layer (cover: 70 to 80 per cent) *Abies alba*, *Picea excelsa*, *Quercus petraea*, *Carpinus betulus*, *Fagus silvatica* etc.; in the shrub layer (cover: 10 per cent) the same species occur. The herb layer of 30 to 40 per cent cover comprises *Oxalis acetosella*, *Galium schultesii*, *Cyclamen europaeum*, *Melica uniflora*, *Luzula pilosa*, *Neottia nidus-avis*, *Hieracium umbellatum*, *H. murorum* etc. This association is especially well developed on the Mount Várhely with *Abies* as absolutely dominant species. Number of species in the mycocoenosis 68, that of pieces per sample quadrate 52 to 163. Constant-dominant species: *Lactarius deliciosus*, *Phallus impudicus*, *Hydnum repandum*, *Gomphidius viscidus*, *Amanita citrina*, *Armillaria mellea*, *Boletus granulatus*, *Cortinarius largus*, *Hebeloma crustuliniforme*, *Laccaria laccata*, *Mycena pura*, *Paxillus atrotomentosus*, *Russula virescens* etc. (Tab. 4).

Fig. 2. *Hypholoma fasciculare* synusia in *Piceetum* (Sopron, September 25, 1965)

Table 4

Abieti-Fagetum, Knapp noricum tip. *Oxalis* et *nudum* Soó	Sb.	S.	F.	Sopron Várhely 22. 9. 1965 A – D, N	Sopron Várhely 22. 9. 1965 A – D, N	Sopron Várhely 22. 9. 1965 A – D, N	Fr.
Agaricus arvensis v. *silvicola*	T.	1	S	1, +	–	–	1
Amanita citrina	T.	1	M	2, +	1, +	1, +	4
Amanita rubescens	T.	1	M	–	1, +	–	1
Amanita vaginata	T.	1	M	–	1, +	1, +	2
Armillaria mellea	Tr.	2	P	50, 1	5, +	8, +	4
Boletus flavus	T.	1	M	–	3, +	1, +	2
Boletus granulatus	T.	1	M	3, +	2, +	1, +	4
Boletus subtomentosus	T.	1	M	–	2, +	–	1
Clitocybe odora	T.	1	S	3, +	–	–	1
Clitocybe phyllophila	T.	1–2	S	–	–	5, +	1
Collybia radicata	T.	1	S	1, +	–	1, +	1
Cortinarius anomalus	T.	1	M	1, +	–	2, +	2
Cortinarius brunneus	T.	1	M	2, +	1, +	1, +	3
Cortinarius caerulescens	T.	1	M	2, +	–	–	1
Cortinarius largus	T.	1	M	4, +	2, +	2, +	4
Cortinarius multiformis	T.	1	M	16, + – 1	–	1, +	3
Fomes annosus	Tr.	3	P	–	2, +	2, +	2
Gomphidius glutinosus	T.	1	M	–	2, +	–	1
Gomphidius viscidus	T.	1	M	2, +	1, +	1, +	4
Hebeloma crustiliniforme	T.	1	S	3, +	2, +	1, +	4
Hydnum repandum	T.	1	M	12, +	2, +	3, +	5
Hypholoma fasciculare	Tr.	2	S	13, +	–	–	2
Laccaria amethystina	T.	1	S	1, +	–	1, +	2
Laccaria laccata	T.	1	S	7, +	2, +	2, +	4
Lactarius deliciosus	T.	1	M	5, +	3, +	3, +	5
Lactarius subdulcis	T.	1	M	2, +	–	2, +	2
Lycoperdon gemmatum	T.	1–2	S	–	–	2, +	1
Lycoperdon pyriforme	T.	1–2	S	–	2, +	2, +	2
Mycena galericulata	Tr.	1–2	S	15, +	–	–	1
Mycena pura	T.	1	S	2, +	1, +	3, +	4
Panus stipticus	Tr.	2	S	–	–	3, +	1
Paxillus atrotomentosus	Tr.	1–2	S	2, +	1, +	1, +	4
Paxillus involutus	T.	1	M	–	2, +	1, +	2
Phaeolus schweinitzii	Tr.	2	P	–	2, +	–	1
Phallus impudicus	T.	1	M	5, +	2, +	1, +	5
Russula atropurpurea	T.	1	M	–	–	2, +	1
Russula lepida	T.	1	M	2, +	–	3, +	3
Russula nigricans	T.	1	M	–	3, +	1, +	3
Russula virescens	T.	1	M	–	2, +	1, +	3
Scleroderma bovista	T.	1–2	M	1, +	–	–	1
Scleroderma vulgare	T.	1–2	M	3, +	1, +	–	2
Stereum hirsutum	Tr.	2	S–P	–	–	M, +	1
Stereum sanguinolentum	Tr.	2	S–P	M, +	–	–	1
Trametes serialis	Tr.	2–3	P–S	–	–	2, +	1
Trametes hirsuta	Tr.	2	S	–	1, +	–	1
Tricholoma acerbum	T.	1	M	–	2, +	1, +	2
Tricholoma rutilans	Tr.	1–2	S	–	3, +	–	1
Total number of species: 46							
Total number of species/Number of fruit bodies				27 : 163	27 : 52	34 : 65	

Most important autumnal aspects: *Lactarius deliciosus – Phallus impudicus; Armillaria mellea – Paxillus atrotomentosus – Tricholoma rutilans; Boletus granulatus – Gomphidius viscidus – Hydnum repandum.* Epixylous aspect: *Tricholoma rutilans – Armillaria mellea.* Epixylous synusia: *Fomes annosus – Stereum sanguinolentum – Trametes serialis* (perennial).

c) Querco petraeae-Carpinetum transdanubicum Soó et Zólyomi. – The tree layer of 60 to 70 per cent cover is built up by *Quercus petraea, Carpinus betulus, Picea excelsa, Betula pendula, Larix decidua* etc. the shrub layer (cover: 40 to 50 per cent) by the same species. The herb layer has a cover of 20 to 25 per cent and

Fig. 3. *Lactarius piperatus* population in *Querco-Carpinetum* (Sopron, September 21, 1965)

contains *Luzula nemorosa, Melica uniflora, Dactylis aschersoniana, Viola silvestris, Cyclamen europaeum, Galium schultesii, Ajuga reptans, Hieracium sabaudum* etc.

Number of fungus species: 51, that of specimens 41 to 227. This mycocoenosis comes, accordingly, after that of *Piceetum* et *Luzulo-Quercetum.* Constant-dominant species: *Lactarius piperatus, Cortinarius multiformis, C. largus, Craterellus cornucopioides, Lactarius subdulcis, Hydnum repandum, Tricholoma columbetta* etc. (Figs 3 and 4).

Autumnal maximum aspects: *Lactarius piperatus – Craterellus cornucopioides – Cortinarius largus; Craterellus cornucopioides – Lactarius scrobiculatus – Russula vesca; Cortinarius hinnuleus – Hebeloma crustuliniforme – Tricholoma columbetta; Amanita pantherina – Craterellus cornucopioides – Lactarius piperatus.* Epixylous aspect: *Pluteus cervinus – Hypholoma fasciculare.* Epixylous synusia: *Stereum hirsutum – Trametes betulina.* This association is worth mentioning as the site producing large masses of *Craterellus cornucopioides* (Tab. 5b).

d) Luzulo-Querco-Carpinetum noricum Soó. – The tree layer consists of *Quercus petraea, Carpinus betulus, Picea excelsa, Pinus silvestris, Larix decidua, Castanea sativa, Tilia cordata* etc. and has a cover of 60 to 70 per cent. No shrub

layer. In the herb layer (cover: 40 to 50 per cent) *Luzula nemorosa*, *Molinia coerulea*, *Deschampsia flexuosa*, *Dactylis aschersoniana*, *Melampyrum cristatum*, *Hieracium sabaudom*, *Dicranum* mosses etc.

Number of fungus species: 49, that of the specimens per minimum area 57 to 310, so this mycocoenosis is – together with that of *Piceetum* – the richest in species.

The resemblance between the two associations of mixed composition is considerable. The former has the highest fruit body production in drier years, the latter, when the weather is more humid.

Fig. 4. *Phallus impudicus*, *Lactarius piperatus* and *Collybia radicata* in *Querco-Carpinetum* (Sopron, September 21, 1965)

Constant-dominant species of the coenosis: *Lactarius subdulcis*, *Russula atropurpurea*, *Collybia dryophila*, *Amanita citrina*, *Cortinarius largus*, *Hebeloma crustuliniforme*, *Russula nigricans*, *R. vesca*, (Table 5a, Fig. 5).

Autumnal aspects examined: *Boletus chrysenteron* – *Clitocybe odora* – *Russula atropurpurea; Collybia fusipes* – *Hydnum repandum* – *Fistulina hepatica; Amanita citrina* – *Russula cyanoxantha* – *Lactarius subdulcis; Cortinarius anomalus* – *Russula nigricans*. Epixylous aspect: *Psathyrella hydrophila* – *Hypholoma sublateritium*. Epixylous synusia: *Stereum hirsutum* – *Trametes versicolor* – *Fistulina hepatica*.

e) Aceri pseudoplatani-Alnetum glutinosae Ubrizsy 1965. – In the tree layer (cover: 60 to 70 per cent) *Acer pseudoplatanus*, *Alnus glutinosa*, *Carpinus betulus*, *Quercus petraea*, *Picea excelsa* etc. In the shrub layer (cover: 10 per cent) *Corylus avellana*, and those of the tree layer. In the herb layer (cover: 60 per cent) *Asperula odorata*, *Impatiens noli-tangere*, *Oxalis acetosella*, *Brachypodium silvaticum*, *Lamium galeobdolon*, *Majanthemum bifolium* etc. This association is the characteristic community of the valleys Kecskepatak and Várvölgy, affording favourable ecological conditions for the fungus vegetation.

Number of species in the mycocoenosis: 50, that of specimens 39 to 154, i.e. very high, demonstrating the favourable feature of the biotope. On the slopes of the valleys the association is mingled with the previous communities. Constant-dominant species: *Xanthochrous radiatus*, *Boletus chrysenteron*, *Russula nigricans*, *R. densifolia*, *Boletus lividus* (characteristic species!), *Armillaria mellea*, *Lepiota procera*, *Boletus subtomentosus* etc. In the humid, wet environment many xylophagous species and epixylous saprophytes (e.g. *Xylaria polymorpha*, *X. hypoxylon*, *Myxomycetes spp.*, *Stropharia aeruginosa*, *Naucoria escharioides* etc.) occur (Table 6).

Fig. 5. *Amanita citrina*, *Russula nigricans*, *R. cyanoxantha* in *Luzulo-Querco-Carpinetum* (Sopron, September 23, 1965)

Autumnal aspects: *Clitocybe pythiophila* – *Cortinarius triumphans; Boletus chrysenteron* – *Russula densifolia* – *Ramaria cinerea; Armillaria mellea* – *Clitocybe infundibuliformis* – *Mycena* ssp. Epixylous aspect: *Hypoxylon coccineum* – *Stropharia aeruginosa* – *Xylaria polymorpha.* Epixylous synusiae: *Xanthochrous radiatus* – *Ganoderma applanatum* – *Stereum gausapatum; Irpex fusco-violaceus* – *Trametes* spp. – *Stereum sulphurosum.* On the stumps of *Alnus glutinosa* abundantly developing "tinder collections" may be found.

f) Querco-Betuletum-Callunetosum Zólyomi. – In the tree and shrub layer (showing together a cover of 60 to 70 per cent) *Betula pendula*, *Carpinus betulus*, *Quercus petraea*, *Q. cerris*, *Picea excelsa* etc. In the herb layer (cover: 50 per cent) *Vaccinium myrtillus*, *Calluna vulgaris*, *Luzula nemorosa*, *Poa nemoralis*, *Melica uniflora*, *Melampyrum cristatum*, *Hieracium umbellatum linariifolium* etc.

Number of species in the mycocoenosis: 33, that of specimens in the examined quadrats: 55 to 71. Constant-dominant species: *Amanita citrina*, *Lactarius turpis*, *Lactarius torminosus*, *Cortinarius largus*, *Russula nigricans*, *R. cyanoxantha*, *Amanita muscaria*, *Boletus scaber*, *B. edulis*, *Lactarius subdulcis*, *Russula atropurpurea* etc. Locally faithful species: *Lactarius torminosus*, *L. turpis*, *Amanita muscaria* (Fig. 6).

Table 5

a) *Luzulo-Querco-Carpinetum* noricum Soó: column No 1–5 b) *Querco petraeae-Carpinetum* Soó and Zólyomi: column No 6–12	Sb.	S.	F.	Deák-kút	Lövérek	Károly magaslat	Dalos emlék
				1	2	3	4
				17.9. 1965	20.9. 1965	25.9. 1965	25.9. 1965
				A–D, N			
Amanita citrina	T.	1	M	–	–	2, +	1, +
Amanita muscaria	T.	1	M	–	–	–	–
Amanita pantherina	T.	1	M	–	2, +	–	–
Amanita phalloides	T.	1	M	2, +	–	2, –	2, +
Amanita vaginata	T.	1	M	–	–	–	1, +
Armillaria mellea	Tr.	1–2	P–S	5, +	–	–	–
Boletus chrysenteron	T.	1	M	9, +	5, +	–	–
Boletus edulis	T.	1	M	–	–	–	1, +
Boletus flavus	T.	1	M	–	–	–	2, +
Boletus luteus	T.	1	M	–	–	–	–
Boletus sanguineus	T.	1	M	3, +	–	–	–
Cantharellus cibarius	T.	1	M	–	–	3, +	–
Clitocybe odora	T.	1	S	8, +	–	–	–
Clitocybe phyllophila	T.	1	S	3, +	–	–	–
Collybia acervata	T.	1–2	S	23, +	9	–	–
Collybia dryophila	T.	1	S	4, +	12, +	–	–
Collybia fusipes	T.	1–2	S–P	5, +	–	–	–
Collybia radicata	T.	1	S	–	–	–	–
Coprinus atramentarius	T.	1	S	–	2, +	–	–
Cortinarius anomalus	T.	1	M	–	–	6, +	8, +
Cortinarius cinnamomeus	T.	1	M	–	–	–	2, +
Cortinarius coerulescens	T.	1	M	–	–	–	2, +
Cortinarius hinnuleus	T.	1	M	–	–	–	–
Cortinarius largus	T.	1	M	–	8, +	–	12, +
Cortinarius multiformis	T.	1	M	3, +	–	–	–
Cortinarius triumphans	T.	1	M	–	–	–	–
Craterellus cornucopioides	T.	2	M	–	–	–	–
Diatrype pulvinata	Sn	2	S	–	–	–	–
Flammula lenta	T.	1–2	S	–	15, +	–	–
Fistulina hepatica	Tr.	1–2	P	2, +	1, +	–	1, +
Hebeloma crustuliniforme	T.	1	S	5, +	62, +–1	–	2, +
Hydnum repandum	T.	1	M	–	–	–	–
Hypholoma fasciculare	Tr.	2	S	28, +–1	–	–	15, +
Hypholoma sublateritium	Tr.	2	S	–	–	–	75, 1
Laccaria amethystina	T.	1–2	S	–	–	–	–
Laccaria laccata	T.	1–2	S	25, +	–	–	–
Lactarius glyciosmus	T.	1	M	–	–	–	3, +
Lactarius piperatus	T.	1	M	–	–	–	–
Lactarius quietus	T.	1	M	–	–	2, +	–
Lactarius scrobiculatus	T.	1	M	–	–	–	–
Lactarius subdulcis	T.	1	M	3, +	4, +	4, +	2, +
Lycoperdon gemmatum	T.	1	S	–	–	–	–
Marasmius peronatus	T.	1	S	4, ++	–	–	–

Károly magaslat	K	Sopron: Fáberrét							Fr.
5		6	7	8	9	10	11	12	
27.9. 1965		22.9. 1965	22.9. 1965	27.9. 1965	27.9. 1965	28.9. 1965	28.9. 1965	28.9. 1965	
				A – D, N					
2, +	3	– +	2, +	2,	–	–	–	–	2
–	–	–	–	–	–	2, +	1, +	–	2
–	1	–	–	–	2, +	2, +	–	–	2
–	2	–	–	2, +	–	–	–	–	1
–	1	–	–	–	1, +	–	–	–	1
15, +	2	–	–	–	–	5, +	15, +	–	2
–	2	–	–	–	–	–	–	–	–
–	1	–	–	–	–	–	–	–	–
–	1	–	–	–	–	–	2, +	–	1
–	–	–	–	–	–	12, +	–	–	1
–	1	–	–	–	–	–	–	–	--
–	1	–	–	–	–	–	–	–	–
–	1	–	–	–	–	–	2, +	–	1
–	1	–	–	–	–	–	–	–	–
30, +	3	–	–	–	–	–	–	–	–
22, +	4	–	–	–	–	–	–	–	–
–	1	–	–	–	–	–	–	–	–
–	–	–	–	–	2, +	2, +	1, +	–	2
–	1	–	–	–	–	–	–	–	–
–	2	–	–	–	3, +	–	4, +	–	2
–	1	3, +	–	–	–	–	–	6, +	2
1, +	2	–	–	–	–	–	–	–	–
–	–	–	3, +	–	4, +	–	–	–	2
2, +	3	5, +	–	3, +	3, +	4, +	1, +	–	4
–	1	3, +	8, +	11, + – 1	1, +	2, +	3, +	8, +	5
–	–	–	–	–	–	12, +	–	–	1
–	–	–	80, 1	150, 1	5, +	125, 1	7, +	–	4
–	–	–	M, +	–	–	–	–	–	1
–	1	–	–	–	–	–	–	–	–
–	3	–	–	–	–	–	–	–	–
–	3	–	–	–	2, +	–	–	–	1
–	–	3, +	–	5, +	25, + – 1	–	3, +	–	3
–	2	–	–	–	2, +	–	–	–	1
52, 1	2	–	–	–	–	40, 1	–	–	1
2, +	1	8, +	–	–	–	–	–	–	–
–	1	–	3, +	–	5, +	–	–	–	2
–	1	–	–	–	–	–	–	–	–
–	–	8, + – 1	3, +	10, + – 1	8, + – 1	5, +	22, 1	9, + – 1	5
–	1	–	–	–	–	–	–	–	–
–	–	5, +	6, +	3, +	2, +	–	–	–	3
1, +	5	–	–	–	3, +	–	2, +	1, +	2
2, +	1	–	–	–	–	3, +	2, +	3, +	2
–	1	–	–	–	–	–	–	–	–

Table 5 continued

a) *Luzulo-Querco-Carpinetum* noricum Soó: column No 1–5 b) *Querco petraeae-Carpinetum* transdanubicum Soó and Zólyomi: column No 6–12	Sb.	S.	F.	Deák-kút	Lövérek	Károly magaslat	Dalos emlék
				1	2	3	4
				17.9. 1965	20.9. 1965	25.9. 1965	25.9. 1965
				A–D, N			
Marasmius wynnei	T.	1	S	5, +	–	–	–
Melamsporidium betulinum	F.	3	P	–	–	–	–
Microsphaera quercina	F.	3	P	–	–	–	–
Mycena pura	T.	1	S	8, +	–	–	–
Panus stipticus	Tr.	2	S	–	–	–	–
Paxillus atrotomentosus	Tr.	1–2	M	–	–	1, +	–
Paxillus involutus	T.	1	M	–	–	–	–
Pluteus cervinus	Tr.	1	S	–	–	–	–
Phallus impudicus	T.	1	P–S	–	–	–	–
Psathyrella candolleana	Tr.	1–2	S	–	2, +	–	–
Psathyrella hydrophila	Tr.	2	S	–	–	–	180, 1
Ramaria cinerea	T.	3	M	–	–	–	–
Russula atropurpurea	T.	1	M	4, +	2°, +	6, +	3, +
Russula cyanoxantha	T.	1	M	–	–	25, + – 1	3, +
Russula fragilis	T.	1	M	–	2, +	–	–
Russula grisea	T.	1	M	–	2, +	–	–
Russula lepida	T.	1	M	–	–	–	–
Russula lutea	T.	1	M	–	–	1, +	–
Russula nigricans	T.	1	M	–	–	3, +	5, +
Russula ochroleuca	T.	1	M	–	–	–	–
Russula vesca	T.	1	M	1, +	–	4, +	1, +
Russula xerampelina	T.	1	M	–	–	–	–
Stereum hirsutum	Tr.	2	S	–	–	–	–
Trametes betulina	Tr.	2	S	–	–	–	–
Trametes hirsuta	Tr.	2	S	–	–	–	–
Trametes versicolor	Tr.	3	S	–	–	–	–
Tricholoma acerbum	T.	1	S	–	–	–	–
Tricholoma album	T.	1	S	–	–	–	–
Tricholoma columbetta	T.	1	S	–	–	–	–
Tricholoma resplendens	T.	1	S	–	–	–	–
Tricholoma sulphureum	T.	1	S	–	6, +	–	–
Total number of species							
Number of species/Number of fruit bodies				20 : 151	14 : 75	11 : 57	20 : 310

This is a very characteristic community in the homogeneous mixed stand around the Károlymagaslat and Dalosemlék. The characteristic species are naturally mycotroph fungi.

Most important maximum aspects: *Lactarius torminosus – L. turpis – Amanita muscaria; Boletus edulis – B. scaber – Russula cyanoxantha; Amanita citrina – Lactarius turpis – Cortinarius largus* and the epixylous synusia: *Hypholoma fasciculare – Stereum hirsutum – Trametes confragosa* (Table 7).

Károly magaslat	K	Sopron: Fáberrét							Fr.
5		6	7	8	9	10	11	12	
27.9.1965		22.9. 1965	22.9. 1965	27.9. 1965	27.9. 1965	28.9. 1965	28.9. 1965	28.9. 1965	
		A–D, N							
–	1	–	–	–	–	–	–	–	–
–	–	M, +	–	–	–	–	–	–	1
–	–	M, +	–	–	–	–	–	–	1
–	1	–	–	–	–	2, +	–	3, +	2
–	–	–	12, +	3, +	M, +	–	–	–	3
–	1	–	–	–	–	–	–	–	–
–	–	–	–	2, +	–	–	–	–	1
–	–	–	–	1, +	–	–	–	–	1
–	–	–	–	–	–	6, +	2, +	–	2
–	1	–	–	–	–	–	–	–	–
–	2	–	–	–	–	–	–	50, 1	1
–	–	–	–	2, +	1, +	5, +	–	–	2
8, +	5	–	–	–	2, +	–	–	–	1
–	2	–	2, +	–	–	–	1, +	–	2
–	1	–	–	2, +	–	–	2, +	3, +	2
–	1	–	–	–	–	–	–	–	–
–	–	–	–	2, +	–	–	–	1, +	2
–	1	–	–	–	–	–	–	2, +	1
2, +	3	–	–	–	–	–	–	–	–
–	–	–	2, +	1, +	–	1, +	–	–	2
–	3	–	–	–	–	–	–	–	–
2, +	1	–	–	–	–	–	–	–	–
M, +	1	M, +	M, +	M, +	M, +	–	M, +	–	4
–	–	–	–	–	–	–	1, +	–	1
–	–	–	–	–	2, +	–	–	–	1
M, +	1	–	–	–	M, +	–	–	–	1
–	–	2, +	–	–	–	–	3, +	2, +	2
–	–	–	5, +	1, +	–	–	–	–	2
–	–	1, +	–	2, +	15, + – 1	–	–	–	3
–	–	–	–	–	–	5, +	–	–	1
–	1	–	–	3, +	–	–	–	–	1
15 : 143	49	12 : 41	13 : 128	19 : 206	22 : 91	16 : 227	19 : 79	12 : 90	51

g) Castaneo-Quercetum noricum Soó. – A considerably mixed type. In the tree layer (cover: 60 to 80 per cent) *Castanea sativa*, *Quercus cerris*, *Q. petraea*, *Carpinus betulus*, *Picea excelsa* etc. The shrub layer is lacking or unimportant. In the herb layer (cover: 50 to 60 per cent) *Luzula nemorosa*, *Deschampsia flexuosa*, *Vaccinium myrtillus*, *Melampyrum cristatum*, *Hieracium sabaudum* etc.

Number of species: 38, that of specimens: 91 to 97. Floristically this association represents a transitional and mixed community between *Luzulo-Quercetum*

Table 6

Aceri pseudoplatani-Alnetum glutinosae Ubrizsy 1965.	Sb.	S.	F.	Sopron			Fr.
				Várvölgy 26.9. 1965	Kecske-patak 26.9. 1965	Kecske-patak 26.9. 1965	
				A–D, N			
Amanita citrina	T.	1	M	2, +	–	1, +	3
Amanita pantherina	T.	1	M	–	1, +	–	1
Armillaria mellea	Tr.	2	P–S	55, 1	8, +	–	4
Boletus castaneus	T.	1	M	1, +	–	–	1
Boletus chrysentheron	T.	1	M	5, +	4, +	2, +	5
Boletus flavus	T.	1	M	–	–	8. +	1
Boletus lividus	T.	1	M	1, +	2, +	–	3
Boletus subtomentosus	T.	1	M	1, +	1, +	2, +	4
Clitocybe cerussata	T.	1	S	–	–	5, +	1
Clitocybe pythiophila	T.	1	S	–	–	35, +–1	2
Clitocybe nebularis	T.	1	S	4, +	–	–	1
Clitocybe infundibuliformis	T.	1	S	6, +	–	8, +	3
Clitocybe inversa	T.	1	S	5, +	–	–	1
Collybia radicata	T.	1	S	1, +	2, +	–	2
Cortinarius anomalus	T.	1	M	–	2, +	–	1
Cortinarius cinnamomeus	T.	1	M	1, +	–	2, +	2
Cortinarius triumphans	T.	1	M	–	–	15, +–1	2
Ganoderma applanatum	Tr.	2	P	1, +	–	–	1
Grifola sulphurea	Tr.	2	P	–	2, +	–	1
Hypoxylon coccineum	Tr.	2	S	–	–	M, +	1
Irpex fusco-violaceus	Tr.	2	P	–	–	M, +	1
Laccaria amethystina	T.	1	S	2, +	–	–	1
Lactarius quietus	T.	1	M	5, +	–	–	1
Lepiota procera	T.	1	S	1, +	2, +	–	3
Leptoporus stipticus	Tr.	2	P	–	–	2, +	1
Lycoperdon gemmatum	T.	1	S	4, +	–	1, +	2
Mycena alcalina	Tr.	2	S	8, +	–	2, +	3
Mycena tintinnabulum	Tr.	2	S	–	–	12, +	2
Naucoria escharoides	T–Tr.	1–2	S	4, +	–	15, +	3
Paxillus atrotomentosus	Tr.	2	S	–	–	2, +	1
Pleurotus dryinus	Tr.	1–2	P	1, +	–	–	1
Peniophora quercina	Su.	2	S	M, +	M, +	–	3
Phallus impudicus	T.	1	M	4, +	–	1, +	3
Pluteus cervinus	Tr.	1	S	1, +	–	–	1
Psathyrella hydrophila	Tr.	2	S	25, +–1	–	8, +	3
Ramaria cinerea	T.	2	S	–	M, +	–	2
Russula cyanoxantha	T.	1	M	2, +	1, +	–	2
Russula densifolia	T.	1	M	–	3, +	–	1
Russula grisea	T.	1	M	2, +	3, +	–	3
Russula heterophylla	T.	1	M	–	–	2, +	1
Russula nigricans	T.	1	M	4, +	2, +	5, +	5
Stropharia aeruginosa	Tr.	1	S	–	–	3, +	1
Stereum gausapatum	Tr.	2	S–P	–	M, +	–	1
Stereum sulphurosum	Tr.	2	P–S	–	M, +	–	1

Table 6 continued

Aceri pseudoplatani-Alnetum glutinosae Ubrizsy 1965.	Sb.	S.	F.	Sopron			Fr.
				Várvölgy 26.9 1965	Kecske-patak 26.9 1965	Kecske-patak 26.9 1965	
				A–D, N			
Trametes gibbosa	Tr.	2	P–S	–	–	2, +	1
Trametes unicolor	Tr.	2	S–P	2, +	–	M, +	3
Trametes versicolor	Tr.	2	S–P	–	–	M, +	1
Tricholoma nudum	T.	1	S	4, +	–	2, +	3
Xanthochrous radiatus	Tr.	2	S–P	M, +	M, +	M, +	5
Xylaria polymorpha	Tr.	2	S	–	M, +	–	2
Total number of species: 50							
Number of species/Number of fruit bodies				29 : 154	19 : 39	27 : 140	

and *Piceetum*. It is not a characteristically developed association, therefore the fungus vegetation is less typical. Constant-dominant species: *Amanita citrina*, *Boletus chrysenteron*, *Collybia acervata*, *Lactarius subdulcis*, *Amanita phalloides*, *Boletus subtomentosus*, *Clitocybe inversa*, *Collybia fusipes*, *Russula atropurpurea* etc. (Table 8).

Autumnal aspects: *Cortinarius largus – Lactarius subdulcis*; *Boletus chrysenteron – Collybia acervata; Collybia fusipes – Hypholoma sublateritium – Laccaria laccata*. Epixylous aspect or synusia of highly transitional feature: *Peniophora quercina – Hypholoma sublateritium – Stereum hirsutum*.

Fig. 6. *Amanita muscaria*, *Lactarius turpis*, *L. torminonosus*, *Russula atropurpurea* in *Querco-Betuletum* (Sopron, September 28, 1965)

Table 7

Querco-Betuletum Callunetosum Zólyomi	Sb.	S.	F.	Sopron: Dalos-emlékmű				Fr.
				25.9.1965	25.9.1965	27.9.1965	27.9.1965	
				A–D, N				
Amanita citrina	T.	1	M	1, +	6, +	2, +	8, +	5
Amanita muscaria	T.	1	M	5, +	–	3, +	–	3
Amanita vaginata	T.	1	M	–	–	–	1, +	1
Armillaria mellea	Fr.	1–2	S–P	–	7, +	–	–	1
Boletus edulis	T.	1	M	2, +	4, +	–	–	3
Boletus scaber	T.	1	M	1, +	2, +	2, +	–	3
Collybia dryophila	T.	1	S	–	–	8, +	–	2
Collybia fusipes	T.	1–2	S–P	–	–	2, +	–	1
Collybia radicata	T.	1	S	–	1, +	–	–	1
Cortinarius cinnabarinus	T.	1	M	–	3, +	–	–	1
Cortinarius coerulescens	T.	1	M	–	1, +	–	–	1
Cortinarius largus	T.	1	M	5, +	4, +	5, +	2, +	5
Cortinarius multiformis	T.	1	M	6, +	–	–	–	2
Fistulina hepatica	Tr.	1–2	S–P	–	2, +	–	–	1
Hebeloma hiemale	T.	1	S	2, +	–	–	–	1
Hypholoma fasciculare	Tr.	2	S	–	–	–	20, +1	2
Laccaria amethystina	T.	1	S	–	–	3, +	–	1
Lactarius scrobiculatus	T.	1	M	–	2, +	3, +	–	2
Lactarius subdulcis	T.	1	M	4, +	–	2, +	2, +	3
Lactarius torminosus	T.	1	M	12, +1	3, +	3, +	–	4
Lactarius turpis	T.	1	M	28, 1	4, +	4, +	3, +	5
Marasmius peronatus	T.	1	S	–	–	3, +	8, +	2
Mycena alcalina	Tr.	1–2	S	–	–	–	8, +	1
Pluteus cervinus	Tr.	1	S	–	2, +	–	–	1
Phallus impudicus	T.	1	P–S	–	2, +	–	–	1
Russula atropurpurea	T.	1	M	4, +	5, +	–	–	3
Russula cyanoxantha	T.	1	M	–	4, +	2, +	1, +	4
Russula exalbicans	T.	1	M	–	–	5, +	–	2
Russula grisea	T.	1	M	–	–	2, +	–	1
Russula nigricans	T.	1	M	–	3, +	4, +	2, +	4
Stereum hirsutum	Tr.	2	S	–	M, +	–	–	1
Trametes betulina	Tr.	1–2	S	–	–	2, +	–	1
Trametes confragosa	Tr.	1–2	S	–	–	8, +	–	2
Total number of species: 33								
Number of species/Number of fruit bodies				11 : 71	18 : 56	18 : 63	10 : 55	

The forests in the environment of Sopron, more thoroughly investigated, would certainly contribute with interesting and important data to the knowledge on the mycocoenoses of Hungarian forest types.

