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Current Plant Virus Research in Hungary*

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Research in Hungary on plant viruses and diseases of viral etiology resulted in increase, not only in our understanding of plant-virus and vector-virus interactions, but also in discovering new sources for resistance, new diagnostic tools, and the use of plant viruses as vectors in biotechnology. There are three institutes in Hungary dealing scientific investigations in the following topics: (i) biological characterization of plant viruses isolated from the Hungarian flora, such as wheat and barley, maize, potato and sugar-beet, tobacco, vegetables; *Brassica* spp.; fruit trees and grapes; forest trees and shrubs; medicinal and ornamental plants; weed plants; aquatic plants from natural water sources (rivers and lakes), (ii) virus resistance in wild plant species, (iii) physiological studies on virus infected plants, (iv) molecular characterization of plant viruses isolated from the Hungarian flora, (v) coat protein mediated resistance in higher plants, (vi) characterization of Cymbidium ringspot *Tombusvirus* (CyRSV), and (vii) plant virus diagnosis based on nucleic acid hybridization.

1. Biological characterization of plant viruses isolated from the Hungarian flora

Last years more than 50 viruses, belonging to 25 virus groups, were isolated and identified from the Hungarian flora. Boussingaultia mosaic *Potexvirus* (BoMV), Datura innoxia Hungarian mosaic *Potyvirus* (HDIV), dulcamara yellow fleck *Tobamovirus* (DYFV), grapevine chrome mosaic *Nepovirus* (GCMV), grapevine line pattern *Ilarvirus* (GLPV), Melandrium yellow fleck *Bromovirus* (MYFV), *Solanum hannemannii Tymovirus* (ShV) are considered as new scientific results not only in Hungary but also in the world as well (Lehoczky and Sárospataki, 1967; Beczner and Vassányi, 1980; Peralta et al., 1981; Hollings and Horváth 1981; Salamon et al., 1987; Horváth et al., 1988a; Lehoczky et al., 1989; Horváth et al., 1993a).

1.1. Gramineae viruses

Until 1988 only barley yellow dwarf *Luteovirus* (BYDV) and barley stripe mosaic *Hordeivirus* (BSMV) was described in Hungarian small grains but their strains were not characterized (Szirmai, 1967; Milinkó and Remete, 1984; Pocsai et al., 1985; Pocsai, 1988). In a five years period the infection a mild, new strain of barley stripe mosaic *Hordeivirus* (BSMV-H) was described (Nagy and Gáborjányi, 1991) and later the occurrence

* Dedicated to Professor Dr. Gy. Sáringer academician, on the occasion of his 70th birthday.

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of other gramineaviruses were detected. According to the last screening the brome mosaic *Bromovirus* (BMV) and BSMV was considered the most common pathogens of maize and barley (Gáborjányi and Nagy, 1988; Papp et al., 1996). The abundance and rapid distribution of BMV in small grains seemed to be correlated to the gradation of *Oulema melanopus* and other *Oulema* species. Positive vector transmission was made from BVM infected wheats to wheat, but not from wheat to corn (Gáborjányi and Szabolcs, 1988). The presence of wheat streak mosaic *Potyvirus* (WSMV) was demonstrated by serological diagnosis and electron microscopy. WSMV was found mainly in the South part of the country (Pocsai and Barabás, 1985; Nyitrai and Gáborjányi, 1988). Infection of wheat dwarf *Geminivirus* (WDV) was found both in wheats and barley (Gáborjányi et al., 1988; Bisztray et al., 1989). Adults and nymphs of the leafhopper *Psammotettix alienus* were able to transmit the pathogen from infected wheats to young wheat seedlings. Hungarian and Czech isolates of WDW were serologically identical. Other three viruses, namely cocksfoot mottle *Sobemovirus* (CfMV), agropyron mosaic *Potyvirus* (AgMV) and ryegrass mosaic *Potyvirus* (RyMV) was also described, without significant economical importance (Gáborjányi, 1991). Antiviral activity of tiazofurine was demonstrated in barley plants after BSMV infection (Nagy et al., 1989).

1.2. Maize (corn) pathogenic viruses

Reactions of different corn varieties to maize dwarf mosaic *Potyvirus* (MDMV) in the fields lead to the discovery of sugarcane mosaic *Potyvirus* (SCMV). In addition to the most common MDMV-A, two SCMV strains caused a consederable yield loss: SCMV-A, which infects *Sorghum halepense*, and the more common SCMV-MB strain, which did not infect the Johnsongrass (Gáborjányi and Hoang, 1991) and overwintered in reeds. The three maize pathogenic *Potyvirus*es were characterized according to the symptoms on *Sorghum* varieties and maize inbred lines (Gáborjányi et al., 1992). Big differences in the susceptibility of Hungarian maize varieties to MDMV and SCMV were established, and the inheritance of resistance was also described (Kovács et al., 1994a, b). It was found that high nitrate nutrition increase the susceptibility to the infection. Lower temperature treatments (10 and 14 °C) altered in different degree the pathogenicity of the three virus strains which manifested in length of incubation period and percentage of diseases plants. SCMV-MB seemed to be less susceptible to lower temperature than MDMV-A and SCMV-A (Kovács et al., 1966).

1.3. Potato and sugar-beet viruses

Between potato and sugar-beet pathogenic viruses the new NTN strain of potato Y *Potyvirus* (PVY) and a strain of beet necrotic yellow vein *Furovirus* (BNYVV) are most important (Beczner et al., 1984; Horváth, 1994a; Horváth et al., 1994a) in Hungary. The identification of a new *Tymovirus* isolated from wild *Solanum hannemannii* is in progress (Horváth et al., 1993). On the basis of DAS-ELISA serological examinations of plants

grown from seeds of potato cultivars (e.g., Aracy, Baronesa, Monte Bonito) showing necrotic rings on berries tobacco rattle *Tobravirus* (TRV) was found to be transmissibly by true potato seed (Horváth et al., 1995a).

1.4. Viruses on tobacco and vegetable crops

We isolated more than twenty viruses from tobacco and different vegetable plants (e.g., bean, pea, pepper, tomato, cabbage, cauliflower, celery, parsley, parsnip, carrot, lettuce, cucumber, patisson etc., reviewed by Horváth and Beczner (1983). Members of *Cucumovirus*, *Geminivirus*, *Nepovirus*, *Potyvirus* and *Tobamovirus* groups are *very important* pathogens in tobacco and vegetable crops. Cucumber mosaic *Cucumovirus* (CMV), tomato leaf curl *Geminivirus* (TLCV), tomato spotted wilt *Tospovirus* (TSWV), and zucchini yellow mosaic *Potyvirus* (ZYMV) has become increasingly important pathogens causing heavy yield losses (Horváth, 1993; Horváth et al., 1986a; Gáborjányi et al., 1994, 1955a,b; Tóbiás and Basky, 1997). The aphid-, seed- and mechanically transmisible CMV is the most significant virus of vegetable plants. The natural occurrence of a new, seed transmitted legume or bean strain of CMV on beans (*Phaseolus vulgaris*) may have role in the ecology of the virus in Hungary (Horváth, 1983). From *Lycopersicon esculentum* cv. Belcanto isolated TLCV was transmitted from tomato to tomato by whitefly (*Trialeurodes* sp.) but not with aphids. *Datura stramonium* was a good host plant of the virus. The TSWV was recorded in tobacco, pepper and tomato plants in fields as well as in plastic tunnels. The virus is transmissible by tobacco thrips (*Thrips tabaci*) and western flower thrips (*Frankliniella occidentalis*). Isolates from different plants did not differ neither in symptoms in various host plants, nor in serological properties. The Hungarian isolates were serologically identical with the BR-01 isolate (serogroup I). The rapid distribution of TSWV and its vectors (*Thrips tabaci* and *Frankliniella occidentalis*) became a potential threat for pepper and tomato production (Gáborjányi et al., 1995b; Jenser et al., 1966). A serious epidemy in cucumber, pumpkin and squash have been observed in the last years. The pathogen was identified as ZYMV. All tested varieties proved to be susceptible to infection (Tóbiás and Basky, 1997). Among pepper pathogenic viruses occurring in Hungary a new *Tobamovirus* strain the Ob was described (Csilléry and Ruskó, 1980; Tóbiás et al., 1982). The Ob strain caused considerably more necrosis in the *Capsicum annuum* cultivars carrying the L^1 gene for resistance under experimental winter conditions, but normally Ob poduces conspicuous, yellow mosaic symptoms. Ob strain was also reported to overcome the L^2 gene for resistance in *Capsicum frutescens* cv. Tabasco. It is interesting to mention that *Lagenaria siceraria* cv. Turbinata, a frequently cultivated plant in the vineyards, can be naturally infected at the same time with three viruses belonging to different virus groups (*Nepovirus*, *Cucumovirus* and *Potyvirus* group). The natural occurrence of grapevine fanleaf *Nepovirus* (GFLV) on herbaceous host *Lagenaria siceraria* is very important (Horváth et al., 1994).

1.5. Brassica *pathogenic viruses*

Cauliflower mosaic *Caulimovirus* which is considered to be one of the most important viral pathogens attacking members of the family *Brassicaceae* (Cruciferae) was first found in Hungary in turnip (*Brassica rapa* var. rapa) and cauliflower (*Brassica oleracea* var. botrytis) plants in 1976 (Horváth et al., 1980c). The deoxyribonucleic acid (DNA) of the Hungarian DH isolate was extracted and examined the sites of restriction endonucleases (R. Hull, Norwich, England). It was demonstrated that the DH isolate has one site each for SalGI and XhoI, three sites for EcoRI and four sites for HhaI. According to Hull (1979, personal communication), all the other known isolates of CaMV contain restriction endonuclease patterns for the first three mentioned enzymes but those for HhaI have only been found in DH isolate. In this respect the Hungarian DH isolate (strain) deviates from other isolates of CaMV. From the *Brassica* pathogenic viruses, the Hungarian turnip yellow mosaic *Tymovirus* (TYMV) was serologically related to but not identical with the type strain of the virus described in formerly Yugoslavia (Juretić et al., 1973; Horváth et al., 1980a). Radish mosaic *Comovirus* (RMV) was identical with the European type isolate but differed from the American strain. Turnip mosaic *Potyvirus* (TuMV) was isolated from turnip, cauliflower and garlic mustard in Hungary (Mamula et al., 1972; Horváth et al., 1975a). According to the symptomatological properties, the cauliflower isolate (JN) belongs to cabbage strain and all isolate from *Alliaria* sp. to ordinary strain of TuMV.

1.6. Viruses on fruit trees and grapes

As far as we know on the major fruit tree species 20 viruses occur in Hungary (Németh, 1986; reviewed by Horváth et al., 1994b). The seven exactly identified and taxonomically well-known viruses belong to the *Clostero-*, *Capillo-*, *Ilar-*, *Nepo-* and *Potyvirus* group. Burgyán et al. (1980) isolated and characterized a strain of tobacco mosaic *Tobamovirus* from plum in Hungary. Plum pox *Potyvirus* (PPV) and cherry leaf roll *Nepovirus* (CLRV) are the most frequent virus pathogens of stone fruits. Transmission efficiency of 32 aphid species were tested. Among them five species were described as new PPV vectors: *Aphis idaei*, *A. sambuci*, *Hyalopterus amygdali*, *Myzus cerasi*, and *Sitobium avenae*. Flight activity of the most known species (*Aphis craccivora*, *Brachycaudus helichrysi*, *Hyalopterus pruni*, *Phorodon humuli* and *Myzus persicae*) was monitored by suction traps and their vector activity was followed by trap plants (*Prunus persicae* GF305) placed in a PPV infected orchard. In transmission experiments of PPV by different aphid species *Hyalopterus pruni* was the most abundant vector of the virus (Gáborjányi and Basky, 1995a, b; Basky et al., 1996). Until know only one Hungarian isolate (SK68) was compared with other European isolates. In these experiments SK68 was classified as a typical M serotype. Recently isolates were characterized by monoclonal antibodies raised against internal, intermediate and external sequences of PPV coat protein and provided the first demonstration of the presence of D type isolates (López Moya et al., 1997). According to the recent study both the M and the D serotype

commonly occurred in the orchards. Walnut trees isolates of CLRV (are distinct from previously elderberry and cherry strains of CLRV (Tóbiás, 1995). To our present knowledge eight *Nepovirus*es, four airborne viruses belonging to various taxonomic groups and three not properly identified viruses infect grapevine in Hungary (reviewed by Horváth et al., 1994b).

1.7. Trees and shrubs as natural virus hosts

Investigations on the viruses of forest trees and shrubs began to come into prominence in Hungary relatively late, in the 1960s only. As far as we know in 21 plant species 13 viruses have been identified so far. The most common viruses are cucumber mosaic *Cucumovirus* (CMV), alfalfa mosaic *Alfamovirus* (AMV), poplar mosaic *Carlavirus* (PMV), cherry leafroll *Nepovirus* (CLRV) and prunus necrotic ringspot *Ilarvirus* (PNRV). The occurrence of tobacco mosaic *Tobamovirus* (TMV) on sweet chestnut and oak is also important (Horváth et al., 1975c). Salamon (1989) isolated a rod-shaped, *carlavirus*-like plant virus c. 640–680 nm × 13–16 nm in size from *Euonymus japonicus* in Hungary. On 18 plant species, virus-, or virus-like symptoms were observed, but the isolation and identification of the pathogens either have not taken place, or was not possible (Horváth et al., 1994b). We believe that viruses and other pathogens (phytoplasmas, rickettsias and others), as predisposing factors are very important in the forest decline.

1.8. Viruses on medicinal plants

Belladonna mottle *Tymovirus* (BeMV) was isolated from medicinal plants *Atropa belladonna* in many mountain regions in Hungary. The virus is closely related serologically to a Yugoslavian isolate of BeMV (Horváth et al., 1976). Dulcamara mottle *Tymovirus* (DuMV) was isolated from naturally infected woody drug plant *Solanum dulcamara* in Hungary (Beczner et al., 1976). The DuMV is closely related to BeMV and distantly related to eggplant mosaic *Tymovirus* (EMV). *Datura innoxia* Hungarian mosaic *Potyvirus* (HDIV) was isolated and characterized from *Datura innoxia* medicinal plants (Peralta et al., 1981).

1.9. Ornamental plants as virus hosts

Cucumber mosaic *Cucumovirus*, broad bean wilt *Fabavirus* (BBWV) and tomato aspermy *Cucumovirus* (TAV), as ornamental pathogenic viruses are commonly found around homes and gardens in Hungary (Horváth and Szirmai, 1975; Horváth et al., 1980b; Simay and Beczner, 1984). Another viruses [e.g., carnation etched ring *Caulimovirus* (CERV), tulip breaking *Potyvirus* (TBV)] and others are distributed. A new *Potexvirus* Boussingaultia mosaic virus, (BoMV) was isolated from *Boussingaultia cordifolia* and *B. gracilis* f. pseudobaselloides in Hungary (Beczner and Vassányi, 1980). This plants are very frequent ornamentals, therefore their virus diseases are very danger-

ous. Salamon and Némethy (1988) isolated the odontoglossum ring spot *Tobamovirus* (ORSV) from ornamental orchids in Hungary. The Hungarian isolates differ slightly from Japan and German isolates in host reactions, but they are serologically identical.

1.10. Wild plants as virus hosts

Wild plants, or weeds are very important hosts and reservoirs of plant viruses. In potato field from *Chenopodium hybridum* and *Datura stramonium* two viruses, sowbane mosaic *Sobemovirus* (SoMV) and henbane mosaic *Potyvirus* (HeMV) were isolated. The Hungarian strain of SoMV is serologically identical with the American and Yugoslavian isolates (Horváth et al., 1993b). From *Datura stramonium* isolated HeMV is pathogenic to some wild *Solanum species* (e.g., *S. canasense*, *S. lesteri*, *S. papita* and others) (Horváth et al., 1988b). *Malva sylvestris*, as perennial plant, is a host of Malva vein clearing *Potyvirus* and cucumber mosaic *Cucumovirus* in Hungary (Horváth et al., 1979, 1995b).

1.11. Aquatic plants as virus hosts

Not only different terrestrial plant communities, but aquatic or uliginous plants have role in the ecology of plant viruses. *Myriophyllum verticillatum* as a water plant, living in the Lake Balaton, Hungary, is a natural reservoir of cucumber mosaic *Cucumovirus* (CMV) (Horváth, 1994b; Horváth and Juretić, 1995). We isolated a *Potyvirus* from *Alisma plantago-aquatica* and a *Nepovirus* from *Trapa natans*.

1.12. Terrestrial viruses in natural waters (rivers and lakes)

We isolated *tobamovirus*es (tobacco mosaic, TMV; tomato mosaic, ToMV), *Potyvirus*es (plum pox, PPV; potato Y, PVY and maize dwarf mosaic, MDMV), one *Luteovirus* (barley yellow dwarf, BYDV) and alfalfa mosaic *Alfamovirus* (AMV) from rivers and lakes in Hungary (Horváth et al., 1986b; Juretić et al., 1986; Pocsai and Horváth, 1987).

2. Virus resistance and resistant sources in wild plant species

2.1. Beta *species*

In the different accessions of *Beta vulgaris* ssp. maritima several resistant plants could be found to beet necrotic yellow vein *Furovirus* (BNYVV). From the point of view of resistance the accessions P.I. 546396, 546413, 868338 deserve special attention. The *Beta vulgaris* accessions P.I. 169023, 169030 (Turkey), 2661 (Utah, USA, California),

266100 (Poland) showed immunity to the virus (Horváth et al., 1994). Considering, that *Beta vulgaris* ssp. maritima is rather easily crossed with *B. vulgaris*, and the virus resistance shows dominant inheritance in the F_1 generation (resistant × susceptible). The importance of *B. maritima* in breeding for virus resistance is very valuable. In production resistant cultivars also the gene technic may also play an important role.

2.2. Capsicum *species*

We have found hypersensitive reaction between eleven *Capsicum* species and four pepper pathogenic viruses (Horváth, 1986a, b, c). Of the new sources of resistance special attention is worth being paid to *Capsicum barbatum* var. pendulum and *C. pubescens* which exhibited resistant to four viruses: potato Y *Potyvirus* (PVY), tobacco mosaic *Tobamovirus* (TMV) and tomato mosaic *Tobamovirus* (ToMV), cucumber mosaic *Cucumovirus* (CMV). Also remarkable is the resistance to PVY in *Capsicum chinense*, *C. eximium*, *C. flexuosum* and *C. pubescens*.

2.3. Cucumis *species*

Between *Cucumis* species, *Cucumis africanus* G1.2302 showed immunity to cucumber green mottle mosaic *Tobamovirus*, CGMMV; cucumber leaf spot *Carmovirus*, CLSV; cucumber mosaic *Cucumovirus*, CMV; watermelon mosaic *Potyvirus*, WMV-2; and zucchini yellow mosaic *Potyvirus*, ZYMV (Horváth, 1993a). Good resistance qualities were shown by *Cucumis melo* (P.I. 217974), which was immune to CLSV, CMV, WMV-2 and ZYMV. The accession P.I. 161375 was immune to CGMMV, melon necrotic spot *Carmovirus* (MNSV), WMV-2 and ZYMV.

2.4. Cucurbita *species*

From thirty accessions of three *Cucurbita* species, 24 accessions of *C. moschata* were immune to cucumber green mottle mosaic *Tobamovirus* (CGMMV), 16 accessions to cucumber mosaic *Cucumovirus* (CMV), 9 to the cucumber fruit streak virus (CFSV), 3 to the cucumber leaf spot *Carmovirus* (CLSV) and 2 to the melon necrotic spot *Carmovirus* (MNSV) and 2 to the zucchini yellow mosaic *Potyvirus* (ZYMV). Four accessions of *Cucurbita ecuadorensis* are immune to CGMMV, 2 accessions to ZYMV, while one of it to CLSV and again one to MNSV. The P.I. 285213 accession of *Cucurbita texana* was immune to CFSV, CGMMV and MNSV (Horváth, 1993b). The investigated *Cucurbita* spp. could be useful for the future breeding programmes.

2.5. Phaseolus *species*

We pointed out immune host-virus relations between ten *Phaseolus* species and ten viruses (Horváth, 1986d, e). Particularly important is the bean yellow mosaic *Potyvirus* immunity of *Phaseolus coccineus* and *P. occoineus*. As it is known

tobacco necrosis *Necrovirus* (TNV) occurs in *Phaseolus* spp. under natural conditions (Szirmai, 1962), therefore the immunity of these *Phaseolus* species to the virus is also important.

2.6. Solanum *species*

Many accessions of different *Solanum* species originated from gene banks (Argentina, Bolivia, Chile, Germany, Mexico, Scotland, United Kingdom, United States of America) were collected and investigated to the reaction of potato viruses. During the investigations resistance was pointed out in the case of three botanically new wild Bolivian *Solanum* species and five viruses: *S. neocardenasii* (PVY), *S. paucisectum* (TRV), *S. violaceimarmoratum* (AMV, CMV, PVX) (Horváth and Hoekstra, 1989). Of the species examined seem particularly valuable as *Solanum stoloniferum* and *S. brevidens*.

Out of the accessions of tuber-bearing *Solanum stoloniferum* examined six (P.I. 239410, 255525, 255548, 275245, 275247, 498005, 498007) were found to be immune to potato Y *Potyvirus* (PVY), five (P.I. 230557, 255548, 275247, 347771, 498005) to cucumber mosaic *Cucumovirus* (CMV) and six (P.I. 161281, 230557, 275247, 338621, 498005, 498007) to henbane mosaic *Potyvirus* (HeMV) (Horváth and Wolf, 1991; Horváth, 1994c). One accession (P.I. 272247), of *Solanum stoloniferum* were equally immune not only to two *Potyvirus*es (PVY, HeMV) but also to CMV and AMV, too.

The non-tuber bearing *Solanum brevidens* is a new source of virus resistance. Two accessions (P.I. 218228 and 245764) showed extreme resistance or immunity (slow cell-to-cell spread) to potato leafroll *Luteovirus* (PLRV). Somatic hybrids (A31 and T16) between virus resistant *S. brevidens* and *S. tuberosum* cv. Gracia gave very low ELISA values, similar to those given by virus-free plants. Recovery of the virus from these hybrids using aphid (*Myzus persicae*) transmission to *Physalis floridana* was unsuccessful. This suggest that virus resistance from *S. brevidens* had been incorporated into the two somatic hybrids. Three another accessions (P.I. 558236, 558239 and 558169) showed extreme resistance to NTN strain of potato Y *Potyvirus* (PVY^{NTN}). The extreme resistant accessions plays an important role in the traditional and somatic hybridization or molecular breeding of resistant potato cultivars.

3. Physiological studies on virus infected plants

In tobacco mosaic *Tobamovirus* (TMV) infected hypersensitive tobacco leaves considerably higher heat production could be detected only during lesion formation. In susceptible plants only upper leaves (showing mosaic symptoms) showed extra heat production. These data suggest that both hypersensitive necrotization and systemic virus infection may lead to energy dissipation of plant cells as heat (Ádám et al., 1993). Sig-

nificant alterations in lipid composition of leaf tissues occurred only as a consequence of decapitation. In the remaining leaves of decapitated plants an increase in phospholipid content and in the phospholipid/sterol molar ratio as well as an enhanced resistance to TMV were observed. Changes in membrane lipids in leaves with systemic acquired resistance (SAR) were not significant, indicating that changes in membrane lipids were not causal factors of SAR (Ádám et al., 1990).

It was suggested that oxygen free radicals may play a significant role in inducing plant cell and tissue death during hypersensitive reaction caused by different pathogens. *In vitro* selected paraquat resistant tobacco plants (resistant to superoxides) were more juvenile and tolerant to abiotic treatments caused local necroses. Necrotic symptoms induced by tobacco necrosis *Necrovirus* (TNV) or other biotic stresses were suppressed, as compared to sensitive control plants. It would seem that the juvenility of plant tissues correlates with resistance of tissues to superoxide. Inhibiton of necrobiosis by induced juvenility should reduce the damaging effect of free radicals. It was concluded that juvenility plays a significant role in tolerance to paraquat and to necrotic diseases and several other stresses (Barna et al., 1993).

Significant changes of glutathione S-transferase activities were found in *Sorghum* varieties representing different forms of resistance. Inoculation with sugarcane mosaic *Potyvirus* (SCMV-MB) resulted in induction of the enzyme in leaves of the immune host, while hitherto unpublished dramatic reductions were detected in the leaves of systemic host (Gullner et al., 1995). In the study of the role of ascorbate-glutathione cycle in diseased plants responses of malonaldehyde (MDA), ascorbate levels, ascorbate peroxidase (AP), dehydroascorbate reductase (DHAR), and glutathion reductase (GR) activities were investigated in hypersensitive tobacco verieties. TMV infections led to considerably increased MDA levels in leaf tissues incubated in the light, but less noticeably in the darkness. AP activities substantially increased, while DHAR activities decreased. In TMV infected leaves elevated GR activities were observed, as well. It is supposed that antioxidative reactions influence the symptom expression in virus infected tobacco plants (Gullner et al., 1997).

Alterations of photosynthetic activity and photosynthetic pigments of tobacco plants infected by three different viruses (TNV; plum pox *Potyvirus*, PPV; and tomato spotted wilt *Tospovirus*, TSWV) were investigated. Electron microscopic results revealed that in the susceptible host to TSWV caused the strongest destruction in chloroplast structures but only at the latest stage of symptom development (Almási et al., 1997). All the three viruses changed the relative 77K fluorescence intensities of the chlorophyll-protein complexes. Fluorescence induction measurements proved that TNV and PPV partially inhibited the electron transfer between Q_A and Q_B in the PSII complex in early stages, before visible symptoms of the infection TSWV, however started to inhibit the above processes only in wilting leaves (Almási et al., 1995, 1996). In systemic host-parasite combinations (PPV and TSWV) there were no significant alterations in superoxide dismutase (SOD) activities neither in the lower inoculated, nor in the upper, non-inoculated leaves. However, in local infection (TNV) qualitative differences were found in SOD profiles in the early stages of the infection. These results indicate that increase of

SOD activity in virus infected tissue correlates only with the development of local necrosis but not in systemic wilting and tisue collapse.

In relation to the physiology of systemic acquired resistance (SAR), Fodor et al. (1997) demonstrated that stimulated antioxidative processes contribute to the suppression of necrotic symptom development in tobacco leaves with SAR. Concomitantly with the development of SAR, the level of glutathione and the activities of GR, GST, and SOD increased in Xanthi leaves having the systemic resistance.

4. Molecular characterization of plant viruses isolated from the Hungarian flora

As early as 1980 along with the development of the molecular techniques, the characterization of plant virus isolates has been initiated by Hungary scientists, too. The first complete primary structure of a Hungarian isolate of cauliflower mosaic *Caulimovirus* (CaMV, Horváth et al., 1980c) has been determined (Balázs et al., 1982). The double stranded DNA of the altered virulence strain of CaMV-D/H contains 8016 bp. The DNA in circular and possesses like the DNA of almost all CaMV strains three sequence interruptions. The sequence comparison leads to the following conclusions: (i) the genom organization of CaMV strains is identical with eight potential genes with two intergenic regions, (ii) the DNA sequences differ from each other by only about 5% with base substitutions accounting for most of the changes. Deletions and insertions could be observed. Characterization of the CaMV strains resulted later to the discovery of biological active plant viral promoters like 35S and 19S promoters, which is commonly used today in plant genetic engineering.

The rapid development in the *in vitro* RNA manipulation promoted the characterization of plant viruses having a RNA genom. The necrotic strain of potato Y *Potyvirus* (PVY^{NTN}) a dominant strong pathogen in the Hungarian potato crop has been cloned and the complete primary structure has been determined (Dalmay and Balázs, 1990; Thole et al., 1993). From the same *Potyvirus* group the well spread plum pox *Potyvirus* (PPV) the SK68 strain has also been cloned and sequenced (Palkovics et al., 1993). The two *Potyvirus* sequences gave us valuable data on the diversity of virus strains in this region. Partial sequences from *Cucumovirus*es like cucumber mosaic *Cucumovirus* (CMV-Trk7 strain) and tomato aspermy *Cucumovirus* (TAV) pepper isolate has been determined and infectious transcripts have been made (Salánki et al., 1994a, b). In a collaboration with French research groups at INRA, Versailles and Montfavet, *Cucumovirus* recombination studies were initiated. Recombinants of CMV/TAV were created and tested for viral gene functions. Using different strains of the two groups it was showed that in this experiments RNA-3 does not play a role in symptom determination whereas the result with *Nicotiana glutinosa* suggest that in this species the capsid protein gene is a major symptom determinant. Results obtained on tomato and *Nicotiana benthamiana* where the pseudorecombinant composed of TAV-RNAs-1 and -2 and CMV-3 induced considerably aggra-

vated symptoms (Salánki et al., 1977). Further analysis of the viral sequences are in progress.

Recently a full length infectious clone of tobacco necrosis *Necrovirus* (TNV) has been prepared and characterised (Molnár et al., 1977).

5. Coat protein mediated virus resistance in higher plants

In the last decade since the first successful demonstration of virus disease resistance in transgenic plants several alternative way has been discovered for engineering virus resistance. The first experiment on coat protein mediated cross protection (Powell et al., 1986) more that a hundred host virus relationships has been involved in this technology. In this case the transgenic plants express the coat protein gene of the introduced virus and confers resistance against the same virus and against its strains (see in details in a recent review Hackland et al., 1994). The coat protein gene of potato X *Potexvirus* (PVX) has been introduced into potato cultivar and expressed in several transgenic lines. Northern and Western analysis of the transgenic potatoes proved the expression of the integrated gene. Transgenic plants showed partial virus resistance after challenge infections. The cross portections manifested by the delay of symptom development and a suppression in the virus replication (Fehér et al., 1992). The coat protein gene from potato Y *Potyvirus* (PVY^{NTN} a Hungarian isolate) was integrated into several different *Nicotiana tabacuum* breeding lines. The integration was detected with PCR technique while the expression of the gene was demonstrated by Northern and Western blots. The efficiency of the protection varied between the different transgenic plants ranging from almost complete to no protection (Kollár et al., 1993). Field experiments strengthened this greenhouse data on cross protection. Coat protein constructs were also prepared from the SK68 isolate of plum pox *Potyvirus* (PPV) and have been integrated into *Nicotiana benthamiana* plants (Palkovics et al., 1995). Some of the transformed plants proved to be resistant to PPV and a new type of resistance has been observed namely several PPV plants initially proved to be sensitive for the challenge infections but a recovery has been observed on the newly developed leaves. Analysis of this phenomenon is in progress. Due to the high efficiency of this coat protein mediated cross protection in plants, several plant virus coat protein are being studies and envisaged to integrate in their economically important crop genome for obtaining virus resistance.

6. Characterization of cymbidium ringspot virus (CyRSV)

An international team was formed for the characterization and genome mapping of cymbidium ringspot *Tombusvirus* (CyRSV). They cloned and sequenced the single-stranded positive sense (4733 nt long) RNA genome of this virus and characterised the

two subgenomic RNAs of 2118 nt 936 nt respectively. CyRSV supports also the replication of other linear RNAs either completely derived from genomic RNA (defecting interfering RNA, DI RNA) or essentially unrelated to it as a satellite RNA (Burgyán and Russo, 1988; Burgyán et al., 1989, 1990). There was also prepared full length infectious clones from the virus. After sequencing the different DI RNAs there was demonstrated that they are linear deletion mutants of the genomic RNA and they have generated *de novo* during virus replications (Burgyán et al., 1991). The CyRSV DI RNAs are mosaic molecules build up from segments of the genomic RNA. The presence of DI RNA in infected plants attenuates symptom severity and prevents the death of the plants. Using deletion mutants of the DI RNAs, specific sequences were identified as cis-acting sequences, necessary for their replication. The study of the 3′ terminal cis-essential domain of both genomic and DI RNAs proved that they can be folded into a stable stem-loop structure composed of three harpins with two short non-base paired regions. None of the three conserved stem-loops can be abolished without loosing the infectivity of DI RNAs (Havelda et al., 1995, 1977). The defective interferring RNA of CyRSV has been tested as a potential RNA vector. The CP gene of the same virus or of the unrelated tomato aspermy *Cucumovirus* (TAV) was inserted in a biologically active clone of CyRSV DI RNA. Both homologous and heterologous CP genes were inserted in differents positions.

A new strategy was used to develop virus resistance against plant viruses. For this purpose the cloned DI RNA was integrated into *Nicotiana benthamiana* plants in both orientations. The integration of DI RNA sequences in the plant genome has been verified using PCR amplification of DNA extract and with Northern blot analysis of RNA extracts. DI RNA related transcripts were detected in uninfected transgenic plants in which DI RNA replicated, and were protected from apical necrosis and death (Kollár et al., 1993). This group are also working on different other *Tombusvirus*es and their replication mechanisms.

7. Plant virus diagnosis based on nucleic acid hybridization test

Current diagnostics for plant viruses are accurate and useful techniques such as indicator plants, serological and electron microscopic analysis. However, in several cases they are inadequate for diagnosis, due to the nature of the pathogenes (i.e. no capsid protein, poor immunogenicity, low titer in infected tissue etc.). A novel solution hybridization text has been adopted and developed for plum pox *Potyvirus* (PPV). Two adjacent, but non-overlapping, clones were made from the PPV genome, subcloned into different vector, and then one of them was immobilized on solid support to act as a catching reagent, while the other was labelled to enable detection (Palkovics et al., 1994).

The rapid development in the field of polymerase chain reaction directed us to introduce this sensitive technique for plant virus diagnosis. A simple and rapid detection of carnation etched ring virus has been developed by using two 20-mer oligonucleotide

primers which sequences were designed to the conserved intergenic region of this virus. The 850 nt amplified fragment was cloned and sequenced showing 95.6% identity with the published sequence. The cloned fragment was used after radioactive labelling to prove the viral origin of the PCR products amplified from nucleic acid samples originated from plant extracts. This PCR method proved to be 20000-fold more sensitive that the commonly used ELISA method. This technique can de adopted to any other DNA viruses due to it's simplicity. Designing primers should be based on selecting conserved non-coding regions from the given virus sequences. This allows the detection of different virus strains with the same high fidelity (Palkovics and Balázs, 1996).

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Transmission of Sowbane Mosaic *Sobemovirus* by Seeds of *Chenopodium* Species and Viability of Seeds[1]

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Investigations were carried out on the seed transmission of sowbane mosaic *sobemovirus* (SoMV) by seeds of *Chenopodium album* L., *Chenopodium quinoa* Willd. and *Chenopodium murale* L. The extent of seed transmission was 6% in the case of *C. album*. Seed transmission varied 2–8 and 2–6% with species *C. quinoa* and *C. murale*, respectively. The viability of *C. album* and *C. quinoa* seeds derived from SoMV infected plants was reduced by 23.1 and 8.1% respectively as compared to the healthy control. SoMV infection did not influence the seed viability of *C. murale*.

Since its discovery (Silva et al., 1958) and description (Bennett and Costa, 1961) sowbane mosaic *sobemovirus* (SoMV) is often in the centre of plant virologists. This fact can be explained with its easy transmission by pollen, seed and mechanical way (Kado, 1971; Horváth, 1972; Schuster et al., 1973; Sehgal, 1981; Francki and Miles, 1985; Francki et al., 1985; Lehoczky and Salamon, 1989). The host range of SoMV includes 14 natural and 59 artificial hosts from 16 families (Teakle, 1968; Bercks and Querfurt, 1969; Quacquarelli, 1971; Šarić, 1971; Šutić et al., 1971; Juretić, 1976; Šutić and Juretić, 1976; Buturović and Juretić, 1980; Šarić and Juretić, 1980; Horváth et al., 1993; Teakle, 1996). Bos and Huijberts (1996) reported the natural infection of spinach (*Spinacea oleracea* L.) by grapevine isolate of SoMV. Natural occurrence of SoMV on *Chenopodium hybridum* L. in Hungary was first reported by Horváth et al. (1993). Serological tests have proved the presence of SoMV in certain pepper cultivars (Gáborjányi et al., 1997). It seems that the host range of SoMV increases in future.

It has been well known for almost 80 years that many viruses can be transmissible with the seeds of certain plant species (Reddick and Stewart, 1918, 1919; Doolittle and Gilbert, 1919). Crowley (1957) reported seed transmission of 45 viruses. Mink (1993) mentioned 108 virueses that are transmitted through pollen and seed. These viruses represents 25 taxonomic groups. Approximately 18% of the described viruses are transmitted in one or more hosts, and it is estimated that one-third of the plant viruses will eventually prove to be seed-transmitted in at least one host (Stace-Smith and Hamilton, 1988; Johansen et al., 1994). A total of 56 seed transmitted plant viruses may occur within 28 vegetable species, belonging to 9 plant families (Schmidt, 1994). The extent of seed transmission varies greatly, e.g. low percentage of corn seeds is able to transmit sugarcane mosaic *potyvirus* (Shepherd és Holdeman, 1965; Teakle et al., 1989) while the ex-

[1] Dedicated to Dr. L. Bos on the occasion of his 70th birthday.

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extent of transmission by seeds of certain pea cultivar is 100% in case of pea seed-borne mosaic *potyvirus* (Hampton and Mink, 1975). The two dominant factors determining whether seed transmission occurs and at what frequency are (a) host-virus interaction and (b) timing of infection. Thus, transmission is successful usually only if plants are infected before fertilization of the ovules and at least before cytoplasmic separation of the developing embryo from maternal tissues. Seed transmission may differ considerably with virus strain and host cultivar as well. Environment, particularly temperature, can influence transmission frequencies but to a lesser extent (Bos, 1977; Mink, 1993).

The percentage of SoMV transmission was 1.6, 21, 30, 45 and 34–62% by seeds of *C. quinoa, Atriplex pacifica* Nels., *C. album, C. murale* and *C. amaranticolor* Coste et Reyn, respectively (Bennett and Costa, 1961; Bancroft and Tolin, 1967; Dias and Waterworth, 1967). Infectivity tests on separate embryos and seed-coats of *S. oleracea* showed that over 30% of the seeds' embryos were infected and c. 80% of the seed-coats contained SoMV (Bos and Huijberts, 1996).

Only few data is availabla about the germination biology and viability of the seeds of the virus infected plants. Horváth (1980) reported that the kernel weight and the germination of the seeds of rape (*Brassica napus* L.) may reduce by 40 and 20%, respectively due to cucumber mosaic *cucumovirus* (CMV) infection. Walkey et al. (1985) have found that the viability of seeds of *Nicotiana* and *Chenopodium* species from virus infected plants were slightly reduced. In an earlier study we have studied the effect of SoMV on the germination biology of some *Chenopodium* species. Germination of seeds from the virus infected plants was reduced by 2–84%, depending on ecological factors. Virus infection altered the phytochrome activity of the seeds as well (Kazinczi et al., 1997). The seed production and viability may be greatly reduced by different pathogens and insects (Crowley and Buchanan, 1982; Neser, 1985; Harris and Hoffman, 1985). It is well known for a long time that certain herbicides may reduce not only seed production but seed viability as well (Fawcett and Slife, 1973; Fernandez-Quintanilla et al., 1987).

The aim of the present study was to determine the extent of seed transmission of SoMV by different *Chenopodium* species and to clear whether the virus infection of the *Chenopodium* species altered only the dormancy of the seeds or seed viability as well.

Materials and Methods

The seeds of *C. album, C. murale* and *C. quinoa* were sown in sterilized boxes in our virological glasshouse free of vectors. The seedlings were planted in plastic pots (12 cm in diameter), containing standard soil mixture (pH: 7.2, humus: 55%). Plants were mechanically inoculated at 8–10 leaves phenological stage with plant tissue sap containing virus diluted with 0.02 M phosphate buffer (pH: 7.2) in the ratio 1:2. Three weeks before inoculation H isolate of SoMV (Horváth et al., 1993) was propagated on *C. quinoa*. Ripened seeds were collected from the controlled healthy and the diseased *Chenopodium* plants infected by SoMV as well. Some batches of the freshly harvested seeds

were stored dry in paper bags at 4 °C and sown in plastic pots next spring. Four hundred plants each of *C. album*, *C. quinoa* and *C. murale* were grown. Infectivity was evaluated on the basis of symptoms and by DAS-ELISA serological method (Clark and Adams, 1977). The presence of the viral antigen was monitored using polyclonal antibody and alkaline-phosphatase (AP)-conjugated antibody. Substrate absorbance was measured forty minutes after adding the substrate, at 405 nm wavelength on Dynatech ELISA reader. Test samples were considered positive if their absorbance values exceeded twice that of the healthy control samples.

In order to determine seed viability, TTC test was used after international standard of International Seed Testing Association (ISTA) (Moore, 1985). 400 seeds were used of both the healthy and virus infected plants of each species.

Results and Discussion

Low percentage of *Chenopodium* plants showed systemic mosaic symptoms, leaf deformation and considerable growth reduction. The extent of seed transmission was 6% in case of *C. album*. The extent of seed transmission varied 2–8 and 2–6% with species *C. quinoa* and *C. murale*, respectively. Our results are not in keeping with the results of previous authors. The frequency of seed transmission was higher in case of *C. quinoa*, while in case of *C. album* and *C. murale* much lower values were obtained as compared to results of earlier studies (Bennett and Costa, 1961; Bancroft and Tolin, 1967; Dias and Waterworth, 1967), suggesting, that the frequency of seed transmission is not a standard value not even in a given host-virus relation but depends on a lot of factors. Seed transmission is very important in the epidemiology of viruses, as it provides a means for virus dispersal over time and distance. Zink et al. (1956) reported that transmission of lettuce mosaic *potyvirus* by lettuce seeds was only 1.6% but 85% of the whole plant population was infected at the time of harvest. Nevertheless, Mink (1993) pays attention that not all virus infected embryos result in virus infected seedlings.

It has been seemed that virus infection altered not only the seed dormancy but seed viability as well. In spite the relatively low percent of seed transmission, SoMV infection reduced greatly (by 23.1%) the seed viability of *C. album* (Table 1). *C. album* is among the worst weeds in the world (Holm et al., 1977) and is a common annual weed in Hungary, too. In order of the importance of the weeds, *C. album* was on the third place in 1987 in Hungary (Tóth et al., 1989). Its seed production may vary between 1300 and 500 000, depending on environmental conditions and competition (Bassett and Crompton, 1978). Stevens (1932) found *C. album* produced approximately 72 450 seeds on an average-sized plant. In case of natural occurrence of SoMV on *C. album* considerable amount of seeds is not able to germinate at all.

The viability of *C. quinoa* seeds derived from SoMV infected plants was reduced by 8.1% as compared with the healthy control. SoMV infection did not influence the seed viability of *C. murale* in spite the fact that virus infection has reduced the germination by

Table 1

Viability of seeds of healthy and SoMV infected *Chenopodium* species

	C. album	*C. quinoa*	*C. murale*
		in %	
Healthy	82	84	82
SoMV	63	77	81
LSD (P=0.05)	3.98	4.44	3.39

18–63%, depending on ecological factors (Kazinczi et al., 1997) (Table 1). Seed dormancy and germination biology is genetically determined for a given species, but may vary greatly (even inside a species) depending on effects of environmental factors on the mother plants (Barralis et al., 1988; Lonchamp and Gora, 1980).

Our results call attention to the fact that virus infection may influence (i) germination characteristics and (ii) viability of weed seeds, therefore – in indirect way – it contributes to the reduction of a weed population.

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Solanum nigrum L. as a New Experimental Host of Melandrium Yellow Fleck *Bromovirus* and Sowbane Mosaic *Sobemovirus*

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Investigations were carried out to study virus susceptibility of *Solanum nigrum* L. Out of the 19 viruses, 10 were pathogenic to *S. nigrum*. This is the first report in which *S. nigrum* is mentioned as a new experimental host of Melandrium yellow fleck *bromovirus* and sowbane mosaic *sobemovirus*.

Solanum nigrum L. is reported as a weed in 37 crops and in 73 countries (Holm et al., 1977). It is a serious weed of sugar beet, corn, cotton, sorghum and – due to its excellent adaptation to high fertility of soils – in horticultural crops, especially in vegetables under moist environments (Binning, 1971; Kempen and Agamalian, 1976; Jones, 1977; Kempen and Lange, 1977; Weller and Phipps, 1979). *S. nigrum* is known as a virophilous species and host of 41 viruses belonging to 21 virus groups (Table 1). Future investigations were carried out to study the virus susceptibility of *S. nigrum*.

Materials and Methods

In our vector free virological glasshouse *S. nigrum* plants were mechanically inoculated at 4–6 leaf stage with 19 viruses (arabis mosaic *nepovirus*, ArMV; belladonna mottle *tymovirus*, BeMV; cauliflower mosaic *caulimovirus*, CaMV; cucumber green mottle mosaic *tobamovirus,* CGMMV; cucumber leaf spot *carmovirus*, CLSV; cucumber mosaic *cucumovirus*, CMV; henbane mosaic *potyvirus*, HeMV; Melandrium yellow fleck *bromovirus*, MYFV; melon necrotic spot *carmovirus*, MNSV; potato aucuba mosaic *potexvirus*, PAMV, potato X *potexvirus*, PVX; potato Y *potyvirus*, PVY, sowbane mosaic *sobemovirus*, SoMV; tomato aspermy *cucumovirus*, TAV; tomato ringspot *nepovirus*, ToRSV, tomato spotted wilt *tospovirus*, TSWV; tobacco ringspot *nepovirus*, TRSV; turnip yellow mosaic *tymovirus*, TYMV and watermelon mosaic *potyvirus*, WMV). Seven plants for each viruses was used. The success of inoculation was evaluated with the method of back inoculation and on the basis of symptoms.

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Table 1

Viruses pathogenic to *Solanum nigrum*

Viruses[1]	Acronym[2]	Literature
Alfalfa mosaic *alfamovirus*	AMV	Schmelzer and Wolf (1977)
Arabis mosaic *nepovirus*	ArMV	Schmelzer and Wolf (1977)
Beet curly top *hybrigeminivirus*	BCTV	Daniel and Tsai (1990)
Beet western yellow *luteovirus*	BWYV	Farrel and Stufkens (1988)
Belladonna mottle *tymovirus*	BeMV	Schmelzer and Wolf (1977)
Broad bean wilf *fabavirus*	BBWV	Schmelzer and Wolf (1977)
Pepper mottle *potyvirus*	PepMoV	Mariappan et al. (1973)
Cucumber mosaic *cucumovirus*	CMV	Schmelzer and Wolf (1977)
Cucumber green mottle mosaic *tobamovirus*	CGMMV	Schmelzer and Wolf (1977)
Cherry leaf roll *nepovirus*	CLRV	Schmelzer and Wolf (1977)
Eggplant mottled dwarf *nucleorhabdovirus*	EMDV	Schmelzer and Wolf (1977)
Grapevine Bulgarian latent *nepovirus*	GBLV	Dimitrievič (1985)
Garden bean crinkle mosaic virus	nm[3]	Schmelzer and Wolf (1977)
Lettuce big vein *varicosavirus*	LBVV	Schmelzer and Wolf (1977)
Pea early browning *tobravirus*	PEBV	Schmelzer and Wolf (1977)
Pea enation mosaic *enamovirus*	PEMV	Schmelzer and Wolf (1971)
Pepper veinal mottle *potyvirus*	PVMV	Alegbejo (1987)
Plum pox *potyvirus*	PPV	Schmelzer and Wolf (1977)
Potato aucuba mosaic *potexvirus*	PAMV	Schmelzer and Wolf (1977)
Potato leaf roll *luteovirus*	PLRV	Schmelzer and Wolf (1977)
Potato mop-top *furovirus*	PMTV	Schmelzer and Wolf (1977)
Potato Y *potyvirus*	PVY	Schmelzer and Wolf (1977)
Potato X *potexvirus*	PVX	Naperkovskaya (1968)
Potato A *potyvirus*	PVA	Schmelzer and Wolf (1977)
Potato T *trichovirus*	PVT	Schmelzer and Wolf (1977)
Tobacco rattle *tobravirus*	TRV	Davis and Allen (1975)
Tobacco mosaic *tobamovirus*	TMV	Schmelzer and Wolf (1977)
Tobacco necrosis *necrovirus*	TNV	Schmelzer and Wolf (1977)
Tobacco etch *potyvirus*	TEV	Schmelzer and Wolf (1977)
Tobacco ringspot *nepovirus*	TRSV	Schmelzer and Wolf (1977)
Tobacco streak *ilarvirus*	TSV	Schmelzer and Wolf (1977)
Tomato yellow leaf curl *bigeminivirus*	TYLCV	Wilson et al. (1981)
Tomato aspermy *cucumovirus*	TAV	Schmelzer and Wolf (1977)
Tomato spotted wilt *tospovirus*	TSWV	Schmelzer and Wolf (1977)
Tomato ringspot *nepovirus*	ToRSV	Schmelzer and Wolf (1977)
Tomato black ring *nepovirus*	TBRV	Schmelzer and Wolf (1977)
Tomato bushy stunt *tombusvirus*	TBSV	Schmelzer and Wolf (1977)
Tomato golden mosaic *bigeminivirus*	TGMV	Horváth (1993)
Turnip mosaic *potyvirus*	TuMV	Schmelzer and Wolf (1977)
Watermelon mosaic *potyvirus*	WMV	Schmelzer and Wolf (1977)

[1] and [2] See Index to Virus Species by Brunt et al. (eds) (1996 onwards) Plant Viruses Online: Descriptions and Lists from the VIDE Database (20th August, 1996)

[3] nm, not mentioned in the Plant Virus Online

Result and Discussion

Out of the 19 viruses ten were pathogenic to *S. nigrum*. Out of them, eight viruses caused both local and systemic symptoms on *S. nigrum*. *S. nigrum* was systemic susceptible to CMV, while only local chlorotic lesions developed due to SoMV infection. *S. nigrum* plants were resistant to the further 9 viruses. Neither the inoculated nor the non-inoculated leaves showed symptoms and the viruses could not be reisolated by back inoculation (Table 2). Out of 10 viruses infected *S. nigrum*, eight (BeMV, CMV, PVY, PAMV, PVX, TAV, ToRSV and TRSV) are well known from the literature, so our present results have confirmed the results of earlier reports (Naperkovskaya, 1968; Schmelzer and Wolf, 1977). *S. nigrum* L. as a new experimental host of Melandrium yellow fleck *bromovirus* and sowbane mosaic *sobemovirus* is mentioned at the first time in the literature. On the basis of previous reports the host range of SoMV includes 14 natural and 59 artificial hosts from 16 families (Horváth et al., 1993) while the host range of MYFV includes 229 species belonging to 24 families (Hollings et al., 1978; Hollings and Horváth, 1978, 1981; Horváth et al., 1988a, b). This study has supplied new data to the experimental host range of both MYFV and SoMV.

Table 2

Reaction of *Solanum nigrum* to different viruses

Viruses[1]	Symptoms[3]	Viruses[1]	Symptoms[3]
ArMV	–	PAMV	Nl/Mo
BeMV	Nl/Mo, Led, Gr	PVX	Nl/Mo, Led
CMV	–/Mo, Led	SoMV[2]	Chl/–
CaMV	–	TAV	Nl/Mo
CGMMV	–	ToRSV	Nl/Mo
CLSV	–	TRSV	Nl/Mo
HeMV	–	TSWV	–
MYFV[2]	Chl/Mo	TYMV	–
MNSV	–	WMV	–
PVY	Chl/Mo		

[1] Virus acronyms see in the footnote of Table 1.
[2] MYFV and SoMV are new pathogens to *Solanum nigrum*
[3] Local/systemic symptoms: Chl, chlorotic lesions; Gr, growth reduction; Led, leaf deformation; Mo, mosaic; Nl, necrotic lesions; –, no symptoms

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Systemic Acquired Resistance in Wheat against Stem and Leaf Rusts

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Systemic acquired resistance was induced in the second leaves of wheat when the first leaves were infected with incompatible stem rust race or *Helminthosporium sativum*, or treated by celite or carborundum. Significant changes were found in proteins extracted with acidic buffer from susceptible wheat leaves after infection. Other changes were observed in protein composition of hypersensitively reacting resistant leaves. Only the compatible infection has systemic effect on leaf proteins, indicating that these changes do not play significant role in inducing SAR in wheat against rusts.

Systemic acquired resistance (SAR) is a nonspecific defence response in many plants, which can be induced by localized infection. In these plants, inoculation by a pathogen (inducer) results in a systemic enhancement of resistance against subsequent (challenge) infections by the same pathogen as well as a number of other bacterial, fungal and viral pathogens (Ross, 1961; Ryals et al., 1996). Not only infection, but certain chemical treatments causing chlorotic or necrotic flecks (Gottstein and Kuć, 1989; Sziráki et al., 1980; Strobel and Kuć, 1995) trigger SAR in plants. In addition, salicylic acid (SA) and 2,6-dichloroisonicotinic acid (INA) have been found to induce systemic resistance (Métraux et al., 1990). However, only a few published data are available on SAR in monocot plants (Hwang and Heitefuss, 1982; Sarhan et al., 1991; Kogel et al., 1994).

In our earlier work we have shown that infection with incompatible stem rust, barley powdery mildew or to a lesser extent mechanical injury of the first leaves of wheat reduced the number of pustules caused by stem rust on the second leaves (Barna et al., 1996). The aim of this paper is to investigate the role of necrotization of the first leaves of wheat in the induction of systemic resistance in the second leaf. Therefore, we compared the effect of incompatible stem rust infection with that of mechanical injury by carborundum or celit treatment and with infection by the necrotrophic pathogen *Helminthosporium sativum*. The systemic effect of the above treatments was measured as the number of stem or leaf rust pustules on the second leaves.

Since we found earlier (Manninger et al., unpublished) that incompatible infection of the apical part of the leaf decreases the number of stem rust pustules on the lower part of the leaf, changes of protein pattern (pathogenesis related proteins) in homogenates and intercellular washing fluids of control and infected leaves were compared after polyacrylamide gel electrophoresis (PAGE).

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Materials and Methods

Plants and pathogens

Wheat cultivars Giza 157, Alcedo and line Bu20, as well as near isogenic line pair without (SrHopeSa) and with stem rust resistance gene (SrHopeRa), kindly provided by Dr. A. P. Roelfs, USDA Cereal Research Laboratory, St. Paul-Minnesotta, were grown in greenhouse (approximately 24/20 °C and 10.000 lux, 14 hours photoperiod). Giza 157 and SrHopeRa are hypersensitively incompatible with *Puccinia graminis tritici* race 1 (reaction type 0;), and compatible with race 218 (reaction type 4). Bu20 and Alcedo are compatible with *Puccinia graminis tritici* race 218 and *Puccinia recondita* race 77, respectively. Inoculation was carried out by brushing or spraying the leaves with uredospore suspension (10 mg spores in 3 ml 4% starch solution for 60 seedlings). The challenge inoculation of the second leaves was made 4–5 days after infection of the first leaves with similar techniques.

The number of developing uredopustules was evaluated 7–12 days after the challenge inoculation. In an other type of experiment the apical part of first leaves was inoculated with incompatible stem rust race and the later growing basal part was challenged with compatible race.

PAGE of proteins in IWF and leaf homogenates

The apical and basal part of first leaves of SrHopeSa and SrHopeRa plants were cut 12 days after inoculation with stem rust race 1 or 218. Distilled water were used for extraction of intercellular washing fluids (IWF). For homogenates the same leaf samples were ground with 0.1 M phosphatecitrate buffer pH 2.8 containing 0.3 M sucrose and 0.005 M dithiotreitol in 1:4 ratio (w/v). Protein content of the samples was measured by the method of Bradford (1976). Aliquots of supernatants containing 40 μg protein were run on SDS anodic 10% polyacrylamide gel (PAGE). Proteins were stained according to the method of Heukeshoven et al. (1983).

Results

As it can be seen from Table 1, infection by *Helminthosporium* or mechanical injury of the first leaves caused a significant reduction in the number of stem or leaf rust pustules on the second leaves. Treatment of leaves with celit or carborund induced about 30%, while *H. sativum* infection a 50.4% decrease in number of colonies, which is similar to the 45.6% decrease caused by incompatible stem rust infection. The reaction type on the second leaves remained susceptible (3–4) according to the Stakman's scale.

After SDS PAGE no differences were detected in the protein pattern of IWFs from healthy leaves of susceptible and resistant near-isogenic lines. There were slight

Table 1

The effect of stem rust (St. r.) or *Helminthosporium sativum* infections, celite or carborundum treatments on the number of colonies of stem or leaf rust (Lf. r.) on the second leaves of wheat cultivars

Cultivar	Inducer/Challenge	Control	Pretreated	D	$LSD_{1\%}$
		(No. of colonies/leaf)			
Giza157	St. r. race1/218	10.7*	5.8	4.8	4.0
Alcedo	Celit/Lf. r. race77	257.0	181.0	76.0	62.7
Alcedo	Carb./Lf. r. race77	257.0	175.0	82.0	56.9
Bu20	*H. s.* /St. r. race218	25.62	12.70	12.92	6.70

* = No. of colonies/cm^2 leaf
D = difference between control and pretreated plants
$LSD_{1\%}$ = Least significant difference at 1% probability

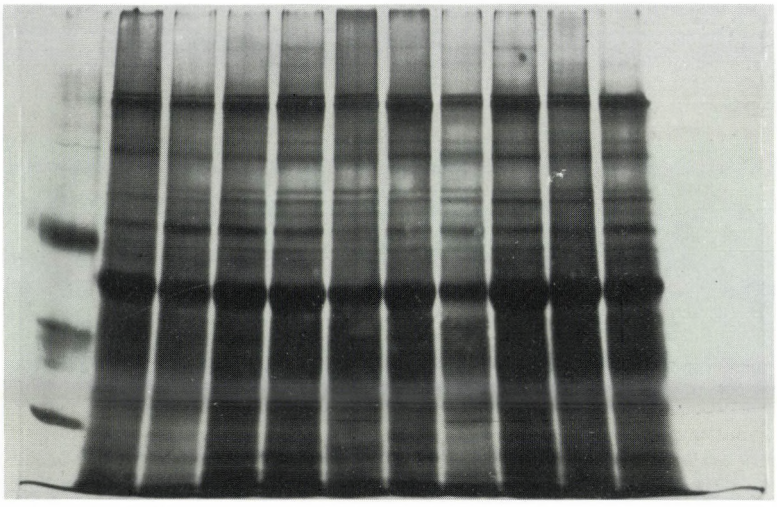
"shift" of some protein bands at the low molecular mass region in the IWFs from the infected apical part of the leaves from both susceptible and resistant plants as compared to the noninfected ones (Fig. 1). However we could not find significant changes in the pattern of IWFs from the uninfected basal part of resistant or susceptible leaves after infection of the apical part (Fig. 1).

More significant changes have been found after SDS PAGE of the leaves extracted with pH 2.8 buffer. There were slight differences between healthy resistant and susceptible leaves in the polypeptide pattern at very low molecular mass region, but it was not consistent (Fig. 2).

Consistent variation was shown between basal and apical leaf extracts. Two bands with about 68 and 60 kD in the older apical leaf extracts appeared as only 1 band of about 64 kD in the younger basal part (Fig. 2).

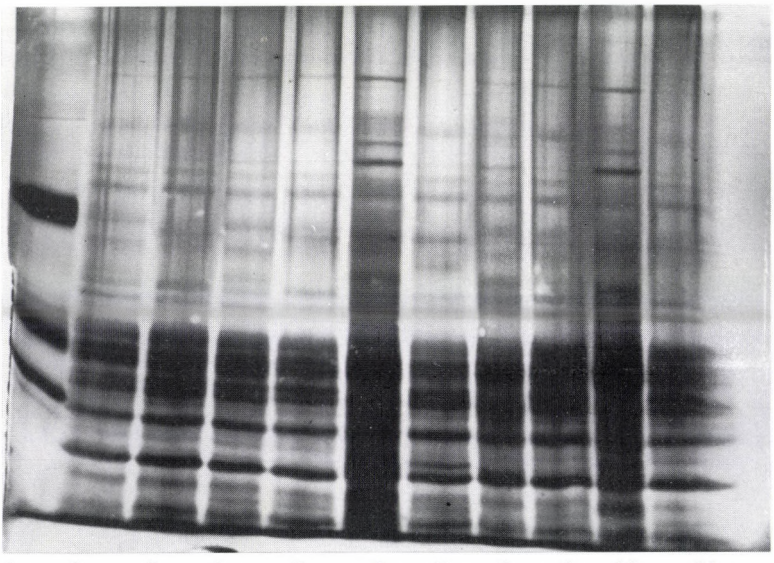
The picture of the protein bands showed the largest alterations after compatible infection. There were very similar changes in both HopeSa/race1 and HopeRa/race218 combinations. The low molecular bands became blurred. Bands appear with about 88 kD, 78 kD, 75 kD and 54 kD after compatible rust infection in the infected apical part of wheat leaves (Fig. 2). There were no similar changes in the uninfected basal part, instead other polypeptide bands of about 57 kD and 22 kD appeared in the polypeptide pattern of the acidic extract of HopeSa leaves, when the apical part was infected with race 1 (Fig. 2).

The incompatible infection caused slight changes in the pattern. There was a much weaker blurring of the bands than in compatible combination, and a slight appearance of the 88 kD band was observed (Fig. 2). We did not find significant changes in the pattern of acidic extract from the noninfected part of leaves with incompatible infection (Fig. 2).



1 2 3 4 5 6 7 8 9 10 11

Fig. 1. Protein paterns after 10% SDS PAGE of the IWF-s from the apical (A) or basal (B) part of primary leaves of SrHopeSa (Sa) and SrHopeRa (Ra) wheat lines, when the apical part was noninfected (C) or infected (I) with stem rust race 1 or race 218. Samples from left to right in order: 1 = molecular mass standards, 2 = SaA-C, 3 = SaB-C, 4 = RaA-C, 5 = RaB-C, 6 = SaA-I-race 1, 7 = SaB-I-race 1, 8 = RaA-I-race 1, 9 = RaB-I-race 1, 10 = RaA-I race 218, 11 = RaB-I-race 218



1 2 3 4 5 6 7 8 9 10 11

Fig. 2. Protein patterns after 10% SDS PAGE of pH 2.8 leaf extracts from the apical (A) or basal (B) part of primary leaves of SrHopeSa (Sa) and SrHopeRa (Ra) wheat lines, when the apical part was noninfected (C) or infected (I) with stem rust race 1 or race 218. Samples from left to right see in Fig. 1

Discussion

According to the above results not only incompatible stem rust infection, but infection with a necrotrophic pathogen and to a lesser extent mechanical injury of the first leaves can induce systemic resistance in the second leaves. This resistance is manifested not in the changes of reaction type but in the reduction of the number of pustules. These data suggest that necrotization of leaf tissue plays an important role in induction of SAR in wheat against rust. Similarly, chlorosis/necrosis were found to be involved in induction of SAR in other host-pathogen systems (Sziráki et al., 1980; Strobel and Kuć, 1995).

As regards mechanisms of SAR there are many hypotheses (Sticher et al., 1997) including the role of PR proteins (Stintzi et al., 1993), salicylic acid (Métraux et al., 1990; Raskin, 1992), higher cytokinin activity (Balázs et al., 1977; Sziráki et al., 1980; Sarhan et al., 1991) and antioxidant systems (Fodor et al., 1997). We found that lignification and activity of lignification enzymes such as phenylalanine ammonia lyase, tyrosine ammonia lyase, 4-cumarate CoA ligase as well as peroxidase are increased in the second leaves of wheat, if the first leaves were infected with stem rust or barley powdery mildew (Barna et al., 1996).

Neither the intercellular fluids, nor the acidic extracts showed significant differences in protein composition of the near isogenic lines with or without resistance gene. Consistent variation was shown between basal and apical acidic leaf extracts, indicating the importance of leaf age in such investigations. Marked changes were found in the pattern of acidic buffer extractable proteins in wheat leaves after compatible rust infection. Changes at the infection site are probably mainly due to proteins from the rust fungus. However changes in protein pattern in the basal part of the leaves are systemic effect of the infection. On the other hand, in resistant combination slight differences were observed in protein pattern of infected and control leaves. There were no systemic effect of the infection. Since systemic acquired resistance was found only after hypersensitive incompatible first infection, protein changes found in the above experiments cannot be responsible for SAR.

In conclusion, systemic acquired resistance was induced in the second leaves of wheat, when the first leaves were infected with incompatible stem rust race or *Helminthosporium sativum*, or treated by celite or carborundum. Significant changes were found in proteins extracted with acidic buffer from susceptible wheat leaves after infection. Other changes were observed in protein composition of hypersensitively reacting resistant leaves. Only the compatible infection had systemic effect on leaf proteins, indicating that these changes do not play significant role in inducing SAR in wheat against rusts.

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Postulation of Resistance Genes to Wheat Stem Rust in Winter Wheat Genotypes from Szeged

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The seedling reaction of thirteen winter wheat genotypes from Szeged was assessed to 12 stem rust (*Puccinia graminis* f. sp. *tritici*) pathotypes with known virulence factors. The postulated genes were Sr5, Sr31 and Sr36 in eight cultivars.

Four cultivars gave low infection types to all isolates. The results of seedling tests and source of stem rust resistance (genetic background) of these cultivars suggested that the resistance gene Sr31 is present in GK Csörnöc and GK Zombor and the resistance gene Sr36 in GK Kincső and GK Góbé.

Cultivars GK István, GK Barna, Yubileynaya 50 and GK Délibáb were postulated to have resistance gene Sr5. On the basis of the pedigree of cultivar GK István it could have the gene Sr31 too, however the results of seedling tests did not confirm that supposition. This cultivar was susceptible to all pathotypes of stem rust races which were virulent to Sr5.

Stem rust (*Puccinia graminis* f. sp. *tritici*) is a major pathogen of wheat (*Triticum aestivum*). Effective chemical control is available but expensive. The regular use of fungicides, however may become an environmental hazard, while the pathogen is often able to become tolerant or resistant to the fungicide. Cultivars, resistant to stem rust, form a good alternative in order to reduce the environmental pollution (Barabás et al., 1987).

High levels of resistance, often based on major genes (race-specific type) can be easily recognized in the field and this type of resistance is therefore widely used in many breeding programmes. A series of such resistance genes are identified and designated other major genes have been detected but still have to be designated (McIntosh et al., 1995).

Knowledge of the genetic basis of resistance to stem rust in wheat cultivars is useful in understanding the distribution of races of the pathogen and also in the breeding for resistance to this disease.

The gene-for-gene hypothesis formulated by Flor (1971), and development of host genotypes differing primarily by a single effective gene for resistance (Green, 1965; 1981; Loegering and Harmon, 1969; Knott and Anderson, 1965; Roelfs and McVey, 1979; Watson and Luig, 1963) made it possible to determine the virulence genes of stem rust population and effective resistance genes of wheat from year to year in Hungary (Manninger, 1994a, b). It is also possible to postulate the presence of resistance gene to wheat rust in cultivars and genotypes by differential pathogen races and infection type data of the cultivars (Manninger et al., 1994). The success of any breeding programme depends primarily upon the availability of the number of different resistant donor parent genotypes.

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The aim of this study was to survey winter wheat genotypes/cultivars from Szeged for resistance to wheat stem rust and to postulate and confirm the presence of stem rust resistance genes in cultivars.

Materials and Methods

Thirteen wheat genotypes/cultivars from Szeged were tested to stem rust resistance in the greenhouse at the Plant Protection Institute in Budapest. The list of cultivars' pedigree are shown in Table 1.

The seedlings of the wheat cultivars were grown at 18–25 °C in the glasshouse. Seven-day-old seedlings were inoculated with 12 pathotypes of Hungarian stem rust population (Table 2). The main difference among the isolates was as the follows: the No. 1, No. 2, No. 3 and No. 4 isolates were avirulent and the other eight isolates (No. 5–No. 12) were virulent to Sr5. Inoculated wheat seedlings were exposed at higher humidity in moist chambers for 24 hours and then transferred to glasshouse by 16 hours daylight and temperature 18–25 °C for development of stem rust. The evaluation of the infection types were made 12–14 days after inoculation according to a 0–4 infection scale.

The stem rust resistance genes of wheat cultivars were identified by the aid of infection types of twelve pathotypes in the cultivars.

Table 1

Pedigree of winter wheat genotypes/cultivars from Szeged

Genotype/Cultivar	Pedigree
GK Barna	D1/Sava//Aquileja
GK Csaba	F2 Population
GK Csörnöc	F1502/W25–2//Tiszatáj
GK Délibáb	DH Mini Mano/3/Yubileynaya 50/SadovoS//Mini Mano/Mv12
GK Gereben	Mini Mano/3/Rousalka/Rannaya 12//Rubin
GK Góbé	Mini Mano/Kincső
GK István	Kremena/Aurora
GK Kata	Yg 884/Mv4//GK3282
GK Kincső	Arthur 71/Sava
GK Őrség	GK F2/Hays 59
GK Öthalom	2*Szeged/Yubileynaya 50
GK Zombor	Kavkaz/Produttore//Sava
Yubileynaya 50	Miranovskaya 808/Bezostaya 1

Table 2

Virulence of Hungarian stem rust isolates

No. of isolates*	Race/pathotypes	Virulence on Sr genes
1	1/1	6, 7b, 21,
2	1/2	6, 7b, 21, 27
3	1/3	6, 7b, 21, 8a, 17, 30
4	1/4	6, 7b, 21, 9g, 8a, 37
5	11	5, 6, 8a, 21
6	34/1	5, 7b
7	34/2	5, 6, 7b
8	34/3	5, 6, 7b, 8a, 9g, 11, 29, 37
9	34/4	5, 7b, 8a, 9g, 11, 29, 30, 37
10	218/1	5, 6, 7b, 8a, 9b, 9e, 11, 21
11	218/2	5, 6, 7b, 8a, 9b, 9e, 11, 21, 27, 29
12	218/3	5, 6, 7b, 8a, 9b, 9e, 11, 21, 26, 27, 29

* Isolates were tested on 23 isogenic lines (Sr5, Sr6, Sr7b, Sr8a, Sr9b, Sr9e, Sr9g, Sr11, Sr13, Sr17, Sr21, Sr22, Sr24, Sr25, Sr26, Sr27, Sr29, Sr30, Sr31, Sr32, Sr33, Sr36, Sr37)

Sr13, Sr22, Sr24, Sr25, **Sr31**, Sr32, Sr33, **Sr36** resistance genes were completely effective

Results and Discussion

The cultivars GK Kata and GK Őrség were susceptible to all pathotypes suggesting the absence of effective resistance genes in these cultivars. The other 11 genotypes/cultivars were classified in two main groups according to their reaction to stem rust isolates (Table 3).

Group I represented by 4 cultivars, showed low infection type to all isolates. That means they are resistant to Hungarian stem rust population.

Group II represented by seven genotypes/cultivars. GK István, GK Barna, Yubileynaya 50 and GK Délibáb were observed to have low infection type to four pathotypes having avirulence to Sr5 and high infection type to other pathotypes, which are virulent to Sr5. These data indicate the presence of the gene Sr5 in these cultivars.

GK Öthalom, GK Csaba and GK Gereben showed low infection type to two different pathotypes which are avirulent to Sr5, but they gave high infection type to other pathotypes. Out of the letter type two were avirulent and the others were virulent to Sr5.

Knowledge of the source of resistance (pedigrees of wheat cultivars and resistance genes of the parents) may help in the identification of resistance genes in cultivars.

According to the results obtained with seedlings, stem rust resistance genes Sr31 or Sr36 are probably present in the first group of cultivars. All these pathotypes were avirulent to isogenic lines carrying the Sr31 and Sr36 genes. The cultivars GK Kincső

Table 3

Postulation of stem rust resistance genes in winter wheat genotypes/cultivars from Szeged

Genotype/Cultivar	Source of resistance	Hungarian stem rust isolates 1	2	3	4	5	6	7	8	9	10	11	12	Postulated Sr Gene
Group I		The genotypes/cultivars give low infections type to all isolates												
GK Kincső	Arthur 71	R	R	R	R	R	R	R	R	R	R	R	R	Sr36
GK Góbé	Kincső (Arthur 71)	R	R	R	R	R	R	R	R	R	R	R	R	Sr36
GK Zombor	Kavkaz	R	R	R	R	R	R	R	R	R	R	R	R	Sr31
GK Csűrös	1BL.1RS+	R	R	R	R	R	R	R	R	R	R	R	R	Sr31
Group II		The genotypes/cultivars give low infections type to some isolates												
GK István	Aurora	**R**	**R**	**R**	**R**	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	Sr5
GK Barna	Sava	**R**	**R**	**R**	**R**	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	Sr5
Yubileynaya 50	Bezostaya–1	**R**	**R**	**R**	**R**	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	Sr5
GK Délibáb	Yubileynaya 50	**R**	**R**	**R**	**R**	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	Sr5
GK Öthalom	?	**R**	*S*	*S*	**R**	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	?
GK Csaba	?	**R**	*S*	*S*	**R**	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	?
GK Gereben	Rousalka	**R**	**R**	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	?

+ Johnson et al. (1996)
R = Resistant (Low infection type 0, ;, 1)
S = Susceptible (High infection type 3, 3+, 4)

and GK Góbé were resistant to all pathotypes. These cultivars originated from cultivar Arthur 71 (Table 1) which carries Sr36 (McIntosh et al., 1995), therefore the presence of the gene Sr36 may occur both in GK Kincső and GK Góbé.

In the case of GK Zombor one of the parents was cultivar Kavkaz, which carries Sr31 (Zeller and Baier, 1973; Bartos, 1984; Luig, 1983; Roelfs, 1988; McIntosh, 1995), consequently this wheat cultivar probably carries the gene Sr31 which provides resistance to stem rust.

According to seedling tests, GK Csörnöc provides resistance to stem rust because it carries either Sr31 or Sr36. However, determination of resistance genes in this cultivar needed further investigations. The stem rust resistance gene Sr31 was derived from rye chromosome, which occurs in wheat either as a 1R(1B) alien substitution or as a 1BL.1RS translocation (Mettin et al., 1973; Zeller and Baier, 1973). The 1BL.1RS translocation in wheat cultivars could be detected by SDS-PAGE (Sozinov et al., 1987). The 1BL.1RS translocation was showed in GK Csörnöc by this method (Johnson et al., 1996), therefore the presence of Sr31 may occur in GK Csörnöc.

In cultivars GK István, GK Barna, Yubileynaya 50 and GK Délibáb the presence of Sr5 was supposed. These cultivars originated from cultivar Aurora, Sava, Bezostaya 1 (Table 1), which are known to carry the stem rust resistance gene Sr5 (Bartos, 1984; Luig, 1983; Knott, 1990). One of the parents of the cultivar GK István is Aurora, which

carries the resistance gene Sr31 too (Roelfs, 1988), thus one can suppose that GK István may have the resistance gene Sr31, however seedlings tests did not confirm this statement. According to the seedling tests, GK István turned to be susceptible to all pathotypes of stem rust which were virulent to Sr5. Furthermore, it was resistant to four pathotypes which exhibited avirulence to the gene Sr5. In the pedigree of GK Öthalom the most important parent seems to be Yubileynaya 50 which carries stem rust resistance gene Sr5. Consequently, GK Öthalom could carry Sr5, however the seedling tests did not confirm that supposition. We were not able to detect resistance genes neither in GK Csaba nor in GK Gereben, although these genotypes exhibit resistance to two stem rust pathotypes. We have to continue our research on this field to identify futher resistance genes to stem rust in these cultivars.

Conclusion

The postulation of stem rust resistance genes following inoculation of wheat cultivars with different known pathotypes is based on the gene-for-gene relationship (Flor, 1971). This approach is not a full-proof way of determining the genetic constitution of cultivars but it is a fast method to identify with reasonable certainty which known genes are present, and if they are effective or not.

We detected three known stem rust resistance genes: Sr5, Sr31 and Sr36 by the aid of infection type data in the eight wheat cultivars from Szeged. Among them the cultivars with Sr31 or Sr36 genes are resistant to stem rust in Hungary.

