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A Simple Semisynthetic Diet for Rearing of the Fall Webworm, *Hyphantria cunea* Drury

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The performance of two semisynthetic diets was compared for laboratory rearing of *Hyphantria cunea* Drury. The better one contained 1200 ml tap water, 200 g wheat germ, 40 g saccharose, 40 g Brewer's yeast, 20 g agar, 4 g Nipagin M (dissolved in approx. 10 ml of 70% ethanol), 4 g ascorbic acid, and 5 ml glacial acetic acid. This diet gave significantly better results than the other one in respect of developmental time (L_1 to pupa, and L_1 to adult), pupal weights, and mortality (L_1 to pupa, and L_1 to adult). Pupal weights of pupae reared on plum leaves were also lower than those on the diet, but here significant difference was found only in females. Using this diet an effective method for laboratory rearing of the insect has been developed.

As a result of its pest status, the fall webworm (*Hyphantria cunea* Drury) is an important experimental insect at our laboratories. As its rearing on natural host plant is very laborious and the insect material obtained usually is not very homogeneous, the necessity of the development of a medium for laboratory rearing of the species has been raised.

Table 1

Composition of semisynthetic diets used for rearing of *Hyphantria cunea* Drury

Ingredients	Diet 1	Diet 2
Tapwater	1200 ml	1200 ml
Wheat germ	200 g	200 g
Saccharose	40 g	40 g
Brewer's yeast	40 g	40 g
Agar	20 g	20 g
Nipagin M	4 g	4 g
Ascorbic acid	4 g	4 g
Glacial acetic acid	5 g	5 g
Wesson's salt mixture	—	1 g
Vitamine mixture *(Polyvitaplex 8)	—	2 pills (crushed)

* Composition of vitamine mixture (per one pill): canocobalmimum 1 μg, ergocalciferolum 1000 IU, axerophtholum 3000 IU, pyridoxinum hydrochloricum 0.5 mg, riboflavinum 0.5 mg, thiaminum hydrochloricum 5 mg, nicotinamidum 10 mg, acidum ascorbicum 50 mg, calcium hypophosphorosum 50 mg

As first trials to apply Nagy's (1970) diet, which had proved to be suitable for the rearing of numerous noctuid and other species (Szőcs and Tóth, 1982, Tóth, 1981, Tóth and Szőcs, 1980), were not successful on this species, it was tried to slightly change the composition of this diet so that it be acceptable to *H. cunea*. In the present paper the development of a simple semisynthetic diet and an effective method for the laboratory rearing of *H. cunea* is reported.

Materials and Methods

Diet

Composition of diets tried out is shown in Table 1. For the preparation of the diets agar was dissolved in hot water, then Brewer's yeast was added and the mixture was cooked for 15 min. Heating then was turned off and the other ingredients were mixed in. After having allowed the blend to cool down to 60 °C, Nipagine M (in an approx. 10 ml solution of 70% ethanol), ascorbic acid, vitamine mixture and glacial acetic acid was added. The diet was stored in closed glass containers at approx. 5 °C until use.

Rearing

Thin (approx. 3 to 5 mm) layers of the cooled diet were smeared onto the inner surfaces of plastic cups (8 × 7 cm). Ten freshly hatched larvae were introduced in a cup (one repetition), the orifice of the cup was covered by linen cloth and the cup was positioned upside down (with the orifice facing downwards). So the larvae could feed on the surface of the diet smeared on the walls, and their excrement fell down without contaminating the diet. When the larvae had grown to L_4, they were transferred to one 1 glass jars. Here the diet was added in small cubes (1 × 2 cm). New cubes were introduced into the jars each week. The larvae wove their pupation chambers on the walls, or preferably where walls of the container and the linen cloth met. Pupation was monitored daily. Pupae were collected, sexed and weighed. They were kept after weighing in Petri dishes between wet papers wadding until emergence. Emergence of adults was also monitored daily.

Experimental insects originated from eggs of moths, the larvae of which were collected (at an approximate age of L_4) from plum trees in the field. These larvae were fed by plum leaves in the laboratory until pupation. The pupae were sexed and weighed, and these pupal weights were used as a basis of comparison with weights of pupae reared on the diets. Naturally, comparison of developmental times and mortality rates could not be performed.

Table 2

Results of rearing of *Hyphantria cunea* Drury on two semisynthetic diets and plum leaves

Rearing substrate	Developmental time (days, $\bar{X} \pm S\bar{X}$)		Pupal weight (mg $\bar{X} \pm S\bar{X}$)		Mortality (%, $\bar{X} \pm S\bar{X}$)	
	L_1 to pupa	L_1 to adult	males	females	L_1 to pupa	L_1 to adult
Diet 1	28.7 ± 0.5 a n = 39	38.1 ± 0.3 a n = 41	121.3 ± 3.3 a n = 22	202.9 ± 6.8 a n = 17	20.0 ± 2.6 a r = 6	30.0 ± 5.8 a r = 6
Diet 2	35.0 ± 0.4 b n = 29	42.8 ± 0.4 b n = 25	106.6 ± 3.9 b n = 16	152.9 ± 6.7 b n = 13	51.4 ± 13.4 b r = 5	57.6 ± 15.3 b r = 5
Plum leaves	—	—	116.7 ± 2.5 ab n = 24	156.4 ± 3.6 b n = 24	—	—

Averages followed by the same letter within one column are not significantly different at P = 1% for pupal weights, P = 0.1% for developmental time, and P = 5% for mortality (*t* test for developmental time and mortality, Duncan's NMRT for pupal weights).

n = number of individuals measured.

r = number of repetitions. In one repetition ten insects were reared.

Results and Discussion

Developmental time

Developmental time from freshly hatched larva to pupa was by about six days shorter on diet 1 than on diet 2 (Table 2). Total development of insects (from freshly hatched larva to adult) was also shorter on diet 1, by about five days. Both differences were found to be significant.

Pupal weights

Heaviest male pupae originated from the group reared on diet 1 (Table 2). Their average weight significantly differed from that of the group reared on diet 2, but not from the larvae reared on plum leaves. The pupal weights did not differ significantly in the groups reared on diet 2 and on plum leaves.

In females outstandingly high average weight was measured in the group reared on diet 1 (Table 2). Averages in groups reared on diet 2 and on plum leaves did not differ from each other, but did differ from that of the group reared on diet 1.

Mortality

Mortality rates were lower in the insects reared on diet 1 (Table 2). In both periods of L_1 to pupa and L_1 to adult mortality rates were about the double of those in the group reared on diet 2. The differences were found to be significant.

There are a few papers published concerning the rearing of *H. cunea* on semisynthetic diets. However, these diets usually contain ingredients which are laborious to prepare – for example mulberry leaf lyophilizate (Kunimi and Aruga, 1974; Starets, 1973). Furthermore, the diet of Injac et al. (1974) contains 13; those of Kunimi and Aruga (1974) 17 or 18; that of Starets (1973) 10; and that of Yearian et al. (1966) 15 ingredients. In contrast, our diet 1 is composed of only eight ingredients, most of which are cheap and easy-to-get substances. Diet 1 was found to perform significantly better than diet 2 in all of the aspects studied. It yielded even heavier pupae than those originating from larvae reared on plum leaves, a natural host plant (although here the difference was significant only in females). On the basis of these findings we established an efficient method for the laboratory rearing of *H. cunea* using diet 1.

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Fungi Associated with a Scale-Insect, *Quadraspidiotus ostreaeformis* (Curtis, 1843) (*Homoptera, Coccoidea: Diaspididae*)

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Fusarium larvarum was isolated from *Quadraspidiotus ostreaeformis* colonizing on *Crataegus oxyacanthae* twigs. The fungus may be a limiting factor for the scale-insect population.

Quadraspidiotus ostreaeformis (Curtis) is a very common scale-insect species with an almost world-wide distribution. It has many different host plants and is regarded as an important pest of fruit and ornamental trees of several genera (*Prunus*, *Cerasus*, *Malus*, *Aesculus*, *Platanus*, *Syringa*, *Tilia*). The density of the species in Hungary is regulated mainly by natural enemies, especially by parasitoids and predators. Fungi may also play an important role in this respect, but we had no data about fungal pathogens until now.

During the early summer of 1982, populations of *Q. ostreaeformis* reached high levels in the western area around Budapest, Hungary. In this time scale-insect colonies on *Crataegus oxyacanthae* twigs showing unusual pinkish colour were frequently observed. Besides older, dead individuals, which were covered by fungal mycelium, young, apparently symptomless but dead females were also found; the mortality of the latters reached 50–60%, a surprisingly high level for this season. Oedematous dead bodies also occurred, these individuals may have died from bacterial infection.

Dead scale-insects were surface-sterilized in 2% sodium hypochlorite solution, rinsed by distilled water and plated onto potato sucrose agar supplied by 100 ppm streptomycin and 50 ppm chloramphenicol to prevent bacterial contamination.

Most of the isolations yielded the same fungus which was identified as *Fusarium larvarum* Fuckel. In cultures the fungus showed the following characters: aerial mycelium was sparse, white, later becoming light brown; only macroconidia were formed in orange pionnote sporodochia scattered over the surface of the agar; the sporogenous cells were simple, cylindrical phialides measuring 20×3 μm; the conidia were strongly curved, fusoid with a moderately marked foot-cell; they were three septate and measured 25×4 μm. Figure 1 shows macroconidia of the fungus. Although the perfect stage of *F. larvarum* is well known (*Nectria aurantiicola* Berk. et Br.) perithecia were not found in the specimens examined.

Two other fungi were also isolated and identified as *Fusarium semitectum* Berk. et Rav. and *Cladosporium sphaerospermum* Penz. Both species are considered

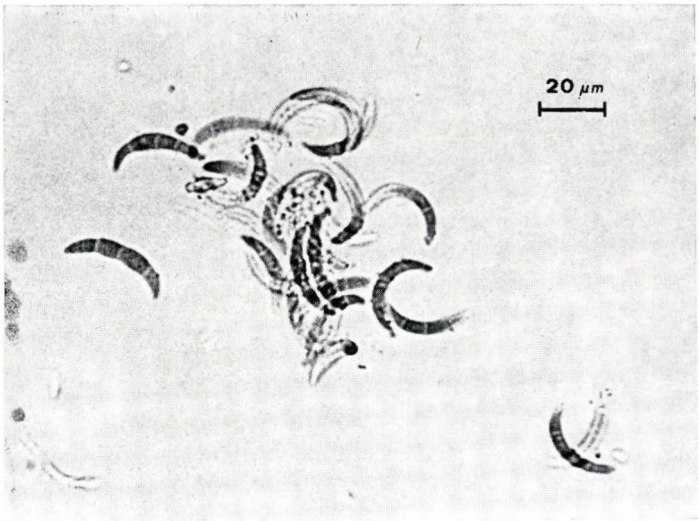

Fig. 1. Macroconidia of *F. larvarum*

to be common saprophytes, the latter frequently occurs as a secondary invader on many different substrates (Ellis, 1971).

Among the fungi that have been reported here *F. larvarum* seems to be responsible for the high percent mortality of *Q. ostreaeformis*. In his classification Booth (1971) listed three *Fusarium* species – *Fusarium coccophilum* (Desm.) Wr. et Rg., *Fusarium juruanum* P. Henn and *F. larvarum* – which are parasites of scale-insects, and only one of these, *F. larvarum* was found to occur in Europe. Besides the empirical records, Claydon et al. (1979) identified several metabolites of *F. larvarum* and some of these (monocerin and fusaretin ethers) showed insecticidal activity in a bioassay against *Calliphora erythrocephala.*

Reshetina (1967) mentioned a synonym, *Fusarium nivale* var. *larvarum* Bilai to be wide-spread in certain parts of the Soviet Union (North Caucasus, East Ukraine) and in Far East. The fungus, however, was not isolated in Hungary until now, and our report is apparently a new record for Central Europe, too.

Regarding the role of *F. larvarum* in the nature, the disease caused by this fungus may be considered as a possible limiting factor for *Q. ostreaeformis* populations, but its use in the man-directed biological control of scale-insects does not seem to be of great promise. When the pathogenicity of two *Fusarium* species – *Fusarium episphaeria* (Tode ex F.) Snyder et Hansen *(= F. larvarum)* and *Fusarium roseum* (Link ex Fr.) Snyder et Hansen *(= nomen confusum)* – was tested towards an armored scale-insect, *Selenaspidus articulatus*, in laboratory conditions, both fungi failed to cause a significant increase in mortality of the adults and of the second-instar nymphs and mortality of the insects was raised only at the crawler stage (Kuno and Ferrer, 1973).

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Click Beetles *(Elateridae)* in the Soils of Central Europe — Their Distribution and Description. Part I. (Gen.: *Agriotes*)

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The author rewiews the click beetles *(Elateridae)* of Central Europe with special regard to species of agriculturalimportance. Based on nationwide surveys carried out for many years in Hungary a thorough description of the species *Agriotes ustulatus* Schaller, *Agriotes obscurus* L., *Agriotes sputator* L., *Agriotes lineatus* L., *Agriotes brevis* Candeze and *Agriotes medvedevi* Dolin is given, together with data on their distribution and bionomics.

Distribution

Click beetles can be found everywhere on Earth. The number of the specief known ranges between 7500 and 8000. The greatest abundance in the number os species occurs in the warm zone. In Hungary their number is around 150. As far as their way of feeding is concerned there are omnivores, phytophags, detritus feeders and predators among them.

From the point of view of plant protection, their most important group includes species causing damage in agriculture, but also those of the predatory group must be considered. Click beetles can permanently be found everywhere in the soils of Europe and considerable losses are caused by them to agriculture in the Soviet Union, France, United Kingdom, and also in Central Europe.

The *Agriotes* species are the most common, but also the *Melanotus*, *Selatosomus*, *Limonius* and *Athous* species are widely distributed.

In the United Kingdom in 80% of the soils examined species belonging to the genus *Agriotes* constitute the largest group. There are some sites, however, where species of the genus *Solatosomus* dominate with the value of 60%, especially in higher altitudes.

In France *Agriotes obscurus* occurs the most frequently (70–80%), followed by *A. sputator* (25%), *Lacon murinus* and *A. lineatus* (Guéniat, 1934). According to Bonnemaison (1955) in the region of Paris *A. ustulatus* occurs. D'Aguilar (1961) reported the same species from the sea-shore.

In the European part of the Soviet Union the *Agriotes* species are widely known (Dolin, 1960, 1964). In the belts of non-black-earth soils *Selatosomus aeneus* is common, while in the blackearth (chernozem) soils *Melanotus brunnipes*, and in Southern Ukraine *Selatosomus latus*. The following species have only a rare occurrence: *Lacon murinus*, *Athous niger*, *A. hirtus* and *A. haemorrhoidalis*. *Agrio-*

tes litigiosus causes damages to agriculture in the region of the Caucasian Mountains and in the Crimean Peninsula. According to Gureva (1972) the following species are known: *A. tauricus* in the region of the Caucasian Mountains, *A. acuminatus* in the Ukraine, *A. meticulosus* from the lower section of the Volga River, *A. brevis* from the Ukraine and *A. medvedevi* on the alkaline alluvial soils. *A. incognitus* may be important in flood-area meadows, and *A. gurgistanus* in the belt of steppe. According to Cosmackevskii (1955) *A. reitteri* is also dangerous to agriculture. In Belorussia the most frequent species are: *Lacon murinus*, *Corymbites sjelandicus*, *Selatosomus aeneus*, *Agriotes* spp., etc.

In the soils of cropland in Poland, *Selatosomus aeneus* is the most common, according te Piekarczyk (1965), other common species are *Agriotes obscurus*, *Athous niger*, *Limonius aeruginosus*, *Selatosomus latus* and *Agriotes sputator*. Piekarczyk mentions altogether 25 species. Ten of these can be found also in Hungary.

In Yugoslavia surveys were made on beet-fields by Vukasovič et al. (1964) and the following order of occurrence has been established: *Agriotes ustulatus*, *A. sputator*, *Melanotus cinerascens*, *A. lineatus*, *A. sordidus*, *ruphipalpis*, *A. obscurus* and *Selatosomus latus*. In some of the districts of north-eastern Yugoslavia Stamankovič and Jovanič (1973) did surveys and they found *A. ustulatus*, *A. sputator* and *A. brevis* to be the dominant species. The following species were also found: *A. obscurus*, *A. sordidus*, *Melanotus cinerascens* and also *Adrastus* spp. (Vukasovič et al., 1969). From the various regions of Yugoslavia Camprag (1977) reported on the following species depending on vegetation and soil type: *Agriotes brevis*, *A. incognitus*, *A. lineatus*, *A. litigiosus*, *A. obscurus*, *A. sordidus*, *A. sputator*, *A. ustulatus*, *Melanotus cinerascens*, *M. fusciceps*, *M. punctolineatus*, *A. brunnipes*, *Selatosomus latus*, *S. aeneus*, *Adrastus spp.*, *Athous spp.*, *Limonius aerunginosus*.

In Czechoslovakia, according to the investigations of Rambousek (1927, 1928, 1929, 1930) *A. obscurus* is dominant in Moravia and Slovakia, while in the Czech regions rather *A. ustulatus* is typical. He mentioned *Melanotus brunnipes* and *Selatosomus aeneus* in the second place. Jagemann (1955) reported on agricultural pests and mentioned *A. pallidulus* and *Corymbites latus* among the pests of sugar beet. He also included *M. brunnipes*, *M. rufipes*, *M. niger*, *Limonius pilosus*, *Adelocera murina* among the pests. Dirlbek and others (1973) found that in the central part of Bohemia *A. ustulatus* was the most frequent and *A. sputator* occurred in 15%. These were followed by the species *Selatosomus*, *Adelocera*, *Athous* and *Melanotus*.

Nikolova (1957) mentioned that in Bulgaria *A. sputator*, *M. brunnipes*, *S. latus* and *S. aeneus* are the most important species. According to the examinations carried out by Popov (1968, 1971) *A. sputator* takes the first place, and is followed by *A. ustulatus*, *A. lineatus*, *A. obscurus* and *M. brunnipes*.

In Romania the most widely distributed species are: *A. lineatus*, *A. ustulatus*, *A. sputator* and *A. obscurus*. In the soils of croplands in many cases very high abundance value (from 36 to 121 adults per m^3) characterizes the species *A. obscurus* and *A. ustulatus* (Manolache-Bogulean, 1961).

As far as Germany in concerned, Klaus (1974) published the most recent data on the larvae of clicking beetles harmful to crop plants. He reported on the frequent occurrence of *Liotrichus affinis*, *Selatosomus latus*, *S. aeneus*, *Cidnopus aeruginosus*, *Athous niger*, *A. haemorrhoidalis* and *A. vittatus*. In the southern and western regions of Germany *Athous bicolor* is important, and of the species of the genus *Agriotes*, *A. obscurus*, *A. lineatus* and *A. ustulatus* are dominant. *A. elongatus* is a pest of sugar beet, grain crops, lettuce and potato especially in Southern Germany. According to Klaus the species *Ctenicera virens* is rarely found. The species *Ctenicera pestinicornis*, *Athous hirtus*, *Melanotus niger*, *M. brunnipes*, *A. brevis*, *Adrastus pallens* and *A. montanus* occur everywhere but their density is not high.

The study of clicking beetles and their larvae living in Hungary has been the subject of many investigations (Emich, 1873; Szaniszló, 1874; Anonymus, 1891; Fáy, 1891; Jablonowski, 1905, 1909; Szombathy, 1910, 1911; Révy, 1929; Baranyovits, 1939, 1942, 1944; Székessy, 1941*a*, 1941*b*; Győrffy, 1942; Bognár, 1954, 1955*a*, 1955*b*, 1955*c*, 1958*a*, 1958*b*, 1958*c*; Nagy, 1968; Szarukán, 1971, 1977).

From the fauna of the agricultural regions in Hungary 565 larvae have been examined by Bognár (1958*b*) and he found that 60.5% belonged to the species *Agriotes obscurus*. Szarukán (1973, 1977) found *Agriotes brevis*, *A. sputator* and *A. ustulatus* as dominant species. He established that also the species *Adrastus rachifer* was common. Beside these species also *Agriotes medvedevi*, *Melanotus brunnipes* and *Limonius pilosus* have to be mentioned. As far as the author's investigations are concerned, 17 species of established occurrence have been found and the first place is taken by *A. ustulatus* followed by other species of the *Agriotes* genus and species belonging to other genera.

It has to be noted that in the lists of species published earlier there were differences, but not by faulty identifications but by the fact that in the earlier taxonomic keys (Roberts, 1921; Korschefsky, 1941) preceding the one of Dolin (1964) the two species of *Agriotes obscurus* and *A. ustulatus* were distinguished based on the angle formed by the inner edge of the mandibles and the supplementary tooth. As a result of this a great number of *A. ustulatus* has been ranged to *A. obscurus*.

Description

In the soils of croplands in Hungary the wireworms of the *Agriotes* genus have the greatest importance. This is why only the species of this genus will be considered. As far as the other species are concerned, the work has to be restricted to the description, distribution and bionomics of genera. The morphological characteristics of the adults have been compiled based on the descriptions of Jagemann (1955), while those concerning the larvae were taken from the works of Dolin (1964), Tóth and Tersztyánszky (1967) and further – for the first time in this study – on the basis of the author's own investigations. Prior to the separation of the species and the morphological characterization the larvae were reared.

Adult

The body size of the beetles may be varying. Among the species living in Hungary *Quasimus minutissimus* is the smallest (from 2 to 2.2 mm), while *Athous rufus* is the largest (25–30 mm). Their exoskeleton is characterized by its surface being hard and smooth or grooved. In most of the cases they are covered by hair. The colour of the beetles is different, but mostly dark. The head is less mobile and the antenna of eleven segments is either filiform, serrated or branched. Of the segments of the thorax the metanotum is the shortest and there are cotyla on its frontal edge wherein the elongation of the pronotum will be fitted at clicking. The legs are generally of medium length, they belong to the "walking" type. The elythra are convex and cover the abdomen almost invariably. The membraneous wings are well-developed. The abdomen always consists of five segments.

Egg

The eggs are either milk-white or cream coloured. They are round, globular, or elliptical (Dolin, 1964). Their size is as follows: the smaller ones (*Agriotes* spp.) are 0.50 mm long and 0.42 mm wide, while the larger ones *(Lacon murinus)* are 0.94 mm long and 0.83 mm wide (Subklew, 1934; Guéniat, 1934).

Larva

Larvae are worm-like, their body is either cylindrical or more or less flattened, consisting of 13 segments. The integument of the species living in the soil and making burrows in it is strongly chitinized and as a result the larvae are similar to pieces of wire (that is where the term "wire-worm" has its origin). The colour of the larvae ranges between light yellow and deep brown. They have three pairs of equally developed legs. Their head is well-developed and prognate, flattened and chitinized. On the upper surface of the head capsule in the central line the epicranial suture runs longitudinally, starting from the basal part. The epicranial suture continues in the frontal suture, clearly delimiting the frontal plate (Fig. 1). The antennae consist of three segments, they are connected to the head capsule near to the mandibles.

Of the mouthparts the labium is rudimentary and is confluent with the maxilla. The mandibles are strongly chitinized and sickle-shaped. The prothorax is the largest segment of the thorax. The abdominal segments are of uniform structure with the exception of the ninth (caudal) and the anal segment. The stigmata are found laterally on the abdominal segments Nos 1–8, while there are no stigmata on the last segment. Segment No. 9 (caudal) of the abdomen may be of various shapes and is important in the identification of the species. There are larvae where it ends in a more or less pointed apex. This segment has a cylindrical, conical shape in the genera *Elater*, *Agriotes*, *Adrastus* and *Dolopius*, while in the larvae of the *Melanotus* and *Synaptus* species the dorsal surface bears an impression. The

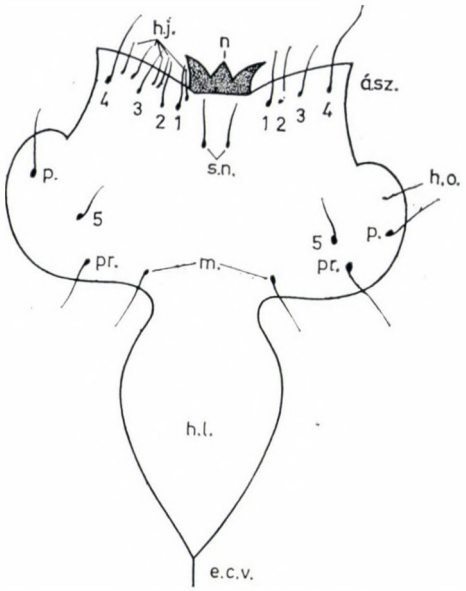

Fig. 1. The frontal plate of a larva of click beetle. a = nasale, á. sz. = sclerite of maxilla, h. o. = the lateral lobe of the frontal plate, h. l. = the posterior lobe of the frontal plate, e.c.v. = epicranial suture, h. j. = additional hairs on the fronthead, 1–5 = ordinary pairs of hairs, s. n. = subnasal pair of hairs, pr. = proximal hairs, n. = nasal hairs (original)

caudal or ninth segment of the *Athous*, *Limonius*, *Lacon*, *Selatosomus* and *Denticollis* species is cut out at the end and ends in two apices, while on the back there is always a well-distinguishable dorsal field.

Pupa

The pupa is a so-called pupa libera and its length ranges generally between 8 and 10 mm depending on the size of the imago. Its colour is ivory white. The pupa can be found in a pupal chamber.

The most important genus and species

Genus: *Agriotes* Eschholtz

The larval body is cylindrical and the colour of the larvae ranges from light yellow to reddish-brown. The head is rectangular or slightly oblique. The rear lobule of the frontal plate is elongated, oval and ends in a pointed or rounded apex. On the frontal plate there are always five pairs and the parietal pair of setae. The nasale is tridental. The maxillar sclerites surpass the peak of the nasale. The

mandibles are well-developed, curved like a sickle. Under the median tooth and the pointed end there is a supplementary tooth. The basis of the antennae of three segments is quite close to the mandibles. The colour of the eyes is dark brown and they are situated laterally under the antennal bases. The epicranial suture is well developed. The prementum is a nearly regular pentagon, and the submentum (Dolin, 1964) is long, elongated, with parallel sides and ends in a blunt angle. The distal end of the stipes gradually tapers, with a straight inner contour and an arched outer one. Among the thoracal segments the prothorax is the longest; it is nearly as long as the meso- and metathorax together. The surfaces of the sternites and tergites are sculptured; either finely or more coarsely. On the tergite of the abdominal segments the pits of muscle attachment points have not been developed, but the longitudinal furrow beside the stigmata can be found. Segment No. 9 is elongated, conical, and is considerably longer than the preceding one. It ends in a chitinized thorn. On the basal part of the segment there is a pair of pits similar to the stigmata, broadening inwards and bordered by a brown colouration. There are two pairs of longitudinal furrows on the back; one of these starts from the pit like the stigmata and the other runs between those of the first pair.

Species

Agriotes ustulatus Schaller

Synonyms: *Agriotes confusus* Dach.
Agriotes foveolatus Rej
Elater blandus Germar

Description

Adult: The body is moderately elongated, its length ranging from 7 to 10.5 mm. The head is convex and is densely an coarsely sculptured. The body is moderately elongated, its length ranging from 7 to 10.5 mm. The head is convex and is densely and coarsely sculptured. The antennae are yellowish brown, their first segment is deeper coloured, the second one is as long as the third one, and they are all light yellow. From the fourth segment onward the segments of the antennae are broader and slightly serrated. The length and breadth of the pronotum are of the same figure. It is strongly convex and is the widest at half of its length. Its rear apices are short, parallel, or moderately divergent, with ridges high, sharp and long. Its sides are thickly and coarsely sculptured. The clypeus is slightly elongated and has always the same colour as the pronotum. The elytra are of the same breadth as the pronotum. Their colour is variegated; they may be yellow or rust-coloured over their whole length or may have dusky ends, while in some cases the whole surface of the elytra is dark. The grooves of the elytra are shallow, and the pits of the grooves are coarse and deep. The fields between the grooves are moderately convex and finely and densely dotted. The legs are yellow or yellowish-brown slightly tapering on their inner edge.

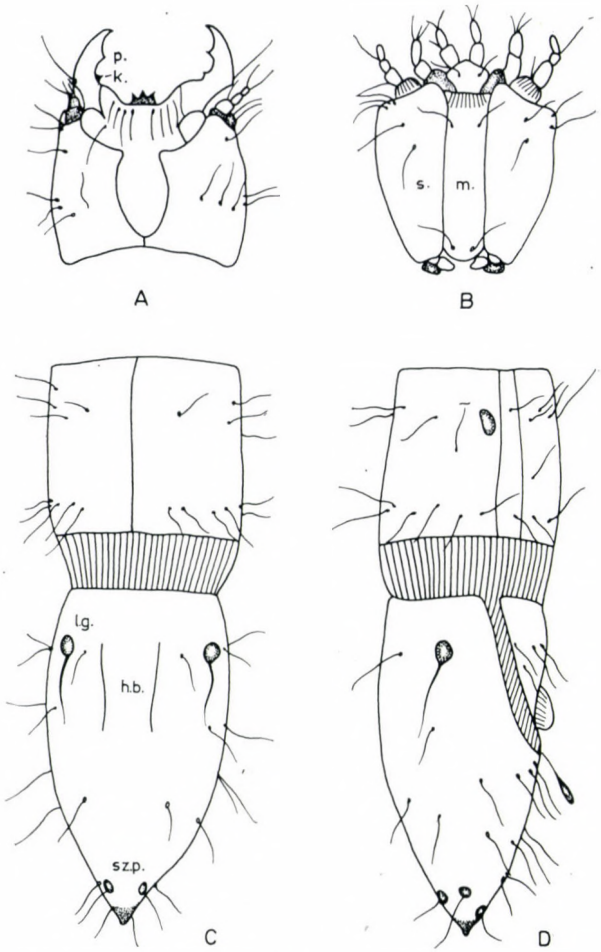

Fig. 2. *Agriotes ustulatus* Schall. (larva). *A* The head seen from above. p. = supplementary tooth of the mandible, k. = medial tooth. *B* Lower maxilla and labium. s. = stipes, m. = mentum. *C* Segments of the abdomen Nos 8 and 9 (seen from above). l. g. = cavity similar to a stigma, h. b. = peres of central position. *D* The end of the abdomen (side view) (original)

Egg: The eggs are slightly oval, greyish-white; their average length is 0.60 and 0.45 mm in diameter.

Larva: the head is broader than its length, the posterior lobe of the frontal plate is elongated, its shape is oval and the apex is rounded. The nasale is short, very strongly transversal with three, equally long teeth. The supplementary tooth of the mandible is barely developed and appears as an eminence (Fig. 2A). The tergites of the thorax and the abdomen are deeply sculptured, slightly and sparsely furrowed, their basis is smooth and shiny to the line of stigmata. The longitudinal

furrows originating from the pits of muscle attachment points are poorly developed. The ninth or caudal segment is nearly twice as long as wide and its apical part is strongly tapering. The apical thorn is well developed and sharp. The longitudinal furrows are short and do not reach to half of the segment. The hairs of the apex originate from large and deep pits with protruding edge. These pits or rather dots are simple and small at the base of the segment (Figs 2C and 2D). The maximum body length of larvae is 27 mm.

Pupa: The pupa is a pupa libera of ivory colour, laying in a pupal chamber. Its length is between 10 and 12 mm.

Distribution

This species has a southern (Mediterranean) distribution. It is frequently found in Central, Southern and Western Europe, in the region of the Caucasian Mountains, in Armenia and in Northern Africa. In Hungary it dominates in the wireworm populations of agricultural soils, with a dominance value of 45.6%.

Biology

Its development is different from that of the other *Agriotes* species as it pupates not in autumn but in early summer, i.e. in June, and so its last over-wintering takes place in larval form (Szarukán, 1971).

Its development takes five years in Central Europe and in the Ukraine, while in Southern Europe it takes only four years. The adult flies in July and August. It feeds on grass leaves and on pollen of *Umbelliferae* (Rambousek, 1927, 1928, 1929, 1930). The larva feeds on germinating seeds and roots. This is the most injurious click beetle species in Hungary.

Agriotes obscurus Linné

Synonyms: *Agriotes hirtellus* Herbst
Agriotes obtusus De Geer
Agriotes variabilis Fabricius
Elater hirtellus Herbst

Description

Adult: The body length ranges between 7 and 10 mm. The upper part of the body is of various colours from rust-brown to brownish-black. The head is convex and very densely and coarsely dotted. The colour of the antennae is light rusty-brown. The second segment is two and a half times longer than its width. It is much longer than the third segment and is of the same length as the fourth segment. From the fourth segment onward the filiform antennae will become broader, forming blunt triangles. The edge on the side of the pronotum curves to the ventral side and therefore it can hardly be seen from above. Its width surpasses its length, and it is

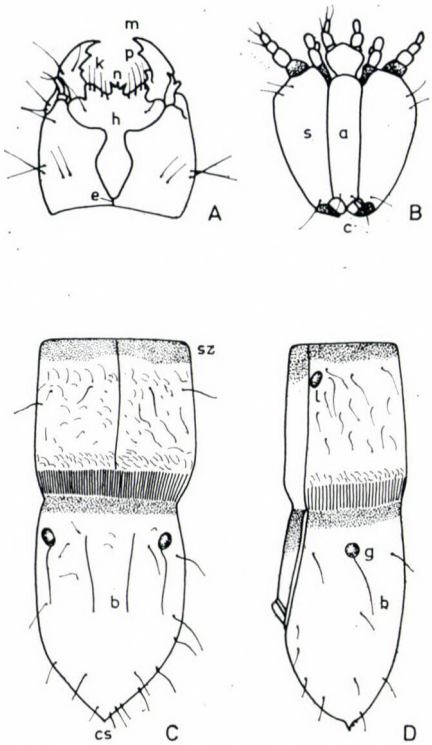

Fig. 3. *Agriotes obscurus* L. (larva) *A* The head seen from above. m. = mandible, p. = supplementary tooth, k. = medial tooth, n. = nasale, h. = frontal plate, e. = epicranial suture. *B* Maxilla and labium. s. =, stipes, a. = maxilla, c. = cardo. *C* The end of the abdomen (seen from above). sz. = granulation, b. = central longitudinal furrow, cs. = apical thorn. *D* The end of the abdomen (side view). l. = stigma, g. = cavity similar to a stigma, b. = longitudinal furrow (original)

strongly convex. It is covered with coarse, dense and deep sculpture. The posterior apixes are broad, slightly divergent, with well developed ridges. The clypeus is always of the colour of the elytra, and its length surpasses only slightly its width. The elytra are as long as they are wide. They are strongly convex and are the broadest at two-thirds of their length. The furrows of the elytra are fine and the interspaces between them are finely and thickly sculptured. The legs are short and usually of the same colour as the antennae; the femurs are frequently deeper coloured.

Egg: The egg is oval, grayish-white, shiny and slightly translucent. Its length varies between 0.4 and 0.6 mm.

Larva: The head is transversal, and broader than its length. The posterior lobe of the frontal plate tapers with nearly straight sides from its half, and it ends in a blunt apex. The frontal thorn (nasale) is well developed, the lateral teeth turn slightly to the side, therefore the basal part is slightly narrower than the apical part.

The medial tooth is slightly protruding. The subapical emergences or supplementary teeth of the two mandibles form a right or a blunt angle (Fig. 3A). The tergal plates of the thorax and of the abdomen are very sparsely and finely sculptured, their surface is shiny. On the basal part they are finely granulated and they do not reach the line of the stigmata. The surface around the frontal coxae is only slightly granulated. The stigmata are not so long as in the case of *Agriotes sputator*, i.e. they are shorter and anteriorly slightly broadening. The lateral longitudinal furrows are poorly developed. The No. 9. (caudal) segment is 1.7 times longer than it is broad; over half of its length it is gradually broadening, then it suddenly tapers with arched sides. The spical thorn is short and broad. The longitudinal furrows reach to the half of the segment. The hairs are sitting growing from simple pits. The surface of the segment is shiny and is sparsely and transversally rugose (Figs 3C and D. The body length of larvae may reach 25 mm.

Pupa: The pupa is similar to that of *Agriotes ustulatus.*

Distribution

This species has an Euro-Siberian distribution. It can be found in Europe, Western Asia, and even in the Lappland (Roberts 1922). In Hungary it is the second species which can be found most frequently.

Bionomics

Pupation occurs in August and then it transforms into adult and spends the winter in the soil. It appears by mid-April or at the end of this month. The adults feed on the plants of the *Gramineae* family on the sides of ditches, in meadows and pastures and in croplands. The total number of eggs, laid in groups of twenty, ranges between 150 and 300. The eggs are placed into the soil surface, to a depth of 1 or 2 cm. The embryonal development takes generally four weeks. The young larva (1.5 mm long) soon begins to feed; depending on humidity and the presence of humus materials the larvae either feed on plants or will become saprophagous. Sometimes they feed on larvae and pupae of other insects (Chrzanowski, 1927), and cannibalism also occurs sometimes. First the larvae remain together in one group and then gradually spread. The total developmental period takes 4 or 5 years as in the case of *Agriotes ustulatus.*

Agriotes sputator Linné

Synonyms: *Agriotes brunnicornis* Gerber
Agriotes corallifer Eschholtz
Agriotes cribosus Eschholtz
Agriotes fusculus Illiger
Agriotes graminicola Redtenbacher
Agriotes productus Rey

Description

Adult: The body is compact, shiny, the colour is dark brown, the body length ranges from 6.5 to 8.5 mm. The elytra and the frontal edge and posterior apices of pronotum are reddish brown. The whole surface of the body is of greyish yellow or rusty grey colour. The antennae are short, light yellowish brown. The second segment of the antenna is considerably longer than the third. The pronotum is longer than its width. The central part of its surface is more finely sculptured than on the edges. The lateral edge is bent towards the ventral side, most of it cannot be seen from above. The greatest width is measured at the half of the length of the pronotum. The posterior apices of the pronotum are long, narrow, with a broad ridge and moderately divergent. The length of the clypeus surpasses only slightly its width, it has the shape of an irregular pentagon and the angles are rounded. The elytra are of the same width as the pronotum but their length is twice as much as their width. The elytra reach their greatest width at the second

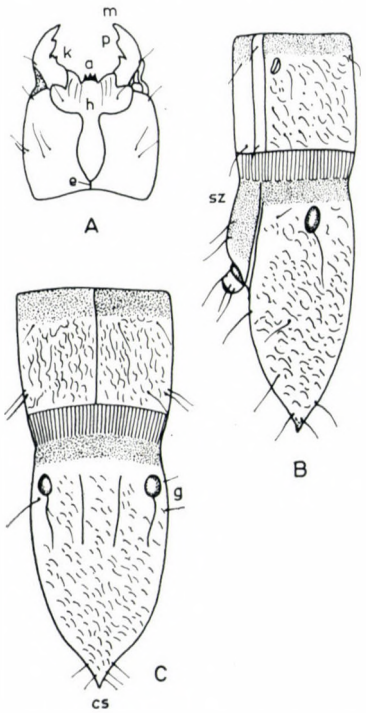

Fig. 4. *Agriotes sputator* L. (larva). *A* The head seen from above. m. = mandible, p. = supplementary tooth, k. = medial tooth, n. = nasale, h. = frontal plate, e. = epicranial suture. *B* The end of the abdomen (seen from above). sz. = granulation, g. = cavity similar to a stigma, b. = .longitudinal furrow. *C* Segments Nos 8 and 9 of the abdomen (seen from above). b. = central longitudinal furrow, g. = cavity similar to a stigma, cs. = apical thorn (original)

third of their length. The lateral edges in the first two thirds are nearly parallel. The furrows of the elytra are of considerable depth and deeply sculptured. The legs of the adults are of the same colour as the elytra.

Egg: The eggs are nearly globular, their colour is white. The average measurements are 0.54×0.43 mm.

Larva: The head of the larvae is rectangular, the posterior lobe of the frontal plate has straightly arched sides and is lance-shaped, with a sharp end. The frontal thorn (nasale) is well-developed, its basal part is broader than the apex. The median tooth is somewhat protruding. The supplementary tooth of the mandible is well developed and usually forms a right angle with its inner edge (Fig. 4A). The mesothorax, the metathorax and the dorsal plates (tergits) of the abdomen are coarsely rugose and densely sculptured. The basal parts of the segments are strongly granulated on the dorsal surface to the upper line of the stigmata, on the ventral side of the ninth (caudal) segment to the pseudopodius and on the surfaces around the coxae. The sides of the stigmata are parallel, they are twice as long as they are wide. The lateral furrows are slightly developed. The ninth (caudal) segment is nearly twice as long as it is wide and it is growing gradually narrower from the line of the pits similar to the stigmata. The thorn of the apex is thin and long. The longitudinal furrows do not reach half of the length of the segment (Figs 4B and C). The dots at the basis of the hairs are simple and small. The body length of the larvae may reach 20 mm.

Pupa: The colour of the pupa is white, its length is 8 mm and its width is 2.5 mm.

Distribution

The species is palearctic. It can be found everywhere in Europe, in Western and Eastern Asia. In Hungary it can be found everywhere but its density is much lower than that of the two previously discussed species.

Bionomics

Pupation takes place in July and August. It swarms from the beginning of May to mid-June. This means that swarming begins about two weeks later than in the case of *A. obscurus*. In daytime it is usually found on the leaves of grasses and on the flowers. It feeds during the night. It feeds more readily on weeds than on leaves of grain crops. This may be the reason for the lower density of this species in the soils of croplands. The species is polyphagous. The larva is especially harmful in the last year of its development. The total developmental time takes 3 to 4 years.

Agriotes lineatus Linné

Synonyms: *Agriotes segetis* Bjerkander
Agriotes striatus Fabricius
Agriotes suecius Gmelin
Elater striatus Fabricius
Elater suecius Linné

Description

Adult: The body is ovally elongated, its colour is brown and its length ranges between 7 and 10.5 mm. The head is moderately convex, densely sculptured. The antennae are light rusty brown. The second segment of the antennae is three times as long as its width, and is about one-fourth longer than the third segment. The first three segments of the antennae are less densely sculptured than the other ones and therefore shiny. From the fourth segment onward the antennae are dull. The lateral edge of the pronotum curves downward to the ventral side and much of it cannot be seen from above. It is somewhat wider than its length and the edges are nearly parallel. The posterior apices are long and sharp, with well-developed ridges. The surface is coarsely and thickly sculptured. The elytra of the male adult are of the same width as the pronotum, while in the female they are broader. The elytra are the widest at half of their length. They are twice as long as their width and very shiny. The inter-row spaces on the elytra are alternating: the broader ones are

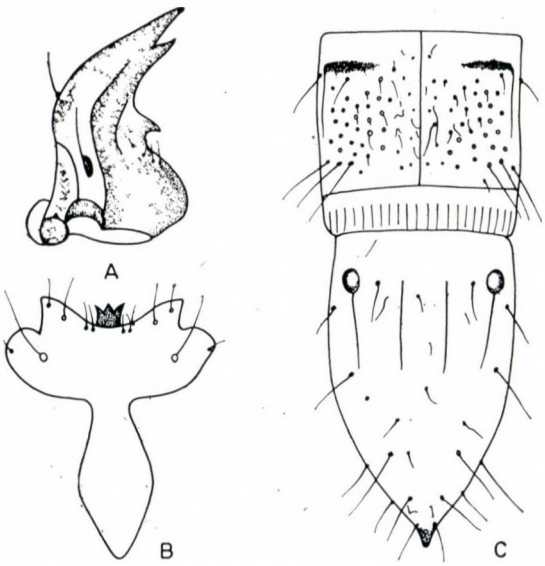

Fig. 5. *Agriotes lineatus* L. (larva). *A* Mandible on the left side. *B* Frontal plate. *C* Segments Nos 8 and 9 of the abdomen (seen from above) (Based on Dolin, 1964)

more densely haired than the narrower intermediate ones appearing thus to be of a lighter colour. The legs are of the same colour as the lighter coloured parts of the body.

Egg: The eggs are a somewhat smaller than those of *A. obscurus*. The size ranges between 0.37 and 0.47 mm.

Larva: The larva is light yellow and the posterior lobe of the frontal plate has a pointed apex. The teeth of the nasale are of the same size. The supplementary tooth of the mandible is pointed and it forms an acute angle with the inner edge (Fig. 5A). The thorax and the first eight tergites of the abdomen are coarsely and densely sculptured, the basal parts of the tergites are smooth and shiny and are only very sparsely granulated. The longitudinal furrows originating from the two cavities of muscle attachment are well developed. The stigmata are 1.5 times longer than their width. The caudal segment is 1.5 times longer than its width. Its surface is smooth, shiny and sparsely rugose. The longitudinal furrows reach to half of the length of the segment. The pits at the bases of the hairs are small, finely ringed, the apical thorn is short and stocky. The length of the larvae may reach 27 mm.

Pupa: The pupa of this species is smaller than those of the *Agriotes* species treated above. The colour is white.

Distribution

This species is palearctic It can be found everywhere in Europe, in Western and Eastern Asia. It occurs also in Hungary, but the individual number is low. The environment suitable to *A. ustulatus* and *A. obscurus* is also favourable for this species, it does not occur, however in about half of the various soil types. This *Agriotes* species is especially sensitive to dry conditions.

Bionomics

The development of this species is nearly identical with that of *A. obscurus*, but its heat and humidity requirements are higher. It is therefore rarely found in the northern regions of Europe. The development takes 4 to 5 years.

Agriotes brevis Candeze

Description

Adult: The body is oval, at the end slightly tapering, the side is arched, the surface is shiny. The colour is brownish black, the elytra are reddish brown, furrowed, or their apical portion is reddish brown. The hairs are yellowish or rust coloured. The head is convex, coarsely sculptured, and the space between the dots formes a narrow wrinkle. The frontal part of the head is frontally straight cut. The antennae are of rusty colour, their length reaches the rear end of the pronotum in the case of the male, while in females they are somewhat shorter. Their first

segment is very stocky, the second one is twice as long as it is wide, and noticeably longer than the third one, while the fourth is thinner and noticeably longer than the second. The antennae from the fourth segment onward are very bluntly serrated. The pronotum is wider than its length and concavely arched on its posterior end. The lateral edges of the frontal half are rounded, while in the rear part they are straight and nearly parallel. The posterior apices of the pronotum are well developed and broad, slightly divergent and the ridge is long and well developed. The central longitudinal furrow usually appears only posteriorly, but in some adults also in the frontal part of the pronotum. The pronotum is heavily sculptured and cut. The distance between the dots is generally equal to half of their diameter. Closer to the edge of the pronotum the dots become larger with smaller interspaces. The clypeus is longer than it is wide, it is flattened with a rounded apex. The elytra are as wide as the basis of the pronotum, they are twice as long as wide, arched, and they are the widest at their first third. The furrows on the elytra are deep, clearly and densely sculptured, and become shorter close to the end of the elytra. The fields between the furrows are nearly flat and smooth, densely and finely sculptured. The colour of the legs is rusty brown, usually with darker femurs. The length ranges between 6.5 and 8 mm. The body of the females is more arched, the antennae are shorter, and the pronotum is narrower than the elytra. This species occurs east of the Mediterranean region. The dorsal surface of their body may be of various colours and the length of the elytra also shows great variability. In Hungary so far no variations of this type have been described.

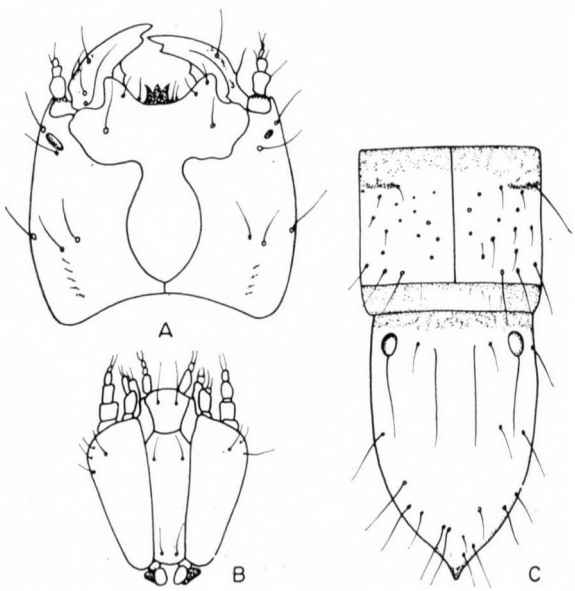

Fig. 6. *Agriotes brevis* Cand. (larva). *A* Head seen from above. *B* Maxilla. *C* The last and the penultimate segment of the abdomen (Dolin, 1964)

Larva: The larva is very similar to that of *A. sputator* L. but its body is broader; the cavities of muscle attachment and the longitudinal lateral furrows of the abdominal tergites are almost fully reduced. They are visible only on the penultimate segment. The caudal segment is more then 1.5 times longer than its width. It is slightly flattened, its apical third becomes gradually conical, very finely and equally rugose and sparsely, finely sculptured. The longitudinal furrows are long, the medial one is longer than half of the length of the segment. As far as the other characteristics are concerned, it is similar to the larva of *A. sputator* L. Its length may reach 22 mm, the width 1.9 mm (Fig. 6.).

Distribution

It occurs in Sub-Carpathia (the lowlands of the Tisza region). The larvae are found in the soils of open biotopes, croplands and gardens.

Bionomics

Its biology is very similar to that of *Agriotes lineatus* L. Its larva is the pest of agricultural plants. Its occurrence in Hungary is very low. It has no economic importance (Dolin, 1964).

Agriotes medvedevi Dolin

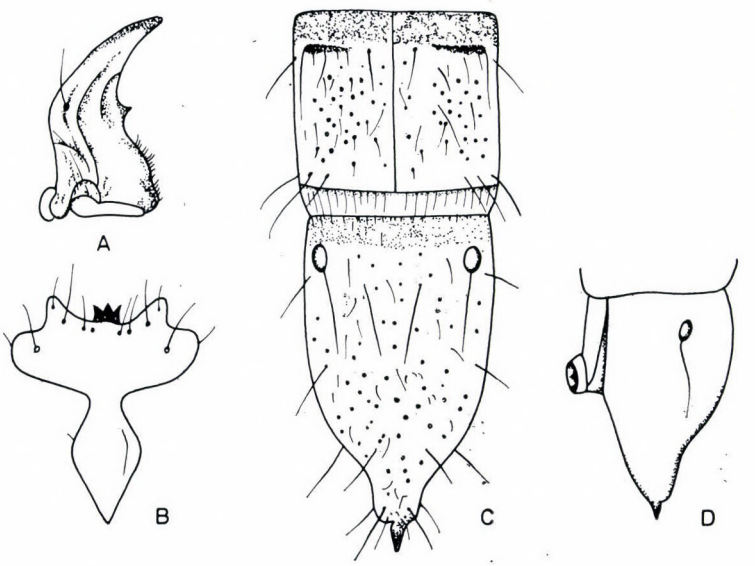

Fig. 7. *Agriotes medvedevi* Dolin. (larva). *A* Mandible on the left side. *B* Frontal plate. *C* The last and the penultimate segment of the abdomen. *D* The last segment (lateral view)

Description

Larva: The colour of its surface ranges from yellow to reddish brown. Near to the apex of the mandibulae a small, rounded protuberance can be found, but there is no tooth (Fig. 7A). The median tooth of the nasale is larger, somewhat protuding. The posterior lobe of the frontal plate is lanciform, tapering from its half to the apex with almost straight sides and terminates in a peak. Its length is twice its width. The parietal pair of hairs on the frontal plate is short, but well developed. The tergite of the prothorax is finely sculptured, while those of the mesothorax, metathorax and of the abdomen are coarsely and densely sculptured along the median line. The basal parts of all tergites (except of the prothorax) are finely granulated and dull. The side of the tergites is slightly developed, is narrow and darkly pigmented, with cavities and longitudinal lateral furrows. The caudal segment is one and a half times longer than its width, it suddenly tapers in its apical third so that the frontal part of the segment looks like a pillow from the side (Figs 7C and D). Further it seems slightly widening and bluntly rounded in the apical part. The longitudinal furrows are slightly developed, the pores at the bases of the setae are small and simple. The apical thorn is well developed but short (Fig. 7C). The body is 22 mm long, its width may be up to 1.6 mm.

Distribution

This species occurs in the south of the European territories of the Soviet Union in the belt of steppe. According to Szarukán (1973) it can be found also in Hungary in alkaline alluvial soils.

Bionomics

Its feeding habits and economic importance are not known. (Dolin, 1964)

Literature

The Literature will be given in Part II of the paper, to be published in Acta Phytopathologica.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 19 (1–2), pp. 31–41 (1984)

Aspects of Mutagenic Nematode Theory

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Mutagenic nematode theory (MNT) is a unified theory of nematode variation and host relations. The theory is a composite model assembled from diverse experimental areas of nematode variation, cytophysiology of host plants attacked by nematode, and radiation effects on seedlings. The theory provides for variation and race formation in nematode reproducing parthogenetically and for the formation of chimeras in clonally reproducing plants such as sweet potato *Ipomoea batatas* Lam. The theory predicts that some nematode feeding activities are mutagenic and causes changes in chromosomes of root cells and localized root chimeras and that in turn chimeric races are formed in the nematodes by mutagenicity and selection pressure of the root chimeras derived from the altered host chromosome complement independent of particular host or host somatoplasm. In contrast with classical genetic theory in nematode and fungus-host relations, MNT proceeds directly to externally induced changes in chromosomes as basis for differences in host plant reactions to disease and eventual production of new biotypes or ecological races of nematodes. In so doing, the theory affords an alternative example to some 35 years of "remote sensing" biochemical approaches to the nature of plant disease, which have not been able to identify distinct heritable biochemical changes in the host mirroring specific plant disease development. MNT points on the theoretical level to cytogenetics as an essential adjunct discipline in biochemical and physiological investigations of cellular phenomena and on the practical level to a method of nursery production of new nematode biotypes for use in prepest breeding of resistant varieties of crop plants before such biotypes appear (postpest) in natural cropping systems.

The basis of mutagenic nematode theory (MNT) lies in a number of correlated events and phenomena which are inconsistent with current theories of variation in organisms based on random mutation and sexual recombination. (*a*) Similarities between radiation and nematode damage in seedlings, stemming from chromosome alterations – known for radiation and hypothetical for nematodes (Johnson, 1936; Stadler, 1936; Hollis 1979*a,b,c;* Hollis 1980*a,b*). (*b*) Morphological and biochemical effects of disease in host cells suggest the possibility of chromosomal changes induced by nematodes (Dropkin and Bird, 1978; Gommers and Dropkin, 1977; Jones and Dropkin, 1975; Jones, Novacky and Dropkin, 1975; Jones and Dropkin, 1976; Reed, Richardson and Russell, 1979) and other agents (Whitney, Shaw and Naylor, 1962; Williams, Aist and Bhattacharya, 1973) (*c*) Considerable heritable variability can occur in cloning organisms in which genetic mechanisms for variation are inoperative; viz., certain plant parasitic nematodes (Martin, 1954; Netscher, 1978; Sasser and Kirby, 1979; Sturhan, 1971; Taylor and Sasser, 1978; Taylor, Cadet and Luc, 1978; Triantaphyllou, 1971)

and in root crops including the sweet potato *Ipomoea batatas* L. (Southern Cooperative Series, 1970).

MNT specifically predicts excessive numbers of variants relative to classical theory in nematodes reproducing by mitotic parthenogenesis, excessive numbers of sweet potato mutants relative to the status of this plant as a clonal organism, that some nematode species are more mutagenic than others, and that formation of new nematode chimeric races is mediated by selection pressure derived solely from the host chromosome complex.

The purpose of this paper is to outline the theory, present a cross section of experimental evidence on which it is based and discuss some of its implications for illumination of current trends in plant pathology and for disciplinary approaches to a broad range of cellular phenomena. Principal elements of the theory have been presented in a series of 5 abstracts.

Experiment Basis of Mutagenic Nematode Theory

The three specific lines of evidence upon which MNT is based relate to comparative effects of radiation and nematodes on plants, effects of nematodes on cell nuclei, and variation in nematodes reproducing by mitotic parthenogenesis.

Duggar assembled relevant information more than 40 years ago on the biological effects of radiation. Observations reported by Johnson (1936), page 968, are typical examples of such effects. They included disturbance in development and growth of root tips of *Vicia* seedlings and retarded development of their lateral roots. In *Vicia faba*, roots were stunted by medium doses of irradiation, root tips became bulbous and lateral roots did not develop in stunted X-rayed specimens.

Johnson, page 969, detailed effects of radiation on *Thunbergia alata*, maize-castor bean *(Ricinus communis)*, radish and narcissus. Both main roots and lateral roots were suppressed in all species. Radiation usually decreased the length of tap roots and the number and length of lateral roots. In some instances there was entire suppression of lateral roots for several days after irradiation. Raying the root end of narcissus bulbs with 3500 r-units caused a necrosis and marked stunting of roots. Three weeks after treatment, the experimental plants exhibited 52% relative decrease in number of roots. Extensive measurements of control and irradiated wheat seedlings summarized by Johnson showed lateral roots 18 times as ray-sensitive as the coleoptile.

Johnson, page 979, reported the observations of Komuro in 1922 on *Vicia faba* following, "irradiation of X-rays upon the seeds, seedlings and young plants . . .". Vacuolization of cytoplasm was apparent in irradiated root tips and all observed mitoses were abnormal. Nine hours after irradiation, binucleate cells, giant nuclei and multinucleolar nuclei were found. Additional degenerative phenomena, occurring in the roots of *V. faba*, were irregular distribution of chromosomes, change in form and contents of the nucleus, appearance of multinucleate cells, a tearing away of the protoplast from the cell wall, vacuolization of both nucleolus

and cytoplasm, dissolution of cell nucleus and thickening of the nucleus by contraction.

Stadler (1936), page 1267, reviewed his own experiments with barley in which mutation was induced by seed treatment with both X-rays and radium. Treatment of both dormant and germinating seeds resulted in mutation but the rate of mutation following treatment of actively germinating seeds was eight times as great as that following similar treatment of dormant seeds. Stadler could recognize about 90% of the induced mutations in the seedling stage, and where high numbers of mutations were required for significant comparisons, seedling mutations alone were used as an index of mutation frequency. About 95% of more than 800 mutations affecting seedling characters were observed to affect chlorophyll characters of diverse types; the remainder included a wide range of morphological abnormalities.

Reconstruction or rearrangement of the chromosome apparatus has been observed by numerous workers following irradiation of root tips of plants. This phenomenon has not been followed up to study heritable effects of the chromosome changes, principally because it has not been possible to recover the entire plant from root tips. Since this type of correlative evidence could have been sought with the sweet potato as test organism, lack of interest must have resulted from absence of motivating ideas such as those now generated by MNT.

Nematological literature is replete with descriptions and pictures of nematode effects on the root systems of plants which bear a general resemblance to the effects of radiation on seedlings.

A considerable number of detailed morphological studies of giant cells and syncytia induced on principal crop hosts by root knot and cyst nematodes have been conducted during the past two decades utilizing light, electron, scanning electron microscopy and histochemical methods. A summary of this work is in original papers and correlated reviews (Dropkin and Bird, 1978; Gommers and Dropkin, 1977; Jones and Dropkin, 1975; Jones, Novacky and Dropkin, 1975; Jones and Dropkin, 1976; Taylor, Cadet and Luc, 1978).

Points of greatest interest to MNT are nuclear and nucleolar condition of syncytia during attack by root knot and cyst nematodes. Syncytia is used here to include both giant cells formed by *Meloidogyne* species and transfer cells formed by *Heterodera* species; recognizing also that the term transfer cells has been applied to both giant cells and syncytia. The most fruitful generalizations at this time are in the diversity of morphological changes and in the limited evidence of histochemical changes in syncytia. These include observations on a great diversity of nuclear changes by mitoses, by fragmentation and by coalescence of nuclei from adjacent cells, after dissolution of their walls, to produce swelling and malformation, increase in numbers and polyploidy of nuclei; extranuclear changes in the cell are proliferation of organelles in the cytoplasm, reduction in size, loss of central cell vacuole and cell expansion with performations in the cell wall (Jones and Dropkin, 1976). There have been no measurements of chromosome damage to the host by nematodes.

Utilizing Lowry's ultra-micro analytical techniques, Gommers and Dropkin (1977) demonstrated that altered morphogenesis in a suitable host of "large multinucleate cells induced by sedentary endoparasitic nematodes" was accompanied by biochemical changes: increase in protein, free amino acids and glucose, as compared with control tissue consisting of cortical cells of actively elongating root tip tissue. Increase in free amino acids was 6-fold in giant cells (*Meloidogyne*-induced) and 1.5 fold in syncytia (*Heterodera*-induced). Implications of selection pressure on the parasites for production of new variants is evident in these data on the basis of mechanisms such as gene replication which have been correlated with development of resistance to drug action in microorganisms (Schimke et al., 1978).

Performing experiments of a similar nature, Dropkin and Bird (1978) mimiced the effects of a chromosome-derived substance on a root knot nematode by what I would label as a centrifugal nuclear mechanism (an inside toward exterior of cell activity). The authors discovered a profound effect of deoxyribonucleic acid (DNA) in minimal concentrations of 0.0075 mg/ml on secretion of gelatinous matrix from rectal gland cells and on morphological changes in nuclei of these cells in females of *Meloidogyne javanica.*

A simple example of centripetal "remote sensing" characteristic of physiological and biochemical approaches to the nature of host-parasite relations is furnished by the paper of Jones, Novacky and Dropkin (1975). Differences were sought in the transmembrane potential of giant cells versus parenchyma; the results were negative in spite of greater metabolic and synthetic activities of the former. These data provide an example of "outside toward interior of cell' centripetal remote-sensing measurements. Implications of centrifugal and centripetal approaches will be treated in the discussion within the broader contents of contrasting disciplines: cytogenetics and biochemistry.

Variations in host reaction characteristics between and within populations of much studied plant parasitic nematodes: *Ditylenchus dipsaci* Kühn, *Meloidogyne* and *Heterodera* species (Martin, 1954; Sasser and Kirby, 1979; Taylor and Sasser, 1978), *Aphelenchoides fragariae* (Ritzema Bos), *Rotylenchulus reniformis* (Linford and Oliveira), *Pratylenchus* species, and others have become legendary (Sturhan, 1971). Variation in root knot nematodes was recognized by Chitwood prior to 1949 (Netscher, 1978). Sturhan (1971) concludes that such variation is genetically controlled and he presents the classical mechanisms of random mutation and recombination; the addition of selection pressure provides for the formation from variants of races = ecological races = biological races = biotypes.

There are two principal flaws in the classical theory with reference to nematodes: (*a*) the very large number of variants within and between populations of nematodes, particularly in *Meloidogyne* species, and (*b*) the cloning reproduction of these species in the absence of gene flow and recombination by a mechanism labeled as ameiotic or mitotic parthenogenesis (Triantaphyllou, 1971). This author demonstrated cytologically that three principal root knot nematode species: *Meloidogyne incognita* Kofoid and White, *M. javanica* Treub. and *M. arenaria* Neal reproduce by mitotic parthenogenesis, and that each species demonstrates

a different degree of polyploidy. Thus, the classical genetic mechanisms are inoperative and cannot be used to interpret the observed variation within these species; MNT provides alternative mechanisms of variation which can function in all parasitic nematode species, with or without intervention of mechanisms of random mutation and recombination.

Discussion

The first symposium on the physiology of parasitism sponsored by The American Phytopathological Society was held 28 years ago at Ithaca during the dawn of molecular biology and dealt with toxin theory in plant diseases. The linking of disease symptoms with interchange of chemical products between pathogen and host had been speculated about for more than 100 years, but concerted efforts in this area have resulted more recently in the stimulus and promise of antibiotics – products of microbial metabolism – in the control of human diseases. The toxin theory was a centripetal and peripheral development divorced as it turned out from the central control mechanisms of the cell, but at the time it was a revolutionary trend which met all criteria for unfettered indeterminate basic research in the finest sense: the area was unknown, the techniques were exploratory and the investigators did not know what results to anticipate.

Toxins, phytoalexins, growth hormones and growth regulators have been characterized but progress in this field over the past 35 years has been unable to identify distinct heritable biochemical changes in the host accompanying specific plant disease development (Wheeler, 1975). Progress has also been slow and somewhat disappointing in linking heritability with other plant phenomena such as germination, growth, differentiation, respiration, dormancy, senescence and death.

These phenomenal areas of research have been approached almost exclusively by centripetal physiological and biochemical remote sensing techniques instead of mixing them with centrifugal cytogenetic or base sequencing methods. We have been wide of the mark; the problems of heritable biological specificity are the problems of DNA structure of central chromosomes. Base sequencing is a close, proximate biochemical method. Biochemical methods can be used for both remote and proximate determinations.

Structure and function are one: structure is potential function, function is actualized structure. Potential function is nonphenomenological and nonexistential in terms of remote sensing. The flaw in remote sensing techniques is that only phenomena (existential aspects of structure) are observed, and we are unable to link them to central structure, unless we determine that central structure by independent morphological or base sequencing methods. It should be recognized that functional aspects of structure not actualized are nonexistential in terms of remote sensing techniques, but their potential existence may be inferred from structure.

A recently published book edited by Daly and Uritani (1979) presents 22 articles of a strict empirical phenomenological existential design. Since structure

and function seem to be separate entities in the minds of the authors, the book is without a unifying theme. It is difficult to arrange orders of events from phenomena alone without recourse to structure. The authors have used the mythological format of the biblical book of genesis – a pot pourri of observations of phenomena with a supportive structural scaffold. By contrast, evidence that structure and function are simply different aspects of the same thing are exemplified clearly in the more advanced earth sciences where sediments (structure) "forecast" phenomena in the past and plates, faults "forecast" phenomena in the future.

Unfortunately, the arrangement of topics into four phenomenal (artificial) categories in this major plant pathological work by eminent authorities serves to ignore the irreducible fact that genomic structure = genomic function = specificity. The scientific roots of plant pathology, which have not yet anchored firmly, would have been much better nourished by a concise unified approach under the four categories of specificity with evidence from: (*a*) remote sensing, (*b*) close sensing, (*c*) structure, (*d*) function.

It is interesting to note that some microorganisms are close sensing entities, which in special instances, have been able to link up with the central structure of the host cell. The viral and bacterial nucleic acid-induced diseases are products of such linkages. Equally, the gene for gene interaction outlined by Flor for flax rust and later detected in other parasite-host relations (Day, 1972, 1974) may be considered evidence for successful natural evolutionary experiments by parasites combining the proper mix of remote sensing and central chromosome cytogenetics, of which man, the scientific investigator, has ot been able to match nor elucidate a mechanism. However, the gene for gene hypothesis is supported by random mutation and recombination theory for highly variable organisms in host-parasite relations; the product of single gene encounters from random mixing among two populations of 100 000 units each is 10^{-10}. A theory of externally induced change (of one gene by another), for example MNT, can provide an explanation for changes in instances where the parasitic component is stable.

It is the special advantage of host-parasite relations that their investigation can provide a window on a variety of cellular phenomena. MNT provides by example a unique way of looking at such areas as differentiation and dormancy in agricultural crops which, for the most part, have been approached only by remote sensing disciplines. The extreme complexity of the genome which has only become apparent with the more recent developments in molecular biology makes MNT and other theories of mutagenesis essential to plant pathology in order to hypothesize structural changes in germplasm of host cells resulting in new phenomena.

Pathotoxins comprise the most important sensing tools yet devised for attacks on problems of heritable specificity in plant disease (Wheeler and Luke, 1955; Wheeler, Williams and Young, 1971; Bruck and Mankon, 1980).

MNT places a severe judgment upon exclusive, single method investigations of phenomena in plant pathology and related disciplines which have encountered the complexities of biological specificity; this judgment points to the central chro-

mosomes of the cell as control substrate, and to investigations of all aspects of their condition in order to link peripheral and other phenomena with the genetic factors controlling their heritability.

The origin of theories of variability of organisms stemming from radical chromosomal alterations are traceable back to the distinction between germplasm and somatoplasm formulated by Weismann (Sinnott and Dunn, 1939; Sturtevant and Beadle, 1940). MNT can be expanded into a general theory of host cell chromosome damage by parasites: nematodes, insects, fungi (Whitney, Shaw and Naylor, 1962; Williams, Aist and Bhattacharya, 1973), bacteria, viruses in order to account for host root, stem or floral chimeras, by reciprocal selective or mutagenic effects of the altered host on the parasite, to produce new biotypes and to render the hosts susceptible to them.

MNT is testable on the sweet potato, *Ipomoea batatas* L., and also on the root crops *Dioscorea* species (yam), *Manihot esculenta* Crantz (cassava), *Solanum tuberosum* L. (white or Irish potato), and *Arachis hypogaea* L. (peanut). The sweet potato is the only species within the author's experience which regenerates the entire plant from individual roots but in addition to the above species, horticulturists have been able to propagate raspberries, apples, cabbages, brussel sprouts, several ornamental trees and various weeds from their roots.

Somatic mutations comprise an important source of new types for sweet potato, yam, cassava and white potato. Mutant forms occur also in the peanut and, in addition there is a limited amount of cross pollination under field conditions. Variants resulting from natural crossing generally breed true and subsequent selections in seed producing fields can be maintained without hereditary changes provided that off-types (mutants) are removed as they appear.

MNT predicts production in a nematode-sweet potato nursery of new sweet potato mutants (root chimeras) differing in their reactions to nematodes, as well as differing in horticultural characteristics; and of new nematode biotypes isolated from new sweet potato lines or soil surrounding them, differing from those originally present in the nursery on basis of differential host reactions.

It is then possible in terms of germplasm potentialities to extend MNT to logical limits set by the chromosomes and to predict that formation of new nematode biotypes is independent of the host; i.e., is dependent upon the chromosomes, and that biotypes produced in a sweet potato patch, in terms of comparative genetics, (by analogy let us say to comparative biochemistry or other comparative sciences where entities can be compared between groups or individuals) will be equivalent to those produced on unrelated host plants in natural cropping systems.

Plant parasitic nematodes represent, in terms of MNT, an extreme physicomechanical example of coevolution involving direct effects on chromosomes. According to MNT, the principal force providing new nematode variants (chimeric races) is external and deterministic. The chaotic reciprocal mutagenesis in the pathogen-host relation is able to produce variants in cloning organisms such as mitotic parthenogenetic nematodes and normally hexaploid sweet potatoes. From this point forward, external selection pressures of host, time, space, temper-

ature and microbiological soil factors provide microhabitats which test the survivability of nematode variants.

In a defensive sense, nematode control has developed into a practice of maintaining high odds against establishment of new ecological races. With respect to MNT, this practice can be reaffirmed and extended to include also the maintenance of high odds against production and survival of variants.

The classical theory of variation based on internal mechanisms of random mutation and sexual recombination, combined with deterministic external selection pressures, provides an acceptable model in nematode species producing small numbers of variants from operative internal mechanisms. However large numbers of variants occur in the common root knot nematode species; the internal mechanisms of variation are not operative in these species, and only a few stable biotypes (ecological races) are known. The classical theory provides no explanation for the observed continuous, repetitive production of extremely large numbers of variants; it provides no explanation for the long term maintenance of a few stable biotypes; certainly it provides no explanation for both; this paradox is resolved by MNT.

The evidence presented for MNT supports a system of variation due to environment; a radical life style in a chaotic environment where variation is nongenetic (outside genetic mechanisms, not due to spontaneous mutation, recombination or hybridization). Only genetically stable (cloning) organisms such as root knot nematodes, reproducing by mitotic parthenogenesis and a cloning polyploid host such as sweet potato can survive to maintain stable ecosystems amid chaos. Preliminary evidence of mutagenicity of sweet potato in production of new nematode variants may be seen in Table 1. The number of interactants (nematode variants) may be seen in Table 1. The number of interactants (nematode variants) is greater for sweet potato than for other hosts, in view of the obvious fact that the sweet potato should be genetically stable as a clonal host and less inclined to stimulate production of new nematode variants.

It is interesting to compare opposing forces: the subjection of somatoplasm to mutagenicity by radical effects on germplasm is opposite in effect to cloning which is the placing, by natural or cultural means, of a somatic cell nucleus within an enucleated germplasm cell. It is apparent that mutagenicity and cloning form an equilibrium condition in an ecosystem. Occasions selecting for mutagenicity, such as following sweet potatoes with another host species for the root knot nematode *M. incognita*, could render the ecosystem unstable and result in new biotype formation.

MNT is not Lamarckian per se; inheritance is not changed by environmentally induced phenotypic characteristics. Heritable change is acquired from externally induced chromosome changes.

Stability of ecological races or biotypes, according to accepted random mutation and recombination theory is a function of the stability of the host, represented by the internal genetic stability of a sweet potato clone, and other ecosystem variables; in the light of MNT, however, stability is perpetuated not by host stability but by the death of unstable units, represented by noncloning host such as cotton,

produced by nematode mutagenic action-root chimeras and by soil conditions, including predation factors, which decimate populations of new chimeric races and prevent their survival, spread and development into a new biotype. Destruction of new chimeric races is thus affected by starvation, predation and other host and soil related variables; in other words by destruction of favorable microhabi-

Table 1

Root-knot nematodes (principal species), interactions with crops[a]

Plant	Nematode interactants[b]	Cultivars	Interactants per cultivar
Tomato	87	58	1.50
Soybean	71	48	1.48
Sweet Potato	61	34	1.79
Tobacco	31	24	1.29

[a] Compiled from data assembled by Sasser, J. N. and Kirby, M. F. International Meloidogyne Project. Apr. 1979.

[b] Interactants are all identified species, races and variants of Meloidogyne in each crop.

tats. Obviously, these variables are subject to manipulation by man; they can be manipulated for defensive pest control.

The principal thrust of MNT, however, is offensive. The use of nurseries as a breeding tool for producing crop plants resistant to plant diseases has long been practiced in Europe, at the University of Minnesota, and in many other parts of the world, but the conceptual nexus has been defensive; we are still laboring on a postpest breeding treadmill, producing cultivars with a useful life of 5 years (Day, 1974).

We can circumvent nematodes and perhaps other agents of disease by making them produce plant mutations and mutants of themselves in a nursery; mutants can be "banked" and plant resistance in our principal crops developed against them and held in readiness for the day when a new biotype appears in a natural cropping system.

Evidence in hand indicates that production of new sweet potato cultivars has been accomplished by man and nematodes working in concert. All consequences of MNT are testable by cytological and genetic methods. Laser technology may provide powerful tools for relating chromosomal to phenotypic change in cellular phenomena. Likewise, a comparison of laser beams, X-rays and nematodes as mutagens relates to areas of both scientific interest and free enterprise with reference to the potential patentability of both nematodes and cultivars in the private domain.

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The Summer Fruit Tortrix Moth*: Sex Pheromone Performance in the Field

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Trials carried out in Hungary to evaluate the effect of different amounts of pheromone on the number of captured summer fruit tortrix males. We tested doses of 2 mg, 1 mg in polyethylene caps, and 1 mg, 0.5 mg, 0.25 mg in sections of polyethylene tube. It was noticed that the dose of 2 mg in polyethylene caps gave the best mean catch. The 1 mg dose in sections of polyethylene tube showed superiority over the other doses and releasers.

After the identification of the sex pheromone of the summer fruit tortrix by Ritter (1971), Tamaki et al. (1971) and Meijer et al. (1972), the sex pheromone traps have largely replaced the light traps in monitoring this pest. Many researchers have stated the effect of pheromone quantity on the catch (Minks and Noordink 1971; Minks and Voerman, 1973). We tested the effect of different pheromone doses and releaser types.

Materials and Methods

Trials were carried out in an abandoned apple orchard at Kamaraerdő, and in commercial apple and apricot orchards at Laki-hegy (research station belonging to the University of Horticulture). The trap type used was Pherotrap 1C (Zoecon Corporation, CA 94304, USA) which is a wing-type paperboard construction with a sticky trapping adhesive on the upper surface of the lower section (Fig. 1). The two sections are joined by a wire frame which also serves as a hanger. The pheromone used was of two sources: standard polyethylene caps (Pherocon TM, Zoecon Corp. Production) and a polyethylene tube (1.4 mm outer diameter, and 0.7 mm inner diameter) impregnated with Adoxomone (9: (Z)-9-TDA, and 1: (Z)-11-TDM, product of International Pheromones Ltd., England) supplied by Dr. P. J. Charmillot, Research Station of Changins, Switzerland. We tested doses of 2 mg (two caps), 1 mg (one cap) and 1 mg, 0.5 mg, 0.25 mg (sections from polyethylene tube). Pheromone sources were replaced every six weeks. The catches were recorded weekly and all insects were removed after recording.

* *Adoxophyes orana* (F. v. R.), *Lepidoptera: Tortricidae.*

Fig. 1. Pherocon-lC trap (photo: M. El-Adl)

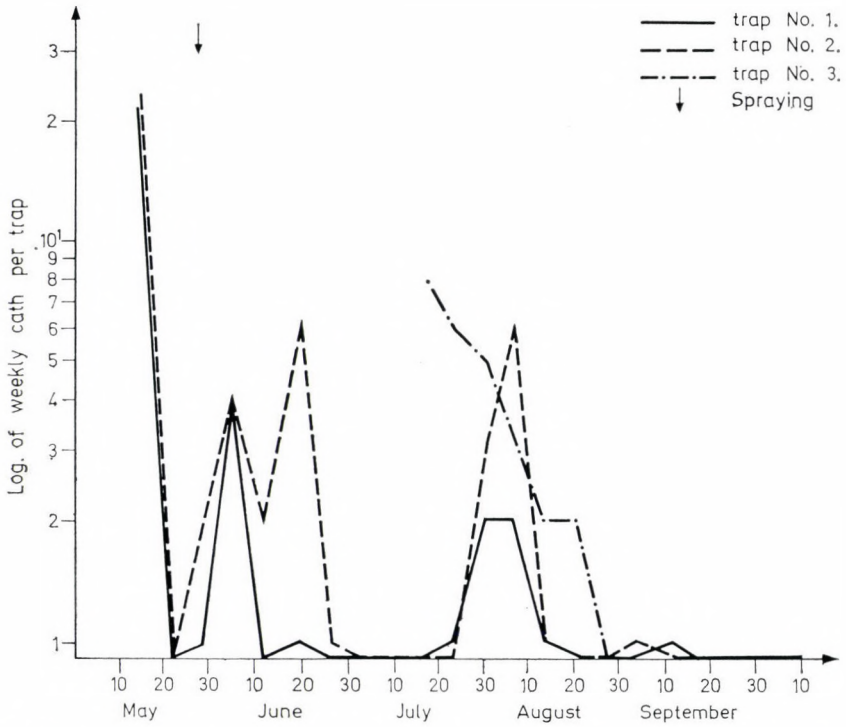

Fig. 2. The summer fruit tortrix moth: seasonal pheromone trapping for 1979 at Kamaraerdő

Table 1

Number of male summer fruit tortrix moths captured in sex pheromone traps with different pheromone doses and releasers at Kamaraerdő in 1979

Date	Trap design, pheromone doses and releaser type			Total	Mean
	Pherotrap 1C baited with 1 mg Adoxomone "Zoecon" in polyethylene cap	Pherotrap 1C baited with 2 mg Adoxomone "Zoecon" in polyethylene cap	Pherotrap 1C baited with 1 mg Adoxomone "Swit" in polyethylene tube		
15.5	21	24	—	45	22.5^{ab}
22.5	0	0	—	0	0.0^{a}
29.5	1	2	—	3	1.5^{a}
6.6	4	4	—	8	4.0^{ab}
13.6	0	2	—	2	1.0^{a}
20.6	1	6	—	7	3.5^{ab}
26.6	0	1	—	1	0.5^{a}
3.7	0	0	—	0	0.0^{a}
10.7	0	0	—	0	0.0^{a}
Total	27	39		66	
Mean of trap catches/week	3^{a}	4.3^{ab}	—	3.66	
17.7	0	0	8	8	2.7^{a}
24.7	1	0	6	7	2.3^{a}
31.7	2	3	4	9	3.0^{a}
7.8	2	6	2	10	3.3^{a}
14.8	1	1	8	4	1.3^{a}
21.8	0	0	2	2	0.7^{a}
28.8	0	0	0	0	0.0^{a}
4.9	0	1	0	1	0.3^{a}
12.9	1	0	0	1	0.3^{a}
18.9	0	0	0	0	0.0^{a}
25.9	0	0	0	0	0.0^{a}
2.10	0	0	0	0	0.0^{a}
16.10	0	0	0	0	0.0^{a}
Total	7	11	24	42	
Mean of trap catches/week	0.5^{a}	0.79^{a}	1.7^{a}	1.0	

— means that the trap was not hung until this date.

Data analyzed by L. S. D. test; means with different letters are significantly different at the 5% level.

Results and Discussion

Kamaraerdő (abandoned orchard)

The trial period lasted 23 weeks (from 8.5.79 to 16.10.79). The weekly catches are presented in Table 1 and graphically in Fig. 2. In the first nine weeks (until 10.7.79) when the pheromone source available was caps of Zoecon, we used doses of 2 mg (two caps) and 1 mg (one cap). There was a significant difference between the means of catches with the two doses (Table 1). The dose of 2 mg captured more moths per week than the dose of 1 mg. In the subsequent 14 weeks (after 10.7.79) beside the afore mentioned pheromone source and doses, we also tested 1 mg in sections of impregnated polyethylene tube. The statistical analysis showed that there was no significant difference in mean catches at the tested doses and releasers, in spite of the high catch of 1 mg in section of polyethylene tube (Table 1).