4. *Buda-Mountains:* Hárshegy, Hárshokorhegy, Jánoshegy, Kecskehegy.

a) Quercetum petraeae-cerris pannonicum Soó. – In a mixed sessile oak – Turkey oak stand on the Hárshegy and Hárshokorhegy (the latter is identical with the forest type examined by Bohus.) The cover of the tree layer ranges from 78

Table 8

Castaneo-Quercetum noricum Soó	Sb.	S.	F.	Sopron: Károly magaslat			Fr.
				17.9. 1965	22.4. 1965	27.9. 1965	
				A–D, N			
Amanita citrina	T.	1	M	2, +	3, +	1, +	5
Amanita phalloides	T.	1	M	–	1, +	3, +	3
Amanita spissa	T.	1	M	1, +	–	–	1
Boletus chrysenteron	T.	1	M	5, +	4, +	3, +	5
Boletus granulatus	T.	1	M	3, +	–	–	2
Boletus subtomentosus	T.	1	M	3, +	2, +	2, +	4
Clitocybe geotropa	T.	1	S	–	–	2, +	1
Clitocybe infundibuliformis	T.	1	S	–	1, +	3, +	3
Clitocybe inversa	T.	1	S	2, +	3, +	–	3
Clitocybe pythiophila	T.	1	S	–	–	22, + – 1	2
Collybia acervata	T.	1–2	S	4, +	52, + 1	3, +	5
Collybia fusipes	T.	1–2	S–P	5, +	1, +	–	3
Collybia inolens	T.	1	S	12, +	–	–	2
Cortinarius largus	T.	1	M	6, +	–	25, 1	3
Hebeloma hiemale	T.	1	S	3, +	1, +	–	2
Hypholoma sublateritium	Tr.	1–2	S	6, +	–	–	2
Laccaria amethystina	T.	1	S	5, +	–	1, +	2
Laccaria laccata	T.	1	S	7, +	2, +	–	3
Lactarius mitissimus	T.	1	M	1, +	–	2, +	2
Lactarius piperatus	T.	1	M	–	1, +	1, +	2
Lactarius rufus	T.	1	M	–	–	1, +	1
Lactarius subdulcis	T.	1	M	6, +	4, +	15, + – 1	5
Lycoperdon gemmatum	T.	1	S	–	2, +	6, +	3
Marasmius candidus	T.	1	S	4, +	–	–	2
Mycena alcalina	Tr.	1	S	2, +	–	–	1
Mycena pura	T.	1	S	3, +	2, +	–	2
Nectria cinnabarina	Sn.	3	S	M, +	–	–	2
Paxillus atrotomentosus	Tr.	1–2	M	–	1, +	–	1
Paxillus involutus	T.	1	M	1, +	–	–	1
Peniophora quercina	Sn.	2	S	M, +	M, +	–	3
Russula atropurpurea	T.	1	M	4, +	5, +	–	3
Russula cyanoxantha	T.	1	M	2, +	–	–	1
Russula aeruginea	T.	1	M	2, +	–	–	1
Russula lepida	T.	1	M	–	–	2, +	1
Russula nigricans	T.	1	M	3, +	–	–	2
Russula vesca	T.	1	M	–	2, +	1, +	2
Scleroderma vulgare	T.	1–2	S	2, +	–	–	1
Stereum hirsutum	Tr.	2	S–P	M, +	–	–	1
Tricholoma acerbum	T.	1	S	–	–	2, +	1
Tricholoma nudum	T.	1	S	–	3, +	–	2
Total number of species: 38							
Number of species/Number of fruit bodies:				28 : 97	19 : 91	18 : 95	

to 80 per cent, in the shrub layer it makes up 30, and in the herb layer (consisting chiefly of *Poa nemoralis)* 50 per cent. Number of pieces found per 500 m^2: 63 to

Table 9

Quercetum petraeae-cerris pannonicum Soó	Sb.	S.	F.	Mátraháza	
				8.7. 1957	23.9. 1958
				A–D, N	
Amanita pantherina	T.	1	M	1, +	–
Amanita phalloides	T.	1	M	–	2, +
Amanita rubescens	T.	1	M	2, +	–
Amanita vaginata	T.	1	M	2, +	–
Armillaria mellea	T.	1–2	P–S	–	8, +
Boletus chrysenteron	T.	1	M	2, +	–
Boletus granulatus	T.	1	M	–	–
Boletus edulis	T.	1	M	–	–
Boletus luridus	T.	1	M	1, +	–
Boletus miniatoporus	T.	1	M	–	–
Boletus subtomentosus	T.	1	M	–	–
Cantharellus cibarius	T.	1	M	–	–
Clitocybe cerussata	T.	1	S	–	3, +
Clitocybe infundibuliformis	T.	1	S	–	4, +
Clitocybe odora	T.	1	S	–	–
Clitocybe phyllophila	T.	1	S	–	–
Clitopilus prunulus	T.	1	S	–	–
Collybia dryophila	T.	1–2	S	–	–
Collybia fusipes	T.	1–2	S–P	–	–
Collybia longipes	T.	1	S–P	–	–
Collybia radicata	T.	1	S	2, +	–
Collybia tenacella	T.	1	S	–	1, +
Collybia velutipes	Tr.	2	P	–	–
Cortinarius collinitus	T.	1	M	–	–
Cortinarius fulgens	T.	1	M	–	–
Cortinarius largus	T.	1	M	–	–
Craterellus cornucopioides	T.	2–3	M	–	–
Daedalea quercina	Tr.	2	P–S	–	1, +
Fistulina hepatica	Tr.	2	P–S	–	2, +
Fomes torulosus	Tr.	1–2	P–S	–	–
Geastrum hygrometricum	T.	1	S	–	–
Hebeloma crustuliniforme	T.	1	S	–	–
Hypholoma fasciculare	Tr.	3	S–P	–	18, +–1
Hypholoma sublateritium	Tr.	3	S–P	–	16, +–1
Inocybe geophylla	T.	1	S	–	2, +
Inocybe patouillardi	T.	1	S	–	–
Laccaria laccata	T.	1	S	–	–
Lactarius acris	T.	1	M	–	–
Lactarius insulsus	T.	1	M	2, +	–
Lactarius piperatus	T.	1	M	–	2, +
Lactarius subdulcis	T.	1	M	–	–
Lactarius uvidus	T.	1	M	–	–
Lepiota cristata	T.	1	S	–	–
Lepiota procera	T.	1	S	–	1, +
Lycoperdon gemmatum	T.	1–2	S	–	8, +

23.9. 1958	Bp. Hűvösvölgy		Budakeszi	Hárshokor-hegy		Mátraháza	
	2.9. 1960	2.9. 1960	6.8. 1965	18.8. 1965	18.8. 1965	6.9. 1965	K
			A–D, N				
2, +	–	–	–	–	–	2, +	2
–	–	–	5, +	11, + – 1	–	1, +	3
–	–	–	–	2, +	–	–	1
–	–	–	2, +	1, +	–	1, +	3
12, +	3, +	15, + – 1	–	–	–	3, +	3
–	–	–	–	2, +	1, +	2, +	2
–	–	–	–	–	3, +	–	1
–	–	–	2, +	1, +	3, +	–	2
–	–	–	–	–	–	–	1
–	–	–	–	3, +	3, +	–	2
–	–	–	–	1, +	–	–	1
–	–	–	3, +	2, +	–	–	1
4, +	2, +	4, +	–	–	–	2, +	3
6, +	–	–	1, +	–	–	2, +	3
–	2, +	–	–	–	–	–	1
–	–	–	–	–	–	5, +	1
–	–	–	1, +	–	–	–	1
2, +	–	–	4, +	–	–	–	2
–	–	–	–	5, +	–	–	1
–	–	–	–	2, +	6, +	–	2
–	–	–	–	1, +	1, +	–	1
–	–	2, +	–	–	–	–	1
–	–	–	–	4, +	–	–	1
–	12, +	–	–	–	–	–	1
–	3, +	–	–	–	1, +	2, +	2
–	2, +	–	–	–	–	–	1
–	–	–	–	52, 1	48, 1	–	2
2, +	–	–	–	–	–	1, +	2
1, +	1, +	–	–	–	–	1, +	2
–	3, +	–	–	–	–	–	1
–	–	–	–	–	1, +	–	1
–	–	5, +	–	–	–	3, +	2
23, + – 1	–	10, +	–	–	–	–	2
18, + – 1	30, 1	8, +	–	–	–	5, +	4
–	–	–	–	–	1, +	1, +	2
–	–	–	–	–	2, +	–	1
–	6, +	2, +	–	8, +	5, +	3, +	3
–	–	–	–	–	4, +	–	1
–	–	–	–	–	2, +	–	1
–	–	–	–	5, +	–	3, +	2
–	–	–	25, 1	12, + – 1	2, +	–	3
–	–	–	2, +	–	2, +	–	1
–	–	–	–	1, +	–	–	1
–	–	–	–	–	–	2, +	1
10, +	1, +	–	–	–	–	–	2

Table 9 continued

Quercetum-petraeae-cerris pannonicum Soó	Sb.	S.	F.	Mátraháza 8.7. 1957 A–D, N	Mátraháza 23.9. 1958 A–D, N
Marasmius peronatus	T.	1–2	S	–	–
Marasmius wynnei	T.	1	S	–	–
Mycena alcalina	Tr.	1–2	S	–	12, +
Mycena galericulata	Tr.	1–2	S	–	6, +
Phellinus igniarius	Tr.	1–2	P	–	2, +
Polyporus adustus	Tr.	3	S–P	M, +	–
Psathyrella gracilis	T.	1	S	–	–
Psathyrella hydrophila	Tr.	1–2	S	–	–
Psathyrella semiglobata	T.	1	S	–	12, +
Rhodophyllus lividus	T.	1	S–M	–	–
Russula aeruginea	T.	1	M	–	–
Russula atropurpurea	T.	1	M	–	–
Russula cyanoxantha	T.	1	M	1, +	–
Russula densifolia	T.	1	M	–	–
Russula foetens	T.	1	M	–	1, +
Russula fragilis	T.	1	M	–	–
Russula lepida	T.	1	M	–	–
Russula lutea	T.	1	M	4, +	–
Russula luteotacta	T.	1	M	–	–
Russula nigricans	T.	1	M	–	–
Russula ochroleuca	T.	1	M	–	–
Russula pectinata	T.	1	M	–	–
Russula vesca	T.	1	M	–	–
Russula virescens	T.	1	M	2, +	–
Scleroderma vulgare	T.	1	M	–	–
Stereum hirsutum	Tr.	3	S–P	–	M, +
Trametes hirsuta	Tr.	2	S	–	2, +
Trametes versicolor	Tr.	2	S	M, +	–
Tricholoma album	T.	1	M	–	–
Tricholoma melaleucum	T.	1	S	–	–
Xanthochrous obliquus	Tr.	2	P	M, +	–
Total number of species: 77					
Number of species/Number of fruit bodies				13 : 22	20 : 104

152, that of species: 77. Constant-dominant species of the mycocoenosis: *Amanita phalloides*, *Boletus subtomentosus*, *Lepiota procera*, *Marasmius confluens*, *Russula atropurpurea*, *R. lepida*, *R. xerampelina*. In summer 1965 *Amanita phalloides* appeared in conspicuously great quantities on various sites of this community. *Russula atropurpurea* grew in large masses as well. Characteristic species: *Lactarius decipiens*, *Marasmius confluens* (BOHUS 1952, 1954).

Seasonal aspects observed so far: *Pholiota praecox – Marasmius confluens* (in June and July); *Amanita phalloides – Lactarius subdulcis – Russula lepida; Amanita phalloides – Craterellus cornucopioides – Russula atropurpurea; Boletus*

23.9. 1958	Bp. Hűvösvölgy		Budakeszi	Hárshokor-hegy		Mátraháza	K
	2.9. 1960	2.9. 1960	6.8. 1965	18.8. 1965	18.8. 1965	6.9. 1965	
	A – D, N						
–	–	–	8, +	–	2, +	3, +	2
–	5, +	–	–	–	–	–	1
16, +	–	–	–	–	–	2, +	2
8, +	–	2, +	–	–	–	4, +	3
–	–	–	–	–	–	–	1
–	–	–	–	–	–	–	1
–	–	12, +	–	–	–	–	1
–	–	45, 1	–	–	–	–	1
–	–	–	–	–	–	–	1
–	–	–	–	–	–	1, +	1
–	–	–	–	–	2, +	–	1
–	1, +	–	–	28, 1	2, +	–	2
–	–	–	–	4, +	–	1, +	2
–	–	–	–	–	2, +	–	1
2, +	–	–	3, +	1, +	–	–	3
–	–	1, +	1, +	–	1, +	–	2
–	–	–	6, +	–	4, +	5, +	3
–	–	–	–	–	2, +	1, +	1
–	–	–	1, +	–	–	–	1
–	–	–	–	2, +	–	–	1
–	–	–	2, +	–	–	1, +	1
–	–	–	1, +	–	1, +	–	1
–	–	–	1, +	–	–	2, +	1
–	–	–	2, +	2, +	1, +	–	2
–	–	–	–	2, +	–	–	1
M, +	–	M, +	M, +	M, +	–	M, +	4
–	–	–	–	–	–	–	1
M, +	–	M, +	M, +	–	–	–	3
–	3, +	–	–	–	–	5, +	2
2, +	–	–	–	–	–	–	1
M, +	–	–	–	M, +	–	–	3
17 : 111	14 : 74	13 : 108	20 : 73	25 : 152	25 : 103	27 : 63	

edulis – *B. miniatoporus* – *Collybia longipes; Russula lepida* – *Marasmius confluens; Boletus chrysenteron* – *Marasmius peronatus; Lepiota procera* – *Collybia dryophila* (in August); *Clitocybe infundibuliformis* – *Lepiota clypeolaria* – *Craterellus cornucopioides* (in September); *Armillaria mellea* – *Hebeloma crustuliniforme* – *Psathyrella gracilis* (in October).

Xanthochrous obliquus – *Trametes* spp. – *Stereum hirsutum* is a frequent epixylous synusia here (Table 9).

b) Genisti tinctoriae-Quercetum petraeae Klika, *subcarpaticum* Soó: Nagyhárshegy, Hárshokorhegy, Jánoshegy and Kecskehegy. – The stand on the Hárs-

bokorhegy is identical with the forest type examined by BOHUS. The cover of the tree layer amounts to 60 per cent, the shrub layer is lacking, the cover of the herb layer (comprising chiefly *Melica uniflora, Luzula nemorosa)* ranges from 20 to 25 per cent. The number of fruit bodies found in the different minimum areas (of 500 m^2) during the maximum aspect was 87 to 516, while in dry summers the so-called rest aspect consisted only of 22 to 63 pieces.

Constant-dominant species in the mycocoenosis of this forest type: *Armillaria mellea, Boletus chrysenteron, B. miniatoporus, Collybia fusipes, C. longipes, Hypholoma sublateritium, Lactarius subdulcis, Marasmius peronatus.* Characteristic species: *Cortinarius collinitus, Limacium russula, Russula nigricans* (BOHUS 1952, UBRIZSY 1959).

The most important, differentiable seasonal aspects are as follows: *Russula nigricans – Collybia fusipes; Marasmius peronatus – Psathyrella hydrophila; Collybia longipes – Boletus miniatoporus – Russula vesca; Boletus chrysenteron – Lactarius subdulcis* (in July and August); *Cortinarius vibratilis – Lactarius vellereus; Armillaria mellea – Psathyrella spadicea* (the latter mainly on the Jánoshegy in September and October); *Armillaria mellea – Lepiota procera; Hypholoma sublateritium – Laccaria laccata; Collybia butyracea – Clitocybe nebularis* (chiefly on the Hárshegy in October); *Limacium arbustivum – Clitocybe nebularis – Limacium olivaceo-album; Cortinarius collinitus – Clitocybe cyathiformis; Limacium arbustivum – Tricholoma album* (the latter on the Kecskehegy in November). The forest types are identical on all three sites, they show roughly similar botanical composition and ecological conditions.

In the above forest types (in the section between Hűvösvölgy and Árpádhegy) the epixylous synusiae developing in the herb layer on the stumps of *Quercus petraea, Carpinus betulus, Pinus nigra* etc. are very characteristic, they are highly rich in species, produce great quantities of fruit bodies. From *Quercus* and *Carpinus* stumps the following species were recorded on the 7th November of 1965. I. *Quercus: Trametes versicolor* M,+−1; *Tr. zonatus*, M,+; *Stereum hirsutum* M,+−1; *Schizophyllum commune* M,+; *Panus stipticus* 5 to 6 pieces, +,. II. Quercus: *Schizophyllum commune* M,+; *Stereum hirsutum* M,+; *St. gausapatum* M,+; *Trametes unicolor* M,+; *Tr. versicolor* M,+−1. III. *Carpinus: Panus stipticus* 8 to 10 pieces M,+; *Polyporus adustus* M,+−1; *Stereum hirsutum* M,+; *Trametes versicolor* M,+; *Tr. hirsuta* 2 pieces+. IV. *Pinus nigra* stump: *Schyzophyllum commune* M,+; *Stereum hirsutum* M,+−1; *Panus stipticus* 5 pieces +; *Trametes serialis* M,+; *Tr. unicolor* 2 pieces+; *Tr. versicolor* M,+.

c) Ceraso (mahaleb)-Quercetum pubescentis Jakucs et Fekete. – The maximum autumnal *Clitocybe nebularis – Tricholoma panaeolum* aspect of the mycocoenosis developed in the association (on the Kecskehegy and Hármashatárhegy) and comprising *Clitocybe inversa, Naucoria furfuracea, Schizophyllum commune* etc. as accompanying species, is very characteristic.

5. *Cotino-Quercetum pubescentis* Soó. – This association in Balatonakali is mixed sporadically with planted Austrian pines *(Pinus nigra)* and was surveyed from the 20th to 30th of August 1957. Due to the Austrian pine the mycocoenosis

is mixed and contains the following species (in pieces): *Boletus granulatus* 1 to 2; *Bolbitius vitellinus* 1 to 2; *Bovista plumbea* 2 to 3; *Clitocybe corda* (characteristic species) 1 to 2; *C. dealbata* 25 to 33 (in fairy-ring); *C. cerussata* 5 to 6; *C. inversa* 6 to 8; *Collybia esculenta* 4 to 5; *C. dryophila* 4 to 6; *Coprinus porcellanus* 1; *Coprinus plicatilis* 1 to 2; *Gomphidius viscidus* 3 to 5; *Marasmius oreades* 30 to 42 (together with *Clitocybe dealbata* aspect-forming species); *Marasmius wynnei* 8 to 10; *Mycena* sp. 1 to 2; *Naucoria semiorbicularis* 1 to 2; *Naucoria* sp. 1; *Psathyrella disseminata* 1; *Ps.gracilis* 1 to 2 and *Tricholoma nudum* 2 to 3 (in the moss layer).

6. Characterization of the fungus population of some rock swards. In the sward of *Caricetum humilis pannonicum* Dost. on the Mount Nagyszénás (near Nagykovácsi) the following fungus populations were found (per 100 m^2) as maximum aspect in September and October 1955. The data given below indicate pieces and are the average of 3 surveys: *Bovista plumbea* 8 to 12; *Clitocybe corda* 56 to 68; *Clitocybe* sp. 6; *Collybia dryophila* 8 to 11; *Galera tenera* 3 to 5; *Marasmius oreades* 11 to 17; *Rhodophyllus pascuus* 6 to 8; *R. sericeus* 17 to 23; *Tricholoma brevipes* 2 to 3; *T. panaeolum* 32 to 34; This aspect can be characterized as the *Rhodophyllus sericeus — Tricholoma panaeolum* maximum aspect.

On the dolomitic rocky Árpádhegy, in the sward *Asplenio rutae-murariae-Melicetum ciliatae* Soó (representing a transition of high degree into the more closed sward *Festucetum glaucae pannonicum* Zólyomi) two surveys were made in August 1955 and a coenosis of following fungi (in pieces) was found per 100 m^2: *Clitocybe dealbata* 6 to 8; *C. eryngii* 5 to 6; *Lepiota erminea* 3 to 4; *Lycoperdon gemmatum* 2 to 3; *Omphalia maura* 8 to 10. The aspect is termed as the *Clitocybe dealbata — Omphalia maura* maximum aspect.

7. In the stands of *Festucetum vaginatae danubiale* Soó *juniperetosum* (— *Junipereto — Populetum* auct.) growing on basic sand near Tatárszentgyörgy, on the bare sand soil a characteristic psammophilous, droughtresistant (xerophilic) geomycophyton fungus vegetation develops which, due to pedologic similarities and partly to equal ecological conditions, reminds of the fungus vegetation of the Mediterranean and South-Atlantic costal dunes. It contains many characteristic species. In the shrub layer (cover: 20 per cent): *Crataegus monogyna, Juniperus communis, Populus alba, P. canescens* etc. In the herb layer (cover 40 to 50 per cent): *Festuca vaginata, Koeleria glauca, Salix rosmarinifolia, Holoschoenus vulgaris, Stipa pennata, Fumana procumbens* etc. Fungi (in pieces) found August 6, 1958: *Pholiota (Agrocybe) dura* 10 to 12; *Tulostoma granulosum* 6 to 8; *T. mammosum* 1 to 2; *Psilocybe ammophila* 2 to 3; *Naucoria semiorbicularis* 1 to 2; *Montagnites radiosus* 1; *Galera tenera* 1. In the shrub layer large masses of *Gymnosporangium clavariaeforme* on *Crataegus*. On the strength of the constant-dominant species and 3 surveys the aspects *Pholiota dura — Tulostoma granulosum* and *Psilocybe ammophila — Tulostoma mammosum — Montagnites radiosus* can be differentiated.

4. Discussion

In some of the most important Hungarian forest communities the mycocoenoses, which appear considerably fluctuating, but with certain homogeneity from year to year, are coenologically analysed by regular permanent surveys on stationary minimum areas of 100, exceptionally of 500 square metres. It was aimed at to continue the investigations on the mycocoenoses of the areas already characterized and described in previous papers (1956, 1959), in order to establish and follow the annual rhythm and aspect conditions of the fungus vegetation still more precisely. As a matter of fact, on the present level of our methods and knowledge the mycocoenosis of a certain area, as biotope, can only be assessed and characterized by the aid of permanent analyses (lasting at least 5 years), exactly so that the seasonal phenomena of fruit body appearance, the so-called mycoaspects, are surveyed with coenological methods. Subsequently the materials gained by the investigations for some years are compared and on this basis it is attempted to establish the probable composition and seasonal rhythm of the maximum aspects, which appear under optimum weather conditions usually from July to September in Hungary (see: the main aspect types). To ascertain the rest aspects of unfavourable years (e.g. in 1956, 1960 to 1964) proved to be useful as well, because these are parts of the stable and maximum aspects and display, on the other hand, not only the peaks but also the whole picture of the fruit body production on a certain site. The author examined the appearance and composition of mycocoenoses developing in different forest communities of the Buda-Mountains, as well as in some rocky and steppe swards both under the very favourable conditions of 1955, 1956, and in the adverse years 1956, 1957, 1958, 1959 and 1960 to 1964. The mycocoenological analyses commenced previously in the Mátra-Mountains were also continued in order to supplement them with the investigations started 1957 in the Bakony-Mountains. Simultaneously the researches were extended to characteristic forest types in areas of the Noricum around Sopron and, between the Danube and Tisza, also to some sand tracts of the Hungarian Great Plain.

The results obtained reveal that such site investigations are indispensable for the proper organization of practical fruit body collection. The average and expectable fungus populations as well as the local and complex fruit body production (i.e. the mycopotential) of the different forest communities and forest types can only be assessed by detailed coenological analyses. According to the investigations of the author and to the establishments of BOHUS and BABOS the fruit body yield is most uniform and reliable in the *Melica* type of the *Querco petraeae-Carpinetum pannonicum* Soó cummunity in the Bükk-Mountains. This association affords culminating yields of 7 to 132 kg in the fairly arid Buda-Mountains and of 8 to 160 kg in the more humid Bükk-Mountains, but the fruit body production of the forest types *Genisti tinctoriae-Quercetum petraeae* Klika *subcarpaticum* Soó and *Quercetum petraeae-cerris pannonicum* Soó is also considerable. To gain precise information on the fruit body production of various forest types further investigations of ecological and coenological character are required, because in the forest types

around Sopron, most advantageous for fungus growth out of all biotopes, the yield exceeds essentially the above values found in the Hungarian Medium Mountains. From the Sopron forest types the highest mycopotential was shown by the associations *Luzulo-Querco-Carpinetum noricum* Soó, *Piceetum excelsae-carpinoso-quercosum* Soó and *Querco petraeae-Carpinetum transdanubicum* Soó et Zólyomi. On the other hand, *Querco-Betuletum-Callunetosum* Zólyomi, *Abieti-Fagetum noricum* Soó and *Aceri pseudoplatani-Alnetum glutinosae* Ubrizsy are floristically the most characteristic forest communities. The mixed *Castaneo-Quercetum noricum* Soó in the environment of Sopron is floristically a community of transitional feature between *Luzulo-Quercetum* and *Piceetum*.

From the coenological point of view the varying and characteristic mycocoenoses of some special biotopes (e.g. the shifting sand between the Danube and Tisza, rock swards etc.) deserve also attention.

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Symbols used in the Tables

Sb = Substratum, ground; T = Terra, soil fungi; Su = Surculus, fungi living on fallen branches; Tr = Truncus, fungi occurring on living and dead truncs or stumps of trees; usually xylophagus species; H = Herba, fungi living on dead or living plants

F = Life form; P = Parasites, parasitic fungi; S = Saprophyton = fungi habiting in the litter; M = Mycotroph, mycorrhizal species

S = Sociability; A-D = Abundance-Dominance according to the scale of BRAUN-BLANQUET; N = Number of pieces (fruit bodies) of fungi; M = Mass occurrence; Fr = Frequency; K = Constancy, according to the scale of BRAUN-BLANQUET

Plant Virus Nucleic Acids: their Role in the Infection Process and Possibilities to Study their Primary Structure[1]

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In recent years plant virus nucleic acids have been given special attention because of their central role in the infection process and because of our increasing understanding of the coding problem. The aim of the present paper is to give a short survey of the recent developments in the study of the infection process at the nucleic acid level (Part I) and in the determination of nucleotide sequences in plant viral nucleic acids (Part II).

PART I

The Infection Process with Plant Viruses[2]

It has been shown that the RNA moiety of TMV (GIERER and SCHRAMM, 1956) and of several other plant viruses (KAPER and STEERE, 1959a, 1959b; DIENER and WEAVER, 1959; HARRISON and NIXON, 1959; KASSANIS, 1960; RUSHIZKY and KNIGHT, 1959; REICHMANN and STACE-SMITH, 1959) is capable of initiating infection on its own and is able to trigger the synthesis of complete virus particles. This means that the whole genetic information of the complete virus particle is contained in its RNA. If this is so, plant virus nucleic acids have to fulfill the following requirements:

1. Once in the host cells they have to separate from their protein coat
2. They have to be capable of self-reproduction, and
3. They have to direct the synthesis of their coat protein.

[1] The following abbreviations have been used: TMV, tobacco mosaic virus; TNV, tobacco necrosis virus; TYMV, turnip yellow mosaic virus; RNA, ribonucleic acid; m-RNA, messenger ribonucleic acid; r-RNA, ribosomal ribonucleic acid; t-RNA, transfer (soluble) ribonucleic acid; DNA, deoxyribonucleic acid; RNase, pancreatic ribonuclease; DEAE-cellulose, diethylaminoethylcellulose; PME, alkaline phosphomonoesterase; A, G, C, U, adenosine, guanosine, cytidine and uridine, respectively, Ap, Gp, Cp and Up, adenosine-(2′), 3′-phosphate. guanosine-(2′)3′-phosphate, cytidine-(2′)3′-phosphate and uridine-(2′)3′-phosphate; pA, adenosine 5'-phosphate. GpUp represents guanylyl-(3′ → 5′) uridine-3′-phosphate. Gp Ap! represents guanylyl-(3′→5′)-adenosine-2′,3′-cyclic phosphate.

[2] No effort toward completeness has been made in view of the excellent reviews by MUNDRY (1963), SIEGEL and ZAITLIN (1964) and WITTMANN and SÄNGER (1965). Only some strictly biochemical aspects of the topic will be dealt with. A special emphasis will be laid on comparing plant virus biosynthesis with normal protein synthesis.

1. *Separation of the viral nucleic acid from the protein coat*

The following observations if taken together point to the fact that TMV–RNA actually separates from the protein at a very early stage of infection:

1. Infiltration of tobacco leaves with RNase prior to or within 2 hours after inoculation with TMV leads to the abortion of the infection (HAMERS-CASTERMAN and JEENER, 1957). Since intact TMV unlike TMV–RNA is not sensitive to the action of RNase the above finding suggests that for about 2 hours after inoculation TMV–RNA occurs in a free state in host cells.