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Susceptibility of Vegetable Crops to *Agrobacterium vitis* Ophel et Kerr

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The susceptibility of 24 annual crop species was tested to seven *Agrobacterium vitis* strains having octopine, nopaline or vitopine Ti plasmids. Four plant species (redbeet, carrot, pepper and tomato) were susceptible to all strains tested, six ones were susceptible only to one or two of the *A. vitis* opine groups, while 14 species did not form any tumor following inoculation. The cultivation of sensitive plant species on grape-growing fields after cutting down the infected plants may promote the survival of the pathogen in the soil leading to infection of new grapevine plantations. Our results provide preliminary data to select non-host plants for interval cultivation on grape fields therefore reducing the risk of the survival of pathogenic *A. vitis* in the soil.

Virulent species of the genus *Agrobacterium* cause tumors or hairy roots on a wide range of plants including several srops. The host range of *A. tumefaciens* and *A. rhizogenes* have already been extensively studied (DeCleene, 1979, 1985; DeCleene and DeLey, 1976, 1981; Tepfer, 1990; Hood et al., 1993), but only limited data are available on *A. rubi* and *A. vitis*, which are responsible for raspberry cane gall and grapevine crown gall, respectively. Agrobacteria are also classified according to their biochemical and metabolic characteristics into three groups as biotype 1, 2 and 3 (Kerr and Panagopoulos, 1977; Süle, 1978). Of these, biotype 3 corresponds to *A. vitis* (Ophel and Kerr, 1990). The virulence of *Agrobacterium* is determined by large plasmids, called tumor-inducing (Ti), or Root-inducing (Ri) plasmids. Besides the tumor-, or hairy root induction these plasmids encode also the synthesis of specific amino acid derivatives (opines) in the plant tumors which are secreted by the plant cells and which are selectively utilized by the inciting bacterium. The opine markers form a third basis for the identification and classification of agrobacteria (Dessaux et al., 1992, 1993).

Of the four *Agrobacterium* species, *A. vitis* is closely associated with grapevines and causes serious economic loss in grape-growing countries. Since the pathogen is systemic in the host plant, the propagating material is a primary source of infection and for spreading of the disease (Lehoczky, 1971; Burr and Katz, 1984; Tarbah and Goodman, 1987; Thies et al., 1991). Until now *A. vitis* has not been isolated from soil collected from virgin fields (Bouzar and Moore, 1987). However is occurs in grapevine soils and in the rhizosphere of infected plants (Burr and Katz, 1984; Burr et al., 1987), as well as in grapevine root and cane remains after cutting down the galled plantation (Burr et al., 1995). This may be a source for infection of replanted grapevines. Reinfection of young

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grapevines from the soil could be extremely important in case of nurseries where a high plant density with an annual turnover is grown.

Growing of sensitive plants in grapevine soils may also promote the survival of the pathogen. It has already been shown that the number of *A. vitis* cells decreases or they quickly disappear from non-host and fallow soils, but they are stably maintained in the presence of grapevine as sensitive host plant (Bishop et al., 1988). Until now little is known about the host range of *A. vitis*. Besides grapevine most isolates were pathogenic on raspberry, tomato, chrysanthemum, *Kalanchoë tubiflora*, tobacco and sunflower. A few strains were also virulent on pea and on jimsonweed (Panagopoulos et al., 1978; Kanuf et al., 1982; Perry and Kado, 1982; Szegedi, 1985; Ma et al., 1987; Sawada et al., 1990). The aim of this study was to obtain additional data on the host range of *A. vitis*.

Materials and Methods

Bacterial strains

A. vitis strains used for experiments (Table 1) were isolated from different locations and grapevine varieties. For inoculations they were grown at 27 °C for two days on AB minimal medium (Lichtenstein and Draper, 1986) supplemented with 1% glucose and 0.5% yeast extract. The non-virulent strain S4(pPM739) was used for control experiments.

Table 1

Agrobacterium vitis strains used for inoculatons

Strain	Opine marker (plasmid number)	References*
AB3	octopine pTi (4)	1, 2
Tm4	octopine pTi (2)	1, 2
AT1	nopaline pTi (3)	1, 2
AT66	nopaline pTi (2)	1, 2
S4	vitopine pTi (4)	1, 2
Sz1	vitopine pTi (2)	1, 2
397/95	vitopine pTi (3)	this work
S4(pPM739)	pTi cured S4 (4)	3

* 1. Szegedi et al., 1988
2. Paulus et al., 1989
3. Szegedi et al., 1996

Production and inoculation of test plants

All plant species involved in the studies (Table 2) were grown as seedlings in garden soil in the greenhouse at 18–25 °C. Stems of young (4–6 week-old) plantlets were inoculated by wounding with a sterile needle dipped into the bacterial culture. The results of inoculations were scored on the basis of tumor formation after 4–5 weeks incubation of the infected plants. Plants showing no symptoms after this time were incubated and observed for an additional three weeks.

Table 2

Plant species tested and their susceptibility to *Agrobacterium vitis* strains*

Family	Species	Agrobacterium vitis strain						
		AB3	Tm4	AT1	AT66	S4	Sz1	397/95
Chenopodiaceae	Red beet	+	+	+	+	+	+	+
	Spinach	–	–	+	+	+	+	–
Polygonaceae	Sorrel	–	–	–	–	–	–	–
Cucurbitaceae	Water melon	–	–	–	–	–	–	–
	Musc. Melon	–	–	–	–	–	–	–
	Pumkin	–	–	–	–	–	–	–
	Cucumber	–	–	–	–	–	–	–
Brassicaceae	Brussels	–	–	–	–	–	–	–
	Cabbage	–	–	–	–	–	–	–
	Radish	–	–	+	+	–	–	–
	Kohlrabi	–	–	–	–	–	–	–
	Cauliflower	–	–	–	–	–	–	n. t.
	Savoy	–	–	–	–	–	–	n. t.
Apiaceae	Parasely	–	–	+	+	+	+	n. t.
	Carrot	+	+	+	+	+	+	–
	Celery	–	–	–	–	–	–	–
Fabaceae	Bean	+	+	–	–	+	+	–
	Pea	–	–	–	–	–	–	n. t.
Solanaceae	Pepper	+	+	+	+	+	+	+
	Tomato	+	+	+	+	+	+	+
	Eggplant	–	–	+	+	+	+	+
Asteraceae	Lettuce	–	–	+	+	+	+	–
Liliaceae	Leekonion	–	–	–	–	–	–	n. t.
	Onion	–	–	–	–	–	–	n. t.

* +: sensitive (tumor forming) reaction, –: non-sensitive reaction, n. t.: not tested
Data on the plant varieties are provided upon request

Result and Discussion

Tumors were observed after an incubation of two weeks. All opine types (octopine, nopaline and vitopine) of *A. vitis* induced tumors on redbeet, carrot, pepper and tomato plants. On the other hand, some plant species, as spinach, radish, parasely, bean, eggplant and lettuce were susceptible only for a limited number of strains. Fourteen of the 24 plant species tested were not susceptible to any of *A. vitis* strains included in our studies. Except strain 397/95, the opine markers and virulence properties were correlating. The results of these experiments are summarized in Table 2.

Our data show that the tested plants could be divided into three groups according to their susceptibility. (1) Four of the 24 species tested were susceptible to all strains. (2) Six were susceptible only to a limited range of *A. vitis* strains and this susceptibility pattern was correlating with opine type of the Ti plasmid with the exception of the Austrian isolate 397/95. Similar host range differences were observed on grapevine varieties when octopine, nopaline and vitopine strains were compared (Szegedi et al., 1989). (3) Fourteen species did not respond with tumor formation following inoculation.

The cultivation of sensitive plant species on grape growing fields may promote the survival of *A. vitis* remaining in grapevine roots, trunks and canes after cutting down an infected plantation. It has already been proven that opines produced in tumors selectively promote the growth of the inducing pathogen (Guyon et al., 1993). Therefore if sensitive crops become galled from the infected soil they may provide a permanent source of pathogenic *A. vitis* cells in soil. This may lead to infestation of grapevines in nurseries or in new plantations. Thus it is useful to consider the potential hosts of *A. vitis* when crops are chosen for the so-called interval cultivation. Our results provide preliminary data for selecting non-sensitive crops, however varietal differences could make this host range pattern more complex.

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Characterization of *Phytophthora infestans* Isolates from Hungary

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Several isolates of *Phytophthora infestans* collected in Hungary in the 1990s were analysed for mating type, response to metalaxyl and allozyme genotypes. Among the isolates there were both A1 and A2 mating types. Radial growth and leaf disk assays revealed either sensitivity or resistance to the systemic fungicide metalaxyl, independently of the mating type. For *Gpi* (glucose-6-phosphate isomerase) the 100/100 homozygous genotype was the only one found. The common type for *Pep* (peptidase) was homozygous, either for the 100 or the 96 allele but one of the isolates (mating type A1) was heterozygous and defined as the 83/96 genotype.

Late blight has become one of the most devastating diseases of potato and tomato in temperate regions of the world (Erwin and Ribeiro, 1996; Érsek and Bakonyi, 1997). Identification of the A2 mating type in addition to the existing A1 in Europe (Hohl and Iselin, 1984) was the first indication of significant change in populations of the late blight fungus, *Phytophthora infestans* (Mont.) de Bary. There was a concomitant increase in complexity of virulence phenotypes and in tolerance to the specific fungicide, metalaxyl (Fry et al., 1991; Daggett et al., 1993; Sujkowski et al., 1994; Martinez, 1995; Schöber-Butin et al., 1995). Analysis of allozyme markers such as peptidase (*Pep*) and glucose-6-phosphate isomerase (*Gpi*) (Tooley et al., 1985; Shattock et al., 1986) and DNA fingerprints (Goodwin et al., 1992a; Goodwin et al., 1992b; Drenth et al., 1993) of isolates from various locations of the world stimulated the hypothesis that a new *P. infestans* population derived from Mexico was displacing an older population in Europe and other continents as well (Spielman et al., 1991; Fry et al., 1993; Koh et al., 1994; Goodwin and Drenth, 1997).

In Hungary, the early report of Mudich (1965) indicated the presence of solely simple races. Since then, no extensive study on the composition of local populations of *P. infestans* has been carried out. Until recently, it was assumed that changes in the diversity of the Hungarian *P. infestans* populations had occurred in concert with the worldwide changes. Indeed, an A2 mating isolate that we found in 1996 exhibited a complex virulence phenotype associated with resistance to metalaxyl (Bakonyi and Érsek, 1997). A more extensive survey, however, is required for better understanding of the present status of the late blight pathogen in Hungary.

Our goal was to see if changes in population structure of *P. infestans*, similar to those found elsewhere, have occurred in Hungary. In this preliminary study, a limited number of isolates of the pathogen that were collected in 1997, in addition to

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those few available from earlier isolations, were analysed. This paper focuses on the determination of mating type, metalaxyl sensitivity and allozyme (*Pep* and *Gpi*) genotypes.

Materials and Methods

Sources of isolates

Blighted plant material was collected in 1997 from commercial fields and kindly supplied by extension experts. *Phytophthora infestans* was isolated onto pea-broth agar (Tuite, 1969; Érsek et al., 1995) containing pimaricin (10 mg l^{-1}), ampicillin (500 mg l^{-1}) and rifampicin (10 mg l^{-1}) for selectivity. Additionally, unknown or partially characterized isolates from earlier collections (one isolate kindly provided by L. Gergely, National Institute for Agricultural Quality Control, Budapest) were also included. Isolates used as standards for mating type determination and/or allozyme analysis were obtained from B. Schöber-Butin (Biologische Bundesanstalt, Braunschweig, Germany) and W. E. Fry (Cornell University, Ithaca, NY, USA). Subsequent culturing of all isolates was carried out on pea-broth agar at 20 °C.

Test for mating type determination

Mating type of each isolate was determined by pairing with German isolates of known mating type. Six-mm-diameter discs were cut from the edge of a 6- to 8-day-old colony of each isolate to be paired and placed 2 cm apart on 1.5% pea-broth agar. Cultures were examined for oospore production at the hyphal interface between the developing colonies after growth for 10 to 14 days at 20 °C. An unknown isolate was designated A1 if it produced oospores in matings with the German isolate 15/93 (A2 mating type). Those isolates forming oospores with the German isolate 4/91 (A1 mating type) were designated A2.

Test for metalaxyl sensitivity

Growth responses of isolates to metalaxyl were evaluated *in vitro* and *in vivo*.The *in vitro* assay was based on measurements of radial growth for 8 days at 20 °C on metalaxyl-amended *versus* fungicide-free pea-broth agar. Metalaxyl (technical grade, Ciba-Geigy) was tested at concentrations of 100 mg a.i. l^{-1} and tenfold dilutions.

In vivo growth was determined by the leaf-disk assay, as recommended by the Fungicide Resistance Action Committee (FRAC) and described by Sozzi et al. (1992). Since all isolates tested were aggressive on tomato, leaf-disks of tomato were used in the assay. Leaf disks, 15 mm in diameter, were cut from mature leaves of 4- to 5-week-old plants (cv. Zömök) and placed abaxial surface up on water agar plates with or without the fungicide. A sporangial suspension (10^5 sporangia ml^{-1}) was sprayed on leaf disks. Inocu-

lated disks were incubated at 18 °C in the dark, for 5 to 6 days. The surface area of disk coverage by fungal structures was scored.

Based on the standard EC_{50} threshold values, an isolate was rated in both assays as resistant at EC_{50} > 10 mg metalaxyl l^{-1} and sensitive in the range of 0.001 and 0.01 mg metalaxyl l^{-1}.

Allozyme analysis

Tissue for cellulose acetate electrophoresis (CAE) consisted of mycelia grown in pea broth. Small pieces of mycelia, ca. 5 mm^3 each, were blotted, washed twice in water, blotted again and then rehydrated. These samples were ground in 1.5-ml microcentrifuge tubes with a tapered pestle and then centifuged at 16,000 *g* for 2 min to pellet cell debris. Gels were loaded with small aliquots (1 to 1.5 µl) of each supernatant. CAE was performed according to the protocol described by Goodwin et al. (1995). Allozyme phenotypes were scored according to the relative mobilities of individual active enzymes, *Pep* and *Gpi*, with the most common allele designated as 100 (Tooley et al., 1985). Allozyme phenotypes of isolates US940501 (clonal lineage US-1), ME93-2A (clonal lineage US-8), JP880001 (clonal lineage JP-1) and PO880033 (clonal lineage PO-6) were used as controls to determine the identity of allozyme alleles. Activities of Pep and Gpi were detected using agar overlays. Supplies and chemicals were purchased from Helena Laboratories and Sigma Chemical Company, respectively.

Results and Discussion

In this preliminary study, 4 isolates collected during 1997 and 3 isolates from earlier collections were studied. Both A1 and A2 mating types were detected among the isolates collected in 1997. Previous report by Bakonyi and Érsek (1997) indicated that the A2 mating type occurred in Hungary as early as 1996. Results of the present study vindicate the presence of A2 mating types as early as 1991 (Table 1).

Growth responses of isolates to the systemic fungicide metalaxyl are shown in Tables 1 and 2. Results of the *in vitro* radial growth assay were comparable to those of the *in vivo* leaf disk assay. Resistance to metalaxyl could be detected in both A1 and A2 isolates. However, two A2 isolates (H-K/91 and H-2/97), both derived from tomato, were sensitive to the fungicide. It should be noted that tomato isolates, in general, seem to be more sensitive to metalaxyl than are isolates from potato (Fry et al., 1991).

Allozyme analysis of Hungarian isolates of *P. infestans* revealed one allele for *Gpi* and three alleles for *Pep*. The 100/100 homozygous genotype of *Gpi* was observed for all isolates (Fig. 1 and Table 1). The common type for *Pep* was either the 100/100 or the 96/96 homozygous genotype. However, one of the isolates, the H-3.1/93 (mating type A1), was heterozygous and defined as the 83/96 genotype (Table 1). This was the same genotype as for isolate 4/91 (A1), collected in Germany and used in mating assays (not shown). Although this genotype is not listed in any of the afore-mentioned publications,

Table 1

Characteristics of *P. infestans* isolates collected in Hungary between 1991 and 1997

Isolate	Year of collection	County/ Region[a]	Host	Growth response to metalaxyl[b]	Mating type	Allozyme *Gpi*	genotype *Pep*
H-2/97	1997	Heves/NE	Tomato	S	A2	100/100	96/96
H-4/97[c]	1997	Nógrád/NC	Potato	R	A1	100/100	100/100
H-6/97[c]	1997	Nógrád/NC	Potato	R	A1	100/100	100/100
H-8/97	1997	Szolnok/EC	Potato	R	A1	100/100	100/100
H-2a/96	1996	Veszprém/WC	Potato	R	A2	100/100	96/96
H-3.1/93	1993	Pest/C	Potato	I	A1	100/100	83/96
H-K/91	1991	Heves/NE	Tomato	S	A2	100/100	96/96

[a] Regions are: North East (NE); North Central (NC); East Central (EC); West Central (WC) and Central (C).
[b] S: sensitive; R: resistant and I: intermediate, as defined in the text.
[c] Collected from the same field.

Table 2

Sensitivity of Hungarian isolates of *P. infestans* to metalaxyl *in vitro* and *in vivo*

Assay	EC_{50} (mg l^{-1}) for isolates						
	H-2/97	H-4/97	H-6/97	H-8/97	H-2a/96	H-3.1/93	H-K/91
In vitro	0.001–0.01	>100	>100	>100	>100	0.1–1	n.m.[a]
In vivo	0.028	175	320	200	>100	n.m.	0.005

[a] Not measured.

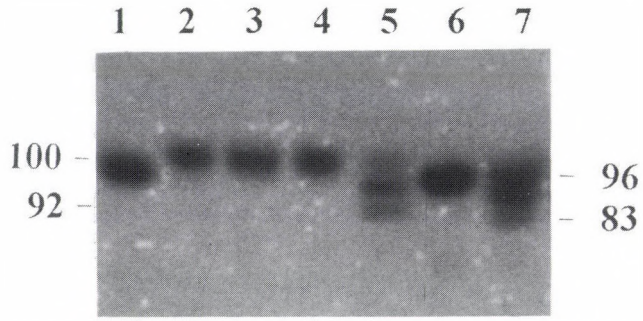

Fig. 1. Electrophoretic phenotypes at the peptidase (Pep) locus of *Phytophthora infestans* isolates on a cellulose-acetate gel. Isolates are as follows (from left to right). 1: H-2/97, 2: H-4/97, 3: H-6/97, 4: H-8/97, 5: US-1 (genotype 92/100), 6: JP-1 (genotype 96/96), 7: H-3.1/93. Locations of the 83, 92, 96 and 100 homodimer bands as based on the migration of bands of the reference isolates, are indicated on the left and right

it seems to correspond to the genotype defined earlier as 83/100 by its appearance on gels other than cellulose acetate (*cf.* clonal lineage PO-6, not shown). The 100/100 genotype first appeared for *Gpi* and *Pep* in 1980 and since then, this has become the predominant genotypic combination among European populations (Daggett et al., 1993; Goodwin and Drenth, 1997). Before the presumed occurrence of A2 in Europe in the early 1980s, the isolates had the dilocus genotype combination *Gpi* 86/100 and *Pep* 92/100 which corresponds to that of isolates of the old clonal lineage, US-1, in the USA (Fry et al., 1993; Goodwin et al., 1994).

The data from this small study do not conclusively resolve the genotypic composition of *P. infestans* populations country-wide. However, it is unambigouosly shown, for the first time, that Hungary did not eschew the world-wide trend of changes that has taken place in populations of *P. infestans* in the past two decades. Recent failures in successful late blight control in the country may, in part, due to increased metalaxyl resistance in populations of *P. infestans*. Changes in fungicide resistance in evolving populations will only be detectable with extended pathogen surveys. On the premise that knowledge of the population genetics of *P. infestans* may contribute to the development of more effective disease management strategies, we have initiated such a study to be conducted in 1998 and thereafter.

Acknowledgements

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Mass Appearance of *Urocystis occulta* (Wallr.) Rabenhorst and the Risk Caused by its Damage

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Authors present symptoms of the diseases on the leaves, leaf sheats, stalks and in the ears (smutted chaff, sterile ears, in the leaf sheath, externally turned diseased ears) as well as in the malformed, 30–40% shorter plants present in the crops.

Centrally performed seed treatment made with careful technical requirements is recommended as the mass of teliospores of the *Urocystis genus* does not disintegrate and the so-called by-cells reduce the efficiency of some seed-dressing agents.

U. occulta is evaluated only with some sentences or pages both in the Hungarian and international literature, though this pathogen has been known for over hundred years on host-plants of *Secale cereale* and *S. montanum* (Schlechtendal, 1824, 1826; Fischer von Waldheim, 1967). During its sporadic incidence in Hungary, Linhart (1910), Husz (1930) and Moesz (1950) collected some species used also by foreign phytopathologists at the Department of Plant Protection, University of Agriculture (Vánky, 1985). The Polish mycological collection (Kochman, 1960) is also stored here containing many species of *Ustilago genus*. All of them together with the collection from Thuringia (Wallroth, 1833) are known as valuable comparative material. At the modern classification of the *Ustilago genus*, the pathogen occurred only in spots both in Hungary and on the Transcarpathian territory (Poland, Rumania, Ukraine) (Vánky, 1983, 1985; Vánky et al., 1982). In other rye-growing countries of Europe, *U. occulta* is, of course, known (Ainsworth, 1995; Fragoso, 1924; Massenot, 1955; Vasyagina, 1977).

In spite of these facts, only little is known about the biology of *Urocystis genus*, the Hungarian practice does not know the most important properties, even contradictions may be found in the literature. A special chapter is made on the resistance and susceptibility mechanisms, as well as on the serological studies concerning the relations of *Ustilago genus* (Vajna, 1987).

The most recent scientific results are considered in evaluating the nutrient accumulation and analyzing the phloem transport (Goodman et al., 1991).

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Materials and Methods

Studies of the spread, change of symptoms and survey of damages of *U. occulta* were made on commercial rye varieties grown on sandy soils in counties Pest, Jász-Nagykun-Szolnok, Bács-Kiskun and Tolna. No data collection was made in county Szabolcs-Szatmár-Bereg, though it has also a significant rye production.

Rye seeds were normally treated in test-tube under laboratory conditions. Prior to treatment, teliospores of the pathogen obtained from the previous year's ecperiments were dispersed (0.5 g/1000 g) on the surface of the seeds. The rate used was uniformly 0.3 1 and kg/100 kg seeds. Untreated seeds infected with head smut were used as control. In autumn, periodic sowing was made at several ocassions.

Studies for detecting damages by *U. occulta* and for testing the seed-dressing agents were made in the laboratory of the Department of Plant Protection of the University of Agriculture (Gödöllő) and in the site of Modul-OK Shareholding Co. (Erdőkertes).

The objective of the experiments was to evaluate the level of germ infection, the favourable time for infection and the efficiency of commercially available seed-dressing agents.

Table 1 shows the seed-dressing agents and active-ingredients used for the experiments.

When studying the effect of the pathogen on the development and growth of rye plants, the height of healthy and infected plants was also measured. Evaluation was made after earing and before ripening. The total number of plants, including the diseased ones was counted in the control rows. Based on these data, the *infection percentage* (*F*%) was generated. Results were statistically evaluated. The average was calculated from the data, then the difference in susceptibility confirmed with variance analysis (Baráth et al., 1996). The height values were expressed in percent.

Table 1

Seed-dressing agents used in the experiments

Seed dressing agents	Active ingredients
Agrocit	50% benomyl
Baytan Universal	15% triadimenol
	2% fuberidazole
	2.5% imazalil
Raxil 2 WS	2% tebuconazole
Biosild BD	150 g/l carbendasim
	15 g/l diniconazole
Buvisild BR	22.5% carbendasim
	7.5% cupperosy-quinolate
Quinolate 15	15% cupperosy-quinolate
Vitavax 200 FF	17% carboxin
	17% thiram
Panoctin 35 EC	35% quazitine

Change in the temperature of healthy and infected rye plants was measured with thermovision in cooperation with the Measurement techniques Dept. of the Institute for Technology of the Ministry of Agriculture (Papp, 1991). For comparison, data of temperature of *Puccinia graminis*, *Tilletia caries* and *U. occulta* were used. At the same time, the measurements were also made in the glasshouse of the Research Institute for Plant Protection of the Hungarian Academy of Sciences.

Results and Discussions

During the experiments, very unexpected data were obtained for the spread of the pathogen. It was found that the sowing date and the soil pH value are not decisive for the establishment of the infection, though the old statement is still valid, i.e. early sowing more frequently shows symptoms of the disease.

After the infection of the germ, hyphae of the pathogen develop together with the plant and the first symptoms appear at the stage of straightening up. They can be observed in the form 1.5–2 mm wide, swollen long bands on the leaves, especially on the internal side of the leaf sheath and on the stalks below the sheath. When after mass propagation of the mycelium, formation of teliospores takes place, the bands are of lead-grey colour, but after the opening of epidermis, black, slightly releasing spores can be found (Fig. 1). Above the flag leaf, the pathogen penetrates the ear from the stalk or delays in development, and causes partial smutting on the glumellae, unfrequently it may deteriorate several spikelets placed on one another (Fig. 3). A typical symptom is (similar to the barley leaf bands) the malformed ears coming out from the sheath, because the husks are tightly retained by the leaf sheath. No fecundation can take place in the ears, thus even no bad seed production is expected (Fig. 2). Infected plants are 40–45% shorter. A dependence of effect upon cause is found between the changes of size and symptoms (Fig. 4).

The mass of spores is black. Shape of the teliospores is rounded and slightly angled, the size ranges between 12 and 18 microns. Their walls are of chestnut colour and smooth. They mostly form spore masses containing 2–3 spores. Size of the mass is 25–40 microns. In this case the spores are flat on the contact surface. The bundle of teliospores is surrounded with various number of hyaline, then yellowish coloured sterile by-cells. They do not tightly surround the teliospores; and their role and the mechanism of formation are not known. Their diameter is 4–6 microns (Fig. 3). For studying the promycelium and sporidia developed from the spore mass, the modified clay-print method, i.e. the use of activated bentonite (clean substance with great swollening capacity, 8 pH) is the best. No difference was found i nthe behaviour of teliospore bundles originating from various sites.

Damage by *U. occulta* is determined by full seed loss and 35–40% loss of green mass, in case of fodder production. The control technology should, therefore, be introduced by all means.

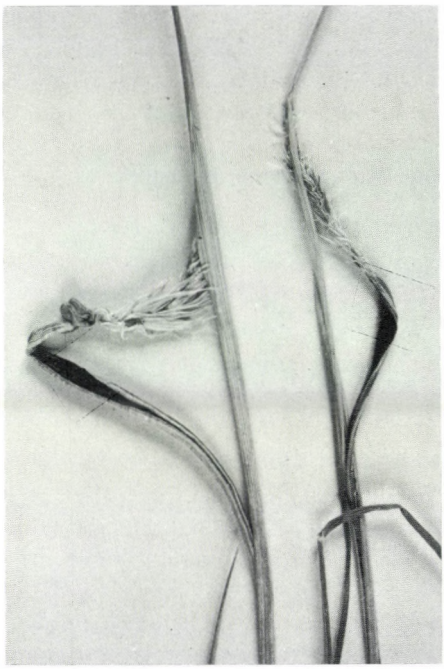

Fig. 1. Typical symptom of *U. occulta*: outcoming ears from the leaf sheath

Fig. 2. Smut(ted) spores appear on the stalk as long bands

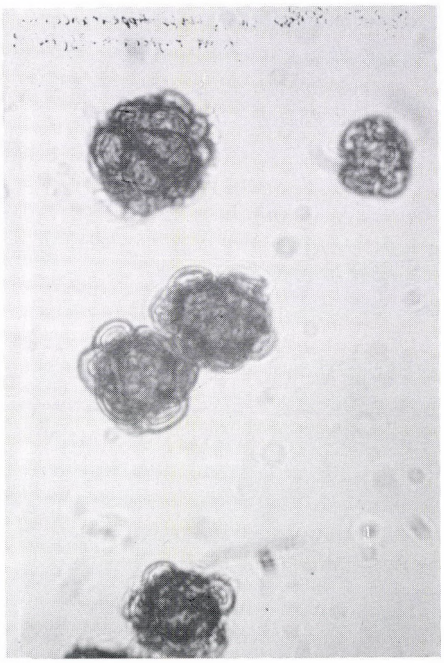

Fig. 3. Microscopic image of the teliospore mass of the pathogen

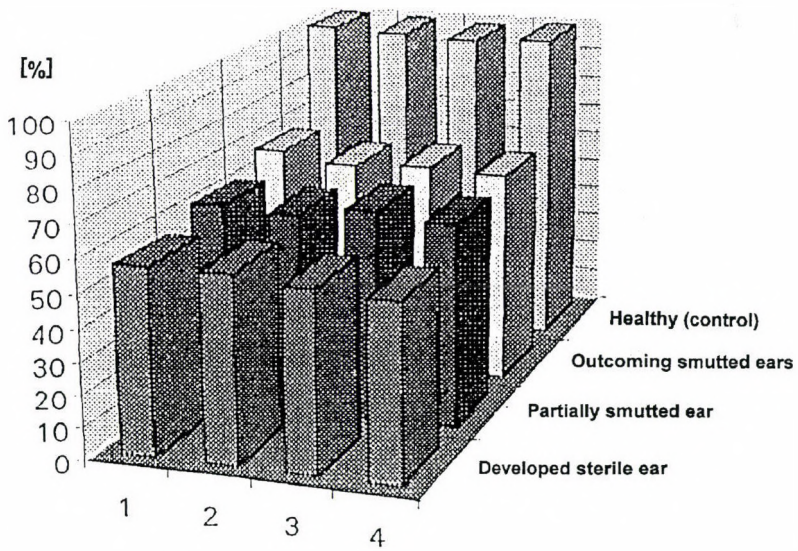

Fig. 4. Effect of *U. occulta* (Wallr.) Rabenhorst on the height of the host-plant

Laboratory test results were checked under field conditions and it was found that the registered seed-dressing agents could be used with good efficiency.

The pathological changes (change of aeration, evaporation, water utilization) can be well demonstrated by means of thermovision. Similarly to the fungi causing rust, the smuts induce also "fever" which can be measured at the beginning of teliospore formation.

By using the camera AGA 782 SW, 0.6–0.8 °C increase has been observed compared to *U. occulta* and *T. foetida*. The complementary tools of the camera allowed visual expression and fixation of the heat radiation by using a special videorecorder. After digitalization of the images, numerical evaluation of the temperature was made by computer and the heat images were recorded by colour printer. It is known that the epidermis (cuticle) breaks on the bands established on the leaf sheath and stalks, the altered transpiration greatly increases the temperature of the diseased plant, therefore on plants showing symptoms of *U. occulta*, the temperature may increase by 0.9–1.2 °C.

When using infected rye plants as green fodder, the 30–40% yield loss is somehow decreased by the fact that the protein content in the infected plant parts is higher. The mycelium of smuts contains 32–35% raw protein, and, among the amino acids, the content of lysin and methyonin raise.

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The Effect of *Macrophomina phaseolina* (Tassi) Goid. and Two Viruses on Pepper (*Capsicum annuum* L.)*

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Investigations were carried out under glasshouse conditions to examine the interactions between three plant pathogens (U/246 strain of cucumber mosaic *Cucumovirus*, NTN strain of potato Y *Potyvirus* and *Macrophomina phaseolina* (Tassi) Goid) and their synergic or antagonist effect on the development of pepper (*Capsicum annuum* L. cv. Keszthelyi Rezisztens). Viruses infected alone did not influence significantly the development of the plants. *M. phaseolina* alone reduced only the fresh weight of both the roots and the shoots, suggesting the disturbance of water uptake of roots due to the infection. In case of several mixed infection of viruses and fungi the injury of the pathogens has increased.

In the 1990s wilting, yellowing symptoms and in severe cases the death of the pepper (*Capsicum annuum* L.) plants occurred in the most pepper growing areas in Hungary mainly under field conditions. The yield losses varied between 10 and 30% depending on years and growing regions (Süle and Viczián, 1995). Besides abiotical and growing technological reasons the different plant pathogens (viruses, phytoplasmas, bacteria, and fungi) are believed to play important role in the forming of complex syndromes (Dula, 1995; Kadlicskó et al., 1995; Csilléry et al., 1996; Kovács et al., 1996; Gáborjányi et al., 1997). In the survey of Edwardson and Christie (1997), 99 viruses have been reported to infect peppers (*Capsicum* spp). *Capsicum* spp. are known as natural hosts of 55 plant viruses, while 44 viruses infect these only under artifical conditions (Horváth, 1981, 1986; Green and Kim, 1991; Green and Kalloo, 1994; Edwardson and Christie, 1997). Among viruses infected pepper plants cucumber mosaic *Cucumovirus* (CMV) was the first to describe in Hungary (Szirmai, 1939, 1941; Petróczi, 1956; Solymosi, 1960) and this virus causes the most severe yield losses even at present (Gáborjányi et al., 1997). Pepper is a natural host of potato Y *Potyvirus* (PVY), too (Horváth, 1967, 1969). The new NTN strain of PVY (PVY^{NTN}) which possesses resistance-breaking characteristic was first described in Hungary by Beczner et al. (1984). This strain belongs to the tobacco veinal necrosis strain group. There is a growing frequency of occurrence of PVY^{NTN} in European countries and is a major problem to potato growers and breeders (Le Romancer and Kerlan, 1991; Weidemann, 1993; Kus, 1995; Milosevič, 1995). The effect of this new strain of PVY on the pepper plants is not known.

* Dedicated to Dr. L. Beczner (1938–1988) on the occasion of his 60th birthday and 10th anniversary of his death.

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The changing of the meteorological conditions (glasshouse effect, dry periods etc.) greatly influenced the host-parasite relations as well. Injury effect of the warm and drought-tolerance polyphagous *Macrophomina phaseolina* Tassi (Goid) has increased not only in Hungary (Békési, 1981; Koppányi et al., 1993; Simay and Kadlicskó, 1993) but abroad (Raut, 1985; Kaiser and Das, 1988; Milam et al., 1990) as well. This pathogen was described also on pepper by Fischl et al. (1995) at the first time in Hungary and it is believed that *M. phaseolina* plays important role in the complex etiology of pepper.

Investigations about the interactions among plant pathogens and their hosts have discussed mainly the effect of different plant pathogens on each other. The effect of the mixed infection on test plant have been rarely taken into consideration. Studies can be devided in two main groups. First group of the studies is about the interactions among plant pathogens e.g. antagonist or synergic effects fungi on fungi (Arsvoll, 1981; Lynch, 1990; Sun and Zeng, 1993); the antagonist effect of different bacteria (mainly different strains of *Bacillus* spp.) against various pathogenic fungi (Red'Kina, 1990; Kelemu and Badel, 1994; Konstantinova et al., 1994; Smirnov et al., 1995; Kozachko et al., 1995). Antagonist effect of bacteria on another ones in reported in work of Jovanovič (1993), Konstantinova et al. (1994) and Kozachko et al. (1995). Antagonist effect of fungi on bacteria is reported by Musroor and Chandra (1989) and Jovanovič (1993). A lot of references are available about mycoviruses (Spire, 1972; Lemke, 1979; Mier, 1979; Ushuyama and Nakai, 1979; Hollings and Woods, 1979; Hollings, 1982; Jamil and Buck, 1984; Ma et al., 1984; Ushiyama, 1985; Liu and Liang, 1988; Khanarasova et al., 1990). Second group of the studies is connected with the plant inhibitors. Plant inhibitors seemed to be effective mainly against viral infection out of the plant pathogens (Okuyama et al., 1978a; Verma and Khan, 1985; Kubo et al., 1990), while extracts of certain plants showed wide antifungal activity as well (Park et al., 1986). Antimicrobial extracts of plants may be natural inhibitors of the healthy plant tissues (Okuyama et al., 1978b, 1978c; Verma et al., 1985; Hansen, 1989). Inhibitors may belong to proteins (Chen et al., 1991), tannins (Zhang et al., 1990) and phenols (Shukla et al., 1989). Plant inhibitors – mainly proteins – may be formed in response to viral, bacterial or fungal infection (Rubin and Ladygina, 1980; Verma and Khan, 1985). Pathogenesis-related proteins (PR) are host proteins induced by various pathogen and stress related factors (Stintzi et al., 1993; Egea et al., 1996). Virus infected plants were investigated for their reaction to challenge-inoculations with fungal pathogen. Extracts from leaves infected with viruses inhibited mycelial growth and conidial production of test fungi (Besada, 1978; Pandey et al., 1989): A little formation is available about the effect of mixed infections on test plants (Nováková, 1977; Banttari, 1988; Khurana et al., 1988).

So far, no data is available about the interactions of the different plant pathogens causing the severe damaging of pepper plants. Therefore the aim of our investigations was to study the interactions between U/246 strain of CMV (CMV-U/246), PVY^{NTN} and *M. phaseolina* on pepper.

Materials and Methods

Seeds of *C. annuum* cv. Keszthelyi Rezisztens were sown in sterilized boxes in the virological glasshouse free of vectors. The seedlings were planted in plastic pots (28 cm in diameter), containing a soil mixture of sand (pH: 6.96, humus%: 0.27): peat (pH: 6.78, humus%: 9.98) 1:3. Pepper plants were inoculated at 6–8 leaf stage with the original Maradona isolate of PVY^{NTN} (Beczner et al., 1984) and CMV-U/246 (Schmidt and Horváth, 1982). Previously the viruses were propagated on *Nicotiana tabacum* L. cv. Xanthi-nc. The pepper plants were inoculated mechanically with tissue sap of *N. tabacum* cv. Xanthi-nc. Sörensen phosphate buffer (pH: 7.2) in the ratio 1:1 was used. To chek the success of infection, back inoculation was also carried out to *N. tabacum* cv. Xanthi-nc and *Nicotiana glutinosa* L., as indicator plants. In order to make inoculation with fungus, pure culture of *M. phaseolina* was produced on potato-dextroze agar (PDA) culture medium. The suspension of ten-days-old microsclerotia culture (3×10^4 microsclerotia/cm^3 suspension) was poured on the surface of plastic pots at 12–14 leaf stage of pepper plants. Twenty ml suspension was applied for each pot and then the pots were covered with 1 cm thick layer of soil mixture. The pots were watered thoroughly and surface irrigation was used in future, too. In order to study the interactions among the pathogens, the following treatments were applied: 1. Control, 2. CMV-U/246, 3. PVY^{NTN}, 4. *M. phaseolina*, 5. CMV-U/246+PVY^{NTN}, 6. CMV-U/246+*M. phaseolina*, 7. PVY^{NTN}+*M. phaseolina*, 8. CMV-U/246+PVY^{NTN}+*M. phaseolina*. There were four plants in a pot and four replicates of each treatments. Tests continued for 90 days, when the fresh and dry weight both of the shoots and roots of pepper plants was measured.

Results and Conclusions

Pepper plants inoculated with the viruses showed systemic mosaic symptoms and considerable leaf deformation. Local and systemic necrotic patterns could be seen, too. Considerable wilting, yellowing and leaf dropping was observed, due to the inoculation with *M. phaseolina*. Fischl et al. (1995) have earlier reported that *C. annuum* cv. Keszthelyi Rezisztens is one of the most susceptible pepper cultivar to *M. phaseolina*.

The height of the plants was not influenced significantly by the different treatments. It means that the height of the plant was unsuitable index number to express the differences between the treatments (Table 1). Neither CMV-U/246 nor PVY^{NTN} alone influenced significantly the fresh weight of the shoots. Synergic effect could be observed, when the plants were simultaneously infected with CMV-U/246 and PVY^{NTN}. The fresh weight of the shoots was reduced by 14.1% as compared to control. This fact pays attention to the importance of the complex virus infections. Infection with *M. phaseolina* reduced the fresh weight of pepper shoots by 13.5%, as compared to healthy plants, while did not influence the dry weight significantly. It is concluded that the fungus alone caused confusions in the water relation of pepper plants. PVY^{NTN} and *M. phaseolina* infection together caused similar reduction in fresh weight of the shoots, than *M. phaseo-*

Table 1

The effect of different pathogens on pepper

Treatments*	Height (cm)	Fresh Weight (g/plant)		Dry Weight (g/plant)	
		Shoot	Root	Shoot	Root
Control	40.4	16.3	10.0	3.7	3.3
CMV-U/246	43.0	17.9	11.3	4.2	4.4
PVY^{NTN}	39.8	16.8	8.8	3.6	3.4
M. phaseolina	40.5	14.1	7.4	3.2	3.3
CMV-U/246 + PVY^{NTN}	40.0	14.0	10.5	3.3	3.8
CMV-U/246 + *M. phaseolina*	41.0	12.6	7.3	2.7	2.9
PVY^{NTN} + *M. phaseolina*	43.6	14.3	8.5	3.3	2.7
CMV-U/246 + PVY^{NTN} + *M. phaseolina*	40.1	12.4	7.2	2.6	2.5
LSD(P = 0.05)	4.83	1.78	1.57	0.51	0.56

* CMV-U/246, U/246 strain of cucumber mosaic *Cucumovirus*; PVY^{NTN}, NTN strain of potato Y *Potyvirus*

lina infection. CMV-U/246 and *M. phaseolina* showed synergic effect in this respect and reduced the fresh weight of shoots to a greater extent, than *M. phaseolina* infection alone. CMV-U/246, PVY^{NTN} and *M. phaseolina* infection together has resulted the most severe (24 and 12%) reduction in the fresh weight of the pepper shoots, as compared to the healthy plants and plants infected with *M. phaseolina*, respectively. Neither CMV-U/246 nor PVY^{NTN} and *M. phaseolina* alone influenced significantly the dry weight of the shoots of pepper plants. Infection of CMV-U/246 and *M. phaseolina* together and that of three pathogens (CMV-U/246, PVY^{NTN} and *M. phaseolina* together) retarded the dry weight of pepper shoots by 27 and 29.7%, respectively, as compared to the healthy plants (see Table 1).

The fresh weight of the roots was not influenced significantly by CMV-U/246 and PVY^{NTN}. *M. phaseolina* alone, CMV-U/246 and *M. phaseolina* together, and the complex infection of CMV-U/246, PVY^{NTN} and *M. phaseolina* reduced the fresh weight of pepper roots to similar extent (26–28%). It is concluded that the reduction in fresh weight of pepper root is a consequence rather of *M. phaseolina* than the virus infection. Significant difference was observed in this respect between infection of PVY^{NTN} and PVY^{NTN} with CMV-U/246 together. PVY^{NTN} alone reduced the fresh weight of the roots of pepper. When infection of PVY^{NTN} occurred together with CMV-U/246 infection the fresh weight of pepper roots significantly enhanced, as compared to that of the pepper plant infected only with PVY^{NTN}, but the fresh weight of the roots was not significantly greater, than the fresh weight of the roots of the control plants (see Table 1). CMV-U/246 has eliminated the inhibitory effect of PVY^{NTN} on the fresh weight of pepper roots (antagonist effect).

Neither CMV-U/246, nor PVY^{NTN} and *M. phaseolina* alone reduced the dry weight of the pepper roots, as compared to the healthy control plants. *M. phaseolina* and PVY^{NTN} together and infection of *M. phaseolina*, CMV-U/246 and PVY^{NTN} together reduced the dry weight of pepper roots by 18 and 24%, respectively. It is interesting that dry weight of pepper roots significantly enhanced due to CMV-U/246 infection (see Table 1). CMV-U/246 is said to be one of the most important viruses of pepper under field conditions, which causes considerable yield losses. Although the most pepper cultivars are resistant to tobacco mosaic *Tobamovirus* (TMV), we have no cultivars resistance to CMV-U/246 so far. Although on the basis of symptoms and back inoculation in suscebtibility of "Keszthelyi Rezisztens" cultivar to CMV-U/246 can be proved, the virus did not inhibited the development of the diseased pepper plants. Our results show that this cultivar is tolerant to CMV. At the same time our results pay attention to the importance of the complex infection. In some cases pathogens, each alone did not retarded the development of pepper plants, but infected the pepper together with other pathogens, considerably inhibited the development of the plants. Viral infection may result in an increase of the exudation of nutrients from roots of infected hosts which may enhance the potentiality of root fungal pathogens of those hosts (Beute and Lockwood, 1968). Nevertheless future investigations are necessary to study the synergic or antagonist interactions of different species and strains of plant pathogens (viruses, phytoplasmas, bacteria fungi) and their injurious effect on the development of the cultivated plants.

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Influence of *Pratylenchus crenatus* Loof, *Fusarium graminearum* Schw. and *Rhizoctonia solani* Kühn. on the Content of Macro and Micro Elements in Maize

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Direct influence of *Pratylenchus crenatus* Loof, *Gibberella zeae* (Schw.) Petch. = *Fusarium graminearum* Schwabe and *Thanatheporus cucumeris* (Frank.) Donk. = *Rhizoctonia solani* Kühn on the content of nitrogen, ammonia and nitrate nitrogen and ammonia and nitrate ions in maize was determined while the influence on the remaining macro and micro elements was not established.

In Slovenia there are two major species of fungi of the genus *Fusarium* causing seed mould and wilting of young maize and, later, root and stem rot and ear rot: *Fusarium moniliforme* Sheld. var. *subglutinans* Wr. Et Reink., telemorph *Gibberella fujikuroi* (Saw.) Wr. var. *subglutinans* and *F. graminearum* Schw., with *Gibberella zeae* (Schw.) Petch. (Milevoj, 1981). All this corresponds with findings of other authors (Smiljaković, 1972). Among the soil fungi the literature (Summer and Minton, 1989) also mentions the species *Rhizoctonia solani* Kuhn. telemorph *Thanatephorus cucumeris* (Frank.) Donk. as causing maize disease that has not been isolated from this plant in Slovenia yet. It is important to know the interactions between fungi and other organisms (e.g., nematodes) in order to get an insight into the less known spreading possibilites of the above-mentioned agents in maize.

Numerous authors mention various *Pratylenchus* species in connection with various fungi and bacterial plant diseases. Egunjobi, Norton and Martinson (1986) in their experiments found interactive influence between *P. scribneri* and *Helminthosporium pedicullatum* in the development of root rot in maize. Riedel and Rowe (1985) reported on synergistic activity of *P. penetrans* with *Verticillium dahliae* at the early potato decay. They proved that the incidence of the above-mentioned disease was more intensive if both pathogens were present and less intensive if the plant was attacked by just one of the pathogens studied. Later they found out that together with the previously mentioned fungus *P. scribneri* had a poor synergistic effect and that *P. crenatus* did not have it at all. Vrain and Copeman (1987) found a significant growth of tumorous swellings caused by bacterium *Agrobacterium tumefaciens* on two raspberry cultivars at the presence of increasing starting population of *P. penetrans*. They also found out the decrease of reproduction of *P. penetrans* in the root system infected by the above-mentioned bacterium. Szczygel and Profic-Alwasik (1986) reported, on the basis of his own results, that *P. penetrans* and *Cylindrocarpon destructans* did not influence the growth of strawberries individually, however, the weight of plants was significanly lower if both pathogens were present at the same time.

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Due to the fact that the incidence of *Pratylenchus crenatus* Loof, 1960, in the field soil of Slovenia was rather frequent it was decided to study the relations between the above-mentioned nematode species and the two fungi, *Fusarium graminearum* Schw. and *Rhizoctonia solani* Kühn, with regard to maize.

Material and Methods

Retrospective inquiry was made in the field of mutual effect of certain pathogens, *Pratylenchus* spp. inclusively, on maize using four international data bases (CAB, BIOSIS, AGRIS, AGRICOLA) from 1990 on. A system of chosen hits was made on the basis of which a desired information on the existing lierature on the above-mentioned topic was obtained.

The experiment was designed in four repetitions and the treatments represented, beside the control, all possible combinations of interactive influence of the three parasites (*P. crenatus, F. graminearum* and *R. solani*) on maize (see Tables 1, 2). Field soil from the surroundings of Ljubljana, sifted and sterilized in autoclave for 3 hours at 121 °C was used as basic substrate. The required quantity of cooled soil was measured and a sterilized very fine cold white sand (the ratio soil : fine sand = 2 : 1) was added. The substrate was homogenized.

Chemical analysis of soil:

pH in KCl	7.3
P2O5 (available) = (mg/100 g)	20.3
K2O (available) = (mg/100 g)	17.3
humus (%)	1.25

In the basis substrate 2.5–2.8 g of sterilized culture medium (coarse ground maize) which contained fungus relevant to particular procedure (*F. graminearum, R. solani*) or the culture medium without fungi (procedures 1 and 2) were added. The above-mentioned mixture was well homogenized and filled in 6 cm high peat pots of 6.5 cm diameter in which the population *P. crenatus* was inoculated (in the procedures 2, 5, 7 and 8). Four maize grains, cv. BF-Lj.-, FAO 200 (1994), washed before sowing under current water for 1 h, were sown in single pots. These pots were then put in the homogeneous basic substrate in PVC containers of 16 cm diameter and height. The experiment began on 06. June 1995 and was conducted in shaded greenhouse. During the experiment the environmental temperature was monitored and moisture was added as necessary.

Fungus culture medium

Test isolate *F. graminearum* Schw. was cultured by isolating the agent from naturally infected wheat ears. The origin of fungus was Jable (Ljubljana) and the wheat cultivars was 'Marija'. The isolation was carried out in the year 1994 according to standard method on PDA culture medium and the fungus was determined following the Booth

Table 1

Influence of pathogenic organisms on dry matter and moisture content in maize

Procedure/Nematode or Fungus	Parameter				
	Root weight (g)	Green forage weight (g)	Dry matter (g/kg)	Moisture (g/kg)	Number of *P. crenatus*/g roots
1. Control	25.8	73.5	189.5	810.5	0
2. *P. crenatus*	25.1	84.8	207.7	729.3	7.3
3. *F. graminearum*	22.8	81.8	207.5	792.3	0
4. *R. solani*	23.8	80.5	216.1	783.9	0
5. *P. crenatus* + *F. graminearum*	23.4	79.5	178.6	819.8	57.7
6. *R. solani* + *F. graminearum*	24.4	78.1	220.8	779.2	0
7. *P. crenatus* + *R. solani*	21.7	83.6	261.9	738.2	37
8. *P. crenatus* + *F. graminearum* + *R. solani*	20.9	91.7	314.4	685.6	11.3

Table 2

Influence of parasitic organisms on the content of macro elements in maize (g/kg/dry matter) ($P \leq 0.05$)

Procedure	Parameters				
	Nitrogen	Calcium	Phosphorus	Magnesium	Potassium
1. Control	10.9	10.9	0.6	5.1	7.7
2. *P. crenatus*	9.6	9.8	0.6	4.7	7.6
3. *F. graminearum*	9.6	10.7	0.6	5.2	8.8
4. *R. solani*	9.3	9.6	0.6	4.3	9.3
5. *P. crenatus* + *F. graminearum*	8.5	9.9	0.6	4.8	8.5
6. *R. solani* + *F. graminearum*	8.9	10.2	0.6	5.0	7.0
7. *P. crenatus* + *R. solani*	9.8	10.3	0.6	5.2	7.2
8. *P. crenatus* + *F. graminearum* + *R. solani*	8.6	8.8	0.5	4.3	8.8

(1971) key and on the basis of comparison with isolate coming from the mycotheca stored at the Institute of Phytomedicine, Agronomy Department, BF (Ljubljana). Until further investigation the fungus was stored on solid PDA culture medium at 5 °C. The fungus *R. solani* Kühn. isolate was obtained from infected cucumbers grown on the location of BF Laboratory Field (Ljubljana). It was submitted to the same treatment as described for the first isolate.

Fungi were grown on a coarse ground maize (Celar, 1992) which was sifted through millimeter sieve and weighed 34 g each in 100 ml Erlenmayer bottle and 34 ml each of distilled water was added. Erlenmayer bottles were covered with aluminium foil

and the content was autoclaved for 20 min. at 121 °C. After one day each fungus was inoculated in three repetitions on sterile culture medium and they were incubated for 10 days at 25 °C so that course ground maize was well overgrown by mycelium.

Inoculation with Pratylenchus crenatus

Soil in which a larger number of *P. crenatus* was found was thoroughly mixed, homogenized and larger skeletal particles were removed in laboratory. 200 cm^3 of soil was placed on 1 mm mesh sieve and washed in a plastic bucket with strong jet of water. This suspension was filled up with clear water to the volume of 8 l, whirled thoroughly, left still for 20 s and slowly poured on four vertically placed, successive, 50 μm sieves with 20 cm peripheral diameter. The sediment which remained on sieves was washed with a weak jet of water in plastic round vessels of 25 cm diameter and 10 cm height and, by means of whirling motion (Hržič, 1973) a pure suspension of nematodes was obtained.

Nematodes collected in a tiny water drop in 12 cm high conic test tube were added 5 cm^3 sterile distilled water and the suspension was thoroughly shaken. After 30 min. nematodes were deposited on the bottom of test tube and the superfluous water was pumped using vacuum pump. The procedure which was carried out in sterile atmosphere (dustless chamber) was repeated five times.

Nematodes which were washed and collected in 5 cm^3 sterile distilled water were poured in the middle of each peat pot filled with basic substrate 2 cm deep. Thus about 840 ± 40 nematodes *P. crenatus* were inoculated in each peat pot.

Method plant sample review

The experiment was evaluated 2 months after the start. The plants were cut and weighed. Then the roots were set apart. The washed roots were inspected visually in each treatment and repetition separately and their health was evaluated. Lengths of necroses in centimeters were measured and counted on all roots in each repetition. Necrotized parts were cut out of roots, each sample and repetition were weighed separately and agents were isolated and reisolated from them.

Analyses of the content of nitrogen substances and micro and macro elements in maize

The content of nitrogen substances was determined from fresh plant material using UV spectrometric methods (Bran+Luebbe Autoanalyzer II). The content of micro and macro elements from plant material was determined by atomic absorption spectrometry (Perkin–Elmer, AAS 2380) exept for phosphorus (UV spectrometric methods). The samples were previously ashed and the ash dissolved in 10% hydrochloric acid (HCl).

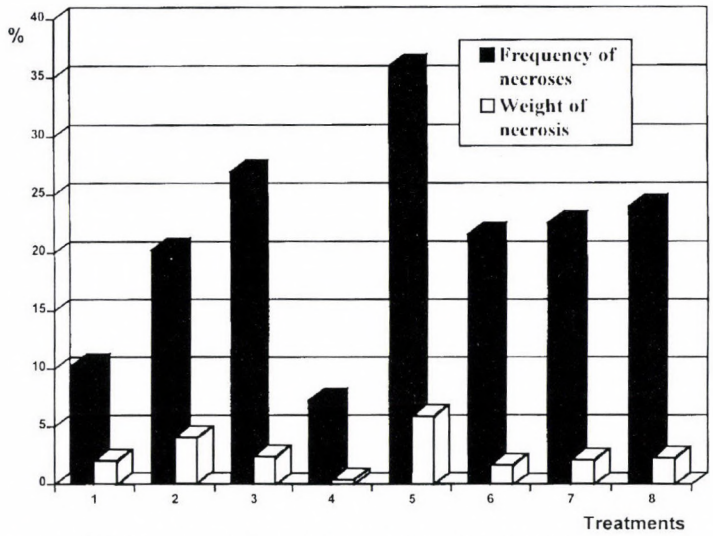

Fig. 1. The frequency of occurrence and the weight proportion of necroses on maize roots at different treatments (see Table 1)

Results

Figure 1 shows the frequency of necroses on maize roots by which the diseased roots were defined. Treatment 5 which includes nematodes shows a great number of necroses than treatment 3 which includes only *F. graminearum*, showing a greater number of lesion spots, probably due to damage of tissue by nematodes, through which the fungus penetrated into the roots. Similar goes to the infection with the fungus *R. solani* which is itself weaker if compared to the traetment 7 which includes nematodes *P. crenatus*. This is also true for the combination of all three organisms, both fungi, *R. solani, F. graminearum,* and nematodes, *P. crenatus*, where more numerous necroses were found in comparison with the treatment 6 which included only the two fungi and no nematodes.

Analysis of variance was used to find out that the discussed parasitic organisms influenced dry matter and moisture content in maize plants statistically significantly ($P \leq 0.05$). Dry matter content in plants which were attacked with all three abovementioned parasites was increasing and moisture content was cereasing in comparison with the control and other procedures (Table 1).

Based on the review of influence of particular parasitic organisms and their interactive influence on the content of macro elements it was found out that the differences between treatments were statistically significant only in the case of nitrogen ($F = 3.50$). The highest amount of nitrogen (10.9 g/kg/dry matter) was recorded in maize which was not infected by any of the studied parasitic organisms – control procedure, and its lowest amount in maize which was attacked by all three parasitic organisms applied in the ex-

Table 3

Influence of the nematode *P. crenatus* and pathogenic fungi on the content of ammonia nitrogen in maize (mg/kg/dry matter)

Procedure	Mean	1	2	3	4	5	6	7	8
1. Control	218.4			*	*	*	*	*	*
2. *P. crenatus*	179.7				*	*	*	*	*
3. *F. graminearum*	137.9								*
4. *R. solani*	132.6								*
5. *P. crenatus* + *F. graminearum*	129.8								*
6. *R. solani* + *F. graminearum*	105.9								
7. *P. crenatus* + *R. solani*	95.8								
8. *P. crenatus* + *F. graminearum* + *R. solani*	75.1								

(*) Denotes pairs of groups significantly differenc at $P \leq 0.05$

Table 4

Influence of the nematode *P. crenatus* and pathogenic fungi on the content of nitrate nitrogen in maize (mg/kg/dry matter)

Procedure	Mean	1	2	3	4	5	6	7	8
1. Control	457.7		*	*	*	*	*	*	*
2. *P. crenatus*	238.4								
3. *F. graminearum*	151.5								
4. *R. solani*	150.1								
5. *P. crenatus* + *F. graminearum*	45.4								
6. *R. solani* + *F. graminearum*	93.6								
7. *P. crenatus* + *R. solani*	69.5								
8. *P. crenatus* + *F. graminearum* + *R. solani*	39.5								

(*) Denotes pairs of groups significantly different at $P \leq 0.05$

periment and in maize attacked with *P. crenatus* and *F. graminearum*. Nitrogen content was decreased by particular parasites in comparison with the control but the difference was not statistically significant. Combinations of two parasites caused the reduction of nitrogen in maize if compared with maize attacked with only one parasite, however, no statistically significant difference was found in these cases, with the exception of the combination *Pratylenchus crenatus* + *Fusarium graminearum*. Other macro elements that were studied did not present any statistically significant influence of parasitic organisms

Table 5

Influence of the nematode *P. crenatus* and pathogenic fungi on the content of ammonia ions in maize (mg/kg/dry matter)

Procedure	Mean	1	2	3	4	5	6	7	8
1. Control	280.8			*	*	*	*	*	*
2. *P. crenatus*	230.9					*	*	*	*
3. *F. graminearum*	177.3								*
4. *R. solani*	169.8								*
5. *P. crenatus* + *F. graminearum*	166.8								*
6. *R. solani* + *F. graminearum*	136								
7. *P. crenatus* + *R. solani*	123.4								
8. *P. crenatus* + *F. graminearum* + *R. solani*	96.5								

(*) Denotes pairs of groups significantly different at $P \leq 0.05$

on their content in maize either, thought it could be affirmed that there was a tendency of calcium decrease in maize if all three parasitic organisms were present (87% reliability) (Table 2).

The presence of particular parasitic organisms and their interactive effect influenced the content of ammonium ($F = 8.46$) and nitrate nitrogen ($F = 4.0$) and the content of ammonium ($F = 8.46$) and nitrate ions ($F = 4.0$) in maize statistically significantly ($P \leq 0.05$).

Certain parasites (*P. crenatus, F. graminearum* and *R. solani*) cause the decrease of the above-mentioned substances in maize. This decrease is intensified at combinations of two pathogens and it is the most pronounced in cases in which maize is attacked with *P. crenatus, F. graminearum* and *R. solani* at the same time.

The content of ammonia nitrogen (LDS = 19.47) was the highest in control procedure in which maize was not attacked by any of the discussed harmful organisms (218.4 mg/kg/dry matter) and it declined when maize was attacked by at least one of the discussed parasites (*P. crenatus, F. graminearum, R. solani*). the decline in the content of ammonia nitrogen was intensified in cases in which maize was attacked by two parasites. The content of ammonia nitrogen in maize was the lowest (75.1 mg/kg/dry matter) when plants were attacked by all the discussed harmful organisms at the same time (Table 3).

The quantity of nitrate nitrogen (LSD = 85.98) was, similar to ammonia nitrogen, the highest in the control procedure (457.7 mg/kg/dry matter) and declined when one of the discussed parasites was present. The decline was intensified at the presence of two and was the highest at the presence of all three pathogenic organisms discussed (39.5 mg/kg/dry matter) (Table 4).

Table 6

Influence of the nematode *P. crenatus* and pathogenic fungi on the content of nitrate ions in maize (mg/kg/dry matter)

Procedure	Mean	1	2	3	4	5	6	7	8
1. Control	2027.1		*	*	*	*	*	*	*
2. *P. crenatus*	1055.4								
3. *F. graminearum*	671.4								
4. *R. solani*	664.2								
5. *P. crenatus* + F. *graminearum*	200.9								
6. *R. solani* + *F. graminearum*	415.4								
7. *P. crenatus* + *R. solani*	307.6								
8. *P. crenatus* + *F. graminearum* + *R. solani*	175.0								

(*) Denotes pairs of groups significantly differenc at $P \leq 0.05$

Also, the content of ammonia ions (LSD = 25.03) in control maize with 280.8 mg/kg/dry matter declined to 96.5 mg/kg/dry matter in maize plants attacked with all three harmful organisms discussed (Table 5).

Similarly, the content of nitrate ions (LSD = 376.51) in maize declined from 2027.1 mg/kg/dry matter in the control procedure at the attack of one, two or all three parasitic organisms. The content of nitrate ions in maize at the presence of nematode and both pathogenic fungi was 175 mg/kg/dry matter (Table 6).

The studied species of phytophagous organisms (*P. crenatus, F. graminearum* and *R. solani*) did not affect the content of the studied micro elements in maize either by themselves or in combinations.

Discussion

Plant parasitic nematodes cause, together with fungi (*Cylindrocladium crotalarie, Fusarium* spp., *Rhizoctonia solani, Verticillium* spp. and others), root diseases but they are also known as co-agents of certain diseases of the above grounds parts of plants (Nicholson et al., 1985).

In the past numerous studies of interactions between *Meloidogyne* spp. and other pathogenic organisms were made as well as studies which dealt with plant diseases as the result of interaction between fungi and other phytoparasitic nematodes (*Belonolaimus* spp., *Pratylenchus* spp., etc.). Revelo Moran (1993) et al. reported on synergistic effect of *P. pratensis* and *Fusarium moniliforme* var. *subglutinans* on the root and stem rot of maize. It was found out that *P. pratensis* influenced the effect of the fungus; in the field trials it caused its augmentation by 12–52% and in greenhouse by 40–100%. Both patho-

gens had a negative influence on the weight of yield of maize grains and on dry weight of roots.

Roth and Boothroyd (1977) reported about large populations of *Pratylenchus penetrans* and *P. crenatus* causing visual damage of root system of maize and about *Fusarium* spp. which did not cause root rot in the presence of the above-mentioned nematodes.

Smiljaković et al. (1975) discussed the interaction between nematodes (*P. thornei, P. crenatus, P. neglectus, P. penetrans, Ditylenchus dipsaci* and *Meloidogyne* spp.) and *Fusarium graminearum* on maize and the incidence of root rot.

Interactive relations between soil fungi and endoparasitic nematodes (*Pratylenchus* sp.) were studied also by Chambers (1987) who found out that the relation between *Pratylenchus* sp., soil fungi and root rot of maize was statistically insignificant.

As a difference from the study of interactive relations between phytophagous organisms and host plants by the previously mentioned authors we have decided to throw light upon the influence of parasitic organisms on plants from the point of view of some physiological changes. We have come to a conclusion that nematodes and pathogenic fungi (*P. crenatus, F. graminearum* and *R. solani*) exerted a different influence on intensity of maize infection and the growth of dry matter and decrease of moisture in plants. At the same time the influence of the above-mentioned parasies on nitrogen content in maize was established of which the highest amount (10.9 g/kg/dry matter) was found in plants which were not attacked by any of the discussed parasitic species, and the lowest amount in plants which were attacked with all three previously mentioned organisms at the same time. The presence of *P. crenatus, F. graminearum* and *R. solani* and their interaction influenced statistically significantly the content of ammonium and nitrate nitrogen and ammonium and nitrate ions in maize plants. The reduction of such nitrogen substances in plants was noticed when particular parasites were present and it was intensified if another one or two parasites were added (cumulative reduction of content of the discussed elements). The influence on the content of other micro and macro elements (with the exception of calcium and magnesium at the presence of all three discussed parasites) was not very pronounced.

Generally, it can be concluded that the isolate *F. graminearum* as the inducer of necroses was more aggressive than the isolate *R. solani,* and that *P. crenatus* stimulated the infection of root part of maize with both fungi and in combination of both. At the content of nitrogen substances the conclusions were similar with the exception of the isolate *R. solani* which was more agressive.

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A Medium for Selective Isolation and Identification of *Fusarium* spp. from Cereal Grains and Maize Kernels

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Isolation and identification of *Fusarium* spp. from cereal grains and maize kernels was studied with the help of a selective medium (Togawa, 1994). Identification and counting of colonies of *F. graminearum*, *F. moniliforme* and *F. subglutinans* was very easy on this medium. Only a few fungal contaminants develop into very small colonies on Togawa agar, whereas colonies of above-mentioned Fusaria grow rapidly. According to our results, this medium was suitable for selective isolation and identification of *F. graminearum* from cereal grains and *F. moniliforme* and *F. subglutinans* from maize kernels. *F. graminearum* and *F. moniliforme* often occurred in same maize kernel. Appearance of the colonies of *F. graminearum*, *F. moniliforme* and *F. subglutinans* is caracteristic due to production of both bright red pigment, pinkish-white dense aerial hyphae, and white powdery colonies, and pale brown skin-like colonies, respectively.

The genus *Fusarium* includes numerous mycotoxin producing species which are commonly found in cereal grains and maize kernels (Marasas et al., 1984). Most *Fusarium* species are plant pathogens occur worldwide on crop plants causing seedling blight, root, stalk, scab, ear, seed and kernel rot, affecting seed quality, and entailing yield losses (Kommedahl et al., 1981; Charmley et al., 1994). Some are able to persist in harvested and stored grain, and grow in storage if moisture contents become favourable (Chelkowski, 1991). In addition to their involvement in crop plant diseases many strains of these fungi produce several mycotoxins which are severe health hazards for animals and humans if strongly infected crop plants are consumed. Two species on considerable interst are *F. graminearum* Schwabe and *F. moniliforme* Sheldon. *F. graminearum* can invade both maize and wheat in the field, causing maize ear rot and wheat scab (Miller and Trenholm, 1994). *F. graminearum* produces trichothecenes (DON, 15-ADON, 3-ADON, 3,15-DADON, NIV, 4-ANIV and 4,15-DANIV) and zearalenone (ZEA) (Seo et al., 1996; Yoshizawa, 1997). *F. moniliforme* invades maize, sorghum and rice. It is seed and soil borne, and very common in maize plants, invading kernels in the field and persisting into storage (Nelson, 1992). *F. moniliforme* produces a number of mycotoxins including moniliformin (Chelkowski et al., 1990), beauvericin (Moretti et al., 1995), and fumonisins (Nelson et al., 1993).

To facilitate the screening of cereals and maize for potentially mycotoxigenic Fusaria, the use of selective media is desirable. Much research effort has gone into the development and standardization of culture media for the isolation, enumeration and identification of *Fusarium* spp. from soil, plants and cereal grains (Nash and Snyder, 1962; Papavizas, 1967; Sharma and Shingh, 1973; Komada, 1975; Hall, 1981; Elad and

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Chet, 1983; Andrews and Pitt, 1986; Van Wyk et al., 1987; Abildgren et al., 1987; Togawa, 1994; Thrane, 1996).

The development of agar media for the selective isolation of specific Fusaria should be approached by one or a combination of the following three methods as mentioned by Tsao (1970), i.e., selective inhibition, selective enhancement and selective differentiation. The only media in which selective enhancement and selective differentiation through pigmentation were employed were those of Komada (1975) and Togawa (1994). Togawa developed a selective medium for the isolation of *F. graminearum* from cereal grains that was based on Komada's medium. Togawa is not used his medium for isolation of Fusaria from maize kernels.

The present study was carried out to use of Togawa-medium for isolation and selective identification of *Fusarium* spp. from cereal grains and maize kernels.

Materials and Methods

Selective medium

The selective medium consisted of the following ingredients/liter of distilled water:

K_2HPO_4	1.0 g
KCl	0.5 g
$MgSO_4.7H_2O$	0.5 g
Fe-EDTA	10.0 mg
D-(+)-xylose	20.0 g
L-glutamic acid monosodium salt	2.0 g
Oxgall	0.5 g
$Na_2B_4O_7 \cdot 10\ H_2O$	1.0 g
Streptomycin sulphate	250.0 mg
Triazine (50%-wp, Nikon Soda Co. LTD, Japan)	1.0 g
PCNB (75%-wp, Terrachlor, USA)	200.0 mg
Agar	15.0 g

pH was adjusted to 10.0–10.5 with NaOH.

Stir and heat medium to dissolve completely. Sterilise at 121 °C for 15 min and cool to 55 °C, then add filter sterilized streptomycin solution. Shake the medium vigorously when pouring into Petri dishes.

Cereal grain and maize kernel samples

Samples of barly grain (six cultivars), maize kernel (from two geographical localities), and wheat grain (two cultivars) originated from different parts of Hungary were used for this study.

Fig. 1. Appearance of colonies of *F. graminearum* – outgrow from wheat grains – on Togawa-medium. View from above

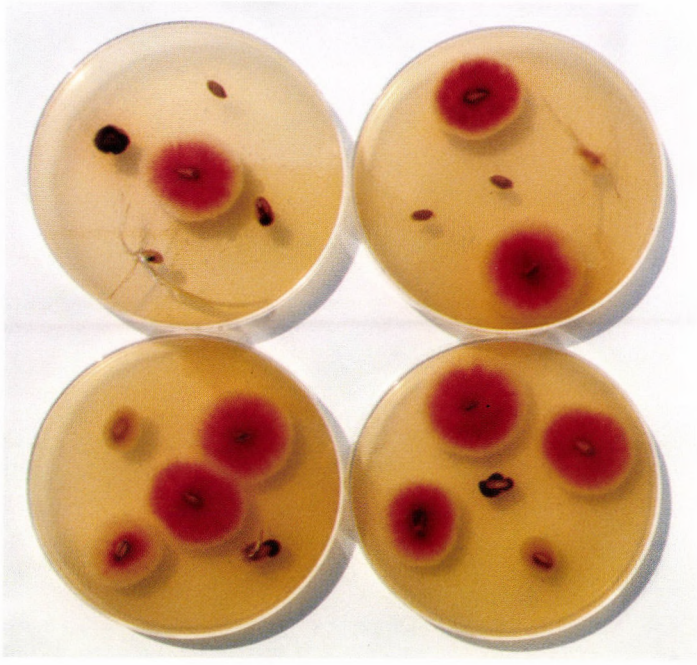

Fig. 2. Appearance of colonies of *F. graminearum* – outgrow from wheat grains – on Togawa-medium. View from below

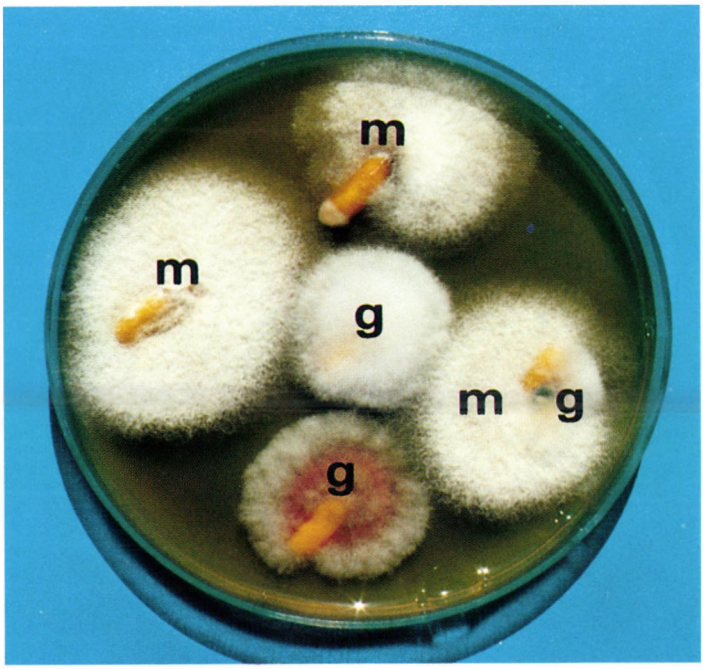

Fig. 3. Appearance of colonies of *F. graminearum* (g) and *F. moniliforme* (m) – outgrow from maize kernels – on Togawa-medium. View from above

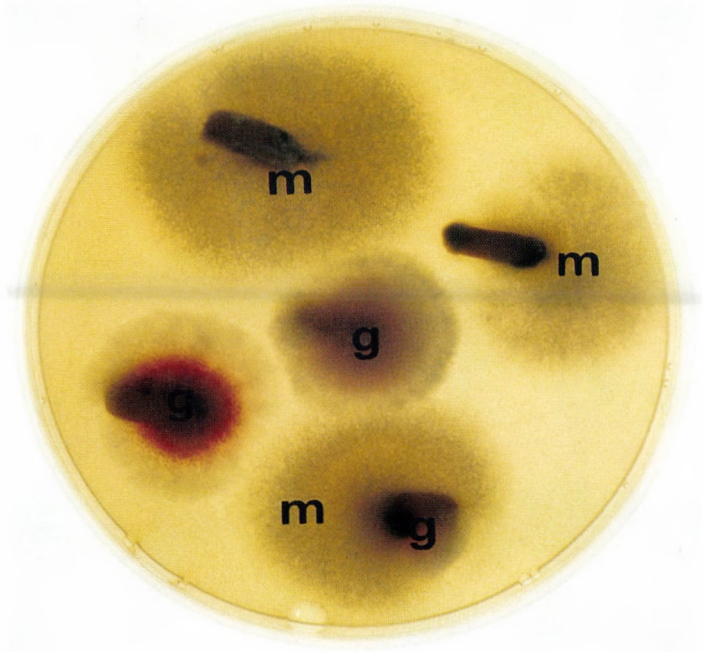

Fig. 4. Appearance of colonies of *F. graminearum* (g) and *F. moniliforme* (m) – outgrow from maize kernels – on Togawa-medium. View from below

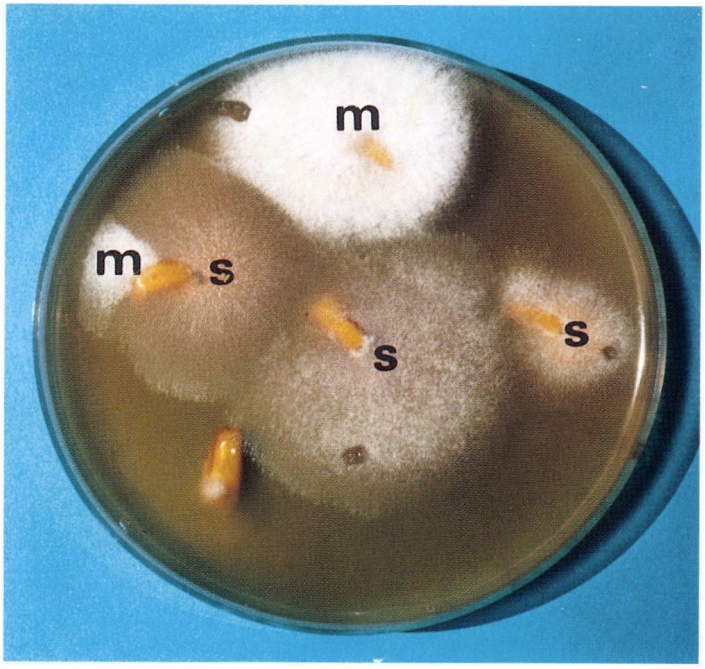

Fig. 5. Appearance of colonies of *F. moniliforme* (m) and *F. subglutinans* (s) – outgrow from maize kernels – on Togawa-medium

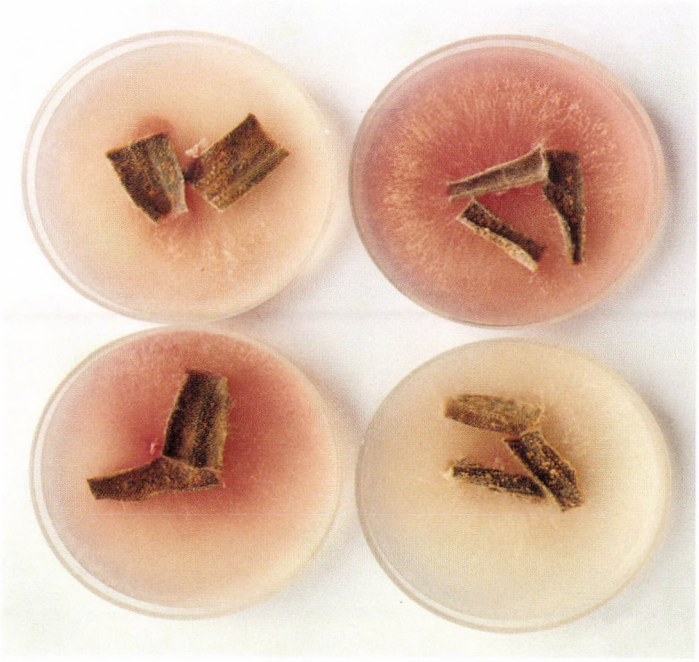

Fig. 6. Single-spore isolates of *F. graminearum* on carnotion-leaf agar

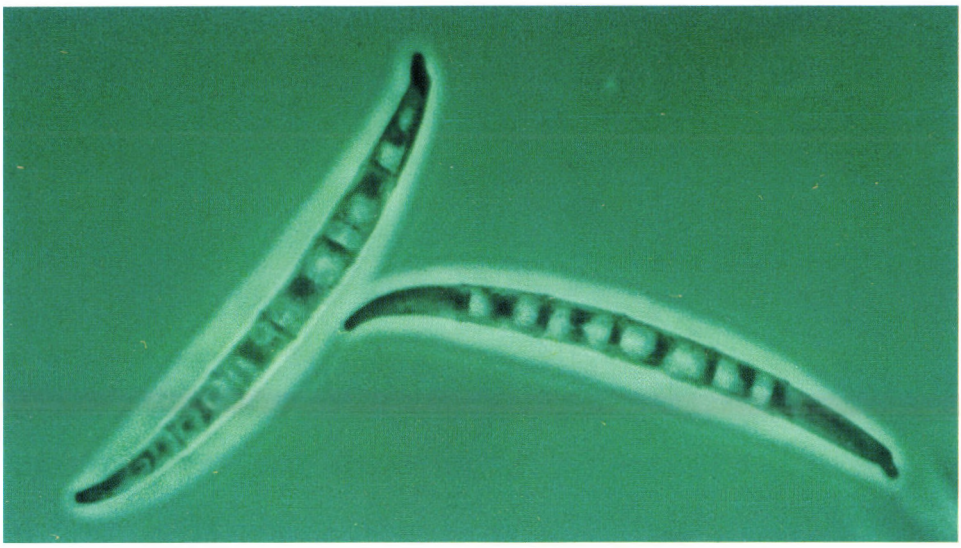

Fig. 7. Macroconidia of *F. graminearum* produced in sporodochia on carnation-leaf agar

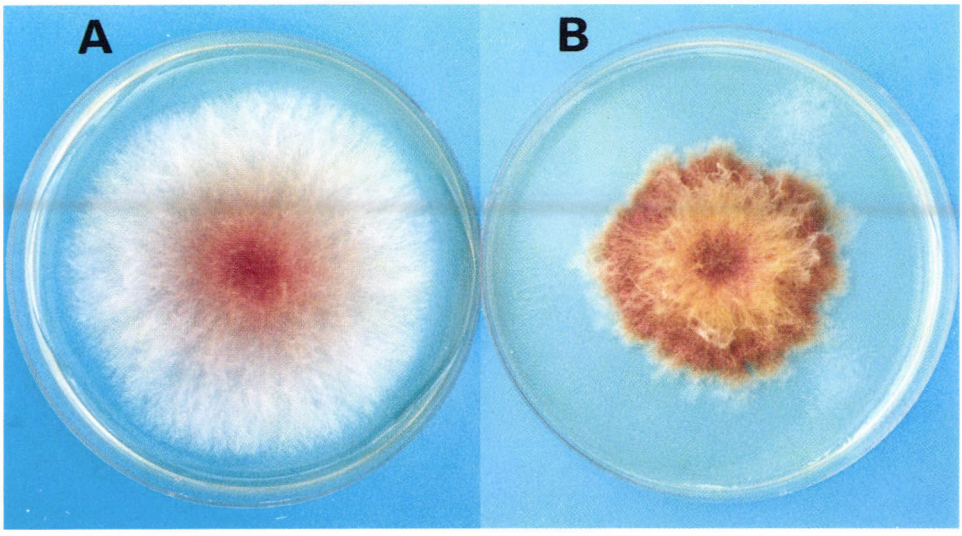

Fig. 8. Four-day-old *Type* A (A) and *Type* B (B) cultures of *F. graminearum* on potato-dextrose agar

Surface disinfection

One hundred cereal grains and maize kernels from each sample were surface disinfected by continouosly shaking with 3.5% sodium hypochlorite (NaOCl) for 2 min, and rinsed three times with sterile water and dried aseptically in a laminar box. Maize kernels were cut off aseptically in half.