Laki-hegy (commercial orchard)

The studies lasted 23 weeks (from 9.5.79 to 16.10.79). The weekly catches are presented graphically in Figs 3, 4) and numerically in Table 2. In the first period

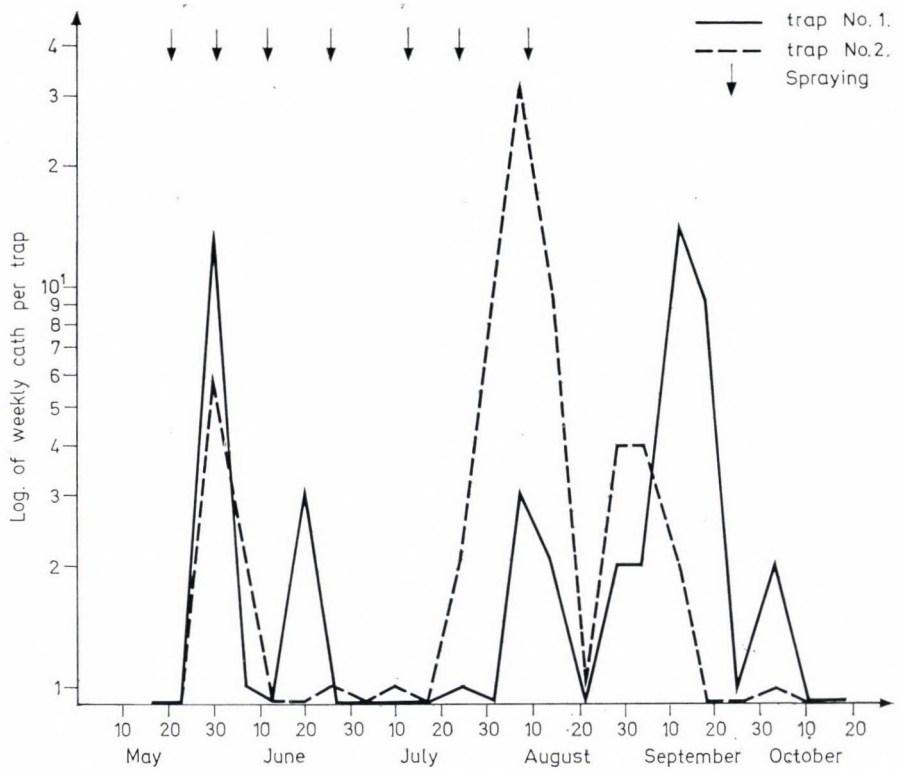

Fig. 3. The summer fruit tortrix moth: seasonal pheromone trapping for 1979 at Lakihegy

Table 2

Number of male summer fruit tortrix moths captured in sex pheromone traps at different pheromone doses and releasers at Laki-hegy in 1979

Date	Trap design, pheromone rate and releaser type					Total	Mean
	Pherotrap 1C baited with 1 mg Adoxomone "Zoecon" in polyethylene cap	Pherotrap 1C baited with 2 mg Adoxomone "Zoecon" in polyethylene cap	Pherotrap 1C baited with 1 mg Adoxomone "Swit" in polyethylene tube	Pherotrap 1C baited with 0.5 mg Adoxomone "Swit" in polyethylene tube	Pherotrap 1C baited with 0.25 mg Adoxomone "Swit" in polyethylene tube		
16.5	0	0	—	—	—	0	0.0[a]
23.5	0	0	—	—	—	0	0.0[a]
30.5	13	6	—	—	—	19	9.5[ab]
7.6	1	2	—	—	—	3	1.5[a]
12.6	0	0	—	—	—	0	0.0[a]
20.6	3	0	—	—	—	3	1.5[a]
26.6	0	1	—	—	—	1	0.5[a]
3.7	0	0	—	—	—	0	0.0[a]
10.7	0	1	—	—	—	1	0.5[a]
Total	17	10	—	—	—	27	
Mean of trap catches/week	1.89[a]	1.1[a]	—	—	—	1.5	
17.7	0	0	7	5	1	73	2.6[a]
24.7	1	2	13	4	2	22	4.4[a]
31.7	0	10	29	11	2	52	10.4[ab]
7.9	3	31	26	15	0	75	15.0[ab]
4.8	2	9	9	7	3	70	6.0[a]
21.8	0	1	1	2	1	5	1.0[a]
28.8	2	4	5	3	1	15	3.0[a]
4.9	2	4	21	11	2	40	8.0[ab]
12.9	14	2	24	19	9	68	13.6[ab]
18.9	9	0	0	0	6	15	3.0[a]
25.9	1	0	0	0	2	3	0.6[a]
3.10	2	1	2	1	0	6	1.2[a]
10.10	0	0	0	0	0	0	0.0[a]
16.10	0	0	0	0	0	0	0.0[a]
Total	36	64	137	78	29	344	
Mean of trap catches/week	2.57[a]	4.57[a]	9.78[ab]	5.57[a]	2.07[a]	X 4.9	

— means that the traps were not hung until this date.

Data analyzed by L. S. D. test; means with different letters are significantly different at the 5% level

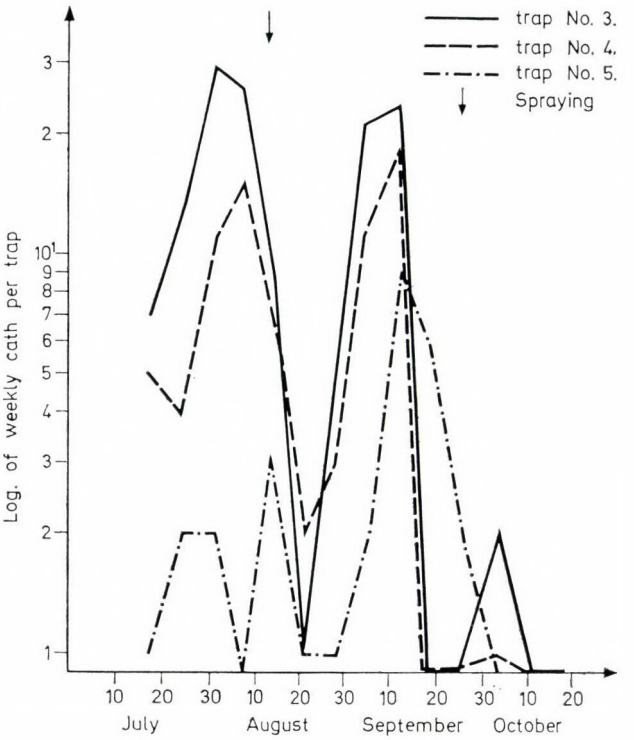

Fig. 4. The summer fruit tortrix moth: pheromone trapping for 1979 at Lakihegy

of the study (until 10.7.79) we tested two pheromone doses, 2 ml and 1mg (caps of Zoecon). There was no significant difference between mean catches with the two doses (Table 2). In the second period (after 10.7.79) which lasted 14 weeks, beside the afore mentioned source and doses we used doses of 1 mg, 0.5 mg, and 0.25 mg in sections of an impregnated polyethylene tube. There was significant difference among the mean catches with the five pheromone doses and the two releasers (Table 2). It was noticed that the dose of 1 mg in a section of the polyethylene tube caught more moths per week than other doses. Also, the dose of 0.5 mg in a section of the polyethylene tube caught more moths per week (without significant difference) than 2 mg and 1 mg in Zoecon caps. This may be due to the difference in age between the two pheromone sources. The polyethylene tube was prepared one week before using it in the field, but the Zoecon caps were kept in a refrigerator for one year.

Acknowledgement

We wish to express our deepest gratitude to Dr. P. J. Charmillot, Research Station of Changins, Switzerland; for supplying the impregnated polyethylene tube.

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Dispersion and Movement Activity of Some Important Moth Pests Living on Stone Fruits*

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From 1975 surveys have been carried out at the Research Institute for Fruit-growing and Ornamentals (Budapest) on the dispersion and movement activity of important moth pests (*Grapholita molesta* Busck, *G. funebrana* Tr., *Adoxophyes orana* F. R., *Anarsia lineatella* Z. and *Enarmonia formosana* Sc.) living on stone fruits. The following conclusions were drawn on the basis of observation of males, with sex pheromone traps in large-scale orchards and in the surrounding territories.

Grapholita molesta does not fly out in significant numbers from the orchard into open areas, and in the absence of its host plants it does not fly to greater distances even in brushland or woody terrain.

Compared with the other examined species (in particular with *G. molesta*), the males of *G. funebrana* show rather intensive movement activity and even the open territories are no such impediment to the dispersal of this moth, as they are for *G. molesta*.

Males of *Anarsia lineatella* and *Enarmonia formosana* fly out from the fruit tree plantations only in limited numbers, however in the case of *A. lineatella* some vegetation types e.g. apple plantations, though they do not present food plant for this moth, do not hinder their dispersal.

Adoxophyes orana does not fly out from orchards in great numbers. The population density is lower in plough-lands variegated with grass, weed strips and roads and in oak forests than in orchards, in spite of the fact that some food plants of this species are present also in the above-mentioned territories.

In recent years an ever increasing demand manifested itself among other countries also in Hungary, to use chemical control methods only when they are really necessary. In other words, efforts were expressed to replace the conventional spraying programmes with a reasonable, "supervised" control system. The demand for a reasonable pest control, however, remains only a desire without a good local forecasting and signalization system. To be able to judge the reliability of the latter, it is necessary to know the vagility and dispersion of local pests, which may (or may not) migrate into the given area from the surrounding territories during a vegetation period.

Any local forecasting or signalization service may help to determine the necessity and optimal time of a given control measure only in case of pests, the dispersal activity of which is limited.

Our knowledge about the dispersion and movement activity of stone fruit pests is rather poor (with the exception of *Adoxophyes orana*). The dispersal of

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A. orana was examined in detail by Barel (1973) and it was established that adults of this microlepidopteran do not fly to greater distances. The same was stated by Maskovich (1930), Fischer (1948), and more recently by Deseő et al. (1971) on the dispersal of *G. funebrana.*

At the Institute for Fruit-growing and Ornamentals surveys have been carried out from 1975 on the dispersal and movement activity of moth pests living on stone fruits (Sziráki, 1979). The examined moth species were (in order of begin of examinations) the following: *Grapholita molesta* Busck, *G. funebrana* Tr., *Adoxophyes orana* F. R., *Anarsia lineatella* Z. and *Enarmonia formosana* Sc.

Methods and Experimental Territories

Our investigations were carried out by utilizing networks of sex pheromone traps for signalization of males.

For trapping of *Grapholita molesta* and *G. funebrana* at first "Orfamone" (Zoecon, USA) afterwards „Atramol" (Roumanian product) pheromone preparations were used (Sziráki, 1978*a*).

For trapping of *Anarsia lineatella* the Roumanian pheromone preparation "Atralin", while in case of *Adoxophyes orana* "Adoxamone" (Zoecon, USA) preparation was used.

For catching males of *Enarmonia formosana* 1 : 1 mixture of Z- and E-9-dodecenyl acetate proved to be suitable (Minks et al., 1976). It is possible that the pheromone preparation "Atramol" used by us in 1980 was contaminated by this compound, as males of *E. formosana* flew in large numbers into the traps baited with "Atramol", these traps could be used for signalization of this moth.

In the first years cylindrical-shaped, later "Pherocon 1C" type traps were used. The pheromone dispensers were changed every 4–6 weeks according to the weather. The sticky surface of the traps was renewed according to necessity. The moths caught were taken from the traps possibly each week and the determination of the microlepidopterous species was carried out on the basis of their genitals (Sziráki, 1978*b*; 1980).

In the first years of the study only traps attracting *G. molesta* (and simultaneously also *G. funebrana*) were placed into different orchards and into territories bordering the orchards at Érd-Elvira (Research Institute for Fruit-growing and Ornamentals), at Törökbálint (Törökbálint State Farm) and at Nagykovácsi Julianna-major (Research Institute for Plant Protection) as it was expounded in an earlier paper (Sziráki, 1979).

In the same territories additional "Atramol" traps were set up after 1978, which were not dealt with in the publication mentioned above. These were the following: $E_{8/a}$ – in a line of poplar trees between plough-lands, NE of the place of trap E_8, at a distance of 250 m; E_{16} – in a peach orchard, near to the place of trap VI; $E_1 f$ – in a peach nursery, NW of the trap E_{16}, at a distance of 450 m; E_{18} – in a peach orchard planted in autumn of 1977, between the places of traps

E_7 and VIII; E_{19} – in a cherry orchard, SW of trap E_9 at a distance of 400 m; E_{20} – in an apple orchard, SW of the place of trap VIII, at distance of 150 m; E_{21} – in an apricot orchard, near to the place of trap "D", at a distance of 150 m; $T_{5/a}$ > in a peach orchard, NE of the place of trap T_5, at a distance of 150 m; $T_{5/b}$ – in a peach orchard, near to the place of trap T_5; $T_{20/a}$ – in an oak forest, in a position similar to trap T_{20} (operated before), NW of its place; $T_{20/b}$ – in an oak forest, SSW from the place of the trap T_{20}, at a distance of 250 m; T_{22} in a peach orchard, NW of the place of the trap T_5, at a distance of 300 m; T_{23} – in a peach orchard, ENE of the trap T_{12}, at a distance of 300 m; T_{24} – in a peach orchard, ESE of the trap T_{12}, at a distance of 400 m; T_{25} – in a peach orchard, SSE of the trap T_{12}, at a distance of 300 m.

At Érd-Elvira and at Törökbálint the traps for *Anarsia lineatella* and *Adoxophyes orana* were placed out to the same places (mainly from 1978), where also the "Atramol' traps operated earlier, or were set up later. So the markings on the traps were similar, but for the exact distinction (both in the text and in Tables 1–6) the traps for *G. molesta* (and for *G. funebrana*) were marked with additional "G", the traps for *Anarsia lineatella* with "Al", and the traps for *Adoxophyes orana* with "Ao" (e.g. E_2G; E_2Al; E_2Ao at Érd-Elvira).

The traps usable for signalization of males of *Enarmonia formosana* (D_{1-5}) were placed into an isolated apricot orchard, and into poplar and Robinia woods bordering the orchard at Dunaharaszti (Fig. 1)

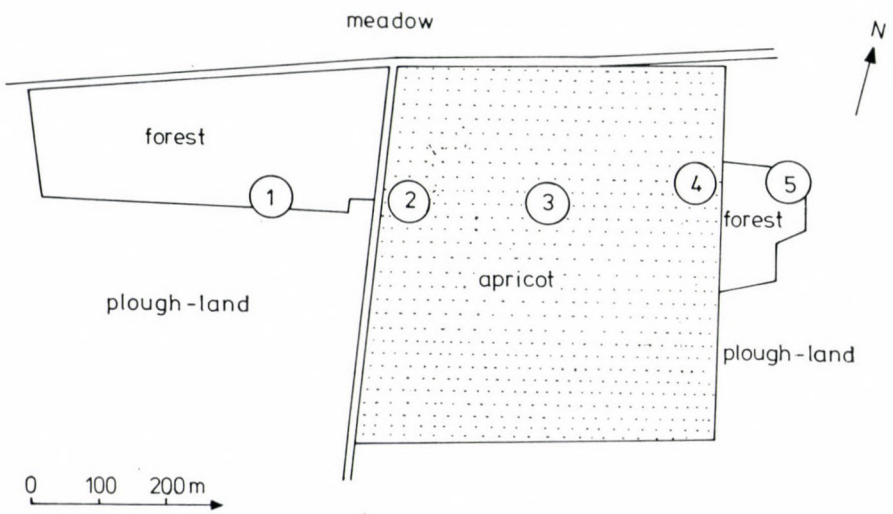

Fig. 1. The distribution of sex pheromone traps at Dunaharaszti

Table 1

The weekly average and the relative value of males of *Grapholita*

Sign of the trap	Fruit species or other vegetation	1977			
		G. molesta		*G. funebrana*	
		Weekly average	Relative value	Weekly average	Relative value
E_1G	Peach	16.1	0.99[a]	11.6	0.31[b]
E_2G	Peach	21.3	1.31[a]	7.9	0.21[b]
E_3G	Peach	14.5	0.90[a]	8.3	0.22[b]
E_4G	Peach	13.0	0.80[a]	11.1	0.29[b]
E_5G	Nut	2.1	0.13[a]	25.5	0.67[b]
E_6G	Plough-land	1.3	0.08[a]	4.7	0.12[b]
E_7G	Pear	0.4	0.02[a]	9.8	0.26[b]
E_8G	Plough-land	0.05	0.003[a]	2.0	0.05[b]
$E_{8/a}G$	Line of trees between plough-land	—	—	—	—
E_9G	Nut	0.5	0.03[a]	9.5	0.25[b]
$E_{10}G$	Sour cherry	1.1	0.07[a]	13.6	0.36[b]
$E_{11}G$	Line of trees next to the orchard	10.8	0.67[a]	10.5	0.28[b]
$E_{12}G$	Plough-land	3.0	0.19[a]	4.5	0.12[b]
$E_{13}G$	Plum	1.0	0.06[a]	35.1	0.93[b]
$E_{14}G$	Plum	2.8	0.17[a]	40.4	1.07[b]
$E_{16}G$	Peach	—	—	—	—
$E_{17}G$	Peach nursery	—	—	—	—
$E_{18}G$	Peach non fruit-bearing	—	—	—	—
$E_{19}G$	Cherry	—	—	—	—
$E_{20}G$	Apple	—	—	–	—
$E_{21}G$	Apricot	—	—	—	—

[a] The unit is the mean catch of the traps in fruit-bearing peach plantations
[b] The unit is the mean catch of the traps in plum plantation

Results and Discussion

Grapholita molesta

The results of sex pheromone trap examinations concerning the dispersal of this pest were reported earlier (Sziráki, 1979).

Most importantly the males of the oriental fruit moth do not fly out in significant numbers from the orchard into open areas even if attracted by sex pheromone, and in the absence of its host plants this microlepidopterous species does not fly to longer distances even in brushland or woody terrain. These statements were confirmed also by our examinations carried out in 1978–1979.

molesta and *G. funebrana* in sex pheromone traps at Érd-Elvira

1978				1979			
G. molesta		*G. funebrana*		*G. molesta*		*G. funebrana*	
Weekly average	Relative value	Weekly average	Relative value	Weekly average	Relative value	Weekly average	Relative value
—	—	—	—	11.3	1.20[d]	4.7	0.53[b]
6.8	1.74[a]	2.8	0.36[b]	11.2	1.19[d]	4.0	0.45[b]
—	—	—	—	—	—	—	—
3.6	0.92[a]	2.8	0.36[b]	1.8	0.62[d]	5.9	0.67[b]
—	—	—	—	—	—	—	—
0.1	0.03[a] 0.02[c]	0.1	0.01[b]	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	0.8	0.09[d]	4.5	0.51[b]
0.2	0.05[a]	1.4	0.18[b]	—	—	—	—
1.4	0.36[a]	2.6	0.34[b]	—	—	—	—
1.1	0.28[a]	1.3	0.17[b]	—	—	—	—
—	—	—	—	—	—	—	—
0.1	0.03[a]	7.7	1.0[b]	1.5	0.16[d]	8.8	1.00[b]
—	—	—	—	—	—	—	—
1.2	0.31[a]	1.9	0.25[b]	—	—	—	—
0.8	0.21[a] 0.67[c]	0.5	0.06[b]	—	—	—	—
0.04	0.01[a]	0.5	0.06[b]	0.7	0.07[d]	3.5	0.40[b]
0.2	0.05[a]	1.2	0.16[b]	—	—	—	—
0.1	0.03[a]	1.9	0.25[b]	1.9	0.20[d]	8.3	0.94[b]
0.6	0.15[a]	2.8	0.36[b]	—	—	—	—

[c] The unit is the mean catch of the traps in the nearest fruit-bearing peach plantation
[d] The unit is the mean catch of the traps E_1G—E_4G in 1979

In 1979 at Érd-Elvira in a fruit-bearing peach plot the traps E_2G and E_4G caught 170 and 91 (weekly average 6.8 and 3.6) males of oriental fruit moth, respectively, while the trap E_6G being on an open area, at a distance of 150 m from the above-mentioned peach plantation, caught only 3 (0.1 weekly average) exemplars. That means a 0.02 relative value, taking the mean catches of the first-mentioned two traps as 1 (Table 1).

In 1979 at Törökbálint in the oak forest bordering the orchard a new trap worked at a distance of 300 m from the plantation. (The other trap $T_{20}/_a$ worked already in 1978 in this forest at a distance of 50 m from the peach orchard.)

Considering the mean catch of traps $T_{14}G$ and $T_{21}G$ operating in the neigh-

Table 2

The weekly average and the relative value of males of *Grapholita*

Sign of the trap	Fruit species or other vegetation	1976				1977	
		G. molesta		*G. funebrana*		*G. molesta*	
		Weekly average	Relative value	Weekly average	Relative value	Weekly average	Relative value
T_1G	Peach	16.6	1.31[a]	21.2	1.75[a]	8.2	1.19[a]
T_2G	Peach	18.4	1.45[a]	10.1	0.83[a]	—	—
T_3G	Apple non fruit-bearing	0.5	0.04[a] 0.03[b]	3.0	0.25[a] 0.19[b]	1.0	0.14[a] 0.12[b]
T_4G	Apple non fruit-bearing	0.7	0.06[a] 0.04[b]	2.4	0.20[a] 0.15[b]	—	—
$T_{5,\ 5/a}G$	Peach	14.1	1.11[a]	7.9	0.65[a]	—	—
$T_{5/b}G$	Peach	—	—	—	—	—	—
T_6G	Peach	10.6	0.83[a]	7.3	0.60[a]	—	—
T_7G	Peach	6.5	0.51[a]	6.3	0.52[a]	—	—
T_8G	Peach	4.9	0.39[a]	8.4	0.69[a]	—	—
T_9G	Peach	5.7	0.45[a]	12.5	1.03[a]	—	—
$T_{10}G$	Peach	6.6	0.52[a]	13.0	1.07[a]	—	—
$T_{11}G$	Peach	9.1	0.72[a]	8.3	0.69[a]	—	—
$T_{12}G$	Peach	9.6	0.76[a]	12.7	1.05[a]	—	—
$T_{13}G$	Peach	14.9	1.17[a]	15.3	1.26[a]	—	—
$T_{14}G$	Peach	10.4	0.82[a]	9.6	0.79[a]	5.6	0.81[a]
$T_{15}G$	Peach	11.7	0.92[a]	16.8	1.39[a]	—	—
$T_{16}G$	Peach	12.4	0.98[a]	13.0	1.07[a]	—	—
$T_{17}G$	Quince	17.8	1.40[a] 0.46[b]	7.4	0.61[a] 0.40[b]	—	—
$T_{18}G$	Peach	38.5	3.03[a]	18.7	1.54[a]	—	—
$T_{19}G$	Apple	—	—	—	—	1.7	0.25[a] 0.21[b]
$T_{20,\ 20/a}G$	Forest (Oak)	—	—	—	—	2.1	0.30[a] 0.38[b]
$T_{20/b}G$	Forest (Oak)	—	—	—	—	—	—
$T_{21}G$	Peach	—	—	—	—	—	—
$T_{22}G$	Peach	—	—	—	—	—	—
$T_{23}G$	Peach	—	—	—	—	—	—
$T_{24}G$	Peach	—	—	—	—	—	—
$T_{25}G$	Peach	—	—	—	—	—	—

[a] The unit is the mean catch of the traps in peach plantations

bouring peach plantations (9.3 males/week) as unit, the relative catch value of trap $T_{20/a}G$ (1.4 males/week) was 0.15 (in the previous year it was similar: 0.20, while in the case of $T_{20/b}G$ only 0.05) (Table 2).

It is characteristic of the relatively low movement activity of oriental fruit moth that – as shown by trap $E_{18}G$ – in a young peach orchard this pest did not settle in high densities even in the second year after plantation. Here 0.7 males/

molesta and *G. funebrana* in sex pheromone traps at Törökbálint

1977		1978				1979			
G. funebrana		*G. molesta*		*G. funebrana*		*G. molesta*		*G. funebrana*	
Weekly average	Relative value	Weekly average	Relative value	Weekly average	Relative value	Weekly average	Relative value	Weekly average	Relative value
21.8	0.87[a]	16.5	1.39[a]	4.1	1.11[a]	7.4	0.86[a]	7.5	0.90[a]
—	—	—	—	—	—	—	—	—	—
7.5	0.30[a] 0.34[b]	4.5	0.38[a] 0.27[b]	1.1	0.30[a] 0.27[b]	3.4	0.40[a] 0.46[b]	1.8	0.22[a] 0.24[b]
—	—	—	—	—	—	—	—	—	—
—	—	7.0	0.59[a]	1.8	0.49[a]	—	—	—	—
—	—	10.5	0.88[a]	4.2	1.14[a]	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	8.9	0.75[a]	3.4	0.92[a]	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	7.2	0.61[a]	3.2	0.86[a]	—	—	—	—
—	—	—	—	—	—	—	—	—	—
20.5	1.3[a]	22.3	1.87[a]	3.2	0.86[a]	10.5	1.22[a]	8.1	0.98[a]
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
12.0	0.48[a] 0.55[b]	2.2	0.18[a] 0.13[b]	1.3	0.35[a] 0.32[b]	—	—	—	—
24.3	0.96[a] 0.85[b]	4.5	0.38[a] 0.20[b]	3.0	0.81[a] 0.61[b]	1.4 0.5	0.16[a] 0.15[b] 0.06[a] 0.05[b]	7.4 5.5	0.89[a] 0.84[b] 0.66[a] 0.63[b]
—	—	—	—	—	—				
—	—	19.9	1.67[a]	6.6	1.78[a]	8.0	0.93[a]	9.4	1.13[a]
—	—	15.3	1.29[a]	2.9	0.78[a]	—	—	—	—
—	—	11.0	0.92[a]	5.7	1.54[a]	—	—	—	—
—	—	8.4	0.71[a]	3.8	1.03[a]	—	—	—	—
—	—	4.0	0.34[a]	1.5	0.41[a]	—	—	—	—

[b] The unit is the mean catch of the traps in the nearest peach plots

week were caught in trap. It means 0.07 relative value, considering the mean result of traps in the fruit-bearing peach orchard (E_2G, E_1G, E_4G) as unit (Table 1).

Grapholita funebrana

As shown in Table 2, males of *G. funebrana* flew regularly and in large numbers into the "Orfamone" or "Atramol" sex pheromone traps worked in the

orchard (mostly of peach) at Törökbálint in spite of the fact that peach is not an important food plant of *G. funebrana.* In the first part of August 1978 at Törökbálint around trap $T_{21}G$ we examined 3000 peach fruits; 0.5% of the fruits were damaged by moths, but in the cases when also the larvae were found, they turned out to belong to *G. molesta.*

This fact indicates that the males of *G. funebrana* have a rather intensive movement activity (at least in comparison with *G. molesta).* The majority of the *G. funebrana* males caught in the peach plantation, very likely emerged not from the peach orchard itself, but might have flown infrom plum trees of back-yard orchards, from a distance of a few kilometers.

This conclusion is supported by the fact that approximately the same number or *G. funebrana* flew into traps $T_{20}G$ and $T_{20/a}G$ placed in the oak forest next to the orchard, as into the traps in the neighbouring plots of the peach orchard.

Considering the mean catch result of all traps in the peach orchard at Törökbálint as a unit, the relative value of the results of traps $T_{20}G$ and $T_{20/a}G$ was 0.96 in 1977; 0.81 in 1978 and 0.89 in 1979. The relative values are similar (0.85, 0.61 and 0.84, respectively) if the mean results of traps $T_{14}G$ and $T_{21}G$ in the nearest peach plantations are regarded as unit. These values are much higher than in the case of *G. molesta* (Table 2).

In 1979 the mean catch result (5.5 males/week) of the trap $T_{20/b}G$ in the oak forest at a distance of 300 m from the edge of the orchard, differs even much more from the very decreased value of *G. molesta* observed in the same sex pheromone trap. The relative value of catch of the trap was 0.66 compared with the mean catch of the whole peach orchard, and 0.63 if the mean result of the traps $T_{14}G$ and $T_{21}G$ in the nearest peach plantations is regarded as unit. Compared with the data of trap $T_{20/a}G$ the catch decrease with the distance from the peach orchard is not significant.

However the catch results were low in traps T_3G and T_4G in a young, non-fruit-bearing apple plantation, (in an almost open terrain compared with the peach plantation or with the forest). Their relative value fluctuated between 0.15 and 0.34 regarding the results of the trap in the nearest peach plantation as unit.

Taking the relatively high movement activity and the high inclination for dispersal into consideration, this observation really does not show that *G. funebrana* does not fly out in significant quantities into open territories, but it indicates that the males of this moth usually do not alight in such terrain, even if attracted by sex pheromone preparations.

The same conclusion may be drawn on the basis of data of sex pheromone traps operated at Érd-Elvira. In the orchard with different fruit plantations *G. funebrana* flew into the traps in a significant number outside the plum plantation as well, if the traps were not in an open territory. The values related to the mean catches of the traps in plum plantation ($E_{13}G$, $E_{14}G$) were rather similar to each other: they ranged from 0.21 to 0.36 in 1977 (Table 1). (The result of trap E_5G in this case has to be left out of consideration, because nearby there was a small, but infested plum plantation.)

Table 3

The weekly average and the relative value of males of *Anarsia lineatella* in sex pheromone traps at Érd-Elvira

Sign of the trap	Fruit species or other vegetation	1978		1979	
		Weekly average	Relative value	Weekly average	Relative value
E_2Al	Peach	2.5	0.68[a]	10.8	1.96[a]
E_4Al	Peach	2.4	0.65[a]	3.0	0.55[a]
E_6Al	Plough-land	0.4	0.11[a] 0.16[b]	1.0	0.18[a] 0.14[b]
$E_{8/a}Al$	Line of trees between plough-lands	0.2	0.05[a]	0.2	0.04[a]
E_9Al	Nut	0.5	0.14[a] 0.50[b]	2.0	0.36[a] 0.34[b]
$E_{10}Al$	Sour cherry	0.7	0.19[a]	2.8	0.51[a]
$E_{11}Al$	Line of trees next to the orchard	0.7	0.19[a] 0.28[b]	2.1	0.38[a] 0.30[b]
$E_{13}Al$	Plum	3.1	0.84[a] 1.24[b]	1.5	0.27[a] 0.22[b]
$E_{16}Al$	Peach	1.3	0.35[a]	5.7	1.04[a]
$E_{17}Al$	Peach nursery	2.1	0.57[a]	—	—
$E_{18}Al$	Peach non fruit-bearing	1.0	0.27[a]	5.9	1.07[a]
$E_{19}Al$	Cherry	0.4	0.11[a]	3.8	0.69[a]
$E_{20}Al$	Apple	1.1	0.30[a] 0.25[b]	4.7	0.85[a] 1.15[b]
$E_{21}Al$	Apricot	5.7	1.54[a]	2.3	0.42[a]
$E_{22}Al$	Apricot	6.4	1.73[a]	—	—

[a] The unit is the mean catch of the traps in fruit-bearing peach and apricot plantations
[b] The unit is the mean catch of the traps in the nearest peach and/or apricot plantations

Only few males of *G. funebrana* flew into the traps in open terrains (E_6G, E_8G, $E_{12}G$) or in the one-year-old peach plantation ($E_{18}G$), which in 1978 had an open character. Considering the mean catch of trap(s) in the plum orchard as unit, the relative value of catches of the above-mentioned traps was 0.05–0.12. On the other hand, in the case of traps E_7G and E_9G in plots of fruit trees which were non-hosts of *G. funebrana* (pear, walnut) the values were higher, even if these plantations were separated from plum by open territories.

The difference between the dispersal of *G. funebrana* and *G. molesta* appears sharply in case of trap $E_{8/a}G$ in an alley of trees between plough lands (consequently separated from the fruit plantations by more or less open territory).

This trap, at a distance of 300 m and 700 m from the plum and peach plantations, respectively, caught 4.5 males of *G. funebrana* weekly in 1979. This relative value – considering the catch result of the trap operated in the plum plantation as 1 – was rather high: 0.51. The same sex pheromone trap in the same year caught 0.8 males of oriental fruit moth weekly. Considering the mean catch result of traps in fruit bearing peach plantations as unit, the relative value was only 0.09.

It is to be noted that trap $E_8/_aG$ was roughly South of the plum (and peach) plantations. Because the main wind direction is north-western in the examined territory, the relatively high catch results in case of *G. funebrana* could not be explained as a result of the positive anemotactic response of males to the effect of sex pheromone.

Anarsia lineatella

On the basis of data shown in the Tables 3 and 4 it seems that the males of *A. lineatella* fly out from the orchards only in a limited quantity.

Table 4

The weekly average and the relative value of males of *Anarsia lineatella* in sex pheromone traps at Törökbálint

Sign of the trap	Fruit species or other vegetation	1978		1979	
		Weekly average	Relative value	Weekly average	Relative value
T_1Al	Peach	2.3	1.21[a]	12.0	1.14[a]
T_3Al	Apple	0.4	0.21[a] 0.17[b]	5.1	0.49[a] 0.43[b]
T_5Al	Peach	1.5	0.79[a]	14.0	1.33[a]
$T_{12}Al$	Peach	2.2	1.16[a]	12.0	1.14[a]
$T_{14}Al$	Peach	1.9	1.00[a]	4.6	0.44[a]
$T_{19}Al$	Apple	1.0	0.53[a] 0.43[b]	3.3	0.31[a] 0.28[b]
$T_{20/a}Al$	Forest (oak)	0.2	0.11[a] 0.11[b]	0.5	0.05[a] 0.10[b]
$T_{20/a}Al$	Forest (oak)	–	–	0.5	0.05[a] 0.10[b]
$T_{21}Al$	Peach	1.6	0.84[a]	5.3	0.50[a]
$T_{22}Al$	Peach	1.8	0.95[a]	11.5	1.10[a]
$T_{23}Al$	Peach	2.3	1.21[a]	12.6	1.20[a]
$T_{25}Al$	Peach	1.4	0.74[a]	12.3	1.17[a]

[a] The unit is the mean catch of the traps in peach plantations
[b] The unit is the mean catch of the traps in the nearest peach plots

At Törökbálint in 1978 0.2–, in 1979 0.5 males/week flew into trap $T_{20}/_{a}Al$ being in an oak forest at a distance of 50 m from the edge of the peach orchard. Considering the mean catches of traps $T_{14}Al$ and $T_{21}Al$ (both in the nearest peach plots) as unit, the relative value of it was 0.11 in 1978, and 0.10 in 1979.

The relative value of catch results of trap E_6Al in a plough-land at Érd-Elvira (compared with the results of the traps in the peach orchard at a distance of 150 m) was 0.16 in 1978, and 0.14 in 1979.

In both years weekly 0.2 males of *A. lineatella* flew into the trap E_8Al in a line of poplar trees between plough-lands, and at a distance of more than 500 m from the peach and apricot plantations. The relative value of the above-mentioned catch results (considering the mean results in peach and apricot plantations as unit) were 0.05 and 0.04 in the two successive years.

In connection with the dispersal ability of *A. lineatella* we have to discuss the catch results of trap $E_{20}Al$. This trap was set up in an apple plot. In 1977 a peach plantation was established next to that orchard. Its other side, which was farther away from the trap, was bordered by an apricot plot. The distance between the trap and the edge of peach plantation was about 80 m. In 1978 1.1 males of *A. lineatella* flew into the above-mentioned trap weekly, while in 1979 4.7 weekly. These results mean 0.30 and 0.85 relative values, respectively – considering the mean catch results of the traps in fruit-bearing peach and apricot plantations as 1.

On one hand, this increase is parallel to the fact that by 1979 *A. lineatella* settled in large numbers in the young peach plantation, while on the other, it shows that apple plantation – although to our knowledge apple is not a food plant of *A. lineatella* – does not form such an obstacle in the dispersal of this pest as forest or open territories do.

Adoxophyes orana

According to our experiments, this pest may be found in a lesser population density than the above-mentioned three moth species. Besides, the attractivity of the sex pheromone traps applied were not so intensive than in the case of the other examined moths, consequently on the basis of the catch results (Tables 5 and 6) we can draw conclusions with less certainty.

However, it is worth mentioning that in 1979 at Érd-Elvira and at Törökbálint not a single male of *A. orana* flew into the traps outside the orchard (E_6–, $E_8/_{a}$–, T_{20}–, $T_{20}/_{a}$–, $T_{20}/_{b}Ao$), and considering the catch results of the traps in fruit-bearing peach orchards as unit, in the case of traps $E_8/_{a}Ao$ and $T_{20}Ao$ the relative value was very low also in 1978: 0.07 and 0.12, respectively.

This fact – in accordance with the findings of Barel (1973) – show that *A. orana* does not fly out of the orchard in significant numbers either, and its population density is higher in the orchards than in plough-lands broken up by strips of weeds, grass and by roads, or than in oak forests, in spite of the fact that some of its food plants occur there as well.

Table 5

The weekly average and the relative value of males of *Adoxophyes orana* in sex pheromone traps at Érd-Elvira

Sign of the trap	Fruit species or other vegetation	1978		1979	
		Weekly average	Relative value[a]	Weekly average	Relative value[a]
E_2Ao	Peach	1.7	1.89	0.8	2.00
E_4Ao	Peach	0.6	0.67	0.2	0.50
E_6Ao	Plough-land	0.4	0.44	0	0
$E_{8/a}Ao$	Line of trees between plough-lands	0.06	0.07	0	0
E_9Ao	Nut	0.1	0.11	0.1	0.25
$E_{10}Ao$	Sour cherry	0.4	0.44	0.1	0.25
E_{11} Ao	Line of tree next to the orchard	0.5	0.56	0	0
$E_{13}Ao$	Plum	0.6	0.67	0.1	0.25
$E_{16}Ao$	Peach	0.4	0.44	0.2	0.5
$E_{17}Ao$	Peach nursery	0.5	0.56	—	—
$E_{18}Ao$	Peach non fruit-bearing	0.1	0.11	0.1	0.25
$E_{19}Ao$	Cherry	0.8	0.89	0.1	0.25
$E_{20}Ao$	Apple	0.2	0.22	0.3	0.75
$E_{21}Ao$	Apricot	1.1	1.22	0.1	0.25

[a] The unit is the mean catch of the traps in fruit-bearing peach plantations

Table 6

The weekly average and the relative value of males of *Adoxophyes orana* in sex pheromone traps at Törökbálint

Sign of the trap	Fruit species or other vegetation	1978		1979	
		Weekly average	Relative value[a]	Weekly average	Relative value[a]
T_1Ao	Peach	0.3	0.60	0	0
T_3Ao	Apple non fruit-bearing	0.1	0.20	0.2	1.00
$T_{5/b}Ao$	Peach	0.6	1.20	0.3	1.50
$T_{12}Ao$	Peach	0.4	0.80	0.2	1.00
$T_{14}Ao$	Peach	1.3	2.60	0.1	0.50
$T_{19}Ao$	Apple	0.3	0.60	0	0
$T_{29/a}Ao$	Forest (oak)	0.06	0.12	0	0
$T_{20/b}Ao$	Forest (oak)	—	—	0	0
$T_{21}Ao$	Peach	0.6	1.20	0.3	1.50
$T_{22}Ao$	Peach	0.2	0.40	0.2	1.00
$T_{23}Ao$	Peach	0.06	0.12	0.7	2.33
$T_{25}Ao$	Peach	0.6	1.20	0.1	0.50

[a] The unit is the mean catch of the traps in peach plantations

Enarmonia formosana

We have data of sex pheromone traps about the dispersal of this pest only from the year 1980, and from Dunaharaszti, a single experimental territory. However, the data demonstrated in Table 7 show very clearly that the males of this microlepidopterous species fly out of the plot of the food plants only to a small percent.

Table 7

The weekly average and the relative value of males of *Enarmonia formosana* at Dunaharaszi

Sign of the trap	Fruit species or other vegetation	Weekly average	Relative value[a]
D_1	Forest (poplar)	0.2	0.04
D_2	Apricot	3.9	0.74
D_3	Apricot	4.3	0.81
D_4	Apricot	7.8	1.47
D_5	Forest (Robinia)	0.4	0.08

[a] The unit is the mean catch of the traps in apricot orchard

Considering the mean catch result (5.3 males/week) or the three traps (D_2, D_3, D_4) in the apricot orchard as unit, the relative value of the catch result of trap D_1 (in a poplar forest W of the orchard, at a distance of 150 m from its edge) was 0.04, while the same value in the case of trap D_5 (operated in a Robinia forest E of the orchard, at a distance of 100 m) was 0.08.

Conclusions

The surveys carried out in the years 1978–79 supported the previous statements of the author (Sziráki, 1979) that the of *Grapholita molesta* Busck do not fly out from the orchard into open territory, even if attracted by sex pheromone, and in absence of its host plants this moth does not fly to greater distances even in brushland or woody terrain.

The males of *G. funebrana* Tr., at least compared with other species examined (in particular compared with *G. molesta)*, show a rather intensive movement activity. Open territories are not such an impediment for the dispersal of this moth than they are for *G. molesta*, even if we consider that the males generally do not fly into the traps placed into open territories.

On the basis of the results, it may be concluded that the males of *Anarsia lineatella* Z. fly out from the fruit plantations only in limited numbers if these are bordered by open territories or by forests (of oak, poplar, or Robinia), while

some other types of vegetation, e.g. apple plantations, do not hinder its dispersal, although they are not food plants for this moth.

As it was already established by Barel (1973), *Adoxophyes orana* F. R. does not fly out of the orchard in significant numbers. Its population density is also higher in orchards than in plough-lands divided by strips of weeds, grass and roads, in spite of the fact that some of its food plants occur there as well.

Observations of a single year (1980) suggest that the males of *Enarmonia formosana* Scop. do not fly out in significant numbers from orchards presenting their food plants either.

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Results of Faunistical Studies in Hungarian Maize Stands (Maize Ecosystem Research No. 16)

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The authors commenced the study of agro-ecosystems in Hungarian maize stands in 1976. The present work contains the list of animal species established during the first 5 years of the series of studies (1976–1980), planned for 10 years. In the maize stands investigated in regular surveys the presence of 582 animal species was registered with different methods.

On the suggestion of Dr. T. Jermy, Academician, the Department of Zoology of the Research Institute of Plant Protection of the Hungarian Academy of Sciences launched a series of surveys in 1976 in agro-ecosystems. The work, planned for a period of 10 years, is being supported by a special grant of the Hungarian Academy of Sciences. The surveys are carried out in two crop plant stands, apple and maize; these are the two plant types in which the considerable changes are the best represented that occurred in the Hungarian agriculture as regards both production technology and agro-ecology. For the research work also the co-operation of many scientists, working in related research institutions, could be obtained.

The basic aim of our studies was the ecological foundation of the integrated plant protection to be introduced in the industrial-scale production systems. For that purpose the animal communities of agricultural areas under treatments of different intensities are studied, with special regard to factors influencing the population dynamics of the most important phytophagous species. These factors themselves underwent many changes under the influence of up-to-date production technologies.

Besides the aims of close practical interest also important conclusions can be drawn as regards the types and significance of controlling mechanisms acting in the Hungarian agro-ecosystems (Jermy, 1977, 1979). For that purpose first the "inventory" of the species occurring in agro-ecosystems had to be assessed. In the following the results of surveys (1976–1980) in maize stands produced in continuous monoculture and rotation system, respectively, are summarized.

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Review of the Literature

By reviewing the faunistical literature related to maize, no work of similar dimension has been found as regards both the wide range of methods applied and the high number of species detected.

Camprag et al. (1971) described the herbivorous species occurring in maize. The enumeration, ranged into systematic order, reported on species of 11 insect orders, five invertebrate and three vertebrate groups, with special regard to Yugoslavian conditions. Tawfik et al. (1974) studied maize plots in Egypt for two years and detected, by sweeping and single collections, phytophagous and zoophagous species from 66 families of 14 insect orders. Wedberg et al. (1975) collected in irrigated maize plots (about 60 ha) in the USA for 2 years, using pitfall traps, sweeping net, plant surveys and vacuum technique; in their work incomplete lists of species from seven insect orders are given. In France, D'Aguilar and Chambon (1977) made surveys in three year's rotation system and reported on the numerical changes in pests, predators and indifferent populations, without details regarding their species composition. Lacatusu et al. (1978) collected in Roumanian maize monocultures for three years; their paper contains data on the most important phytophagous and predatory species belonging to eight insect orders.

Sites of Assessment

The experimental areas are situated in the Department Fejér. By the beginning of the surveys no more traditional, small maize plots existed in this part of the country, so the comparative studies had to be made in two large-scale farms with different methods of cultivation.

2.1. *Continuous monoculture:* Agárd State Farm, Tükröspuszta, about 400 ha. The cultivation of the maize is carried on with maximal mechanization and intensive chemical treatment. In the area maize has been grown since 1965. Surrounding area: cultivated cropland, on one side of the plot a neglected park. The soil is medium-heavy loam.

2.2. *Maize grown in rotation system:* Vörösmarty Cooperative, Kápolnásnyék. The site of surveys changed according to a three-year rotation system. The preceding crop was never maize and with the exception of one year (1977) the maize was preceded by winter wheat. According to local conditions the area of experimental plots varied between 20 and 100 ha. Surrounding area: cultivated cropland and meadows. The soil is medium-heavy loam.

Methods

A short summary of methods and their serial numbers is given in Table 1. In the lists of species (p. 71) the serial numbers of the methods were written into the individual columns, thus these do not refer to quantitative relationships.

Table 1

Methods used in the survey of maize ecosystems

1. Light trap	collects from air space
2. Pheromone trap	collects from air space
3. Yellow pan trap	collects from air space
4. Individual plant survey, rearing	collects from maize plants
5. Mite brushing	collects from maize plants
6. Stem trap	collects from maize plants
7. Sweeping on maize	collects from maize plants
8. Sweeping from weeds	collects from weed level
9. Individual weed survey, rearing	collects from weed level
10. Soil pitfall trap	collects on soil level
11. Individual collection on soil surface	collects on soil level
12. Soil sampling	collects in soil upper level

Some of the methods contained in the list were not used in the first part of the surveys. As these will be used in the second part of the experimental period it seemed worth while to mention them.

The methods are summarized as follows:

1. Light trap. A simple, Jermy-type light trap with 100 W normal light bulb as described by Jermy (1961). The light trap was operated in area 2.1. from maize sowing until harvest, emptied daily.

2. Sex trap. In the maize plots no pheromone traps were operated in the period 1976–1980.

3. Yellow pan trap. No yellow pan traps were operated in the period 1976–1980.

4. Individual plant assessment, rearing. In this column are mentioned the adults and larvae reared to adults, collected in the weekly surveys on individual plants. Also parasites reared and predators found on the individual plants are mentioned here.

5. Mite brushing. No mite-brushing was carried out from maize leaves during the period 1976–1980.

6. Stem trap. The traps serve for catching the insects moving upward or downward on the maize stems.

7. Sweeping on maize plants. The maize is swept weekly, by applying 100 strokes with the sweeping net.

8. Sweeping of weeds. The weed border and weedy areas neighbouring the maize plots are weekly surveyed by a sweeping net.

9. Study of individual weed plants, rearing. Identical with the method mentioned under No. 4.

10. Pitfall trap. Plastic cups are sunk into the soil, with their brim at soil level and containing salt solution or ethylene glycol. The traps are emptied weekly.

If small mammals, like voles are killed in the trap, the content is discarded from hygiene reasons.

11. Individual collection on soil surface. Single animals, occurring on the soil surface of maize plots are hand-picked.

12. Soil sampling. For coleopterological studies square holes are dug (100 × × 100 cm, 50 cm deep), for nematological studies 5 × 100 cm^3 soil samples are taken from the upper soil layer.

Review of Taxons Included into the Collections

Phylum: Nemathelminthes

Class: Nematoidea

Nematological studies were carried out in both areas. The surveys and determinations were made by Ilona M. Benedek. The whole collected material was worked up and from the areas surveyed 22 species were demonstrated.

Phylum: Arthropoda

Class: Insecta

Super-ordo: Orthopteroidea (orders *Saltatoptera, Dermaptera)*

Orthopterological studies were made in both areas. The determination was made by László Nagy and the collected material was worked up. In the maize plots 11 *Orthopteroidea* species were detected.

Ordo: Homoptera

Aphidological studies were carried out in both areas. The determinations were made by László Szalay-Marzsó and András Meszleny. Coccidological studies were also carried ont in both areas, the material was worked up by Ferenc Kozár. In the maize plots studied 21 *Aphidoidea* and 8 *Coccoidea* species were detected.

Ordo: Heteroptera

The survey of *Heteroptera* was made in both areas. All material was worked up, the determination was made by Vera Rácz. In the maize plots 73 species were found.

Super-ordo: Neuropteroidea (orders *Raphidioptera, Planipennia = Neuroptera)*

Neuropterological studies were made in both survey areas, by using different methods. The materials were worked up by Ferenc Szentkirályi and Sándor Szabó. In the maize plots 20 species were detected.

Ordo: Coleoptera

Coleopterological studies were made in both areas. The material was worked up by László Ádám and Gábor Lővei. The *Coleoptera* material collected by the light trap is still partly determined. In the maize plots studied 114 species were detected.

Ordo: Lepidoptera

The lists presented are based on light trap catches and rearings. The materials were completely worked up. The "Microlepidoptera" were determined by Csaba Szabóky, with the corn borer catches Ferenc Szentkirályi dealt with. The larvae of "Macrolepidoptera" were reared by Zoltán Mészáros and determined by Zoltán Mészáros and László Ronkay. In the maize plots studied 92 "Microlepidoptera" and 118 "Macrolepidoptera" were detected.

Ordo: Diptera

Dipterological studies were carried out in both areas but only one part of the material collected was worked up. The determinations were made by Ágnes D. Draskovits, Ferenc Mihályi, László Papp and Árpád Soós. In the maize plots studied 34 species were detected.

Ordo: Hymenoptera

Hymenopterological studies were carried out in both areas but only one part of the material was worked up. The determinations were made by Jenő Papp, László Polgár and Gusztáv Szelényi. In the maize plots 57 species were detected.

Class: Arachnoidea

Ordo: Acaridea

Acarological studies were made in both areas but the material is still being worked up. The soil mites are determined by Sándor Mahunka.

Discussion

In course of the five years the presence of 582 animal species was observed in the maize plots regularly surveyed (Table 2.). The work is far from being finished as the list may considerably increase by the completion of determinations in some groups (Arachnoidea, Thysanoptera, Cicadoidea etc.). From other groups, like Diptera and Hymenoptera only some parts of the collected materials were determined so far.

The authors are well aware of the fact that a considerable part of the species reported herein does not depend on the maize plants as energy sources but belong to the fauna of their environment and were brought into the area studied by active or passive movement. However, it is also a fact that these species are, at least as adults, present in the maize plots and may become parts of trophic chains either as consumers or preys. Their occurrence is thus by no means indifferent to the community of a given area.

As comparison it may be mentioned that in Hungary only in two protected areas were extensive surveys carried out so far, that had aimed to explore the fauna as completely as possible. One of those is the ancient marshland Bátorliget, the faunistical survey of which has been carried out in the 1950-ies (Székessy et al., 1953), the other is the Hortobágy National Park. The faunistical exploration of the latter was recently finished, with special regard to secondary alkaline (szik) prairies, reeds and scattered forests (Mahunka, 1981, personal communication). These areas can be regarded as practically undisturbed, "natural" ecosystems.

In the Bátorliget marshland 4432, in the Hortobágy National Park 7667 animal species were detected. These data render especially interesting the 582

Table 2

Number of animal species collected in maize ecosystems

	Survey areas		
	2.1	2.2	Total
Nematoidea	17	14	22
Orthopteroidea	9	9	11
Homoptera — Aphidoidea	13	17	21
— Coccoidea	4	6	8
Heteroptera	68	21	73
Neuropteroidea	17	14	20
Coleoptera	102	54	114
Lepidoptera — "Microlepidoptera"	92	1	92
— "Macrolepidoptera"	116	7	118
Diptera	31	7	34
Hymenoptera	34	38	57
Acaridea	12	6	12
Animal species total	515	194	582

species established in the maize stands, the more as the material collected during the surveys is still being worked up. It has also to be considered that the plants of a maize plot are destroyed during the harvest and autumn cultivation measures, which also destroy a portion of the faunal elements.

The numbers indicate that even in intensively cultivated agricultural areas both beneficial, noxious and indifferent species may occur in considerable numbers. Integrated pest management systems can be worked out only if the living community of the given crop is well known. Our present paper aimed to furnish data to the knowledge of maiz ecosystems.

Acknowledgements

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List of animal species observed in the maize stands surveyed

	2.1	2.2
NEMATOIDEA		
RHABDITIDEA		
Rhabditidae		
Rhabditis sp.	12	12
Mesorhabditismonhystera Bütschli	12	—
Diploscapter coronata Cobb	12	—
Cephalobidae		
Cephalobus nanus De Man	12	—
Eucephalobus triatus Bast.	12	12
— sp.	12	12
Chiloplacus sp.	12	—
Acrobeles ciliatus Linst.	12	12
Teratocephalidae		
Teratocephalus terrestris Bütschli	12	—
TYLENCHIDEA		
Tylenchidae		
Pratylenchus pratensis De Man	12	12
Criconematidae		
Criconemoides sp.	12	12

(Continued 1)

	2.1	2.2
Aphelenchidae		
Aphelenchus avenae Bast.	—	12
Aphelenchoides sp.	12	12
Paraphelenchus pseudoparietinus Micol.	12	12
ENOPLIDEA		
Alaiminidae		
Alaimus sp.	12	—
Dorylaimidae		
Dorylaimus obtusicaudatus Bast.	12	12
— *monohystera* De Man	—	12
— *vestibulifer* Nicol.	12	—
— *granuliferus* Cobb	—	—
Dorylaimus sp.	12	12
Aporcelaimus laetificans Andrássy	—	12
Discolaimus sp.	—	12
ORTHOPTEROIDEA SALTATOPTERA		
Tettigoniidae		
Phaneroptera falcata Poda	8, 9	8, 9
Leptophyes albovittata Koll.	8, 9	9
Conocephalus dorsalis Latr.	—	9
Metroptera roeseli Hgb.	—	8, 9
Gryllidae		
Oecanthus pellucens Scop.	8, 9	—
Gryllus campestris L.	10	10
Acrididae		
Chorthippus albomarginatus De Geer	8, 9	—
— *apricarius* L.	8, 9	9
— *brunneus* Th.	8, 9	8, 9
Eucorthippus declivus Br.-Ba.	8, 9	8, 9
DERMAPTERA		
Forficulidae		
Forficula auricularia L.	8, 9	8, 9
HOMOPTERA, APHIDOIDEA		
Callaphididae		
Therioaphis sp.	7	7
Aphididae		
Ammiaphis sii Koch	—	4, 8
Aphis fabae Scop.	—	8

(Continued 2)

	2.1	2.2
Acyrtosiphon pisum Harris	7	—
Brachycaudus cardui L.	—	8
— *helichrysi* Kalt.	8	—
Dactynotus cichorii HB.	—	8
— *jaceae* CB.	8	—
Dysaphis plantaginea Pass.	4	4
Hayhurstia atriplicis L.	8	8
Hyadaphis foeniculi HRL.	8	—
Hyperomyzus lactucae L.	—	8
Macrosiphoniella sp.	8	8
Macrosiphum avenae CB.	4	4
— *granulatum*	—	4
Metopolophium dirhodum Walk.	4, 7	4, 7
Myzus persicae Sulz.	—	8
Rhopalosiphum maidis Fitch.	4	4
— *padi* L.	4	4
Sitobion avenae F.	—	7
Pemphigidae		
Tetraneura ulmi L.	7	7
HOMOPTERA, COCCOIDEA		
Pseudococcoidae		
Chaetococcus phragmitidis March.	—	9
Heterococcus nudus Green	—	9
Phenacoccus bicerarius Borchs.	9	—
Trionymus tomlini Green	—	9
Eriococcidae		
Greenisca glyceriae Green	9	—
Rhizococcus cynodontis Kiritsch.	—	9
Coccidae		
Eriopeltis festucae Fonsc.	9	9
Parthenolecanium corni Bouché	9	9
HETEROPTERA		
Corixidae		
Callicorixa concinna Fieb.	1	—
— *praeusta* Fieb.	1	—
Hesperocorixa linnei Fieb.	1	—
Sigara falleni Fieb.	1	—
— *lateralis* Leach.	1	—
— *striata* L.	1	—
Miridae		
Deraeocoris ruber L.	1	—
— *serenus* Dougl. et Scott	1	—

(Continued 3)

	2.1	2 2
Alloeotomus germanicus E. Wagn.	1	—
Leptopterna ferrugata Fall.	1	—
Stenodema calcaratum Fall.	1, 7	4
Notostira erratica L.	7	4
Trigonotylus coelestialium Kirkaldy	1, 4, 7	4,7
— *pulchellus* Hahn.	1, 4	—
Phytocoris meridionalis H. Sch.	—	7
— *dimidiatus* Kirsch.	1	—
— *novickyi* Fieb.	1	—
— *varipes* Boh.	1	—
— *insignis* Reut.	1	—
Adelphocoris seticornis F.	1, 4	—
— *annulicornis* Sahlb.	1	—
— *lineolatus* Goeze	1, 4, 7	7
Calocoris quadripunctatus Vill.	1	—
— *norvegicus* Gmel.	1	4
Lygus lucorum Meyer-Dür	1	7
Exolygus rugulipennis Popp.	1, 4, 7, 10	4, 7
— *pratensis* L.	1	7
— *gemellatus* H. Sch.	1, 7	—
Orthops campestris L.	1	—
— *kalmi* L.	1	—
Polymerus nigritus Fall.	—	4
— *vulneratus* Panz.	1	7
— *cognatus* Fieb.	1	—
— *asperulae* Fieb.	1	—
— *palustris* Reut.	1	—
— *unifasciatus* F.	1	—
Orthotylus marginalis Reut.	1	—
— *tenellus* Fall.	1	—
— *flavoparsus* C. Sahlberg	1, 4, 7	—
Cyllocoris histrionicus L.	1	—
Macrotylus horvathi Reut.	1	—
Harpocera thoracica Fall.	1	—
Plagiognathus albipennis Fall.	1	—
Stenarus rottermundi Scholtz	1	—
Oncotylus setulosus H. Sch.	1	—
Icodema infuscatum Fieb.	1	—
Megalocoleus molliculus Fall.	1	—
Psallus sp.	1	—
Miridae sp.	1	—
Anthocoridae		
Anthocoris sibiricus Reut.	—	7
Orius niger Wolff	4, 7	4, 7
— *majusculus* Reut.	1, 4, 7	4, 7
Lyctocoris campestris F.	1	—

(Continued 4)

	2.1	2.2
Nabidae		
Nabis ferus L.	1, 4, 7	4, 7
— *punctatus* Costa	1, 4, 7	4, 7
— *pseudoferus* Rem. ♂	4, 7	4, 7
— *pseudoferus* Rem. or		
— *punctatus* Costa) ♀	1, 4, 7	4, 7, 10
Reduviidae		
Reduvius personatus L.	1	—
Tingidae		
Tingis auriculata Costa	1	—
Saldidae		
Saldula pilosella Thoms.	1	—
Lygaeidae		
Nysius senecionis Schill.	4	—
Emblethis griseus Wolff.	1	—
Sphragisticus nebulosus Fall.	1	—
Pachybrachius fracticollis Schill.	1	—
Rhyparochromus vulgaris Schill.	1	—
Coreidae		
Coreus marginatus L.	4	—
Brachycarenus tigrinus Schill.	1	—
Pentatomidae		
Holcostethus vernalis Wolff.	4	—
Dolycoris baccarum L.	7	4
Eurydema oleraceum L.	4	7
Scutelleridae		
Eurygaster maura L.	—	4
Cydnidae		
Sehirus sexmaculatus Rambur	4	-
NEUROPTEROIDEA		
PLANIPENNIA (NEUROPTERA)		
Chrysopidae		
Chrysoperla carnea Steph.	1, 4, 6, 7, 9	4, 7, 9, 10
Chrysopa phyllochroma Wesm.	1, 4, 6, 7, 10	4, 7, 9
— *formosa* Brauer	1, 4, 6, 7, 9, 10	4, 7, 9
— *septempunctata* Wesm.	1, 4	4, 9
— *perla* L.	1, 4	4, 7, 9
— *abbreviata* Curtis	1, 4, 7	4, 7, 9
— *commata Kis et* Ujhelyi	1	—

(Continued 5)

	2.1	2.2
Anisochrysa ventralis Curtis	1, 4	4
Chrysotropia ciliata Wesm.	4, 9	—
Nineta flava Scop.	4	7
Hemerobiidae		
Micromus angulatus Steph.	1, 4, 6, 7, 10, 12	4, 7, 9, 10
— *variegatus* F.	1, 4, 6, 7, 10, 12	9, 11
Wesmaelius subnebulosus Steph.	1, 4, 6, 10	4, 10
— *nervosus* F.	10	7, 10
— *quadrifasciatus* Reut.	10	—
Hemerobius humulinus L.	1, 4, 10	4, 7, 9
— *nitidulus* F.	—	4, 7
— *atrifrons* Mc. Lachlan	—	4, 9
— *pini* Steph.	1	—
Sympherobius elegans Steph.	1	—
COLEOPTERA		
Carabidae		
Cicindela germanica L.	10	—
Acupalpus meridianus L.	1	—
— *suturalis* Dej.	1	—
Aechmites terricola Herbst	10	10
Agonum micans Nicolai	1	—
— *gracilipes* Duft.	—	10
Amara bifrons Gyll.	10	10
— *communis* Panz.	10	10
— *consularis* Duft.	10	10
— *familiaris* Duft.	10	10
— *ingenua* Duft.	10	10
Anisodactylus binotatus F.	1	—
— *poeciloides* Steph.	1	—
— *signatus* Panz.	1, 10	—
Badister unipustulatus Bon.	1	—
— *sodalis* Duft.	1	—
Bembidion lampros Herbst	10	10
— *properans* Steph.	10	—
— *varium* Oliv.	1	—
Broscus cephalotes L.	10	10
Calathus ambiguus Payk.	10	10
— *erratus* C. R. Sahlb.	10	10
— *fuscipes* Goeze	—	10
— *melanocephalus* L.	10	10
Calosoma auropunctatum Herbst	10	10
Carabus scheidleri Panz.	—	10
Clivina fossor L.	1	10
Dolichus halensis Schall.	10	10
Harpalus anxius Duft.	—	10
— *calceatus* Duft.	10	10

(*Continued 6*)

	2.1	2.2
— *griseus* Panz.	1, 10	10
— *psittaceus* Fourcroy	10	10
— *rubripes* De Geer	—	10
— *rufipes* De Geer	1, 10	10
— *tardus* Panz.	10	—
— *zabroides* Dej.	10	—
Olisthopus rotundatus Payk.	10	—
Ophonus diffinis Dej.	1, 10	10
Microlestes minutulus Goeze	—	10
Platynus dorsalis Pont.	10	10
Poecilus cupreus L.	—	10
— *sericeus* Fisch.	10	10
Pterostichus melanarius Illig.	10	10
— *niger* Schall.	10	10
— *macer* Marsh.	10	10
— *vernalis* Panz.	—	10
Trechus pilisensis Csiki	10	10
— *quadristriatus* Duft.	10	10
Zabrus tenebrionides Goeze	10	10
Dytiscidae		
Coelambus parallelogrammus Ahr.	1	—
Ilybius subaeneus Er.	1	—
Hydrophilidae		
Berosus spinosus Stev.	1	—
Cercyon quisquilius L.	1	—
Hydrobius fuscipes L.	1	—
Philydrus quadripunctatus Herbst	1	—
Silphidae		
Necrophorus germanicus L.	10	10
— *vespillo* L.	10	—
Silpha carinata L.	10	—
Thanatophilus sinuatus F.	10	—
Staphylinidae		
Bledius tricornis Herbst	1	—
Ocypus olens O. F. Müll.	10	—
Oxytelus rugosus F.	1, 10	—
Paederus fuscipes Rossi	1	—
Histeridae		
Atholus duodecimstriatus Schrank	10	—
Cantharidae		
Cantharis rufa L.	1	—
— *rustica* L.	1	—
Rhagonycha fulva Scop.	1	—

(Continued 7)

	2.1	2.2
Elateridae		
Adrastus rachifer Fourcroy	1, 7, 10	7
Agriotes ustulatus Schall.	7	7
Athous haemorrhoidalis Fabr.	1	—
Heteroceridae		
Heterocerus fenestratus Thunb.	1	—
Dermestidae		
Dermestes laniarius Illig.	—	10
Phalacridae		
Olibrus millefolii Sturm.	7	7
Mycetophagidae		
Typhaea stercorea L.	1	—
Nitidulidae		
Glischrochilus hortensis Fourcroy	1	—
Meligethes aeneus Fabr.	7	—
Coccinellidae		
Adalia bipunctata L.	1, 7	7
Adonia variegata Goeze	1, 7	7
Coccinella septempunctata L.	1, 7, 10	7
Coccinula quattuordecimpustulata L.	7	10
Propylaea quattuordecimpunctata L.	1, 7	7
Scymnus rubromaculatus Goeze	7	7
Subcoccinella vigintiquattuorpunctata L.	7	—
Anobiidae		
Xyletinus laticollis Duft.	7	—
Tenebrionidae		
Alphitobius diaperinus Panz.	1	—
Blaps halophila Fisch.	10	—
Lagriidae		
Lagria hirta L.	10	—
Anthicidae		
Anthicus humilis L.	10	—
Formicomus pedestris Rossi	10	—
Notoxus monoceros L.	10	—
Melolonthidae		
Amphimallon solstitialis L.	1	—
Anisoplia austriaca Herbst	7	—
— *segetum* Herbst	7	—

(*Continued 8*)

	2.1	2.2
Scarabeidae		
Aphodius circumcinctus W. Schm.	1	—
— *distinctus* O. F. Müll.	10	—
— *granarius* L.	1	—
— *kraatzi* Har.	1	—
— *lugens* Creutz.	1	—
— *plagiatus* L.	1	—
— *varians* Duft.	1	—
Pleurophorus caesus Creutz.	1	—
Trox hispidus Pont.	1	—
Chrysomelidae		
Aphthona euphorbiae Schrank	1	7
Asiorestia ferruginea Scop.	1	—
Cassida nebulosa L.	—	7
Dlochrysa fastuosa Scop.	7	—
Oulema lichenis Voet.	7	—
— *melanopus* L.	7	7
Phyllotreta nemorum L.	—	7
— *undulata* Cutts.	1	7
Curculionidae		
Balanobius salicivorus Payk.	7	—
Sitona crinitus Herbst	—	7
— *sulcifrons* Herbst	7, 10	7
Tanymecus dilaticollis Gyll.	4	4
LEPIDOPTERA "MICROLEPIDOPTERA"		
Hepialidae		
Triodia sylvina L.	1	—
Cossidae		
Zeuzera pyrina L.	1	—
Phragmataecia castanea Hbn.	1	—
Tineidae		
Monopis monachella Hbn.	1	—
Ateliotum hungaricellum Z.	1	—
Plutellidae		
Plutella maculipennis Curt.	1	—
Epermeniidae		
Ochromolopis ictella Hbn.	1	—

(Continued 9)

	2.1	2.2
Cosmopterygidae		
Stagmatophora serratella Tr.	1	—
Limnaecia phragmitella Stt.	1	—
Oecophoridae		
Agonopteryx propinquella Tr.	1	—
— *applana* F.	1	—
Carcina quercana F.	1	—
Gelechiidae		
Gomphrocrates rasilella HS.	1	—
Metzneria paucipunctella Z.	1	—
Tortricidae		
Pandemis dumetana Tr.	1	—
— *heparana* Den. et Schiff.	1	—
Argyrotaenia pulchellana Haw.	1	—
Aphelia viburnana Den. et Schiff.	1	—
— *paleana* Hbn.	1	—
Clepsis strigana Hbn.	1	—
— *spectrana* Tr.	1	—
— *semialbana* Gn.	1	—
Cnephasia communana HS.	1	—
Aleimma loefflingiana L.	1	—
Tortrix viridana L.	1	—
Celypha striana Den. et Schiff.	1	—
Argyroploce lacunana Den. et Schiff.	1	—
Olethreutes decrepitana HS.	1	—
Bactra furfurana Haw.	1	—
— *lanceolana* Hbn.	1	—
Endothemia quadrimaculana Haw.	1	—
Hedya nubiferana Haw.	1	—
— *salicella* L.	1	—
Zeiraphera isertana F.	1	—
Epiblema scutulana Den. et Schiff.	1	—
Pseudeucosma caecimaculana Hbn.	1	—
Eucosma metzneriana Tr.	1	—
— *tundrana* Kenn.	1	—
— *lacteana* Tr.	1	—
— *conterminana* HS.	1	—
— *cana* Haw.	1	—
— *expallidana* Haw.	1	—
Thiodia citrana Hbn.	1	—
Lathronympha strigana F.	1	—
Collicularia microgrammana Gn.	1	—
Cochylidae		
Agapete zoegana L.	1	—
— *hamana* L.	1	—

(Continued 10)

	2.1	2.2
Aethes dipoltella Hbn.	1	—
Lozopera bilbaensis Rössl.	1	—
Eupoecilia angustana Hbn.	1	—
Hysterosia inopiana Haw.	1	—
Pterophoridae		
Agdistis adactyla Hbn.	1	—
Aciptilia pentadactyla L.	1	—
Phycitidae		
Myelois cribrumella Hbn.	1	—
Acrobasis tumidana Den. et Schiff.	1	—
Salebria semirubella Sc.	1	—
Divona illignella Z.	1	—
Etiella zinckenella Tr.	1	—
Nytegretis achatinella Hbn.	1	—
Trissonca oblitella Z.	1	—
Homoesoma sinuellum F.	1	—
— *nebulellum* Den. et Schiff.	1	—
Ematheudes punctella Tr.	1	—
Prinarastia lotella Hbn.	1	—
Crambidae		
Schoenobius gigantellus Den. et Schiff.	1	—
Chilo phragmitellus Hbn.	1	—
Argyria cerusella Chrét.	1	—
Calamothropa paludella Hbn.	1	—
Pediasia luteella Schiff.	1	—
— *contaminella* Hbn.	1	—
Agriphila culmella L.	1	—
Crambus perlellus Sc.	1	—
— *hortuellus* Hbn.	1	—
Galleriidae		
Mellisoblaptes zelleri De Joann	1	—
Lamoria anella Den. et Schiff.	1	—
Pyralidae		
Pyralis costalis F.	1	—
— *perversalis* HS.	1	—
Actenia honestalis Tr.	1	—
Pyraustidae		
Evergestis aenelis L.	1	—
— *frumentalis* L.	1	—
— *extimalis* Sc.	1	—
Pyrausta cespitalis Den. et Schiff.	1	—
Psammotis pulveralis Hbn.	1	—

(Continued 11)

	2.1	2.2
Perinephela rubiginalis Hbn.	1	—
— *verbascalis* L.	1	—
Eurrhypara hortulata L.	1	—
Sclerocona acutella Ev.	1	—
Sitochroa palealis Den. et Schiff.	1	
— *verticalis* L.	1	—
Loxostege sticticalis L.	1	—
Ostrinia nubilalis Hbn.	1, 4	4
"MACROLEPIDOPTERA"		
Geometridae		
Pelurga comitata L.	1	—
Perizoma alchemillata L.	1	—
— *lugdunaria* H. Sch.	1	—
Xanthorhoe ferrugata Cl.	1	—
— *spadicearia* Den. et Schiff.	1	—
Costaconvexa polygrammata Bkh.	1	—
Camptogramma bilineata L.	1	—
Lithostege farinata Hufn.	1	—
Lythria purpuraria L.	1	—
Eupithecia innotata Hufn.	1	—
— *centaureata* Den. et Schiff.	1	—
Idaea muricata Hufn.	1	—
— *fuscovenosa* Goeze	1	—
— *dimidiata* Hufn.	1	—
— *aversata* L.	1	—
Calothysanis amata L.	1	—
Cyclophora sp.	1	—
Scopula immorata L.	1	—
— *rubiginata* Hufn.	1	—
Rhodostrophia vibicaria Cl.	1	9
Lomaspilis marginata L.	1	—
Semiothisa alternaria Hbn.	1	—
— *chlathrata* L.	1	—
Narraga fasciolaria Hufn.	1	—
Tephrina arenacearia Den. et Schiff.	1	—
Angerona prunaria L.	1	—
Biston betularia L.	1	—
Peribatodes rhomboidaria Den. et Schiff.	1	—
Boarmia roboraria Den. et Schiff.	1	—
— *punctinalis* Scop. (danieli Whrl.)	1	—
Ascotis selenaria Den. et Schiff.	1	9
Synopsia sociaria Hbn.	9	9
Ectropis bistortata Goeze	1, 4	—
Tethidia smaragdaria F.	1	—
Chlorissa viridata L.	1	—
Thalera fimbrialis Scop.	1	—

(Continued 12)

	2.1	2.2
Noctuidae		
Euxoa aquilina Den. et Schiff.	1	—
— *temera* Hbn.	1	—
Agrotis segetum Den. et Schiff.	1	—
— *exclamationis* L.	1	—
— *ipsilon* Hufn.	1	—
Ochropleura plecta L.	1	—
Axylia putris L.	1	—
Noctua interposita Hbn.	1	—
— *janthina* Den. et Schiff.	1	—
Xestia c-nigrmu L.	1	—
— *rhomboidea* Esp.	1	—
— *xanthographa* Den. et Schiff.	1	—
Discestra trifolii Hufn.	1	9
Mamestra brassicae L.	1, 4	4
— *persicariae* L.	1	—
— *suasa* Den. et Schiff.	1	—
— *oleracea* L.	1, 4	—
Hadena luteago Den. et Schiff.	1	—
Tholera cespitis Den. et Schiff.	1	—
— *decimalis* Poda	1	—
Hyssia cavernosa gozmanyi Kov.	1	—
Mythimna albipuncta Den. et Schiff.	1	—
— *pallens* L.	1	—
Leucania obsoleta Hbn.	1	—
Senta "stenoptera" Stgr.	1	—
Calophasia lunula Hufn.	1	—
Eupsilia transversa Hufn.	1	—
Simyra albovenosa Goeze	1	—
Amphipyra tragopogonis Cl.	1	—
Rusina ferruginea Esp.	1	—
Thalpophila matura Hufn.	1	—
Trachea atriplicis L.	1	—
Eucarta virgo Tr.	1	—
Oligia latruncula Den. et Schiff.	1	—
— *strigilis* L.	1	—
Photedes extrema Hbn.	1	—
— *minima* Haw.	1	—
Luperina testacea Den. et Schiff.	1	—
Celaena leucostigma Hbn.	1	—
Archanara sparganii Esp.	1	—
— *algae* Esp.	1	—
Hoplodrina alsines Brahm.	1	—
— *ambigua* Den. et Schiff.	1	—
Caradrina morpheus Hufn.	1	—
Athetis gluteosa Tr.	1	—
— *furvula* Hbn.	1	—
— *lepigone* Möschl.	1	—

(Continued 13)

	2.1	2.2
Aegle koekeritziana Hbn.	1	—
Pyrrhia umbra Hufn.	1	—
Heliothis maritima Grasl.	1	—
Jaspidia pygarga Hufn.	1	—
Eustrotia bankiana F.	1	—
— *candidula* Den. et Schiff.	1	—
Emmelia trabealis Scop.	1	—
Acontia luctuosa Den. et Schiff.	1	—
Earias chlorana L.	1	—
Abrostola triplasia L.		9
Macdunnoughia confusa Steph.	1	—
Autographa gamma L.	1, 4	—
Euclidia glyphica L.	1	—
Hypaenina sp.	1	—
Nolidae		
Celama centonalis Hbn.	1	—
Lithosiidae		
Comacla senex Hbn.	1	—
Eilema complana L.	1	—
— *lutarella* L.	1	—
— *pallifrons* Z.	1	—
Arctiidae		
Phragmatobia fuliginosa L.	1	—
Spilarctia lutea Hufn.	1	—
Spilosoma menthastri Esp.	1	—
— *urticae* Esp.	1	—
Hyphantria cunea Drury	1	—
Arctia caja L.	1	—
Notodontidae		
Gluphisia crenata Esp.	1	—
Spatalia argentina Den. et Schiff.	1	—
Lophopteryx camelina L.	1	—
Pterostoma palpinum L.	1	—
Clostera curtula L.	1	—
Lasiocampidae		
Epicnaptera tremulifolia Hbn.	1	—
Sphingidae		
Amorpha populi L.	1	—
Lymantriidae		
Dasychira fascelina L.	1	—
Orgyia recens Hbn.	—	9

(Continued 14)

	2.1	2.2
DIPTERA		
Bibionidae		
Dilophus febrilis L.	1, 7, 8	—
— *femoratus* Meig.	1	—
— *hortulanus* L.	1	—
Cecidomyidae		
Cecidomyidae indet.	1, 7, 8	7, 8
Mycetophilidae		
Mycetophila fungorum Deg.	1	—
Culicidae		
Culicidae indet.	1	—
Chironomidae		
Chironomidae indet.	1, 7, 8	7, 8
Tipulidae		
Tipulidae indet.	1	—
Limoniidae		
Limoniidae indet.	1	—
Asilidae		
Asilidae indet.	—	7, 8
Dolichopodidae		
Dolichopodidae indet.	1	—
Syrphidae		
Syrphidae indet.	1	7, 8
Sciomyzidae		
Ditaeniella grisescens Meig.	7, 8	—
Micropezidae		
Micropeza sp.	1	—
Lauxaniidae		
Calliopum aeneum Fall.	1	—
Ephydridae		
Ephydra riparia Fall.	1	—
Paracoenia fumosa Stenh.	1	—
Sphaeroceridae		
Sphaerocera curvipes Latr.	1	—
Copromyza equina Fall.	1	—

(Continued 15)

	2,1	2.2
Leptocera lutosoidea Duda	1	—
Limosina pullula Zett.	1, 7, 8	—
Drosophilidae		
Scaptomyza pallida Zett.	7, 8	—
Chloropidae		
Elachiptera cornuta Fall.	—	7, 8
Tricimba cincta Meig.	1	—
Oscinella frit L.	7, 8	7, 8
Scathophagidae		
Scathophaga stercararia L.	1	—
— sp.	1	—
Anthomyidae		
Craspedochaeta pullula Zett.	1	—
Paregle radicum L.	1	—
Delia platura Meig.	1	7, 8
Muscidae		
Muscidae indet.	1	—
Calliphoridae		
Pollenia sp.	1	—
Lucilia sp.	1	—
Calliphoridae indet.	1	—
HYMENOPTERA		
Cephidae		
Cephus pygmaeus L.	—	8
Tenthredinidae		
Athalia circularis Klug.	—	8
— *rosae* L.	—	8
Ichneumonidae		
Amblyteles armatorius Först.	8	8
Chirotica insignis Grav.	7	—
Eniscus ramidulus L.	1	—
Eutanycarpa picta Schranck	8	8
Ichneumon pallidicornis Wesm.	8	8
— *zonalis* Grav.	8	—
Paniscus luteus L.	1	—
Temelucha confluens Grav.	1	—

(Continued 16)

	2.1	2.2
Braconidae		
Amyras clandestina Halid.	—	7
Blacus ruficornis Nees	12	8
Homolobus truncator Say	1	—
Macrocentrus collaris Spin.	1	—
Microplitis tuberculifer Wesm.	—	8
Rogas circumscriptus Nees	1	—
Synaldis concinna Halid.	8	8
Syntretus vernalis Wesm.	1	—
Zele nigricollis Thoms.	1	—
Aphidiidae		
Aphidius gregarius Marsh.	7, 8	8
— *picipes* Nees	—	4
— *ervi* Halid.	4	4
Ephedrus plagiator Nees	—	4, 8
Trioxys brevicornis Halid.	8	—
Chalcididae		
Asaphes vulgaris Walker	—	4
Cynipidae		
Allotris sp.	—	4
Pteromalidae		
Pachyneuron aphidis Bouché	8	8
— *cremafaniae* Delucci	8	8
— *formosum* Nees	—	8
Stenomalina muscarum L.	8	—
Encyrtidae		
Bothriothorax clavicornis Dalm.	8	—
Eulophidae		
Chrysocaris sp.	—	8
Diglyphus isaea Walker	8	8
Entedon fufius Walker	—	8
Hemitarsenus unguicellus Zett.	—	8
Necremnus leucarthros Nees	8	8
— *tidius* Walker	8	8
Tetrastichus coccinellae Kurdj.	—	8
— *ecus* Walker	—	8
— *evonymellae* Bouché	8	—
Proctotrupidae		
Proctotrupes gravidacor L.	—	8
Diapriidae		
Diapria conica F.	—	8

(Continued 17)

	2.1	2.2
Scelionidae		
Asolcus semistriatus Nees	8	—
Platygastridae		
Inostemma contariniae Szelényi	—	8
Megaspilidae		
Conostigmus sp.	8	—
Lygocerus sp.	8	—
Ceraphronidae		
Ceraphron sp.	—	8
Lygocerus sp.	4	4
Bethylidae		
Pristocera depressa F.	1	—
Sphecidae		
Diodontus minutus F.	8	8
Mellinus arvensis L.	—	8
Halictidae		
Halictus fulvipes Klug.	—	8
— *interruptus* Panz.	—	12
— *maculatus* Smith	—	8
Formicidae		
Formica rufa L.	8	8
Lasius niger L.	8	—
ARACHNOIDEA		
Tarsonemidae		
Scutacarus quadrangularis	12	—
Tarsonemus belemnitoides	12	—
Steneotarsonemus sp.	12	—
Acaridae		
Rhyzoglyphus echinopus	12	12
Tyrophagus putrescentiae	12	—
Oribatidae		
Oppiella nova Oudemans	12	12
Protoribates lophotrichus Berlese	12	—
Scheloribates laevigatus C. L. Koch	12	12
Zygoribatula exarata Berlese	12	12
Ceratozetes mediocris	12	12
— *conjunctus* Mihelcic	12	—
Punctoribates punctum C. L. Koch	12	12

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1. Rácz, V. (1979): Heteropterológiai vizsgálatok kukoricásban (Heteropterological studies in maize). Állattani Közl. 66, 1–4, 131–134.
2. Lővei, G. (1980): Kukoricásban élő futóbogár-közösség szerkezete és dinamikája (Structure and dynamics of a Carabid community in maize.) VEAB Értesítő, I, 304.
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Results of Faunistical and Floristical Studies in Hungarian Apple Orchards (Apple Ecosystem Research No. 26)

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The authors commenced the study of agro-ecosystems in Hungarian apple orchards in 1976. The present work contains the lists of animal and plant species established during the first five years of the series of studies (1976–1980), planned for ten years. In the apple stands investigated in regular surveys, 1759 animal and 137 plant species were registered during this period.

On the suggestion of Dr. T. Jermy, academician, the Department of Zoology of the Research Institute of Plant Protection of the Hungarian Academy of Sciences commenced a series of surveys in 1976 in agro-ecosystems. The work, planned for a period of ten years, is being supported by a special grant of the Hungarian Academy of Sciences. The surveys are carried out in two crop plant stands, apple and maize; these are the two plant types in which the considerable changes are the best represented that had occurred in Hungarian agriculture as regards both production technology and agro-ecology. For the research work also the co-operation of many scientists, working in related research institutions, could be obtained.

The basic aim of our studies was the ecological foundation of the integrated plant protection to be introduced in the industrial-scale production systems. For that purpose the animal communities of agricultural areas under treatments of different intensities are studied, with special regards to factors influencing the population dynamics of the most important phytophagous species. These factors themselves underwent many changes under the influence of up-to-date production technologies.

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Besides the aims of close practical interest also important conclusions can be drawn as regards the types and significance of controlling mechanisms acting in the Hungarian agro-ecosystems (Jermy, 1977, 1979). For that purpose first the "inventory" of the species occurring in the agro-ecosystems had to be assessed. In the following study the results of surveys (1976–1980) in apple orchards of different cultivation type are summarized.