2. If Nicotiana glutinosa leaves are inoculated with TMV and TMV–RNA, respectively, and UV-irradiated at different intervals after inoculation, the UV-resistance of both TMV and TMV–RNA starts to increase after a certain period of time as judged by the number of local lesions which later appear (SIEGEL et al. 1957). The length of the lag period preceding the increase in resistance, however, is different with TMV and TMV–RNA. With TMV it lasts for about 2½ and 5 hours, respectively, depending on the virus strain used, whereas with TMV–RNA it is much shorter than that or is practically non-existing. These results have been interpreted in the following way: Since the viral RNA is much more sensitive to UV-irradiation than the intact virus, the UV-sensitive phase observed suggests that during this lag period viral nucleic acid exists in a free state in the host cell. The increase in UV-resistance, on the other hand, shows that the nucleic acid macromolecules have been bound by some receptor in the host cell, losing thereby their sensitivity to UV-irradiation. The fact, that infection with intact TMV results in a rather long UV-sensitive period indicates that nucleic acid separates in vivo from the protein moiety of the virus particle and occurs in a free state in the cells. TMV–RNA, on the other hand, if introduced into host cells, will be bound immediately by the cell-receptor(s). The same results are obtained with the TNV-bean virus-host combination (KASSANIS, 1960) as well.

3. Maximum number of local lesions (FRAENKEL-CONRAT et al., 1958; KASSANIS, 1960) with both TMV and TNV, infective TMV–RNA in a free state (SCHRAMM and ENGLER, 1958) and infective intact TNV (KASSANIS, 1960) appear in the infected tissues earlier upon inoculation with the viral RNA than with the intact virus. These time differences presumably correspond to the time periods needed by the viral RNA to separate from its proteins coat.

The mechanism of the in vivo separation of the viral nucleic acid from the protein moiety of the virus particle is not known. The results of GORDON and SMITH (1960, 1961), however, suggest that the enzymatic machinery of the host cells may be involved. The above authors have namely found that at low light intensities Rhoeo discolor is immune from infection with intact TMV, but can be infected with TMV–RNA. This indicates that at least at low light intensities cells of Rhoeo discolor, though able from support virus multiplication once it has been started, are unable to cooperate in the initiation of the infection process, most probably in the separation of TMV–RNA from the viral protein.

2. *Self-reproduction of plant viral RNA*

Once in the host cell in a free state plant virus RNA has to induce the synthesis of complete virus (nucleoprotein) particles i.e. of both RNA and protein.

The problems involved in this process are very similar to those of normal protein biosynthesis. There are, however, some basic differences. In the synthesis of normal proteins (e.g. enzymes) the message coded in nuclear DNA is transferred by the m-RNA to the cytoplasmic ribosomes and read by t-RNA to which the specific amino acids are attached:

DNA → RNA → Protein
Nucleus Cytoplasm
Ribosomes

The characteristics of this process at the nucleic acid level are as follows:

1. The original information is contained in the double stranded nuclear DNA which has a double function: on the one hand it reproduces itself according to the Watson-Crick model and on the other hand it serves as a template for m-RNA (DNA-dependent RNA synthesis). Nuclear DNA does not leave the nucleus and does not become a structural component of the product of the synthesis (enzyme).

2. The short-lived m-RNA serves only to transmit information and does not get incorporated into the product either.

In the case of plant virus biosynthesis we have the following situation:

1. The original information is contained in the single stranded viral RNA which is introduced into the cytoplasm. This single stranded RNA has to play the role of the double stranded DNA: on the one hand it has to reproduce itself and on the other hand it has either to serve as a template for some sort of m-RNA or to act as a m-RNA itself.

2. In addition, viral RNA has to give rise to alike RNA-strands which will become the structural components of the intact virus particles.

The question arises how viral RNA can cope with these requirements. Most of the pertaining experiments have been performed with TMV–RNA. These will be summarized below.

As mentioned earlier the results of SIEGEL et al. (1957) suggest that after TMV–RNA has separated from its protein coat, it is absorbed by some "receptor" in the host cell. It seems very likely that this "receptor" is the cell nucleus. Examination of nuclei isolated from tobacco leaves infected with P^{32} labeled TMV revealed the presence of a radioactive RNA fraction, which was nonsedimentable at 105 000 g and digestible with RNase (REDDI, 1966). Its nucleotide composition was similar to that of TMV–RNA. These results seem to indicate that the radioactive RNA present in the nuclear fraction was parental TMV–RNA. As a next step of TMV biosynthesis this parental RNA is expected to multiply. The earliest report suggesting that shortly after infection with TMV there is an accumulation of RNA in the nuclei of infected

cells comes from ZECH and co-workers (ZECH, 1954; ZECH and VOGT-KÖHNE, 1955). By the use of UV microscopy, they have shown that in TMV-infected tobacco leaf hair cells the absorbancy at 260 mμ of the nucleus increases shortly after the cell has been infected. The hypothesis that the initial site of viral RNA synthesis may be the nucleus and/or nucleolus has been substantiated by later studies. HIRAI and WILDMAN (1963) have shown by cytochemical methods involving differential staining of RNA and DNA that it is RNA indeed which accumulates in the nuclei of TMV-infected cells. BALD (1964) came to the same conclusion by using phase contrast microscopy and digestion with RNase. Further suggestion that cell nuclei might be the initial site of TMV–RNA synthesis was given by YASUDA and HIRAI (1964). They have examined the incorporation of H^3-uracil into TMV infected tobacco leaf epidermis by means of microautoradiography and have found that the rate of incorporation into the nuclei was much higher with TMV-infected cells than with healthy ones.

All these results clearly show that shortly after infection with TMV there is an accumulation (ZECH, 1954; ZECH and VOGT-KÖHNE, 1955; HIRAI and WILDMAN, 1963; BALD, 1964) or rather synthesis (YASUDA and HIRAI, 1964) of RNA in the nucleus. Two important questions, however, still remain unanswered, namely:

1. What are the steps involved in RNA-synthesis in the nucleus, and
2. Is it viral RNA indeed which is synthesized.

As to the first question there are two possibilities: viral RNA is synthesized either via the "normal" pathway, i.e. there is a DNA-dependent RNA-synthesis, or by some other mechanism in which the participation of DNA is excluded. The results of SÄNGER and KNIGHT (1963) point to the second possibility. They have demonstrated that actinomycin D, a potent inhibitor of DNA-dependent RNA synthesis, inhibits normal nucleic acid metabolism in plant cells, but not TMV–RNA synthesis. Some further data also show that the participation of host nuclear DNA is not needed for TMV–RNA synthesis and throw some more light on the mechanism of this type of RNA-directed RNA synthesis, which consists in the formation of double-stranded RNA.

SHIPP and HASELKORN (1964) were the first to report the occurrence of the so-called replicative form of TMV–RNA. In the nucleic acid fraction of TMV-infected plants, they have found a component which when mixed with P^{32}-labeled TMV–RNA made the latter resistant to the action of RNase probably by hybridization. The product thus formed was resistant also to DNase, could be heat-denatured and reannealed with P^{32}-labeled TMV–RNA and exhibited upon melting a sharp thermal transition curve of RNase susceptibility. These data have been confirmed and the double-stranded RNA has been further characterized by BURDON et al. (1964). Their results on the GC content, melting point, buoyant density and sedimentation constant of this particular, partially purified nucleic acid fraction of infected leaves fully support the idea that it is a double-stranded, TMV-specific RNA.

As to the second question, i.e. whether it is a virus-specific RNA which is synthesized in the nuclei of virus-infected cells the paper by SMITH and SCHLEGEL

(1965) seems to give an unequivocal answer. In order to distinguish between host cell RNA synthesis and virus RNA synthesis the above authors have made use of the demonstration by SÄNGER and KNIGHT (1963) that actinomycin D inhibits normal nucleic acid metabolism in plant cells without affecting the synthesis of TMV–RNA. Using autoradiographic techniques SMITH and SCHLEGEL (1965) have shown that tritiated uridine was selectively incorporated into the nucleus and nucleolus of cells of actinomycin D-treated tobacco leaf disks infected with TMV and Vicia faba root tips infected with clover yellow mosaic virus. Healthy tobacco and V. faba cells treated with actinomycin D did not show such an incorporation. With long periods of incorporation, the label appeared in virus inclusion bodies in TMV-infected cells. Especially with virus-infected and actinomycin D-treated Vicia faba roots it was clearly visible that the label was incorporated primarily in the nucleolus. Neither RNase nor DNase nor a combination of the two removed this label entirely. This phenomenon is compatible with the hypothesis that it was a double stranded RNA which had incorporated the labeled uridine. On the basis of the above results the authors have suggested that virus-directed RNA synthesis takes place in the nucleolus and that at least some of this RNA is virus RNA.

The role in plant virus multiplication of the supposedly nucleolar double stranded virus specific RNA has not been established yet, but on the basis of our present concepts on nucleic acid self-duplication the following asymmetric, semiconservative mechanism can be hypothesized:

After the penetration of the complete virus particle the single stranded RNA (plus strand) separates from the protein coat and gets absorbed by the cell nucleolus. Here, serving as a template, it induces the synthesis of a complementary minus strand. The plus and minus strands combine to give the double stranded replicative form. After that the minus strand serves as a template for the formation of more plus strands.

In the following the plus strands formed have to leave the nucleolus and nucleus, get into the cytoplasm and induce the synthesis of specific virus protein.

The fact that the virus-specific RNA actually diffuses from the nucleus into the cytoplasm has been shown in the papers cited above (ZECH, 1954; ZECH and VOGT–KÖHNE, 1955; HIRAI and WILDMAN, 1963; BALD, 1964). On the other hand, as early as 1960 (ENGLER and SCHRAMM, 1960) the accumulation of infectious RNA in a free state in the cells of TMV-infected tobacco leaves was established and later confirmed by DIENER (1962). The amount of infectious RNA has been shown to increase for about 40 hours post inoculationem (ENGLER and SCHRAMM, 1960). After about 40 hours a sudden decrease sets in. In the authors' opinion this is the time when the free viral RNA assembles into virus nucleoprotein particles.

The experimental results mentioned so far seem to give a nice coherent picture about the early events of TMV biosynthesis at the nucleic acid level. One has to bear in mind, however, that this applies only to TMV and even here the results are sometimes questioned. RALPH and CLARK (1966) were unable to demonstrate the presence of double stranded TMV–RNA in the cell nuclei. Instead

they suggest on the basis of sedimentation studies that the viral duplex RNA may be associated with mitochondria. TYMV duplex RNA has been shown to replicate viral RNA by an asymmetric semiconservative mechanism (RALPH et al., 1965) and to appear in TYMV-infected Chinese cabbage leaves 3 days after inoculation, i.e. during the latent period preceding the appearance of complete virus particles (RALPH and CLARK, 1966). These findings strongly support the view that TYMV duplex RNA is a replicative form, as also postulated for TMV. It has also been shown (RALPH and CLARK, 1966) however, that in leaves systemically infected with TYMV P^{32} labeled double stranded RNA is associated with the chloroplast fraction and not with the nuclei which is the case with the duplex RNA of TMV.

There are furthermore some aspects concerning the role of viral RNA in the infection process of which extremely little is known. Two of them deserve special attention.

First, as every process in a living organism, the biosynthesis of plant viral RNA has to be catalyzed by specific enzymes. The genetic code for these enzymes has to be contained in the single stranded plant virus RNA, since it is able alone to initiate the entire infection process. The size and structure of TMV–RNA are such as to allow for this. Since the protein subunits of TMV consist of 158 amino acid residues (ANDERER et al., 1960; TSUGITA et al., 1960), assuming a triplet code, 474 nucleotides out of the 6500 nucleotides present in TMV–RNA are enough to code for the virus protein. The rest would suffice to code for a number of enzymes. Oddly enough, no virus-specific enzyme has been found so far in virus-infected plants, except for a synthetase in TYMV-infected Chinese cabbage (ASTIER-MANIFACIER and CORNUET, 1965). These authors claim that the enzyme is able to synthesize in vitro single stranded polynucleotides which are identical with TYMV–RNA. These results have to be confirmed. One of the reasons for the failure to find virus specific enzymes in infected cells may be the fact that they occur in such small quantities as to escape detection by present methods.

The other problem for which no definite answer can be given yet is the origin of the building blocks of the newly synthesized virus-specific nucleic acids. A logical initial step for the elucidation of this problem is to fractionate nucleic acids in both infected and healthy leaves and to see whether there is any remarkable shift in the amounts of the individual fractions. KUBO et al. (1965) using MAK column chromatography have carried out such experiments with leaves harvested 2 days after inoculation. The overall chromatographic pattern of nucleic acids (s-RNA, DNA, 18 S and 28 S r-RNA) from healthy and TMV infected tissues except, of course, the occurrence of TMV–RNA in the latter was practically the same. REDDI (1963a), focusing his attention primarily on r-RNA has shown that upon infection of tobacco leaves with TMV, r-RNA is rapidly degraded and this degradation is accompanied by a concomitant increase in TMV–RNA (REDDI, 1963a). From this and from the results of labeling experiments using tritiated uridine he has concluded that the breakdown products of ribosomal RNA are actually utilized in the synthesis of TMV–RNA. Further studies with P^{32} labeling have suggested (REDDI, 1963b) that the breakdown products which are used in TMV–RNA

synthesis are ribonucleosides. KUBO (1966) using P^{32} labeling at various time intervals after inoculation and separating nucleic acid fractions by means of MAK column chromatography has shown that before the highest relative rate of TMV–RNA synthesis (48 hours p. i.) was reached an enhanced synthesis of host-RNA had taken place as judged by the amount of P^{32} incorporated into nonviral RNA. This finding is in agreement with RÖTTGER's data (1965) on a dramatic increase in RNA which had a base composition similar to normal leaf RNA, in TMV-infected tobacco leaves at early stages of infection.

The same problem, i.e. the origin of the building blocks for viral RNA synthesis has been recently reinvestigated by BABOS (1966) using sucrose density gradient centrifugation for the separation of ribosomes and virus particles. Using tobacco leaves which were growing by cell expansion only, unlike the young leaves which were growing by both cell division and cell expansion used by REDDI (1963a, b), he has shown that the decrease of ribosomal content was independent of the amount of virus synthesized in the infected leaves. The rate of incorporation of P^{32} into ribosome was also the same with both virus-infected and healthy leaves. In BABOS's opinion the discrepancy between his results and those by REDDI (1963a, b) may be due to the fact that "the source of materials for the synthesis of virus RNA is variable and depends on the physiological state of the cells in which the virus multiplies".

SHIGEMATSU et al. (1966), however, did not find any reduction in the amount of ribosomal RNA two days after infecting young tobacco leaves with TMV. The yields of s-RNA and 17 S RNA even showed a four and twofold increase, respectively, upon infection. On the basis of the experimental results cited above it is very hard to come to any definite conclusion about the origin of the building blocks of TMV–RNA. We have no clear picture about the shifts upon TMV-infection in the pathways of nucleic acid metabolism either.

Furthermore, it has to be stressed that most of the information we have are gathered from studies on TMV. The infection process may well be quite different with other plant viruses. It has been reported, e.g. that the RNA moieties of both wound tumor virus (BLACK and MARKHAM, 1963) and rice dwarf virus (MIURA et al., 1966) are double-stranded. Consequently, multiplication of these viruses will involve steps different from those of TMV biosynthesis.

3. *Synthesis of virus protein*

In the preceding chapter we have seen that the experimental evidence strongly favours the view that plant virus RNA starts to multiply alone and that the multiplication goes through a step which involves the formation of a so-called replicative, double stranded form. We have also seen that before the bulk of infective complete virus particles can be detected protein-free infectious RNA accumulates in the infected cells in large amounts. Another line of evidence points to the fact that the protein of plant viruses also accumulates at a certain stage of the infection process and in this stage it is not associated with RNA.

As early as 1952 TAKAHASHI and ISHII (TAKAHASHI and ISHII, 1952) have shown that in TMV-infected tobacco leaves there is an accumulation of a protein which is missing in healthy tobacco leaves. This has been called X-protein (by others also abnormal protein, soluble antigen). Later they have found (TAKAHASHI and ISHII, 1953) that the X-protein consists of RNA-free spherical particles 15 mμ in diameter, has an antigenic structure similar to that of intact TMV and polymerizes in vitro to form rod shaped particles which resemble intact TMV particles. The amino acid composition of the X-protein and of the protein moiety of TMV is the same (NEWMARK and FRAZER, 1956). Electrophoretic mobility of the X-protein is characteristic for the TMV-strain which produces it (TAKAHASHI, 1955), indicating that the X-protein is strictly virus-specific. This is shown also by the fact that the host does not seem to have any influence on the serological properties of the X-protein (TAKAHASHI and ISHII, 1953). van RYSSELBERGE and JEENER (1957) by showing that the abnormal protein incorporates C^{14} provided in the form of $C^{14}O_2$ at a much higher rate than the virus, came to the conclusion that it is actually the immediate precursor of the protein part of the virus. The specific radioactivity of the virus and that of the X-protein were in agreement with this hypothesis.

On the basis of the results mentioned above it is not difficult to visualize TMV biosynthesis as a process in which viral RNA and protein first accumulate separately and are then assembled as a final step of TMV multiplication. This hypothesis is supported by three independent lines of evidence.

1. TAKAHASHI (1959) has demonstrated that anomalous noninfectious protein isolated from diseased tobacco plants and nucleic acid isolated from TMV could be reconstituted in vitro to form highly infectious TMV particles. There is no reason to suppose that TMV could not assemble in vivo by the same mechanism.

2. As early as 1949 MARKHAM and SMITH (MARKHAM and SMITH, 1949) demonstrated that purified preparations of turnip yellow mosaic virus contained two types of particles. One was a nucleoprotein containing RNA packed in a roughly spherical shell of protein. The other was an apparently identical protein containing no RNA. In the ultracentrifuge the infectious nucleoprotein sedimented at a higher rate (bottom component) than the non-infectious protein (top component). This shows that the synthesis of virus particles does not necessarily imply the simultaneous presence of proportionate amounts of both RNA and protein but may involve a mechanism in which viral protein is being produced in excess. It has to be mentioned that neither with TMV nor with TYMV were pools of detectable quantities of X-protein (COMMONER et al., 1953; JEENER, 1954) and top component, respectively, found before the appearance of the virus. This, however, does not mean that they cannot be in a way precursors of the virus. It may just signify that at the time of their formation in excess there was not enough viral RNA present to combine with. Subunits of the protein of TYMV are able to form virus-like particles in vivo giving rise to the "top component" whereas subunits of TMV protein for some reason are unable to do so and occur in the infected plant as nonaggregated X-protein.

Later MATTHEWS (1960) using density gradient centrifugation was able to demonstrate the presence of altogether five virus-like nucleoprotein fractions (in addition to the top component containing no RNA) with approximate RNA contents of 6%, 10%, 20%, 35%, and 36%, respectively. Only the fraction containing the highest amount (36%) of RNA was infectious. When virus-infected plants were labeled with P^{32} for short periods the specific radioactivity of RNA in the individual fractions decreased with increasing RNA contents (MATTHEWS et al., 1963). This suggests that the fractions may represent steps in the assembly of the complete virus nucleoprotein.

Although it is difficult to reconcile this interpretation with our present concepts on virus multiplication, the results nevertheless suggest that the synthesis of nucleic acid and of protein are probably separately controlled and do not always run in step. A tentative explanation of the origin of these fractions would be to suppose that owing to some disturbance in RNA biosynthesis some RNA threads not fully formed yet are detached from their site of synthesis and coated by the excess protein. This interpretation, however, would not give any explanation as to the results of the labeling experiments mentioned above. As BOLLARD and MATTHEWS have put it recently (BOLLARD and MATHEWS, 1966): "So far, there is no conclusive evidence as to the role of any of these anomalous virus proteins in the infected cell".

Macromolecules differing in their RNA content have been found also in plants infected with squash mosaic virus (MAZZONE et al., 1962), cherry yellow virus (WILLISON et al., 1961), necrotic ring spot virus (WILLISON et al., 1961) and wild cucumber mosaic virus (YAMAZAKI and KAESBERG, 1961).

3. The evidence to be outlined below shows that in some cases plant viral RNA is able to multiply in the infected cell without assembling with or inducing the formation of its protein coat.

CADMAN (1962) has shown that the NM form of tobacco rattle virus, in spite of causing severe symptoms, exists in the form of free nucleic acid strands probably attached to cell nuclei. BABOS and KASSANIS (1962) have described unstable variants of tobacco necrosis virus. These also possibly exist in the cell as uncoated nucleic acid. SIEGEL et al. (1962) reported the isolation of two defective nitrite mutants of TMV, one of which (PM_1) failed to induce the synthesis of viral protein whereas plants infected with the other one (PM_2) synthesized a virus-like protein, which, however, failed to aggregate in vivo with the viral nucleic acid to form complete virus particles.

As to the site of virus protein synthesis there is much controversy. SCHRAMM and RÖTTGER (1959) using fluorescent antibodies for in situ investigations showed that TMV-protein in infected tobacco plants appeared first in a zone around the nucleus and then spread all over the cytoplasm. In this later stage of infection most virus protein was found in the microsomal fraction. No virus protein could be demonstrated from either the nuclei or the chloroplasts. From these observations SCHRAMM and RÖTTGER (1959) have concluded that virus protein is synthesized in the microsomes. REDDI (1964) on the other hand claims to have been able to isolate

complete virus particles from the nuclei of infected tobacco cells. He concludes that the site of virus synthesis (probably that of viral protein synthesis, too) is the nucleus. REDDI (1964) found the chloroplast fraction of TMV-infected tobacco leaves to be free from TMV. In contrast to this ZAITLIN and BOARDMAN (1958) showed that small amounts of TMV could be released from the chloroplast fraction of TMV-infected tobacco leaves by extraction with an appropriate buffer. Labeling experiments with C^{14}-aspartic acid suggested that TMV could be synthesized within the chloroplasts and the virus might move from its site of synthesis into the cytoplasm (BOARDMAN and ZAITLIN, 1958). This suggestion was later substantiated by MATSUSHITA (1965). He has shown that most of the infectivity was observed in the chloroplast fraction 40 hours after inoculation. Thereafter infectivity in the chloroplast fraction decreased and that of the cytoplasm increased with time.

As indicated above nuclei, microsomes and chloroplasts have equally been suggested to be the organelles in which TMV particles or their protein moieties are synthesized. The reason for this discrepancy may be the difficulty to distinguish between the site of viral protein synthesis and the site of virus assembly.

Recent reports, however, strongly favour the view that viral protein is synthesized within the chloroplasts. It has been shown by SPENCER and WILDMAN (1964) that cell-free extracts of tobacco leaves were capable of incorporating amino acids into protein and that more than 80 per cent of this activity was associated with particles which sedimented at 1000 g. The 1000 g pellet consisted mainly of nuclei and chloroplasts but most of the activity was associated with this latter fraction. Later it was found (BOARDMAN et al., 1965) that the ability of chloroplasts to incorporate amino acids was due to ribosomes. These ribosomes have a sedimentation constant of 70s and their physical properties are different from the 80s cytoplasm ribosomes (BOARDMAN et al., 1966). 70s ribosomes are 10 to 20 fold more active in protein synthesis (in vitro) than 80s ribosomes (BOARDMAN et al., 1966). Since in addition to the 70s ribosomes the chloroplasts seem to contain all the materials including t-RNA and/or activating enzymes necessary for protein synthesis (FRANCKI et al., 1965) they may well be the site of active viral protein synthesis.

In extracts of Chinese cabbage too, two species of ribosomes have been found (CLARK et al., 1964): the 68s ribosomes appear to be confined to the chloroplasts and the 83s ribosomes to the cytoplasm. It has been shown recently (REID and MATTHEWS, 1966) that TYMV infection has little effect on the concentrations of 83s ribosomes and fraction II protein (both cytoplasmic) but the concentration of fraction I protein (which is in the chloroplasts) is reduced and 68s (chloroplast) ribosomes are eliminated in TYMV-infected tissues. Cytologically, TYMV causes marked abnormalities in the ultrastructure of chloroplasts of infected cells without affecting any other cell structures (CHALCROFT and MATTHEWS, 1966).

PART II

Primary Structure of Plant Virus Nucleic Acids

In view of the central role of plant virus nucleic acids in the infection process, as outlined in the preceding chapter, the elucidation of their primary structure is of paramount importance. Oddly enough, very little has been achieved in this respect with nucleic acids as compared to the results of structural analyses of plant virus proteins. The reason for this is, without question, a methodical one.

Both proteins and nucleic acids are macromolecules consisting of building blocks, amino acids and nucleotides, respectively. This means that the fundamental principles involved in their structural analysis are similar. The corresponding methods consist of either breaking up the macromolecule by specific enzymes into smaller fragments and analysing the individual fractions (partial sequences) or chopping off the building blocks from one end of the macromolecule one by one and identifying them (stepwise degradation). In most cases the two methods are used in conjunction.

There are, however, two basic differences in the structure of proteins and nucleic acids of plant viruses, and these are responsible for the peculiar difficulties encountered in the elucidation of the structure of nucleic acids:

1. Whereas there are 20 different amino acids in proteins, in plant virus nucleic acids only the four "classical" nucleotides have been found so far. It is not difficult to visualize that the less variability there is in the building blocks the less choice in specific enzymes and the more probability we have getting fractions of the same sequence.

2. The proteins of plant viruses studied so far consist of subunits, each with a comparatively limited number of amino acid residues (e.g. 158 with TMV), whereas the RNA moiety of TMV particles and probably of other plant viruses too is a single-stranded macromolecule (cf. GIERER, 1959) without any apparent repetition in its structure (about 6000 nucleotides in TMV – RNA). Obviously it is much easier to elucidate the sequence of 158 building blocks than that of 6000.

In spite of these drawbacks structural studies on plant virus nucleic acids were begun and are being continued on the basis of the above-mentioned two principles (partial sequence studies and stepwise degradation). Of course, the methods used so far do not permit the elucidation of the complete nucleotide sequence of plant viral nucleic acids but may be useful in detecting some rough differences between viruses and virus strains with respect to the structure of their nucleic acids.

1. *Partial sequences in plant virus nucleic acids*

Although the principle involved in partial sequence studies is the same for both proteins and nucleic acids, the amount of information one can get is quite different with these two types of molecules. With subunits of plant virus proteins

several specific enzymes can be used independently and by analysing individual oligopeptides obtained with enzymes of varying specificities the overlapping portions can be identified and the complete sequence established. With plant virus RNAs this approach does not work, except for t-RNA (HOLLEY et al., 1965) which consists of about 70 to 80 nucleotide residues, due to the very large number of building blocks in a single molecule and to the limited variability in the building blocks. All one can do is to split specifically some of the internucleotide bonds and to get in this way a quantitative liberation of oligonucleotides of different length and different composition. By a reasonable separation of the resulting oligonucleotides and by establishing their amounts the *relative frequency in which certain sequences occur in ribonucleic acids* can be established.

To achieve this two requirements have to be fulfilled:

a) To get reproducible fractions

b) To get a reproducible separation of as many of the fractions obtained as possible.

a) Getting reproducible fractions of plant virus RNA is the less difficult problem of the two. It can be achieved by using highly specific enzymes. The two most widely used nucleolytic enzymes are RNase and ribonuclease T_1 (SATO and EGAMI, 1957). RNase is known to hydrolyze uridine-3′ phosphoryl and cytidine 3′-phosphoryl bonds in ribonucleic acids giving rise to cytidylic acid, uridylic acid and fragments of varying lengths terminated by either cytidylic acid or uridylic acid. Ribonuclease T_1 specifically cleaves the secondary phosphate ester bonds of guanosine 3′ phosphate giving rise to guanylic acid and several oligonucleotides of different lengths terminated by guanylic acid.

b) For the reproducible separation of the fractions obtained paper chromatography combined with electrophoresis and column chromatography are the methods most widely used.

As early as 1952 MARKHAM and SMITH (MARKHAM and SMITH, 1952) by using paper chromatography followed by the electrophoretic separation of the eluates of the chromatographic bands were able to detect some of the smaller products of ribonuclease digestion of TYMV–RNA. RUSHIZKY and KNIGHT (1960a, 1960b) have developed a mapping procedure similar in its lay-out to finger printing as used with peptides. After enzymic digestion the fragments were separated first by paper electrophoresis in one direction and then with the paper turned 90° from the direction used in electrophoresis by paper chromatography in the other. In this way RNase digests of TMV–RNA were resolved into eighteen spots all of which were identified. The advantage of this method lies in the fact that comparatively small amounts of RNA are needed. The disadvantage, however, is, as MARKHAM (1963) has put it, that "it is almost impossible to correct for paper blank variations". At any rate, the method proved to be quantitative enough for the demonstration of the preferential specificity of RNase (RUSHIZKY et al., 1961), and of the striking differences in partial nucleotide sequences between strain HR, and two other strains (M and common strain) of TMV upon digestion with either RNase (RUSHIZKY and KNIGHT, 1960b) or ribonuclease T_1 (RUSHIZKY and SOBER

1962). This method was used also for the elucidation of the mechanism of action of micrococcal nuclease employing TMV–RNA as a substrate (RUSHIZKY et al., 1962).

The other most promising method for the separation of oligonucleotide fragments obtained by enzyme digestion is ion exchange chromatography. The advantage of such methods is that it is much easier to estimate the amounts of the individual fractions than with methods involving the use of chromatographic papers. The disadvantage, however, is that fairly large amounts of starting material are needed.

As early as 1953 VOLKIN and COHN (VOLKIN and COHN, 1953) succeeded in separating products of ribonuclease action by ion exchange column chromatography under acidic conditions using Dowex-1 and stepwise increments of NaCl for elution. By this procedure a fairly large number of fractions could be separated, there were, however, considerable overlappings (e.g. ApUp came off together with ApGpCp in a single peak) making quantative estimations difficult. In addition, not all of the possible sequences (up to the pentanucleotide level) could be detected because of a considerable overlapping of the peaks. STAEHELIN's procedure (1961a, 1961b, 1961c) in which DEAE-cellulose is used as a resin and elution is carried out under slightly alkaline conditions with increasing concentrations of ammonium bicarbonate offers some advantages over the method mentioned above in that polyvalent anions are not bound so tightly by DEAE-cellulose than by Dowex-1 and therefore less time is needed for the elution procedure. Even with this method, however, there are overlappings. In cases where two oligonucleotides were not completely separated additional techniques such as rechromatography at a lower pH and paper electrophoresis had to be used to obtain the exact amounts of the individual components. SYMONS et al. (1963) used the above method to analyze six strains of TYMV and one each of wild cucumber mosaic virus and turnip crinkle virus for the products of pancreatic ribonuclease digestion of their RNA, and reported the amounts of the mono-, and dinucleotides, most of the trinucleotides and of one of the tetranucleotides in the individual virus strains.

It is easy to realize that the higher oligonucleotides can be isolated and identified the more information one can get on the structural characteristics of an RNA molecule.