Plating procedure

After surface disinfection, grains and kernels (5–10/Petri-dishes) were set into the medium before agar solidified to prevent germination. Plates were incubated upright at 25 °C for 4 days in the dark, after which they were placed under a combination of black light/incandescent light with a 12 h light/12 h dark cycle for 4 days at 20 °C. Fusaria which grew were identified to species level on the basis of identification protocol of Nelson et al. (1983) and Liddel and Davis (1991).

Results and Discussion

The advantage of Togawa-medium is that different *Fusarium* species are more readily recognised by their pigmentation and cultural appearance. Cultures on Togawa-medium are of more uniform appearance. This medium permits the examiner to recognise *F. graminearum*, *F. moniliforme*, and *F. subglutinans* by their cultural appearance, it reduces the amount of subculturing necessary for subsequent identification, as only representative isolates need to be cultured. For routine purposes it may even be possible to identify above-mentioned Fusaria directly on Togawa-medium. Togawa (1994) did not use his medium for isolation of *Fusarium* spp. from maize kernels. According to our results this medium is suitable for selective isolation and identification of *F. graminearum* from cereal grains and maize kernels, but *F. moniliforme* and *F. subglutinans* only from maize kernels.

Appearance of colonies of *F. graminearum* is characteristic due to production of both bright red pigment and pinkish-white dense aerial hyphae (Figs 1 and 2). Furthermore, it was easy to distinghuish *F. graminearum* from *F. moniliforme* and *F. subglutinans*. Togawa-medium yielded characteristic white powdery colonies of *F. moniliforme* (Figs 3 and 4) and pale brown skin-like colonies of *F. subglutinans* (Fig. 5). In some case, *F. graminearum* and *F. moniliforme* outgrow from same meize kernel (Figs 3 and 4). On this basis, grains and kernels of a series of cereal and maize varieties were evaluated for contamintion by *Fusarium* spp. Of the *Fusarium* species isolated, *F. graminearum* occurred in all cultivars of barley, wheat and maize, but *F. moniliforme* and *F. subglutinans* only in maize samples.

Two distinct colony-morphological forms (Cullen et al., 1982) of *F. graminearum* were isolated from naturally infected wheat grains. These two forms could not distinguished from each other on the basis of production of sporodochia (Fig. 6) and shape of

macroconidia (Fig. 7). The *Type A* isolates were fast growning, produced abundant white aerial mycelium, and produced regular circular colonies on freshly prepared PDA (Fig. 8 A). In contrast, *Type B* isolates were slower growing, produced yellowish-red pigment, and irregularly shaped appressed colonies on PDA (Fig. 8 B).

In conclusion, Togawa-medium is very suitable for routine isolation and identification of *F. graminearum*, *F. moniliforme* and *F. subglutinans* from cereal grains and maize kernels.

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Induced Resistance in Tomato Plants against *Fusarium* Wilt Invoked by *Fusarium* sp, Salicylic Acid and *Phytophthora cryptogea*

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A *Fusarium* species (Fsp) non-pathogenic to tomato (*Lycopersicon esculentum* Mill.) was shown, using *in vitro* experiments, to be an effective inhibitor of the tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Induction of resistance to FOL in tomato plants was performed with Fsp, salicylic acid (Sa) and *Phytophthora cryptogea* (Pc) under greenhouse conditions. The inducers were applied to the root systems three weeks after sowing. The plants were challenge inoculated with FOL two days after induction. The resistance was evident as significant inhibition of disease incidence in the case of Fsp and Sa. Although, these inducers initially caused some deleterious and phytotoxic effects, respectively, the plants later recovered and no wilt symptoms could be detected. On the other hand, when Pc was added to tomato roots, it caused pathogenic effects and no induced resistance was obtained.

Fusarium oxysporum f. sp. *lycopersici* (FOL) is the causal agent of wilt in tomato. Research worldwide so far is centred on the use of resistant varieties, but new pathogenic races may arise that can overcome plant resistance (Tello and Lacasa, 1988). As Kuć (1990) suggests, since induced resistance utilizes mechanisms for resistance present in plants, it may be considered natural and just as safe for man and the environment as diseaseresistant plants. Systemic induced resistance (SIR) has been described in different plant-pathogen systems, including most of the *Solanaceae* family, tobacco (Pont, 1959), tomato (Heller and Gessler, 1986), and potato (Doke et al., 1986; Strömberg and Brishammar, 1993). Unfortunately, the mechanisms of SIR in plants are not fully understood. Kroon et al. (1991) have shown that *F. oxysporum* f. sp. *dianthi* can induce resistance to FOL. Likewise, a non-pathogenic isolate of *Fusarium oxysporum* strain Fo47 can induce resistance to *Fusarium* wilt in tomato (Fuchs et al., 1997). The objective of the current investigation was to determine whether a *Fusarium* species, salicylic acid and *Phytophthora cryptogea* could be used to induce resistance to *Fusarium* wilt in tomato plants when added exogenously to the root system.

Materials and Methods

Plant material

The tomato cultivar, Danish Export, susceptible to race 2 of FOL was chosen. Fertilizer (Osmocote Plus mikro N-P-K-Mg 5-5-11-1.2) at the rate of 1.5 kg m^{-3} was used to water the plants three times a week with 50 ml per pot.

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Growth conditions

The experiments were performed in a greenhouse maintained at 26 °C during daytime and 20 °C at night and 60–70% R.H. Daylength was 12 h and the light intensity 7–10 kLux at the top level of the plant (irradiance 30–35 Wm^{-2}, light source Osram HPTI/HQI 400W).

Cultures

An isolate of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen race 2 (FOL) was obtained from Centraalbureau Voor Schimmelcultures, Baarn-Delft (the Netherlands) and maintained on potato dextrose agar (PDA). Conidial suspensions were obtained by culturing the fungus in Czapek Dox medium (Oxoid CM95) on a reciprocal shaker for 7 days at 26 °C. Mycelial fragments were removed by filtering through sterile glasswool. After washing in sterile water, the conidial suspension was adjusted to the desired concentration. The aggressiveness of this strain of FOL was previously tested and recorded as type 3 according to Pineau (1976).

Fusarium species (Fsp) isolated from the roots of broad beans taken from the south of Sweden was maintained on V8-juice agar. Ten-day-old fungus cultures were used as inoculum and prepared in the same manner as FOL-isolate.

Phytophthora cryptogea (Pc) Pethyb. and Laff. was isolated from wheat and maintained on V8-juice agar and zoospore suspensions prepared according to Larsson and Gerhardson (1990).

Inducers

As inducers, *Fusarium* species (Fsp), salicylic acid (Sa) and *Phytophthora cryptogea* (Pc), were used. Fsp and Pc were obtained as described above. Sa was prepared as an aqueous solution with distilled water at a concentration of 15mM prior to inoculation.

In vitro experiments: inhibition of FOL *by using* Fsp

The method of Padmodaya and Reddy (1996) was applied. An isolate of *Fusarium* sp. was tested for antagonistic activity in four culture media: V8-juice agar (V8), Kings medium (KB), Trypton soy broth (TSA) and potato dextrose agar (PDA) in Petri dishes. Both Fsp and FOL were inoculated at the same time. Discs of 5 mm cut from the margin of young vigorously growing cultures were placed at opposite points of Petri dishes, 4 cm apart from each other. The plates were incubated at room temperature 26 ± 2 °C. The Fsp isolate was selected for the potential after contact with FOL on the basis of their overgrowth and smothering of the FOL colony. The percentage inhibition of radial growth (PIRG) was determined after 5, 7 and 10 days as an estimate of the growth inhibition of FOL by the antagonist. Mycelium of FOL was periodically observed under a light microscope until day 10 of incubation. Three replicates were set up per treatment. The data were analyzed statistically.

In vivo experiments: induction and challenge inoculations

The trials were designed in a randomized block design with 5 blocks. The inductions were made with Fsp, Pc as well as with Sa. Tomato seedlings, 21-day-old with 2–4 leaves, were grown in 10 cm diameter pots, i.e. one seedling per pot containing standardized soil (Enhetsjord P) mixed with sand 80:10. All inductions as well as the challenge inoculation were performed by adding the suspensions into the root system using a syringe. The volume of suspension used was 30 ml in the case of the Fsp and Pc, and only 5 ml in the case of Sa. After 48 h, 30 ml of FOL-suspension was added to the root-system. Untreated plants and plants with the respective inducers without FOL, were used as controls. Plants with merely FOL-additions served as disease controls. The complete experiment was repeated twice.

Evaluation of disease incidence and statistical analysis

Disease incidence was visually estimated during a period of 60 days after challenge inoculation. The rating scale used was from 0–100%; 0 means no wilt disease observed and 100 means all plants diseased. Statistical analyses were conducted with two-way Anova with two replications. Differences between means were analyzed with Duncan's test ($P = 0.05$).

Results

The effect of *Fusarium* sp. on the radial growth of FOL is depicted in Fig. 1. The antagonistic fungus inhibited the radial growth of FOL significantly ($P = 0.05$) and had effect on its mycelium (Table 1). V8-juice agar was found to be a suitable medium for Fsp. Plant roots inoculated with FOL showed disease symptoms 25–30 days after inoculation. Plants treated with Fsp in the same way showed some deleterious effects 20–30 days after the treatment but subsequently, the plants recovered and became healthy. When plants were treated with Fsp and challenge inoculated with FOL no wilt disease symptoms were obtained but Fsp could be isolated from the leaves. During Sa-induction, some phytotoxic signs were observed in the plants 20–30 days after treatment but FOL-inoculation did not cause any wilt disease symptoms in this case either. Plants pretreated with the fungus Pc and challenge inoculated with FOL showed wilt disease symptoms after 30 days. Plants treated with Pc only showed disease symptoms but not that of the wilt type. The Anova test for all the material, revealed significant differences for factor treatments and time (Table 2).

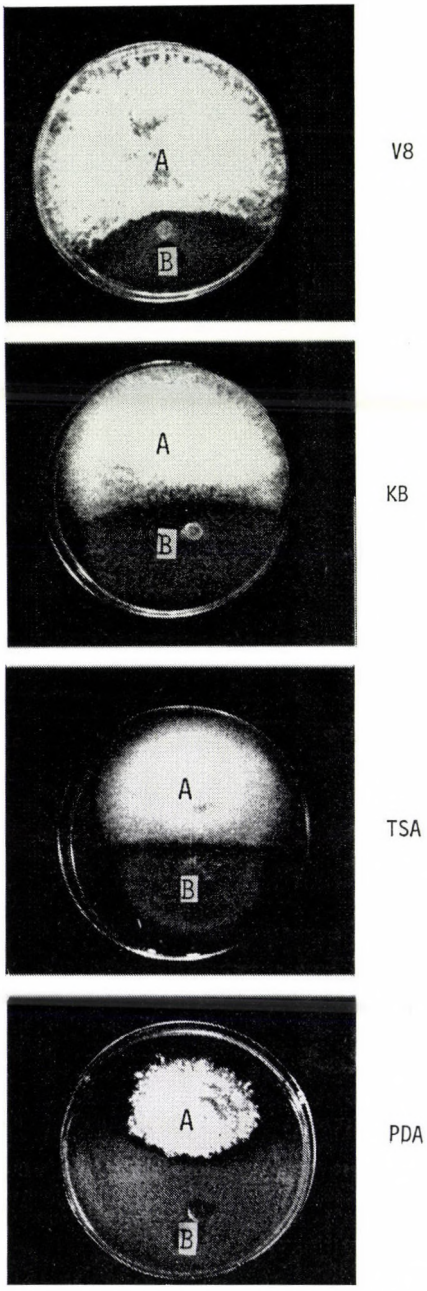

Fig. 1. *In vitro* experiments on inhibition of FOL by using Fsp on different culture media: V8-juice agar (V8), Kings media (KB), Trypton soy broth (TSA) and potato dextrose agar (PDA). A: Radial growth of *Fusarium* species (Fsp), B: Inhibition of a *Fusarium oxysporum* f. sp. *lycopersici* (FOL) colony

Table 1

Percentage Inhibition of Radial Growth (PIRG) in mm after different treatments in different culture media *in vitro*

Treatment	PIRG	No. of tests
	Different days independent of media and fungus	
5 days	28.8 c	16
7 days	47.6 b	16
10 days	69.4 a	16
	Different media independent of days and fungus	
KB	50.0 ab	12
V8	51.7 ab	12
TSA	53.4 a	12
PDA	39.2 b	12
	Different fungus independent of days and media	
Fsp Control	67.5 a	12
FOL Control	63.3 a	12
Fsp	38.3 b	12
FOL	25.0 c	12

Means with the same letter are not significantly different

Table 2

Effects of the respective inducers: Fsp, Sa and Pc on the disease incidence causing by FOL after up to 60 days *in vivo*

Treatment	Disease incidence* after					
	10 d	20 d	30 d	40 d	50 d	60 d
Fsp + FOL	0 a	0 1) a	0 1) b	0 b	0 b	0 b
Fsp Control	0 a	0 1) a	0 1) b	0 b	0 b	0 b
Sa + FOL	0 a	0 2) a	0 2) b	0 b	0 b	0 b
Sa Control	0 a	0 2) a	0 2) b	0 b	0 b	0 b
Pc + FOL	0 a	0 a	70 a	80 a	80 a	90 a
Pc Control	0 a	0 a	0 3) b	0 3) b	0 3) b	0 3) b
FOL Control	0 a	0 a	60 a	90 a	90 a	100 a
Control Plants	0 a	0 a	0 b	0 b	0 b	0 b

Means with the same letter are not significantly different
The analyses are done on arscine transformed values
1) = Deleterious effect 2) = Phytotoxic sign 3) = Pc disease symptoms
* Disease incidence = Number of wilted plants out of 100 treated

Discussion

In the present study, the growth of FOL obviously was retarded when paired with Fsp 4 cm apart on different media in Petri dishes. Within five days Fsp had grown over the FOL colony. De Cal et al. (1995) reported that *Penicillium oxalcium*, *P. purpurogenum* and *Aspergillus nidulans* can inhibit radial growth and had some effect on the mycelium of FOL *in vitro*. Furthermore, these organisms could reduce disease severity *in vivo* and even act as inducers of SIR (De Cal et al., 1995, 1997). Our results confirmed that Fsp is also an effective bio-agent capable of reducing tomato wilt disease and can be used as an inducer.

Sa and Pc were used as comparative inducers. Marked reducing effects of disease incidence in potato against the late blight fungus *P. infestans* were obtained by Sa-treatment (Quintanilla and Brishammar, 1998). Pc has successfully been used as an inducer in potato (Strömberg and Brishammar, 1991; Quintanilla and Brishammar, 1998) and in tobacco (Kamoun et al., 1993). However, in tomato, which is a member of *Solanaceae*, like potato and tobacco, Pc caused pathogenic effects when added to the roots and therefore was not useful to induce SIR. Though aware of any hazardous effects associated with the use of Pc, its degree of aggressiveness seems to vary from one cultivar to another (Ilieve et al., 1992). Furthermore, Kratka and Kalinova (1995) not consider Pc to be a pathogen on tomato plants. Fsp appeared in whole plant but did not harm the tissues. It may have an effect similar to that of an endophyte (Chen et al., 1994, 1995). This should be regarded as a special type of SIR and has similarities with cross protection since Fsp is present and also since neither FOL could be detected nor any apparent damage. Induction with 15 mM Sa in the susceptible cv. Danish Export drastically increased resistance. Currently, experiments are going on to test inductions and state if lower concentrations of Sa can be used. Further experiments will be conducted to correlate the observed induction of resistance obtained by Fsp and Sa in tomato plants and the biochemical and physiological mechanisms responsible, and also to identify whether this type of induced resistance can be regarded as an increase in general resistance (Heller and Glessler, 1986). In addition, it will be important to know if induced resistance obtained by Fsp is active against other tomato plant pathogens, and if Fsp can be used as an inducer in other kinds of plants as well that belong to different plant families.

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The Influence of Nico-, Primi-, Rim- and Thifensulfuron-Methyl Herbicides on the Mycelial Growth of *Fusarium graminearum*

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The effects of four sulfonylurea herbicides on the mycelial growth of *Fusarium graminearum* were studied in three concentrations on potato dextrose agar. The inhibition of the mycelial growth of the fungus by Nicosulfuron (Motivell) was statistically significantly proportional to the concentration of the herbicide. Primisulfuron (Tell) exerted similar inhibition but on a lower level and the results were not statistically significant. Rimsulfuron (Tarot) stimulated the growth of the fungus in the two higher concentrations (partly statistically significantly) while the lowest concentration inhibited it. Thifensulfuron (Harmony) exerted inhibition of the mycelial growth proportional to concentration however on a middle level.

Nicosulfuron, primisulfuron, rimsulfuron and thifensulfuron-methyl belong to the group of sulfonylurea herbicides, they are relatively new herbicide substances and their application rates are very low. Their physicochemical reactions in soil, e.g. absorption, translocation, degradation, leaching and several biotests have been intensively investigated (Iwanzik et al., 1988; Goatley et al., 1990; Ivanainen and Heinonen-Tanski, 1991; Mc Dovel et al., 1997) while the publications about their effects on soil organisms are very scarce (Oppong and Sagar, 1992). Their effect on parasitical soil fungi under the conditions of soil residues was first of all described for chlorsulfuron on *Rhizoctonia* sp. and *Gaeumannomyces graminis* var. *tritici* (Rovira and Mc Donald, 1986; Altman and Rovira, 1989), and then on *R. solani* and *R. oryzae* in the case of winter wheat (Smiley and Wilkins, 1992). In the last years some works were published about the effects of primi- and triasulfuron of *F. moniliforme, R. solani, Sclerotinia sclerotiorum* and *Trichoderma longibrachiatum* (Maček and Lešnik, 1994), about the effects of the same herbicides on mycelial growth and sporulation of *Fusarium avenaceum, F. moniliforme* var. *subglutinans, F. sambucinum* and *F. solani* (Maček and Berden, 1994), about the effects of the same herbicides and of prosulfuron on the same fungal properties on some *Fusarium* and *Helminthosporium* species (Maček and Angelini, 1994), those of the same herbicides on the same fungal properties for *Alternaria solani*, *Fusarium nivale*, *F. oxysporum* var. *phaseoli* and *Stemphylium vesicatorium* (Maček and Leben, 1996). The present work is a further contribution to the rather underinvestigated field of the side effects of this herbicide group on the (soil) parasitic fungi.

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Materials and Methods

Four sulfonylurea herbicides were investigated: nicosulfuron ($C_{15}H_{18}N_6O_6S$) as Motivell, primisulfuron ($C_{15}H_{12}N_4O_7F_4S$) as Tell, rimsulfuron ($C_{14}H_{17}N_5O_7S_2$) as Tarot and thifensulfuron-methyl ($C_{12}H_{13}N_5O_6S_{2)}$ as Harmony (CIBA-GEIGY, 1986–1989, The Pesticide Manual, 1995).

Because of the very low application rates in question and as we wanted to approach field conditions as much as possible, we decided to use commercial preparations, and not pure substances. Table 1 gives the concentrations used. The fungus chosen in this study was *Fusarium graminearum*.

The fungus was cultivated by standard methods on potato dextrose agar at 24 °C, 2 hours of exposure to daylight daily. After the sterilization of the medium the corresponding quantities of the herbicide solution were added and the Petri dishes thoroughly shaked. The thermal stability of the herbicides was biotested with garden chess (*Lepidium sativum* L.). The results obtained show that the reduction of the herbicidal affect because of mixing with hot potato-dextrose agar is not worth mentioning.

The fungus was cultivated for 6 days. The mycelial growth was followed daily on the lower side of the Petri dishes. Because of the very regular radial growth of the mycelium the plane geometry was not carried out. Each ring was outlined and then two rectangular lines (diameters) were drawn on the bottom. So the mean radius was determined and the daily overgrown area was then calculated.

Results

Table 2 gives the influence on the herbicides on the mycelial growth of *Fusarium graminearum*. Only statistically significant statements will be discussed. The mycelial growth of the mentioned fungus was strongly inhibited by nicosulfuron (Motivell) in all concentrations. Thifensulfuron-methyl inhibited the growth only at the highest (C_1) and at the middle (C_2) concentration. Rimsulfuron stimulated the growht at the middle (C_2) concentration. Primisulfuron exerted no significant influence.

Discussion

The discussion of the present results is difficult because there are no experimental findings exist about the side effects of nicosulfuron, thifensulfuron-methyl or rimsulfuron on the parasitic fungi that could serve for comparison. But it is interesting that nico- and thifensulfuron-methyl exert inhibition of mycelial growth of *Fusarium graminearum* at all concentrations, while rimsulfuron stimulates it significantly at C_2 and not significantly at C_1 while inhibited it at C_3.

Table 1

Herbicide concentration in the nutrient medium

Herbicide Field doses g/ha Concentration in the medium	Harmony 15		Motivell 40		Tarot 15		Tell 30	
	%	ppm	%	ppm	%	ppm	%	ppm
C_1	0.0281	281.0	0.133	1333.0	0.0375	375.0	0.075	750.0
C_2	0.00281	28.1	0.0133	133.0	0.00375	37.5	0.0075	75.0
C_3	0.000281	2.81	0.00133	13.3	0.000375	3.75	0.00075	7.5

Note: C_1 = Field conc. × 10^1, c_2 = Field conc., C_3 = Field conc. × 10^{-1}

Table 2

The influence of sulfonylurea herbicides on the mycelial growth of *Fusarium graminearum* (in cm^2 5th day)

Concentration resp. control	Harmony	Motivell	Tarot	Tell
	$LSD_{0,05}$ = 10.509 $HSD_{0,05}$ = 14.186	$LSD_{0,05}$ = 8.212 $HSD_{0,05}$ = 11.085	$LSD_{0,05}$ = 5.745 $HSD_{0,05}$ = 7.755	$LSD_{0,05}$ = 8.293 $HSD_{0,05}$ = 11.195
C_1	43.49**	12.12**	66.49	63.71
C_2	42.42**	24.76**	70.60**	58.47
C_3	48.51	44.27**	57.30	54.92
Control	57.96	57.96	61.22	61.22

* Statistically significant after HSD (Tukey) test at $\alpha = 0.05$
** Statistically significant after LSD test at $\alpha = 0.05$

The discussion is possible in the case of primisulfuron because there are some results. In this study the herbicide showed no significant influence on the mycelial growth of *F. graminearum* which differs from the data about *F. solani* (Maček and Berden, 1994) *F. moniliforme* and *Helminthosporium graminearum* (Maček and Angelini, 1994). For some other fungi and different concentrations mostly inhibition was observed for this herbicide, but in some cases even stimulation was reported. In general, the results of this study are very much like those already known the investigated sulfonylurea herbicides showed (Maček and Lešnik, 1994; Maček and Berden, 1994; Maček and Angelini, 1994; Leben and Maček, 1996), a rather moderate influence on (soil) parasitic fungi.

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Pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* Isolates on Tomato Cultivars and Solanaceous Crops in Libya

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Pathogenicity tests were carried out under greenhouse conditions using eight common tomato cultivars inoculated with twenty isolates of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. and Hans., isolated from different regions of Libya. Tested tomato cultivars were susceptible to all isolates of the pathogen but the responses of cultivars to the individual isolate varied. Results indicated the possible presence of differential pathogenic races of the pathogen. Host range of *Fusarium oxysporum* f. sp. *lycopersici* isolates was studied using various solanaceous plant species including tomato, pepper, eggplant and potato. Results indicated that all isolates tested were virulent to all hosts tested except potato. Tomato, pepper and eggplant cultivars showed high susceptibility to all isolates while potato cultivar was slightly susceptible to some isolates and resistant to others.

Fusarium wilt is a destructive disease affecting tomato as well as a large number of other plant species (9). In Libya the disease was first recorded in 1960 and 1962 (6, 10). It occurs in the major tomato production areas and it is generally severe during August and September (7). The pathogen consists of numerous host specific formae speciales, one previously identified as *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. and Hans. (3, 9, 12). To prove the pathogenicity of this pathogen, twenty isolates of *Fusa-rium oxysporum* f. sp. *lycopersici* have been tested to eight tomato cultivars and other solanaceous crops commonly used by growers in Libya.

Materials and Methods

Fusarium isolates collected from different tomato cultivars and soil samples from Ain Zarah, Benghazi, Garabouli, Zawia and Maraj regions were identified as *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. and Hans. (3). Tomato cultivars Roma VF, Walter, Earliana, San Marzano, Ace VF55, VF515, VF270, and VF145-21-4 – Select. and three solanaceous crops such as pepper (Long Hot), eggplant (Black Beauty), and potato (Ajax) were used to test the virulence of these isolates and to evaluate plant responses to the pathogen. Four replicates of each cultivar (3-plants each) of test plants were used and arranged in split design under greenhouse conditions.

Conidial suspensions were harvested from culture plates of each isolate and suspended in 500 ml of sterile distilled water. Suspensions were filtered by several layers of sterile cheese cloths to remove mycelial fragments (1).

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Root tips of 4-week-old seedlings were washed with tap water, wounded and placed into the conidial suspensions for 24 hours, while other dipped in sterile distilled water served as control. Then the seedlings replanted in sterile soil and fertilized with 25 ml diluted (half strength) Hoagland solution (5).

Inoculated plants were grown for 12 days at 25–30 °C and then visual disease rating was taken by the following scale: 0 = symptomless, 1 = epinasty, 2 = clearing of veinlets, 3 = yellowing of lower leaves, 4 = wilting and death of plants.

Cross sections of roots and stems of tested plants were used for detection of vascular discoloration. Isolations have been done for confirmation of the presence of the pathogen in plant tissues. Plants were classified into the following categories:

0.0 – 0.9 = Resistant (r)
1.0 – 1.9 = Slightly susceptible (ss)
2.0 – 2.9 = Susceptible (s)
3.0 – 4.0 = Highly susceptible (hs)

Table 1

Reaction of tomato cultivars to different isolates of *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditons

Tomato Cultivars											
Roma VF			VF145-21-4-Select			San Marzano			Walter		
I	M	H. R.	I	M	H. R.	I	M	H. R.	I	M	H. R.
GP2*	2.25		MSI	1.5	SS	GS1	2.0		ZS2	1.75	SS
AP2	2.75		GPI	2.25		ZS2	2.25		GS1	2.0	
MSI	2.75	S**	BP4	2.5	S	GP1	2.25		MS1	2.5	S
ZP2	2.75		ZS2	2.5		MS1	2.25		MP1	2.75	
AP3	3.0		BS5	2.5		AS4	2.5		ZP2	3.0	
BS5	3.25		ZP2	3.0		MS2	2.5		AS1	3.0	
ZP3	3.25		BP2	3.25		ZP2	2.5		AS2	3.0	
GS1	3.25		ZP3	3.25		MP1	2.75		AS4	3.0	
AP1	3.25		GP2	3.25		ZP3	3.0		BP4	3.0	
BP7	3.5		AP1	3.25		GP2	3.0		MS2	3.0	
GP1	3.5		GS1	3.25		AP3	3.0	S	AP1	3.25	
MS2	3.5	HS	MP1	3.25		BS5	3.0		AP4	3.25	HS
ZS2	3.5		AP2	3.5	HS	AP1	3.25		GP2	3.25	
MP1	3.5		AP4	3.5		AP4	3.25		BP7	3.5	
BP4	3.75		AS2	3.5		AS1	3.25		ZP3	3.5	
AS1	3.75		AS4	3.5		BP2	3.5		GP1	3.5	
AS2	3.75		MS2	3.5		BP4	3.5		AP3	3.5	
AS4	3.75		BP7	3.75		BP7	3.5		BP2	3.75	
BP2	4.0		AP3	3.75		AP2	3.75		BS5	3.75	
AP4	4.0		AS1	3.75		AS2	3.75		AP2	3.75	

Table 1 (cont.)

Tomato Cultivars											
VF 515			Ace VF 55			Earliana			VF 270		
I	M	H. R.	I	M	H. R.	I	M	H. R.	I	M	H. R.
AP1	2.25		MP1	2.75	S	MS1	2.5	S	GP2	2.75	S
ZP3	2.5		BP2	3.0		MS2	3.0		BP4	3.0	
GP2	2.5	S**	GP1	3.0		MP1	3.25		MS1	3.0	
BP7	2.75		GP2	3.0		BP2	3.25		ZP2	3.25	
MS2	2.75		AP4	3.0		BP7	3.25		ZP3	3.25	
BS5	3.0		AS4	3.0		AS2	3.25		GS1	3.25	
BP2	3.0		BS5	3.0		ZP2	3.25		AP1	3.25	
AP4	3.0		BP4	3.25		GP1	3.25		AP2	3.25	
AS1	3.0		MS1	3.25		AP4	3.25		AS4	3.25	
ZS2	3.0		ZS2	3.25		AS4	3.5		MP1	3.25	
AS2	3.0		ZP2	3.5	HS	BP4	3.5	HS	MS2	3.25	HS
GS1	3.25		ZP3	3.5		ZP3	3.5		BP2	3.5	
BP4	3.5	HS	GS1	3.5		AP1	3.5		BP7	3.5	
ZP2	3.5		AP2	3.5		GS1	3.75		BS5	3.5	
GP1	3.5		AS2	3.5		BP5	3.75		AS1	3.5	
AS4	3.5		MS2	3.5		GP2	3.75		AS2	3.5	
MP1	3.5		BP7	3.75		AP2	3.75		GP1	3.75	
AP2	4.0		AS1	3.75		AP3	3.75		AP3	3.75	
AP3	4.0		AP1	4.0		AS1	3.75		AP4	4.0	
MS1	4.0		AP3	4.0		ZS2	4.0		ZS2	4.0	

I = Isolates

M = means (average of readings)

H. R. = host reactions

* = Key of isolates : Locations A (AinZarah), B (Benghazi), G (Garabouli), M (Maraj), Z (Zawia), P (isolated from plants), S (isolated from soil)

** = Cultivar reacted to the isolates signed: S = susceptible, HS = highly susceptible, SS = slightly susceptible

Results and Discussion

Responses of eight tomato cultivars to twenty isolates of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. and Hans. were tested (Table 1). Results indicate that all cultivars were susceptible to all isolates of the pathogen to different degrees. Plant response was evaluated between 1.5–4.0 categories. Cultivar Roma VF rated from moderately to highly susceptible, cultivars ACE VF, Earliana, VF 270 were highly susceptible, San Marzano, VF 515 were moderately susceptible, cultivar Walter was slightly susceptible, while VF 145–21–4 Select. was rated slightly to highly susceptible to different isolates of the pathogen.

Table 2

Responses of solanaceous host plants to different isolates of *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditons

Host Plants											
Pepper			Eggplant			Tomato			Potato		
I	M	H. R.	I	M	H. R.	I	M	H. R.	I	M	H. R.
BP2	2.65		MS2	2.325		GP1	2.35		GP2	0.325	
BP7	2.750		MS1	2.475		BP7	2.5		AS2	0.425	
AP4	2.750		GP1	2.675		ZP2	2.65		MP1	0.50	
AS4	2.750		AP1	2.725		AS4	2.675		ZP3	0.575	
MP1	2.750	S**	MP1	2.825	S	MP1	2.675		GS1	0.575	
AP1	2.825		BS5	2.925		BP2	2.70		AP3	0.675	
AP3	2.825		AP3	2.925		AP2	2.825		AS4	0.675	R
ZP2	2.90		AS1	2.975		AP3	2.825	S	ZS2	0.75	
GP2	2.925		AS4	2.975		ZS2	2.825		BS5	0.825	
AP2	3.0		BP4	3.075		GS1	2.85		AP2	0.90	
AS2	3.0		GP1	3.075		AP1	2.85		GP1	0.925	
ZS2	3.0		GS1	3.075		MS2	2.925		AS1	0.925	
ZP3	3.075		AP4	3.075		AP4	2.925		MS1	1.00	
MS1	3.075		ZS2	3.10		ZP3	2.925		MS2	1.10	
MS2	3.075	HS	ZP2	3.15	HS	BP4	2.925		ZP2	1.15	
BP4	3.150		ZP3	3.15		GP2	3.00		BP2	1.225	SS
GP1	3.150		AP2	3.15		AS1	3.075		BP7	1.25	
AS1	3.150		BP2	3.25		AS2	3.075	HS	AP4	1.25	
BS5	3.25		BP7	3.25		BS5	3.15		AP1	1.325	
GS1	3.25		AS2	3.25		MS1	3.175		BP4	1.325	

I = Isolates

M = means (average of readings)

H. R. = host reactions

* = Key of isolates: Locations A (Ain Zarah), B (Benghazi), G (Garabouli), M (Maraj), Z (Zawia), P (isolated from plants), S (isolated from soil)

** = S = susceptible, HS = highly susceptible, R = resistant, SS = slightly susceptible

Results indicate that all isolates of *Fusarium oxysporum* f. sp. *lycopersici* were virulent to all tested hosts (Table 2). However, potato was resistant to 9 isolates and slightly susceptible to 11 isolates. Isolates on pepper showed no significant differences in their virulence, while on tomato and eggplant several degrees of susceptibility were noted to the tested isolates.

Tomato cultivars were susceptible to all isolates of *Fusarium oxysporum* f. sp. *lycopersici* but their responses to individual isolates varied. These results indicated different degrees of virulence of the isolates and refers to the existance of races of *F. oxysporum* f. sp. *lycopersici* (2, 4, 11).

Table 3

Virulence of *Fusarium oxysporum* f. sp. *lycopersici* isolates to different solanaceous host plants

Isolates	Host response Potato	Tomato	Pepper	Eggplant
BP2	1.225	2.70 c*	2.65 c	3.25 d*
BP4	1.325	2.925	3.15	3.075
BP7	1.25	2.5	2.75	3.25
BS5	0.825	3.15	3.25	2.925
ZP2	1.15	2.65	2.9	3.15
ZP3	0.575	2.925	3.075	3.15
ZS2	0.75	2.825	3.0	3.1
GP1	0.925	2.35	3.15	3.075
GP2	0.325	3.0	2.925	2.675
GS1	0.575 a*	2.85	3.25	3.075
AP1	1.325	2.85 b*	2.825 b	2.725 b
AP2	0.9	2.825	3.0	3.15
AP3	0.675	2.825	2.825	2.925
AP4	1.25	2.925	2.75	3.075
AS1	0.925	3.075	3.15	2.975
AS2	0.425	3.075	3.0	3.25
AS4	0.675	2.675	2.75	2.975
MP1	0.5	2.675	2.75	2.825
MS1	1.0	3.175 c	3.075 bc*	2.475
MS2	1.1	2.925	3.075 c	2.325

* = Means within each column followed by different letter(s) are significantly different at 5% level

Potato was more resistant to some isolates tested. Tomato, pepper and eggplant exhibited susceptibility to most isolates (Table 3). All isolates were virulent on a wide range of solanaceous crops. Results are in accordance with other author's results (2, 4, 8).

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Distribution of *Fusarium* spp. of Tomato from Different Regions in Libya

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This study was conducted on *Fusarium* wilt disease of tomato in some coastal regions of Libya. The causal pathogen was *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. and Hans. in all regions. Morphology of the pathogen on PDA and selective media was different. The disease incidence was high on plants (28–57%) and in soil (14–42%). Several *Fusarium* spp. were also isolated and identified from tomato plants and soils.

Tomato (*Lycopersicon esculentum*) considered one of the most important crops in Libya. It is cultivated along the coastal areas as well as in southern regions in greenhouses and field conditions (2, 3). Among the important diseases of this crop *Fusarium* wilt was reported first in 1960 in Tripoli (10, 11) and in 1962 in Benghazi (6, 7). The causal pathogen was determined as *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. and Hans. This study refers to the disease incidence in some coastal regions of Libya and identification of the causal pathogen of this disease.

Materials and Methods

Surveying of *Fusarium* wilt was conducted in seven farms from locations of Ain Zarah, Benghazi, Garabouli, Zawia and Maraj. Random samples from greenhouses and fields of soils and tomato plants (roots and stems) were collected for isolations. Underground samples were taken at 20–30 cm depth. Transverse sections of stems and roots were used for isolation of the pathogen, sterilized with 5% chlorax solution and cultivated on PDA and selective media. Two g of soil in 200 ml of 1% water agar were used after a centrifugation at 3000 rpm./10 minutes, the supernatant was diluted to 10^{-3} (1) and laid on PDA and on selective media with fungicide and streptomycin sulphate (8). The plates were incubated during 7–14 days at 26 °C. Pure cultures of these isolates were obtained using single spore isolation technique (12). Stocks were maintained in PDA slants covered with mineral oil and kept at 3 °C. Isolates were identified by specific keys (4, 12). The identity was confirmed by Dr. C. Booth of CMI, England.

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Table 1

Incidence of *Fusarium* wilt fungus on tomato and in soil in different regions of Libyan Jamahiriya

Source of Samples Regions	Fields surveyed 1		2		3		4		5		6		7		Disease occurrence%*	
	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P
Ain Zarah	+	+	+	+	–	+	+	+	–	–	–	–	–	–	42	57
Benghazi	–	–	–	+	–	–	–	+	+	–	–	–	–	+	14	42
Garabouli	+	+	–	+	–	–	–	–	–	–	–	–	–	–	14	28
Maraj	+	+	+	–	–	–	–	–	–	–	–	–	–	–	28	14
Zawia	–	–	+	+	–	+	–	–	–	–	–	–	–	–	14	28

S = soil
P = plants
+ = infected
– = not infected
* = Disease severity and presence of the pathogen in soil samples and plants

Growth of *Fusarium* on both media was compared, and disease incidence was determined by the following scale:

0 = free from disease
1 = clearing of veinlets
2 = epinasty
3 = yellowing of lower leaves
4 = wilting and death of plants

Results and Discussion

Results indicate that the disease was prevalent on tomato plants in all regions surveyed (6, 10). The pathogen was identified as *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. and Hans.

Disease incidence was highest on plants (28–57%) as in soil (14–42%). The most infected region was Ain Zarah (Table 1) because it has more cultivated rich soils, while in Zawia region the soil has less cultivations and less rich than in Ain Zarah. The disease symptoms of the infected tomato plants was determined with vascular examinations. Browning was evident in vessels of all infected parts (7, 9, 11). Growth of *Fusarium* isolates on PDA and selective media was compared. On PDA the hyphal growth was unlimited and the colony colour varied from dark-pink to pinkish yellow or white (Fig. 1). On the selective medium the colony colour was white-pale pink and hyphal growth was restricted due to the presence of PCNB and streptomycin in this medium (1). Conidial germination was not inhibited. *Fusarium* moniliformae, *Fusarium solani*, *F. fusarioides* and *Fusarium equiseti* were also isolated (Table 2) and identified in some places. *Fusarium fusarioides* and *F. equiseti* were first reported from these locations (4, 5).

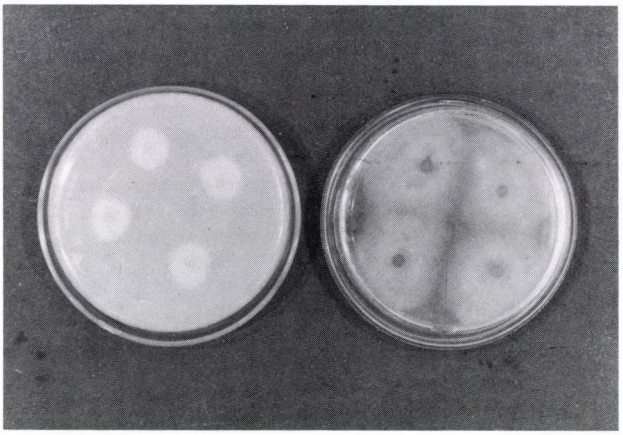

Fig. 1. Cultures of *Fusarium oxysporum* after 10 days cultivations: Right on selective (PCNB) medium. Left on PDA medium

Table 2

Identification of *Fusarium* spp. on tomato from different regions of Libyan Jamahiriya

Regions	*Fusarium* spp.									
	F. oxysporum		*F. solani*		*F. moniliformae*		*F. equiseti*		*F. fusarioides*	
	S	P	S	P	S	P	S	P	S	P
Ain Zarah	+	+	+	–	–	–	–	–	–	–
Benghazi	+	+	+	+	+	+	–	+	+	–
Garabouli	+	+	–	–	+	–	–	–	+	–
Maraj	+	+	–	–	–	+	–	–	–	–
Zawia	+	+	+	+	–	–	–	–	–	–

S = soil samples
P = plant samples
+ = present
– = absent

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Curvularia protuberata, a New Seed-borne Pathogen of Rice

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Curvularia protuberata was isolated from rice seeds exhibiting spotting symptoms following the blotter test method. Pathogenicity tests were conducted in the field on panicles of varieties Irga 409, Irga 410, Irga 412, Villaguay, Colonia Macías, San Miguel, Lucas and Te Bonnet. Sterilized and non-sterilized seeds from the field inoculated plants were tested by the blotter assay technique to determine the pathogen incidence. For both treatments the percentage of infected grains that did not germinate was very high (47 to 100). These grains showed discoloration and a dark brown mass of conidia and conidiophores covering their surfaces. The evidence that *C. protuberata* was related to spotting symptoms confirmed the Koch's postulates.

This is the first report of *Curvularia protuberata* as an associated microorganism to the spotting of rice grains.

Rice (*Oryza sativa* L.) grains may be infected by various organisms before or after harvest causing reduction in viability and grain quality, seedling blight, production of toxins during storage, and discoloration (Ou, 1972). Due to this last symptom, grain quality has been decreased in many Latin American countries since 1980 (Ferrer et al., 1980; Salive and Vargas, 1985; Soave et al., 1985). However, discoloration frequently has no effect on the quality of seeds for sowing, although this discoloration may reduce the commercial value of the grain (Jayaweera et al., 1988). In Argentina spotting of rice is increasing at an alarming rate, and in many cases reaches an incidence of 60% (Sisterna et al., 1994).

Among the fungi associated with discoloration of rice grain there are several *Curvularia* spp. that also produce the typical black kernel symptoms (Ou, 1972).

Analyzing rice grain quality, some samples with grain spotting symptoms were found. From them *Curvularia protuberata* Nelson and Hodges, together with other fungi were isolated (Sisterna et al., 1994). This species has been observed on leaves of *Axonopus affinis* Chase, *Cynnodon dactylon* (L.) Pers., *Dactylis glomerata* L., *Deschampsia flexuosa* (L.) Trin., *Eleusine indica* (L.) Gaertn., *Lolium multiflorum* Lam., *Oryza sativa* L., *Saccharum officinalis* L., *Sorghum vulgare* (L.) Moench., *Zea mays* L. (Nelson and Hodges, 1965), *Juncus* sp. L., *Panicum* sp. L. (Farr et al., 1989), and wheat seeds (Carranza, 1979).

In this research the pathogenicity of *C. protuberata* and its effect on germination of rice grains were studied.

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Material and Methods

Rice seed samples of variety Irga 409 from Corrientes and Formosa provinces, Argentina, were analyzed following the rules given by ISTA for the blotter test method (Neergaard, 1974): non-surface sterilized seeds were sown in Petri dishes on moistened absorbent paper. They were incubated one week in a growth chamber at 20 ± 2 °C and a 12 h artificial daylight NUV/12 h dark cycle. After this incubation period, the development of a dark brown mycelium over some seeds was observed with a low power stereomicroscope at 50 to 60 X. This fungus was transferred PDA in slant tubes and incubated at 25 ± 1 °C.

To determine the fungus species, the morphological and cultural characteristics were considered, and compared with the *Curvularia* spp. descriptions mentioned by Ellis (1966).

In the field, pathogenicity tests were carried out on panicles of varieties: Irga 409, Irga 410, Irga 412, Villaguay, Colonia Macías, San Miguel, Lucas and Te Bonnet.

Inoculum consisted of a mycelium and conidial suspension in sterile distilled water adjusted to 4.5×10^5 spores/ml. Four or five drops/L of Triton-X 100 were added. The suspension was applied with a sprayer and controls were treated only with distilled water.

Panicles were inoculated at anthesis stage, when plants were 134 days old. They were cultivated under irrigation in a 5 × 5 m plot at the Julio Hirschhorn Experimental Station (Los Hornos, Buenos Aires Province), in a typic Argiudoll soil. Two panicles each from 3 plants (total 6/variety) were infected artificially for each of the 5 repetitions and 3 controls. These panicles were covered with polyethylene bags for 48 h.

One hundred independent samples of sterilized and non-sterilized seeds were analyzed for each repetition by the blotter method.

To complete Koch's postulates, reisolates from contaminated grains were done by standard phytopathological methods.

Curvularia protuberata was obtained from infected grains and transferred to PDA slant tubes.

Results and Discussion

A fungus, later identified as *C. protuberata*, was initially isolated from infected seeds of variety Irga 409. This species forms colonies that are effused, dark grey or very dark greyish brown and velvety on PDA. Stromata formed readily on rice grains, usually in groups, sometimes branched black. Conidiophores arose singly or in groups, terminally and laterally on the hyphae, also on stromata, simple or branched, straight or flexuous, rather pale brown up to 500 µm long. Conidia were acropleurogenous, straight or slightly curved, cylindrical to ellipsoidal, with a markedly protuberant hilum at the base, almost always 4 septate, the central cell the largest, the cell at each end usually subhyaline or pale brown, intermediate cells brown or dark brown. Measuring 500 conidia, the dimensions were: 22.5 – 33.7 (29.55) × 8.4 – 13 (11) µm.

Table 1

Seed assay results from pathogenicity test

Cultivar	Contaminated seeds with *C. protuberata*					
	Sterilized (%)			No sterilized (%)		
	Control	Total	No germinated	Control	Total	No germinated
Irga 409	0	20	70	0	38	84
Irga 410	0	33	51	0	42	80
Irga 412	0	25	72	0	38	65
Villaguay	0	34	47	0	48	58
C. Macías	0	52	69	0	54	100
S. Miguel	0	46	65	0	40	85
Lucas	0	50	62	0	47	72
Te Bonnet	0	30	49	0	36	80

Within each column values are the average of data from the 5 replications.

On analyzed samples of variety Irga 409 the fungus incidence was 2%. According to the rice grading standards, samples must not have more than 0.5% imperfect kernels, including discolorations (Fazli and Schroeder, 1966). So, in this case the values found were higher than the tolerance. The presence of any of several types of kernel spot or discoloration reduce the product quality (Atkins, 1974).

Data from the pathogenicity tests indicated a high number of discolored grains related with *C. protuberata* contamination. The infection incidence under inoculated field of the different varieties was between 36 and 54% for non-sterilized seeds and between 20 and 52%, for sterilized grains (Table 1).

On the lemma and palea of inoculated grains, light to dark brown dots and circular spots were observed. Later lesions coalesced into large necrotic areas covering almost 50% of the kernels surface.

When these grains were analyzed by the blotter test *C. protuberata* was observed growing on them. Infection values of the fungus registered from inoculations show that natural seed infections could probably reduce the plant stand. This is because of the great number of discolored seeds that did not germinated.

As it is observed in Table 1, the fungus developed not only over the non-sterilized seeds but on the sterilized ones, showing that the pathogen is established into the internal tissues and on the surface, too.

Fungus infection appears as a discoloration affecting severely to a high percentage of seeds and inhibiting its germination. These results support the involvement of *C. protuberata* as a causal agent of the disease.

This is the first record of *C. protuberata* as a component of the pathogen complex causing discoloration of rice kernels.

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Effect of Cultural Conditions on the Growth and Amylolytic Enzyme Production by *Rhizopus oryzae*

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Rhizopus oryzae achieved a peak amylase production within 6 days of culture for starch yeast extract (SYE) medium and 8 days for yeast extract (YE) medium through the 20 days period of incubation. Maximum amylase activity by the fungus occurred prior to maximum production of mycelial biomass both in the SYE and YE media. Production of amylase was not related to total mycelium produced but was directly related to the type of carbon source in the medium. Starch and glucose were the best carbon sources for amylase production whereas maximum mycelial biomass was recorded in the medium containing sucrose as the sole carbon source. The maximum mycelial biomass and amylase activity were recorded at 30 °C and pH 6. The fungus as a prolific amylase producer could be of industrial use in some fermentation processes.

In Nigeria *Rhizopus* rot is the most serious disease of root crops, fruits and vegetables (Adisa, 1983; Ikotun, 1983; Fajola, 1979). The rot causing organism has been reported to secrete extracellular pectolytic enzymes (Amadioha, 1994) and cellulolytic enzymes (Amadioha, 1993). The reduction in the quantity of starch and subsequent increase in the quantity of glucose during pathogenesis by pathogenic organisms have been attributed to the hydrolisis of disaccharides and oligosaccharides by amylases (Arinze et al., 1975; Ogundana et al., 1975). The increased production of a given splitting enzyme by a pathogen grown in a particular medium has been termed induction and such enzymes are often said to be inducible. Strong cellulase and polygalacturonase (PG) activity were recorded in medium containing CMC and citrus pectin respectively (Ogundana et al., 1971). Spalding (1963) reported that a pectin-polypectate mineral salts medium was useful for the production of pectolytic and cellulolytic enzymes by *Rhizopus stolonifer*. The effectiveness of the enzymes and their existence in active forms are affected by the environment into which they are produced as temperature and pH of the environment are known to affect the production and stability of a given enzyme (Ogundero, 1978; Adisa, 1980). Eugbee (1973) concluded that regulation of the controlled systems governing the synthesis or production of enzymes is of significance in host-parasite relationships.

This investigation was conducted to determine the growth and production of amylolytic enzyme by *Rhizopus oryzae* under different cultural conditions in relationship to their roles in nature.

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Materials and Methods

Source and culture of pathogen

The inoculum was *Rhizopus oryzae* previously obtained in Nigeria from stored potato tubers (Amadioha, 1993) and grown on potato dextrose agar (PDA) in Petri dish at 30 °C for 5 days.

Growth and amylase synthesis in culture media

One disc (5 mm dia) of the fungus was grown on Starch Yeast Extract (SYE) medium whose composition was starch 5.0 g, yeast extract: 2.0 g; KH_2PO_4 : 1.0 g; $MgSO_4 \cdot 7H_2O$: 0.5 g; made up to one litre with distilled water and yeast extract (YE) medium which contained all the components above except soluble starch (Champan et al., 1975). The pH of both SYE and YE media after autoclaving was 6.4. The inoculated flasks containing the medium (30 ml) were incubated for 20 days at 30 °C. At 2 days intervals, three flasks of each culture medium were removed from incubator and the pH, amylase activity and mycelial biomass of each culture determined. The mycelial dry wight was determined by filtering the contents of the flasks through a preweighed filter paper. The culture filtrate was used for enzyme assay. The filter paper with mycelium was then oven dried at 80 °C for 24 h and weighed to the nearest mg to obtain the mycelial biomass. The initial oven-dry weight of the filter paper was substracted from the oven-dry weight of the filter paper and harvested mycelium to determined the amount of growth.

The effect of different concentrations of starch (0, 3, 5, and 10 g/litre) and yeast extract (0, 1, 2, and 4 g/litre) on the growth and amylase activity was also investigated.

Effect of temperature and pH on the growth and amylase activity

The culture medium (SYE) dispensed 30 ml/flask was inoculated with a disc (5 mm dia) of the fungus and incubated in incubators set at 5, 10, 15, 20, 25, 30, 35, and 40 °C for 10 days. The pH after autoclaving was 6.4. Cultures for other experiments were incubated at 30 °C because the peak activity of amylase by the fungus was obtained at this temperature.

Effect of pH was achieved by adjusting the culture medium to pH 2, 3, 4, 5, 6, 7 or 8 as described by Nolan (1976) and then inoculated with a fungal disc (5 mm dia). The culture was incubated at 30 °C for 10 days. The amount of growth and amylase activity were deterined as described.

Effect of carbon sources on the growth and amylase activity

The basal medium employed was a carbon-free sterilized (121 °C for 15 min) medium according to Adams (1976), containing KNO_3 : 3.0 g; KH_2PO_4 : 1.0 g; $MgSO_4 \cdot$

· $7H_2O$: 0.5 g; biotin: 5 µg; thiamine: 0.1 mg; microelement solution (Celerin and Fergus, 1971): 2 ml; distilled water to make one litre. A 1% w/v carbon source which included glucose, sucrose, fructose, maltose, starch, CMC and pectin was separately prepared and autoclaved and aseptically added after cooling to sterile 250 ml Erlenmeyer flask containing the basel medium (20 ml), thus giving a total of 40 ml/flask. Each flask was inoculated with a mycelial disc (5 mm dia) of the test organism adn incubated for 10 days at 30 °C. Controls were prepared in a similar fashion except that they lacked the carbon source.

Enzyme assay

The amylase activity was determined using the method of Chapman et al. (1975). One ml of culture was mixed with 9 ml of starch solution (1.0% soluble starch in 0.02 M $NaHPO_4$ and 0.006 M NaCl as pH 6.9) and incubated for 1 h at 30 °C after which 1 ml of the filtrate/starch reaction mixture was transfered into a test tube. To this was added 3 ml of dinitrosalicylic acid (DNSA) reagent containing 1 g of DNSA, 20 ml of 2 M NaOH and 20 g of potassium acdium tartarate/100 ml of water. The mixture was boiled for 5 min in water bath at 100 °C and cooling under running tap water. The optical density was read at 540 nm using SP 600 spectrophotometer against a reagent blank. The reducing sugar released by the action of the culture filtrate on starch molecule was estimated quantitatively by reference to a standard curve constructed with different concentrations of maltose (0.1–2.0 mg/ml). One unit of amylase activity is defined here as that amount of enzyme catalyzing the formation of 1.0 mg of those reducing sugars detectable by colour formation with DNSA per 1 h at 30 °C. Each value is an average of three measurements from two separate experiments.

Results

Results in Table 1 show the mycelial production and amylase activity by *Rhizopus oryzae* culture media. Mycelial formation was more in the medium containing starch (SYE) than in the medium lacking starch (YE) and peak volue was obtained after 18 days of culture and the mycelial dry weight of the fungus reduced appreciably after this period. The maximum amylase activity was greater in SYE than in YE. Maximum enzyme activity was reached after 6 days for SYE and 8 days for YE. The amylase production by the organism after 20 days of culture in both SYE and YE culture media was significantly less than that attained after 6 days and 8 days for SYE and YE respectively. Maximum amylase activity was detected before the fungus had achieved maximum mycelial growth in both SYE and YE media.

Various concentrations of starch and yeast extract affected the mycelial growth and amylolytic enzyme activity (Table 2). There was no detectable amylase activity in medium containing only yeast extract but no starch eventhough a small amount of mycelial growth was recorded. With maximum concentration of yeast extract (4 g/l), almost

Table 1

Effect of culture media on the growth and amylase activity of *Rhizopus oryzae* after 20 d of culture at 30 °C

Days of incubation	Starch yeast extract			Yeast extract		
	MDW (mg)	Amylase unit	pH	MDW (mg)	Amylase unit	pH
2	25	0.19	5.6	10	0.05	5.7
4	79	0.56	5.9	52	0.07	5.6
6	84	0.76	5.5	63	0.07	5.7
8	86	0.69	5.5	65	0.10	5.8
10	102	0.66	6.0	75	0.05	6.4
12	106	0.43	6.2	75	0.03	6.2
14	109	0.36	7.1	76	0.01	6.9
16	114	0.21	7.1	78	0.00	6.7
18	120	0.18	7.2	82	0.00	7.4
20	89	0.10	7.6	51	0.00	6.6

Data are average of three replicates in two seperate experiments
MDW = Mycelial dry weight.

Table 2

Effect of various concentrations of starch and yeast extract in SYE medium of the growth and extracellular amylase activity of *Rhizopus oryzae*

Starch (g/l)	Yeast extract (g/l)	MDW (mg)	Amylase unit
0	1	0	0
0	2	12	0
0	4	14	0
3	0	24	0.44
3	1	35	0.38
3	2	42	0.41
3	4	44	0.48
5	0	53	0.50
5	1	36	0.46
5	2	48	0.49
5	4	93	0.52
10	0	78	0.85
10	1	46	0.65
10	2	87	0.86
10	4	188	1.00

Value are average of three replicates in two seperate experiments

Table 3

Growth and amylase activity of *Rhizopus oryzae* after 10 d of culture in SYE medium at different temperature and pH levels

	Temperature (°C)							
	5	10	15	20	25	30	35	40
Growth	0	21	54	78	94	107	74	36
Amylase unit	0	0.07	0.33	0.46	0.68	0.86	0.49	0.08

	pH						
	2	3	4	5	6	7	8
Growth	0	35	74	96	100	61	46
Amylase unit	0	0.25	0.53	0.65	0.88	0.55	0.30

Data are average of three replicates in two separate experiments

Table 4

Growth and amylase activity of *Rhizopus oryzae* when grown for 10 d at 30 °C on SYE medium supplemented with different carbon sources

Carbon source	MDW (mg)	Amylase unit
Maltose	103.4	0.77
Sucrose	110.8	0.75
Glucose	100.0	0.79
Fructose	100.0	0.78
Starch	107.0	1.02
Carboxy methyl cellulose (CMC)	84.6	0.68
Pectin	91.6	0.74
Control	78.6	0.59

Data are average of three replicates in two separate experiments

equal amylase activity resulted when the organism was supplied 3 and 5 g of starch whereas 10 g resulted in near doubling the enzyme activity. When yeast extract was absent, increasing the starch concentration from 3 to 10 g/l resulted in the increase of both mycelial biomass and amylase activity. When the concentration of starch was kept constant, increasing the concentration of the yeast extract resulted in an increase of mycelial biomass and amylase activity. Maximum mycelial growth and amylase activity occurred

when the maximum concentrations of a starch and yeast extract, 10 g/l and 4 g/l respectively were used. When the effects of temperature and pH on the growth and amylase activity of the fungus were determined, 30 °C and pH 6 were found to favour the maximum production of both mycelial biomass and amylase activity (Table 3). The growth and activity of the amylolytic enzyme decreased thereafter through the 10 days incubation period.

The fungus grew and produced amylase when incubated in media containing any of the seven carbon sources tested during the 10 days culture (Table 4). The amount of amylase produced by the organism was not related to the mycelial growth. The maximum amounts of amylase produced by the fungus were detected in extracellular filtrates containing starch whereas the maximum mycelial growth was recorded in the medium containing sucrose as the sole carbon source.

Discussion

Rhizopus oryzae grew and produced extracellular amylase more in cultures containing starch than those lacking in starch. Maximum mycelial activity was detected after 6 days for SYE and 8 days for YE (Table 1).This disparity in the production pattern of amylase by the fungus due to differences in substrate supplement, indicates that starch in necessary in the growth and extracellular amylase production by the fungus. Doubling of the concentration of a starch in the growth medium resulted in a near doubling of the amylase activity. Ogundero (1978) also came to this conclusion. It was observed in this investigation that the mycelial biomass of the fungus after 10 days of culture in both SYE and YE media was less than that attained after the 15 days, suggesting that autolysis occurred between 18 and 20 days of incubation.

The fungus produced extracellular amylase in culture medium containing starch and other carbon sources but the highest amount of amylolytic activity was detected in medium containing starch as the sole carbon source. The amylase of *R. oryzae* might be described as induced enzyme. The detection of induced and constitutive extracellular amylase in other pathogenic fungi had earlier been reported (Ogundero, 1978; Adams, 1976; Barnett and Fergus, 1971).

The growth and amylase production by the fungus were influenced by the temperature and pH of the culture medium. This investigation has shown that there was no correlation between the amount of growth and amylase production and that the pH and temperature of the medium were of primary factor regulating the growth and production of the enzyme. The results from this study suggest that amylase will accumulate in a culture medium that supports the growth of the fungus when the temperature and the pH of the medium is between 25–30 °C and 5–6 respectively.

The fungus could be of industrial use in some fermentation processes because of its prolific production of amylase at the early stage of incubation period in medium containing starch.

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Antagonism of *Trichoderma atroviride* and *Trichoderma viride* Strains against *Sclerotinia minor* as Influenced by Mancozeb, Benomyl and Vinclozolin

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The influence of fungicides on the antagonistic effect of four previously selected *Trichoderma* strains against *S. minor* was investigated *in vitro*. Benomyl hindered most strongly the mycelial growth of antagonists (EC_{50} between 0.1–1.0 mg litre^{-1}), followed by vinclozolin (EC_{50} between 1.0–10.0 mg litre^{-1}), and mancozeb (EC_{50} around 100.0 mg litre^{-1}). The inhibitory effect of the antagonists was significant at lower concentrations of fungicides, but it was masked by higher concentrations of benomyl and vinclozolin which fully inhibited the growth of *S. minor*. *Trichoderma* strains alone totally prevented the sclerotium formation of *S. minor*, while on fungicide-amended media more or less sclerotia were produced. Significantly higher number of sclerotia were counted on plates treated with *Trichoderma* and 1.0 mg litre^{-1} benomyl, because the antagonists were more sensitive to benomyl than *S. minor*. In addition to vinclozolin, mancozeb is also proposed for combined application with *Trichoderma* fungi against *S. minor*.

Reducing the amount of agricultural chemicals applied to the soil is an increasing trend nowadays (Cook, 1993). On the other hand, the protection of plants against soil borne pathogenic fungi, for example against *Sclerotinia minor* Jagger, a world-wide spread polyphagous pathogen (Purdy, 1979) requires high amount of fungicides for economical production (Steadman, 1979) which increases also the risk of development of fungicide tolerance (Waard, et al., 1993) beneath environmental pollution. The application of biocontrol agents with lower doses of compatible chemicals seems to be a promissory way (Papavizas, 1985; Chet, 1987; Cook, 1993).

Undoubtedly, the *Trichoderma* spp. are effective antagonists of *Sclerotinia minor* (Davet 1979; Imolehin and Grogan, 1980; Artigues and Davet, 1982; Vanacchi and Pechia, 1987; Naár and Kecskés, 1995). But the compatibility of antagonists with fungicides used in the chemical control of *S. minor*, is not yet clarified. Some works suggest that dicarboximide fungicides (e.g. vinclozolin) are not adverse to *Trichoderma* (e.g. Davet and Martin, 1985; Elad et al., 1993), thus, they are combinable with microbial antagonists. Benomyl, however, was found to be lelss toxic to *Sclerotium rolfsii* than to *Trichoderma*, the natural antagonist of this pathogenic fungus, therefore the application of this fungicide resulted in lower yield because of higher disease incidence (Backman and Roriguez-Kabana, 1977; Backman et al., 1975). Although the compatibility of some often used fungicides (e.g. benomyl, vinclozolin) with *Trichoderma* fungi were extensively studied, we have very few information about the compatibility of biocontrol agents with some other fungicides (e.g. dithiocarbamates) which are applied also in higher

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quantities in agricultural practice. Thus, there no data about the effect of mancozeb, an old, but effective dithiocarbamata fungicide on antagonistic activity of *Trichoderma* fungi against *S. minor*.

The present study was conducted to compare the effect of three different fungicides (mancozeb, benomyl, and vinclozolin) on the antagonism of selected *Trichoderma* strains on *S. minor* to select compatible combinations for further biocontrol studies.

Materials and Methods

Trichoderma strains

Two strains of *T. atroviride* Karsten (TK13 isolated from bark, TK14 from soil) and two strains of *T. viride* Pers. ex Gray (TK28 from compost, TK60 from sclerotium of *Sclerotinia sclerotiorum* Lib. de Bary) were investigated. They were chosen in previous tests according to their high parasitic activity on *S. minor* determined on water agar, in autoclaved and in natural soil (Naár and Kecskés, 1995). *Trichoderma* strains were maintained on 2% malt extract agar slants.

Sclerotinia minor strain

An isolate from a diseased chrysanthemum was applied as target organism for *Trichoderma* strains and fungicides. Phytopathogenic fungus was maintained on potato dextrose agar (PDA, Oxoid) slants.

Fungicides

Three fungicides with different modes of action were tested: benomyl (1-butylcarbamoyl-benzymidazole-2-methyl-carbamate) as Chinoin Fundazol 50 WP (a.i. 50%) mady by CHINOIN, Hungary, a frequent subject of fungicide tolerance tests of biocontrol agents (e.g. Papvizas et al., 1982; Hau and Beute, 1983; Adams and Wong, 1991), vinclozolin (3-(3,5-dichloro-phenyl)-5-methyl-vinyl-1,3-oxazolydin-2,4-dione) as Ronilan 50 WP (a.i. 50% of BASF AG (Germany), as one the most effective fungicides against *S. minor*, which provide little inhibition on non-target microorganisms in doses commonly applied (Brenneman et al., 1987; Smith et al., 1991); and mancozeb ((Mn + Zn)-ethylene-bis-dithiocarbamate) as Dithane-M 45 (a.i. 50%) of Rohm and Haas S. A. (France), which represented dithicarbamates, a rarely tested fungicide against biocontrol agents.

In vitro toxicity test

The toxicity of fungicides to *Trichoderma* strains and *S. minor* was determined as reduction of mycelial growth on fungicide-amended salty glucose-peptone agar (SGPA: NaCl – 5.0 g, peptone – 1.0 g, glucose – 5.0 g, yeast extract – 0.5 g, agar – 20.0 g in

1,000 cm^3 distilled water). Fungicides were suspended in sterile, distilled water. Desired quantities of these stock solutions were pipetted into flasks containing an appropriate volume of cooled (about 40 °C) SGPA. The final concentrations were 0.1, 1.0, 10.0 and 100.0 mg a.i. $litre^{-1}$ by each fungicide. The content of flasks was stirred during the addition of the fungicide and poured into Petri dishes (100 mm diam.). Solidified media were inoculated with 5 mm diam. mycelial agar plugs taken from the periphery of intensively growing colonies of fungi. An antagonist and *S. minor* were seeded on the opposite sides of plates 1 cm distance from the edge. Two controls were used: antagonist + *S. minor* and *S. minor* alone both on non-amended medium. Dishes were sealed with plastic trips to prevent loss of volatile metabolites. Incubation was carried out at 25 °C in the dark. Radial growth both of *Trichoderma* and *S. minor* was measured after 2 days of incubation. Changes in sporulation of *Trichoderma* was observed microscopically when it was needed. Reproduction of phytopathogenic fungus was determined by counting the number of sclerotia produced per plate after two weeks. Three replicates were used, ant two separate tests were performed with similar results.

Results and Discussion

Toxicity of fungicides to Trichoderma *strains*

Tested fungicides differed considerably as to the extent of the inhibitory effect on *Trichoderma* strains (Table 1). The radial growth of antagonists was most strongly hindered by benomyl of which EC_{50} value proved to be between 0.1–1.0 mg $litre^{-1}$. It was in accordance with the results of Papavizas et al. (1982), who found that value 0.5 mg $litre^{-1}$ for a wild type strain. Vinclozolin showed a moderate effect having the EC_{50} value between 1.0–10.0 mg $litre^{-1}$. To obtain vinclozolin tolerant *Trichoderma* isolates, Abd-El Moity et al. (1982) began the exposure of a wild strain at 5.0 mg $litre^{-1}$, which caused a medium growth inhibition. Davet and Martin (1985) proposed the application of vinclozolin in a *Trichoderma*-selective medium at 2.5 mg $litre^{-1}$ concentration providing less inhibitory effect for *Trichoderma* fungi than for other ones. Even lower reduction of mycelial growth of antagonists was caused by mancozeb of which EC_{50} value proved to be around or slightly above 100 mg $litre^{-1}$, depending on strain. Thus, the rank of fungicides regarding their increasing toxicity for *Trichoderma* fungi was a follows: mancozeb < vinclozolin < benomyl. From the tested fungicides, benomyl is not proposed for combination with wild type *Trichoderma* fungi because its EC_{50} value for antagonists was markedly lower than 0.1% (dose used in agricultural practice). Benomyl-tolerant mutants would be very effective in combinations (Abd-El Moity et al., 1982), but its release in the field has not been allowed by laws till now.

Trichoderma strains significantly ($P = 0.05$) differed in their fungicide sensitivity as it was showed by different colony sizes at the same fungicide treatments. Mancozeb had little effect on the *T. viride* TK60 strain at concentrations lower than 100.0 mg $litre^{-1}$, while other strains were deterred in 22.0–33.9% as low as 10.0 mg $litre^{-1}$. *T. atroviride*

Table 1

Inhibitory effect of fungicides on mycelial growth of *Trichoderma* strains*

Fungicide	Concentration [mg litre^{-1}]	*Trichoderma* strains**			
		TK13	TK14	TK28	TK60
Mancozeb	0.1	-1.8^{ab}	10.1^{a}	2.8^{ab}	6.0^{bc}
	1.0	4.8^{bc}	21.7^{b}	2.8^{ab}	9.7^{b}
	10.0	22.0^{d}	33.9^{c}	23.2^{c}	7.4^{bc}
	100.0	51.2^{f}	56.6^{d}	45.3^{d}	37.3^{d}
Benomyl	0.1	8.3^{c}	21.2^{b}	6.6^{b}	-0.8^{ab}
	1.0	87.5^{g}	93.1^{e}	79.5^{e}	98.5^{e}
	10.0	100.0^{h}	100.0^{h}	100.0^{h}	100.0^{h}
	100.0	100.0^{h}	100.0^{h}	100.0^{h}	100.0^{h}
Vinclozolin	0.1	-3.0^{a}	9.0^{a}	-2.8^{a}	-8.2^{a}
	1.0	29.2^{e}	39.1^{c}	25.4^{c}	9.7^{c}
	10.0	100.0^{h}	100.0^{h}	100.0^{h}	100.0^{h}
	100.0	100.0^{h}	100.0^{h}	100.0^{h}	100.0^{h}

* Values are percent of radial growth reduction rated to non-amended control; negative values show some, but not significant stimulation

** Values followed by the same letter do not differ significantly (P = 0.05) in a column

TK14 strain proved to be the most sensitive to all three fungicides. In some cases, slight stimulations were observed at the lowest dose of chemicals. *T. atroviride* TK14 strain was the only exception, which growth was neither accelerated. One of four *Trichoderma* strains (*T. viride* TK60) could be adapted to benomyl in one week. Some mycelial growth of this strain was observed in sectors, which could not sporulate even after two weeks. Although 10 and 100 mg litre^{-1} of vinclozolin completely inhibited the growth of antagonists in two days, the inoculum was not killed, as indicated it the appearance of some white mycelium on agar plugs after one week incubation. This mycelium did not grow onto fungicide amended medium, and did not become green (did not sporulate), even after two weeks.