Review of the literature

In course of the past 80 years many literature data were published on the phytophagous and zoophagous groups of animal communities in apple orchards. There are, however only few comprehensive works in which the authors tried to assess the complete fauna or even only the Arthropod fauna of an apple orchard, for at least one year. From this point of view the work of Oatman et al. (1964) is of special interest. The authors detected in the USA, in an orchard of 1.4 ha, consisting of insecticide-treated and untreated parts, in four years 736 species, belonging to 158 families of 14 insect orders. Malevez (1976) studied in France in an apple orchard consisting of 0.6 ha plots (treated with insecticides, untreated and plots treated with integrated control methods) the most important arthropod pests and predators for one year, as regards phenology and population dynamics. The paper contained the most important species of mites, spiders and five insect orders, collected by plant assessment, beating and sexpheromone traps. Baeschlin and Taksdal (1979) followed in Norway the changes in the Arthropod fauna for three years by plant assessment and beating in a 0.6 ha apple orchard, kept free of insecticides for the duration of the observations. Their paper only contains the comparative data of more important pests and predators of the 11 insect orders, arachnoids and mites collected by the two methods. MacLellan presented the insect pests and predators collected in 68 apple orchards in Canada in the period 1953–1977, without meeting the claim of completeness.

Mathys and Baggiolini (1965) carried out the comparative study of four methods of assessment for 3 years. They compared the effectivities of plant assessment, beating, light trap and sucking trap, concerning seven insect orders, spiders and mites. Steiner et al. (1970) compared the methods of collection by beating and Stuttgart funnel in phytophagous, zoophagous and other insect groups, both on untreated and insecticide-treated apple trees.

Sites of Assessment

The experimental orchards are situated in the department Szabolcs-Szatmár and Pest (the latter near Budapest).

1.1. Scattered orchard: Nyíregyháza–Sóskút, about 0.2 ha. Receiving no treatment of any kind, the ground cover by weeds is practically 100%. Surrounding area: brushland and scattered trees, further cultivated cropland.

1.2. Backyard-orchard: Nyíregyháza–Füzesbokor, about 0.5 ha. Receiving regular chemical treatment (11–12 sprayings per year, about 8 or 10 of which are insecticides). Weed cover is 20–40%. Surrounding area: backyard gardens, cropland, scattered little farms.

1.3. Traditional farming-scale orchard: Újfehértó, about 5 ha. Receiving regular chemical treatment (12–19 sprayings per year, 7–9 of which are insecticides). Weed cover is 10–50%. Surrounding area: farming-scale orchards, cropland, meadows.

1.4. Intensive farming-scale orchard: Nyíregyháza–Ilonatanya, 100 ha. Receiving regular chemical treatment (11–19 sprayings per year, of which 7–11 are insecticides). Weed cover is 10–50%. Surrounding area: farming-scale orchards, cropland.

The soil of the four sites above is brown sandy soil, the apple variety grown in the orchard is Jonathan (in the farming-scale orchards Starking is used as pollinating variety).

1.5. Experimental orchard: Nagykovácsi–Julianna major (near the city limits of Budapest, north from the centre). About the half of the 5.8 ha orchard (2.5) ha is kept as insecticide-free control area (1.5.1.) and 3.3 ha receive regular chemical treatment (1.5.2. area). The treated part is sprayed 4–6 times per year (fungicides + insecticides) whereas the experimental area receives only fungicides. The weed cover is variable, 40–80%. The surrounding area is mostly forest (about 50-years old oak forest: Querceto petraeae-cerris and Ceraso-Quercetum pubescentis) and cropland plots surrounded themselves by forests. The soil is brown forest soil.

Methods

The short summary of methods and their serial numbers are contained in Table 1. In the lists of species the serial numbers of methods were written into the individual columns, these do not refer thus to quantitative relationships. In the lists of birds and floral elements instead of serial numbers + signs are used, indicating the occurrence of the species. The methods are summarized as follows:

1. Light trap. A simple, Jermy-type light trap with 100 W normal light bulb as described by Jermy (1961). The traps were operated in the areas 1.2, 1.3, 1.4 and 1.5.1. during the whole growing season and were emptied daily.

2. Lamp catch. As in the area 1.1 no electric current is available, since 1980 occasionally lamp catches have been made during the night. The electric source is a Honda electric generator type E 300, the bulb is a Tungsram 160 W HMLI bulb ("blended light lamp").

3. Sucking trap. The sucking traps (one in each of 1.3 and 1.5.1. areas) are operated during the whole growing season, with their orifice in the height of the tree crowns. The traps are emptied weekly.

4. Yellow pan traps are operated continuously during the growing season in the areas 1.1, 1.2, 1.3, 1.4, 1.5.1., 1.5.2., emptied once or twice a week.

Table 1

Review of methods used in the survey of apple ecosystems

1. Light trap	collects from air space
2. Lamp catch	collects from air space
3. Sucking trap	collects from air space
4. Yellow pan trap	collects from air space
5. Bait and scent trap	collects from air space
6. Sex pheromone trap	collects from air space
7. Beating	collects from tree crown
8. Individual plant assessment, rearing	collects from tree crown
9. Mite brushing	collects from tree crown
10. Trunk trap	collects between tree crown and soil- and grass level
11. Corrugated paper belt	collects between tree crown and soil- and grass level
12. Sweeping of weeds	collects in grass level
13. Study of individual weed plants, rearing	collects in grass level
14. Pitfall trap	collects on soil surface
15. Individual collection on soil surface	collects on soil surface
16. Soil sampling	collects on soil surface
17. Food of nestlings	collects from the whole area

5. Bait and scent traps. Traditional baits for Lepidoptera adults (sugar syrup + beer + fruit aroma, absorbed into plastic foam) are used occasionally in the areas 1.1. and 1.5.1. Scent trap containing fermented apple juice is used in the areas 1.1, 1.2, 1.3 and 1.4, mostly to assess the population of *Synanthedon myopaeformis* Bkh. (Lep. Aegeriidae).

6. Sex pheromone traps are operated with the following Zoecon preparations: Codling moth: Pheromon cap CM; *Adoxophyes orana:* Adox-C; oriental fruit moth: CF-M.

7. Beating. Total fauna of apple trees is assessed bi-weekly, by beating branches and limbs of apple trees and collecting the insect material into a beating umbrella (84 cm in diameter) or 8 m^2 plastic sheet placed under the tree.

8. Individual plant assessment, rearing. This column contains the names of species which were established during the weekly surveys of individual trees (adults and larvae of herbivorous populations, their reared parasites and predators observed to prey on them).

9. Mite brushing. Leaves of apple trees are brushed (100 leaves per week) and the mites collected thus are being worked up.

10. Trunk trap. Serves to collect insects moving upward or downward on the tree trunk. The traps are operated in the areas 1.5.1. and 1.5.2. and are emptied weekly.

11. Corrugated paper belts. In the first years of the study in all areas corrugated paper belts were placed around the tree trunks to collect overwintering and pupating insect larvae. The belts were often destroyed by birds, so the method was abandoned in the 1.5. area.

12. Use of sweeping net on weeds. In the grass level of apple orchards collections are carried on weekly by sweeping net

13. Study of individual plants (weeds). Practically the same as in method No. 8 but carried out weekly in the grass level of the orchard.

14. Pitfall trap. Plastic cups are sunk into the soil, with their brim at soil level. Insects walking on the surface fall into the cups and are conserved in salt solution or ethylene glycol. (If small mammals like voles or shrews fell into the trap they usually rot and the trap content is discarded from hygiene reasons).

15. Individual collections on soil surface. Single animals, occurring on the soil surface are hand-picked during given time periods.

16. Soil sampling. For coleopterological studies square holes are dug ($100 \times \times 100 \times 50$ cm), for nematological studies 5×100 cm^3 soil samples are taken from the upper soil layer.

17. Bird nestlings. In the area 1.5 and close environment J. Török ligatures the neck of young titmouse birds for short periods and takes out of their mouth cavity the insects brought by the mother bird. After removing the ligature the nestlings are fed.

Review of Taxons Included into the Collections Regnum Animale

Phylum: Nemathelminthes

Class: Nematoidea

Nematological studies were carried out in the areas 1.1, 1.2, 1.3 and 1.4. The surveys and determinations were made by Ilona M. Benedek. The whole material collected was worked up and from the areas surveyed 57 species were demonstrated.

Phylum: Arthropoda

Class: Insecta

Super-ordo: Orthopteroidea (Saltatoptera, Dermaptera, Mantodea, Blattoptera)

Orthopterological surveys were carried out with different intensities in all surveyed areas. The materials were worked up, the determinations were made by László Nagy. In the apple orchards 18 Orthopteroidea species were detected.

Ordo: Physopoda

The Physopoda material collected by sucking traps is still being worked up.

Ordo: Homoptera

The aphidological studies were carried out in all survey areas, the materials were determined by András Meszleny and László Szalay-Marzsó, some data were collected in the Nyírség area by Csaba Csikai. The determination of the material has not been finished yet.

Coccidological studies were carried out in all survey areas, the materials were worked up, determined by Ferenc Kozár. In the apple orchards surveyed 53 Aphidoidea, 15 Coccoidea and 1 Psylloidea species were detected.

Ordo: Heteroptera

The survey of Heteroptera was carried out in all survey areas. The materials were worked up, determined by Vera Rácz. In the apple orchards 184 species were detected.

Super-ordo: Neuropteroidea (Raphidioptera, Planipennia = Neuroptera)

The neuropterological studies were made in all survey areas, by different methods. The materials were worked up and determined by Ferenc Szentkirályi and Sándor Szabó. In the apple orchards 34 species were detected.

Ordo: Coleoptera

The coleopterological studies were carried out in all survey areas, the determinations were made by László Ádám, Gábor Lővei, István Szarukán and Zeinab Radwan. The Coleoptera material collected by the light traps is still partly worked up. In the apple orchards 205 Coleoptera species were found.

Ordo: Lepidoptera

The lists presented are based first of all on collections made by light traps and pheromone traps and rearings of larval materials. The whole material was worked up.

The "Microlepidoptera" were reared by Klára Balázs and Krisztina Mihályi, the pheromone-trap studies were made by György Sziráki, the determinations were carried out by Csaba Szabóky, Klára Balázs and György Sziráki.

The "Macrolepidoptera" were reared by Zoltán Mészáros and Klára Balázs, determined by Zoltán Mészáros and László Ronkay. Some data from the Nyírség region were collected by Csaba Csikai. In the apple orchards studied 345 "Microlepidoptera" and 441 "Macrolepidoptera" were found.

Ordo: Diptera

Dipterological studies were carried out in all areas surveyed, but the material was only partly worked up. Most parasitic Diptera reared from phytophagous insects (mostly lepidopterous larvae) were determined. The Diptera material was

determined by Ágnes D. Draskovits, Ferenc Mihályi, László Papp and Árpád Soós. The parasites were reared by Klára Balázs and Krisztina Mihályi. In the apple orchards 133 species were detected.

Ordo: Hymenoptera

Hymenopterological studies were made in all survey areas, but only one part of the material, mostly reared hymenopterous parasites, were worked up. The parasites were reared by Klára Balázs, Ferenc Kozár, Krisztina Mihályi and László Szalay-Marzsó; the determination was made by Jenő Papp, László Polgár and Gusztáv Szelényi. Some data were furnished by Ferenc Kozár. In the apple orchards studied 207 Hymenoptera species were found.

Class: Arachnoidea
Ordo: Acaridea

Acarological studies were carried out in most of the areas, but only one part of the material was worked up. The soil-inhabiting mites were determined by Sándor Mahunka, some data were given by Ferenc Kozár.

Phylum: Vertebrata
Class: Aves

The ornithological observations were carried out in the areas 1.2, 1.3, 1.4, but mostly in 1.5. The observations were made by János Török. In apple orchards 39 bird species were noted.

Class: Mammalia

No regular observations were made on mammals occurring in the apple orchards; the data presented were collected by Ferenc Kozár.

Regnum plantarum
Phylum: Lychenes

The observations on lychens were made by Péter Solymosi in the areas 1.1 1.2, 1.4 and 1.5. In the apple orchards 22 lichen species were found.

Phylum: Pteridophyta and Angiospermatophyta

The weed surveys were carried out in the areas 1.1, 1.2, 1.4 and 1.5, by Péter Solymosi, Béla Oláh and Lajos Szőke. In the orchards studied 1 Pteridophyton and 114 Angispermatophyta species were detected (Dicotyledonopsida: 93 species, Monocotylenodopsida: 21 species).

Discussion

In course of the five years the presence of 1759 animal species and 137 plant species was observed in the apple orchards studied (Table 2.). This work has not been finished, as the list will undoubtedly be increased by the working up of further materials and groups (Arachnoidea, Thysanoptera, Cicadoidea etc.). From other groups, like Diptera and Hymenoptera, only some parts of the collections have been determined so far.

The authors are well aware of the fact that a considerable part of the species reported herein are not depending on the apple tree as energy source but belong to the fauna of its environment and were brought into the areas studied by active or passive movement. However, it is also a fact that these species are, at least as adults, present in the orchards and may become parts of trophic chains either as consumers or preys. Their occurrence is thus by no means indifferent to the community of a given area.

As a comparison, it may be mentioned that in Hungary extensive surveys that had aimed to explore the fauna as completely as possible have been carried

Table 2

Number of animal — and plant species detected in apple orchards

	Survey areas						
	1,1	1.2	1.3	1.4	1.5.1	1.5.2	Total
Nematoidea	28	23	22	14	0	0	57
Orthopteroidea	12	1	1	0	9	5	18
Homoptera-Aphidoidea	3	1	19	1	52	16	53
-Coccoidea	15	4	0	0	5	2	15
-Psylloidea	0	0	0	0	1	1	1
Heteroptera	69	55	47	33	122	52	184
Neuropteroidea	6	13	14	14	33	0	34
Coleoptera	116	59	32	53	97	54	205
Lepidoptera-"Microlepidoptera"	45	189	137	130	199	37	345
"Macrolepidoptera"	66	228	127	130	313	13	441
Diptera	51	73	54	31	13	4	133
Hymenoptera	82	58	43	38	96	37	207
Acaridea	24	10	10	10	2	2	26
Aves	0	12	7	13	33	33	39
Mammalia	0	0	0	0	1	1	1
Animal species total	517	726	513	467	976	257	1759
Lychenes	22	6	0	6	6		22
Equisetales	1	0	0	0	0	0	1
Dicotyledonopsida	65	17	0	12	26	21	93
Monocotyledonopsida	17	5	0	5	5	5	21
Plant species total	105	28	0	23	37	32	137

out only in two protected areas so far, one of those areas is the ancient marshland Bátorliget, the faunistical survey of which has been carried out in the 1950-ies (Székessy et al., 1953), the other is the Hortobágy National Park. The faunistical exploration of the latter has recently been finished, with special regard to secondary alkaline (szik) prairies, reeds and scattered forests (Mahunka et al., 1981,). These areas can be considered as practically undisturbed, "natural" ecosystems.

In the Bátorliget marshland 4432 and in the Hortobágy National Park 7667 animal species were detected. These data give special interest to the 1759 species established in the apple orchards, the more so as the collected material is still being worked up. Even in the most intensively treated farming-scale orchard (1.4, Nyíregyháza–Ilonatanya) 517 species were detected.

The numbers clearly indicate that even in agricultural areas treated intensively chemically beneficial, noxious and indifferent species may be present in considerable numbers. Integrated pest management systems can be worked out only if the living world of the given crop is well known. Present paper aimed to furnish data to the knowledge of apple ecosystems.

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List of animal and plant species observed in apple orchards

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
NEMATOIDEA						
RHABDITIDEA						
Rhabditidae						
Protorhabditis tristis Hirsch.	—	16	—	16	—	—
— sp.	—	—	16	—	—	—
Rhabditis octopleura Stein.	—	16	—	16	—	—
— sp.	16	16	16	16	—	—
Diplogasteridae						
Diplogaster sp.	—	16	—	—	—	—
Cephalobidae						
Panagrolaimus sp.	—	16	—	—	—	—
Eucephalobus paracornutus De Coninck	16	—	—	—	—	—
— *striatus* Thorne	—	16	16	16	—	—
— *mucronatus* Kozl. et Rog.	—	—	16	16	—	—
— *oxyuroides* Stein.	16	16	—	16	—	—
— sp.	—	16	16	—	—	—
Cephalobus persegnis Bast.	—	16	16	16	—	—
— *nanus* De Man	—	—	16	—	—	—
— sp.	16	16	16	16	—	—
Heterocephalobus nanus Stein.	—	—	16	—	—	—
— *elongatus* De Man	—	—	16	—	—	—
— *bisimilis* Thorne	—	—	16	—	—	—
Acrobeloides bütschlii De Man	—	—	16	—	—	—
— *enoplus* Stein.	—	—	16	—	—	—
— *variabilis* Stein.	—	—	16	—	—	—
— sp.	—	—	16	—	—	—
Chiloplacus trilineatus Stein.	—	16	—	—	—	—
— *symmetricus* Thorne	—	—	16	—	—	—

Acrobeles ciliatus Lins.	16	16	–	–	–	–
– *prominens* Andrássy	–	–	16	16	–	–
Teratocephalidae						
Teratocephalus terrestris Bütschli	16	–	–	–	–	–
TYLENCHIDEA						
Tylenchidae						
Tylenchus polyhypnus Stein. et Alb.	–	–	16	–	–	–
– sp.	16	–	–	–	–	–
Tylenchorhynchus dubius Bütschli	–	16	–	–	–	–
– *tesselatus* Goodey	–	16	–	–	–	–
Rotylenchus robustus De Man	–	16	–	–	–	–
Pratylenchus pratensis De Man	16	16	16	–	–	–
Criconematidae						
Paratylenchus macrophalus De Man	–	16	–	–	–	–
Aphelenchidae						
Aphelenchus avenae Bast.	16	16	16	16	–	–
Aphelenchoides sp.	16	–	–	–	–	–
CHROMADORIDEA						
Plectidae						
Plectus granulosus Bast.	16	–	–	–	–	–
– *cirratus* Bast.	16	–	–	–	–	–
– sp.	16	–	–	–	–	–
Camacolaimidae						
Bastiania gracilis De Man	–	–	–	16	–	–

(Continued 2)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
ENOPLIDEA						
Triphylidae						
Tryphyla arenicola De Man	16	—	—	—	—	—
Mononchidae						
Mononchus sp.	16	—	—	—	—	—
Sporonchulus dentatus Cobb	16	—	—	—	—	—
Anatonchus sp.	16	—	—	—	—	—
Mylonchulus parabrachyurus Thorne	16	—	—	—	—	—
— *polonicus* Andrássy	16	—	—	—	—	—
Dorylaimidae						
Labronema ferox Thorne	16	—	—	—	—	—
Dorylaimus obtusicaudatus Bast.	16	16	16	16	—	—
— *pratensis* De Man	16	16	—	16	—	—
— sp.	16	16	—	—	—	—
Aporcelaimus laetificans Andrássy	16	—	—	16	—	—
Discolaimus texanus Cobb	16	16	—	—	—	—
— *major* Thorne	—	16	—	—	—	—
— sp.	16	—	—	—	—	—
Nygolaimus sp.	—	—	16	—	—	—
Leptonchidae						
Leptonchus sp.	16	—	—	—	—	—
Dorylaimoides sp.	16	—	—	—	—	—
Diphtherophoridae						
Trichodorus primitivus De Man	—	—	16	—	—	—
ORTHOPTEROIDEA						
SALTATOPTERA						
Tettigoniidae						
Phaneroptera falcata Poda	12, 13	—	—	—	—	—

Leptophyes albovittata Koll.	12, 13	—	—	—	—	—
Meconema thalassinum De Geer	—	—	—	—	5, 8, 10	—
Tettigonia viridissima L.	—	—	—	—	8, 10	—
Platycleis grisea F.	—	—	—	—	12, 13	12, 13
Ephippiger ephippiger Fieb.	—	—	—	—	8, 10	—
Gryllidae						
Gryllus campestris L.	14	—	—	—	14, 15	14, 15
Gryllotalpidae						
Gryllotalpa gryllotalpa L.	14	—	—	—	—	—
Tetrigidae						
Tetrix subulata L.	13	—	—	—	—	—
Acrididae						
Dociostaurus brevicollis Ever.	12	—	—	—	—	—
Chorthippus parallellus Zett.	12, 13	—	—	—	—	—
— *mollis mollis* Charp.	12, 13	—	—	—	—	—
— *brunneus* Th.	12, 13	—	—	—	12, 13	12, 13
Euchorthippus declivus Br.-Ba.	12, 13	—	—	—	—	—
DERMAPTERA						
Labiduridae						
Labidura riparia Pall.	14	—	14	—	—	—
Forficulidae						
Forficula auricularia L.	12, 13	12, 13	—	—	1, 5, 7, 10 12, 13, 11 14	7, 10, 12 13, 14
MANTODEA						
Mantidae						
Mantis religiosa L.	—	—	—	—	13	—

(Continued 3)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
BLATTOPTERA						
Ectobiidae						
Ectobius sylvestris Poda	–	–	–	–	1, 13	13
HOMOPTERA, APHIDOIDEA						
Lachnidae						
Cinara pinea Mord.	–	–	–	–	3, 4	–
Eulachnius riley Will.	–	–	–	–	4	–
Chaitophoridae						
Chaitophorus populeti Panz.	–	–	–	–	3, 4	–
Periphyllus lyropictus Kessl.	–	–	–	–	3	–
– *testudinaceus* Fern.	–	–	–	–	3, 4	–
Sipha maidis Pass.	–	–	–	–	3, 4	–
Callaphididae						
Callaphis juglandis Goetz.	–	–	–	–	3, 4	–
Drepanosiphum dixoni Hrl.	–	–	–	–	4	–
Eucallipterus tiliae L.	–	–	–	–	4	–
Hoplocallis ruperti Pint.	–	–	–	–	3, 4	–
Myzocallis castanicola Baker	–	–	–	–	3, 4	–
– *boerneri* Stroyan	–	–	–	–	3	–
Phyllaphis fagi L.	–	–	–	–	3, 4	–
Therioaphis trifolii	–	–	3, 4	–	3, 4	4
Tuberculoides annulatus Hart.	–	–	–	–	3, 4	4
– *eggleri* Börn.	–	–	–	–	3, 4	–
– *querceus* Kalt.	–	–	–	–	4	–
Aphididae						
Acyrtosiphon pisum Harris	–	–	3	–	3, 4	–
Amphorophora rubi Kalt.	–	–	3, 4	–	4	–

Aphis craccivora Koch	—	—	3, 4	—	3, 4, 8	8
— *fabae* Scop.	—	—	3, 4	—	3, 4, 8	8
— *pomi* Deg.	8	—	8	—	3, 4, 8	4, 8
— *rumicis* L.	—	—	—	—	4	—
Aulacorthum solani Kalt	—	—	4	—	3, 4	—
Brachycaudus cardui L.	—	—	—	—	3, 4	—
— *helichrysi* Kalt	—	—	3, 4	—	3, 4, 8	4, 8
Brevicoryne brassicae L.	—	—	3, 4	—	3, 4, 8	4, 8
Capitophorus eleagni Del Guer.	—	—	3	—	3, 4, 8	8
— *hippophaes* Walk.	—	—	—	—	3, 4	—
Cavariella aegopodii Scop.	—	—	—	—	3, 4	—
— *theobaldi* Gill. et Br.	—	—	—	—	3, 4	—
Cryptomyzus galeopsidis Kalt.	—	—	—	—	3	—
— *ribis* L.	—	—	—	—	3, 4	—
Dysaphis plantaginea Pass.	—	—	—	—	3, 4, 8	4, 8
— *devecta* Walk.	8	—	—	—	8	4, 8
— *pyri* Boy. et Fons.	—	—	—	—	4	—
Hayhurstia atriplicis L.	—	—	—	—	3, 4	—
Hyalopterus pruni Geoffr.	—	—	3, 4	—	3, 4, 8	—
Hyperomyzus lactucae L.	—	—	3, 4	—	3, 4, 8	4, 8
— *picridis* Börn.	—	—	—	—	4	—
Macrosiphum euphorbiae Theob.	—	—	4	—	—	—
— *rosae* L.	—	—	—	—	3, 4, 8	4, 8
Metopolophium dirhodum Walk.	—	—	3	—	3, 4	—
Myzus ascalonicus Donc.	—	—	—	—	4	—
Myzus cerasi F.	—	—	4	—	3, 4, 8	—
— *lythri* Schrank	—	—	—	—	3, 4	—
— *persicae* Sulz.	—	—	3, 4, 8	—	3, 4, 8	4, 8
Nasonovia ribisnigri Mosl.	—	—	—	—	3, 4	—
Anoeciidae						
Anoecia corni F.	—	—	3	—	3, 4	4
Pemphigidae						
Eriosoma lanigerum Hausm.	8	8	8	8	3, 4, 8	4, 8

(Continued 4)

	1.1	1.2	1.3	1.4	1.5.1	1.5 2
Paracletus cimiciformis Heyd.	—	—	—	—	3	—
Pemphigus bursarius L.	—	—	—	—	3, 4	—
Phylloxeridae						
Phylloxera glabra Börn.	—	—	3, 4	—	3, 4, 8	8
HOMOPTERA, COCCOIDEA						
Pseudococcidae						
Phenacoccus aceris Geoffr.	8	—	—	—	8	—
— *angustatus* Borchs.	13	—	—	—	—	—
— *hordei* Lind.	13	—	—	—	—	—
Trionymus perrisi Sign.	13	—	—	—	—	—
— *tomlini* Green	13	13	—	—	—	—
Eriococcidae						
Acanthococcus munroi Borat.	13	—	—	—	—	—
Greenisca glyceriae Green	13	—	—	—	—	—
Rhizococcus cynodontis Kiritsch.	13	—	—	—	—	—
Coccidae						
Eriopeltis festucae Fonsc.	13	—	—	—	—	13
Palaeolecanium bituberculatum Targ. — Tozz.	8	—	—	—	—	—
Parthenolecanium corni Bouché	8	8	—	—	8	—
Sphaerolecanium prunastri Fonsc.	8	—	—	—	—	—
Diaspididae						
Epidiaspis leperii Sign.	8	8	—	—	8	—
Quadraspidiotus perniciosus Comst.	8	8	—	—	8	8
— *ostraeformis* Curt.	8	—	—	—	8	—

HOMOPTERA, PSYLLOIDEA						
Psyllidae						
Psylla mali Schmiebd.	–	–	–	–	8	8
HETEROPTERA						
Corixidae						
Cymatia rogenhoferi Fieb.	–	–	1	–	–	–
Callicorixa concinna Fieb.	–	–	1	–	–	–
– *praeusta* Fieb.	–	–	1	1	–	–
Hesperocorixa linnéi Fieb.	–	–	1	1	–	–
Sigara falleni Fieb.	–	–	1, 3	–	–	–
– *lateralis* Leach.	–	–	1	1	–	–
– *striata* L.	–	–	1	1	–	–
– *limitata* Fieb.	–	–	1	–	–	–
Miridae						
Deraeocoris olivaceus F.	–	–	–	–	1	–
– *trifasciatus* L.	–	–	1	1	1, 7	–
– *annulipes* H. Sch.		–	–	–	1	–
– *rutilus* H. Sch.	–	–	–	–	1	–
– *ruber* L.	–	–	–	–	1, 3, 7, 8, 12	7, 12
– *serenus* Doug. et Scott	–	–	–	–	–	12
– *punctulatus* Fall.	–	–	–	–	11	11
– *putoni* Mont.	–	–	7	–	7	–
– *lutescens* Schill.	–	–	–	–	1	–
Alloeotomus gothicus Fall.	–	1	1	–	–	–
– *germanicus* E. Wagn.	–	–	–	–	1	–
Dicyphus globulifer Fall.	–	–	–	–	12	12
Leptopterna dolobrata L.	–	–	–	–	8	7
– *ferrugata* Fall.	12	–	1	1	1	–
Teratocoris antennatus Boh.	–	1	1	–	–	–

(Continued 5)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Stenodema calcaratum Fall.	12	1, 12	1	1	1	–
– *laevigatum* L.	12	–	–	–	12	–
– *sericans* Fieb.	–	1	–	–	–	–
Notostira elongata Geoffr. ♂	12, 14	–	–	–	–	–
– *elongata* Geoffr. (or *N. erratica* L.) ♀	12	12	–	–	12	–
– *erratica* L.♂	12	–	–	–	12	12
Megaloceroea recticornis Geoffr.	–	–	–	–	–	12
Trigonotylus coelestialium Kirk.	12	1, 12	1	1, 12	1	12
– *pulchellus* Hahn	–	1	1	1	–	–
Phytocoris meridionalis H. Sch.	–	–	–	–	1	–
– *longipennis Flor*	–	–	–	1	1	–
– *tiliae* F.	7	–	–	–	1	–
– *dimidiatus* Kirsch.	–	–	–	–	1	–
– *thrax* Josifov	–	–	–	–	1	–
– *reuteri* Saund.	–	–	–	–	1, 3, 7	7
– *ulmi* L.	–	–	–	–	1, 7, 8, 12	7, 12
– *varipes* Boh.	–	–	–	–	1	–
– *insignis* Reut.	–	–	1	–	1, 12	–
Pantilius tunicatus F.	–	–	–	1	1	–
Megacoleum beckeri Fieb.	–	–	–	–	1	–
Adelphocoris seticornis F.	12	1	1	1	1	–
– *ticiensis* Meyer-Dür	–	–	–	–	1	–
– *lineolatus* Goeze	12	1	1	1, 12, 14	1, 12	7, 12
– *annulicornis* Sahlb.	–	–	–	–	12	–
Calocoris quadripunctatus Vill.	–	–	–	–	1, 8	–
– *biclavatus* H. Sch.	–	–	–	–	1	–
– *norvegicus* Gmel.	–	12	–	–	1, 3, 12	12
Alloeonotus fulvipes Scop.	–	–	–	–	1	–
Miris striatus L.	–	–	–	–	1	–
Brachycoleus decolor Reut.	–	–	–	–	1	–
Stenotus binotatus F.	–	–	–	–	1, 3	–

Lygus lucorum Meyer-Dür	–	–	–	–	1	–
Exolygus rugulipennis Popp.	7, 12	1, 12, 14	1, 3	1, 7, 12	1, 7, 8, 12	7, 12
— *pratensis* L.	12	1, 12	1	1	1, 12	7, 12
— *gemellatus* H. Sch.	12	1	1	1	1, 3, 7, 8, 12	8
Orthops campestris L.	–	1	1	–	–	–
— *kalmi* L.	12	1, 12	1	1	1	7
Liocoris tripustulatus F.	–	–	–	–	11, 12	11
Charagochilus gyllenhali Fall.	–	1	–	–	–	–
Polymerus vulneratus Panz.	–	1	1	1	1	–
— *cognatus* Fieb.	–	–	1	1	–	–
— *asperulae* Fieb.	–	–	–	–	1	–
— *palustris* Reut.	–	1	1	1	–	–
— *unifasciatus* F.	–	1	1	1	1	–
Capsus ater L.	12	–	–	–	–	–
Capsodes cingulatus F.	–	–	–	–	12	–
Piezocranum simulans Horváth	–	–	–	–	1	–
Orthocephalus ferrarii Reut.	–	–	–	–	1	–
— *vittipennis* H. Sch.	–	–	1	1	1	–
Orthotylus marginalis Reut.	–	–	1	–	–	–
— *tenellus* Fall.	–	–	–	–	1	–
Globiceps flavomaculatus F.	–	–	–	–	1	–
— *sphegiformis* Rossi	–	–	–	–	7	–
Dryophilocoris luteus H. Sch.	–	–	–	–	1, 8	–
— *flavoquadrimaculatus* De Geer.	–	–	–	–	1, 7	–
Cyllocoris histrionicus L.	–	–	–	–	8	–
Pilophorus perplexus Doug. et Scott	–	–	–	–	7	7
Macrotylus herrichi Reut.	–	–	–	–	1	–
— *horvathi* Reut.	–	1	1	–	–	–
— *solitarius* Meyer-Dür	–	–	–	–	1	–
Harpocera thoracica Fall.	–	–	–	–	1, 7	–
Chlorillus alpinus Reut.	–	1	–	–	1	–
Plagiognathus chrysanthemi Wolff.	–	–	–	–	7, 12	12
— *fulvipennis* Kirsch.	–	1	–	–	1, 12	–
Clamidatus pullus Reut.	–	–	–	–	7	–
Sthenarus rottermundi Scholtz	–	–	1	–	–	–
Atractotomus mali Meyer-Dür	–	–	–	–	7, 8, 12	7, 12

(Continued 6)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Psallus lepidus Fieb.	—	—	—	—	1	12
Oncotylus setulosus H. Sch.	—	—	—	—	1	—
Phylus melanocephalus L.	—	—	—	—	1	—
Icodema infuscatum Fieb.	—	—	—	—	1	—
Amblytylus concolor Jakovl.	—	1	—	—	1, 12	12
Megalocoleus molliculus Fall.	—	—	—	—	1	—
Orthops sp.	—	12	—	—	—	—
Psallus sp.	—	—	—	—	1	—
Miridae sp.	12	1, 12	1	1	1, 12	7, 12
Anthocoridae						
Anthocoris nemorum L.	—	—	—	—	7	7
— *sibiricus* Reut.	—	—	—	—	7, 8	—
Orius niger Wolff	12	12	—	—	7, 12	12
— *minutus* L.	8	14	—	—	7, 8, 12	7, 8, 12
— *majusculus* Reut.	—	14	—	—	—	—
Orius sp.	—	12	—	12	—	—
Nabidae						
Himacerus apterus F.	—	14	—	—	7, 8	7
Aptus myrmecoides Costa	—	—	—	—	7, 11	—
Nabis ferus L.	12	1, 12	1	1, 12, 14	1, 12, 14	7,12
— *pseudoferus* Rem.♂	12, 14	12	—	12, 14	12	12
— *pseudoferus* Rem. (or — *punctatus* Costa) ♀	12	7, 12, 14	1, 7	1, 12, 14	1, 7, 8, 12	7, 12, 14
— *punctatus* Costa ♂	7, 12	12, 14	1, 7	—	1, 7, 12	12, 14
— *rugosus* L.	—	—	—	—	1	7
Reduviidae						
Reduvius personatus L.	—	—	—	1	—	—
Pirates hybridus Scop.	—	—	—	—	12	—

Tingidae						
Dictyonota tricornis Schrank	12	–	–	–	–	–
Stephanitis pyri F.	7, 12	–	–	–	–	–
Dictyla echii Schrank	12, 14	–	–	–	–	–
Saldidae						
Saldula pilosella Thoms.	–	1	1	–	–	–
Piesmidae						
Piesma capitatum Wolff	–	–	–	–	12	–
– *maculatum* Lap.	12	–	–	–	–	–
Berytidae						
Neides tipularis L.	12, 14	–	–	–	–	–
Berytinus minor H. Sch.	12, 14	12	–	–	–	–
Lygaeidae						
Lygaeus equestris L.	–	–	–	–	7, 12	–
Nysius senecionis Schill.	7, 12	12	–	–	1, 7, 12	7, 12
– *thymi* Wolff	7, 12, 14	12	–	–	–	–
Geocoris dispar Waga	12, 14	–	–	–	–	–
Chilacis typhae Perr.	–	–	1	–	–	–
Heterogaster urticae F.	–	–	1	–	11	7
Platyplax salviae Schill.	–	–	–	–	7	–
Metopoplax origani Kolen.	12	–	1	–	–	7
Oxycarenus pallens H. Sch.	–	–	–	–	–	11
Pachybrachius fracticollis Schill.	–	–	1	1	–	–
Stygnocoris rusticus Fall.	–	–	–	–	12, 14	–
– *fuligineus* Geoffr.	14	–	–	–	14	14
– *pedestris* Fall.	12	–	–	–	12	–
Drymus silvaticus F.	12	–	–	–	12, 14	–
Scolopostethus affinis Schill.	14	–	–	–	–	–
Emblethis denticollis Horváth	–	–	–	–	14	–
– *griseus* Wolff	12, 14	–	–	–	–	–
Trapezonotus arenarius L.	12, 14	–	–	–	–	–
Sphragisticus nebulosus Fall.	12, 14	1, 14	1	1	7, 12, 14	11, 12
Rhyparochromus vulgaris Schill.	–	–	–	–	3, 7, 11, 12	7, 11, 12

(Continued 7)

1.1	1.2	1.3	1.4	1.5.1	1.5.1				
— *alboacuminatus* Goeze				—	—	—	—	11	—
— *lynceus* F.				12	—	—	—	—	—
Beosus maritimus Scop.				12	12	—	—	11, 14	12
Peritrechus gracilicornis Puton				—	—	1	—	7, 12, 14	—
— *nubilus* Fall.				7	—	—	1	—	—
— *geniculatus* Hahn				—	—	—	—	12	—
Megalonotus chiragra F.				—	14	—	—	—	—
Pyrrhocoridae									
Pyrrhocoris apterus L.				14	14	14	—	—	—
Coreidae									
Syromastes rhombeus L.				12	—	—	—	8, 12	—
Coreus margiratus L.				—	—	—	—	1, 12	—
Bathysolen nubilus Fall.				12	—	—	—	—	—
Alydidae									
Alydus calcaratus L.				12	—	—	—	—	—
Corizidae									
Corizus hyoscyami L.				—	—	—	—	—	12
Lyorrhyssus hyalinus F.				—	1	—	—	1	—
— *parumpunctatus* Schill				12	12	—	—	12	12
Brachycarenus tigrinus Schill.				12	12	1	—	12	7, 12
Stictopleurus punctatonervosus Goeze				12	12	—	—	12	—
— *crassicornis* L.				12	—	—	—	12	—
— *abutilon* Rossi				7, 12	12	—	—	7	—
Myrmus miriformis Fall.				12	12	—	12	—	—
Chorosoma schillingi Schill.				12	—	—	—	—	—
Pentatomidae									
Aelia acuminata L.				7, 12	12	—	—	7, 12	7
Neottiglossa leporina H. Sch.				12	—	—	—	—	—

— *pusilla* Gmel.	12	—	—	—	—	—
Eusarcoris aeneus Scop.	12	12	—	—	12	—
Holcostethus vernalis Wolff	12	—	—	—	12	—
Carpocoris purpureipennis De Geer	—	—	—	—	—	12
Dolycoris baccarum L.	12	12	—	—	3, 7, 8, 12	7, 12
Palomena prasina L.	—	—	—	—	3, 7, 12	7
Rhaphigaster nebulosa Poda	7	7, 14	—	—	—	—
Pentatoma rufipes L.	—	—	—	—	1, 7	7
Eurydema ornatum L.	—	—	—	—	—	7
— *oleraceum* L.	12	12	—	—	11, 12	7
Picromerus bidens L.	7, 12, 14	—	—	14	—	—
Arma custos F.	—	—	—	7	7	—
Scutelleridae						
Eurygaster austriaca Schrank	—	—	14	—	1	12
— *maura* L.	12	—	—	—	—	—
Cydnidae						
Aethus nigritus F.	14	—	—	—	—	—
Tritomegas bicolor L.	7, 14	—	—	—	—	—
— *sexmaculatus* Rambur	7	—	—	—	—	—
Ochetostethus nanus H. Sch.	12	—	—	—	—	—
Plataspidae						
Coptosoma scutellatum Geoffr.	12	—	—	—	—	—
NEUROPTEROIDEA						
RAPHIDIOPTERA						
Raphidiidae						
Raphidia flavipes Stein	—	—	—	—	1, 8, 17	—
— *ophiopsis alcoholica* Aspöck et Aspöck	—	—	—	—	17	—
— *xanthostigma* Schum.	—	—	—	—	17	—
— *major* Burm.	—	—	—	—	17	—

(Continued 8)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Inocelliidae						
Inocellia braueri Albarda	—	—	—	—	8	—
— *crassicornis* Schum.	—	—	—	—	17	—
PLANIPENNIA (NEUROPTERA)						
Hemerobiidae						
Drepanepteryx phalaenoides L.	—	—	—	—	1, 17	—
Wesmaelius nervosus F.	—	14	—	—	1, 7	—
— *subnebulosus* Steph.	—	1	1, 3	—	1, 3, 4 5, 7, 17	—
Hemerobius humulinus L.	12	1, 12, 14	14, 3	1, 12, 14	1, 3, 4, 7, 17, 8	—
— *atrifrons* Mc Lach.	—	—	3	14	3, 4	—
— *handschini* Tjed.	—	—	—	—	1	—
— *nitidulus* F.	—	—	3, 14	—	1, 3, 4, 17	—
— *micans* Oliv.	—	1	14	14	—	—
— *lutescens* F.	—	—	—	—	1	—
Micromus variegatus F.	—	14	1	1, 14	1, 8	—
— *angulatus* Steph.	—	—	1, 3, 4	1, 12, 14	1, 3, 4, 5, 7, 17	—
Sympherobius elegans Steph.	—	—	—	—	1	—
— *pygmaeus* Rambur	—	—	—	—	1	—
Chrysopidae						
Chrysoperla carnea Steph.	7, 12	1, 12, 14	1, 3, 4, 7, 14	1, 7, 12, 14	1, 2, 3, 4, 5, 7, 8, 10, 12, 17	—
Chrysopa formosa Brauer	14	1	1, 3, 4	1, 14	1, 2, 3, 4, 5, 7, 8, 12	—

— *phyllochroma* Wesm.	2, 12	1, 14	1, 3, 4, 14	1, 14	1, 3, 7, 8, 12	—
— *perla* L.	12	1	—	1	1, 3, 4, 5, 7, 8, 12, 14, 17	—
— *abbreviata* Curt.	2,12	1	1, 3	1	1, 4, 7, 8, 12, 17	—
— *septempunctata* Wesm.	—	1	1, 3, 14	1	1, 2, 3, 4, 7, 8, 12	—
— *walkeri* Mc Lach.	—	—	—	—	1	—
— *dorsalis* Burm.	—	—	—	—	8	—
Anisochrysa ventralis Curt.	—	1, 14	1, 3	1	1, 2, 3, 4, 5, 8, 12	—
— *prasina* Burm.	—	—	—	—	3, 8, 12	—
— *flavifrons* Brauer	—	—	—	—	1	—
Chrysotropia ciliata Wesm.	—	1	3	1	1, 3, 4, 5, 7, 8, 17	—
Hypochrysa elegans Burm.	—	—	—	—	17	—
Nineta flava Scop.	—	—	—	1	1, 5, 2, 7, 12	—
— *vittata* Wesm.	—	—	—	—	3, 4, 7, 14	—
COLEOPTERA						
Carabidae						
Abax ater Vill.	—	—	—	—	14	—
— *ovalis* Duft.	—	—	—	—	14	—
— *paralellepipedus* Pill.	—	—	—	—	14	—
Acupalpus meridianus L.	—	—	—	—	14	14
— *teutonus* Schrank	16	—	—	—	—	14
Aechmites terricola Herbst	—	—	—	—	14	14
Agonum krynickii Sperk	16	—	—	—	—	—
— *moestum* Sturm	—	—	—	—	14	—
Amara anthobia Villa et V.	12	—	—	—	14	—
— *bifrons* Gyll.	12, 14, 16	—	—	—	14	14
— *aenaea* Heer	16	—	—	16	14	14
— *apricaria* Payk.	—	—	—	—	14	—
— *aulica* Panz.	—	—	—	—	14	—
— *communis* Panz.	—	—	—	16	—	—

(*Continued 9*)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
— *consuralis* Duft.	—	—	—	—	14	14
— *convexior* Steph.	16	—	—	—	—	—
— *eurynota* Panz.	—	—	14	—	14	—
— *familiaris* Duft.	14, 16	—	14	14, 16	14	14
— *ingenua* Duft.	—	—	—	—	14	14
— *ovata* F	—	—	—	—	14	14
— *similata* Gyll.	16	—	16	16	14	14
Anisodactylus binotatus F.	16	—	14, 16	14, 16	14	—
— *signatus* Panz.	14	14, 16	14	14, 16	14	14
Asaphidion flavipes L.	—	—	—	—	14	—
Badister lacertosus Strum.	—	—	—	—	14	—
— *meridionalis* Puel.	—	—	—	—	14	—
Bembidion lampros Herbst	—	—	—	—	14	14
— *properans* Steph.	—	—	16	—	14	—
Brachinus crepitans L.	—	—	—	—	14	—
— *explodens* Duft.	—	—	—	—	14	14
Bradicellus harpalinus Serv.	—	—	—	—	14	—
Broscus cephalotes L.	—	—	14	12	14	14
Calathus ambiguus Payk.	—	—	—	—	14	—
— *erratus* C. R. Sahlb.	—	—	—	—	14	14
— *fuscipes* Goeze	12, 14	—	—	—	14	14
— *melanocephalus* L.	14, 16	—	—	—	14	—
Calosoma aruopunctatum L.	—	14	—	—	—	14
Carabus coriaceus L.	—	—	—	—	—	14
— *hortensis* L.	—	—	—	—	14	—
— *scabriusculus* Ol.	—	—	—	—	14	—
— *ullrichi* Germ.	—	—	—	—	14	—
— *violaceus* L.	16	—	—	—	14	14
Dolichus halensis Schall.	—	—	—	14	14	—
Drypta dentata Rossi	—	—	—	—	14	—
Dyschirius globosus Herbst	14	—	—	—	—	—

Harpalus affinis Schrank	–	14	–	14	–	–
– *anxius* Duft.	14, 16	–	–	–	–	–
– *calceatus* Duft.	–	–	14	–	14	14
– *griseus* Panz.	16	–	14	–	14	14
– *neglectus* Serv.	16	–	–	–	–	–
– *picipennis* Duft.	14	14, 16	–	–	14	–
– *psittaceus* Fourc.	–	16	14, 16	14, 16	14	14
– *rufipes* De Geer	14	14	14, 16	14, 16	14	14
– *serripes* Quens.	–	–	–	16	–	–
– *servus* Duft.	16	–	–	–	–	–
– *signaticornis* Duft.	–	–	–	–	14	–
– *tardus* Panz.	16	14	14, 16	16	14	14
Leistus rufomarginatus	–	–	–	–	14	14
Licinus cassideus Ill. Duft.	–	–	–	–	14	–
Metabletus pallipes Dej.	–	–	–	–	14	14
Microlestes maurus Redt.	–	–	–	–	14	–
Panagaeus crux-major L.	–	–	–	–	14	–
Parophanus complanatus Dej.	–	–	–	–	14	–
– *maculicornis* Duft.	–	–	–	–	14	–
Platynus dorsalis Pont.	–	–	–	–	14	14
Platyderus rufus Duft.	–	–	–	–	14	–
Poecilus cupreus L.	–	–	–	16	14	14
Pterostichus macer Marsh.	–	–	–	–	14	–
– *melanarius* Ill.	–	–	14	14	14	14
– *melas* Cremtz.	–	–	–	–	14	–
– *niger* Schall.	–	–	–	–	14	14
– *oblongopunctatus* F.	–	–	–	–	14	14
– *strennuus* Duft.	–	–	–	–	–	14
– *striatopunctatus* D.	–	–	–	–	14	–
– *versicolor* Sturm.	–	–	–	–	14	14
Stomis pumicatus Panz.	–	–	–	–	14	14
Synuchus nivalis Panz.	–	–	–	–	–	14
Trechus quadristriatus Duft.	–	–	–	–	14	–
Silphidae						
Silpha carinata L.	14	–	–	14	–	14

(Continued 10)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Staphylinidae						
Ocypus olens O. F. Müll.	—	—	—	—	14	14
— *ophthalmicus* Scop.	14	—	14	—	14	14
Ontholestes tesselatus Fourc.	—	—	—	—	14	—
Paederus fuscipes Rossi	12, 14	—	—	—	—	—
Platydraticus parumtomentosus Stein.	—	—	—	—	14	14
Histeridae						
Margaringotus purpurascens Herbst	14	14	14	14	14	14
Lampyridae						
Lamporhiza splendidula L.	—	—	—	—	14	—
Cantharidae						
Cantharis fusca L.	14	12, 14	—	14	—	—
— *rustica* L.	7	12, 14	—	12, 14	—	14
Rhagonychus fulva Scop.	12	—	—	—	14	—
Silis nitidula Fabr.	—	—	—	14	—	—
Malachiidae						
Anixotarsus marginalis F.	12	—	—	—	—	—
Malachius aeneus L.	—	12	—	—	—	—
— *geniculatus* Germ.	12, 14	—	—	12	—	—
— *vulneratus* Ab.	12	—	—	—	—	—
Elateridae						
Adrastus rachifer Fourc.	7, 12	7, 12	—	—	—	—
Agriotes sputator L.	12	12, 16	—	12	—	—
Agrypnus murinus L.	7, 12, 14	—	—	—	—	—
— *murinus* L. (larva)	16	—	—	—	—	—
Cidnopus pilosus Leske	12	—	—	—	—	—
Melanotus crassicollis L.	—	—	—	16	—	—
— *crassicollis* L. (larva)	—	—	—	16	—	—

Melanotus niger F.	16	—	—	—	—	—
— *niger* F. (larva)	16	—	—	16	—	—
Selatosomus latus F. (larva)	—	16	—	16	—	—
Buprestidae						
Anthaxia cichorii L.	12	—	—	—	—	—
Dermestidae						
Attagenus schaefferi Herbst	—	—	—	14	—	—
Dermestes laniarius Ill.	12, 14	—	—	—	—	—
Colydiidae						
Synchita humeralis Fabr.	—	—	—	7	—	—
Phalacridae						
Olibrus millefolii Sturm.	12	12	—	—	—	—
Phalacrus fimetarius Payk.	12	—	—	—	—	—
Stilbus testaceus Panz.	7, 12	—	—	—	—	—
Mycetophagidae						
Typhaea stercorea L.	—	14	—	—	—	—
Nitidulidae						
Glischrochilus hortensis Fourc.	—	—	—	14	—	—
Meligethes aeneus F.	8, 12	12, 14	7	12	14	—
— *coracinus* Sturm.	12	12	—	—	—	—
Nitidula quadrimaculata L.	—	12	—	—	—	—
Coccinellidae						
Adalia bipunctata L.	7, 12	12	7, 14	—	7, 8, 14	7, 14
— *decempunctata* L.	—	—	—	—	7, 8	7
Adonia variegata Goeze	—	—	—	14	7, 8	7, 14
Calvia quattuordecimguttata L.	—	—	—	—	7, 8	7
Chilocorus bipustulatus L.	7, 8, 14	—	—	—	—	—
Coccinella septempunctata L.	7, 12, 14	7, 12, 14	7, 14	7, 12, 14	7, 8, 14	7, 14
Coccinula quattuordecimpustulata L.	7, 12	12	—	7, 12, 14	7, 14	7
Exochomus quadripustulatus	7	14	—	—	7, 8	7

(Continued 11)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Halyzia sedecimguttata L.	7, 12	—	—	—	—	—
Harmonia quadripunctata Pont.	—	—	—	—	8	—
Hippodamia quadripunctata L.	—	—	—	—	—	7
— *tredecimpunctata* L.	14	12	7	12	8	7
Platynaspis luteorubra Goeze	12	12	—	—	—	—
Propylaea quattuordecimpunctata L.	7, 12, 14	7, 12, 14	7, 14	7, 12, 14	—	—
Semidalia undecimnotata Schn.	—	—	—	—	8	—
Scymnus apetzi Muls.	—	—	—	—	8	—
— *rubromaculatus* Goeze	12	—	—	—	—	—
Stethorus punctillum Weise	7	7, 12	7	7, 12	—	—
Subcoccinella vigintiquattuorpunctata L.	7, 12, 14	12	—	—	—	—
Synharmonia conglobata L.	—	—	—	—	7, 8	—
Thea vigintiduopunctata L.	12	14	—	—	7	7
Tenebrionidae						
Opatrum sabulosum Panz.	16	—	—	—	—	—
Lagriidae						
Lagria hirta L.	7, 12, 14	—	—	14	—	—
Anthicidae						
Anthicus antherinus L.	—	12, 14	14	12	14	—
— *humilis* L.	—	—	—	—	14	—
Notoxus monoceros L.	12, 14	12, 14	14	12, 14	—	—
Oedemeridae						
Oedemera lurida Marsh.	12	—	—	—	—	—
— *virescens* L.	12	14	—	—	—	—
— *podagrariae* L.	12, 14	—	—	—	—	—
Melolonthidae						
Amphimallon solstitialis L. (lar.)	16	—	—	—	—	—
Anisoplia segetum Herbs.	12	—	—	—	—	—

— *segetum* Herbst (larva)	16	—	—	—	—	—
Homaloplia alternata Küst.	12	—	—	—	—	—
— *ruralis* (larva)	16	—	—	—	—	—
Hoplia hungarica Burm.	12, 14	—	—	—	—	—
— sp. (larva)	16	—	—	—	—	—
Maladera holoserica Scop.	14	—	—	14	—	—
— *holoserica* Scop. (larva)	16	—	—	—	—	—
Melolontha melolontha L.	7, 8	14	7	7, 14	—	—
— *melolontha* (larva)	16	16	16	16	—	—
Phyllopertha horticola L. (larva)	16	—	—	—	—	—
Polyphylla fullo L. (larva)	16	—	—	—	—	—
Rhizotrogus aestivus Oliv.	—	—	—	—	14	—
Serica brunnea L.	14	—	—	—	—	—
— *brunnea* L. (larva)	16	—	—	—	—	—
Valgus hemipterus L.	14	—	14	—	—	—
Scarabeidae						
Aphodius distinctus O. F. Müll.	—	14	—	—	—	—
— sp. (larva)	16	16	—	16	—	—
Geotrupes vernalis L.	—	—	—	—	14	14
Trox hispidus Pont.	—	—	—	—	14	14
Cerambycidae						
Anoplodera livida pecta Dan.	12	—	—	—	—	—
Chlorophorus varius O. F. Müll.	—	12	—	—	—	—
Chrysomelidae						
Aphthona euphorbiae Schrank	12	12	—	7, 12, 14	—	—
Asiorestia ferruginea Scop.	12, 14	12	—	—	—	—
Cassida nebulosa L.	7, 12	12	—	14	—	—
— *nobilis* L.	7, 12	12	—	—	—	—
Chrysolina staphylea L.	—	—	—	12	—	—
Cryptocephalus connexus Oliv.	12	—	—	12	—	—
— *sericeus* L.	12	—	—	—	—	—
Dlochrysa fastuosa Scop.	12	—	—	—	—	—
Galerucella calmariensis L.	—	12	7	—	—	—
Hispa atra L.	12	—	—	—	—	—

(Contiuned 12)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Hyppocassida subferruginea Duft.	12	—	—	—	—	—
Labidostomis longimana L.	12	—	—	—	—	—
Leptinotarsa decemlineata Say	12, 14	—	—	12, 14	—	—
Oulema melanopus L.	12, 14	12	—	12, 14	—	—
— *lichenis* Voet.	12	—	—	—	—	—
Phyllotreta nemorum L.	12	12, 14	—	—	—	—
— *undulata* Kutsch.	12, 14	12	—	12	14	—
Bruchidae						
Bruchus pisorum L.	12	12	—	—	—	—
Euspermophagus sericeus Fourc.	7	—	—	—	—	—
Apionidae						
Apion apricans Germ.	—	12	—	—	—	—
— *pisi* Fabr.	12	—	—	12	—	—
— *trifolii* L.	7, 12, 7, 12	—	—	—	—	—
Curculionidae						
Anthonomus pomorum L.	7, 8, 12, 14	—	—	—	8, 10, 11	—
Balanobius salicivorus Payk.	7	—	—	—	—	—
Baris timida	12	—	—	—	14	—
Ceutorhynchus caeruleus	12	—	—	—	—	—
— *contractus* Marsh.	12	12	—	—	—	—
Eusomus ovulum Germ.	—	12	—	14	—	—
Mylacuss seminulum Fabr.	12	12	—	—	—	—
Otiorhynchus ligustici L.	7	7	7	—	14	—
— *ovatus* L.	12, 14	14	—	—	—	—
Peritelus familiaris Boh.	12, 14	12	7, 14	—	—	14
Phyllobius argentatus L.	8, 12	12	12	—	—	—
— *oblongus* L.	7, 12, 14	12, 14	7, 12, 14	—	—	—
Sitona crinitus Herbst	7, 12	—	12	—	—	—
— *sulcifrons* Herbst	7, 12	12	—	—	—	—

Smicronyx jungermanniae Reich	12	–	–	–	–	–
Tanymecus palliatus F.	–	–	7	–	–	–
Scolytidae						
Scolytus mali Bechst.	7, 12, 14	–	–	–	–	–
Anthribidae						
Anthribus nebulosus Först.	8	8	–	–	–	–
LEPIDOPTERA "MICROLEPIDOPTERA"						
Eriocraniidae						
Mnemonica subpurpurella Haw.	–	–	–	–	1	–
Hepialidae						
Triodia sylvina L.	–	1	1	1	1	–
Incurvariidae						
Adela degeerella L.	–	1	1	–	1	–
Nepticulidae						
Nepticula pomella Vaug.	8	–	–	8	8	8
– *malella* Stt.	8	8	8	8	8	8
Cossidae						
Cossus cossus L.	–	–	–	–	–	1, 8
Dyspessa ulula Bkh.	–	–	–	–	1	–
Phragmataecia castaneae Hbn.	–	–	1	–	1	–
Tineidae						
Nemapogon granellus L.	–	1	–	–	–	–
Triaxomera prasitella Hbn.	–	–	–	1	–	–
Neurothaumasia ankerella Mn.	–	1	–	1	–	–
Cephimallota simplicella HS.	–	1	–	–	–	–
Monopis monachella Hbn.	2	1	1	1	1	–
Morophaga boletti F.	–	1	1	–	1	–
Talaeporiidae						
Talaeporia tubolosa Retz.	–	–	1	–	–	–

(Continued 13)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Psychidae						
Rebelia perlucidella Brd.	–	1	–	–	–	–
Bijugis bombycella Den. et Schiff.	–	–	–	1	–	–
Psyche viciella Schiff.	–	–	–	–	1	–
Sterrhopteryx gozmanyi Kovács	–	–	–	1	–	–
Lyonetiidae						
Lyonetia clerkella L.	–	–	–	–	8	8
Plutellidae						
Acrolepia valeriella Snell.	–	1	–	–	–	–
Orthotelia sparganella Thnbg.	–	1	–	–	–	–
Ypsolophus vitellus L.	–	–	–	–	1	–
– *lucellus* F.	–	–	–	–	1	–
– *alpellus* Den. et Schiff.	–	–	–	–	1	–
– *chazariellus* Mn.	–	–	1	–	–	–
– *scabrellus* L.	–	–	–	–	8	–
Plutella maculipennis Curt.	–	1	1	1	1	–
Eidophasia messingiella F.	–	1	–	–	1	–
Leucopteridae						
Leucoptera scitella Z.	8	8	8	–	8	8
Lithocolletidae						
Lithocolletis betulae Z.	–	–	–	–	1	–
– *corylifoliella* Haw.	8	8	8	8	8	8
– *blancardella* F.	8	8	8	8	8, 11	8
Gracilariidae						
Parornix caudulatella Z.	–	1	–	–	–	–
– *petiolella* Frey	8	8	8	8	8	8
Callisto denticulella Thnbg.	8	–	–	–	8, 11	8

Euspilapteryx phasianipennella Hbn.	–	1	–	–	–	–
Gracilaria anastomosis Haw.	–	1	–	–	–	–
Caloptilia alchimiella Sc.	–	–	1	–	–	–
– *stigmatella* F.	–	1	–	–	–	–
Coleophoridae						
Coleophora serratella L.	8	–	–	–	8	–
– *frischella* L.	–	1	–	–	–	–
– *alcyonipennella* Koll.	–	1	–	–	–	–
– *lixella* Z.	–	–	–	–	1	–
– *ochrea* Haw.	–	–	–	–	1	–
– *squalorella* Z.	–	1	1	–	–	–
– *anatipennella* Hbn.	–	–	–	–	1, 8, 11	8
Epermeniidae						
Epermenia illigerella Hbn.	–	–	–	1	–	–
Hyponomeutidae						
Scythropia crataegella L.	–	1	–	–	–	–
Hyponomeuta vigintipunctatus Retz.	–	1	–	–	–	–
– *evonymellus* L.	–	1	–	1	–	–
– *padellus* L.	–	1	–	–	–	–
– *malinellus* L.	–	–	–	–	8	8
– *plumbellus* Den. et Schiff.	–	–	–	–	1	–
Swammerdamia pyrella Vill.	8	–	–	–	8	8
Ethmiidae						
Ethmia pusiella Roemer	–	–	–	–	1	–
– *decemguttella* Hbn.	–	–	–	–	1	–
– *terminella* Fletcher	–	–	–	–	1	–
– *bipunctella* F.	–	–	–	–	1	–
Aegeriidae						
Synanthedon myopaeformis Bkh.	5	5	5	5	–	–
Glyphipterygidae						
Aechmia thrasonella Sc.	–	1	1	1	–	–

(Continued 14)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Cosmopterygidae						
Pyroderces argyrogrammos Z.	–	–	–	–	1	–
Blastodacna atra Haw.	–	–	–	–	8	–
Stagmatophora serratella Tr.	–	–	–	–	1	–
Limnaecia phragmitella Stt.	–	1	1	–	–	–
Oecophoridae						
Bratia unitella Hbn.	6	6	–	–	–	–
Diurnea phryganella Hbn.	8	–	–	–	1, 8	8
— *fagella* F.	8	–	1	–	1, 8	8
Cheimophila salicella Hbn.	8	1	–	–	–	–
Henicostoma lobellum Den. et Schiff.	–	1	–	–	–	–
Depressaria nervosa Haw.	–	–	1	–	–	–
— *heracliana* de Geer	–	–	–	–	1	–
Agonopteryx costosa Haw.	–	–	–	–	1	–
— *alstroemeriana* Cl.	–	1	1	–	–	–
— *furvella* Tr.	–	–	–	–	1	–
— *liturella* Hbn.	–	–	–	–	1	–
— *propinquella* Tr.	–	–	–	1	1	–
— *yeatiana* F.	–	–	–	–	1	–
— *applana* F.	–	–	1	–	1	–
— *ciliella* Stt.	–	1	–	–	–	–
Hypercallia citrinalis Sc.	–	–	–	–	1	–
Carcina quercana F.	–	–	–	–	1	–
Pleurota bicostella Ci.	–	–	–	–	1	–
— *pyropella* Den. et Schiff.	–	1	–	–	–	–
Epicallima formosella F.	–	1	–	–	1	–
Bisigna procerella Den. et Schiff.	–	–	–	–	1	–
Schiffermülleria schaefferella L.	–	1	–	–	–	–
Oecophora bractella L.	–	–	–	–	1	–

Symmocidae						
Oegoconia quadripuncta Haw.	–	–	–	–	1	–
Gelechiidae						
Gomphocrates rasilella HS.	–	1	–	–	1	–
Brachmia rufescens Haw.	–	–	–	–	1	–
– *dimidiella* Den. et Schiff.	–	1	1	–	–	–
– *albinervis* Geras	–	1	1	1	–	–
– *inornatella* Dgl.	–	1	–	1	–	–
Dichomeris limosella Schlag.	–	1	1	1	1	–
Anarsia spartiella Schrk.	–	–	–	–	1	–
– *lineatella* Z.	–	1	–	–	–	–
Mesophleps silacellus Hbn.	–	–	–	–	1	–
Acompsia cinerella Cl.	–	1	–	–	–	–
Nothris verbascella Hbn.	–	1	1	1	1	–
Gnorimoschema ocellatellum Boyd.	–	1	–	–	–	–
Gelechia rhombella Den. et Schiff.	–	–	–	–	11, 8, 1	–
Pexicopia malvella Hbn.	–	1	–	–	1	–
Aratrognathosia vilella Z.	–	1	–	–	–	–
Bryotropha terrella Hbn.	6	–	–	–	–	–
Recurvaria leucatella Cl.	8	1	8	–	1, 8	–
– *nanella* Hbn.	8	–	–	–	–	8
Stenolechia nigrinotella Z.	–	–	–	–	1	–
Argyritis pictella Z.	–	–	1	–	1	–
Microsetia hermannella F.	–	1	–	1	1	–
– *neuropterella* Tngstr.	–	–	–	–	1	–
– *paucipunctella* Z.	–	–	–	–	1	–
Metzneria metzneriella Stt.	–	1	–	–	–	–
Isophrictis striatella Hbn.	–	1	–	1	–	–
Tortricidae						
Sparganothis pilleriana Den. et Schiff.	–	1	1	–	–	–
Pandemis dumetana Tr.	–	1, 6	1	1	–	–
– *heparana* Den. et Schiff.	8, 5	1, 8	1, 6, 8	1	1, 11, 8	8
– *corylana* F.	–	1	–	–	–	–
– *ribeana* Hbn.	8, 5	1	1, 8	1, 8	1, 8	8

(Continued 15)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
— *ribeana var. cerasana*	—	1, 6	1, 6, 8	—	1, 8	8
Argyrotaenia pulchellana Haw.	8	1	1	1	8, 11	—
Archips sorbiana Hbn.	—	—	—	—	1, 8	—
— *rosana* L.	—	—	—	—	1, 8	8
— *crataegana* Hbn.	—	—	—	5	1, 8	8
— *xylosteana* L.	—	1	8	—	1, 8	8
— *podana* Sc.	5	1, 5	1	1, 5, 8	1	—
Syndemis musculana Hbn.	—	—	—	1	—	—
Aphelia ochreana Hbn.	—	—	—	1	1	—
— *viburnana* Den. et Schiff.	—	1	1	1	1	—
— *paleana* Hbn.	—	1	1	1	—	—
Clepsis strigana Hbn.	—	1	1	1	1	—
— *spectrana* Tr.	—	1	1	1	—	—
— *semialbana* Gn.	—	1	—	1	—	—
Adoxophyes orana Fr.	2, 6, 8	1, 6, 8	1, 6	1, 6	1, 6, 8	6, 8
Ptycholoma lecheanum L.	—	—	—	—	1, 8	8
Paramesia gnomana Cl.	—	1	—	—	1	—
Epagoge grotiana F.	5	1	—	—	—	—
Doloploca punctulana Den. et Schiff.	—	1	—	—	—	—
Eana argentana Cl.	—	1	—	—	—	—
Neosphaleroptera nubilana Stph.	—	—	—	—	1	—
Cnephasia alternella Stph.	6	6	6	6	—	—
— *communana* HS.	—	1	1	1	1	—
Isotrias hybridana Hbn.	—	—	—	—	1	—
Aleimma loefflingiana L.	—	1	—	—	—	—
Tortrix viridana L.	—	1	1	1, 5	1, 8	—
Croesia holmiana L.	—	—	—	—	1, 8	8
— *forskaleana* L.	—	—	—	—	1	—
— *bergmanniana* L.	—	—	—	—	1	—
Acleris conterminana Den. et Schiff.	—	—	1	1	1, 8	—
— *variegana* Schiff.	—	—	—	—	8	8

— *permutana* Dup.	—	—	—	—	1	—
Celyphoides flavipalpanus HS.	—	1	1	1	—	—
— *rufana* Sc.	—	—	—	—	1	—
Celypha striana Den. et Schiff.	—	1, 8	1	1	1	—
Paracelypha rivulana Sc.	2, 5	1	1	1	1	—
Argyroploce lacunana Den. et Schiff.	2	1	1	1	1, 6	—
Orthotaenia undulana Den. et Schiff.	6	—	—	—	—	—
Bactra robustana Chr.	—	1	1	—	—	—
— *furfurana* Haw.	—	1	1	1	—	—
— *lanceolana* Hbn.	—	1	1	1	—	—
Endothenia quadrimaculana Haw.	—	1	1	1	—	—
— *sellana* Gn.	—	1	1	—	—	—
Apotomis lineana Den. et Schiff.	6	—	—	—	—	—
— *turbidana* Hbn.	—	—	—	6	—	—
Hedya nubiferana Haw.	5, 8	1, 6	—	1	1, 6, 8	8
— *salicella* L.	—	1	1	1	—	—
Ancylis paludana Barr.	—	1	—	—	—	—
— *unculana* Haw.	—	—	1	1	—	—
— *lundana* F.	—	1	1	—	—	—
Zeiraphera diniana Gn.	—	—	1	1	—	—
— *isertana* F.	—	6	1, 6	6	—	—
Notocelia uddmanniana L.	—	1	—	—	—	—
Pardia cynosbatella L.	—	1	—	—	1	—
Epiblema scutulana Den. et Schiff.	6	1, 6	1, 6	6	6	6
— *foenella* L.	—	1	1	1	1	—
Pseudeucosma caecimaculana Hbn.	—	1	—	—	—	—
Eucosma metzneriana Tr.	—	1	1	1	1	—
— *conterminana* HS.	—	1	1	—	1	—
— *lugubrana* Tr.	—	—	—	—	1	—
— *cana* Haw.	—	1	1	1	1	—
— *expallidana* Haw.	—	1	—	—	—	—
— *hohenwartiana* Den. et Schiff.	—	1	—	—	—	—
Thiodia citrana Hbn.	—	1	—	—	1	—
Spilonota ocellana F.	8	—	—	—	1, 8	8
Rhyacionia pinicolana Dbld.	—	—	1	—	—	—
— *buoliana* Den. et Schiff.	—	—	—	1	—	—

(Continued 16)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Enarmonia formosana Sc.	–	–	–	1	–	–
Lathronimpha strigana F.	–	–	–	–	1	–
Pammene insulana Gn.	–	–	–	6	6	–
– *aurantiana* Stgr.	–	–	–	–	6	–
– *spiniana* Dup.	–	–	–	6	6	–
– *albuginana* Gn.	6	–	–	6	6	6
– *argyrana* Hbn.	–	–	–	–	6	6
– *inquilina* Fletch.	–	–	–	–	6	6
– *gallicolana* Z.	6	6	–	6	6	6
– *suspectana* Z.	–	–	–	–	6	–
Grapholitha sinana Feld.	–	–	–	1	–	–
– *molesta* Busck.	6	6	6	6	–	–
– *funebrana* Tr.	6	6	6	6	–	–
– *tenebrosana* Dup.	–	–	–	–	6	6
– *janthinana* Dup.	–	–	–	–	6	6
Laspeyresia pomonella L.	6, 8, 11	6, 8	1, 6, 8	6, 1	6, 8, 11	6, 8, 11
– *splendana* Hbn.	–	1	1	–	–	–
– *querceti* Gozm.	–	–	–	–	6	6
– *gallicana* Gn.	–	–	–	–	6	–
– *aurana* F.	–	–	–	–	6	6
Cochylidae						
Cochylis hybridella Hbn.	–	1	–	–	1	–
– *posterana* Z.	–	1	–	–	1	–
Falseuncaria ruficiliana Haw.	–	1	–	–	–	–
– *epiliana* Z.	–	–	1	–	–	–
Phalonodia permixtana Den. et Schiff.	–	1	–	1	–	–
– *affinitana* Dgl.	–	–	1	–	–	–
Agapete zoegana L.	–	1	1	1	1	–
– *hamana* L.	–	1	1	1	1	–
Euxanthoides straminea Haw.	–	–	–	1	1	–
Aethes dipoltella Hbn.	–	1	–	1	1	–

— *tesserana* Den. et Schiff.	—	1	—	—	—	—
— *badiana* Hbn.	—	1	—	—	—	—
— *kindermanniana* Tr.	2	1	1	1	1	—
— *hartmanniana* Cl.	—	—	—	—	1	—
Lozopera flagellana Dup.	—	1	—	1	—	—
Eupoecilia angustana Hbn.	—	—	—	—	1	—
Clysia ambiguella Hbn.	—	1	—	—	—	—
Pterophoridae						
Platyptilia gonodactyla Z.	—	—	—	—	1	—
Gillmeria ochrodactyla Den. et Schiff.	—	—	—	—	1	—
Eucnemidophorus rhododactylus F.	—	—	—	—	1	—
Adkinia pelidnodactyla Stein.	—	1	—	—	—	—
Emmelina monodactyla L.	—	1	—	1	1, 8	—
Porrittia galactodactyla Hbn.	—	1	—	—	—	—
Aciptilia pentadactyla L.	—	1	—	—	1	—
Merrifieldia tridactyla L.	—	—	—	—	1	—
Phycitidae						
Myelopsis tetricella Den. et Schiff.	—	1	—	1	1	—
Myelois cribrumella Hbn.	—	—	1	—	1	—
Eurhodope rosella Sc.	—	—	—	—	1	—
Acrobasis sodalella Z.	—	—	—	—	1	—
— *consociella* Rag.	—	—	—	—	1	—
— *tumidella* Zck.	5	—	—	—	—	—
Catacrobasis obtusella Hbn.	—	—	—	—	1	—
Phycita spissicella F.	—	—	—	1	1	—
Dioryctria splendidella HS.	—	—	1	—	—	—
Salebria semirubella Sc.	—	1	1	—	1	—
— *formosa* Haw.	—	—	1	1	—	—
— *adelphella* Fr.	—	1	—	—	—	—
Asalebria fumella Ev.	—	—	—	—	1	—
Selagia spadicella Hbn.	—	—	—	1	1	—
Epischnia prodromella Hbn.	—	—	—	—	1	—
Etiella zinckenella Tr.	—	—	1	—	1	—
Hypochalcia ahenella Den. et Schiff.	—	—	—	—	1	—

(Continued 16)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Nytegretis achatinella Hbn.	—	1	1	—	1	—
Synallorema triangulella Rag.	—	1	—	—	—	—
Euzophera bigella Z.	—	—	1	1	—	—
Pempelia ornatella Den. et Schiff.	—	—	—	—	1	—
Trissonca oblitella Z.	—	—	1	1	—	—
Ephestia elutella Hbn.	—	—	1	—	—	—
Cadra furcatella Mn.	—	—	—	1	—	—
Homoesoma sinuellum F.	—	1	1	—	—	—
— *nebulellum* Den. et Schiff.	—	—	1	1	1	—
— *nimbellum* Z.	—	1	1	1	—	—
Rotruda binaevella Hbn.	—	1	1	1	1	—
Ematheudes punctella Tr.	—	1	1	—	—	—
Prinanerastia lotella Hbn.	—	1	1	1	—	—
Crambidae						
Schoenobius gigantellus Den. et Schiff.	—	1	1	1	—	—
Donacaula mucronella Den. et Schiff.	—	1	1	—	—	—
— *forficella* Thnbg.	—	1	1	1	—	—
Chilo phragmitellus Hbn.	—	1	1	1	1	—
Argyria cerusella Chret.	—	1	1	1	1	—
Calamothropha paludella Hbn.	—	1	1	1	—	—
— *aureliella* Fr.	—	—	1	—	—	—
Pediasia fascelinella Hbn.	—	1	—	—	—	—
— *luteella* Schiff.	—	1	1	1	—	—
— *contaminella* Hbn.	—	1	1	1	1	—
— *aridella caradjaella* Rbl.	—	1	—	—	—	—
Agriphila tristella Den. et Schiff.	—	1	1	1	1	—
— *selasella* Hbn.	—	1	—	—	—	—
— *culmella* L.	—	1	1	—	1	—
— *hungarica* Schmidt	—	1	—	—	—	—
— *inquinatella* Den. et Schiff.	2	1	1	1	1	—
— *genicuela* Haw.	—	1	1	1	1	—

Crambus perlellus Sc.	–	1	1	1	1	–
– *hortuellus* Hbn.	–	1	1	1	1	–
– *pratellus* L.	–	1	1	1	1	–
– *pascuellus* L.	–	1	1	1	–	–
Chrysocrambus craterellus Sc.	–	1	1	1	1	–
Xanthocrambus saxonellus Zck.	–	–	–	–	1	–
Thisanotia chrysonuchella Sc.	–	1	–	1	–	–
Catoptria lythargyrella Hbn.	–	–	–	–	1	–
– *falsella* Den. et Schiff.	–	1	1	1	1	–
– *pinella* L.	–	–	–	–	1	–
Galleriidae						
Aphomia sociella L.	–	–	–	–	1	–
Melissoblaptes zelleri De Joann	–	–	1	1	1	–
Lamoria anella Den. et Schiff.	–	–	1	1	–	–
Acentropidae						
Acentropus niveus Oliv.	–	–	1	–	–	–
Pyralidae						
Pyralis costalis F.	5	1, 5	1	1, 5	1	–
– *regalis* Den. et Schiff.	–	–	–	–	1	–
– *farinalis* L.	–	–	1	1	1	–
Herculia glacinalis L.	–	1	1	1	1	–
– *incarnatalis* Z.	5	–	–	–	–	–
Actenia honestalis Tr.	–	–	–	–	1	–
– *brunnealis* Tr.	–	–	–	–	1	–
Synaphe angustalis Den. et Schiff.	–	1	1	1	1	–
Endotricha flammealis Den. et Schiff.	–	1	–	1	1	–
Pyraustidae						
Witlesia pallida Stph.	–	1	–	–	–	–
– *mercurella* L.	–	1	–	–	–	–
– *centurionalis* Hbn.	–	1	–	–	–	–
Scoparia arundinata Thnbg.	–	1	–	–	–	–
– *basistrigalis* Knaggs.	–	–	–	–	1	–
Nymphula nymphaeata L.	1	1	1	1	1	–

(Continued 17)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Parapoynx stratiotata L.	–	1	1	1	–	–
– *nivalis* Den. et Schiff.	–	1	–	–	–	–
Cataclysta lemnata L.	–	1	1	1	–	–
Evergestis aenealis L.	–	1	–	1	1	–
– *forficalis* L.	–	1	–	–	1	–
– *frumentalis* L.	–	1	1	1	1	–
– *pallidata* Hufn.	–	1	1	1	–	–
– *extimalis* Sc.	–	1	1	1	1	–
– *limbata* L.	–	–	–	–	1	–
Cynaeda dentalis Den. et Schiff.	–	–	–	–	1	–
Epacestria pustulalis Hbn.	–	–	–	–	1	–
– *cingulata* L.	–	–	–	–	1	–
– *cespitalis* Den. et Schiff.	2	1	1	1	1	–
– *aurata* Sc.	–	–	1	–	–	–
– *purpuralis* L.	–	1	1	1	1	–
Nascia cilialis simplalis Car.	–	1	–	–	1	–
Psammotis purveralis Hbn.	–	–	1	–	–	–
Perinephela lancealis Den. et Schiff.	–	–	–	–	1	–
– *coronata* Hufn.	–	1	–	–	–	–
– *perlucidalis* Hbn.	–	1	–	1	–	–
– *rubiginalis* Hbn.	–	1	1	–	1	–
– *verbascalis* Den. et Schiff.	–	1	1	–	–	–
Eurrhypara hortulata L.	–	1	–	1	1	–
Sclerocona acutella Ev.	–	1	1	1	–	–
Microstege pandalis Hbn.	–	1	–	–	–	–
– *hyalinalis* Hbn.	–	1	1	1	–	–
Sitochroa palealis Den. et Schiff.	–	–	–	–	1	–
– *verticalis* L.	2	1	1	1	1	–
Opsibotys fuscalis Den. et Schiff.	–	–	1	–	–	–
Loxostege sticticalis L.	–	–	1	–	1	–
Epicorsia repandalis Den. et Schiff.	–	1	–	–	–	–

Udea martialis Gn.	–	1	1	1	–	–
– *accolalis* Z.	–	1	–	–	–	–
Haritala ruralis Sc.	–	1	1	1	1	–
Ostrinia nubilalis Hbn.	–	1	1	1	1	–
Dolichartria punctalis Den. et Schiff.	–	–	–	–	1	–
Diasemia litterata Sc.	–	1	1	1	–	–
Nomophila noctuella Den. et Schiff.	–	1	1	1	–	–
"MACROLEPIDOPTERA"						
Geometridae						
Alsophila quadripunctaria Esper	–	–	–	–	1, 8	8
– *aescularia* Den. et Schiff.	–	–	1	–	1, 8	8
Minoa murinata Esp.	–	–	–	–	1	–
Epirrita dilutata Den. et Schiff.	–	–	–	–	–	8
Operoptera brumata L.	8	1	1, 8	1	1, 8	8
Pelurga comitata L.	2	1	1	1	1	–
Cosmorhoe ocellata L.	–	–	–	–	1	–
Eulithis pyraliata Den. et Schiff.	–	–	–	–	1	–
Cidaria fulvata Forst.	–	–	–	–	1	–
Horisme vitalbata Den. et Schiff.	–	–	–	–	1	–
– *corticata* Tr.	–	–	–	–	1	–
Melanthia procetalla Den. et Schiff.	–	–	–	–	1	–
Philereme vetulata Den. et Schiff.	–	–	–	–	1	–
– *transversata* Hufn.	–	–	–	–	1	–
Perizoma flavofasciata Thnbg.	–	1	–	–	1	–
– *alchemillata* L.	–	1	–	1	1	–
– *lugdunaria* H. Sch.	–	–	–	1	–	–
Euphya unangulata Haw.	–	–	–	–	1	–
Orthonama vittata Bkh.	–	1	–	–	1	–
– *obstipata* F.	–	1	–	1	–	–
Xanthorhoe ferrugata Cl.	–	1	1	1	1	–
– *spadicearia* Den. et Schiff.	–	1	–	1	1	–
– *fluctuata* L.	–	1	–	–	1	–
Scotopteryx moeniata Sc.	–	–	–	–	–	–
– *bipunctaria* Den. et Schiff.	–	–	–	–	1	–

(Continued 18)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
— *chenopodiata* L.	—	—	—	—	1	—
— *mucronata* Sc.	—	—	—	—	1	—
— *luridata* Hufn.	—	—	—	—	1	—
Catarhoe rubidata Den. et Schiff.	—	1	—	—	1	—
— *cuculata* Hufn.	—	1	—	—	—	—
Epirrhoe alternata Müll.	—	1	1	1	1	—
— *rivata* Hbn.	—	—	—	1	1	—
— *galiata* Den. et Schiff.	—	—	—	—	1	—
Costaconvexa polygrammata Bkh.	—	1	—	1	1	—
Camptogramma bilineata L.	—	—	—	—	1	—
Cataclysme riguata Hbn.	—	—	—	—	1	—
Mesotype virgata Hufn.	—	1	—	—	—	—
Aplocera plagiata L.	—	—	—	—	1	—
Lithostege farinata Hufn.	—	1	1	1	1	—
— *griseata* Den. et Schiff.	—	1	1	1	—	—
Lythria purpurata L.	12	—	—	—	1	—
Lobophora sexalata Retz.	—	1	—	—	—	—
Eupithecia linariata Den. et Schiff.	—	1	1	1	1	—
— *centaureata* Den. et Schiff.	—	1	1	1	1	—
— *veratraria* H. Sch.	—	—	—	—	1	—
— *absinthiata* Cl.	—	1	—	1	1	—
— *castigata* Hbn.	—	—	—	—	1	—
— *innotata* Hufn.	—	1	1	—	8	—
— *millefoliata* Rössl.	—	1	—	—	1	—
— *icterata* Vill.	—	—	—	—	1	—
— *graphata* Tr.	—	—	—	—	1	—
— *dodoneata* Guén.	—	—	—	—	1	—
— *ochridata* Pinker	—	1	—	—	—	—
— *catharinae* Vojnits	—	1	—	—	—	—
— *inornata* Hufn.	2, 5	—	—	—	—	—
— sp.	2	—	—	—	—	—