SOLYMOSY et al. (1965) have set out to find a method which would permit the isolation of oligomers larger than trinucleotides. In their work the ribonucleic acid of TYMV was digested with RNase and the resulting oligonucleotides were separated by combining two column chromatographic methods. In the first column (DEAE-cellulose) elution by neutral salt solutions containing 7 M urea to suppress secondary binding forces between the oligonucleotides and the DEAE-cellulose (TOMLINSON and TENER, 1963a, 1963b) led to the separation of the mixture into fractions containing oligomers of equal length. These individual fractions were then subfractionated under acidic conditions on Dowex-1 resins: a separation which depends not only on the charge carried by each oligomer but also on hydrophobic

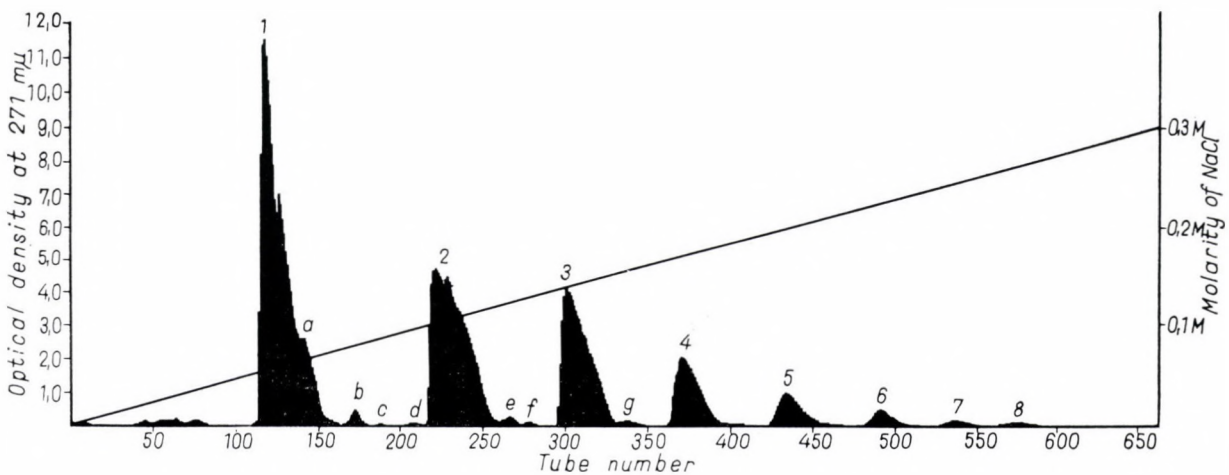


Fig. 1. Column chromatographic separation of a pancreatic RNase digest of TYMV—RNA on a DEAE-cellulose column (SOLYMOSY et al., 1965)

interaction between the resin and the base residues (VOLKIN and COHN, 1953). An extensive fractionation of the digest thus resulted. The fractions were then characterized with respect to both their base composition and the portion of the total epresented by them.

Fig. 1 shows a representative elution pattern of the oligonucleotides obtained by ribonuclease digestion of TYMV–RNA. The arabic numbers at the peaks in Fig. 1 show the number of nucleotides per oligonucleotide molecule in the corresponding fraction. The letters designate minor fractions the composition of some of

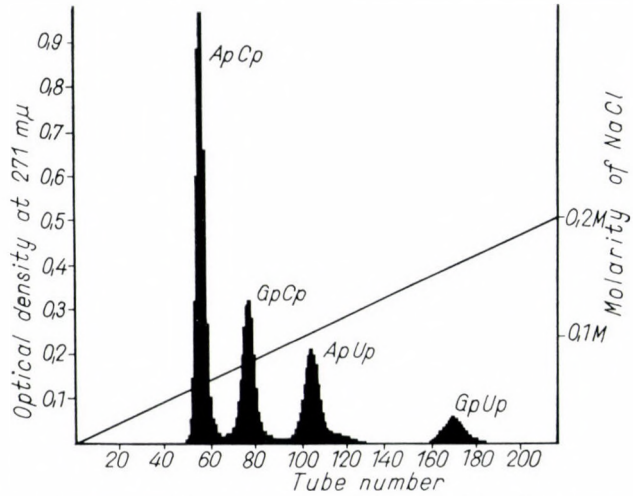


Fig. 2. Column chromatographic separation of dinucleotides from a pancreatic RNase digest of TYMV—RNA on a Dowex-1 column (SOLYMOSY et al., 1965)

which has been investigated (SOLYMOSY et al., 1965). Figs 2, 3, 4 and 5 show the elution patterns of the di-, tri-, tetra-, and penta-nucleotides, respectively. Tables 1, 2, and 3 represent the compositions of the individual oligomers and the portions of the total represented by them.

It may be seen that all of the dinucleotides (Fig. 2) and all but one of the trinucleotides (Fig. 3 and Table 1) have been clearly resolved by this combined column chromatographic method. Among the trinucleotides the isomers GpApCp and ApGpCp did not separate. Fig. 4 and Table 2 show that unlike the trinucleotides the isomeric tetranucleotides were no longer resolved. However, the tetranucleotides of different base compositions were separated and reproducibly determined. The only exceptions were the two tetranucleotides containing three Gp residues which seemed to have appeared as aggregates in the form of small peaks (a, b, c, d in Fig. 4). The sum total of the O. D. units found in the two analyzed digests was the same within the experimental error. The analysis of the minor peaks (Table 2), although not very accurate because of the very small amounts of material, indicates the high Gp content in these fractions. In order to avoid the probable

aggregation of oligomers rich in Gp as experienced with the tetranucleotides, the fractionation of pentanucleotides was carried out in the presence of 7 M urea (LIPSETT and HEPPEL, 1963). It may be seen in Fig. 5 and Table 3 that in spite of the use of 7 M urea in the elution GpGpGpGpU and GpGpGpGpC oligonucleotides were not detected in discrete peaks. Disregarding isomers the number of peaks

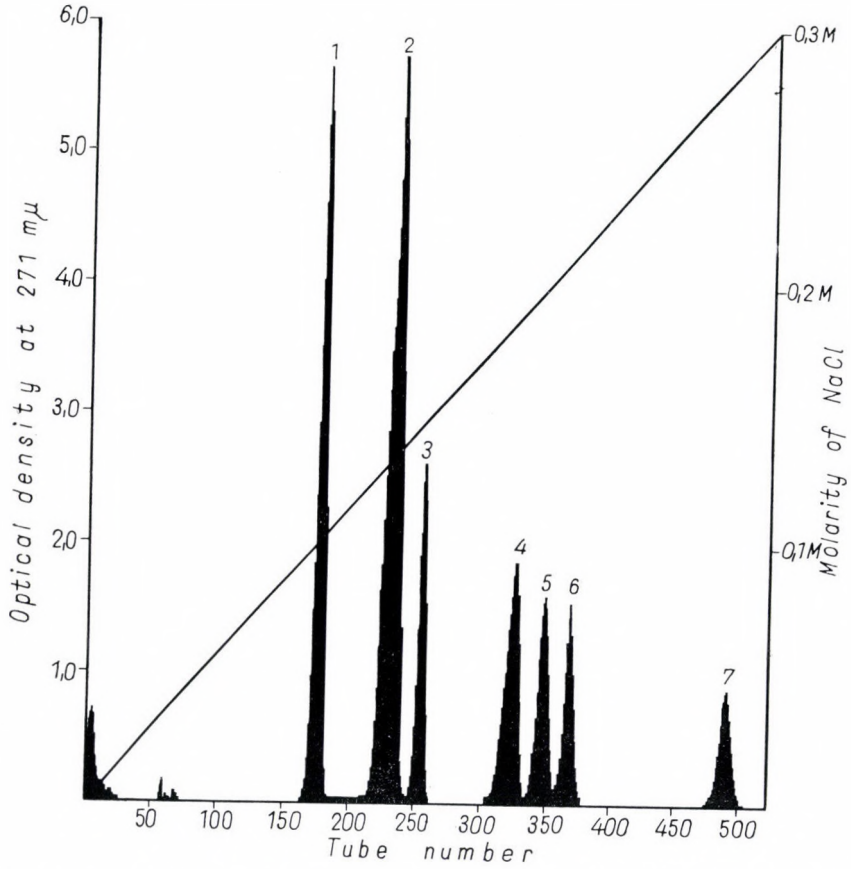


Fig. 3. Column chromatographic separation of trinucleotides from a pancreatic RNase digest of TYMV—RNA on a Dowex-1 column (SOLYMOSY et al., 1965)

expected for the rest of the pentanucleotides should be 8. However, only 6 major peaks were obtained. A detailed analysis of the individual peaks showed that the fractions 3 and 4 contained both pyrimidines, indicating the presence of two molecular species. Assuming the order of elution to be in the sequence of increasing negative charge, peak 3 should have contained ApApApApU followed by ApApGpGpC. From the quantitative determination of the bases in peak 3 it was

Table 1

Trinucleotide content in pancreatic ribonuclease digests of TYMV—RNA (SOLYMOSY et al., 1965)

Peak No.	Composition	Base ratios A : G : C : U	Moles of trinucleotides per 100 moles of nucleotide residues		
			Preparation I	Preparation II	Random distribution
1	ApApCp	2.03 : 0.00 : 1.00 : 0.00	1.14	1.13	1.16
2	GpApCp ApGpCp	1.06 : 0.97 : 1.00 : 0.00	1.79	1.74	1.78
3	ApApUp	2.03 : 0.00 : 0.00 : 1.00	0.51	0.51	0.67
4	GpGpCp	0.00 : 1.91 : 1.00 : 0.00	0.76	0.64	0.68
5	GpApUp	—	0.46	0.48	0.51
6	ApGpUp	—	0.45	0.44	0.51
7	GpGpUp	0.00 : 1.88 : 0.00 : 1.00	0.35	0.35	0.39

obvious that the two oligonucleotides were eluted together. A similar analysis showed peak 4 to be a mixture of the two pentanucleotides given in Table 3.

The above procedure (SOLYMOSY et al., 1965) makes it possible to separate and evaluate most of the oligonucleotides in pancreatic RNase digests of RNA, up to the pentanucleotide level. However, no separation of the isomeric tetra- and

Table 2

Tetranucleotide content in pancreatic ribonuclease digests of TYMV—RNA (SOLYMOSY et al., 1965)

Peak No.	Composition	Base ratios A : G : C : U	Moles of tetranucleotides per 100 moles of nucleotide residues		
			Preparation I	Preparation II	Random distribution
1	ApApApCp	3.33 : 0.00 : 1.00 : 0.00	0.188	0.198	0.260
2	(ApApGp)Cp	2.06 : 0.97 : 1.00 : 0.00	0.571	0.612	0.599
3	ApApApUp	3.19 : 0.00 : 0.00 : 1.00	0.103	0.101	0.150
4	(ApGpGp)Cp	0.99 : 1.53 : 1.00 : 0.00	0.487	0.489	0.460
5	(ApApGp)Up	1.79 : 1.02 : 0.00 : 1.00	0.278	0.317	0.346
a		0.00 : 6.26 : 0.00 : 1.00	0.013	0.022	—
6	(ApGpGp)Up	1.26 : 2.44 : 0.00 : 1.00	0.185	0.224	0.265
b		0.00 : 5.35 : 0.00 : 1.00	0.011	0.022	—
c		0.31 : 4.43 : 0.00 : 1.00	0.059	0.031	—
	GpGpGpCp	Not found			0.118
	GpGpGpUp				0.068

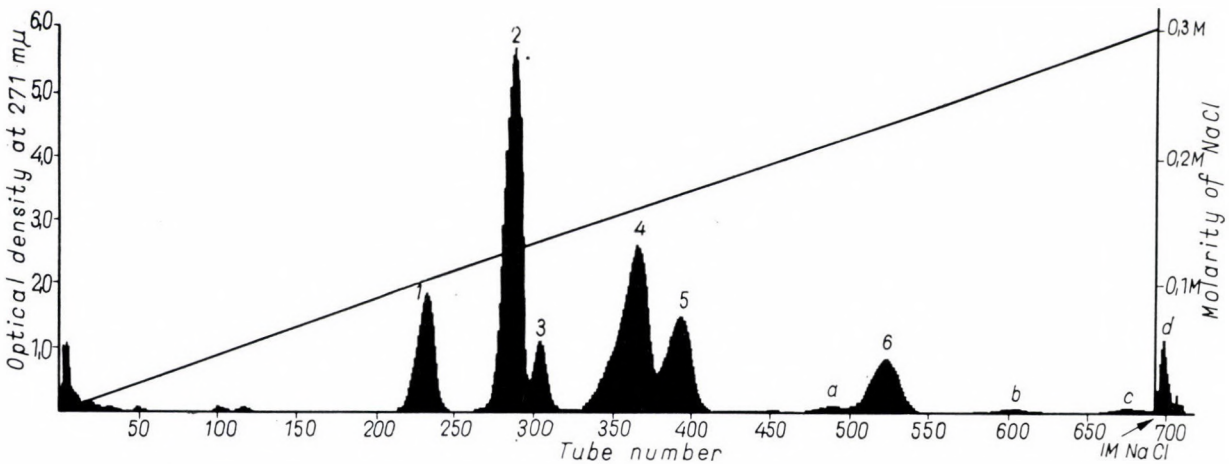


Fig. 4. Column chromatographic separation of PME-treated tetranucleotides from a pancreatic RNase digest of TYMV—RNA on a Dowex-1 column (SOLYMOSY et al., 1965)

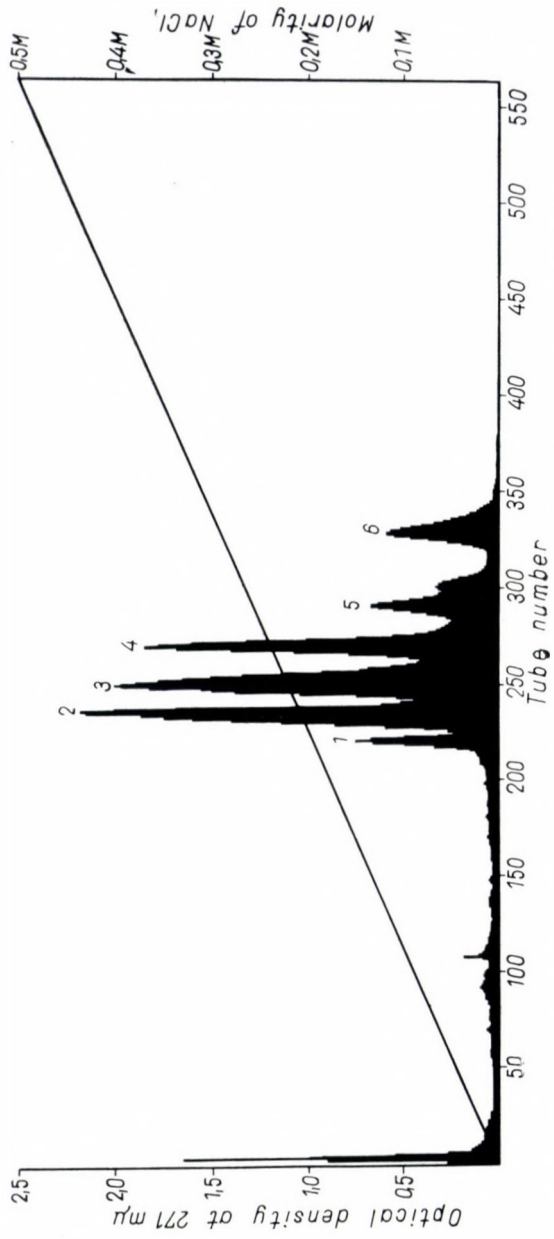


Fig. 5. Column chromatographic separation of PME-treated pentanucleotides from a pancreatic RNase digest of TYMV—RNA on a Dowex-1 column (SOLYMOSY et al., 1965)

Table 3

Pentanucleotide content in a pancreatic ribonuclease digest of TYMV—RNA (SOLYMOSY et al., 1965)

Peak No.	Composition	Base ratios A : G : C : U	Moles of pentanucleotides per 100 moles of nucleotide residues	
			Preparation I	Random distribution
1	ApApApApCp	3.86 : 0.00 : 1.00 : 0.00	0.036	0.058
2	(ApApApGp)Cp	3.01 : 0.86 : 1.00 : 0.00	0.178	0.179
3	ApApApApUp	4.00 : 0.00 : 0.00 : 1.00	0.056	0.034
	(ApApGpGp)Cp	2.20 : 2.10 : 1.00 : 0.00	0.189	0.137
4	(ApApApGp)Up	3.00 : 1.00 : 0.00 : 1.00	0.088	0.103
	(ApGpGpGp)Cp	1.14 : 3.26 : 1.00 : 0.00	0.078	0.106
5	(ApApGpGp)Up	2.13 : 2.11 : 0.00 : 1.00	0.107	0.079
6	(ApGpGpGp)Up	1.23 : 3.09 : 0.00 : 1.00	0.082	0.061
		Not found		
	GpGpGpGpCp			0.020
	GpGpGpGpUp			0.012

pentanucleotides can be achieved, and also one pair of trinucleotides remains unresolved. Potentially, however, the method can be extended further for establishing the molar ratios of some of the isomers. For example the oligomer (ApApGp)C consisting of the isomers ApApGpC, ApGpApC and GpApApC could be treated with ribonuclease T_1 which would give rise in this case to the following products: ApApGp + C; ApGp + ApC; Gp + ApApC. These could be easily separated and the amounts of the products could be used for determining the molar percentages of the isomers in question.

2. *End group determination and stepwise degradation with plant virus nucleic acids*

Before discussing this topic one has to be reminded again of the formidable difficulties involved in such experiments. The aim of such experiments is to determine the sequence of at least some of the terminal nucleotides each of which represents one out of about 6000 to 6500 nucleotides. For this type of work mostly labeled (P^{32} or C^{14}) viral RNA is used. In many cases only the determination of the terminal nucleoside or nucleotide at either end of the chain is aimed at. The following methods used alone or in conjunction have been used so far:

A. Enzymatic methods

a) Removal of terminal phosphate groups with alkaline phosphomonoesterase.

b) Digestion with snake venom phosphodiesterase. Snake venom phosphodiesterase is an exonuclease which attacks poly- and oligonucleotides from the 3′-hydroxyl end in a stepwise fashion giving rise to nucleoside 5′-phosphate units. Upon exhaustive digestion of RNA with this enzyme one diphosphate will be released from the 3′-end of the molecule, if it is phosphorylated and one nucleoside from the 5′-end if it is not phosphorylated.

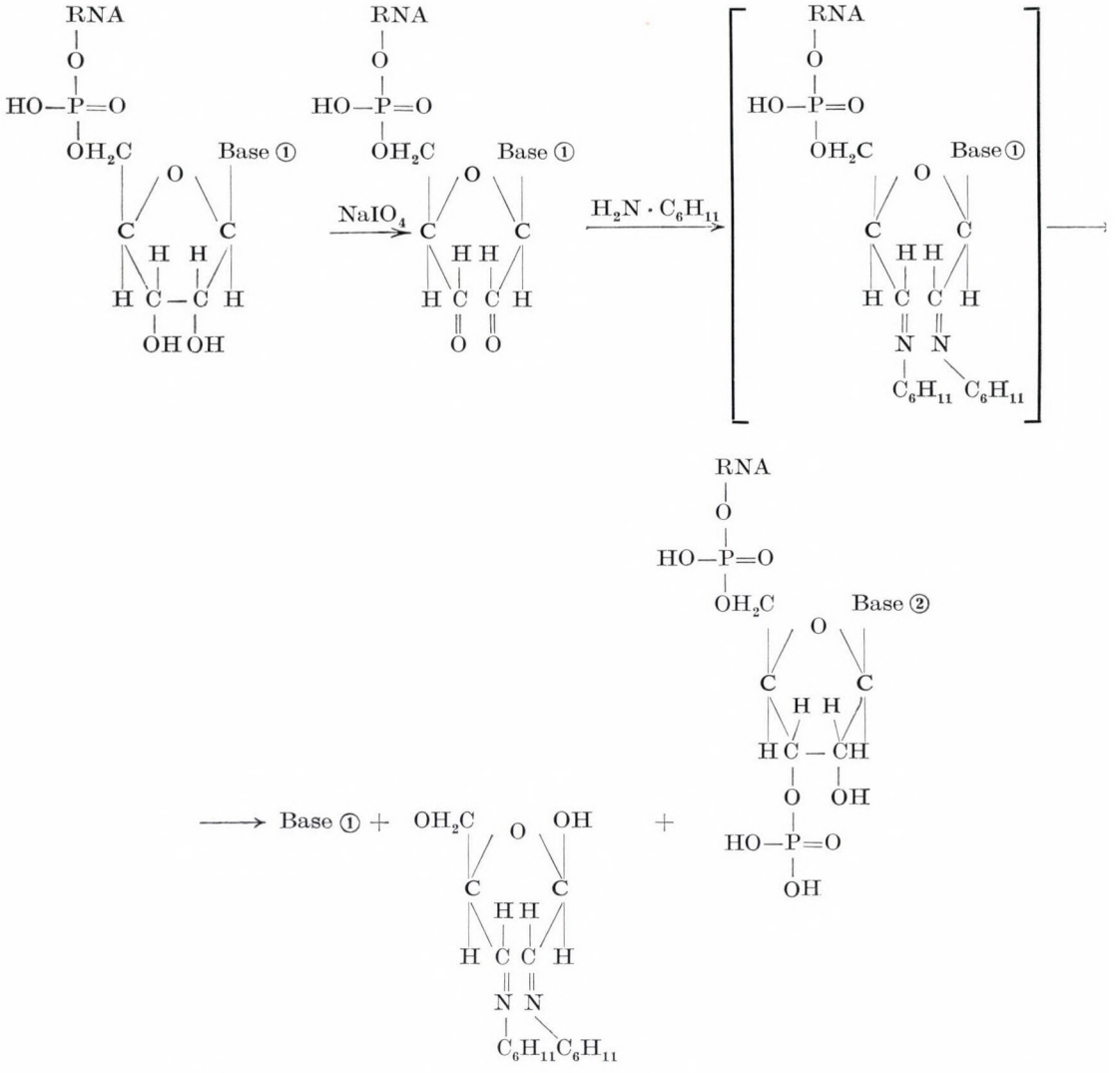
c) Digestion with spleen phosphodiesterase. The action of this enzyme on oligo- and polynucleotides is complementary to that of venom phosphodiesterase. Thus the mode of action is stepwise from the end bearing the 5′-hydroxyl group and results in the successive release of nucleoside 3′-phosphate units. Upon exhaustive digestion of RNA with this enzyme the same end groups can be determined as by alkaline degradation.

d) Another approach to end group analysis of nucleic acids is that proposed by TOMLINSON and TENER (1963b): The nucleic acid is degraded with an appropriate nuclease such that the polynucleotide containing the original terminal nucleotide residue has either no, or two, terminal phosphate groups, whereas the rest of the polynucleotides, which arise from inside the nucleic acid chain, contain only one terminal phosphate residue. The mixed polymers are separated according to their charge on DEAE-cellulose at pH 7.5 with 7 M urea in the eluting solution and an increasing concentration of sodium chloride. Each fraction is isolated and treated with phosphomonoesterase. The polymer derived from the end now differs by two charges from the other polymers in the fraction and can be separated from them by rechromatography in the same system.

B. Chemical methods

a) Alkaline degradation. Alkali is known to split the phosphate ester bond to the 5′-OH group of the ribose of RNA and to give rise to a mixture of 2′ and 3′-nucleoside phosphates from the middle part of the chain and to one nucleoside diphosphate from the 5′-end of the molecule if that is phosphorylated and to one nucleoside from the 3′-end if that is unphosphorylated.

b) Periodate oxidation followed by cleavage with an amine. Theoretically this method is suitable for the stepwise degradation of an oligonucleotide chain from the 3′-end. If the 3′-end is phosphorylated the terminal 3′-phosphate is first removed by phosphomonoesterase. After this the oligonucleotide chain is treated with periodate which oxidizes the cis-hydroxyls on carbon atoms 2 and 3 of the terminal ribose. Upon subsequent treatment with an amine (cyclohexylamine and lysine have been most successfully used by YU and ZAMECNIK, 1960 and NEU and HEPPEL, 1964, respectively) an unstable amine complex (Shiff-base) is being formed which is cleaved to produce the free base of the first nucleotide residue and the exposed 3′-phosphate of the second. Repetition of these steps results in the stepwise liberation of bases from the 3′-end. The mechanism of the reaction is supposed to be as follows (YU and ZAMECNIK, 1960):



Even to determine whether or not the 5′-end or the 3′-end nucleoside of TMV–RNA is phosphorylated was a task difficult to solve. In an earlier paper GORDON et al. (1960) reported that under the influence of phosphomonoesterase TMV–RNA releases inorganic phosphate per 3000 to 5000 nucleotides. This was interpreted as signifying that each intact RNA molecule of about 6500 nucleotides carries, at most, 1 terminal phosphate, and that additional phosphate ends are due to chain breakage. It turned out later (FRAENKEL-CONRAT and SINGER, 1962) by employing specifically purified (by the use of bentonite or sucrose density gradient centrifugation) RNA preparations in particular in regard to freedom from

nucleases, that the phosphate released from TMV–RNA upon digestion with E. coli-phosphomonoesterase was much less than mole/mole RNA. This means that the 3′-end of TMV–RNA is not phosphorylated. This conclusion was supported by the finding that degradation with snake venom phosphodiesterase yielded under optimum conditions less than one mole of nucleoside 2′(3′)5′-diphosphates per mole RNA. Alkaline degradation of TMV–RNA yielded no nucleoside 2′(3′)5′-diphosphates indicating the absence of 5′-phosphorylated chain ends. On the basis of these data FRAENKEL-CONRAT and SINGER (1962) concluded that neither the terminal 5′-position nor the terminal 2′(3′)-position of the TMV–RNA chain carried a phosphate group. Using C^{14}-labelled TMV–RNA it was further shown that upon alkaline hydrolysis the nucleoside liberated was adenosine (SUGIYAMA and FRAENKEL-CONRAT, 1961) suggesting that the 3′-end of TMV–RNA is an adenosine residue. Exhaustive digestion of C^{14}-labeled TMV–RNA by snake venom phosphodiesterase usually yielded about 0.5 moles of guanosine, cytidine and uridine and 1.5 moles of adenosine (SUGIYAMA and FRAENKEL-CONRAT, 1963). The authors have interpreted these findings as suggesting that adenosine represents the 5′-end of the intact RNA chain.

SOLYMOSY and REICHMANN (1965) have used snake venom phosphodiesterase (VDE) as a tool for the stepwise degradation of TYMV–RNA and the RNA of the satellite tobacco necrosis virus. Their method consisted in digesting the viral RNA with a large amount of the enzyme for a very short period. After digestion the undegraded RNA was precipitated with alcohol in the presence of some drops of Na-acetate, centrifuged off and the supernatant after desalting by paper chromatography was subjected to two-dimensional paper chromatography in order to determine the amounts of the individual mononucleotides liberated from the 3′-end. It was assumed that 1) the 3′-end of the RNA molecule was not phosphorylated and that 2) the relative amounts of the mononucleotides liberated would give an indication as to their sequence at the 3′ end.

In one experiment 1414 O. D. units of TYMV–RNA were digested for 5 minutes with VDE. This amounts to 1875 O. D_{260} units if one takes into consideration a hyperchromic effect 32,62 per cent resulting from total digestion (HASELKORN, 1962). Taking an average molar extinction coefficient of 10.488×10^3 for the nucleotides in TYMV–RNA and assuming the number of nucleotide residues in the RNA strand to be 6000 we arrive to a total amount of 180 μ moles of nucleotide residues. This means that 0.03 μ moles will correspond to one nucleotide residue liberated. The quantitative estimation of the mononucleotides liberated under the influence of snake venom phosphodiesterase gave the following results:

0.060 μ moles of pU corresponding to 2 nucleotide residues
0.047 μ moles of pC corresponding to 1.5 nucleotide residues
and 0.015 μ moles of pA corresponding to 0.5 nucleotide residues
no pG was found

These results suggest the 3′-end to be pApCp(CpUpU). The sequence in brackets is not known.

In an attempt to find out the sequence of the 3 terminal nucleotides another experiment was carried out with a TYMV–RNA preparation of 2674 O. D_{260} units. First it was digested for 2 minutes with VDE and then (after precipitation and solubilization) for another 3 minutes. The supernatants obtained in both reactions were analyzed as to their mononucleotide content. The following results were obtained:

Nucleotide	Number of nucleotide residues released		
	after a digestion		Total
	for 2 min	for 5 min	
pU	0.30	0.55	0.85
pC	0.35	0.45	0.80
pA	—	0.08	0.08

Apparently in this experiment the recovery was not quantitative for some reason as compared to that in the former experiment. If we suppose, however, that losses with each nucleotide were the same, a 3′-end sequence . . . ApCpUpUpC can be tentatively suggested.

Using the same method a 3′-end sequence of . . . pCpApU was obtained for the RNA of the satellite tobacco necrosis virus.

It has to be stressed that the above-mentioned data of SOLYMOSY and REICHMANN (1965) have to be regarded as preliminary observations. There are a number of factors which could have interfered making the results unreliable. Several control experiments and some different approaches are needed to eliminate these factors.

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Studies on Strains of Potato Virus Y

2. Normal Strain

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Our experiments showed that Capsicum annuum L. var. Markgärtner, Datura stramonium L. var. tatula and Lycopersicon pimpinellifolium (Jusl.) Mill. are not susceptible to infection with different isolates of the so-called normal strain (PVY^N) of potato virus Y. The virulence of different isolates of PVY^N was determined on the basis of the degree of synergism observed when PVY^N was inoculated to plants together with the ringspot strain (PVX^{RS}) or the normal strain (PVX^N) of potato virus X. The symptoms produced on different host plants and the results of cross protection tests served as a basis for dividing the isolates into three groups: mild, medium and severe. With the different isolates the thermal inactivation point varied between 56 and 72 °C, the dilution end point between 10^{-4} and 2×10^{-6} and the longevity in vitro between 18 and 31 days. In dried leaf-tissues of tobacco some isolates remained active even after 426 days. No correlation was found between the physical properties and the virulence of the individual isolates.