Joint effect of Trichoderma *strains and fungicides on the growth of* S. minor

Colony size of *S. minor* measured after two days of incubation (before mecrobes grew together) was significantly larger in control without *Trichoderma* and fungicide than in the presence of antagonists (Table 2). No deformation of *S. minor* colonies was observed, thus the growth reduction may be explained by effect of volatile metabolites of *Trichoderma* fungi. A volatile metabolite, 6-n-pentil-2H-piran-on (active against wide spectrum of fungi and have a typical coconut-smell) might account for this inhibitory

Table 2

Inhibitory effect of fungicides and *Trichoderma* strains on mycelial growth of *S. minor**

Fungicide	Concentration [mg litre^{-1}]	*Trichoderma* strains**			
		TK13	TK14	TK28	TK60
Control***		12.0^{a}	21.5^{cde}	12.3^{a}	9.8^{a}
Mancozeb	0.1	28.2^{f}	17.7^{bc}	24.7^{def}	26.3^{efc}
	1.0	21.5^{cde}	28.2^{f}	19.9^{cd}	19.9^{cd}
	10.0	44.0^{g}	42.1^{g}	51.9^{h}	51.6^{h}
	100.0	88.3^{k}	93.0^{n}	93.7^{n}	86.7^{kl}
Benomyl	0.1	29.4^{f}	14.6^{ab}	17.7^{bc}	11.4^{a}
	1.0	78.5^{j}	72.2^{i}	84.2^{k}	78.8^{j}
	10.0	100.0^{o}	100.0^{o}	100.0^{o}	100.0^{o}
	100.0	100.0^{o}	100.0^{o}	100.0^{o}	100.0^{o}
Vinclozolin	0.1	88.3^{klm}	89.6^{lmn}	88.3^{klm}	89.6^{lm}
	1.0	100.0^{o}	100.0^{o}	100.0^{o}	100.0^{o}
	10.0	100.0^{o}	100.0^{o}	100.0^{o}	100.0^{o}
	100.0	100.0^{o}	100.0^{o}	100.0^{o}	100.0^{o}

* Values are percent of radial growth reduction rated to non-amended control treatment: *S. minor* alone at the same conditions

** Values followed by the same letter do not differ significantly (P = 0.05)

*** Radial growth of *S. minor* in dual culture with *Trichoderma* on non-amended medium

effect because the production of this compound is characteristic for most *T. viride* strains (Ghisalberti and Sivasithamparam, 1991) and both tested *T. atroviride* strains produced that coconut-smell, too. Vanacci and Pecchia (1987) found a similar rate of inhibition of *S. minor* by *Trichoderma* fungi (12–21%) which was slightly exceeded by our strongest antagonist *T. atroviride* TK14. This strain has been able also parasitize aggressively the propagula (both sclerotium and mycelium) of *S. minor* (Naár and Kecskés, 1995), thus it was treated as an excellent antagonist of this phytopathogenic fungus.

Compared to the non-amended control, fungicides increased the reduction of growth of *S. minor* by *Trichoderma* fungi (Table 2.) The effect of antagonists was considerable in all combinations at which the phytopathogenic fungus developed more or less colonies as it was indicated by significantly different *S. minor* colony sizes observed by different strains at the same fungicide treatment. The only exception was 0.1 mg litre^{-1} vinclozolin of which combination with any of *Trichoderma* strains gave the same result. The sensitivity of antagonists to this fungicide dose was also the same, whereas they responded differently to other fungicide treatments which allowed their growth. These findings suggested that the different sensitivity of *Trichoderma* strains might account for

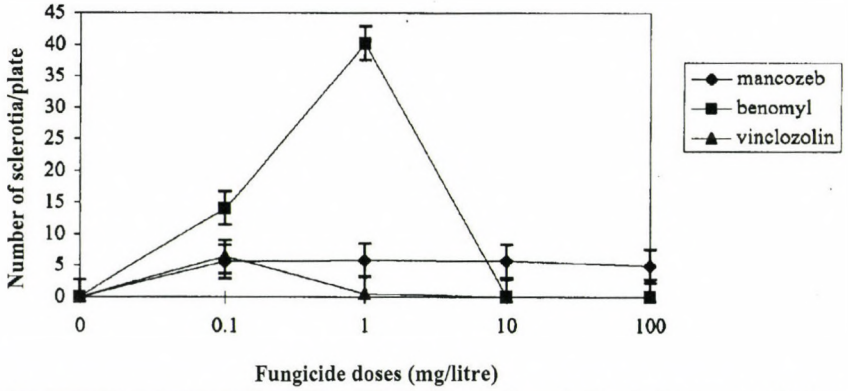

Fig. 1. Influence of fungicides on the number of *S. minor* sclerotia production in the presence of *Trichoderma* fungi

different extent of inhibitory effect of *Trichoderma* + fungicide combinations. However, no clear correlation was revealed between sensitivity and changes in inhibitory effect as influenced by fungicides indicating that the fungicide sensitivity test of biocontrol agents alone may not be enough to assess the compatibility against a target microbe. The influence of fungicides during antagonistic action of biocontrol agents needed also to investigate.

Reproduction ability of *S. minor* determined as number of sclerotia per agar plate was also strongly influenced both by antagonists and fungicides. No sclerotia was observed by any of tested strains in non-amended control which confirmed the previous selection of these *Trichoderma* strains (Naár and Kecskés, 1995). In contrast to their different inhibitory effect on mycelial growth of *S. minor* in fungicide combinations, statistically the same number of sclerotia was developed in the presence of any *Trichoderma* strains at a given fungicide treatment. Therefore, data were processed in ANOVA only by fungicide treatments and the result is demonstrated by Figure 1. Generally, fungicides decreased the inhibitory effect of antagonists on sclerotium development because only those combinations prevented totally the sclerotium formation which fully blocked the vegetative growth of *S. minor*. Exceptionally high number of sclerotia was found in combinations with 1.0 mg litre^{-1} benomyl which was more toxic to *Trichoderma* strains than to phytopathogenic fungus.

Regarding of sensitivity of *Trichoderma* strains to fungicides and their combined effect on the development of *S. minor*, vinclozolin was chosen for further studies as compatible chemical, however, mancozeb may be also useful when a broad spectrum fungicide is needed in the plant protection program.

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Chestnut Blight *Cryphonectria parasitica* (Murr.) Barr and its Biological Control in Hungary

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During the last few years destruction in Hungarian chestnut (*Castanea sativa* Mill.) populations by the fungus chestnut blight [*Cryphonectria parasitica* (Murr.) Barr] has increased. One main aspect of this research is to use hypovirulent strains of the pathogen as a biological control agent against this parasite. A total of 36 strains of this pathogen (from 19 observed chestnut stands, in south- and west-Hungary) were examined for laccase activity, virulence, classification of vegetative compatibility groups (VCGs) and for the presence of hipovirulence associated dsRNA.

Up to now 13 VCGs were found in Hungarian chestnut stands. Long dsRNA fragments (with 6.2×10^6 molecular weight) were found in the cytoplasm of 10 Hungarian hypovirulent strains. These dsRNAs were also similar in their electropohoretic characteristics. These hypovirulent strains demonstrated reduced virulence and no significant correlation between hypovirulence and low laccase activity was observed. Virulence of each strains was analyzed using two different types of virulence tests. Both investigations were found to be efficient methods for testing *in vitro* virulence of different *C. parasitica* strains.

The chestnut tree blight fungus *Cryphonectria parasitica* (Murr.) Barr [syn.: *Endothia parasitica* (Murr.) Anderson] caused almost total destruction of the American (*Castanea dentata*) populations after its introduction into the United States in the beginning of this century (Anagnostakis, 1987). This pathogenic fungus was reported first in Europe in an Italian chestnut forest near Genova, in 1938. A similar epidemic and destruction in European chestnut (*Castanea sativa*) stands was observed (Biraghi, 1946). Once established in a wound the fungus invades and destroys the surrounding bark and cambial tissue, forming a visible sunken canker. Death of the cambium of this ring-porous tree prevents formation of the xylem vessels needed for liquid transport, and this causes wilting of the leaves above (beyond) the canker. European chestnut trees generally with one or more cankers, die within 1 or 2 years after cankers are apparent.

In many regions (such as in Italy, France and Switzerland) spontaneous recovery was reported and chestnut stands recovered from the blight by producing naturally calluse "healing" cankers. This has been attributed to the natural occurrence of hypovirulent *C. parasitica* strains (Grente, 1965).

Hypovirulent strains of *C. parasitica* carry double-stranded RNA viruses, located in the cytoplasm of the fungal cells. They are classified as a *Cryphonectria hypovirus* recently (Hillman et al., 1995) in the genomes of a novel family of nonencapsidated viruses. Hypovirulent strains produce nonlethal, superficial cankers that are restricted to the outer parts of the bark and do not destroy the vascular cambium of the host. Beside reducing fungal virulence, *Cryphonectria hypovirus* affects other fungal features, such as

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sporulation and pigmentation (Hillman et al., 1990) laccase and cutinase expression (Rigling et al., 1989), and other proteins and mRNAs normally produced (Powell and Van Alfen, 1987). Acquisition of dsRNA often affects the fungus cultural phenotype, resulting in slow growth, lobate margins or reduced aerial mycelium. The hypovirus can be transmitted via hyphal anastomosis, resulting in conversion of the recipient one to the hypovirulent morphology.

Transmission of the hypovirus among fungal strains is controlled by the vegetative compatibility system, involving five to seven vc-loci (Anagnostakis, 1977). Compatible fungal strains which have the same alleles at all vc-loci are in the same vegetative compatibility group (VCG). The hypovirus is transmitted among strains belonging to the same VCG (Anagnostakis and Wagonner, 1981). Transfer of the hypovirus can also happen between strains belonging to different VCGs, but more slowly and at lower rates (Kuhlman et al., 1984).

Sexual recombination in the fungal genome is the main source of VCG diversity. Two mating type alleles (MAT1 and MAT2) at a single locus control sexual compatibility in *C. parasitica* (Anagnostakis, 1988). Sexual recombination takes place between strains carrying different alleles at the mating type locus. Both sexual and asexual reproductions take place in populations of *C. parsitica* (Anagnostakis and Kranz, 1987). In a population with predominantly sexual reproduction, a mating type ratio 1:1 is expected. Sexual reproduction in populations of chestnut blight has a negative impact on the natural dissemination of hypovirulence, because ascospores do not carry the hypovirus (Chen et al., 1993). If the mating type ratio is not 1:1 then asexual reproduction could play a major role in the fungal population and realize the spread of the mycovirus that causes hypovirulence. Therefore the population structure, with special regards to VCGs and mating types, is an important factor for the success of hypovirulence in chestnut blight fungus populations.

The chestnut blight fungus threatens chestnut stands of Central Europe, including Austria (Donaubauer, 1964), Hungary (Körtvély, 1970) and Slovakia (Juhasova, 1992). Hungarian chestnut stands have already been seriously damaged by this parasite. The presence of hypovirulence in Hungary has not been reported up to now. One main aspect of this research: is the application of hypovirulent fungal strains from different Hungarian regions, including extensive collection and sampling of fungal isolates, physiological investigations, determination of VCGs and analysis for presence of dsRNA.

Materials and Methods

Isolation and characterization of C. parasitica strains

Bark samples were removed with sterilized cork borer (5 mm in diameter) from the 300 randomly selected cankers. Within 24 hours all bark samples were surface strerilized in 70% ethanol, washed in steril, destillated water and placed on Difco PDA-medium at 25 °C. After some days small agar blocks with visible hyphae were removed

and grown on PDA supplied with L-methionine (100 mg/l) and biotin (1 mg/l) (PDAmb) as described by Anagnostakis and Day (1979) in 15 × 100 mm Petri dishes with 25 ml of medium/dish.

One *C. parasitica* isolate per canker was randomly selected for futher analysis. All isolates were stored on slants of PDAmb at 4 °C in the dark.

Culture phenotype

Observations of *C. parasitica* isolates morphology were made on PDAmb. Plates were inoculated with 6 mm plugs of mycelia removed from the margins of 7 day-old cultures with sterile cork borer. Two diagnostic criteria were used in this study to test for virus infection of *C. parasitica*:

– the white culture type and,

– the transmissibility of the white culture type to an orange "normal" strain.

The white cultural appearance of *C. parasitica* is correlated with the infection of the fungus by the *Cryphonectria hypovirus*. All isolates were grown on PDAmb plates at 25 °C in the dark for 7 days, followed by incubation under daylight at room temperature for another 7 days. Under these conditions, virus-free isolates produced visible orange pigments, whereas virus-infected isolates remained white.

Each white strain was tested for its ability to convert "normal" orange strains during the course of the vegetative compatibility tests.

Vegetative compatibility

The fungal strains were paired on PDAmb (Anagnostakis and Day, 1979) and PDA supplemented with 7 g/l malt extract, 2 g/l yeast extract, 0.8 g/l tannic acid, 0.1 g/l L-methionine, 1 mg/l biotin, 2 mg/l thiamine, 5 g/l extra agar and 50 mg/l bromocresol green (Powell, 1995). Agar plugs containing mycelia were removed from the margins of 7 day-old cultures and were paired 3 mm apart on the two media for week photoperiods as described above.

Vegetative compatibility was assesed according to the marging/barrage response (Anagnostakis, 1977). Merging of the two colonies indicated compatible strains, whereas the formulation of a barrage zone between the colonies, indicated incompatibility. Vegetative incompatibility and mycelial death of *C. parasitica* was detected with a pH indicator (bromocresol green) in the PDA-Powell media. Compatibility pairings were analyzed by Systat statistical software and dendrograms also generated. All pairings were repeated twice.

Laccase activity

Laccase activity of the *C. parasitica* strains were examined by growing the isolates on agar containing malt extract (3%) and tannic acid (2%) [Bavendamm-test, modified by Rigling et al. (1989)] Four replicates per strain were inoculated onto individual

Petri-dishes and incubated at 25 °C in the dark. Nine days after inoculation, individual plates were back-lighted with a fluorescent lightbox. Media color was grouped in three classes such as: low, medium and high laccase activity.

Virulence assays

Virulence was assessed by inoculation of Granny Smith apple fruits using Elliston's method (1985). Each apple was surface sterilized by 70% ethanol and the skin at 5 mm in diameter was removed by using a cork borer. Each fruit was inoculated (PDA plugs containing mycelium) with EP155 and 3 additional isolates were chosen at random. Apples were incubated in sterilized transparent plastic storage boxes in daylight (not direct sun) at room temperature. Nine and 15 days after inoculations the diameter of each wound was measured along two axes. Radial growth was also expressed as a percentage of the radial growth of EP155.

The virulence of the *C. parasitica* strains was also tested on dormant chestnut sticks using Figl's method (1991) with a minor modification. The surface of the sticks were desinfected by rinsing with NaOCl (approx. conc.: 10%). The bark was removed by using a cork borer (diam.: 5 mm). PDA plugs in the same size containing mycelium were put on the bark wounds (infection sites). The sticks were stored in plastic, sterilized containers supplied with sections that prevented the sticks to get in touch with each other. The test was performed at room temperature in the dark. After 15 and 30 days of the inoculations each lesion was measured by using a planimeter. These tests were repeated twice.

Mating type tests

All strains were crossed with each of the two mating type testers EP146 (MAT1) and EP155 (MAT2) of *C. parasitica* on sterilized pieces of *Castanea sativa* placed on PDAmb media as described by Anagnostakis (1988). Isolates were inoculated with the mating tester (one strain on each site of the stem) and incubated at 25 °C under 16 h photoperiod for 14 days. Sterile destillated water was added to the plots and the conidia produced by both strains were suspended and distributed over the stem to indicate mating. Plates were incubated at 18 °C under 8 h photoperiod then examined for the presence of perithecia following 2, 3 and 4 months the suspension. Each cross was performed twice.

Detection of dsRNA

DsRNA was isolated from 7 to 10 day-old cultures esentially as described by Morris and Dodds (1979). Quality and quantity of dsRNA from each isolate was assessed by agarose gel electrophoresis. The dsRNA was electrophoresed using 1.2% agarose gels in 1 × TBE at 80 Volts. For visualisation ethidium bromide was used (0.25 mg/ml gel) and 300 nm UV-light. For photography Polaroid MP-4, black and white film was used.

The result was checked by using EP713 and EP155 as positive and negative control strains in each series and performing DNA digestion with DNAse (2 ml Promega DNAse enzyme + 400 ml DNAse digestion buffer/sample) on the eluates containing all types of nucleic acids of the extraction phase from the fungal tissue.

Results

Culture phenotype

The culture morphologies of Hungarian *C. parasitica* isolates are described in Table 1. Ten of the 300 isolates exhibited the white phenotype typical of other European hypovirulent strains. All the Hungarian hypovirulent isolates were white or white orange. DsRNA-containing isolates were easily distinguished from dsRNA-free isolates by their colony morphologies. The reduction of growth rates and aerial mycelium was evident in these Hungarian hypovirulent isolates.

Vegetative compatibility

The VCG of each Hungarian *C. parasitica* isolates collected since 1993 was determined. The test showed that there exist at least 13 VC-Groups in Hungary (Table 1 and Fig. 1). These 13 different VCGs were found in 19 plots. The three VCGs (*10, 19* and *36*) were codominant. A few VCGs were represented by only one or two isolates (*9, 11, 56* and *M919*). Four VCGs (*12, 19, 36* and *40*) including the most frequent ones, contained both orange and white isolates. Pairing between strains of VCG *12* and VCG *19* resulted in weak barrage reactions indicating overlappings in these groups (VCG-network).

The dendrogram in Fig. 2 was drawn from alignments of the results of the vegetative compatibility tests. It is one of the three possible trees. The other two were identical in their grouping of most of the investigated strains. An exception was Z2, V8, R5 and S2 which were varied slightly in grouppings.

Laccase activity

The laccase activity of the strains was constant in all replications. No exact correlation between hypovirulence and laccase activity was found. EP713, C2, R20, IH2 and G1 hypovirulent (dsRNA-containing) strains had medium activities. Few hypovirulent strains (S3 and G1) showed high activity, while some Hungarian virulent (dsRNA-free) strains such as: C10, ZG1, S2 and Z11 were laccase-negative but had normal virulence.

Table 1

Description of *Cryphonectria parasitica* strains

Strain	Origin/number of isolation plot[1]	Laccase activity[2]	Mating type[3]	DsRNA (yes/no)	Vc-groups for Hungarian strains[4]
EP713	USA	M	–	yes	–
EP155	USA	H	–	no	–
HN1	Germany	L	–	yes	–
A1	Hungary/1	M	1	no	*19*
A3	Hungary/1	H	2	no	*19*
B3	Hungary/2	H	1	no	*19*
BA1	Hungary/3	M	1	no	*9*
F2	Hungary/4	M	1	no	*19*
FS1	Hungary/5	H	2	no	*19*
FS4	Hungary/5	H	1	no	*13*
C2	Hungary/6	M	–	yes	*19*
C10	Hungary/6	L	1	no	*19*
CG3	Hungary/7	H	1	no	*19*
CG5	Hungary/7	H	1	no	*19*
CK2	Hungary/8	M	1	no	*10*
CK5	Hungary/8	H	1	no	*10*
V4	Hungary/9	L	1	yes	*12*
V8	Hungary/9	H	1	no	*12*
SZ1	Hungary/10	M	1	no	*10*
R2	Hungary/11	L	1	yes	*36*
R5	Hungary/11	L	1	yes	*36*
R6	Hungary/11	M	1	no	*36*
R9	Hungary/11	L	1	yes	*36*
R11	Hungary/11	L	1	yes	*36*
R20	Hungary/11	M	–	yes	*36*
N3	Hungary/12	M	1	no	*36*
ZG1	Hungary/13	L	2	no	*10*
S1	Hungary/14	M	1	no	*40*
S2	Hungary/14	L	1	no	*36*
S3	Hungary/14	H	2	yes	*40*
IH2	Hungary/15	M	2	yes	*56*
IH5	Hungary/15	M	1	no	*M779*
Z2	Hungary/16	M	1	no	*30*
Z6	Hungary/16	H	1	no	*19*
Z9	Hungary/16	H	2	no	*19*
Z11	Hungary/16	L	1	no	*30*
Z13	Hungary/16	M	1	no	*30*
P3	Hungary/17	M	1	no	*33*
G1	Hungary/18	H	1	yes	*11*
G4	Hungary/18	M	1	no	*M779*
NM3	Hungary/19	H	2	no	*M919*

[1]The numbers of isolation plots correspond to Fig. 1. (for Hungarian strains)

[2]The letters describe the extent of laccase activities: L – low; M – medium; H – high

[3]Strains EP713, EP155 and HN1 were not included in this test. The numbers describe mating types such as: 1-MAT1 and 2-MAT2. The strains C2 and R20 were incompatible with both testers

[4]The VCGs were classified according to the vegetative compatible tester strain from the collection of the Connecticut Agricultural Experiment Station, New Haven, USA. Strains EP713, EP155 and HN1 were not included in this test

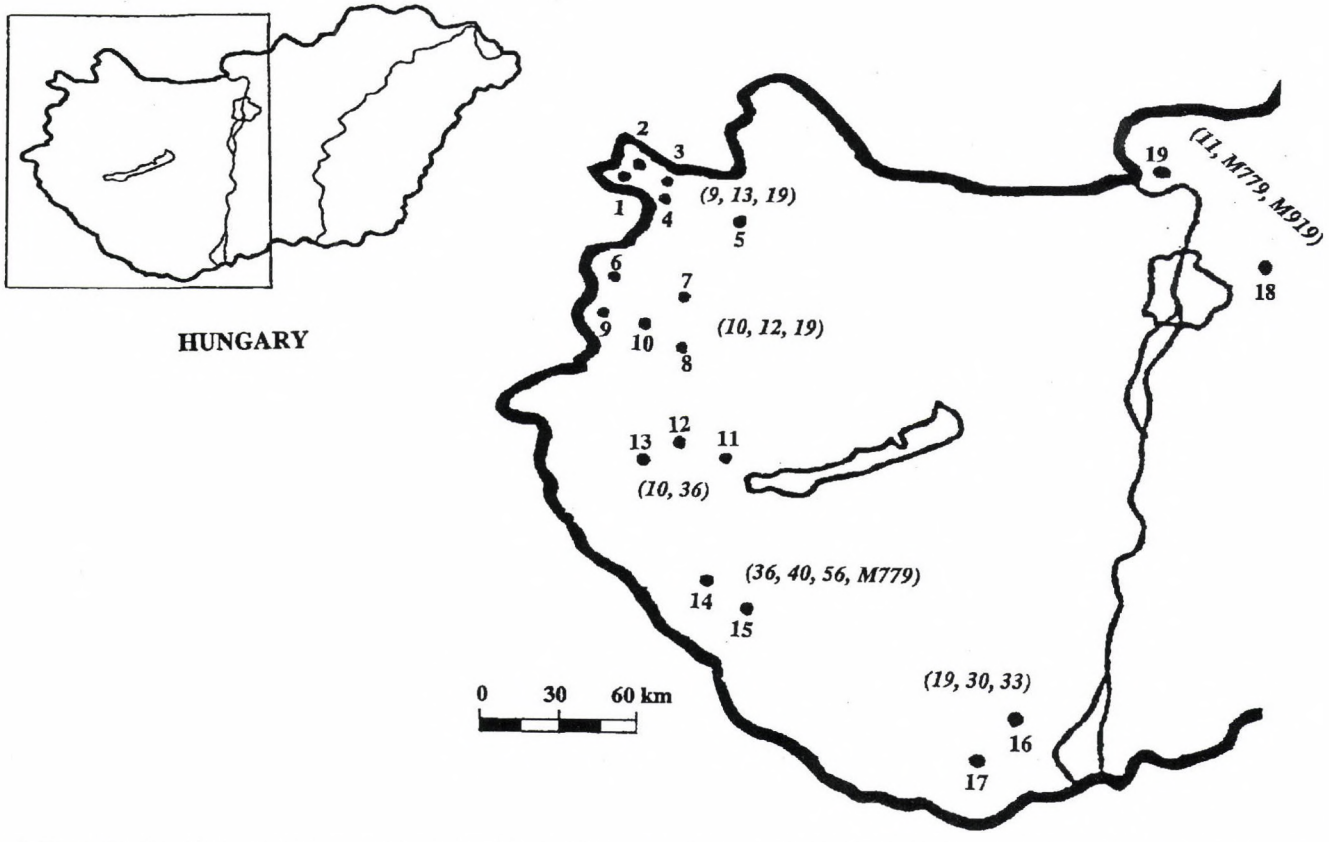

Fig. 1. Hungarian sites of Cryphonectria parasitica strain collection.VC -groups (9, 10, 11, 12, 13, 19, 30, 33, 36, 40, 56, M779, M919) and number of plots 1–19 same as in Table 1

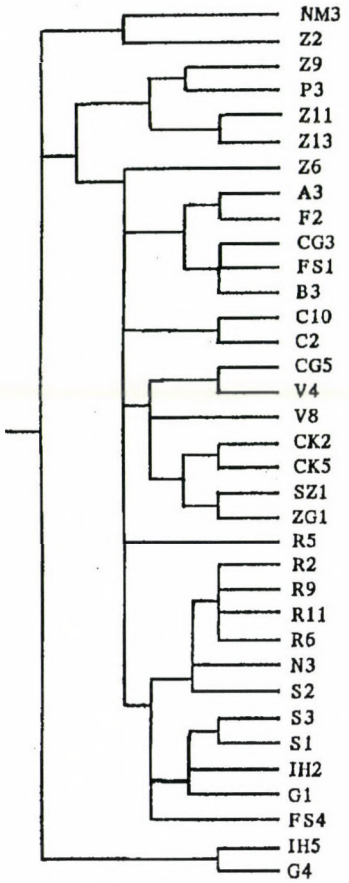

Fig. 2. Dendrogram of the 35 Hungarian strains of the fungus *Cryphonectria parasitica* (Compete linkage clustering was generated based on vegetative compatibility tests data)

Virulence assays

Average lesion size on Granny Smith apples and chestnut sticks, expressed as a percentage of the radial growth of EP155, ranged from 2.7% to 109.8% (Table 2 and Fig. 3). Data were transformed by taking the natural logarithm of the percent radial growth to correct for heterogenicity of sample variences. Hypovirulent (dsRNA-containing) isolates (number from 1 to 12) produced lesions that were significantly smaller ($P < 0.05$) than the virulent ones.

Table 2

Virulence of *Cryphonectria parasitica* strains on chestnut sticks and Granny Smith apple fruits

Number/ Strain[1]	Lesion area (cm^2)[2]	Relative virulence%[3]	Wound area (cm^2)[4]	Relative virulence%[3]
1/ EP713	0.3 (0.2)	2.7%	2.8 (0.8)	23.5%
2/ HN1	0.3 (0.1)	2.7%	4.5 (0.6)	37.8%
3/ C2	1.7 (0.4)	15.2%	5.0 (1.6)	42.0%
4/ R9	2.4 (0.8)	21.4%	1.8 (0.3)	15.1%
5/ IH2	2.9 (0.1)	25.9%	4.1 (1.5)	34.5%
6/ R20	3.2 (0.3)	28.6%	3.4 (0.7)	28.6%
7/ R2	3.6 (0.6)	32.1%	3.1 (0.7)	26.0%
8/ S3	3.6 (0.6)	32.1%	4.5 (0.3)	37.8%
9/ R5	3.8 (1.0)	33.9%	3.5 (0.9)	29.4%
10/ R11	5.5 (0.7)	49.1%	4.8 (0.8)	40.3%
11/ V4	6.3 (1.3)	56.3%	6.2 (0.7)	52.1%
12/ G1	6.5 (0.6)	58.0%	5.5 (0.4)	46.2%
13/ Z2	7.3 (0.7)	65.2%	10.3 (0.6)	86.6%
14/ SZ1	7.9 (0.6)	70.5%	9.5 (1.2)	79.8%
15/ Z9	8.3 (1.3)	74.1%	9.3 (1.2)	78.2%
16/ CK5	8.4 (0.9)	75.0%	9.9 (0.8)	83.2%
17/ R6	8.6 (0.6)	76.8%	7.7 (0.2)	64.7%
18/ CG5	8.7 (1.8)	77.7%	9.7 (0.6)	85.5%
19/ P3	8.8 (0.4)	78.6%	7.9 (0.3)	66.4%
20/ IH5	8.9 (1.3)	79.5%	9.9 (0.8)	83.2%
21/ C10	9.1 (0.7)	81.3%	9.1 (0.3)	76.5%
22/ B3	9.3 (0.8)	83.0%	8.6 (1.3)	72.3%
23/ S1	9.5 (0.5)	84.8%	7.3 (0.9)	61.3%
24/ N3	9.6 (1.8)	85.7%	9.1 (0.9)	76.5%
25/ Z11	9.8 (1.0)	87.5%	8.6 (0.7)	72.3%
26/ V8	9.9 (0.9)	88.4%	6.7 (1.3)	56.3%
27/ Z13	10.6 (1.6)	94.6%	8.9 (1.5)	74.8%
28/ FS4	10.7 (0.8)	95.5%	10.3 (1.1)	86.6%
29/ A3	10.7 (1.2)	95.5%	11.1 (0.7)	93.3%
30/ G4	10.7 (1.2)	95.5%	11.3 (1.3)	95.0%
31/ Z6	11.1 (0.9)	99.1%	11.8 (1.8)	99.2%
32/ EP155	11.2 (0.9)	100.0%	11.9 (1.5)	100.0%
33/ CG3	11.8 (2.5)	105.4%	10.3 (1.3)	86.6%
34/ ZG1	12.1 (2.9)	108.0%	8.9 (0.3)	74.8%
35/ CK2	12.2 (1.3)	108.9%	10.3 (1.1)	86.6%
36/ NM3	12.3 (2.3)	109.8%	10.8 (1.7)	90.8%

[1] Numbers correspond to Fig. 3.

[2] Average untransformed size of lesions on dormant chestnut sticks, 30 days postinoculation. Standard deviation is written in brackets

[3] Expressed as a percentage of the radial growth of virulent control strain EP155

[4] Average untransformed size of wounds on Granny Smith apple fruits, 15 days postinoculation. Standard deviation is written in brackets

1- EP713
2- HN1
3- C2
4- R9
5- IH2
6- R20
7- R2
8- S3
9- R5
10- R11
11- V4
12- G1
13- Z2
14- SZ1
15- Z9
16- CK5
17- R6
18- CG5
19- P3
20- IH5
21- C10
22- B3
23- S1
24- N3
25- Z11
26- V8
27- Z13
28- FS4
29- A3
30- G4
31- Z6
32- EP155
33- CG3
34- ZG1
35- CK2
36- NM3

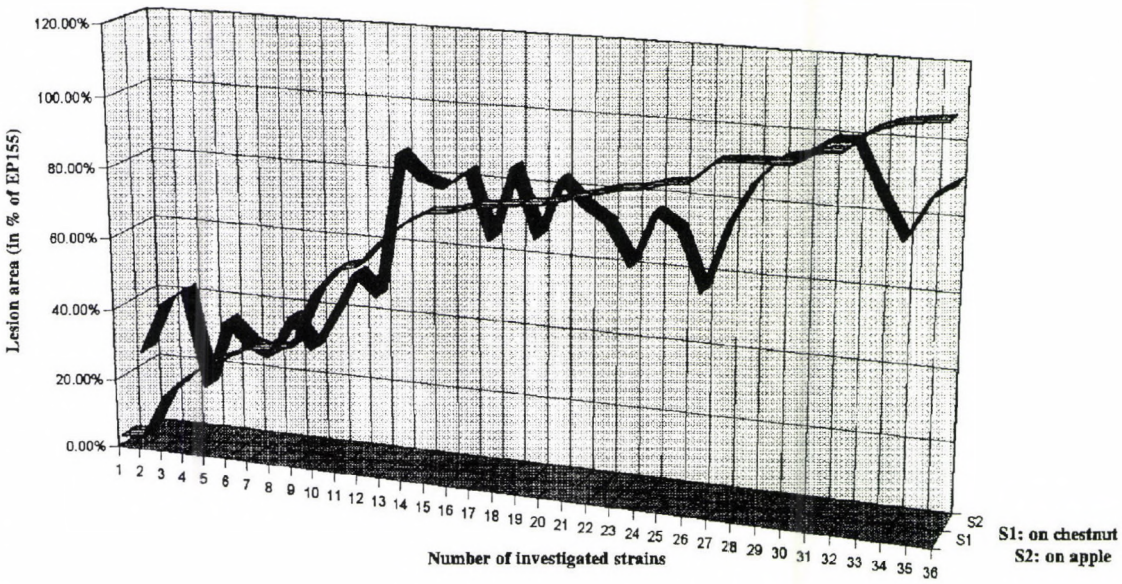

Fig. 3. Relative virulence of *Cryphonectria parasitica strains*. Number and relative virulence% of the strains correspond to Table 2. Series 1: relative virulence of the fungal strains on chestnut sticks. Series 2: relative virulence of the fungal strains on apple fruits

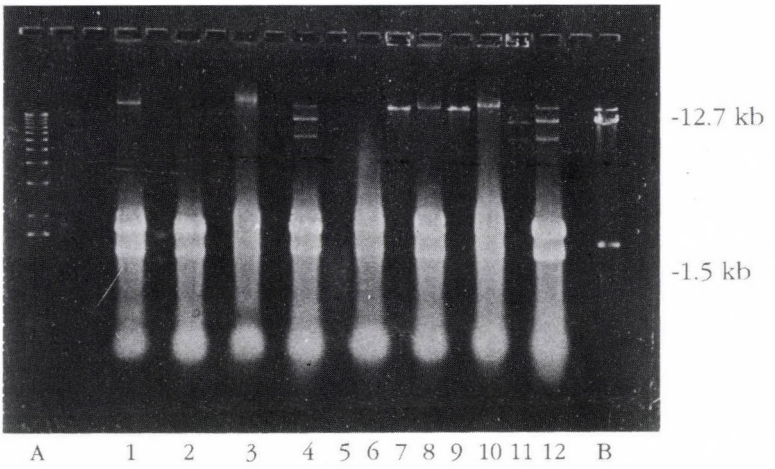

Fig. 4. Ethidium bromide stained gel of 10 double-stranded (dsRNA) containing Hungarian strains of the fungus *Cryphonectria parasitica*. DsRNAs were isolated as described in the text from the following fungal strains; Lanes from left to right: A-1kb DNA-ladder, 1-C2, 2-V4, 3-R2, 4-R11, 5 and 6-EP155 (negative control), 7-G1, 8-S3, 9-IH2, 10-R5, 11-R9, 12-R20 and B-EP713 (positive control). Samples of C2, V4, R2, R11, S3, R5, R20 in the lanes 1, 2, 3, 4, 8, 10, 12 were DNA-digested as it described in the text

Mating types

The distribution of the two mating types of *C. parasitica* among white and orange isolates is summarized in Table 1. The mating type of all orange- and more than 80% of all white strains were identified. One mating type (MAT1) was dominant in Hungarian chestnut stands. The two mating types were found in five test sites (Hungary 1, -5, -14, -15 and -16). So far self-fertilization was not observed among the Hungarian strains of chestnut blight fungus.

Detection of dsRNA

Ten Hungarian strains with atypical colony morphology and reduced virulence were considered to be potentially hypovirulent. After phenol extraction and gel electrophoresis, long dsRNAs (approx. 12.7 kb) were detected from the fungal tissue of each white, Hungarian strains (Fig. 4).

Discussion

This study represents the first molecular examination of the Hungarian populations of the chestnut blight fungus. Total of 13 VCGs were identified from the 19 study sites. Hungarian hypovirulent strains of *C. parasitica* (carrying dsRNA) showed differ-

ences in culture morphology, virulence and laccase activity. These white strains were detected at variable frequences in all major VCGs. This suggest that vegetative incompatibility is probably not a major barrier for the natural dissemination of the *Cryphonectria hypovirus* in Hungary.

During the course of our 4 year-study we found an increase of vegetative compatibility diversity and the total number of VCGs. This might be because of (a) sexual recombination among local VCGs; (b) introduction of new VCGs, probably from neighbouring countries; or (c) mutations that generated new VC-types (Bissegger et al., 1997).

(a) The sexual reproduction producing ascospores which are the main source of genetic recombination of the vc-loci in the fungal genome. Much fewer VCGs have been reported from Europe than from North-America. This is explained by low vc-polymorphism and limited sexual progeny (Heiniger and Rigling, 1994).

(b) VC-Groups *10, 19* and *36* are the most frequent ones in Hungary. They also represent the most widespread groups in the test sites. It may therefore be possible that strains belonging to VCGs *19* and *36* (Italian related) were probably the first to attack Hungarian chestnut stands. VC-Groups *9* and *13* (Austrian and Swiss related) are represented by very few strains and could be detected only from the western part of the country. This observation suggest that two different waves of the pathogen entered Hungary, from the south (earlier-around 1970s) and from west (later-around 1980s). As sampling of chestnut blight cankers was random, the number of VC-Groups determined in this study cannot be used as an unequivocal state. An international classification system for the *C. parasitica* VCGs would contribute considerably to these observations.

(c) Instability of VCGs was observed in *C. parsitica* after ultraviolet treatment (Rizwana and Powell, 1992). However it is not known if this phenomenon occurs under natural conditions and in virus-infected fungal strains.

Although the role of cytoplasmic dsRNA in the fungal physiology is unclear, laccase activity is usally reduced in hypovirulent strains (Rigling, 1995). Rigling's hypothesis that hypovirulence resulted in reduced phenoloxidase activity was partly confirmed. Although the low laccase activity was significantly more frequent in hypovirulent – than in virulent strains, there was no correlation between virulence and laccase activity among hypovirulent strains. These support the same observations by Choi and Nuss (1992) and Wronski et al. (1997). However C10, ZG1, S2 and Z11 had low laccase activities and normal virulence, demonstrating that hypovirulence is not always correlated with low laccase activity (Table 1).

In addition to VCG, we also included the mating type as a phenotypic marker to analyze the fungal populations. The two mating types were found in five Hungarian plots (Hungary 1, -5, -14, -15 and -16) suggesting that sexual reproduction is occurring in these populations. In fact perithecia the sexual fruiting bodies of *C. parasitica* have been observed, but they were rare (Radócz et al., 1997). Isolates sexually compatible with both mating type testers (self-fertilization), were not found among Hungarian strains. This phenomenon may be the result of heterokaryosis (Anagnostakis, 1981) or mixed cultures. Self-fertilization was found in the United States and in Switzerland. Current investiga-

tions suggest that self-fertilized perithecia produce both mating types in a 1:1 ratio, and do not affect the mating type ratio in a fungal population (Bissegger et al., 1997).

The Hungarian strains which produced lesions larger than 60% of the virulent control (EP155) were classified as virulent ones. We found both methods (on chestnut sticks and on apples) were good tools to detect reduced virulence of the fungal strains under study. Virulence tests on apple fruits are faster but could be gained more accurate data, especially about the virulence of the hypovirulent strains, by inoculating chestnut sticks.

Elliston (1985) noted that hypovirulence-associated phenotypes exhibited by *C. parasitica* isolates are most dependent on dsRNAs rather than host genotype. Studies by L'Hostis et al. (1985) demonstrate the similarity of European hypovirulent isolates to each other and the differences from North-American strains used in the study. Examination of Hungarian hypovirulent strains confirms these observations. Hungarian hypovirulent strains showed homogenicity in electrophoretic characteristics of their dsRNAs and similarity in their effect on the phenotype of the host fungus.

The European chestnut blight epidemic is still not fully understood. Hypovirulence is a potentially important factor in limiting destruction in European chestnut populations and the control of virulent cankers caused by the fungus chestnut blight. Observations of genetic diversity and population dynamics of the fungus are therefore important in European chestnut blight populations. In some countries (like France, Italy and Slovakia) mixtures of hypovirulent strains from different VCGs (multiconverter-inocula) is used. The introduction of a new genotype of the fungus into small, scattered chestnut stands (with regularly one or two VCGs) could be potentially dangerous and requires continuous monitoring. There are also other studies reporting an increase in the number of VCGs in different European countries (Heiniger and Rigling, 1994). To obtain more accurate results in describing population structure and dynamics of *C. parasitica* cross-border collaboration is necessary. Because of its simplicity and suitability for large-scale investigations, the use of RAPD could be an excellent method for detecting genetic changes in the fungal as well as in mycoviral populations. It is also necessary to find primers for the molecular amplification which produce adequat markers for grouping *Cryphonectria parasitica* strains.

In several European countries (for example in France, Italy and Switzerland) and in the United States hypovirulence has been used as a biological control agent in the field in recent years. We hope that this type of control which has been shown to be both efficient and economical will fit in a program for the recovery of Hungarian chestnut trees from blight.

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Oxygen Consumption as an Indicator of Diapause Intensity in Pupae of *Lacanobia oleracea* and *Mamestra brassicae* Reared at Different Inductive Photoperiods

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The term 'diapause intensity' is often used as the expression of the length of 'diapause development' or diapause in itself under constant environmental conditions. In authors' opinion it should be applied first of all to the overall metabolic state or level of a diapausing insect. Since the rate of oxygen consumption reflects very well the basal metabolism of a dormant phase, it is also an appropriate indicator of diapause intensity. In present studies larvae of two noctuid species, *Lacanobia oleracea* and *Mamestra brassicae* were reared at constant 25 °C and at two diapause-inducing photoperiods, LD 14:10 and LD 12:12. The oxygen consumption of diapause pupae was measured manometrically with a Warburg respirometer at 25 °C. In both species it was demonstrated that daylength had a positive influence on the rate of respiration. In pupae reared in the whole period of larval development at the shorter daylength (LD 12:12), significantly lower rates of oxygen consumption could be detected than in the other group of diapausing pupae raised at LD 14:10. Therefore, it seems that in the sensitive period of larval development photoperiods 'preprogram' somehow, possibly via endocrine mechanisms, the intensity of pupal diapause.

Most authors use the term 'diapause intensity' for the rate of diapause completion or for the duration of so-called 'diapause development', a complex physiological process necessary for the termination of a dormant state (Danks, 1987; Beck, 1989). Some of them emphasize the significance of prediapause developmental conditions which not only determine the incidence of diapause but may 'preprogram' also its intensity. From ecological factors photoperiod (Beck et al., 1963; McLeod and Beck, 1963) and temperature (Sieber and Benz, 1980; Denlinger and Bradfield, 1981) have been found to influence also the length of diapause under constant conditions. It has been demonstrated that longer exposition to inductive factors results in more intense dormancy (Tauber and Tauber, 1970; Bodnaryk, 1977). Genetical control of diapause intensity also occurs in other species (Sims, 1983).

It is well known that the progressive suppression of endocrine functions during diapause is connected with lowered metabolic rate (Harvey, 1962). As a consequence, diapause metabolism is merely 10 to 20% of the level recorded in the non-dormant state of the same developmental stage of an insect species (Danks, 1987). Depression of the rate of oxygen consumption has been reported as one of the most typical physiological changes associated with the 'intensification' of diapause (Tauber et al., 1986). We are convinced that the respiratory activity of an otherwise inactive, practically immobile dormant insect as a diapausing pupa, is a characteristic feature, reliable indicator of the depth, strength or – simply – the intensity of diapause. It is one of the best reflexion of

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basal metabolism which, in turn, can determine the length of diapause under given environmental conditions (e.g. constant temperature).

The aim of present investigation was to make a comparison between the rates of oxygen consumption, measured with Warburg's manometric method, of diapause pupae of two noctuid species reared as larvae at two inductive photoperiods.

Materials and Methods

Caterpillars of the tomato moth, *Lacanobia oleracea* L. and the cabbage moth, *Mamestra brassicae* L. (*Lepidoptera*; *Noctuidae*; *Hadeninae*) were reared on a semi-synthetic artificial diet (Nagy, 1970) at 25 ± 1 °C and at two constant (stationary) photoperiods, LD 14:10 and LD 12:12. These short-day conditions induced pupal diapause in the whole population. The approx. 10-day-old pupae were removed from the loose cocoons laying in the medium (slightly wet mixture of soil and turf) given for pupation. Until measurements, for a period of cca. 4 to 5 weeks, these animals were kept then in Petri dishes (with a piece of wet cotton) at 25 °C.

The oxygen consumption of diapause pupae was measured with a manometric method by means of a Warburg respirometer, type Wa 0110/66 (VEB MLW Prüfregäte-Werk, Medingen, Germany). Vessels of 17 to 18.5 cm^3 in volume were used and the manometers were filled with 'traditional' Brodie solution. Potassium hydroxide solution served as CO_2 absorbent. Warburg vessels, containing a single female or male pupa, were not shaken in the water-bath maintaining constant temperature of 25 ± 0.2 °C. The O_2 consumption was measured in incubation periods of 4 or 5 hours. Body mass of each pupa was measured prior to the respirometry with an electronic analytical balance, type Sartorius 5020 (accuracy: 0.1 mg).

Results

Data on the oxygen consumption of diapause pupae of *L. oleracea* are presented in Table 1. At least 26 male or female pupae were used in each experimental variant and on average 14 to 17 mm^3 pupa/h oxygen uptake could be determined being equivalent to 38 to 45 mm^3/g b. m./h values on body mass basis. Though the respiration rates of female pupae proved to be slightly lower than those of males, the differences were not significant. On the other hand, the O_2 consumption of diapause pupae which had developed from larvae exposed to photoperiods LD 14:10 and LD 12:12, respectively, exhibited good dependence on daylengths. In both sexes shorter days (longer nigths) induced pupal diapause accompanied with lower respiration rates. The differences, detected in present studies with Warburg's manometric method were moderate, approx. 4 to 5 mm^3/g b. m./h but, nevertheless, significant at least at $p = 0.1$ level.

Similar results were obtained when diapause pupae of the related noctuid species, *M. brassicae* were used (Table 2). In this case values of O_2 consumption fluctuated between 9 to 11 mm^3/pupa/h and 23 to 28 mm^3/g b. m./h, respectively. Therefore, *Mamestra* pupae took up less oxygen/hour than *Lacanobia* pupae. Nevertheless, the dependence on rearing conditions is unequivocal is this species, too. In individuals (both sexes) raised at shorter daylength (LD 12:12) less intensive respiration was found than in those kept at LD 14:10 during their larval development. Since in these investigations less (up to 12) insects could be used in experimental variants, the differences, being 3 to 4 mm^3/g b. m./h, were significant, if they were calculated on body mass basis only. In *M. brassicae* the sex-related differences of pupal respiration (slightly lower rates in females) were also small and not significant.

Discussion

Measuring oxygen consumption of diapause pupae has the great advantage that this developmental stage is a resting form without any 'disturbing' activity or physiological function (e.g. movement, feeding, energy-consuming developmental process) that can significantly modify the results of respirometry. However, due to the very low rate of metabolism characteristic for such a dormant stage, comparable data on respiration can be obtained, depending also on the method of measurement, only during a prolonged incubation, registration period as it was the case in our studies. In order to reveal individual variations in O_2 consumption and also to exlude possible extreme values, respiration of a single pupa was studied in each Warburg vessel. Specimens with outstanding low or high breathing rate occurred in cca 20% of the whole population in both species. We did not use their data in further calculations.

For the sake of a good comparison of the corresponding respiration rates, it was an important requirement to use developmentally synthronous insects, all of them resting in a 'deep', intense phase of pupal diapause. The photoperiods maintained during the rearing of caterpillars, from hatching of first instar larvae until entering the pupation medium, were in the regime of 'natural' daylengths. The difference between them was only 2 hours (as to the duration of photophase or scotophase). Thus we did not expect great differences between average respiration rates of the two main groups of noctuid pupae. Nevertheless, we could detect significantly altered values in oxygen consumption (see Tables 1 and 2). Here we want to stress that in each measurement pupae (of the same sex) were used in equal numbers from the two insect groups raised at different photoperiods.

The results of present study on the levels of oxygen consumption registered in diapause pupae of *L. oleracea* and *M. brassicae*, respectively, correspond well to similar data of other authors reported for diapause pupae of *Deilephila euphorbiae* (Heller, 1926), *Celerio lineata* (Keeley, 1970), *Pieris rapae* (Kono, 1970) and *Helicoverpa (Heliothis) armigera* (Fytizas et al., 1974).

Table 1

Oxygen consumption of diapause pupae of *Lacanobia oleracea* developed as larvae at different inductive photoperiods

Photoperiod	Oxygen consumption ± SD (N)*			
	mm^3/pupa/h		mm^3/g b.m./h	
	male	female	male	female
LD 14:10	17.3 ± 3.6 (26)	16.8 ± 2.5 (26)	45.4 ± 9.7	43.0 ± 6.9
LD 12:12	15.1 ± 2.6 (27)	13.9 ± 2.6 (30)	41.1 ± 7.3	37.7 ± 7.2
	p = 0.05	p = 0.001	p = 0.1	p = 0.01

* Measured with a Warburg respirometer at 25 °C

Means in each column are significantly different. Levels of significance are indicated below the columns. Students *t*-test

Table 2

Oxygen consumption of diapause pupae of *Mamestra brassicae* developed as larvae at different inductive photoperiods

Photoperiod	Oxygen consumption ± SD (N)*			
	mm^3/pupa/h		mm^3/g b.m./h	
	male	female	male	female
LD 14:10	11.4 ± 2.4 (12) a	10.9 ± 1.5 (10) a	28.2 ± 3.9 a	26.9 ± 3.2 a
LD 12:12	10.0 ± 2.3 (10) a	9.3 ± 2.7 (9) a	24.8 ± 4.9 b	23.1 ± 5.6 b

* Measured with a Warburg respirometer at 25 °C

Means in a column followed by the same letter are not significantly different at p = 0.1 level. Students *t*-test

Especially when calculated on the basis of body mass, *Lacanobia* pupae consumed 1.4 to twice more oxygen/hour than *Mamestra* pupae. It is difficult to explain this difference since we do not know whether diapause pupae of the two noctuids were actually in the same phase of dormancy at the time of measurements. The sexual difference in O_2 uptake did not prove to be significant. Nevertheless, it is worth mentioning that females, containing in general more food-reserves, may have slightly reduced rates of metabolism.

The most interesting of our investigations was the positive relationship found between the daylengths (nightlengths) of diapause inducing photoperiods and the average

levels of oxygen consumption in diapausing pupae. Shorter days (longer nights) induced slightly but significantly lower rates in respiration as compared to data of other pupae raised at 2 hours shorter scotophases (longer photophases).

Respiratory activity (oxygen consumption) can be considered as a very informative indicator of diapause intensity. In this sense, results of present study support the assumption that different photoperiods acting during the sensitive period of larval development have some influence on the intensity of diapause. The physiological mechanism of this 'preprograming' is not known but the contribution of some endocrine factors (e.g. juvenile hormone, ecdysteroids) can be supposed.

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New Data to the Knowledge of the Aphid Fauna (Homoptera: Aphidoidea) on Ornamental Trees and Shrubs in Hungary

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Authors give report on the results of studies done in 1990–1996 on street trees, in parks, in green spaces of housing estates and in arboreta. Out of the 301 woody plant species studied, aphids were found on 213 species belonging to 53 plant families. A total of 160 aphid species were found of which 7 species belong to the family Adelgidae, 3 species to family Phylloxeridae and 150 species to family Aphididae. The following 24 of them are new for the Hungarian fauna: *Adelges cooleyi* (Gillette); *Pineus strobi* (Hartig); *Phylloxerina capreae* Börner; *Phylloxerina salicis* (Lichtenstein); *Eriosoma grossulariae* (Schüle); *Glyphina betulae* (Kaltenbach); *Chaitophorus capreae* (Mosley); *Chaitophorus mordvilkoi* Mamontova; *Chaitophorus ramicola* (Börner); *Periphyllus bulgaricus* Tashev; *Periphyllus rhenanus* (Börner); *Periphyllus venetianus* Hille Ris Lambers; *Drepanosiphum oregonensis* Granovsky; *Appendiseta robiniae* (Gillette); *Monelliopsis caryae* (Monell); *Tinocallis platani* (Kaltenbach); *Tinocallis saltans* (Nevsky); *Aphis hederae* Kaltenbach; *Aphis mammulata* Gimingham et Hille Ris Lambers; *Aphis passeriniana* (del Guercio); *Aphis versicolor* (Börner); *Chaetosiphon tetrarhodum* (Walker); *Cryptomyzus korschelti* Börner; *Trichosiphonaphis polygonifoliae* (Shinji).

The most frequent aphid species were as follows: *Periphyllus testudinaceus* (Fernie), *Periphyllus obscurus* Mamontova, *Periphyllus lyropictus* (Kessler), *Eucallipterus tiliae* (Linnaeus), *Chaitophorus leucomelas* Koch, *Pemphigus spyrothecae* Passerini, *Aphis farinosa* Gmelin, *Aphis pomi* De Geer and *Aphis fabae* Scopoli.

The highest number of aphid species (29) occurred on the plants of both Rosaceae and Salicaceae families. Among the 7 ant species occurred together with the aphids *Lasius niger* (Linnaeus) was the most frequent.

In discovering the aphid fauna in Hungary, works by Horváth (1897), Szelegiewicz (1968, 1977), Andrásfalvy (1978) and Szalay-Marzsó (1969, 1971, 1989) were significant. Halmágyi (1969, 1974) reported mostly about results of studies on aphid species of forestry tree species, while Haltrich et al. (1992) and Ripka et al. (1993) about those concerning aphid species of ornamental trees and shrubs.

Materials and Methods

Between 1990 and 1996, an aphid survey was made on ornamental trees and shrubs of the traffic roads, squares, green areas of housing estates, parks, botanical gardens and home gardens in all districts of Budapest, few specimens were collected also in other region of Hungary and in Croatia in 1996. Plant samples from 301 woody plant species (minimum 5 shoots, or 25 leaves/plant) were taken in polythene bags. During the study of the surfaces made with binocular microscope, all the aphids found on the plant

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samples were put in 70% ethylalcohol. After boiling the aphids for some minutes in water they were put in 10% sodium-hydroxide. The animals' body were cleaned by boiling them again in water and by pressing them to the necessary extent. Having rinsed the animals with distilled water, they were mounted in Hoyer's medium, in Hoyer's medium containing sorbitol (Keifer, 1975) and in Heinze's medium of polivinil-alcohol (Schmutterer, 1959). The microscope-slide preparations were dried in a thermostat at 32 °C and then sealed with nail varnish. Aphids were studies with phase contrast microscope. In order to determine the aphid species works by Börner (1952), Rupais (1969), Stroyan (1977), Szelegiewicz (1977, 1978), Blackman and Eastop (1984, 1994), Binazzi (1984), Remaudiere et al. (1992) were used.

Results

Table 1

Aphid species collected from woody ornamentals
(* = new species for the Hungarian fauna)

Aphid species	Plant species	Sampling place	Sampling date
Adelgidae			
Adelges (Sacchiphantes) abietis (Linnaeus, 1758)			
	Picea abies	Rákospalota	13. 05. 1992
**Adelges (Gilletteella) cooleyi* (Gillette, 1907)	*Pseudotsuga menziesi*	Pesterzsébet	09. 03. 1992
Adelges laricis Vallot, 1836	*Larix decidua*	Mártonhegy	24. 05. 1991
		Széchenyihegy	22. 08. 1991
Adelges (Dreyfusia) nordmannianae (Eckstein, 1890)			
	Abies nordmanniana	Csillaghegy	27. 03. 1996
Adelges (Sacchiphantes) viridis (Ratzeburg, 1843)			
	Picea abies	Margitsziget	11. 05. 1992
Pineus pini (Macquart, 1819)	*Pinus mugo*	Vérmező	22. 04. 1992
**Pineus strobi* (Hartig, 1837)	*Pinus strobus*	Vérmező	22. 04. 1992
		Vár	10. 03. 1993
Phylloxeridae			
Phylloxera glabra (von Heyden, 1837)	*Quercus robur*	Hűvösvölgy	22. 09. 1993
		Rákoskeresztúr	14. 05. 1995
**Phylloxerina capreae* Börner, 1942	*Salix caprea*	Rákospalota	30. 08. 1993
		Rákospalota	08. 09. 1993
	Salix viminalis	Sasad	11. 06. 1993
**Phylloxerina salicis* (Lichtenstein, 1884)	*Salix alba*	Vérmező	16. 04. 1993
		Rákospalota	07. 05. 1993
		Kőbánya	16. 05. 1993
		Rákospalota	30. 08. 1993
		Rákospalota	08. 09. 1993

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
Aphididae Pemphiginae	**Eriosomatini**		
Colopha compressa (Koch, 1856)	*Ulmus laevis*	Józsefváros	29. 06. 1994
		Józsefváros	15. 05. 1996
**Eriosoma grossulariae* (Schüle, 1887)	*Ulmus minor*	Zöldmál	18. 05. 1993
		Pasarét	25. 05. 1996
Eriosoma lanigerum (Hausman, 1802)	*Malus* sp.	Óbuda	23. 04. 1992
Eriosoma lanuginosum (Hartig 1839)	*Ulmus minor*	Zöldmál	18. 05. 1993
		Fárkasrét	04. 05. 1995
Eriosoma ulmi (Linnaeus, 1758)	*Ulmus* × *elegantissima*	Gellérthegy	09. 05. 1995
	Ulmus minor	Zöldmál	18. 05. 1993
	Ulmus scabra	Törökvész	11. 06. 1992
Kaltenbachiella pallida (Haliday, 1838)	*Ulmus minor*	Farkasrét	04. 05. 1995
	Ulmus × *elegantissima*	Gellérthegy	09. 05. 1995
Tetraneura nigriabdominalis (Sasaki, 1899)	*Ulmus scabra*	Törökvész	11. 06. 1992
	Ulmus minor	Pasarét	25. 05. 1996
Tetraneura caerulescens (Passerini, 1856)	*Ulmus scabra*	Rézmál	09. 05. 1995
Tetraneura ulmi (Linneaus, 1758)	*Ulmus scabra*	Rózsadomb	18. 05. 1994
Aphididae Pemphiginae	**Pemphigini**		
Pemphigus populi Courchet, 1879	*Populus nigra* 'Italica'	Sasasd	13. 09. 1991
Pemphigus protospirae Lichtenstein, 1885	*Populus nigra*	Sasad	01. 09. 1994
Pemphigus spyrothecae Passerini, 1856	*Populus nigra*	Pestszentlőrinc	24. 09. 1992
	Populus nigra 'Italica'	Sasad	13. 09. 1991
Prociphylus bumeliae (Schrank, 1801)	*Fraxinus angustifolia*	Kőbánya	22. 05. 1992
	Fraxinus pennsylvanica	Rákoskeresztúr	14. 05. 1995
Prociphylus fraxini (Fabricius, 1777)	*Fraxinus angustifolia*	Óbuda	23. 05. 1991
Aphididae Phloeomyzinae			
Phloeomyzus passerini (Signoret, 1875)	*Populus nigra*	Kispest	15. 05. 1992
	Populus × *canadensis*	Törtel (Pest m.)	17. 08. 1995
Aphididdidae Anoeciinae			
Anoecia corni (Fabricius, 1775)	*Cornus glabrata*	Gellérhegy	04. 05. 1994
	Cornus sanguinea	Belváros	02. 05. 1992
		Törökvész	15. 05. 1993
Anoecia vagans Koch, 1856	*Cornus sanguinea*	Belváros	02. 05. 1992
		Törökvész	15. 05. 1993
Aphididae Thelaxinae			
**Glyphina betulae* (Kaltenbach, 1843)	*Betula pendula*	Ferencváros	09. 06. 1992
		Városmajor	04. 06. 1993
		Törökvész	29. 06. 1994
		Gellérthegy	21. 06. 1995
Thelaxes dryophila (Schrank, 1801)	*Quercus robur*	Rákoskeresztúr	14. 05. 1995
Thelaxes suberi (del Guercio, 1911)	*Quercus ilex*	Lanterna (Croatia)	05. 07. 1996

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
Aphididae Lachninae	**Cinarini**		
Cinara brauni Börner, 1940	*Pinus nigra*	Pünkösfürdő	16. 03. 1995
Cinara cupressi (Buckton, 1881)	*Thuja occidentalis*	Gellérhegy	07. 06. 1994
		Lágymányos	22. 10. 1996
Cinara nuda (Mordvilko, 1895)	*Pinus sylvestris*	Óbuda	14. 07. 1995
Cinara pilicornis (Hartig, 1841)	*Picea pungens*	Zugló	03. 06. 1991
		Törökvész	21. 04. 1992
Cinara pinea (Mordvilko, 1895)	*Pinus mugo*	Vérmező	22. 04. 1992
	Pinus nigra	Balatonalmádi (Veszprém m.)	27. 10. 1993
	Pinus sylvestris	Balatonudvari (Veszprém m.)	18. 07. 1996
Cinara pini (Linnaeus, 1758)	*Pinus sylvestris*	Őrmező	15. 06. 1993
Cinara tujafilina (del Guercio, 1909)	*Thuja occidentalis*	Gazdagrét	02. 01. 1994
	Juniperus virginiana	Pesterzsébet	28. 08. 1996
Eulachnus brevipilosus Börner, 1940	*Pinus mugo*	Őrmező	03. 06. 1994
Eulachnus riley (Williams, 1911)	*Pinus mugo*	Kőbánya	14. 05. 1993
Aphididae Lachninae	**Lachnini**		
Lachnus roboris (Linnaeus, 1758)	*Quercus ilex*	Lanterna (Croatia)	05. 07. 1996
Tuberolachnus salignus (Gmelin, 1790)	*Salix alba*	Rómaifürdő	08. 10. 1990
Aphididae Chaitophorinae	**Chaitophorini**		
**Chaitophorus capreae* (Mosley, 1841)	*Salix viminalis*	Sasad	11. 06. 1993
		Sasad	20. 05. 1996
Chaitophorus leucomelas Koch, 1854	*Populus* × *berolinensis*	Gellérthegy	08. 06. 1994
		Vérmező	21. 06. 1994
	Populus × *canadensis*	Gellérthegy	02. 06. 1994
	Populus nigra	Kispest	15. 05. 1992
		Törökvész	18. 06. 1994
	Populus simonii	Békásmegyer	11. 06. 1992
		Óbuda	10. 06. 1993
Chaitophorus longisetosus Szelegiewicz, 1959	*Populus alba*	Óbuda	04. 10. 1991
**Chaitophorus mordvilkoi* Mamontova, 1960	*Salix purpurea*	Törökvész	05. 06. 1993
		Törökvész	07. 08. 1993
Chaitophorus nassonowi Mordvilko, 1895	*Populus alba*	Óbuda	10. 06. 1993
	Populus nigra	Sasad	13. 09. 1991
Chaitophorus niger Mordvilko, 1929	*Salix alba*	Devecser (Veszprém m.)	01. 07. 1993
	Salix babylonica	Gellérthegy	08. 06. 1994
	Salix viminalis	Kispest	06. 05. 1991
		Óbuda	31. 05. 1993
Chaitophorus populeti (Panzer, 1801)	*Populus alba*	Vérmező	02. 07. 1991
		Óbuda	04. 10. 1991
		Óbuda	10. 06. 1993
	Populus × *berolinensis*	Gellérthegy	08. 06. 1994

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
	Populus × *canescens*	Óbuda	04. 07. 1991
		Vérmező	05. 05. 0992
		Rákoskeresztúr	22. 05. 1992
		Kőbánya	22. 05. 1992
		Óbuda	10. 06. 1993
Chaitophorus populialbae (Boyer de Fonscolombe, 1841)			
	Populus alba	Tabán	27. 06. 1991
		Óbuda	04. 10. 1991
		Őrmező	12. 05. 1995
	Populus × *canescens*	Óbuda	05. 07. 1991
		Kőbánya	22. 05. 1992
		Kispest	08. 05. 1993
		Gazdagrét	08. 06. 1993
	Populus grandidentata	Józsefváros	03. 05. 1995
		Józsefváros	15. 05. 1996
**Chaitophorus ramicola* (Börner, 1949)	*Salix aegyptiaca*	Gellérthegy	13. 06. 1994
Chaitophorus salicti (Schrank, 1801)	*Salix aegyptiaca*	Pasarét	23. 05. 1992
	Salix daphnoides	Törökvész	12. 08. 1993
	Salix 'Mesuneco'	Gellérthegy	20. 05. 1996
	Salix viminalis	Kispest	06. 05. 1991
		Óbuda	31. 05. 1993
Chaitophorus tremulae Koch, 1854	*Populus grandidentata*	Józsefváros	03. 05. 1995
		Józsefváros	15. 05. 1996
	Populus tremula	Újpalota	13. 06. 1993
		Gellérthegy	02. 06. 1994
Chaitophorus viminalis Monell, 1879	*Salix viminalis*	Sasad	11. 06. 1993
Periphyllus acericola (Walker, 1848)	*Acer pseudoplatanus*	Józsefváros	16. 05. 1991
		Lágymányos	23. 05. 1991
		Pasarét	02. 05. 1992
		Törökvész	07. 05. 1994
Periphyllus aceris (Linnaeus, 1761)	*Acer platanoides*	Józsefváros	16. 05. 1991
	Acer opalus	Gellérhegy	07. 06. 1994
	Acer saccharinum	Kőbánya	16. 05. 1991
		Kőbánya	27. 06. 1991
**Periphyllus bulgaricus* Tashev, 1964	*Acer monspessulanum*	Gellérthegy	29. 04. 1994
Periphyllus coracinus (Koch, 1854)	*Acer platanoides*	Kispest	06. 05. 1991
		Törökvész	26. 04. 1992
		Rákosfalva	28. 04. 1992
		Budaörs (Pest m.)	15. 06. 1992
		Kispest	27. 05. 1993
		Alsórákos	04. 08. 1993
Periphyllus hirticornis (Walker, 1848)	*Acer campestre*	Zugló	11. 07. 1991
		Németvölgy	20. 07. 1993
		Törökvész	02. 06. 1996
Periphyllus lyropictus (Kessler, 1886)	*Acer campestre*	Belváros	02. 05. 1992
	Acer platanoides	Belváros	02. 05. 1992

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
		Lipótváros	02. 05. 1992
		Rákosfalva	28. 04. 1992
		Budaörs (Pest m.)	15. 06. 1992
		Kispest	11. 07. 1993
		Alsórákos	04. 08. 1993
	Xanthoceras sorbifolium	Józsefváros	01. 05. 1994
Periphyllus minitus Shaposhnikov, 1952	*Acer ginnala*	Kispest	08. 05. 1993
	Acer tataricum	Rézmál	10. 06. 1994
Periphyllus obscurus Mamontova, 1955	*Acer campestre*	Őrmező	02. 05. 1991
		Mártonhegy	24. 05. 1991
		Belváros	02. 05. 1992
		Pasarét	06. 10. 1992
		Kispest	08. 05. 1993
		Törökvész	01. 05. 1995
		Törökvész	02. 06. 1996
**Periphyllus rhenanus* (Börner, 1940)	*Acer opalus*	Gellérthegy	07. 06. 1994
Periphyllus testudinaceus (Fernie, 1852)	*Acer campestre*	Őrmező	02. 05. 1991
		Zugló	11. 07. 1991
		Istenhegy	22. 08. 1991
		Belváros	02. 05. 1992
		Németvölgy	27. 05. 1992
		Józsefváros	09. 06. 1992
		Keszthely (Zala m.)	25. 07. 1994
		Törökvész	01. 05. 1995
		Törökvész	02. 06. 1996
	Acer ginnala	Kispest	08. 05. 1993
		Németvölgy	23. 05. 1993
	Acer monspessulanum	Gellérthegy	29. 04. 1994
		Városmajor	09. 05. 1995
	Acer negundo	Belváros	02. 05. 1992
		Zugló	25. 08. 1992
		Városmajor	09. 05. 1995
	Acer opalus	Gellérthegy	07. 06. 1994
	Acer platanoides	Kispest	06. 05. 1991
		Törökvész	26. 04. 1992
		Lipótváros	02. 05. 1992
		Rákosfalva	28. 04. 1992
	Acer pseudoplatanus	Józsefváros	16. 05. 1991
		Lágymányos	23. 06. 1991
		Pasarét	21. 04. 1992
		Törökvész	02. 05. 1992
		Pestszentlőrinc	22. 09. 1992
		Pestszentlőrinc	05. 08. 1993
		Rózsadomb	08. 10. 1993
		Újlak	13. 10. 1993
		Józsefváros	01. 05. 1994

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
	Acer saccharinum	Óbuda	30. 04. 1991
		Kőbánya	16. 05. 1991
		Kőbánya	27. 06. 1991
		Belváros	02. 05. 1992
		Zugló	04. 08. 1993
	Acer tataricum	Németvölgy	23. 05. 1993
	Aesculus neglecta	Gellérthegy	29. 04. 1994
	Aesculus pavia	Gellérthegy	04. 05. 1994
	Xanthoceras sorbifolium	Józsefváros	01. 05. 1994
**Periphyllus venetianus* Hille Ris Lambers, 1966			
	Acer campestre	Törökvész	01. 05. 1995
Aphididae Drepanosiphinae Drepanosiphini			
**Drepanosiphum oregonensis* Granovsky, 1939	*Acer opalus*	Gellérthegy	07. 06. 1994
Drepanosiphum platanoidis (Schrank, 1801)	*Acer platanoides*	Belváros	02. 05. 1992
	Acer pseudoplatanus	Józsefváros	16. 05. 1991
		Lágymányos	23. 06. 1991
		Pasarét	21. 04. 1992
		Rózsadomb	08. 10. 1992
		Újlak	13. 10. 1992
Aphididae Drepanosiphinae Phyllaphidini			
**Appendiseta robiniae* (Gillette, 1907)	*Robinia pseudoacacia*	Kispest	25. 06. 1991
		Belváros	17. 07. 1992
	Populus × *berolinensis*	Gellérthegy	08. 06. 1994
Betulaphis quadrituberculata (Kaltenbach, 1843)			
	Betula pendula	Ferencváros	09. 06. 1992
		Gellérthegy	02. 06. 1994
Calaphis flava Mordvilko, 1928	*Betula pendula*	Ferencváros	09. 06. 1992
Callipterinella calliptera (Hartig, 1841)	*Betula pendula*	Ferencváros	09. 06. 1992
		Gellérthegy	02. 06. 1994
Callipterinella tuberculata (von Heyden, 1837)	*Betula pendula*	Rézmál	10. 06. 1992
		Ferencváros	09. 06. 1992
		Városmajor	04. 06. 1993
		Törökvész	29. 06. 1994
Chromaphis juglandicola (Kaltenbach, 1843)	*Juglans nigra*	Kőbánya	07. 07. 1995
	Juglans regia	Széchenyi hegy	15. 06. 1994
	Juglans sp.	Gellérthegy	24. 05. 1995
Eucallipterus tiliae (Linnaeus, 1758)	*Tilia americana*	Józsefváros	29. 06. 1994
	Tilia cordata	Ferencváros	09. 06. 1992
	Tilia × *europaea*	Józsefváros	29. 06. 1994
	Tilia × *euchlora*	Gellérhegy	01. 06. 1994
	Tilia × *moltkei*	Gellérthegy	01. 06. 1994
	Tilia petiolaris	Gellérhegy	01. 06. 1994
	Tilia platyphyllos	Újpest	27. 06. 1991
		Krisztinaváros	05. 05. 1992

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
		Pasarét	12. 05. 1992
		Tabán	09. 06. 1992
	Tilia tomentosa	Soroksár	27. 06. 1991
		Újpest	27. 06. 1991
		Tabán	26. 05. 1992
		Terézváros	26. 08. 1992
Euceraphis betulae Koch, 1855	*Betula pendula*	Rézmál	10. 06. 1992
		Ferencváros	09. 06. 1992
		Gellérthegy	26. 04. 1994
Euceraphis punctipennis (Zetterstedt, 1828)	*Betula pendula*	Rézmál	10. 06. 1992
Hoplocallis pictus (Ferrari, 1872)	*Quercus ilex*	Lanterna (Croatia)	05. 07. 1996
Monaphis antennata (Kaltenbach, 1843)	*Betula pendula*	Ferencváros	09. 06. 1992
		Gellérthegy	26. 04. 1994
**Monelliopsis caryae* (Monell, 1879)	*Juglans nigra*	Kőbánya	07. 07. 1995
		Kőbánya	10. 08. 1996
Myzocallis carpini (Koch, 1855)	*Carpinus betulus*	Tabán	26. 05. 1992
Myzocallis coryli (Goetze, 1778)	*Corylus avellana*	Törökvész	30. 05. 1992
		Pesterzsébet	28. 05. 1993
	Corylus colurna	Budafok	20. 07. 1993
	Corylus maxima	Gellérthegy	02. 06. 1994
Panaphis juglandis (Goetze, 1778)	*Juglans regia*	Széchenyihegy	15. 06. 1994
Phyllaphis fagi (Linnaeus, 1767)	*Fagus sylvatica*	Vérmező	08. 05. 1991
	Acer opalus	Gellérthegy	07. 06. 1994
Pterocallis alni (De Geer, 1773)	*Alnus glutinosa*	Tabán	16. 05. 1992
Pterocallis maculata (von Heyden, 1837)	*Alnus glutinosa*	Pesterzsébet	28. 05. 1993
Symydobius oblongus (von Heyden, 1837)	*Betula pendula*	Rézmál	10. 06. 1992
		Gellérthegy	26. 04. 1994
**Tinocallis platani* (Kaltenbach, 1843)	*Ulmus laevis*	Józsefváros	29. 06. 1994
		Józsefváros	15. 05. 1996
**Tinocallis saltans* (Nevsky, 1929)	*Zelkova serrata*	Felhévíz	14. 10. 1996
Tuberculatus annulatus (Hartig, 1841)	*Quercus robur*	Pesterzsébet	19. 06. 1992
Aphididae Aphidinae	**Pterocommatini**		
Pterocomma pilosum Buckton, 1879	*Populus* × *berolinensis*	Kispest	03. 10. 1994
Pterocomma pilosum konoi Hori, 1939	*Salix alba*	Rákospalota	07. 05. 1993
Pterocomma populeum (Kaltenbach, 1843)	*Populus* × *berolinensis*	Kispest	19. 11. 1992
Pterocomma salicis (Linnaeus, 1758)	*Salix aegyptiaca*	Gellérthegy	13. 06. 1994
Aphididae Aphidinae	**Aphidini**		
Aphis arbuti Ferrari, 1872	*Arbutus unedo*	Lanterna (Croatia)	05. 07. 1996
Aphis chloris Koch, 1854	*Hypericum* 'Hidcote'	Gellérthegy	21. 06. 1995
Aphis clematidis Koch, 1854	*Clematis vitalba*	Törökvész	04. 08. 1995
Aphis craccivora Koch, 1854	*Amorpha fruticosa*	Kőbánya	01. 07. 1995
	Caragana arborescens	Németvölgy	28. 06. 1992
		Törökbálint (Pest m.)	06. 07. 1995
	Catalpa bignonioides	Kelenföld	11. 07. 1991