Gymnoscelis rufifasciata Haw.	–	–	–	–	1	–
Chloroclystis v-ata Haw.	–	1	–	–	–	–
Calliclystis rectangulata L.	–	1	8	–	1, 8	8
Idaea rufaria Hbn.	–	–	–	–	1	–
— *sericeata* Hbn.	–	–	–	–	1	–
— *ochrata* Sc.	–	–	–	–	1	–
– *serpentata* Hufn.	–	1	–	–	–	–
– *aureolaria* Den. et Schiff.	–	–	–	–	1	–
— *muricata* Hufn.	–	1	1	1	1	–
— *rusticata* Den. et Schiff.	–	1	–	–	1	–
— *filicata* Hbn.	–	–	–	–	1	–
— *moniliata* Den. et Schiff.	–	–	–	–	1	–
— *sylvestraria* Hbn.	–	1	–	–	–	–
— *biselata* Hufn.	–	–	–	–	1	–
— *dilutaria* Hbn.	–	–	–	–	1	–
— *fuscovenosa* Goeze	–	1	–	–	–	–
— *humiliata* Hufn.	–	1	–	–	1	–
— *seriata* Schrk.	–	1	–	–	–	–
— *dimidiata* Hufn.	2	1	1	1	1	–
— *subsericeata* Haw.	–	–	–	–	1	–
— *trigeminata* Haw.	–	–	–	–	1	–
— *nitidata* H. Sch.	–	1	1	–	–	–
— *aversata* L.	12	1	1	1	1, 5	–
— *degeneraria* Hbn.	–	–	–	–	1	–
— *inornata* Haw.	–	1	–	–	1	–
— *deversaria* H. Sch.	2	–	–	–	1	–
Calothysanis amata L.	2	1	1	1	1	–
Cyclophora annulata Schulze	–	1	–	–	1	–
— *ruficiliaria* H. Sch.	–	1	–	–	1	–
— *quercimontaria* Bast.	–	1	–	–	–	–
— *porata* L.	–	–	–	–	1	–
— *punctaria* L.	–	1	1	–	1	–
— *linearia* Hbn.	–	1	–	–	–	–
Scopula immorata L.	–	1	1	1	1	–
— *corrivalaria* Krt.	–	1	1	1	–	–
— *nigropunctata* Hufn.	–	1	–	–	–	–

(Continued 19)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
— *virgulata* Den. et Schiff.	—	1	—	—	1	—
— *ornata* Sc.	—	—	—	—	1	—
— *rubiginata* Hufn.	—	1	1	1	1	—
— *marginepunctata* Goeze	—	1	—	—	1	—
— *incanata* L.	—	—	—	—	1	—
— *immutata* L.	2	1	1	1	—	—
— *flaccidaria* Z.	2	1	1	—	—	—
— *subpunctaria* H. Sch.	—	1	1	1	—	—
Rhodostrophia vibicaria Cl.	—	—	1	—	—	—
Lomaspilis marginata L.	—	1	1	1	—	—
Ligdia adustata F.	—	1	—	—	1	—
Stegania dilectaria Hbn.	—	1	1	—	—	—
Semiothisa notata L.	—	1	—	—	—	—
— *alternaria* Hbn.	2	1	1	1	1	—
— *clathrata* L.	2	1	1	1	1	—
— *glarearia* Brahm.	—	—	—	—	1	—
— *wauaria* L.	—	—	—	—	1	—
Narraga tessularia Metz.	—	1	1	—	—	—
Tephrina murinaria Den. et Schiff.	—	1	—	—	—	—
— *arenacearia* Den. et Schiff.	—	1	1	1	1	—
Plagodis pulveraria L.	—	1	—	—	1	—
— *dolabraria* L.	—	—	—	—	1	—
Opisthograptis luteolata L.	—	—	—	—	1, 8	—
Epione repandaria Hufn.	—	1	—	—	—	—
Pseudopanthera macularia L.	—	—	—	—	1	—
Therapis flavicaria Den. et Schiff.	2	1	—	—	—	—
Apeira syringaria L.	—	—	—	—	1	—
Ennomus autumnaria Wrbg.	—	1	—	—	1	—
— *quercinaria* Hufn.	—	—	—	—	1, 8	—
— *erosaria* Den. et Schiff.	—	1	—	—	—	—
Selenia lunularia Hbn.	8	1	—	—	1	—

— *tetralunaria* Hufn.	–	–	–	–	1	–
Crocallis elinguaria L.	–	–	–	–	1	–
Olrapteryx sambucaria L.	–	–	–	–	1	–
Colotois pennaria L.	–	–	–	–	1, 8	8
Angerona prunaria L.	2	1	1	1	1	–
Apocheima pilosaria Hbn.	–	–	–	–	1, 8	8
— *hispidaria* Den. et Sch.	–	–	–	–	1, 8	8
Lycia zonaria Den. et Schiff.	–	–	–	–	1	–
— *hirtaria* Cl.	8	1	1	–	1, 8	–
Biston strataria Hufn.	–	–	–	–	1, 8	–
— *betularia* L.	8	1	–	–	–	–
Agriopis leucophaearia Den. et Schiff.	–	–	–	–	1	–
— *bajaria* Den. et Schiff.	–	–	–	–	1, 8	–
— *aurantiaria* Hbn.	–	–	–	–	1	–
— *marginaria* Bkh.	–	–	–	–	1, 8	–
Erannis defoliaria Cl.	–	1	–	–	1, 8	8
Peribatodes rhomboidaria Den. et Schiff	–	1	1	1	1	–
Selidosema brunnearia Vill.	–	–	–	–	1	–
Cleora cinctaria Den. et Schiff.	–	–	–	–	1	–
Boarmia roboraria Den. et Schiff.	–	–	–	–	1	–
— *punctinalis Sc.* (or *danieli* Whrl.)	2, 5, 8	1	1	1	1	–
— *arenaria* Hufn.	–	–	–	–	1	–
Ascotis selenaria Den. et Schiff.	2	1	1	1	1	–
Ectropis bistortata Goeze	8	1, 8	1	1, 8	1, 8	8
— *extersaria* Hbn.	8	1, 5	1	1	–	–
Ematurga atomaria L.	–	1	1	1	–	–
Cabera pusaria L.	–	1	–	–	–	–
— *exanthemata* Sc.	–	1	–	–	1	–
Lomographa bimaculata F.	–	1	–	–	1	–
— *temerata* Den. et Schiff.	–	–	–	–	1	–
Theria primaria Haw.	–	–	–	–	8	–
Campaea margaritata L.	–	–	–	–	1	–
Siona lineata L.	–	–	–	–	1	–
Pseudoterpna pruinata Hufn.	–	–	–	–	1	–
Aplasta ononaria Fssl.	–	1	–	–	–	–
Comibaena pustulata Hufn.	–	–	–	–	1	–

(Continued 20)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Thetidia smaragdaria F.	2	1	—	1	1	—
Chlorissa viridata L.	—	1	—	—	1	—
— *cloraria* Hbn.	—	1	—	—	1	—
Thalera fimbrialis Sc.	—	1	—	—	1	—
Hemistola chrysoprasaria Esp.	—	—	—	—	1	—
Noctuidae						
Euxoa obelisca Den. et Schiff.	—	—	—	—	1	—
— *tritici eruta* Hbn.	—	—	1	—	—	—
— *temera* Hbn.	—	—	—	—	1	—
— *aquilina* Den. et Schiff.	—	—	—	—	1	—
Agrotis cinerea Den. et Schiff.	—	—	—	1	1	—
— *vestigialis* Hufn.	2	—	1	1	—	—
— *segetum* Den. et Schiff.	2, 5	1	1	1	1, 5	—
— *clavis* Hufn.	—	—	—	—	1	—
— *exclamationis* L.	—	1, 5	1	1	1, 5	—
— *ipsilon* Hufn.	5	1	—	—	1	—
— *crassa* Tr.	—	1	—	—	—	—
Ochropleura praecox L.	—	—	1	—	—	—
— *plecta* L.	2	1, 5	—	1	1	—
Axylia putris L.	2	1	1	1	1	—
Noctua pronuba L.	5	1, 5	1	1	—	—
— *orbona* Hufn.	—	—	—	—	—	1
— *fimbriata* Schreb.	5	—	—	—	—	—
Epilecta linogrisea Den. et Schiff.	—	—	—	—	—	1
Spaelotis ravida Den. et Schiff.	—	—	1	—	—	—
Diarsia rubi View.	2	1, 5	1	1	—	—
Xestia c-nigrum L.	2, 5	1, 5	1	1, 5	1	—
— *triangulum* Hufn.	—	—	—	—	1	—
— *rhomboidea* Esp.	—	—	—	—	1, 5	—
— *xanthographa* Den. et Schiff.	—	1, 5	—	1, 5	1	—

Cerastis rubricosa Den. et Schiff.	–	–	–	–	1	–
– *leucographa* Den. et Schiff.	–	–	–	–	1	–
Mesogona acetosellae Den. et Schiff.	–	–	–	–	1, 5	–
Discestra trifolii Hufn.	–	1	1	1	1	–
Hada nana Hufn.	2, 5	1	–	–	1	–
Polia bombycina Hufn.	–	–	–	–	1	–
Pachetra sagittigera Hufn.	–	–	–	–	1	–
Sideridis albicolon Hbn.	–	–	–	–	1	–
Heliophobus reticulata Goeze	–	1	–	–	1	–
Mamestra brassicae L.	2, 5	1, 5	1	1, 5	1, 5	–
– *persicariae* L.	–	–	–	–	1	–
– *contigua* Den. et Schiff.	–	–	–	–	1	–
– *w-latinum* Hufn.	–	–	–	–	1	–
– *thalassina* Hufn.	–	1	–	–	–	–
– *suasa* Den. et Schiff.	2, 5	1	1	1	1	–
– *oleracea* L.	2, 5	1	1	1, 5	–	–
– *pisi* L.	–	1	–	–	–	–
– *bicolorata* Hufn.	–	–	1	–	–	–
Hadena rivularis F.	–	1	–	1	–	–
– *perplexa* Den. et Schiff.	–	1	–	–	1	–
– *luteago* Den. et Schiff.	–	1	1	–	1	–
– *bicruris* Hufn.	–	1	–	–	–	–
Cerapteryx graminis L.	–	1	1	1	1	–
Tholera cespitis Den. et Schiff.	–	1	1	–	–	–
– *decimalis* Poda	–	1	1	1	1	–
Xylomiges conspicillaris L.	–	–	–	–	1	–
Orthosia cruda Den. et Schiff.	–	–	–	–	1, 5, 8	8
– *miniosa* Den. et Schiff.	–	–	–	–	1	–
– *gracilis* Den. et Schiff.	–	–	–	–	8	–
– *stabilis* Den. et Schiff.	–	–	–	–	1, 5	–
– *incerta* Hufn.	–	1, 8	1	1	1, 5, 8	8
– *munda* Den. et Schiff.	–	–	–	–	1, 5, 8	–
– *gothica* L.	–	1	1	1, 8	1, 8	–
Hyssia cavernosa gozmanyi Kov.	–	1	1	1	–	–
Perigrapha i-cinctum Den. et Schiff.	–	–	–	–	1	–
Mythimna turca L.	2, 5	1	1	1	–	–

(Continued 21)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
— *conigera* Den. et Sch.	—	—	—	—	1, 5	—
— *ferrago* F.	—	—	—	—	1, 5	—
— *albipuncta* Den. et Schiff.	2, 5	1	1	1	1	—
— *pudorina* Den. et Schiff.	—	1	—	1	1	—
— *pallens* L.	2, 5	1	1	1, 5	1	—
— *l-album* L.	—	1	1	1	1	—
Leucania obsoleta Hbn.	—	1	—	—	—	—
Senta flammea Curt.	—	1	—	—	—	—
Cucullia fraudatrix Ev.	—	1	—	—	—	—
— *artemisiae* Hufn.	—	—	—	1	1	—
— *chamomillae* Den. et Schiff.	—	—	—	—	1	—
— *umbratica* L.	—	1	1	—	1	—
— *scopariae* Dorfm.	—	1	—	—	—	—
Calophasia lunula Hufn.	—	—	—	—	1	—
Ompalophana antirrhini Hbn.	—	—	—	—	1	—
Episema glaucina Esp.	—	—	—	1	—	—
— *tersa* Den. et Schiff.	—	—	—	—	1	—
— *scoriacea* Esp.	—	—	—	—	1	—
Brachionycha sphinx Hufn.	—	—	—	—	1, 8	—
Litophane ornitopus Hufn.	—	—	—	—	1, 5, 8	—
Xylina vetusta Hbn.	—	1	1	1	—	—
— *exsoleta* L.	—	—	—	—	1	—
Allophyes oxyacanthae L.	—	—	—	—	1, 5	—
Dichonia convergens Den. et Schiff.	—	—	—	—	1	—
Dryobotodes eremita F.	—	—	—	—	1, 5	—
Blepharita satura Esp.	—	—	—	—	1	—
Ammoconia caecimacula Den. et Schiff.	—	—	—	—	1, 5	—
Eupsilia transversa Hufn.	—	1	—	—	1, 5	—
Conistra vaccinii L.	—	—	—	—	1, 5, 8	—
— *ligula* Esp.	—	—	—	—	1	—
— *rubiginosa* Scop.	—	—	—	—	1, 5	—

Chilodes maritima Tausch.	–	1	–	1	–	–
Athetis gluteosa Tr.	–	1	1	1	1	–
– *pallustris* Hbn.	–	1	–	1	–	–
– *furvula* Hbn.	–	1	1	1	–	–
– *lepigone* Möschl.	–	1	1	1	1	–
Aegle koekeritziana Hbn.	–	–	–	–	1	–
Hapalotis venustula Hbn.	2	1	–	–	1	–
Pyrrhia umbra Hufn.	–	1	–	1	1, 5	–
Heliothis viriplaca Hufn.	–	–	–	–	1	–
– *maritima* Grasl.	–	–	1	–	–	–
– *peltigera* Den. et Schiff.	–	–	–	–	1	–
Periphanes delphinii L.	–	–	–	–	1	–
Porphyrinia purpurina Den. et Schiff.	–	–	–	–	1	–
Jaspydia pygarga Hufn.	–	1	1	1	1	–
– *deceptoria* Sc.	–	–	–	–	1	–
Eustrotia uncula Cl.	–	1	1	1	–	–
– *bankiana* F.	–	1	1	1	1	–
– *candidula* Den. et Schiff.	–	1	1	1	1	–
Emmelia trabealis Sc.	–	1	1	1	1	–
Acontia luctuosa Den. et Schiff.	–	1	1	–	1, 5	–
Nycteola asiatica Krul.	–	1	–	–	–	–
Earias chlorana L.	–	1	1	1	–	–
Bena prasinana L.	–	–	–	1	–	–
Colocasia coryli L.	8	1, 8	–	–	–	–
Abrostola triplasia L.	–	–	1	–	–	–
– *asclepiadis* Den. et Schiff.	–	–	–	–	1	–
– *trigemina* Werneb.	–	1	–	1	–	–
Diachrysia chrysitis L.	2	1	1	1	1	–
Macdunnoughia confusa Steph.	2	1	1	–	1	–
Autographa gamma L.	2, 12	1	1	1	1	–
Ephesia fulminea Sc.	–	–	–	5	–	–
Anua lunaris Den. et Schiff.	–	–	–	–	1	–
Euclidia glyphica L.	–	5	1	–	–	–
Scoliopteryx libatrix L.	–	1	–	–	–	–
Lygephila craccae Den. et Schiff.	–	–	–	–	1, 5	–
– *pastinum* Tr.	2	–	–	–	–	–

(Continued 22)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Enargia ypsillon Den. et Schiff.	—	1	—	—	1	—
Dicycla oo L.	—	—	—	—	1	—
Cosmia affinis L.	—	—	—	—	1	—
Calymnia trapezina L.	2, 5, 8	1	1, 8	1, 5	1, 5, 8	8
— *pyralina* Den. et Schiff.	8	—	—	1	—	—
Actinotia polyodon Cl.	—	—	—	—	1	—
Apamea monoglypha Hufn.	—	1	—	—	—	—
— *sordens* Hufn.	—	1	—	1	1	—
Oligia strigilis L.	—	1	—	—	1	—
— *latruncula* Den. et Schiff.	—	1	—	1	1	—
— *furuncula* Den. et Schiff.	5	1	—	—	1	—
Mesapamea secalis L.	—	1	—	—	1, 5	—
Photedes minima Haw.	—	—	—	—	1	—
— *extrema* Hbn.	—	—	—	1	—	—
— *fluxa* Hbn.	—	1	—	—	1	—
— *pygmina* Haw.	—	1	—	1	—	—
Luperina testacea Den et Schiff.	—	1	1	1	1	—
Hydroecia micacea Esp.	—	1	—	—	—	—
Gortyna flavago Den. et Schiff.	—	1	—	—	1	—
Calamia tridens Hufn.	—	1	—	—	—	—
Celaena leucostigma Hbn.	—	1	—	—	—	—
Archanara algae Esp.	—	1	—	—	—	—
— *geminipuncta* Haw.	—	1	1	—	—	—
— *sparganii* Esp.	—	1	—	—	—	—
Rhizedra lutosa Hbn.	—	1	—	—	—	—
Sedina buettneri Her.	—	—	1	—	—	—
Meristis trigrammica Hufn.	—	—	—	—	1	—
Hoplodrina alsines Brahm.	—	1	1	1	1	—
— *blanda* Den. et Schiff.	—	1	—	—	1	—
— *ambigua* Den. et Schiff.	—	1	1	1	1	—
Caradrina morpheus Hufn.	—	1	1	1	1	—

Dasycampa erythrocephala Den. et Schiff.	–	–	–	–	1, 5	–
Agrochola circellaris Hufn.	–	1	1	1	1, 5	–
– *macilenta* Hbn.	–	–	–	–	1, 5	–
– *nitida* Den. et Schiff.	–	–	–	–	1	–
– *helvola* L.	–	–	1	1	1	–
– *humilis* Den. et Schiff.	–	–	–	–	1	–
– *litura* L.	–	–	–	1	1	–
– *lychnidis* Den. et Schiff.	–	1	1	–	1, 5	–
– *laevis* Hbn.	–	–	–	–	1, 5	–
Parastichtis suspecta Hbn.	–	1	1	–	–	–
Atethmia ambusta Den. et Schiff	–	–	–	–	1	–
Xanthia aurago Den. et Schiff.	–	–	–	–	1, 5	–
– *fulvago* Cl.	–	–	–	–	1	–
– *gilvago* Den. et Schiff.	–	1	–	–	–	–
– *ocellaris* Bkh.	–	1	–	–	–	–
Simyra albovenosa Goeze	–	1	1	1	–	–
Apatela psi L.	8	1	–	5	–	–
– *tridens* Den. et Schiff.	–	1	–	–	–	–
Subacronicta megacephala Den. et Schiff.	–	–	1	–	–	–
Pharetra rumicis L.	5	1, 5	1	1, 5	1	–
– *auricoma* Den. et Schiff.	–	–	–	–	8	–
Cryphia fraudatricula Hbn.	–	1	–	–	–	–
Amphipyra pyramidea L.	–	–	–	–	1, 5	–
– *berbera* Rungs.	5	–	–	–	–	–
– *livida* Den. et Schiff.	5	–	–	1	1	–
– *tragopogonis* L.	–	–	1	1	1	–
Dipterygia scabriuscula L.	–	–	1	1	1	–
Rusina ferruginea Esp.	–	1	–	1	1	–
Polyphaenis sericata Esp.	–	–	–	–	1	–
Thalpophila matura Hufn.	–	–	–	1	1	–
Trachea atriplicis L.	2, 5	1	–	1	–	–
Euplexia lucipara L.	–	1	1	1, 5	–	–
Phlogophora meticulosa L.	–	1	–	1	–	–
Eucarta amethystina Hbn.	–	1	1	–	–	–
– *virgo* Tr.	–	1	1	1	1	–
Ipimorpha subtusa Den. et Schiff.	2	1	–	–	–	–

(Continued 23)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Aedia funesta Esp.	–	–	–	–	1	–
Parascotia fuliginaria L.	–	1	–	1	–	–
Phytometra viridaria Cl.	–	–	–	–	1	–
Rivula sericealis Sc.	2	1	1	1	1	–
Simplicia rectalis Ev.	–	1	–	–	–	–
Macrochilo tentacularia L.	2, 12	1	–	–	1	–
Zanclognatha tarsipennalis Tr.	–	1	–	–	–	–
– *lunalis* Sc.	–	1	1	–	1	–
– *grisealis* Den. et Schiff.	–	1	–	–	–	–
Paracolax glaucinalis Den. et Schiff.	–	1	–	–	1	–
Hypena proboscidalis L.	2, 5	1	–	1	1	–
Schrankia costaestrigalis Steph.	–	1	–	–	–	–
Dilobidae						
Diloba caeruleocephala L.	–	–	–	1, 8	8	–
Nolidae						
Nola cuculatella L.	–	1	–	1	–	–
Roeselia strigula Den. et Schiff.	–	–	–	–	1	–
– *albula* Den. et Schiff.	–	1	–	–	1	–
Celama centonalis Hbn.	–	1	1	1	–	–
Lithosiidae						
Comacla senex Hbn.	2	1	1	1	–	–
Cybosia mesomella L.	–	1	–	1	1	–
Eilema lurideola Zinck.	–	–	–	–	1	–
– *complana* L.	–	–	1	1	1	–
– *unita* Hbn.	–	–	–	–	1	–
– *lutarella* L.	–	–	–	–	1	–
– *pallifrons* Z.	–	–	–	–	1	–
Pelosia muscerda Hufn.	–	1	1	1	1	–

Arctiidae						
Ocnogyna parasita Hbn.	–	–	–	–	1	–
Chelis maculosa Gern.	–	–	–	–	1	–
Phragmatobia fuliginosa L.	–	1	1	1	1	–
Eucharia casta Esp.	–	–	–	–	1	–
Spilarctia lutea Hufn.	2	1	1	1	–	–
Spilosoma menthastri Esp.	2, 13	1	1	1	1	–
– *urticae* Esp.	2	1	–	–	1	–
Hyphantria cunea Drury	2, 8	1	1	1	1	–
Arctinia caesarea Goeze	–	1	1	1	1	–
Cycnia mendica L.	–	–	–	–	1	–
Rhyparia purpurata L.	–	–	–	–	1	–
Diacrisia sannio L.	–	1	–	–	1	–
Arctia caja L.	–	1	1	1	–	–
– *villica* L.	–	–	–	–	1	–
Ammobiota festiva Hbn.	–	–	–	–	1	–
Panaxia quadripunctata Poda	–	–	–	–	1	–
Ctenuchidae						
Dysauxes ancilla L.	–	1	1	1	1	–
Notodontidae						
Exaereta ulmi Den. et Schiff.	–	–	–	–	1	–
Gluphisia crenata Esp.	–	1	–	–	1	–
Drymonia querna F.	–	–	–	–	1	–
– *trimacula dodonea* Hbn.	–	–	–	–	1	–
– *ruficornis* Hufn.	–	–	–	–	1	–
Pheosia tremula Cl.	–	–	1	–	–	–
Notodonta ziczac L.	–	1	–	–	–	–
Peridea anceps Goeze	–	–	–	–	1	–
Spatalia argentina Den. et Schiff.	–	–	–	–	1	–
Pterostoma palpinum L.	–	1	1	–	1	–
Ptilophora plumigera Esp.	–	–	–	–	1	–
Phalera bucephala L.	–	–	–	–	1	–
Clostera anastomosis L.	–	1	1	–	–	–

(Continued 24)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
— *curtula* L.	—	1	1	—	—	—
— *pigra* L.	—	—	1	—	—	—
Lymantriidae						
Dasychira fascelina L.	—	—	—	—	1	—
— *pudibunda* L.	—	—	—	—	1	—
Orgyia gonostigma L.	8	—	—	—	—	—
— *recens* Hbn.	8	8	8	1	—	—
Lymantria dispar L.	2, 8	1	1, 8	1, 8	1, 8	8
Euproctis chrysorrhoea L.	—	1	—	1	—	—
Thyatiridae						
Habrosyne pyrithoides Hufn.	—	1	1	1	1	—
Thyatira batis L.	—	1	—	—	—	—
Tethea or F.	—	1	1	—	—	—
— *ocularis* L.	—	5	1	—	—	—
Polyploca ridens F.	—	—	—	—	1	—
— *ruficollis* F.	—	—	—	—	1	—
— *diluta* F.	—	—	—	—	1	—
Drepanidae						
Platypteryx binaria Hufn.	—	—	—	—	1	—
Cilix glaucatus Scop.	—	1	—	1	1, 8	8
Lasiocampidae						
Malacosoma neustrium L.	8	1	1	1	—	—
— *castrensis* L.	—	—	—	—	1	—
Poecilocampa populi L.	—	—	—	—	1	—
Eriogaster rimicola Hbn.	—	—	—	—	1	—
Lasiocampa quercus L.	—	—	—	1	—	—
Macrothylacia rubi L.	—	—	—	—	1	—
Epicnaptera tremulifolia Hbn.	—	—	—	—	1	—

Sphingidae						
Smerinthus ocellata L.	—	1	—	—	—	—
Amorpha populi L.	—	1	1	—	1	—
Celerio euphorbiae L.	—	—	1	—	1	—
Pergesa porcellus L.	—	—	—	—	1	—
DIPTERA						
Anisopodidae						
Anisopus fenestralis Scop.	—	—	—	1	—	—
Bibionidae						
Dilophus febrilis L.	—	1	1, 7	—	—	—
— *hortulanus* L.	—	1	—	—	—	—
— *marci* L.	—	—	7	1	—	—
Cecidomyiidae						
Cecidomyiidae indet.	—	1, 12, 14	1	1, 14	—	—
Sciaridae						
Sciaridae indet.	1, 12, 14	12, 14	1	1, 14	7, 12	—
Mycetophilidae						
Mycetophila fungorum Deg.	—	1	1	1	—	—
— sp.	—	1	1, 14	1	—	—
Psychodidae						
Psychoda sp.	—	1	1	—	—	—
Culicidae						
Culicidae indet.	—	1, 14	1	1	—	—
Ceratopogonidae						
Ceratopogonidae indet.	—	1, 14	1	—	—	—
Chironomidae						
Chironomidae indet.	12	1, 12, 14	1	1	—	—

(Continued 25)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Simulidae						
Simulidae indet.	—	1	—	—	—	—
Tipulidae						
Tipulidae indet.	—	1	1	1	—	—
Limoniidae						
Limoniidae indet.	12	1, 12	1	1, 14	—	—
Tabanidae						
Tabanus bromius L.	-	—	1	—	—	—
Stratiomyidae						
Chloromyia formosa Scop.	12	—	—	—	—	—
Rhagionidae						
Rhagio lineola F.	12	1	1	—	—	-
Therevidae						
Thereva apicalis Wied.	12	—	—	—	—	—
Scenopidae						
Scenopinus fenestralis L.	-	—	—	7	—	—
Empididae						
Empididae indet.	12	1, 12	1	1	—	—
Dolichopodidae						
Dolichopodidae indet.	12, 14	1, 12	1	1	—	—
Lonchopteridae						
Lonchoptera furcata Fall.	12	1, 12	1	1	—	—
— sp.	—	12	—	—	—	—

Syrphidae						
Syrphidae indet.	—	1, 12	1	1	—	—
Pipunculidae						
Pipunculidae indet.	12	—	—	—	—	—
Phoridae						
Phoridae indet.	12, 14	1, 12	7	—	—	—
Sciomyzidae						
Pherbina coryleti Scop.	12	—	—	—	—	—
Trypetoptera punctulata Scop.	12	12		—	—	—
Euthycera chaerophylli F.	—	—	—	—	14	—
Coremacera marginata F.	12	—	—	—	—	—
— *catenata* Lw.	12	—	—	—	—	—
Sciomyzidae indet.	12, 14	—	—	—	—	—
Sepsidae						
Themira putris L.	—	1, 12	—	—	—	—
— *superba* Halid.	—	—	1	—	—	—
— *annulipes* Meig.	—	12	—	—	—	—
Sepsis bifleuxuosus Strobl.	—	12	—	—	—	—
— *fulgens* Hoffm.	12	12	—	—	—	—
— *punctum* F.	12	—	—	—	—	—
— *thoracica* Rob.-Desv.	—	12	—	—	—	—
— *violacea* F.	12	—	—	—	—	—
— *neocynipsea* Mel. et Spul.	—	12	—	—	—	—
Micropezidae						
Micropeza corrigiolata L.	12, 14	—	—	—	—	—
— *brevipennis v.* Ros.	12	—	—	—	—	—
Ulididae						
Physiphora demandata F.	—	—	12	—	—	—
Ulididae indet.	—	—	1	—	—	—

(Continued 26)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Tephritidae (Trypetidae)						
Tephritidae (Tryp.) *indet.*	—	12	—	—	—	—
Lauxaniidae						
Minettia lupulina	12	—	—	—	—	—
— *rivosa* Meig.	12	—	—	—	—	—
Calliopum aeneum Fall.	—	1	—	—	—	—
Chamaemyzidae						
Leucopis sp.	—	12	—	—	—	—
Heliomyzidae						
Oecothea fenestralis Fall.	—	1	1	—	—	—
Tephroclamys sp.	—	12	—	—	—	—
Anthomyzidae						
Anthomyzidae indet.	12	—	—	—	—	—
Opomyzidae						
Opomyza florum F.	12	12, 14	—	12	—	—
Geomyza tripunctata L.	12	—	—	—	—	—
— *venusta* Meig.	—	—	12	—	—	—
Opomyzidae indet.	12	—	1	1	—	—
Euphydridae						
Psilopa compta Meig.	—	12	—	—	—	—
— *nitidula* Fall.	—	12	—	—	—	—
— *polita* Macq.	12, 14	12	—	—	—	—
Hydrellia griseola Fall.	—	1, 12	—	—	—	—
Philygria trilineata de Meij.	—	12	—	—	—	—
Notiphila riparia Meig.	—	1	1	1	—	—
Parydra fossarum Halid.	—	—	1	—	—	—

Ephydra riparia Fall.	–	1	1	1	–	–
Setacera aurata Stenh.	–	1	–	–	–	–
– *micans* Halid.	–	1	1	1	–	–
Coenia palustris Fall.	–	–	–	1	–	–
Paracoenia fumosa Stenh.	–	1	1	1	–	–
Scatella paludum Meig.	–	1	1	–	–	–
– *stagnalis* Fall.	–	1	14	–	–	–
Sphaeroceridae						
Copromyza nigra Meig.	–	1	–	–	–	–
– *atra* Meig.	–	12	–	–	–	–
– *equina* Fall.	–	1	1	1	–	–
Coproica ferruginata Stenh.	–	14	1	–	–	–
– *vagans* Halid.	–	1	1	1	–	–
Opacifrons coxata Stenh.	-	1	1	–	–	–
– *humida* Halid.	–	1	–	1	–	–
Pteremis fenestralis Fall.	12	–	–	–	–	–
Leptocera curvinervis Stenh.	–	1	–	–	–	–
– *fuscipennis* Halid.	–	1	1	–	–	–
– *limosa* Fall.	–	1	1	–	–	–
– *lutosa* Stenh.	–	–	1	–	–	–
– *lutosoidea* Duda	–	1	1	1	–	–
– *modesta* Duda	–	1	–	–	–	–
Limosina clunipes Meig.	–	1, 12	–	–	–	–
– *ochripes* Meig.	–	1	–	–	–	–
– *parapusio* Dahl.	12	–	–	–	–	–
Diastatidae						
Diastata fuscula Fall.	12	–	–	–	–	–
Drosophilidae						
Scaptomyza pallida Zett.	12	12	1	–	–	–
– *graminum* Fall.	–	–	1, 14	–	–	–
Drosophila andalusiaca Strobl	–	–	1	–	–	–

(Continued 27)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Agromyzidae						
Ceratomyza denticornis Panz.	12	12	1	—	—	—
— *lateralis* Macq.	12	—	—	—	—	—
Liriomyza? *congesta* Beck.	—	12	—	—	—	—
— ?*pedestris* Hend.	—	12	—	—	—	—
Amauromyza luteiceps Hend.	—	12	—	—	—	—
Pseudonapomyza atra Meig.	12	—	—	—	—	—
Phytomyza fuscula Zett.	12	12	1	—	—	—
— *horticola* Goureau	—	—	1	—	—	—
— sp.	—	12	—	—	—	—
Chloropidae						
Elachiptera tuberculifera Corti	—	—	14	—	—	—
Tricimba cincta Meig.	—	—	1	—	—	—
Aphanotrigonum fasciellum Zett.	—	12	—	—	—	—
Trachysiphonella scutellata v. Ros.	12	—	—	—	—	—
Tropidoscinis albipalpis Meig.	12, 14	—	—	—	—	—
Oscinella albiseta Meig.	12	—	—	—	—	—
— *frit* L.	1, 12, 14	1, 12	1	—	—	—
— *pusilla* Meig.	12	—	—	—	—	—
Camarota curvipennis Latr.	12	—	—	—	—	—
Meromyza sp.	12	—	—	—	7	—
Cetema cereris Fall.	12, 14	—	—	—	—	—
— *neglecta* Tonnoir	12	—	—	—	—	—
Scatophagidae						
Scatophaga stercoraris L.	—	1	1	—	—	—
— sp.	—	—	—	1	—	—
Anthomyidae						
Paregle radicum L.	—	1	—	—	—	—
Anthomyia pluvialis L.	—	—	—	1	—	—

Chelisia monilis Meig.	12	–	–	–	–	–
Delia platura Neig.	12, 14	1, 12	1	1	7	–
Muscidae						
Muscidae indet.	12	1. 12	1	1	–	–
Calliphoridae						
Calliphoridae indet.	–	1, 14	1	1	–	–
Sarcophagidae						
Sarcophagidae indet.	–	–	1	–	7	–
Tachinidae						
Athrycia trepida Meig.	–	–	–	–	–	8
Actia pilipennis Fall.	–	–	–	–	8	–
Bessa parallela Meig.	8	–	–	–	8	8
Blepharomyia pagana Meig.	–	–	–	–	8	–
Blondelia nigripes Fall.	–	–	–	–	8	–
Peribaea fissicornis Strobl	8	–	–	–	–	–
Platymyia mitis Meig.	–	–	–	–	8	8
– *westermanni* Zett.	–	–	–	–	8	–
Triarthria spinipennis Meig.		–	–	–	8	8
Tachinidae indet.	12	12, 14	1	–	12	–
HYMENOPTERA						
Tenthredinidae						
Athelia circularis Klug	–	–	–	–	12	–
– *cordata* Lepet.	–	7	–	–	–	–
– *rosae* L.	7	–	–	–	–	–
Dolerus germanicus F.	12	–	–	–	–	–
– *puncticollis* Thom.	–	–	–	12	–	–
Fenella minuta Thom.	12	–	–	–	–	–
Hoplocampa minuta Christ.	14	–	–	–	–	–
– *testudinea* Klug.	8	–	–	–	–	–
Monophadnus pallescens Gmel.	12	–	–	–	–	–

(Continued 28)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Nematus myosotidis F.	—	—	—	12	—	—
Priophorus pallipes Brullé	—	12	—	—	—	—
Caliroa limacina Ret.	8	—	—	—	—	—
Ichneumonidae						
Amblyteles armatorius Först.	—	—	—	1	—	—
Chirotica insignis Grav.	—	—	—	1	—	—
Eniscospilus ramidulus L.	—	1	—	1	—	—
Eutanycarpa picta Schrank	—	—	1, 8	—	—	—
Gelis sp.	14	—	—	—	—	—
Ichneumon pallidicornis Wesm.	—	—	—	—	12	—
— *zonalis* Grav.	—	—	—	12	—	—
Lampronota piceator Thnbg.	—	—	—	14	—	14
Paniscus luteus L.	—	1	1	1	1	—
Pimpla instigator F.	—	14	—	—	—	14
Scambus brevicornis Grav.	—	—	1, 12	—	—	—
Temelucha confluens Grav.	—	1	1	—	—	—
Tryphon signator Grav.	—	7	—	—	—	—
Braconidae						
Agathis nigra Nees	—	—	—	—	12	—
Apanteles tibialis Curt.	12	12	1, 12	12	—	—
— *viminetorum* Wesm.	12	—	7	—	—	—
— *arisba* Nixon	8	8	—	8	8	—
— *ater* Ratz.	—	—	—	—	8	—
— *cheles* Nixon	—	—	—	—	8	—
— *circumscriptus* Nees	—	—	—	—	8	—
— *jucundus* Marsh.	—	—	—	—	8	—
— *juniperate* Bouché	—	—	—	—	8	—
— *longicauda* Wesm.	8	—	—	—	8, 11	—
— *pedias* Nixon	8	8	8	8	8	—

— *praepotens* Halid.	—	—	—	—	8	8
— *solitarius* Ratz.	—	—	—	—	8	—
— *xanthostigma* Hal.	—	—	—	—	8	8
Aphaereta minuta Nees	12	—	—	—	—	—
Ascogaster annularis Nees	8, 12	—	—	—	—	—
— *rufipes* Latr.	7, 12	—	—	—	—	—
Aspilota sp.	—	1	—	—	—	—
Alysia soror Halid.	—	—	—	14	—	14
Biosteres haemorrhoeus Halid.	12	—	—	—	—	—
Biosteres spinaciae Thom.	—	1	—	—	—	—
Bracon obscurator Nees	—	1	—	—	—	—
— *variator* Nees	12	—	—	—	—	—
Charmon extensor L.	—	—	—	1	—	—
Chorebus affinis Nees	12	12	—	—	—	—
Coelinius niger Nees	12	—	—	—	—	—
Dacnusa sp.	12	—	—	—	—	—
Eubazus parvulus Ruthe	7, 12	—	1	—	—	—
Homolobus annulicornis Nees	—	1	1	1	—	—
— *truncator* Say	—	1	1	1	—	—
Leiophron pallipes Curt.	—	—	—	—	12	—
Macrocentrus collaris Spin.	—	1, 12	1	1	1, 7, 12	—
— *pallipes* Nees	—	—	—	—	8	8
— *buoliane* Eady et Clark	—	—	—	—	8	—
— *linearis* Nees	8	—	8	—	8	8
Meteorus rubens Nees	—	1	1	1, 14	—	—
— *gyrator* Thnbg.	8	—	—	—	—	—
— *ictericus* Nees	8	—	—	—	—	—
— *striatus* Thoms.	—	—	—	—	—	8
Microctonus aethiops Nees	—	—	—	—	12	12
Microdus dimidator Nees	8	—	—	—	8	8
Microgaster globata L.	—	—	—	—	—	8
Microplitis sordipes Nees	8	—	—	—	12	—
— *tuberculifer* Wesm.	—	—	12	—	8	—
Opius laevis Wesm.	7	1	—	—	—	—
— *ochrogaster* Wesm.	—	3	—	—	—	—
— *rotundiventris* Thom.	—	12	—	—	—	—

(Continued 29)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Phaenocarpa ruficeps Nees	12	12	1	—	—	—
Phanerotoma minor Snofl.	—	1	—	—	—	—
Pygostolus falcatus Nees	—	—	1	—	—	—
Rizarcha areolaris Nees	—	—	—	—	7, 12	—
Rogas circumscriptus Nees	—	—	1	1	—	—
— *ductor* Thnbg.	12	1	1	—	—	—
Spathius rubidus Rossi	—	1	—	—	—	—
Synaldis concinna Halid.	—	14	—	—	—	—
Syntretus vernalis Wesm.	—	1	—	—	—	—
Zele nigricollis Thom.	—	1	1	—	1	—
Aphidiidae						
Aphidius gregarius Marsh.	—	14	1	10, 14	1	—
Trioxys brevicornis Halid.	—	1	—	—	1	—
Chalcididae						
Brachymeria intermedia Nees	—	—	8	—	—	—
Pteromalidae						
Asaphes suspensus Nees	—	—	—	—	—	8
Catolaccus ater Ratz.	—	—	—	—	8	—
Chlorocytus longicapus Graham	—	—	—	—	12	—
Cyrtogaster vulgaris Walk.	12	—	—	—	—	—
Dibrachys cavus Walk.	—	—	—	—	8, 11	8, 11
Chrysolampus thenae Walk.	—	12	—	—	12	—
Habrocytus chrysos Walk.	—	—	—	—	8	—
— *vibulenus* Walk.	—	—	—	—	12	—
— sp.	—	—	—	12	—	—
Halticoptera aenea Walk.	12	—	—	—	—	—
Homoprus apharetus Walk.	—	—	—	—	12	—
— *fulviventris* Westw.	—	1	—	—	—	—

Mesopolobus aequus Walk.	12	–	–	–	–	–
– *fasciiventris* Westw.	–	–	12	–	–	–
– sp.	–	–	–	12	–	–
Miscogaster rufipes Walk.	12	–	–	–	–	–
Pachyneuron aphidis Bouché	–	–	–	–	12	–
– *cremifaniae Delucchi*	–	–	–	–	8	–
– *formosum* Nees	–	12	–	–	12	–
Pseuderimus pratensis Erdős	–	–	–	–	12	–
Psychophagus omnivorum Walk.	–	–	–	–	12	–
Stenomalina muscarum L.	–	–	–	–	12	–
Systasis encyrtoides Walk.	–	–	–	–	12	–
Eupelmidae						
Eupelmus urozonus Dalm.	8	–	–	–	–	–
Encyrtidae						
Bothiothorax clavicornis Dalm.	–	13	–	–	8	–
Copidosoma sp.	–	–	–	–	12	–
Homalotylus flaminius Dalm.	–	–	–	–	7	–
Holcothorax testaceipes Ratz.	8	8	8	8	8	8
Blastorix confusa Erdős	8	8	–	–	–	–
Trichogrammidae						
Trichogramma evanescens Westw.	8	–	–	–	–	–
Aphelinidae						
Aphytis proclia Walk.	8	8	–	–	8	8
Prospaltella perniciosi Tower	8	8	–	–	8	8
Aphelinus mali Hald.	–	–	–	–	8	8
Eurytomidae						
Bruchophagus ononis Mayr.	–	12	–	–	–	–
Eurytoma flavimana Thom.	12	–	–	12	12	–
– *robusta* Mayr	–	–	–	–	12	–
– *strigifrons* Thom.	–	–	–	–	12	–
Tetramesa linearis Walk.	12	–	–	–	–	–
– sp.	12	12	–	12	–	–

(Continued 30)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Torymidae						
Megastigmus spermotrophus Wachtl.	—	—	—	—	7	—
Torymus sp.	12	12	—	—	—	—
Eulophidae						
Achrysocharoides atys Walk.	8	8	—	—	8	—
Chrysocharis sp.	—	—	—	—	—	8
— *nephereus* Walk.	—	—	—	—	8	—
— *pubens* Delucchi	—	—	—	—	8	8
Cirrospilus lyncus Walk.	8	—	—	—	8	8
Colpoclypeus florus Walk.	—	—	—	—	8	8
Achrysocharella chlorogaster Erdős	—	—	—	—	8	—
— *sericea* Erdős	—	—	—	—	8	—
Diglyphus isaea Walk.	—	12	—	—	—	8
Elachertus inunctus Nees	8	—	—	—	—	—
Entedon costalis Dalm.	12	—	—	—	—	—
— *fufius* Walk.	—	—	—	—	12	8
Euplector bicolor Swed.	—	—	—	—	8	—
Eulophus larvarum L.	—	—	—	—	8	8
— *pennicornis* Nees	—	—	—	—	8, 11	—
Kratochviliana nitetis Walk.	—	8	—	—	8	8
Necremnus leucarthros Nees	—	—	8	12	—	—
— *tidius* Walk.	—	—	—	—	—	8
Pediobius eubius Walk.	—	12	—	12	12	—
— *cassidae* Erdős	8	—	—	—	—	—
— *chilaspidis* Boŭcek	8	—	—	—	—	—
— *facialis* Giraud	8	—	—	—	—	—
Pnigalio pectinicornis L.	12	8	8	8	8	8
— *agraules* Walk.	8	—	—	—	—	—
— *smerinthicida* Boŭcek	—	—	—	—	8	—
— *soemius* Walk.	8	8	8	—	8	—

Sympiesis gordius Walk.	8, 12	8	8	8	8	—
— *sandanis* Walk.	12	—	—	—	—	—
— *viridula* Thom.	—	12	—	—	—	—
— *acalle* Walk.	8	—	—	—	8	8
— *albiscapus* Erdős	—	—	—	—	—	8
— *gregori* Boucek	8	—	8	—	—	—
— *euspilapteris* Erdős	—	—	—	—	8	—
— *sericeicornis* Nees	8	8	—	8	8, 11	8
Tetrastichus coccinellae Kurdj.	—	—	—	—	8, 12	—
— *ecus* Walk.	8	—	8	—	8, 12	8
— *?eupatorii* Kurdj.	—	—	—	—	12	—
— *evonymellae* Bouché	12	—	—	—	8	—
— *palustris* Walk.	—	—	—	—	7	8
Proctotrupidae						
Codrus ligatus Nees	—	—	—	1	—	—
Cryptoserphus brevimanus Kieffer	—	12	—	—	—	—
Phaenoserphus calcar Halid.	—	—	1	—	—	—
— *pallipes* Latr.	12	12	1	1	—	—
Proctotrupes gravidator L.	1	—	—	—	1	—
Diapriidae						
Diapria conica F.	12	—	—	—	12	—
Loxotropa sp.	12	—	—	—	12	—
Scelionidae						
Asolcus semistriatus Nees	—	—	—	—	1	—
Scelio sp.	—	—	—	—	7	—
Trimorus sp.	8	—	—	—	—	8
Platygastridae						
Inostemma contariniae Szelényi	—	—	8	—	—	8
Platygaster sp.	12	12	—	—	—	—
Ceraphronidae						
Ceraphron sp.	—	—	—	—	—	8

(Continued 31)

	1-1	1.2	1.3	1.4	1.5.1	1.5.2
Bethylidae						
Pristocera depressa F.	—	—	1	—	—	—
Mutiliidae						
Smicromyrme montana Panz.	—	13	—	—	—	—
Chrysididae						
Chrysis succincta L.	—	—	14	—	—	—
Pseudochrysis neglecta Schuck.	—	12	—	—	12	—
Sphecidae						
Alysson bimaculatus Panz.	12	—	—	—	—	—
Diodontus minutus F.	—	—	12	—	—	—
Lindenius sp.	—	—	—	—	12	—
Mellinus arvensis L.	—	—	12	—	—	—
Oxybelus sp.	—	—	1	—	—	—
Vespidae						
Paravespula germanica F.	—	—	8	—	—	—
— *vulgaris* L.	—	—	—	—	7, 12	—
Colletidae						
Prosopis annulata L.	—	—	—	—	12	—
Andrenidae						
Andrena ovulata Kirby	—	—	—	—	12	—
Panurgus calcaratus Sc.	12	—	—	7	—	—
Halictidae						
Halictus fulvipes Klug	12	—	—	12	—	—
— *interruptus* Panz.	12	—	—	12	8	—
— *maculatus* Vachal	12	—	—	12, 14	12	14

— *smaragdulus* Vachal	—	—	—	7, 12	—	—
Lasioglossum pauxillum Schenck	12	12	—	12	—	—
— *politum* Schuck.	12	—	12	8	—	—
Megachilidae						
Megachile lagopoda L.	7	—	—	—	—	—
Osmia cornuta Latr.	12	—	8	—	—	—
Apidae						
Apis mellifera L.	7	—	1, 7, 10	14	7, 12	—
Ceratina cucurbitina Rossi	12	—	—	—	—	—
Eucera clypeata L.	—	—	14	—	—	—
Formicidae						
Formica rufa L.	12	—	—	—	8	—
Lasius brunneus Latr.	12	12	—	—	7, 12	—
— *niger* L.	7, 12, 14	12, 14	7	14	22	—
ARACHNOIDEA						
Oribatidae						
Atropacarus striculus C. L. Koch	16	—	—	—	—	—
Rhysotritia ardua C. L. Koch	16	16	16	16	—	—
Epilohmannia cylindrica Berlese	16	—	—	—	—	—
Camisia horrida Herm.	16	16	16	16	—	—
Platynothrus grandjeani Sitnikova	16	—	—	—	—	—
Eremaeus oblongus C. L. Koch	16	—	—	—	—	—
Liacarus coracinus C. L. Koch	16	—	—	—	—	—
Xenillus tegeocranus Herm.	16	16	16	16	—	—
Tectocepheus sarekensis Träg.	16	16	16	16	—	—
Oppia insculpta Paoli	16	—	—	—	—	—
— *minus* Paoli	16	—	—	—	—	—
— *obsoleta* Paoli	16	—	—	—	—	—
— *nova* Oudemans	16	16	16	16	—	—
Suctobelba trigona Mich.	16	16	16	16	—	—
Scutovertex sculptus Mich.	16	—	—	—	—	—

(Continued 32)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Oribatula tibialis Nicol.	16	—	—	—	—	—
Scheloribates latipes C. L. Koch	16	16	16	16	—	—
— *laevigatus* C. L. Koch	16	16	16	16	—	—
Zygoribatula exarata Berlese	16	16	16	16	—	—
— *exilis* Nicol.	16	—	—	—	—	—
Protoribates capucinus Berlese	16	—	—	—	—	—
— *lophotrichus* Berlese	16	16	16	16	—	—
Ceratozetes conjunctus Mihelcic	16	—	—	—	—	—
Trichoribates trimaculatus C. L. Koch	16	—	—	—	—	—
Tetranychidae						
Panonychus ulmi Koch	8	8	8	8	8	8
Phyllocoptidae						
Phyllocoptes schlechtendali Nal.	—	—	—	—	8	8
AVES						
GALLIFORMES						
Phasianidae						
Phasianus colchicus L.	—	—	—	+	+	+
COLUMBIFORMES						
Columbidae						
Streptopelia turtur L.	—	-	—	+	—	—
— *decaocto* Friv.	—	+	—	—	—	—

PICIFORMES						
Picidae						
Jynx torquilla L.	–	–	–	–	+	+
Dendrocopos major L.	–	–	–	–	+	+
– *medius* L.	–	–	–	–	+	+
PASSERIFORMES						
Alaudidae						
Alauda arvensis L.	–	–	–	–	+	+
Hirundinidae						
Delichon urbica L.	–	–	–	–	+	+
Oriolidae						
Oriolus oriolus L.	–	–	–	+	–	–
Corvidae						
Pica pica L.	–	+	–	–	–	–
Garullus glandarius L.	–	+	+	+	+	+
Paridae						
Parus major L.	–	+	+	+	+	+
– *caeruleus* L.	–	+	+	+	+	+
– *palustris* L.	–	–	–	–	+	+
Aegithalos caudatus L.	–	–	–	–	+	+
Turdidae						
Turdus viscivorus L.	–	–	–	–	–	+
– *pilaris* L.	–	–	–	–	+	+
– *merula* L.	–	+	+	+	+	+
Phoenicurus ochrusos Gm.	–	+	–	+	+	+
Erithacus rubecula L.	–	–	–	–	+	+

(Continued 33)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Sylviidae						
Sylvia atricapilla L.	–	–	–	–	+	+
– *corruca* L.	–	–	–	–	+	+
Phylloscopus collybita V.	–	–	–	+	+	+
Regulidae						
Regulus regulus L.	–	–	–	–	+	+
Muscicapidae						
Muscicapa striata Pall.	–	–	–	–	+	–
Motacillidae						
Motacilla alba L.	–	–	–	–	+	+
Laniidae						
Lanius excubitor L.	–	–	–	–	+	+
– *collurio* L.	–	+	–	+	+	+
Sturnidae						
Sturnus vulgaris L.	–	–	–	–	+	+
Passeridae						
Passer domesticus L.	–	+	–	–	–	–
– *montanus* L.	–	+	+	+	+	+
Fringillidae						
Coccothraustes coccothraustes L.	–	–	–	–	+	+
Chloris chloris L.	–	–	+	–	+	+
Carduelis carduelis L.	–	+	–	–	+	+
– *cannabina* L.	–	–	–	–	+	+
Pyrrhula pyrrhula L.	–	–	–	–	+	+

Fringilla coelebs L.	−	−	−	+	+	+
— *nontifringilla* L.	−	−	−	−	+	+
Emberiza ci,rinella L.	−	+	+	+	+	+
MAMMALIA						
RODENTIA						
Muridae						
Microtus arvalis L.	−	−	−	−	+	+
LYCHENES						
Arthonia dispersa Schr.	+	+	−	+	+	−
— *punctiformis* Ach.	+	+	−	+	+	−
— *radiata* Pers.	+	+	−	+	+	−
Buellia disciformis Fr.	+	−	−	−	−	−
Caloplaca cerina Ehrht.	+	−	−	−	−	−
— *pyracea* Ach.	+	−	−	−	−	−
Candelaria concolor Dic.	+	−	−	−	−	−
Diplotomma alboatra Hoffm.	+	−	−	−	−	−
Lecidea parasema Ach.	+	−	−	−	−	−
Lecania cyrtella Ach.	+	−	−	−	−	−
Lecanora carpinea L.	+	−	−	−	−	−
— *pallida* Schreb.	+	−	−	−	−	−
Lepraria candelaris L.	+	−	−	−	−	−
Opegrapha atra Pers.	+	+	−	+	+	−
Parmelia sulcata Tayl.	+	−	−	−	−	−
Pertusaria amara Ach.	+	−	−	−	−	−
— *leioplaca* Ach.	+	+	−	+	+	−
Physcia ascendens Bitter	+	−	−	−	−	−
— *orbicularis* Neck.	+	−	−	−	−	−
Pyrenula nitida Weig.	+	+	−	+	+	−
Rinodina exigua Ach.	+	−	−	−	−	−
Xanthoria candelaria L.	+	−	−	−	−	−

(Continued 34)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
EQUISETALES						
Equisetaceae						
Equisetum arvense L.	+	–	–	–	–	–
ARISTOLOCHIALES						
Aristolochiaceae						
Aristolochia clematitis L.	+	–	–	–	–	–
ROSALES						
Rosaceae						
Rubus caesius L.	+	–	–	–	–	–
Potentilla anserina L.	+	–	–	–	–	–
– *reptans* L.	+	–	–	–	–	–
– *argentea* L.	+	–	–	–	–	–
Aphanes arvensis L.	+	–	–	–	–	–
Fragaria viridis L.	+	–	–	–	–	–
– *vesca* L.	+	–	–	–	–	–
FABALES						
Fabaceae						
Trifolium repens L.	+	–	–	–	–	–
– *pratense* L.	+	–	–	–	–	–
Vicia hirsuta L.	+	–	–	–	–	–
– *villosa* Roth	+	–	–	–	–	–
– *lathyroides* L.	+	–	–	–	–	–

MYRTALES						
Onagraceae						
Oenothera biennis L.	+	−	−	−	−	−
Epilobium tetragonium L.	−	−	−	−	+	−
UMBELLALES						
Umbelliferae						
Falcaria vulgaris Bernh.	+	−	−	−	−	−
Pimpinella saxifraga L.	+	−	−	−	−	−
Tordylium maximum L.	+	−	−	−	−	−
Daucus carota L.	+	−	−	−	−	−
DIPSACALES						
Caprifoliaceae						
Sambucus ebulus L.	+	−	−	−	−	−
MALVALES						
Malvaceae						
Hibiscus trionum L.	+	+	−	+	−	−
GERANIALES						
Geraniaceae						
Geranium molle L.	+	−	−	−	−	−
— *pusillum* Burm. f.	+	−	−	−	−	−
Erodium cicutarium L.	−	−	−	−	−	+
GENTIANALES						
Rubiaceae						
Galium parisiense L.	−	+	−	−	−	−
— *aparine* L.	−	−	−	−	+	+

(Continued 35)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
BORAGINALES						
Convolvulaceae						
Convolvulus arvensis L.	−	+	−	+	+	+
Boraginaceae						
Lithospermum arvense L.	+	−	−	−	−	−
Labiatae						
Glechoma hederacea L.	+	−	−	−	−	−
Lamium amplexicaule L.	+	−	−	−	−	−
− *purpureum* L.	+	−	−	+	−	−
Ballota nigra L.	+	−	−	−	−	−
SOLANALES						
Solanaceae						
Solanum nigrum L.	+	−	−	−	−	−
Scrophulariaceae						
Veronica triphyllos L.	+	−	−	−	−	−
− *verna* L.	+	−	−	−	−	−
− *arvensis* L.	+	−	−	−	−	−
− *hederifolia* L.	+	−	−	−	+	+
− *persica* Poir.	−	−	−	−	+	+
Verbascum nigrum L.	+	−	−	−	−	−
− *phlomoides* L.	+	−	−	−	−	−
Linaria vulgaris Mill.	−	−	−	−	+	+
Plantaginaceae						
Plantago lanceolata L.	+	−	−	−	−	−

PAPAVERALES						
Papaveraceae						
Chelidonium majus L.	+	−	−	−	−	−
Papaver rhoeas L.	+	−	−	−	−	−
Fumariaceae						
Fumaria schleieri Say-Will.	+	−	−	−	−	−
CAPPARALES						
Cruciferae						
Cardaria draba Desv.	−	−	−	−	+	+
Raphanus raphanistrum L.	−	−	−	−	+	+
Capsella bursa-pastoris L.	+	+	−	+	+	+
Diplotaxis tenuifolia Jusl.	−	−	−	−	+	+
Arabidopsis thaliana L.	−	−	−	+	−	−
Thlaspi arvense L.	−	+	−	−	−	−
Myagrum perfoliatum L.	+	−	−	−	−	−
Berteroa incana L.	+	−	−	−	−	−
Sysimbrium officinale L.	+	−	−	−	−	−
— *sophia* L.	+	−	−	−	−	−
Resedaceae						
Reseda lutea L.	−	−	−	−	+	+
ASTERALES						
Compositae						
Erigeron canadensis L.	+	+	−	+	+	+
Cirsium arvense L.	−	−	−	−	+	+
Taraxamum officinale Weber	+	+	−	+	+	+
Achillea distans W.	−	−	−	−	+	+
— *millefolium* L.	+	−	−	−	−	−
Sonchus arvensis L.	+	−	−	−	+	−
— *oleraceus* L.	−	−	−	−	+	−

(Continued 36)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
Solidago gigantea Ait.	−	−	−	−	+	−
Matricaria maratima inodora Soó	−	−	−	−	−	+
Tussilago farfara L.	−	−	−	−	+	+
Ambrosia elatior L.	+	−	−	−	−	−
Galinsoga parviflora Cav.	−	+	−	−	−	−
Anthemis arvensis L.	+	−	−	−	−	−
Artemisia vulgaris L.	+	−	−	−	−	−
− *annua* L.	+	−	−	−	−	−
Centaurea jacea L.	+	−	−	−	−	−
Leontodon autumnalis L.	+	−	−	−	−	−
− *hispidus* L.	+	−	−	−	−	−
Picris hieracioides L.	+	−	−	−	−	−
Tragopogon dubius Scop.	+	−	−	−	−	−
CARYOPHILLALES						
Portulacaceae						
Portulaca oleracea L.	−	−	−	+	−	−
Caryophillaceae						
Stellaria media L.	+	+	−	+	+	−
Melandrium album Mill.	+	−	−	−	−	−
Cerastium semidecandrum L.	+	−	−	−	−	−
Chenopodiaceae						
Chenopodium album L.	+	+	−	+	+	−
Amaranthaceae						
Amaranthus retroflexus L.	−	+	−	+	+	−
− *chlorostachys* Willd.	+	+	−	+	+	+

PRIMULALES						
Primulaceae						
Anagallis arvensis L.	−	−	−	−	+	+
− *arvensis azurea* Hyl.	−	−	−	−	+	+
POLYGONALES						
Polygonaceae						
Rumex obtusifolius L.	−	+	−	−	−	−
− *acetosella* L.	+	+	−	−	−	−
Polygonum lapathifolium L.	−	+	−	−	−	−
− *persicariae* L.	−	+	−	−	−	−
− *aviculare* L.	+	−	−	−	−	−
Fallopia convolvulus L.	+	−	−	−	−	−
URTICALES						
Cannabinaceae						
Cannabis sativa L.	+	−	−	−	−	−
Urticaceae						
Urtica dioica L.	−	−	−	−	+	+
POALES						
Gramineae						
Bromus arvensis L.	+	−	−	−	−	−
− *sterilis* L.	+	−	−	−	+	+
− *mollis* L.	−	−	−	−	+	+
Poa pratensis L.	+	−	−	−	−	−
− *annua* L.	+	+	−	+	−	−
− *bulbosa* L.	+	−	−	−	−	−

(Continued 37)

	1.1	1.2	1.3	1.4	1.5.1	1.5.2
— *trivialis* L.	−	−	−	−	+	+
Lolium perenne L.	+	−	−	−	−	−
Agropyron repens L.	+	+	−	+	+	+
Cynodon dactylon L.	+	−	−	−	−	−
Digitaria sanguinalis L.	+	+	−	+	−	−
Echinocloa crus-galli L.	−	+	−	+	−	−
Setaria glauca L.	+	+	−	+	−	−
— *viridis* L.	+	−	−	−	−	−
Festuca rubra L.	+	−	−	−	−	−
Dactylis glomerata L.	+	−	−	−	−	−
Arrhenaterum elatius L.	+	−	−	−	−	−
— *flavescens* L.	+	−	−	−	−	−
Corynephorus canescens L.	+	−	−	−	−	−
Festuca pseudovina Hack	+	−	−	−	−	−
Calamagrostis epigeios L.	−	−	−	−	+	+

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Application of Modified Silica gels in the Pesticide Analysis I. Preparation and Preliminary Investigations

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Silica gels with covalently bonded organic substituents were tested as reversed-phase thin-layer chromatographic sorbents for the separation of dinitro-octyl-phenyl-crotonate and its impurities. Silica gels with chloro-propyl and chloro-methyl groups bonded on the surface showed similar retention characteristics as the traditional impregnated reversed-phase plates. The amino-propyl group has too great adsorptive power to be used for the separation.

To comply the growing requirements of the up-to-date separation science a large number of silica derivatives have been prepared and tested under different chromatographical conditions as thin-layer chromatography (Butte et al., 1981), gas chromatography (Aue and Wickramanayake, 1980) and high performance liquid chromatography (Rittich and Dubsky, 1981; Little et al. 1979*a*; Little et al. 1979*b*). The silica derivatives have been applied not only to enhance the efficiency of separations but to measure the lipophilicity of compounds as the lipophilicity is one of the physico-chemical parameters used most often in the Quantitative Structure-Activity Relationship (QSAR) studies (Andrew et al., 1979; Rekker, 1977). Besides of the classical partition method in water : n-octanol system the lipophilicity has been determined chiefly by reversed-phase thin-layer chromatography (RPTLC) (Ogierman and Silowiecki, 1981; Guerra et al., 1981) and high performance liquid chromatography HPLC) (Rittich and Dubsky, 1981; Aten and Bourke, 1977). The R_M value characterizing the liphophilicity of compounds is calculated from its R_f value in RPTLC according to Eq. 1.

$$R_M = \log\left(\frac{1}{R_f} - 1\right) \tag{1}$$

As the R_f values depend not only on the molecular lipophilicity but on the experimental conditions (quantity and quality of organic solvent in the eluent) too (Guerra et al., 1981), Eq. 2 describes the behaviour of compounds under RPTLC conditions:

$$y = a + b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 \tag{2}$$

where y = R_f value
x_1 = lipophilicity of organic phase (π_1) (Hansch et al., 1973)
x_2 = lipophilicity of compounds (π_2)
x_3 = concentration of organic phase in the eluent %

For a given compound x_2 = constant therefore Eq. 2. simplifies to Eq. 3.

$$y = a + b_1 \cdot x_1 + b_3 \cdot x_3 \tag{3}$$

where the symbols are identical with the symbols of Eq. 2

Our aim was to prepare some new silica derivatives and to test their applicability in pesticide analysis. Due to its practical importance the dinitro-octyl-phenyl-crotonate and its impurities were choosed as test compounds.

The "dinocap" is a fungicide widely used in agricultural practice (Byrde et al., 1964; Kirby, 1964; Kirby et al., 1966). The active ingredient is a mixture of dinitro-octyl-phenyl-crotonate isomers containing dinitro-octyl-phenol and mononitro-octyl-phenol isomers as impurities. Spectrophotometry (Crossley and Lynch, 1968), column and thin-layer chromatography (Byrde et al., 1966; Clifford et al., 1965) and gas chromatography (Kurz and Baum, 1969; Kurz et al., 1970) were applied for the analysis of commercial formulations. However, full separation of isomers has not been achieved even by the gas chromatographic method.

For the common analytical practice separation to three chief fractions (dinitro-octyl-phenyl-crotonates, dinitrooctyl-phenols, mononitro-octyl-phenols) is generally sufficient.

Materials and Methods

The sorbent to be modified was Kieselgel HR reinst nach Stahl (Merck) for thin-layer chromatography, the sililating agents were as follows:

1. N-(2-aminoethyl)-3-amino-propyl-trimethoxi-silane
$H_2NCH_2CH_2NHCH_2CH_2CH_2Si(OCH_3)_3$ (Wacker Silane GF91)
2. chloromethyl-methyl-diethoxi-silane
$ClCH_2(CH_3)Si(OC_2H_5)_2$
3. chloropropyl-methyl-diethoxi-silane
$ClCH_2CH_2CH_2(CH_3)Si(OC_2H_5)_2$
4. chloropropyl-methyl-dichloro-silane
$ClCH_2CH_2CH_2(CH_3)SiCl_2$

Compounds 2, 3 and 4 were prepared at the Department for Inorganic Chemistry of the Technical University of Budapest.

Fixation of organofunctional groups

Sililation was carried out in the following way: 30 g silica was suspended under stirring in 300 cm^3 xylene and it was refluxed for 24 h to remove the water adsorbed on the surface (azeotrop distillation in Marcusson apparatus). After this procedure two drops of 0.2 N NaOH solution were added to the mixture and it was refluxed again for an hour. Then the organic silicon compound (15 g/300 cm^3 xylene) was added slowly to the suspension. To complete the chemical reaction

between the support and the organofunctional sililating agent a six h reflux was applied.

The B.E.T. surface area of the modified silicas was determined by a sorptometer made at the Department for Physical Chemistry of Technical University of Budapest. It applies the same principle as Sorptometer 212D (Ettre, 1966). A nitrogen-hydrogen gas mixture of known composition was used. The measurements

Table 1

Eluents for reversed phase TLC separation of dinitro-octyl-phenyl-crotonate and its impurities

Eluent composition	Concentrations of organic phase, %
Water : acetone	50, 70, 75
Water : methanol	50, 70, 75
Water : ethanol	40, 55, 70
Water : n-propanol	20, 30, 40, 70
Water : i-propanol	30, 40, 70
Water : tetrahydrofurane	50, 60, 70

were carried out at the boiling point of liquid nitrogen. The concentration change of nitrogen was followed by the change of thermocapacity of gas mixture. The specific areas were calculated by the B.E.T. "one point" method.

The modified silicas were blended with water (1 : 4 weight ratio) and the slurry was applied in a thickness of 0.25 mm to glass plates of 20 × 5 cm. The ready plates were dried overnight at room temperature. Because of their mechanical instability the layers prepared without binders have to be handled very carefully.

A Karathan 50EC formulation containing 50% dinitro-octyl-phenyl-crotonate was applied in our investigations: 5 μl of a solution of 10 mg active ingredient/cm^3 acetone was spotted to the plates. Untreated Kieselgel layers of same thickness. impregnated with a solution of 5% paraffin oil in n-hexane served as controls. The eluents used are listed in Table 1.

To avoid the uncertainty of visual evaluation for the exact determination of R_f values a video densitometer (Telechrom OE-976, Chinoin, Hungary) was applied.

Our experiments were carried out with three parallels in three repetitions that is the R_f values in the calculations are the means of nine determinations.

To obtain information on the eventual proton-donor or proton-acceptor ability of tetrahydrofurane and acetone Eq. 2 was fitted to all R_f values and separately to R_f values measured in eluents containing only n-alcohols. Similar calculations were carried out separately for the dinitro-octyl-phenyl-crotonate and also for its two impurities (Eq. 3).

Results and Discussion

The specific surface area of silica measured before and after the modifying process are shown in Table 2. The surface area of modified silica decreased specially in the case of sililating agent 1. As the performance of a TLC sorbent depends

Table 2

B. E. T. Specific surface area of modified silica gels

Sample number	Surface area, m^2/g
Reference	325
1	109
2	263
3	256
4	293

Table 3

Correlations between the R_f values of dinitro-octyl-phenyl-crotonate and its impurities and the concentration and lipophilicity of the organic phase (for symbols see text)

Parameter	Equation 2		Equation 3					
			Normal alcohols			All eluents		
	Normal alcohols	All eluents	dinitro--octyl--phenyl--crotonate	dinitro--octyl--phenol	mono-nitro--octyl--phenol	dinitro--octyl--phenyl--crotonate	dinitro--octyl--phenol	mono-nitro--octyl--phenol
n	39	59	13	13	13	20	20	20
b_1	0.25	0.50	1.37	1.42	1.83	0.49	0.45	0.56
b_2	−0,08	−0.08						
b_3	1.55	1.42	1.44	1.73	1.89	1.32	1.42	1.50
a_2	−2.06	−2.05	−2.42	−2.46	−2.88	−2.05	−1.74	−1.97
R^2	0.6602	0.5857	0.8611	0.8089	0.7981	0.6839	0.6426	0.5915
R	0.8125	0.7653	0.9280	0.8994	0.8934	0.8270	0.8016	0.7691
s	0.21	0.23	0.10	0.14	0.16	0.15	0.18	0.21
s_1	0.06	0.14	0.26	0.38	0.43	0.16	0.18	0.21
s_2	0.02	0.02						
s_3	0.22	0.19	0.18	0.26	0.30	0.22	0.26	0.30
F	22.67	25.91	30.99	21.17	19.76	18.39	15.29	12.31
$F_{99.9\%}$	7.05	6.60	12.55	12.55	12.55	8.73	8.73	8.73
t_1	4.17	3.69	5.23	3.72	4.25	3.19	2.47	2.62
t_2	4.20	4.90						
t_3	7.09	7.36	7.87	6.48	6.28	6.06	5.52	4.96
$t_{99.9\%}$	3.59	3.49	4.59	4.59	4.59	4.02	4.02	4.02
b_1'	28.56	24.16	39.94	36.51	40.37	34.48	30.95	30.54
b_3'	22.93	27.75						
b_3'	48.51	48.09	60.06	63.49	59.63	65.52	69.05	69.46

chiefly on the average particle size and particle size distribution the lower surface enhances only the possibility to overload the TLC plates. At the quantities applied we did not observe any spreading of spots as a result of overloading and the performance of modified silicas was the same as that of the original sorbent. The results of our calculations are summarized in Table 3,

where n = sample number
a = intercepts of Eq. w and 3
b_1, b_2, b_3 = slopes of Eqs 2 and 3
R = regression coefficient
R^2 = coefficient of determination
s = standard deviation of y dependent variable
s_1, s_2, s_3 = standard deviation of slopes b_1, b_2, b_3
F = calculated value of F test
$F_{99\cdot9\%}$ = tabulated value of F test
t_1, t_2, t_3 = calculated values of t test of slopes b_1, b_2, b_3
$t_{99\cdot9\%}$ = tabulated t values
b'_1, b'_2, b'_3 = path coefficients

The R^2 values of Eq. 2 show that the changes in the three independent variables are responsible for the 60% of the change of R_f values. The path coefficients indicate that the relative importance of organic phase concentration is about twice as high as that of lipophilicities which have quasi the same relative importance. The higher R^2 and b'_1 values in Eq. 2 for normal alcohols show clearly that in the case of non homologous organic phases not only their lipophilicity but also their other characteristics (proton-donor, proton-acceptor ability) have to be taken into consideration. The data of Eq. 3 support our previous statement. The best fitting was achieved for dinitro-octyl-phenyl-crotonate because it does not contain any dissociable and highly polar hydroxi groups which are sensitive to the proton donor or proton acceptor ability of organic solvent in mobile phase.

Table 4

R_f values of dinitro-octyl-phenyl-crotonate and dinitro-octyl-phenole on different supports (eluent water : methanol, 1 : 3)

Sample number	Dinitro-octyl-phenyl-crotonate		Dinitro-octyl-phenole		ΔR_f
	R_f	s	R_f	s	
Reference	0.36	0.04	0.78	0.07	0.42
2	0.17	0.04	0.65	0.04	0.48
3	0.07	0.04	0.56	0.06	0.49
4	0.43	0.05	0.83	0.07	0.40

n = 9; s = standard deviation of R_f values

A specially good fitting was obtained for normal alcohols because in this case is the possibility of interactions independent of (or partially dependent on) lipophilicity the lowest. The relative importance of organic phase lipophilicity decreases in the case of all eluent systems supporting again our view that characteristics other than lipophilicity of organic phase have to be taken into consideration in RPTLC.

Some comparative data obtained on modified silicas and on impregnated silica (traditional RPTLC) are compiled in Table 4. The lipophilicity order is $3 > 2 >$ reversed-phase > 4. The modified silica 4 has practically the same characteristics as the Kieselgel impregnated with paraffin oil. Sample 1 was not suitable to separate the dinitro-octyl-phenyl-crotonate from its impurities because of its very strong adsorptivity. The separating power of silicas 2, 3 and 4 was very similar to that of classical RPTLC.

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DISEASES

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 19 (3—4), pp. 183—191 (1984)

Biochemical Changes in Barley Plants in the Preparasitic Stage of Powdery Mildew-Barley Interaction

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Physiological and biochemical responses of both resistant and susceptible barley cultivars to powdery mildew (*Erysiphe graminis* f. sp. *hordei* Marchal) were studied in the preparasitic stage of their interaction.

Increased proteosynthesis, activation of enzymes and changes in cell membrane permeability were demonstrated in this early stage of plant-pathogen interaction. Metabolical response of resistant barley cultivars to germinating conidia starts sooner and is more intensive than in the susceptible ones. This phenomenon does not seem to play any substantial role in plant resistance.

The results indicate that these physiological changes in the cells of inoculated leaves are induced by substances produced by the germinating conidia of powdery mildew.

Generally little attention is paid to physiological and biochemical events in plant cells initiated by "physical" contact of the epidermal plant cells with an obligate parasite, during the preparasitic stage of plant-pathogen interaction. The study of these early events in plant metabolism may be very important for understanding the metabolical principles of plant resistance or susceptibility.

It can be supposed that these very early biochemical events in an attacked plant organ are consequences of gene activations, which at the end are responsible for the host-parasite compatibility or incompatibility.

We have already shown that in the leaves of susceptible as well as of resistant barley cultivars, significant changes in some enzyme activities can be observed as early as a few hours after inoculation with conidia of powdery mildew (Frič and Wolf, 1979, 1980).

The present study was designed to elucidate the activation course of some enzymes in leaves, epidermis and intercellular spaces of inoculated barley leaves in the preparasitic stage of host-pathogen interaction.

Materials and Methods

Two compatible (Peruvian — *E. graminis* f. sp. *hordei* Marchal, race C 17, Slovenský dunajský trh — *E. graminis*, race C 6) and two incompatible (Rupee — *E. graminis*, race C 17, Korál — *E. graminis*, race C 6) genotype combinations of

barley-powdery mildew were chosen for investigation. Seedlings of barley were grown in phytotron under controlled conditions.

Light period: 16 h illumination (12 400 lux), 20 °C ± 1 °C, 70% relative humidity. Dark period: 8 h, 16 °C ± 1 °C 70% relative humidity.

The primary leaves in two leaf-stage were inoculated with freshly harvested conidia of powdery mildew. The inoculated leaves were taken for analysis in appropriate time intervals.

Extraction

Proteins from the intercellular spaces were obtained by vacuum infiltration of water into the leaves and subsequent centrifugation, according to Klement (1965). The obtained solution was directly used for analysis. Protein extracts from leaves and leaf-epidermis strips were obtained in the usual manner. The sample was homogenized (Potter – Elvehjam homogenizer) in an appropriate amount of 0.1 M Tris-HCl buffer pH 7.5 at 0 °C. The buffer-tissue homogenate was then centrifuged at 100 000 *g* at 2 °C for 30 minutes. The supernatant was assayed for enzyme activities and was also used for protein separations by gradient polyacrylamide gel electrophoresis.

Assays

Total protein content of the samples was determined according to the method of Bradford (1976). Determinations of peroxidase (E.C.1.11.1.7), phosphatase (E.C.3.1.3.2), unspecific nucleases were described previously (Frič and Fuchs, 1970; Frič, 1975a).

Esterase-aryl-ester hydrolase (E.C.3.1.1.2) activity was determined spectrophotometrically. Reaction mixture: 2.7 ml 0.1 M imidazol-buffer, pH 7.2; 0.2 ml p-nitrophenylacetate (5 mg/ml); 0.1 ml sample. Absorbancy increase at 400 nm was followed for 30 min at 30 °C. Activity expression: OD/30 min. μg protein. Proteins were separated on cylindrical polyacrylamide gels (2–30%) according to Wolf (see Hwang, Wolf and Heitefuss, 1982). The electrode compartments contained 0.1 M Tris-Borate buffer, pH 8.6. Protein bands were stained by Coomassie Brilliant Blue R 250. Peroxidase was detected by incubating the gels with benzidine-H_2O_2 solution according to Liu (1973) but without ascorbic acid. Acid phosphatase was detected by incubating the gels in a solution of α-naphtyl-phosphate (0.5 mg/ml), Fast Blue B (0.3 mg/ml) in 0.1 M acetate buffer, pH 5.2. Esterase was detected in a solution of α-naphtylacetate (0.5 mg/ml), Fast Blue RR (0.3 mg/ml) in 0.1 M Tris-HCl buffer, pH 7.2. Radiocarbon labelling of proteins was performed by applying $^{14}CO_2$ (8 MBq) for 1 h at 20 °C and 12 400 Lux to barley plants (Frič 1975b). The plants were labelled 1 h before inoculation. Afterwards the plants were kept under the same conditions as mentioned above. The labelled proteins from intercellular spaces or leaves were then fractionated by polyacrylamide gel electrophoresis. The cut gel slices were combusted in Tri Carb

Sample Oxidizer of Packard Instr. Ltd. The radioactivity of the samples were determined in a Packard Liquid Scintillation Spectrophotometer Model 3390. The counting efficiency of the samples was calculated by means of external standard channel ration technique. Sugars and phenolic substances obtained (washed out) from the intercellular spaces were determined spectrophotometrically by methods described by Erdelský and Frič (1979) and Frič (1969), respectively.

All values given in this paper are result from at least three experiments with three replications each.

Results and Discussion

As early as in the preparasitic stage of barley-powdery mildew interaction (20 h after inoculation) a significant increase of leaf protein ^{14}C-labelling was ascertained (Fig. 1). This phenomenon can be observed in both resistant and susceptible barley cultivars. It is worth mentioning that the rate of radiocarbon incorporation into proteins occurred earlier, and was found higher in incompatible host-pathogen genotype combinations than in the compatible ones. This finding confirms also our earlier observation (Frič and Wolf, 1979).

Enhanced radiocarbon incorporation into proteins was noticed in epidermal cells, too. However in these cells which were in contact with appresoria of the

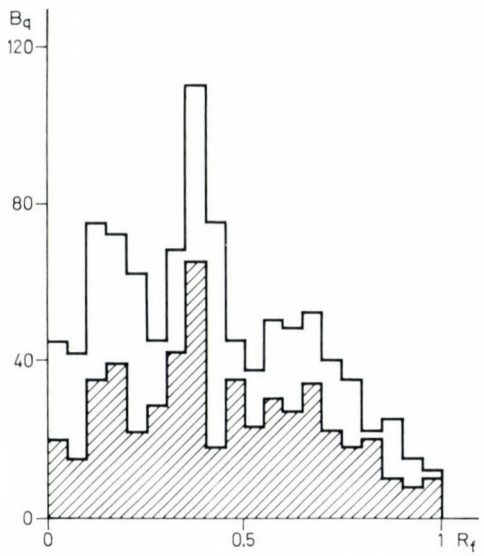

Fig. 1. Radiocarbon labelled barley leaf proteins separated by polyacrylamide gel electrophoresis; 24 h after the inoculation of barley (susceptible cultivar) with powdery mildew conidia. Hatched area: radioactivity of proteins (gel segments) from healthy plants; unhatched area: radioactivity of proteins from inoculated plants. Abscissa: R_f values. Ordinate: radioactivity (Bq)

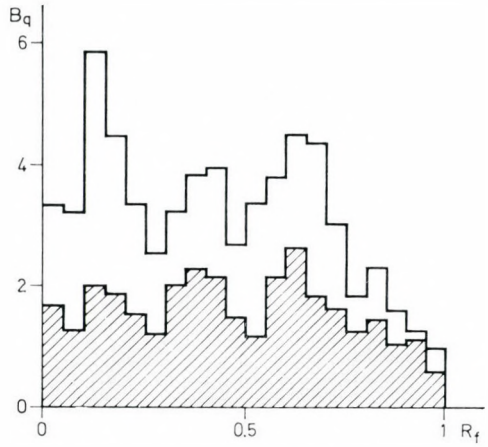

Fig. 2. Electrophoretogram of radiocarbon labelled proteins isolated from barley leaf intercellular spaces. Legend as in Fig. 1

fungus, the rate of radiocarbon protein labelling was less expressive than in mesophyll cells. This observation suggests that the pathogen must produce some metabolites by which the plant proteosynthesis is stimulated. It seems that this phenomenon is rather a nonspecific response of the plant than a specific one. On the other hand, it cannot be excluded that at the same time some metabolic events occur in the resistant cultivars which may be responsible for plant resistance in the later stages of host-parasite interaction.

The pathogen stimulated proteosynthesis in plants is correlated with other physiological processes. It was also shown that the physiological efflux of some proteins, i.e. enzymes, from leaf cells into intercellular spaces is significantly influenced by the parasite before the fungus enters the epidermal cell. Proteins isolated from inoculated leaf-intercellular spaces of susceptible and resistant barley cultivars were strongly labelled as a consequence of increased protein efflux of the cells (exocytose) (Fig. 2).

There were no qualitative changes in electrophoretic patterns of periplasmatic proteins neither in susceptible nor in the resistant barley cultivars. On the other hand, significant quantitative differences were demonstrated in some protein bands. These changes from a representative experiment are shown in Fig. 3. The increased protein concentration in the intercellular spaces may depend on the degree to which the pathogen affects processes controlling the efflux of definite proteins (enzymes) from cytoplasm. It is to mention that in this early stage of host-parasite interaction the cytoplasmatic proteins of mesophyll and epidermal cells showed no quantitative changes.