Introduction

SMITH (1931) after having separated PVY by aphids, reported that the virus caused different symptoms in various potato varieties. In the first year after infection usually necrotic symptoms appeared which led to leaf drop, whereas in further years intensive mosaic symptoms prevailed. Simultaneously with SMITH's (1931) work similar observations were made in Germany by KÖHLER and his group (BÖHME, 1933a, b; KÖHLER, 1934, 1965). Some other potato virus diseases, e.g. those originally described as potato stipple streak (ATANASOFF, 1922) and rugose mosaic (ORTON, 1920; MURPHY, 1921; SCHULTZ and FOLSOM, 1922; QUANJER, 1923; JOHNSON, 1925) were in all probability also due to PVY. Strains of PVY seemed to be much less variable than those of PVY (SMITH, 1931). SMITH (1931) concluded therefore that in England the variability of mosaic symptoms in potatoes doubly infected with PVY and PVX is due to the PVX component rather than to PVY. This opinion has later been refuted by Bawden and KASSANIS (1947) and by DARBY et al. (1951). In case of a double infection the effect of PVX always depends on the individual reaction of the potato variety to this particular virus. In their studies on synergism ROSS (1950) and ROSS et al. (1952) pointed out that in tobacco plants doubly infected with PVX and PVY the concentration of PVX reached a higher level and the symptoms became more severe than in tobacco

plants infected with PVX alone. ZACHOS (1954) reported similar results with PVX and tobacco mosaic virus (TMV). BERCKS (1955) on the other hand, has found that the severity of symptoms produced upon simultaneous infection with both PVX and PVY depends on the environmental conditions under which the plants are kept and the observed increase in the concentration of PVX is only temporary. FORD and ROSS (1962) in a recent publication regard the increase in the concentration of PVX as being due to the effect of temperature rather than to the presence of PVY. KÖHLER and KLINKOWSKI (1954) have pointed out that mixed infections involving both PVX and PVY do not always result in an additive effect.

After the appearance of the excellent papers by DARBY et al. (1951) and by EASTON et al. (1958) a German agrobotanical expedition was being organized in 1959 under the leadership of Prof. Dr. H. Ross (Max–Planck–Institut für Züchtungsforschung, Köln-Vogelsang) to investigate among others viruses which infect potato crops grown in the Andes. Screening of the potato samples in their collection revealed the spontaneous occurrence of PVY strains differing from each other in virulence [(severe chlorotic, regular chlorotic, necrotic, regular, chlorotic, weak necrotic) (ROSS, 1959; SILBERSCHMIDT, 1961)]. Similar findings have been reported by MCKEE (1963) in connection with the expedition of the John Innes Institute to South America and by COCKERHAM (1963) from an expedition of the University of Birmingham to Mexico and to Central America.

Material and Methods

For the experiments, in addition to PVX^{RS} and PVX^{N} which have been employed previously (Horváth, 1966) to study synergism with PVY^{C}, a number of isolates of PVY^{N} were used, partly isolated by us and partly collected from different countries (Table 1).

For the designation of the isolates the following rules were followed:

1. When the isolate was designated by the sender, no alteration was made (PVY–L, PVY–N, PVY–R).

2. When the isolate was not designated by the sender the following rules were followed with isolates from tobacco:

a) The strain from England has been called PVY–W (after Dr. M. A. WATSON).

b) One isolate from Israel has been called PVY–P, because it had been isolated from red pepper (NITZANY, 1964).

c) Another strain isolated from tomato in Israel (NITZANY, 1964) has been called PVY–LL because in the literature (NITZANY and SELA, 1962; Nitzany and TANNE, 1962) it figures under the same designation.

3. When the isolate was not designated by the sender the following rules were followed with isolates from potato:

a) For the abbreviation of one word names of varieties the first, second and last letters of the word were used (e.g. Bie = Bintje).

Table 1

Sources of potato virus Y isolates

Designation of the isolate	Potato variety or other host	Isolated in	Supplied by[1]
Adg 43	Adg 43 × Aquila 2/2	Belgium	V. Mélard
BdN	Bonotte de Noirmontier	Belgium	V. Mélard
Bie	Bintje	Austria	H. Wenzl
CSW	Craigs Snow—White	Scotland	G. Cockerham
Ine	Industrie	Belgium	V. Mélard
Lü 72	Fink	Germany	author
Lü 86	Fink	Germany	author
PK	Paul Krüger	Holland	A. Rozendaal
PVY—L	Tobacco	California	R. G. Grogan S. D. Sanchez
PVY—LL	Tobacco	Israel	F. E. Nitzany
PVY—N	Tobacco	California	R. G. Grogan S. D. Sanchez
PVY—P	Tobacco	Israel	F. E. Nitzany
PVY—R	Tobacco	California	R. G. Grogan S. D. Sanchez
PVY—W	Tobacco	England	M. A. Watson
UM	Ulster Magnet	Belgium	V. Mélard
Von	Voran	Austria	H. Wenzl

[1] Thanks are due to Dr. G. Cockerham, Dr. R. G. Grogan, Dr. V. Mélard, Dr. F. E Nitzany, Dr. A. Rozendaal, Dr. Santiago Delgado-Sanchez, Dr. M. A. Watson and Dr. H. Wenzl for sending the strains of potato virus Y and potato plants, respectively.

b) For the abbreviation of two word names of varieties the first letters of both words were used (e.g. PK = Paul Krüger).

c) For the abbreviation of three word names of varieties the first letters of each of the three words were used (e.g. CSW = Craigs Snow—White).

4. Adg 43 × Aquila 2/2 was abbreviated by the symbol "Adg 43".

5. The isolates obtained by the author in Gross Lüsewitz have been called Lü 72 and Lü 86.

The methods used in the experiments presented in this paper (growing of test plants, inoculation methods, determination of virulence, determination of physical properties, serology, cross protection test etc.) were the same as those described in our previous publication (HORVÁTH, 1966).

Results

Determination and virulence of isolates of potato virus Y

As shown in our previous paper (HORVÁTH, 1966) the severity of the spot necroses produced as a result of synergism between PVX^{RS} and PVX^{N} on the one hand and the isolate EP (strain PVY^{C}) and some PVY^{N} isolates on the other,

Table 2

Type of reaction of PVX^{RS} and PVX^{N} with potato virus Y isolates on tobacco

Virus culture	Type of the reaction with	
	PVX^{RS}[1]	PVX^{N}[2]
Adg 43	additive	additive
BdN	additive	additive
Bie	additive	additive
CSW	additive	additive
Ine	additive	additive
Lü 72	additive	no effect
Lü 86	additive	no effect
PK	additive	additive
PVY—L	additive	antagonistic
PVY—LL	additive	no effect
PVY—N	additive	no effect
PVY—P	additive	additive
PVY—R	additive	antagonistic
PVY—W	additive	no effect
UM	additive	additive
Von	additive	additive

Key: [1] Ringspot strain of potato virus X.
[2] Normal strain of potato virus X.

did not agree with the virulence of the PVY isolates if inoculated alone to test plants. With the isolate PVY—L there was no synergism whatsoever. It has been observed that PVX^{RS} had an additive effect on the symptom production by PVY^{N} isolates. This phenomenon, however, could be used neither for quantitative tests nor for establishing the differences in the virulence among the individual isolates. The failure of the spot necroses to show the differences in virulence among our isolates was apparently due to the unsuitable temperature, as pointed out by DARBY et al. (1951). Between PVX^{N} and isolates of PVY usually an additive effect was observed (Table 2). Some isolates did not exhibit either additive or antagonistic effects as compared to the controls. With two isolates (PVY—L, PVY—R), however, there was a characteristic antagonistic effect which resulted in a weakening of the symptoms. Because of the difficulties in evaluating the spot necroses grouping of the isolates was achieved according to the severity of the symptoms produced by them on individual test plants.

Comparison of the isolates on different test plants upon mechanical transmission

Gomphrena globosa L.:

Symptomless with all isolates tested. Isolates PVY—L, PVY—N, PVY—R, Lü 72 and PK could be recovered, however.

Capsicum annuum L.:

Systemic symptoms consisting of a characteristic vein-clearing and mosaic mottling. "Severe" isolates had a shorter incubation period (22 to 31 days) than "mild" and "medium" isolates. As far as severity of symptoms is concerned, the individual isolates showed pronounced differences (Fig. 1).

Capsicum annuum L. var. Markgärtner:

Symptomless with all isolates tested. No virus was recovered (Fig. 1).

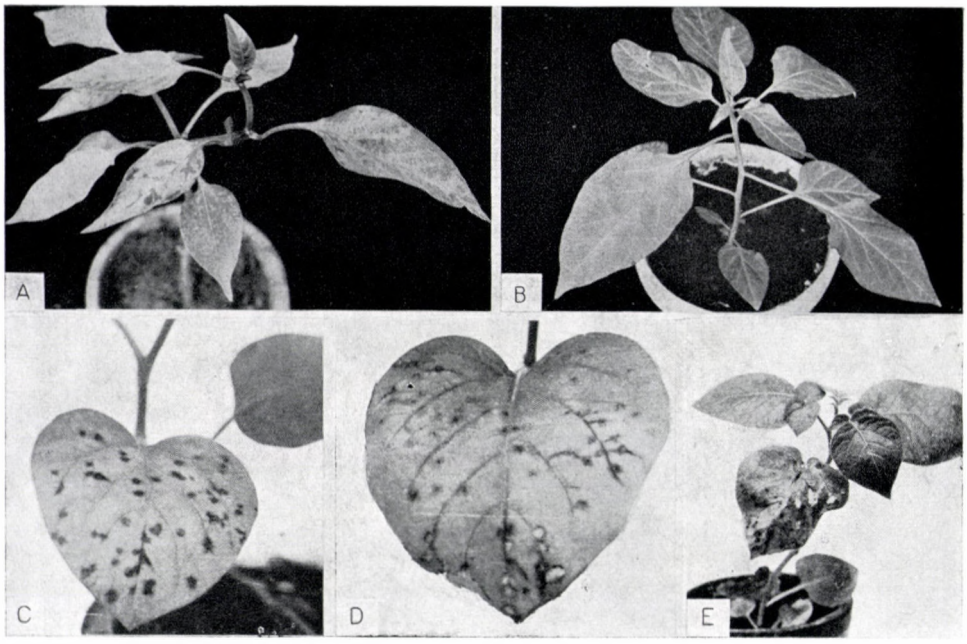

Fig. 1. Plants infected with various isolates of potato virus Y. A: Symptoms caused by the isolate PVY—N on Capsicum annuum L. plants. B: Capsicum annuum L. var. Markgärtner does not show symptoms upon infection with isolate PVY—L. Symptoms caused by isolates C: BdN, D: CSW and E: Lü 86 on Solanum tuberosum Seedling No. 59/558

Datura metel L.:

The severity of symptoms reflected very accurately the virulence of the isolates applied. The symptoms consisted of vein-clearing, mosaic mottling and leaf deformations, which appeared in about 10 to 15 days after inoculation (Fig. 2).

Datura stramonium L. var. tatula:

Not susceptible to any of the PVY isolates tested. No virus was recovered from the inoculated plants.

Lycium halimifolium Mill.:

One of the most reliable test plants with various isolates. The symptoms consisted of brown, circular local lesions. In the case of "medium" and "severe" isolates there was in addition a rapid leaf drop. Only two isolates (BdN, CSW) did not induce leaf drop. Unfortunately with the virulent strains leaf drop occurred so suddenly that no quantitative evaluation was possible. The incubation period lasted in general for 5 to 9 days.

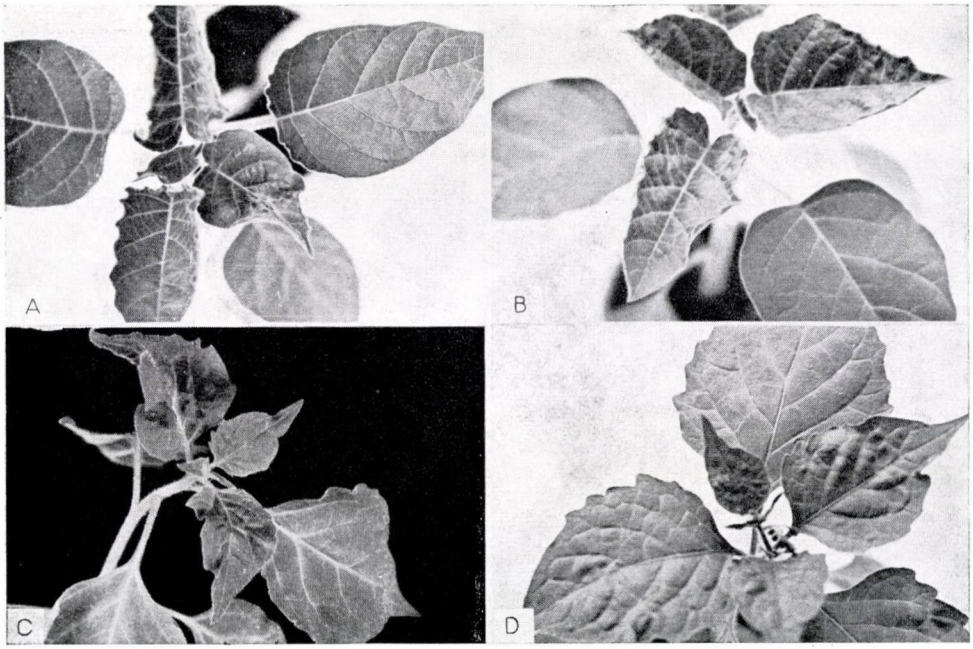

Fig. 2. Datura metel L. (A—B) and Solanum nigrum L. (C—D) plants mechanically infected with potato virus Y isolates. A: PVY—N; B: PVY—L; C: PVY—P; D: PVY—N

Lycopersicon esculentum Mill.:

The symptoms consisted of vein-clearing and of mild, slowly developing mosaic pattern. It is an unreliable host for the demonstration of differences in the virulence of different isolates. The incubation period varied from 15 to 18 days.

Lycopersicon pimpinellifolium (Jusl.) Mill.:

Symptomless with all PVY isolates tested. No virus could be recovered from the inoculated plants.

Nicotiana glutinosa L.:

Depending on the virulence of the individual isolates the plants showed accordingly more or less severe vein-clearing, diffuse mosaic mottling, leaf defor-

mations and reduction in growth. With the "severe" isolates reduction in growth was especially pronounced, and the incubation period was also shorter. Isolate PVY–LL gave circular local necrotic lesions. In this respect isolate PVY–LL differs from all the other isolates tested which induce systemic symptoms (Fig. 3).

Fig. 3. Nicotiana glutinosa L. plants infected with potato virus Y isolates. A: PVY–L; B: PVY–R; C: PVY–N; D: PVY–L; E: PVY–LL

Nicotiana repanda Willd.:

Symptoms usually consisted of systemic vein-clearing and mosaic pattern. Some isolates (Bie, Ine, Lü 72, PVY–LL, UM, PVY–W), however, produced local necrotic symptoms. In addition, isolates Ine, Lü 72, Lü 86, PVY–LL caused a characteristic leaf-roll. The incubation period varied in general between 12 and 16 days.

Nicotiana tabacum L. var. "V 20".:

With most isolates no symptoms were obtained although the virus could always be recovered from the infected plants. Isolates Bie, Von and PVY–L, however, produced a characteristic, steadily increasing circular mosaic pattern. The three isolates had fairly long incubation periods of 25, 27 and 28 days, respectively.

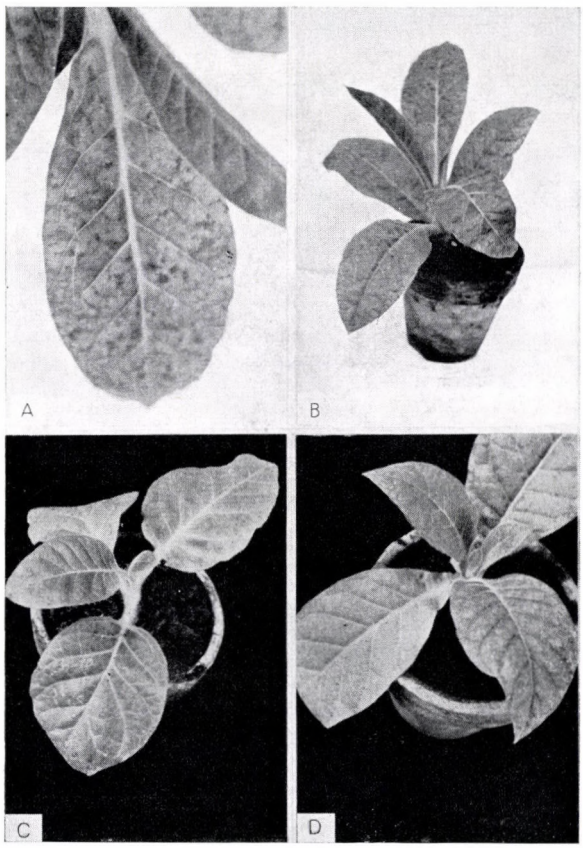

Fig. 4. Nicotiana tabacum L. var. White Burley (A, B, D) and Nicotiana tabacum L. var. Samsun (C) plants infected with an isolate of potato virus Y. A and B: Symptoms upon mechanical transmission. C and D: Symptoms upon aphid transmission. A: PVY–L; B: PVY–L; C: PVY–L; D: PVY–L

Nicotiana tabacum L. var. Havana 38.:

All isolates caused characteristic vein-clearing and vein banding, which were more pronounced with the "severe" isolates. The incubation period varied between 12 and 14 days.

Nicotiana tabacum L. var. Havana 425.:

Susceptible to all isolates. Symptoms consisted of vein-clearing and vein banding. The incubation period varied between 10 and 14 days.

Nicotiana tabacum L. var. Samsun:

The top leaves showed characteristic vein-clearing the intensity of which was in agreement with the virulence of the isolate which caused it. The incubation period lasted for 11 to 14 days (Fig. 4).

Nicotiana tabacum L. var. Sanderae:

Characteristic systemic vein-clearing and vein banding which were formed in about 12 to 14 days, depending on the isolate.

Nicotiana tabacum L. var. White Burley:

As with the tobacco varieties mentioned above the symptoms consisted of vein-clearing and vein banding. The incubation period varied between 12 and 14 days (Fig. 4).

Petunia hybrida Vilm.:

Vein-clearing and diffuse mosaic mottling. Infection with isolates CSW, Bie and PVY – LL led to the formation of large yellow spots in addition to vein-clearing. None of the PVY^N isolates produced necrotic symptoms. The incubation period lasted for 14 to 19 days.

Physalis floridana Rydb.:

Susceptible to PVY^N isolates. Local symptoms consisted of small necrotic lesions, systemic symptoms of mosaic pattern and leaf drop. With the virulent isolates (PVY – L, PVY – N, PVY – R, PVY – W) the symptoms were very severe and dropping of the leaves occurred very soon. There were considerable differences in the virulence of the individual isolates. The virulence proved to be very high with isolates Lü 72, Lü 86, PVY – L and PVY – N, medium with isolates Adg 43, Bie, Ine, PK, UM, Von, PVY – R and PVY – W, and low with isolates PVY – LL, PVY – P, BdN and CSW. The incubation period varied between 10 to 14 days depending on the isolates (Fig. 5).

Solanum nigrum L.:

This test plant, to which usually not too much attention is being paid, was susceptible to all the isolates tested and showed characteristic systemic symptoms which consisted of a systemic vein-clearing, mosaic mottling and a crinkling of the leaves. Some isolates (PVY – LL, PVY – N, PVY – R) produced a systemic leaf deformation. In this respect S. nigrum behaves similarly to some Capsicum spp., which also show leaf deformation upon infection with some virulent isolates.[1]

[1] HORVÁTH, J. (1967): Virulenzdifferenzen verschiedener Stämme des Kartoffel-Y-Virus an Capsicum-Arten und Varietäten. Acta Phytopath. Sci. Hung. (in press).

The incubation period was quite long, varying from 23 to 35 days depending on the isolate (Fig. 2).

Solanum tuberosum L. (Seedling No. 59/558):

Local and systemic symptoms usually consisted of circular necroses, linear vein necroses and mosaic pattern, vein-clearing, respectively. With virulent isolates there was a rapid leaf drop. Isolates Ine and PK did not produce any symp-

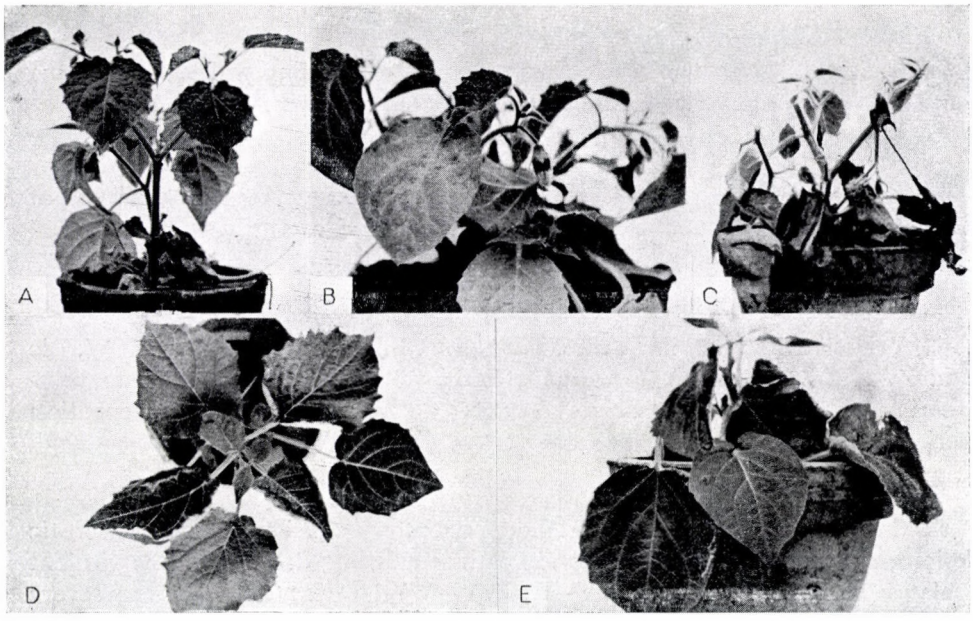

Fig. 5. Physalis floridana Rydb. plants infected with potato virus Y isolates. A—C: Symptoms upon mechanical transmission. D and E: Symptoms upon aphid transmission. A: PK; B: PVY—R; C: PVY—L; D: BdN; E: Bie

toms, but the virus could be recovered from the infected plants. The incubation period varied between 19 to 22 days depending on the isolate (Fig. 1.).

The incubation period, pathogenicity, infectivity and the severity of the symptoms on different test plants of the individual isolates are shown in Table 3. According to the severity of symptoms on different host plants the isolates have been divided into three groups (mild, medium and severe).

Comparison of the isolates on different test plants upon aphid transmission

The incubation period of the individual isolates was usually a few days longer in the case of aphid transmission than with mechanical transmission (Table 4). The only exception to this was the behaviour of some "severe" isolates (PVY—L,

Table 3

Comparison of potato virus Y isolates

Virulence	Virus culture	Incubation period days	Pathogenicity[1]	Infectivity	Severity of symptoms[2]
Mild	BdN	16	28	94	15
	CSW	16	28	93	14
Medium	Adg 43	15	28	93	18
	Bie	16	28	93	18
	Ine	15	27	94	19
	Lü 72	14	27	97	21
	Lü 86	14	27	97	24
	PK	14	27	93	17
	PVY—LL	15	28	96	24
	PVY—P	15	28	96	19
	UM	16	28	95	18
	Von	18	28	92	18
Severe	PVY—L	15	27	97	34
	PVY—N	14	27	100	38
	PVY—R	14	28	99	34
	PVY—W	15	28	98	34

Key: [1] Pathogenicity: 10 = severe; 20 = medium; 30 = mild
[2] Severity of symptoms: 10 = mild; 20 = medium; 30 = severe; 40 = very severe

PVY—N, PVY—R, PVY—W). Aphid transmission did not influence the pathogenicity of the isolates, but considerably lessened their infectivity as compared to that upon mechanical transmission. This may be in connection with the decreased amount of virus injected into the plants by the aphids (Fig. 6). The percentage of successful transmissions was higher with the "severe" isolates than with "weak" ones. This suggests that as far as transmissibility by vectors is concerned there are differences among the isolates of PVY. With regard to the symptoms produced the only difference between the two types of transmissions was that upon aphid transmission of some strains (Adg 43, Bie, Lü 72, Lü 86). Physalis floridana Rydb. exhibited severe crinkling of leaves.

Determination of physical properties

With the 16 PVY^N isolates tested the thermal inactivation point varied from a minimum of 56 °C to a maximum of 72 °C, the average value being 61 °C (Table 5). The dilution end point varied between 10^{-4} and 2×10^{-6}, the longevity in

Table 4

Comparison of potato virus Y isolates on different test plants upon aphid transmission[a]

Virus culture	Pest plants					
	Capsicum annuum L.	N. tabacum L. var. Havana 38.	N. tabacum L. var. Havana 425.	N. tabacum L. var. Samsun	N. tabacum L. var. White Burley	Physalis floridana Rydb.
Adg 43	Not inv.[b]	+/□/×+[c] Vc, Vb[d] 16/30/80/20[e]	+/□/×+ Vc, Vb 16/30/60/20	+/□/×+ Vc, Vb 17/30/80/30	+/□/×+ Vc, Vb 15/30/80/30	+/○ □/×+ I LNSp, II LeAb 13/20/60/20
BdN	Not inv.	+/□/×+ Vc, Vb 15/30/60/10	+/□/×+ Vc, Vb 17/30/60/20	+/□/×+ Vc, Vb 16/30/80/20	+/□/×+ Vc, Vb 16/30/60/20	+/○ □/×+ I LNSp, II LeAb 14/30/60/20
Bie	Not inv.	+/□/×+ Vc, Vb 14/30/80/20	+/□/×+ Vc, Vb 15/30/60/20	+/□/×+ Vc, Vb 18/30/40/30	+/□/×+ Vc, Vb 16/30/40/20	+/○ □/×+ I LNSp, II LeAb 15/20/60/30
CSW	Not inv.	+/□/×+ Vc, Vb 17/30/40/10	+/□/×+ Vc, Vb 18/30/40/10	+/□/×+ Vc, Vb 17/30/40/10	+/□/×+ Vc, Vb 15/30/40/20	+/○ □/×+ I LNSp, II Mo, LeAb 18/30/40/10
Ine	Not inv.	+/□/×+ Vc, Vb 18/30/60/20	+/□/×+ Vc, Vb 17/30/60/20	+/□/×+ Vc, Vb 18/30/60/30	+/□/×+ Vc, Vb 15/30/60/20	+/○ □/×+ I LNSp, II Mo, LeAb 13/20/60/10
Lü 72	Not inv.	+/□/×+ Vc, Vb 13/30/80/30	+/□/×+ Vc, Vb 13/30/80/30	+/□/×+ Vc, Vb 12/30/100/40	+/□/×+ Vc, Vb 13/30/80/30	+/○ □/×+ I LNSp, II Mo, LeAb 13/10/80/20
Lü 86	Not inv.	+/□/×+ Vc, Vb 12/30/80/30	+/□/×+ Vc, Vb 13/30/100/40	+/□/×+ Vc, Vb 14/30/100/40	+/□/+= Vc, Vb 13/30/100/40	+/○ □/×+ I LNSp, II Mo, LeAb 13/10/100/30
PK	+/□/×+ Vc, Vb, Mo 24/30/80/30	+/□/×+ Vc, Vb 18/30/60/30	+/□/×+ Vc, Vb 17/30/40/10	+/□/×+ Vc, Vb 11/30/60/20	+/□/×+ Vc, Vb 15/30/40/30	+/○ □/×+ I StN, Vn, II Mo LeAb 16/30/40/20

PVY–L	+/□/×+ Vc, Vb, Mo 12/30/100/40	+/□/×+ Vc, Vb 12/30/100/40	+/□/×+ Vc, Vb 12/30/100/40	+/□/×+ Vc, Vb 10/30/100/40	+/□/×+ Vc, Vb 10/30/100/40	+/○□/×+ I LNSp, II Mo, LeAb 12/10/100/30
PVY–LL	Not inv.	+/□/×+ Vc, Vb / 12/30/80/30	+/□/×+ Vc, Vb 15/30/80/30	+/□/×+ Vc, Vb 13/30/80/30	+/□/×+ Vc, Vb 13/30/80/40	+/○□/×+ I LNSp, II Mo, LeAb 14/30/60/20
PVY–N	Not inv.	+/□/×+ Vc, Vb 13/30/100/40	+/□/×+ Vc, Vb 12/30/100/40	+/□/×+ Vc, Vb 12/30/100/40	+/□/+ Vc, Vb 12/30/100/40	+/○□/×+ I LNSp, II Mo, LeAb 12/10/60/30
PVY–P	Not inv.	+/□/×+ Vc, Vb 15/30/80/30	+/□/×+ Vc, Vb 15/30/80/30	+/□/×+ Vc, Vb 16/30/60/20	+/□/×+ Vc, Vb 13/30/60/30	+/○□/×+ I LNSp, II Mo, LeAb 13/30/60/20
PVY–R	Not inv.	+/□/×+ Vc, Vb 13/30/80/30	+/□/×+ Vc, Vb 12/30/80/30	+/□/×+ Vc, Vb 12/30/80/30	+/□/×+ Vc, Vb 12/30/80/30	+/○□/×+ I LNSp, II Mo, LeAb 13/20/60/20
PVY–W	Not inv.	+/□/×+ Vc, Vb 13/30/80/30	+/□/×+ Vc, Vb 13/30/80/30	+/□/×+ Vc, Vb 12/30/60/30	+/□/×+ Vc, Vb 12/30/80/30	+/○□/×+ I LNSp, II Mo, LeAb 14/20/60/20
UM	Not inv.	+/□/×+ Vc, Vb 16/30/60/20	+/□/×+ Vc, Vb 17/30/40/10	+/□/×+ Vc, Vb 18/30/40/20	+/□/×+ Vc, Vb 17/30/60/20	+/○□/×+ I LNSp, II Mo, LeAb 16/20/40/20
Von	Not inv.	+/□/×+ Vc, Vb 16/30/60/20	+/□/×+ Vc, Vb 18/30/60/20	+/□/×+ Vc, Vb 17/30/40/20	+/□/×+ Vc, Vb 16/30/60/30	+/○□/×+ I LNSp, II Mo, LeAb 16/20/40/20

Key: [a] with 5 test plants
[b] not investigated
[c] + = symptom production; □ = systemic symptom; ○ = local symptom; ×+ = virus recovered
[d] The letters indicate the following symptoms: LeAb = leaf abscission; LNSp = local necrotic spots; Mo = mosaic; StN = stem necrosis; Vb = vein banding; Vc = vein clearing; Vn = vein necrosis
[e] Time of incubation in days/pathogenicity/infectivity/severity of symptoms
Pathogenicity: 10 = severe; 20 = medium; 30 = mild. Severity of symptoms: 10 = mild, 20 = medium; 30= severe; 40 = very severe

vitro between 18 and 31 days, depending on the isolate. Some isolates, when kept in dried tobacco leaf tissues over $CaCl_2$ at 0 °C remained active even after 426 days, whereas some others lost their infectivity between 192 and 365 days (Table 5). Eight out of the 16 isolates remained active for over a year.