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
	Colutea arborescens	Törökbálint (Pest m.)	06. 07. 1995
	Gleditsia triacanthos	Angyalföld	12. 06. 1993
	Juglans nigra	Kőbánya	07. 07. 1995
	Robinia pseudoacacia	Kispest	25. 06. 1991
		Pesterzsébet	19. 06. 1992
		Sasad	19. 06. 1992
		Kispest	27. 05. 1993
	Robinia viscosa	Sasad	15. 07. 1993
	Wisteria sinensis	Törökvész	01. 06. 1993
		Gazdagrét	14. 07. 1995
Aphis cytisorum Hartig, 1841	*Cytisus supinus*	Gellérthegy	07. 06. 1994
	Colutea arborescens	Békásmegyer	25. 06. 1992
		Gazdagrét	07. 06. 1993
	Laburnum anagyroides	Békásmegyer	11. 06. 1992
		Gazdagrét	28. 05. 1993
	Petteria ramentacea	Gellérthegy	02. 06. 1994
	Spartium junceum	Pasarét	04. 07. 1992
		Törökvész	09. 06. 1993
Aphis euonymi Fabricius, 1775	*Euonymus europaeus*	Kőbánya	17. 06. 1994
Aphis fabae Scopoli, 1763	*Acer tataricum*	Németvölgy	23. 05. 1993
	Ailanthus altissima	Kispest	25. 06. 1991
		Lágymányos	28. 05. 1994
	Amelanchier canadensis	Lágymányos	28. 05. 1994
		Gödöllő (Pest m.)	03. 06. 1994
	Amorpha fruticosa	Gazdagrét	13. 05. 1993
		Gazdagrét	04. 08. 1993
	Aronia melanocarpa	Gellérthegy	01. 06. 1994
	Buddleia alternifolia	Óbuda	19. 06. 1993
		Gellérthegy	01. 06. 1994
	Buddleia davidii	Gellérthegy	13. 05. 1994
	Buddleia davidii var. *nanhoensis*	Gellérthegy	07. 06. 1994
	Callicarpa bodinieri	Gellérthegy	01. 06. 1994
	Campsis radicans	Maglód (Pest m.)	13. 06. 1993
	Campsis × *tagliabuana*	Gellérthegy	13. 06. 1994
	Celastrus orbiculatus	Gellérthegy	20. 05. 1994
	Celtis occidentalis	Pesterzsébet	28. 05. 1993
		Lágymányos	28. 05. 1994
	Cistus laurifolius	Gellérthegy	28. 05. 1994
	Cornus stolonifera	Gazdagrét	28. 05. 1993
		Gazdagrét	11. 06. 1995
	Cotinus cogyggria	Rézmál	10. 06. 1992
	Cydonia oblonga	Óbuda	11. 06. 1992
	Deutzia × *hybrida*	Gellérthegy	08. 06. 1994
	Deutzia × *magnifica*	Pasarét	12. 06. 1993
	Escallonia × *langleyensis*	Gellérthegy	08. 06. 1994

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
	Euonymus alatus	Józsefváros	04. 05. 1995
	Euonymus europaeus	Rákoskeresztúr	21. 05. 1991
		Vérmező	22. 04. 1992
	Euodia hupehensis	Kőbánya	27. 06. 1993
		Rákoskeresztúr	27. 06. 1993
	Euodia velutina	Gellérthegy	02. 06. 1994
	Ficus carica	Törökvész	30. 05. 1994
	Fontanesia fortunei	Gellérthegy	28. 05. 1994
	Forsythia suspensa	Kispest	13. 06. 1993
		Gazdagrét	05. 24. 1994
	Gleditsia triacanthos	Törökvész	09. 06. 1994
	Hibiscus syriacus	Rézmál	08. 06. 1993
	Koelreuteria paniculata	Pasarét	12. 06. 1993
	Kolkwitzia amabilis	Őrmező	22. 05. 1995
	Maclura pomifera	Törökvész	01. 06. 1993
	Magnolia kobus	Víziváros	05. 06. 1994
	Morus alba	Pasarét	22. 05. 1993
	Periploca graeca	Pasarét	22. 05. 1993
		Gellérthegy	13. 06. 1994
	Phellodendron amurense	Gellérthegy	01. 06. 1994
	Philadelphus coronarius	Rákoskeresztúr	22. 05. 1992
	Philadelphus × lemoinei	Gellérthegy	26. 04. 1994
	Philadelphus × virginalis	Törökvész	14. 05. 1993
	Polygonum orientale	Törökvész	21. 05. 1994
	Potentilla fruticosa	Törökvész	19. 06. 1993
		Őrmező	24. 06. 1993
	Populus alba	Óbuda	10. 06. 1993
	Populus × berolinensis	Gellérthegy	08. 06. 1994
	Pseudocydonia sinensis	Gellérthegy	13. 06. 1994
	Ptelea trifoliata	Törökvész	10. 06. 1992
		Törökvész	07. 06. 1996
	Punica granatum	Rézmál	17. 06. 1993
		Gellérthegy	29. 05. 1995
	Reynoutria aubertii	Törökvész	05. 06. 1993
	Rhamnus catharticus	Törökvész	10. 07. 1992
	Rhodotypos scandens	Gellérthegy	29. 05. 1995
	Rhus typhina	Rákoskeresztúr	14. 05. 1995
	Smilax excelsa	Gellérthegy	08. 06. 1994
	Sophora japonica	Pasarét	08. 06. 1993
	Spiraea × bumalda	Gellérthegy	28. 05. 1994
	Spiraea × salicifolia	Tata (Komárom-Esztergom m.)	22. 07. 1993
	Symphoricarpos albus	Lágymányos	28. 05. 1994
	Ulmus laevis	Józsefváros	15. 05. 1996
	Vitex agnus-castus	Víziváros	30. 05. 1993
		Víziváros	05. 06. 1994

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
	Vitis vinifera	Törökvész	26. 05. 1993
	Zanthoxylum americanum	Gellérthegy	02. 06. 1994
	Zanthoxylum simulans	Gellérthegy	01. 06. 1994
	Yucca filamentosa	Pasarét	11. 07. 1992
Aphis farinosa Gmelin, 1790	*Salix aegyptiaca*	Pasarét	23. 05. 1992
		Gellérthegy	13. 06. 1994
	Salix alba	Sasad	19. 05. 1993
		Őrmező	25. 05. 1993
	Salix babylonica	Gellérthegy	08. 06. 1994
	Salix 'Mesuneco'	Gellérthegy	20. 05. 1996
	Salix viminalis	Sasad	20. 05. 1996
Aphis frangulae Kaltenbach, 1855	*Catalpa bignonioides*	Kelenföld	11. 07. 1991
	Rhamnus × *hybrida*	Gellérthegy	24. 05. 1995
	Rhamnus japonica	Józsefváros	04. 05. 1994
Aphis genistae Scopoli, 1763	*Genista tinctoria*	Józsefváros	01. 06. 1995
Aphis gossypii Glover, 1877	*Campsis radicans*	Maglód (Pest m.)	13. 06. 1993
	Catalpa bignonioides	Pünkösfürdő	03. 07. 1991
		Lipótváros	02. 05. 1992
		Ferencváros	09. 06. 1992
	Celastrus orbiculatus	Gellérthegy	20. 05. 1994
	Citrus aurantiaca	Józsefváros	15. 05. 1996
	Hibiscus syriacus	Rézmál	14. 05. 1993
		Törökvész	08. 06. 1993
	Photinia serrulata	Józsefváros	15. 05. 1996
	Punica granatum	Rézmál	17. 06. 1993
	Sophora japonica	Rézmál	09. 06. 1993
	Symphoricarpos albus	Pasarét	13. 06. 1992
		Víziváros	30. 05. 1993
	Ulmus × *elegantissima*	Gellérthegy	09. 05. 1995
	Zelkova carpinifolia	Józsefváros	15. 05. 1996
**Aphis hederae* Kaltenbach, 1843	*Hedera helix*	Pasarét	15. 05. 1993
**Aphis mammulata* Gimingham et Hille Ris Lambers, 1949			
	Rhamnus catharticus	Pasarét	11. 07. 1992
Aphis nasturtii Kaltenbach, 1843	*Rhamnus alaternus*	Gellérthegy	24. 05. 1995
	Rhamnus × *hybrida*	Gellérthegy	07. 06. 1994
	Rhamnus imeretinus	Józsefváros	01. 05. 1994
	Solanum dulcamara	Újlak	05. 06. 1994
	Zanthoxylum americanum	Gellérthegy	02. 06. 1994
	Zanthoxylum simulans	Gellérthegy	01. 06. 1994
Aphis nerii Boyer de Fonscolombe, 1841	*Nerium oleander*	Rézmál	08. 08. 1991
**Aphis passeriniana* (del Guercio, 1900)	*Salvia officinalis*	Gellérthegy	16. 05. 1995
Aphis pomi De Geer, 1773	*Amelanchier canadensis*	Törökvész	21. 05. 1993
		Lágymányos	28. 05. 1994
		Gödöllő (Pest m.)	13. 06. 1994
	Aronia melanocarpa	Gellérthegy	01. 06. 1994
	Chaenomeles speciosa	Törökvész	15. 05. 1993

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
	Cotoneaster divaricata	Törökvész	13. 06. 1992
	Cotoneaster horizontalis	Kispest	11. 07. 1993
	Cotoneaster microphylla	Őrmező	25. 05. 1993
	Cotoneaster multiflorus	Országút	03. 08. 1993
	Cotoneaster salicifolius	Óbuda	11. 06. 1992
		Városmajor	23. 08. 1994
	Crataegus laevigata	Kispest	12. 06. 1991
		Rézmál	13. 06. 1991
		Horváth-kert	26. 05. 1992
	Crataegus × lavallei	Rézmál	30. 05. 1992
	Crataegus pedicellata	Víziváros	11. 06. 1991
	Cydonia oblonga	Óbuda	11. 06. 1992
	Malus domestica	Gazdagrét	13. 05. 1993
	Malux × purpurea	Kelenföld	22. 04. 1992
		Békásmegyer	11. 06. 1992
		Vérhalom	21. 05. 1993
	Photinia serrulata	Józsefváros	15. 05. 1996
	Pyracantha coccinea	Békásmegyer	11. 06. 1992
		Kispest	24. 06. 1992
		Gazdagrét	13. 05. 1993
	Pyrus pashia	Gellérthegy	07. 06. 1994
	Rhaphiolepis indica	Józsefváros	01. 06. 1995
	Sorbus aucuparia	Vérmező	21. 06. 1994
Aphis ruborum (Börner, 1932)	*Rubus fruticosus*	Széchenyihegy	15. 06. 1994
Aphis sambuci Linnaeus, 1758	*Sambucus nigra*	Pasarét	25. 05. 1992
	Syringa vulgaris	Törökvész	07. 05. 1994
Aphis schneideri (Börner, 1940)	*Ribes aureum*	Gazdagrét	11. 06. 1992
	Ribes alpinum	Gellérthegy	13. 06. 1994
Aphis spiraephaga Müller, 1961	*Spiraea × arguta*	Gellérthegy	02. 06. 1994
	Spiraea × billiardii	Gazdagrét	01. 06. 1994
	Spiraea × bumalda	Gellérthegy	28. 05. 1994
	Spiraea cantoniensis	Gellérthegy	02. 06. 1994
	Spiraea × cinerea	Gellérthegy	02. 06. 1994
	Spiraea japonica	Gellérthegy	01. 06. 1994
	Spiraea media	Gellérthegy	01. 06. 1994
	Spiraea nipponica	Gellérthegy	01. 06. 1994
	Spiraea salicifolia	Tata (Komárom-Esztergom m.)	22. 06. 1993
	Spiraea × schinabeckii	Gellérthegy	02. 06. 1994
	Spiraea trichocarpa	Gellérthegy	02. 06. 1994
	Spiraea trilobata	Gellérthegy	02. 06. 1994
	Spiraea × vanhouttei	Törökvész	03. 06. 1991
		Rézmál	14. 05. 1993
	Symphoricarpos albus	Pasarét	13. 06. 1992
Aphis verbasci Schrank, 1801	*Buddleia davidii*	Békásmegyer	11. 06. 1992
		Gazdagrét	28. 05. 1993

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
		Gellérthegy	13. 05. 1994
		Gellérthegy	21. 06. 1995
	Buddleia davidii var. *nanhoensis*	Gellérthegy	07. 06. 1994
**Aphis versicolor* (Börner, 1950)	*Rhamnus catharticus*	Törökvész	10. 07. 1992
Aphis viburni Scopoli, 1763	*Viburnum carlesii*	Szombathely (Vas m.)	29. 07. 1993
		Gellérthegy	07. 06. 1994
	Viburnum lantana	Gellérthegy	01. 06. 1994
	Viburnum opulus	Törökvész	30. 05. 1992
		Óbuda	01. 05. 1993
		Gazdagrét	28. 05. 1993
	Viburnum tinus	Gellérthegy	08. 06. 1994
Brachyunguis tamaricis (Lichtenstein, 1885)	*Tamarix gallica*	Őrmező	24. 06. 1993
	Tamarix tetrandra	Kispest	08. 05. 1993
		Törökvész	19. 06. 1993
Brachyunguis tamaricophila (Nevsky, 1828)	*Tamarix tetrandra*	Törökvész	19. 06. 1993
Hyalopterus amygdali (Blanchard, 1840)	*Prunus dulcis*	Zöldmál	21. 05. 1993
	Prumus persica	Tabán	09. 06. 1992
Hyalopterus pruni (Geoffroy, 1762)	*Prunus persica*	Budaörs (Pest m.)	01. 07. 1991
		Törökvész	12. 06. 1996
	Prunus cerasifera	Tabán	09. 06. 1992
Melanaphis donacis (Passerini, 1862)	*Arundo donax*	Gellérthegy	22. 08. 1994
Melanaphis pyraria (Passerini, 1861)	*Pyrus pyraster*	Gellérthegy	24. 05. 1995
Rhopalosiphum nimphaeae (Linnaeus, 1761)	*Prunus tenella*	Óbuda	01. 05. 1993
		Gazdagrét	04. 05. 1994
Rhopalosiphum padi (Linnaeus, 1758)	*Prunus* × *davidiopersica*	Gellérthegy	12. 04. 1994
	Prunus padus	Gellérthegy	15. 04. 1994
	Prunus persica	Gellérthegy	17. 05. 1994
	Tilia platyphyllos	Pasarét	12. 05. 1992
Aphididae Aphidinae Macrosiphini			
Aulacorthum solani (Kaltenbach, 1843)	*Campsis* × *tagliabuana*	Gellérthegy	13. 06. 1994
	Populus × *berolinensis*	Gellérthegy	08. 06. 1994
	Solanum dulcamara	Újlak	05. 06. 1994
Brachycaudus amygdalinus (Schouteden,1905)	*Prumus dulcis*	Zöldmál	21. 05. 1993
	Prunus persica	Gellérthegy	17. 05. 1994
	Prunus tenella	Gazdagrét	24. 05. 1994
Brachycaudus cardui (Linnaeus, 1758)	*Prunus* × *blireana*	Rézmál	14. 05. 1993
	Prunus cerasifera	Kelenföld	22. 04. 1992
		Gazdagrét	28. 05. 1993
		Törökvész	20. 05. 1995
		Gellérthegy	20. 05. 1996
	Prunus spinosa	Vérmező	26. 04. 1993
	Prunus tenella	Óbuda	01. 05. 1993

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
Brachycaudus helichrysi (Kaltenbach, 1843)	*Baccharis halimifolia*	Józsefváros	01. 05. 1994
	Prunus cerasifera	Kelenföld	22. 04. 1992
	Prunus spinosa	Vérmező	26. 04. 1993
	Prunus triloba	Gazdagrét	12. 05. 1993
		Maglód (Pest m.)	23. 05. 1994
Brachycaudus schwartzi (Börner, 1931)	*Prunus cerasifera*	Kelenföld	22. 04. 1992
	Prunus persica	Törökvész	09. 06. 1993
Brevicoryne brassicae (Linnaeus, 1758)	*Tamarix tetrandra*	Törökvész	19. 06. 1993
Capitophorus elaeagni (del Guercio, 1894)	*Elaeagnus angustifolia*	Vérmező	26. 09. 1994
		Kispest	12. 04. 1995
	Elaeagnus commutata	Rákoskeresztúr	29. 03. 1995
	Elaeagnus multiflora	Józsefváros	03. 05. 1995
	Elaeagnus umbellata	Józsefváros	03. 05. 1995
Capitophorus hippophaes (Walker, 1852)	*Hippophaë rhamnoides*	Tabán	22. 04. 1992
		Gazdagrét	06. 04. 1993
Cavariella aegopodii (Scopoli, 1763)	*Salix alba*	Kőbánya	16. 05. 1993
		Sasad	19. 05. 1993
	Salix daphnoides	Gellérthegy	26. 04. 1994
	Salix × *erythroflexuosa*	Zöldmál	27. 04. 1993
	Tamarix tetrandra	Törökvész	19. 06. 1993
**Chaetosiphon tetrarhodum* (Walker, 1849)	*Rosa rugosa*	Kispest	24. 06. 1992
Corylobium avellanae (Schrank, 1801)	*Corylus avellana*	Törökvész	30. 05. 1992
	Corylus colurna	Budafok	20. 07. 1993
		Sasad	14. 06. 1994
	Corylus maxima	Gellérthegy	02. 06. 1994
**Cryptomyzus korschelti* Börner, 1938	*Ribes* sp.	Józsefváros	04. 05. 1995
Cryptomyzus ribis (Linnaeus, 1758)	*Ribes rubrum*	Maglód (Pest m.)	08. 05. 1992
Dysaphis crataegi (Kaltenbach, 1843)	*Crataegus laevigata*	Vérmező	22. 04. 1992
		Pasarét	02. 05. 1992
	Crataegus monogyna	Gazdagrét	04. 05. 1993
Dysaphis devecta (Walker, 1849)	*Malus* × *purpurea*	Kelenföld	22. 04. 1992
Dysaphis plantaginea (Passerini, 1860)	*Malus domestica*	Gazdagrét	13. 05. 1993
Dysaphis pyri (Boyer de Fonscolombe, 1841)	*Pyrus communis*	Maglód (Pest m.)	27. 06. 1991
	Pyrus pashia	Gellérthegy	07. 06. 1994
Elatobium abietinum (Walker, 1849)	*Picea pungens*	Zugló	03. 06. 1991
Hyadaphis foeniculi (Passerini, 1860)	*Lonicera caprifolium*	Gazdagrét	13. 05. 1993
		Gellérthegy	02. 06. 1994
	Symphoricarpos orbiculatus	Gellérthegy	13. 06. 1994
Hyadaphis tataricae Aizenberg, 1935	*Lonicera caprifolium*	Gazdagrét	13. 05. 1993
	Lonicera × *purpusii*	Sasad	24. 06. 1993
	Lonicera tatarica	Pasarét	13. 06. 1992
		Gellérthegy	15. 04. 1993
Hyperomyzus picridis (Börner et Blunck, 1916)	*Ribes* sp.	Józsefváros	04. 05. 1995
Macrosiphum euphorbiae (Thomas, 1878)	*Acer tataricum*	Rézmál	10. 06. 1994
	Ailanthus altissima	Lágymányos	28. 05. 1994

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
	Campsis × tagliabuana	Gellérthegy	13. 06. 1994
	Escallonia × langleyensis	Gellérthegy	08. 06. 1994
	Forsythia suspensa	Gazdagrét	24. 05. 1994
	Populus alba	Óbuda	04. 10. 1991
	Solanum dulcamara	Újlak	05. 06. 1994
	Spiraea × bumalda	Gellérthegy	28. 05. 1994
	Symphoricarpos albus	Lágymányos	28. 05. 1994
Macrosiphum rosae (Linnaeus, 1758)	*Fontanesia fortunei*	Gellérthegy	28. 05. 1994
	Gleditsia triacanthos	Törökvész	09. 06. 1994
	Rosa sp.	Őrmező	25. 05. 1992
	Symphoricarpos albus	Pasarét	04. 06. 1994
	Symphoricarpos orbiculatus	Gellérthegy	13. 06. 1994
	Vitex agnus-castus	Víziváros	05. 06. 1994
Myzus cerasi (Fabricius, 1775)	*Prunus avium*	Rózsadomb	03. 06. 1993
	Prunus × blireana	Rézmál	14. 05. 1993
	Prunus padus	Vérmező	29. 04. 1993
		Rákoskeresztúr	06. 08. 1993
	Prunus serrulata	Horváth-kert	26. 05. 1992
		Óbuda	11. 06. 1993
Myzus ligustri (Mosley, 1841)	*Ligustrum vulgare*	Törökvész	30. 05. 1992
Myzus lythri (Schrank, 1801)	*Prunus × davidiopersica*	Gellérthegy	12. 04. 1994
	Prunus mahaleb	Törökvész	09. 06. 1992
		Törökvész	15. 05. 1993
Myzus ornatus Laing, 1932	*Euonymus alatus*	Józsefváros	04. 05. 1995
Myzus persicae (Sulzer, 1776)	*Baccharis halimifolia*	Józsefváros	01. 05. 1994
	Buddleia davidii	Gellérthegy	13. 05. 1994
	Campsis radicans	Maglód (Pest m.)	13. 06. 1993
	Cydonia oblonga	Óbuda	11. 06. 1992
	Deutzia × hybrida	Gellérthegy	08. 06. 1994
	Lycium halimifolium	Krisztinaváros	24. 05. 1993
	Populus nigra	Törökvész	14. 10. 1995
	Prunus mahaleb	Törökvész	15. 05. 1993
	Prunus persica	Gellérthegy	17. 05. 1994
	Solanum dulcamara	Újlak	05. 06. 1994
Myzus varians Davidson, 1912	*Clematis vitalba*	Törökvész	29. 05. 1993
	Populus nigra	Törökvész	14. 10. 1995
	Prunus × davidiopersica	Gellérthegy	12. 04. 1994
	Prunus persica	Gellérthegy	17. 05. 1994
		Gellérthegy	28. 05. 1994
Ovatus crataegarius (Walker, 1850)	*Crataegus laevigata*	Kispest	12. 06. 1991
		Horváth-kert	26. 05. 1992
	Cydonia oblonga	Óbuda	11. 06. 1992
	Pseudocydonia sinensis	Gellérthegy	13. 06. 1994
Ovatus insitus (Walker, 1849)	*Cydonia oblonga*	Óbuda	11. 06. 1992
Phorodon humuli (Schrank, 1801)	*Celtis occidentalis*	Pesterzsébet	28. 05. 1993

Table 1 (cont.)

Aphid species	Plant species	Sampling place	Sampling date
		Gellérthegy	28. 05. 1994
	Cydonia oblonga	Óbuda	11. 06. 1992
	Prunus cerasifera	Gazdagrét	28. 05. 1993
		Törökvész	30. 04. 1995
		Törökbálint (Pest m.)	30. 05. 1995
	Prunus spinosa	Vérmező	29. 04. 1993
	Symphoricarpos albus	Pasarét	04. 06. 1994
Rhopalomyzus lonicerae Siebold, 1839	*Lonicera tatarica*	Gellérthegy	15. 04. 1994
		Gellérthegy	29. 04. 1994
Rhopalosiphoninus staphyleae (Koch, 1854)	*Staphylea pinnata*	Gellérthegy	29. 04. 1994
		Gellérthegy	02. 06. 1994
Roepkea marchali (Börner, 1931)	*Prunus mahaleb*	Ferencváros	09. 06. 1992
		Törökvész	19. 05. 1993
**Trichosiphonaphis polygonifoliae* (Shinji, 1944)			
	Lonicera tatarica	Pasarét	21. 05. 1994
		Pasarét	25. 05. 1996
		Pasarét	26. 10. 1996

Table 2

Simultaneous occurrence of aphids and ant species on ornamental trees and shrubs

Ant species	Aphid species	Plant species
Lasius niger (Linnaeus)	*Aphis fabae*	*Aronia melanocarpa*
		Buddleia alternifolia
		Buddleia davidii
		Callicarpa bodinieri
		Campsis × *tagliabuana*
		Celastrus orbiculatus
		Cistus laurifolius
		Cornus stolonifera
		Deutzia × *hybrida*
		Magnolia kobus
		Phellodendron amurense
		Polygonum orientale
		Pseudocydonia sinensis
		Rhus typhina
		Smilax excelsa
	Aphis chloris	*Hypericum* 'Hidcote'
	Aphis cytisorum	*Cytisus supinus*
	Aphis gossypii	*Celastrus orbiculatus*
	Aphis nasturtii	*Rhamnus* × *hybrida*

Table 2 (cont.)

Ant species	Aphid species	Plant species
	Aphis pomi	*Amelanchier canadensis*
		Aronia melanocarpa
		Rhaphiolepis indica
		Sorbus aucuparia
	Aphis schneideri	*Ribes alpinum*
	Aphis verbasci	*Buddleia davidii*
	Aphis viburni	*Viburnum carlesii*
		Viburnum tinus
	Brachycaudus amygdalinus	*Prunus tenella*
		Prunus persica
	Brachycaudus helichrysi	*Prunus triloba*
	Callipterinella tuberculata	*Betula pendula*
	Chaitophorus populialbae	*Populus grandidentata*
	Chaitophorus tremulae	*Populus grandidentata*
	Glyphina betulae	*Betula pendula*
	Macrosiphum euphorbiae	*Campsis* × *tagliabuana*
	Myzus persicae	*Buddleia davidii*
		Prunus persica
	Myzus varians	*Prunus persica*
	Ovatus crataegarius	*Pseudocydonia sinensis*
	Periphyllus testudinaceus	*Acer negundo*
Lasius brunneus Latr.	*Aphis fabae*	*Euodia velutina*
Lasius emarginatus Ol.	*Aphis fabae*	*Symphoricarpos albus*
	Aphis ruborum	*Rubus fruticosus*
	Macrosiphum euphorbiae	*Symphoricarpos albus*
	Periphyllus testudinaceus	*Acer monspessulanum*
Lasius fuliginosus Latr.	*Aphis clematidis*	*Clematis vitalba*
	Periphyllus obscurus	*Acer campestre*
	Periphyllus testudinaceus	*Acer campestre*
Formica rufibarbis F.	*Aphis fabae*	*Rhodotypos scandens*
	Cryptomyzus korschelti	*Ribes* sp.
	Hyperomyzus picridis	*Ribes* sp.
Plagiolepis pygmaea (Latr.)	*Aphis nasturtii*	*Rhamnus alaternus*
Pagliolephis sp.	*Aphis fabae*	*Cistus laurifolius*
		Ficus carica
		Periploca graeca
		Phellodendron amurense
	Aphis cytisorum	*Cytisus supinus*
	Aphis nasturtii	*Rhamnus* × *hybrida*
	Brachycaudus helichrysi	*Prunus triloba*
Prenolepis nitens Mayr	*Anoecia corni*	*Cornus glabrata*

Discussion and Conclusions

A total of 160 aphid species were determined; 7, 3 and 150 species of the Adelgidae, Phylloxeridae and Aphididae families were identified, respectively. The following 24 species were new for the Hungarian fauna: *Adelges cooleyi; Pineus strobi; Phylloxerina capreae; Phylloxerina salicis; Eriosoma grossulariae; Glyphina betulae; Chaitophorus capreae; Chaitophorus mordvilkoi; Chaitophorus ramicola; Periphyllus bulgaricus; Periphyllus rhenanus; Periphyllus venetianus; Drepanosiphum oregonensis; Appendiseta robiniae; Monelliopsis caryae; Tinocallis platani; Tinocallis saltans; Aphis hederae; Aphis mammulata; Aphis passeriniana; Aphis versicolor; Chaetosiphon tetrarhodum; Cryptomyzus korschelti; Trichosiphonaphis polygonifoliae.*

Most aphid species (29 species) occurred on plants of both Rosaceae and Salicaceae families. Fifteen species were determined from plants of both Aceraceae and Pinaceae families and 14 aphid species from the plants of Ulmaceae family. *Aphis fabae* was collected from the most woody host plants (62). The most aphid species (10) occurred on *Betula pendula*. Seven species were found on each of *Prunus persica*, *Populus nigra* and *Salix alba*. On species of maple *Periphyllus testudinaceus*, *Periphyllus lyropictus* and *Periphyllus obscurus*, on species of linden *Eucallipterus tiliae*, on species of poplar *Chaitophorus leucomelas*, *Chaitophorus populeti*, *Chaitophorus populialbae* and *Pemphigus spyrothecae*, on species of willow *Aphis farinosa* and *Chaitophorus salicti* and on species of the Rosaceae family *Aphis pomi* occurred the most frequently and with the highest population.

On greatly pruned and thinned trees and shrubs, infestation by aphids was always higher than on unpruned plants. *Aphis fabae* particularly favoured the shoots more intensively growing after thinning, e.g. *Celtis occidentalis*, *Cornus stolonifera*, *Maclura pomifera*, *Morus alba*, *Rhus typhina*. After thinning/pruning, *Aphis farinosa* on *Salix* species, *Chaitophorus leucomelas* on *Populus* species, and *Aphis craccivora* on *Robinia pseudoacacia* and *Caragana arborescens* were present with high population.

Together with the aphid species the following ant species occurred: *Lasius niger*, *Lasius brunneus*, *Lasius emarginatus*, *Lasius fuliginosus*, *Formica rufibarbis*, *Plagiolepis pygmaea* and *Prenolepis nitens*. *L. niger* being the most frequent, occurring associated to 20 aphid species.

Acknowledgements

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Management of Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte) in Corn Based on Survey Information from Previous Soybean Crop

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Reports of western corn rootworm (WCR) larval feeding damage in first-year corn following soybean have primarily occurred in northwestern Indiana and east central Illinois. There is speculation that WCR may be adapting its behavior to the corn/soybean rotation system by laying its eggs in areas that result in the greatest potential for larval survival the following growing season, that being corn following soybean (Edwards et al. 1996). During the summers of 1996 and 1997 experiments were carried out on various farms in Indiana. In the first study, 17 pairs of corn/soybean fields were used. Sampling to determine numbers of WCR adults emerging in corn was accomplished by using emergence traps. The traps were placed in the corn fields, all of which were in soybean the previous year. The data collected in this portion of the study provided important information on egg deposition in soybean fields and on adult emergence the following year in corn fields.

The second study was designed to compare counts of WCR adults caught on traps placed in both corn and soybean fields. Pherocon AM® yellow sticky traps were used for trapping adults. In the third study, four trap types (Pherocon AM traps, cone traps, cucurbitacins insecticide-baited vial traps and Olson Yellow Stiky® Cards) were compared to determine the numbers of WCR adults caught for each trap type in soybean and corn. The cone trap was replaced in 1997 by a new WCR attracticide trap which contained cucurbitacins, carbaryl, and paraffin.

Results showed that significant numbers of WCR adults emerged from corn fields where soybean was planted the previous year. Results also showed that an adult WCR survey in soybean can potentially be used for predicting larval damage in the following year's corn crop, although the specific economic threshold numbers were not determined in this study. For WCR adult survey in soybean, Pherocon AM traps seem to be the most efficient, although Olson Yellow Stiky Cards have good potential.

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, is the most serious insect pest of corn, *Zea mays* L., in the northwestern 'corn-belt' in the USA (Stamm et al., 1985). Moreover, it is one of the most economically damaging insect pests in North America with crop losses and control costs reaching nearly USA $1 billion annually (Metcalf, 1986). Rootworm larvae inhabit the soil and feed almost exclusively on corn roots (Branson and Ortman, 1971), thus interfering with water and nutrient uptake. When root pruning is extensive, or when feeding damage coincides with high amounts of precipitation and strong winds, plant lodging can occur. Lodged plants make mechanical harvesting very difficult.

Shortly after emerging in corn fields, WCR beetles begin to feed. The beetles primarily feed on corn pollen, but can also feed on corn silk, kernels, and/or leaf tissue. Within a short time after emerging, beetles begin to mate. Females lay their eggs in the soil in the corn field where they emerged, and/or move to other fields for egg laying.

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Until recently in parts of Indiana, Illinois, Michigan, and Ohio, USA, WCR beetles moved primarily to other corn fields to feed and lay eggs. Thus, rootworm larval predictions for the following season were generally based on the previous year's adult counts in corn.

However, agriculturists in the states of Indiana and Illinois have observed a substantial increase in economic WCR larval damage to corn following soybean (Edwards et al., 1996). This means that WCR beetles are laying significant numbers of eggs in soybean fields. This represents a significant change from what was observed in the past. The exact reason(s) for this sudden and dramatic increase in damage in first-year corn is unknown. The problem has expanded from a few fields in north central Illinois in 1987 to hundreds of Indiana fields in 1995. Reports of WCR larval feeding damage in first-year corn following soybean have primarily occurred in northwestern Indiana and east central Illinois. Rootworm damage to first-year corn has also been observed in other parts of northern Indiana, in southern Michigan and in northwestern Ohio. There is speculation that WCR may be adapting its behavior to the corn/soybean rotation system by laying its eggs in areas that result in the greatest potential for larval survival the following growing season, that being corn following soybean (Edwards et al. 1996).

This change in WCR behavior has resulted a significant increase in the use of soil insecticides in first-year corn. It is not uncommon for farmers to treat 80% or more of their first-year corn acreage, whereas approximately 4 years ago it was less than 15%. Because of the lack of a sampling protocol for WCR beetles in soybean, and the lack of crop loss figures for larval feeding in the following year's corn crop based on number of beetles observed in the previous year's soybean, farmers have started to apply soil insecticides as insurance treatments to their first-year corn fields. This has reactivated the pesticide treadmill that was deactivated more than a decade ago through an increase in use of crop rotation. Under crop rotation, there was little need for a soil insecticide since the planting of soybean broke the rootworm cycle.

The research reported on in this study was designed to obtain some of the information that is needed to develop a management decision model for WCR in first-year corn. The parameters studied were 1) the emergence of WCR beetles from first-year corn fields following soybean, 2) WCR adult populations in corn and soybean fields through time, and 3) the efficacy of various trap types for catching WCR beetles over time.

Materials and Methods

The experiments were conducted in farmers' corn and soybean fields from about 30 to 62 km west, northwest and north of Purdue University, W. Lafayette, Indiana, USA, in 1996 and 1997. These studies were part of a larger study referred to as the Indiana Multi-County WCR Study.

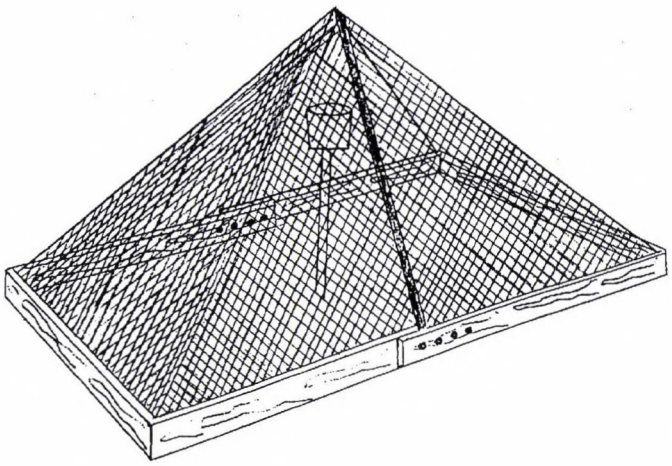

Fig. 1. Adult corn rootworm emergence trap (after Krysan and Miller 1986)

Emergence of WCR adults from corn fields previously in soybean

By using emergence cages, the appearance of WCR adults in corn fields from eggs laid the previous year in soybean could be observed and the numbers of emerging adults recorded. The emergence cages were made of 1.9 cm × 8.9 cm wood strips that were formed in the shape of a 76 cm × 35 cm frame (0.27 m^2) and covered with aluminum screen (7.1 squares of mesh/cm) formed in the shape of a 50 cm high pyramid (Krysan and Miller, 1986) (Fig. 1). A paper cup was located in the top of each emergence trap. These cups were coated with Tanglefoot® Insect Trap Coating (The Tanglefoot Company, Grand Rapids, Michigan). The cups were changed each week. Captured WCR adults were counted and numbers recorded. Two pairs of untreated (no insecticide) rows were designated as the sampling unit in each corn field. Six traps were placed in each of 2 untreated rows for a total of 12 emergence traps per field. The entrance to the rows containing the emergence cages was flagged along the field border for easy identification. The traps were operated from 8 July to 28 August 1996 and 7 July to 25 August 1997.

Survey of WCR adults in neighboring soybean and corn fields

Six Pherocon AM traps (Fig. 2) were placed in each of 11 paired corn and soybean fields. In 1996, 3 traps were placed in each of corn rows 12 and 24, with corn row 1 being next to the soybean field. Soybean fields were divided into thirds lengthwise, creating two transects running the length of the field between the three sections. Three Pherocon AM traps were placed in each transect and were separated by equal distance to cover the length of the field. Traps were attached to 1.2 m wooden stakes. The traps were re-

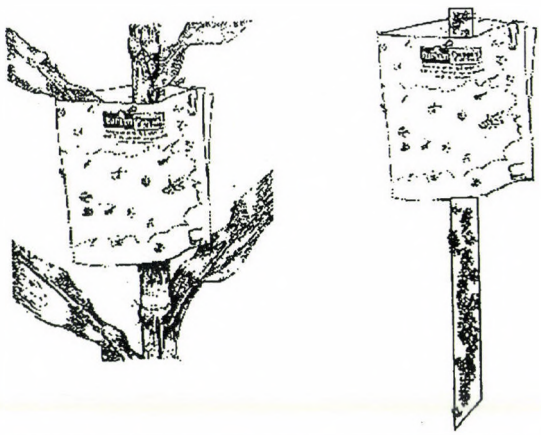

Fig. 2. Pherocon AM® trap on a corn plant (Krysan and Miller 1986) and on wooden stake in soybean field

placed each week and the height of traps was adjusted at that time so as to keep the traps slightly above the soybean canopy. The trapping season in 1996 was from 31 July to 3 September.

In 1997, the arrangement of traps in both corn and soybean was changed based on observations of trapping results from 1996. The data from the 1996 trapping activity showed that there was no difference between the two trapping rows in soybean and in corn. Therefore, trapping was simplified in 1997. The same number of traps, that being 6 per crop, were utilized for both years. In corn in 1997, all 6 traps were placed at an equal distance from each other in the corn row nearest the soybean test field. In soybean, 6 traps were placed at equal distance in the middle of the field. The traps in soybean were arranged on wooden stakes as in 1996, and were changed and the height adjusted weekly. The traps in corn were wrapped around the corn stalk at about ear height. The 1997 trapping season was from 28 July to 2 September.

As a part of the above study, sweep net samples were taken each week in each soybean field so as to collect 30 beetles for sex ratio and stage of female ovarial development determinations. In corn fields, 30 beetles were also collected for the same purpose. The beetles in corn were collected by knocking them into a vial containing ethyl-acetate.

Efficacy of various trap types and trap placement for WCR adult detection

The efficacy of four types of traps for capturing WCR adults was examined in 6 paired corn and soybean fields in 1996 and 1997. In each field, 6 each of Pherocon AM traps, cone traps, cucurbitacins insecticide-baited vial traps, and Olson Yellow Stiky Cards were used. Each trap type was randomly placed in corn rows 12, 24, 36, 48, 60,

and 72, with row 12 being closest to the soybean field. This arrangement was used to determine the efficiency of each trap for trapping WCR adults, and to look at variability among traps within a field and variability of WCR across a field. As a result of the analysis of data generated in 1996, it was decided that only one type of trap was needed in corn to provide WCR population data sufficient for relating to populations in soybean. The trap of choice was the Pherocon AM trap. In 1996, the trapping season was from 31 July to 4 September.

In the soybean fields, 6 traps of each trap type, as noted above for corn in 1996, were placed randomly in one of 4 trapping lanes in 1996. In 1997, the same types of traps were used in soybean, except the cone trap was replaced by the new WCR attracticide trap which contained cucurbitacins as the feeding stimulant, carbaryl as the toxin, and paraffin as the carrier. Since the results from 1996 were basically the same in each row in the corn, it was not necessary to place traps in multiple rows in 1997. The first row of corn next to each soybean test field was selected for WCR trapping in 1997. Also, since the primary trapping was to be concentrated in soybean, it was not necessary to use all trap types in corn. Therefore, the Pherocon AM trap was selected for use in the corn. The trapping season was from 29 July to 3 September in 1997.

As with the previous study, sweep net samples were taken each week in each soybean field so as to collect 30 beetles for sex ratio and stage of female ovarial development determinations. Thirty beetles were also collected in corn for the same purpose. The beetles were collected in corn by knocking them into a vial containing ethyl-acetate.

Statistical analysis (ANOVA) of data in the trap placement trial was carried out using the Microsoft Excel Analysis ToolPak (GreyMatter International, Inc., Cambridge, Massachusetts).

Results and Discussion

Emergence of WCR adults from corn fields previously in soybean

Figures 3 and 4 summarize the results of the capture of WCR adults in emergence cages in corn following soybean for the two years of the study. Although peak emergence occurred latter in 1996 than in 1997, the dates of the beginning of emergence and completion of emergence for the two years were similar. In 1996, the first beetles emerged in the middle of July with the majority of beetles being trapped from 31 July to 12 August. In 1997, the first beetles emerged on the same date as in 1996, 15 July, and the beginning of significant levels of beetle emergence, 21 July, was similar to that in 1996, 22 July. However in 1997, the number of beetles emerging after the peak emergence became gradually less and less while the number captured in 1996 built up gradually and fell off sharply after 12 August. The highest number of beetles captured in 1996 was 23 beetles/m^2 while 15 beetles/m^2 were captured in 1997.

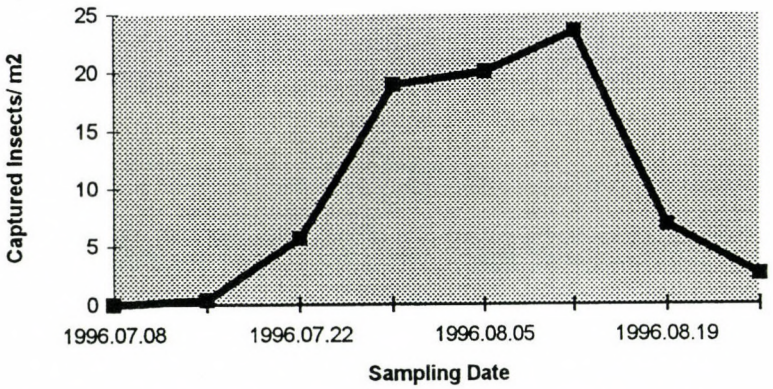

Fig. 3. Western corn rootworm beetle captures in emergence cages, 1996

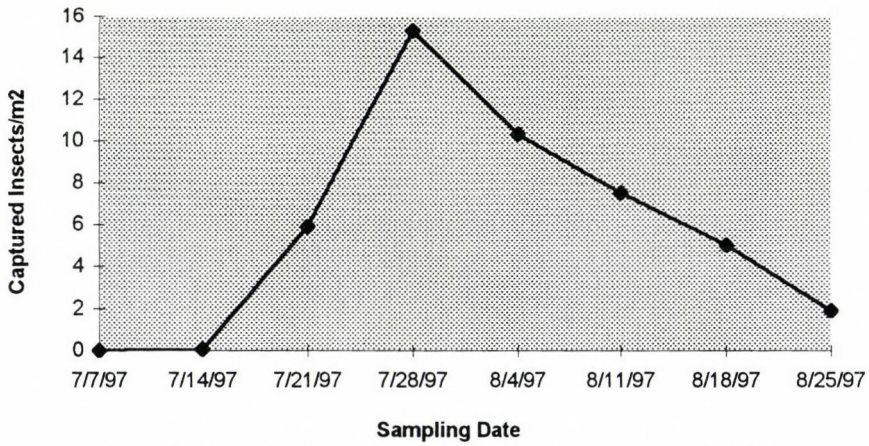

Fig. 4. Western corn rootworm beetle captures in emergence cages, 1997

Survey of WCR adults in neighboring soybean and corn fields

Using data of captured WCR beetles in the Indiana Multi-County WCR Study, diagrams showing the incidence-rates of WCR adults in corn and soybean fields were produced (Figs 5 and 6). WCR beetles were present in corn and soybean fields during the same time period. The build-up in the two crops followed a similar pattern with slightly higher numbers in the corn fields. The majority of beetles present in corn and soybean fields was present in the last 3 week of August for both years. During this period of increasing WCR beetle numbers, approximately 75% of the beetles were females.

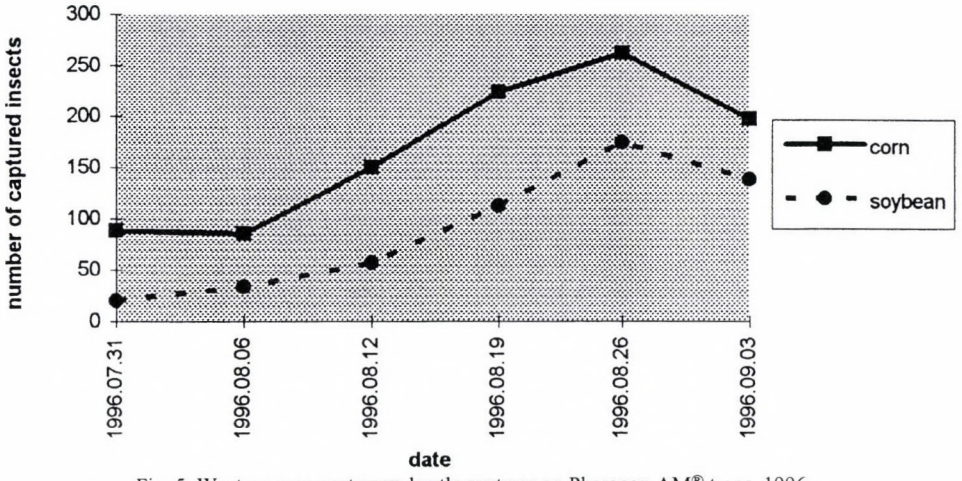

Fig. 5. Western corn rootworm beetle captures on Pherocon AM® traps, 1996

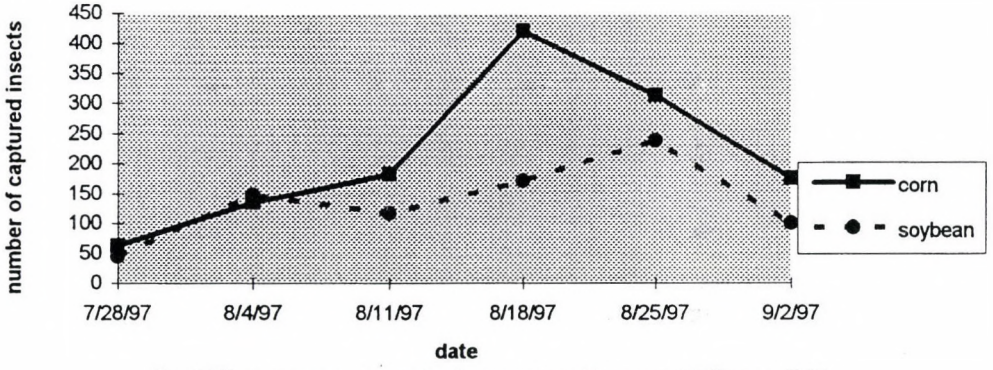

Fig. 6. Western corn rootworm beetle captures on Pherocom AM® traps, 1997

Efficacy of various trap types and trap placement for WCR adult detection

From the data of the trapping study, the efficiency of the various traps in each examined corn row can be observed (Fig. 7). Statistical analysis was conducted to find out if there are relationships between traps and row-placement in corn. The results indicate that there is close a relationship between types of traps (P = 0.731), but no relationship between rows (P = 1.54E–09). These results show that it is not necessary to place traps in multiple rows and thus sample throughout a field (Fig. 8). The mean value of each trap's captures is shown in Fig. 9.

The most efficient traps used in soybean and corn in 1996 were the Olson Yellow Stiky Card and Pherocon AM traps (Fig. 9). Cone and cucurbitacins insecticide-baited vial traps were not as efficient in capturing adults in 1996 when compared to the other traps. When comparing the data in Figs 9 to 10, it is apparent that in 1997 the Olson

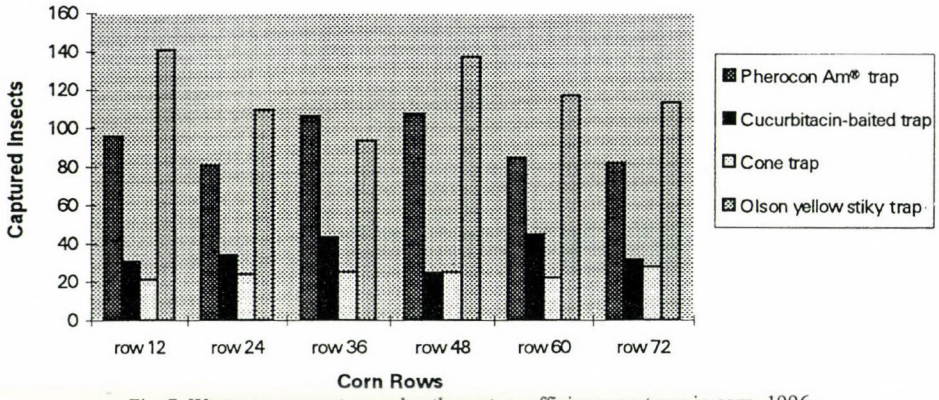

Fig. 7. Western corn rootworm beetle capture efficiency on traps in corn, 1996

ANOVA

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
distance	36945.05	3	12315.02	82.2137	1.54E-09	3.287383
traps	417.5971	5	83.51942	0.557567	0.730791	2.901295
Error	2246.891	15	149.7927			
Total	39609.54	23				

Fig. 8. Analysis of variance for Western corn rootworm beetle capture efficiency of traps by row, 1997

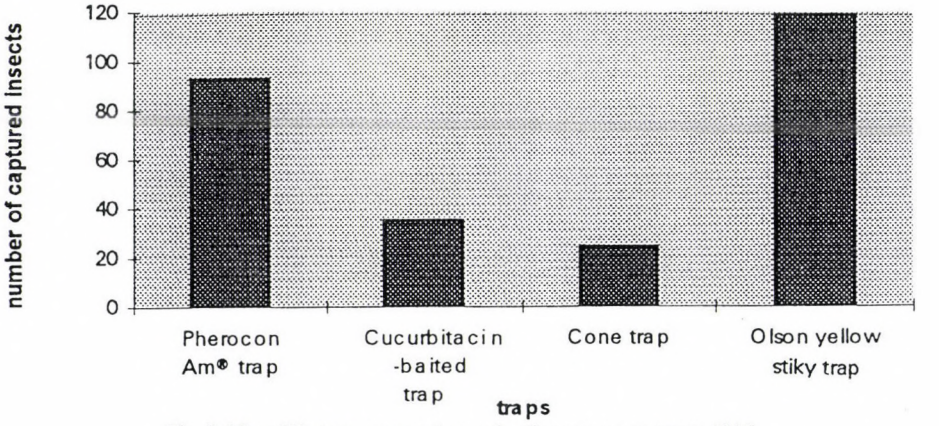

Fig. 9. Mean Western corn rootworm beetle captures on traps, 1996

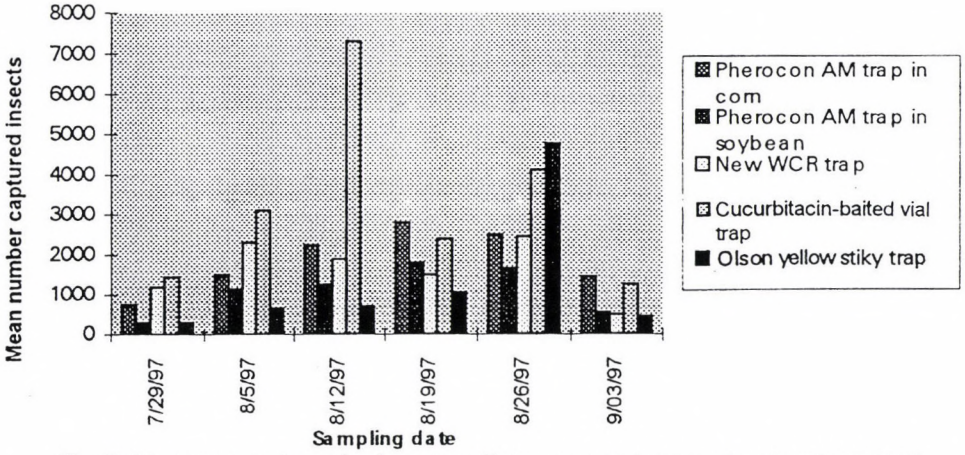

Fig. 10. Western corn rootworm beetle capture efficiency on traps in corn and soybean fields, 1997

Yellow Stiky Card did not work as well as it did in 1996. This was due to the dislodging of the Olson traps from the stakes in soybean by numerous periods of heavy rainfall and strong winds. The fact that more beetles were present in the study area in 1997 should have resulted in higher captures on the Olson trap in 1997 when compared to 1996. Although the efficiency of cucurbitacins insecticide-baited vial trap was not very high in 1996, it performed much better in 1997 (Fig. 10). It is not known as to the reason for this occurrence. In the corn fields in 1997, Pherocon AM traps were placed in the first corn row on the side next to the soybean field. The data show that beetle captures on Pherocon AM traps in corn were higher than those for the Pherocon AM trap in soybean (Fig. 10). These data show the same trend for this trap type as the results presented in Figs 5 and 6 show.

Conclusions

Initially, these studies were established in northwestern Indiana to determine the level of movement of WCR beetles into soybean from corn fields. However, this study also presented the opportunity to evaluate the significance of egg laying in soybean fields by evaluating WCR beetle emergence in the corn the following year (soybean/corn rotation). Also, it allowed for the examination of different types of traps to determine WCR adult trapping efficiency. Results showed that there was a significant level of emergence of WCR beetles in the following year's corn crop based on emergence cage trappings. Results also showed that adult WCR surveys made in soybean using one or more trapping techniques can be used for predicting potential larval damage in the following year's corn crop.

The data show that overall the Pherocon AM trap is the most efficient trap to use in soybean of the trap types evaluated. Although the Olson Yellow Stiky Cards are as good if not better than the Pherocon AM trap for capturing WCR beetles, the fact that they are easily dislodged from the stakes that hold them is of concern.

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The Coccoidea Fauna and their Host Plants in Cultivated and Non-cultivated Areas in the East Mediterranean Region of Turkey

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The Coccoidea fauna of the east Mediterranean region of Turkey and their host plant range was studied in cultivated and non-cultivated areas in 1994 to 1997. Special emphasis was placed to sample individuals in different climatic region and various ecosystems such as perennial crop plants and woody plants adjacent to agricultural fields, ornamental and urban plants, as well as forests and macchia.

During this study 51 Coccoidea species including 30 from Diaspididae and 22 species from Coccoidae, Pseudococcidae, Eriococcidae, Asterolecaniidae, Kermesidae, Ortheziidae, Cerococcidae, and Margarodidae were identified. Six diaspidid species, one species from Pseudococcidae and Coccoidae, and the genus Acanthoccosus from Eriococcidae were determined as new records for the Turkish fauna. Most species encountered were from the family Diaspididae (30) followed by Coccidae (9). Scale insects were more frequently determined on wild plants, especially on those bordering agricultural fields, than on crop plants. Since scale insects on wild plants were often associated with different parasitoids and predators there is a strong indication that these habitats play an important role in the naural control of scale insects in agricultural crops.

Species of superfamily Coccoidea (Homoptera) and in particular armoured scale insects (Diaspididae) are most important pests damaging not only agricultural, urban, and ornamental plants but also forest trees and plants of natural ecosystems (Rosen, 1990). In Turkey several surveys on Coccoidea fauna were carried and 163 species have been identified up to now (Bodenheimer, 1949, 1952–1953; Soylu, 1976; Öncüer, 1974 and 1977; Çanakcıoğlu, 1977; Selim, 1979; Kozár et. al., 1979; Erler et. al., 1996). The world-wide importance of Coccoidea as pest of agricultural, ornamental, and urban plants is due to difficulties in control and the increasing risk of spreading these pests by infested plant material.

Geographical areas characterised by a rich and divers flora are likely to habituate many different scale insect species including their natural enemies like predators and parasitoids. The east Mediterranean region is such a region located at the conjunction of two faunistic subregion: the Mediterranean and Irano-Turanian. In additions, this area consists of many different climatic regions, ranging from subtropical-mediterranean to boreal. Even though several studies on Coccoidea fauna have been conducted in fruit plantations in the east Mediterranean region (Karaca and Uygun, 1990, 1993; Erkılıç and Uygun, 1995; Uygun et al., 1995), none of these surveys considered natural habitats, ornamentals or urban trees.

In the present paper the Coccoidea fauna of agricultural crops, ornamental and ur-

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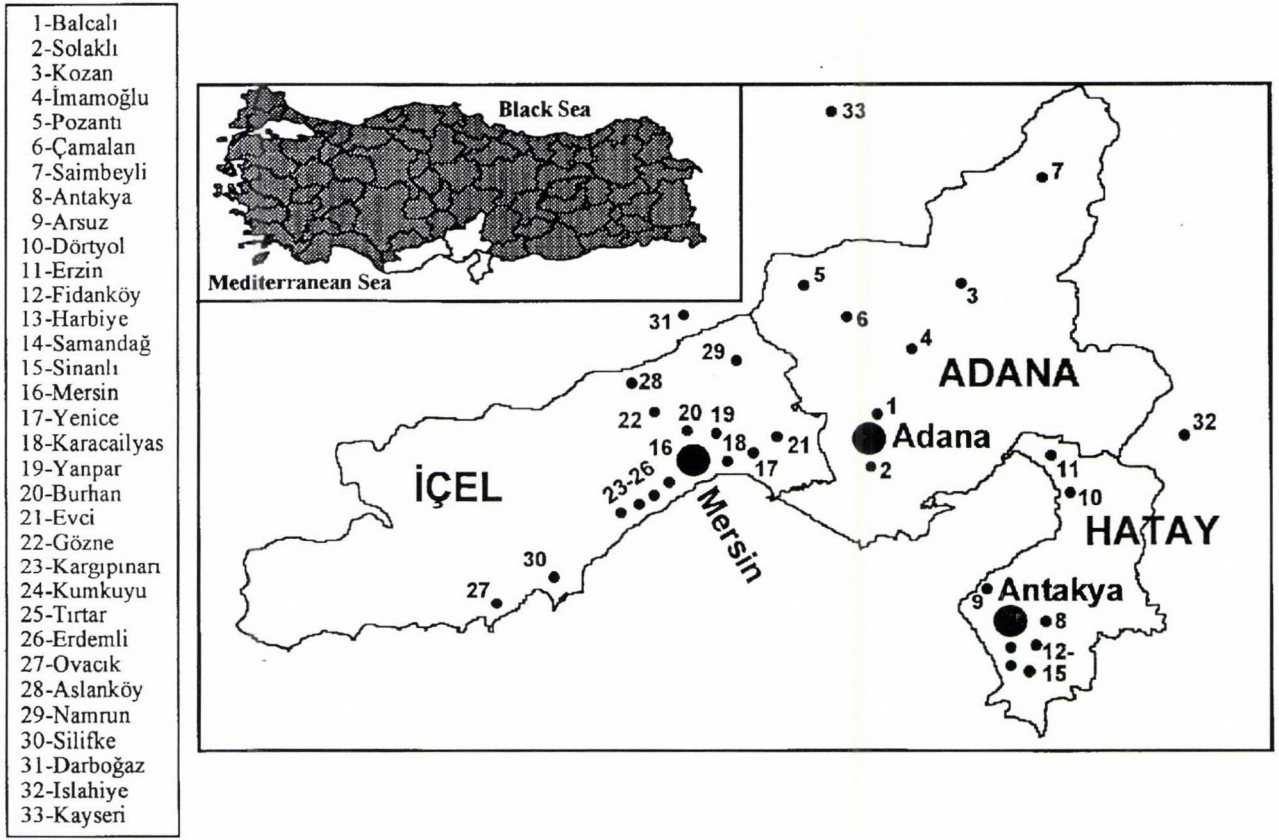

Fig. 1. Sampling sites of scale insects in the east Mediterranean region of Turkey

ban plants, forests and macchia was studied in the east Mediterranean region of Turkey and their importance as alternative hosts of scale insect parasitoids and predators is discussed.

Materials and Methods

Coccoidea were sampled in different ecosystems in east Mediterranean region of Turkey in 1994 to 1997 (Fig. 1). These ecosystems consisted of mainly perennial crop plants and woody plants adjacent to agricultural fields, ornamental and urban plants, as well as forests and macchia as an example of less disturbed biotopes.

Sampling was conducted in irregular surveys at different locations of the study area. Following roads, every 20 km scale insects were collected after visual examination of different plant parts like leaves, branches, trunks, fruits and some cases also roots. The sampling sites were recorded according to their habitat, their plant community and altitude. Samples were wrapped in paper, packed in polyethylene bags and brought to laboratory in an ice chest.

In the laboratory, scale insects were carefully removed from the plant surface with an insect needle and slides prepared according to Kosztarab and Kozár (1988). Specimens were identified using the keys of Balachowsky (1948), Kosztarab and Kozár (1988), Danzig (1993), Hodgson (1993), and Ben-Dov (1995). In addition, samples were compared with those of the scientific scale insect collection of Dr. F. Kozár, Plant Protection Institute, Budapest, Hungary, and with material in several naturaly history museums. Voucher specimens of all scale insects are kept in Plant Protection Research Institute in Adana, Turkey.

Host plants of scale insects were identified by the plant taxonomist at the Institute of Botany, University of Çukurova.

Results and Discussion

During this study 51 Coccoidea species including 30 from Diaspididae and several species from Coccidae, Psuedococcidae, Eriococcidae, Asterolecaniidae, Kermesidae, Ortheziidae, Cerococcidae, and Margarodidae were identified in east Mediterranean region of Turkey.

In fruit plantations 13 diaspidid species were determined. Among these, 11 species are known pests of various agricultural plants, while the remaining two species, *Aulacaspis rosae* (Bouché) and *Parlatoria crotonis* Douglas, may be considered as potential pests (Table 1). The majority with 21 diaspidid species, however, was sampled from woody plants in non-cultivated areas as forests or macchia. Only four species, *Aonidiella aurantii* (Maskell), *Aulacaspis rosae* (Bouché), *Parlatoria oleae* (Colvée), *Epidiaspis leperii* (Signoret) were common in cultivated areas and natural habitats, 17 species were restricted to wild plants (Table 2).

Table 1

Scale insects and their host plants in cultivated areas in the east Mediterranean region of Turkey in 1994–1997
For location see Figure 1

Scale insect species	Host plant	Location
Diaspididae		
Aonidiella aurantii (Maskell)	*Citrus* spp.	1, 3, 8, 10, 12, 14, 14, 26
Aonidiella citrina Coquillet	*Citrus* spp.	26
Aulacaspis rosae (Bouché)	*Malus communis*	31
Chrysomphalus dictyospermi (Morgan)	*Citrus* spp.	1
Epidiaspis leperii (Signoret)	*Prunus* sp.	13
Lepidosaphes beckii (Newmann)	*Citrus* spp.	1, 8, 9, 10, 12, 13, 14
Leucaspis ricae Targioni-Tozzetti	*Oleae europeae*	20, 32
Nilotaspis halli (Green)	*Prunus* sp.	32
Parlatoria crotonis Douglas	*Citrus* spp.	10
Parlatoria oleae (Colvée)	*Eriobotrya japonicus Malus communis, Oleae europae, Prunus* sp.	1, 13, 24, 32
Parlatoria pergandii Comstock	*Citrus* spp.	8, 10, 14
Pseudaulacaspis pentagona Targioni-Tozzetti	*Morus alba, Prunus persicae*	1, 11, 13, 23, 30
Quadraspidiotus perniciosus (Comstock)	*Eriobotrya japonicae, Malus communis*	1, 28
Pseudococcidae		
Planococcus citri (Risso)	*Citrus* ssp., *Vitis vinifera*	1, 8, 28
Planococcus ficus (Signoret)	*Ficus* sp., *Malus* sp., *Vitis vinifera*	1
Pseudococcus viburni (Maskell)	*Citrus* spp.	14
Coccidae		
Ceroplastes floridensis Comstock	*Citrus* spp.	1, 10, 26
Coccus hesperidum Linneaus	*Citrus* spp.	1, 9, 10, 14
Coccus pseudomagnoliarum (Kuwana)	*Citrus* spp.	3, 11, 12, 16
Eulecanium rugulosum (Archangelskaya)	*Prunus persicae*	28
Palaeolecanium bituberculatum Targioni-Tozetti	*Malus communis*	5
Shaerolecanium prunastri (Fonscolombe)	*Prunus persicae*	28, 31
Cercoccidae		
Pollinia pollini (Costa)	*Oleae europaea*	1
Margarodidae		
Icerya purchasi Maskell	*Citrus* spp.	1, 8

Table 2

Scale insects and their host plants in non-cultivated areas in the east Mediterranean region of Turkey in 1994–1997 (For location see Figure 1)

Scale insect species	Host plant	Location
Diaspididae		
Aonidia lauri (Bouché)	*Laurus nobilis*	8, 13, 15
Aonidiella aurantii (Maskell)	*Acacia* sp., *Euonimus* sp., *Nerium oleander*	1, 11, 26
Aspidiotus nerii Bouché	*Acacia cyamophylla, Erica verticiallata, Hedera helix, Melia azadraci, Nerium oleander*	1, 11, 13, 26, 29, 32
Aulacaspis rosae (Bouché)	*Rubus cuesus*	22
Carulaspis carueli (Signoret)	*Cupressus* sp.	18
Chionaspis salicis (Linneaus)	*Cornus mas*, *Populus* sp.	22
Diasidiotus distinctus (Leonardi)	*Gonocytisus* sp.	1
Diaspis syriaca Lindiger	*Pistaceae terebinthus, Populus* sp.	27, 28
Dulachionaspis stanotophri Cooley	*Phragmites australis*	1
Epidiaspis leperii (Signoret)	*Rosae* sp.	1
Lepidosaphes cochyformis Gmelin	*Ulmus* sp.	15
Lepidosaphes malicola Borchsenius	*Prunus* sp.	33
Lepidosaphes pistaciae (Archangelskaya)	*Pistaceae terbinthus*	27
Lepidosaphes ulmi (Linneaus)	*Populus* sp., *Prunus* sp., *Rosea* sp., *Rubus* sp.,	17, 22, 27, 33
Leucaspis knemion Hoke	*Pinus* sp.	32
Leucaspis pini (Hargit)	*Pinus nigra*	6, 11
Leucaspis pusilla Loew	*Pinus nigra*	1, 4
Lineaspis cilicae (Coleman)	*Cupressus* sp., *Thuja* sp.	4, 12, 14, 18, 25, 26, 32
Parlatoria oleae (Colvee)	*Rosae* sp., *Syringa vulgaris*	1, 24, 26
Rhizaspidiotus donacis (Leonardi)	*Phragmites australis*	1, 18
Salicola kermanensis Lindinger	*Populus* sp.	11, 13, 19
Coccidae		
Bodenheimera rachelae Bodenheimer	*Vitex agnus castus*	30, 32
Ceroplastes floridensis (Comstock)	*Herdera helis, Myrtus communis, Laurus nobilis, Nerium oleander*	1, 21, 25, 26
Ceroplastes rusci (L.)	*Laurus nobilis, Myrtus communis, Nerium oleander*	1, 13, 25, 26
Coccus hesperidum L.	*Hedera helix, Laurus nobilis*	13, 26
Coccos pseudomagnoliaruum (Kuwana)	*Nerium oleander*	13
Parthenolecanium corni (Bouche)	*Cornus mas*	7
Pseudococcidae		
Phenacoccus aceri (Signoret)	*Acer* sp.	33
Planococcus vovae (Nasanov)	*Cupressus* sp.	2, 4, 10, 11
Eriococcidae		
Acanthococcus sp.	*Laurus nobilis*	13
Gossyparia spuria (Modeer)	*Ulmus* sp.	13
Kermesidae		
Kermes sp.	*Quercus* sp.	28
Ortheziidae		
Ortheziae urticae (Linneaus)	*Erica verticillata*	11
Asterolecaniidae		
Asterodiaspis quercicola (Bouché)	*Quercus* sp.	11

Six diaspidid species were new record for the Turkish armoured scale insect fauna, however, two of them were determined outside of east Mediterranean region. Because these locations are close to the sutdy area and the species were new records, they were included in this study; material examined:

Diaspidiotus distinctus Leonardi – Adana, 3. 7. 1994, *Gonocytisus* sp.,
Duplachionaspis stanotophri Cooley – Adana, 3. 7. 1996, *Phragmites austriacus*,
Rhizaspidiotus donacis (Leonardi) – Içel/Karacailyas, 8. 8. 1995, *P. austriacus*,
Lepidosaphes malicola Borchsenius – Kayseri, 23. 7. 1994, *Prunus* sp.,
Leucaspis knemion Hoke – Gaziantep/Islahiye, 25. 4. 1995, *Pinus* sp.,
Parlatoria crotonis Douglas – Hatay/Dörtyol, 12. 9. 1995, *Citrus* sp.

The highest number of diaspidid species was encountered on citrus, including important pests like *A. aurantii*, *A. citrina* Coquillet, *Chrysomphalus dictyospermi* (Morgan), *Lepidosaphes beckii* (Newmann) and *Parlatoria pergandii* Comstock. With regard to the geographical area, citrus cultivated in Hatay displayed the richest scale insect fauna with 11 species while only seven and four species were found on citrus in Adana and İçel, respectively.

Other diaspidid species, namely *Quadraspidiotus perniciosus* (Comstock), *Pseudaulacaspis pentagona* Targioni-Tozzetti, *Nilotaspis halli* (Green), *Parlatoria oleae* (Colvée), *Epidiaspis leperii* (Signoret) found in cultivated areas are most destructive scale insects on fruit trees in many countries (Düzgüneş, 1940; Schmutterer, 1957; Erkam, 1981; Kozár et al., 1979; Kosztarab, 1990; Miller and Davidson, 1990; Erkılıç and Uygun, 1995). In olive plantations, *P. oleae* and *Lepidosaphas riccae* Targioni-Tozzetti were the only diaspidid species found during this study. *L. riccae* is almost monophag on olive and not found on any other cultivated plant (Borchsenius, 1966). In addition, *A. rosae* has to be considered as potential pest for plants in the family Rosaceae, which is the only host plant group of this species. *Parlatoria crotonis* (Colvée) is a new record for citrus scale insect fauna and was up to now only reported from Palmae, Orchidaceae, Moraceae, Magnoliaceae, Lauraceace, Leguminoceae, Euphorbiaceae, Aceraceae (Borchsenius, 1966).

A higher number of diaspidid species was determined in non-cultivated areas compared to cultivated areas. *Aspidiotus nerii* Bouché although rare on cultivated plants was determined on a wide range of wild plants and often bordering fruit plantations. Since many parasitoids and predators were determined on *A. nerii* during this study, this scale insect species and its respective host plants are important factors in biological control of scale insect. *Lepidosaphes ulmi* (Linneaus) was common on wild plants and in contrast only a few specimens of were determined on cultivated plants. This species is known as an important pest of fruit plantations in Central Anatolia (Okul et. al., 1983). Common and widely distributed plants like *Pinus* sp., *Cupressus* sp., *Thuja* sp., *Rubus* sp., *Laurus nobilis*, *Acacia cyamophylla*, *Nerium oleander*, or *Populus* sp. hosted many different scale insect species. Thus, these plants and their scale insect fauna may serve as important habitat for natural enemies associated with Diaspididae.

In total 21 species were identified from Coccidae, Pseudococcidae, Eriococcidae, Asterolecaniidae, Kermesidae, Ortheziidae, Cerococcidae, and Margorodidae, including two new species for the Turkish fauna. Twelve species were from crop plants (Table 1) and 13 species were sampled on wild plants in non-cultivated areas (Table 2). However, only three species were common, namely *Coccus hesperidum* (Linneaus), *C. pseudomagnoliarum* (Kuwana) and *Ceroplastes floridensis* Comstock (Coccidae). Two species sampled in cultivated area are new for the Turkish fauna: *Eulecanium rugulosum* Archangelskaya and *Pseudococcus viburni* (Maskell). Despite *P. viburni* is known as a forest pest (Ben-Dov, 1995), in this study *P. viburni* was recorded on citrus for the first time. Among pseudococcids, *Planococcus citri* (Risso) and *P. ficus* (Signoret) were found only on cultivated plants. They are widespread on cultivated plants in all Mediterranean countries (Ben-Dov, 1995). Species determined from Coccidae, Cerococcidae and Margarodidae during this study are all known from plantation-like ecosystems (Nizamlioğlu and Gökmen, 1964, Öncüer, 1974; Soylu, 1976).

Beside armoured scale species, Coccidae samples were dominating among the other Cocciodea families in non-cultivating areas. Two Ceroplastes species, *C. floridensis* and *C. rusci* (Linnaeus) were found on typical Mediterranean vegetation such as *Laurus nobilis*, *Myrtus communis*, *Nerium oleander*. These widely distributed host plants may served on one hand as an infestation source, but are on the other hand important refuges for coccids and their natural enemies. Also several Coccus species were frequently found on *L. nobilis, Hedera helix, Nerium oleander*. Only a single species was determined each from Asterolecaniidae, Cerococcidae and Ortheziidae. Eriococcidae is little known family for Turkey, the genus, *Acanthococcus* sp. was recorded from the samples collected on *L. nobilis. Planococcus vovae* (Nasarov) was frequently encountered on *Cupressus* sp., a plant used as wind break around citrus orchards. It was observed that *P. vovae* is an important alternative host of *Leptomastix dactylopii* Howard (Hymenoptera: Aphelinidae) and *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (Yiğit, 1995). Both natural enemies are annually released for the control of *P. citri* in citrus. Thus, *P. vovae* plays an important role as alternative host of *L. dactylopii* and *C. montrouzieri* and *Cupressus* sp. is an important refugee for natural enemies.

Kermesidae is one of the most difficult group for identification, since the crawler stage is needed. This is the reason that the single species determined can be given only as genus.

This survey on the Coccoidea fauna of the east Mediterranean region of Turkey uncovered many different scale insects on various cultivated and non-cultivated plants in several different geographical and climatic regions. The wide distribution of Coccoidea on plants adjacent to crop plants indicates that these habitats play an important role tin the natural control of scale insects in agricultural crops.

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A Revision of the Parasitoids of Whiteflies from Egypt

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Twenty whitefly parasitoid species are reviewed with data on their geographical distribution, hosts and their host plants and abundance. Five parasitoids are new records from Egypt, of which *Euderomphale ezzati* sp. n. During this work, six parasitoids species introduced into Egypt for the whitefly control. Key to parasitoids of whieflies are given.

Many authors have dealt with hymenopterous parasitoids of whiteflies, the most recent author is Gerling (1990), who listed parasitoids in six genera under two superfamilies of parasitic Hymenoptera namely: *Amitus* Haldeman, *Ablerus* Howard, *Cales* Howard, *Encarsia* Forester, *Eretmocerus* Haldeman and *Euderomphale* Girault.

Hymenopterous parasitoids of whiteflies in Egypt belong to four genera, *Amitus* Haldeman, *Encarsia* Forester, *Eretmocerus* Haldeman and *Eudermphale* Girault. The parasitoids of whiteflies in Egypt were identified and studied by many workers, such as Priesner and Hosny (1940) (*Encarsia elegans* Masi, *E. inaron* (Walker) and *Eretmocerus corni* Haldeman); Khalifa and El-Khidir (1965) (*Eretmocerus diversicilatus* Silvestri); El-Helaly et al. (1971) (*Eretmocerus mundus* (Mercet)); Abdel-Fattah et al. (1984) (*Encarsia lutea*); and Abd-Rabou (1996) (*Amitu hesperidum* Silvestri, *Encarsia davidi* Viggiani, *E. galilea* Rivnay and Gerling and *Eretmocerus cadabae* Viggiani).

The present contribution aims at providing a preliminary revision of the parasitoids of whiteflies from Egypt. It is based largely on the data studied by me and that obtained from the literature.

Materials and Methods

The revision of the parasitoids of whiteflies in Egypt was carried out, by examining the specimens collected during the present work (about 350 male and female specimens). The specimens were prepared for microscopic examination according to the method described by Noyes (1982) for slide mounting of Chalcidoidea. Morphological terminology and synonymies are after La Salle and Schauff (1994), Polaszek et al. (1992), Hayat (1989), Rivnay and Gerling (1987), Viggiani (1985) and Hayat (1972). Samples of parasitoids of whiteflies were collected from 16 species of host plants infested 20 species of whiteflies in 16 localities in Egypt. Abunance of these parasitoids was carried out on 7 host plants in 6 regions infested by 8 species of whiteflies. Host

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plants in the six regions did not receive any chemical control. Whitefly species, second and third larval stages and pupae were sampled on the plant leaves collected monthly (20 infested leaves/sample) from one site representing each of six regions in Egypt. Plant leaves were transferred to the laboratory in well ventilated boxes. At the laboratory, whiteflies species eggs and first larval stages were eliminated as well as other insects. Total number of whiteflies species stages were recorded per leaf. Each leaf was stored in well ventilated glass emergence tube and monitored daily for parasitoids emergence. The parasitoid adults were slide mounted in Hoyer's medium and identified to species. Percent parasitism was calculated using the formula: Percent parasitism (total number of parasitized immature/leaf)/(total number of susceptible whiteflies immature stages/leaf). Susceptible stages considered as the third larval and the early pupal stages, with unpigmented eyes (Vet et al., 1980).