In spite of this observation significant quantitative changes in some isoenzyme patterns especially of periplasmatic peroxidase were observed in analyzed samples. Resistant as well as susceptible barley cultivars showed increases in both anodic

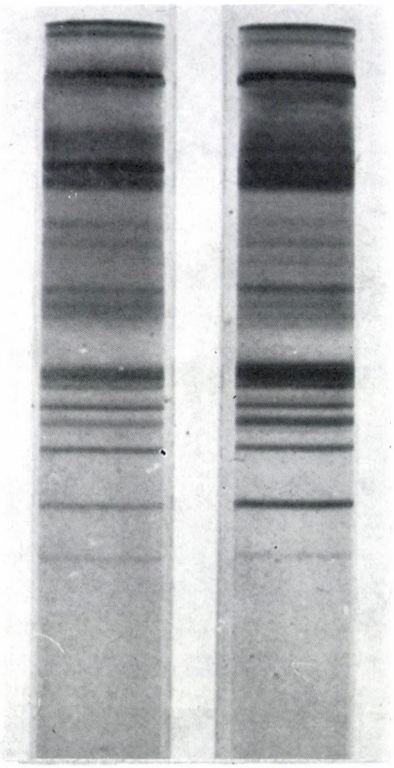

Fig. 3. Electrophoretic separation of leaf-intercellular proteins isolated from healthy (left) and inoculated (right) susceptible cultivar (24 h after the inoculation)

and cathodic isoperoxidases. The increase of individual cathodic isoperoxidase activity was always larger than the anodic one. Generally cathodic isoperoxidase activities are always more significantly affected by disease or injury. Increased activity of peroxidase in leaves, epidermal cells and in intercellular spaces after leaf inoculation with powdery mildew conidia is evident from Fig. 4.

The rise of peroxidase activity in the leaves of both resistant and susceptible barley cultivars is especially high. Up to 24 h, no qualitative changes in cytoplasmatic isoperoxidase pattern can be observed in the susceptible cultivar, while in the resistant one a "new" macromolecular isoperoxidase appears 24 h after inoculation. It has also been confirmed that mechanical injury did not induce any new isoperoxidase. In the intercellular spaces of the resistant barley cultivar the activity of peroxidase is more expressive than in the susceptible one (Fig. 5). In both cases the rate of peroxidase activity enhancement induced by the pathogen is higher in the periplasma than in the cytoplasma. This is caused by the release of de novo synthetized peroxidase by the plant cells which is significantly increased by substances produced by the germinating conidia.

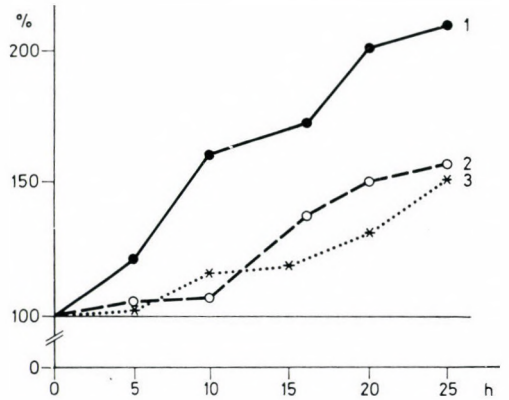

Fig. 4. Activity of peroxidase in intercellular spaces (1), epidermis (2) and in leaves (3) of inoculated barley (cv. Slovenský dunajský trh). Abscissa: per cent of uninoculated healthy plants. Ordinate: hours after inoculation

The idea that these preinfectional changes in isoperoxidase activities lead to a high degree of resistance is very attractive. In many cases it is thought that peroxidase protects the attacked plant cells against pathogens in various ways. It may enhance oxidation of phenolic substances to toxic compounds (quinones), which are toxic to both host and pathogen cells. Peroxidase can be implicated in lignin formation in the cell walls as a resistance response (Johnson and Cunningham, 1972; Stafford, 1974; Ohguchi and Asada, 1975; Ride, 1975; Vance et al., 1976). From the point of view of lignin biosynthesis the extracellular peroxidases are of interest. Increased lignification of the epidermal cell walls may be closely related to plant defence reactions. Little is known about the effect of the

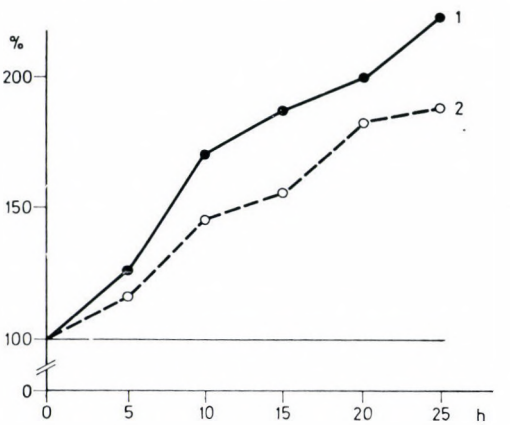

Fig. 5. Peroxidase activity in the intercellular spaces of resistant (1) and susceptible (2) barley cultivars inoculated with powdery mildew conidia. Legend as in Fig. 4

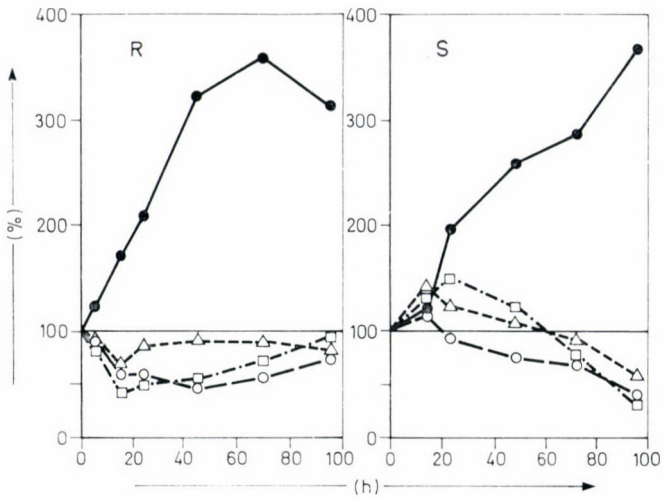

Fig. 6. Activity of enzymes isolated from the intercellular spaces of resistant (R) and of susceptible (S) barley cultivars. ● peroxidase, ○ esterase, △ nuclease, □ acid phosphatase. Abscissa: per cent of uninoculated healthy plants. Ordinate: hours after inoculation

increased periplasmatic peroxidase activity on the process of lignification in the inoculated leaves.

The fact that some stress factors (wounding, herbicides etc.) also enhance peroxidase activity in plants, and often stimulate increased lignin deposition in cell walls, confirms the view that these processes are not specific for resistance response of the plants to pathogens (Harwey et al., 1975; Asada and Matsumoto, 1972; Ride, 1975).

It is to stress that the peroxidase activity or the rate of its increase in attacked plant is not necessarily linked with host defence reaction to a pathogen. The intensity of peroxidase-activity enhancement in plants may depend on the degree to which the pathogen affects physiological and biochemical processes in which peroxidase is involved.

In the preparasitic stage of plant-pathogen interaction not only the peroxidase, but also other enzyme activities are influenced, especially in the intercellular spaces (Fig. 6). Enzymes extracted from the leaf intercellular spaces of susceptible barley cultivar show an increased activity as early as 5 to 8 h after the inoculation of the leaves. At the same time the enzyme activities in the cytoplasma of mesophyll and epidermal cells show a very slight increase in their activities. In the resistant barley variety the mentioned periplasmatic enzymes show a decrease in their specific activity. These specific activity decrease of enzymes (Fig. 6) in the preparasitic stage of plant is only appearent, caused by the increased concentration of total protein in the intercellular spaces.

Concentration changes of sugars and phenolic compounds were also studied in the intercellular spaces of barley leaves after powdery mildew inoculation. In

Fig. 7. Appresoria of powdery mildew with the induced "halo" of the epidermal cell

the preparasitic stage of plant-pathogen interaction no concentration changes of sugars were noticed in resistant or in susceptible barley cultivars. The only change which was noticed, was the increased concentration of phenolic substances of resistant barley cultivar. This finding is very interesting. Aromatic substances may play a definite role in plant defence reactions (lignification, peroxidase-catalyzed cross-linking of cell-wall-bound phenols, etc.) (Friend, 1976). It was also proved that in the preparasitic stage of powdery mildew-barley interaction phytoalexin production was more prominent in incompatible than in compatible host genotype (Oku et al., 1975). It seems that the metabolism of cyclic or aromatic substances is stimulated as early as in the preparasitic stage of plant-parasite interaction. Their enhanced synthesis in the later parasitic stage has been well known for a long time (Farkas and Király, 1962).

All these observations indicate that the germinating conidia on leaf surface must produce some physiological active metabolites which have a marked influence on proteosynthesis and on cell membrane permeability in surrounding plant cells. Effect of fungal metabolites on epidermal cells can be easily visualized by staining, as it was shown in a previous paper (Wolf and Frič, 1981). Around the appresoria typical concentrical areas known as "halo" can be stained by Coomassie Brilliant Blue R-250 (Serva) (Fig. 7).

Our results support the view that the studied physiological and biochemical processes in barley leaves in the preparasitic stage of plant-pathogen interaction do not play a substantial role in the process of plant resistance. It seems that the metabolic plant response leading to resistance occurs as late as after the penetration of infection structures.

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Pilzliche Zuckeralkohole und ihre Beziehung zur Sporenproduktion bei Rostpilzen der Gerste

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In primary leaves of barley plants (*Hordeum vulgare* L.) infected with yellow rust (*Puccinia striiformis* West.) or with brown rust (*Puccinia hordei* Otth.) the level of mannitol and arabitol increased during pathogenesis. It could be demonstrated that the polyols are mainly occurring in maturing and mature uredospores. The procedure of gaschromatographic analysis of mannitol and arabitol, therefore, is suitable to determine the spore production in different resistant barley varieties.

Die Sporenproduktion von Rostpilzen an Einzelpflanzen und im Pflanzenbestand gilt als ein sicheres Maß zur Erfassung partiell resistenter Formen des Getreides. Epidemiologisch wirksam sind: der Zeitpunkt der ersten Sporenproduktion, die Menge gebildeter Sporen pro Zeiteinheit, Sporulationsdauer und Keimfähigkeit der gebildeten Sporen. Sporenproduktion und Keimfähigkeit werden durch Umweltbedingungen und die Zusammensetzung des Pflanzenbestandes wesentlich beeinflußt.

Zur Messung der Sporenproduktion sind auch biochemische Verfahren eingesetzt worden (Mitchell et al., 1978; Whipps et al., 1979; Reiss, 1981; Reiss und Hartleb, 1984). Die Zuordnung der geprüften Pilzinhaltsstoffe zu den in der Wirtspflanze nachweisbaren Infektionsstrukturen der pilzlichen Pathogene war nicht immer eindeutig. Wir überprüften daher, welcher Zusammenhang zwischen der Konzentration pilzlicher Zuckeralkohole (Arabitol, Mannitol) und der Ausbildung bestimmter Infektionsstrukturen von Rostpilzen der Gerste besteht.

Material und Methoden

Die Untersuchungen wurden mit der für Zwergrost (*Puccinia hordei* Otth.) und Gelbrost (*Puccinia striiformis* West.) anfälligen Gerstensorte 'Xenia' und mit der für Gelbrost anfälligen Sorte 'Valja' und der resistenten Sorte 'Bigo' durchgeführt.

Anzucht, Infektion und Inkubation der Pflanzen erfolgten in Klimakammern (14 °C, 70% relative Luftfeuchte, Beleuchtung mit Leuchtstofflampen in Röhrenform Narva LS 65 weiß und Narva LS 65 Lumoflor, die im Verhältnis 1 : 1 angeordnet waren, Beleuchtungsstärke etwa 1 400 lx, Beleuchtungsdauer 16 h, Dunkel-

heit 8 h). Keimpflanzen mit entfaltetem ersten Blatt wurden 11 Tage nach der Aussaat mit frisch geernteten und im Verhältnis 1 : 10 mit Talkum gemischten Uredosporen behandelt, anschließend mit Wasser fein besprüht und 3 Tage unter einer lichtdurchlässigen Haube bei hoher Luftfeuchte gehalten. Die Probenahme der infizierten Primärblätter erfolgte zu verschiedenen Zeiten bis zu 28 dpi.

Die Infektionsstrukturen der Rostpilze wurden rasterelektronenmikroskopisch abgebildet (Schmidt et al., 1985). Die Extraktion, Fraktionierung und gaschromatographische Bestimmung der pilzlichen Zuckeralkohole erfolgten nach Reiss (1981). Zur selektiven Färbung der Rostpilze verwendeten wir den optischen Aufheller Wobital BBK (VEB CKB Bitterfeld) als Fluorochrom (Schlegel, Noll und Opel, 1982).

Ergebnisse

Die summarisch als Pilzzuckeralkohole (PZ) erfaßten Arabitol und Mannitol wurden in Abbildung 1 als Prozentanteil der gesamten wasserlöslichen Zucker (GZ) dargestellt, und zwar der zeitliche Verlauf ihrer Bildung in Tagen nach der Infektion (dpi). Die Polyole steigen in ihrer Konzentration kurz vor dem Aufbrechen der Uredosporenlager an. Sie folgen im Konzentrationsverlauf den auf den Blättern ermittelten Pustelzahlen (Reiss und Hartleb, 1984). Für die gegen Gelbrost Rasse 24 resistente Gerstensorte 'Bigo', die keine Pusteln bildet, konnten auch keine Konzentrationserhöhungen der PZ nachgewiesen werden.

Die erhöhten Werte zu Versuchsbeginn sind vermutlich auf die im Inokulum enthaltenen Pilzzucker zurückzuführen, doch kommen auch in nicht inokulierten Kontrollpflanzen schwache Peaks an den für Arabitol und Mannitol im Gaschromatogramm bestimmten Positionen vor. Eine Identifizierung mit den in der Cochromatographie eingesetzten Zuckeralkoholen Mannitol und Arabitol war

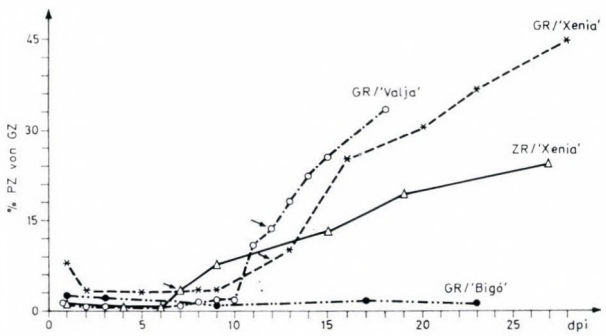

Abb. 1. Gehalt an Pilzzuckeralkoholen (PZ) in % vom Gesamtzucker (GZ) infizierter Primärblätter der Gerstensorten 'Valja', 'Xenia' und 'Bigo' in Tagen nach der Infektion (dpi). ○–·–·–○ Gelbrost auf 'Valja', ×- - - -× Gelbrost auf 'Xenia', □–··–··–□ Gelbrost auf 'Bigo', und △———△ Zwergrost auf 'Xenia' Die Pfeile bezeichnen den Zeitpunkt des Aufbrechens der Uredosporenlager. GR = Gelbrost, ZR = Zwergrost

Tabelle 1

PZ-Werte an stark befallenen Primärblättern der Sorte 'Xenia'
Die Konzentrationen stellen Mittelwerte aus drei Versuchen dar

Proben	mg PZ/g TS	SD	% PZ von GZ	SD
Unbehandelt	9.4	0.62	34.21	1.05
Nach Abschaben von Sporen	6.79	1.2	31.10	0.46

SD = Standardabweichung
TS = Trockensubstanz

bei den geringen Konzentrationen nicht sicher. Auch andere Autoren (Mitchell, Fung und Lewis, 1978; Whipps, Clifford, Roderick und Lewis, 1979) ordnen diese in gesunden Getreidepflanzen nachweisbaren Peaks den genannten Zuckeralkoholen zu.

In einem weiteren Versuch wurde überprüft, welcher Anteil PZ in den Blättern verbleibt, wenn man die reifen Sporen der Pusteln abschüttelt und zusätzlich mit einem Spatel weitere Sporen vom Blatt abstreift. In der Tabelle 1 sind die Werte dargestellt. Daraus ergibt sich, daß bis zu 72% der PZ im Blatt verbleiben.

Licht-, fluoreszenz- und rasterelektronenmikroskopische Untersuchungen, die zu verschiedenen Zeiten nach der Infektion mit Gelbrost an den Gerstensorten 'Valja' und 'Xenia' durchgeführt wurden, ermöglichten die Zuordnung der in Abbildung 1 dargestellten PZ-Konzentrationen zu den entsprechenden Pilzstrukturen. Der Gelbrost entwickelt nach Penetration der Spaltöffnungen zunächst substomatäre Vesikeln, meist drei Primärinfektionshyphen mit den entsprechenden primären Haustorienmutterzellen und dann ein ausgeprägtes Myzel (»runner«- und »feeding«-Hyphen), das sich in den interzellulären Räumen der Pflanze ausbreitet (Opel, Wolffgang, Schmidt und Müller, in Vorbereitung). Dieser Vorgang läuft vorwiegend bis zum 8. Tag nach der Infektion ab (Abb. 2) und ist nicht mit einer Erhöhung der PZ verbunden.

Die Entwicklung von Uredosporenlagern beginnt sehr vereinzelt zwischen 6 und 8 dpi und zeigt nur frühe Phasen der Uredosporenentwicklung. Der Reifeprozeß der Uredosporen vollzieht sich zwischen 8 und 12 dpi (Abb. 3) und verläuft dem Anstieg der PZ-Konzentration proportional (Abb. 1). Es wird daraus geschlossen, daß die PZ vorwiegend in reifenden und reifen Uredosporen gespeichert werden. Folglich ist die Bestimmung der PZ-Konzentration ein geeignetes Verfahren zur Messung der Sporenproduktion. Erfaßt werden dabei nicht nur die reifen Sporen, die aus den geöffneten Uredosporenlagern abfallen oder durch den Wind übertragen werden können, sondern auch die in den tieferen Schichten der Uredosporenlager reifenden und reifen Sporen (Abb. 3), wie auch aus der Tabelle 1 ersichtlich ist.

Der Gelbrost unterscheidet sich durch seine systemische Ausbreitung im Wirt deutlich vom Zwergrost. Eine erfolgreiche Infektion führt beim Zwergrost

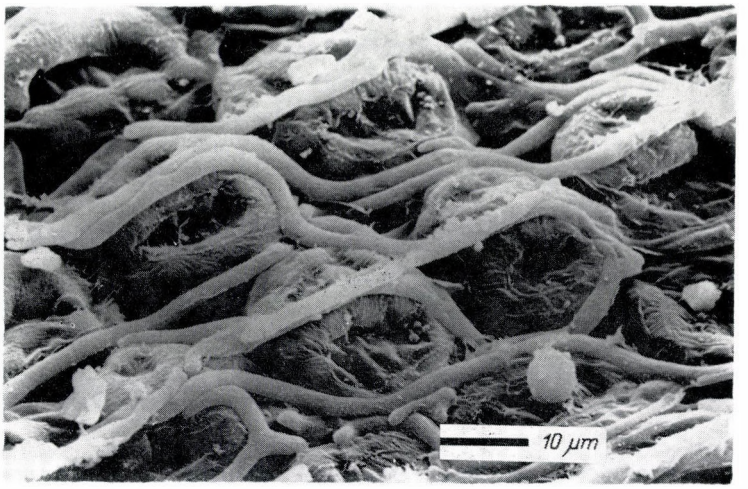

Abb. 2. Myzelentwicklung des Gelbrostes in den Interzellularräumen des Gerstenblattes nach Entfernung der unteren Epidermis 6 Tage nach der Inokulation der Primärblätter der Gerstensorte 'Valja' auf der Blattunterseite

nur zu einer Pustel, während der Gelbrost 100 und mehr Pusteln von einer Infektionsstelle ausgehend bilden kann. Der länger anhaltende Anstieg der PZ in der Produktion von Zuckeralkoholen bis zum Aufbrechen der Uredosporenlager beim Gelbrost in Abbildung 1 könnte damit in Zusammenhang gebracht werden.

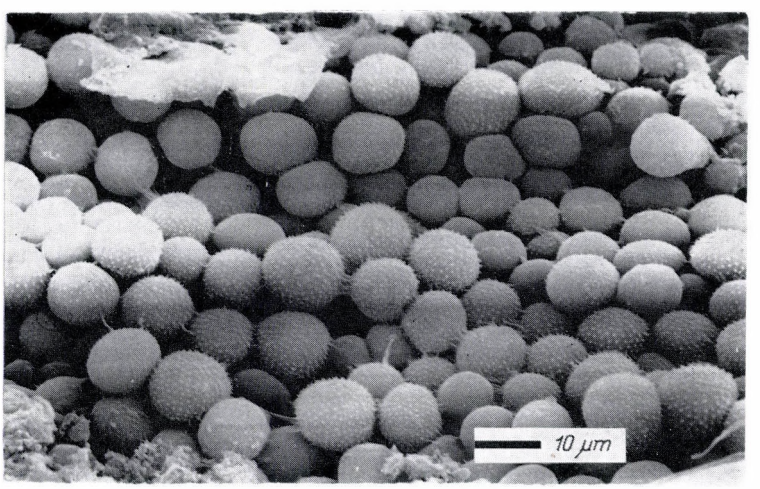

Abb. 3. Uredosporenlager des Gelbrostes nach Epidermisabriß der Blattunterseite 12 Tage nach der Inokulation der Blattoberseite von Primärblättern der Gerstensorte 'Valja'. Es sind Uredosporen unterschiedlichen Reifegrades zu erkennen

Diskussion

Mitchell, Fung und Lewis (1978) wiesen beim Kronenrost des Hafers und beim Schwarzrost des Weizens nach, daß während der Flecken- und Pustelbildung der Gehalt an Polyolen zunimmt und zwischen der Anzahl aufgebrochener Pusteln und dem Gehalt an Mannitol und Arabitol ein direkter Zusammenhang besteht. Beim Flachs konnte durch radioaktive Markierung nach Infektion mit Flachsrost ebenfalls eine Zunahme der PZ-Konzentration während der Pathogenese festgestellt werden (Chancy und Coffey, 1980). Hohe Konzentrationen an Zuckeralkoholen (vorwiegend Mannitol) sind bei vielen fakultativen Pilzparasiten aber auch im Myzel nachgewiesen worden (Lewis und Smith, 1967; Rast, 1963), wobei die qualitative und quantitative Zuckerzusammensetzung des Myzels stark von der Ernährung des Pilzes abhängt. Für biotrophe Pathogene sind Untersuchungen dieser Art wesentlich schwieriger durchzuführen. Doch konnten wir in der Zeit vor der Sporenbildung trotz des elektronenmikroskopisch beobachteten starken Pilzwachstums (Abb. 2) keine Pilzzuckerakkumulation im gelbrostinfizierten Blattgewebe feststellen. Es mag mit der ausschließlich auf den Wirt orientierten Lebensweise des Parasiten zusammenhängen, daß die für nichtgrüne Pflanzen als Kohlenstoffreserve und zur Speicherung von Reduktionsvermögen besonders geeigneten Zuckeralkohole (McLean und Scott, 1976) sich erst in der Fruktifikationsphase bilden. Mannitol und Arabitol bestimmen im wesentlichen den Gehalt an wasserlöslichen Zuckern in den Uredosporen des Gelb- und Zwergrostes (Reiss, 1985) Whipps, Clifford, Roderick und Lewis (1979) wiesen auf Zumensamhänge zwischen dem Phänomen des »slow-rusting« bei Gerste/Zwergrost und der Konzentration von PZ hin und konnten direkte Beziehungen zwischen dem Mannangehalt infizierter Gerstenblätter und der Entwicklung des Zwergrostes (*Puccinia hordei* Otth.) feststellen. Neben der durch Hydrolyse freigesetzten Mannose wird nach der beschriebenen Methode unter »Mannan« aber auch das gesamte Mannitol erfaßt. Es wurde nicht untersucht, in welchen Infektionsstrukturen des Pilzes das Mannitol vorkommt. Der stärkste Anstieg erfolgte während der Fleckenbildung bis zum Beginn der Sporulation.

Unsere Ergebnisse zeigen, daß während der »vegetativen Phase« der Pilzentwicklung keine wesentliche Zunahme der PZ erkennbar ist und erst mit der Entwicklung der Uredosporenlager der Gehalt an Mannitol und Arabitol zunimmt. Wir schließen daraus, daß diese Polyole vorwiegend in reifenden und reifen Uredosporen vorkommen und somit ihre Konzentration als ein Maß zur Bestimmung der Sporenproduktion des Gelb- und Zwergrostes auf Gerstenpflanzen verwendet werden kann.

Zusammenfassung

In Primärblättern von Gerstenpflanzen (*Hordeum vulgare* L.), die mit Gelbrost (*Puccinia striiformis* West.) oder Zwergrost (*Puccinia hordei* Otth.) infiziert waren, nahm der Gehalt an Mannitol und Arabitol während der Pathogenese zu. Es konnte nachgewiesen werden, daß die Polyole vorwiegend in reifenden und reifen Uredosporen vorkommen. Das Verfahren der gaschromatographischen Analyse von Mannitol und Arabitol eignet sich daher zur Messung der Sporenproduktion in unterschiedlich resistenten Gerstensorten.

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Secretion of Cytokinins by *Mycovellosiella concors* during its Infection on *Solanum tuberosum*

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Green islands observed at infection sites of *Mycovellosiella concors* on leaves of *Solanum tuberosum* were found to contain higher amounts of chlorophyll a and b, soluble sugars and total dry matter as compared to the surrounding healthy leaf tissue. A higher cytokinin activity was found to be associated with these infection sites. Possible role of cytokinins in inducing green islands and providing nutrition to the pathogen during its growth in the host is suggested.

Potato leaves infected with *Mycovellosiella concors* are observed to exhibit delayed senescence in nature frequently at the sites of infection and as a consequence green islands develop there. Green islands associated with rusts, powdery mildews, *Helminthosporium* leaf spots (Brian, 1967; Király et al., 1967; Vizárová, 1974; Mandahar and Arora, 1978; Ariora and Mandahar, 1979) and in many other plant pathogens (Dekhuijzen, 1976; Green, 1980) are known to occur because of a higher cytokinin activity at infection sites, but no such studies have been reported earlier with *M. concors*. Therefore, cytokinin activity at infection sites of *M. concors* was determined here using three different bioassays and is reported.

Materials and Methods

Leaves of variety — Kufri Chandramukhi (*Solanum tuberosum*) with distinct spots of *Mycovellosiella concors* were collected from potato fields at Kufri (Simla Hills, India). Leaf discs were cut with 5 mm cork borer from the spotted portion and the surrounding uninfected tissues. Seven gm of the leaf tissues in each case was processed for cytokinins extraction or physiological analysis.

Dry weight of leaf tissues was determined after drying it at 80 °C for 24 h. Carbohydrates were determined by the method of Dubois et al. (1956). Total chlorophylls and chlorophyll a and b were determined by method of Arnon (1949).

Cytokinins were extracted by the method of Vizárová (1975). The residue obtained in this process was dissolved in 5 ml of sterile distilled water. Cytokinin activities of the extract were determined by the chlorophyll retention test, root elongation inhibition bioassays (Király et al., 1967) and radish cotyledon expansion bioassay (Letham, 1968).

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Results and Discussion

Green islands found at the infection sites of *M. concors* contained 3.22 percent higher total dry weight; 286.46 percent chlorophyll a; 181.60 percent chlorophyll b, 238.81 percent total chlorophylls; 96.46 percent soluble sugars and 28.57 percent total carbohydrates over the healthy surrounding leaf tissues.

Higher amounts of dry weight, carbohydrates and chlorophylls in green islands of *M. concors* possibly suggest that these areas acted as the sinks of host's metabolites. This is first report with *M. concors*, however, such reports exist with some other fungi (Shaw, 1961; Wang, 1961; Livne and Daly, 1966).

Data on the results of three bioassays performed with extracts obtained from green islands caused by *M. concors* and the surrounding leaf tissue are given in Table 2 and are discussed as under: –

Amounts of chlorophyll retention in radish cotyledonary leaves increased considerably by application of cytokinin extract from infected tissues as compared to those from the healthy tissues. There was an increase of 257.43, 93.93 and 199.90 percent in retention of chlorophyll a, b and total chlorophylls by application of cytokinin extract from green islands over to the extract from the surrounding uninfected tissues.

Data obtained from barley root elongation inhibition test were also positive. As compared to control a 21.82 percent higher inhibition in the length of barley roots was observed by extract from the infected tissues.

Letham's radish cotyledon expansion test was also positive. An increase in the weight of radish cotyledons (14.64 percent in fresh weight and 14.20 percent in dry weight) was observed by extract from infected tissue as compared to the control.

All the three bioassays showed the presence of a higher cytokinin activity in green islands than the surrounding uninfected leaf tissues. The assumption that a higher cytokinin activity exists at infection sites of *M. concors* is, therefore, confirmed.

Secretion of cytokinins during pathogenesis of *M. concors* possibly facilitate the fungus to mobilize the host's metabolites from the surrounding regions to the infection sites and also benefits the pathogen by inducing green islands in the host where the pathogen continues its activities much later even when the surrounding leaf tissue start senescence. Movement of metabolites by cytokinins from area of lower cytokinin activity to the areas of higher cytokinin activity is known (Leopold and Kriedmann, 1975). Formation of green islands and accumulation of higher amounts of metabolites at infection sites during pathogenesis of some other fungi is reported previously (Király et al., 1966; 1967; Pozsár and Király, 1966; Bushnell, 1967).

Table 1

Dry weight, chlorophylls and carbohydrates in green islands on potato leaves infected with *Mycovellosiella concors*

Treatment	Total dry weight (percent)	Chlorophylls (mg/g dry weight)			Carbohydrates (mg/g dry weight)		
		a	b	Total	Sugar	Starch	Total carbohydrates
Infected tissue	15.97	0.436	0.245	0.681	102.80	49.30	152.10
Healthy tissue	13.75	0.114	0.087	0.201	51.80	66.50	118.30
Per cent increase/dicrease over control	3.22	282.46	181.60	238.81	96.46	−34.89	28.57
C.D. 0.01%	0.11**	0.046**	0.063**	0.103**	NS	16.83	NS

** Significant at probability level 0.01 percent
NS = Non-significant at probability level 0.01 percent

Table 2

Cytokinin activity of the extracts from green islands on potato leaves infected with *Mycovellosiella concors*

Treatments	Bioassays											
	Chlorophyll retention						Root elongation inh.		Radish cotyledonary expansion			
	Chlorophylls mg/m² area			Percent increase over control (healthy tissue)			Length of roots (cm)	Percent inhibition over control (healthy tissues)	Actual weight of 8 cotyledons after 3 days of exposure in continuous light (mg)		Percent increase over onctrol (healthy tissues)	
	a	b	Total	a	b	Total			Fresh	Dried	Fresh	Dried
Infected tissue	47.16	14.08	61.69	257.43	93.93	199.90	4.30	21.82	320	64.33	14.64	14.20
Healthy tissue	13.32	7.26	20.57	0	0	0	5.50	0	280	56.33	0	0
Water (control)	4.74	5.49	10.29				6.63		256	51.67		
S. E.	0.027	0.11					0.39		3.43	0.81		
C. D. (0.05)	—	—					1.53*		13.46*	2.52		
C. D. (0.01)	0.166**	0.72**					2.55**		22.43**	5.29**		

** Significant at probability level 0.01 percent
* Significant at probability level 0.05 percent

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A Laboratory Method to Predict Pathogenicity of *Fusarium graminearum* in Field and Resistance of Wheat to Scab

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14–21 isolates of *F. graminearum* were tested for pathogenicity on two wheat genotypes (Bezostaya-1 for susceptible, and 2/1974 for moderate resistance) in 1978–1980. Inoculations were made parallel in the laboratory (Petri dish), greenhouse, and the field (head blight).

The differences in the frequency of disease between the two varieties changed from isolate to isolate. Therefore the differences in disease could not be considered as differences in resistance. This stresses the use of more parallel isolates in resistance tests.

As the general frequency of disease depends also on the pathogenicity of the inoculum, the use of more isolates to receive a more acceptable approach of genetically fixed resistance in unavoidable. The same is true of the pathogenicity tests. As the level of pathogenicity depends also on the level of resistance, its evaluation needs more varieties with different resistance. Pathogenicity results on one variety have about the same value as those of the resistance test with one isolate.

The pathogenicities in the seedling and head blight tests correlated well, even if the closest relationship was not always obtained for the same parameter. The pathogenicity of the inoculum can be forecasted for head blight tests via seedling tests. This is a tendency and it helps to eliminate the least pathogenic inocula from experimental work.

The seedling reaction of 2/1974 was superior in the three years' study over Bzt-1. This was true also for the head blight test.

The improvement of wheat resistance to fusarial head blight (scab) was an important task in the past hundred years and is still an unsolved task today (Cook, 1981; Cassini, 1981; Burgess et al., 1981; Maric, 1981; Munteanu et al., 1972; Sutton, 1982). Its main pathogens are *F. graminearum* and *F. culmorum*, but also other *Fusarium* spp. of minor importance can infect wheat (Nelson et al., 1981; Mesterházy, 1974, 1978a). Numerous reports deal with variety differences (Capetti, 1974; Christensen et al. 1929; Jovicevich, 1970; Sindrova, 1970; McNight and Hart, 1966; Purss, 1966, 1971), but the data are often contradictory; the same variety seldom gives the same reaction in different years (Andersen, 1948; Atanasoff, 1920; Cook, 1980, 1981; Cassini, 1981; Christensen and Stakman, 1925; Greaney et al., 1938; Hanson et al., 1950; McNight and Hart, 1966; Scott, 1927). This means for us that basic problems should be in the background of this situation.

Recent findings show that powdery mildew infection increases disease severity in head blight (Mesterházy and Rowaished, 1977). I have similar data in this relationship also for leaf rust.

Another earlier observation was that the individual isolates gave various differences for the same varieties (Mesterházy, 1981). Such results were published for head blight by Tu (1930), but he was not aware of the importance of his results in variety testing, he did not even mention the results in the text, the data were only presented in a table. Therefore, the first important task of this paper was to investigate the effect of different isolates on the differences in the frequency of disease between varieties not only in the greenhouse, but also in the head blight test, retesting Tu's (1930) results.

Even in the case of very good replicability in the field, the number of genotypes to be tested is very limited. The method is suitable only for uniform population, segregating generations and individual plant screening tests are not possible. Therefore, we need an indirect method to predict the head blight reaction. Unfortunately the literature does not have any positive information in this context. Cook (1980) and Cassini (1981) are rather pessimistic. Parameters such as presence of awns, plant height, size of the leaf and nature of the leaf surface cannot be used in a selection program. The only morphological character is the flowering in boot stage (Cook, 1981) as being connected to the head blight field resistance, but he considers this as an escape mechanism. Therefore, we decided to investigate the possible role of the seedling tests. The basis for this were experimental results where the same isolates had similar pathogenicities in fusarial seedling and scab tests (Mesterházy, 1975, 1978b). Therefore, the second task of the paper is to investigate the relationships between the fusarial seedling and adult stage (scab) resistance by using the methodological advantages of earlier research work (Mesterházy, 1977, 1978a, 1978b, 1981; Mesterházy and Rowaished 1977).

Materials and Methods

For the three year's study a susceptible (Bezostaya-1) and a moderately resistant (2/1974, selected by us) genotype was used. The isolates of *F. graminearum* were isolated and identified by the author between 1970 and 1976 according to the manual of Booth (1971). Altogether 23 isolates were used, for several reasons only eleven of them were tested every year. Sixteen were isolated from wheat seeds, two from wheat glumes and five from corn seeds (Table 1).

The methods for raising inoculum and applying inoculation have earlier been presented by Mesterházy (1978b).

As powdery mildew and leaf rust increases significantly the disease severity to scab, the plants must be free of it. In the first year no fungicides were at hand; in autumn 1977 the plots received no fertilizer. In earlier years they were supplied with large amount of fertilizers and their postponed effect was enough to provide a near normal crop development with very limited amount of powdery mildew and leaf rust. From autumn 1978 we had Bayleton, so the plots could be given NPK fertilizer 80 : 110 : 110 kg active agent/ha. Two sprayings were given

Table 1

Characteristics of the isolates used in 1978–80

Isolate	Date of the isolation	Source	Conidium concentration $\times 10^6$		
			1978	1979	1980
648/1a	1970	wheat seed	0.01 M*	0.73	0.90
648/1b	1970	wheat seed	1.58	—	—
2856	1974	wheat seed	0.27	0.40	5.05
3682	1975	wheat glume	0.68	2.67	1.20
3715	1975	wheat seed	—	1.20	0.20 M
3905	1975	wheat seed	0.27 M	—	—
4041	1975	wheat seed	0.01 M	2.53	—
4059	1975	wheat glume	0.25	—	0.75
4366	1975	corn seed	0.30	—	0.45
4502	1975	corn seed	0.83	8.47	2.12
4530	1975	corn seed	0.90	—	—
7073	1976	corn seed	0.00 M	0.60	0.60 M
7558	1976	wheat seed	—	—	0.35
7752	1976	wheat seed	0.63 M	—	—
7874/1	1976	wheat seed	1.73	0.53	0.55
7874/2	1976	wheat seed	—	2.60	—
7905	1976	wheat seed	0.01 M	3.27 M	0.55 M
8179	1976	wheat seed	0.00 M	—	0.13 M
8760	1976	wheat seed	0.45	3.07 M	0.55
9 800	1976	corn seed	0.28 M	—	—
10 758	1976	wheat seed	1.32 M	—	1.20
10 791	1976	wheat seed	1.47	1.80	0.60 M
10 885	1976	wheat seed	1.63	0.47	0.22 M
10 887	1976	wheat seed	2.80	0.33	0.65
10 897/1	1976	wheat seed	0.00 M	0.80	1.76
10 897/2	1976	wheat seed	—	0.33	—

M* = high mycelium concentration

(0.4 kg/ha), the first at tillering, the second after heading. The plants were free of diseases or they occurred only in traces.

The inoculation was made at flowering stage of wheat (about the end of May, beginning of June), together with the laboratory and greenhouse test. For the three tests the same inocula were used.

In Petri dish tests four replicates were performed every year and for every isolate tested. 25 surface sterilized seeds (NaClOCl, 1 % solution, 20 minutes, after that washing three times with distilled water) were sown in Petri dishes on double layer filter paper infested with inoculum (Petri dish diameter was 120 mm, the amount of inoculum 10 ml). The number of living healthy germs was counted on the 2nd, 3rd, 4th, 5th and 6th days'after sowing. The data were averaged and related to the respective noninoculated controls.

In the greenhouse tests (Mesterházy, 1978b) ton pots 7 cm of diameter were used, filled 2/3rd with perlit. This was watered first and then infested with 10 ml

inoculum, uniformely on the whole surface. In a pot 15 surface sterilized seeds were sown and covered by 2 cm perlit. The number of replicates was four. Germination and number of killed plants were recorded every second day. Day-time tempera-

Table 2

Infectivity of *Fusarium graminearum* isolates on a tolerant and a susceptible wheat genotype with different inoculation techniques. 1978 data
All are expressed in % of the control

Isolates	Genotypes							
	2/1974				Bezostaya-1			
	Petri dish	Greenhouse	Field		Petri dish	Greenhouse	Field	
	Surviving germs %	Plant height %	Yield %	1000 grain mass %	Surviving germs %	Plant height %	Yield %	1000 grain mass %
648/1a	42.18	22.27	62.61	60.45	16.38	17.55	86.47	64.30
648/1b	24.60	53.02	37.52	50.72	20.55	44.83	22.16	21.96
2 856	17.25	23.10	14.18	29.49	10.20	27.32	20.10	25.16
3 682	3.35	5.90	19.99	19.35	2.58	1.86	15.28	24.72
3 905	0.00	0.00	11.08	16.48	0.00	1.93	8.44	9.82
4 041	12.00	33.59	25.53	40.32	9.58	12.90	40.07	52.09
4 059	40.63	20.15	23.67	41.38	20.53	16.62	28.34	27.95
4 366	11.23	2.17	14.08	34.11	2.03	5.22	22.57	26.41
4 502	97.03	80.85	44.50	46.65	86.68	63.87	79.38	43.98
4 530	10.15	6.99	4.51	12.98	3.10	7.66	19.09	34.26
7 073	33.50	1.83	18.36	19.06	27.83	8.70	32.29	46.28
7 752	29.33	4.53	10.09	22.20	15.85	3.39	25.13	41.77
7 874	16.20	27.25	19.38	31.84	10.38	14.90	25.58	35.84
7 905	21.73	29.43	12.96	33.65	11.96	23.15	18.17	30.49
8 179	28.28	0.47	38.35	47.39	18.95	5.92	41.86	59.56
8 760	4.13	0.99	9.51	18.52	0.50	5.30	10.04	15.34
9 800	0.00	0.00	9.15	15.09	0.00	0.36	13.82	14.23
10 758	28.00	36.59	15.88	23.67	18.98	25.07	27.98	31.24
10 791	17.00	7.15	24.28	31.21	12.98	15.00	17.97	32.97
10 885	4.15	0.66	22.69	32.03	1.50	5.19	16.02	21.14
10 887	15.68	14.74	12.99	34.17	17.15	11.11	33.94	43.85
10 897	19.63	7.24	24.06	36.05	15.05	7.32	28.95	32.66
Mean	21.64	17.22	21.61	31.67	14.67	14.78	28.35	33.49
LSD 0.1 %	15.23	18.28	26.57	28.26	16.09	21.11	18.73	11.76
1.0 %	11.77	14.13	20.38	21.73	12.43	16.31	14.47	9.09
5.0 %	8.84	10.61	15.42	16.26	9.33	12.25	10.86	6.83
LSD values between individual data pairs for the parameter considered								
0.1 %	18.79	17.02	22.51	11.09				
1.0 %	14.64	13.26	17.50	8.61				
5.0 %	11.11	10.06	13.23	6.53				
LSD values between means for the parameter considered								
0.1 %	3.75	3.34	4.95	2.36 (1 %)				

Correlation analysis

Parameters	1	2	3	4	5	6	7	8
1	—	0.72****	0.61***	0.55***	0.95****	0.76****	0.76****	0.51**
2	—	—	0.48	0.53**	0.76****	0.95****	0.54***	0.21
3	—	—	—	0.87****	0.51**	0.49*	0.83****	0.60***
4	—	—	—	—	0.44	0.52**	0.72****	0.57***
5	—	—	—	—	—	0.80****	0.70****	0.41
6	—	—	—	—	—	—	0.51**	0.15
7	—	—	—	—	—	—	—	0.78****

**** P = 0.1% *** P = 1% ** P = 2% * P = 5%

ture was 25 °C, night temperature 20 °C, relative humidity was kept at 80%. When the number of killed plants was stabilized (three weeks after sowing), the experiment was evaluated. Every plant's height was measured, than the plants were washed out of the perlit and evaluated for root infection on a scale 0–10 and the dry matter production per pot was checked, too. From these parameters only plant height was considered for this paper in 1978, because this showed the closest correlation with the Petri dish test. In 1979 the relationships between plant height and the Petri dish test were less close, therefore we cite beside plant height also dry matter production and germination. In 1980 again only plant height was taken into account.

In the head blight test (Mesterházy, 1977, 1978b) yield and 1000 kernel weight were measured, because these characteristics are considered to characterize partial resistance or tolerance (Robinson, 1969). For inoculation of heads developmental stage 21 according to Romig is optimal (Calpouzos et al., 1976; Mesterházy, 1978b). In 1979 the inoculation could be done several days later because of the cold and rainy weather (Romig scale 23–24). Therefore additional tests were made from the seeds threshed from the artificially inoculated ears taken from the 1979 trial. First we made a germination test in Petri dishes (humid chamber), and after that the *Fusarium* infection of the threshed seeds was controlled on Papavizas (1967) PCNB medium. From the seeds of every field replicate (their number was four in every year, in one replicate 15–20 ears were inoculated) 3 × 50 seeds were checked. The healthy germs were counted on the 7th day after sowing; in the first rating all germs were recorded, in the second only the healthy ones.

In 1980 the inoculation could be done at growth stage 21 according to Romig.

In the evaluation of harvested ears 10 average ears were selected from every replicate (15–20 ears). These were threshed for seeds by hand and the yield as well as 1000 kernel weight were measured. In 1980 beside seed weight also the weight of the 10 selected ears were measured. The question was wether this could replace the laborious hand threshing.

For biostatistical evaluation correlation and variance analyses were used.

Results

Pathogenic variation as a factor in detecting cultivar differences

The varieties reacted very differently to different isolates. This was true not only for the seedling results which were known from earlier tests (Mesterházy,

Table 3

Infectivity of *Fusarium graminearum* isolates on wheat with different inoculation techniques Summarized data of 1978. All data are expressed in % of the control

Isolates	Petri dish	Greenhouse	Field	
	1 Surviving germs %	2 Plant height %	3 Yield %	4 1000-grain weight %
648/1a	29.28	19.91	74.54	62.38
648/1b	22.58	48.93	29.84	36.34
2 856	13.73	25.21	17.14	27.33
3 682	2.97	3.88	17.64	22.04
3 905	0.00	0.97	9.72	13.15
4 041	10.79	23.25	32.80	46.21
4 366	6.63	3.70	18.32	30.26
4 059	30.58	18.39	26.00	34.67
4 502	91.86	72.36	57.44	45.32
4 530	6.63	7.33	11.84	23.62
7 073	30.67	5.27	25.33	32.67
7 752	22.59	3.96	17.61	31.98
7 874	13.29	21.08	22.48	33.84
7 905	16.84	26.29	15.57	32.07
8 179	23.62	3.20	40.37	53.48
8 760	2.32	3.15	9.78	16.93
9 800	0.00	0.18	11.47	14.66
10 758	23.49	30.83	21.93	27.46
10 791	14.99	11.08	21.37	32.09
10 885	2.83	2.93	22.11	26.98
10 887	16.42	12.93	23.46	39.01
10 897	18.88	7.28	26.50	34.35
Mean	18.23	16.01	22.15	32.58
LSD 0.1%	13.30	12.05	15.84	7.82
1.0%	10.36	9.39	12.30	6.07
5.0%	7.86	7.12	9.32	4.61

Correlation analysis

Parameters	1	2	3	4
1	—	0.77****	0.67****	0.55***
2	—	—	0.50**	0.39
3	—	—	—	0.88****

**** P = 0.1% *** P = 1% ** P = 2%

Table 4

Variance analyses of experiments conducted in 1978–1980 with different inoculation techniques and different parameters. Mean square values

1978

Source of variance	Petri dish tests	Greenhouse tests	Field tests	
	Surviving germs	Plant height	Yield	1000 kernel w.
Isolates (A)	2189.1[a]	2391.4[a]	2020.4[a]	1128.9[a]
Varieties (B)	123.3[a]	2085.3[a]	2411.5[a]	147.2[a]
A × B interaction	172.0[a]	473.7[a]	245.4[a]	311.8[a]
Error	51.7	63.0	89.5	21.7

1979

	Petri dish tests	Greenhouse test			Field test		
	Surviving germs %	Plant height	Germination	Dry matter content	Yield	Germination in humid chamber	Living germs on PCNB medium 7th day
Isolates (A)	1401.3[a]	2013.7[a]	84.3[a]	2069.3[a]	419.5[a]	4458.1[a]	7062.0[a]
Varieties (B)	14596.8[a]	12835.7[a]	1084.3[a]	16061.1[a]	17548.3[a]	3292.3[a]	1985.3[a]
A × B	420.7[a]	627.0[a]	28.7[a]	297.3[a]	402.1[a]	347.3[a]	102.3[a]
Error	27.6	83.3	3.4	79.8	117.7	125.4	40.3

1980

	Petri dish tests	Greenhouse test	Field test	
	Surviving germs	Plant height	Yield Whole ears	Yield Threshed
Isolates (A)	2741.1[a]	2117.8[a]	1292.7[a]	658.8[a]
Varieties (B)	117815.8[a]	1215.1[a]	4085.0[a]	216.5[b]
A × B	901.5[a]	164.0[a]	948.6[a]	171.0[a]
Error	70.0	112.3	80.9	45.2

[a] $P = 0.1\%$; [b] $P = 1\%$

1981), but also for head blight reaction. To demonstrate this I present the 1978 results in detail (Tables 2, 3). If we take any of the data pairs, we see that the amount of disease depends actually on the isolate used. From no difference to 4 – 5 fold deviation every possibility occurs. Therefore it is impossible to accept the difference in disease received for one isolate as difference in resistance. At the same time two varieties cannot be genetically equal or very different in resistance. This variability is the source of the isolate-genotype interaction, which was significant for every parameter considered in the three-year study (Table 4). Another problem of

Table 5

Infectivity of *Fusarium graminearum* isolates on wheat with different inoculation techniques Original data were expressed in the % of the control. Correlation analysis

Parameters	Inoculation methods							
	Petri dish	Greenhouse				Field		
							Germination	
	Surviving germs	Plant height	Dry matter content	Germination	Yield	1000 grain mass	2nd day (humid chamber)	7th day PCNB
	1	2	3	4	5	6	7	8
1	—	0.37	0.30	0.18	−0.02	0.19	0.62***	0.46***
2	—	—	0.93****	0.86****	−0.18	−0.28	−0.10	−0.02
3	—	—	—	0.91****	−0.25	−0.26	−0.23	−0.08
4	—	—	—	—	−0.38	−0.49*	−0.47	−0.34
5	—	—	—	—	—	0.64	0.26	0.39
6	—	—	—	—	—	—	0.56*	0.54*
7	—	—	—	—	—	—	—	0.78***

**** P = 0.1%; *** P = 1%; ** P = 2%; * P = 5%

measuring resistance according to one simple isolate is the very different absolute level of disease for different isolates. Theoretically every variety can be between 0 and 100% of the not inoculated control according to the pathogenicity of the isolate. Therefore, the amount of the disease to one isolate should not mean the level of the genetically determined resistance. The same consequences were concluded from the 1979–80 trials.

Therefore, by using one isolate in variety tests we can never be sure that the results will show genetically fixed variety differences, and the genetically determined amount of resistance.

Relationships between methods of inoculation

The results achieved by two seedling methods gave very close relationships in 1978 (r = 0.72 for 2/1974, 0.80 for Bzt-1 and 0.77 for the means of the two cultivars, P = 0.1%) (Tables 2, 3). The Petri dish test correlated closer with the yield and 1000 kernel weight results than with the plant height data. The Petri dish test gave r = 0.67 (P = 0.1%) with yield results higher than the greenhouse tests (r = 0.50, P = 2%). Similar relationships were calculated for the two varieties, too (Table 2). This means that a low pathogenic isolate in the greenhouse or Petri dish tends to have low pathogenicity in head blight tests and vica versa. It is remarkable that 1000 kernel weight correlates worse with seedling tests than yield data do.

In 1979 the picture was somewhat different (Table 5). No seedling results were in accordance with yield and 1000 kernel weight results. The reason for that

Table 6

Correlation coefficients between pathogenicity of *Fusarium graminearum* isolates at different inoculation techniques. 1980. Number of data pairs (isolates) is 18

Parameters		Inoculation techniques			
		Petri dish	Greenhouse	Head blight	
		Surviving germs	Plant height	Yield 1[a]	Yield 2[b]
		1	2	3	4
A. Tolerant genotype, 2/1974	1	—	0.32	0.58***	0.55**
	2	—	—	0.04	0.17
	3	—	—	—	0.93****
B. Susceptible genotype, Bzt-1	1	—	0.15	0.53*	0.47*
	2	—	—	0.07	0.09
	3	—	—	—	0.79****
C. Mean of the two genotypes[c]	1	—	0.25	0.62***	0.59***
	2	—	—	0.04	0.17
	3	—	—	—	0.87****

**** P = 0.1%; *** P = 1%; ** P = 2%; * P = 5%
[a] Yield of the whole ears
[b] Yield of the threshed seeds
[c] Correlation counted between means (2/1974, Bzt-1) of date pairs

was the relatively late inoculation. Therefore we controlled the germination of seeds from the inoculated ears. Here we received significant correlations (r = 0.62 for the humid chamber, and r = 0.66 for the 7th day on PCNB medium) with the Petri dish tests, but the greenhouse tests gave no correlation. This is very important, because we can estimate the resistance of the materials even in the case of a mild infection, where yield or 1000 kernel weight are no more suitable to detect differences. The greenhouse data had no correlation with the Petri dish results, e.g. the pathogenicities by the two tests did not correlate.

In 1980 the two seedling tests gave no relationship, as happened in 1979 (Table 6), but the relation with yield was significant. As in previous years the 1000 kernel weights gave lower relationships with seedling test results than yield did. We measured instead of this the whole ear weights before threshing. This is about as exact as the threshed yield data (the correlations with the Petri dish data are about the same) but in the case of variety tests it was not so good, because the ratio of seeds and rest ear is different from variety to variety (Mesterházy, 1983). In the case of a screening, however, where less exactness is needed, the whole ear weight is acceptable sparing time and labour.

Performance of the two genotypes

The more tolerant 2/1974 was superior to Bzt-1 in nearly every case (Table 7), the difference between the two lines was not consistent for the different methods

Table 7

Pathogenicity of 14 *Fusarium graminearum* isolates on a tolerant and a susceptible wheat genotype with different inoculation techniques, 1978–1980

Inoculation technique	Genotype				
	Tolerant 2/1974	Susceptible Bzt-1	LSD	Tolerant 2/1974	Susceptible Bzt-1
	in % of the control			100% =	
1978					
Petri dish					
Surviving germs	21.6	14.7	3.7***	95.3	96.0
Greenhouse					
Plant height	17.2	14.8	3.3***	10.8 cm	15.2 cm
Head blight					
Threshed yield	21.6	28.3	4.9***	10.2 g	14.4 g
1000 grain weight	31.7	33.5 g	2.4***	24.7 g	34.9 g
1979					
Petri dish					
Surviving germs	34.2	12.0	3.1***	100.00	96.8
Greenhouse					
Plant height	31.7	8.0	5.3***	17.2 cm	18.4 cm
Dry matter prod.	42.7	20.8	5.2***	0.8 g	0.7 g
Germination	67.0	30.2	6.9***	100.00	97.0
Head blight					
Yield	82.2	57.4	6.1***	14.1 g	16.2 g
1000 grain weight	83.6	60.6	2.5***	32.5 g	35.9 g
Germination on[a]					
2nd day (humid chamber)	33.1	28.4	3.6***	100.0	100.0
7th day (PCNB medium)	12.8	8.1	2.1***	100.0	100.0
1980					
Petri dish					
Surviving germs	65.2	15.4	3.9***	96.0	86.0
Greenhouse					
Plant height	34.1	28.7	4.65**	20.1	23.7
Head blight					
Yield, whole ears	56.4	48.8	3.5***	20.6	18.9
Yield, threshed	24.6	21.8	2.2*	17.8	14.9

[a] on the 2nd day every germ was considered, on the 7th day only the healthy ones.
*** P = 0.1%; ** P = 1%; * P = 5%

and symptoms. In seedling tests the differences are consequently larger than in the field investigations. This means that the better variety in seedling stage was better in head blight resistance, but not proportionally. The differences were always significant (Table 4), however. The field data of 1978 need a special explanation.

The 2/1974 received, in spite of our efforts, normal amounts of N, but Bzt-1 received none as it was intended. Bzt-1 was free of leaf diseases, and 2/1974 was severly infected by leafrust and powdery mildew. As these diseases increase heavily the *Fusarium* head blight severity, this variety could not realize its higher resistance; it even gave a more susceptible reaction than the not fertilized Bzt-1. A further proof for the correctness of this way of thinking is that in the same field we had a plot of 2/1974 without fertilization, inoculated with the isolate 10 897 in variety resistance test. Here 2/1974 was significantly better, its yield was 79.06% compared to the 63.21% of Bzt-1, for 1000 kernel weight the numbers are 79.74 and 52.56%, respectively. Without leaf diseases the 2/1974 could bring its usual high resistance. This agrees with the data of 1979 and 1980 results as well as with earlier experimental data.

Discussion

The results show that the differences, which were found in seedling stage between genotypes, are not stable for different isolates even in head blight tests. We have to emphasize that variablity of the *Fusarium* species has a very serious impact on the resistance tests. It is theoretically impossible to accept that a single isolate can represent a species which distributes for innumerable levels of pathogenicities. Maybe, this finding will not be very new for theoretical plant pathologist, as Hooker (1977) emphasized the importance of the problem, but as the practical resistance investigations show, the consequences were not successfully applied in practical breeding work. In order to influence the practical resistance testing work, however, we should stress the need of using more isolates with different pathogenicity levels. By this way we are significantly closer to the population of the fungus and the results will rather reflect the performance of the genotype than working with one single isolate. The second argument for using more isolates is that the amount of the disease depends not only on the resistance of the genotype, but also on the pathogenicity of the fungus. At low pathogenicity every variety, will be "resistant", at high pathogenicity every cultivar will be "susceptible". For a genotype these two statements are not acceptable at the same time.

A third argument for the use of more isolates is that controlling of pathogenicity of the inoculum for head blight is not fully exact. In these tests the pathogenicities correlated well between seedling and adult stage reactions, but the closeness varied. The tendency is true that isolates having good pathogenicities in seedling tests will have good pathogenicity also in head blight investigations and vica versa, but not valid for every inoculum. The seedling test is, therefore, suitable for a preselection of the inocula to be used in the field, the use of more inocula excludes the loss of years as a consequence of too low or too high pathogenicity in variety testing and other experiments.

The problems arising from the use of a single isolate are largely responsible for the inconsequent experimental results and low interannual correlations. But these are responsible (at least in part) for the low inheritance values for head blight,

and for the fact that the environmental effects were very high in inheritance traits (Nakagawa et al., 1966).

As we have stressed, the use of more isolates in resistance tests raises the question what we should do in the case of large scale screening tests, where only a preselection for the extreme plusvariants is needed.

Based on the present material, we cannot answer the question. For having a better insight, not two but at least 20 or more varieties are needed to be treated with different isolates. This will be a task of the future.

The correlations between seedling and head blight tests show that pathogenicities correlate well with each other, but the relationships are only of medium level. This is true first of all for the Petri dish tests, the greenhouse test gave an acceptable relationship only in 1978, so we decided to use the Petri dish test as standard seedling method. The exception is the year 1979, when the head inoculation was later and the yields were only moderately influenced. From 1975 (Mesterházy and Rowaished, 1977) we know that the earliest inoculation is the best, later inoculations bring significantly less differences. The same can happen with the use of a low pathogenic inoculum; yield or 1000 kernel weight reaction will be only moderate. But even in this case we can detect differences, as in 1979 the germination of the seeds from the inoculated ears gave good relationship with seedling data. This means that not always the same parameter of head blight will provide the closest relationship with seedling data. Therefore, it seems to be reasonable to evaluate more parameters, and, if needed, also germination, *Fusarium* infection of harvested seeds can be investigated.

Following the three years' study for variety differences, we see that the more resistant variety was better in seedling, and head blight test, too. The mean performances show that the largest variety differences were recorded in seedling tests (Petri dishes), but less in field parameters. The seedling resistance correlated with head blight resistance. This urges us to investigate further the possible role of seedling tests in the selection for head blight resistance. This will be another important research topic in the future.

If the biotrophic leaf diseases can increase the amount of *Fusarium* by 50%, we have a ground not to belive in the traditional *Fusarium* head blight resistance tests. Therefore the third important task will be to determine how far we can improve the interannual replicability of field results applying these new findings. Until now we achieved very close ($r = 0.78$ $P = 0.1\%$) interannual correlation in seedling tests, but the head blight relationships were neither better nor worse than $r = 0.50$, which is about the international practice (Hanson et al., 1950; Mesterházy, 1983), and not very encouraging for the breeder or pathologists.

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Lectins in Germinating Seeds and Their Effect on Fungi

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Lectins from *Arachis hypogaea*, *Cajanus cajan*, *Cicer arietinum*, *Dolichos lab-lab*. *Glycine max*, *Phaseolus vulgaris*, *Pisum sativum* and *Vigna unguiculata* were isolated. Except *C. cajan* lectin, which agglutinated specifically trypsinized rabbit erythrocytes, other lectins agglutinated untrypsinized rabbit erythrocytes. Lectins from *A. hypogaea*, *D. lab-lab*, *G. max*, *Ph. vulgaris* and *P. sativum* induced mitogenesis and cell lysis in rabbit as well as in human leukocytes. *C. cajan* and *V. unguiculata* lectins did not cause any nuclear change. Lectins from *C. arietinum* induced blasting of rabbit leukocytes.

During seed germination, lectin level declined. Moderate seed infestation did not affect lectin content of germinating seeds but heavy seed infestation reduced lectin level. In the germinating seeds of *A. hypogaea*, *G. max* and *P. sativum*, the nature of lectins was altered.

Trypsinized and germinating spores of *Col. capsici* and *F. solani* were more sensitive to the lectins than untrypsinized conidia. *D. lab-lab* and *Ph. vulgaris* lectins were bound weakly with *Col. capsici* conidia. Similarly, *P. sativum* lectins showed weak binding with *F. solani* conidia. *D. lab-lab* seed lectins induced blasting of untrypsinized *F. solani* conidia and inhibited spore germination but did not agglutinate them. However, lectins isolated from unsterilized germinated seeds of *D. lab-lab* agglutinated untrypsinized conidia.

Lectins from seeds and germinated seeds affected the development of conidia and influenced their development even after the removal of lectins. Lectins isolated from surface sterilized germinated seeds of *D. lab-lab*, *Ph. vulgaris*, *P. sativum* and *V. unguiculata* bound weakly with untrypsinized *Col. capsici* conidia. However, *Col. capsici* conidia germinated after transfering them from lectins. Lectins isolated from germinated seeds of *C. arietinum*, *Ph. vulgaris* and *V. unguiculata* bound weakly with trypsinized *Col. capsici* conidia. *F. solani* conidia germinated after removal of *P. sativum* lectins. Although lectins inhibit pathogenic fungi, their role in resistance or recognition is not clear.

Lectins are sugar binding proteins or glycoproteins of non-immune origin which are devoid of enzymatic activity towards sugar to which they bind and do not require free glycosidic hydroxyl groups on these sugars for their binding (Kocourek and Horejsi, 1981). Legume seeds contain reserve proteins stored in

Abbreviations used: CPl — chick pea lectin; Con. A — concanavalin-A; DLL — *Dolichos lab-lab* lectin; PBS — Phosphate buffer saline; PVL — *Phaseolus vulgaris* lectin; PL — potato lectin; PNL/PNA — Pea nut lectin/agglutinin; PPL — Pigeon pea lectin; PSL — *Pisum sativum* lectin; RCA — *Ricinus communis* agglutinin; SBL/SBA — soybean lectin/agglutinin; SPA — sweet potato agglutinin; VUL — *Vigna unguiculata* lectin; WGA — wheat germ agglutinin.

parenchymatous cells of cotyledons and about 10–25% of proteins occur as 'lectins' (Bollini and Chrispeels, 1979). Despite wide interest and much research on lectins (Agrawal and Mahadevan, 1983; Barondes, 1981; Lis and Sharon, 1977; Mahadevan et al., 1982) investigators have lavished their efforts in search of a suitable function for lectin. Experiments on lectins as determinants of specificity of plant parasites are not convincing (Agrawal and Mahadevan, 1983; Kojima et al., 1982; Mahadevan et al., 1982).

Distribution of lectins in plants varies. In soybean and peanut plants, lectins were present in root, shoot, leaf and throughout their development (Bowles et al., 1979). In roots and hypocotyls of peanut seedlings, lectins were present up to 4 weeks of development (Pueppke, 1979). During soybean seed germination, lectin level declined (Bhuvaneswari et al., 1977, Pueppke, 1979). Seedlings collected after 2 weeks of germination did not contain any lectin (Pueppke et al., 1978). Lectins were present up to 8 weeks of growth in wheat plants with maximum in adventitious roots and basal portion of shoot (Mishikind et alc, 1980).

We have very little information on the influence of seed microflora on the lectins of germinating seeds. Alternatively, we know nothing about the effect of seed lectins on seed microflora. However, toxicity of lectins to microorganisms is known. PL/tobacco lectin agglutinated a virulent B-1 strain of *Pseudomonas solanacearum* but not the virulent K-60 strain (Sequeira and Graham, 1977). Bradshaw-Rouse et al., (1981) observed the agglutination of avirulent strains of *Erwinia stewartii* by lectins from susceptible corn cultivars. Lectins from resistant bean cultivars agglutinated virulent races 1 and 2 of *Ps. phaseolicola* but not from susceptible cultivar (El-Banoby and Rudolph, 1980). Apple agglutinin from seed, leaf and stem agglutinated both E-8 (avirulent) and E-9 (virulent) strains of *E. amylovora*, although it was more active against avirulent strain (Romeiro et al., 1981). Fett and Sequerira (1980b) found that virulent strains of *Xanthomonas phaseoli* var. *sojensis* were agglutinated by SBL but not avirulent strains of *Ps. glycine* and *Rhizobium japonicum*. Similarly, Young and Kauss (1982) did not find any correlation between the agglutination of *Colletotrichum lindemuthianum*/*Phaseolus vulgaris* lectins isolated from either resistant or susceptible varieties.

WGA inhibited hyphal extension and spore germination of *Botrytis cinerea* (Allen and Neuberger, 1973). It was bound to the hyphae of *Trichoderma viride* and inhibited hyphal growth and spore germination (Mirelman et al., 1975). Barkai-Golan et al. (1978) found that PNA, SBL and WGA inhibited spore germination of *Aspergillus niger*, *A. Ochraceus* and *Penicillium italicum* as well as incorporation of (3H) acetate, D-(1-^{14}C) galactose and N-acetyl-D-(1-^{3}H) glucosamine in to hyphae. The conidia of strains of *Ceratocystis fimbriata* which were agglutinated by SPA, did not germinate (Kojima et al., 1982). Gibson et al. (1982) showed that SBA from resistant soybean seeds inhibited the growth of *Phytophthora infestans sojae*. Lectin treated zoospores developed small germ tubes and high concentrations were inhibitory. Seeds of resistant soybean varieties Pl 200, 5A4, Pickett 71, H.24, Govan and Sohoma contained more lectins than susceptible varieties Shore, Rokusun, Dorchstoy, 2A, P1, 171.

In this paper, we report our results on the presence of lectins in germinating legume seeds, the effect of seed microflora on the lectin content of germinating seeds and effect of lectins on *Colletotrichum capsici* and *Fusarium solani.*

Materials and Methods

Cajanus cajan (var. CO 4), *Cicer arietinum* (var CO 2) and *Dolichos lab-lab* (var. CO 6) seeds were obtained from Tamilnadu Agriculture University, Coimbatore. *Arachis hypogaea* (var. TMV-2, TMV-7, TMV-10 and TMV-12), *Vigna unguiculata* (var. CO 152), *Glycine max* were purchased from local market. *Phaseolus vulgaris* and *Pisum sativum* were the gift from Central Plant Protection Training and Research Institute, Hyderabad.

Extraction of lectins

One lot of seeds was surface sterilized with 0.1% $HgCl_2$ for 1 min, washed thoroughly with sterile water, and germinated in petri dish containing moist filter paper. Another lot of seeds was germinated without surface sterilization. A third lot of seeds was extracted for lectins before germination. Seeds with radicle length of 2 cm were collected and fungal flora developed on seed coat of germinating seeds were enumerated by examining semipermanent mounts. After decoating, the seeds were thoroughly washed, crushed, air dried, and defatted in petroleum ether (b.p. 80–100 °C) in a Soxhlet apparatus. A preliminary survey of the varieties of *A. hypogaea* (TMV-2, TMV-7, TMV-10 and TMV-12) revealed that the ammonium sulphate precipitate of TMV-10 possessed highest hemagglutinating activity against rabbit erythrocytes (Liener, 1955), and therefore variety TMV-10 was used.

A modified method of Takahashi et al., (1967) was followed to extract lectin from 10 g defatted seeds of *C. cajan*, *C. arietinum*, *D. lab-lab*, *Ph. vulgaris*, and *V. unguiculata*. Polyvinyl-polypyrrilidone (Sigma Chemicals Co.), 10 g, was added to the extract to precipitate phenols.

The method of Hupricar and Sohonie (1964) was adopted to isolate *P. sativum* lectins, using 10 g of defatted meal. The lectin was extracted in 60% and 100% ammonium sulphate saturation level.

Lectin from *A. hypogaea* was isolated using 10 g of defatted meal (Pueppke et al., 1979). Phosphate buffer saline, 0.05 M and pH 7.2 + 50 mM ascorbic acid was used to extract the lectins. Ammonium sulphate precipitate was obtained at 50% and 100% saturation levels, dialysed, lyophilized and stored at −10 °C.

Crude extract of SBL was prepared in 0.05 M PBS as described above and was further purified in Sepharose 4B column (15 × 3.5 cm). The column was equilibrated with 0.05 M PBS pH 7.2 at 4 °C; 15 mg of protein was loaded at the top of the column and eluted with the buffer with a flow rate of 1 ml/min. The fractions (30 in each case) were dialysed against 0.005 M PBS. Each fraction was tested for hemagglutination.

Protein was estimated using bovine serum albumin as standard (Lowry et al., 1951), and total sugar (Mahadevan and Sridhar, 1982) using glucose standard.

Hemagglutination test

Preparation of blood samples, trypsin treatment and hemagglutination test were conducted (Liener, 1955). Using serial dilution technique minimum detectable concentration of lectins required for hemagglutination was made.

Agglutination of conidia

Fungi were grown on PDA medium. Conidia were collected and filtered through layers of cheese cloth to remove mycelial fragments. *F. solani* spores were washed thoroughly and used for agglutination test. Conidia of *Col. capsici* frequently occurred in lumps. They were washed in 0.005 M PBS, pH 7.2.

Agglutination test was performed with (1) ungerminated conidia (2) germinated conidia and (3) 1% trypsin treatment on ungerminated conidia at 30 °C for 2 h, washed thoroughly and used. Agglutination was tested by incubating the conidia at 30 °C in 1 ml of lectin solution (1 mg lyophilized powder/ml) in 0.005 M PBS and 1 ml of conidial suspension in water (1×10^6) with occasional stirring up to 18 h.

Effect of agglutination on conidial germination and germ tube growth

Treated conidia were washed thoroughly in sterile distilled water and incubated in 1% Richard's medium. After 6 h incubation, germination and germ tube growth were examined.

Results

The unsterilized seeds of *A. hypogaea*, *C. cajan*, *Cicer arietinum*, *G. max* and *V. unguiculata* harboured less number of fungi than *D. lab-lab*, *Ph. vulgaris* and *P. sativum*. The most common fungi were species of *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*. *D. lab-lab* and *Ph. vulgaris* harboured *Colletotrichum* spp. Radicle length of 2 cm (second day of germination) was considered as fully germinated seed.

Except from soybean, lectins from other seeds were purified with ammonium sulphate and about 95% purification was achieved. Groundnut varieties TMV-2, TMV-7, TMV-10 and TMV-12 were screened for lectin. It was maximum as revealed by hemagglutination activity in TMV-10 variety and least in TMV-2. Therefore, TMV-10 was used for further study.

Hemagglutination by lectins from seeds

Rabbit erythrocytes were most sensitive to the lectins of *A. hypogaea.*, *Cicer arietinum*, *C. cajan*, *D. lab-lab*, *Ph. vulgaris*, *P. sativum* and *V. unguiculata* (Table 1.). *D. lab-lab*, *Ph. vulgaris* and *P. sativum* lectins agglutinated A, O, B types of human erythrocytes. *A. hypogaea* lectins agglutinated only A and B types of human blood cells. *C. cajan* lectins even at 20 mg/ml did not agglutinate untrypsinized rabbit erythrocytes (Table 2). But it agglutinated trypsinized rabbit erythrocytes at 10 μg/ml. Lower concentrations were not effective. Trypsinized and untrypsinized human blood cells were not agglutinated by the lectins from *C. cajan.*

Lectin level declined both in germinated and surface sterilized germinated *A. hypogaea* seeds, as revealed by hemagglutination (Table 1).

Table 1

Hemagglutination of rabbit erythrocytes by lectins

Lectin source		Concn (μg) required for agglutination
A. hypogaea.	S	1×10^{-12}
	G	1×10^{-6}
	SG	1×10^{-6}
C. cajan[a]	S	
	G	1×10
	SG	
C. arietinum	S	1×10^{-4}
	G	1×10^{-2}
	SG	1×10^{-2}
D. lab. lab	S	1×10^{-30}
	G	1×10^{-23}
	SG	1×10^{-26}
Ph. vulgaris	S	1×10^{-26}
	G	1×10^{-10}
	SG	1×10^{-10}
P. sativum	S	1×10^{-16}
	G	1×10^{-14}
	SG	1×10^{-14}
V. unguiculata	S	1×10^{-18}
	G	1×10^{-8}
	SG	1×10^{-14}

S = Seed lectins
G = Germinated seed lectins
SG = Surface sterilized germinated seed lectins.
[a] *C. cajans* lectins did not agglutinate untrypsinized rabbit erythrocytes; but only trypsinized erythrocytes.

Table 2

Hemagglutination of rabbit and human erythrocytes, mitogenesis and lysis caused by lectins

Lectin source		Agglutination				Mitogenesis				Lysis			
		R	A	O	B	R	A	O	B	R	A	O	B
Arachis hypogaea													
	S	++++	++++	−	++++	+	+	−	+	+	+	−	+
	GS	+	−	−	+	−	−	−	−	−	−	−	−
	SG	+	−	−	+	−	−	−	−	−	−	−	−
Cajanus cajan°													
	S	−	−	−	−	−	−	−	−	−	−	−	−
	GS	−	−	−	−	−	−	−	−	−	−	−	−
	SG	−	−	−	−	−	−	−	−	−	−	−	−
Cicer arietinum°°													
	S	++++	−	−	−	−	−	−	−	−	−	−	−
	GS	+++	−	−	−	−	−	−	−	−	−	−	−
	SG	+++	−	−	−	−	−	−	−	−	−	−	−
Dolichos lab-lab													
	S	++++	++++	++++	++++	+	+	+	+	+	+	+	+
	GS	+++	+++	+++	+++	+	+	+	+	+	+	+	+
	SG	+++	+++	+++	+++	+	+	+	+	+	+	+	+
Phaseolus vulgaris													
	S	++++	++++	++++	++++	+	+	+	+	+	+	+	+
	GS	+++	++	++++	++	+	+	+	+	+	+	+	+
	SG	+++	++++	++	++++	+	+	+	+	+	+	+	+
Pisum sativum													
	S	++++	++++	++++	++++	+	+	+	+	+	+	+	+
	GS	+++	++++	++++	++++	−	−	−	−	−	−	−	−
	SG	+++	++++	++++	+++	−	−	−	−	−	−	−	−
Vigna unguiculata													
	S	++++	−	−	−	−	−	−	−	−	−	−	−
	GS	+	−	−	−	−	−	−	−	−	−	−	−
	SG	+++	−	−	−	−	−	−	−	−	−	−	−

S = Seed; GS = Germinated seed; SG = Surface sterilized germinated seeds; − = No agglutination; + = 25% agglutination; R = Rabbit erythrocytes; A, O, B = Human erythrocytes

° *C. Cajan* lectin agglutinated only trypsinized rabbit erythrocytes

°° *C. arietinum* lectin induced blasting of a few blood cells

Lectins from *D. lab-lab*, *Ph. vulgaris* and *P. sativum* induced mitogenesis, cell lysis and nuclear deformities in rabbit and A, O, B types of human leukocytes (Table 2). *A. hypogaea* lectin did not cause any cellular change in 0 type of human blood cells. Similarly, lectins isolated from unsterilized and sterilized germinated seeds of *A. hypogaea* and *P. sativum* did not induce mitogenesis or cell lysis in blood cells.

Separation of soybean lectins

Soybean seed lectins were separated in Sepharose 4B column without ligand. Five major fractions of lectins were separated (Fig. 1). Peaks A, B and C agglutinated erythrocytes, caused lysis and mitogenesis. Peaks D and E induced mitogenesis, caused lysis but did not agglutinate erythrocytes (Fig. 1). Peak B was maximally active.

Lectins from unsterilized germinated soybean seeds were separated into 5 major fractions (Fig. 1). Peaks A and B caused both agglutination and cell-lysis but peaks C, D and E induced mitogenesis on leukocytes. However, erythrocytes appeared turgid. All the fractions agglutinated B type of human erythrocytes but did not induce any lysis or mitogenesis. However, peak E induced agglutination, lysis and mitogenesis on A and O types of blood cells. Lectin concentration declined in germinated seeds.

Lectins from surface sterilized germinating soybean seeds were purified into 6 major fractions (Fig. 1). They differed from the lectins of unsterilized germinated seeds. Peaks A and B caused cell lysis as well as agglutination of rabbit erythrocytes and B type of blood cells. Peaks C and D induced mitogenesis in both rabbit

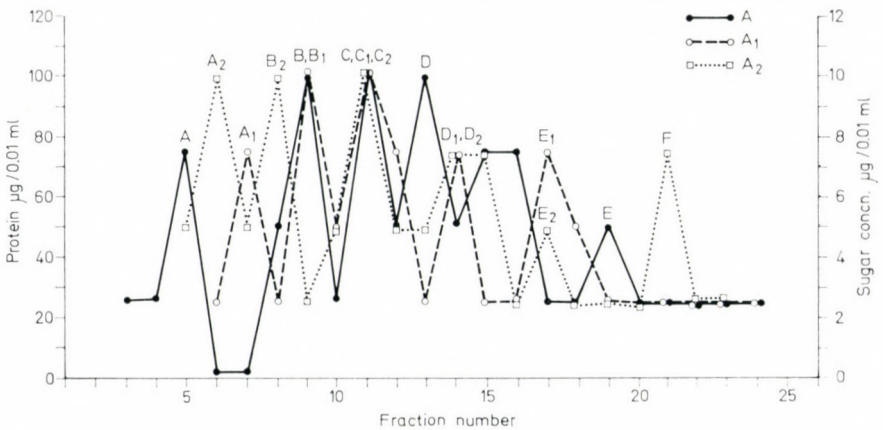

Fig. 1. Fractionation of soybean seed lectins in Sepharose 4B column. A = Unsterilized soybean seeds, Al = unsterilized germinated seeds, A2 = surface sterilized germinated seeds

and B type of leukocytes but fractions E and F induced only blasting and nuclear deformities in rabbit blood cells. A and O types of blood cells were completely unaffected by the lectins.

Agglutination of conidia by seed lectins

Lectins from *D. lab-lab*, *Ph. vulgaris* and *V. unguiculata* agglutinated *Col. capsici* conidia after 6 h of exposure and they did not agglutinate up to 18 h of observation (Table 3). The agglutination was however, 25%. In contrast CPL, PNL, PPL and PSL did not agglutinate the conidia, which germinated after 18 h, the conidia treated with CPL and PSL germinated but those treated with PNL and PPL did not develop further. In DLL and PVL treatment, the unagglutinated conidia germinated by the 18th of observation. No conidia germinated in VUL.

Only PNL agglutinated the conidia of *F. solani* after 6 h of treatment. No conidia germinated till 18 h in the treatment, but in the control, conidia germinated by 6 h. After 18 h, PNL, PPL and PSL caused 75% germination while CPL, PVL

Table 3

Agglutination of *Col. capsici* and *F. solani* conidia

Source of lectin	Conidia						Trypsinized	
	Col. capsici			*F. solani*			*Col.*	
	2	6	18	2	6	18	2	6
Arachis hypogaea (PNL)	–	GE	GI	–	+	GE 75%	–	–
Cajanus cajan (PPL)	–	GE	GI	–	–	GE 75%	GE	GE 25%
Cicer arietinum (CPL)	–	GE	G	–	–	GI	GE 25%	GE 25%
Dolichos lab-lab (DLL)	–	+	GE, +	–	–	BL GE 25%	GE 50%	GE 50%
Phaseolus vulgaris (PHA)	–	+	GE, +	–	–	GI	GE 75%	GE 75%
Pisum sativum (PSL)	–	GE	G	–	–	GE 75%	GE 50%	GE 50%
Vigna unguiculata (VUL)	–	+	++	–	–	GI	GE 25%	GE 25%
Control	–	GE	GE 75%	–	–	GE 75%	GE 75%	GE 75%

– = No agglutination; + = 25% agglutination; BL = Blasted cells; G = Growing germ

and VUL completely inhibited them. Conidia treated in DLL germinated poorly and developed swelling.

Treatment of trypsinized *Col. capsici* conidia with lectins did not result in agglutination till 6th h. By 18 h, CPL, PNL, PPL and VUL agglutinated the germinating conidia. DLL, PVL, and PSL did not agglutinate the conidia and continued to develop. The quantity of conidia that germinated in presence of lectin was less and the length of germ tube was always smaller than in the control.

Trypsinized conidia of *F. solani* were not agglutinated by the lectins till 6th h. At 18 h, only PNL caused 25% agglutination of the conidia. In presence of these lectins, the conidia germinated. At 6th h, DLL inhibited germ-tube growth. PHA caused blasting of germinating conidia. However, development of the conidia continued in CPL, DLL, PPL, PSL and VUL.

Germinated conidia of *Col. capsici* were agglutinated by CPL at 6th h which did not develop further. PNL caused inhibition of germ tube elongation. DLL, PHA, PPL, PSL and VUL were not effective. Only PNL agglutinated the germinated conidia by 18th h, but developed unabatedly in other lectins.

by seed lectins after incubation for 2, 6 and 18 h

conidia	Germinated conidia								
capsici	*F. solani*			*Col. capsici*			*F. solani*		
18	2	6	18	2	6	18	2	6	18
+	BL	BL	+, BL	−	−	++	++++	++++	++++
++	GE	GE	GE 75%	−	G	G	G	G	GI
++	GE	GE	GE 50%	−	++	++++	++++	++++	++++
GE 75%	GE	GI	GI	−	G	G	++++	++++	++++
GE 75%	GE	BL	BL	−	G	G	++++	++++	++++
GE 50%	GE	GE	GE 75%	−	G	G	G	G	GI 50%
++	GE	GE	GI	−	G	G	G	G	G
GE 90%	GE	GE	GE 75%	−	G	G	G	G	G

tube; GE = Germ tube emergence; GI = Growth inhibition

Table 4

Agglutination of *Col. capsici* and *F. solani* conidia by germinated

Source of lectin	Conidia						Trypsinized	
	Col. capsici			*F. solani*			*Col.*	
	2	6	18	2	6	18	2	6
Arachis hypogaea (PNL)	−	++	+++	−	−	+++	−	+
Cajanus cajan (PPL)	−	++	++	−	+	++	−	++
Cicer arietinum (CPL)	−	+, GE	G	−	+ GE	G, +	−	+, GE
Dolichos lab-lab (DLL)	−	GE	G	−	++	++	−	GE
Phaseolus vulgaris (PHA)	−	GE	G	−	GE	++	−	++ GE
Pisum sativum (PSL)	−	GE	G	−	GE, +	++	−	+
Vigna unguiculata (VUL)	−	−	GE 25%	−	−	++	−	−
Control	−	GE	100% GE	−	GE	90% G	−	50% GE

− = No agglutination; + = 25% agglutination; BL = Blasted cells; G = Growth;

CPL, DLL, PNL and PVL agglutinated germinated conidia of *F. solani* within 2 h of treatment and did not germinate even by 18th h. In PPL, PSL, and VUL, the germinated conidia developed till 6th h only.