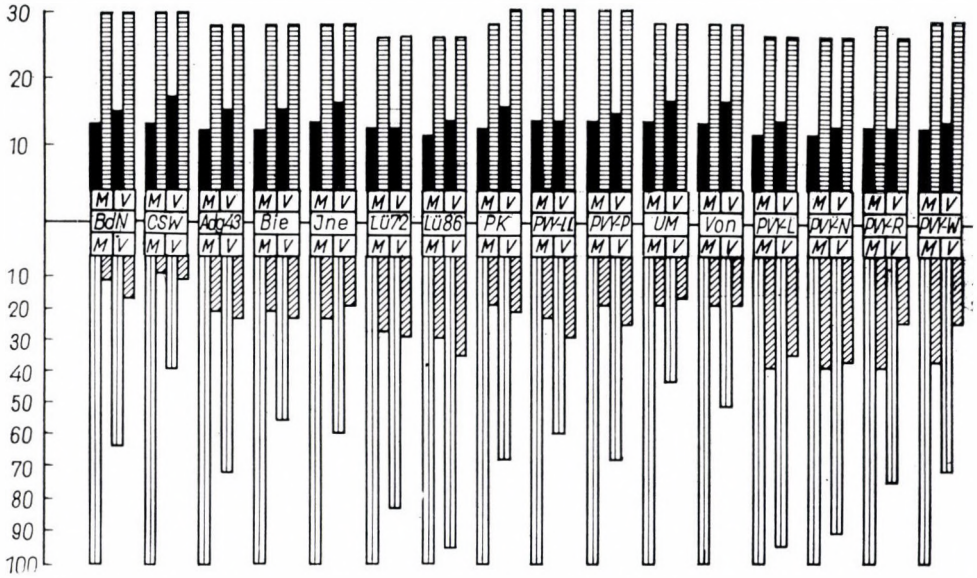

Fig. 6. Incubation period, pathogenicity, infectivity and severity of symptoms on different test plants upon mechanical and aphid transmission of potato virus Y isolates. M: Mechanical transmission. V: Aphid transmission

■: Incubation period in days; ▤: Pathogenicity; ▦: Infectivity; ▨: Severity of symptoms. Test plants used: Nicotiana tabacum L. var. Havana 38; Nicotiana tabacum L. var. Havana 425; Nicotiana tabacum L. var. Samsun; Nicotiana tabacum L. var. White Burley; Physalis floridana Rydb.

Serological relationship

Isolates of PVY^N did not react with antisera prepared against other virus species (PVX, PVS, PVM, PAMV). The individual isolates differed from each other in the intensity of the serological reaction (Table 6), which roughly paralleled the severity of the symptoms produced on different Nicotiana spp. MÉLARD (1964) in a letter to the author described the results of the serological tests carried out with the isolates sent to the author. They were the same as those obtained by the author with these isolates.

Cross protection tests between isolates of PVY^C and PVY^N on tobacco

In one of our previous publications (HORVÁTH, 1966) we have shown that strain PVY^C (isolate EP) shows a premunity effect against infection with some PVY^N strains, whereas it gives no cross protection with the strains PVY^R and

Table 5

Physical properties of potato virus Y isolates

Virus culture	Thermal inactivation point °C	Dilution end point	Longevity in vitro days	Storage over calcium chloride days[1]
Adg 43	56	2×10^{-5}	24	192—365
BdN	60	10^{-4}	21	over 426
Bie	60	2×10^{-5}	21	365—426
CSW	60	10^{-4}	18	192—365
Ine	60	10^{-4}	27	365—426
Lü 72	58	2×10^{-6}	24	over 426
Lü 86	60	2×10^{-6}	31	192—365
PK	58	10^{-4}	24	365—426
PVY—L	72	10^{-5}	24	365—426
PVY—LL	62	2×10^{-5}	27	192—365
PVY—N	64	2×10^{-5}	18	365—426
PVY—P	62	10^{-4}	27	192—365
PVY—R	62	2×10^{-5}	21	192—365
PVY—W	62	10^{-4}	21	192—365
UM	62	10^{-4}	24	365—426
Von	72	10^{-4}	21	192—365

Key: [1] Examined on the 120th, 192nd, 365th and 426th day, respectively.

Table 6

Serological reaction of potato virus Y isolates with antiserum[1] prepared against potato virus Y

Virus culture	Severity of the reaction[2]	Virus culture	Severity of the reaction[2]
Adg 43	++	PVY—L	+++
BdN	+	PVY—LL	++
Bie	++	PVY—N	+++
CSW	+	PVY—P	++
Ine	++	PVY—R	+++
Lü 72	+++	PVY—W	+++
Lü 86	++	UM	++
PK	++	Von	+

Key: [1] In each case an extract of N. tabacum L. var. Samsun was used
[2] + = mild; ++ = medium; +++ = severe.

PVY^{An}. This fact clearly shows that premunity tests important as they are in the identification of virus strains and in the determination of their interrelationships have to be interpreted with caution as pointed out by SILBERSCHMIDT (1957). The results reported in several papers on cross protection and some of his own results led SCHMELZER et al. (cf. 1960) to make the following conclusions:

"1. Normale Y-Stämme wehren die Rippenbräune-Stämme im allgemeinen nicht ab. 2. Die Rippenbräune-Stämme üben keine Schutzwirkung gegenüber normalen Stämmen aus, selbst wenn reziprok gelegentlich Prämunitätserscheinungen auftreten. 3. Die Prämunitätswirkung kann von der Pflanzenart abhängen (Variante "C" schützt die Kartoffel, nicht jedoch den Tabak, vor Rippenbräune). 4. Auch zwischen normalen Y-Stämmen können die Prämunitätserscheinungen ausbleiben."

With the above conclusions in mind and with the isolates exhibiting a wide range of virulence in our hands we expected to find out more about the phenomena involved in cross protection between our "mild" and "severe" isolates. In our experiments two methods were used:

1) 5 tobacco plants *(Nicotiana tabacum* L. var. *Samsun)* were infected first with each isolate. When they showed well developed symptoms they were superinoculated with the challenge isolate. Tobacco plants infected only with the challenge isolate served as a control. After four weeks some of the plants infected first with strain PVY^C (isolate EP) or the "mild" isolates of PVY^N and then with "severe" isolates showed cross protection (Table 7). The degree of cross protection has been expressed similarly to the system developed by KLINKOWSKI and SCHMELZER (1957). From Table 7 it may be seen that there was a complete cross protection with the combinations EP/PVY – N, EP/PVY – W, BdN/PVY – L and BdN/PVY – N and a partial premunity with CSW/PVY – L, CSW/PVY – N, CSW/PVY – W, EP/PVY – R, EP/PVY – L, EP/PVY – LL, EP/Lü 72, EP/Lü 86, BdN/PVY – R, BdN/PVY – LL and BdN/ PVY – W.

Table 7

Cross protection between potato virus Y isolates in *Nicotiana tabacum L. var. Samsun*

Inoculation on August 14, 1964 with	Superinoculation on September 2, 1964 with						
	Lü 72	Lü 86	PVY–L	PVY–LL	PVY–N	PVY–R	PVY–W
	Cross protection[2]						
BdN	–	–	CCP	PCP	CCP	PCP	PCP
CSW	–	–	PCP	–	PCP	–	PCP
EP[1]	PCP	PCP	PCP	PCP	CCP	PCP	CCP

Key: [1] Strain PVY^C

[2] PCP = Partial cross protection; CCP = Complete cross protection.

2) From tobacco plants infected for three weeks with mild isolates (EP, BdN, CSW), mild + severe isolates (EP + PVY−L, EP + PVY−N, EP + PVY−R, EP + PVY−W, BdN + PVY−L, BdN + PVY−N, BdN + PVY−R, BdN + PVY−W, CSW + PVY−L, CSW + PVY−N, CSW + PVY−R, CSW + PVY−W) and severe isolates (PVY−L, PVY−N, PVY−R, PVY−W) inoculum was prepared and applied to *Nicotiana tabacum* L. var. *Samsun* plants. After five weeks it was established that tobacco plants inoculated with sap containing both mild and severe isolates always showed symptoms which were characteristic for the mild isolate. Tobacco plants infected with severe isolates exhibited, of course, the severe symptoms expected. The above observation can be explained on the basis of a partial cross protection between the mild and severe isolates.

Unfortunately because of technical difficulties and other reasons the premunity tests could not be repeated in various seasons of the year and with different host plants.

Summary and Conclusions

16 PVY^N strains partly collected from different countries and partly isolated by the author in Gross Lüsewitz were studied with respect to their virulence, host range, physical and serological properties. Cross protection between individual isolates was also tested. Virulence was determined on the basis of synergism between the PVY^N strains on the one hand and PVX^{RS} or PVX^N on the other. All the isolates of PVY^N had an additive effect on PVX^{RS}, the reactions, however, were not suitable for quantitative evaluation presumably because of unfavourable temperature conditions. Most PVY^N isolates had an additive effect also on PVX^N, with some of them, however, there was an antagonistic effect. Similar observations have been made by KÖHLER and KLINKOWSKI (1954) in the potato variety Erdgold. In our experiments some isolates did not show any interference. Similar observations have been reported by McWhorther and PRICE (1949) between strains of tobacco etch virus (TEV) and tobacco mosaic virus (TMV). They have found that these two viruses were able to multiply in the same plant cell without showing any sign of interference.

Differences in the composition of the RNA and protein moieties of plant virus strains seem to offer a useful tool for their differentiation. Because of the methodical difficulties encountered in the chemical analyses, however, the differentiation of virus strains on the basis of their host range is the quickest and most promising way to follow (BALD, 1960, cf. SCHMELZER, 1961, WEIL, 1963). For this reason our isolates have also been grouped according to the severity of symptoms produced in different host plants. They were divided into three groups: mild, medium and severe.

Transmission experiments have shown that *Capsicum annuum* L. var. *Markgärtner*, *Datura stramonium* L. var. *tatula* and *Lycopersicon pimpinellifolium* (Jusl.) Mill. are immune to all the isolates tested, whereas *Gomphrena globosa* L. proved to be a symptomless carrier of some of the isolates. These observations

are in line with the earlier results of PAUL (1956). *Nicotiana tabacum* L. "V 20" did not show symptoms upon infection with most isolates, the virus, however, could be recovered. These observations contradict to some extent those of SCHMELZER and KLINKOWSKI (1959), who have reported *Nicotiana tabacum* L. "V 20" to be resistant to some PVY isolates (Y III, Y I), which cause non-necrotic symptoms on tobacco. It is worth to notice that *Solanum tuberosum* L. Seedling No. 59/558 which is susceptible to PVY strains proved to be a symptomless carrier of two PVY^N isolates but showed characteristic symptoms with the other isolates. *N. rependa* Willd. gave local lesions with some isolates. This finding is in agreement with the earlier observations by NITZANY (1964), NITZANY and SELA (1962) and NITZANY and TANNE (1962). It is remarkable that *Solanum nigrum* L. was susceptible to all the isolates tested, and that there were characteristic differences in the symptom production of various isolates. The above observations are in contradiction with the experimental data of EASTON et al. (1958) according to which *Solanum nigrum* L. is immune to PVY. In our opinion this discrepancy may be due to the facts that the authors cited above did not take into consideration the long incubation period for the final evaluation of the reaction or were working with the varieties Gorden Huckleberry or Quensence, which are immune indeed. With some isolates aphid transmission resulted in an increase of the incubation period and in a decrease of the infectivity as compared to the same characteristics upon mechanical transmission. As far as the physical properties of our isolates are concerned we usually found values which exceeded those reported in earlier publications and books. DARBY et al. (1951) for example, comparing about 18 isolates of PVY found thermal death point to vary between 56 °C and 62 °C, ageing in vitro between 8 and 18 days and dilution end point between 1 : 10 000 and 1 : 750 000. In our experiments thermal inactivation point varied from 56 °C to 72 °C, dilution end point from 10^{-4} to 2×10^{-6} and longevity in vitro from 18 to 31 days. Some isolates remained active in dried leaf tissues of tobacco even after 426 days, though most of them lost their infectivity in about a year.

The intensity of serological reactions was different with various isolates, and roughly paralleled the severity of symptoms caused on tobacco by the individual isolates.

In premunity tests plants infected with some mild isolates became partly or completely protected from infection with severe strains.

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Effect of Photoperiod and Temperature on the Diapause of the Alfalfa Weevil (*Hypera variabilis* Herbst.).

By

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In this paper experimental results obtained by studying the effect of photoperiod and temperature on the activity of overwintered adults of the first generation of *Hypera variabilis* Herbst. are reported.

In South-Western Hungary less than 50 percent of the overwintered adults undergoes aestivation. After aestivation the adults will lay eggs again, and 100 per cent of the adults survived will begin their autumn or winter diapause.

At 18 and 23 °C and at photoperiods of 13, 14 and 17 hours, respectively, after maturation feeding the adults of the first generation diapause for 30 to 60 days and become activated again. This new active phase lasts for about 30 to 50 days and is followed again by diapause.

Adults kept at 28 °C and at the photoperiods mentioned above begin their definitive diapause after maturation feeding.

Both summer and autumn or winter diapauses are of an obligate nature. The experiments have shown that during the period between the two diapauses the activity of adults depends on the temperature: at high temperatures the beetles remain inactive.

Introduction

H. variabilis is first of all a pest of alfalfa. The way it causes damage is well known: it feeds on leaf and flower buds as well as on young shoots, and leaves (TITUS, 1910; LEHMANN, 1933; YAKHONTOV, 1943; BALACHOWSKY and MESNIL 1936; LEHMANN and KLINKOWSKI, 1942; HEY, 1945; SORAUER, 1954; PONOMARENKO, 1956; SCHMIDT, 1962; BALACHOWSKY, 1963).

In Hungary too *H. variabilis* is regarded as an important pest of alfalfa (GRÓF, 1936; SZELÉNYI, 1941; 1942; 1960, MANNINGER 1960, ANONYMUS, 1962; HUZIÁN, 1963) although no detailed studies have been made yet on its development, especially on the factors affecting the diapause of adults.

The present paper is mainly concerned with the factors involved in the diapause of adults. Therefore of the vast number of publications available on the alfalfa weevil only those bearing on this special topic will be shortly discussed.

On the basis of the results obtained by the Hungarian authors mentioned above the development of *H. variabilis* can be outlined as follows: Overwintering of adults occurs either on the soil surface under a layer of dropped and dried plant residues or in the upper soil layer of bulks near alfalfa stands. The adults

start to appear on the first spring day. In 1948 near Keszthely MANNINGER observed adults which appeared as early as February 2. Egglaying usually starts in April, when the temperature is about 15 to 20 °C. Larvae from the first eggs pupate by the middle of June and by the end of June the adults of the first generation appear. The beetles of this generation after maturation feeding begin to overwinter and do not appear before next spring. Thus, according to the above authors, in Hungary the alfalfa weevil has only a single generation.

Earlier ecological analyses in Hungary (DESEŐ, 1961) have yielded the following data of interest: Alfalfa weevil had a constancy degree of 5, indicating its occurrence in every alfalfa stand. The number of adults, however, showed remarkable variations during the vegetation period. Table 1 shows the numbers of adults and larvae collected by sweeping with a net 250 times each time for two successive years at various locations of Hungary in different alfalfa stands before the first, second and third mowing, respectively. Table 1 contains also the number of adults of *H. variabilis* collected by the use of 15 soil traps. The data in Table 1 clearly show that the number of both adults and larvae is greatly reduced at the time of the second mowing (June and

Table 1

Number of adults and larvae of *H. variabilis* Herbst. collected at various locations and in different years

Location and year	Before 1st Mowing		Before 2nd Mowing		Before 3rd Mowing	
	Adults	larvae	Adults	larvae	Adults	larvae
Sweeping with a net						
Alsóbogát (1956)	87		0		4	
Barattyos (1955)	9	20	0	0	1	47
Boly (1956)	5		1		6	
Gorzsa (1955)	14	2	1	0	4	3
Hajdúszoboszló (1956)	94	36	2	5	67	243
Kanota (1956)	24		2		7	
Klára (1955)	7		6		1	
Klára (1956)	69		5		9	
Klára (irrigated) (1955)	8	8	2	5	0	15
Klára (irrigated) (1956)	64		4		22	
Kutas (1955)	26	2	7	10	0	0
Kutas (1956)	38		0		7	
Nagyszentjános (1956)	10		2		1	
Szőllős (1955)	0		1		4	
Ujváros (1955)		11		10		0
Soil traps						
Zsámbék		155		23		12

July) and increases again to a higher or lesser extent only when the third mowing is due.

No experimental results from either Hungary or abroad give an unequivocal explanation of the reasons for these quantitative changes observed with *H. variabilis*.

As to the role of the number of generations in the quantitative changes observed there are two hypotheses opposing each other. Adherents of the first view hold that *H. variabilis* has one or more additional partial generations yearly (PARKS, 1914; YAKHONTOV, 1934; LEHMANN and KLINKOWSKI, 1942; MANGLITZ and APP, 1957). According to the other hypothesis the alfalfa weevil has but one generation in a year and eggs to be found in autumn originate from overwintered adults (ESSIG and MICHELBACHER, 1933; LEHMANN and KLINKOWSKI, 1942; MANGLITZ, 1958).

The above authors, irrespective of regarding eggs laid in autumn as originating from overwintered adults or from those of a new generation, agree in that egglaying starts only after quiescence induced by the ecological conditions prevailing in summer.

The effect of temperature and relative humidity on *H. variabilis* has been thoroughly studied by REEVES (1917), SWEETMAN (1932) and SWEETMAN and WEDEMEYER (1933). They have established both the optimum and mortality values.

This short literary survey will suffice to indicate that even in countries where losses caused by *H. variabilis* in alfalfa are of major importance, there is no general agreement as to the biology of the pest in question. This is probably connected with its considerable ecological plasticity. These discrepancies led us to carry out detailed studies on the development of this species in Hungary with a special emphasis on the factors influencing the diapause of the adult population.

The aim of our experiments was to study:

1. The feeding habits, egglaying and quiescence of the overwintered adult population both under laboratory conditions at constant temperatures and in the field.

2. The effect of differences in the photoperiod affecting the larval progeny of overwintered adults and the adults developed from them (first generation) on the activity of the adults i.e. on their feeding habits, egglaying and quiescence both at constant temperatures and in the field.

Materials and Methods

The adults used in the experiments were collected in South-Western Hungary on May 18, 1965. The experiments were performed in constant temperature chambers, in laboratory rooms and in outdoor cages in the field. Adults were kept in glass cylinders covered with cheesecloth. Alfalfa shoots serving as food and for the purpose of egglaying were changed daily in the cylinders. The stem of the alfalfa shoot was immersed into water through a perforated glass plate.

Egglaying was established by dissecting the stem pieces and by checking them in the microscope. By the use of a camel's hair brush the eggs were placed in a hygrostate on a sheet of filter paper which was in touch with the water contained in the hygrostate by a thin paper strip. The use of a hygrostate was necessary because of the great sensitivity of eggs to air humidity.

The larvae hatched were kept in a hygrostate in which the relative air humidity was about 100 per cent. The larvae were fed with top leaves as long as their development was completed. Pupation also took place in a hygrostate. The beetles developed from the pupae were again placed in cheese-cloth covered glass cylinders in which filter paper was layered to create a hiding place for the diapausing adults. In each experiment 25 to 110 adults were included. The constant temperature chambers were illuminated by two parallel fluorescent lights (Tungsram) of 40 watts each, yielding a total light intensity of about 1500 lux.

Results and Discussion

In the first part of our experiments *the activity of the overwintered adult population* was studied. At a constant temperature of 23 °C and a photoperiod of 13 hours the following results were obtained: Adults collected in the field by the middle of May were laying eggs on the average for 46 days. After this a considerable portion of the population (about 42 per cent comprising both males and females) began to aestivate. During this period the nondiapausing females layed eggs only sporadically. Diapause was followed again by a period of intensive oviposition lasting for 56 days. The last egg was layed on October 29. After this 100 per cent of the adults began their diapause. Egg production was much higher and more uniform during the spring period than in autumn. Mortality of adults started at the time of aestivation (end of July) and continued for the period of oviposition in autumn. About 21 per cent of the starting population was still alive by the time the present paper was completed (February, 1966).

Adults bred in the field and in laboratory rooms respectively, to serve as a control for the above experiment behaved exactly in the same way. Under both conditions adults the breeding of which started in the middle of May laid eggs regularly until the beginning of July. From then on egg production suddenly decreased. 37 per cent of the adults was quiesceing in an immobil state between the filter paper sheets layered loosely. From the beginning of July until the beginning of August non-diapausing adults were laying eggs only sporadically. By the middle of August adults which stopped quiesceing became activated again, were feeding and laying eggs. The last eggs were laid on September 17. These adults, however, also began to hibernate again at the beginning of September. Egg production by the end of summer was considerably less than that of spring or early summer. Only 27 per cent of the adults survived until the beginning of the autumn diapause.

The developmental stages of the alfalfa weevil in the laboratory and in the field are represented in Fig. 1.

The results indicated that the alfalfa weevil has both a summer and an autumn or winter diapause. The question arises whether either of them is an obligate diapause or merely quiescence. Summer diapause is apparently an obligate diapause, since in spite of the optimum temperature and food plants the animals did not become active. As far as autumn diapause is concerned we may not be quite so sure. KAUFMANN (1939) has reported in his monograph that as the temperature decreases the adults begin their quiescence and as it increases again they

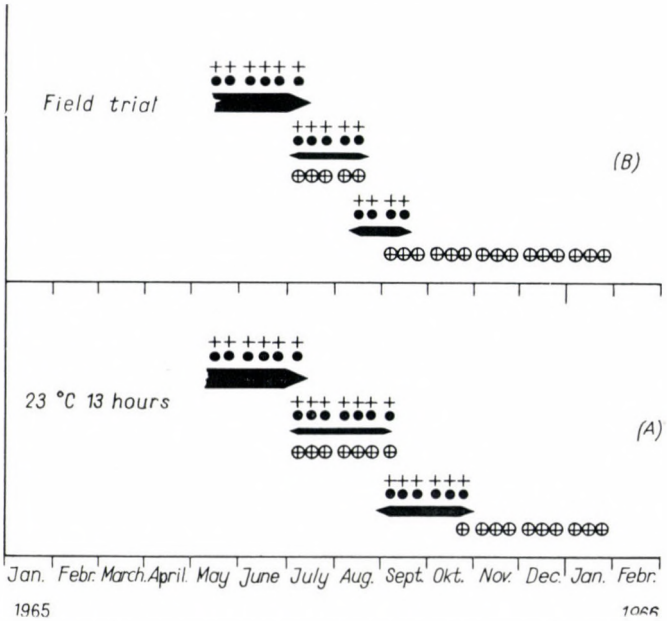

Fig. 1. Behaviour of overwintered adults at 23 °C and at a photoperiod of 13 hours (A) and in outdoor cages (B). + active adult, ● egg, ⊕ diapausing adult, — relative frequency of eggs

become active. Furthermore MANNINGER's February observation mentioned above also suggests that as soon as the temperature increases the overwintering adults interrupt their quiescence and shortly start their activity. From these data one would conclude that the autumn or winter diapause is merely quiescence. To decide whether this is so indeed, adults which began to hibernate in the field for about 20–25 days were exposed to photoperiods of 13, 14 and 17 hours and to temperatures of 18, 23 and 28 °C, respectively. None of these factors induced the adults to leave their place of hibernation. They kept on quiesceing. Consequently both the summer and the autumn or winter diapauses are to be regarded as being of an obligate nature. The appearance of adults in February may be explained in turn by the fact that at that time the adults already underwent their obligate diapause. With the alfalfa weevil the time period of this obligate quiescence is about 50 to 70 days.

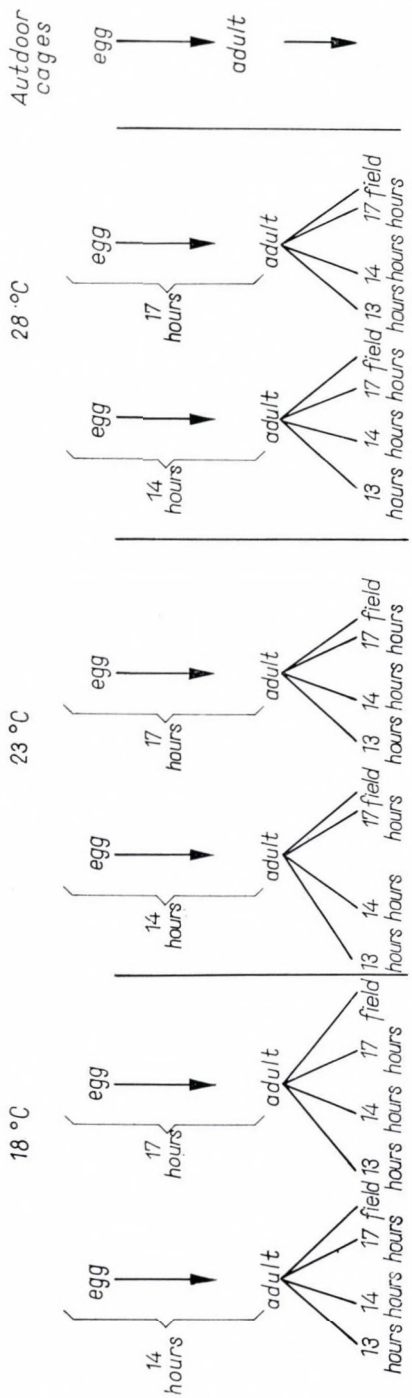

Fig. 2. Sketch of the photoperiod treatment of adults of the first generation

In the second part of our experiments the effect of different temperatures and photoperiods on the behaviour of the progeny (first generation) of overwintered adults was investigated. The experiments were carried out in constant temperature chambers at 18, 23 and 28 °C, respectively and in outdoor cages in the field. The freshly laid eggs of the overwintered adults were exposed to photoperiods of 14 and 17 hours, respectively, at each of the three temperatures until they developed into adults. Each group of adults thus obtained was divided into four groups. Three groups were raised at photoperiods of 13, 14 and 17 hours, respectively, and one in outdoor cages. As a control to this experimental series eggs hatched by adults which had overwintered in the field were kept in outdoor cages until they developed into adults. The behaviour of these adults was then further observed. A sketch of the experiment described above is given in Fig. 2 and the results obtained are shown in Fig. 3.

Adults kept at *18 and 23 °C* and at the photoperiods indicated behaved very similarly except for the differences in the durations of the active and quiescence periods. Therefore the results obtained with these two temperatures will be discussed together. The adults of the first generation showed the following behaviour at the three photoperiods: Adults which appeared during June the first half of July began their diapause after a maturation feeding for 25 to 40 days. The diapause came to an end in about 35 to 90 days, depending on the temperature and photoperiod, and the adults started to feed and lay eggs again. The egglaying period took about 20 to 50 days and was followed again by diapause. Egglaying was not intensive at all. In one case adults which originated from larvae raised at 23 °C and a photoperiod of 17 hours again interrupted their diapause after 30 days, but were feeding only without laying eggs.

Adults kept at 18 and 23 °C and then in outdoor cages after a maturation feeding for 35 to 45 days began quiesceing for 31 to 40 days, then became active again, layed some eggs and finally after having spent about 15 to 30 days on the surface began their autumn diapause by the beginning of October.

Adults kept at 28 °C behaved quite differently from those raised at the two other temperatures. Both in the field and in the laboratory with different photoperiod treatments they all began their final diapause by the end of July and beginning of August after a maturation feeding for about 15 to 35 days. Only a small percentage of the adults kept at a photoperiod of 13 hours interrupted their diapause but even these layed no eggs and started to diapause again after a feeding period of 21 days.

The progeny of the adults which had overwintered in outdoor cages was also being observed as a simultaneous control to the laboratory experiments. They behaved in the same way as the adults raised at a constant temperature of 28 °C, i.e. after a maturation feeding they began to diapause from the middle of August.

The experiments with both the overwintered adults and those of the first generation can be evaluated as follows:

Adults of the first generation originating from larvae raised at 18, 23 and 28 °C and at different photoperiods behaved similarly at both 18 and 23 °C. This points

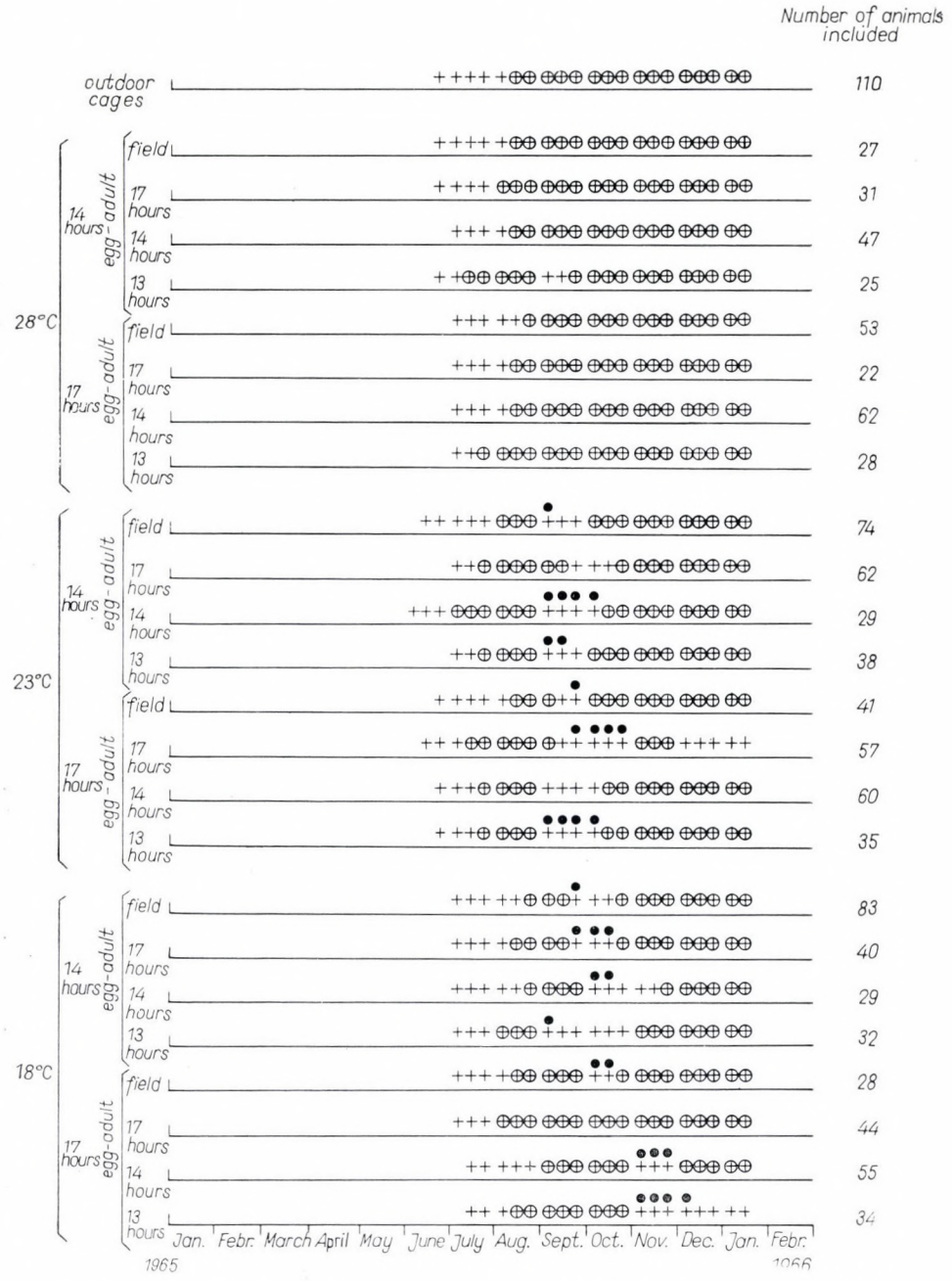

Fig. 3. Behaviour of adults of the first ger eration at different temperatures and photoperiods and in the field. + active adult, • egg, ⊕ diapausing adult

to the fact that under the experimental conditions used photoperiod is of no significance as far as diapause is concerned. Temperature, however, seems to have a decisive influence on the behaviour of adults. The deciding role of temperature is further shown by the behaviour of adults kept at 28 °C. In this case the adults at the same photoperiods as those used with the temperatures 18 and 23 °C did not interrupt their diapause once begun but kept on diapausing without interruption. This observation suggests that a temperature of 28 °C has an inhibitory effect on the becoming active of adults. This suggestion is supported by the observations of YAKHONTOV (1934), GUERRA and BISHOP (1962) and TOMBES (1964). The inhibitory effect of high temperature on the activation may explain the observation by ARMENGOL (1963) who found that in South-Western France under mediterranian climatic conditions the alfalfa weevil started to lay eggs as late as the beginning of October. Oviposition lasted until the end of next spring. Then a diapause set in till the end of September. PONOMARENKO (1956) also refers to the inhibitory action of high temperature on activity by reporting that Phytonomus transsylvanicus Petri (a synonym for *Hypera variabilis*) at temperatures of about 28 to 30 °C borrows into the soil. SHCHEGOLEV (1951) has found a retardation of the development of ovaries at temperatures higher than 25 °C.