Key to the parasitoid species of whiteflies in Egypt

1. Pronotum quadrate, not reaching tegulae; antennae usually elbowed, number of antennal segments (5–13); fore wing with five or fewer cells.. ..... Chalcidoidea 2

– Pronotum triangular in a lateral view, reaching tegulae; antennae elbowed or fili-form; number of antennal segments (7–15); fore wings with five or fewed closed cells or front wings with six or more closed cells; female club 3-segmented, male antennae without club..... .....*Amitus hesperidum* (Silvestri)

2.(1) Gaster subsessile, i.e. its base almost as broad as the propodeum; the petiole, if present, very difficult to see; postendophragma extending at least slightly, usually for, into the gaster; postmarginal vein absent or rudimentary; stigmal vein usually forming a very acute angle with the costal edge of the wing; body nonmetallic, black, or partly to entirely yellow or brown, scutellum without submedian impressed longitudinal lines .....*Aphelinidae* 3

– Gaster distinctly constricted at its junction with the propodeum, and a petiole always present even though sometimes strongly transverse; postendophragma not extending into the greater; postmarginal vein present; stigmal vein forming an angle of not less than about 35° with the costal edge of the wing; body most often at least partly metallic, if nonmetallic then the scutellum usually with a pair of impressed submedian longitudinal lines .....*Eulophidae* 19

3.(2) Antenna 5-segmented, with one elongated club segment; tarsi 4 segmented; fore wing with slightly curved asetose stripe (speculum); male antenna 3 segmented ..... *Eretmocerus* Haldeman 4

– Antenna 8-segmented, all tarsi 5 segmented, or only mid tarsi 4 seg-

mented; fore-wing unifermly setose; male antenna 8 or 7 segmented .... *Encarsia* Forester 10

4.(3) First funicle segment quadrate ..... *Eretmocerus mundus* (Mercet)

– First funicle segment different 5

5.(4) Marginal vein subequal to stigmal vein..... 6

– Marginal vein longer than stigmal vein..... 8

6.(5) Mesoscutum with 4 long setae ..... *Eretmocerus roseni* Gerling

– Mesoscutum with 6–8 long setae 7

7.(6) Submarginal vein with 3 setae; tibial spur of middle leg twice as long as basitarsus ..... *Eretmocerus diversicilatus* Silvestri

– Submarginal vein with 2 setae; tibial spur of middle leg 0.5 times as long as basitarsus..... *Eretmocerus cadabae* Viggiani

8.(5) Female antennal club 5 times as long as wide..... ..... *Eretmocerus siphonini* Viggiani and Battaglia

– Female antennal club 6–8 cimes as long as wide 9

9.(8) Mesoscutum with 10 setae; pedicel 2.4–3.1 times as long as wide and 0.25–0.30 times as long as club..... *Eretmocerus corni* Haldeman

– Mesoscutum with 6 setae, pedicel 3–4 times as long as wide and 0.3–0.39 times as long as club.. *Eretmocerus eremicus* Rose and Zolnerowich

10.(3) Antennal club 3-segmented..... 11

– Antennal club 2-segmented..... 16

11.(10) Gonostyli dark-brown 12

– Gonostyli yellow 13

12.(11) Gonostyli short, about 1/2–2/3 of the inner plate length; first 2 funicular segments longer than wide (each about one, 2–2.5 times as long as wide); mid-tibial spur shorter than basitarsus..... *Encarsia lutea* (Masi)

– Gonostyli long, about 3/4–4/5 ofthe inner plate length; first 2 funicular segments square or wider than long each about 1–2/3 times as long as wide); mid-tibial spur subequal to basitarsus .....*Encarsia davidi* Viggiani

13.(11) Mesoscutum bears 7 pairs of long setae; body yellowish with few brown spots ..... *Encarsia galilea* Rivnay and Gerling

– Mesoscutum bears 2–4 pairs of long setae; body entirely yellow..... 14

14.(13) Marginal fringe about 1/6 of wing of width...*Encarsia strenua* (Silvestri)

– Marginal fringe more than 1/6 of wing of width..... 15

15.(14) F_1 very slightly shorter than F_2; marginal fringe of fore wing at least about 1/3 of the wing of width; tibial spur of middle leg 1/2 as long as the corresponding basitarsus ..... *Encarsia transvena* (Timberlake)

– F_1 clearly longer than F_2; marginal fringe 3/5 of the wing of width; tibial spur of middle leg 1/3 as long as the corresponding basitarsus..... ..... *Encarsia lahorensis* Howard

16.(10) Female with gaster largely yellow, remainder of body largely brown or black 17

– Female body yellowish or dark-brown..... 18

17.(16) Mid tarsi 4-segmented, male antennae 7-segmented..... ..... *Encarsia formosa* Gahan

– Mid tarsi 5-segmented, male antennae 8-segmented..... .....*Encarsia inaron* (Walker)

18.(10) Mesoscutum with only 2 pairs of setae; third funicle segment of male antennae without sensorial complex.....*Encarsia mineoi* Viggiani

– Mesoscutum with 6–7 pairs of setae; third funicle segment of male with sensorial complex.....*Encarsia elegans* Masi

19.(12) Length body less than 1 mm; first funicular segment shorter than second funicular segment; length of fore wing 0.8–1.0 mm .... *Eudermophale chelidonii* Erdős

– Length body 1 mm; first funicular segment subequal second funicular segment; length of fore wing 0.7 mm.....*Euderomphale ezzati* sp. n.

A review of parasitoid species with data on their geographical distribution, hosts and their host plants and abundance

1-*Amitus hesperdium* Silvestri (Family: Platygastridae) (Figs 1–3)

Amitus hesperdium Silvestri, 1927

This species was recorded for the first time in Egypt associated with *Parabemisia myricae* (Kuwana) on *Citrus* sp. by Abd-Rabou (1996).

Diagnostic features: Club as narrow basally as segment VII, broadening a little distally, 2.5 times as long as wide, 3-segmented. Tergite I with a median, carinate elevation, with submedian pits and irregular carinulae at the sides. Male, essentially like the female; lateral plate of antennal fourth segment about 2/3 as long as the segment; male flagellar segments longer, more loosely connected, without antennal club.

Distribution: Qalyubiya.

Host whiteflies: *P. myricae* on *Citrus* sp.

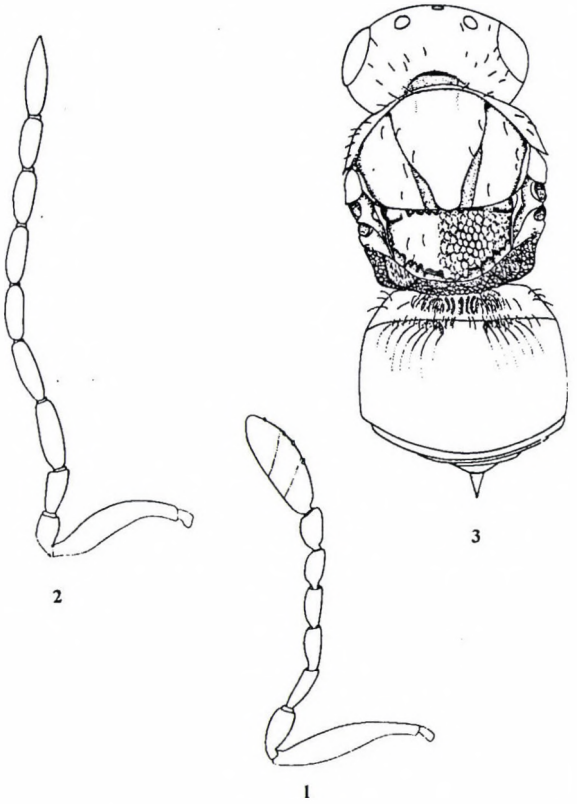
Abundance: Parasitism rates by this species on Qalyubiya region on the whitefly, *P. myricae* on *Citrus* sp. with average 2.2% during May 95–May 96 (Fig. 40).

2-*Encarsia davidi* Viggiani (Family: Aphelinidae) (Fig. 4)

Prospaltella lutea Rosen, 1966

In the present work, this species was recorded here for the first time in Egypt associated with *Aleurolobus niloticus* on *Zizyphus spinachristi*. For several years, this species was incorrectly identified as *Encarsia lutea*, Rosen (1962, 1966).

Diagnostic features: Head and thorax light-lemon yellow, gaster brown. Female with antennal club 3-segmented, first 2 funicular segments square or wider than long (each about 1–2/3 times as long as wide). Mesoscutum with 10 long setae. Mid tarsi 5-segmented. Tibial spur of middle leg subequal in length of the corresponding basitarsus.



Figs 1–3. *Amitus hesperidum*: 1. Female antenna, 2. Male antenna, 3. Whole body (after Masner and Huggert, 1989)

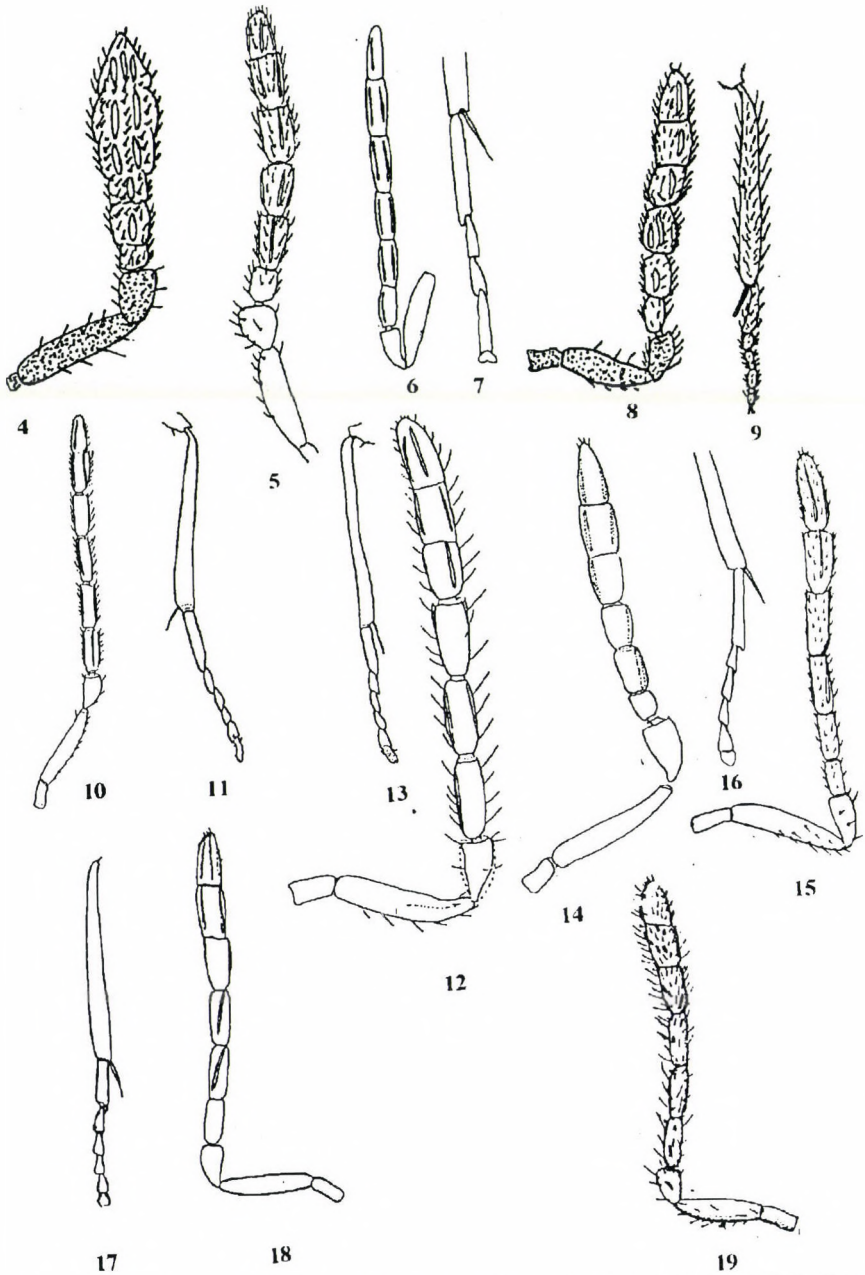
Gonostyli dark brown, long, about 3/4–4/5 of the inner plate length. Male antenna with strongly developed sensorial complex on funicular segments.

Species-group placement: *E. lutea* group.

Distribution: El-Arish, El-Tor and Giza.

Host whiteflies: *Acaudaleyrodes citri* on *Citrus* sp.; *A. niloticus* on *Z. spinachristi*; *Aleyrodes proletella* (Linn.) on *Lawsonia alba*; *Bemisia tabaci* (Genn.) on *Solanum nigrum*; *Siphoninus phillyreae* (Hal.) on *Punica granatum*.

Abundance: Parasitism rates by this species El-Arish region on the whitefly, *S. phillyreae* on *P. granatum* with average 1.9% during June 1994–June 1995 (Fig. 41).



Figs 4–19. Antennae and middle tibial spur and basitarsus of genus *Encarsia*: 4. *E. davidi*, 5. *E. elegans*, 6–7. *E. formosa*, 8–9. *E. galilea*, 10–11. *E. inaron*, 12–13. *E. lahorensis*, 14. *E. lutea*, 15–16. *E. mineoi*, 17–18. *E. strenua*, 19. *E. transvena* (after Abd-Rabou, 1994; Hayat, 1989 and Viggiani, 1982)

3-*Encarsia elegans* Masi (Family: Aphelinidae) (Fig. 5)

Encarsia elegans Masi, 1911

This species was recorded for the first time from Egypt associated with *A. niloticus* by Priesner and Hosny (1940).

Diagnostic features: Head and thorax brown, gaster dark-brown. Female with antennal club 2-segmented. Mesoscutum with 13 setae. Mid tarsi 5-segmented. Tibial spur of middle leg subequal in length of the corresponding basitarsus. Male antenna with sensorial complex on funicular segments.

Species-group placement: *E. elegans* group.

Distribution: Aswan, Assiut, Cairo, El-Fayoum, Qalyubiya and Sharqiya.

Host whiteflies: *A. niloticus* on *Z. spinachristi*; *Aleurolobus olivinus* Silvestri on *Olea* sp.; *B. tabaci* on *Altheae rosae*, *Lantana camara*; *Ramsesseus follioti* Zahradnik on *Acacia nilotica*, *A. tortilis*.

Abundance: Parasitism rates by this species in Sharqiya region on the whitefly, *A. niloticus* on *Z. spinachristi* with average 41.9% during August 95–August 96 (Fig. 42).

4-*Encarsia formosa* Gahan (Family: Aphelinidae) (Figs 6–7)

Encarsia formosa Gahan, 1924

E. formosa was introduced into Egypt for the control of *B. tabaci* and *Trialeurodes vaporariorum* (Westwood) by me from Biolab Company in Italy and Ciba Bunting Company in U. K.

Diagnostic features: Head and thorax largely brown or black, gaster largely yellow. Female with antennal club 2-segmented. Mesoscutum with 24 setae. Mid tarsi 4-segmented. Tibial spur of middle leg about 1/2 as long as the corresponding basitarsus. Each axilla with at least 6 reticulated cells longitudinally. Male antenna 7-segmented. Fourth and fifth funicular segments of male antenna separate.

Species-group placement: *E. formosa* group.

Distribution: Cairo, Giza and Qalyubiya.

Host whiteflies: *B. tabaci* on *Lycopersicum esculentum*, *L. camara*; *T. vaporariorum* on *Ricinus communis*.

Abundance: Parasitism ratex by this species in Qalyubiya region on the whitefly *T. vaporariorum* on *L. camara* with average 29.5% during May 1996–May 1997 (Fig. 43).

5-*Encarsia galilea* Rivnay and Gerling (Family: Aphelinidae) (Figs 8–9)

Encarsia galilea Rivnay and Gerling, 1987.

This species was recorded for the first time in Egypt associated with *S. phillyreae* on *P. granatum* by Abd-Rabou (1996).

Diagnostic features: Head, thorax and gaster largely yellow with brown spots. Female with antennal club 3-segmented. Mesoscutum with 14 long setae. Mid tarsi 5-segmented. Tibial spur of middle leg about 2/3 as long as the corresponding basitarsus. Gonostyli yellow.

Species-group placement: *E. inaron* group.

Distribution: El-Arish and El-Tor.

Host whiteflies: *A. citri* on *P. granatum*, *S. phillyreae* on *P. granatum*.

Abundance: Parasitism rates by this species in El-Arish region on the whitefly, *S. phillyreae* on *P. granatum* with rates 0.4% during June 94–June 95 (Fig. 44).

6-*Encarsia inaron* (Walker) (Family: Aphelinidae) (Figs 10–11)

Aphelinus inaron Walker, 1839
Aphelinus idaeus Walker, 1839
Encarsia inaron (Walker); Graham, 1976
Encarsia partenopea Masi, 1909
Encarsia partenopea Mercet, 1930

This species was recorded for the first time from Egypt associated with *B. tabaci* and *S. phillyreae* by Priesner and Hosny (1940).

Diagnostic features: Head, thorax and gaster brown to black. Female with antennal club 2-segmented. Mesoscutum with 12 setae. Sculpture of thorax reticulate, simple. Mid tarsi 5-segmented. Tibial spur of middle leg less than 1/2 length of the corresponding basitarsus. Male antenna 8-segmented. Fifth and sixth funicular segments of male antenna separate.

Species-group placement: *E. inaron* group.

Distribution: Assiut, Beni-Suef, El-Kharga, El-Minya, El-Arish and El-Tor.

Host whiteflies: *A. proletella* on *S. nigrum*; *B. tabaci* on *L. camara*; *S. phillyreae* on *P. granatum*; *T. vaporariorum* on *R. communis*.

Abundance: Parasitism rates by this species in Asiiut region on the whitefly, *S. phillyreae* on *P. granatum* with average 32.2% during June 94–June 95 (Fig. 45).

7-*Encarsia lahorensis* Howard (Family: Ahelinidae) (Figs 12–13)

Prospaltella lahorensis Howard, 1911
Prospaltella lahorensis Viggiani and Mazzone, 1979

E. lahorensis was introduced into Egypt, 1996 for the control of *Dialeurodes citri* (Ashmead) on citrus plants by me from Prof. Harold Browning, University of Florida.

Diagnostic features: Head and thorax yellow, gaster pale-yellow, gonostyli yellow. F_1 clearly longer than F_2. Female with antennal club 3-segmented. Mesoscutum with 4 long setae. Mid tarsi 5-segmented. Tibial spur of middle leg 1/3 as long as the corresponding basitarsus. Longest cilia of marginal fringe 1/2 time of the width of disc. Male funicular segments subequal in length.

Species-group placement: *E. lahorensis* group.

Distribution: Qalyubiya

Host whiteflies: *D. citri* on *Citrus* sp.

Abundance: Parasitism rates by this species after six months from the introduction was 22%.

8-*Encarsia lutea* (Masi) (Family: Aphelinidae) (Fig. 14)

Prospaltella lutea Masi, 1909

Encarsia lutea Ferriere, 1965

This species was recorded for the first time from Egypt by Abdel-Fattah et al. (1984).

Diagnostic features: Head and thorax light-lemon yellow, gaster pale-brown. Female with antennal club 3-segmented, first 2 funicular segments longer than wide (each about one, 2–2.5 times as long as wide). Mesoscutum with 10 setae. Mid tarsi 5-segmented. Tibial spur of middle leg 1/3 the length of the corresponding basitarsus. Longest cilia of marginal fringe as long as 1/3 time of the width of disc. Gonostyli dark-brown, short, about 1/2–2/3 of the inner plate length. Male antenna with strongly developed sensorial complex on funicular segments.

Species-group placement: *E. lutea* group.

Distribution: El-Arish, Aswan, Assiut, Qalyubiya, Cairo, Dakhla Oasis, El-Fayoum, Giza and Siwa Oasis.

Host whiteflies: *A. niloticus* on *Z. spinachristi; Aleuroplatus acaciae* Bink-Moenen on *A. tortilis*; *B. tabaci* on *L. camara*; *P. myricae* on *Citrus* sp.; *S. phillyreae* on *P. granatum*.

Abundance: Parasitism rates by this species in Qalyubiya region on the whitefly, *B. tabaci* on *L. camara* with average 15.5%, during September 95–September 96 (Fig. 46).

9-*Encarsia mineoi* Viggiani (Family: Aphelinidae) (Figs 15–16)

Encarsia mineoi Viggiani, 1982

This species was recorded for the first time in Egypt associated with *B. tabaci* on *L. alba* by Polaszek et al. (1992).

Diagnostic features: Head, thorax and gaster yellow. Female with antennal club 2-segmented. Mesoscutum with 4 setae. Longest cilia of marginal fringe 1/2 time as long as the width of disc. Male antenna with fifth and sixth funicular segments partly fused, third funicle segment without sensorial complex.

Species-group placement: *E. parvella* group.

Distribution: El-Arish, Assiut, Giza, Qalyubiya and Siwa Oasis.

Host whiteflies: *A. citri* on *P. granatum*; *B. tabaci* on*L. camara*, *L. alba*.

Abundance: Parasitism rates by this species in Qalyubiya region on the whitefly, *B. tabaci* on *L. camara* with average 5.5% during September 95–September 96 (Fig. 47).

10-*Encarsia strenua* (Silvestri) (Family: Aphelinidae) (Figs 17–18)

Encarsia strenua (Silvestri), 1927.

E. strenua was introduced into Egypt in 1996 for the control of *D. citri* on citrus plants by me from Prof. Harold Browning, University of Florida.

Diagnostic features: Pedicel distinctly shorter than F_1, F_1 and F_2 each about 2.5 times as long as wide, club 3-segmented. Mesoscutum with 10 setae; marginal fringe about one-sixth of wing width; basal cell with 10 setae; stigmal vein with narrow neck.

Species-group placement: *E. strenua* group.

Distribution: Qalyubiya.

Host whiteflies: *D. citri* on *Citrus* sp.

Abundance: Parasitism rates by this species after six months from the introduction was 1.3%.

11-*Encarsia transvena* (Timberlake) (Family: Aphelinidae) (Fig. 19)

Prospaltella transvena Timberlake, 1926

Prospaltella sublutea Silvestri, 1931

Prospaltella flava Shafee, 1973

E. transvena was introduced in Egypt in 1997 for the control of *B. tabaci* by me from U. S. A.

Diagnostic features: Body yellow, F_1 very slightly shorter than F_2. Female with antennal club 3-segmented. Mesoscutum with 8 long setae. Mid tarsi 5-segmented. Tibial spur of middle leg 1/2 as long as the corresponding basitarsus. Longest cilia of marginal fringe 3/5 time of the width of disc.

Species-group placement: *E. lahorensis* group.

Distribution: Qalyubiya.

Host whiteflies: *B. tabaci* on *L. camara*.

12-*Eretmocerus cadabae* Viggiani (Family: Aphelinidae) (Figs 20–21)

Eretmocerus cadabae Viggiani, 1982

This species was recorded for the first time from Egypt associated with *Aleuroplatus cadabae* Priesner and Hosny by Abd-Rabou (1996).

Diagnostic features: Body entirely yellowish. The first funicle segment triangular. Female antennal club 3–4 times as long as wide. Mesoscutum with 8 setae. Marginal vein subequal to stigmal vein, submarginal vein with 2 setae. Tibial spur of middle leg 0.5 time as long as basitarsus. Male antennal club 10–11 time as long as wide.

Distribution: Eastern Desert.

Host whiteflies: *A. cadabae* on *Salvadora persica.*

13-*Eretmocerus corni* Haldeman (Family: Aphelinidae) (Figs 22–23)

Eretmocerus corni Haldeman, 1850

This species was recorded for the first time from Egypt, associated with *B. tabaci* by Priesner and Hosny (1940).

Diagnostic features: The first funicle segment triangular. Female antennal club 6–7 times as long as wide. Mesoscutum with 10 setae. Marginal vein longer than stigmal vein, submarginal vein with 3 setae, marginal fringe twice the width of disc. Tibial spur of middle leg 1.5 times as long as basitarsus. Male antennal club 9–10 times as long as wide.

Distribution: Assiut, El-Arish, Beni-Suef, Cairo, Giza and Qalyubiya.

Host whiteflies: *B. tabaci* on *L. camara*, *L. alba*; *S. phillyreae* on *P. granatum*; *T. vaporariorum* on *R. communis*.

Abundance: Parasitism rates by this species in Assiut region on the whitefly, *S. phillyreae* on *P. granatum* with average 8.1% during June 94–June 95 (Fig. 48).

14-*Eretmocerus diversicilatus* Silvestri (Family: Aphelinidae) (Figs 24–25)

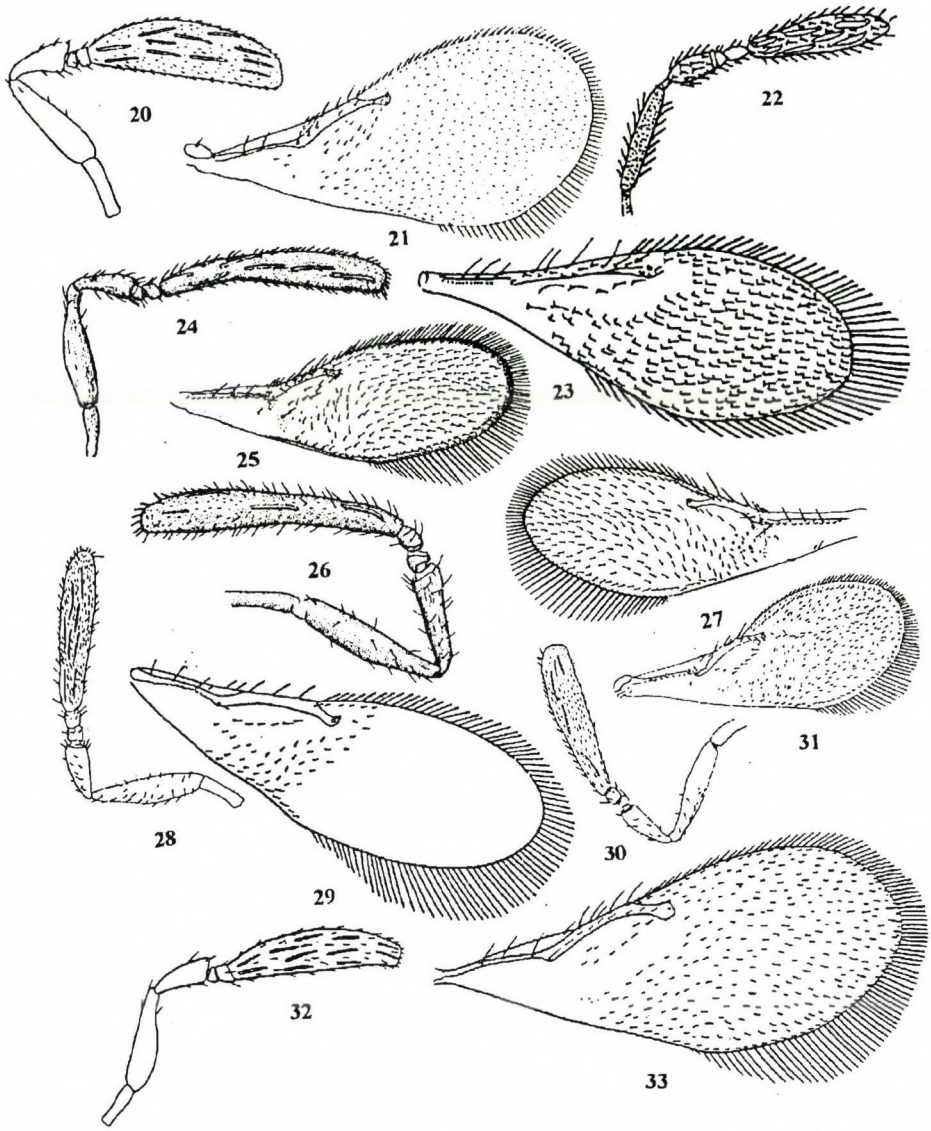
Eretmocerus diversicilatus Silvestri, 1915

This species was recorded for the first time from Egypt associated with *B. tabaci* by Khalifa and El-Khidir (1965).

Diagnostic features: The first funicle segment triangular. Female antennal club 5 times as long as wide. Mesoscutum with 6 setae. Marginal vein subequal to stigmal vein, submarginal vein with 3 setae, marginal fringe about 2.4 times the width of disc. Tibial spur of middle leg twice as long a basitarsus. Male antennal club 10 times as long as wide.

Distribution: Cairo, El-Arish, El-Minya, El-Tor, Giza and Qalyubiya.

Host whiteflies: *A. citri* on *Citrus* sp.; *B. tabaci* on *L. camara*, *L. alba*; *S. phillyreae* on *P. granatum*.



Figs 20–33. Antennae and fore wing of genus *Eretmocerus*: 20–21. *Eretmocerus cabadae*, 22–23. *E. corni*, 24–25. *E. diversicilatus*, 26–27. *E. eremicus*, 28–29. *E. mundus*, 30–31. *E. roseni*, 32–33. *E. siphonini* (after Rose and Zolnerowich, 1997; Abd-Rabou, 1994; Myartseva and Yasnosh, 1994 and Viggiani, 1982)

15-*Eretmocerus eremicus* Rose and Zonerowich (Family: Aphelinidae) (Figs 26–27)

Eretmocerus eremicus Rose and Zolnerowich, 1997

E. eremicus was introduced into Egypt in 1996 for the control of *B. tabaci* by me from the Netherlands.

Diagnostic features: Female antennal club 7.3 times as long as wide. Pedicel that is 3–4 times as long as wide and 0.3–0.39 times as long as the club. Mesoscutum with 1 pair, but occasionally 2 pairs of lateral setae.

Distribution: Qalyubiya.

Host whiteflies: *B. tabaci* on *L. camara*.

Abundance: Parasitism rates by this species after six months from the introduction was 20.1% during September 95–September 96 (Fig. 49).

16-*Eretmocerus mundus* (Mercet) (Family: Aphelinidae) (Figs 28–29)

Eretmocerus corni Masi, 1909

Eretmocerus massi Silvestri, 1934

This species was recorded for the first time from Egypt on *B. tabaci* by El-Helaly et al. (1971) and was introduced as a new Mediterranean strain in Egypt in 1996 for the control of *B. tabaci* by me from Biolab Company in Italy.

Diagnostic features: The first funicle segment quadrate, second funicle segment longer than wide. Female antennal club 8 times as long as wide. Mesoscutum with 6 setae. Marginal vein longer than stigmal vein, submarginal vein with 3 setae, marginal fringe 1/3 width of disc. Tibial spur of middle leg twice as long as basitarsus. Male antennal club 7.5 times as long as wide.

Distribution: Alexandria, Qalyubiya, Cairo, Demyaat, El-Khatatba, El-Kharga Oasis, Giza and Siwa Oasis.

Host whiteflies: *A. citri* on *citrus* sp.; *A cadabae* on *S. persica*; *A. proletella* on *Brassica oleracea*; *B. tabaci* on *Gossypium barbadense*, *L. camara*, *L. alba*; *Dialeurodes kirkaldyi* (Kot.) on *Jasminum sambac*.

Abundance: Parasitism rates by this species in Demyaat on the whitefly, *B. tabaci* on *G. barbadense* with average 16.7% during September 95–September 96 (Fig. 50).

17-*Eretmocerus roseni* Gerling (Family: Aphelinidae) (Figs 30–31)

Eretmocerus roseni Gerling, 1972

In the present work, this species was recorded here for the first time in Egypt associated with *A. citri* on citrus plants.

Diagnostic features: The first funicle segment trapezoidal, second funicle segment

longer than wide. Female antennal club 4 times as long as wide. Mesoscutum with 4 setae. Marginal vein subequal to stigmal vein. Marginal fringe 1/4 width of disc. Male antennal club 10 times as long as wide.

Distribution: Beni-Suef and Giza

Host whiteflies: *A. citri* on *Citrus* sp.

Abundance: Parasitism rates by this species in Giza on the whitefly, *A. citri* on *Citrus* sp. with average 18.6% during May 95–May 96 (Fig. 51).

18-*Eretmocerus siphonini* Viggiani and Battaglia (Family: Aphelinidae) (Figs 32–33)

Eretmocerus siphonini Viggiani and Battaglia, 1983

In the present work, this species was recorded here for the first time in Egypt associated with *A. niloticus* on *Z. spinachristi*.

Diagnostic features: The first funicle segment triangular. Female antennal club 5 times as long as wide. Marginal vein longer than stigmal vein. Marginal fringe about 1/5 width of disc.

Distribution: Sharqiya.

Host whiteflies: *A. niloticus* on *Z. spinachristi*.

Abundance: Parasitism rates by this species in Sharqiya on the whitefly, *A. niloticus* on *Z. spinachristi* with average 11.2% during August 95–August 96 (Fig. 52).

19-*Euderomphale chelidonii* Erdős (Family: Eulophidae) (Figs 34–35)

Euderomphale chelidonii Erdős, 1966

In the present work, this species was recorded here for the first time in Egypt associated with *A. proletella* on *S. nigrum*.

Diagnostic features: Postmarginal vein present, first funicle segment shorter than second funicle segment, length of fore wing 0.8–1.0 mm, length of marginal fringe at least 20% of the greatest width of the wing (Hulden, 1986).

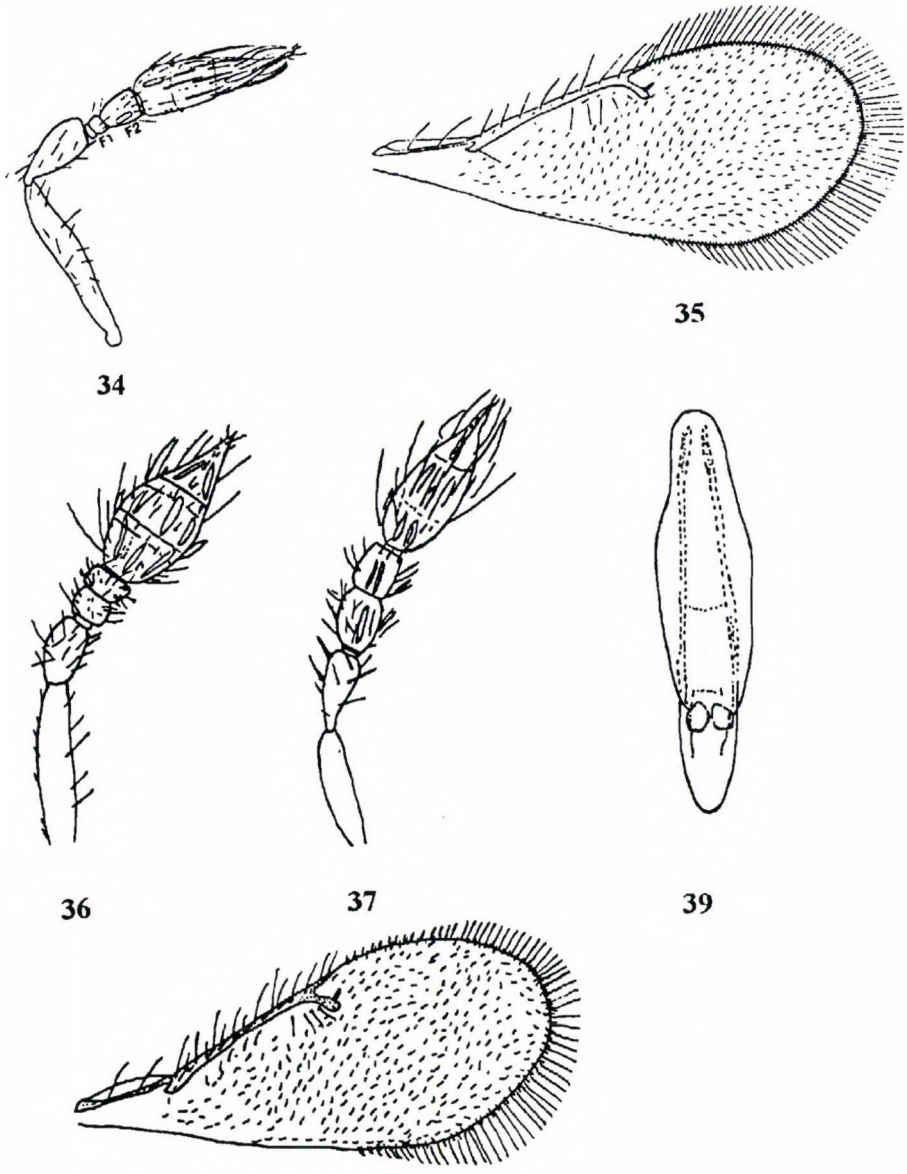
Distribution: Giza.

Host whiteflies: *A. proletella* on *S. nigrum*.

20-*Euderomphale ezzati* Abd-Rabou sp. n. (Family: Eulophidae) (Figs 36–39)

Description:

Female: Coloration. Black with metallic reflections, antennae fuscous, legs black except cephalic tarsi, the proximal three segments of the middle and hind tarsi pale; fore wings hyaline, a comparatively narrow infumation of clouding beneath the marginal vein at about the middle, this clouding does not cross the entire wing but reaches only to the



Figs 34–39. 34–35: *Euderomphale chelidonii*, 34. Female antenna, 35. Forewing. 36–39. *E. ezzati* sp. n. 36. Female antenna. 37. Male antenna. 38. Forewing. 39. Male genitalia

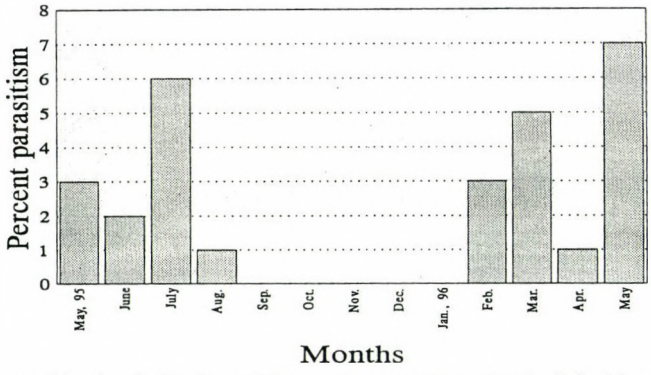


Fig. 40. Percent parasitism by *Amitus hesperidum* on *Parabemisia myricae* in Qalyubiya region during May 95–May 96

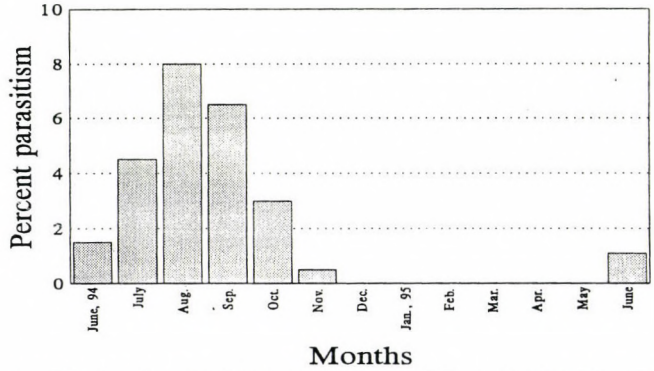


Fig. 41. Percent parasitism by *Encarsia davidi* on *Siphoninus phillyreae* in El-Arish region during June 95–June 96

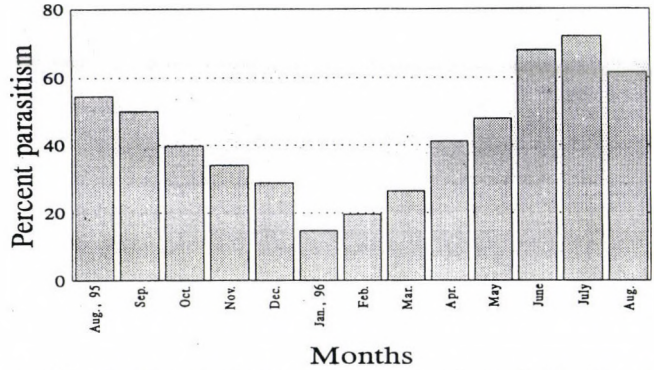


Fig. 42. Percent parasitism by *Encarsia elegans* on *Aleurolobus niloticus* in Sharqiya region during August 95–August 96

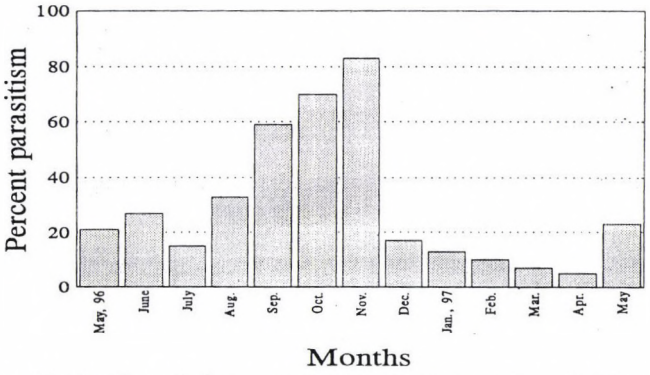


Fig. 43. Percent parasitism by *Encarsia formosa* on *Trialeurodes vaporariorum* in Qalyubiya region during May 96–May 97

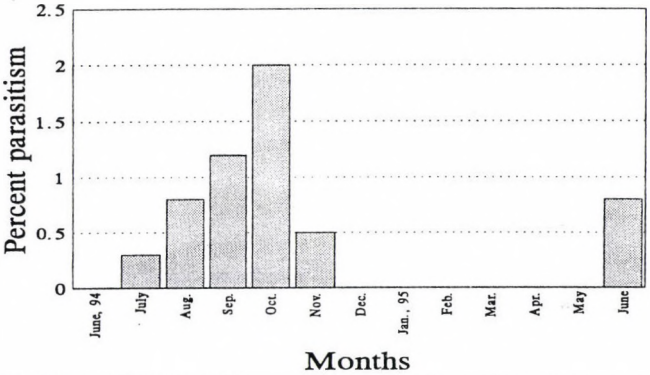


Fig. 44. Percent parasitism by *Encarsia galilea* on *Siphoninus phillyreae* in El-Arish region during June 94–June 95

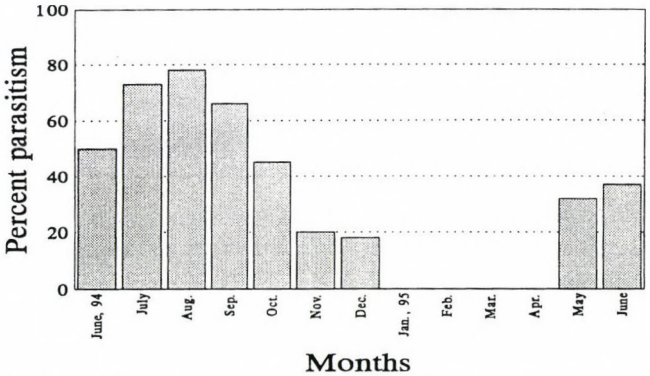


Fig. 45. Percent parasitism by *Encarsia inaron* on *Siphoninus phillyreae* in Assiut region during June 94–June 95

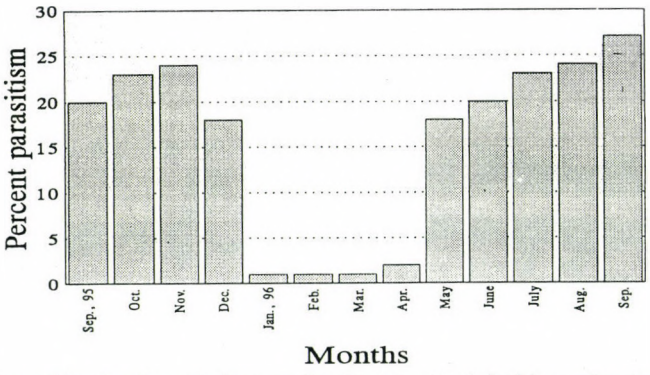


Fig. 46. Percent parasitism by *Encarsia lutea* on *Bemisia tabaci* in Qalyubiya region during September 95–September 96

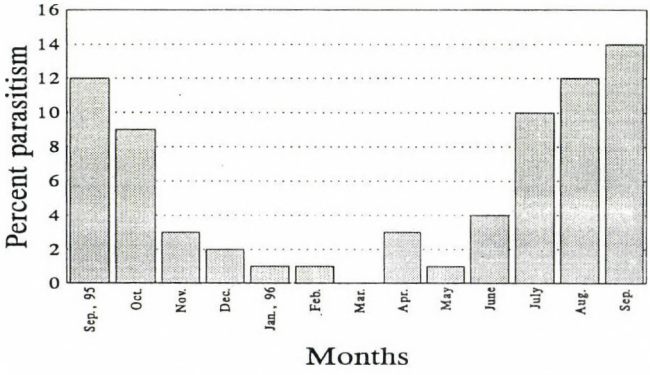


Fig. 47. Percent parasitism by *Encarsia mineoi* on *Bemisia tabaci* in Qalyubiya region during September 95–September 96

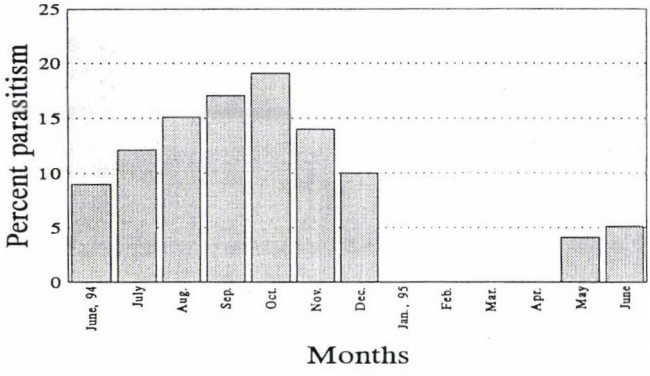


Fig. 48. Percent parasitism by *Eretmocerus corni* on *Siphoninus phillyreae* in Assiut region during June 94–June 95

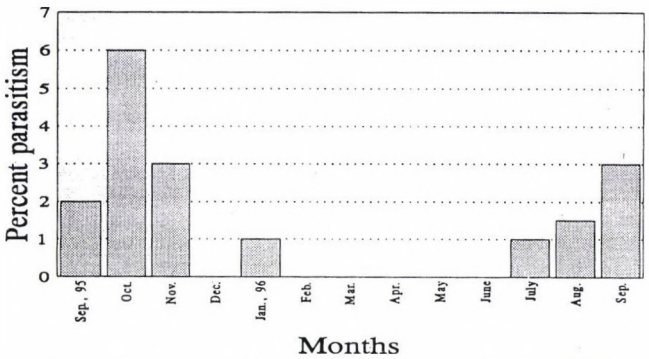


Fig. 49. Percent parasitism by *Eretmocerus diversicilatus* on *Bemisia tabaci* in El-Minia region during September 95–September 96

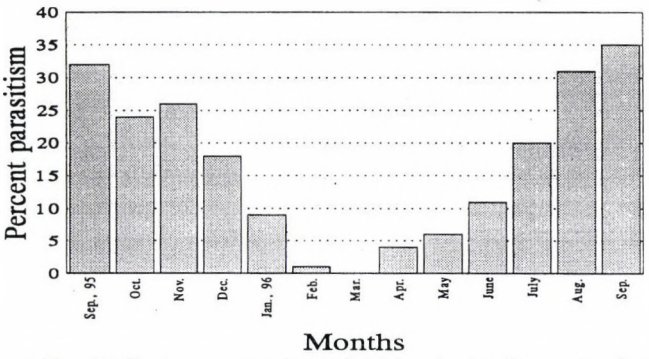


Fig. 50. Percent parasitism by *Eretmocerus mundus* on *Bemisia tabaci* in Demyaat region during September 95–September 96

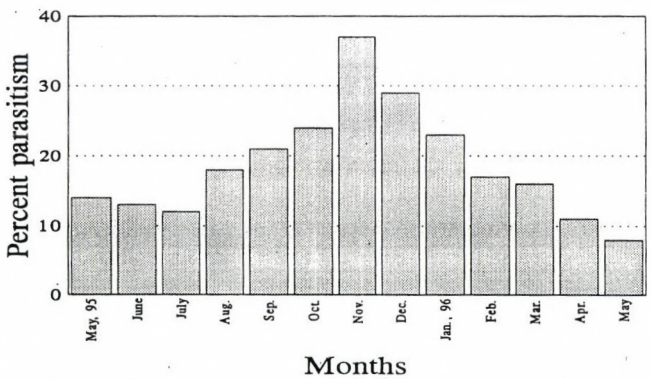


Fig. 51. Percent parasitism by *Eretmocerus roseni* on *Acaudaleyrodes citri* in Giza region during May 95–May 96

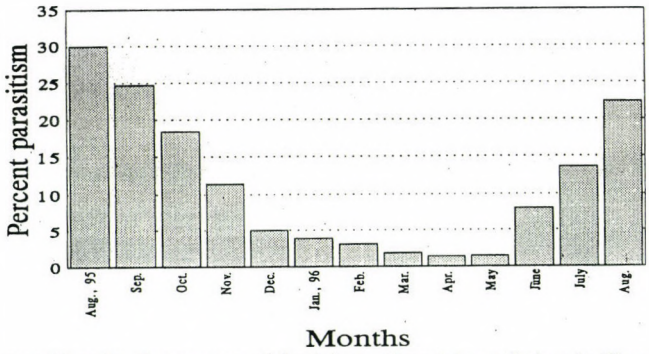


Fig. 52. Percent parasitism by *Eretmocerus siphonini* on *Aleurolobus niloticus* in Sharqiya region during August 95–August 96

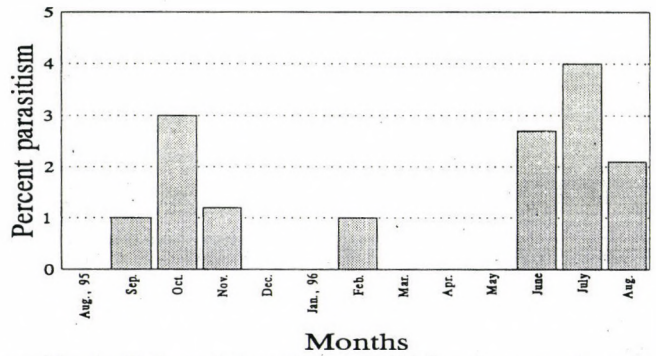


Fig. 53. Percent parasitism by *Euderomphal ezzati* sp. n. on *Trialeurodes vaporariorum* in Qalyubiya region during August 95–August 96

row of setae that runs along close to the lower border of the wing; venation brown; the veine pale brown. Length 1 mm.

Head: Normal, eyes naked, the margin of the vertex with a number of prominent setae. Antennae with the scape long; pedicel elongate, narrow at base and gradually enlarging, pedical longer than first funicle segment, first and second funicle segments subequal in length and without sensoria; club 3-segmented, widest near distal end of the basal segment, with 8 sensoria.

Thorax: Mesoscutum (Middle lobe) with 4 pairs of setae; axilla 3 pairs of setae; scutellum two pairs of setae; prododeum 3 pairs of setae and one pair of spiracles; Fore wing broader, 2.1 times as long as broad; marginal vein very long, more than 3 times as long as the marginal; submarginal and marginal vein 2 and 6 setae; postmarginal vein present, disc proximed of parastigma without setae; the length of marginal fringe 1/3 (33%) the width of disc. Hind wing 8.8 times as long as wide; cilia of marginal fringe longer than wing width. Tarsi 4 segmented, the distal segment the longest.

Gaster: Slightly more than 1.2 times longer than thorax, sessile conic-ovate. Ovipositor originating from about the beginning of fourth segment and exerted at apex, ovipositor slightly more than 1.3 times longer than hind tibia.

Male: Length subequal to female and distinguished immediately by difference in antennae. Club 3 segmented with 6 sensoria; first funicle segment with one sensorium and second funicle segment with two sensoria. Gaster not sessile as in the female but with petiole, distinctly narrower at base than the thorax, narrowing to tip where the genitalia are distinctly exerted.

Material examined: Holotype female and male, Egypt: Qalyubiya, El-Qanater El-Khiryia, X. 94, from *Trialeurodes vaporariorum* (Westwood) on *Ricinus communis*. Reservation in Plant Protection Research Institute, Egypt.

Host: *T. vaporariorum* on *R. communis*.

Distribution: Qalyubiya.

Abundance: Parasitism rates of this species in Qalyubiya on the whitefly, *T. vaporariorum* on *R. communis* with average 1.2% during August 95–August 1996 (Fig. 53).

Remarks: This species resembles *E. chelidonii* but in can be separated from the latter by the following: Total length 1.1 mm, the length of fore wing 0.7 mm, first funicle segment subequal to second funicle segment, and first and second funicle segment of male with sensoria.

Etymology: The species is named for Prof. Dr. Y. M. Ezzat, the former coccidologist in Egypt, Azhar University, Cairo, Egypt.

Acknowledgement

I am very gratitude to Prof. Dr. Galal Moawad, Director of the Plant Protection Research Institute, for encouragement and for providing all the necessary facilities for carrying out this work.

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Acta Phytopathologica et Entomologica Hungarica 33 (1–2), pp. 217–221 (1998)

BOOK REVIEWS

Genetics Manual by George P. Rédei. World Scientific, Singapore, New Jersey, London, Hong-Kong, 1998. 1–1152 pp.

This is a formidable, unique and comprehensive work on modern genetics, cell and molecular biology, physiology, pathology, biometry etc. The author treats the most important plant pathological terms in relation to genetics such as host-pathogen relations, hypersensitive reaction, virulence genes, genetic transformation, Agrobacterium tumefaciens, T-DNA, antisense RNA, signal transduction, Flor's model, plant defense, salicylic acid, systemic acquired resistance etc. In addition, many terms are explained in connection with botany, zoology, plant breedig, plant and animal physiology. The most important feature of this book is that all concepts are explained not just defined. The cross references help the reader to get a comprehensive information on questions of modern biology. This book covers cca. 18,000 topics and in spite of that it is more than a dictionary because it explains modern theories and concepts.

This giant volume is recommended to those who are in contact with genetical aspects of questions in their field of research or practice. The book contains 600 illustrations, over 950 book and text references and web addresses of almost 100 relevant databases. The style of the author is clear even for a beginner. Graduate students can obtain integrated information for exams or lectures.

Zoltán Király

Schoonhoven, L.M., T. Jermy and J.J.A. van Loon. Insect-Plant Biology. From Physiology to Evolution. Chapman & Hall, London, etc., 1998, p. x+409, ISBN: 0-412-80480-8 (hb), £ 70.00, ISBN: 0-412-58700-8 (pb), £ 29.99.

One who is fond of books might not resist inspecting and touching the cover, perhaps taking a smell at pages before starting to become acquainted with the very content. All stimuli are important when judging consumables. So it is for the book in question. The binding is impressive. The cover picture is almost symbolic: both senior authors have spent much time experimenting with the large cabbage butterfly, *Pieris brassicae* whose larvae are shown on the cover as they are devouring a piece of cabbage leaf. While becoming almost a domestic animal at Wageningen and Budapest, respectively, the species served general and electrophysiology, as well as behavioural experiments for years. Although this species is frequently mentioned, the book is not about the large white.

Akadémiai Kiadó, Budapest

This book is probably the 5th among the most important *not-edited* volumes dealing with herbivorous organisms, among others phytophagous insects. [The other four covered community ecology of phytophagous insects (Strong et al., 1984), herbivory in general (Crawley 1983), ecology of herbivores (Howe and Westley, 1988), and behaviour in relation to host selection (Bernays and Chapman, 1994). The reviewer dares to predict at least one more yet to come: physiology of phytophagous insects. Although the present volume has the subtitle "From Physiology to Evolution", the topics covered are somewhat different. Physiology in this volume is largely restricted to sensory physiology and the endocrine system (a very short chapter) as influenced by secondary plant substances.]

Why is it that phytophagous insects receive so much attention in recent years? The simplest explanation would be J. B. S. Haldane's well-known answer for the preponderance of beetles, however, I suspect that the really important reason is the immense number and the endless morphological and behavioural variability of phytophagous insects, the varieties of their life-histories, of their adaptations and so forth. Phytophagous insects are present everywhere where plants are having a chance to live. Strong et al. (1984) estimated their numbers as ca. 26% of all living species on Earth. Since then this ratio has probably grown much higher due to studies of giant tropical trees that stimulated much debates on biodiversity as well.

Schoonhoven et al.'s book is unique from several aspects. It is written by scientists who have been engaged with the topic throughout their lives. They worked on problems they discuss. They are also known to have a special reputation for ideas not easily chewed and digested by the scientific community studying phytophagous insects. As a consequence, some New World colleagues might even call this volume a European synthesis of the field that, after all should be considered a positive discrimination.

The book is divided into 12 chapters. In addition there are three appendices and various indices supplied. The Introduction discusses the whys of increased attention paid to insects in general and to phytophagous species in particular. Chapter 2 (*Herbivorous insects: something for everyone*) deals with the multitude of life-forms, host-plant range and factors shaping the damage exerted to plants by phytophages. Chapter 3 (*Plant chemistry: endless variety*) describes the fascinating chemical characteristics of plants and the factors influencing their productions. The largest and most important chemical groups are surveyed and Appendix B usefully supports understanding with structural formulae.

Discussion of secondary plant substances are usually connected with plant resistance to phytophages. Early at the start of Chapter 3 the authors define the difference between resistance and defence reserving the second for evolutionary responses only. Interestingly, although the problem of plant resistance to herbivorous insects is treated in a separate chapter (No. 12, *Insects and plants: how to apply our knowledge*), induced resistance is discussed in Chapter 3. On the one hand, it is possible that the authors might have wished to emphasize its intimate relationship with the plasticity of secondary metabolism. On the other, Chapter 12 accentuates applied aspects more. The authors draw attention to the confusion of terminology in the literature and make some points to define

induced resistance. However, it is still not quite clear how important time scale is in the process. By time scale both induction- and effect-time can be considered. Should the elevated phenol level in a dwarf *Betula* that lasts for three years be defined as induced or constitutive resistance? Is it possible that the same chemical compound that provides constitutive resistance for a perennial is rapidly induced in an annual plant species, or *vice versa*? Cost-benefit analysis is outstandingly important to assess the importance of resistance of plants to phytophagous insects, therefore it receives a thorough treatment in Chapter 3. On the other hand, a more detailed discussion of the recent controversy with turn-over of secondary plant metabolites within the plants would have been very useful.

Chapter 4 (*Plants as insect food: not the ideal*) is mainly nutritional physiology. Problems of food utilisation get stressed here. [This, to some extent, is contradicting to p. 2 where the diversity and success of extant insect taxa are discussed. Is it possible that such a large and successful group is evolutionarily malnourished, or is there no causal relationship between the two at all?] This chapter is, however, not attempting to cover all aspects. For instance, physiology of fat accumulation and use, or special features in relation to developmental stadia are not discussed, only to mention two problems of rather secondary importance. Instead, the chapter is focusing on the consequential side of feeding, namely how secondary plant substances once entered the insect body are handled.

It is somewhat curious that, among the logical steps of getting into contact with a plant, feeding precedes food selection and food finding. Yet, a three-chapter group follows emphasizing cohesiveness by the first part of each title "Host plant selection". Chapter 5 covers the first topic (*Host plant selection: how to find a host plant*). In this chapter the importance of various modalities participating in host finding is discussed including olfactory coding and host finding in nature. The chapter is a selection of the most important relevances from the existing vast literature. Chapter 6 (*Host plant selection: when to accept a plant*) concentrates on plant chemistry as it affects contact chemoreception and results in a given behaviour. This chapter is an excellent synthesis of plant chemistry, sensory physiology and behaviour. Chapter 7 has a curious subtitle (*Host plant selection: why insects do not behave normally*). The thoughts presented here must have rooted in Chapter 4 that stressed the idea that insects' food is not ideal, i.e., they live on a suboptimal diet (based mostly on low N-content and high structural carbohydrate ratio).

So, why do not insects behave normally? The authors seek the answers in individual behavioural variability that is, after all, seems to be normal and not the exception. However, why is individual variability not normal? We do not get a clear answer. This chapter also gives an adequate cover of what we know about learning processes shown by insects and the plasticity of this behaviour.

Chapter 8 (*The endocrine system of herbivores listens to host plant signals*) is a short summary of how food type and quality affect frequencies of morphs, diapause, maturation and reproduction. These are essential to the general subject although discussing them in a separate chapter may not be justified. It could have been a special part of chapter 6.

Chapter 9 (*Ecology: living apart together*) is one of the chapters where the available vast literature needed careful selection of data. This has been rightly achieved. Problems are discussed along increasing organisational levels, from population interactions to communities. The chapter concludes that the overall importance of interspecific competition among herbivorous insects in shaping community structure is still insufficiently known, but at the same time it seems rare enough and may be more intensified among guild members.

This book [by Chapter 10 (*Evolution: who drives whom?*)] probably is the only example where interested readers can obtain a comprehensive and comparative treatment of insect-plant evolution. The chapter contains all important evolutionary ideas, old and new, on the evolutionary aspects of insect-plant interactions. Since the publication of Thompson's (1994) book on coevolution this is also the first opportunity to answer criticisms aimed at the sequential evolution theory, and to evaluate the newest hypothesis, such as the geographic mosaic theory of coevolution set forth by Thompson. While a recent hypothesis on how predators might have driven the evolution of food-plant specialisation of herbivorous insects receives serious criticism, the very question, that is why there are so many specialised phytophagous insect species, remains still unanswered. The chapter concludes that (a) "an acceptable explanation may be sought in some constraints on the evolution of the insects' nervous system ...", and (b) that "there is no equivocal evidence, ..., for evolutionary changes in plants that might have resulted from selection by herbivorous insects."

Perhaps due to the fascinating features of the topic, a whole chapter is devoted to pollination (Chapter 11, *Insects and flowers: the beauty of mutualism*). It is doubtless that it is an important relationship providing ample opportunities to discuss population and community level issues, as well as evolutionary thoughts. The topic is finely presented. The title is, however, promising a bit more by referring to mutualism than it can provide. There is ample evidence for equally important asymmetrically mutualistic relationships not discussed in the book, e.g., ant-fungus, ant-plant, insect-microbe, etc. relations. It is, however, very refreshing to notice that in an area such as pollination traditionally considered to be a coevolved system, asymmetric characters get strong emphasis. I remember participating in a seminar at an American university held on insect-plant relations in 1991, and the suddenly frozen atmosphere around me, when I made a remark on the mostly asymmetric nature of pollination, on the lack of reciprocal selection processes, cheating orchids and the like. As it has been increasingly evident (e.g., Thompson 1994), relationships can be arranged along an antagonism-mutualism axis. An interaction losing from its antagonistic character can be a candidate for mutualism. However, this still does not necessarily mean that participants in the relationship are also coevolving.

The importance of thoughts disseminated by the pollination chapter may counterbalance the lack of separate discussions of equally interesting and significant topics, such as gall formation, seed predation, mycorrhizal connection and herbivory, or insect communities living in the digestive fluid of carnivorous plants, a very special insect-plant relationship, to mention only some. Indeed, an all-inclusive book is a dream, and the more voluminous the less accessible to those who are targeted.

The literature is arranged at the end of each chapter. The reviewer is not sure whether the reader will be happy with this solution that might have been the publisher's decision. On the one hand, there is no doubt that the relevant literature is more concentrated by this way and it strengthens the independence of chapters. On the other, generally more space is necessary because duplication is unavoidable. The average "age" of the literature is low and on the basis of three chapters it distributes as follows: ca. 40% of references are later than 1990, ca. 50% originates from between 1970 and 1990, and the rest is shared by years before 1970. For some chapters (e.g., Ch. 3) this must be in connection with the development of analytical techniques in chemistry.

A remark on the title of the book is timely. It is without doubt that insect-plant "relationship" and "interaction" are the two most frequently used expressions to describe the special connection between these groups of organisms. Both are used as standard indexing words too. The symposia on insect plant interaction, and a recent CRC series on the subject also used the same. However, the substitution of interaction/relationship with "biology" generates extra expectations. It has a wider meaning than the formers, involving genetics, morphology, anatomy, etc. that are not discussed as separate chapters in the book. Still, it is true that most topics are presented in the light of these.

The text is easy to read. The pages are arranged in two columns. Figures are clear and adequate to the subject. Models, more formal approaches are not characteristic of the text. However, this may qualify the field rather than the book.

Who are the potential "consumers" of the book? Most probably no single reader-group can be defined. For those who could profit most (graduate students or young post-docs) some of the chapters, e.g., receptor physiology and insect-plant evolutionary relationship, are professionally written. But these are the very chapters from which even colleagues with the same specific interest can learn. At the same time Chapter 12 (*Insect and plants: how to apply our knowledge*) and Appendix C on methodological ideas may tell most to younger scientists. This book is also highly recommended to seminar discussions and for anyone who likes to know something new about an old scientific field. I am glad that I have a copy of it.

Árpád Szentesi

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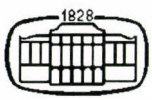

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Erwinia carotovora ssp. *carotovora* Cannot Induce Systemic Acquired Resistance to Bacterial Growth and Necrotic Symptoms in a Transgenic Tobacco that Expresses Salicylate Hydroxylase

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We have investigated the possibility of systemic acquired resistance (SAR) directed against multiplication of *Erwinia carotovora* ssp. *carotovora*, a causal agent of bacterial soft rot of plants in transgenic *nahG* tobaccos that cannot accumulate salicylic acid (SA) and in nontransformed control plants. Our further goal was to find out whether the putative SAR induced by *E. carotovora* ssp. *carotovora* or its pectolytic enzymes is effective against necrotic symptoms caused by other bacterial and viral phytopathogens? The results of our study show that in 40 and 60 days old tobacco plants the putative induction of SAR is ineffective against both multiplication of *E. carotovora* ssp. *carotovora* and necrosis caused by a bacterial and a viral pathogen (*Pseudomonas syringae* pv. *syringae* and tobacco mosaic virus, respectively), regardless whether or not the plants are able to accumulate SA.

A plant's ability to survive pathogen attack depends both on preformed barriers and on induced active defense mechanisms. Induced resistance or systemic acquired resistance (SAR) has been characterized in a variety of plant species (Chester, 1933; Ross, 1961a; Kuć, 1982; Uknes et al., 1992 – for recent reviews see Ryals et al., 1996; Sticher et al., 1997). SAR is induced by most pathogens that cause tissue necrosis ranging from a hypersensitive response (HR) (Ross, 1961b) to a necrotic disease lesion (Kuć and Richmond, 1977). The SAR response is characterized by the induction of long lasting, systemic resistance that is often effective against bacterial, viral and fungal pathogens. For example, in tobacco (*Nicotiana tabacum*), SAR provides a significant level of protection against *Pseudomonas syringae* pv. *tabaci*, tobacco mosaic virus (TMV), *Cercospora nicotianae*, *Phytophthora parasitica*, and *Peronospora tabacina* (Vernooij et al.,1995; Friedrich et al., 1996).

In tobacco, the development of SAR is associated with the activation of SAR-related genes (eg. Ward et al., 1991), most of which encode so-called pathogenesis-related (PR) proteins. Expression of certain PR genes are often used as molecular markers of SAR-induction (Ryals et al., 1996). Although little is known about the signal transduction pathway that leads to SAR in tobacco, one step apparently involves the accumulation of salicylic acid (SA). Levels of SA rise dramatically at the onset of SAR (Malamy et al., 1990; Enyedi et al., 1992) and exogenously applied SA leads to the induction of the same set of PR genes as with pathogen-induced SAR (Ward

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et al., 1991), as well as resistance to pathogen infection (reviewed by Malamy and Klessig, 1992). The most direct evidence for the role of SA in SAR comes from experiments with transgenic tobacco that expresses a salicylate hydroxylase (*nahG*) gene from *Pseudomonas putida* (Gaffney et al., 1993). These plants cannot accumulate SA and are unable to manifest an SAR response (Gaffney et al., 1993; Delaney et al., 1994), nor can they induce PR protein synthesis in response to SA. Therefore, SA seems to play a pivotal role in the signal transduction pathway leading to SAR, although it is not likely to be the systemically transmitted signal (Vernooij et al., 1994).

Erwinias are gram-negative enterobacteria that cause soft rot symptoms in many plant species (Pérombelon and Kelman, 1980). Members of this group such as *Erwinia carotovora* ssp. *carotovora* have a very wide host range, attacking a number of plant species if conditions are favorable. The virulence of *E. carotovora* ssp. *carotovora* is dependent on the production and secretion of a large arsenal of plant cell wall-degrading enzymes, including pectinases, cellulases and proteases that macerate the plant tissue and release nutrients for the pathogen (Collmer and Keen, 1986; Kotoujansky, 1987; Pirhonen et al., 1991). Genetically defined resistance that is specific to infection of *E. carotovora* ssp. *carotovora* and is determined by the interaction of plant resistance gene(s) and bacterial avirulence gene(s) has not been described (Collmer and Keen, 1986; Kotoujansky, 1987; Keen, 1990). On the other hand, many of the cell wall-degrading enzymes of *E. carotovora* ssp. *carotovora* have been shown to induce defense mechanisms in plants, probably by releasing cell wall fragments active as elicitors of host defense responses (Davis et al., 1984; Yang et al., 1992; Palva et al., 1993). Recent research has demonstrated that acellular preparations that contain cell wall-degrading enzymes of *E. carotovora* ssp. *carotovora* induce the expression of genes encoding PR proteins (Palva et al., 1993; Vidal et al., 1997,1998). Furthermore, pretreatment of young tobacco seedlings with pectolytic enzymes of *E. carotovora* ssp. *carotovora* as well as with SA was reported to induce resistance to plant tissue maceration caused by the pathogen (Palva et al., 1993, 1994). In a recent study by Vidal et al. (1998) it was suggested that cell wall-degrading enzymes of *E. carotovora* ssp. *carotovora* might induce a SAR response in tobacco to subsequent bacterial infections by affecting the growth of bacteria *in planta* and that this induced resistance does not require SA accumulation. Indeed, pretreatment of tobacco with bacterial pectolytic enzymes caused a slight inhibition of growth of *E. carotovora* ssp. *carotovora* in distal plant parts (Vidal et al., 1998). However, the plants used in this study were rather young, only 21 days old *in vitro* grown seedlings. Such a putative SAR response should be functional in older plants as well, in order to have practical significance at all.

We have investigated the possibility of SAR directed against multiplication of *E. carotovora* ssp. *carotovora* in 40 and 60 days old transgenic *nahG* tobaccos that cannot accumulate SA and in nontransformed control plants. Our further goal was to find out whether the putative SAR induced by *E. carotovora* ssp. *carotovora* or its pectolytic enzymes is effective against necrotic symptoms caused by other bacterial and viral phytopathogens? The results of our study show that in older tobacco plants the putative in-

duction of SAR is ineffective against both multiplication of *E. carotovora* ssp. *carotovora* and necrosis caused by *Pseudomonas syringae* pv. *syringae* and TMV, regardless whether or not the plants are able to accumulate SA.

Materials and Methods

Plant material and plant growth conditions

Transgenic *nahG* tobacco (*Nicotiana tabacum* cv. Xanthi-nc) that expresses salicylate hydroxylase (Gaffney et al., 1993) and nontransformed Xanthi-nc plants were used. Plants were grown for 40 or 60 days under greenhouse conditions. Two days before treatments plants were transferred to a growth chamber set for 16 hour days with a light intensity of 150 $\mu E/m^2/s$ at 26 °C.

Bacterial inoculations and determination of bacterial growth in planta

Erwinia carotovora ssp. *carotovora* strain SCC3193 (Pirhonen et al., 1988) and *Pseudomonas syringae* pv. *syringae* (Hungarian isolate) were used for bacterial inoculations. *E. carotovora* ssp. *carotovora* was cultured for 72 hours at 28 °C in L-medium (Miller, 1972), harvested by centrifugation (10 min at 4000 *g*) and resuspended into 10 mM $MgSO_4$. To evaluate symptom development in tobacco in response to *E. carotovora* ssp. *carotovora* , bacteria were resuspended at 12 different concentrations ranging from 10^6 to 4.0×10^9 cfu (colony forming units)/ml (see Table 1). Interveinal segments of the five uppermost leaves of plants were inoculated by injection with a hypodermic syringe and a 26 gauge needle.

For induction of a putative SAR, *E. carotovora* ssp. *carotovora* was resuspended at 5.0×10^9 cfu/ml, while cell-free culture filtrate (CF) was prepared from bacteria as follows: *E. carotovora* ssp. *carotovora* was cultured for 7 days in L-medium and CF was separated from a 5.0×10^8 cfu/ml bacterial suspension by sterile filtration (pore size 0.2 µm). Interveinal segments of lower leaves were injected with bacteria, CF or water.

To test the ability of *E. carotovora* ssp. *carotovora* strain SCC3193 to macerate plant tissue, potato tuber slices were treated with 0.5 ml of a 5.0×10^9 cfu/ml bacterial suspension, CF prepared from a 5.0×10^8 cfu/ml bacterial suspension as described above or water. Tuber slices were put in sterile Petri dishes in a water bath and incubated at 28 °C for 1–2 days, until the development of tissue maceration.

For evaluation of a putative SAR to hypersensitive necrosis caused by *P. syringae* pv. *syringae* in tobacco, upper leaves were challenged with 9 different concentrations of the bacterium (ranging from 10^5 to 10^9 cfu/ml, see Tables 2 and 3) following pretreatments of lower leaves. In order to induce SAR in the upper leaves interveinal segments of upper, challenged leaves were inoculated by injection.

Evaluation of *in planta* growth of *E. carotovora* ssp. *carotovora* in upper leaves

Table 1

Symptom development in transgenic *nahG* (cv. Xanthi-nc) and nontransformed Xanthi-nc tobacco in response to inoculation of leaves with different concentrations of *Erwinia carotovora* ssp. *carotovora*

Plant	*nahG* (60 d.)[a]					*nahG* (40 d.)					Xanthi-nc (40 d.)				
Leaf No.[b]	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Inoculum concentration (cfu/ml)															
4.0×10^9	–	–	–	–	–[c]	+	+	+	+	–	+	+	+	+	–
2.0×10^9	–	–	–	–	–	+	+	+	+	–	+	+	±	±	–
1.0×10^9	–	–	–	–	–	±	±	±	±	±	+	–	–	–	–
5.0×10^8	–	–	–	–	–	±	±	±	±	±	–	–	–	–	–
2.5×10^8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1.0×10^8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5.0×10^7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2.0×10^7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1.0×10^7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5.0×10^6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2.0×10^6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1.0×10^6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Age of plants in days at the time of bacterial inoculations

[b] The five uppermost leaves of *nahG* (40 and 60 days old, respectively), and Xanthi-nc (40 days old) plants were inoculated in the interveinal segments with different concentrations of *E. carotovora* ssp. *carotovora* strain SCC3193 as described in Materials and Methods. Leaf No. 5 represents the top leaf of each plant

[c] Symptom development was scored 24 hours after inoculation using the following scale: – no visible symptoms, ± slightly discolored, pale green maceration lesions, + pale green maceration lesions with more pronounced appearance

in response to different pretreatments of lower leaves was done as follows: 3 or 7 days after pretreatments, upper leaves were inoculated with a 10^7 cfu/ml suspension of *E. carotovora* ssp. *carotovora* . At the indicated time points after inoculation (see Figs 1, 2 and 3), plant tissue was homogenized and numbers of viable bacteria determined in the following manner: using a 1 cm^2 sterile cork borer, 4 discs from each inoculated leaf were homogenized in 400 µl sterile distilled water. A 10-fold serial dilution was made and 0.1 ml of each dilution was plated onto nutrient agar plates and incubated at 28 °C for 48 hours. Bacterial colonies were counted and used to determine numbers of viable bacteria/cm^2 leaf tissue.