Agglutination of conidia by germinated seed lectins

Germinated seeds which were not surface sterilized harboured abundant microbial growth. Lectins were extracted from the seeds after carefully removing the microorganisms and assayed.

PNL and PPL agglutinated *Col. capsici* conidia which did not germinate even up to 18 h (Table 4). CPL caused 25% agglutination by 6th h but the unagglutinated conidia germinated. DLL, PVL and PSL did not agglutinate the conidia but caused germination. VUL had no effect on the conidia till 6th h of observation; only on 18th h, did they germinate. The germinated conidia suspended in CPL, DLL, PHA and PSL continued to grow up to 18th h of observation.

DLL, PNL, PPL and VUL agglutinated *F. solani* conidia, which did not germinate. CPL and PSL caused 25% agglutination but a few conidia germinated.

seed lectins after incubation for 2, 6 and 18 hours

conidia			Germinated conidia						
capsici			*F. solani*			*Col. capsici*			*F. solani*
18	2	6	18	2	6	18	2	6	18
++, GE	–	–	++++	–	GI	GI	–	++	++, BL
++++	–	–	BL	–	++	GI	–	++	++
+, GE 75%	–	–	BL	–	GI	GI	–	–	BL, G
GE 75%	–	–	+++ GE	–	G	G	–	G	G
++, GE 25%	–	–	+++	–	G	G	–	+	GI
++	–	–	+++	–	G	GI	–	G	G
GE	–	–	+++ GE	–	++	++	–	+	GI
100% GE	–	50% GE	100% GE	G	G	G	G	G	G

GE = Germ tube emergence; GI = Growth inhibition

By 18th h, the germinated conidia suspended in PSL were agglutinated, but with CPL, germinated conidia continued to develop.

Trypsinized *Col. capsici* conidia were agglutinated by CPL, PVL, PNL, PPL and PSL; agglutination was 25% with CPL and PSL but it was 50% with PPL and PVL. DLL and VUL did not cause any agglutination. With PPL and PSL, the agglutinated conidia did not germinate. But the unagglutinated conidia of *Col. capsici* suspended in CPL, PHA and PNL germinated. Germination of conidia incubated in PVL and VUL was poor.

Lectins had no effect till 6th h of incubation on trypsinized *F. solani* conidia. By 18th h, PVL, PNL and PSL agglutinated the trypsinized *F. solani* conidia. DLL and VUL agglutinated the conidia but a few germinated. CPL and PPL induced only blasting of conidia.

Germinated conidia of *Col. capsici* were agglutinated by CCL and VUL by 6th h. CPL and PNL inhibited germ tube elongation. DLL and PVL had little effect on the growth of germinated conidia, however, the germ tube length was reduced. PSL inhibited the germ tube growth only after 6 h of incubation.

Germinated *F. solani* conidia were agglutinated by PNL, PPL, PVL and VUL at 6th of incubation which did not develop further. But in CPL treatment germ

Table 5

Agglutination of *Col. capsici* and *F. solani* conidia by surface

Source of lectin	Conidia						Trypsinized	
	Col. capsici			*F. solani*			*Col.*	
	2	6	18	2	6	18	2	6
Arachis hypogaea (PNL)	−	−	++++	−	−	++	−	+
Cajanus cajan (PPL)	−	−	+++	−	−	++++	−	+
Cicer arietinum (CPL)	−	++	++++	−	−	+++	−	++, GE
Dolichos lab-lab (DLL)	−	+++	+++	−	GE	++	−	++++
Phaseolus vulgaris (PHA)	−	+++	+++	−	+++	+++ GE	−	+++
Pisum sativum (PSL)	−	−	++ GE	−	GE	GE 75%	−	++
Vigna unguiculata (VUL)	−	++	++ GE	−	GE	++ GE	−	−
Control	−	GE	GE 100%	−	GE	GE 100%	−	GE

− = No agglutination; + = 25% agglutination; G = Growth; GI = Growth

tube elongation continued and a few conidia were blasted. Conidia treated in DLL and PSL, initiated germ tube.

Agglutination of conidia by lectins from surface sterilized germinated seeds

CPL, DLL, PVL, PNL and PPL agglutinated *Col. capsici* conidia by 6th h which did not germinate (Table 5). PSL and VUL caused 50% agglutination of the conidia by 6th h. The unagglutinated conidia slowly germinated and required 18 h for completion.

CPL, PNL and PPL agglutinated the conidia of *F. solani* which did not germinate. DLL, PSL and VUL did not agglutinate the conidia at 6th h but induced germination. By 18th h, the germinated conidia in DLL and VUL agglutinated. Although, PVL agglutinated most of the conidia, a few germinated by 18th h. In the PSL treated conidia, per cent germination and germ-tube length were less than the control.

Trypsinized *Col. capsici* conidia were agglutinated by CPL, DLL, PVL, PNL, PPL and PSL and did not germinate even by 18th h. VUL only inhibited germination.

sterilized germinated seed lectins for 2, 6 and 18 hours

conidia	Germinating conidia								
capsici	*F. solani*			*Col. capsici*			*F. solani*		
18	2	6	18	2	6	18	2	6	18
++	−	+	++	−	++	++, G	−	++	+++, G
++	−	GE	++	−	+	GI	−	+	++
++	−	GE	+++	−	++	GI	−	++	++
++++	−	−	++, GE, 50%	−	G	G, 50%	−	+	+++
+++	−	++, GE	+++	−	G	G, 50%	−	+	++
+++	−	+++	++++	−	+++	GI	−	++	+++
−	−	+++	++++	−	+++	GI	−	−	++++
GE 100%	−	GE	G	G	G	G	G	G	G

inhibition; GE = Germ tube emergence

Trypsinized *F. solani* conidia were agglutinated by PNL, PSL and VUL and failed to germinate. PVL agglutinated trypsinized *F. solani* conidia at 6th h but a few conidia germinated. However the germinated conidia were agglutinated by 18th h. CPL and PPL did not agglutinate the conidia by 6th h but induced germination. However, by 18th h, these lectins agglutinated the germinated conidia.

CPL, PPL, PSL and VUL agglutinated the germinated conidia of *Col. capsici* and inhibited germ tube elongation. PNL caused 50% agglutination at 6th h of incubation but by 18th h a few conidia developed. DLL and PVL did not cause agglutination but inhibited germ tube growth by 50%.

Germinated *F. solani* conidia were agglutinated by the lectins at 6th h, and no conidia developed by 18th h. Only in PNL treatment, did a few conidia initiate germ tubes.

Lectins on growth of agglutinated Col. capsici and F. solani conidia

Untrypsinized *Col. capsici* conidia rapidly germinated after removal of DLL, PVL, PSL and VUL, from seeds. Germination of trypsinized *Col. capsici* conidia treated in DLL, PVL and PSL, from seeds increased after their removal.

DLL, PNL, PSL and VUL from germinating, surface sterilized seeds allowed the germination of untrypsinized unagglutinated *Col. capsici* conidia. The trypsinized *Col. capsici* conidia germinated after the removal of CPL, PNA and VUL isolated from the surface sterilized germinated seeds.

Removal of PSL from incubation medium containing *F. solani* conidia caused germination and germ-tube elongation. In contrast, removal of CPL, DLL, PVL, PNL, PPL and VUL did not restore germination, indicating permanent damage to the conidia.

Discussion

Peanut varieties differ in lectin content, which confirms the report of Pueppke (1979). Bowles et al. (1979) and Mishikind et al. (1980) claimed that after seed germination, most of the lectins are located in plasma membrane and hypocotyl is the major site for lectins. As we did not separate the lectins from cotyledons and radicle, we are not certain which part of the seedling contained maximum amount of lectins.

We noted that during seed germination, lectin concentrations declined. Bhuvaneswari et al. (1976), found similar changes in soybean. In contrast, levels of total protein and total sugars did not show any correlation with lectin changes.

Lectins induce mitogenesis in blood cells (Hupricar and Sohonie, 1964). Besides mitogenesis, we observed cell lysis and nuclear deformities in the treated blood cells. But PNL and PSL from germinating seeds lacked mitogenic effect which might be due to isolectins in the lectin preparation, as suggested by Newman (1977).

Fungi differ in their sensitivity to lectins. Alternatively, lectins differ in their capacity to bind with fungal conidia. Furthermore, lectins caused deformities in conidia, especially blasting in *F. solani*. Lectins bind with fungal cell-wall or hyphal tips, as noted by Barkai-Golan et al. (1978) with *Aspergillus ochraceus* and might inhibit chitin synthesis (Mirelman et al., 1975).

Untrypsinized, trypsinized and germinated conidia differed in their binding capacity with lectins. Trypsinized and germinated conidia bound easily with lectins compared with untrypsinized conidia. Klienschuster and Baker (1974) observed that untrypsinized *F. solani phaseoli* did not bind with RCA and WGA but Con A caused strong agglutination. They suggested that exposure of conidia of *F. solani* to trypsin resulted in the unmasking of a large number of WGA receptors but released moderate amount of RCA and Con A receptors. These results show that carbohydrate core for lectin binding is available at different layers of fungal cell wall and lectin binding depends on the availability of carbohydrate core. These findings also explain the growth of *Col. capsici* conidia after the removal of DLL and PVL. The weak binding and poor agglutination of *Col. capsici* conidia with DLL and PVL might have been due to less availability of carbohydrate core in the fungal cell wall. During washing, conidia might have separated themselves from lectins and restored germination Unfortunately, structure of cell wall of the conidia of *Colletotrichum* and DLL and PVL happens are not known.

Except PSL, other lectins agglutinated *F. solani* conidia which did not germinate even after their removal. Presumably nonpathogenicity of *F. solani* to legumes other than *P. sativum* might be due to its agglutination by lectins, as implicated by Kojima et al. (1982) in strains of *Ceratocystis fimbriata* with sweet potato agglutinin. Avirulent strains of *C. fimbriata* conidia agglutinated strongly with SPA but virulent strains agglutinated very weakly. But Kawakita and Kojima (1983) could not correlate the agglutination of *C. fimbriata* strains by *Colocasia esculenta* agglutinin and pathogenicity. Similarly, PL lysed the zoospore of 5 races of *Phytophthora infestans* and the ability to lyse was not related to the resistance of cultivars (Garas and Kuc, 1981).

In the field, *D. lab-lab* and *Ph. vulgaris* are susceptible to *Colletotrichum* spp. The weak binding and transient inhibitory effect of the lectins on *Col. capsici* suggested that lectins do not limit its pathogenicity. We do not know whether *Colletotrichum* spp. utilize lectins as energy source. Nor do we know, whether proteolytic enzymes of the fungi cleave the lectins. Protein inhibitors could also play an important role in pathogenicity by influencing the production and activity of proteolytic enzyme, which may destroy lectin. Peng and Black (1976) observed that protein inhibitors decreased in tomato plants infected by compatible races of *Phy. infestans*.

From our data we suggest the following:

(a) Sensitive spores are agglutinated after coming in contact with lectins, immobilized and would not germinate.

(b) Virulent spores would germinate in presence of lectins, however growth is restored. But in susceptible variety, if the concentration is not adequate, growth of the parasite is not suppressed.

(c) Lectins might get transported from one tissue to another or utilized by parasites. It might be inactivated by proteolytic enzymes (Fett and Sequeira, 1989a) or may remain in inactive form (Gibson and Etzler, 1979). We found that conidial germination and enhanced growth of germ tubes occurred after the removal of lectins.

Whether lectins are present at the infection site is not known. Assuming that lectins accumulate at the infection site, these may agglutinate the infecting parasite if the binding site of the parasite is exposed. There could be three reasons for the exposure of binding sites of fungal cell wall:

1. change in pH which alters the characteristics of outer cell-wall layer,
2. proteolytic enzymes produced by pathogen itself acting on cell-wall,
3. after spore germination, carbohydrate core present in germ tube may provide binding site to lectins.

Our results indicate that lectins may not be the primary determinant of specificity but they might play secondary role. For, the essential factor for the action of lectins will be available only after pathogen gets established on the host.

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Phytoalexin Production in Rice-*Pyricularia oryzae* Interaction: Induction by Wounding

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Phytoalexin production in compatible and incompatible rice-*Pyricularia oryzae* relationships was examined using uninjured and injured detached leaves of susceptible (Karuna) and resistant (IR 8) cultivars by drop-diffusate technique. Extracts of water drops and infection drops from uninjured leaf blades of either Karuna or IR 8 incubated up to 72 h were not toxic to *P. oryzae* spore germination. However, extracts of both water and infection drops from uninjured leaf blades of IR 8 were feckly toxic to the germ tube growth of *P. oryzae*.

Injuring the leaf blades of IR 8 resulted in tissue necrosis and browning and it triggered the accumulation of fungitoxic substance(s) in the water drops; the toxicity to *P. oryzae* spores increased from 7 to 100% during 24 and 72 h of incubation, respectively. Pathogen intensified the tissue browning and markedly augmented the accumulation of fungitoxic substance(s) in the infection drops from the injured leaf blades of resistant cultivar even during 48 h of incubation. The response of the susceptible cultivar to injury in accumulating antifungal substance(s) in the water drops was insignificant. Nevertheless, extracts of infection drops collected from injured leaf blades of Karuna were slightly more toxic to *P. oryzae* spores when compared to the extracts of water drops, despite browning of the injured tissues. We propose to consider the injury-induced post-infectionally accumulating antifungal substance(s) as "wound-mediated phytoalexin(s)" and question its participation in resistance to initial establishment of *P. oryzae*.

Phytoalexins (post-infectionally formed antimicrobial substances) form a vital component of plants' arsenal to protect themselves against parasites (Kuc, 1976; Mahadevan, 1979a,b; Cruickshank, 1980). Production of phytoalexins in rice-*Pyricularia oryzae* system has been established (Uehara, 1958, 1962; Ohata and Kozaka, 1967). Langcake and associates (Cartwright et al., 1977; Langcake et al., 1978; Cartwright and Langcake, 1980) claimed its isolation and characterization. These workers used wound-inoculated tissues. However, in nature the parasite does not require injury to enter the tissue and penetration in most cases is accomplished through bulliform cells (Ito and Shimada, 1937; Yoshii, 1936; Hashioka, 1950) which are highly vacuolated and contain water (Esau, 1965). The critical difference between the models used by early workers (Uehara, 1958, 1962; Ohata and Kozaka, 1967; Cartwright et al., 1977; Langcake et al., 1978; Cartwright and Langcake, 1980) to detect phytoalexins and the natural infection process of the blast fungus prompted us to reexamine the phytoalexin production.

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We examined the accumulation of antifungal substance(s) in water and infection drops in compatible and incompatible host-parasite combinations as influenced by injury.

Materials and Methods

Phytoalexin production. Rice (*Oryza sativa* L.) cultivars Karuna susceptible and IR 8 resistant to blast were grown in 20-cm diameter earthen pots. Production of phytoalexins was assessed by drop-diffusate technique (Cruickshank, 1980). Fully expanded top two leaf blades of 28-day-old seedlings (4 leaf stage) were harvested and washed gently in tap water to remove the adhering dust. The apical 10-cm segments of these leaf blades were placed with their adaxial side up in groups of 10 in 18-cm Petri dishes lined with moist filter paper. Leaf blade segments in one set were wounded at various points approximately one cm apart by gently pressing their adaxial surface with the cut end of a 2-mm diameter glass rod. The other set was kept intact. These were again divided into two sets and 25 μl of either distilled water drops or infection drops (8 to 10 drops/segment) containing *P. oryzae* (isolate P 258, compatible to cv. Karuna and incompatible to cv. IR 8) spores (ca. 3×10^4 spores ml^{-1}, obtained from 10-day-old cultures maintained on oat meal agar slopes) were placed on the leaf blade segments using hypodermic syringe. The Petri dishes were incubated on laboratory benches for 72 h at 28 $\pm$ 2 °C and at 12 h alternating light (Philips cool-daylight fluorescent tubes TL 40 W/54, intensity 8.4 W. m^{-2}) and dark.

Phytoalexin extraction. At the end of 24, 48 and 72 h of incubation, the drops were collected by a syringe and centrifuged at 3000 g for 30 min. Five ml portions of the diffusates were extracted thrice with equal volumes of distilled ethyl acetate. The solvent fractions were pooled and evaporated to dryness at room temperature (28 $\pm$ 2 °C). The residue was dissolved in one ml of ethyl acetate (Mahadevan and Sridhar, 1982).

Bioassay of phytoalexin. The antifungal activity of the extracts were bio-assayed by slide germination method (Horsfall, 1956) using *P. oryzae* spores. One hundred μl of the extracts was placed in three replicate cavity slide wells and allowed to dry at room temperature. Into each well were pipetted with 50 μl of spore suspension (ca. 3×10^4 spores ml^{-1}) in water. After incubation of the slides at 28 $\pm$ 2 °C for 6 h in moist Petri dishes, a drop of lactophenol cotton blue was added to each well and the number of germinated spores were counted at random from 10 microscopic fields. Suitable controls were kept and the test was done in triplicate.

Results and Discussion

Langcake and his associates (Cartwright et al., 1977; Langcake et al., 1978; Cartwright and Langcake, 1980) demonstrated that treatment of susceptible rice (cv. Sasahigure) with a blast-specific systemic fungicide (2,2,-dichloro-3,3-dimethyl-

Table 1

Influence of injury on the toxicity of extracts from water and infection drops collected from leaf blades of susceptible (Karuna) and resistant (IR 8) cultivars to *P. oryzae* spore germination (Data are per cent inhibition over control based on the observation of 300 spores)

Extract	Treatment	Cultivar					
		Susceptible			Resistant		
		Incubation period (h)					
		24	48	72	24	48	72
Water drops	Uninjured	2.33	3.05	3.86	5.21	4.57	2.60
Water drops	Injured	1.85	4.47	10.60	7.13	4.80	100**
Infection drops	Uninjured	3.21	4.34	4.23	4.60	3.81	4.55
Infection drops	Injured	3.42	5.84	14.09	5.04	93.06**	99.24**

** Significant at P = 0.01

cyclopropane carboxylic acid) induced the resistance of plants to *P. oryzae* by accumulating diterpene phytoalexins momilactone A and B at the wound-inoculated sites in the leaves. Ultraviolet irradiation of leaves and dark-grown coleoptiles of rice also induces the accumulation of phytoalexins (Cartwright et al., 1977). The untreated wound-inoculated tissues accumulate only traces of the less toxic momilactone A. However, neither compounds occur in the uninfected leaves even after treating with the fungicide (Cartwright and Langcake, 1980).

We found that extracts of water drops collected from uninjured leaf blades of neither susceptible nor resistant cultivar were inhibitory to the spores of *P. oryzae* (Table 1). However, the extracts of water drops collected from the uninjured leaf blades of resistant cultivar were weakly inhibitory to germ tube growth of *P. oryzae* (Table 2). In contrast, extract of water drops collected from injured tissues of resistant leaves 72 h after incubation was highly toxic to both spores and germ tube growth, while those from the susceptible leaf blades exhibited markedly low level of inhibition. Cartwright et al. (1977) and Cartwright and Langcake (1980) did not detect phytoalexins in the uninfected leaves of either fungicide treated or untreated plants. Presumably, these workers analyzed the uninjured tissues as their control.

Extracts of infection drops collected from uninjured susceptible and resistant leaf blades were not significantly inhibitory to either spores (Table 1) or germ tube growth of *P. oryzae* (Table 2). Despite the fact that the extracts of infection drops from uninjured leaf blades of resistant cultivar were less toxic to germ tube growth than those of water drops from similar leaf blades up to 48 h of incubation, the toxicity of the former extract (24%) was greater than that of the latter (15%) at 72 h after incubation. Nevertheless, the level of toxicity was low. Perhaps, it may be possible to exaggerate the *in vitro* toxicity of the extract of diffusate collected from uninjured resistant leaf blades by increasing the volume of diffusate

Table 2

Influence of injury on the toxicity of extracts from water and infection drops collected from leaf blades of susceptible (Karuna) and resistant (IR 8) to germ tube growth of *P. oryzae* (Data are per cent stimulation (+) or inhibition (−) over control based on the observation of 50 sporelings)

Extracts	Treatment	Cultivar					
		Susceptible			Resistant		
		Incubation period (h)					
		24	48	72	24	48	72
Water drops	Uninjured	−4.53	−1.91	−9.76	−34.03	−36.30	−15.36
Water drops	Injured	−3.13	+0.26	−13.46	−31.47	−7.84	N. D.
Infection drops	Uninjured	−8.89	+2.44	−8.36	−11.33	−8.71	−24.42
Infection drops	Injured	−8.89	−5.05	−19.16	−18.32	−65.84**	−71.56**

** Significant at P = 0.01
N. D. = No determination (Due to total inhibition of spore germination, assessment of germ tube growth does not arise)

and by manipulating the concentration used for bioassay. If that be done, its participation in host resistance would remain in doubt.

Infection initially results in injury at submicroscopic level. The inability of uninjured leaf blades especially that of resistant cultivar to accumulate antifungal substance(s) shows that injury caused by the parasite was not sufficient enough to trigger the accumulation of antifungal substance(s) compared to that of physically injured tissues. We, however, noticed that pathogen in the injured leaf blade tissue of susceptible cultivar meagrely enhanced its weak potentiality of accumulating antifungal substance(s). But, the extracts of infection drops collected from the injured resistant leaves 48 h and 72 h after incubation were highly toxic to both spore germination and germ tube growth of *P. oryzae*. We made no attempt to ascertain the chemical nature of the substance(s) involved at this stage.

The physically injured resistant leaf blades turned light brown by 48 h due to cellular necrosis and the intensity of browning increased by 72 h (Table 3). Browning of the resistant tissues was accelerated by the parasite. Only faint browning of the injured tissues of susceptible cultivar occurred after 72 h. Although pathogen intensified the browning of injured susceptible leaves by 72 h, the extracts of infection drops collected from these tissues lacked substantial amount of antifungal activity. According to Deverall (1976) and Mahadevan (1979b) hypersensitively reacting tissues synthesise high amounts of phytoalexins. Phytoalexin accumulation accompanies cellular death during host-parasite interactions (Bailey and Deverall, 1971; Rahe, 1973; Mansfield and Deverall, 1974). Cellular death caused by physical injury of *Vicia faba* leaves induces phytoalexin (Deverall and Vessey, 1969). We noticed that accumulation of antifungal substance(s) due to injury was

Table 3

Tissue browning in response to injury alone and as influenced by pathogen in blast susceptible (Karuna) and resistant (IR 8) cultivars (+ = visible browning, — = no visible browning)

Presence or absence of pathogen	Treatment	Cultivar					
		Susceptible			Resistant		
		Incubation period (h)					
		24	48	72	24	48	72
No pathogen	Uninjured	—	—	—	—	—	—
No pathogen	Injured	—	—	—	—	+ ?	++
Pathogen	Uninjured	—	—	—	—	—	—
Pathogen	Injured	—	+ ?	++	—	++	+++

restricted only to the resistant cultivar. The absence of antifungal activity in infection drops collected 72 h after incubation from injured susceptible tissues despite browning of tissues indicates that browning of the tissues in this case was not associated with the accumulation of antifungal substance(s).

Phytoalexin accumulation in apparently healthy cells surrounding the lesions caused by *Botrytis fabae* or *B. cinera* (Deverall and Vessey, 1969) and by *Colletotrichum lindemuthianum* in french bean leaves (Theodorou et al., 1982) suggests that phytoalexins are produced by apparently healthy cells and by cells undergoing necrosis in advance of the fungi (Deverall and Vessey, 1969). However, it appears that the phytoalexin accumulation in healthy tissues of rice leaves adjacent to the necrotic infected wounds (Cartwright and Langcake, 1980) seems to be due to diffusion of certain chemical stimuli originating from the wounded sites whose synthesis or release might be accelerated by the pathogen, for our results show that injury alone elicited marked accumulation of antifungal substance(s) and the parasite further hastened and augmented their production in incompatible host.

We draw three major conclusions from this study. Firstly, injury alone induces marked accumulation of antifungal substance(s) in resistant leaf blades. Secondly, presence of parasite in the physically injured leaf tissues of resistant cultivar further accelerated the accumulation of antifungal substance(s), but not in the uninjured leaves. Thirdly, the response of susceptible leaves either to injury alone or to the combined effect of injury plus pathogen was of less significance. Therefore, we propose that the antifungal substance(s) detected by us may be considered 'wound-mediated phytoalexins'.

In the absence of injury, the interaction between host and parasite might be delayed especially, in rice since the pathogen or its metabolites diffusing out of infective spores have to initially pass through the highly vacuolated, water-containing bulliform cells. This raises a question whether the phytoalexin reported with wound-inoculated models (which would facilitate an easy and immediate contact

between the synthesizing host cells and the pathogen to interact with each other) in rice has a definite role in resistance, especially during the initial stages of pathogenesis. Furthermore, the extract of infection drops collected from uninjured leaves of resistant cultivar even 72 h after incubation showed only traces of antifungal activity. In other words, these 'wound-mediated phytoalexins' do not influence the resistance of tissues to initial establishment of the pathogen, but may be of importance in restricting its spread in tissues.

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Do Germinating Paddy Seeds Produce Phytoalexins?

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Production of phytoalexins by germinating paddy seeds exposed to native microflora was examined. Although the extract from ungerminated seeds stimulated the germination of spores of *Curvularia lunata* at 10 and 50 μl and that of *Drechslera oryzae* at 5 μl, it was toxic to germ tube growth of spores at 5 to 50 μl and 10 and 50 μl, respectively. Spores of *Pyricularia oryzae* were highly sensitive to the extract. Toxicity of the extract from germinated seeds which were exposed to native seed microflora during germination was markedly greater than that from the ungerminated seeds. Paper chromatograms of both ungerminated and germinated seed extracts bioassayed with *C. lunata* revealed the presence of a single toxic principle. Dialysis removed the toxicity of extracts. Germinating paddy seeds challenged by native microflora did not produce any phytoalexins.

Seeds harbour a variety of microorganisms, both pathogenic and nonpathogenic (Mahadevan, 1975; Neergaard, 1977). During seed germination, the spermosphere microflora change and the germinating seeds are also exposed to a plethora of microorganisms (Pollock, 1972). While the seed expels most of the microorganisms through its chemical defenses, seed borne pathogenic microorganisms, if present, seem to tolerate the toxicants and colonise them under favourable conditions. Both prohibitins (preformed antimicrobial substances, Mahadevan, 1982) and phytoalexins (post-infectionally synthesized antimicrobial substances, Mahadevan, 1979) effectively influence the development of parasites.

Germinating paddy seeds challenged with native microflora (incompatible host-parasite interaction) do not produce phytoalexins (Keen, 1975). In contrast, Zuber and Manibhushanrao (1979) reported its production by rice seeds inoculated with *Rhizoctonia solani* (compatible host-parasite interaction). We re-examined the production of phytoalexins by germinating paddy seeds exposed to natural microflora and the results are presented in this paper.

Materials and Methods

Seed material. Paddy seeds (200 g) of cv. Ratna free from detectable infection were soaked in tap water for 16 h, spread in enamel trays lined with moist filter

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paper, covered with a layer of moist filter paper and incubated at 28 ± 2 °C for 24 h. During incubation, the germinating seeds were exposed to the native microflora. An identical lot of water soaked unincubated seeds (ungerminated and unchallenged by native microflora) was maintained as control.

Extraction. The seeds were homogenized in a blendor with ca. 500 ml of distilled and chilled ethanol for about 5 min. The homogenate was held at cold (4 – 5 °C) over night, filtered through Whatman No. 41 filter paper, concentrated in a rotary vacuum evaporator at 50 °C and partitioned thrice with equal volumes of distilled ethyl acetate. Solvent fractions were pooled and evaporated at 30–35 °C to 3 ml (Mahadevan and Sridhar, 1982).

Bioassay. The fungitoxicity of the extract was assessed by the slide germination method (Horsfall, 1956) using *Curvularia lunata*, *Drechslera oryzae* (syn. *Helminthosporium oryzae*) and *Pyricularia oryzae*. Five, 10, and 50 µl of the extract were placed in cavity slides and the solvent was evaporated. Spore suspension, 100 µl of either *C. lunata* (ca. 5000 spores), or *D. oryzae* (ca. 2000 spores) or *P. oryzae* (ca. 4000 spores) was placed in the cavities incubated at 28 ± 2 °C in Petri dishes lined with moist filter paper for 3.5, 4, and 5 h, respectively. The spores were killed by the addition of a drop of lactophenol cotton blue stain. Ten microscopic fields were observed at random for spore germination and germ tube growth of the sporelings.

The crude extracts (400 µl) were chromatographed on paper using chloroform-ethanol (95 : 5, v/v) as developing solvent. The dried chromatograms were bioassayed for antifungal compounds as described by Mahadevan and Sridhar (1983) using *C. lunata* as the test organism.

Dialysability of the toxicants. To assess the relative molecular size of the inhibitor, a known aliquot of the extract diluted with excess distilled water was dialysed for 24 h. The dialysate was reextracted with ethyl acetate and 50 µl of the extract (representing the original proportion of 200 g of seeds in 3 ml of ethyl acetate) was bioassayed using *P. oryzae* spores.

Results and Discussion

A condition similar to incompatible host-parasite interaction was simulated during germination of seeds that were exposed to their native microflora. This would encourage the germinating seeds to synthesize and accumulate phytoalexins (Keen, 1975; Gnanamanickam, 1979). Paddy seeds contain a variety of phenolic prohibitins (Jayachandran-Nair and Sridhar, 1975) with a wide spectrum of antimicrobial properties and two 9-β-pimaradiene diterpenes, momilactones A and B which are toxic to *P. oryzae* (Cartwright et al., 1977; Watanabe et al., 1979; Cartwright and Langcake, 1980). These might protect the seeds against seed borne and soil microflora during germination.

The extracts from ungerminated seeds stimulated the spore germination of *C. lunata* and *D. oryzae* at 10 and 50 µl and 5 µl, respectively (Table 1). The ability

of *C. lunata*, a seed parasite and *D. oryzae*, a seed transmitted parasite to infect the glumes (Ou, 1972; Rangaswami, 1972) and under favourable conditions to mould and colonize the seeds (Ou, 1972) is perhaps related to the stimulatory effect of the extract. Nevertheless *P. oryzae* spores were highly sensitive to the extract. It is of interest to mention that *P. oryzae* is not usually transmitted through seeds (Ou, 1972; Rangaswami, 1972). The extract from ungerminated seeds was toxic to germ tube growth of *C. lunata* and *D. oryzae* (Table 2). While its toxicity to *D. oryzae* increased with increase in concentration from 10 to 50 μl, the inhibitory effect on germ tube growth of *C. lunata* decreased with increase in concentration. However, this extract stimulated the germ tube growth of *D. oryzae* at 5 μl. The extract obtained from the germinated seeds exposed to microorganisms at 10 and 50 μl caused total inhibition of the germination of *C. lunata* and *D. oryzae* spores (Table 1). *C. lunata* bioassay on paper chromatograms revealed the presence of a single inhibitory zone occurring at R_f 0.9 with extracts from both ungerminated and germinated seeds (Fig. 1). The extracts completely lost their toxic effect upon dialysis indicating low molecular weight nature of the inhibitory principle.

Zuber and Manibhushanrao (1979) failed to detect any antimicrobial activity in the extract from germinated seeds exposed to native microflora. But, germinated seeds exposed to native microflora. But, germinated seeds challenged with *R. solani* produced phytoalexins. This is surprising since *R. solani* heavily parasitises the germinating seeds, yet the seeds produced extractable phytoalexins. Our results show the presence of a dialysable antimicrobial substance in seeds both challenged and unchallenged by native microorganisms. We did not detect any antimicrobial

Table 1

Toxicity of paddy seed extracts to spore germination of three pathogens (Data are percent spore germination on the basis of 200 spores; figures in parentheses represent percent stimulation (+) or inhibition (−) over water control)

Treatment	Test organism	Control	Concn of extract (μl)		
			5	10	50
Ungerminated seeds	*C. lunata*	59	43 (−27)	67 (−14)	96 (+63)
	D. oryzae	79	88 (+11)	25 (−68)	10 (−87)
	P. oryzae	91	0 (−100)	0 (−100)	0 (−100)
Germinated seeds	*C. lunata*	59	15 (−75)	0 (−100)	0 (−100)
	D. oryzae	79	78 (−1)	0 (−100)	0 (−100)
	P. oryzae	91	0 (−100)	0 (−100)	0 (−100)

Table 2

Toxicity of paddy seed extracts to germ tube growth of three pathogens (Data are mean germ tube length in μm of 40 sporelings; figures in parentheses represent percent stimulation (+) or inhibition (−) over water control)

Treatment	Test organism	Control	Concn of extract (μl)		
			5	10	50
Ungerminated seeds	*C. lunata*	33	12 (−64)	15 (−55)	24 (−27)
	D. oryzae	135	165 (+22)	75 (−44)	42 (−69)
	P. oryzae	39	n. d.	n. d.	n. d.
Germinated seeds	*C. lunata*	33	18 (−45)	n. d.	n. d.
	D. oryzae	135	138 (+2)	n. d.	n. d.
	P. oryzae	39	n. d.	n. d.	n. d.

n. d. = No determination (Due to total inhibition of spore germination, assessment of germ tube growth does not arise)

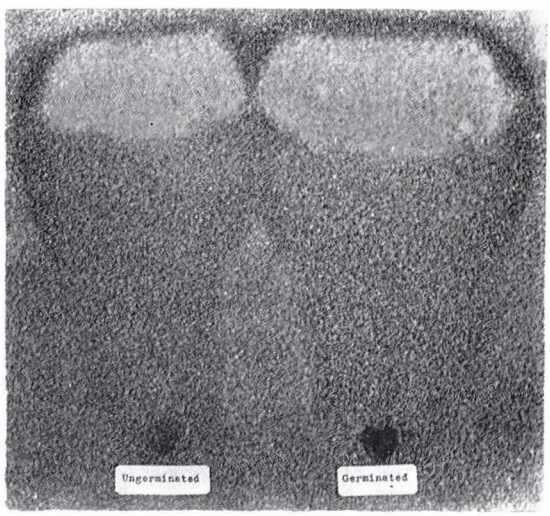

Fig. 1. *Curvularia* bioassay of extracts from ungerminated (left) and germinated (right) rice (cv. Rathna) seeds. The white zones correspond to areas of inhibition of test fungus showing the presence of prohibitins

activity in the extracts from germinating seeds exposed to microorganisms which is absent in the ungerminated control seeds. Its concentration increased during seed germination presumably due to synthesis as a result of incompatible host-parasite interaction caused by the seed microflora. Therefore, the inhibitory principle in these extracts be considered prohibitin. The tolerance of seed pathogen *C. lunata* and the seed transmitted pathogen *D. oryzae* to high and low concentrations of the extract and the sensitivity of *P. oryzae* require critical investigation. The chemical properties of the prohibitin are under investigation.

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Changes in Phenolics and Enzymes of Phenol Metabolism in Sheath Blight Disease of Rice Caused by *Rhizoctonia solani**

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Changes in phenol contents in resistant (IR 20) and susceptible (TKM 9) rice cultivars were studied during different stages of sheath blight disease progress. Contrary to the expected rise in the resistant plants, ortho-dyhydroxy (OD) phenols accumulated earlier and faster in TKM 9 than in IR 20. Similar was the case with increase of total phenols in TKM 9. Resistant and susceptible cultivars failed to present a consistent pattern in the accumulation of total phenols and flavonoids. Flavonoids increased in IR 20 only in the later stages of infection. Both resistant and susceptible *R. solani* inoculated rice seedlings showed decreased levels of phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) excepting the TAL increase in susceptible TKM 9. No DOPA-oxidase activity was detected in uninoculated and inoculated plants. Peroxidase decreased in both the varieties as a result of infection.

Sheath blight disease incited by *Rhizoctonia solani* is one of the major diseases of rice. It causes heavy yield losses and has become a potential threat in all the rice growing countries. Among the fungal diseases of rice it is second in importance next to blast (Marshall and Rush, 1980).

Natural infection usually occurs at the seedling, tillering and boot-leaf stages of the rice plant. However, the disease is more prevalent in Dapog nurseries, where the soaked seeds are allowed to germinate and grow in plastic lined beds under high humid conditions. These are regularly adopted by farmers to cut down the area and cost of raising nurseries. Seedling infection is also very common in upland rice cultivation immediately after direct sowing (Kannaiyan and Prasad, 1978a). A comprehensive review on the sheath blight disease of rice has been published (Manibhushanrao et al., 1979).

In view of the widespread prevalence of the disease and the serious yield losses it causes, the physiological responses accompanying disease development in sheath blight-infected rice seedlings are worth investigating.

It is known that certain phenolic compounds and their oxidation products play a role in disease resistance by blocking fungal growth in the process of necrotic lesion development (Kosuge, 1969), or by inhibiting the fungal pectolytic enzyme

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(Hunter, 1974, 1978). Further, the changes in phenol content may depend upon alterations in the activity of the enzymes responsible for their synthesis. Phenylalanine ammonia lyase (PAL) is probably important in the synthesis of various phenolics including the flavonoids, isoflavonoids and hydroxycinnamic acids. The biosynthesis of phenylpropanoids from phenylalanine and tyrosine is initiated through the action of PAL and TAL, respectively. The reaction catalysed by PAL is the deamination of phenylalanine to transcinnamic acid and ammonia, while TAL deaminates tyrosine to p-coumaric acid. This deamination mechanism has been consequently linked with disease resistance in view of its importance in aromatic compound metabolism (Young et al., 1966). The various aspects of PAL have been reviewed by Camm and Towers (1973).

The oxidation of phenolic compounds in the infected tissue by polyphenol (Dihydroxyphenylalanine; DOPA) oxidase (PPO) and the relatively high toxicity of these oxidation products have long drawn attention. Despite several reported failures to detect PPO (Rao and Nayudu, 1979; Sridhar, 1972), Muralidharan (1974) detected the enzyme activity in blast-infected rice leaves.

Increased peroxidase (PO) activity has often been studied in connection with the oxidation of phenolic substances in diseased plants and resistance of the host was attributed to the toxicity of these oxidation products. Results from studies on the role of PO in resistance or susceptibility have been summarized (Frić, 1976; Stahmann and Demorest, 1973).

This paper reports the changes in phenolic and enzyme activities of phenol biosynthesis and oxidation products in *R. solani*-inoculated resistant and susceptible rice seedlings with the disease progress.

Materials and Methods

Method of raising plants and inoculation

Rice seeds (*Oryza sativa* L.) of TKM 9 and IR 20 cultivars were obtained from the Paddy Experiment Station, Tirur-602 025, Tamil Nadu. The seeds were soaked in tap water for one day and were allowed to germinate, after decanting the water, for 24 h. Such pregerminated seeds were sown just below the surface of garden soil in plastic troughs. The seedlings were raised under green house conditions and were irrigated with tap water on alternate days.

A virulent isolate of *R. solani* isolated from rice was used in the present study. The inoculum preparation using rice-sand medium and inoculation of plants were followed as detailed by Kannaiyan and Prasad (1978b). The rice-sand medium was mixed with garden soil at an inoculum level of 25% and the pregerminated rice seeds (50 g) were sown in such fungus-infected moistened soil contained in plastic troughs (8 × 19 cm). Control seedlings were grown only in normal garden soil.

Sample collection and extraction

Collection of samples was commenced on 7th day, when the symptoms were first visible and was continued at 48 h intervals till 15th day. Only lesions and areas adjoining the lesions appearing on sheaths and leaves were used for all analyses.

Healthy and infected tissue (sheath and leaf material) were collected and chopped into small bits, extracted in boiling 80% ethanol for 5 min and homogenized. The homogenate was filtered through four layers of cheese cloth and the residue extracted once again. The extracts were combined and filtered through Whatman No. 41 filter paper.

Estimation of total phenols

The amount of total phenols was determined colorimetrically using Folin-Ciocalteau reagent (Bray and Thorpe, 1954). Standards prepared with catechol were used to calculate the total phenols.

Estimation of ortho-dihydric (OD) phenols

Arnow's reagent was used to estimate the OD phenol content (Johnson and Schall, 1952). Catechol was used as the standard.

Estimation of flavonoid compounds

The flavonoids were measured colorimetrically following the method of Shinoda (1928). Standards prepared with catechin hydrate were used to calculate the flavonoid content.

Preparation of acetone powders

Acetone powders of uninoculated and inoculated rice plants were prepared following the method of Umbriet et al. (1972).

Extraction and assay of PAL and TAL

The acetone powder was extracted with cold 0.1 M borate buffer (pH 8.8) for 3 h at 4 °C. The extract was squeezed through four layers of cheese cloth and clarified by centrifugation at 12,000 rpm for 30 min at 4 °C and the clear supernatant was used as enzyme extract (Neisch, 1961).

PAL and TAL were assayed spectrophotometrically by measuring the cinnamic and coumaric acids, at 268 and 333 nm respectively (Higuchi, 1966).

Assay of PPO

Reaction mixture consisted of 2 ml of enzyme extract (in M/15 phosphate buffer, pH 7.0) and 3 ml of 5×10^{-3} M DOPA dissolved in the same buffer. The

mixture was shaken frequently to ensure the availability of oxygen. The formation of O-quinone of DOPA by the enzyme was followed for 1 h by determining OD of the mixture at 470 nm at 15 min intervals. Enzyme extract and buffer without the substrate served as the blank. Enzyme activity was expressed as increase in OD/h/mg protein (Hampton and Fulton, 1961).

Assay of PO

The enzyme was extracted into 0.05 M Tris-HCl buffer, pH 7.0, containing 20% sucrose, 0.1% cysteine HCl and 0.1% ascorbic acid and assayed using pyrogallol as the substrate (Fehrmann and Dimond, 1967). The reaction mixture consisted of 2.5 ml of 0.05 M pyrogallol, 0.2 ml of enzyme extract and 0.1 ml of 0.01% H_2O_2. An equivalent volume of buffer was substituted for H_2O_2 in the reference cuvette. The enzyme reaction was initiated with the addition of H_2O_2. The increase in absorbance at 420 nm was recorded for 5 min. Maximal initial slope of the recorded curves was used for calculation of peroxidase activity. 0.01 increase in OD/min/mg protein was taken as one enzyme unit and the results expressed in terms of specific units.

Protein determination

The amount of protein present in a given sample was estimated following the method of Lowry et al. (1951).

Results

Total phenols

The total phenol contents of uninoculated and *R. solani*-inoculated rice seedlings are given in Table 1. Infection caused an initial decrease in total phenol content in both the resistant and susceptible varieties. The decrease was, however, double in the susceptible TKM 9 than that in the resistant IR 20. Total phenol content in TKM 9 fluctuated in the subsequent stages of infection. The resistant variety showed an increase and decrease till final stages of the disease. Thus, in the susceptible variety, in general, was observed an initial decrease and then considerable increase in total phenols on 9th and 11th day followed by decrease on 13th day of infection. By contrast, no consistent increase or decrease was evident in the resistant variety.

OD phenols

The changes in OD phenol contents of uninoculated and inoculated rice seedlings are given in Table 2. Resistant and susceptible varieties differed markedly in the accumulation of OD phenols in response to *R. solani* infection. The suscep-

Table 1

Total phenols[δ] in uninoculated and *R. solani*-inoculated rice seedlings

Age in days	Resistant (IR 20)			Susceptible (TKM 9)		
	UI	I	% of control	UI	I	% of control
7	3.1	2.6	−16.1	3.0	2.0	−33.3
9	1.8	2.8	+55.5	2.7	3.6	+33.3
11	3.0	2.7	−10.0	2.9	3.2	+10.3
13	2.3	3.4	+47.8	2.7	1.9	−29.6
15	2.7	2.4	−11.1	2.5	2.5	0

δ : mg catechol equivalents/g dry weight
UI : Uninoculated
I : Inoculated

tible TKM 9 showed a marked phenol accumulation on 7th and 9th day followed by a decrease in the later stages of infection. On the other hand, the resistant IR 20 recorded an initial decrease and a consistent increase in subsequent disease development (except on 11th day). Thus, OD phenols accumulated earlier and faster in the susceptible than in the resistant variety following inoculation.

Flavonoids

Table 3 gives the flavonoid contents of uninoculated and inoculated rice seedlings. In response to infection, the resistant and susceptible varieties showed an initial decrease of flavonoids. TKM 9 subsequently exhibited marked fluctuations in flavonoid accumulation. The resistant variety IR 20, however, showed a consistent increase in flavonoids in later stages of infection (except on 11th day).

Table 2

OD phenols[δ] in uninoculated and *R. solani*-inoculated rice seedlings

Age in days	Resistant (IR 20)			Susceptible (TKM 9)		
	UI	I	% of control	UI	I	% of control
7	3.8	3.3	−13.1	1.9	3.6	+89.5
9	2.9	3.6	+24.1	4.1	4.8	+17.0
11	4.0	4.0	0.0	4.0	3.7	−7.5
13	3.2	4.3	+34.4	3.5	3.2	−8.6
15	2.7	2.8	+3.7	2.4	2.6	+8.3

δ : mg catechol equivalents/g dry weight
UI : Uninoculated
I : Inoculated

Table 3

Flavonoid content$^{\delta}$ in uninoculated and *R. solani*-inoculated rice seedlings

Age in days	Resistant (IR 20)			Susceptible (TKM 9)		
	UI	I	% of control	UI	I	% of control
7	9.4	8.3	−11.7	10.9	6.5	−40.4
9	6.5	8.8	+35.4	9.1	13.4	+47.2
11	12.3	9.1	−26.0	10.5	9.2	−12.4
13	6.1	10.6	+73.8	6.9	8.9	+29.0
15	4.6	6.5	+41.3	7.1	6.4	−9.9

δ : mg catechin hydrate equivalents/g dry weight
UI : Uninoculated
I : Inoculated

Thus, while the flavonoids accumulated only at later stages of infection in IR 20, the susceptible TKM 9 failed to present a definite pattern throughout the samplings investigated.

PAL

The PAL activity in uninoculated and inoculated resistant and susceptible rice seedlings is shown in Fig. 1. Both the inoculated varieties, in general, registered a significant decrease in PAL activity over the uninoculated plants. The enzyme activity rose to maximum level in uninoculated 9-day old resistant and susceptible varieties. In response to infection, IR 20 presented simultaneous increase, though to a lesser extent over the uninoculated controls and the susceptible TKM 9, however, failed to do so.

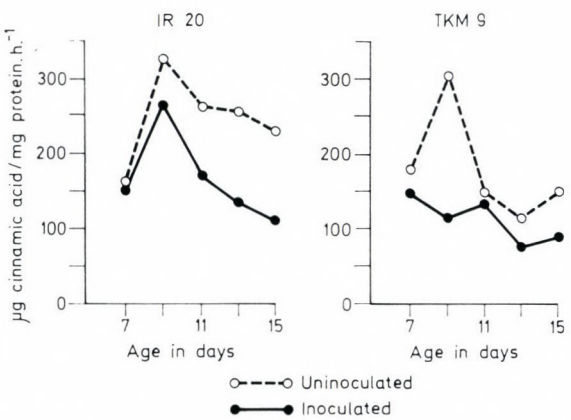

Fig. 1. PAL activity in uninoculated and *R. solani*-inoculated resistant (IR 20) and susceptible (TKM 9) rice seedlings

TAL

Figure 2 shows the TAL activity in uninoculated and inoculated rice varieties. TAL activity, in general, was appreciably high in uninoculated resistant IR 20 than in healthy susceptible TKM 9 (except on 15th day). Infection, however, decreased the enzyme activity in IR 20 but induced significant increase in enzyme levels in TKM 9 over the uninoculated controls. Thus, TAL rather than PAL appeared to increase in the susceptible variety as an immediate response to infection by *R. solani*. However, TAL activity was lower than the PAL activity in both varieties.

PPO

Results presented in Fig. 3 clearly show that the uninoculated and inoculated resistant and susceptible rice seedlings registered little or no DOPA-oxidase activity.

PO

The inoculated resistant and susceptible varieties, in general, showed relatively lower peroxidase activities than the uninoculated controls (except on 11th and 9th days in IR 20 and TKM 9, respectively). During the early infection (7-day-old seedlings), the resistant IR 20 however, showed an increase in enzyme activity (Fig. 4).

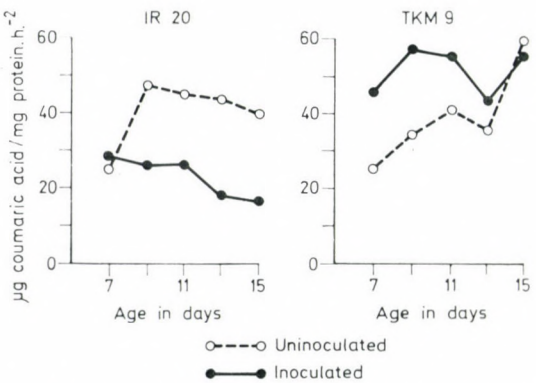

Fig. 2. TAL activity in uninoculated and *R. solani*-inoculated resistant (IR 20) and susceptible (TKM 9) rice seedlings

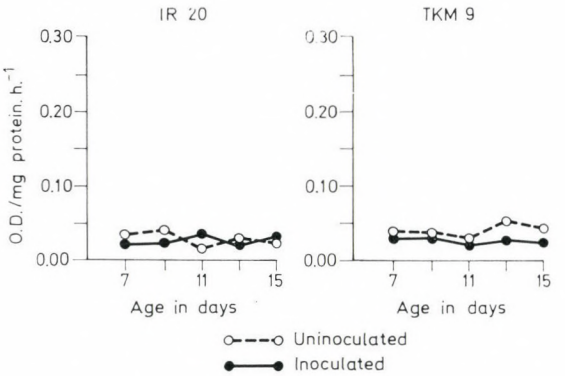

Fig. 3. DOPA oxidase activity in uninoculated and *R. solani*-inoculated resistant (IR 20) and susceptible (TKM 9) rice seedlings

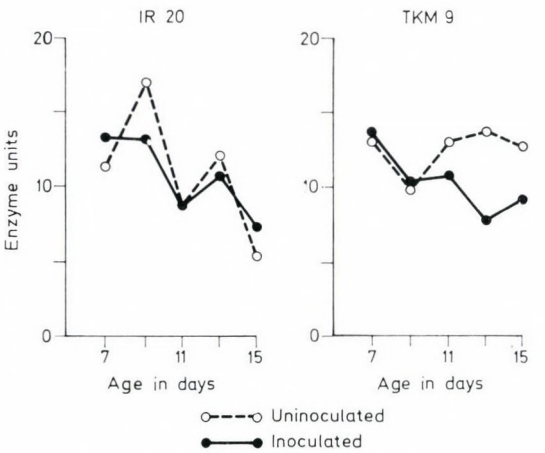

Fig. 4. Peroxidase activity in uninoculated and *R. solani*-inoculated resistant (IR 20) and susceptible (TKM 9) rice seedlings

Discussion

The resistant varietal response in rice to *R. solani* inoculation was characterized by an initial necrotic browning reaction (Zuber and Manibhushanrao, 1981). Since cell browning has been attributed to irreversible oxidation of polyphenols (Suzuki, 1965), earlier browning in the resistant rice varieties is likely to be reflected due to an earlier increase of phenolic compounds. On the other hand, OD phenols accumulated earlier and faster in the susceptible TKM 9 than in the resistant IR 20

following *R. solani* infection, despite the absence of browning reaction in the former. Similar was the case with increase in total phenols in TKM 9. However, flavonoids increased in the resistant IR 20 during the later stages of infection. The cultivars failed to present a consistent pattern in the accumulation of total phenols and flavonoids, respectively. Interestingly, both the varieties exhibited an initial decrease in total phenol and flavonoid contents. Similar results have been reported in wheat stem rust (Daly et al., 1971) and in rice plants infected with *Xanthomonas translucens* f. sp. *oryzicola* (Reddy and Sridhar, 1975). Failure to detect phenolic increase in the resistant rice cultivar might possibly be related to the fact that the metabolic rates for this particular host-pathogen combination do not reach as high a level as in compatible systems. Nor this anomaly can be explained by a fundamental difference in analytical techniques, because the procedures followed were basically the same as those used in other host-pathogen systems (see Materials and Methods). It should, however, be noted that environmental conditions and soil nitrogen levels can influence aromatic biosynthesis in normal plants. Environmental factors may condition tissues so that in some instances phenolics are synthesized in greater amounts as a consequence of disease (Daly et al., 1971; Seevers and Daly, 1970a). Instead, accumulation of phenols may result from damage to cell integrity of susceptible plants (Edreva, 1977). Reduction in phenolic contents in the brown zone in the resistant rice variety may be due to inhibition of their synthesis (Cohen and Ibrahim, 1975), or due to polymerisation and transformation (Tomiyama, 1963). The possibility also should not be overlooked that the host metabolic activity is suppressed during pathogenesis, resulting in the reduction of phenolic contents in IR 20 (Matta et al., 1970).

It is difficult to explain the marked fluctuations noticed in the accumulation of these cell constituents in both the susceptible and resistant plants. Reduction in sugar contents (Zuber and Manibhushanrao, 1983) associated with simultaneous increase in phenolics in the susceptible TKM 9 suggests that a major part of the sugars is shunted to polyphenol synthesis (Reddy and Sridhar, 1975). However, simultaneous decreases in both carbohydrate and phenol contents are also evident in the resistant rice cultivar. According to Lukens (1968) reduction in sugar contents will lead to decrease in phenolic contents of plants since the latter are synthesized from the former through shikimic acid pathway.

Attendant with phenol accumulation, there was a rapid increase in the activity of TAL in the susceptible TKM 9 (Fig. 2). However, phenol and flavonoid increases (Tables 2 and 3) in the resistant IR 20 were not accompanied by simultaneous enhancement of TAL or PAL. The enzymes indeed presented a decreasing pattern (Figs 1 and 2). Although TAL activity, in terms of μg of product formed, was considerably lower than PAL activity in both resistant and susceptible varieties, the former only appears to have contributed to the phenolic increases. Decreased enzyme levels are possibly due to PAL inhibition by indole acetic acid (IAA) and its possible plant precursor, tryptophan (Innerarity et al., 1972). However, IAA and tryptophan concentrations in healthy and diseased rice plants were not investigated. Recently, Creasy (1976) reported that a high molecular weight fraction

extracted from sunflower leaves inactivates PAL from sunflower. It could be possible that healthy rice plants had a lower level of PAL-inactivating system which increased upon infection. It is pertinent to quote here the existence of a lyase-inactivating system in rice cultivars (Purushothaman, 1974b).

No DOPA-oxidase activity could be detected in the present investigation in uninoculated or inoculated resistant and susceptible rice seedlings (Fig. 3). PPO indeed has been related to resistance in *Pyricularia oryzae* (Muralidharan, 1974) and *Helminthosporium oryzae*-infected (Chattopadhyay and Bera, 1980) rice leaves. However, it should be noted that this study does not preclude the possibility of some other phenol oxidase being involved, because DOPA was the only substrate used in the present investigation.

Not only the ammonia lyases but also PO decreased in rice leaves and sheaths as a consequence of infection (Fig. 4). It is generally assumed that PO activity in infected tissues is a reflection of metabolic demand for hydroxylation reactions in the synthesis of aromatic compounds (Seevers and Daly, 1970b). However, the decreased enzyme levels are highly inconsistent with enhanced phenolic levels in *R. solani*-inoculated rice plants. It is tempting to suggest that PO activities measured *in vitro* cannot be related directly to resistance or susceptibility of IR 20 and TKM 9 as measured by differential symptom development. Seevers and Daly (1970a, b) in wheat stem rust also reported a situation parellel to these results.

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Study of Cellulolytic and Pectolytic Enzymes in Nine Biological Forms of *Cercospora beticola* Sacc

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Production of cellulolytic and pectolytic enzymes in the representative isolates of nine biological forms of *Cercospora beticola* obtained by classifying 58 monosporic isolates collected from different sugar-beet-growing regions of India was studied. The results showed a marked variation among these forms in their ability to produce these enzymes in vitro and a positive correlation between the activity of these enzymes and the pathogenicity was established.

The disintegration of plant tissue by successful plant pathogenic entity implies that the particular pathogenic entity possesses enzymes that degrade the complex polysaccharides and other components of the cell membrane of host plant. These enzymes are implicated almost routinely as a feature of host-pathogen interactions and the significant production of a lesion depends how rapidly a pathogen produces certain cell wall degrading enzymes. Various investigators have studied the ability of many plant pathogenic fungi to produce cellulolytic and pectolytic enzymes (Ashour, 1954; Tribe, 1955; Kamal and Wood, 1956; Gupta, 1956; Cole, 1956; Wood and Gupta, 1958; Brown, 1965 and Bateman and Millar, 1966). Rautela and Payne, 1971 reported that *Cercospora beticola* the incitant of Cercospora leaf spot of sugar beet, produces the polygalacturonase *in vitro* and *in vivo*. It is reasonable that various other cellulolytic and pectolytic enzymes are being produce by *C. beticola* and might be involved in the development of the lesions. The present investigation was thus tried to find out the difference, if any, in the ability to produce various cell wall degrading enzymes in different isolates representing nine biological forms of the fungus, to establish the correlation, if it exists, between the activity of these enzymes and pathogenicity and finally to provide a strong base for distinguishing the various biological forms/physiological races of the fungus.

Materials and Methods

Nine biological forms of *C. beticola* were established based on morphological, cultural, biochemical and pathological characters of 58 monosporic isolates of the

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fungus collected from different sugar beet growing regions of India. One representative isolate of each biological form was grown in 50 ml Erlenmeyer flask containing 20 ml of Czapeck's Dox broth (K_2HPO_4, 1.0 g; $NaNO_3$, 2.0 g; $MgSO_4$, 0.5 g; KCl, 0.5 g; ferrous chloride 0.01 g and sucrose 20.0 g/litre, pH 6.5) in which sucrose was replaced by 2% cellulose and 2% pectin for cellulolytic and pectolytic enzymes, respectively. Each flask was inoculated with 7 mm mycelial agar disc cut from the margins of one week old culture grown on PDA. After incubation for 20 days at room temperature (28 ± 2 °C), the culture filtrates were obtained by filtering this material through Whatman No. 42 filter paper. The filtrates were then dialyzed in cellophane tubing against distilled water at 0–4 °C for 24 hours by changing water at every 8 hours and the activity of cellulase, polygalacturonase (PG), polymethygalacturonase (PMG), pectin transeliminase (PTE) and polygalacturonase transeliminase (PGTE) was determined by viscometric method of Bell et al. (1955) with slight modification. The reaction mixture contained 2 ml enzyme preparation, 1 ml respective buffer and 4 ml respective buffered substrates. In case of cellulase 4 ml of 0.5% carboxymethyl cellulose (CMC) and 1 ml of sodium acetate-acetic acid buffer, for polygalacturonase 4 ml of 0.75 sodium polypectate and 1 ml of acetate buffer, for polymethylgalacturonase 4 ml of 1% pectin and 1 ml of acetate buffer, for pectin transeliminase 4 ml of 1% pectin in boric acid borax buffer and for polygalacturonate transeliminase 1.2% sodium polypectat with boric acid borax buffer were pipetted into 300 Ostwald-fenske viscometer kept in water bath at 30 °C. Two ml of respective enzyme preparation was added and the contents were mixed by drawing air gently through the large arm of the viscometer. The efflux time of the mixture was recorded at 0 and 30 min and the activity of each enzyme was determined by following formula:

$$V = \frac{T_0 - T}{T_0 - T_{H_2O}} \times 100$$

where V = percent loss in viscosity, T_0 = flow time in seconds at zero time, T = flow time of the reaction mixture in seconds after 30 min, T_{H_2O} = flow time of distilled water in seconds

The results are expressed as percent reduction in viscosity of respective substrates.

Results

Cellulolytic and pectolytic enzyme production itae by the nine biological forms of *C. beticola* as determined by viscometric method are presented in Table 1 and Figs 1 and 2.

Cellulase

The activity of this enzyme was found maximum in bio-form 9 followed by bioforms 6, 7, 5, 3, 8, 1 and 2. The bio-form 4 had the lowest cellulase activity.

Table 1

Cellulolytic and pectolytic enzymes of nine biological forms of *C. beticola*

Bio forms	Activity in terms of percent reduction in viscosity after 30 min.				
	Cellulase	PG	PMG	PTE	PGTE
1	21.70 (27.76)	14.26 (22.16)	12.16 (20.33)	9.50 (17.93)	2.20 (8.53)
2	16.36 (23.86)	(15.92 (23.53)	16.87 (24.20)	11.08 (19.40)	2.60 (9.33)
3	34.33 (35.86)	33.33 (35.30)	34.74 (36.13)	15.06 (22.76)	3.24 (10.30)
4	11.16 (19.53)	10.26 (18.90)	11.07 (19.36)	4.73 (12.06)	1.40 (6.83)
5	40.00 (39.23)	41.50 (40.13)	20.21 (26.70)	14.53 (22.36)	4.08 (11.63)
6	50.00 (45.00)	48.58 (44.20)	37.50 (37.80)	18.08 (25.16)	5.56 (13.63)
7	44.83 (42.03)	33.27 (35.23)	16.33 (23.53)	13.27 (21.33)	2.65 (9.34)
8	23.50 (28.86)	20.16 (26.76)	14.83 (22.66)	10.50 (18.86)	2.10 (8.30)
9	77.50 (61.73)	78.66 (62.53)	69.00 (56.12)	24.07 (29.33)	7.87 (16.33)
C D at 5%	0.79	0.62	0.55	0.49	0.37

PG Polygalacturonase
PMG Poly-methyl-galacturonate
PTE Pectin-transeliminase
PGTE Polygalacturonate transeliminase
( ) Transformed values of percent reduction in viscosity

Polygalacturonase

The bio-form 9 tended to have the maximum activity of polygacturonase among all the nine bio-forms followed by bio-forms 6, 5, 3, 7, 8, 2 and 1. The minimum activity of this enzyme was recorded in bio-form 4.

Polymethylgalacturonase

Again bio-form 9 showed the maximum activity of this enzyme and followed by bio-forms 6, 3, 5, 2, 7, 8 and 1. The bio-form 4 had the lowest activity.

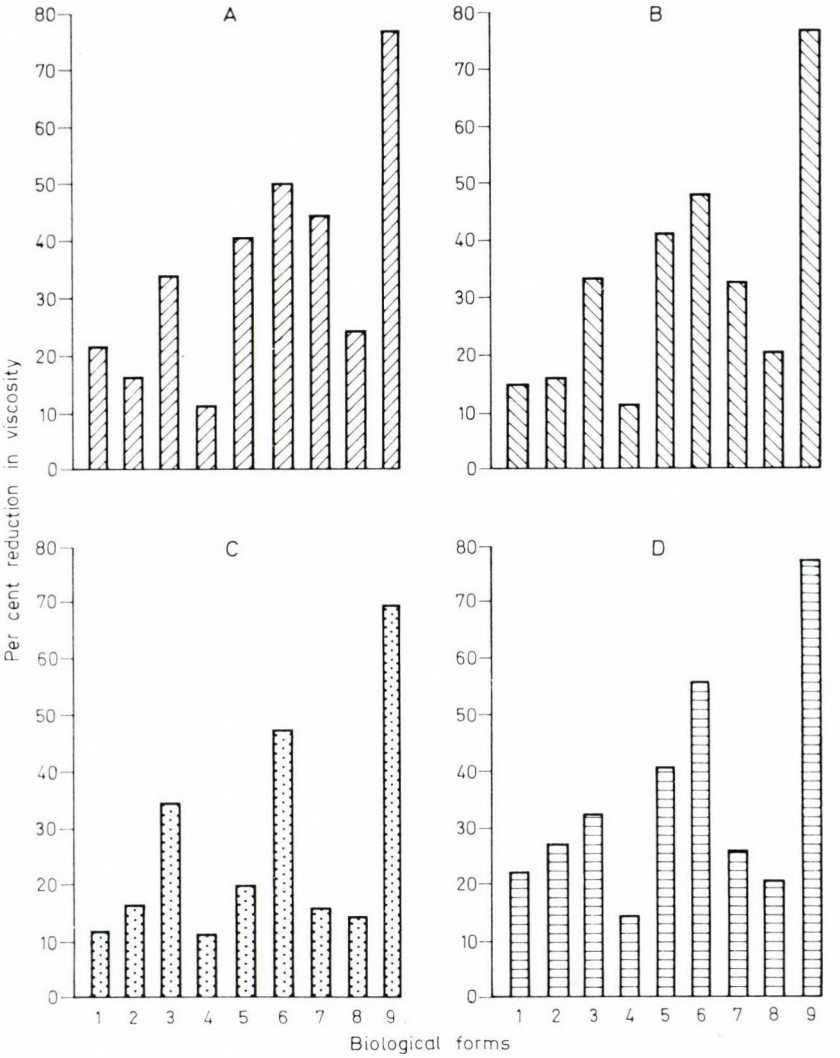

Fig. 1. Cellulolytic and pectolytic enzyme activity of nine biological forms of *C. beticola* as expressed in terms of percent reduction in viscosity of respective substrates. A: Cellulase. B: Polygalacturonase. C: Poly-methyl-galacturonase. D: Polygalacturonase transeliminase

Polygalacturonate transeliminase

The pattern of the production of this enzyme in all the bio-forms was more or less similar to above enzymes. However, the activity of this enzyme was very low as compared to other enzymes.

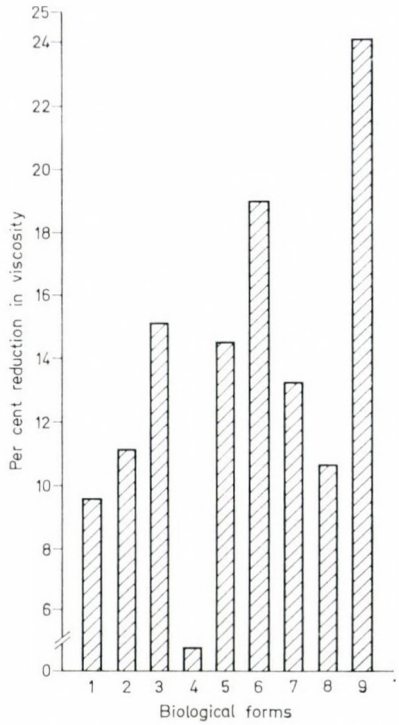

Fig. 2. Pectin transeliminase activity of nine biological forms of *C. beticola* as expressed in terms of percent reduction in viscosity of 1% pectin in boric acid borax buffer

Pectin transeliminase

The activity of this enzyme varied sufficiently in different nine bioforms of this fungus. The bio-forms 9 and 4 exhibited maximum and minimum activity respectively and followed by the bio-forms 6, 3, 5, 7, 2, 8 and 1.

An analysis of variance of transformed values of percent reduction in viscosity indicated the significant difference in ability to produce these enzymes by nine bio-forms of *C. beticola* in vitro (Table 1).

Discussion

The results reported here indicate clearly that 9 isolates of *C. beticola* (representing 9 bio-forms) are not similar in their ability to produce cellulolytic and various pectolytic enzymes in vitro. This criterion may provide one of the strong bases for distinguishing/identification of bio-forms/physiological forms of other fungi including *C. beticola*. Similar investigations in vivo may provide a valuable

support to extend these findings. The concept that cellulolytic and pectolytic enzymes may be involved in pathological manifestation induced in plant tissues by biotic entities was substantiated by the research findings of Brown (1915) and numerous research papers which appeared in the literature in the past, indicated that the pathogenicity of an organism is correlated with its ability to produce cell wall degrading enzymes, e.g. *Rhizoctonia solani* (Barker and Walker, 1962), *Colletotrichum falcatum* (Singh and Husain, 1964), *Fusarium oxysporum* f. sp. *lycopersici* (Paquin and Coulombe, 1962) and *Verticillium* spp. (Leal and Villaneuva, 1962). Rautella and Payne (1971) reported the only production of polygalacturonase itae and itae by *C. beticola*. But they did not correlate this enzymic activity to the pathogenicity of the fungus. However, there seems to be a positive correlation between the cellulolytic and pectolytic production ability and the pathogenicity of these bio-forms of this fungus reported herein. The bio-forms 9 and 4 which showed maximum and minimum production of cellulase, PG, PMG, PTE and PGTE were found most virulent and least virulent, respectively, as tested on sugar beet cv. Dobrovicka under artificial inoculation conditions. More likely, the bio-forms 6, 5, 3 and 7 which also exhibited a comparatively high activity were found more aggressive than the other bio-forms 1, 8 and 2 which had lesser activity of these enzymes under the similar environmental conditions (unpublished information). In contrast to our findings, Sladka and Brillova (1978) reported the production of endopolygalacturonase, pectin methylesterase and cellulase by U.V. mutants of *C. beticola* without positive correlation between the activity of these enzymes and virulence. It may be attributed to the U.V. light treatment of the isolates, which might have altered the pathway of the production of some other pectin degrading enzymes as they did not recover the activity of pectin lyases in U.V. mutants and only these enzymes may be responsible for the development of the lesion.

Finally, it may be concluded from the results obtained so far that nine bio-forms of *C. beticola* have differed in their ability to produce cellulose and pectin degrading enzymes, which may provide an additional base for detecting the bio-forms/physiological races of *C. beticola* and other fungi also. A positive correlation between the activity of these enzymes and virulence of the 9 different bio-forms is established.

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Pathogenicity of *Pseudomonas aeruginosa* for Plants and Animals

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The pathogenicity of a series of 54 bacterial strains of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Pseudomonas syringae* pv. *syringae* was tested on carrot and parsley roots, onion, bulbes of kohlrabi, potato tubers and tobacco leaves. The group of *P. aeruginosa* comprised strains of clinical, plant and soil origin. The clinical strains were evidenced to possess a high phytopathogenicity, characterised by their rotting capacity. The ratio of pathogenic : non-pathogenic strains for plants was 5 : 1. Also the plant strains showed a marked phytopathogenicity. Strains from soils were non-pathogenic on plants. A positive correlation was demonstrated between production of pyocyanine and rotting capacity within the whole series of *P. aeruginosa* strains. Carrot appeared as the most susceptible. In many cases great differences were observed in the pathogenicity of the different strains of *P. aeruginosa* on plants tested. Both the clinical and plant strains of *P. aeruginosa* were pathogenic for animals, the soil ones were non-pathogenic. No pathogenicity for animals was found in strains of *P. fluorescens* and *P. syringae* pv. *syringae*. In most cases, the pathogenic or non-pathogenic capacity of *P. aeruginosa* strains for animals was correlated with the production of protease. On the basis of the results obtained *P. aeruginosa* is considered as an opportunistic plant pathogen.

In human medicine, *Pseudomonas aeruginosa* is considered as a potential pathogen or sometimes even as a primary pathogen (Jedličková, 1981). Recently this bacterium has become an increasingly frequent source of infections and epidemics in hospital environment (Green et al., 1974).

So far, very few experimental data are available on the pathogenicity of *P. aeruginosa* for plants. The bacterium is often found on various organs as an epiphytic microorganism (Cho et al., 1975). For example, rather large numbers of *P. aeruginosa* have been demonstrated on tomato, celery, endivia and cucumber (Kominos et al., 1977). The fact that *P. aeruginosa* may act as a plant pathogen was first pointed out in the twenties and thirties of this century by Paine and Branfoot (1924), Mehta and Berridge (1924), Baldacci and Ciferri (1934) and, several years later, also Elrod and Braun (1942). A reliable evidence of the pathogenicity of *P. aeruginosa* for plants was provided by Cho et al. (1975) who found that some strains originating from clinical material were strongly pathogenic to celery, lettuce and potato. The authors consider this bacterium as a "quasi-pathogen" with a pathogenic potential in limited special conditions only.

The objective of our study was to verify whether strains of *P. aeruginosa*, coming from clinical material, could be pathogenic for certain kinds of root vege-

tables, onion, kohlrabi, potato and tobacco. Furthermore, we tested whether strains of *P. aeruginosa* originated from plant material could be pathogenic for animals. The present paper is a sequel to our previous communication (Lebeda et al., 1982).

Materials and Methods

Bacterial species and strains

For pathogenicity tests we used a total of 54 strains of three species of bacteria, viz., *Pseudomonas aeruginosa* (Schroeter 1872) Migula 1900; *Pseudomonas fluorescens* Migula 1895; and *Pseudomonas syringae* pv. *syringae* van Hall 1902.

Most of the strains of *P. aeruginosa* (in all, 37) came from clinical material, most frequently from cystic fibrosis, nosocomial infection and septic burns. The strains were isolated in the Institute of Hygiene and Epidemiology in Praha. Strains of *P. aeruginosa* CCM 1960 = ATCC 10145 (source: probably clinical), CCM 1961 = ATCC 9027 (source: outer-ear infection) and CCM 1968 = NCIB 950 (source: probably clinical) were obtained from the Czechoslovak Collection of Microorganisms (CCM) in Brno. *P. aeruginosa* DAR 26702 was originally isolated from *Allium cepa* cv. "Cream Gold" (Cother, Darbyshire and Brewer, 1976) and was kindly supplied by Dr. E. J. Cother, of the Agricultural Research Centre, Yanco, N.S.W., Australia. Strains of *P. aeruginosa* denoted as UCPPB 16 (source: chrysanthemum), UCPPB 35 (source: soil in chrysanthemum pot), UCPPB 60 (source: petunia soil), UCPPB 61 (source: chrysanthemum), UCPPB 62 (source: maize stem) were kindly supplied by Dr. M. N. Schroth, of the Department of Plant Pathology, University of California, Berkeley, USA.

Strains of *P. fluorescens* CCM 1969 = NCIB 3756 (source: cerebrospinal fluid), CCM 2659 = NCIB (source: probably clinical) and CCM 2799 (source: plants for silage) from the CCM. Also the strains of *P. syringae* pv. *syringae* CCM 2114 = NCPPB 1072 (source: *Pyrus communis)*, CCM 2868 (source: cherry) and CCM 2870 (source: French prune) come from this collection. The strain of *P. syringae* pv. *syringae* F 97 A was isolated from *Phaseolus vulgaris* (Kůdela et al., 1982) and the strain T 34 from a cherry (Kůdela, unpublished).

Culture media

All bacterial strains described above were kept on King's medium B (King et al., 1954) or on meat-peptone agar at temperatures around 5 °C.

Serological typing of strains of P. aeruginosa

The serological typing of the strains of *P. aeruginosa* used in this study was carried out according to Zahradnický and Jedličkova (1979) whose system is based on the original scheme of Habs (1957). The serotypes of *P. aeruginosa* iden-

tified are denoted as s 01 to s 012. Strains not denoted in this way could not have been typed.

Pathogenicity assay on plants

For pathogenicity tests all the above strains were propagated on King's medium B at laboratory temperatures (20–24 °C). The inoculum was prepared from cultures about 48 h old. From the surface of the cultivation media the bacterial culture was sampled with a microbiological wire loop and transferred to the surface of the tested parts of plant organs.

The tests were made on root discs of carrot (*Daucus carota* ssp. *sativus* (Hoffm.) Hay, cv. "Nantais") and parsley (*Petroselinum hortense* Hoffm., cv. "Hanácká"), on discs of kohlrabi (*Brassica oleracea* var. *gongyloides* L., cv. "Moravia"), onion (*Allium cepa* L., cv. "Hiberna"), and potato (*Solanum tuberosum* L., cv. "Radka"). The discs of all the plant species tested were about 5 mm thick. The tests were triplicated in each plant species or cultivar, each replication containing 2 to 4 discs. Each disc received four drops of inoculum (nondiluted bacterial culture). Discs on which a drop of sterilized distilled water was placed with a pipette served as controls.

The treated discs were placed in Petri dishes on moistened filter paper. Incubation took place in darkness at laboratory temperatures and 100% relative air humidity. Manifestations of pathogenicity were evaluated on the 7th and 14th day after inoculation according to the following scale:

− (0) = bacteria do not multiply, tissues remain undamaged;
\+ (1) = visible but limited growth of bacteria, tissues remain undamaged or are water-soaked only in place of contact with bacterial culture;
\+ + (2) = considerable growth of bacteria, tissues heavily water-soaked both in places of contact with bacterial culture and elsewhere;
\+ + + (3) = heavy growth of bacteria, tissues water-soaked on most or the whole of their surface and undergoes a total collapse (soft rot).

Some of the strains were tested for hypersensitivity on leaves of tobacco (*Nicotiana tabacum* L., cv. "White Burley") according to Klement (1963). Tobacco plants were cultivated at temperatures of 20–24 °C and a 12 h photoperiod. In all, five to six infiltrations with one strain were carried out. The inoculum concentration was 10^7–10^9 cells · ml^{-1}. 48 h after inoculation the symptoms were evaluated according to the following scale:

0 = no symptoms;
1 = minute localised lesions in places of infiltration, sometimes accompanied by weak chlorosis;
2 = larger necrotic spots in place of infiltration but their total area is smaller than the originally infiltrated one;

3 = necrotic lesion of the same size or larger than the originally infiltrated area of leaf.

Pathogenicity assay on animals

The pathogenicity was tested on laboratory white mice (strain ICR). For the assay, we used 0.2 ml of 18 h culture suspended in Ringer's solution (Černá et al., 1973), corresponding by its density to degree 6 according to McFarland (Király et al., 1974). The suspension was injected to mice 18 to 20 g in body weight intraperitoneally. Mortality of the experimental animals was recorded 6, 19, 30 and 48 h after inoculation. Most of the mice died within 24 h after inoculation.

Results

Pathogenicity of P. aeruginosa strains for plants

The results obtained from our study of the pathogenicity of *P. aeruginosa* strains are summarized in Table 1. It is evident that we tested a total of 46 strains. Of these 40 were of clinical origin, 4 of plant origin and two from soils. The effect of a series of clinical strains of *P. aeruginosa* on plant tissues tested gave both extreme types of pathogenicity, i.e., strong pathogenicity, and non-pathogenicity. The strains tested could be divided into three groups (Table 1) according to their pathogenic specificity (i.e. whether their pathogenicity became apparent in all or only some of the plant species tested) and according to the degree of their pathogenicity (i.e. their relative capability of destroying plant tissues).

The pathogenicity was evaluated by computing the relative frequency of the different degrees of infestation which manifested themselves in the interaction of a particular strain with all plant species tested. For this evaluation the symbols of the intensity scale (−, +, ++, +++) were replaced by numerical values (0. 1, 2, 3). The degree of relative pathogenicity of particular strain was obtained by summing all numerical values of individual interactions of that strain with the various plant species and dividing the sum by the total number of interactions. By this characteristic the series of strains was divided into the following groups:

1. 0.0−0.9 = non-pathogenic or slightly pathogenic;
2. 1.0−1.9 = medium pathogenic;
3. 2.0−3.0 = strongly or very strongly pathogenic.

Evaluating the whole series of *P. aeruginosa* strains according the above scale, it becomes apparent that the ration of pathogenic : non-pathogenic or slightly pathogenic strains (i.e. 2 and 3 : 1) was 4 : 1. Considering only the clinical strains, the resulting ratio was 5 : 1. This general evaluation indicates that a major part of the series caused symptoms of soft rot in the plant species tested.

The first group comprises nine strains (14.5% of the total number), seven of which are clinical and two come from soils. All these strains were characterized

Table 1

Pathogenicity of strains of *Pseudomonas aeruginosa* and other *Pseudomonas* sp. on carrot, parsley, onion, kohlrabi and potato

Bacteria, strain	Fluorescein, pyocyanin	Serotype	Plant species				
			carrot	parsley	onion	kohlrabi	potato
P. aeruginosa							
1. 2424 M	F	010	−	−	−	−	−
UCPPB 35	F	.	−	−	.	.	.
UCPPB 60	F	.	−	−	.	.	.
121	F	011	−	+	−	.	−
0322474	F	.	+	−	+	+	−
128	F	06	+	−	−	−	++
21622	F	03	−	+	+	−	+
CCM 1961	F	03	++	−	+	−	−
22474	F	03	+	++	−	+	−
2. 032431 Mp	F	.	++	−	+	++	−
148	P	011	+	++	++	−	−
2465	F	01	−	++	+	+	+
CCM 1960	F	06	++	−	++	−	+
CCM 1968	F	06	+++	+	+	−	−
UCPPB 62	P	06	+	+	.	.	.
2407 R	F	05	++	−	+	+	++
2431 M	F	03	+	++	−	+	++
147	F	011	+++	+	+	++	−
76	F	011	++	+	−	++	++
2486	P	03	+	++	++	+	+
22307	P	group 2	+	+	++	+	++
2497	P	06	+++	++	++	+	−
22562	P	01	+	++	+	+++	+
2414	F	03	+	+++	+	−	+++
21626	P	05	+	+++	++	+	+
0122562	P	.	++	+	+++	++	+
72	P	02	++	+	++	++	++
21574	P	04	+++	+	−	++	+++
22783	P	011	+++	+	+	+++	+
2525	F	06	+++	−	+++	+	++
22956	F	06	++	+	+	+++	++
3. 111	P	08	+++	++	++	++	+
2407 M	F	05	+++	+	+	+++	++
21628	P	01	++	+	++	++	+++
032431 Rp	P	.	+++	+++	+++	+	++
129	P	06	+++	+++	+++	++	+
22560	F	group 2	+++	++	+	+++	+++
DAR 26702	P	09	+++	+++	++	++	++
UCPPB 16	F	06	+++	++	.	.	.
UCPPB 61	F	04	+++	++	.	.	.
2506	P	010	+++	++	+++	+++	++
2490 R	P	01	++	+++	+++	++	+++

Table 1. continued

Bacteria, strain	Fluorescein, pyocyanin	Serotype	Plant species				
			carrot	parsley	onion	kohlrabi	potato
2431 R	F	03	+++	+++	+++	+	+++
2424 R	P	010	+++	+++	++	++	+++
2490 M	P	01	+++	+++	+++	+++	++
2432 M	P	03	+++	+++	++	+++	+++
P. flurorescens							
CCM 1969	F		+	+	−	+	−
CCM 2659	F		+	+	+	−	−
CCM 2799	F		−	−	+	+	+
P. syringae pv. *syringae*							
CCM 2114	F		++	+	+	−	−
T 34	F		+	+	+	.	++
CCM 2868	F		+++	+	+	−	+
CCM 2870	F		+++	+	+	−	+
F 97 A	F		+	+	+	.	+++

F = fluorescein, P = pyocyanin, . = not tested

by their being incapable of destroying plant tissues or capable of causing a slight destruction in some of the plants tested. For example, strain 121 grew feebly on parsley discs; strain 128, on carrot ones and somewhat more so on potato (Table 1). Strains 2424 M, UCPPB 35 and UCPPB 60 were not pathogenic in any cases tested.