Our experimental results suggest that in Hungary if the second half of summer is cold and humid enough the adults of the first generation will interrupt their diapause and feed again for a while and will even lay eggs. In all probability most of the larvae from these eggs are about to perish. When, however, a long lasting autumn of favourable weather conditions sets in they may develop into adults. In this case one has to reckon with the appearance of a second generation. This generation, however, is practically of very little economic importance.

If weather conditions in autumn are such that the larvae cannot develop into adults, and winter is also mild, the fully developed larvae may overwinter in a state of quiescence. This was the case e.g. in 1964.

Fully developed overwintered larvae, however, may represent not only the progeny of the first generation but may originate from overwintered adults, too. Therefore it is impossible to determine which generation such larvae found in the field belong to.

Conclusions

1. In South-Western Hungary, the *overwintered* adult population of the alfalfa weevil has two diapauses in a year. Whereas the summer diapause involves less than 50 per cent of the population, the autumn or winter diapause includes 100 per cent of the population.

Hence the population still alive in autumn may begin to overwinter for a second time.

2. In the becoming active of adults of the first generation by the end of summer temperature plays a decisive role. High temperatures prevent adults from becoming active.

3. Both summer and autumn diapauses are obligate.

4. In Hungary if the end of summer is cold and humid enough adults which had already begun their diapause may become active again for a while and may even lay eggs. If weather conditions are favourable for larval development a second generation may develop or the developed larvae may overwinter in a state of quiescence and finish their development next spring.

Fully developed larvae collected late in the autumn may represent the progeny of either the first generation or the overwintered adult population. The reason for this is that egglaying by the overwintered adults can reach far into autumn and go on thereby simultaneously with the oviposition of the adult population of the first generation.

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Phytopathogenic and Saprophytic Fungi from Hungary, III.

Contributions to the Rust Fungus *(Uredinales)* Flora of Hungary

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During the course of the collection of microscopic fungi in almost the entire territory of Hungary between the years 1947 and 1966, 127 species of rust fungi were found. 9 of them are new records from Hungary while 16 species occurred on new host plants. The collection presented here are deposited in the herbarium of the Research Institute for Plant Protection, Budapest. The nomenclature and systematic arrangement of fungi applied in this paper are generally identical with the conception of G. MOESZ (Fungi Hungariae IV. *Basidiomycetes*. Pars 1. *Uredineae*, I—II. Ann. Mus. Nat. Hung. XXXIII—XXXIV. Budapest, 1940—1941. p. 128—200.; p. 72—158.), except in the case of new species and in the case of important nomenclatural changes. In these cases Traian SAVULESCU: Monografia Uredinalelor din RPR, I—II. Bucuresti, 1953. was followed. New records are signed with**, and new host plants with*.

I. *Basidiomycetes: Uredineae.*

1. *Pucciniaceae.*

Gymnosporangium clavariaeforme (JACQ.) DC. — Roestelia (aecidium) formation is frequent on the fruits, branches and petioles of *Crataegus monogyna*, Szarvas, Arboretum, 6. 29. 1948. (aecidiospores: 20.5—28 × 18.6—23.5 micron, peridium-cells: 74.4—93—130.2 × 18.6—23.5 micron); Ágasegyháza, 7. 12. 1954. (aecidiospores: 26—33.5 × 20—25.5 micron); Tatárszentgyörgy, (aecidiospores: 31.4—36 × 26—27.5 micron). (Figure 1)

Gymnosporangium sabinae (DICKS.) WINTER — Aecidia are frequent on *Pyrus pyraster* and on *P. sativa* all over the country, where *Junipures sabina* is present, too.

Gymnosporangium confusum PLOWR. — Aecidia are frequent on *Cydonia oblonga*, Szank, Bács-Kiskun (leg. LEHOCZKY, J.) 7, 2, 1966.

Phragmidium disciflorum (TODE) JAMES — On *Rosa canina*, Szarvas, Erzsébetliget, 9. 28. 1947. (uredospores: 18.6—27.9 × 14—18.6 micron, teleutospores: 65.1—102.3 × 28.7—35.3 micron, teleutospore-stalks: 158.1—213.9 micron); l.c. 5. 5. 1948. (aecidiospores: 18.6—27.9 × 14—16.7 micron); On *Rosa centifolia*, Szarvas, Arboretum, 10. 12. 1948. (uredospores: 22—27.5 × 18—21 micron, teleutospores: 68—105 × 32—41 micron); On**Rosa rugosa* l.c. 5. 2. 1948. (aecidiospores: 22—28 × 18—21.5 micron).

Phragmidium phragariastri (DC.) SCHROETER – On *Potentilla alba*, Mátraháza, 10. 2. 1950. (ureodospores: 17.5–25 × 16–20 micron, teleutospores: 51.5–62.3 × 22.5–29.4 micron).

Phragmidium potentillae (PERS.) KARST. – On *Potentilla argentea*, Szarvas, Bikazug, 9. 17. 1947. (with uredo- and teleutospores); Szarvas, Arboretum, 8. 23. 1948. (uredospores: 18.6 × 19.1–20.9 micron, teleutospores: 51–59.2 × × 19.5 micron, teleutospore-stalks: 123–144–148.8 micron teleutosori: 186–465 micron – in company with the perithecia of *Sphaerotheca macularis* (WALLR.) JACZ.).

Fig. 1. Aecidia of *Gymnosporangium clavariaeforme* (JACQ.) DC. on the leaves and twigs of *Crataegus monogyna*

Phragmidium rosae-pimpinellifoliae (RABH.) DIETEL – On *Rosa pimpinellifolia*, Budapest, Árpádhegy, 6. 4. 1955. (uresodspores: 20–25 × 17.5–19.8 micron).

Phragmidium rubi (PERS.) WINTER – On *Rubus caesius* var. *agrestis*, Szarvas, 9. 18. 1947. (uredospores: 18.6–23.1 × 14–16.7 micron, teleutospores: 60.4–84.5 × 26–27.9 micron, teleutospore-stalks: 79 × 9.3–19.5 micron, length of the teleutospores with the stalk together: 130.2–195.3 micron); On *Rubus candicans*, Mátraháza, 10. 12. 1954. (teleutospores: 65.5–109 × × 25.5–30.5 micron).

Phragmidium rubi-idaei (PERS.) KARST. – On *Rubus idaeus*, common all over the country, with caeoma, uredo- and teleutosori.

Phragmidium sanguisorbae (DC.) SCHROETER – On *Sanguisorba minor*, Budapest, Jánoshegy, (uredospores: 16 – 24.5 × 12 – 18.5 micron, teleutospores: 48 – 67.5 × 19.5 – 27.5 micron).

Puccinia absinthii DC. – On *Artemisia absinthium* and on *A. vulgaris*, frequent all over the country, with uredo- and teleutosori; On *Artemisia monogyna* and on *A. salina*, Mezőtúr, 10. 4. 1947. (uredospores: 22.5 – 25 × 20 micron,

Fig. 2. Teleutospores of *Puccinia absinthii* DC. (1000×)

teleutospores: 40 – 47.5 × 25 – 27.5 micron – measurements from the host plant *A. salina* – on both hosts in company with *Darluca filum*, conidia: 10 – 12.5 × 3.7 micron); On *Artemisia pontica*, Szarvas, Bikazug, 9. 25. 1947. (uredospores: 27.5 – 30 × 26.5 micron, teleutospores: 37.5 – 52.5 + 22.5 × 25 micron, teleutospore-stalks: 185 – 200 micron – in company with *Leptosphaeria artemisiae* (FUCK.) AUERSW., ascospores: 22.5 – 25 × 7.5 micron, and with *Darluca filum*) (Figures 2, 3).

Puccinia actaeae-elymi E. MAYOR – On *Elymus europaeus*, Békéscsaba, 7. 12. 1953. (uredospores: 18.5 – 24 × 18 – 23 micron).

Puccinia aegopodii (SCHUM.) MART. – On *Aegopodium podagraria*, Lillafüred, 8. 31. 1951. (teleutospores: 31 – 48 × 16 – 22 micron).

Puccinia agropyri ELL. et EV. – On *Clematis integrifolia*, Szarvas, Köröspart

(aecidiospores: 18–31 × 18–28 micron); On *Clematis vitalba*, Visegrád, Várhegy, 6. 7. 1950. (aecidispores: 17.5–32 × 18–27 micron).

Puccinia agropyrina ERIKSS. – On *Agropyron repens*, (uredospores: 22.5–25 × × 21.2–22.5 micron, globular, wall thickness: 0.6–0.8 micron).

Puccinia antirrhini DIET. et HOLW. – On *Antirrhinum majus*, frequent, all over the country, e.g. Szentes, 6. 23. 1948. (uredospores: 9–10 × 7–8.5 micron,

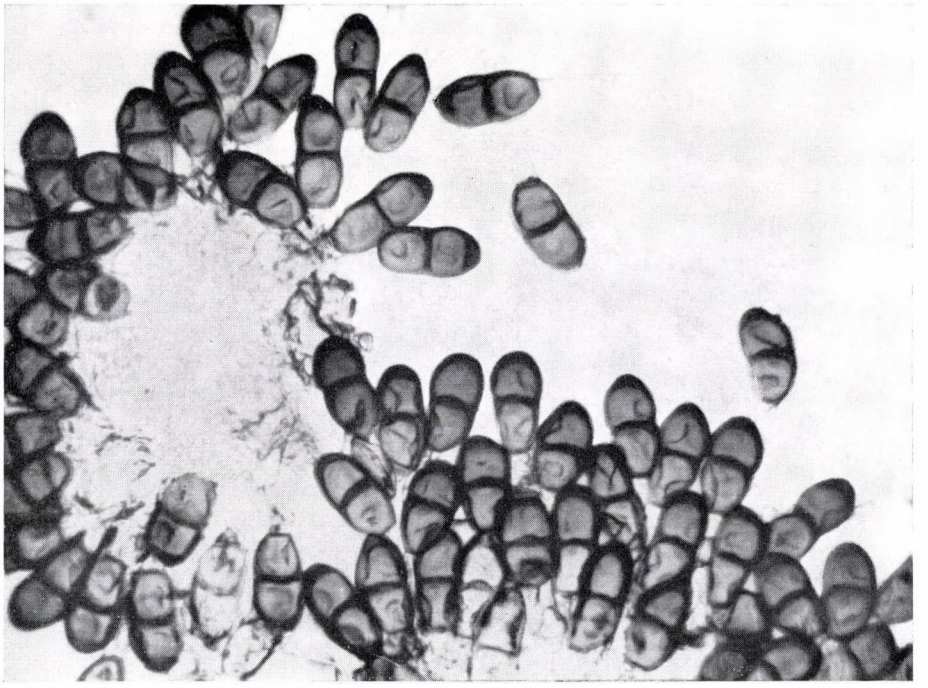

Fig. 3. Teleutosorus of *Puccinia absinthii* DC. (250×)

teleutospores: 17.5–18 × 9–10.5 micron); Sopron, 9. 12. 1955. (uredospores: 10.5–11.2 × 7–8 micron), etc.

Puccinia arenariae (SCHUM.) WINTER – On *Arenaria sepyllifolia*, Nagykovácsi, 6. 23. 1953. (teleutospores: 32–50 × 13–20 micron); On *Stellaria aquatica*, Nagykanizsa, 6. 21. 1950. (teleotuspores: 28–44 × 12–18.5 micron).

Puccinia arrhenatheri (KLEB.) ERIKSSON – On *Arrhenatherum elatius*, Szarvas, 6. 17. 1947. (uredospores: 20–28 × 17–25.5. micron).

Puccinia asarina KUNZE – On *Asarum europaeum*, Nagykovácsi, Zsíroshegy, 6. 17. 1953. (teleutospores: 35–42 × 17.5–25 micron).

Puccinia asparagi DC. – Frequently all over the country on cultivated and wild *Asparagus officinalis*.

Puccinia bardanae CDA. – On *Arctium lappa*, frequent, e.g. Szarvas, Bikazug,

Erzsébetliget, 7. 19. 1948., 9. 24. 1948. (uredospores: 25–30 × 22–27 micron, teleutospores: 33–38 × 22–25 micron); On *Arctium minus*, common, e.g. Szarvas, 9. 24. 1948. (uredospores: 27.5–32.5 × 25–27.5 micron, teleutospores: 35–36–(47.5) × 25–27.5 micron).

***Puccinia bromina* ERIKSS. (Syn.: *Aecidium lithospermi* THUEM.) – On *Lithospermum arvense*, Nagykovácsi, 5. 5. 1954 (aecidiospores: 19.2–28.5 × 18–25 micron).

Puccinia bupleuri-falcati (DC.) WINTER – On *Bupleurum falcatum*, Budapest, Árpádhegy, 5. 17. 1965. (uredospores: 20.5–24.5 × 18–24 micron).

Puccinia Celakovskyana BUBÁK – On *Galium cruciata*, Pogányszentpéter, 6. 21. 1950. (uredospores: 21–30.5 × 18–26 micron).

Puccinia centaureae DC. – On *Centaurea pannonica*, Szarvas, Arboretum, 7. 23. 1948. (uredospores: 27.5–32.5 × 26.2 micron, teleutospores: 32.5–40 × × 22.5–30 micron).

Puccinia Cesatii SCHROETER – On **Chrysopogon gryllus*, Szigetmonostor, 9. 23. 1951. (teleutospores: 30–34 × 19–22 micron).

Puccinia chaercphylli PURT. – On **Chaerophyllum temulum*, Kadarkut, 6. 20. 1951. (uredospores: 22–30 × 18–25 micron).

Puccinia chondrillina BUB. et SYD. – On *Chondrilla juncea*, Nagykovácsi, 9. 8. 1953. (uredospores: 22–28 × 16–24 micron, teleutospores: 32–37.5 × 20–22 micron).

Puccinia cichorii (DC.) BELL. – On *Cichorium intybus*, Szarvas, 9. 29. 1948. (uredospores: 25–26.2 × 22.5 micron, teleutospores: 35–40 × 20–22.5 micron); Tápiószele, 6. 24. 1960. (uredospores: 23–27.5 × 19–21 micron).

Puccinia circaeae PERS. – On *Circaea lutetiana*, Keszthely, Georgikon-park, 7. 13. 1954. (teleutospores: 23–34 × 11–16 micron).

Puccinia cirsii-lanceolati SCHROETER – On *Cirsium lanceolatum*, Szarvas, Bikazug, 9. 17. 1947. (uredospores: 25–31.2 × 25–27.5 micron, teleutospores: 37.5–45 × 22.5–25 micron).

Puccinia convolvuli (PERS.) CAST. – On *Calystegia sepium*, Vácrátót, Arboretum, 8. 12. 1949. (uredospores: 24–35 × 19–27.5 micron, teleutospores: 42–51 × 24–30 micron).

Puccinia coronata CDA. f. *agrostis* ERIKS. – On *Agrostis alba*, 9. 3. 1947. (uredospores: 17.5–20 × 15–17.5 micron, teleutospores: 40–66.2 × 12.5–16.6 micron).

Puccinia coronifera KLEB. – Caeoma-sori are frequent on *Rhamnus cathartica*, e.g. Budapest, Jánoshegy, 5. 12. 1848.; Keszthely, 6. 22. 1950., etc.; On *Alopecurus pratensis: f. alopecuri* ERIKSS., Mezőtúr, 10. 4. 1947. (uredospores: 19.5–25 × 17.5–18.6 micron, teleutospores: 45–55–60.5 × 12.5–15 micron); On *Lolium perenne: f. lolii* (NIELS.) ERIKSS. causing teratologic symptoms, Szarvas, 7. 21. 1947. (uredospores: 20–25 × 17.5–20 micron).

Puccinia crepidicola SYDOW – On **Crepis setosa*, Szarvas, Bikazug, Erzsébetliget, 5. 19. 1947. (uredospores: 20–24 × 17.5–20 micron).

Puccinia cyani (SCHLEICH.) PASS. – On *Centaurea cyanus*, frequent, e. g. Nagyko-

vácsi, 6. 2. 1954., Keszthely–Kisbalaton, 5. 25. 1952. (teleutospores: 32.5–38.7×24–28 micron); Nagykőrös, 6. 27. 1951. (teleutospores: 33–40×25–29 micron).

Puccinia cynodontis DESM. – On *Cynodon dactylon*, Szarvas-Halásztelek, 9. 3. 1947. (ureodospores: 25–28×25–27 micron, teleutospores: 47.5–57.5× × 17.5–22.5 micron); Szigetmonostor, (teleutospores: 33–58×16–24 micron); Makád, 9. 30. 1955. (with teleutosori).

Puccinia dispersa ERIKSS. – Very common, all over the country in *Secale cereale*. It is epidemic in some years, e. g. in Nyírség, in 1954–1955.; On *Secale silvestre*, Kecskemét-Miklóstelep, 6. 15. 1966 (with uredosori).

Puccinia drabae RUD. (1829). – On *Draba lasiocarpa*, Budapest, Árpádhegy, 5. 27. 1965. (teleutospores: 25–44×14–20 micron).

***Puccinia dracunculina* FAHRENDORFF (1941) – On *Artemisia dracunculus*, Szarvas, Tangazdaság, 9. 13. 1947. (uredospores: 26.2–37.5×17.5–22.5 micron, teleutospores: 45–63×22.5–25 micron, teleutospore-stalks: 45–90 micron, hyperparasited by *Darcula filum*, conidia: 16.2–17.5×5 micron).

Puccinia echinopsis DC. – On *Echinops multiflorus*, Siófok, 7. 16. 1950. (uredospores: 21.5–29.5×22–28 micron).

Puccinia epilobii-tetragoni (DC.) WINTER – On **Epilobium lamyi*, Szarvas, 8. 16. 1947. (uredospores: 18.7–22.5×18.1–20 micron, teleutospores: 27–30× ×17.5–20 micron).

Puccinia falcariae (PERS.) FUCKEL – On *Falcaria vulgaris*, very common, e.g. Szarvas-Erzsébetliget, Halásztelek, 6. 28. 1948. (aecidiospores: 20–37.5× ×17.5–20 micron); Pomáz-Kőhegy, 4. 29. 1953. (aecidiospores: 23–36× 19–20.5 micron).

***Puccinia festucae* SYDOW (1912) – On *Muscari racemosum*, Pomáz-Kőhegy, 4. 29. 1953. (aecidiospores: 22–30×16–24 micron).

Puccinia galii WINTER – On *Galium mollugo*, Szarvas-Arboretum, 9.24. 1948. (uredospores: 22.5–25×21.5–22.5 micron); On *Galium verum*, Szarvas, 8. 13. 1947.) uredospores: 25–30×20–22.5 micron, teleutospores: 46–78×18–25 micron).

Puccinia galii-silvatici OTTH. – On *Galium schultesii*, Visegrád-Várhegy, 6. 8. 1950. (aecidiospores: 18–23×18–22 micron).

Puccinia gentianae (STRAUSS.) MART. – On *Gentiana cruciata*, Mátraháza, 9. 5. 1959. (uredospores: 21–33×20–24 micron, teleutospores: 27.5–42.5×20–28.6 micron).

Puccinia glechomatis DC. – On *Glechoma hederacea*, Szarvas, 9. 25. 1947. (teleutospores: 42.5–50×15–16.3 micron, teleutospore-stalks: 45–57.3 micron); Lillafüred, 9. 3. 1955. (with teleutosori); Pogányszentpéter, 6. 21. 1950. (teleutosori, in company with *Ramularia calcea* (DESM.) CES.).

Puccinia graminis PERS. – Aecidia are common on *Berberis*, *Mahonia* and on *Mahoberberis* spp., all over the country, – f. sp. *secalis* ERIKSS. et HENN. – On *Agropyron repens*, Szarvas, 7. 11. 1948. (uredospores: 27.5×15 micron,

teleutospores: 43.7–60 × 15–17.5 micron); Halásztelek, 6. 23. 1949. (teleutospores: 42.5–60 × 15–22.5 micron, teleutospores in another specimen: 50–62.5 × 15–20 micron); Zebegény, 7. 22. 1950. (teleutospores: 36.9–57.9–79.4 × 18–19.6 micron).

Puccinia helianthi SCHWEINITZ – On *Helianthus annuus*, frequent all over the country, e.g. Szarvas, 10. 14. 1947., 5. 6. 1948. etc., (aecidiospores: 20–27 × 18–22 micron, uredospores: 22.5–25 × 21.2–22.5 micron, teleutospores: 40–50 × 20–28.7 micron); Kisvárda, 7. 27. 1950. (uredo- and teleutosori in company with *Plasmopara halstedii* (FARL.) BERL. et DE TONI.)

Puccinia hieracii (SCHUM.) MARTIUS – On *Hieracium pilosella*, Szarvas-Arboretum, 9. 26. 1948. (uredospores: 28.7 × 23.7 micron, teleutospores: 29–35 × 23–25 micron).

***Puccinia hordei-murini* BUCHWALD (1943) – On *Hordeum murinum*, Budapest, Rózsadomb, 6. 22. 1957. (uredospores: 23–27.5 × 18–25 micron); Balatonakarattya, 6. 9. 1966. (with uredosori).

Puccinia lampsanae (SCHULTZ.) FUCK. – On *Lapsana communis*, very frequent, e.g. Szarvas-Erzsébetliget, Arboretum, etc. 6. 28. 1947., 7. 3. 1948. (aecidiospores: 17.5–22.4 × 12.5–16.5 micron, uredospores: 15–20 × 15–16.5 micron, teleutospores: 27.5–31.8 × 20–25 micron).

Puccinia magnusiana KÖRN. – On *Phragmites vulgaris*, frequent, e.g. Szarvas, 8. 3. 1947. (uredospores: 23–32.5 × 17.5–19.2 micron, teleutospores: 33–52 × 17–25.5 micron).

Puccinia malvacearum MONT. – On *Malva neglecta*. Szarvas, 6. 2. 1947. (teleutospores: 25–68 × 17–25 micron); On *Malva pusilla*, Szarvas, 7. 17. 1949. (teleutospores: 30–70 × 16–24 micron); On *Malva silvestris*, Naszály (Vác), 5. 27. 1950. (teleutospores: 39.2–66.4 × 17.5–25.2).

Puccinia maydis BÉRENG. – On *Zea mays*, occurring everywhere in the country, e.g. Keszthely, 10. 3. 1965. Júliamajor, 10. 4. 1966. (with uredo- and teleutosori).

Puccinia menthae PERS. – On *Mentha aquatica*, Szarvas-Bikazug, 8. 23. 1948. (teleutospores: 26–34 × 20–25 micron); On *Mentha viridis*, Szarvas, 8.23. 1948. (uredospores: 25–30 × 17.5–20 micron, teleutospores: 27–33 × 20–24 micron, hyperparasited by *Darluca filum*); On *Satureia vulgaris*, Budapest, Hűvösvölgy, 8. 21. 1951. (with uredosori).

Puccinia perplexans PLOWR. – On *Alopecurus pratensis*, Szarvas, 9. 15. 1948. (uredospores: 22–30 × 15.5–22.5 micron, teleutospores: 35–55.2 × 15.5–23.5 micron).

Puccinia phlei-pratensis ERIKSS. et HENN. – On *Phleum pratense*, Budapest, Rózsadomb, 6. 20. 1961. (leg.: J. PODHRADSZKY), (uredospores: 18–27 × 16–19 micron).

Puccinia phragmitis (SCHUM.) KOERN. – On *Rumex crispus*, common, e.g. Szarvas, 5. 2. 1948., 5. 25. 1948., etc. (aecidiospores: 18–25 × 15–19 micron); On *Rumex patientia*, Szarvas-Erzsébetliget, 4. 10. 1947. (aecidiospores: 19–25.5 × × 16–20 micron); On *Phragmites communis*, frequent, e.g. Szarvas, 4. 10. 1948., Hortobágy, 9. 3. 1948. (uredospores: 25–34 × 20–22 micron,

teleutospores: 50–75 × 21.2–23.5 micron, teleutospore-stalks: 50–92.5 micron).

Puccinia picridis HAZSL. – On *Picris hieracioides*, Szarvas-Arboretum, (uredospores: 25–30 × 22.5 micron, teleutospores: 30–35 × 22.5–25 micron).

Puccinia poarum NIELSEN – Aecidia are common on *Tussilago farfara*, all over the country, e.g. Zebegény, 8. 21. 1950. (aecidiospores: 18–27 × 16–20 micron);

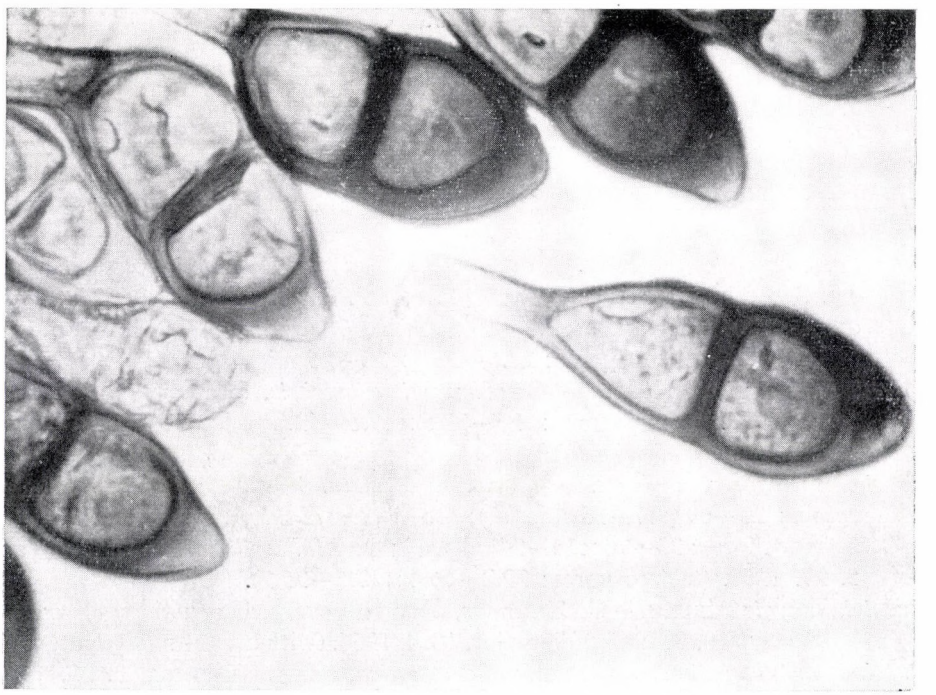

Fig. 4. Teleutospores of *Puccinia pygmaea* ERIKSS. (1000×)

On *Poa nemoralis*, Szarvas-Erzsébetliget, 8. 21. 1948. (uredospores: 17.5–25 × 17.5–20 micron, teleutospores: 25–27.5 × 25 micron, paraphysis: 60–75 × 12.5 micron).

Puccinia podospermi DC. – On *Scorzonera cana*, Szarvas, 4. 17. 1947. (aecidiospores: 19–24.6 × 19–20 micron).

Puccinia polygoni ALB. et SCHW. – On *Polygonum convolvulus*, Szarvas-Bikazug, 6. 3. 1947. (uredospores: 20–28 × 15–20 micron).

Puccinia polygoni-amphibii PERS. – On *Polygonum amphibium*, Szarvas, 9. 8. 1947. (uredospores: 20–25 × 15–20 micron, teleutospores: 40–50 × 17.5–20 micron).

Puccinia porri (SOW.) WINTER – On **Allium porrum*, Vácrátót, 5. 13. 1951. (uredospores: 23.5–32 × 19.8–27 micron); Csopak, 5. 28. 1964. (aecidiospo-

res 19–30.5 × 18.5–22.5 micron); On **Allium scorodoprasum*, Martonvásár, 5. 20. 1960. (leg.: J. PODHRADSZKY), (with aecidia).

Puccinia praecox BUBÁK – On *Crepis biennis*, Turkeve-Templomzug, 4. 28. 1950. (aecidiospores: 17–34 × 17–22 micron).