Evaluation of *in planta* growth of *P. syringae* pv. *syringae* in upper, challenge inoculated leaves prior to the development of hypersensitive necrosis (Table 4) was done as described above for *E. carotovora* ssp. *carotovora* except that an inoculum of 5×10^6 cfu/ml was used.

Table 2

Erwinia carotovora ssp. *carotovora* does not induce systemic acquired resistance (SAR) to hypersensitive necrosis caused by *Pseudomonas syringae* pv. *syringae* in 60 days old transgenic *nahG* and nontransformed Xanthi-nc tobacco

Plant	*nahG*			Xanthi-nc		
Pretreatment[a] / Challenge inoculum[b] (cfu/ml)	CF	Ecc	control	CF	Ecc	control
1.0×10^5	–[c]	–	–	–	–	–
5.0×10^5	–	–	–	–	–	–
1.0×10^6	–	–	–	–	–	–
2.5×10^6	–	–	–	–	–	–
5.0×10^6	–	–	–	–	–	–
1.0×10^7	+	–	–	±	–	–
5.0×10^7	++	+	+	+	+	+
1.0×10^8	++	+	++	++	+	++
1.0×10^9	++	++	++	++	++	++

[a] Pretreatments consisted of inoculations of 5.0×10^6 cfu/ml *E. carotovora* ssp. *carotovora* (Ecc) or treatments with cell-free culture filtrate (CF) or water (control) in lower leaves as described in Materials and Methods

[b] Leaves above the pretreated ones were challenged with different concentrations of *P. syringae* pv. *syringae* by inoculation of interveinal leaf segments 7 days after pretreatments as described in Materials and Methods

[c] The extent of hypersensitive necrosis caused by *P. syringae* pv. *syringae* was scored 24 hours after inoculation using the following scale: – no visible symptoms, ± slight chlorosis, + necrosis occasionally surrounded by a slightly chlorotic halo, ++ necrosis occasionally surrounded by a pronounced chlorotic halo, +++ necrosis only

Virus inoculations

A putative SAR to hypersensitive necrosis caused by tobacco mosaic virus (TMV) was evaluated as follows: plants were pretreated with *E. carotovora* ssp. *carotovora*, CF or water as described above. Seven days after pretreatments leaves above the pretreated ones were inoculated with TMV (U1 strain) by homogenizing 0.5 g leaf tissue from Samsun tobacco plants systemically infected with the virus using 10 ml of tap water. Celite was used as an abrasive. The number of TMV-elicited hypersensitive necrotic lesions in inoculated leaves was counted 4 days after virus inoculation.

Table 3

Erwinia carotovora ssp. *carotovora* does not induce systemic acquired resistance (SAR) to hypersensitive necrosis caused by *Pseudomonas syringae* pv. *syringae* in 40 days old transgenic *nahG* and nontransformed Xanthi-nc tobacco

Plant Pretreatment[a] Challenge inoculum[b] (cfu/ml)	*nahG*			Xanthi-nc		
	CF	Ecc	control	CF	Ecc	control
1.0×10^6	–[c]	–	–	–	–	–
2.5×10^6	–	–	±	–	–	–
5.0×10^6	+	+	+	+	±	+
1.0×10^7	++	++	+	++	+	+
5.0×10^7	+++	+++	+++	+++	+++	+++

[a] Pretreatments consisted of inoculations of 5.0×10^9 cfu/ml *E. carotovora* ssp. *carotovora* (Ecc) or treatments with cell-free culture filtrate (CF) or water (control) in lower leaves as described in Materials and Methods.

[b] Leaves above the pretreated ones were challenged with different concentrations of *P. syringae* pv. *syringae* by inoculation of interveinal leaf segments 7 days after pretreatments.

[c] The extent of hypersensitive necrosis caused by *P. syringae* pv. *syringae* was scored 24 hours after inoculation using the following scale: – no visible symptoms, ± slight chlorosis, + necrosis occasionally surrounded by a slightly chlorotic halo, ++ necrosis occasionally surrounded by a pronounced chlorotic halo, +++ necrosis only.

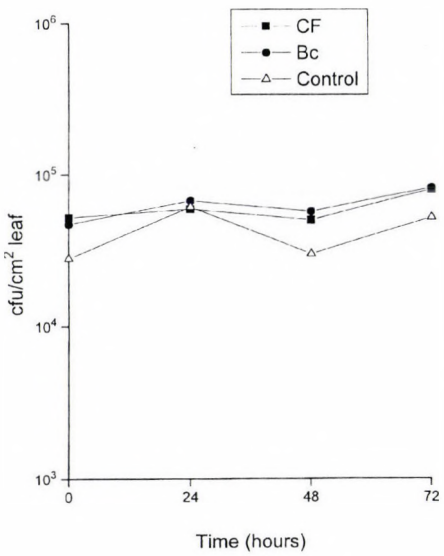

Fig. 1. Neither *Erwinia carotovora* ssp. *carotovora* nor its cell wall-degrading enzymes induce systemic acquired resistance (SAR) to the pathogen in transgenic *nahG* tobacco (*Nicotiana tabacum* cv. Xanthi-nc). Plants were grown under greenhouse conditions. 60 days old plants were either inoculated with a 5.0×10^9 cfu(colony forming units)/ml suspension of *E. carotovora* ssp. *carotovora* strain SCC3193 (Bc) or treated with cell-free culture filtrate (CF) in a lower leaf as described in Materials and Methods. Three days later leaves above the pretreated ones were challenged with a 10^7 cfu/ml *E. carotovora* ssp. *carotovora* suspension. At the indicated time points (hours) after inoculation leaf tissue was homogenized and numbers of viable bacteria were determined as described in Materials an Methods. Bacterial growth in leaves above the ones pretreated with CF or *E. carotovora* ssp. *carotovora* was compared to water-pretreated controls. Data points represent means of three independent experiments.

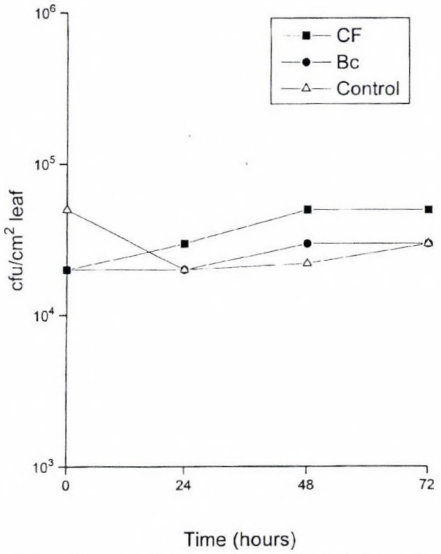

Fig. 2. Neither *Erwinia carotovora* ssp. *carotovora* nor its cell wall-degrading enzymes induce SAR to the pathogen in 60 days old transgenic *nahG* tobacco with a time interval of 7 days between pretreatments and challenge inoculations. Plant growth conditions, treatments, and determination of bacterial growth were as described in Fig. 1. and Materials and Methods.

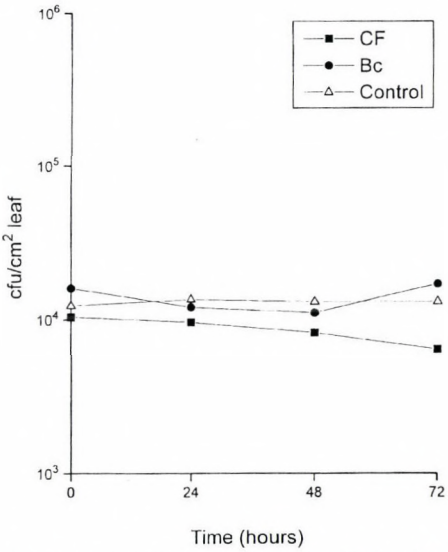

Fig. 3. Neither *Erwinia carotovora* ssp. *carotovora* nor its cell wall-degrading enzymes induce SAR to the pathogen in 40 days old transgenic *nahG* tobacco with a time interval of 7 days between pretreatments and challenge inoculations. Plant growth conditions, treatments, and determination of bacterial growth were as described in Fig. 1. and Materials and Methods.

Table 4

Erwinia carotovora ssp. *carotovora* does not induce systemic acquired resistance (SAR) to multiplication of *Pseudomonas syringae* pv. *syringae* in transgenic *nahG* and nontransformed Xanthi-nc tobacco prior to the development of hypersensitive necrosis

Plant and Pretreatment[a] Time (hours)[b]	*nahG* (Ecc)	*nahG* (control)	Xanthi-nc (Ecc)	Xanthi-nc (control)
0	2.0×10^5[c]	2.5×10^5	3.2×10^5	2.5×10^5
24	4.3×10^5	5.3×10^5	4.8×10^5	6.7×10^5

[a] Pretreatments consisted of inoculations of 5.0×10^7 cfu/ml *E. carotovora* ssp. *carotovora* (Ecc) or water (control) in lower leaves as described in Materials and Methods

[b] Time after challenging leaves above the pretreated ones with an 5.0×10^6 cfu/ml inoculum of *P. syringae* pv. *syringae* 7 days after pretreatments. Hypersensitive necrosis appeared 24 hours after challenge inoculations

[c] Numbers (cfu/cm^2) of viable bacteria in challenged leaves were determined as described in Fig. 1 and Materials and Methods

Results

According to previous research, pretreatment of tobacco with pectolytic enzymes of *E. carotovora* ssp. *carotovora* as well as with SA can induce resistance to plant tissue maceration caused by the pathogen (Palva et al., 1993, 1994). Furthermore, in a recent study by Vidal et al. (1998) it was suggested that cell wall-degrading enzymes of *E. carotovora* ssp. *carotovora* might induce systemic acquired resistance (SAR) in tobacco to subsequent bacterial infections by affecting the growth of bacteria *in planta* and that this induced resistance does not require SA accumulation, because it is also functional in transgenic *nahG* tobacco (cv. Xanthi-nc) that cannot accumulate SA. Indeed, pretreatment of young, 21 days old tobacco seedlings with bacterial pectolytic enzymes caused a slight inhibition of growth of *E. carotovora* ssp. *carotovora* in distal plant parts (Vidal et al., 1998).

Symptom development in transgenic nahG *(cv. Xanthi-nc) and nontransformed Xanthi-nc tobacco in response to inoculation of leaves with different concentrations of* Erwinia carotovora *ssp.* carotovora

In the present study we have investigated the possibility of the development of SAR induced by *E. carotovora* ssp. *carotovora* or its cell wall-degrading enzymes that would effectively inhibit growth of the pathogen in older, 40 and 60 days old tobacco plants. In order to induce an effective SAR with *E. carotovora* ssp. *carotovora* suspensions, different concentrations of the bacterium were tested for localized symptom in-

duction in transgenic *nahG* tobaccos that cannot accumulate SA and in nontransformed Xanthi-nc plants (Table 1). 24 hours after inoculation (PI) visible symptoms developed only in response to inoculum concentrations greater than 5.0×10^8 cfu/ml. These symptoms consisted of pale green lesions with a diameter of ca. 0.5–1 cm. Within 1 or 2 days these maceration-type alterations became transparent and 5 days PI necrotic in appearance. Younger (40 days old) plants were more sensitive to bacteria-induced maceration symptoms than older (60 days old) plants. Also, transgenic *nahG* plants were more sensitive to this type of symptoms than nontransformed Xanthi-nc plants (Table 1).

To ensure development of a putative SAR response in the plant, a 5.0×10^9 cfu/ml inoculum of *E. carotovora* ssp. *carotovora* was used as an inducer in all subsequent experiments. In addition, the acellular culture filtrate (CF) of *E. carotovora* ssp. *carotovora* that contains cell wall-degrading enzymes and caused similar maceration-type symptoms in tobacco leaves as the pathogen (data not shown) was also used as an inducer of SAR. The ability of the bacterium (*E. carotovora* ssp. *carotovora* strain SCC 3193) to macerate plant tissue was tested by treatment of potato tuber slices with a 5.0×10^9 cfu/ml bacterial suspension or CF. Both treatments resulted in tuber tissue maceration within 2 days following applications (data not shown).

Neither E. carotovora *ssp.* carotovora*, nor its cell wall-degrading enzymes can induce systemic acquired resistance to bacterial growth*

Induction of SAR to *in planta* multiplication of *E. carotovora* ssp. *carotovora* by the pathogen itself or its CF was investigated in 40 and 60 days old *nahG* tobacco plants with an induction time of 3 and 7 days between pretreatments and challenge inoculations. Following pretreatments of lower leaves with *E. carotovora* ssp. *carotovora* (5.0×10^9 cfu/ml), CF or water, leaves above the pretreated ones were challenged with a 10^7 cfu/ml inoculum of *E. carotovora* ssp. *carotovora*. In 60 days old *nahG* plants an induction time of 3 days resulted in no apparent reduction in bacterial growth in upper leaves following pretreatment of lower leaves with *E. carotovora* ssp. *carotovora* and CF as compared to water-pretreated controls (Fig. 1). Allowing a longer induction time of 7 days for the development of SAR was still ineffective in reducing bacterial growth in challenge-inoculated upper leaves of 60 days old *nahG* tobacco plants (Fig. 2). In order to see whether the development of such a SAR is age dependent, we pretreated younger, 40 days old plants and challenged them with *E. carotovora* ssp. *carotovora* 7 days after pretreatments as described above. Again, no reduction in bacterial growth occurred in challenge-inoculated leaves following pretreatments with *E. carotovora* ssp. *carotovora* and CF as compared to water-pretreated controls up to 72 hours after challenge (Fig.3). Furthermore, no reduction in bacterial growth occurred even between 7 to 10 days after challenge. Similar experiments carried out with 40 days old nontransformed Xanthi-nc plants gave essentially the same results: no SAR that effectively inhibits growth of *E. carotovora* ssp. *carotovora* developed within an induction time of 7 days up to 10 days after challenge inoculations (data not shown). Therefore, the above results demonstrate

that neither *E. carotovora* ssp. *carotovora*, nor its cell wall-degrading enzymes can induce SAR to multiplication of the pathogen in tobacco, regardless whether or not the plants are able to accumulate SA.

Neither E. carotovora *ssp.* carotovora*, nor its cell wall-degrading enzymes can induce systemic acquired resistance to necrosis caused by a bacterial and a viral pathogen*

Previous research showed that pretreatment of tobacco with pectolytic enzymes of *E. carotovora* ssp. *carotovora* as well as with SA can induce resistance to plant tissue maceration caused by the pathogen (Palva et al., 1993, 1994). In this study we were interested in whether *E. carotovora* ssp. *carotovora* or its cell wall-degrading enzymes are able to induce systemic acquired resistance (SAR) to localized (hypersensitive) necrotic symptoms caused by a bacterial and a viral pathogen in transgenic *nahG* tobacco that cannot accumulate SA and in nontransformed Xanthi-nc plants.

First, we investigated the possibility of the development of SAR in transgenic *nahG* and nontransformed Xanthi-nc plants to hypersensitive necrosis caused by the bacterial phytopathogen *Pseudomonas syringae* pv. *syringae* following induction by either *E. carotovora* ssp. *carotovora* or its CF that contains cell wall-degrading enzymes. SAR-development was monitored in 40 and 60 days old transgenic *nahG* and nontransformed Xanthi-nc tobacco after an induction time of 7 days between pretreatments and challenge inoculations. Following pretreatments of lower leaves with *E. carotovora* ssp. *carotovora* (5.0×10^9 cfu/ml), CF or water, leaves above the pretreated ones were challenged with different concentrations of *P. syringae* pv. *syringae*.

In 60 days old plants, 9 different concentrations of *P. syringae* pv. *syringae* (ranging from 10^5 to 10^9 cfu/ml) were applied as challenge inocula (Table 2). However, no apparent reduction of bacterial hypersensitive necrosis occurred in challenged, upper leaves following pretreatment of lower leaves with *E. carotovora* ssp. *carotovora* and CF as compared to water-pretreated controls 24 hours after challenge (Table 2). Furthermore, no reduction of necrosis occurred in upper leaves even up to 5 days after challenge (data not shown). The absence of SAR to bacterial necrosis was evident in both transgenic *nahG* and nontransformed Xanthi-nc plants (Table 2).

In order to see whether the development of a putative SAR to bacterial necrosis is age dependent, we pretreated younger, 40 days old transgenic *nahG* and nontransformed Xanthi-nc plants as described above and challenged them with different concentrations of *P. syringae* pv. *syringae*. In general, younger (40 days old) plants appeared more susceptible to hypersensitive necrosis caused by *P. syringae* pv. *syringae* than older (60 days old) plants in a similar manner as seen with maceration-like symptoms caused by *E. carotovora* ssp. *carotovora* (see Table 1). Therefore, in case of 40 days old tobacco, concentrations of *P. syringae* pv. *syringae* challenge inocula applied to upper leaves ranged from only 10^6 to 5.0×10^7 cfu/ml (Table 3). Again, as seen with older (60 days old) plants, no reduction of hypersensitive necrosis occurred in challenged, upper leaves following pretreatments with *E. carotovora* ssp. *carotovora* and CF as compared to

Table 5

Erwinia carotovora ssp. *carotovora* does not induce systemic acquired resistance (SAR) to hypersensitive necrosis caused by tobacco mosaic virus (TMV) in transgenic *nahG* and nontransformed Xanthi-nc tobacco

Plant Pretreatment[a] Plant age[b]	*nahG*			Xanthi-nc		
	CF	Ecc	control	CF	Ecc	control
40 days	350±160[c]	392±70	385±135	477±130	437±90	442±130
60 days	447±90	462±134	450±30	417±148	434±66	410±50

[a] Pretreatments consisted of inoculations of 5.0×10^9 cfu/ml *E. carotovora* ssp. *carotovora* (Ecc) or treatments with cell-free culture filtrate (CF) or water (control) in lower leaves. Leaves above the pretreated ones were challenged with TMV 7 days after pretreatments as described in Materials and Methods.

[b] Age of plants at the time of inoculation with TMV.

[c] Number of hypersensitive necrotic lesions/leaf (from an average of 4 plants/pretreatment) 4 days after inoculation with TMV.

water-pretreated controls 24 hours after challenge (Table 3) and even up to 7 days after challenge (data not shown). Furthermore, the absence of SAR to bacterial necrosis was evident in both transgenic *nahG* and nontransformed Xanthi-nc plants (Table 3). The above results demonstrate that neither *E. carotovora* ssp. *carotovora* nor its cell wall-degrading enzymes can induce SAR to bacterial hypersensitive necrosis caused by *P. syringae* pv. *syringae* in tobacco regardless whether or not the plants are able to accumulate SA.

In order to find out if *E. carotovora* ssp. *carotovora* can induce SAR to multiplication of *P. syringae* pv. *syringae* prior to the development of hypersensitive necrosis, transgenic *nahG* and nontransformed Xanthi-nc tobacco plants were pretreated with *E. carotovora* ssp. *carotovora* (5.0×10^9 cfu/ml) or water. Seven days after pretreatment leaves above the pretreated ones were challenged with an 5.0×10^6 cfu/ml inoculum of *P. syringae* pv. *syringae*. Hypersensitive necrosis appeared 24 hours after challenge inoculation. Limited bacterial growth occurred in leaves challenged with *P. syringae* pv. *syringae*, however, no apparent reduction in bacterial growth was measurable up to the development of hypersensitive necrosis following pretreatment with *E. carotovora* ssp. *carotovora* as compared to water-pretreated controls (Table 4). Such an absence of SAR to *P. syringae* pv. *syringae* growth occurred in both transgenic *nahG* and nontransformed Xanthi-nc tobacco plants. Taken together, these results indicate that *E. carotovora* ssp. *carotovora* can induce SAR neither to symptoms, nor to multiplication of a bacterial pathogen in tobacco, regardless of the plants ability to accumulate SA.

To test whether *E. carotovora* ssp. *carotovora* is capable of inducing SAR to hypersensitive necrosis caused by a viral pathogen, transgenic *nahG* and nontransformed

Xanthi-nc tobacco plants were pretreated with *E. carotovora* ssp. *carotovora* (5.0×10^9 cfu/ml), CF or water. Leaves above the pretreated ones were challenged with tobacco mosaic virus (TMV) 7 days after pretreatments. In transgenic *nahG* plants, the size of TMV-elicited, hypersensitive necrotic lesions was almost twice as large as in nontransformed Xanthi-nc tobacco, in accordance with previous observations (Delaney et al., 1994) (data not shown). On the other hand, lesion numbers/leaf remained essentially the same in transgenic *nahG* and nontransformed Xanthi-nc tobacco plants in response to all treatments (Table 5). This indicates that neither *E. carotovora* ssp. *carotovora* nor its cell wall-degrading enzymes are capable of inducing SAR to hypersensitive necrosis caused by TMV in tobacco regardless whether or not the plants are able to accumulate SA.

Discussion

Ineffective systemic acquired resistance to growth of plant pathogenic bacteria

We have investigated the possibility of the development of systemic acquired resistance (SAR) directed against multiplication of *E. carotovora* ssp. *carotovora*, a causal agent of bacterial soft rot of plants in transgenic *nahG* tobacco that cannot accumulate salicylic acid (SA) and in nontransformed Xanthi-nc plants. In order to induce an effective SAR, both *E. carotovora* ssp. *carotovora* and its culture filtrate (CF) that contains cell wall-degrading enzymes were applied as inducers in concentrations that are able to cause visible symptoms (Table 1). These symptoms consisted of pale green lesions indicative of limited tissue maceration and turned necrotic only 5 days after inoculation. It is known that SAR is induced by pathogens that cause a necrotic reaction in the host (Ross, 1961b; Kuć and Richmond, 1977). Therefore, the absence of SAR to the growth of *E. carotovora* ssp. *carotovora* in 60 days old *nahG* plants following only 3 days after induction was expected, since necrotic symptoms did not develop in the lower leaves following induction (Fig.1). However, a longer induction time of 7 days was still ineffective in reducing bacterial growth in upper, challenge inoculated leaves of *nahG* plants, in spite of the development of necrosis in the lower leaves within the induction period (Fig. 2). These results demonstrated that SAR directed against bacterial growth does not develop in these plants following induction by *E. carotovora* ssp. *carotovora* or its CF. Furthermore, such a SAR did not develop in younger, 40 days old plants as well (Fig. 3), even up to 10 days after challenge which strongly suggests that plant age has no significant role in the development of this putative SAR. Although Vidal et al. (1998) reported a slight inhibition of growth of *E. carotovora* ssp. *carotovora* and *Xanthomonas campestris* in distal plant parts following pretreatment of lower leaves with pectolytic enzymes of *E. carotovora* ssp. *carotovora* in very young 21 days old tobacco seedlings, bacterial multiplication was never lower than 1 order of magnitude as compared to non-pretreated controls. Similarly, in our experiments bacterial growth, in response to all pretreatments, remained essentially the same within the range of 1 order of magnitude. Such a slight difference in bacterial multiplication should not be sufficient to account for an effective

resistance mechanism against bacterial growth in tobacco. For example, resistance to plant pathogenic bacteria during a hypersensitive response (HR) in tobacco is manifested as a reduction of 3–4 orders of magnitude in bacterial growth compared to a compatible (disease) plant-bacterium interaction (Klement et al., 1964). Thus, SAR may not be effective at all in inhibiting bacterial growth *in planta*, only in suppressing necrotic symptoms. It was shown that in cucumber SAR to the bacterium *Pseudomonas lachrymans* is manifested as a reduction of necrotic symptoms, but not of bacterial growth (Doss and Hevesi, 1981).

The absence of SAR to multiplication of *E. carotovora* ssp. *carotovora* was evident in both nontransformed Xanthi-nc and transgenic *nahG* plants that cannot accumulate SA, a key component of the signal transduction pathway that leads to SAR in tobacco (Gaffney et al., 1993; Delaney et al., 1994). Interestingly, cell wall-degrading enzymes of *E. carotovora* ssp. *carotovora* have been shown to induce SAR related genes encoding PR proteins in tobacco in an SA-independent manner (Palva et al., 1993; Vidal et al., 1997, 1998). However, systemic induction of PR proteins may not necessarily indicate the existence of an SAR response that effectively inhibits bacterial growth *in planta* (see Vidal et al., 1998). Accumulation of PR proteins could be only a correlative phenomenon which has nothing to do with SAR. In this paper, we have not studied systemic induction of PR proteins following pretreatments with *E. carotovora* ssp. *carotovora* or its cell wall-degrading enzymes. On the other hand, our results clearly demonstrate that in 40 and 60 days old tobacco neither *E. carotovora* ssp. *carotovora*, nor its cell wall-degrading enzymes can induce SAR to in planta bacterial growth regardless whether or not the plants are able to accumulate SA.

Ineffective systemic acquired resistance to necrotic symptoms caused by a bacterial and a viral pathogen

It was shown that neither *E. carotovora* ssp. *carotovora*, nor its cell wall-degrading enzymes can induce systemic acquired resistance (SAR) to *in planta* bacterial growth regardless of the plant's ability to accumulate salicylic acid (SA). Our further goal was to find out whether *E. carotovora* ssp. *carotovora* or its cell wall-degrading enzymes are able to induce SAR to localized (hypersensitive) necrotic symptoms caused by a bacterial and a viral pathogen in transgenic *nahG* tobacco that cannot accumulate SA and in nontransformed Xanthi-nc plants.

First, we investigated the possibility of the development of SAR to hypersensitive necrosis caused by the bacterial phytopathogen *Pseudomonas syringae* pv. *syringae* following induction by either *E. carotovora* ssp. *carotovora* or its culture filtrate (CF) that contains cell wall degrading enzymes. No apparent reduction of necrosis occurred in challenged upper leaves of 40 and 60 days old transgenic *nahG* and nontransformed Xanthi-nc plants following inducer pretreatments, as compared to water-pretreated controls (Tables 2 and 3). These results indicate that in tobacco neither the soft rot bacterium, nor its cell wall-degrading enzymes can induce SAR to hypersensitive necrotic symptoms caused by *Pseudomonas syringae* pv. *syringae*. Previous studies show that

local acquired resistance (as opposed to SAR) to symptoms of plant tissue maceration caused by *E. carotovora* ssp. *carotovora* can be induced in tobacco by pretreatments of the inoculated leaves with either SA (Palva et al., 1994; Chivasa et al., 1997) or pectolytic enzymes of the pathogen (Palva et al., 1993). On the other hand, the existence of SAR directed against pathogen-elicited symptoms in tobacco following pretreatments with *E. carotovora* ssp. *carotovora* or its cell wall-degrading enzymes has not been investigated so far. Vidal et al. (1998) studied a putative SAR to growth of *E. carotovora* ssp. *carotovora* following the above mentioned pretreatments, but the question of systemic resistance to bacterial symptoms was left unanswered. These workers used transgenic *nahG* tobacco that cannot accumulate SA, a key component of the signal transduction pathway that leads to SAR in tobacco (Gaffney et al., 1993; Delaney et el., 1994). Our results clearly demonstrate that in tobacco neither *E. carotovora* ssp. *carotovora*, nor its cell wall-degrading enzymes can induce SAR to hypersensitive necrosis caused by *Pseudomonas syringae* pv. *syringae* regardless of the plants ability to accumulate SA (Tables 2 and 3).

E. carotovora ssp. *carotovora* cannot induce SAR to growth of *Pseudomonas syringae* pv. *syringae* prior to the development of hypersensitive necrosis in challenge-inoculated leaves of transgenic *nahG* and nontransformed Xanthi-nc plants (Table 4). This is not surprising in light of an earlier work which shows that in cucumber, SAR does not develop to growth of *Pseudomonas lachrymans* even if an SAR to bacterial necrosis is functional (Doss and Hevesi, 1981). Taken together, the results of our work show that the soft rot bacterium or its CF can induce SAR neither to symptoms, nor to multiplication of a bacterial pahogen in tobacco, regardless whether or not the plants are able to accumulate SA.

Both *E. carotovora* ssp. *carotovora* and its cell wall-degrading enzymes are also unable to induce SAR to hypersensitive necrosis caused by a viral pathogen, tobacco mosaic virus (TMV) in the upper challenged leaves of transgenic *nahG* and nontransformed Xanthi-nc tobacco (Table 5). The number of hypersensitive necrotic lesions in upper leaves challenge inoculated with TMV remained essentially the same in response to all treatments in both 40 and 60 days old transgenic *nahG* and nontransformed Xanthi-nc plants (Table 5). It is very likely that virus replication was also not affected. According to Balázs et al. (1977) an SAR to TMV-elicited hypersensitive response in tobacco is manifested as a reduction of visible necrosis, but not of virus replication.

In conclusion, the results of the present study demonstrate that in 40 and 60 days old Xanthi-nc tobacco *E. carotovora* ssp. *carotovora* and its cell wall-degrading enzymes are unable to induce SAR to both bacterial growth and necrotic symptoms caused by a bacterial and viral pathogen. The absence of such an SAR-response occurs regardless whether or not the plants can accumulate SA, a key component of the signal transduction pathway that leads to SAR in tobacco. From a practical point of view, our results indicate that instead of *E. carotovora* ssp. *carotovora* and its cell wall-degrading enzymes other, potent inducers of SAR should be considered which effectively inhibit multiplication of pathogens or symptom development in tobacco plants following challenge infections.

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Bacteriological Characteristics of Some Yugoslavian *Erwinia* Soft Rot Strains Originating from Lettuce Head Leaves

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Many *Erwinia* soft rot strains were isolated from rotted heads of lettuce. Three of them were investigated using corresponding bacteriological tests. On the basis of biochemical and physiological characteristics they belong to *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al.

However, according to some tests, the lettuce strains showed differences comparing to usually biochemical and physiological properties given for *E. c.* ssp. *carotovora* subspecies.

Bacteriological soft rot is spread and very frequent disease on many plants cultivated in Yugoslavia. Three bacteria: *Erwinia carotovora* subsp. *carotovora, E. c.* ssp. *atroseptica* and *E. chrysanthemi* were found as causal agents of the disease.

Among them the first one (*E. c.* ssp. *carotovora*) prevailing on various plant species: sunflower stock, cauliflower and lettuce heads, potato plants and tubers, seed cabbage plants and cabbage heads, pepper and eggplants fruits and others (Arsenijević, 1970, 1990; Arsenijević et al., 1993, 1996a, b, c; Obradović, 1994; Jovanović, 1997).

The second bacterium (*E. c.* ssp. *atroseptica*) prevailing as potato pathogen causing black-leg of the plants diseased and tubers soft rot (Arsenijević et al., 1994a; Obradović, 1994; Jovanović, 1997).

The third bacterium (*E. chrysanthermi*) was isolated only from maize plants wilting (Arsenijević et al., 1992).

The strains of the first two bacteria (*E. c.* ssp. *carotovora* and *E. c.* ssp. *atroseptica*) investigated in Yugoslavia expressed the bacteriological properties characteristics for these pathogens.

However, sometimes instead of expected data the opposite results appeared. This frequently was noticed regarding the reducing substances from sucrose and erythromycin resistance tests as well as in some other characteristics (Arsenijević et al., 1994b, 1996a, b; Arsenijević and Obradović, 1996; Obradović, 1994; Jovanović, 1997).

Therefore, it was a motive to investigate the properties of the *Erwinia* soft rot strains (*E. c.* ssp. *carotovora*) originating from the leaves of lettuce heads, which expressed more differences from the results expected.

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Materials and Methods

Isolation of the bacterium and the strains used

The bacterial strains isolation was carried out according by usual procedure using small fragments taken between healthy and diseased plants tissue and by the macerate streaking onto nutrient agar (NA) plates. Single bacterial colonies were transferred onto agar slants after 2 days of incubation at 26 °C (Klement et al., 1990).

From many isolates, obtained three representatives once (Sa-29, Sa-31, Sa-32) were used for further investigations. As reference strains, three identified bacteria were added, mentioned above: *E. c.* ssp. *carotovora* (Kr-154), *E. c.* ssp. *atroseptica* (Kr-20) and *E. chrysanthemi* (K-54) which were subject of the previous investigations in Yugoslavia (Arsenijević et al., 1992, 1993, 1994a, b; Obradović, 1994; Jovanović, 1997).

As the check tests for fluorescence and Gram stain the bacteria *Pseudomonas syringae* pv. *syringae* van Hall and *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al., were also used.

Morphological and cultural characteristics

Shape and dimensions of the bacterial cells as well as the flagellation of the bacteria investigated were examined by light and electron microscopy and Gram stain using 3% KOH solution (Ryu, 1938; loc. cit. Fahy and Hayward, 1983).

Growth characteristics of the isolates were studied by cultivating the bacteria on nutrient agar (NA), potato dectrose agar (PDA) and Logan's differential medium. Blue pigment production was determined on yeast extract dextrose agar (YDCA) and fluoresce on King's B medium using *P. s.* pv. *syringae* as a check strain (Fahy and Hayward, 1983; Lelliott and Stead, 1987; Arsenijević, 1997).

Growth ability at 37 °C and tolerance to 5% NaCl were investigated according to usual procedure (Fahy and Hayward, 1983).

Biochemical and physiological properties

The follow identification tests corresponding for *Erwinia carotovora* group were used: reducing substance from sucrose, phosphatase, lecithinase and indole production and erythromycin sensitivity (Lelliott and Stead, 1987; Klement et al., 1990), oxidase acitivity (Kovasc, 1956; loc. cit. Lelliott and Stead, 1987; Klement et al., 1990), catalase production (Dye, 1968; loc. cit. Arsenijević, 1997) and glucose (O/F) metabolism (Hugh and Leifson, 1953; loc. cit. Lelliott and Stead, 1987; Klement et al., 1990).

Acid production from carbohydrates was carried out according Lelliott and Stead (1987) and Klement et al. (1990). Dulcitol, lactose, maltose, trehalose and α-methyl glucoside were used (Table 1).

Table 1

The properties of the *Erwinia carotovora* subsp. *carotovora* isolates investigated comparing with other soft rot *Erwinias* and with literature data

Tests	Lettuce soft rot strains			Reference strains			L. D.
	Sa-29	Sa-31	Sa-32	Kr-154 (Ecc)	Kr-20 (Eca)	K-54 (Echr)	(Ecc)
Gram stain	–	–	–	–	–	–	–
Fluorescence on King's medium B	–	–	–	–	–	–	–
Blue pigment on YDCA	–	–	–	–	–	–	–
Growth on Logan's medium	(2)	(2)	(2)	(2)	(1)	(3)	(2)
Glucose metabolism	O/F	O/F	O/F	O/F	O/F	O/F	O/F
Tolerance to 5% NaCl	+	+	+	+	+	–	+
Growth at 37 °C	+	+	+	+	–	+	+
Reduc. subst. from sucrose	+	+	+	–	+	–	–
Erythromycin sensitivity	R	R	R	S	R	S	R
Phosphatase	–	–	–	–	–	+	–
Lecithinase	–	–	–	–	–	+	–
Catalase	+	+	+	+	+	+	+
Oxidase	–	–	–	–	–	–	–
Acid form: dulcitol	–	–	–	–	–	–	–
lactose	+	+	+	+	+	–	+
maltose	–	–	–	–	+	–	V
trechalose	+	+	+	+	+	–	+
α–met.gluc.	+	+	+	–	+	–	–
Indole production	–	–	–	–	–	+	–

– negative reaction; + positive; V – variable; (1) colonies small (1–2 mm) and red; (2) colonies middle large (3–4 mm) light-red and pink in the centre; (3) colonies large (4–5 mm) and dark–red; S – susceptible; R – resistant; Ecc (*Erwinia carotovora* subsp. *carotovora*); Eca (*E. c.* ssp. *atroseptica*); Echr (*E. chrysanthemi*); L. D.–literature data (Cother and Sivasithamparam, 1983)

Results

No differences were noticed among the lettuce *Erwinia* soft rot strains (Table 1). Bacterial cells were gramnegative, asporogenous, rode-shaped, with peritrichous flagellation, single or in pairs and rarely in short chains. The bacteria measured 1.92–0.84 μm. The colonies on NA were round, slightly convex, glistening and grayish-white. They do not produce green fluorescent pigment on King's medium B. On PDA the colonies of the strains investigated as well as the chek strains (Ecc, Eca, Echr) are cream and have a "fried egg" outlook.

No blue pigment on YDCA was noticed (Table 1). On Logan's medium they form light-red colonies with pink center as well as the check strain Kr-154 (Ecc).

The check strain Kr-154 (Ecc) and lettuce strains grow at 37 °C and also in liquid medium with 5% NaCl. Potato check strain Kr-20 (Eca) exhibits no growth at 37 °C and maize strain K-54 (Echr) does not grow in the presence of 5% NaCl (Table 1).

The lettuce *Erwinia* soft rot strains (Sa-29, Sa-31, Sa-32) produced catalase and acids from lactose, trehalose and α-methyl glucoside, but not from dulcitol and maltose. They were oxidase, phosphatase, lechitinase and indole production negative and reducing substances from sucrose were positive; glucose metabolism was oxidative and fermentative. Except check strain Kr-154 (Ecc) all lettuce *Erwinia* strains were erythromycin resistant (Table 1).

Discussion

The results obtained indicated that lettuce *Erwinia* soft rot strains expressed the same biochemical and physiological characteristics (Table 1).

Their properties the most correspondent to *E. c.* ssp. *carotovora* bacterium what is in accordance, with literature data (Tanii and Akai, 1975; Togashi, 1988; Tsuchiya et al., 1987; Karnjanarat et al., 1987).

The colonies of the pathogen incubated in PDA had a "fried egg" outlook characteristic for *E. chrysanthemi* bacterium (Reisfneider and Lopes, 1982).

Tolerance to 5% NaCl and their growth on Logan's differential medium and at 37 °C differ our lettuce *Erwinia* soft rot strains from *E. chrysanthemi* and from *E. c.* ssp. *atroseptica* (Table 1).

However, the *Erwinia* soft rot strains from lettuce have produced reducing substances from sucrose and acid from α-methyl glucoside expressing the most specificity because, as it is known, such results are typical for *E. c.* ssp. *atroseptica* bacterium (Table 1).

But, in the last time certain authors gave the opposite data for *Erwinia* soft rot strains comparing with usual results (Tanii and Akai, 1975; Thomson et al., 1987; Karnjanarat et al., 1987; De Boer et al., 1987; Togashi, 1988; Dela Cruz et al., 1988; Janse and Spit, 1989). Regarding to Yugoslav *Erwinia carotovora* ssp. *carotovora* strains of various origin they also expressed differences in some characteristics mentioned above. So, in reducing substances from sucrose test the *E. c.* ssp. *carotovora* strains isolated from pepper and eggplants fruits and from seed cabbage plants were negative, but the *Erwinia* soft rot strains from cabbage heads were positive (Arsenijević et al., 1996c, 1997; Arsenijević and Obradović, 1996).

The Yugoslav *E. c.* ssp. *carotovora* strains from pepper and eggplant fruits and from cabbage heads were resistant or susceptible in erythromycin test what depends from the strain investigated. The isolates from seed cabbage plants were erythromyin resistant (Arsenijević and Obradović, 1996) and from pepper and eggplant fruits were susceptible or resistant (Obradović, 1994; Arsenijević et al., 1997).

The authors from abroad gave also the uneven results in erythromycin sensitivity and reducing substances from sucrose tests as well as acid production from maltose, the

reaction for which is known to be variable (Tanii and Akai, 1975; Karnjanarat et al., 1987; Togashi, 1988).

In indole production test the lettuce *E. c.* ssp. *carotovora* strains were negative (Table 1) and those from pepper and eggplant fruits positive (Arsenijević et al., 1997) what was also noticed by Japanese authors Tanii and Akai (1975) and Togashi (1988).

Other biochemical and physiological characteristics of the lettuce *Erwinia* soft rot pathogen investigated here, were in accordance with properties of *E. c.* ssp. *carotovora* reference bacterium (Table 1).

The differences in some characteristics of *E. c.* ssp. *carotovora* strains investigated comparing to those from the literature data (Tanii and Akai, 1975; Thomson et al., 1981; Karnjanarat et al., 1987; Togashi, 1988; Dela Cruz et al., 1988; Janse and Spit, 1989) indicate on population variability of *E. c.* ssp. *carotovora* subspecies.

Therefore, the presence of intermediate strains or biovars are also possible containing both, *E. c.* ssp. *carotovora* and *E. c.* ssp. *atroseptica*, biochemical and physiological characteristics (Arsenijević et al., 1989, 1994a, 1996a, b, 1977; Tsuchyia et al., 1987; Karnjanarat et al., 1987; Jabuonski et al., 1987).

For more contemporary details about relationships and bacteriological properties of these two pathogens using their fatty acid profiling, genome analyisis, and other tests are necessary to investigate (Persson and Sletten, 1995) in the future.

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Occurrence of *Pseudomonas syringae* Pathovars on Soybean in Hungary and their Differentiation

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Biochemical, serological and pathogenic properties of 58 randomly selected hypersensitive positive fluorescence pigment producing strains isolated from soybean seed and plant samples originated from Hungary were studied. Based on biochemical properties 34 isolates were identified as *Pseudomonas syringae* pv. *glycinea*, while the remaining part was identified as *Pseudomonas syringae* pv. *syringae*, which were isolated exclusively from seeds. From the 95 compounds studied δ-amino-n-valeric acid, betaine, meso-erythritol, D-galacturonic acid, glutaric acid, α-ketoglutaric acid, L(+)-lactic acid, L-serine, D(+)-xylose could be chosen for differentiation of the two pathovars from each others. In Ouchterlony agar diffusion and ELISA test the *Pseudomonas syringae* pv. *glycinea* isolates were divided into 2 serogroups.

Twenty-one *Pseudomonas syringae* pv. *glycinea* isolates were determined as race 4, 2 belonged to race 6, 1 to race 7, 5 to race 9, 4 to race 10 while one isolate did not fit in any defined races. The isolates identified in biochemical tests as *Pseudomonas syringae* pv. *syrignae* did not produce symptoms on any of the varieties used for race differentiation.

For the routine isolation a semiselective levane-borate medium was found to be the best. Based on several years experiences, optimal conditions for routine seed assay are proposed.

According to literature data (Klement, 1965; Szili, 1975; Kocsisné Csontos E. 1979; Hevesi and Érsek, 1981) and our surveys in the past two decades, the main bacterial pathogen of soybean in Hungary is *Pseudomonas syringae* pv. *glycinea*. However, literature data show the possible occurrence of two other important pathogenic bacteria in soybean: *Xanthomonas campestris* pv. *glycines* and *Curtobacterium flaccumfaciens* subsp. *flaccumfaciens* (Dunleavy, 1963; Beltjukva et al., 1974; Smith et al., 1986). According to the surveys carried out by the bacteriological laboratory of the Hungarian Plant Health Organization the presence of the latter two pathogens has not been proven.

Several papers have been published on the races of *Pseudomonas syringae* pv. *glycinea*. Cross et al. (1966) separated seven pathogenic races by the reaction of the seven differential soybean varieties. Thomas and Leary (1980) reported about the 8. race. Gnanamanickam and Ward (1982) described the 9. one. Kucharek and Stall (1985) examining the pathogen causing disease symptoms on a new variety, Centennial, concluded, that symptoms were caused by a new race which they called race 0. Abo-Moch et al. (1995) studying races of this pathogen in Europe found a new 10. race.

In Hungary studies carried out by Hevesi and Érsek (1981) suggested the presence of two different races but in the lack of complete series of differential soybean cultivars the exact race determination was not completed.

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The object of the recent study was to clear up the presence of possible *Pseudomonas syringae* pathovars other than pv. *glycinea,* and to find simple tests, suitable for rapid differentiation of them.

Materials and Methods

Samples

In the seed assay samples from commertial seed lots originated from Hungary were divided into 5 subsamples each of 1,000 seeds and were incubated in sterile tap water at 4 °C overnight, followed by shaking in a rotary shaker at room temperature for 30 min at 250 rev min^{-1}. Decimal dilutions of the suspension were made up to 3, 4 dilutions. One hundred µl of each dilution was plated in two replicates from each subsamples on King's medium B (King et al., 1954), nutrient-peptone agar (peptone, 10 g; beef extract, 5 g; NaCl, 5 g; glycerol, 18 g; agar, 15 g; distilled water, 1000 ml), M-71 (Leben, 1972) and on a semiselective levane-borate medium with the following composition: sucrose, 50 g; yeast extract, 3 g; Difco proteose peptone No 3,5 g; boric acid, 1.5 g; agar, 18 g; distilled water, 1000 ml (Grondeau et al., 1992). After autoclaving 200 mg cycloheximide and 80 mg cephalexin per litre were added. Plant samples were originated from Baranya, Bács-Kiskun, Szabolcs-Szatmár-Bereg, Szolnok, Tolna, and Somogy counties. Diseased leaves were macerated in sterile tap water and the resulting suspensions were streaked onto the media described above.

The inoculated plates were incubated at 25–27 °C for 3–4 days. Colonies with the phenotype of *P. syringae* pv's were transferred to King's medium B slants for preparation of single cell isolates and for further analysis. Following isolation, pathogenic strains were selected on tobacco hypersensitive reaction (HR), and isolates giving a positive HR reaction were further characterized. Several strains isolated in the period of 1982–1990 were also included into the study.

Characterization by LOPAT

For characterization of the pathogenic isolates, LOPAT tests were carried out as follows.

To check for levan production, cultures were grown for 3 days on agar containing 5.0% sucrose. Isolates that produced large white domed mucoid colonies were considered to be levan positive (Lelliott et al., 1966).

The oxidase test (Kovács, 1956) was done on filter paper soaked with a 1.0% (w/v) aqueous solution of N,N-dimethyl-p-phenylenediamide. A loopful of bacteria from 2–3-days-old cultures grown on King's medium B were smeared on the filter paper with a platinum needle. Production of dark-purple colour in 30 sec was recorded as positive.

For the potato soft-rot test, 1 cm thick slices prepared from surface disinfected peeled tubers were placed on filter paper in Petri dishes. A loop of bacterial slime from

1–2-days-old King's medium B culture was smeared on the surface of the slices. Test was recorded positive if the slices were soft rotted 2 days after inoculation.

Arginine dihydrolase production was tested in the medium of the following composition: peptone, 1.0 g; NaCl, 5.0 g; K_2HPO_4, 0.3 g; pehnol red, 10 mg; L-arginine HCl, 10 g; agar, 3 g; distilled water, 1000 ml (Lelliott et al., 1966). The pH was adjusted to 6.2 with 1 N NaOH. 4 ml of the medium were added to test tubes and sterilized. The tubes were stab-inoculated from 2-day-old cultures grown on King's medium B and sealed with sterile mineral paraffine oil. The production of alkali from arginine was determined by the change in colour of the indicator after 4 days, which suggested the presence of arginine dihydrolase enzyme (Thornley, 1960).

Induction of tobacco hypersensitive reaction was checked by injection of a suspension prepared from 24 h old culture containing 10^9 CFU/ml into the intercellular space of tobacco leaf (Klement et al., 1964). Further tests were carried out only with the isolates characterized by the LOPAT test as *P. syringae* pv's (levan +, oxidase –, potato soft rot –, agrinine dihydrolase –, tobacco HR +).

Nutritional requirements of the strains

For the nutritional tests Misaghi and Grogan (1969) basic medium was used. Substrate containing both carbon and nitrogen was added singly to the mineral base to test for utilization as source of both carbon and nitrogen. Organic substrates with no nitrogen were tested in mineral base containing 0.1% (w/v) ammonium sulphate. Sugars were used at 0.2% (w/v), while other compounds were used at 0.1% (w/v). Test media were solidified adding 1.5% Noble agar. All substrates were filter sterilized. When the substrates could not be sterilized, they were added aseptically to the base. pH of the media was adjusted to 7.2 before plates were poured. Eightly ml medium was added to a Petri dish of 150 mm diameter. Plates after pouring were kept for 3 days at room temperature to allow drying.

Droplets of suspensions (approximately 5 µl) containing 10^9 cells/ml prepared from 2-day-old King's medium B cultures were placed by syringe on the agar surface. This method ensured the uniformity of number of bacterial cell of the different strains tested, furthermore possibility of exhaustion and cross feeding were reduced significantly as well.

Plates were kept at 25–27 °C after inoculation and were checked visually for growth on days 7, 14 and 21. All compounds were tested at least 2 twice.

The 95 substrates tested are listed in Table 1.

Serological tests

Double antibody sandwich (DAS) ELISA and Ouchterlony agar diffusion tests (ODD) (Ouchterlony, 1948) were carried out as serological tests. Polyclonal antibodies were prepared in rabbits against living bacterial cells, IgG was purified as described by Clark and Adams (1977) and conjugated with peroxidase according to Avrameas and Ternynck (1971).

Table 1

Nutritional tests for identification of *P. s.* pv. *glycinea* and *P. s.* pv. *syringae* strains

Substrates	*P. s.* pv. *glycinea*	*P. s.* pv. *syringae*
Adonitol, amino butyric acid, β-aminobenzoic acid, D-arabinose, L-arabitol, arbutin, D-aspartic acid, D-cellobiose, L-cisteine, L-cistine, citraconic acid, dulcitol, formic acid, D-glutamic acid, glycolic acid, hippuric acid, DL-homoserine, inulin, L-isoleucine, 2-keto-D-gluconic acid, 5-keto-D-gluconic acid, D-lactose, levulinic acid, D-maltose, D-melezitose, D-melibiose, mesaconic acid, D-methionine, L-methionine, α-methyl-D-glucoside, nicotinic acid, nitrilotriacetic acid, palatinose, D-pantothenic acid, L-β-phenylalanine, polygalacturonic acid, propionic acid, D-raffinose, L-rhamnose, salicin, sorbic acid, L-sorbose, L-tartaric acid, thioproline, L-threonine, D-trehalose, L-tryptophan, uric acid, L-valine	N	N
δ-amino-n-valeric acid, betaine, meso-erythritol, D-galacturonic acid, glutaric acid, α-ketoglutaric acid, L-lactic acid, L-serine, D-xylose	N	P
DL-glyceric acid, glycine, succinic acid, D-tartaric acid, L-tyrosine	N	d
DL-asparagine, L-asparagine, L-aspartic acid, citric acid, fumaric acid, D-gluconic acid, glucose, mucic acid, protocatechuic acid	P	P
L-glutamic acid	P	d
β-alanin	d	N
L-α-alanin, L-arabinose, L-arginin, caprylyc acid, D-fructose, D-galactose, L-glutamine, glycerol, L-histidine, 4-hydroxybenzoic acid, DL-β-hydroxybutyric acid, myo-inositol, DL-malic acid, malonic acid, D-mannitol, D-mannose, pyruvic acid, L-quinic acid, D-ribose, D-sucrose, D-sorbitol	d	P

N = negative
P = positive
d = strain variability

Race differentiation

Virulence of HR positive isolates was checked first on the host variety from which the strain was isolated, than on the seven soybean cultivars for race identification (Acme, Chippewa, Flambeau, Harasoy, Lindarin, Merit, Norchief), which were obtained from the Hungarian Institute of Agrobotany, Tápiószele. Seeds were sown in perlite, layered between two layers of peat sand and brown forest soil mixture in plastic trays. Plants were grown in greenhouse at 20–24 °C. For inoculation, a bacterial suspension containing 10^8 CFU/ml prepared from a 2-day-old King's medium B culture was used. The suspension was smeared onto the lower surface of unfolded trifoliate leaves with painting brush in age of the leaves when they were of 1/2–2/3 part of final size. The inoculated plants were incubated at 20–24 °C in a humid chamber. Symptoms were re-

corded 7–11 days after inoculation and classified as resistant, intermediate or susceptible (Cross et al., 1966). Race determinations was repeated twice. In the separate assays 6–8 plants of the differential cultivars were inoculated. Reference strains GSPB 1835, GSPB 1837, GSPB 1974, GSPB 1985, GSPB 2006, GSPB 2011, were obtained from the collection of Dr. K. Rudolph (Germany, Göttingen).

Results

For isolation of pathogenic *Pseudomonas* spp., King's medium B proved to be better than nutrient-peptone and M-71 agar. On King's medium B the fluorescent pigment producing *Pseudomonas* spp. could be easily distinguished from other saprophytes, while on nutrient-peptone agar it was sometiems difficult. Recovery of *P. s. glycinea* was significantly lower on M-71, than on King's medium B and in routine isolation procedure the differentiating qualities of this medium are not always expressed. On King's medium B and nutrient-peptone agar the saprophytes occurring usually in great number ins suspensions, prepared from seed or plant samples, often overgrow the pathogenic *Pseudomonas* spp. The semiselective levane-borate medium (Grondeau et al., 1992) used in the study proved to be the best. Compounds providing selectivity suppressed many saprophytes, furthermore typical levan positive colonies could be easily identified.

The results of the LOPAT test for all 58 strains were: levan +, oxidase –, potato soft rot –, arginine dihydrolase production –, HR +.

The results of biochemical tests are demonstrated in Table 1. Based on the nutritional tests the studied HR positive strains could be divided into 2 distinct groups. Thirty-four isolates were determined as *P. s.* pv. *glycinea*, while 24 could be identified as *P. s.* pv. *syringae*. From 95 compounds tested *P. s.* pv. *glycinea* strains could grow only on DL-asparagine, L-asparagine, L-aspartic acid, citric acid, fumaric acid, D-gluconic acid, D(+)-glucose, D-glutamic acid, mucic acid, and protocatechuic acid. On L-α-alanine, β-alanine, L(+)-arabinose, L-arginine, caprylic acid, D(–)-fructose, D(+)-galactose, L-glutamine, glycerol, L-histidine, 4-hydroxybenzoic acid, DL-β-hydrosybutyric acid, myo-inositol, DL-malic acid, malonic acid, D-mannitol, D(+)-mannose, pyruvic acid, (–)-quinic acid, D(–)-ribose, D-sorbitol, sucrose, only part of the *P. s.* pv. *glycinea* isolates could grow while on the remaining substrates all of them failed to grow. From the tested substrates δ-amino-n-valeric acid, betaine, meso-erythritol, D-galacturonic acid, glutaric acid, α-ketoglutaric acid, L(+)-lactic acid, L-serine, D(+)-xylose could be chosen for differentiation purposes (Table 2). All of *P. s.* pv. *syringae* isolates showed growth on these compounds, while *P. s.* pv. *glycinea* strains were not able to grow on these substrates. One Hungarian strain identified earlier as *P. s.* pv. *glycinea* (No. 637) and one foreign standard strain (GSPB 2007) were not able to grow on any of the substrates, although they proved to be virulent in host plant test.

In the artificial inoculation test of the host plant, symptoms appeared on days 5–7 in form of small water soaked translucent spots bordered by veins sometimes surrounded by a pale-yellow halo. Spots became brown to black later, but failed to produce ragged

Table 2

Nutritional tests for differentiation of *P. s.* pv. *glycinea* and *P. s.* pv. *syringae* strains from each others

No.	Substrates	*P. s.* pv. *glycinea*	*P. s.* pv. *syringae*
1	δ-amino-n-valeric acid	N	P
2	betaine	N	P
3	meso-erythritol	N	P
4	D-galacturonic acid	N	P
5	glutaric acid	N	P
6	α-ketoglutaric acid	N	P
7	L-lactic acid	N	P
8	L-serine	N	P
9	D-xylose	N	P

N = negative
P = positive

appearance, which is rather typical phenomenon under natural infection conditions. The resistant type of reaction appeared in form of pinhead-size necrotic spots, which were visible 3 to 4 days after inoculation. The intermediate type of reaction (small water-soaked spots which becoming necrotic rapidly) were scored as sensitive reaction.

In our preliminary studies, artificial inoculation tests carried out in the period between November and February did not result in typical water soaked symptoms usual for the sensitive reaction – the sensitive reaction was almost indistinguishable from the intermediate one, despite of the proper temperature regime and artificial light. All the *P. s.* pv. *glycinea* strains, caused typical symptoms on the homologous variety from which they were isolated.

Twenty strains belonged to the race 4. Some of the strains determined as race 4 produced real sensitive reaction on variety Chippewa. Five isolates belonged to 9, 4 isolates to race 10. Two isolates were identified as race 6 and one isolate as race 7. One strain was very similar to race 3, but differed in the reaction on variety Chippewa. One freshly isolated strain produced intermediate reaction on all varieties. It was determined as race 4 with a low virulence.

Some inconsistency among the replicated trials recorded were due to the unproper age of the inoculated primordial leaves especially if they were inoculated in almost the fully expanded stage.

The isolates identified in biochemical tests as *P. s.* pv. *syringae* did not produce symptoms on any of the varieties. In ODD and ELISA tests the *P. s.* pv. *glycinea* isolates could be divided into 2 serogroups (Table 3). The majority of the strains proved to be belonging to serogroup 1. Some *P. s.* pv. *syringae* isolates reacted with serum raised against *P. s.* pv. *glycinea* giving high extinction values in ELISA, while some others reacted with serum of serogroup 2 in ODD.

Table 3

Serological relationship between *P. s.* pv. *glycinea* strains in ELISA and Ouchterlony agar diffusion (OOD) tests

Isolates	Serum I. (raised against strain 475)		Serum II (raised against strain 599)		Serogroups of *P. s.* pv. *glycinea*
	ELISA*	ODD	ELISA	ODD	
115	1.380	+	0.452	–	1
132	1.400	+	0.300	–	1
134	1.250	+	0.440	–	1
211	1.235	+	0.505	–	1
274	1.260	+	0.332	–	1
315	0.291	–	1.170	+	2
368	0.293	–	0.965	+	2
372	1.430	+	0.383	–	1
473	1.350	+	0.420	–	1
474	1.225	+	0.480	–	1
475	1.527	+	0.480	–	1
599	0.270	–	1.080	+	2
601	1.510	+	0.412	–	1
603	1.265	+	0.331	–	1
621	1.156	+	0.486	–	1
624	0.258	–	0.976	+	2
626	0.289	–	1.075	+	2
627	0.330	–	1.250	+	2
631	1.410	+	0.330	–	1
636	0.313	–	0.980	+	2
637	1.274	+	0.347	–	1
638	1.235	+	0.460	–	1
			P. s. pv. *syringae* strains		
240	0.221	–	0.490	–	
312	0.500	–	0.860	–	
623	0.350	–	0.980	+	
629	1.280	–	0.500	–	
643	0.236	–	0.348	–	
blank	0.129		0.161		

* extinction values
\+ positive
– negative

Discussion

Based on biochemical analysis of 58 randomly selected hypersensitive positive strains, 34 proved to be *P. s.* pv. *glycinea*, while 24 were *P. s.* pv. *syringae*. The *P. s.* pv. *syringae* strains were isolated exclusively from seeds. The *P. s.* pv. *glycinea* strains all proved to be virulent on their host soybean varieties and on some of the set of varieties for race identification, and were determined as isolates belonging to races 4., 6., 7., 9. and 10. The *P. s.* pv. *syringae* strains did not produced necrotic spots or water-soaked lesions neither on primordial nor on trifoliate leaves. This latter results did not conform with results of other researchers (Abo-Moch et al., 1995) reporting that *P. s.* pv. *syringae* isolates were able to produce symptoms on varieties Chippewa and Harasoy. Our results underline our previous data (Kovács and Németh, 1993) and results of Abo-Moch et al., (1995), that in Europe there are at least 5 races of *P. s.* pv. *glycinea* present. The composition of races can change during years most probably due to the change in variety composition produced in a certain region. Our previous results, confirmed by Abo-Moch et al. (1995) and the present study show that from the late seventies to early eighties (Hevesi and Érsek, 1981) new races appeared in Hungary, but the dominant one is still race 4.

The serological study showed that in Hungary two serogroups of the pathogen exist, which is in agreement with the results of Samson and Saunier (1987).

The pathogenic role of *P. s.* pv. *syringae* isolates were not studied. They might be part of the epiphytic population, which under special circumstances can contribute to diseases during germination or cause symptoms on soybean plants. Their role should be clarified. Due to the usually low infection level of the commercial seed lots in routine seed assay minimum 5 × 1,000 or even 10 × 1,000 seeds should be analyzed. The overnight soaking of the seeds in buffer solution is necessary to get the bacteria into suspension. It is vital to keep the soaked seeds at temperature close to zero (0–4 °C) to avoid the multiplication the saprophytic bacteria. To increase the release of pathogenic bacteria from out of the seed coat shaking of the seed sample is recommended. Besides of the general King's medium B use of selective media is recommended to suppress the growth of the saprophytic bacterial population which very often overgrows the target bacteria. *P. s.* pv. *syringae* and *P. s.* pv. *glycinea* can be easily distinguished from each other by a few biochemical test (meso-erythritol, D-xylose, L-lactic acid, α-ketoglutaric acid, L-serine etc.). Where the technique is available the fatty acid profiling can be recommended for rapid separation of *P. s.* pv. *syringae* and *P. s.* pv. *glycinea* as well (Németh and Janse, 1993). As the confirmatory test ODD can be used instead of ELISA test. As a final confirmatory test the artificial inoculation of the primordial or the trifoliate leaf of the varieties for race identification can be used, or the Harasoy and Flambeau, which are sensitive to all 10 races known at this moment. The first choice is very laborious, while the second alternative has some risk in case when a new race – which produce resistant reaction on these varieties – should be identified. To overcome this obstacles the artificial inoculation of the primordial or the trifoliate leaf of the host variety from which the strain was isolated can be proposed. In this case it could happen that the isolated pathogenic

strain was living on the host variety as part of the epiphytic population not affecting it. However this seems to be very rare. During our 20 years laboratory experience we have never experienced a case like this.

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Detection and Identification of Stolbur Phytoplasma in Hungary by PCR and RFLP Methods*

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Malformed and yellows-diseased plants of pepper, tomato, tobacco, carrot, parsley, celery, grape, rape, jimsonweed (*Datura stramonium*), dandelion (*Taraxacum officinale*) and bladder campion (*Silene vulgaris*) collected in Hungary were examined for phytoplasma infection. DNA was extracted from midribs by an MLO enrichment method and subjected to polymerase chain reaction (PCR) using universal (fP1/rP7) and specific primer pairs (fSTOL/rSOLS) directed to ribosomal sequences. Phytoplasmas detected by PCR were characterized using restriction fragment length polymorphism (RFLP) analysis of PCR amplified DNA. Phytoplasmas identified in all examined plants were typical stolbur phytoplasmas. RFLP analysis of the PCR amplified ribosomal DNA from all plant species revealed an uniform pattern that was identical to that of stolbur phytoplasma collected in Serbia from pepper. Special malformations were observed on several crops and weeds suggesting hormonal imbalance. Even the overwintering roots of carrot were infected that indicates that the disease overwinters not only in weeds but also in the roots of cultivated plants. Phytoplasma was transmitted from celery leaves to periwinkle plants with dodder (*Cuscuta campestris*). This is the first report detecting stolbur phytoplasma on dandelion (*Taraxacum officinale*) and bladder campion (*Silene vulgaris*).

Infection of stolbur phytoplasma causes generally spectacular symptoms on several crops and weeds. Symptoms vary within plant species. In consequence of the disease, yellowing, reddening, reduced leaf size, shoot proliferation, virescence and finally death could be observed.

Occurrence of the disease was first described by Suhov and Vovk (1949) in the Soviet Union. Data on the occurrence of stolbur are from Europe (Martelli et al., 1969; Alivizatos 1989; Schneider et al., 1993). Similar symptoms were observed in Australia (Samuel et al., 1933) and in the U. S. A. (Dana, 1940; Coe and Alstaff, 1947; Grannet and Provvidenti, 1974; Dale and Smith, 1975) on tomato called tomato big bud (TBB). In the U. S. A. TBB is associated with the beet leafhopper-transmitted virescence phytoplasma (Shaw et al., 1992). Due to the development of the PCR (polymerase chain reaction) and RFLP (restriction fragment length polymorphism) technics, the European stolbur and TBB could be distinguished (Schneider et al., 1992; Smart et al., 1996). Stolbur infection in Hungary on potato, tomato, pepper, tobacco and jimsonweed (*Datura stramonium*) was first reported by Szirmai (1956). Occurrence of stolbur phytoplasma in

* Dedicated to Dr. János Szirmai who first described this disease in Hungary for this 90th birthday.

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many Hungarian regions was surveyed by Petróczy (1962), Milinkó et al. (1966), Gáborjányi and Lönhard (1967) and Horváth (1969). The disease is spread by leafhoppers (Fos et al., 1992). Data about the biology of the main vector (*Hyalesthes obsoletus*) spreading stolbur phytoplasma in Hungary was collected by Kuroli (1970). Stolbur phytoplasma in grapevine was detected recently by Szendrey et al. (1996).

Diseased plants with different symptoms suggesting a hormonal imbalance were found recently on several plant species and many of them (pepper, tomato, tobacco, carrot, parsley, celery, grape, rape, jimsonweed (*Datura stramonium*), dandelion (*Taraxacum officinale*) and bladder campion (*Silene vulgaris*) proved to be infected with stolbur phytoplasma.

In this study, molecular technologies were used to characterise the causal agent infecting the above-mentioned diseased plants. The disease of some of the plants examined were known, but their causal agents were not yet accurately determined.

Materials and Methods

Plant samples and reference phytoplasma strains

Symptomatic and healthy plant samples were collected from several parts of Hungary at the end of July and August. American aster yellows (AAY), stolbur of pepper (STO, Serbia), apple proliferation (AP15), European stone fruit yellows (ESFY) phytoplasmas strains previously transmitted to periwinkle (*Catharanthus roseus*) originated from Dr. Seemüller's collection [BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft) D-69216 Dossenheim, Germany] were used as reference strains.

DNA isolation

DNA was extracted from petiols and midribs of healthy and diseased plant samples using a phytoplasma enrichment procedure (Ahrens and Seemüller, 1992).

Polymerase chain reaction

Both universal [P1/P7 (Kirkpatrik et al., 1994)] and stolbur specifife [fSTOL/rSTOLS (Maixner et al., 1995)] primer pairs derived from ribosomal sequences were used for amplification. The universal primer pairs amplify approximately 1.800 bp and the specific primer pairs a 580bp DNA fragments. The reaction mixture in a total volume of 40 µl contained 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 200 uM (each) deoxynucleoside triphosphate, 2 pmol of each primer, 2.5 µl of template, 2.5 U of *Taq* DNA polymerase (Pharmacia). PCR was performed in a DNA thermal cycler PDR1, using the following protocol: initial denaturation at 95 °C for 1 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at

71 °C for 2 min, and an additional extension at 72 °C for 7 min. After PCR, 10 µl of the amplification product was separated by electrophoresis in a 1% agarose gel in TAE buffer and the amplified DNA fragment was visualised by ethidium bromide staining.

RFLP analysis of PCR products

For identification 20 µl of the positive PCR products were digested with two restriction endonucleases [(*Alu*I and *Rsa*I), Pharmacia] and separated in a 12% polyacrylamide-gel. After electrophoresis, the DNA was stained with ethidium bromide and visualised under UV light.

Results and Discussion

Eleven samples (pepper, tomato, tobacco, carrot, parsley, celery, grape, rape, jimsonweed, dandelion and bladder campion) from many investigated symptomatic diseased plant species were proved to be infected with phytoplasma. Following the digestion of the PCR products with *Alu*I and *Rsa*I restriction endonucleases, all phytoplasma positive samples showed the same restriction profiles as strain stolbur (STO).

Symptoms

Symptoms observed on diseased pepper (*Capsicum annuum*), tomato (*Lycopersicon esculentum*), jimsonweed (*Datura stramonium*) and tobacco were similar to those previously reported (Szirmai, 1956). Diseased pepper plants showed general chlorosis firstly on the young shoots then on the entire foliage. The leaves rolled and folded and finally the whole plant defoliated. Berrys were spongy and desiccated. Symptoms on tomato (*Lycopersicon esculentum*) are more characteristic than on pepper. The affected shoots proliferated and deformed with malformed and rolled leaves. The most typical symptoms appeared on flowers. They were deformed with enlarged calyxes without petals. Fruits were undersized and stringy. Diseased celery (*Apium graveolens*) leaves became yellow with green midribs. Later depending on the varieties, the diseased leaves became red or remained yellow. They were stunted and dwarfed. Leaves of carrot (*Daucus carota sativus*) and parsley (*Petroselinum crispum*) showed yellowing and reddening of leaves similarly to celery. There were no symptoms on roots of carrot although in spring (6–7 months after harvest) presence of phytoplasma could be detected, indicating that the pathogen was able to overwinter in this host. As soon as new leaves emerged and the phloem developed phytoplasmas appeared in young shoots indicating that the aboveground part of plants was reinfected from the roots. Phytoplasmas seemed to overwinter in roots and in insect vectors may transmit them to healthy plants. Grape plants showed yellowing or reddening depending on the varieties.

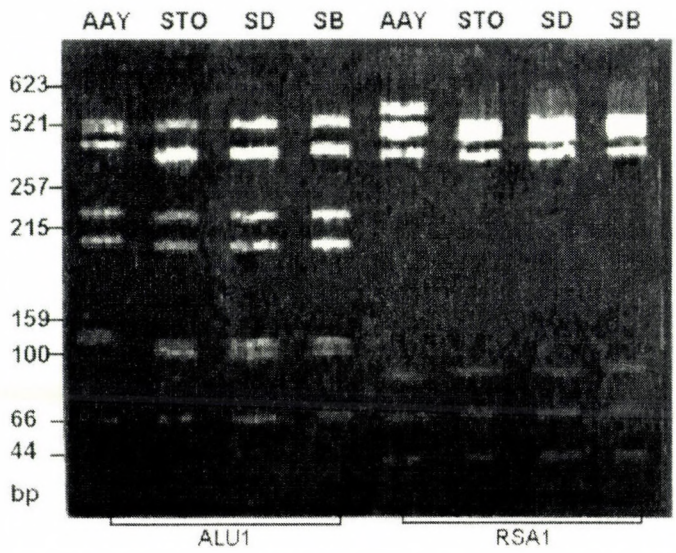

Fig. 1. *Alu*I and *Rsa*I restriction profiles of 16S ribosomal phytoplasma DNA amplified by polymerase chain reaction using universal primer pair fP1/rP7. The target DNA was obtained from dandelion (SD) and bladder campion (SB). AAY and STO were used as reference strains

On the upper part of rape (*Brassica napus convar. napus*) shoot proliferation was the typical symptom. Flowers were green and infertile. Leaves of tobacco (*Nicotiana tabacum*) were folded, yellowed and wrinkled. The shoots stunted, deformed and sometimes proliferated. The influorescence turned to green and there were no seed production. In case of bladder campion (*Silene vulgaris*) and dandelion (*Taraxacum officinale*) the most prominent symptoms appeared on the flowers. Petals were malformed and almost entirely green. Seeds were not formed.

Identification of the causal agent

DNA was extracted from plants suggesting phytoplasma infection and phytoplasma DNA was amplified by PCR. Samples showing typical symptoms of stolbur infection showed positive results using universal (P1/P7) as well as specific primer pairs (STOL/STOLS). In all case healthy plants were negative. RFLP analysis of the examined samples revealed that plants showing the written symptoms are affected by stolbur phytoplasma corresponding to STO strain isolated in Serbia (Fig. 1) (Schneider et al., 1993; Gunderson et al., 1994; Seemüller et al., 1994).

Detectability of the organism depends on the date of sampling and the stage of the disease. Symptoms appear at the end of July. Samples collected at this time were positive but later in dying plants phytoplasmas could not be detected any more.

There were not data about stolbur infection on carrot, celery and parsley, in Hungary and dandelion (*Taraxacum officinale*) and bladder campion (*Silene vulgaris*) were hitherto unknown natural host plants of the disease.

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Role of Virus- and Phytoplasma Infections in Pepper Decline in Hungary: An Overview*

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Last some years serious yield losses of pepper production have been observed even in the traditional growing areas of Hungary, similarly the situation noticed Szirmai in 1936. The most common symptoms of the so-called "pepper decline" were: stunting, general yellowing of the plants, leaf abscission and reduced size of newly developed leaves. We consider that pepper decline has complex etiology, in which the unfavorable growing conditions (hot summers), infections of different viruses, bacteria (*Xanthomonas campestris* pv. vesicatoria), fungi (*Fusarium solani, Verticillium dahliae, Sclerotinia sclerotiorum, Macrophomina phaseolina*), and phytoplasma (*stolbur phytoplasma*) take part. Infections of different *tobamoviruses,* cucumber mosaic *cucumovirus (CMV),* potato Y *potyvirus* (PVY) and tomato spotted wilt *tospovirus* (TSWV) were the most common viral pathogens in the field. Plants affected first by viruses were often reinfected by other pathogens. Last year an epidemy of stolbur phytoplasma was also stated. Some Hungarian pepper varieties had some resistance to different viruses in different level, however almost all ones were susceptible to CMV. In our opinion virus- and phytoplasma infections are the most important factors in the degenerative process.

Serious yield loss and degeneration of pepper plantations has been observed by Szirmai in the traditional pepper growing areas of the South Plain region of Hungary in 1936. Diseased plants were reduced in their growth, a lot of new branches developed with short internodia, and the plants became bushy in their appearance. Many flowers developed on diseased plants, but remained sterile, or only malformed fruits developed from the fertile ones. By mechanical inoculation and aphid transmission experiments the pathogen was identified as cucumber mosaic *cucumovirus* (CMV), a first viral pathogen described in Hungary on pepper (Szirmai, 1941).

The definition "pepper decline" originated also from Szirmai (1941), indicating the slow degradation process of pepper plants where the production occurs for long years at the same area. Later new pepper pathogen viruses, as well as bacteria [*Pseudomonas siringae* pv. siringae, *P. viridiflava, Xanthomonas campestris* pv. vesicatoria (Hevesi, 1991)], fungi [*Fusarium solani, Verticillium dahliae, Sclerotinia sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, Phaeoramularia capsicicola* (Fischl et al., 1995; Kazinczi et al., 1998)] and stolbur *phytoplasma* (Szirmai, 1956) have been also described. All of them caused necrotic spots on leaves, wilting and phloem necrosis or

* Dedicated to Dr. János Szirmai for his 90th birthday.

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dying of the young seedlings. The aim of this paper was to overview the available informations from the Hungarian literature about the pepper pathogen viruses to calculate the specific role of this important component in the pepper decline.

Pepper-pathogen viruses in Hungary

More than 45 plant viruses have been reported to infect peppers, among that 35 have been isolated from naturally infected plants (Horváth, 1981, 1983, 1986 a, b, c; Green and Kim, 1990; Green and Kalloo, 1994; Edwardson and Christie, 1997). In Hungary 12 viruses and one phytoplasma have been isolated from infected plants. First descriptions are listed in Table 1.

Disease incidences in the field

Recently the frequency of these viruses were analysed by ELISA tests from plants showing disease symptoms (Gáborjányi et al., 1997; Gáborjányi, 1998). The majority of plants were infected in a complex by three or more viruses. Former similar studies showed that the general incidence of virus infection in the field was about 15–60%, but reached the 100% at the end of the vegetation period. It was generally accepted, that among pepper pathogen viruses tobacco mosaic *tobamovirus* (TMV) and other *tobamoviruses,* CMV, potato Y *potyvirus* (PVY) and tomato spotted wilt *tospovirus* (TSWV) could cause considerable yield losses in the fields. In the greenhouses the most frequent viruses are: tomato mosaic *tobamovirus* (ToMV), CMV and TSWV. Others, e.g. alfalfa mosaic *alfamovirus* (AMV), broadbean wilt *fabavirus* (BBWV), potato X *potexvirus* (PVX) or tomato aspermy *cucumovirus* (TAV) had no economic impact.

Leaf samples of about 40 Hungarian pepper varieties (independently from the visible symptoms) were analysed by DAS-ELISA tests at the end of the vegetation period. According to this study the majority (65%) of the varieties were infected. Incidence of infections was the followings: CMV (22%), PVY (20%), AMV (10%) and PVX (8%). In this field test only 6 from 40 varieties were free from infections; 19 were infected only by one virus; 11 by two different viruses and 4 suffered in a complex of three viruses (Gáborjányi, 1998).