The second group is the most extensive as to the number of strains comprised. It comprises 47.8% of the total number of strains and 52.5% of the clinical ones. This group is the most diverse as to pathogenicity. It comprises a number of a strains which showed a strong pathogenicity, e.g., on carrot and weak or no pathogenicity on parsley, onion, and potato (strains 147, CCM 1960, CCM 1968, 21574, 22783). In a limited extent even contrary responses were observed, i.e. a strong pathogenicity for onion or parsley and a feeble one for carrot. The second group also comprises strain UCPPB 62, isolated from maize, which was tested on parsley and carrot only, producing negligible lesions.

The third group, characterized by strong to very strong pathogenicity of strains for all or most plant species tested, comprises altogether 15 strains (32.7% of the total number), 12 of which are of clinical and three of plant origin (DAR 26702, UCPPB 16 and UCPPB 61). Inoculation of test plants with these strains was followed by intensive bacterial growth, accompanied by distinct symptoms of soft rot. In cases of strong pathogenicity for a particular plant species the first signs of tissue decay were observed on the 3rd to 4th day after inoculation (or even sooner in single cases). In carrot and parsley the decay was accompanied

by the tissues turning brown to black. In some cases a complete decay of the tissues occurred around the 7th day after inoculation. This fact was especially typical for the carrot. Onion showed predominantly symptoms of water-soaking which proceeded along the circumference of the layers but even centripetally and centrifugally. The affected tissues sometimes turned green-blue, which was particularly typical of strains producing pyocyanine. Some of the clinical strains of this group attained almost maximum values of pathogenicity (e.g., 2490 R, 2431 R, 2424 R, 2490 M, 2432 M), i.e. the tissues of all plant species tested were completely disorganised or heavily damaged.

Very interesting responses were observed in strains originating from plants. DAR 26702 showed a strong or very strong pathogenicity for all plants tested. UCPPB 16 and UCPPB 61 were tested on carrot and parsley only. Also these strains were strongly pathogenic for these plant species (Table 1).

From Table 1 can derive one more interesting correlation concerning differences in pathogenicity between *P. aeruginosa* strains, depending on whether they produce fluorescein (F) or pyocyanine (P). By summing the values of relative degree of pathogenicity of the F and P strains and dividing the sums by the number of cases we obtained the degree of pathogenicity of the two series (including strains of plant or soil origin). This value is 1.19 for the F strains, and 1.97 for the P ones. Table 1 also shows that the highest frequency of the F strain is found in the first group. Hence the P strains appear to be more strongly pathogenic than the F strains. No correlation was demonstrated between growth phase (R, M), serotype and pathogenicity of *P. aeruginosa*.

Differences in susceptibility of plants to P. aeruginosa

From the mean degree of infestation of the test plants after inoculation by the different strains of *P. aeruginosa* we inferred the relative susceptibility of the plant species tested. Figure 1 shows that carrots were the most and potatoes the least susceptible. The susceptibility of parsley, onion and kohlrabi was practically at the same level as that of potato.

Pathogenicity of P. fluorescens and P. syringae pv. syringae

All three strains of *P. fluorescens* tested showed a low degree of pathogenicity for all plant species tested (Table 1). A few of the plant/strain interactions resulted in a feeble multiplication of the bacteria, the plant tissues remaining undamaged or showing feeble water-soaking only in places of contact with the bacterial culture. There were no significant differences in the susceptibility or resistance of the plant species tested. Onion showed more marked water-soaking in places of contact of the bacteria with the tissues than did the other plants.

All five strains of *P. syringae* pv. *syringae* tested were feebly pathogenic for parsley and onion. However, they differed as to the degree of pathogenicity for carrot and potato. Strains CCM (from pear, cherry and French prune) showed a

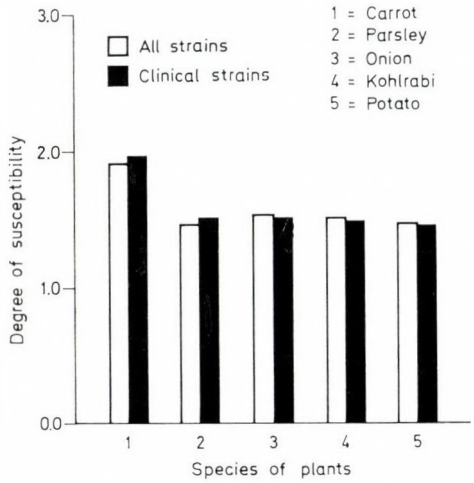

Fig. 1. Susceptibility of plant species to *P. aeruginosa*

higher degree of pathogenicity for carrot than for potato whereas a contrary was found for the remaining strains (from beans and cherry).

Tobacco hypersensitivity test of Pseudomonas sp. strains

Thirty strains of all three *Pseudomonas* sp. under study were selected for comparative assays. The results obtained are summarized in Table 2. In the assays the manifestations of hypersensitivity in tobacco were confronted with the response of the most susceptible plant species (carrot) of the series tested. The relative degrees of pathogenicity of the different strains were used as another comparative criterion.

According to the results of the hypersensitivity tests the series of strains was divided into four groups (Table 2). The first and second groups comprise strains which did not produce any hypersensitive response. Comprised here are all three strains of *P. fluorescens*, the members of which species are mostly considered as saprophytic bacteria. Thus their response agrees with the assumption of Klement (1963) on the response of pathogenic and non-pathogenic bacteria on the indicator plant. However, the first and second groups also comprise a number of *P. aeruginosa* strains (UCPPB 61, DAR 26702, 2431 R, 2424 R) which were strongly pathogenic for carrot as well as other plant species tested but failed to produce the theoretically expected symptoms on tobacco. The third and fourth groups comprise strains that produced intensive symptoms of hypersensitivity on tobacco leaves. Comprised here are *P. aeruginosa* strains (21628, 129, UCPPB 16, 2506) which were pathogenic for carrot and the remaining plants tested, as well as strains (121, 148, 2465) which were non-pathogenic or feebly pathogenic for carrot and the other plants tested.

Table 2

Pathogenicity of strains of *P. aeruginosa* and other *Pseudomonas* sp. for tobacco

Bacteria, strain	Plant species		Relative degree of pathogenicity for the five plant species tested
	tobacco	carrot	
1. *P. aeruginosa*			
CCM 1960	0	2	1.0
UCPPB 61	0—1	3	2.5
P. fluorescens			
CCM 1969	0	1	0.6
CCM 2799	0—1	0	0.6
2. *P. aeruginosa*			
UCPPB 35	1	0	0.0
UCPPB 60	1	0	0.0
0322474	1	1	0.6
21622	1	0	0.6
22562	1	1	1.6
2497	1	3	1.6
DAR 26702	1	3	2.4
2431 R	1	3	2.6
2424 R	1	3	2.6
P. fluorescens			
CM 2659	1	1	0.6
P. syringae pv. *syringae*			
F 97 A	1	1	1.2
CM 2870	1	3	1.2
3. *P. aeruginosa*			
121	2	0	0.25
148	2	1	1.0
UCPPB 62	2	1	1.0
76	2	2	1.4
129	2	3	2.4
UCPPB 16	2	3	2.5
2414	2—3	1	1.6
21628	2—3	2	2.0
P. syringae pv. *syringae*			
T 34	2	1	1.0
4. *P. aeruginosa*			
2465	3	0	1.0
22783	3	3	1.8
2506	3	3	2.6
P. syringae pv. *syringae*			
CCM 2114	3	2	0.8
CCM 2868	3	3	1.2

Comparison of pathogenicity of Pseudomonas sp. strains for plants and animals

To test the pathogenicity for animals we selected twelve strains, representing the three *Pseudomonas* sp. under study and all three groups of *P. aeruginosa* showing different pathogenicity for plants. The results are summarized in Table 3. Very interesting results were obtained for *P. aeruginosa*, with all clinical as well as plant strains pathogenic for animals. The soil strains showed no pathogenicity, the same as the strains of *P. fluorescens* and *P. syringae* pv. *syringae*.

In Table 3 the pathogenicity or non-pathogenicity is also compared with the presence of certain enzymes in the different strains. It is the case of extracellular enzymes known to participate in the pathogenicity for animals. Table 3 rather clearly shows connections between the presence of protease and pathogenicity for animals and, to a certain extent, also plants.

Table 3

Pathogenicity of *Pseudomonas* sp. for animals and their enzymatic capacity

Bacteria, strain, source	Pathogenicity for		Enzymes*		
	animals	plants	alcaline phosphatase	esterase	protease
P. aeruginosa					
Clinical strains					
CCM 1960	+	1.0	−	+	+
CCM 1961	+	0.6	+	−	−
CCM 1968	+	1.0	−	+	+
Plant strains					
UCPPB 16	+	2.5	−	+	+
UCPPB 61	+	2.5	−	+	+
UCPPB 62	+	1.0	−	−	+
DAR 26702	+	2.4	−	−	+
Soil strains					
UCPPB 35	−	0.0	−	−	−
UCPPB 60	−	0.0	+	−	−
P. fluorescens					
CCM 2799	−	0.6	+	−	−
CCM 2659	−	0.6	+	−	−
P. syringae pv. *syringae*					
CCM 2868	−	1.2	−	−	+

Pathogenicity for animals: + = positive; − = negative.
Pathogenicity for plants: 0 = non-pathogenic; 3 = highly pathogenic.
Enzymes: + = present; − = absent.
* Zemek et al. (unpublished); the pertaining data were kindly provided by Ing. J. Zemek, CSc., of the Chemical Institute, Slovak Academy of Sciences, Bratislava. They are the results of a collective study directly joining the present communication.

Discussion

The observations presented in this paper indicate that clinical strains of *P. aeruginosa* show an extensive pathogenic potential and can produce symptoms of soft rot of storage tissues in a number of plant species. As far as we know from the literature, the phytopathogenicity of clinical strains of *P. aeruginosa* was first demonstrated on tomato fruits (Baldacci and Ciferri, 1934). Having studied 17 strains of *P. aeruginosa* from various sources, Elrod and Braun (1942) found that some clinical strains were capable of producing soft rot on tobacco, onion, cucumber, potato and lettuce. Recently reliable evidence was provided of the pathogenicity of seven clinical strains of *P. aeruginosa* for celery leaf peduncles, lettuce leaves and potato discs (Cho et al., 1975). Furthermore, the pathogenicity of *P. aeruginosa* was confirmed for sugarcane (Desai, 1935) and chrysanthemum (Stapp, 1934). In those cases, however, clinical strains were not involved. Serrada, Sanchez Serrano and Beltrá (1982) observed wilting of beans, sunflower and tomato following inoculation with a strain of *P. aeruginosa* (of unstated origin).

The possible pathogenicity of *P. aeruginosa* for root vegetables was suggested by Lebeda et al. (1982). In the present study the pathogenicity of clinical strains of *P. aeruginosa* has been demonstrated experimentally on roots of carrot and parsley and on stems of kohlrabi. Besides, the pathogenicity of these strains has been confirmed for onion and potato. Our series of 40 clinical and six further strains is probably the most extensive tested on plants so far. A number of strains have shown pathogenic specificity for certain plant species, which is in full accordance with the observations of Cho et al. (1975). Carrot was found most susceptible for the whole series of strains.

Having tested 14 strains (8 clinical and 6 from plants) of *P. aeruginosa*, Cho et al. (1975) concluded that it was impossible to determine a connection between production of pyocyanine or origin of the strain and their rotting capacity. The authors note, however, that such a connection could be found in a larger series of strains. Our results indicate that such a connection does exist for both criteria stated above. Strains producing pyocyanine generally showed greater pathogenicity for the plants tested than those producing fluorescein. A correlation was also found between the origin of the strains and their pathogenicity. Most strains originated from plants (DAR 26702, UCPPB 16 and UCPPB 61) were pathogenic to strongly pathogenic for the plants tested. On the other hand, strains isolated from soils (UCPPB 35 and UCPPB 60) were non-pathogenic for carrot and parsley and produced indistinct necroses on tobacco.

As for the hypersensitivity responses on tobacco, we observed a number of deviations from the theory of Klement (1963). In our observations, many strongly pathogenic strains (e.g. UCPPB 61, DAR 26702, 2431 R, 2424 R) could be classified, in view of the responses produced on tobacco, as non-pathogenic or saprophytic bacteria. It appears that it may be necessary to revise the differentiation system of Klement (1963).

Substantial differences have been evidenced in the pathogenicity of *P. aeruginosa* for animals. This fact has so far been pointed out only by Elrod and Braun (1941). In our case this capability has been unequivocally confirmed in strains markedly differing in their origin, i.e. in plant species from which they had been isolated (chrysanthemum, maize, onion) but also geographically (USA, Australia). Soil strains were non-pathogenic for animals, the same as strains of *P. fluorescens* and *P. syringae* pv. *syringae*. We strated to look for an explanation of these theoretically very interesting differences in the enzyme activity of the various strains. Our hitherto experiments (Zemek et al., unpublished) suggest that such close connections do exist. For example, protease was found in all but one strains pathogenic for animals. On the contrary, the enzyme does not occur in non-pathogenic strains (with a single exception again). A detailed explanation of this mechanism, however, will have to be looked for in a much wider range of enzymes as well as toxins or secondary metabolites.

To characterize the pathogenicity of the clinical but also other strains of *P. aeruginosa* from the plant species tested it is necessary to emphasize that it was manifested by a capability of producing symptoms typical of soft rot. The pathogenicity of the soft rot causing Erwinia carotovora, is correlated with a high activity level of polygalacturonase, pectatlyase, and cellulase (Beraha and Garber, 1971; Zucker et al., 1972), phosphatidase (Beraha et al., 1974) or with increased proteolytical activity (Friedman, 1962). Evidence has also been provided of the importance of pectolytical enzyme production for the pathogenicity of *Pseudomonas* sp. causing soft rot (Heuther and McIntyre, 1969; Hildebrand, 1972; Nasuno and Starr, 1966).

It is probable that even the pathogenicity of *P. aeruginosa* strains for plants could be explained by their capability of reproducing on plant tissues and producing enzymes indispensable for the breakdown of cell walls and membranes. The presence of pectolytical enzymes in *P. aeruginosa* was demonstrated by Coles (1926) and Prunier and Kaiser (1964). On the other hand, Zucker et al. (1972) failed to demonstrate pectatlyase activity in *P. aeruginosa* strain C 59. The above differences could account for the substantial differences in the pathogenicity of the different strains. Our preliminary results (Zemek et al., unpublished) suggest that a close correlation does exist between production of proteolytic enzymes and capability of producing soft rot in *P. aeruginosa*. At the same time, it is evident from our observations that this is no simple relation but a complex phenomenon in which some other enzymes such as protease, glucosidase etc. may participate. Besides enzymes also various metabolites may participate in the phytopathogenicity of *P. aeruginosa*. Serrada et al. (1982) connect this property with the capability of producing HCN. In relation to the cultivation media and animal tissues, this fact has been experimentally demonstrated by Patty (1921). *P. aeruginosa* also produces a number of secondary metabolites (Leisinger and Margraff, 1979) which could also participate in its pathogenicity for plants.

Although *P. aeruginosa* is abundant in soils and on plants (Green et al., 1974), in water, mainly sewage (Mašínová, 1979), on animals and humans (Jed-

ličková, 1981), its pathogenic potential can manifest itself under certain conditions only (mechanical lesions of plant tissues) higher temperatures and relative humidity of air).

On the basis of our results as well as observations of other authors *P. aeruginosa* can be considered as an opportunistic plant pathogen.

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Progress of Bacterial Leaf Blight Disease in Mixed Population of Resistant and Susceptible Rice Cultivars

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Progress of bacterial leaf blight disease caused by *Xanthomonas campestris* pv. *oryzae* was studied in mixed plantings of susceptible (Taichung Native 1) and resistant (Ramakrishna and CR 44-117-1) rice cultivars. A significant decrease in the infection rate was observed in 1–50% susceptible host due to the presence of 50–99% resistant host. The infection rate of resistant host was not influenced by any proportion of susceptible host in the mixture. Based on the average score value in the mixed stand, 30 S : 70 R proportion appeared to serve as equilibrium mixture. The implications of these findings in host pathogen equilibrium are discussed.

Bacterial leaf blight disease of rice caused by *Xanthomonas campestris* pv. *oryzae* has emerged as one of the major constraints in rice production, as evidenced by the recent outbreak of epidemic in the Punjab and Haryana states of India (Reddy, 1980). So far no satisfactory control measure has been evolved against this disease and therefore host resistance is given priority in disease management. Continuous use of resistance genes for control of several plant diseases has caused a shift in pathogen population, in terms of virulence or races, which could be prevented or delayed by use of broad based resistance in multiline varieties, composed of a number of different host lines, each with different genes for resistance against different pathogenic races. Even though such an attempt has not been made to evolve multiline cultivars against bacterial blight disease of rice, mixture of host plants with different degrees of resistance might lead to minimise disease incidence and stabilise pathogen population. Measurement of the rate of disease increase in host mixtures in field plots provide a basis for better understanding the effect of these mixtures on disease increase, rate of evolution of new strains of the pathogen and maintenance of host-pathogen equilibrium. Mode (1956) recognized the effect of diverse host populations on the reduction in incidence of neck blotch of barley in understanding the dynamics of host-pathogen relationship. An attempt has been made, through the present experiment, to measure the rate of disease increase in host mixtures of resistant and susceptible varieties.

Materials and Methods

Twenty five-day-old healthy seedlings of the highly susceptible variety Taichung Native 1 (TN-1) were planted in individual plots mixed at different pro-

Table 1

Arrangement of susceptible and resistant plants in the mixture

Percent of susceptible plants	Plant arrangements
1	One susceptible plant at the centre of the plot containing 99 resistant plants
10	Ten susceptible plants randomly distributed in the plot containing 90 resistant plants
30	Thirty susceptible plants randomly distributed in the plot containing 70 resistant plants
50	Alternate rows of susceptible and resistant plants
70	Thirty resistant plants randomly distributed in the plot containing 70 susceptible plants
90	Ten resistant plants randomly distributed in the plot containing 90 susceptible plants
99	One resistant plant at the centre of the plot containing 99 susceptible plants

portions of the resistant varieties CR 44-122-1 (Ramakrishna) and CR 44-117-1 separately as two field experiments. Each experiment constituted seven treatments of different proportions of host mixture (Table 1) planted in randomised complete block design replicated thrice. Each treatment consisted of one plot of ten lines, each line with ten plants, planted in a spacing of 20×20 cm. Each plot was separated from the neighbouring plot by one meter strip of cultivated fallow. Ammonium sulphate was applied at the rate of 100 kg N/ha in two split doses. Natural incidence of the disease was scored at weekly intervals, using 1–9 scale of the SES developed by the International Rice Research Institute, Philippines (Anonymous, 1980), beginning from the first day of the disease appearance till the susceptible variety got highest score value. The average score for the mixed stand was computed by sum of score values of individual plants in the mixed stand divided by total number of plants. The increase or decrease in the disease reaction in one variety due to the influence of the other over the respective 99% stand was calculated as per cent reduction or increase in score value from that of 99%. The apparent infection rate ('r') was computed as regression coefficient of $\log_e(x/1 - x)$ on time (Van der Plank, 1963) and is presented here as per unit per day.

Results

Average disease incidence

The disease first appeared in the susceptible variety in both the experiments. The initiation of the disease was delayed by a fortnight in the variety Ramakrishna and by 21 days in CR 44-117-1. The progress of epidemic was more rapid in the

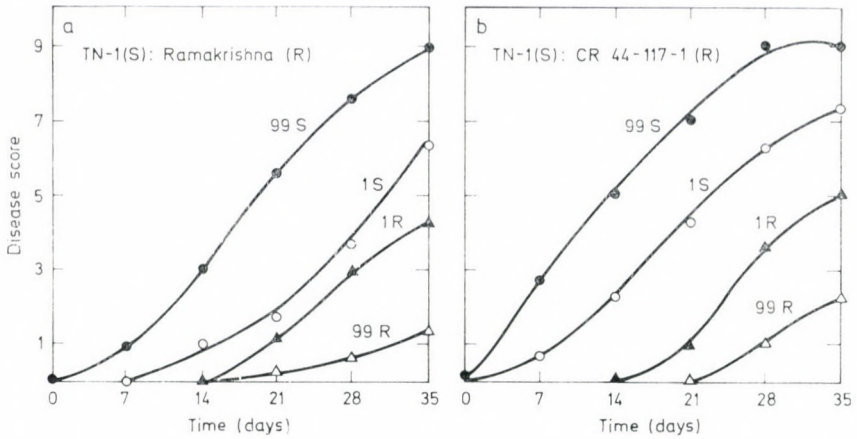

Fig. 1. Disease progress curves in each component of the mixture, Taichung Native 1 (S) : Ramakrishna (R) (*a*) and Taichung Native 1 (S) : CR 44-117-1 (R) (*b*)

susceptible variety than both the resistant varieties, resulting in maximum score between 21 to 28 days. The average disease score of both the resistant varieties remained low during the period (Fig. 1). Disease progress in the susceptible variety in 1 S : 99 R was more rapid than in the resistant variety in 1 R : 99 S mixture. Comparatively late initiation and slow development of the disease imparted both the resistant varieties to act like horizontal resistant types.

Disease increase in the mixed stand

The disease incidence in mixed stand increased with increase in the proportion of susceptible host in the mixture (Fig. 2). The maximum reduction in disease

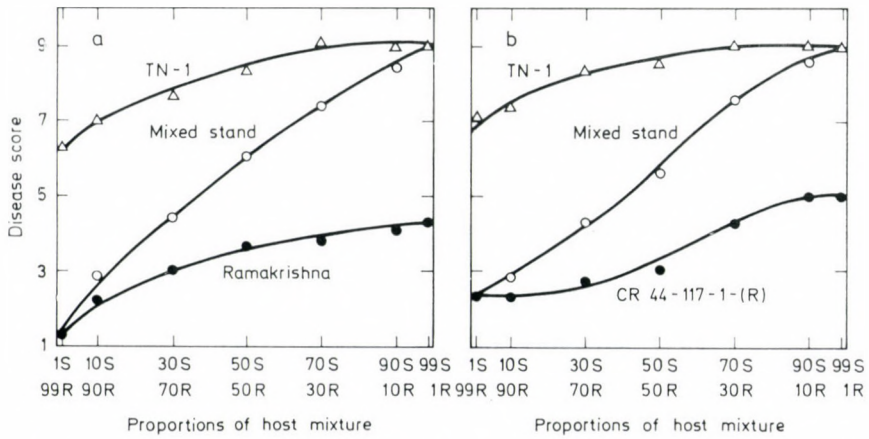

Fig. 2. Disease incidence in different proportions of individual stand and mixed stand in TN-1: Ramakrishna (*a*) and TN-1 : CR 44-117-1 (*b*)

Table 2

Disease score and infection rate (r) in each component of the mixture between Taichung Native 1 (S) and Ramakrishna (R)

Components of mixture	Disease incidence						Infection rate	
	Susceptible stand		Resistant stand		Mixed stand			
S R	Score	% decrease over 99 S	Score	% increase over 99 R	Average score	% decrease over 99 S	S	R
1 : 99	6.33a[1]	29.67	1.33a	0	1.38	84.67	0.143a	0.098
10 : 90	7.00ab	22.22	2.33ab	75.19	2.80	68.89	0.159a	0.138
30 : 70	7.67abc	14.78	3.00bc	125.56	4.40	51.11	0.248ab	0.165
50 : 50	8.33bc	7.44	3.67c	176.70	6.00	33.33	0.267ab	0.154
70 : 30	9.00c	0	3.67c	176.70	7.42	17.55	0.321b	0.121
90 : 10	9.00c	0	3.67c	176.70	8.47	0.59	0.321b	0.121
99 : 1	9.00c	0	4.33c	225.56	8.95	0.55	0.321b	0.143 NS

S = Susceptible, R = Resistant
[1] Means in a column followed by the same letter do not differ significantly at P = 0.05
NS = Not significant

incidence in the mixed stand was 84.67 and 73.55% at 1 S : 99 R proportion for Ramakrishna and CR 44-117-1, respectively. A corresponding reduction of 51.11 and 51.55% was obtained at 30 S: 70 R host mixture thus indicating that this proportion might serve as equilibrium mixture (Tables 2 and 3).

Table 3

Disease score and infection rate (t) in each component of the mixture between Taichung Native 1 (S) and CR 44-117-1 (R)

Components of mixture	Disease incidence						Infection rate	
	Susceptible stand		Resistant stand		Mixed stand			
S R	Score	% decrease over 99 S	Score	% increase over 99 R	Average score	% decrease over 99 S	S	R
1 : 99	7.33a[1]	18.55	2.33a	0	2.38	73.55	0.132a	0.145
10 : 90	7.33a	18.55	2.33a	0	2.83	68.55	0.129a	0.112
30 : 70	8.33ab	7.44	2.67a	14.16	4.36	51.55	0.254ab	0.112
50 : 50	8.33ab	7.44	3.00a	28.75	5.67	26.00	0.237ab	0.132
70 : 30	9.00b	0	4.33b	85.83	7.59	15.64	0.301b	0.143
90 : 10	9.00b	0	5.00b	114.59	8.60	4.44	0.278b	0.165
99 : 1	9.00b	0	5.00b	114.59	8.96	0.44	0.278b	0.165 NS

S = Susceptible, R = Resistant
[1] Means in a column followed by the same letter do not differ significantly at P = 0.05
NS = Not significant

Disease increase in each component of the mixture

Although there was a significant reduction in disease incidence in the 1 to 30% susceptible stand under the influence of 99 to 70% resistant host of Ramakrishna; with CR 44-117-1 mixture, a significant reduction was observed from 1 to 50% susceptible stand (Tables 2 and 3). Similarly, the increase in disease incidence in Ramakrishna was observed to be significant for 1 to 70% stand while that for CR 44-117-1 it was from 1 to 30% stand. The amount of disease increase compared to the 99% stand of resistant host was upto 225.56% and 114.59% for 99 S : 1 R mixed stand in Ramakrishna and CR 44-117-1 respectively. Thus high proportion of susceptible host in the mixture induced the resistant host to act as moderately resistant, when the analysis was based on the final score value.

Infection rate

Analysis of infection rate, computed on the basis of entire period of disease development, revealed no significant difference in 1 to 50% susceptible stand under the influence of both the corresponding resistant stands. The infection rates in both the resistant varieties on the other hand were not influenced by any of the proportion of susceptible stand even though there was a significant influence when the analysis was based on the final score value (Tables 2 and 3).

Discussion

Bacterial blight infection originates in a few foci in a large plant population of susceptible rice cultivars under field condition (Nayak and Ranga Reddy, unpublished). In the present study infection originated only on the plants of the susceptible cultivar in a mixed population of two different rice cultivars. The probability of the inoculum reaching the next susceptible plant depends on the proportion and distribution of susceptible plants in the host mixture. In other words, the resistant plants in the host mixture act as a barrier to production of inoculum as well as spread of the disease. Hence, higher proportions of resistant plants in the host mixture were observed to reduce the rate of infection even in the susceptible host plants compared to those with higher proportions of susceptible stand. This is more reflected in the reduction in disease incidence due to the mixed stand over the 99% stand of susceptible host (Tables 2 and 3).

The significant increase in disease development in the lower proportions of resistant host, grown with higher proportions of the susceptible hosts in the mixture, indicated that these varieties might not be genetically stable for resistance. But, when the entire period of epidemic was taken into consideration, the same resistant varieties did not show any significant difference between any proportions in the mixed stand, possibly because the regression coefficient ('r') of $\log_e(x/1-x)$ upon time takes care of the factors responsible for such differences.

Bacterial blight pathogen can multiply at a faster rate on a susceptible host than on a resistant host thereby causing more damage to the former than the latter (Reddy and Kauffman, 1973). The disease intensity can also be influenced to a great extent if infected by mixture of strains of the pathogen possessing different degrees of virulence (Devadath, 1970; Reddy and Kauffman, 1974). In an equilibrium mixture, the rate of reproduction of different strains of the pathogen should be the same, and such a mixture may lead to diversification of resistance as a method of stabilizing the pathogen population by delaying or preventing the build up of new strains (Leonard, 1969). The level of equilibrium mixture is also subject to variation depending upon the degree of susceptibility of individual components of the mixture as well as the rate of reproduction of the strains of the pathogen on each component. Even though host pathogen equilibrium can be achieved by careful manipulation of host mixtures, at present such a practice is impracticable in rice cultivation from production point of view till isogenic lines are evolved and till the pathogen is differentiated into distinct pathotypes. The implication of the present findings in (*i*) the rate of development of bacterial blight disease in multiline cultivars, (*ii*) in screening nurseries and (*iii*) in vast areas of farmers field covered by different varieties with varying degrees of resistance grown as crop mosaic, is obvious and should be studied critically.

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The Transmission of Arabis Mosaic Virus to Peach (*Persica vulgaris* Mill.) and Apricot (*Armeniaca vulgaris* Lam.) Seedlings by Using the Nematode *Xiphinema diversicaudatum* (Micoletzky) Thorne

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According to the studies the individuals of the nematode *Xiphinema diversicaudatum* (Micoletzky) Thorne transmitted the arabis mosaic virus originating from strawberry, to stone fruit seedlings, i.e. peaches (*Persica vulgaris* Mill. cv. Elberta) and apricots (*Armeniaca vulgaris* Lam. cv. CGY 580).

The host range of arabis mosaic virus (AMV) is extremely broad, containing both herbaceous and woody plants (Cropley, 1961; Harrison and Winslow, 1961; Dorst and Van Hoof, 1965; Lister and Murant, 1967; Murant and Lister, 1967; Taylor and Thomas, 1968; Lister, 1970; Berks et al., 1976). From among the woody species belonging to the family Rosaceae the virus was isolated so far only from cherry (Cropley, 1961) and peach (Vértesy and Schuster, 1979). Also the host range of *Xiphinema diversicaudatum* is extremely wide, comprising both mono- and dicotyledonous species (Harrison and Winslow, 1961; Cohn, 1969; Fritzsche and Hofferek, 1969; Prota et al., 1971; McElroy, 1972; Arias, 1975; Cotten, 1977). From among the Rosaceae only *Malus domestica* Borkh., *Cerasus avium* Mönch, *Persica vulgaris* Mill., *Prunus cerasifera* Ehrh., *Prunus domestica* L. and *P. spinosa* L. was known (Harrison and Winslow, 1961; Fritzsche and Hofferek, 1969; Pitcher et al., 1974). It was considered as justified to study the potential of *X. diversicaudatum* in the transmission of AMV to some stone fruits.

Methods

The possibility of AMV transmission by *X. diversicaudatum* nematodes was studied on peach- and apricot seedlings that emerged in the same year and had been planted into a soil infected by AMV.

In the soil used for the experiment earlier strawberry was grown for many years, infected by AMV. The nematodes were extracted from the soil samples for monitoring purposes by using the modified Cobb-system (Flegg, 1967). In samples of 250 ml 32—46 individuals were found. Of the presence of AMV we ascertained ourselves by using the bait-plant method described by Cadman and Harrison (1960).

The peach and apricot seedlings were grown from seeds germinated in the spring and were planted into the soil by the begin of June; 10 seedlings from each

species were planted into soil samples infected by AMV and containing *X. diversicaudatum* nematodes and 10 from both species into soil free of both agents. Early in autumn (22 September) the seedlings were transplanted into larger pots and sterilized soil and the plants were placed into field isolators. The percentages of virus transmission were established in the spring of the third experimental year.

In course of the survey leaves of cucumber (*Cucumis sativa* cv. Delicatesse) and *Chenopodium murale* plants were inoculated with the sap of young shoots of peach and apricot seedlings, to which also phosphate buffer was added.

The infection of seedlings was controlled also by ELISA test. This study was carried out by using the method of Clark et al. (1976), modified in some points by György (1980).

Results

From the 10 seedlings of each species 4 peach and 3 apricot seedlings was found infected. The results gained with the indicator plants were in accordance with the data of the ELISA tests. The check proved to be free of infection also by ELISA test.

According to the knowledge gathered so far regarding the host plants both of AMV and *X. diversicaudatum* it could be assumed by good reason that AMV could be transmitted also to stone fruits by its vector. It was proved experimentally, however, the first time in our studies that AMV, originating from strawberry, was transmitted by *X. diversicaudatum* individuals to stone fruits i.e. peaches and apricots. From these, apricot (*Armeniaca vulgaris*) is a new host of AMV.

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Study of the Multiplication of Potato Virus X in Variously Aged Leaves of *Datura stramonium* L.

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The reproduction of potato virus X (PVX) was studied in detached leaves of *Datura stramonium* L. during the investigation of infectivity of tissues and in ultrathin sections. In very young upper leaves the virus multiplies much less than in more mature and moderately old leaves. In parenchyma cells of tip parts of upper leaves the virus was mainly present in dispersed form and small aggregates were observed rarely. In cell areas with scattered poorly stained virus particles, a large number of PVX-specific laminated inclusions were usually observed. In mature cells, in addition to scattered PVX particles associated with laminar inclusions, aggregates of virions of various size and density were often observed. In cells of moderately old leaves sometimes small compact PVX aggregates were found that might be bordered by laminated bodies. In this case rather large virus groups were usually found often in an unusual flake-like form. Large masses of PVX in mature and moderately old cells were not associated with laminar inclusion components.

One of the important factors influencing the reproduction of plant viruses is the tissue age of the plant host. It has been shown in a number of papers on systemically infected plant hosts (Solberg and Bald, 1962; Faed and Matthews, 1972; Reunov et al., 1980) that fewer viruses accumulate in very young tissues than in more mature ones. According to Takahashi (1971) the distribution of TMV in leaves of various tiers of *N. tabacum* cv. Bright Yellow showed a U-shaped form: maximal virus content was found in the inoculated leaf (third leaf from the bottom) and in the young developing leaf, which showed the first symptoms, while the intermediate leaves had a relatively small virus concentration. In experiments by Fraser (1972), the largest TMV content was found in leaves of *N. tabacum* L. cv. Samsun which were 1.5–8 cm in length at the moment of infection.

It should be noted that in systemically infected leaves the accumulation of virus particles in them is caused both by replication and by the transport of virus with assimilates. In young upper leaves the role of translocated infectious material with photosynthate in virus accumulation is especially large, as these leaves have a high demand for assimilates (Kursanov, 1976). Evaluation of the ability of tissue of various age to support virus replication is apparently only possible on detached leaves.

The present work presents data on the study of PVX multiplication in detached leaves of various age of *Datura stramonium* L.

Materials and Methods

The study was conducted on the tip parts of very young upper leaves (about 3 cm long), fully developed leaves and moderately old leaves of five-week old plants of *D. stramonium* L. grown in a greenhouse. Detached leaves were cut in half along the center vein, dusted with Carborundum, and one half was inoculated with sap of *D. stramonium* leaves infected with a severe strain of PVX ("Tayozhny") (Reifman and Kolesnikova, 1973) and the other half with sap of healthy leaves. The leaves were washed with water and placed in a moisture chamber for four days.

In experiments on determination of tissue infectivity, four days after inoculation five disks 5 mm in diameter were punched from the variously-aged half-leaves. The disks were homogenized in 0.05 M phosphate buffer, pH 7.0, and after suitable dilution (10^{-2}), the homogenates obtained were titrated on 10–15 half-leaves of *Gomphrena globosa* in relation to purified control preparations of PVX (10^{-2} mg/ml). Experiments were repeated five times.

For evaluation of the amount of viral inhibitors in the experimental tissues, homogenates prepared from half-leaves of various age rubbed with sap of healthy leaves of *D. stramonium* with Carborundum after suitable dilution (10^{-2}) were mixed with a purified PVX preparations whose final concentration was 10^{-2} mg/ml. After retention at room temperature for ten minutes, the homogenate mixtures of variously-aged tissues with the virus were titrated in relation to each other on 15 half-leaves of *G. globosa.*

Small pieces of infected tissue intended for study under the electron microscope were fixed for 3 h in 6.5% solution of glutaraldehyde prepared on phosphate buffer, pH 7.4. The samples were postfixed for two h in 1% osmium tetroxide followed by dehydration in acetone and alcohol and were embedded in Epon epoxy resin. Sections were stained with uranyl acetate and lead citrate and observed on an EMV-100 L (USSR) electron microscope.

Results

Our results show that in very young detached leaves of *D. stramonium* very much less PVX accumulates, judging by infectivity, than in mature and aging leaves (Fig. 1). The highest level of infectivity was observed in moderately old leaves. The inhibitory qualities of the homogenates from tissues of upper, mature and moderately old leaves in dilution of 10^{-2}, in which tissue infectivity was evaluated in relation to purified PVX preparations (10^{-2} mg/ml) did not differ essentially. The results of infectivity determination correlate with the data obtained in investigating ultrathin sections of infected tissues.

In cells of very young tip parts of upper leaves the virus showed mainly in the form of dispersed virions in the cytoplasm and only rarely were small particle aggregates seen. In areas containing dispersed virions, a large quantity of PVX-

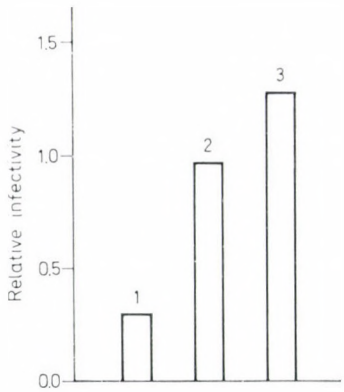


Fig. 1. Relative infectivity* of homogenates obtained from upper (1), mature (2) and aging (3) leaves of *D. stramonium* four days after PVX inoculation.+

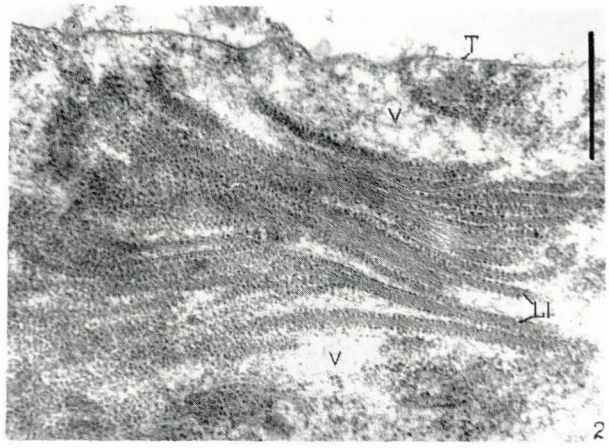
specific laminated inclusion bodies were observed, such as has been reported by numerous authors (Kozar and Sheludko, 1969; Stols et al., 1970; Shalla and Shepard, 1972; Reunov and Reunova, 1977). Often these bodies were present on sections as observed earlier (Reunov and Reunova, 1977) in bundles of laminar structures, which might be limited by ribosome-like particles (Fig. 2). We very often observed twisted laminar structures, within which virions were located (Fig. 3). Viral particles, dispersed along laminated inclusion components, usually were seen as faintly stained filaments, the diameter of which was much less than the normal diameter of PVX (Figs 2 and 3). In cell areas where accumulations of laminar bodies were observed, the PVX particles might take flake-like form and were difficult to distinguish (Fig. 2).

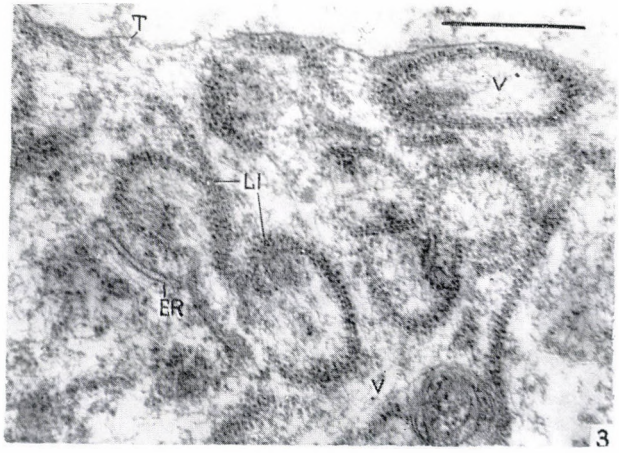
Likewise in cells of mature leaves of middle tiers, areas of cytoplasm were often observed that contained a relatively small quantity of PVX particles associated with laminated inclusions. Such areas of infected mature cells were similar to those presented in Figs 2 and 3. However, in contradistinction to cells of upper leaves, in cells of mature leaves aggregates of PVX particles were often observed of various size and density not usually associated with laminar structures (Fig. 4). The diameter of virions in the aggregates were close to normal.

In some cells of moderately old leaves of lower tiers in electron dense areas of the cytoplasm, comparatively small compact aggregates of PVX particles of

* Expressed as average value of N/N_0 obtained on 15 leaves of *G. globosa*, where N is the number of local lesions on the experimental half-leaves of *G. globosa* inoculated with the sap of infected *D. stramonium* leaves (diluted to 10^{-2}); N_0 is the number of local lesions on the control half-leaves of *G. globosa* inoculated with purified PVX preparation (10^{-2} mg/ml). The value of N_0 varied from 30 to 60 in the experiment

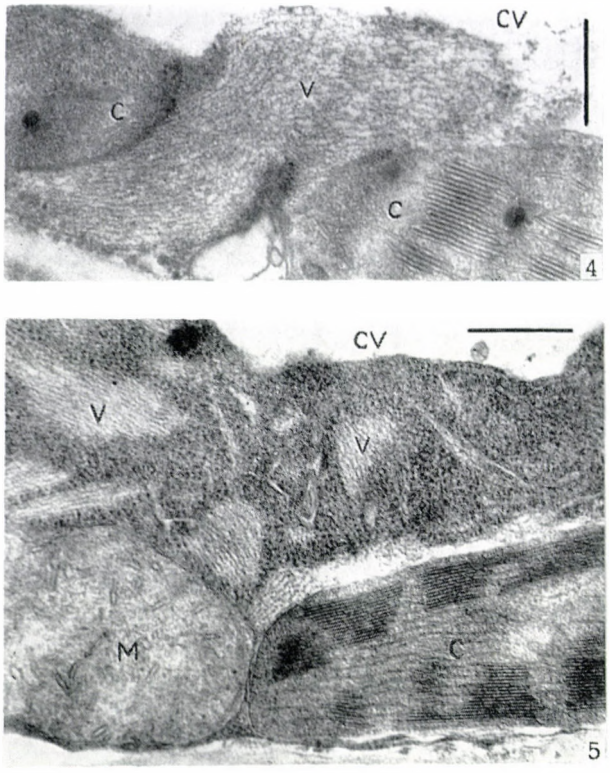
\+ Results of a representative experiment are presented.





Figs 2, 3. PVX particles (V) and laminar inclusions (LI) in parenchyma cells of young upper leaves of *D. stramonium*. T — tonoplast, ER — endoplasmic reticulum. Bar = 500 nm

normal diameter were observed, which might be limited laminar structures (Fig. 5). In most of the cells of aging leaves examined, PVX was seen as particle aggregates similar to those found in mature leaf cells (see Fig. 4) or in large masses of unusual flake-like structures (Figs 6 and 7). Virus accumulations in such cells were usually accompanied by progressive development of destructive processes. Frequently, electron dense collapsed areas of cytoplasm were observed in contact with masses of PVX (Fig. 7).

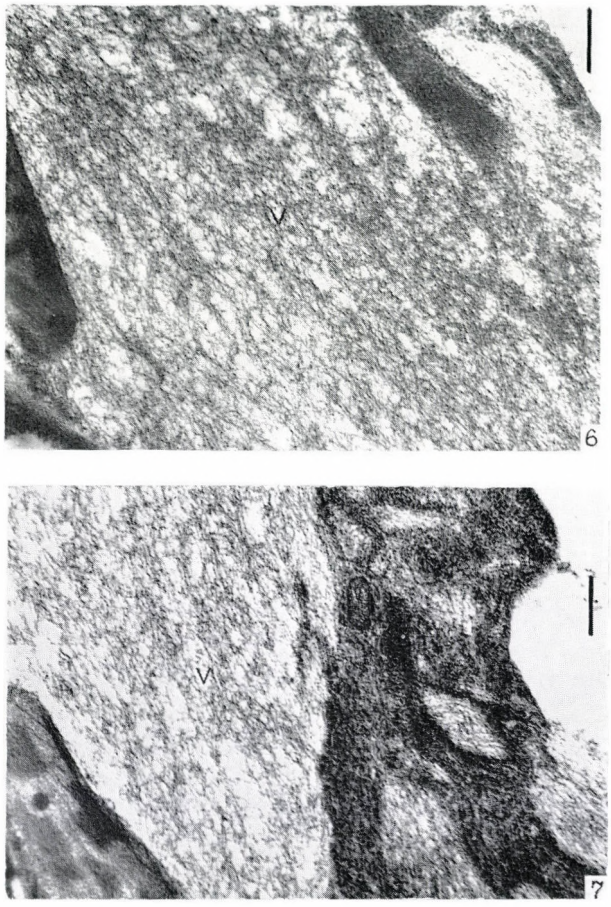


Figs 4, 5. PVX virions (V) in aggregate form in parenchyma cells of mature (Fig. 4) and moderately old (Fig. 5) leaves of *D. stramonium*. CV — central vacuole, C — chloroplast, M — mitochondria. Bar = 500 nm

Discussion

The accumulated PVX in tissues of detached leaves of *D. stramonium* observed in our experiments did not depend on the translocation of infectious material throughout the plant, as in the systemically infected tissues of young growing leaves (Solberg and Bald, 1962; Takahashi, 1971; Fraser, 1972; Faed and Matthews, 1972; Reunov et al., 1980) and, therefore, was caused only by the multiplication of the virus.

In evaluating virus content in plant tissue in terms of infectivity, it is necessary to take into account that the infectivity of leaf homogenates may be influenced by inhibitors contained in them (Gendon and Kassanis, 1954; Bawden and Pirie, 1957). In our experiments, the inhibitory qualities of homogenates from very young tissues of upper leaves and from more mature leaves of *D. stramonium* in relation to purified PVX preparations in dilution of 10^{-2}, used to evaluate tissue



Figs. 6, 7. Amorphous accumulations of PVX (V) in parenchyma cells of moderately old leaves of *D. stramonium*. Bar = 500 nm

infectivity, did not essentially differ, and, therefore, could not affect the virus content determination. Thus, the results obtained in determining the infectivity of tissue of varying age indicate that on the fourth day after inoculation PVX multiplies much less in very young tip parts of upper leaves of *D. stramonium* than in mature and aging leaves.

The data from electron microscope observations is in good agreement with the data on tissue infectivity, indicating that the cells of very young leaves of *D. stramonium* apparently were less able to support PVX synthesis than those of mature and aging leaves. The question of what factors limit PVX replication in cells of very young tissue requires further study.

Data on the relation between quantities of PVX particles and PVX-induced laminar structures in cells may excite a certain interest. In our experiments on sec-

tions of cells of upper, and sometimes even of mature leaves, where relatively few PVX particles were found, we observed a large number of laminated bodies; in areas densely filled with these bodies there were only individual faintly tinted virions. On the contrary, in cells of aging leaves large masses of PVX particles which were not associated with laminated inclusions were usually observed. These data may be considered as evidence in favor of the hypothesis advanced earlier (Reunov and Reunova, 1977) concerning the protective role of laminated bodies. In this connection, recent data (Lapshina and Reunov, 1979) has been advanced on the inhibiting effect of cell fractions containing PVX-induced laminated structures on PVX infectivity.

Attention should be focused on some variations in the morphology of PVX particles that we found in the sections of investigated tissues. While virions in aggregate form were dyed very effectively and appeared sufficiently electron dense with a diameter nearly that of normal PVX, the same as on the microphotographs of other authors (Kozar and Sheludko, 1969; Stols et al., 1970; Shalla and Shepard, 1972; Doraiswamy and Lesemann, 1974), the PVX particles scattered among the laminar bodies had a poorly contrasting appearance and their diameter was much less than normal.

This fact that PVX could have a diameter much less than usual on sections was noted by us in our studies of the ultrastructure of systemically infected with PVX young upper leaves of *D. stramonium* (Reunov, 1979). Earlier an analogous fact was established for TMV (Kolehmainen et al., 1965; Esau, 1968) and was explained by the fact that capsid protein on sections may be faintly stained with uranyl acetate and lead, and the basic contribution to the diameter of virions observed is given by the more effective stain nucleic acid. This explanation is to a certain extent acceptable for PVX in our experiments, but it is not completely satisfactory. The very pale appearance of the virions on a background of electron dense protein laminar bodies and the occasional flake-like form which they assume raises the question: might not such PVX particles have undergone destructive changes?

The unusual amorphous flake-like PVX masses which we observed frequently in the degenerating cells of aging leaves of *D. stramonium* (Figs 6 and 7) also suggest that in similar accumulations virus particles could undergo degradation.

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Reaction of *Physalis* Species to Plant Viruses. VIII. *Physalis glabripes* Pojark, as a New Host of Various Plant Viruses

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As for the virus susceptibility of *Physalis glabripes* Pojark, a plant hardly known in the literature on plant virology, 15 new host-virus relations and 5 new incompatible relations have been pointed out. Of the compatible host-virus relations one proved local (tobacco necrosis virus) and 14 local and systemic (alfalfa mosaic virus, *Arabis* mosaic virus, belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, *Melandrium* yellow fleck virus, potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus, tobacco ring spot virus, tomato aspermy virus, tomato mosaic virus, turnip mosaic virus). The only literary reference to the susceptibility of *Physalis glabripes* to tobacco rattle virus was confirmed in comparative trials. In our experiments the *Physalis glabripes* proved resistant to bean common mosaic virus, bean yellow mosaic virus, carnation ring spot virus, lettuce mosaic virus and watermelon mosaic virus.

Various *Physalis* species have recently been playing an increasingly important role in plant virology as natural virus hosts, artificial virus indicators, differential plants (virus separators) and production hosts, respectively. *Physalis floridana* Rydb., one of the best known species, which according to our knowledge is susceptible to some 45 viruses and suitable for the differentiation of numerous viruses (reviewed by Horváth, 1970, 1974b, 1976) has recently been found to be – as a common weed – good production host to satsuma dwarf virus (Tanaka and Imada, 1974) and natural host e.g., to groundnut eyespot virus (Dubern, 1981). In the course of investigations in the last several years *Physalis floridana* played an important role in the identification of potato viruses occurring in the Andes (e.g., Andean potato mottle virus, Andean potato calico strain of the tobacco ring spot virus, Andean potato latent virus and wild potato mosaic virus) too (Fribourg, 1977; Fribourg et al., 1977a, b; Jones and Fribourg, 1979). Furthermore, those results obtained lately which show the species *Physalis floridana* and other *Physalis* species (e.g., *Physalis angulata* L., *P. pubescens* L.) to be suitable for the postharvest indexing of certain potato viruses are also remarkable (Singh et al., 1979; Sing and Smith, 1981; Sing, 1982). The importance of the *Physalis* species is increased by the fact that many of them (e.g., *Physalis alkekengi* L., *P. angulata*, *P. floridana*, *P. heterophylla* Nees, *P. ixocarpa* Brot., *P. mendonica* Phil., *P. minima* L., *P. pruinosa* L., *P. subglabrata* MacKenzie et Bush.) are known as natural virus hosts (Feldman and Gracia, 1973; Schmelzer et al., 1973; Lovisolo and Bartels, 1970; Moline and Fries, 1974; Peters and Dercks, 1974; Horváth, 1975b, 1983; Joshi and Dubey, 1976).

In the course of further studies on the artificial relations between *Physalis* species and viruses – covering about 26 *Physalis* species, one *Physalis* varieties and 90 viruses according to our present knowledge (cf. Horváth, 1983) – investigations have been made with *Physalis glabripes*, a species not long since known in plant virology. According to data by Ambrosaj and Davidchuk (1978) this plant is susceptible to tobacco rattle virus. Considering that there are no data concerning its susceptibility to other viruses, we have carried out examinations in order to establish the responses of *Physalis glabripes* to various plant viruses.

Materials and Methods

In our experiments young *Physalis glabripes* plants were inoculated with 20 plant viruses to which the responses of *Physalis glabripes* had not been known so far (Table 1). As to the maintenance of the viruses, the methods of inoculation, the re-isolation of viruses from inoculated, non-inoculated, or subsequently developed leaves, and the test- or indicator plants detailed data can be found in our earlier publications (cf. Horváth, 1974a, 1976, 1977, 1981, 1983).

Results and Conclusions

In the course of investigations on the virus susceptibility of *Physalis glabripes* the plant under discussion proved to be local host to one virus, and local and systemic host to 14 viruses, while showing resistance to further 5 viruses (see Table 1). Plants inoculated with tobacco necrosis virus exhibited no symptoms, although from the inoculated leaves of *Physalis glabripes* the virus could be re-isolated to indicator plants, so that the plant in question can be regarded as latent local host to tobacco necrosis virus. We mention here that other *Physalis* species (e.g., *Physalis aequata* Jacq., *P. floridana*, *P. ixocarpa*, *P. pubescens*, *P. viscosa* L.) tested in our earlier experiments were also found to be locally susceptible to inoculation with tobacco necrosis virus, but they responded with manifest disease symptoms (Horváth, 1974c, 1975a, b). Other *Physalis* species (e.g. *Physalis angulata*, *P. curassavica* L., *P. peruviana*, *P. pruinosa* var. *macrocarpa*), on the other hand, proved immune of tobacco necrosis virus (Horváth, 1974a, b, 1981). Thus, the host-virus relations between the *Physalis* species and the tobacco necrosis virus seem to be highly heterogenous. Considering that we used one and the same strain of tobacco necrosis virus in our experiments, we are of the opinion that the differences in susceptibility between the *Physalis* species can be traced back to the diverse characteristics of the test plants rather than to virus strain differences.

As to the local and systemic relations between *Physalis glabripes* and various viruses (see Table 1) the results obtained in connection with the alfalfa mosaic virus are first mentioned. In the course of experiments with the so-called systemic strain of the alfalfa mosaic virus (MvS70) we found that while the inoculated leaves

Table 1

Reaction of *Physalis glabripes* Pojark to some plant viruses

Viruses	Symptoms[1]	Results of the reisolation of the investigated viruses
Alfalfa mosaic virus	IL: no symptoms NIL: yellow mosaic	IL: positive NIL: positive
Arabis mosaic virus	IL: no symptoms NIL: no symptoms	IL: positive NIL: positive
Bean common mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Bean yellow mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Belladonna mottle virus	IL: necrotic rings NIL: chlorotic and necrotic rings	IL: positive NIL: positive
Broad bean wilt virus	IL: chlorotic lesions NIL: yellow mosaic	IL: positive NIL: positive
Carnation ring spot virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cucumber mosaic virus	IL: chlorotic and necrotic lesions NIL: severe mosaic	IL: positive NIL: positive
Lettuce mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Melandrium yellow fleck virus	IL: chlorotic and necrotic lesions NIL: severe mosaic	IL: positive NIL: positive
Potato aucuba mosaic virus	IL: chlorotic lesions NIL: mosaic	IL: positive NIL: positive
Potato virus X	IL: no symptoms NIL: severe mosaic	IL: positive NIL: positive
Potato virus Y	IL: chlorotic lesions NIL: yellow mosaic	IL: positive NIL: positive
Tobacco mosaic virus	IL: chlorotic and necrotic lesions NIL: mosaic	IL: positive NIL: positive
Tobacco necrosis virus	IL: no symptoms NIL: no symptoms	IL: positive NIL: negative
Tobacco ring spot virus	IL: necrotic rings NIL: necrotic lesions	IL: positive NIL: positive
Tomato aspermy virus	IL: chlorotic lesions NIL: mosaic	IL: positive NIL: positive
Tomato mosaic virus	IL: chlorotic and necrotic lesions NIL: mosaic	IL: positive NIL: positive
Turnip mosaic virus	IL: chlorotic and necrotic lesions NIL: mosaic	IL: positive NIL: positive
Watermelon mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative

[1] IL: Inoculated leaves, NIL: non-inoculated leaves, or subsequently developed leaves.

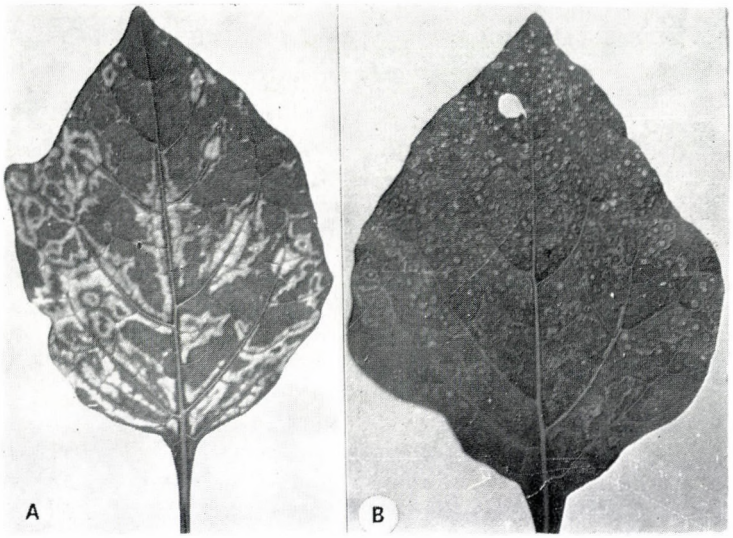

Fig. 1. Systemic symptoms on *Physalis glabripes* Pojark inoculated with alfalfa mosaic virus (A) and local lesions on the inoculated leaves of *P. glabripes* inoculated with tobacco rattle virus (B)

of the experimental plants did not show disease symptoms, the virus was easy to re-isolate from them, and so the *Physalis glabripes* is regarded as such a local and systemic host of the alfalfa mosaic virus in which the disease is locally latent. The systemic infection of the plant was manifested in conspicuous ochre mosaic spots (Fig. 1A). *Physalis glabripes* plants inoculated with *Arabis* mosaic virus did not display either local or systemic symptoms – unlike the manifest disease of *Physalis minima* (cf. Horváth, 1983) –, still the virus could be reisolated from both the inoculated and non-inoculated leaves. The *Physalis glabripes* can thus regarded as a latent local and latent systemic host to arabis mosaic virus. Other local and systemic relations (belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, *Melandrium* yellow fleck virus, potato aucuba mosaic virus, potato virus Y, tomato aspermy virus, tobacco mosaic virus, tomato mosaic virus, tobacco ring spot virus and turnip mosaic virus) were characterized by chlorotic-necrotic lesions on the inoculated leaves and mosaic on leaves formed after the inoculation. Speaking of local and systemic host-virus relations we call attention to potato virus X which did not induce symptoms in the leaves of inoculated *Physalis glabripes* plants, although it was easily re-isolated from them. The non-inoculated leaves and the axillary shoots exhibited severe mosaic symptoms. As to the nature of the local and systemic host-virus relations certain differences were observed between the responses of *Physalis glabripes* to broad bean wilt virus and *Melandrium* yellow fleck virus and those of *Physalis minima* and *P. curassavica* to the same viruses. According to the results of our earlier experiments *Physalis minima*

and *P. curassavica* while only systemically susceptible to broad bean wilt virus were both locally and systemically susceptible to *Melandrium* yellow fleck virus, although the systemic disease in them was latent (cf. Horváth, 1981, 1983).

The data by Ambrosaj and Davidchuk (1978) – concerning the susceptibility of *Physalis glabripes* to tobacco rattle virus – were confirmed in our experiments. We found the experimental plant under discussion to respond to the above virus with small, necrotic circular and semi-circular lesions as well with mosaic and ringspot symptoms even affecting the non-inoculated leaves and the axillary shoots (Fig. 1B).

Physalis glabripes – like the other *Physalis* species examined so far – proved resistant to bean common mosaic virus, bean yellow mosaic virus, carnation ring spot virus, lettuce mosaic virus and watermelon mosaic virus. Its experimentally proved compatible and incompatible host-virus relations make the species suitable for being used as indicator and virus separator in various virological laboratories.

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Isolation of Cucumber Mosaic Virus from Pomegranate (*Punica granatum* L.) in Yugoslavia

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From pomegranate (*Punica granatum* L.) growing in the Botanical Gardens of Zagreb University (Yugoslavia) an isolate of cucumber mosaic virus (CMV-Pg) has been isolated. It has been established on basis of test plant reactions, serology, virus stability in sap, and size of the virus particle. In addition, some observations concerning cell virus inclusions are presented. This is the first report on virus infection of pomegranate.

Pomegranate (*Punica granatum* L., Family: *Punicaceae*) is a natively grown shrub in many regions of Asia. As a cultivated plant it is bred in many warm zones of the world. Also, this shrub is cultivated in southern parts of Yugoslavia especially along the coast. The pomegranate can be found in botanical gardens all over the world as well.

In 1982 virus symptoms on an exemplar of pomegranate growing in the Botanical Gardens of Zagreb University were observed. In order to establish which virus causes these symptoms some experiments were carried out. The results of these investigations are presented herein. It is interesting that till now no virus have been isolated from this plant.

Materials and Methods

An infective material of naturally infected *Punica granatum* was collected in 1982 in the Botanical Gardens of Zagreb University (Yugoslavia). Virus symptoms were observed only on one of two bushes cultivated in the garden.

Inocula were prepared by grinding the young leaves just developed from buds in 0.01 M phosphate buffer pH 7.0 which contained 0.02 percent thioglicolic acid. The prepared inocula were mechanically inoculated to some herbaceous plants early in spring (March).

The isolated virus was partially purified after the method of Habili and Francki (1974) including some slight modifications which will be described later. The purified virus was examined by means of electron microscope. The serological experiments were carried out by means of the agar gel double diffusion test.

Results and Discussion

Symptoms of the naturally infected pomegranate

The virus symptoms could be observed only on some branches of the infected plant. The leaves of these shrubs were deformed and the majority of them showed a variegation and a pronounced yellowing (Fig. 1A). The flowering on the branches with symptoms was significantly reduced.

Symptoms on test plants

The isolate was cleaned by local lesion transmissions using *Chenopodium amaranticolor* Coste et Reyn. and then it was inoculated on other test plants. In total 12 herbaceous plants were mechanically inoculated with the virus. In addition, young healthy seedlings of pomegranate were mechanically inoculated. The symptoms on test plants are shown in Table 1 and Fig. 1B. Ten plants reacted positively to the virus. No symptoms were produced in *Ocimum basilicum* L. and *Spinacia oleracea* L.

In order to establish wheather observed symptoms on pomegranate were caused by the investigated virus, 20 seedlings of young *Punica granatum* in the stage of 3–4 leaves were inoculated with the virus. However, the attempts of transmission of the virus to healthy pomegranate were unsuccessful.

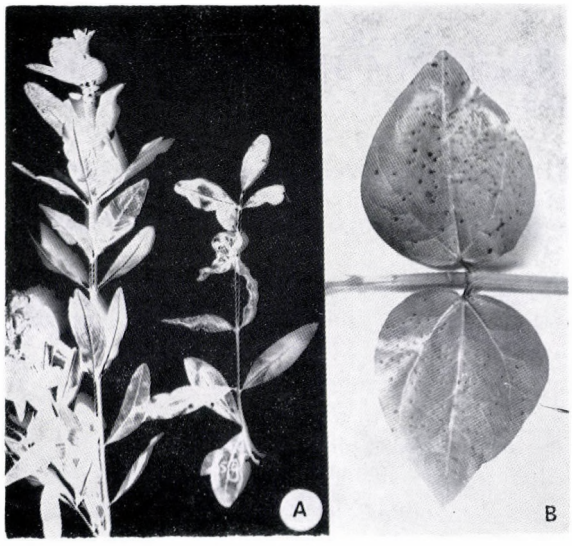

Fig. 1. A: Healthy branch (left) and naturally infected branch of *Punica granatum* L. (right) B: Local lesions on inoculated leaves of *Vigna sinensis* (L.) Savi. caused by the virus isolate (CMV-Pg) from pomegranate

Table 1

Reactions of test plants infected with virus isolate from pomegranate (*Punica granatum* L.)

Hosts	Symptoms
AIZOACEAE	
Tetragonia expansa Murr.	Systemic mild mottling and leaf deformation
CHENOPODIACEAE	
Beta vulgaris L.	Systemic mottling and blistering
Chenopodium amaranticolor Coste et Reyn.	Local chlorotic-necrotic lesions
C. quinoa Willd.	Local chlorotic lesions
Spinacia oleracea L.	No symptom reaction
CUCURBITACEAE	
Cucumis sativus L.	Local chlorosis and systemic mosaic
FABACEAE	
Vigna sinensis (L.) Savi.	Local necrotic lesions (Fig. 1B)
LAMIACEAE	
Ocimum basilicum L.	No symptom reaction
SOLANACEAE	
Datura stramonium L.	Systemic mottling
Lycopersicon esculentum Mill.	Systemic mottling
Nicotiana megalosiphon Heurck. et Muell.	Systemic mosaic
N. tabacum L. cv. *White Burley*	Systemic mild mottling

As it can be seen from the Table 1 the host range and symptoms are very well in concordance with those of cucumber mosaic virus (CMV, cf. Francki et al., 1979). Therefore, on the basis of reactions on test plants it was preliminarily concluded that the investigated isolate could be an isolate of CMV (CMV-Pg). Since our isolate caused only local infection on cowpea [*Vigna sinensis* (L.) Savi.] it can most likely not be considered to be an isolate of peanut stunt virus (PSV, see Mink, 1972; Francki et al., 1979).

Stability in sap

The experiments showed that the Pg isolate of CMV had the thermal inactivation point between 60 and 65 °C and the infectivity of the virus was lost in 4 days at room temperature. These data also show that the investigated virus can represent an isolate of CMV (cf. Gibbs and Harrison, 1970).

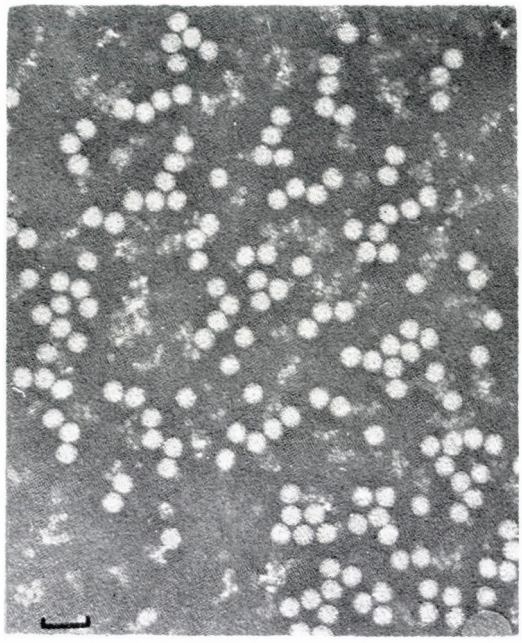

Fig. 2. Particles of the virus isolate (CMV-Pg) from *Punica granatum* L. in a partially purified preparation. Bar represents 60 nm

Purification, ultraviolet absorption and electron microscopy

The virus was partially purified by the method of Habili and Francki (1974). In contrast to these authors we resuspended the virus pellet after the first and second high-speed centrifugations in 0.03 M phosphate buffer pH 7.0 which contained 1 percent of formaldehyde. Further purification by density gradient centrifugation was not undertaken. A_{260}/A_{280} of the purified virus suspension was 1.65.

The virus has icosahedral particles about 30 nm in diameter (Fig. 2).

Serological reactions

Antiserum to CMV titer 1/64 was used in serological tests carried out by agar gel double diffusion method. Our isolate reacted with the used serum (Fig. 3). Since the serum contained some antibodies against normal protein it was absorbed with sap of healthy plants. Serological tests also indicated that the Pg isolate belong to CMV.

Light microscope analysis

In the cytoplasm of the infected tobacco hair cells virus cytoplasmatic inclusions have been detected. The inclusions were not similar to the ones of CMV

described by Christie and Edwardson (1977). The detected inclusions have a form of the irregular aggregates consisting of minute needle-shaped crystals which were irregularly arrayed. Sometimes individual prolongated needle crystals were observed.

On the basis of all the above quoted data it seems that the investigated isolate belongs to CMV. It is necessary to point out that exemplare of pomegranate growing along Yugoslav Adriatic coast often exhibit yellowing symptoms. It would be interesting to establish what is a causal factor of these symptoms.

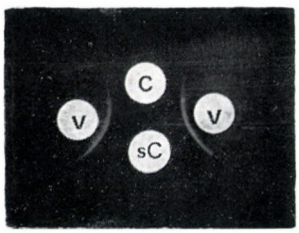

Fig. 3. Agar gel double diffusion precipitin pattern. V = Sap of plant infected with virus isolate (CMV-Pg) from *Punica granatum* L., C = Healthy sap, sC = Immune serum to cucumber mosaic virus (CMV) absorbed with healthy plant sap

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Use of Bioluminescence to Study the Inhibition of Algal Photosynthetic Oxygen Evolution, Using Diuron as a Test Chemical

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A mixture of cells of a green alga and a luminous bacterium was sealed in a glass vial and exposed to a cycle of alternating illumination and darkness. The concentration of oxygen produced photosynthetically during the illuminated phase of each cycle was estimated from the intensity of bacterial luminescence in the dark phase.

Cutting off the light caused the oxygen level to decline asymptotically to zero, due to respiration of the algal-bacterial mixture. A similar decline was observed following injection of varying amounts of diuron (3-[3,4-dichlorophenyl]-1, 1-dimethyl urea), the final oxygen concentration depending on the diuron concentration.

Photosynthetic rates at various times following addition of diuron were calculated by comparison of the asymptotic curves produced by termination of illumination and by injection of diuron. The results compare favourably with those obtained by conventional methods and suggest that the bioluminescence method will be useful for studying the effects of other photosynthesis-inhibiting compounds.

A variety of methods has been used to follow the uptake and action of the photosynthesis-inhibiting herbicides, including diuron (3-(3,4-dichlorophenyl)-1, 1-dimethyl urea). The herbicide-induced increase in red fluorescence (wavelength 689 nm) of cells or chloroplasts illuminated with blue light (exciting wavelength from 420 to 480 nm) has been used to study the uptake (Zweig and Greenberg, 1964) and binding (Lien et al., 1977) of these compounds. This method is sensitive and the response to changes is rapid, but it suffers from the disadvantages that it does not directly measure photosynthesis and that the sample must be illuminated with blue light, which does not include the optimal wavelengths for photosynthesis.

Changes in the rate of reduction of ferricyanide have been used as an indicator of the effect of herbicides on the photosynthetic system (Izawa and Good, 1965; Lien et al., 1977; Ramaswamy and Nair, 1979). This method is very sensitive but does not directly measure photosynthesis; ferricyanide reduction involves diversion of electrons from the normal photosynthetic chain into a different pathway (Whitmarsh and Cramer, 1978).

Methods involving CO_2 uptake or O_2 evolution have the advantage of measuring parameters which are directly related to photosynthetic activity. Carbon dioxide uptake has been measured both polarigraphically and by changes in radioactivity in a $^{14}CO_2$ atmosphere, whereas O_2 concentrations have only been determined polarigraphically (Gould and Bassham, 1965; Lien et al., 1977; Ramaswamy and Nair, 1979). These methods provide adequate sensitivity but elaborate apparatus, which may not be available in some laboratories, is needed.

The bioluminescence method of Tchan et al. (1975, 1977) offers a sensitive alternative technique for following changes in the rate of photosynthetic oxygen production. Originally developed to monitor photosynthesis-inhibiting substances in the environment, the method is based upon oxygen-dependent luminescence of a luminous bacterium in a sealed system containing the bacteria plus a photosynthesizing green alga. The reaction cell is placed in an instrument, the 'photobioluminometer' (Tchan et al., 1977), which enables rapid cyclical illumination of the cell followed by measurement of the intensity of the light emitted. Thus the amount of oxygen produced photosynthetically during the illumination phase is measured in terms of luminous intensity during the measurement phase. In this system, a diuron concentration of 0.2 μM gave 50% inhibition of luminescence and levels as low as 0.02 μM caused a detectable decrease (Chiou, 1980; Tchan and Chiou, 1977).

This paper reports the application of the bioluminescence technique to study the effect of diuron on photosynthetic oxygen evolution by the green alga, *Dunaliella tertiolecta*, to illustrate the application of the technique to investigations of plant physiology.

Materials and Methods

Organisms and culture methods

The luminous bacterium, *Photobacterium phosphoreum* strain T3, was grown in seawater-yeast extract-peptone medium (SWYP) at 20 °C (Tchan et al., 1975). Stock cultures were grown on slopes of SWYP medium solidified with 1.5% agar and stored at 4 °C. Cultures with maximum light emission were obtained for experiments by adding 0.5 ml of a liquid culture to 100 ml fresh medium and incubating without shaking for 18 h. Such cultures had an A_{700} of approximately 0.30, when measured in a Hitachi Model 101 spectrophotometer using a cuvette with a 1 cm light path.

The green alga, *Dunaliella tertiolecta*, was grown at 20 °C with continuous illumination at a level of approximately 7000 lux in a medium containing (per litre): NaCl, 9.86 g; $MgSO_4 \cdot 7\,H_2O$, 2.05 g; $CaCl_2$, 0.15 g; NH_4NO_3, 0.57 g; Fe–Na–EDTA, 0.024 g; NH_4VO_3, 0.023 mg; $NiCl_2 \cdot 6\,H_2O$, 0.041 mg; Na_2MoO_4, 0.252 mg; $ZnSO_4 \cdot 7\,H_2O$, 2.20 mg; $MnSO_4 \cdot 4\,H_2O$, 2.30 mg; $CuSO_4 \cdot 5\,H_2O$, 0.078 mg. KH_2PO_4 and Na_2HPO_4 solutions were autoclaved separately and added to the sterile medium to give respectively 1.25 and 0.89 $g.l^{-1}$. The pH was adjusted to 7.5 with 1N NaOH. Cultures for experiments were obtained by inoculating 150 ml of medium with 15 ml of culture, and incubating without shaking for 5 days, when an A_{662} of 0.28 was attained, corresponding to a concentration of approximately 3×10^6 cells ml^{-1} and a chlorophyll $a + b$ content (Arnon, 1949) of approximately 2 $\mu g.ml^{-1}$.

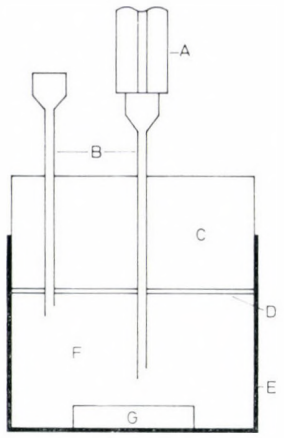

Fig. 1. Sample container and arrangement for injecting diuron. A, Hamilton syringe containing mixture of diuron plus luminous bacteria; B, hypodermic needles; C, rubber stopper; D, aluminium foil; E, glass vial; F, mixture of *D. tertiolecta* and *P. phosphoreum;* G, glass stirring bar

Measurement of photosynthesis

A 1 : 1 (v/v) mixture of algal and bacterial cultures was placed in a glass vessel which was fitted with a stopper with provision for rapidly injecting the herbicide while excluding oxygen (Fig. 1), and placed in the specimen chamber of the photobioluminometer (Tchan et al., 1977). This instrument was used in the 'constant run' mode, in which a continually rotating mirror exposes the specimen chamber to a cycle of illumination and darkness. During the dark phase of each cycle, light emitted by the sample vessel is measured by a photomultiplier and recorded as a narrow peak on a recorder chart (Fig. 2). A microscope lamp (in-

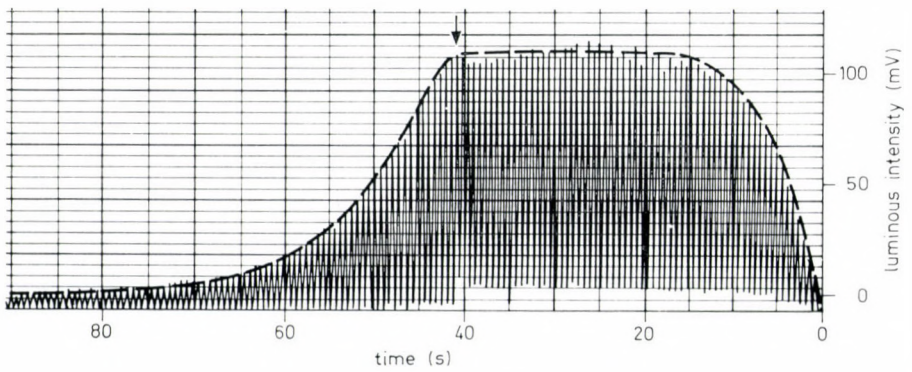

Fig. 2. Chart recording showing fitted "dark" curve. Light was cut off at 41 s (see arrow)

tense lamp, Vickers Ltd., York, England) with a heat filter was used to focus white light on the specimen chamber. The luminous intensity of the light received in the specimen chamber was set at 1500 lux, which was saturating for algal photosynthesis, by means of the aperture diaphragm of the lamp.

The sample container was a vial of 2.0 cm diameter and 2.5 cm height, which was sealed with a rubber stopper so as to exclude any air bubbles (Fig. 1). Two number 17 hypodermic needles inserted through the stopper acted as an inlet for herbicide solution and an outlet for excess culture. The inlet needle was connected to a microsyringe (Hamilton Co., Reno, Nevada) containing a 9 : 1 (v/v) mixture of 0–85.8 μM diuron in 0.9% NaCl and the photobacterial culture. The latter was included in order to exhaust all the dissolved oxygen in the mixture. An adjustable stop (Chaney adaption) fitted to the syringe enabled accurate injection of a 200 μl volume. Both the conventional teflon-coated magnetic stirring bar and the rubber stopper released enough adsorbed oxygen to permit bioluminescence by the bacteria; this problem was solved by gluing a sheet of aluminium foil to the bottom of the stopper and by using a glass-coated stirring bar. The volume of liquid in the sample container, which varied depending on how far in the stopper had been pushed, was measured at the completion of every run.

A large volume of the algal-bacterial mixture was prepared for use at the start of each experiment, a fresh aliquot of this mixture being used for each diuron treatment. Each experiment was completed within the time during which algal photosynthesis and intensity of bacterial luminescence are known to remain constant.

Results

Response of photosynthesis to darkness and to diuron

The algal–bacterial mixture was placed in the sample chamber of the photobioluminometer above a magnetic stirrer which was operating continuously, and left in darkness until all dissolved oxygen was exhausted and light emission had ceased. The mixture was then exposed to a 0.31 sec cycle of alternating illumination and darkness until the peaks of luminous intensity had reached a steady value. The illuminating light was then suddenly cut off by placing an opaque object in the light path, and the luminous intensity began to decline (Fig. 2). After the luminescence had reached zero the light source was uncovered and, when the maximum luminous intensity was reached, a 200 μl aliquot of diuron solution was swiftly injected.

When the light was cut off there was an immediate response in the intensity of bacterial luminescence which, apart from minor fluctuations due to electronic interference by the magnetic stirrer and electrical parts of the photobioluminometer, decreased asymptotically until it reached zero (Fig. 3*a*, *c*). When the same mixture was again exposed to the light-dark cycle, the luminous intensity returned to its original maximum steady state, corresponding to the maximum photosyn-

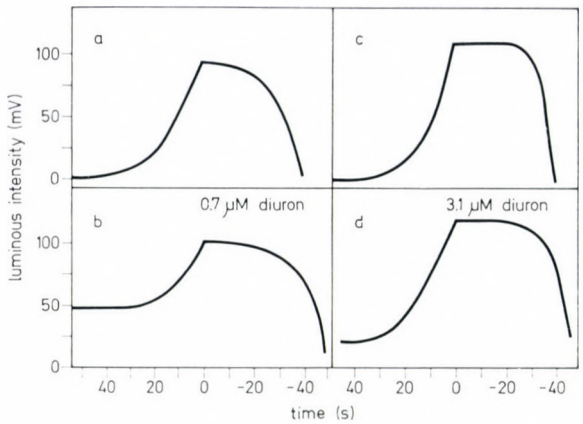

Fig. 3. Pairs of "light" and "dark" curves for two algal-bacterial samples (*a* and *b*, *c* and *d*), showing the effect of cutting off the light or injecting diuron on the luminous intensity of the same sample. The light was cut off *(a, c)* or diuron was injected *(b, d)* at zero time. Diuron concentrations were *b*, 0.7 μM; *d*, 3.1 μM

thetic rate of the algae. Cutting off the light again resulted in an identical decline in luminous intensity.

The immediate decline in luminous intensity, when the light was cut off, was due to continual removal of oxygen from the reaction vessel by the combined respiration of bacteria and algae. Since the curve of luminous intensity *v.* time is asymptotic rather than linear, it follows that the respiration rate is not constant, but varies with the concentration of dissolved oxygen.

As soon as diuron was injected into the mixture, the intensity of luminescence began to decline asymptotically until a new, lower steady value was obtained, depending on diuron concentration: the higher the concentration, the faster the decline and the lower the new value (Fig. 3*b*, *d*). When the diuron concentration was equal to or greater than 5.2 μM, the chart recordings were identical with those obtained for the same mixture when the light was cut off. The same result was obtained by simultaneously cutting off the light and adding 5.2 μM of diuron. The decline in luminescence at all diuron concentrations began immediately following herbicide addition and no lag could be detected, even when the rotation rate of the mirror was increased to give a light-dark cycle length of 0.21 or 0.16 s.

Calculation of photosynthetic rate

Assuming the luminous intensity of the bacterial-algal mixture to be proportional to the concentration of dissolved oxygen in the system, photosynthetic rates can be determined by comparison of the "light" and "dark" curves obtained from the chart recordings of luminous intensity *v.* time.

The "dark" curve is obtained by drawing a line through the peaks corresponding to the period of declining intensity after the light was cut off. A corre-

sponding "light" curve for the same bacterial-algal mixture is obtained in the same way for the period of decline following injection of diuron (Fig. 3). The "dark" curve represents the curve for the falling oxygen concentration due to respiration. Its slope (S_D) at any given oxygen concentration (expressed as luminous intensity) is proportional to the respiration rate (R) of the mixture at that oxygen level:

$$R = k\, S_D \tag{1}$$

where k is the constant of proportionality.

The slope of the "light" curve (S_L), at any given oxygen concentration, is proportional to the net respiration rate (R_n), which is a function of continuous respiratory oxygen uptake (R′) minus photosynthetic oxygen production during the brief periods of illumination, occupying a small fraction (1/x) of every light-dark cycle. (The fraction $1/x < 1/2$, because the mirror reflects the light beam onto the specimen chamber for less than half the time in every revolution.) That is:

$$R_n = k\, S_L \tag{2}$$

and

$$R_n = R' - \frac{P}{x} \tag{3}$$

where P is the rate of photosynthetic oxygen production. If respiration of the bacterial-algal mixture is unaffected by darkness or light, or by diuron at the concentrations used, the respiration rate at a given oxygen concentration (luminous intensity) in the "light" curve is the same as that calculated from the "dark" curve at the same oxygen concentration. Therefore, at the same luminous intensity:

$$R' = R \tag{4}$$

Substituting equations 1, 2 and 4 in equation 3:

$$k\, S_L = k\, S_D - \frac{P}{x}$$

$$P = xk(S_D - S_L)$$

$$P' = S_D - S_L$$

where $P' = P/xk$ and is the rate of photosynthetic oxygen production expressed in terms of changing luminous intensity (units are mV. s^{-1}).