Puccinia prenanthis-purpureae (DC.) LINDR. – On *Prenanthes purpurea*, Lillafüred, 8. 22. 1951. (uredospores: 18–21.2 micron, teleutospores: 32–36.5 ×

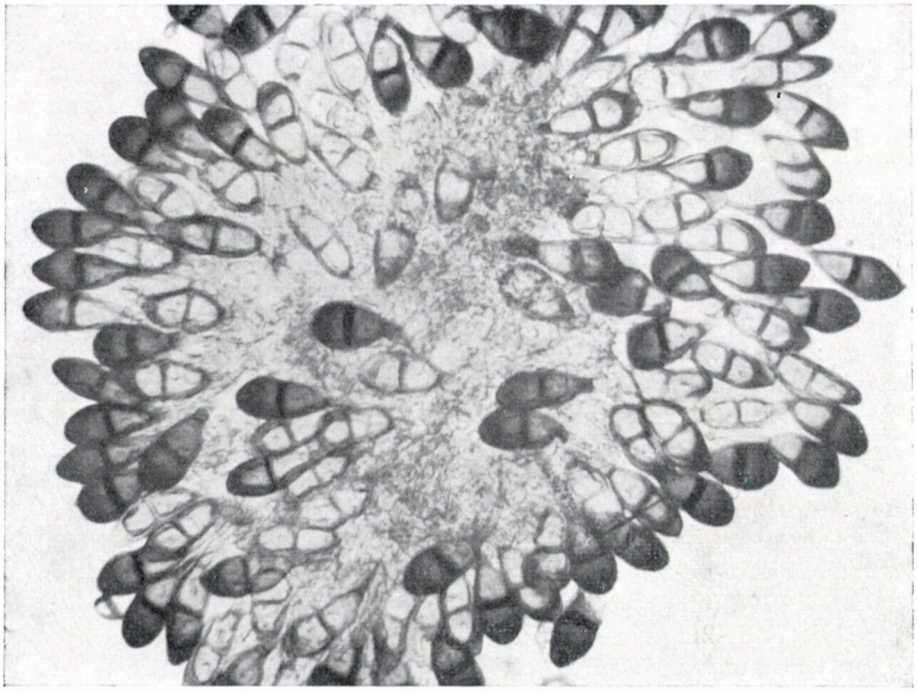

Fig. 5. Teleutosorus of *Puccinia pygmaea* ERIKSS. (250×)

× 19.7–23.3 micron, in company with the perithecia of *Erysiphe cichoracearum* DC.).

Puccinia pygmaea ERIKSS. – On *Calamagrostis epigeios*, Tatárszentgyörgy, 8. 27. 1950. (teleutospores: 34.4–46–51.6 × 14.2–18.9 micron). (Figures 4, 5.)

Puccinia silvatica SCHROETER – On *Taraxacum officinale*, Szarvas, 4. 28. 1947. (aecidiospores: 12–16 × 11–14 micron); Halásztelek, 4. 19. 1948. (with aecidia); On *Carex stenophylla*, Halásztelek, 9. 21. 1947. (uredospores: 20–27.5 × 15–20 micron).

Puccinia simplex (KOERN.) ERIKSS. et HENN. – On *Hordeum distichon*, found all over the country, e.g. Mór, 6. 15. 1950. (with uredo- and teleutosori).

Puccinia suaveolens (PERS.) ROSTR. – Very common on *Cirsium arvense*, e .g. Szarvas, 5. 7. 1949., Budapest, Rózsadomb, 4. 21. 1951., etc.

Puccinia symphyti-bromorum F. MÜLLER – On *Bromus mollis*, Nagykovácsi, 7. 19. 1954. (with uredo- and teleutosori); Balatonakarattya, 6. 9. 1966. (uredospores: 19.5–28.8 × 18–24 micron); On *Bromus sterilis*, Szarvas, 7. 3. 1948. (uredospores: 25–26.2 × 20–22.5 micron, teleutospores: 43.7–55 × 15–20 micron); it was epidemic in Balatonakarattya, 5. 5. 1966. (with uredo- and teleutosori).

Puccinia tanaceti DC. – On *Chrysanthemum vulgare*, Bp. Jánoshegy, 9.24. 1950 (with uredo- and teleutosori); Szarvas-Halásztelek, 9. 3. 1947. uredospores: 23–25 × 15–20 micron; teleutospores: 35–50 × 17.5–20 micron.

Puccinia taraxaci (REBENT.) PLOWR. – On *Taraxacum officinale*, very common, e.g. Szarvas, 8. 5. 1947. (uredospores: 22.5–27.5–(32) × 20–25 micron, teleutospores: 24.1–41.2 × 15–23.5 micron).

Puccinia thesii (DESV.) CHAILLET. – On *Thesium intermedium*, Budapest, Árpádhegy, 6. 20. 1965. (uredospores: 22–30 × 25–27.5 micron).

Puccinia tinctoriicola P. MAGNUS. – On *Serratula tinctoria*, Pomáz, Kőhegy, 9. 6. 1953. (teleutospores: 29–44 × 23–29.5 micron).

Puccinia vincae (DC.) BERK. – On *Vinca herbacea*, in large numbers, Budapest, Árpádhegy, 4. 22. 1966. (uredospores: 25–31 × 20–22.5 micron, teleutospores; 34–50 × 19–26 micron).

Puccinia violae (SCHUM.) DC. On **Viola kitaibeliana*, Szarvas, Halásztelek, 4. 19. 1948. (aecidiospores: 25–26 × 18 micron); On *Viola odorata*, Szarvas, Arboretum, 4. 11. 1947. (aecidiospores: 15–25.5 × 11–18.5micron); Szarvas–Erzsébetliget, 6. 20. 1948. (uredospores: 20–30 × 17.5–22.5 micr.)

Tranzs. discolor (Fuck.) Tranzsch. et Litv. on *Pr. domestica*, Szarvas, 9. 18. 1947. teleutosp. 25–40 × 16–20 micr.

Tranzschelia pruni-spinosae (PERS.) DIETEL (1922) – On *Prunus domestica*, Szarvas, 9. 18. 1947. (uredospores: 25–32.5 × 15–17.5 micron, teleutospores: 40–42.5 × 17.5–21.2 micron, paraphysis: 25–55 × 8.7–11.2 micron, hyperparasited by *Darluca filum)*; On *Prunus spinosa*, Szarvas, 8. 18. 1948 (uredospores 22–28 × 16–18 micron, in company with *Polystigma rubrum* (PERS.)DC.). On *Prunus armeniaca*, Lengyeltóti, 9. 28. 1959. (uredo- + teleutosori).

Triphragmium filipendulae (LASCH.) PASS. – On *Filipendula hexapetala*, Gyenesdiás, 6. 22. 1952. (uredospores: 21–33.5 × 14.5–20.5 micron).

Triphragmium ulmariae (SCHUM.) LINK – On *Filipendula ulmaria*, Nagykanizsa, 6. 22. 1951. (uredospores: 25.5–32.5+18–23.5 micron, teleutospores: 36.5–48 × 25–45.5 micron).

Uromyces acetosae SCHROET. – On *Rumex acetosa*, Szarvas, 6.3 1948. (with uredosori); On *Rumex acetosella*, Debrecen, Nagyerdő, 7. 12. 1948. (with uredo- and teleutosori).

Uromyces ambiguus (DC.) LÉV. – On *Allium scorodoprasum*, Martonvásár, 5. 20. 1960. (leg.: J. PODHRADSZKY), uredospores: 20–27 × 17.5–22.5 micron).

Uromyces anthyllidis (GERV.) SCHROETER – On *Anthyllis polyphylla*, Budapest, Árpádhegy, 6. 25. 1965. (with uredosori).

***Uromyces coronillae-variae* VIENNOT–BOURGIN (1950) – On *Coronilla varia*, Budapest, Zugliget, 6. 12. 1951. (uredospores: 23–27 × 21–23.5 micron).

Uromyces bäumlerianus BUBÁK – On *Melilotus officinalis*, Szarvas, Bikazug, 9. 23. 1948. (uredospores: 19.5–30 × 17–22 micron, teleutospores: 20–25 × × 17.5–21 micron).

Uromyces behenis (DC.) UNGER – On *Silene vulgaris*, Visegrád, Várhegy, 6. 8. 1950. (aecidiospores: 16–22.5 micron).

Uromyces caryophyllinus (SCHRANK) WINTER – On **Dianthus serotinus*, Ágasegyháza, 10. 2. 1958. (teleutospores: 22.5–29 × 17.5–22.5 micron).

Uromyces dactylidis OTTH – On *Ranunculus polyanthemos*, Vizesfás, 4. 21. 1950. (aecidiospores: 18–25 × 15.5–20 micron); On *Dactylis glomerata*, Keszthely, 6. 22. 1950. (teleutospores: 17.5–28.8 × 14–17.5 micron).

Uromyces euphorbiae-astragali (OPIZ) E. JORDI. – On *Astragalus cicer*, Iregszemcse, 6. 16. 1950. (uredospores: 16–24.5 × 15–21.2 micron); On *Astragalus glyciphyllos*, Putnok, 7. 11. 1948. (with uredosori); Leányfalu, 7. 26. 1949. (with uredo- and teleutosori); Pusztavacs, 8. 27. 1950. (teleutospores: 16.8–19–21.6 × 14.4–16.8 micron).

Uromyces viciae-fabae (PERS.) JÖRSTAND. – On *Lathyrus niger*, Pogányszentpéter, 6. 21. 1950. (with uredosori); Nagykovácsi, 8. 6. 1965. (uredospores: 22–30 × 17.5–25.2 micron); On *Vicia sepium*, Nagykovácsi, 6. 26. 1950. (with uredo- and teleutosori).

Uromyces ficariae (SCHUM.) LÉV. – On *Ranunculus ficaria*, Budapest, Zugliget, 4. 21. 1951. (teleutospores: 22–40 × 18–27.5 micron).

Uromyces genistae-tinctoriae (PERS.) WINTER – On *Laburnum anagyroides*, Szarvas-Arboretum, 9. 26. 1948. (uredospores: 17.5–22.5 × 17.5–20 micron).

Uromyces geranii (DC.) OTTH et WARTM. – On *Geranium phaeum*, Pogányszentpéter, 6. 22. 1950. (aecidiospores: 21–30.2 × 17.5–23.5 micron); On *Geranium pratense*, Nagykovácsi, 4. 20. 1966. (aecidiospores: 23–30 × 23–27.5 micron); On *Geranium pusillum*, Szarvas-Halásztelek, 4. 29. 1950. (aecidiospores: 23.1–29–39 × 28.5 micron, uredospores: 22.2–29.8 × 15–19 micron).

Uromyces laevis KÖRN. – On *Euphorbia gerardiana*, Kecskemét, Ágasegyháza, 7. 23. 1953. (teleutospores: 21.2–27.5 × 17.5–24 micron).

Uromyces lilii (LK.) FUCK. – On *Fritillaria meleagris*, Tarpa, 3. 21. 1951. (leg.: S. JÁVORKA), (with aecidia).

Uromyces limonii (DC.) LÉV. – On *Statice Gmelini*, frequent, e. g. Gyula-Galbácskert, 10. 6. 1943., Szarvas-Halásztelek, 9. 3. 1947., 8. 18. 1948., Mezőgyán, 9. 11. 1948. (uredospores: 26–30.5 × 18.6–26.8 micron, teleutospores: 28–52 × 16–25 micron).

***Uromyces lathyri-latifolii* GUYOT (1938) – On *Lathyrus megalanthus* (=*L. latifolius*), Nagyszénás (Nagykovácsi), 9. 29. 1953. (urodespores: 25.2–33.6 × 22.1–25.2 micron).

Uromyces scirpi (CAST.) BURR. – On *Bolboschoenus maritimus*, frequent, e.g. Szarvas-Bikazug. 8. 28. 1959. (with uredosori); Szarvas-Kopáncs, 8. 30. 1961. (with uredo- and teleutosori); Hódmezővásárhely, 9. 23. 1959. (with uredosori); Hortobágy, 8. 21. 1959. (with uredosori); (uredospores: Szarvas-Koppáncs – 21–32.5 × 17.5–21 micron, teleutospores: 27.2–48.2 × 17–22 micron); On *Daucus carota*, Szarvas-Bikazug, 5. 3. 1948. (aecidiospores: 17.5–23.5 × 15–21.5 micron).

***Uromyces lupinicolus* BUBÁK (1902) – On *Lupinus hirsutus*, Kisvárda, 6. 16. 1951. (uredospores: 17.5–23.5 × 16–18.6 micron).

Uromyces ononidis PASS. – On *Ononis spinosa*, Szarvas-Bikazug, 9. 25. 1947. (uredospores: 19.5–27.5 × 15–22 micron, teleutospores: 20–25 × 17.5–22.5 micron).

Uromyces phaseoli (PERS.) WINTER – On *Phaseolus vulgaris*, frequent all over the country, e.g. Debrecen, 8. 12. 1948. (with uredosori).

Uromyces pisi (PERS.) SCHROETER – On *Euphorbia cyparissias*, everywhere common; On *Euphorbia virgata*, Szarvas-Halásztelek, 4. 19. 1948. (with aecidia); On **Lathyrus latifolius*, Nagyszénás, 9. 29. 1953. (teleutospores: 22–28 × 17–22 micron); On **Lathyrus nissolia*, Sarkad, 9. 12. 1948. (uredospores: 21–25.5 × 18–21 micron, teleutospores: 20–30 × 15–22 micron); On *Lathyrus tuberosus*, Tiszasüly, 6. 18. 1966. (with uredo- and teleutosori), On *Pisum sativum*. frequent all over the country, e.g. Füged, 6.16. 1950. (uredospores: 21–26 × 18–21 micron, teleutospores: 21–30 × 16–22 micron) (Figure 6).

Uromyces poae RABH. – On *Ranunculus ficaria*, Szarvas, 6. 16. 1948. (aecidiospores: 17–24 × 10–19.5 micron); On **Poa nemoralis*, Szarvas-Erzsébetliget, 8. 28. 1947. (uredospores: 17.5–22.5 × 13–18 micron, teleutospores: 18.2–31.5 × 14–20 micron).

Uromyces polygoni (PERS.) FUCKEL – On *Polygonum aviculare*, Szarvas, 6. 10. 1948. (teleutospores: 22 × 17 micron); Mezőhegyes, 6. 15. 1951. (with uredo- and teleutosori); On **Polygonum lapathifolium*, Szarvas, 9. 21. 1947. (teleutospores: 18–30 × 15–20 micron).

Uromyces rumicis (SCHUM.) WINTER – On *Rumex crispus*, Szarvas-Erzsébetliget, 8. 27. 1948. (uredospores: 20–27 × 17.5–22.5 micron, teleutospores: 22.5–32.5 × 22.5 micron); On *Rumex limosus*, Nyiregyháza, 8. 16. 1948. (with uredosori); On **Rumex stenophyllus*, Szarvas-Bikazug, 8. 23. 1948, Mezőtúr, 8. 25. 1948. (uredospores: 20–28 × 17–22 micron, teleutospores: 25–35.2 × 22.5 micron, in company with the perithecia of *Erysiphe polygoni* DC.).

Uromyces scillarum (GREV.) WINTER – On *Muscari comosum*, Vác-Naszály mount, 5. 26. 1950. (teleutospores: 21–25.2 × 15.9–17.6 micron, in company with *Ustilago vaillantii* TUL); Pomáz-Kőhegy, 4. 28. 1953. (teleutospores: 18.5–26.3 × 15–21.2 micron).

Uromyces striatus SCHROETER – On *Medicago sativa*, Martonvásár, 6. 11. 1953. (uredospores: 17.5–21 × 17.5–20 micron).

Uromyces sublevis TRANZSCHEL – On *Euphorbia pannonica*, in large numbers, Nagykovácsi, Nagyszénás, 6. 22. 1953. (teleutospores: 22–30. 5 × 20–25 micron).

Uromyces trifolii (HEDW. f.) LÉV. – On *Trifolium fragiferum*, Szarvas, 9. 21. 1947. (uredospores: 20–25 × 17–23 micron, teleutospores: 21–28 × 17.5–23.5 micron, in company with the pycnidia of *Polythrincium trifolii* KZE. et SCHM.).

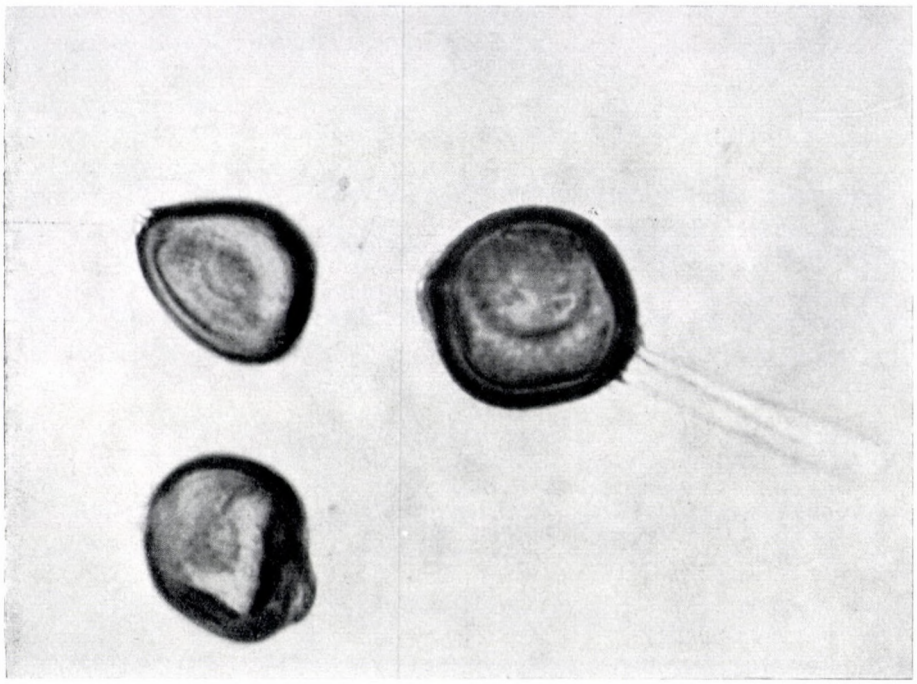

Fig. 6. Teleutospores of *Uromyces pisi* (PERS.) SCHROETER. (1000×)

Uromyces trifolii-repentis (CAST.) LIRO – On *Trifolium repens*, Szarvas, 9. 27. 1947. (uredospores: 20–25.5 × 19–20 micron, teleutospores: 23.7–27.5 × 20–22.5 micron).

Uromyces veratri (DC.) SCHROETER – On *Adenostyles alliariae*, Magas Tátra, Ótátrafüred (Stary Smokovec), Tarpatak, 8. 28. 1962. (aecidiospores: 18–23.5 micron).

Uromyces viciae-craccae CONSTANTINEAU – On *Lens culinaris*, Fürged, 6. 16. 1950. (uredospores: 19.2–23.5 × 78–22 micron).

Cummunsiella sanguinea (PECK.) ARTH. – On *Mahonia aquifolium*, on *Mahoberberis ilicifolia* and on *Berberis vulgaris*, common all over the country, e.g. Szarvas-Arboretum, 9. 29. 1948., 5. 6. 1948., etc. (aecidiospores: 17.5–22. 5 × 15.3–20 micron, uredospores: 25–40 × 16.2–20 micron, teleutospores: 30–32.5 × 20–22.5 micron).

2. *Melampsoraceae.*

Cronartium flaccidum (A. et S.) WINTER – On *Cynanchum vincetoxicum*, Balatongyörök, Szentmihályhegy, 5. 25. 1954. (uredospores: 19–27.5 × 16–21 micron, teleutospores: 28–56 × 12–16 micron).

Endophyllum euphorbiae-silvaticae (DC.) WINTER – On *Euphorbia amygdaloides*, Nagykevély, 5. 15. 1955. (aecidiospores: 30–34.5 × 19–25 micron).

Endophyllum sempervivi (ALB. et SCHW.) DE BARY – On **Sempervivum hirtum*, Nagykovácsi, Nagyszénás, 5. 12. 1953. (aecidiospores: 26–31.5 × 22–27.5 micron).

Melampsora euphorbiae-dulcis OTTH – On *Euphorbia virgata*, Szarvas, 9. 9. 1948. (uredospores: 17.5–23.7 × 15–20 micron, paraphysis: 57.5–62.5 × 16.3–17.5 micron); Sarkad, 8. 31. 1948.; Öcsöd, 9. 23. 1948. (with uredosori, in company with the perithecia of *Sphaerotheca euphorbiae* (CAST./SALM.).

Melampsora euphorbiae (Schub.) Cast. f. sp. *euphorbiae-exiguae* W. MÜLLER. – On *Euphorbia exigua*, Nagykovácsi, 10. 12. 1955. (with teleutosori).

Melampsora larici-populina KLEB. – On *Populus canadensis*, frequent, e.g. Szarvas, 9. 28. 1947. (uredospores: 25–32.5–35 × 17–20 micron); On *Populus italica*, Szarvas-Erzsébetliget, 9. 25. 1947. (with uredo- and teleutosori).

Melampsora larici-tremulae KLEB. – On *Populus alba*, frequent, e.g. Szarvas-Arboretum, 8. 21 1947., 10. 2. 1947., 4. 29. 1948., etc. (uredospores: 20–23.5 × 18–19 micron).

Melampsora lini (PERS.) DESM. – On *Linum usitatissimum*, Martonvásár, 4. 16. 1951. (uredospores: 16–25.2 × 14–18.8 micron).

***Melampsora ribesii-viminalis* KLEBAHN – On *Salix viminalis*, Szarvas, 9. 21. 1947. (uredospores: 15–17.5 × 12.5–15 micron).

Melampsora betulinum (PRES.) KLEB. – On *Betula pendula*, in large numbers, Sopron, Fáberrét, 9. 22. 1965. (uredospores: 25–38 × 10–16 micron).

Pucciniastrum circaeae (SCHUM.) SPEG. – On *Circaea lutetiana*, Keszthely, 8. 12. 1951. (uredospores: 20.2–23.1 × 12–13 micron). Szentgál (uredo+teleuto), 9. 27. 1966.

Coleosporium campanulae (PERS.) LÉV. – On *Campanula rapunculoides*, Erdőbénye, 8. 8. 1948. (uredospores: 22–34 × 13–23 micron, teleutospores: 62–98 × 18–27 micron); Csesznek, 6. 26. 1950. (with uredo- and teleutosori, in company with *Placosphaeria campanulae* (DC.) BÄUMLER); On *Campanula trachelium*, Szarvas-Arboretum, 6. 28. 1947. (uredospores: 19.5–27 × 18–18.6 micron).

Coleosporium euphrasiae (SCHUM.) WINTER – On *Euphrasia stricta*, Nagykanizsa, 6. 21. 1950. (with uredosori); On *Odontites rubra*, Nagykovácsi, Nagyszénás, 8. 2. 1959. (uredospores: 18–27 × 14–18 micron, teleutospores: 70–85 × 18–24 micron).

Coleosporium melampyri (REBENT.) KLEB. – On *Melampyrum arvense*, Nagyvázsony, 6. 25. 1950. (with uredosori); Lillafüred, 8. 30. 1959. (uredospores:

19–28 × 14–23 micron); On *Melampyrum nemorosum*, Budapest, Hűvösvölgy, 7. 12. 1950. (uredospores: 22–30 × 16–21 micron); On *Melampyrum pratense*, Mátraháza, 9. 5. 1959. (leg: J. PODHRADSZKY), (with uredo- and teleutosori), Kékestető, 10. 2. 1952. (uredospores: 18–28 × 12–20 micron, teleutospores: 85–106 × 16–27 micron); On **Melampyrum barbatum*, Nagyvázsony, 6. 25. 1950. (uredospores: 22–27.5 × 16–24 micron).

Coleosporium petasitis (DC.) FISCHER – On *Petasites hybridus*, Szarvas-Arboretum, 10. 2. 1947. (with uredo- and teleutosori); Mátraháza, 10. 3. 1952. (uredospores: 23.2–37.2–46.5 × 27.8–37.2 micron, teleutospores: 79–98 × 20–26 micron, teleutospore-stalks: 74.4–93 micron).

Aecidium doronici RBH. – On *Doronicum hungaricum*, Pomáz, Kőhegy, 6. 3. 1954. (aecidiospores: 18–20 × 16–18.5 micron, peridium cells: 26–33 × 18–24 micron).

Book Review

A. GULYÁS: *A dohány betegségei és kártevői* (Krankheiten und Schädlinge des Tabaks). Mezőgazdasági Könyvkiadó, Budapest, 1965. p. 245.

Der Doyen der ungarischen phytopathologischen Wissenschaft, der 82 Jahre alte Professor Dr. Antal Gulyás fasste am Vorabend seines Lebens seine sich auf mehrere Jahrzehnte erstreckenden Forschungen betreffs der phytopathologischen und Pflanzenschutzprobleme die er in jugendlichem Alter begann, in einem wertvollen und beachtenswerten Werk zusammen. Er war der erste, der sich monographisch mit den in Ungarn verbreiteten Viruskrankheiten des Tabaks befasste (1936), während er die Tabak-Bakteriosen schon im Jahre 1927, die verschiedenen Mykosen im Jahre 1930 besprach. Er veröffentlichte auch mehrere Bücher kleineren Umfangs über die Treibbettkrankheiten des Tabaks (1951) sowie die nicht infektiösen sog. physiologischen Krankheitserscheinungen (1936). Er besass alle Gegebenheiten dazu, um das reiche Erfahrungsmaterial eines in wissenschaftlicher Forschung und Praxis verbrachten arbeitsreichen Lebens in einem zusammenfassenden Werk zu veröffentlichen. So entstand dieses Buch von 245 Seiten mit 32 farbigen Tafeln, 78 photographischen Abbildungen und Zeichnungen. Das sorgfältig ausgewählte und zusammengestellte Bildermaterial ergänzt vortrefflich den Text und macht die leichte und sichere Erkenntnis der Symptome auch für Anfänger möglich.

Das Werk gibt in der Einführung eine Übersicht über die die Erkrankungen des Tabaks auslösenden physiologischen und pathologischen Ursachen und klassifiziert symptomatologisch die wichtigsten Krankheiten. Die unter Einwirkung der meteorologischen Faktoren auftretenden pathologischen Erscheinungen sowie die Wirkung der ungünstigen Bodenverhältnisse auf die Tabakpflanze werden eingehend erörtert. Die in der Literatur enthaltenen Angaben über die zahlreichen Viruskrankheiten des Tabaks sowie die wertvollen Feststellungen des Verfassers und anderer einheimischen Forscher sind in einem besonderen Kapitel besprochen und bewertet. Auch den bakteriellen Krankheiten sowie Mykosen des Tabaks werden separate Kapitel gewidmet. Die an der Tabakpflanze parasitierenden Blüten-Epiphyten, besonders der Tabak-Sommerwurz werden ausführlich dargestellt.

Die tierischen Schädlinge des Tabaks sind nach den Körperregionen der Pflanze erörtert und ihre Schäden gewertet. Ein besonderes Kapitel ist der Problematik der Krankheiten und Schädlinge, im allgemeinen den Lagerschäden gewidmet. Der Wegweiser über die allgemeinen Regeln des Pflanzenschutzes sowie die besonderen Bekämpfungsmassnahmen bieten eine wertvolle Hilfe vor allem den mit dem Tabakbau beschäftigten Fachleuten der Praxis. Das ausführliche Literaturverzeichnis am Ende des Bandes ermöglicht die weitere Orientierung und umfasst die wichtigste in- und ausländische Fachliteratur unter mehreren Hunderten von Stichworten und in der folgenden fachtechnischen Einteilung: 1. Physiologische Krankheiten, 2. Virosen, 3. Bakterielle Erkrankungen, 4. Pilzkrankheiten, 5. Insektenschädlinge und 6. Allgemeiner Pflanzenschutz.

Das in schöner Ausstattung erschienene nützliche Werk von A. Gulyás befriedigt wichtige Ansprüche und mag nicht nur die wissenschaftlichen Forscher, sondern auch die breiten Kreise der Praxis weitgehend zu interessieren. Die wertvolle Monographie ist die erste seit dem Erscheinen des Werkes von A. Juhász—Gy. Kadocsa »A dohány állati kártevői« (Die tierischen Schädlinge des Tabaks), die die Pflanzenschutzprobleme des berühmten und auf eine grosse Vergangenheit zurückblickenden ungarischen Tabakbaus in ihrer Gänze umfasst.

G. UBRIZSY

Report on the XVI. Scientific Conference on Plant Protection, Budapest

The XVI. session of the Scientific Conference on Plant Protection which became already traditional, was held from February 22 to 25, 1966 in the House of Technique. It was organized by the Hungarian Association of Agricultural Sciences and the Research Institute of Plant Protection. Scientists both from Hungary and from abroad showed considerable interest in the Conference. In the plenary sessions mainly the present worries and basic problems of plant protection in Hungary were discussed, among others the methods and significance of an up-to-date prognosis of pests and diseases in practical plant protection. The second topic which was the subject of intensive disputes was integral plant protection, a way out of the increased difficulties of the harmful side- and after effects of plant protection and pesticides, the increasing importance of biological protection and of biotherapy generally (autocidal, e.g. male sterilization methods, use of attractants, repellents, utilization of entomopathogenic factors etc.) in addition to the one-sided chemotherapy. Similar urgent questions were discussed in a whole-day debate on the mode of action and toxicology of pesticides, on residues and on carency periods, topics, about which a number of lectures were held by foreign participants.

On the scientific session 92 papers were presented by 20 foreign workers (Great Britain, Austria, Egypt, German Democratic Republic, German Federal Republic, Italy, Switzerland, USA) and by 87 Hungarian specialists. The distribution of the lectures according to topics was as follows: 32 papers on phytopathological problems, 22 on entomological ones, 14 connected with herbicide research, 7 on the chemical aspects of plant protection and 17 general lectures. At the special phytopathological sessions papers were presented on plant virology (e.g. stolbur virus, virus diseases of fruit-trees, virus diseases of maize etc.), on mycology and pathophysiology-biochemistry) e.g. the role of cytokinins in pathophysiological processes, effect of benzyladenine on virus infected plants, nitropyrasol derivatives as systemic fungicides etc.), phytobacteriology (hypersensitive reaction induced by avirulent bacteria), and on antibiotics that can by used in phytotherapy (e.g. the use of cycloheximide in Hungary). The entomological papers were related to insect-ethological, etiological, gradological and methodical questions of protection. In the section for chemical weed control results obtained with new herbicides and modes of action were dealt with (e.g. the binding of triazin derivatives by the organic and inorganic colloids of the soil, greenhouse model experiments with herbicides, soil sterilization combined with the use of herbicides etc.). In the section dealing with the chemical aspects of plant protection papers were presented on new dithiocarbamate derivatives, aminochlorotriazine and triasol derivatives, mode of action of the isomers of new systemic insecticides and their breakdown in plants.

Summarizing the significance of the XVI. scientific conference on plant protection in Budapest it may be stated that it covered the most actual problems of basic research in plant protection, methods of research work and plant protection and that in a number of lectures and contributions valuable new results were presented. The intensive discussion of integral plant protection, and of the toxicological, hygienic and nutrition hygienic problems indicated the proper direction in the endeavour to supplant off plant protection in Hungary within a possibly short period the chlorinated hydrocarbons of persistent and cumulative effect and to adopt, in the place of these, pesticides of slight chronical toxicity readily metabolizing and disturbing the agrobiocenose (ecosystem) to a lesser extent. In the complex and harmonic systems of protection on the other hand the autocidal processes especially male sterilizing gain increased importance.

The material of the scientific meeting was published with the rotaprint process in two volumes and is available for all who are interested in the Hungarian Association of Agricultural Sciences and in the Research Institute of Plant Protection (Budapest, II. Herman Ottó út 15.).

G. Ubrizsy

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