Kinetics of action of diuron

Rates of photosynthesis (P′) were plotted against time after injection of diuron for each concentration of diuron. The overall pattern is clear: injection of

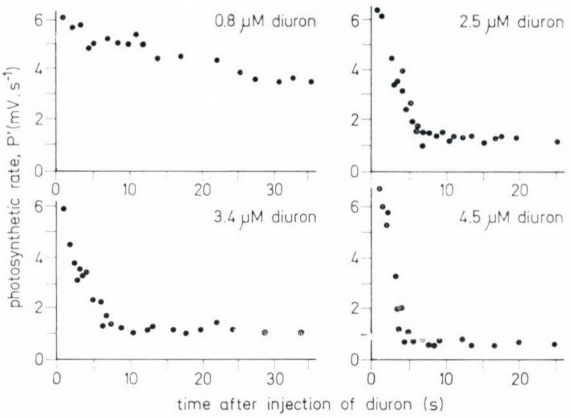

Fig. 4. Change in photosynthetic rate following injection of 0.8, 2.5, 3.4 or 4.5 μM diuron. Photosynthetic oxygen evolution is expressed in terms of luminous intensity (in mV) of the algal-bacterial mixture

diuron is followed by a decline in photosynthetic oxygen production until a steady, lower rate is reached (Fig. 4). The higher the concentration of diuron, the steeper the decline, the lower the equilibrium rate of photosynthesis and the shorter the time required to reach it (t_{eq}) (Table 1). The graph of log diuron concentration *v.* t_{eq} approximated a straight line of slope -0.030 and vertical intercept 0.728 (Fig. 5).

Discussion

The intensity of bacterial luminescence in a mixture of algae and photobacteria provides a very sensitive means of determining the concentration of dissolved oxygen in the mixture, and there are reasonable grounds for assuming that the relationship is linear, even though in this case the dissolved oxygen concentrations

Table 1

Effect of diuron concentration on the equilibrium photosynthetic rate and the time taken to attain it, in *Dunaliella tertiolecta*

Diuron concentration (μM)	0.8	1.4	2.0	2.5	2.6	3.4	4.5	5.2
Equilibrium photosynthetic rate (mV. s^{-1})	3.61	n. d.	n. d.	1.26	n. d.	1.11	0.71	0
Time to attain equilibrium, t_{eq} (s)	27	20.5	12.5	9.5	8.5	7.5	4.5	0

n. d. = not determined

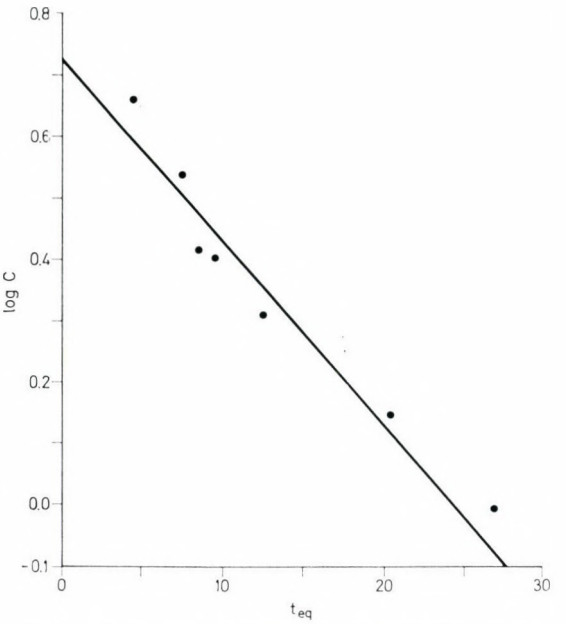

Fig. 5. Graph of Log C ($\log_{10}$ diuron concentration in μM) *v.* t_{eq}. Regression equation is Log C = 0.728−0.030 t_{eq}. Correlation coefficient, r = −0.98; standard error of slope, S_b = 0.052

were less than 0.06 μM, which lies below the range for which linearity has been experimentally proved. Chiou (1980), using the same mixture, demonstrated a linear relationship for oxygen levels between 12.5 μM and the lower limit of sensitivity of the dissolved oxygen electrode (approximately 1.5 μM), and Funnell (1975), using a pure culture of the same luminous bacterial strain, found that luminous intensity in his system was directly proportional to dissolved oxygen concentration down to the lowest level tested, 0.09 μM.

The results of Shapiro (1934) provide further support for the basic assumption that the intensity of bacterial luminescence (I) is directly proportional to dissolved oxygen concentration ($[dO_2]$). Using a different luminous bacterium, he found that

$$\frac{I}{(I_{max} - I)} \alpha [dO_2]$$

where I_{max} is the maximum luminous intensity of the *Vibrio phosphorescens* culture. This expression differs from the relationship found by Chiou (1980), but when applied to the present system I is so much smaller than I_{max} ($I < 120mV$; $I_{max} > 8V$) that the approximation, $I \alpha [dO_2]$ may still be used. Therefore, in the calculation of photosynthetic oxygen evolution, oxygen levels were expressed indirectly as bacterial luminescence (measured as mV by the photomultiplier).

Using this sensitive means of monitoring oxygen concentrations, it was found that the combined respiration rate of the algal-bacterial mixture was not constant at low oxygen levels, but varied with the oxygen concentration. Thus when the rate of photosynthetic oxygen production was reduced by cutting off the light or adding a photosynthesis-inhibiting herbicide, the concentration of dissolved oxygen declined until a new equilibrium was reached, when respiration rate equalled photosynthetic oxygen evolution.

Rates of photosynthetic oxygen evolution at various intervals after diuron treatment were calculated from the difference between the slope of the "dark" curve generated by cutting off the light and the slope, at the same oxygen concentration, of the "light" curve generated by injecting diuron. The curves obtained following injection of diuron at 5.2 μM or greater concentrations, with or without simultaneous termination of illumination, were identical with the corresponding "dark" curves, indicating that photosynthetic oxygen production is immediately and completely inhibited by these levels of herbicide. In addition, these results strongly support the assumptions upon which the calculations of photosynthetic rate are based, namely that respiration of the algal-bacterial mixture is unaffected by diuron and is the same in intermittent light as in darkness, and that diuron does not alter the relationship between luminous intensity and oxygen concentration. Chiou (1980) has also shown that respiration of the algal-bacterial mixture is insensitive to diuron up to 12.9 μM, using a dissolved oxygen electrode.

Plots of photosynthetic oxygen production *v.* time after diuron injection show that the response to diuron injection was rapid, the rate of photosynthesis decreasing steadily until a new, lower rate was reached (Fig. 4). These curves are similar in shape to those found by Zweig and Greenberg (1964), for diuron uptake by cells of *Chlorella pyrenoidosa*, using the increase in red fluorescence as an indicator of uptake.

The graph of log diuron concentration (log C) *v.* time to reach equilibrium photosynthetic rate (t_{eq}) was a reasonably good straight line (Fig. 5) which is consistent with the hypothesis (Zweig and Greenberg 1964) that movement of diuron from the extracellular solution to its site of action within the algal cells is governed by first order kinetics. Our results fit the equation

$$\log C = k\, t_{eq} + A$$

where k and t are constants (Zweig and Greenberg, 1964) – in this case $k = -0.030$ and $A = 0.728$.

The antilog of A, 5.35 μM, is the theoretical concentration of diuron required to cause instantaneous inhibition of photosynthesis to its equilibrium rate (i.e. $t_{eq} = 0$), and this value compares favourably with the observation that concentrations in excess of 5.2 μM caused instantaneous cessation of photosynthesis. The comparable results for *C. pyrenoidosa* were $k = 0.083$ and the antilog of $A = 13$ μM (Zweig and Greenberg, 1964), the different values being due to the different test organism as well as the different parameter being measured. The time required

for photosynthesis of *D. tertiolecta* to reach equilibrium with 1 μM diuron was estimated from Fig. 5 to be 24.1 s, compared with equilibrium times for *C. pyrenoidosa* of 13.4 sec by the fluorescence method (Zweig and Greenberg, 1964) or 18 minutes as determined by polarigraphic measurement of oxygen and carbon dioxide (Gould and Bassham, 1965).

These results show that the bioluminescence method provides data on diuron-induced changes in the rate of photosynthesis that are comparable with those obtained by the fluorescence method and superior to those obtained by polarigraphy by Gould and Bassham (1965).

Although diuron was used in these studies, similar investigations could be carried out using other chemicals affecting photosynthetic oxygen evolution. The photobioluminometer is cheaper and more portable than the equipment needed for polarigraphic or fluorimetric monitoring of photosynthesis inhibition, and this should make the bioluminescence method particularly useful for *in situ* measurement of physiological changes in the field in ecological studies or for environmental protection investigations. The applicability of the bioluminescence method is not limited to studies of *Dunaliella tertiolecta*, but other salt-tolerant algae have been tested (e.g. *Chlorella salina* – Y. T. Tchan, personal communication) and, with suitable adaptations the technique may be extended to other salt-tolerant plants, including higher plants.

Acknowledgements

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Click Beetles (*Elateridae*) in the Soils of Central Europe—Their Distribution and Description. Part II* (Gen: *Melanotus*, *Adrastus*, *Selatosomus*, *Athous*, *Lacon*, *Limonius*, *Synaptus*, *Cardiophorus*)

Z. TÓTH

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Genus: *Melanotus* Eschscholtz

The body of young larvae is cylindrical, the one of older larvae is more flat. Their cuticula is strongly chitinized. The colour of young larvae is light yellow, the more mature ones are of deeper colour while the head and anal segment are dark yellow. The head is from above rectangular, slightly tapering anteriorly, from side-view flattened. The frontal plate is characteristically cup-shaped, the distal end of its posterior lobe broadly rounded, its lateral lobes (maxillar plate) are not reaching beyond the apex of the frontal spine. The nasale is cuneiform. In some species the anterior edge of the frontal plate bears beside the nasale additional teeth. On the frontal plate the 6 pairs of setae are always present, four of them are found on the anterior edge, the fifth pair on the lateral lobe and the 6th pair is behind the 5th one. In some species also the mesal pair of setae is present, the mandibles are sturdy, with a median tooth (retinaculum). The antennae are three-segmented, the ultimate segment is very short. No eyes and epicranial suture developed. The stipes, cardo and subcardo are distally tapering; the submentum is prolonged, with parallel sides. The length of thoracal- and abdominal segments increases from the second segment. The first abdominal segment is as long as the second and third segments together. The pleurae and tergites form an united plate, divided only by a suture. From the 2. thoracal segment on the basal part of sternites an edge-like crest is found; on the middle of the second and third thoracal- and first abdominal tergite the same crest developed. On the sides of tergites piths, serving as muscle attachment points are found with the exception of the first tergite. On the tergites a longitudinal groove runs along the distal edge, starting on the first abdominal segment.

The 9th, caudal, segment is long, gradually tapering, with two longitudinal grooves on the prolonged tergite. In the apical half a more or less delimited, depressed area is found. The segment is terminated in a median and two lateral spines. The presence and shape of muscle-attachment points (piths) varies according to species groups.

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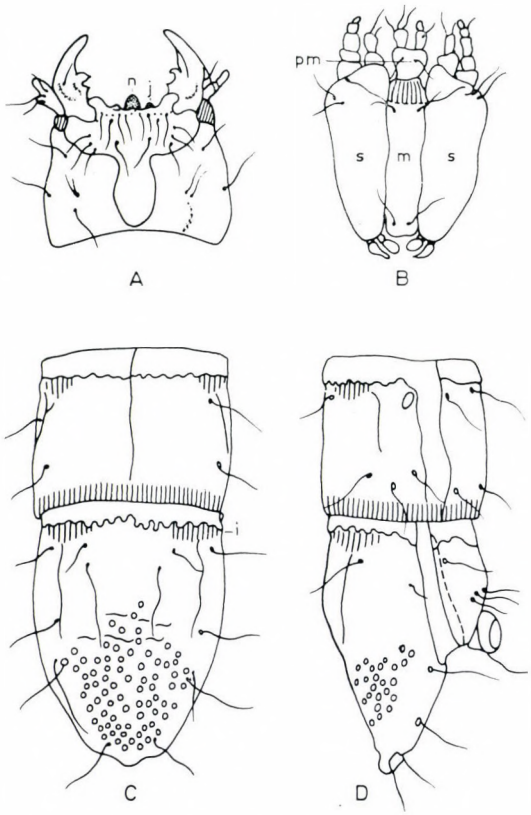

Fig. 8. *Melanotus niger* Fabricius, larva. A = head from above, n = tooth of nasale, j = additional tooth; B = head from below, p.m. = praementum, s = stipes, m = mentum; C = the 8th and 9th abdominal segments viewed from above, i = depression, point of muscle attachment; D = 8th, 9th and 10th abdominal segments, lateral view (original)

Melanotus niger Fabricius (Fig. 8)

Synonyms: *Melanotus ater* Eschscholtz
Melanotus punctolineatus Pelirin

Distribution

This species has an Eurasial distribution. It occurs in Central, Southern and East Europe, in Asia Minor and Turkestan. In Hungary its occurrence is characteristic for the chernozem soils but occurs also in peaty soils.

Biology:

Its way of life is mostly unknown; the larva is omnivorous that feeds on plant seeds but is mostly predatory.

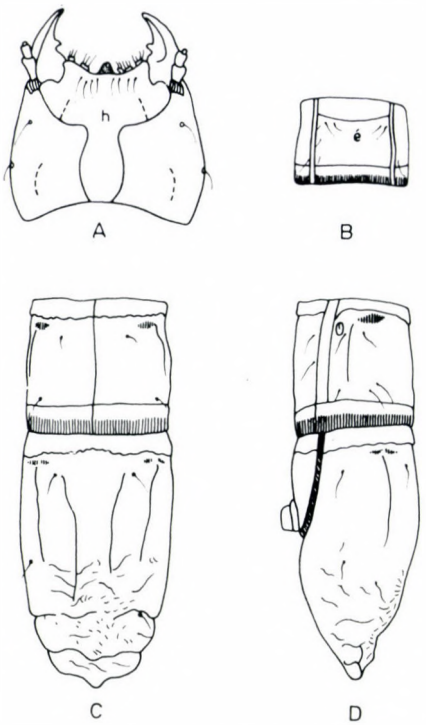

Fig. 9. *Melanotus tenebrosus* Erichson, larva. A = head from above, m = mandible, h = frontal plate; B = second abdominal segment from below, e = crest-like edge; C = 8th and 9th abdominal segments, viewed from above; D = 8th, 9th and 10th abdominal segments, lateral view (original)

Melanotus tenebrosus Erichson (Fig. 9)

Synonym: *Melanotus aspericollis* Mulsant – Guillebeau

Distribution

The species is distributed in South Europe. It occurs in South- and Central Europe, Crimean Peninsula and Caucasus. In Hungary we have found this species in low-lying clay, loam and loamy meadow soils.

Biology

Its biology is partially unknown. According to the observation of Dolin (1960) the larvae are not particularly sensitive to the drying-out of soils as they are found in the upper soil layer even in dry weather. The larvae are predators and do not feed on plants even if they starve.

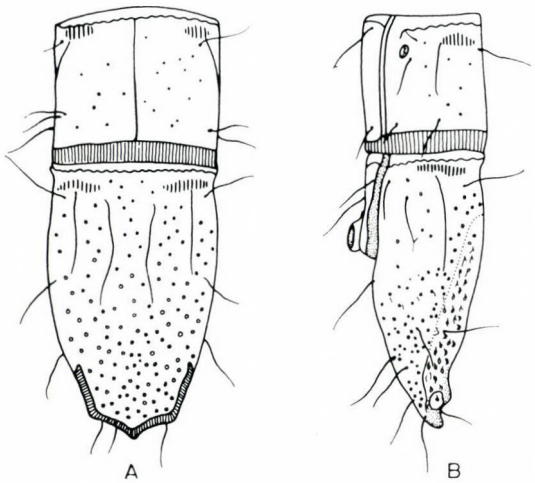

Fig. 10. *Melanotus crassicollis* Erichson, abdominal end of larva. A = 8th and 9th abdominal segments, viewed from above, e = crest-like edge; B = 8th, 9th and 10th abdominal segment, lateral view (original)

Melanotus crassicollis Erichson (Fig. 10)

Synonyms: *Melanotus brunnipes* Boisduval – Lacordaire
Melanotus subrugatus Rey

Distribution

A South-European species, distributed in Central- and South Europe. In Hungary it was found in forest- and chernozem soils.

Biology

The development takes very likely 3 years. In the experiments of Dolin (1960) it did not feed on plants but preyed upon wire-worms and other coleopterous larvae. Its predatory action is remarkable in the Hungarian agricultural soils. It is a thermophilous species.

Melanotus brunnipes Germar (Fig. 11)

Synonyms: *Melanotus fasciculatus* Küster
Melanotus subvestitus Brulle
Melanotus subvillosus Erichson

Distribution

A South-European species, distributed in Central- and South-Europe, Asia Minor and Turkestan. It is the least common Melanotus species in the agricultural soils of Hungary.

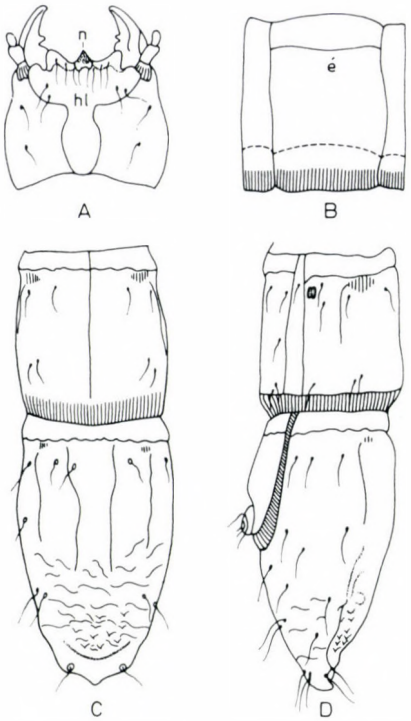

Fig. 11. *Melanotus brunneipes* Germar, larva. A = head, viewed from above, n = nasale, h. l. = frontal plate; B = ventral plate of second abdominal segment, e = crest-like edge; D = 8th, 9th and 10th abdominal segments, lateral view (original)

Biology

The females lay their eggs in May–June, the pupation takes place in August–September. The larva is in the Ukraine a dangerous pest of crop plants (Dolin, 1960), but preys also on earthworms and larvae of Diptera. Its development takes 4 years. It is quote resistant to drought, similarly to many species of this group.

Genus: *Adrastus* Eschscholtz

The body is cylindrical, their colour varies from light yellow to deep brown. The head is nearly rectangular, with parallel or slightly arched sides. The posterior lobe of the frontal plate is lanceiform, gradually tapering from the middle. The nasale bears 3 teeth, it is transversal and broader than the base of the posterior lobe. On the anterior part of the frontal plate four pairs of setae are found. The mandibles are powerful, without an additional tooth on the subapical part. The

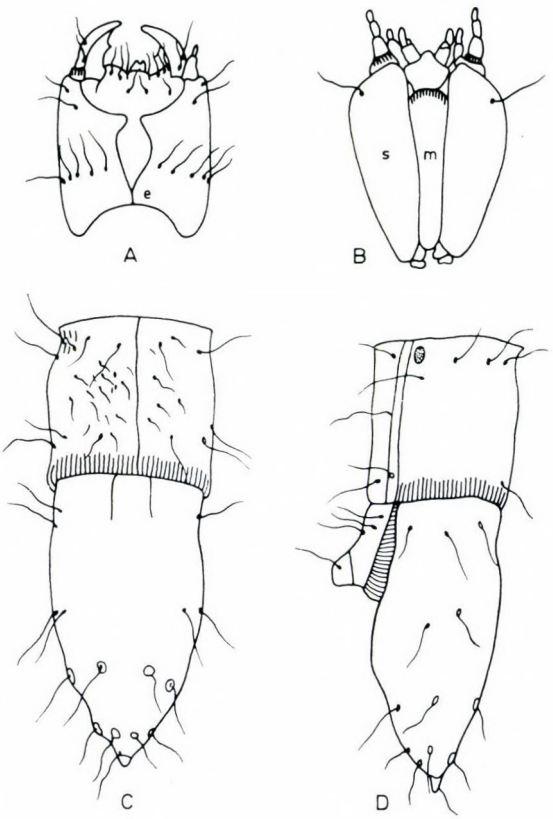

Fig. 12. *Adrastus montanus* Scopoli, larva. A = head, viewed from above, e = epicranial suture; B = maxilla and labium, s = stipes, m = mentum; C = 8th and 9th abdominal segments, viewed from above; D = abdominal end, lateral view (original)

median tooth is well developed. The tergites of thorax and abdomen are finely sculptured and wrinkled, in some cases entirely smooth; with 3–5 setae on their sides; the latter are situated transversally, in the posterior third of the tergites. The edge-like crest did not develop on the basal part of the segment, the muscle attachment points (piths) are foud in some species. The caudal segment is longer than its width, conically tapering and terminating in a chitinous thorn. There is no delimited area on the caudal segment and the longitudinal grooves are hardly visible.

Adrastus muntanus Scopoli (Fig. 12)

Synonym: *Adrastus humilis* Erichson

Distribution

The species occurs in Central- and South Europe. It is quite common in the plains and low mountains in sparse deciduous forests, clearings and forest edges. According to the reared materials it is the most common Adrastus species in the Hungarian agricultural areas.

Biology

Its feeding habits and economic significance are not known.

Genus: *Selatosomus* Stephens

The body of larvae is strongly flattened, their colour is yellowish brown. The head is markedly transversal, nearly twice as wide as long, anteriorly tapering. The frontal plate is sturdy, its posterior plate is broader than its length, with a broadly cut end. The maxillary sclerites are markedly protruding. The nasale is powerful, wedgeform, with one apex. On the frontal plate five pairs of setae are constantly present. The mandibles are powerful, with well developed median tooth. The thoracal and abdominal tergites are finely and sparsely sculptured. Towards the end of the abdomen the sculptures and crinkles become rougher. The distal cranial suture is absent. On the abdominal tergites the crest-like edge is interrupted at the median line. The prothoracal sternite is divided by a well visible suture to two large lateral sclerites and a median, smaller, rhomboid plate. The abdominal sternites are divided to three plates. On the caudal (9th) abdominal segment laterally three pairs of well-developed protuberances are found. The caudal area is transversal-oval, well delineated and markedly depressed. In the area three longitudinal and many transversal grooves are found. The smaller diameter of the embayment is identical with the width of apices, the end is slightly tapering. The apices are two-pointed, short, equal in size.

Selatosomus latus Fabricius (Fig. 13)

Synonyms: *Corymbites latus* Fabricius
Elater germanus Olivier
Elater pectinicornis Fourcroy

Distribution

It is an Eurasian species. It is distributed in Europe, West-Asia and in Asia Minor. It occurs in the forest- and chernozem soils in Hungary, with a dominance value of 1.5%.

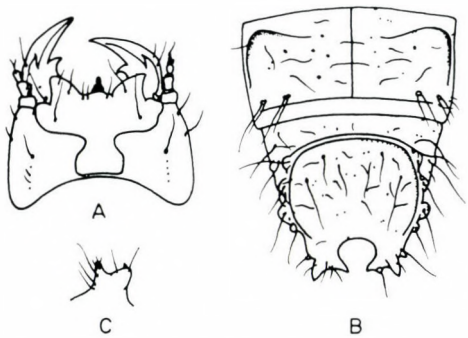

Fig. 13. *Selatosomus latus* Fabricius, larva. A = head, viewed from above; B = ultimate and penultimate abdominal segments; C = right apex of the last abdominal segment (Dolin, 1964)

Biology

The significance of the species is increased by its local outbreaks, followed by an extensive damage in agricultural plants (Znamensky, 1926; D'Aguilar, 1961). The adults swarm from mid-May until June. The females lay about 200 eggs. The larvae are especially dangerous in the spring because later they descend into deeper soil layers; in the autumn the larvae damage mostly the potato tubers. The pupation begins by the end of July and continues for 25–30 days (Masaitis, 1929). The whole development takes 3.5 to 4.5 years. The larvae are omnivorous as they feed on seeds and subterranean plant parts but attack also the larvae of other soil-inhabiting insects.

Selatosomus aeneus Linnaeus (Fig. 14)

Synonyms: *Corymbites aeneus* Linnaeus
Corymbites impressus Marsham
Elater aeneus-rufipes De Geer

Distribution

It is an Eurasian species. It is distributed in Europe, West- and East Asia, Caucasus. In Hungary its significance is similar to *S. latus*. It occurs in nearly all soil types.

Biology

The overwintered adults appear by the end of March. Their flight activity is especially conspicuous on warm days. The adults feed on flowers and on leaves of monocotyledonous plants; the females lay their eggs in April–May. The larvae hatch in June. Their development takes 4 or 5 years, as the mature larvae overwinter in some cases. The pupation takes place by the end of August–begin of

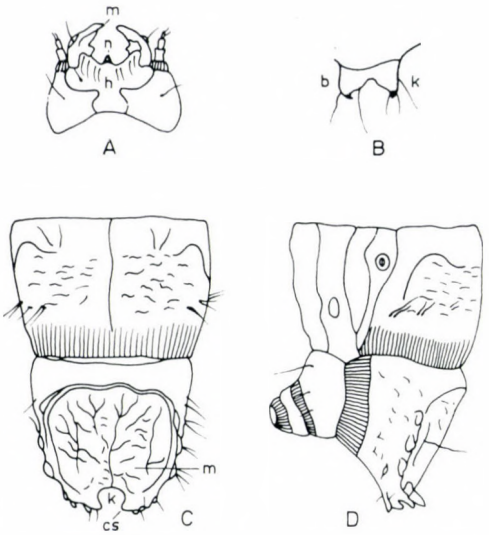

Fig. 14. *Selatosomus aeneus* Linnaeus, larva. A = head, viewed from above, m = mandibula, n = nasale, h = frontal plate; B = apex of caudal segment, b = internal apex, k = external apex; C = abdominal end, viewed from above, m = area, k = indentation, cs = apex; D = abdominal end, lateral view (original)

September. The species belongs to the most dangerous soil-inhabiting pests in the non-chernozem area of the Soviet Union (Dolin, 1964). According to the data of Subklew (1934) and Schaerffenberg (1942) the larvae are temporarily predaceous.

Genus: *Athous* Eschscholtz

The body of the larvae is flat, their colour is yellowish brown. The head is markedly transversal, 1.5-times broader than its length. On the frontal plate 5 pairs of setae, besides two pairs of rudimentary setae (proximal and parietal) are found. The apex of the posterior lobe is broadly cut. The maxillary sclerites are well developed and protruding. The nasale is wide, well developed and bears 3 teeth. The mandibles are powerful, with a well developed median tooth. The sutura epicranialis does not appear on the head. The stipes is oblong-shaped, with parallel sides. The lacinia is triangular with a pointed apex, the distal part of the submentum is tapering with direct sides. From among the thoracal segments the first one is nearly as long as the second and third segment together. The length of the segments gradually increases from the prothorax. The tergites of abdominal segments are strongly sculptured with marked transversal crinkles, with crestlike edge on their base and sides. The morphology of the 9th, caudal segment is very variegated with diagnostic characteristics and the separation of species is based on

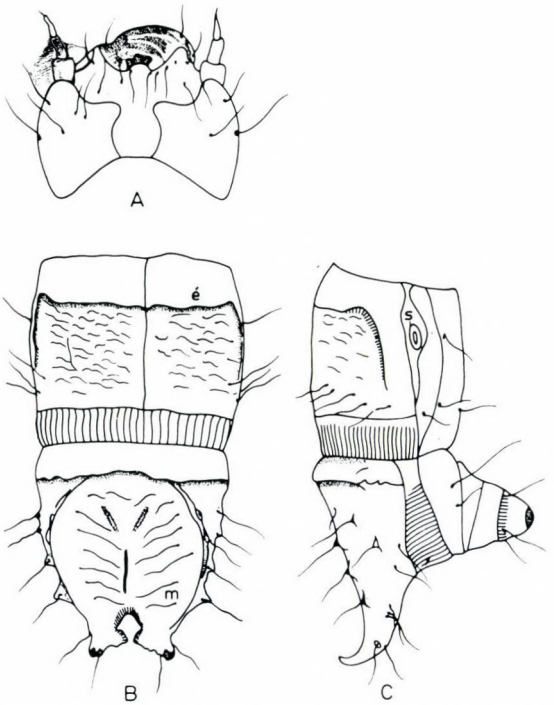

Fig. 15. *Athous haemorrhoidalis* Fabricius, larva. A = head, viewed from above; B = 8th and 9th abdominal segments, viewed from above, e = crest-like edge, m = area; C = 8th, 9th and 10th abdominal segments, lateral view, s = stigma (original)

its features. The end of the segment is indented, forming thus two apices. The area is well defined; laterally well developed, chitinized protuberances are found on this segment.

Athous haemorrhoidalis Fabricius (Fig. 15)

Synonyms: *Athous cariniscapus* Dubuysson
Athous fuscusminor De Geer
Athous interpositus Rey
Athous nigropiceus Ivan
Athous obscurus Paykuli

Distribution

It is an Eurasian species. It is distributed throughout Europe, West- and East Asia and Asia Minor. In Hungary it occurs mostly in silt-loam soils whereas it is rather uncommon in other soils.

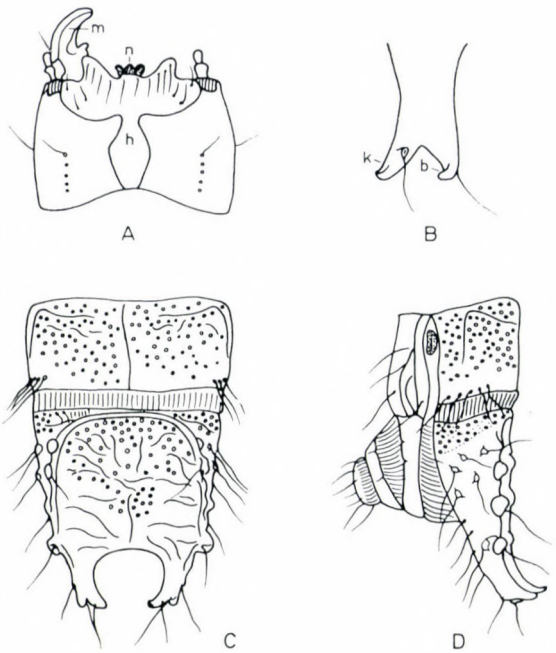

Fig. 16. *Athous niger* Linnaeus, larva. A = head, viewed from above, m = mandibula, n = nasale, h = frontal plate; B = apex, k = external apex, b = internal apex; C = 8th and 9th abdominal segments from above; D = 8th, 9th and 10th abdominal segments, lateral view (original)

Biology

The adults swarm in May–June and the females lay their eggs mostly on the root systems of monocotyledonous plants. The meadows and pastures are always more infested than other cropland types. The adults are good flyers and are found usually on flowers and leaves; the larvae feed on roots. Sometimes vegetables are damaged, so Bognár (1958b) observed heavy damage in tomato. According to Langenbuch (1932) the larvae may be also predaceous. The development takes 3 years.

Athous niger Linnaeus (Fig. 16)

Synonyms: *Athous alpinus* Redtenbacher
Athous deflesus Thomson
Athous hirtus Bach
Athous pubescens Mannerheim

Distribution

It is an Eurasian species. It is distributed in Central-, North- and West-Europe, East-Asia and Caucasus. In Hungary the species is of minor importance;

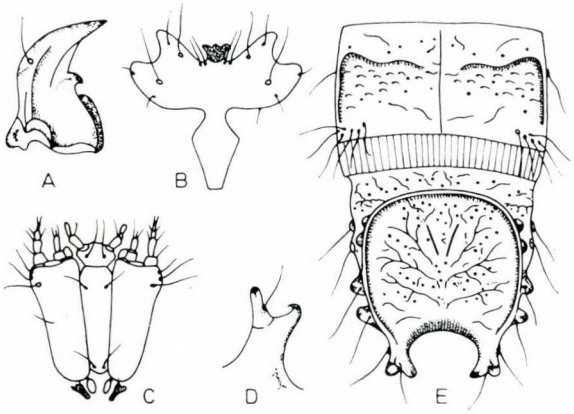

Fig. 17. *Athous hirtus* Herbst, larva. A = left mandible; B = frontal plate; C = maxilla; D = right apex of the last abdominal segment; E = penultimate and last segments (Dolin 1964)

it was found only occasionally in different soil types. The species seems to prefer soils with high organic material content.

Biology

The adults fly mostly in July and prefer for egglaying the meadows and pastureland. The larvae develop on plant roots but feed mostly on other soil-inhabiting insects. The development takes 3—4 years. The last winter is spent still as larvae and the pupation takes place in the spring.

Athous hirtus Herbst (Fig. 17)

Synonyms: *Athous niger* De Geer
Athous porrectus Thomson
Elater ater Geoffroy
Elater nigrinus Marsham

Distribution

It is an Eurasian species. It is distributed throughout Europe, East-Asia and Asia Minor.

Biology

Similar to the one of Athous niger.

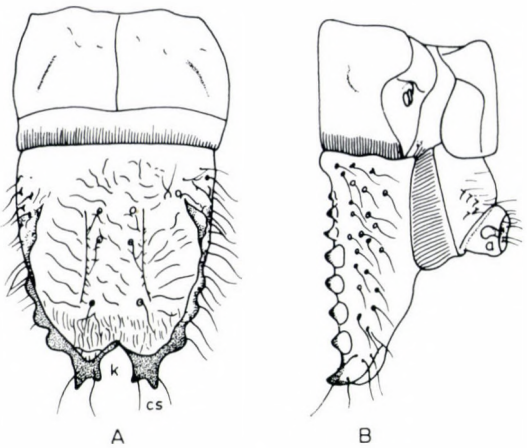

Fig. 18. *Lacon murinus* Linnaeus larva. A = penultimate and last abdominal segments, viewed from above, k = indentation, cs = apex; B = the last two abdominal segments, lateral view, a = anal thorn (original)

Genus: *Lacon* Castelnau

The body of larvae is very flat. The head, prothorax and the caudal segment are deep brownish-red, strongly chitinized. The other segments are light yellow and less chitinized. The posterior lobe of the frontal plate is broadly rounded. On the tergal side of the caudal segment a well-defined area is found with chitin teeth on the bordering edge. The end of the segment is deeply invaginated, terminating in two apices. On the side of the anal segment two powerful thorns are found.

Lacon murinus Linnaeus (Fig. 18)

Synonyms: *Adelacora murina* Linnaeus
Brachylacon murinus Linnaeus
Elater rufipes De Geer
Elater thoracicus Scopoli
Lacon mucoreus Leconte
Lacon nebulosus Razoumowsky
Lacon rufipes De Geer
Lacon thoracicus Scopoli

Distribution

It is a holarctic species, distributed throughout Europe, West- and East Asia, Caucasus and North America. In Hungary it is quite uncommon in the agricultural soils.

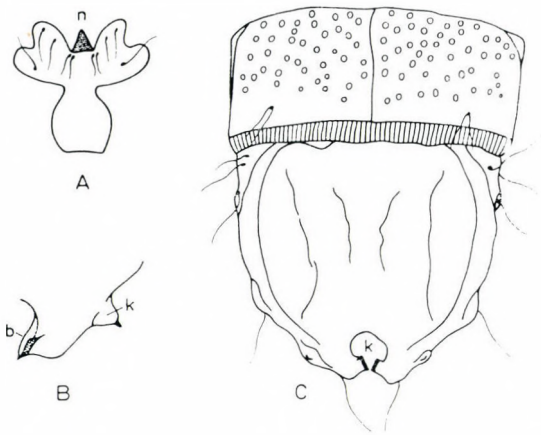

Fig. 19. *Limonius pilosus* Leske, larva. A = frontal plate, n = nasale; B = apex, a = internal apex, b = external apex; C = abdominal end, viewed from above, k = indentation (original)

Biology

The literature data show considerable differences regarding the appearance of adults in the spring: Horst (1922) reported the end of March, Guéniat (1934) June. In Hungary, Németh (1971) collected the adults of this species by the begin and mid-May. The adults feed on umbelliferous plants, on leaves of trees and grasses. The females lay their eggs in May-June, the pupation takes place in June. The biology of larvae was extensively studied by Dolin (1964). The larvae are predominantly predaceous and feed on their own kind as on larvae of other species. Tscherepanov (1957) reported only on their damage on agricultural crops. According to the author's opinion the larvae are exclusively predaceous.

Genus: *Limonius* Eschscholtz

The larvae are uniformly light yellow or dark yellow. The head is transversal broader than its length. On the mandibles a well developed tooth (median tooth), is found. The indentation of the 9th, caudal segment is small, circular, nearly closed. The apices are short, powerful. The external apices are short, resembling to protuberances, the internal ones are more developed, flat. The area on the segment is well delimited.

Limonius pilosus Leske (Fig. 19)

Synonyms: *Elater cylindricus* Rossy
Limonius nigripes Gyllenhal

Distribution

It is an Eurasian species, distributed throughout Europe, West-Asia, Caucasus and Asia Minor. It is quite uncommon in Hungary, occurring mostly in chernozem soils.

Biology

Its way of life is only partially known; according to Jagemann (1955) it may damage agricultural crops, nursery trees. In the author's observations the larvae fed readily on plant materials.

Genus: *Synaptus* Eschscholtz

The body of larvae is cylindrical, reddish-brown. The head is nearly rectangular, somewhat broader than its length. On the frontal plate the four pairs of setae are always visible, the posterior lobe is lanceiform, narrowing from the middle into an apex with direct sides. The nasale is broad, pointed, with an additional tooth below the apex, therefore visible only from the ventral view. No eyes develop, the external part of the sutura epicranialis is well developed. The submentum is long, with parallel sides, the end is rounded. The stipes resembles to that of *Adrastus montanus*. The tergites of the thoracal- and abdominal segments are slightly sculptured with fine, longitudinal wrinkles. The piths serving as muscle attachment points are well developed and broad. The tergites are depressed around the piths. The lateral grooves are shallow. The 9th, caudal segment is 1.5 times longer than it is wide. The segment is directly tapering and terminates in a powerful, pointed apex. From among the two longitudinal grooves the external ones run from the middle of the muscle-attachment points (depressions), the internal ones from the lower end of these depressions. On the dorsal side of the segment a slightly delineated area is found, the surface of the latter is transversally wrinkled. On the apical part of the segment the setae are set on wart-like protuberances, surrounded by a pith. The body length of the larvae attains 22 mm.

Synaptus filiformis Fabricius (Fig. 20)

Synonyms: *Elater cinereus* Illinger,
Synaptus ungulliseris Gyllenhal

Distribution

It is more common in the forest- and forest-steppe zone of the Soviet Union, in the Carpathian Mountains and Caucasus, occurs rather rarely in agricultural soils. It is also uncommon in Hungary.

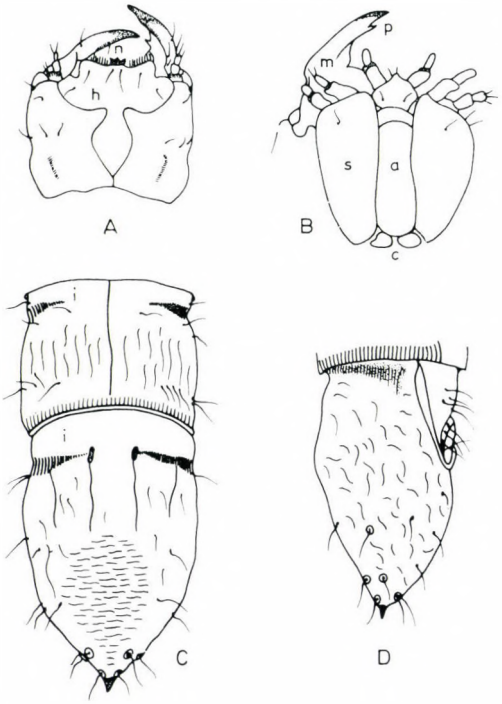

Fig. 20. *Synaptus filiformis* Fabricius, larva. A = head, viewed from above, n = nasale, h = frontal plate; B = head, from below, m = mandible, p = additional tooth, s = stipes, a = sub-mentum, c = cardo; C = 8th and 9th abdominal segments, viewed from above, i = depression, serving as muscle attachment point; D = caudal segment, lateral view (original)

Biology

Its trophic relationships are unknown. In laboratory rearings Dolin (1964) observed predation and cannibalism.

Genus: *Cardiophorus* Eschscholtz

The larvae live in the soil and in the litter layer. The species is quite common in forest soils but occurs rarely in agricultural soils. The larvae are predatory and feed on small insects and other invertebrates but may also feed on decaying plant substances (Dolin, 1964). In Hungary the species was only occasionally found in agricultural soils and in very low numbers.

In the Hungarian literature dealing with *Elateridae* (Móczár et al., 1969; Bognár and Huzián, 1979) also other species were mentioned (*Melanotus rufipes* Herbst, *Athous vittatus* Fabricius, *Athous subfuscus* Müller, *Dolopius marginatus*

Linnaeus etc.) but these may not be considered as they did not occur during the studies of the author. Their occurrence in Hungary may be possible but their agricultural significance is rather low.

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Correlation Between the Biological Activity and Thin-layer Chromatographic Behaviour of Some Benzonitrile Derivatives

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The effect of 10 substituted benzonitrile derivatives on 8 in vivo and in vitro photosynthetic characteristics, and thin-layer chromatographic behaviour of the same compounds on reversed-phase, on silica, on cellulose and on aluminiumoxide were determined. The chromatographic parameters showed high intercorrelations. Due to the intramolecular interactions of substituents and to the hydrophilic —OH group the lipophilicity of compounds increased drastically in ionic environment. The biological activities differed considerably from each other. This phenomenon can be explained by the differences between the in vivo and in vitro conditions. To correlate the biological activities and chromatographic behaviour of compounds polynomial functions were applied. In some cases the molecular adsorptivity correlated better to the biological effect than the lipophilicity suggesting that the biological efficiency of benzonitrile derivatives depends rather on their adsorption on hydrophilic surfaces. Higher adsorption energy decreased the biological activity in vivo.

Computer assisted methods have a growing acceptance in the up-to-date drug design (Cavallito, 1973; Andrew, 1979). The applicability of great number of molecular parameters has been studied to predict biological efficiency, however in most cases the lipophilicity was the most successfully applied (Fujita et al., 1964; Hansch and Dunn, 1972; Hansch and Clayton, 1973). To determine lipophilicity the classical partition in water: n-octanol (Hansch and Anderson, 1967), reversed-phase thin-layer chromatography (Biagi et al., 1969; Biagi et al., 1970; Boyce and Milborrow, 1965), high performance liquid chromatography (Haggerty and Murrill, 1974; McCall, 1975; Ellgehausen et al., 1981) and gas-liquid chromatography (Clifford and Watkins, 1971; János et al., 1982) have been applied.

Since the first contact between the bioactive compound and target organism is of adsorptive character we assumed that the biological efficiency may correlate not only to the lipophilicity but also to the adsorptivity of a compound. The adsorptivity of a compound is determined by the chemical structure and by the characteristics of sorbent. It is well known that the interaction of bioactive molecule and target organism takes place in ionic environment. On the other hand the ions can modify the lipophilicity (Cserháti et al., 1982), e.g. the effect of membrane damaging antibiotics depends on the ion concentration of media (Hancock, 1962; Hurwitz and Rosano, 1962; Kavanagh, 1963; Davis et al., 1969; Pache and Zahner, 1969; Pittinger and Adamson, 1972; Tamás et al., 1974).

The herbicidal character of 3,5-dihalogeno-4-hydroxi-benzonitriles has been known since 1963 (Wain, 1963; Carpenter and Heywood, 1963). These compounds

inhibit the photosynthetic electron transport chain on the reducing side of photosystem II in the region between Q and plastoquinone (Friend and Olsson, 1967; Paton and Smith, 1965; Katoh, 1972). Other derivatives as the 3-nitro-5-halogeno-4-hydroxi compounds have a similar mode of action (Szigeti et al., 1981). It is known that the herbicidal effect of 3,5-dihalogeno- and 3-nitro-5-halogeno derivatives depends on the halogeno substituents and decreases in the order: I > Br > Cl (Ferrari and Moreland, 1969; Trebst et al., 1979; Szigeti et al., 1982).

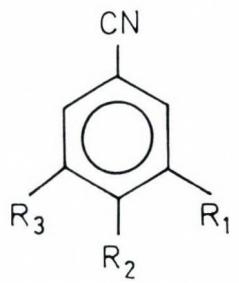
As the biological activity and chromatographic behaviour of compounds may be governed by the same molecular characteristics the aim of our work was to determine the lipophilicity and adsorptivity (Cserháti et al., 1982/b) of some benzonitrile derivatives by reversed-phase (RPTLC) and adsorptive thin-layer chromatography (TLC) and to study the effect of pH value and ion environment on these molecular parameters and to correlate them to the biological effect.

Materials and Methods

The benzonitrile derivatives were synthetized in the Research Centre of Chinoin Pharmaceutical and Chemical Works Ltd. (Budapest, Hungary). The chemical structure of compounds are shown in Table 1, the RPTLC and TLC

Table 1

Chemical structure of benzonitrile derivatives investigated



No. of compound	R_1	R_2	R_3
I	H	OH	H
II	H	OH	Br
III	H	OH	NO_2
IV	NO_2	OH	NO_2
V	Br	OH	Br
VI	Cl	OH	NO_2
VII	Br	OH	NO_2
VIII	I	OH	NO_2
IX	Br	O - CO - OCH_3	NO_2
X	Br	C - CO - $OCH(CH_3)_2$	NO_2

systems applied are compiled in Table 2. Layer thickness was always 0.25 mm. From solutions of 2 mg compound per cm^3 acetone 5 μl were spotted on the plates. After development the exact localization of spot maximums was carried out by video-densitometry (Telechrom OE 976, Chinoin, Hungary). The lipophilicity and the adsorptivity of compounds were characterized by R_M and R_f value respectively. For RPTLC the plates were impregnated in a 5% solution of paraffin oil in n-hexane for a night.

Because of the acidic character of $CaCl_2$, 2M $Ca(OH)_2$ suspension was titrated to pH = 7 value by 2M $CaCl_2$ solution and the resulting neutral solution was used as eluent.

Before correlating the biological activity (dependent variables) to the chromatographic parameters (independent variables) intercorrelation was investigated among the dependent and independent variables, respectively. We calculated

Table 2

Chromatographic systems to study the RPTLC and TLC behaviour of benzonitrile derivatives (BR = Britton–Robinson buffer)

No. of system	Layer	Eluent composition
1	reversed-phase	water : methanol 1 : 1
2		water : methanol 4 : 1
3		2M NaCl : methanol 4 : 1
4		2M NaCl
5		water
6		BR pH = 7.96
7		BR pH = 5.02
8		2M CaOHCl pH = 7.0
9	silica	water : methanol 1 : 1
10		water : methanol 4 : 1
11		water
12		2M NaCl
13		BR pH = 7.96
14		BR pH = 5.02
15		2M CaOHCl pH = 7.0
16	aluminiumoxide	water
17		2M NaCl
18		BR pH = 7.96
19		BR pH = 5.02
20		2M CaOHCl pH = 7.0
21	cellulose	water : methanol 4 : 1
22		water
23		2M NaCl
24		BR pH = 7.96
25		BR pH = 5.02
26		2M CaOHCl pH = 7.0

linear correlations between the chromatographic parameters (R_f or R_M values) measured in different eluents and between the biological activities:

$$y = a + b \cdot x \tag{1}$$

where y and x are R_f or R_M values, in case of biological systems y and x are inhibitory effect of compounds in percent. The simultaneous effect of organic solvent and ion concentration on the R_M value was described by the function:

$$y = a + b_1 \cdot \log x_1 + b_2 \cdot \log x_2 \tag{2}$$

where $y = R_M$, x_1 = concentration of methanol in percent, x_2 = molality of NaCl in eluent.

A polynomial equation was applied to correlate the biological activity to RPTLC or TLC parameters:

$$y = a + b_1 \cdot x + b_2 \cdot x^2 \tag{3}$$

where y = inhibition in percent, $x = R_f$ or R_M values.

Because of the relatively low number of compounds the application of more sophisticated QSAR methods could have increased the probability of occurrence of fortuitous correlations (Topliss and Edwards, 1980).

In vivo CO_2 fixation of spinach and of 7-day-old wheat leaves, 2,6-dichlorophenol-indophenole (DCPIP) reduction by isolated spinach or wheat chloroplast were determined as described earlier (Szigeti et al., 1981). The activity of ribulose-1,5-bisphosphate-carboxylase (RUBPC) and phospoenol-pyruvate-carboxylase (PEPC) isolated from spinach and wheat leaves was determined in vitro by the method of Nagy et al., 1973. The final concentration of benzonitriles was 30 μM in all cases.

Table 3

$100 \cdot R_M$(columns 1–8) and $100 \cdot R_f$(columns

No. of compound	No. of chromatographic									
	1	2	3	4	5	6	7	8	9	10
I	−25	4	−7	99	46	36	12	90	87	90
II	−37	−36	35	127	36	71	115	152	89	88
III	−76	−79	−26	85	−39	11	−5	57	93	96
IV	−72	−83	−21	82	−48	−7	−16	58	96	96
V	−75	−58	12	121	−21	26	74	105	nd	nd
VI	−65	−75	−13	78	−47	9	−12	78	92	97
VII	−65	−66	−5	94	−34	8	−4	87	94	95
VIII	−63	−57	9	117	−15	22	30	117	96	91
IX	28	117	nd	93	s	s	s	s	83	75
X	66	168	163	s	s	s	s	s	nd	nd

nd = non-detected, f = spot on front, s = spot on start
For other symbols see Tables 1 and 2

Results and Discussion

The R_f and R_M values compiled in Table 3 are the averages of five independent parallel determinations. The chromatographic behaviour of benzonitrile derivatives deviates considerably from that of antibiotics (Cserháti and Szőgyi, 1979), steroids (Cserháti et al., 1981), trisubstituted symmetric triazine derivatives (Cserháti and János, 1981), phospholipids (Cserháti et al., 1981) and heterocyclic quaternary ammonium salts (Cserháti et al., 1982) studied up till now. The benzonitrile derivatives move well in distilled water as eluent in the majority of chromatographic systems applied. According to our earlier results with other compounds, molecules containing hydrophilic substituents have a lower lipophilicity in ionic environment than in ion free one (Cserháti and Szőgyi, 1981). However the benzonitrile derivatives contain highly hydrophilic substituents they have higher liphophilicity in ionic environment. We assume that this phenomenon can be explained by the strong intramolecular forces between the substituents which are modified by the ions. Similar results were obtained by other authors (Butte et al., 1981; Draffen et al., 1981). Ions increase the lipophilicity of the compounds to a much more higher extent than that of other compounds investigated up-till now.

Similar results were obtained concerning the adsorption of benzonitriles on silica. Due to the competition of ions and hydrophilic substituents of compounds for the polar adsorption sites on silica surface a decrease of adsorptivity of molecules containing hydrophilic substituents can be expected and was found to be true for all other compounds studied. In case of benzonitrile derivatives – in spite of hydrophilic substituents – their adsorptivity is enhanced by ions. This effect is extremely great compared to the behaviour of other bioactive compounds in presence of ions but it is not so great as the ion effect observed in RPTLC.

9–26) values of some benzonitrile derivatives

system												
11	12	13	14	15	16	19	21	22	23	24	25	26
87	77	82	86	76	nd	85	nd	nd	nd	68	66	44
89	64	83	86	63	nd	87	nd	nd	nd	nd	nd	nd
91	56	94	f	74	90	92	78	75	nd	74	69	nd
97	82	f	f	82	87	91	85	91	53	76	73	55
90	53	94	f	61	nd	89	nd	nd	nd	nd	nd	nd
96	71	f	f	76	89	95	nd	85	42	71	67	nd
96	74	f	f	71	88	89	83	85	37	69	69	nd
95	73	f	f	nd	90	88	81	84	31	66	60	nd
42	s	41	41	17	93	85	nd	nd	nd	38	36	nd
s	s	nd	nd	s	nd	94	nd	nd	s	3	6	s

In systems 17, 18 and 20 the spots were too streaked making the determination of R_f values impossible.

Table 4

Linear correlations between the chromatographic parameters of some benzonitrile derivatives

No. of function	y	x	n	$r_{calculated}$	Significance level, %
I	1	2	10	0.9898	99.9
II	1	3	9	0.9205	99.9
III	1	5	8	0.9286	99.9
IV	2	3	9	0.9387	99.9
V	2	5	8	0.9441	99.9
VI	3	4	8	0.9263	99.9
VII	6	7	8	0.8755	99
VIII	8	4	8	0.9017	99
IX	9	10	8	0.8496	99
X	9	11	8	0.8401	99
XI	9	12	8	0.7427	95
XII	9	16	6	0.8310	95
XIII	10	11	8	0.9396	99.9
XIV	10	12	8	0.8560	99
XV	10	16	6	0.8268	95
XVI	11	12	10	0.9170	99.9
XVII	11	16	6	0.8454	95
XVIII	12	16	6	0.9103	95
XIX	24	25	8	0.9963	99.9
XX	15	12	9	0.9679	99.9
XXI	1	9	8	0.9457	99.9
XXII	2	10	8	0.9630	99.9
XXIII	5	11	8	0.8170	95

$y = a + bx$, y and x = R_M or R_f values measured under different conditions
For symbols see Table 2.

On aluminium oxide the benzonitrile derivatives do not give sharp spots in ionic eluents, however they show the same extremely low adsorptivity in distilled water as on silica surface. On cellulose the adsorptivity is similarly negligible in distilled water and it increases considerably in ionic environment. The lipophilicity enhancing effect of ions can be observed also at a buffer concentration of 0.1 M.

The regression coefficients of significant linear correlations between R_f and R_M values measured in various chromatographic systems are listed in Table 4. Following the general rule the R_M values measured at different organic solvent concentrations correlate well (Table 4, functions I–V). At higher ion concentration the correlation diappears showing that the ion sensitivity of benzonitrile derivatives deviates considerably from each other. The good correlation between the R_M values measured at pH values 5 and 8 (function VII) indicates that in this interval the pH value does not affect the lipophilicity order of compounds. The lipophilicity values determined in presence of sodium and calcium ions also corre-

Table 5

Dependence of lipophilicity of some benzonitrile derivatives on methanol and NaCl concentration of eluent

Parameters of function	No. of compound		
	I	V	VIII
b_1	−0.17	−0.16	−0.16
b_2	0.07	0.32	0.29
a	0.30	0.45	0.43
r^2	0.8948	0.9485	0.9454
r	0.9459	0.9739	0.9723
b_1'	−0.86	−0.50	−0.53
b_2'	0.26	0.74	0.71
s	0.23	0.25	0.24
s_{b_1}	0.05	0.05	0.05
s_{b_2}	0.06	0.07	0.07
F	8.51	18.42	17.32
t_1	3.67	3.07	3.15
t_2	1.10	4.50	4.23

$y = a + b_1 \cdot \log x_1 + b_2 \cdot \log x_2$, $y = R_M$, x_1 = methanol %, x_2 = NaCl molality, $n = 8$, $F_{95\%} = 5.79$, $t_{95\%} = 2.57$

late well pointing out the insignificant role of ion quality (function VIII). However, we have to stress that the high effect of ion concentration may overshadow the eventually low effect of ion quality. Neither the quantity of organic solvent nor the ion concentration influence considerably the adsorption order of benzonitrile derivatives on silica (functions IX–XI, XIV–XVI). The adsorption orders on silica and on aluminiumoxide are similar (functions XII, XV, XVII, XVIII) illustrating that the acidity or alkalinity of adsorption sites has a negligible importance in the binding order of benzonitriles.

The adsorptivity orders measured at different pH values and ion environments correlate well on silica and on cellulose (functions XIX–XX) supporting our data obtained under RPTLC conditions that the 5–8 pH range and the difference between the effect of sodium and calcium ions do not influence differently the order of parameters investigated. Between lipophilicity and adsorptivity on silica very good correlations were found (functions XXI–XXIII). This fact emphasizes the possibility to apply the adsorptivity as a molecular parameter in QSAR studies. The adsorptivity can be determined more easily by TLC than the lipophilicity by RPTLC. The lipophilicity did not correlate to the adsorptivity on cellulose and on aluminium oxide.

Function 2 describing the simultaneous effect of organic solvent and ion concentration fits well to the experimental data (Table 5). The change of the two

Table 6

Biological activity of some benzonitrile derivatives (inhibition %)

No. of compound	Biological parameter							
	A	B	C	D	E	F	G	H
I	14.20	4.38	−6.96	35.06	2.78	9.26	3.86	34.86
II	8.31	31.12	6.25	18.83	16.67	4.63	1.65	33.94
III	2.88	35.98	0.71	43.51	11.11	9.26	9.09	28.44
IV	98.69	−12.32	1.61	32.47	51.39	−21.30	5.79	32.11
V	91.23	89.30	0.00	27.92	51.39	52.78	10.47	30.38
VI	80.89	23.66	8.04	16.88	52.78	−2.78	0.00	7.34
VII	91.56	84.93	−3.04	55.84	62.50	4.63	1.38	41.28
VIII	91.69	33.55	12.86	27.27	76.39	16.67	7.16	15.60
IX	28.60	88.98	6.07	0.00	45.83	8.33	15.43	−10.09
X	44.18	74.55	19.29	12.34	45.83	−5.56	21.49	6.42

A. DCPIP-reduction by spinach chloroplast
C. RUBPC-activity of spinach
E. DCPIP-reduction by wheat chloroplast
G. RUBPC-activity of wheat
For other symbols see Table 1
B. CO_2 fixation of spinach leaves in vivo
D. PEPC-activity of spinach
F. CO_2 fixation of wheat seedlings in vivo
H. PEPC-activity of wheat

independent variables explains about 90% of the change in R_M value. It can be concluded from the normalized slope values that the two independent variables have similar impact on lipophilicity. It must to be noted that in case of heterocyclic quaternary ammonium salts the ionic effect (Cserháti et al., 1981), in case of trisubstituted symmetric triazine derivatives the solvent effect is the determinative one (Cserháti and János, 1981; János and Cserháti, 1982).

The biological activities summarized in Table 6 were discussed in details in our earlier publications (Szigeti et al., 1981; Szigeti and Nagy, 1980). While in chromatographic systems the behaviour of benzonitrile derivatives exhibits similarities, their biological activities correlate hardly to each other (Table 7). This is understandable since the data represent biological activities measured on in vivo and in vitro systems respectively. Under in vivo conditions the molecules on their path from external application to the final site of action are affected by several factors causing a decrease in their effectivity, so e.g. chemical or photochemical degradation, incomplete penetration, biochemical degradation, binding by unspecific receptors (Draber et al., 1974). During the relatively short time of in vitro measurements only this last factor can play a definitive role. We assume that the other factors mentioned above can be responsible for the possible deviation between in vivo and in vitro results. The deciding role of these other factors is

Table 7

Parameters of significant linear correlations between biological activities of some benzonitrile derivatives

y	x	a	b	s_b	$r_{calculated}$
A	E	−4.85	1.44	0.29	0.8681
C	H	11.42	−0.33	0.12	0.6844
D	H	9.06	0.82	0.19	0.8338

$y = a + b.x$, $n = 10$, $r_{95\%} = 0.6319$, $r_{99\%} = 0.7646$, y and x = inhibition % measured on various biological objects. For symbols see Table 6

manifested also in the different in vivo sensitivity of spinach and wheat leaves to the benzonitrile derivatives.

Due to the high intercorrelation of RPTLC and TLC data function 3 was applied to each biological activity but only to some chromatographic parameters. The calculated F values are compiled in Table 8. The two DCPIP reductions (A and E) do not show correlation to any of molecular parameters investigated. Maybe one or several factors mentioned above are responsible for this finding. Both in vivo activities (B and F) correlate mainly to the adsorption capacity of compounds on hydrophilic surface in presence of ions. It indicates that adsorptive processes (adsorption energies between bioactive compound and target) may influence considerably the biological efficiency. The interaction of benzonitrile derivatives and

Table 8

Calculated F values of polynomial functions

No. of chromatographic system	Biological parameter							
	A	B	C	D	E	F	G	H
1	1.93	0.94	3.00	2.88	0.95	0.18	*11.47*	2.46
2	0.74	1.07	1.98	2.76	1.27	0.42	*9.46*	*4.63*
4	0.06	1.04	*4.54*	0.62	0.03	2.63	2.44	0.58
5	1.89	0.65	1.12	*3.40*	0.77	0.94	*5.08*	*4.76*
6	1.80	1.12	1.66	*4.54*	0.57	2.73	*4.99*	*3.28*
7	0.45	1.79	1.65	*5.21*	0.00	*5.26*	3.32	2.05
8	0.78	1.71	2.76	*4.67*	0.22	1.40	*5.39*	2.44
11	1.28	1.00	*3.33*	*3.52*	0.31	0.49	*10.95*	*3.45*
12	0.74	*4.04*	1.67	3.09	0.24	*3.66*	*11.49*	*4.76*

$y = a + b_1 \cdot x + b_2 \cdot x^2$, y = biological activity, x = chromatographic parameter, $F_{90\%} = 3.26$, $F_{95\%} = 4.74$, $F_{99\%} = 9.55$

For symbols see Tables 1 and 6

the target (the actual site of action) can be governed by hydrophilic binding forces. In some cases the RUBPC and PEPC activites show different correlation patterns. This fact indicates that the inhibition of the same enzyme isolated from spinach or wheat may require different structural parameters. In general the change in chromatographic parameters explained 50–75% of the change of biological efficiency. The normalized slope values were similar for the linear and quadratic member of function 3 showing the applicability of polynomial approximation.

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Complexing of Some Bioactive Heterocyclic Quaternary Ammonium Salts by Beta-cyclodextrin Polymer

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The 3,3,5-trimethyl-1-azacycloheptane and 1,3,3-trimethyl-6-azabicyclo-(3,2,1)-octane derivatives showing marked growth retardant and anti-feeding activity can be easily complexed by β-cyclodextrin polymer. The effect of environmental factors such as pH value and adsorption on hydrophilic and lipophilic surfaces on the complex stability was studied by thin-layer chromatography. The results established that the inclusion complexes have a higher hydrophilicity than the original quaternary ammonium salts. The β-cyclodextrin decreases their adsorption strength on basic and on acidic surfaces. The stability of inclusion complexes depends considerably on the pH value, the substituents exert different effects at different pH value.

Due to the favourable physico-chemical characteristics of cyclodextrin inclusion complexes of bioactive compounds their rapidly broadening application is expected in the future also in the up-to-date agrochemistry (Szejtli, 1982; Chiba and Yonemura, 1982). As beta-cyclodextrin form inclusion complexes with aromatic amino acids (Szejtli, 1982; Solms and Egli, 1965; Wiedenhof, 1969; Zsadon et al., 1979; Hartmann et al., 1978) and also with the polypeptide antibiotics polymixine containing aromatic amino acids (Cserháti et al., 1983) it was promising to extend the investigations to study the inclusion complex formation of some bioactive heterocyclic quaternary ammonium salts because their heterocyclic ring has similar size as the rings of aromatic amino acids therefore they will probably fit to the cavity of beta-cyclodextrin.

The heterocyclic quaternary ammonium salts show marked antifeeding and growth retarding activity. To correlate their biological activity to their physico-chemical parameters as lipophilicity and adsorptivity thin-layer and reversed-phase thin-layer chromatography (TLC and RPTLC) were applied. The pH value, ion strength and ion quality (ion charge and ion radii) influence considerably their chromatographic behaviour (Cserháti et al., 1982a). As good correlation was found between the biological activity and physico-chemical parameters measured by TLC and RPTLC (Darwish et al. 1982a,b) we assume that the change of chromatographical behaviour caused by beta-cyclodextrin will correlate with the change of biological activity.

The TLC and RPTLC methods are suitable not only for the better separation of compounds, but also they are applied to determine interaction energies of two molecule species. Generally one species forms the layer and the second one is run on it (Cserháti et al., 1982b) or one species is mixed in the eluent and the second

one is run on this (Cserháti et al., 1983) or the complexing agent is mixed in the silicagel (Slifkin et al., 1982). In RPTLC the effect of complexing agent is measured by the differences of R_M values:

$$B = R_{M_1} - R_{M_2} \tag{1}$$

where B = binding constant characterizing the interaction energies of molecules,
R_{M_1} = lipophilicity value of one molecule measured in RPTLC system in absence of second molecule,
R_{M_2} = lipophilicity value of one molecule measured in RPTLC system in presence of second molecule.

However, in TLC the evaluation methods are not uniformly agreed. To calculate the effect of interaction, the relative differences of R_f values (Slifkin et al., 1982):

$$B = \frac{R_{f_1} - R_{f_2}}{R_{f_1}} \cdot 100 \tag{2}$$

where B = as in Eq. 1,
R_{f_1} = R_f value of one molecule measured in TLC system in absence of second molecule,
R_{f_2} = R_f value of one molecule measured in TLC system in presence of second molecule,
or the differences in the logarithms of R_f values (Cserháti et al., 1982b) are applied:

$$B = \log R_{f_1} - \log R_{f_2} \tag{3}$$

where the symbols are the same as in Eq. 2.

Materials and Methods

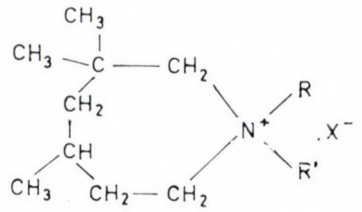
Chemical structure of heterocyclic quaternary ammonium salts is shown in Fig. 1.

Layers of 0.25 mm thickness were prepared on glass plates of 20 × 20 cm from the following supports:

I. Kieselgel G nach Stahl (Merck)

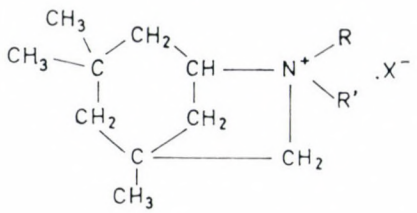
II. MN-Aluminiumoxide G (Macherey Nagel)

To determine lipophilicity the plates were impregnated with 5% paraffin oil in n-hexane and dried at room temperature. The strength of adsorption (adsorptivity) was measured on non impregnated plates because recent research indicates that the results obtained on non impregnated plates showed a better correlation with the biological activity than the lipophilicity values determined on impreg-



3,3,5-trimethyl-1-aza-cycloheptane derivatives

Compound number	R	R'	X^-
1	CH_3	CH_3	J
2	CH_3	H	J
3	CH_3CH_2	CH_3	J
4	$CH_2=CHCH_2$	CH_3	Br
5	$C_6H_5CH_2$	CH_3	Cl
6	$(CH_2)_4Br$	—	—



1,3,3-trimethyl-6-aza-bicyclo (3.2.1.)-octane derivates

Compound number	R	R'	X^-
7	CH_3	CH_3	J
8	CH_3	H	J
9	CH_3CH_3	H	J
10	$CH_2=CHCH_2$	H	Br

Fig. 1. Chemical structure of heterocyclic quaternary ammonium salts

nated ones (Guerra et al., 1981). The compounds (2 μl of solutions of 10 mg of heterocyclic quaternary ammonium salts in 1 cm^3 of methanol) were spotted on the plates. All determinations were run in four parallels. Because of the fairly low solubility of beta-cyclodextrin monomer in the eluent, its water soluble polymer (5300 dalton weight-average molecular weight prepared by cross-linking beta-cyclodextrin with epichlorohydrine) (Fenyvesi et al., 1982) was applied in our investigations. Methanol was chosen as an organic solvent miscible with water because it does not form stable inclusion complexes with beta-cyclodextrin (Budai and Szejtli, 1981) consequently it does not modify the character of interaction between beta-cyclodextrin polymer and heterocyclic quaternary ammonium salts. However, for other type of compounds it was proved that the interaction strength decrease at increasing concentration of organic solvent in eluent (Cserháti et al., 1983). The TLC and RPTLC systems applied are listed in Table 1. After development the plates were dried at 105 °C, the spots were revealed with Dragendorff reagent (Stahl, 1962).

Results and Discussion

The binding constants calculated by Eqs 1, 2 and 3 are compiled in Table 2.

The beta-cyclodextrin polymer change the chromatographic behaviour of heterocyclic quaternary ammonium salts in all TLC and RPTLC system proving unambigously the interaction (probably inclusion complex formation). However, our experiments could not exclude the possibility of adsorption of quaternary

Table 1

TLC and RPTLC systems to study the interaction of some heterocyclic quaternary ammonium salts with beta-cyclodextrin polymer (βCD)

Layer	Impregnation	Eluent composition		βCD g	Sign of binding constant calculated
		water cm^3	methanol cm^3		
Silica	no	17	17	—	B_1
		17	17	2	
	no	17[a]	17	—	B_2
		17[a]	17	2	
	no	17[b]	17	—	B_3
		17[b]	17	2	
	yes	17	17	—	B_4
		17	17	2	
	yes	17[a]	17	—	B_5
		17[a]	17	2	
	yes	17[b]	17	—	B_6
		17[b]	17	2	
Aluminiumoxide	yes	34	0	—	B_7
		34	0	2	

[a] = Britton–Robinson buffer, pH 9.15.
[b] = Britton–Robinson buffer, pH 6.09.

ammonium salts on the various polar or apolar groups on beta-cyclodextrin polymer surface. We have to take into consideration that in consequence of polymerization the access to the sites of inclusion complex formation (central cavities in cyclodextrin moieties) are hindered in various degree modifying the retention of

Table 2

Binding constants of interaction between heterocyclic quaternary ammonium salts and beta-cyclodextrin polymer

No. of compound	No. of binding constants									
	$B_{1(2)}$	$B_{1(3)}$	$B_{2(2)}$	$B_{2(3)}$	$B_{3(2)}$	$B_{3(3)}$	$B_{4(1)}$	$B_{5(1)}$	$B_{6(1)}$	$B_{7(1)}$
1	−479.2	−0.76	−29.0	−0.11	−116.1	−0.33	−1.12	−0.31	−0.26	−0.70
2	−804.6	−0.96	−26.4	−0.10	−107.0	−0.32	−1.07	−0.24	−0.23	−0.60
3	−657.1	−0.88	−25.2	−0.10	−82.3	−0.26	−1.17	−0.27	−0.13	−0.70
4	−624.9	−0.87	−24.7	−0.09	−79.9	−0.26	−1.19	−0.20	−0.23	−0.83
5	−522.3	−0.80	−10.7	−0.04	−64.3	−0.22	−1.13	−0.32	−0.24	−0.78
6	−591.5	−0.84	−6.1	−0.03	−72.8	−0.24	−1.16	−0.23	−0.21	−0.68
7	−403.6	−0.70	−7.5	−0.03	−57.2	−0.20	−1.21	−0.19	−0.33	−0.63
8	−511.0	−0.79	−10.6	−0.04	−64.5	−0.22	−1.50	−0.11	−0.25	−1.62
9	−370.6	−0.67	−10.9	−0.04	−39.8	−0.15	−1.21	−0.23	−0.32	−0.86
10	−367.8	−0.67	−11.3	−0.05	−127.1	−0.36	−1.14	−0.21	−0.32	−0.57

Second index (in parentheses) of B values refers to Eq. applied in the calculation

complexable molecules. Furthermore, secondary cavities of different dimensions are formed within the polymer network. These dimensions are at least partly commensurable with the dimensions of beta-cyclodextrin cavity resulting in different inclusion complex formation energies and consequently in different retention characteristics.

Adsorptivity

The binding constants of adsorptivity change are the highest in non buffered ion free system. This observation was somewhat unexpected because the ions of buffer present in the eluent are bound to the available silanol groups of silica surface reducing its adsorption capacity. Consequently the competitive equilibrium between the dissolved beta-cyclodextrin polymer and the silanol groups of solid phase should be shifted to the favour of complexation of heterocyclic quaternary ammonium salts by the polymer.

Our data however strongly contradict this supposition. The deteriorating effect of ions on the stability of inclusion complex can be higher than that which follows from our data because we could measure only the resultant of two effects: the diminution of adsorption strength of silica and the decrease of stability of inclusion complex caused by the ions of buffer in eluent.

The binding constants of buffered system pH 6.09 are higher than the corresponding values at pH 9.15, indicating that the degree of dissociation of quaternary ammonium groups influences considerably the inclusion complex formation that is also ionic processes take part in the interaction. The correlation between the binding constants of various adsorptive systems are fairly poor indicating that the different environmental conditions as adsorber characteristics, pH value of media influence differently the inclusion complex formation.

The binding constants of 3,3,5-trimethyl-1-azacycloheptane derivatives are higher in all adsorptive systems than the binding constants of 1,3,3-trimethyl-6-azabicyclo(3,2,1)-octane derivatives. It indicates that the bulkier bicyclo-derivatives have a lower probability to enter in the beta-cyclodextrin cavity than the smaller cycloheptane derivatives. The conclusions drawn from the binding constant calculated by Eqs 2 and 3 are the same, that is in our case, both Equations gave the same results.

Lipophilicity

The effect of beta-cyclodextrin polymer on the lipophilicity of heterocyclic quaternary ammonium salts is similar to the effect on adsorptivity. The non impregnated systems show the highest differences, however on impregnated aluminiumoxide the impact of beta-cyclodextrin is a little lower than on impregnated silica. This observation can be explained by the assumption, that the paraffin oil does not cover all adsorptive sites of supports therefore the original adsorptive characteristics prevail also after impregnation.

The lower impact of beta-cyclodextrin polymer on the lipophilicity of heterocyclic quaternary ammonium salts in buffered systems is explained by the facts that the anions of buffers form ion pairs of different bonding strength with the quaternary ammonium groups, thus influencing the lipophilicity of the molecule and the ions can inhibit the complexable molecules to enter in the cavity of beta-cyclodextrin polymer.

The correlations between the various binding constants are poor proving once more again that the ionization of quaternary ammonium groups and the remaining adsorptive capacity of supports play a deciding role in the inclusion complex stability of heterocyclic quaternary ammonium salts.

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Book Review

R. K. S. Wood (editor): *Active Defense Mechanisms in Plants*. Plenum Press, New York and London, 1982, 381 + X p.

This is the 37th volume of the NATO Advanced Study Institutes Series and contains the papers presented at the symposium held in Cape Sounion, Greece, 21 April–3 May, 1981. Sixteen lectures were held and all participants read contributed papers. Only the summary of the contributed papers are published in this volume, in addition to the whole texts of the lectures. The discussions are not included in the book. All of the papers are dealing with those defence mechanisms that follow changes in the host caused by the pathogen. The passive mechanisms that are independent of the pathogen, are not included in the text.

The book begins with an account on the general aspects of active defence mechanisms (Heitefuss). The subsequent lectures deal with the structural view of active defence (Ingram), physiological and biochemical events associated with expression of resistance to disease (Bailey), mechanisms conferring specific recognition in gene-for-gene plant-parasite systems (Keen), determinants of plant response to bacterial infection (Sequeira), defence mechanisms of plants against varietal non-specific pathogens (Touzé and Esquerré-Tugayé), mechanisms in compatible host-pathogen interactions (Heath), plant immunization-mechanisms and practical implications (Kuć), genetical aspects of active defence (Ellingboe), active resistance of plants to viruses (Harrison), localized resistance and barrier substances (Loebenstein, Speigel and Gera), the protective effects of systemic virus infection (Fulton), regulation of changes in proteins and enzymes associated with active defence against virus infection (Van Loon), antiviral agents and inducers of virusresistance: analogies with interferon (Gianinazzi), the effect of defence reactions on the energy balance and yield of resistant plants (Smedegaard-Petersen).

The book is extremely useful for researchers in the fields of plant pathology, genetics, plant breeding and plant physiology. At the end of the book the subject index helps the reader to find quickly themes, definitions and pathogens. The editor has done a perfect work in arranging the texts of the lectures and contributions. This volume will be a valuable piece of a series of books recently published on plant pathophysiology and disease resistance.

Z. KIRÁLY

I. J. Misaghi: *Physiology and Biochemistry of Plant-Pathogen Interactions*. Plenum Press, New York and London, 1982, 287 + XVI p.

This useful book brings together the literature on the biochemistry and physiology of plant parasite interactions. It is an up-to-date reference to the current state of pathophysiology of plants. Thus, advanced students, researchers and teachers of the biochemistry of healthy and infected plants can use it successfully. The recent results accumulated in the seventies and eighties are included into the text but some important early literature is also mentioned.

Treating the subject, the author begins with the penetration and infection processes, then discusses the pathogen produced metabolites and the alterations in plant structure and function. Emphasis has been made on the recognition process between plant and pathogen (surface-surface interraction between the two organisms).

Chapter 1 deals with the definitions (symptoms, pathogenicity, virulence, tolerance, compatibility, hypersensitivity, biothrops, etc.). The subsequent chapters with the penetration of pathogens, the role of cell-wall-degrading enzymes, toxins, altered permeability, water relations in diseased plants, pathological carbohydrate metabolism, alterations in transcription and translation, phenol metabolism, growth regulators, physiology of tumors mechanism of disease resistance, induced resistance and specificity of host-parasite interactions. At the end of the book some useful books are recommended for further studies. There is an extremely useful and modern collection of references and a subject index in the book.

The text is readable. The topics are very well summarized and the author pointed out several uncertainties whenever it was possible and necessary.

This fine summary is highly recommended for those who are working in the fields of plant pathology, plant physiology, biochemistry and plant breeding.

Z. KIRÁLY

Y. Asada, W. R. Bushnell, S. Ouchi and C. P. Vance: *Plant Infection: The Physiological and Biochemical Basis*. Japan Scientific Soc. Press, Tokyo and Springer Verlag, Berlin, Heidelberg and New York, 1982. XVIII + 362 p.

This symposium volume contains the proceedings of a U.S. — Japan meeting held May 17–22, 1981 in Brainerd, Minnesota on the physiological basis of plant infection and to commemorate the contributions of two internationally known Japanese professors, K. Tomiyama and I. Uritani. The book covers the recent findings of broad areas of disease physiology: penetration, the hypersensitive reaction, the role of phytoalexins and preformed compounds in disease resistance, specificity of host-pathogen interactions, toxins, metabolic alterations in diseased plants, induced resistance and induced susceptibility, the development of symptoms in infected plants.

Y. Asada summarized the recent advances in Japan in the physiology of plant infection. The subsequent papers and discussions are dealing with the sites of action of disease determinants (Durbin), initial events during penetration (Sherwood and Vance), primary germ tubes of powdery mildew conidia (Kunoh), infections by *Rhizobium* (Bauer), elicitation and suppression of the hypersensitive reaction (Doke, Tomiyama and Furuichi), hypersensitivity in rusts and powdery mildews (Bushnell), physiology of induced susceptibility (Ouchi and Oku), immunization of plants against virus, fungus and bacterial diseases (Kuć), metabolic alterations in response to wounding and infection (Oba et al.), metabolic regulation in plant pathogens (Kosuge and Comai), mitochondrial DNA associated with cytoplasmic male sterility and disease susceptibility (Kemble na Pring), host specific toxin of *Alternaria alternata* (Nishimura et al.), the host specific toxins of *Helminthosprium* spp. (Daly), chemical basis of host recognition by *Alternaria* spp. (Ueno et al.), action sites for AM-toxin (Kohmoto et al.), biochemical mechanism of glyceollin accumulation in soybean (Yoshikawa and Masago), phytoalexins (Keen), phytoalexins as preformed antifungal substances (Tani and Mayama), detoxification of phytoalexins (VanEtten), hypersensitive cell death (Tomiyama), biochemical approaches to general principles in plants underlying plant disease phenomena.

This symposium volume is highly recommended to those who are working on the fields of plant pathology, plant biochemistry and physiology and plant breeding.

Z. KIRÁLY

E. Klapp: *Taschenbuch der Gräser. Erkennung und Bestimmung, Standort und Vergesellschaftung, Bewertung und Verwendung.* 11. überarbeitete Auflage von P. Boeker. Verlag Paul Parey, Berlin und Hamburg. pp. 261.

Soeben erschien die 11. Auflage des bewährten Werkes, die im wesentlichen die Vorteile der früheren Ausgaben beibehalten hat, so die kurzen Darstellungen, sichere Bestimmungsschlüssel und nützliche ergänzende Beschreibungen, Abbildungen und Wertungen. Das Werk dient zum Bestimmen mitteleuropäischer Gräser in blühendem und blütenlosem Zustand.

Kapitel II befaßt sich in klarer, kurzer Darstellung mit der Lebensdauer, dem Wuchs sowie vegetativen und Blütenmerkmalen der behandelten Gräser.

Kapitel III gibt einen kurzen, tabellarischen Überblick über die Unterscheidungsmerkmale der Süßgräser von Sauergräsern, Simsen und Binsen.

Kapitel IV enthält den Bestimmungsschlüssel blühender Gräser. Dabei werden nach dem Typ des Blütenstandes 6 Gruppen gebildet, innerhalb dieser sind Arten oder Artengruppen aufzufinden. Die Artengruppen und artenreichen Gattungen werden in Gruppenschlüsseln weiter bis auf die Arten abgeleitet, z. B. die Queckenarten, Schwingelgruppe, Rotschwingel usw.

Kapitel V besteht aus dem Schlüssel für blütenlose Gräser. Dabei werden drei gut unterscheidbare Gruppen gebildet und in diesen der übliche dichotomoe Schlüssel praktiziert. Zum Schluß sind die vier Hauptgetreidearten abgebildet und in Schlüssel gefaßt.

Kapitel VI stellt die Gräser im Bilde vor. 143 Arten sind von Teilblütenstand bis zum zusammengesetzten charakteristischen Blütenstand (Ähren, Rispen usw.) mit den zum Erkennen wichtigen vegetativen Merkmalen, z. B. Blattquerschnitten, Öhrchen, Blatthäutchen usw. abgebildet.

Kapitel VII enthält Beschreibungen jeder einzelnen Art hinsichtlich ihres Verhaltens, ihrer Verwendbarkeit, landwirtschaftlichen Wertes, Verbreitung, Standort und Vergesellschaftung.

Kapitel VIII besteht aus nützlichen Tabellen, in denen charakteristische Entwicklungsdaten (Blütezeit, Lebensdauer, Wuchsform, Zeigerwert, Verhalten zum Standort) zusammengefaßt wurden.

Kapitel IX befaßt sich mit ansaatwürdigen Futtergräsern und ihrer Verwendung.

Ein weiteres Kapitel wurde von P. Boeker bearbeitet und behandelt Rasenansaaten.

Das Bestimmungsbuch ermöglicht in bewährter Weise das Erkennen und Bestimmen der mitteleuropäischen Gräser. Es ist all denen zu empfehlen, die Gräser in Mitteleuropa und den angrenzenden Gebieten in blühendem oder nicht blühendem Zustand bestimmen wollen. Als besonders nützlich wird es sich für alle erweisen, die sich mit Krankheitserregern an Gräsern befassen und sonstige tiefere Kenntnisse des Fachgebiets benötigen.

L. Almádi

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