Susceptibility of pepper varieties to viruses

Before 1968 all the tested varieties were susceptible to TMV, CMV, PVX and PVY infections (Horváth, 1967; Beczner and Horváth, 1969; Horváth, 1969), but after this time a sightly breeding program was started to built the resistance gene into different pepper varieties (Zatykó, 1982). Resistance of varieties and sources of resistance to vi-

Table 1

First descriptions of pepper-pathogenic viruses and the stolbur *phytoplasma* in Hungary

Viruses	References
Cucumber mosaic *cucumovirus* (CMV)	Szirmai, J. (1941)
Alfalfa mosaic *alfamovirus* (AMV)	Szirmai, J. (1944)
Tobacco mosaic *tobamovirus* (TMV)	Szirmai, J. (1950)
Potato X *potexvirus* (PVX)	Szirmai, J. (1950)
Stolbur *phytoplasma* (SP)	Szirmai, J. (1956)
Potato Y *potyvirus* (PVY)	Horváth, J. (1967)
Tomato aspermy *cucumovirus* (TAV)	Beczner et al. (1979)
Broadbean wilt *fabavirus* (BBWV)	Salamon et al. (1980)
Dulcamara yellow fleck *tobamovirus* (DYFV)	Salamon et al. (1987)
Tomato mosaic *tobamovirus* (ToMV)	Csilléry et al. (1983)
Tomato spotted wilt *tospovirus* (TSWV)	Gáborjányi et al. (1995a, b)
Henbane mosaic *potyvirus* (HeMV)*	Gáborjányi et al. (1997)
Sowbean mosaic *sobemovirus* (SoMV)*	Gáborjányi et al. (1997)

* Detected only by serological means

ruses was first summarized by Horváth (1983). In 1985 about only 10%, but in 1995 31% of registered Hungarian pepper varieties had some degree of virus resistance. Eleven varieties contained the L^1 and two the L^2 gene. Three varieties (Suptol, Szintetikus Cecei, Táltos) were tolerant to CMV infection (Fehér and Kristóf, 1995; Fehér, 1996).

Breeding strategies

Tobamoviruses

The breeding program started with the incorporation of L genes into commercial pepper varieties. TMV resistance was depended on only one gene and inherited dominantly. Different *Capsicum* species served as sources of resistance: L^1 originated from *Capsicum annuum,* L^2 from *C. frutescens*, L^3 from *C. chinense* and finally the L^4 from *C. chacoense* (Zatykó, 1993). Possibility of crossing with *Capsicum* species was limited. *C. annum* as mother plant could be easily hybridized with *C. chacoense*, but it was difficult to cross with *C. chinense* and *C. frutescens*. Reciprocal crossing were more easily done (Csilléry, 1978; Csilléry, 1985a). Now in 1979 interspecific hybride were made between *C. annuum* and *C. chinense* (Csilléry, 1983), and later between *C. annuum* and *C. frutescens* or *C. chacoense* (Csilléry, 1985a, b, c) giving the possibility to introduce TMV resistance. First resistant (L^2) varieties were made by crossing sweet pepper varieties with

Keyston Resistant Giant variety (Csilléry, 1982). Now in 1970 three varieties (Fehérözön, D. Cecei, Rezisztens Keszthelyi) and a red spice variety were mentioned to be resistant to TMV infection (Szirmai, 1970; Zatykó, 1982). Occurrence of a new pathotype (P_{1-2}) was reported in 1980. The so-called "Ob strain" (ToMV-Ob) isolated from pepper in greenhouses was breaking the L^1 resistant gene (Csilléry and Ruskó, 1980; Tóbiás et al., 1982; Csilléry et al., 1983). Hybrids of *Capsicum annuum* × *C. chinense* (P. I. 159236) proved to be a good source of the resistance (Csilléry, 1985a, b, c). First varieties (Novares F_1 and Rapires F_1) containing the L^3 gene were introduced in 1995 (Zatykó and Moór, 1995). Recently a new variety, Ciklon F^1 was reported to have the L^3 gene (Sági and Salamon, 1998). First hybrid (H 19–6F_1) containing the L^4 gene was created first by Csilléry in 1983 using *Capsicum chacoense* as a source of resistance (Zatykó and Moór, 1995) and a green hot pepper variety (Himes F_1) which was registered in 1997 (Salamon, 1997; Sági and Salamon, 1998).

Hungarian *tobamoviruses* (formerly called as TMV strains) showed a large-scale of variations in their symptoms and serological properties (Burgyán et al., 1978). The first resistance breaking *tobamovirus* (ToMV-Ob strain) was observed in 1978 (Csilléry and Ruskó, 1980). Later a new pathotype called "Dulcamara yellow fleck virus" was also described (Salamon et al., 1987). On the other hand, still there are no reliable informations about the distribution and pathogenity of different *tobamoviruses* occurring in Hungary.

Cucumber mosaic virus (CMV)

The problem of CMV resistance is the most serious. The degree of the infection varies from year to year, depending on ecological factors (Tóbiás et al., 1978, Tóbiás and Molnár, 1983). Between the two main serotypes (To and D) the severe To serotype was the more frequent in the fields (Beczner and Burgyán, 1983). Susceptibility of different varieties were tested previously (Tóbiás et al., 1978), but the majority of the varieties proved to be susceptible or at least tolerant, like variety Táltos Synthetic (Zatykó, 1982). The tolerance of this variety was temporal, young seedlings were susceptible to CMV. In spite of the fact that the CMV resistance is polygenic and inherited in a recessive form, now the sources of CMV resistance are available, and in few years new CMV resistant varieties will be introducted into the production.

Symptoms caused by CMV infections were extremly variable. Experiments in the last two decade have led to the conclusions, that complex infections in pepper are very frequent. CMV was usually accompanied by PVY, AMV TMV, ToMV and in certain places sporadically by BBWV, TAV, and PVX (Horváth and Beczner, 1983). Similar survey has been made in 1996 and 1997 also showed that CMV and AMV infections were the most frequent ones, and the complex infections reached 30% (Gáborjányi et al., 1997; Gáborjányi, 1998). Among ten varieties only one (Óriás cseresznye) proved to be resistant to CMV infection, the others showed a large-scale of susceptibility, determined by ELISA tests in a greenhouse experiment, indicating that the susceptibility of Hungarian varieties to CMV remained still an unsolved problem.

Alfalfa mosaic virus (AMV)

Infection of AMV in the fields was always represented in a high percentage. However, appropriate experiments has been never made to calculate the direct yield loss. In a provocative inoculation experiments AMV-N (necrotic in beans) and AMV-S (systemic symptoms on beans) strains equally infected all pepper cultivars without any differences in resistance to the virus (Beczner, 1969; Beczner and Horváth, 1969; Horváth and Beczner, 1983).

Tomato spotted wilt virus (TSWV)

The pathogen was known in northeastern part of Hungary, infecting tobacco plantations by *Thrips tabaci* (Ligeti and Nagy, 1972; Gáborjányi et al., 1994). Naturally infected peppers were also found in the neighbourhood of tobacco fields but sporadically. By the rapid distribution of *Frankliniella occidentalis* TSWV infection seemed to be a new threat for pepper production especially in the greenhouses and plastic tunells (Gáborjányi et al., 1995a, b; Jenser et al., 1996). At the moment one serotype (BR-01) was detected. After mechanical inoculation of several wild *Capsicum* species some died because of systemic necrosis, others produced local lesions. Among ten varieties three proved to be resistant, others were susceptibles to different degree.

Other, economically non-important viruses

The occurrence of TAV in pepper fields had a special interest (Beczner et al., 1979). It was found first in pepper under glasshouse conditions. PVX and PVY were relatively abundant in the pepper fields. The importance of PVY has increased for some years, as the occurrence of the tobacco veinal necrosis strain (PVY^N) has become more frequent. Only one or two of ten varieties were resistant to PVX (Sobor, Kalocsai V2) and PVY (Szintetikus cecei) infection. Last year the presence of henbane mosaic *potyvirus* (HeMV) and sowbane mosaic *sobemovirus* (SoMV) was detected by indirect ELISA tests (Gáborjányi et al., 1997). Because of complex infection we were not able to separate these viruses from other components. However, in experimental conditions SoMV inoculation produced no visible symptoms, but the pathogen was detectable in seven varieties. SoMV was formerly isolated from *Chenopodium hybridum* (Horváth et al., 1993), which could have play an important role in the natural infection of peppers.

Role of phytoplasma infection in pepper decline

Infection of pepper plants by stolbur *phytoplasma* (SP) was firstly observed by Szirmai (1956); this pathogen was believed as a virus at that time. Epidemy occurs every year at the end of summer which keeps its distribution ranges also in the cooler years and it decreaes only in percentage value (Gáborjányi and Lönhard, 1967). Last two years the

relatively warm winters and hot sommers supported the overwintering and large-scale gradation of leafhoppers. Traditional pepper growing areas were totally affected by stolbur disease. The pathogen was identified by PCR (Viczián et al., 1998). Disease symptoms, like general yellowing and wilting, defoliation were described many times in details, in spite of it the growers have often mistaken these symptoms for the infection of different fungi *(Fusarium, Verticillium* and *Macrophomina* species). In our opinion stolbur disease plays as important role as the viruses in the degradation process of pepper production in the fields, especially in hot, dry summers.

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Sublethal Water Saturation Deficit of the Healthy and Virus Infected Black Nightshade (*Solanum nigrum* L.)*

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Investigations were carried out to study drought resistance of *Solanum nigrum* L. The sublethal water saturation deficit (WSDsubl) of the healthy *S. nigrum* leaves was low (35.6%). It means that the leaves can lose only 35.6% of their maximum water content without irreversible injuries. The WSDsubl of the leaves, showing severe leaf deformation and mosaic symptoms due to the cucumber mosaic *cucumovirus* (CMV) infection was lower (30%), suggesting that the virus infection caused disturbance in the water relation of the species. The results pay attention to the fact that viruses unfavourably influence physiological processes of the weed species and therefore – in indirect way – they contribute to the reduction of their competitive ability in the intra- and interspecific competition.

Solanum nigrum L. is reported as a weed in 37 crops and in 73 countries (Holm et al., 1977). It is a serious weed of sugar beet, corn, cotton, sorghum and – due to its excellent adaptation to high fertility of soils – in horticultural crops, especially in vegetables (Binning, 1971; Kempen and Agamalian, 1976; Jones, 1977; Kempen and Lange, 1977; Weller and Phipps, 1979). Its competitive injurious effect is considerable in tomato (Maillet and Fatah, 1983; Caussanel et al., 1989; Damato and Montemuro, 1986; Gonzalez et al., 1995), broccoli (Agamalian, 1983) and bean (Fenninmore et al., 1984) In Hungary *S. nigrum* does not belong to the serious weeds, in spite of this, under moist environments in late-sown cultures it is considered to be a noxious weed.

The weeds are able to influence the quality and quantity of crops not only in direct way (e.g. nutrient and water uptake), but they are important as alternative hosts of different pathogens as well. Alternative hosts serve as food for the vectors of viruses, while the seeds and vegetative reproductive organs of certain wees species can play important role in the epidemiology and overwintering of viruses, therefore the weeds may be primary infection sources at the beginning of vegetation period. *S. nigrum* is known as a virophilous species and host of 43 viruses (Naperkovskaya, 1968; Mariappan et al., 1973; Davis and Allen, 1975; Holm et al., 1977; Schmelzer and Wolf, 1977; Wilson et al., 1981; Dimitrievič, 1985; Alegbejo, 1987; Farrel and Stufkens, 1988; Daniel and Tsai, 1990; Kazinczi and Horváth, 1998). From practical view it is important to know the competitive ability of the diseased weeds (with their reduced ability of nutrient and water

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uptake) as compared to the healthy ones. So far only few data is available about the changes of biology of virus infected plants. The aim of our study was to examine the virus susceptibility and water relation of the diseased plants of *S. nigrum*. Sublethal (critical) water saturation deficit (WSDsubl) is a good index to evaluate drought tolerance of a given species (Oppenheimer, 1963). At present time WSDsubl is accepted as a saturation deficit when plant parts (most often the leaves) are able to re-saturate in 90% of their maximum water content after desiccation. Considering that formerly the physiological examinations of the virus diseased plants were connected mainly with the cultivated plants, therefore our present work supplies new data to the knowledge of the biological changes of the virus diseased weed plants.

Materials and Methods

In our virological glasshouse (free from vectors) *S. nigrum* plants were mechanically inoculated at 4–6 leaf stage with cucumber mosaic *cucumovirus* (CMV). Seven plants for each viruses was used. The success of inoculation was evaluated with the method of back inoculation and on the basis of symptoms.

From the CMV infected plants 30–40 leaves were collected at the beginning of flowering. In order to compare the drought resistance of the healthy and the diseased plants, the index of WSDsubl was determined after Weinberger et al. (1972). First the leaves were saturated to their maximum water content in wet chambers and then desiccated to different degrees. After desiccation the leaves were saturated again. The saturation deficit after desiccation (X) and re-saturated dificit (Y) were represented in a system of coordinates. With the help of regression analysis linear function was set to the values, where the re-saturated deficits (Y) were between 65 and 95%. The simple equation was solved at Y = 90. The pairs of number (X and Y) were given with the formulae below:

$$X = \text{WSDdesiccated (\%)} = \frac{(\text{Wsaturated} - \text{Wdesiccated}) \times 100}{(\text{Wsaturated} - \text{Wdried})}$$

$$Y = \text{WSDre-saturated (\%)} = \frac{(\text{Wsaturated} - \text{Wdried}) \times 100}{(\text{Wsaturated} - \text{Wdried})}$$

(W = weight)

Results and Conclusions

S. nigrum was systemic susceptible to CMV; severe mosaic symptoms occurred due to the infection.

The WSDsubl of the healthy *S. nigrum* leaves was low (35.6%), as compared to other weed species. It means that the leaves can lose only 35.6% of their maximum water

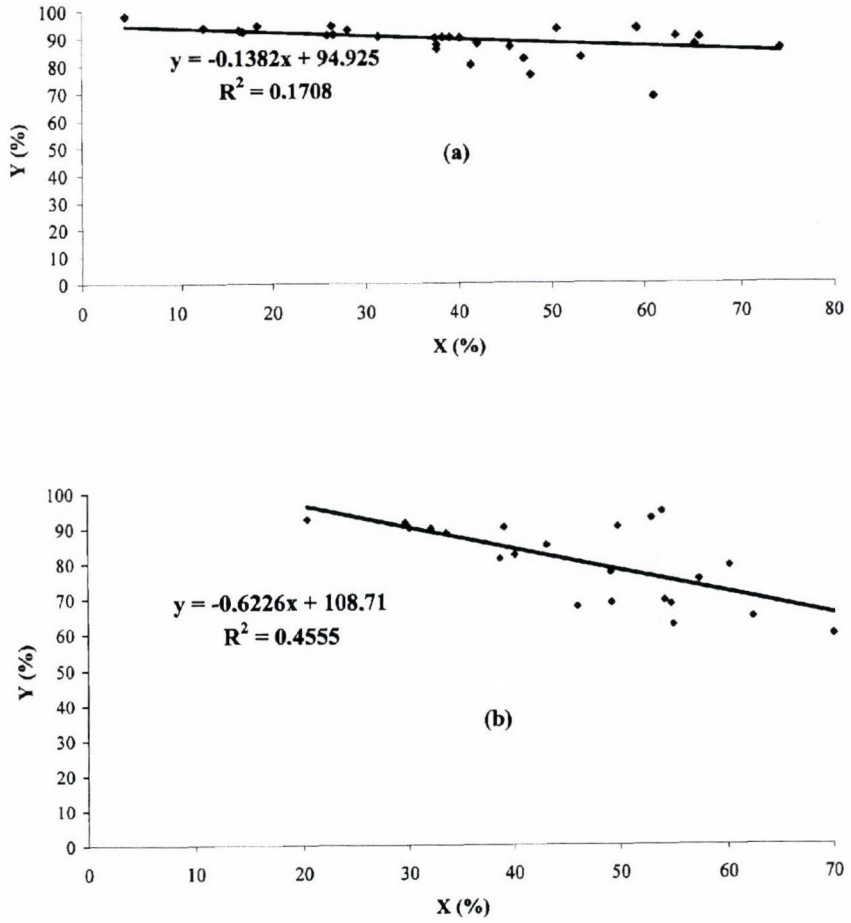

Fig. 1. Correlation between the WSDdesiccated (X) and WSDre-saturated (Y) of *Solanum nigrum* (a: healthy; b: CMV infected plants)

content without irreversible injuries. The WSDsubl of the leaves, showing systemic mosaic symptoms and severe leaf deformation due to the CMV infection was lower (30%), suggesting that the virus infection caused disturbance in the water relation of the species (Fig. 1a, b). In previous experiments high values of WSDsubl (71 and 75%) were obtained with species of *Digitaria sanguinalis* (L.) Scop. and *Ambrosia elatior* L., respectively (Almádi, 1976; Kazinczi and Hunyadi, 1992). *Papaver rhoeas* L. had similar index (66.4%), while other species – the important weeds of winter wheat – had lower (44–56%) values. The seeds of *D. sanguinalis* and *A. elatior* germinate relatively late and their development demands warmth, therefore they adapted better to drought conditions.

Oppositely the weeds of winter wheat have already germinated in autumn, finished their life cycle by the beginning of summer and practically disappeared from the fields before drought period. In spite the fact, that WSDsubl is genetically determined for a given species its values always decrease by the end of vegetation period (Arvidsson, 1951; Pisek and Larcher, 1954; Florineth, 1974). Rychnovska and Ulehlova (1975) did not experience seasonal changes of WSDsubl. WSDsubl is always high (70–75%) in case of those species which have unstable water balance (Almádi, 1986). Rychnovska and Ulehlova (1975) reported in *Stipa* genus that actual water saturation deficit (WSDact) often exceeds the value of WSDsubl causing irreversible injuries of the plants.

Drought tolerance of certain species can be derived from different biological causes. One reason is the reduced stomatal transpiration with minimal cuticular transpiration at the same time (Stocker, 1967). In case of other species the considerable water consumption is compensated with enormous root-system (Rychnovska and Ulehlova, 1975). Almádi (1976) reported that drought tolerant species have a high WSDsubl. In the terminology of Levitt (1958) drought resistance is the result of desiccation avoidance and desiccation tolerance. Larcher (1983) reported, that only the succulents have drought tolerance due to their considerable water storage capacity.

S. nigrum has no suitable strategy to bear drought stress, therefore it adapted well mainly to moist conditions, such as irrigated farmland (Holm et al., 1977). Singh (1971) noted that *S. nigrum* avoids open areas in warmer months. Sen (1973) found that this species does not control stomatal aperture effectively under drought stress. *S. nigrum* is generally less tolerant of drought stress than a variety of common crop and weed species and – compared with a variety of crop and weed species – it has a relatively high shoot:root ratio, indicating a relatively small root system. It means that the leaves can transpirate large amount of water because of the large leaf area and the water loss is not compensated due to the reduced root system (Burgert and Burnside, 1972a, b). Our results has confirmed the observations mentioned above.

The results pay attention to the fact that viruses unfavourably influence physiological processes of the weed species and therefore – in indirect way – they contribute to the reduction of their competitive ability in the intra- and interspecific competition.

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Comparison of *in vitro* Testing Methods for Screening of Resistant Peas to *Fusarium* spp.

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A set of experiments focused on interaction of *Fusarium oxysporum* and *F. solani* isolates and their culture filtrates with peas (*Pisum sativum* L.) were carried out on the levels of intact plants (tube test) and *in vitro* cultures (multiple shoot and dual culture). Degree of resistance and/or susceptibility was evaluated as the infection degree or the rooting frequency on rooting media. Results obtained from a set of exact screening methods should contribute to improvement of the peas resistance breeding process.

The main requirement for successful and meaningful breeding in general is variation of the trait genetic basis. Some literature data concerning the variation in resistance/susceptibility to *Fusarium* spp. were summarised by several authors (Hagedorn, 1984; Jacobsen, 1992; Jedryczka, 1995; Lebeda et al., 1988). Various methods *in situ, ex situ, in vivo* and *in vitro* for screening of resistance were used in these studies. Until now in literature there is no available comparison of various *in vitro* screening methods for *Fusarium* spp. resistance in peas.

The main purpose of recent study was to compare different *in vitro* screening approaches for resistance selection of pea genotypes. Because of a quite simple basis of resistance (single dominant gene) and an idea about possible advantageous replacing of the fungus with filtrate or toxin for *in vitro* cultures (Lebeda, 1995) the investigation was started with *Fusarium* spp.

Materials and Methods

Plant material

The list and taxonomical characterization of 17 cultivars and accessions belonging to two *Pisum* spp. is summarized in Table 1. This set is represented by pea genotypes included in the Czech National List of Cultivars, some genotypes from abroad and also three accessions of wild *Pisum* spp. forms. All genotypes were maintained and multiplied at AGRITEC *Pisum* Collection (Šumperk, Czech Republic).

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Table 1

List of *Pisum* species (AGRITEC *Pisum* Collection) used for experiments

Cultivar/Accession (Genebank Number)	Species	Subspecies, convar., var.
Adept (01/762)	*Pisum sativum* L.	
Arvika (02-354)	*Pisum sativum* L.	ssp. *sativum* convar. *speciosum* (DIERB.) ALEF. var. *griseo-coloratum* GOV.
AZ 26 (01-841)	*Pisum sativum* L.	
Bohatýr (01-530)	*Pisum sativum* L.	ssp. *sativum* convar. *sativum* var. *episcopi*
DP 1059 (01-854)	*Pisum sativum* L.	
Dundale (02-410)	*Pisum sativum* L.	ssp. *sativum* convar. *speciosum* (DIERB.) ALEF. var. *concolor* GOV.
Janus (01-732)	*Pisum sativum* L.	
Komet (01-736)	*Pisum sativum* L.	
Lantra (01-757)	*Pisum sativum* L.	
Maja (01-855)	*Pisum sativum* L.	
Sirius (02-366)	*Pisum sativum* L.	ssp. *sativum* convar. *speciosum* (DIERB.) ALEF. var. *sanquivita* ALEF.
Smaragd (01-265)	*Pisum sativum* L.	ssp. *sativum* convar. *sativum* var. *nanoviride* ALEF.
Tyla (02-404)	*Pisum sativum* L.	ssp. *sativum* convar. *speciosum* (DIERB.) ALEF. var *griseo-coloratum* GOV.
Tyrkys (01-603)	*Pisum sativum* L.	ssp. *sativum* convar. *sativum* var. *nanoviride* ALEF.
(W 226)	*Pisum elatius* (M.B.) STEV.	
(W 807)	*Pisum sativum* L.	ssp. *asiaticum*
(W 1979)	*Pisum sativum* L.	ssp. *sativum* convar. *medullosacharatum* var. *koernickei*

W designation of *Pisum* spp. accessions express that they originated from the *Pisum* Gene Bank at Weibullsholm (Sweden)

Pathogen

Isolates of *Fusarium solani* f.sp. *pisi, Fusarium oxysporum* f.sp. *pisi,* were isolated from peas harvested from the infection field (located in the area of the experimental plots of AGRITEC). More detailed characterisation of *Fusarium* spp. isolates used is given in Table 2. The isolates were maintained, multiplied and cultured on Czapek-Dox agar (CDA) in laboratory conditions.

Filtrate preparation

Fungus isolates multiplied on CDA in rough amount a quarter of Petri dish (100 mm in diameter) content were placed onto surface of liquid medium (2% sucrose, 0.8% KNO_3, 0.05% KH_2PO_4) in 1000 ml Erlenmeyer flasks and cultured for a period of a month. Content of flasks was then filtered to remove mycelium and macroconidia. Filtrate was stored under –18 °C.

Table 2

Characteristics of *Fusarium* spp. isolates

Isolate No.	*Fusarium* spp.	Original host plant	Date of collection/ isolation	Cultivation media
Fox/96/IV	*Fusarium oxysporum* f.sp. *pisi*	*Pisum sativum* cv. Dundale	95-05-21/ 95-05-23	CDA
Fsol/96/IV	*Fusarium solani* f.sp. *pisi*	*Pisum sativum* cv. Bohatýr	95-05-10/ 95-05-13	CDA

Resistance screening methods

TUBE TEST

An adapted method according to Lebeda and Buczkowski (1986) complemented with 10% filtrate variant was used. Variants used in this experimental set were: 1) control plants – without treatment; 2) filtrate – 10% filtrate addition in media and Knop's solution; 3) inoculation – inoculation with fungus *Fusarium oxysporum* and. *F. solani* grown on the CDA surface (Lebeda and Buczkowski, 1986).

For the evaluation of the experiment, the value of infection degree (ID) was calculated as a maximum score in %: $ID = \sum(n.V).100 / x.N$; ID = total infection degree; n = number of plants in each category of evaluation scale; V = infection degree (0–3); x = range of evaluation scale (4); N = whole number of plants evaluated in each replicate (Lebeda and Buczkowski, 1986). Infection degree of roots and aboveground parts was evaluated by using a scale 0–3 according to Lebeda and Buczkowski (1986).

Roots:

0 = symptomless, roots free of any visual symptoms;

1 = limited occurrence of local necrosis, discoloration (browning) on the tap and lateral roots;

2 = mild necrotization and reduction of the tap roots, reduced development of lateral roots;

3 = severe necrotization and growth depression of tap roots, no development of lateral roots, complete collapse of the root.

Aboveground parts:

0 = symptomless, stems and leaves free of any visual symptoms of wilting;

1 = limited growth depression and wilting, plant is more or less turgescent;

2 = mild growth depression, wilting and chlorotization of leaflets;

3 = severe wilting and chlorotization, complete collapse of the plant.

Final evaluation was made 21 days after inoculation. The sum of infection degrees of roots and aboveground parts served to the assessment of the response to the effect of filtrate and inoculation in the tube test conditions (see legend – Table 3).

Table 3

Response of *Pisum* spp. in the tube test to *Fusarium oxysporum* f.sp. *pisi*

Cultivar/Accession Variant	Infection degree (ID) in %*							
	Roots (IDR)		Aboveground parts (IDA)		Σ (IDR+ IDA)		Response in the tube test**	
	Filtrate	Inoculation	Filtrate	Inoculation	Filtrate	Inoculation	Filtrate	Inoculation
Adept	0.00	50.00	0.00	0.00	0.00	50.00	R	R
Arvika	30.00	30.00	0.00	14.82	30.00	44.82	S	R
AZ 26	0.00	80.00	6.67	70.00	6.67	150.00	R	S
Bohatýr	–3.34	54.44	0.00	53.33	–3.34	107.77	R	T
DP 1059	0.00	73.33	6.67	40.00	6.67	113.33	R	S
Dundale	16.66	66.66	0.00	41.67	16.66	108.33	T	T
Janus	23.33	67.77	0.00	44.44	23.33	112.21	S	S
Komet	0.00	46.67	3.33	56.67	3.33	103.34	R	T
Lantra	–10.00	16.67	10.83	10.00	0.83	26.67	R	R
Maja	0.00	73.33	3.33	66.67	3.33	140.00	R	S
Sirius	26.66	53.33	0.00	33.34	26.66	86.67	S	T
Smaragd	0.00	46.67	0.00	60.00	0.00	106.67	R	T
Tyla	16.67	66.67	3.33	66.67	20.00	133.34	T	S
Tyrkys	0.00	75.75	0.00	63.33	0.00	139.08	R	S
W 226	33.33	66.67	0.00	0.00	33.33	66.67	S	R
W 807	–10.00	31.47	33.33	48.14	23.33	79.61	S	T
W 1997	9.52	62.50	–2.38	45.83	7.14	108.33	R	T
Min.	–10.00	16.67	–2.38	0.00	–3.34	26.67		
Max.	33.33	80.00	33.33	43.33	33.33	150.00		
Mean	7.81	56.59	3.83	42.05	11.64	98.63		

* ID – calculated as a difference to control
** Response was assessed according to the values of the sum IDR + IDA
filtrate – Σ (IDR+ IDA): < 10 = resistant (R), 10–20 = tolerant (T), >20 = susceptible (S)
inoculation – Σ (IDR+ IDA): < 70 = resistant (R), 70–110 = tolerant (T), >110 = susceptible (S)

Multiple shoot culture

Clusters of multiple shoots precultured on multiplication medium (Griga et al., 1986) were transferred on the same media complemented with 10% *Fusarium* filtrate (v/v). Effect of filtrate was evaluated as a rooting frequency on rooting media (1/2 MS + 1 μM NAA) and compared to control. The decline between rooting frequency in control and filtrate variants was used for cultivar sorting into three groups: resistant, tolerant and susceptible.

Dual culture

An analogous method to Ahmed et al. (1991) was used. Inoculum was cultured in flasks on CDA, inactivated by autoclaving, overlayed with multiplication medium (Griga et al., 1986). Multiple shoot culture was transferred onto double layer and cultured fur-

Table 4

Response of *Pisum* spp. in the tube test to *Fusarium solani* f.sp. *pisi*

Cultivar/Accession Variant	Infection degree (ID) in %*							
	Roots (IDR)		Aboveground parts (IDA)		Σ (IDR+ IDA)		Response in the tube test**	
	Filtrate	Inoculation	Filtrate	Inoculation	Filtrate	Inoculation	Filtrate	Inoculation
Adept	0.00	51.11	12.50	40.00	12.50	91.11	T	T
Arvika	–15.21	60.54	13.34	44.82	–1.87	105.36	R	T
AZ 26	10.00	53.33	6.67	44.17	16.67	97.50	T	T
Bohatýr	0.00	53.33	0.00	53.33	0.00	106.66	R	T
DP 1059	–7.41	14.81	0.00	21.37	–7.41	36.18	R	R
Dundale	6.34	67.44	–18.00	57.00	–11.66	124.44	R	S
Janus	9.17	46.67	23.33	27.04	32.50	73.71	S	T
Komet	4.17	53.33	10.00	46.67	14.17	100.00	S	T
Lantra	12.64	60.00	2.34	49.40	14.98	109.40	S	T
Maja	0.00	52.33	3.33	54.16	3.33	106.49	R	T
Sirius	6.66	60.00	10.00	48.15	16.66	108.15	T	T
Smaragd	30.00	53.34	0.00	73.33	30.00	126.67	S	S
Tyla	16.67	73.33	10.00	66.67	26.67	140.00	S	S
Tyrkys	16.67	60.00	–16.67	30.00	0.00	90.00	R	T
W 226	33.33	53.33	33.33	33.33	66.66	86.66	S	T
W 807	–2.50	37.50	3.33	48.14	0.83	85.64	R	T
W 1997	6.67	33.33	–2.98	30.83	3.69	64.16	R	R
Min.	–15.21	14.81	–18.00	21.37	–11.66	36.18		
Max.	33.33	73.33	33.33	73.33	66.66	140.00		
Mean	7.49	51.99	5.83	45.21	12.81	97.18		

Legend: see Table 3

ther 4 weeks. Viability of survived shoots was evaluated as rooting frequency and further assessed similarly to multiple shoot culture.

Plantlets obtained from the tube tests, multiple-shoot and dual cultures were transferred into pots filled with non-sterile soil in glasshouse. Seeds of fertile ones were produced and harvested.

Results

Tube test

Filtrates in 10% (v/v) dilution in studied conditions were not very effective. Plants inoculated with fungal culture were severely damaged by the pathogen, genotypic variability in infection degree was observed. No genotype could be indicated as absolutely resistant in the sense of symptomless habit (more details in Tables 3 and 4). For *F. oxy-*

Table 5

Response of *Pisum* spp. multiple shoot culture (MSC) grown on *Fusarium* spp. filtrates (10% v/v) (plantlet viability evaluated as rooting frequency in %)

Cultivar/Accession	Control	*F. oxysporum* filtrate			*F. solani* filtrate		
	Rooting frequency (%)*	Rooting frequency (%)*	Difference to control	Response in MSC **	Rooting frequency (%)*	Difference to control	Response in MSC **
Adept	50	20	30	S	30	20	T
Arvika	50	30	20	T	20	30	S
AZ 26	60	40	20	T	20	40	S
Bohatýr	40	30	10	T	40	0	R
DP 1059	40	10	30	S	30	10	T
Dundale	50	45	5	T	20	30	S
Janus	30	40	–10	R	20	10	T
Komet	50	30	20	T	30	20	T
Lantra	80	20	60	S	15	65	S
Maja	60	10	50	S	15	45	S
Sirius	50	30	20	T	20	30	S
Smaragd	40	30	10	T	15	25	S
Tyla	40	20	20	T	30	10	T
Tyrkys	30	20	10	T	15	15	T
W 226	40	30	10	T	30	10	T
W 807	50	40	10	T	35	15	T
W 1997	60	10	50	S	25	35	S
Min.	30	10			15		
Max.	80	45			40		
Mean	48.23	26.76			24.11		

* Rooting frequency is the mean of 2 replicates, each per 10 plants. ** Response was evaluated according to the difference between rooting frequency in filtrate and control variant: < 10 = resistant reaction (R); 10-20 = tolerant (T); > 20 = susceptible (S)

sporum only in four cases (Adept, Dundale, Janus, Lantra) the response to the filtrate and inoculation were alike, for *F. solani* agreed these two variants seven times (Adept, AZ 26, DP 1059, Sirius, Smaragd, Tyla, W1997). Substantial differences between the effect of filtrate and inoculation e.g. resistant x susceptible response were also recorded several times.

Multiple-shoot culture

The effect of the two *Fusarium* species filtrates was variable. Both *F. oxysporum* and *F. solani* filtrates reduced the rooting frequency of shoots substantially (Table 5). Resistant reaction was very rare in these conditions (Janus × *F. oxysporum* filtrate; Bohatýr × *F. solani* filtrate). The values of rooting frequency in MSC should be considered in relation to the control variant which never reached 100%.

Table 6

Response of *Pisum* spp. dual culture to inactivated *Fusarium* spp. cultures (plantlet viability evaluated as rooting frequency in %)

Cultivar/Accession	Control	*F. oxysporum* filtrate			*F. solani* filtrate		
	Rooting frequency (%)*	Rooting frequency (%)*	Difference to control	Response in MSC **	Rooting frequency (%)*	Difference to control	Response in MSC **
Adept	40	45	–5	R	30	20	T
Arvika	60	30	30	S	20	30	S
AZ 26	50	30	10	T	20	40	S
Bohatýr	60	15	45	S	40	0	R
DP 1059	70	50	20	T	30	10	T
Dundale	60	20	40	S	20	30	S
Janus	40	0	40	S	20	10	T
Komet	50	10	40	S	30	20	T
Lantra	40	30	10	T	15	65	S
Maja	50	40	10	T	15	45	S
Sirius	60	50	10	T	20	30	S
Smaragd	35	30	5	R	15	25	S
Tyla	80	15	65	S	30	10	T
Tyrkys	30	25	5	R	15	15	T
W 226	55	15	40	S	30	10	T
W 807	60	20	40	S	35	15	T
W 1997	40	10	30	S	25	35	S
Min.	30	0			15		
Max.	80	50			40		
Mean	51.76	25.58			25.29		

*, ** - see Table 5

Dual culture

Rather different reaction to pathogen inactivated by autoclaving comparing to the filtrate variant in multiple-shoot culture was noticed (Table 6). Both *Fusarium* species used in this set of experiments decreased the mean of rooting capacity nearly equally. The variation in the response was broader; some cultivars or accessions could be recorded as resistant ones to *F. oxysporum* (cvs. Adept, Smaragd, Tyrkys) and to *F. solani* (cv. Bohatýr).

Simple generalization and comparison of the results of these three *in vitro* experimental approaches is given in Table 7. About one-third of the cultivars gave similar response to the different experimental treatments. Cvs. Adept, Dundale and Janus to *F. oxysporum* and *F. solani*; cv. Sirius to *F. oxysporum*; cvs. Lantra, Smaragd, Tyrkys, and accessions W 226, W 807 to *F. solani*. The response of the other genotypes varied too much. From this reason the generalization of the results was limited. Among quite a broad variation in the response was very surprising the complete agreement in *F. solani* variants – multiple shoot culture grown on filtrate and on dual culture (see last two columns in Table 7).

Table 7

Comparison of *Pisum* spp. cultivars and accessions response in different *in vitro* cultures to *Fusarium oxysporum* and *F. solani*

Cultivar / Accession	*F. oxysporum*				*F. solani*			
	TT – filtrate	TT – inoculation	MSC – filtrate	DC – inactivated inoculation	TT – filtrate	TT – inoculation	MSC – filtrate	DC – inactivated inoculation
Adept	***R***	*R*	S	***R***	***T***	***T***	***T***	***T***
Arvika	S	R	T	S	R	T	S	S
AZ 26	R	S	T	T	T	T	S	S
Bohatýr	R	T	T	S	R	T	R	R
DP 1059	R	S	S	T	R	R	T	T
Dundale	*T*	*T*	***T***	S	R	***S***	***S***	***S***
Janus	*S*	*S*	R	***S***	S	***T***	***T***	***T***
Komet	R	T	T	S	S	***T***	***T***	***T***
Lantra	R	R	S	T	*S*	T	***S***	***S***
Maja	R	S	S	T	R	T	S	S
Sirius	S	*T*	***T***	***T***	T	T	S	S
Smaragd	R	T	T	R	***S***	***S***	***S***	***S***
Tyla	T	S	T	S	S	S	T	T
Tyrkys	R	S	T	R	R	***T***	***T***	***T***
W 226	S	R	T	S	S	***T***	***T***	***T***
W 807	S	T	T	S	R	***T***	***T***	***T***
W 1997	R	T	S	S	R	R	S	S

TT = tube test; MSC = multiple shoot culture; DC = dual culture

R = resistant, T = tolerant, S = susceptible

Bold italics indicate high degree of coincidence in the responsive reaction in four or three culture variants within cultivar / accession tested

Progeny of fertile regenerated plants obtained from our experiments will be used for screening in infection field to detect level of susceptibility under natural infection conditions.

Discussion

The application of tissue cultures and toxins or pathogen filtrates as the selective agents in biotechnological approaches is routinely used (Bulk, 1991). Theoretical schemes are presented frequently in biotechnological textbooks (Ingram and Helgeson, 1983; Isaac, 1992). Fundamental demands for *in vitro* selection are: (1) techniques allowing induction and regeneration of tissue culture, (2) an effective selective agent representing at cellular level the desired trait (Remotti et al., 1995).

Variable influence of filtrates on the different plant levels – intact plants in tube

tests and multiple shoot culture *in vitro* was recorded. While the intact plants were not very sensitive to the filtrates, multiple shoots differed in the responsiveness considering the filtrate used. Dual culture conditions provided different results as compared to both – tube test and multiple shoot culture. The differences might be caused by the presence of resting mycelium as well as the content of more complexed substances than in the case of filtrates. Some correlating results were recorded.

Searching of the plant response is necessary to improve at each experimental level by exact biochemical methods which allow to follow some of the multicomponent defence mechanisms: accumulation of pathogenesis-related proteins (chitinases, glucanases), phytoalexins (pisatin, medicarpin, avenacin, etc.), cell wall proteins and fenolics (hydroxyproline-rich glycoproteins, lignins) (Dixon et al., 1996; Lebeda, 1995).

The role of mycotoxins in plant pathogenesis was defined similarly by several authors. Desjardins and Hohn (1997) defined mycotoxins as probable virulence factors. If production of a toxin increases pathogen virulence, then increased host resistance to the toxin should increase host resistance to the disease. Nevertheless, even in each chemical group of mycotoxins (e.g. trichothecenes, fumonisins, aflatoxins, etc.) differences in importance in disease from one plant species to another do exist. Recently only few examples are available on probable correlations between the mycotoxin content and resistance – fumonisin × *Giberella fujikuroi* (Desjardins and Hohn, 1997), fusaric acid × *Fusarium oxysporum* f.sp. *gladioli* (Remotti et al., 1996).

Limited side-effects are one of the advantages in *in vitro* conditions which allow to carry out the screening tests more precisely. *Vice versa* a big disadvantage is a need for fungus substitution in tissue cultures. Literature references about fungus substitution for some other substances which can possibly trigger the pathogenicity process mentioned filtrates, toxins, chitinases, β-1,3 glucanases (Dumas-Gaudot et al., 1997; Fukamizo et al., 1995; Miller, 1995; Moraru et al., 1995). The attention must be paid to the fact if phytotoxic or phytostatic effects of these substances are connected with the same impact as the pathogen does, e.g. if the defence response on the intact plant level is similar to either the fungus or the filtrate, toxin effect on the tissue or cell culture level.

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Seed Borne Mycoflora of Cowpea [*Vigna unguiculata* (L.) Walp.] and their Effect on Seed Germination under Different Storage Conditons

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The following fungi *viz., Macrophomina phaseolina, Fusarium oxysporum* f.sp. *tracheiphilum, Alternaria alternata, Curvularia lunata, Aspergillus flavus, Aspergillus niger, Penicillium* sp. and *Rhizopus stolonifer* were isolates from cowpea seeds collected from eight districts in Tamil Nadu. Among them *M. phaseolina* was noticed to the maximum extent of 31.9% while *Rhizopus stolonifer* showed the least association (0.33%). *M. phaseolina* caused maximum reduction in germination of cowpea seeds when stored at 100% relative humidity followed by *F. oxysporum* f.sp. *tracheiphilum, A. flavus* and *A. niger*. As the relative humidity and days of storage increases the reduction in germination also increases. Germination of cowpea seeds inoculated with *A. niger* was less affected when stored at 10 °C compared to seeds inoculated with other seed-borne fungi *viz., A. flavus, F. oxysporum* f. sp. *tracheiphilum* and. *M. phaseolina*. A negative correlation was observed between germination percentage and increase in storage temperature.

Cowpea [*Vigna unguiculata* (L.) Walp] is an ancient neolithic African crop, now grown throughout the tropics and subtropics as a pulse, vegetable, fodder and as a cover crop. In world agriculture, cowpea occupy more than five million hectares annually under cultivation (Ferry, 1981). One of the major constraints in increasing the productivity of cowpea is seed-borne pathogens. Microorganisms usually invade the seed before harvest and reduce the quality and yield. Germination was badly reduced due to infection of seed-borne mycoflora. Various moulds and pathogens cause spoilage of cowpea seeds in storage and reduce the viability of seeds and seedling vigour. Forty-one fungal species were isolated from cowpea seeds of which *Aspergillus* spp. and *Penicillium* sp. were predominant (Muthe Gowda and Sullia, 1987). *Fusarium moniliforme, Fusarium oxysporum* and *Rhizoctonia solani* isolated from cowpea seeds inhibited the germination and retarded the seedling growth (Saad Shama et al., 1988). Seed health in storage is influenced by environmental factors like moisture content, length of storage period, temperature and different storage conditions. Temperature and humidity are the most congenial factors for seed deterioration. With a view to understand the role of relative humidity and temperature on the retardation of germination of cowpea seeds infected by seed-borne fungi, the persent study was conducted to isolate and identify the seed-borne mycoflora of cowpea seeds collected from different places and to asses their influence on seed germination at different relative humidity and temperature under storage conditions.

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Materials and Methods

Detection and identification of seed-borne fungi of cowpea

Cowpea seed samples were collected from different parts of Tamil Nadu Blotter method (ISTA, 1985) was followed for the detection of seed-borne fungi of cowpea. The seeds in Petri dishes on three layers of Whatman No. 1 filter paper moistened with sterile water were incubated at 20 ± 2 °C under alternate cycles of 12 h of near ultraviolet light and 12 h of darkness for seven days. The seeds were examined on the eighth day under stereo-binocular microscope (Wild MPS 45) and the fungi were identified. The identify of the fungi was further checked by preparing slides and examined under microscope.

Isolation and purification of seed-borne fungi

The fungi were isolated using potato-dextrose agar (PDA) medium and purified by single spore and single hyphal tip methods (Riker and Riker, 1936) and maintained under laboratory conditions.

Effect of storage conditions on germination

RELATIVE HUMIDITY

The effect of different level of relative humidity on germination of cowpea seeds inoculated with seed-borne fungi was assessed by treating apparently healthy seeds with spore suspensions (1×10^6 spores/ml) of *M. phaseolina, F. oxysporum* f.sp. *tracheiphilum, A. flavus* and *A. niger* isolated from cowpea seeds and stored in the desiccators maintained at 35, 50, 75 and 100% relative humidity using different concentrations of potassium hydroxide solution (Solomon, 1951). Seeds incubated at room relative humidity served as control.

TEMPERATURE

To study the effect of temperature on seed germination a set of seeds treated with the spore suspensions of the above fungi was also stored in incubators maintained at 10, 20 and 30 °C. Seeds stored at room temperature (25 ± 2 °C) served as control. Seed samples were with drawn at 30, 60 and 90 days of storage and the germination was assessed.

Results

The fungi isolated from cowpea seeds include *Macrophomina phaseolina, Fusarium oxysporum* f.sp. *tracheiphilum, Alternaria alternata, Curvularia lunata, Aspergillus flavus, A. niger, Penicillium* sp. and *Rhizopus stolonifer.* Maximum infection of *M. phaseolina* (31.9%) was observed in seed samples collected from Coimbatore district

Table 1

Survey on seed-borne fungi of cowpea in Tamil Nadu

Seed-borne fungi	% infection Districts of Tamil Nadu surveyed							
	Coimbatore	North Arcot	Periyar	Ramnad	Salem	Tirunelveli	Thiruvanna malai sambuvarayar	Trichy
M. phaseolina	31.9	19.3	25.9	11.6	21.0	12.0	11.0	19.0
	(34.4)	(25.9)	(30.6)	(19.83)	(27.27)	(20.12)	(19.32)	(25.81)
F. oxysporum f.sp.	8.98	0.00	13.6	12.0	12.3	6.65	15.3	12.0
tracheiphilum	(15.52)	(1.28)	(21.54)	(20.27)	(20.41)	(14.99)	(22.82)	(20.27)
Alternaria alternata	6.65	8.98	0.33	0.00	2.66	0.00	0.99	5.32
	(14.72)	(17.45)	(2.65)	(1.28)	(9.33)	(1.28)	(4.7)	(13.44)
Curvularia lunata	3.99	0.00	0.00	0.00	0.00	1.99	0.99	1.66
	(10.41)	(1.28)	(1.28)	(1.28)	(1.28)	(6.16)	(4.7)	(6.75)
Aspergillus flavus	1.99	3.33	5.32	6.32	4.66	1.66	12.6	11.64
	(7.43)	(10.54)	(13.44)	(14.54)	(12.55)	(5.68)	(20.55)	(19.59)
Aspergillus niger	11.9	7.32	13.3	2.33	11.6	4.32	11.6	14.0
	(19.8)	(15.39)	(21.3)	(8.65)	(19.97)	(9.21)	(19.81)	(21.29)
Penicillium sp.	3.00	13.6	1.33	0.33	17.3	5.32	3.99	5.99
	(8.15)	(21.6)	(6.07)	(2.65)	(24.6)	(13.44)	(11.52)	(14.37)
Rhizopus stolonifer	0.33	1.33	0.67	1.00	0.33	1.00	0.33	0.33
	(2.65)	(4.31)	(4.02)	(4.7)	(2.65)	(4.7)	(2.65)	(2.65)
CD(p=0.05)	8.09	4.35	3.29	3.59	2.17	7.04	4.15	3.33

(Figures in parentheses are arc sine transformed values)

followed by seeds from Periyar district (25.9%). *F. oxysporum* f.sp. *tracheiphilum* infection was maximum in seed samples collected from Thiruvannamalai Sambuvarayar district (15.3%) followed by that of Periyar district (13.6%). *F. oxysporum* f.sp. *tracheiphilum* infection was not seen in the seed samples of North Arcot district. The association of *A. alternata* was maximum (8.98%) in seed samples of North Arcot district and absent in seed samples of Ramnad and Tirunelveli districts. Occurrence of *C. lunata* was negligible and it ranged from 0.99 to 3.99% in seed samples of Thiruvannamalai Sambuvarayar, Trichy, Tirunelveli and Coimbatore district and its infection was not observed in seed samples of Periyar, Ramnad, Salem and North Arcot districts (Table 1).

A. flavus infection was observed in 1.66 to 12.6% of the seed samples of eight districts of Tamil Nadu. *A. niger* was found to be minimum (2.33%) and maximum (14.0%), respectively, in the seed samples of Ramnad and Trichy districts. Occurrence of *Penicillium* sp. ranged from 0.33 to 17.3% in seed samples of eight districts of Tamil Nadu. *R. stolonifer* infection was negligible in seed samples of all the districts surveyed (Table 1).

Table 2

Effect of relative humidity on the germination of cowpea seeds affected by seed-borne fungi

Treatments	Germination (%)											
	35%-RH			50%-RH			75%-RH			100%-RH		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
M. phaseolina	93.4	89.9	88.4	91.2	88.2	87.1	73.4	71.9	67.8	67.2	55.0	45.4
	(75.11)	(71.47)	(70.05)	(72.72)	(69.62)	(68.95)	(58.92)	(57.96)	(55.43)	(55.03)	(47.84)	(42.35)
F. oxysporum f.sp.	95.2	92.9	90.7	93.5	91.4	89.1	78.7	72.9	70.2	68.7	58.8	47.2
tracheiphilum	(77.30)	(74.56)	(72.27)	(75.23)	(72.96)	(70.72)	(62.52)	(58.64)	(56.92)	(55.95)	(50.04)	(43.42)
A. flavus	97.0	95.6	92.8	94.5	94.2	91.2	82.0	75.9	70.0	76.3	63.4	61.3
	(80.03)	(77.85)	(74.38)	(76.44)	(76.08)	(72.70)	(64.90)	(60.57)	(56.8)	(70.07)	(52.74)	(51.56)
A. niger	97.7	96.9	93.3	95.0	94.3	91.8	83.5	80.1	72.2	77.6	63.2	62.7
	(81.24)	(79.79)	(74.99)	(77.08)	(76.19)	(73.31)	(66.03)	(63.51)	(58.15)	(61.72)	(56.66)	(52.36)
Control	99.2	98.7	98.3	98.9	97.1	96.6	92.1	89.9	88.6	83.5	82.5	79.6
	(85.32)	(83.71)	(82.40)	(83.97)	(80.24)	(79.40)	(73.68)	(71.49)	(70.27)	(66.04)	(65.23)	(63.19)

CD (p=0.05)

t–Treatment	t-1.18	hxt-2.36
rh–Relative Humidity	rh-1.05	dxt-2.04
d–Days	d-0.91	hxtxd-4.08
tx rhxd-Interactions		hxd-1.82

(Figures in parantheses are arc sine transformed values)

A gradual decrease in the germination % of cowpea seeds treated with spore suspensions of seed-borne fungi namely, *M. phaseolina, F. oxysporum* f.sp. *tracheiphilum, A. flavus* and *A. niger* as well as in control was noticed when the seeds were stored at 35 to 100% relative humidity. Similar trend of results was observed when germination was assessed at 30, 60 and 90 days of storage at all relative humidity (35, 50, 75 and 100%) level. Germination was minimum and significantly less in *M. phaseolina* treated seeds followed by *F. oxysporum* f.sp. *tracheiphilum* treated seeds when stored at 35, 50, 75 and 100% relative humidity on 30, 60 and 90 days of storage (Table 2). This was followed by seeds treated with *A. flavus* and *A. niger* stored at 35, 50, 75 and 100% relative humidity.

Germination of cowpea seeds treated with spore suspension of seed-borne fungi was drastically reduced when they were stored at 30 °C sompared to storage at 10 °C. *M. phaseolina, F. oxysporum* f.sp. *tracheiphilum, A. flavus* and *A. niger* treated seeds recorded, respectively, 63.2, 64.4, 79.5 and 81.0% germination after 90 days of storage at 30 °C as against 89.4% germination in control (Table 3). As the storage temperature increases the reduction in germination % also increases. The reduction in germination per cent due to the treatment of spores of seed-borne fungi was significant when seeds were stored at 10 °C even after 30 days of storage. After 30 days of storage at 20 °C *M. phaseolina* treated seeds recorded 72.2% germination compared to 97.5% germination in control (Table 3).

Table 3

Effect of temperature on the germination of cowpea seeds affected by seed-borne fungi

Treatments	Germination (%)								
	10 °C			20 °C			30 °C		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
M. phaseolina	91.5	90.5	88.6	72.2	69.3	66.0	68.3	64.6	63.2
	(73.01)	(72.10)	(70.26)	(58.17)	(56.35)	(54.3)	(55.73)	(53.48)	(52.65)
F. oxysporum f.sp.	94.6	91.2	90.8	73.5	70.0	67.5	71.6	69.5	64.4
tracheiphilum	(76.56)	(72.74)	(72.34)	(59.02)	(56.8)	(55.24)	(57.79)	(56.48)	(53.36)
A. flavus	96.5	96.5	94.5	89.1	84.6	82.0	84.8	84.2	79.5
	(79.25)	(79.24)	(76.40)	(70.40)	(66.89)	(65.27)	(97.05)	(66.57)	(63.07)
A. niger	97.2	96.5	94.8	89.0	84.9	84.0	86.0	85.0	81.0
	(80.36)	(79.24)	(76.81)	(70.63)	(67.10)	(66.42)	(68.04)	(67.20)	(64.16)
Control	99.0	98.1	98.0	97.5	96.5	95.0	94.5	92.1	89.4
	(84.26)	(81.86)	(81.86)	(80.94)	(79.24)	(78.14)	(76.45)	(73.67)	(70.99)

CD (p = .05)

t-054	txtr-1.21	t–Temperature
d-0.54	dxtr-1.21	tr–Treatment
tr-0.7	txdxtr-2.1	d–Days
txd-0.96	txtrxd-interactions	

(Figures in parentheses are arc sine transformed values)

Discussion

Seeds play a vital role in crop health. They are known to carry pathogens with potential to cause heavy yield losses. Seed-borne fungi have been reported to reduce seed germination and field emergence of soybean (Chamberlain and Gray, 1974). In the present study the following eight seed-borne fungi namely, *Macrophomina phaseolina, Fusarium oxysporum* f.sp. *tracheiphilum, Alternaria alternata, Curvularia lunata, Aspergillus flavus, A. niger, Penicillium* sp. and *Rhizopus stolonifer* were isolated from cowpea seeds collected from various parts of Tamil Nadu. Similarly Saad Shama et al. (1988) isolated *A. alternata, A. flavus, A. niger, C. lunata, Fusarium* spp. and *Penicillium* sp. from cowpea seeds. The infection of *M. phaseolina* and *F. oxysporum* f.sp. *tracheiphilum* was maximum. This is in conformity with the findings of Sinha et al. (1978), who observed that 23 out of 43 seed lots of cowpea were infected with *M. phaseolina* and 20 lots were infected with *F. equiseti.* They also reported 45 and 35% association of *M. phaseolina* and *F. equiseti* respectively. In the present study also the infection of *M. phaseolina* (31.9%) and *F. oxysporum* f.sp. *tracheiphilum* (15.3%) was more compared to other fungi.

Germination of cowpea seeds was maximum at low relative humidity and minimum at 100% relative humidity at 90 days after storage in *M. phaseolina* treated seeds

(45.4%) followed by seeds treated with *Fusarium oxysporum* f.sp. *tracheiphilum* (47.2%). This may be due to excessive growth of pathogens at high relative humidity thus resulting in reduced germination of cowpea seeds. Lopez and Christensen (1962) reported that at lower humidity levels the invasion by seed-borne fungi was negligible, and at higher relative humidity their activities were pronounced resulting in germination failures. Germination of cowpea seeds decreased with increase in temperature and was low at 30 °C at 90 days of storage. This might be due to higher temperature (30 °C) favouring the growth and infection of fungi. Christensen (1973) reported that the growth of various fungi was very slow at 10 °C. The deterioration of sorghum grains stored at 10 °C and at 11% moisture content was negligible while, the deterioration of grains by *A. niger, A. flavus* and *F. moniliforme* was more when stored at 30 °C and at 18% moisture content. In the present study also maximum reduction in germination was observed at 30 °C in seeds treated with *M. phaseolina* and *F. oxysporum* f.sp. *tracheiphilum*. Hence it may be concluded that cowpea seeds could safely be stored at 35% relative humidity and 10 °C without much reduction in germination even though if they are infected by seed-borne fungi.

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Host Range of *Gnomonia rostellata* (Fr.) Wehm., the Pathogen of Blackberry *(Rubus fruticosus* L.*)* Plants

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Studying the host range of *G. rostellata* we have shown that this species occurs in nature as a pathogen of wild and cultivated blackberry, raspberry, tayberry, strawberry and rose plants. No symptoms have been observed on inoculated gooseberry and red currant plants (Table 1).

Apple, pear, apricot, peach, plum, sweet and sour cherry and walnut trees as well as *Potentilla* plants also did not react to *G. rostellata* infection (Table 2).

Among the numerous pathogens of the blackberry plant established in Yugoslavia, *G. rostellata* occupies a significant position (Arsenijević and Veselić, 1995, 1997). Owing to its frequency and striking aggressiveness to cultivated blackberry this fungus has become one of the most significant pathogens observed during the past few years in blackberry plantations.

As a result of severe necrosis and the intensive spread of the necrotic process, the wilting and die-back of blackberry canes occurred (Arsenijević and Veselić, 1995). As a result of such a pronounced virulence of this pathogen to blackberry canes and the insuficcient knowledge about this fungus in general, we wanted to study its behavior towards other, related or botanically more distant, fruit species.

Materials and Methods

Fungus cultures and plant inoculation

Considering the slow growth of *G. rostellata* colonies in cultures (Arsenijević and Veselić, 1995), 30-day-old strains, which were grown on potato-dextrose agar (PDA), were used. Two strains of the fungus (Kp-3 and Kp-107) isolated from diseased blackberry canes in 1984 and 1987 respectively, were used (Arsenijević and Veselić, 1997). All the inoculations were carried out in the field at the end of March 1992.

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Table 1

The response of small berry fruits and rose plants to *Gnomonia rostellata*

Plant species	Reading after days	Tissue necrosis in cm (Φ for 6 repetitions)		
		Fungus strains		
		Kp-3	Kp-107	Check
Blackberry (*Rubus*	30	3.6	5.8	–
fruticosus L.)	60	8.2	8.9	–
Cv. Thornfree	90	11.4	13.3	–
Wild blackberry	30	5.1	5.1	–
(*Rubus sp.*)	60	7.5	7.5	–
Domestic population	90	8.2	12.4	–
Raspberry (*Rubus*	30	4.6	4.9	
idaeus L.)	60	6.0	5.1	–
Cv. Willamette	90	10.8	12.6	–
Tayberry	30	2.8	3.8	–
(*Blackberry* x	60	4.8	5.2	–
Raspberry)	90	4.9	6.5	–
Strawberry	55	1.9	1.5	–
(*Fragaria vesca* L.)	10	2.5	2.3	–
Cv. Senga Sengana	15	*	*	–
Rose (*Rosa canina*	30	2.5	1.7	–
L.)	60	5.0	3.9	–
Cv. Evening Star	90	6.0	4.6	–
Gooseberry (*Ribes*	30	–	–	–
grossularia L.)	60	–	–	–
Cv. Triumph	90	–	–	–
Red currant (*Ribes*	30	–	–	–
rubrum L.)	60	–	–	–
Cv. Versailles red	90	–	–	–

– negative reaction; * wilting and die-back of inoculated plants

The artificial inoculation of caneberry plants and related species

The artificial inoculation of wild and cultivated blackberry, raspberry, tayberry, gooseberry, red currant, strawberry and rose plants was conducted according to the procedure applied earlier while working with *Cytospora concta*, a pathogen of the peach tree (Arsenijević et al., 1973). Fungus colony fragments, about 1.0 cm in diameter were placed under the cut bark of two-year-old canes of caneberry plants directly on the xylem. The inoculated places were wrapped into moist cotton wool and aluminum foil, which were removed 30 days after inoculation.

The inoculations of all berry plants were carried out using 2 canes of each plant species and 3 inoculations on each cane. Control canes were tested in the same way, but instead of fungus colonies medium fragments without the pathogen were applied.

Table 2

The response of other plant species than small fruit plants, pome and stone fruit trees, Walnuts and *Potentilla* to *Gnomonia rostellata*

Plant species	Reading after days	Tissue necrosis in cm (Φ for 6 repetitions)		
		Fungus strains		
		Kp-3	Kp-107	Check
Apple (*Malus*	30	–	–	–
domestica Borkh.)	60	–	–	–
Cv. Idared	90	–	–	–
Pear (*Pyrus domestica*	30	–	–	–
Med.)	60	–	–	–
Cv. Curé	90	–	–	–
Apricot (*Prunus*	30	–	–	–
armeniaca L.)	60	–	–	–
Cv. Magyar Kajszi	90	–	–	–
Peach (*Prunus persica*	30	–	–	–
Batsch)	60	–	–	–
Cv. Redhaven	90	–	–	–
Plum (*Prunus*	30	–	–	–
domestica L.)	60	–	–	–
Cv. Stanley	90	–	–	–
Cherry (*Prunus*	30	–	–	–
avium L.)	60	–	–	–
Cv. Bing	90	–	–	–
Sour cherry (*Prunus*	30	–	–	–
cerasus L.)	60	–	–	–
Cv. Kellerüs 14	90	–	–	–
Walnut (*Juglans regia*	30	–	–	–
L.) spontaneous	60	–	–	–
population	90	–	–	–
Potentilla (*Potentilla*	30	–	–	–
palustris L.)	60	–	–	–
	90	–	–	–
Blackberry (*Rubus*	30	–	5.0	–
fruticosus L.)	60	5.9	8.7	–
Cv. Thornfree	90	8.2	10.2	–

Changes were evaluated after 30, 60 and 90 days by measuring the length of necrotic tissue. The reisolation of the fungus was carried out using necrotic tissue from the inoculated plants.

Unlike other species, strawbarry plants were inoculated into the leaf petioles. A similar procedure was applied, but with considerably less injury to the plant's tender tissue.

Inoculation of pomaceous, stone fruit and walnut trees and Potentilla *plants*

Inoculation of pomaceous, stone fruit and walnut trees was carried out using 8-years-old fruit-bearing trees. Two-year-old branches were used for inoculation. The same method was applied as in the case of small fruit trees. Here too, incisions were made in the tissue under the bark and 30-day-old fungus colony fragments, grown on PDA, were applied.

Three branches of every fruit tree were inoculated in two places, amounting to 6 repetitions for each plant species (Table 2). "Thornfree" cv. of blackberry plants were inoculated as a positive control group. Results were checked 30, 60 and 90 days after inoculation (Table 2).

Potentilla plants were inoculated in a greenhouse by pricking the stem and applying the pathogen's mycelia with perithecia. The places of inoculation were wrapped in moist cotton wool and aluminum foil.

Results

Small berry species and rose plants

When the first results were checked on April 25th 1992, 30 days after inoculation, large dark-purple spots could be observed on wild and cultivated blackberry as well as on raspberry and tayberry plants. The sizes of the spots ranged between 2.8 and 5.1 cm. After 60 days, tissue necrosis increased and after 90 days it spread over a significant area of the canes causing phloem, cambium and xylem necrosis (Table 1). The cortical tissue of the inoculated plants became whitish-grey.

The necrotic process spread longitudinally as well as round the canes causing the die-back of inoculated plants. Perithecia formation on the necrotic tissue of cultivated blackberry was abundant. No fructification could be observed on tayberry and raspberry plants even 90 days after inoculation. Similar changes could also be observed on inoculated roses.

However, in this case the necrotic tissue became deformed and bulging, forming cankreous wounds, which was not the case with other inoculated plant species. In the spring of the following year, the inoculated canes displayed no signs of vitality since they were completely dead. Mass perithecia formation on them during the April of 1993, 13 months after inoculation. Microscopic examination revealed the presence of *G. rostellata* asci and ascopores.

Inoculated strawberry plants reacted most quickly. Severe tissue necrosis appeared after only 8 to 10 days from the inoculation, followed by wilting and die-back of the plants.

Pomaceous, stone fruit and walnut trees and Potentilla *plants*

Pome, stone fruit and walnut trees as well as *potentilla* plants did not display any tissue necrosis even after 90 days from inoculation (Table 2). Unlike them, blackberry plants used as positive control displayed severe tissue necrosis spreading along and around the canes (Table 2). The necrotic tissue of blackberry plants became greyish-white with the presence of numerous fungus perithecia. Examining the perithecia under a microscope revealed asci and ascospores typical of the *G. rostellata* species.

Discussion

According to data given by Monod (1983), several species of the genus *Gnomonia* have been described as pathogens of cultivated blackberry plants. Past research of blackberry pathogens in Yugoslavia revealed the presence as well as the striking aggressiveness, only of the *G. rostellata* fungus, a very frequent and widespread species in almost all blackberry plantations (Arsenijević, 1988, 1989; Arsenijević et al., 1992; Arsenijević and Veselić, 1995). Considering its frequency and high level of virulence to cultivated blackberry, it presented a matter of great interest to study the behavior of this pathogen towards other plant species more or less closely related to blackberry.

As it turned out, apart from cultivated blackberry plants, *G. rostellata* is also a pathogen of wild blackberry, raspberry, tayberry, strawberry and rose plants (Table 1). Each of these plants displayed a high degree of infection and severe tissue necrosis followed by the appearance of the pathogen's perithecia. The necrotic process spread to the phloem, cambium and xylem resulting in the wilting and die-back of inoculated plants. Strawberry plants displayed changes in the shortest period of time, because necrosis and wilting appeared only 8–10 days after inoculation.

Inoculated gooseberry and red currant plants displayed no changes, because the appearance of tissue necrosis on these plants was left out altogether (Table 1). It is interesting to mention that *G. comari* has been studied as a pathogen of strawberry plants in Yugoslavia (Stojanović and Borić, 1976). In his study of this fungus as a pathogen of strawberry, Bolay (1971) lists several representative genera of its host species such as: *Agrimona, Alchemilla, Comarum, Fragira, Geum, Potentilla* and *Sanguisorgba.*

Although the host range of *G. rostellata* is, as far as we know, relatively understudied, apart from cultivated blackberry, wild blackberry is also mentioned as a host of this fungus both in America and Yugoslavia (Fischer, 1956; Schneider et al., 1969; Arsenijević et al., 1992; Arsenijević and Veselić, 1995).

There is no literature data on the behavior of *G. rostellata* towards species of the following genera: *Juglans, Malus, Pyrus, Prunus, Potentilla.* Judging by our experiments, apple, pear, apricot, peach, plum, sour cherry, sweet cherry and walnut trees as well as *potentilla* plants had a negative reaction to *G. rostellata* (Table 2).

Therefore, apart from cultivated blackberry, its main host, *G. rostellata* is also a pathogen of wild blackberry, raspberry, tayberry, strawberry and rose plants. Inoculated

species of the genera: *Malus, Pyrus, Prunus* and *Potentilla* cannot be considered to be hosts of the *G. rostellata* fungus.

Fours strains (Kp-33, Kp-72, Kp-107 and Kp-132) of the *G. rostellata* pathogen were deposited in July 1995 at the American Type Culture Collection, Maryland, USA.

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Role of Oxidative Enzymes in Sugarcane and *Colletotrichum falcatum* Went Interaction

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The role of oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO) in disease resistance has been reported in many crops. An attempt was made to ascertain the defense role played by PO and PPO in sugarcane against red rot pathogen (*Colletotrichum falcatum* Went). Sugarcane varieties CoC 671 and BO 91 with absolute susceptibility and resistance to red rot disease respectively, were used to carry out the experiments. The observations indicated that there was considerable spurt in activity of PO in the calli from resistant variety as well as in calli infiltrated with the pathogen toxin material, though the trend was similar in case of PPO, the rate of activity was less. More of PO activity was induced in the tissue (both internodal and calli) from BO 91 when compared to CoC 671. However in both varieties a corresponding manifold increase of PO activity was assayed in case of callus tissue as compared to the internodal samples. The maximum PO activity peak was observed between 0 and 90 sec in both calli and internodal tissues of these varieties. Toxin induced PO activity was prominently more in the resistant variety. The PPO activity assay responded with a negligible change in absorbance over a period of time. The results showed that the inherent PO activity can serve as an useful biochemical marker for the selection of sugarcane genotypes, resistant to red rot.

Red rot disease caused by *Colletotrichum falcatum* Went (Perfect stage: *Glomerella tucumanensis* Arx and Muller) is the major constraint for sugarcane cultivation in most parts of India. This baffling disease is of great economic importance and was responsible for the failure of many ruling commercial varieties in India. It continues to be a serious threat to the Indian sugar industry, particularly in the State of Tamil Nadu, Andhra Pradesh, Orissa, Gujarat, Rajasthan, Punjab, Haryana, Uttar Pradesh and Bihar (Alexander and Viswanathan, 1996). The exact biochemical mechanism that governs resistance to red rot disease is not fully understood. The role of phenolic compounds in biochemical resistance was demonstrated by several workers. High levels of phenolics in resistant and moderately resistant varieties were reported by Rao et al. (1986). However later studies by Singh et al. (1976) revealed that there was no positive correlation between total phenols in sugarcane varieties and degree of resistance to red rot. Their studies also showed that in resistant varieties phenol content increased after infection and maintained, while in susceptible varieties, the level dropped after an initial increase. Recent studies indicated that red rot pigments (Viswanathan et al., 1994) and 3-deoxyanthocyanidin phytoalexins have a role (Viswanathan et al., 1996a) in red rot disease resistance.

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The role of oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO) in disease resistance has been reported in many crops. In general plants respond to infection by pathogen with a series of changes in gene expression and cellular metabolism (Bowles, 1990). Among the protein components of host cell wall, which confer defense response, PO is an important, versatile and ubiquitous group of enzymes. Implication of POs and other oxidative enzymes in a variety of defense related process has been well documented in other crops (Nicholson, 1987). Previous studies on oxidative and phenyl propanoid pathway enzymes were performed with the tissue samples derived from leaf tissue or stalk tissues.

Investigations were not carried out to ascertain these enzyme activities at cellular level. Attempts were made to raise sugarcane callus and enzyme activity was measured and compared with enzyme activity of intact tissues. In order to find out the triggering response in the pathogen to induce PO and PPO activity in the host at cellular level, partially purified toxin of the pathogen was used to induce the enzyme activity.

Materials and Methods

For the enzyme assay, one standard resistant variety BO 91 and a standard susceptible variety CoC 671 used in all plant pathology trials were taken throughout the study.

Callus indiction

Sugarcane callus was raised using the innermost leaf sheath as the explant. The explants were surface sterilized for 2 minutes in 0.1% mercuric chloride and then subsequently in 10% sodium hypochlorite (with two drops of Tween 20) for 10 minutes. Three washes were given using sterile distilled water. The callus induction medium contained MS basal (Murashige and Skoog, 1962) supplemented with 0.5 mg/l each of Nicotinic acid, Pyridoxine and Thiamine HCl, 2 mg/l of Glycine and 4 mg/l of 2,4–D. The explants placed on the medium were incubated in darkness and callus induction and proliferation was observed between 15 and 20 days. Subsequent callus multiplication (subculture) and maintenance was carried out on the same medium but with 2 mg/l of 2,4–D. This callus was used as a representative sample for the enzyme assays.

Red rot toxin isolation and purification

Monoconidial cultures of the red rot pathogen *Collectotrichum falcatum* were isolated from infected stalks (variety CoC 671), multiplied in oat meal agar medium. Eight mm mycelial discs were aseptically transferred to 250 ml Erlenmeyer flasks, containing 100 ml of Czapeks liquid medium in which sucrose was substituted with host extract (as carbon source) so that 1000 ml of medium would contain extract from 30 g of young sugarcane stalk tissues prepared by homogenization and filtration. After 21 days

of inoculation, the mycelial mats were discarded and the culture filtrate was used as the source material of the toxin.

The filtrate containing the toxin metabolites were purified (Nair and Ramakrishnan, 1973). The volume of the filtrate is reduced to 1/10th of its total volume under reduced pressure, added equal volume of methanol, stored at 5 °C overnight and precipitates removed by filtration. The methanol was removed by vacuum evaporation at 45 °C, the pH of the aqueous phase adjusted to 3.5 with dilute HCl and shaken well with equal volume of diethyl ether. The ether phase was separated, mixed well with equal volume of 5% Na_2CO_3 and the aqueous phase discarded. The ether phase was evaporated to dryness by air drying. The residue forms part of the partially purified toxin which is used for induction of the oxidative enzymes.

Toxin treatment

The partially purified red rot toxin was found to be capabel of exhibiting part of the symptoms at a particular concentration (2000 µg/l). This toxin preparation was used to study the induction of oxidative enzymes in callus tissues of both the sugarcane varieties CoC 671 and BO 91. Calli (100 mg) were rinsed with sterile distilled water, enclosed in washed muslin cloth and placed in glass vials. The calli were vacuum infiltrated with 3 ml of the toxin solution or deionised water (for control) for 30 minutes, and the subjected to the enzymes assay.

Assay of oxidative enzymes

Plant tissues were homogenized in a chilled pestle and mortar in 0.1 M phosphate buffer (pH 6.5) in the ratio of 1:5 (W/V), strained through several layers of cheese cloth. The buffer tissue homogenates were then centrifuged at 6000 rpm at 4 °C for 15 minutes. The clear supernatant served as the enzyme source. The activity of PO and PPO was determined at 420 nm and 495 nm respectively in spectrophotometer (Boesch and Lamb). 0.05 M Pyrogallol (substrate for PO), 1% H_2O_2 and catechol (substrate for PPO) were the components involved in the assay. Change in absorbance was recorded for 3 minutes at 30 seconds interval and the enzymes activity was expressed as absorbance values (mean of four replications) given in standard units. Heat killed enzyme extract served as control and was used to set zero absorbance.

Results

In the present study, it was observed that among the sugarcane internodal and callus tissues assayed for their inherent peroxidase activity, as a whole the callus tissue exhibited a very high peroxidase activity than the internodal tissue irrespective of the test varieties used. Among the two varieties, the resistant variety BO 91 had a pronounced increase in the enzyme activity than that of the susceptible variety CoC 671. A sudden

Table 1

Changes in inherent peroxidase activity in sugarcane varieties CoC 671 (susceptible to red rot) and Bo 91 (resistant to red rot)

Time (sec)	Internodal tissue Mean abs. value		Time (sec)	Callus tissue Mean abs. value	
	CoC 671 (S)	Bo 91 (R)		CoC 671 (S)	Bo 91 (R)
0	38	97	0	1355	1976
30	414	814	30	1392	2148
60	452	895	60	1444	2199
90	488	929	90	1478	2263
120	520	964	120	1512	2310
150	540	992	150	1543	2358
180	573	995	180	1571	2395

S = Susceptible variety to red rot
R = Resistant variety to red rot
0.001 Absorbance = 1 unit

Table 2

Changes in PO activity in sugarcane callus induced by red rot toxin treatment (Expressed as OD of reaction mixture at 420 nm from 0 to 180 sec)

Time (sec)	Mean abs. value (CoC 671) S			Mean abs. value (Bo 91) R		
	Control	RRT	% inc. over control	Control	RRT	% inc. over control
0	355	2025	49.5	1926	3471	75.7
30	1392	2052	47.4	2148	4420	105.8
60	1444	2714	88.0	2199	4914	123.5
90	1478	2950	99.6	2263	5352	136.5
120	1512	3094	104.6	2310	5585	141.5
150	1543	3218	108.6	2358	5633	138.9
180	1571	3246	106.6	2395	5690	137.6

S = Susceptible to red rot
RRT = Red rot toxin treatment
R = Resistant to red rot
Optical Density of 0.001 = 1 unit

spurt in activity was observed between 0 and 30 sec, maintained up to 1 minute and the rate of increase in activity was gradual thereafter up to 3 minutes (by which the assay was terminated) (Table 1). As the callus tissue responded with a pronounced enzyme activity, this was chosen as the representative tissue for studying the induced enzyme activity on infusion with the partially purified red rot toxin.

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Table 3

Red rot toxin induced changes in PPO activity of sugarcane callus tissue (CoC 671 and BO 91)

Time	Mean abs. value (CoC 671) S			Mean abs. value (Bo 91) R		
(sec)	Control	RRT	% inc. over control	Control	RRT	% inc. over control
0	247	294	19.0	316	355	12.3
30	249	298	19.7	412	436	5.8
60	256	301	17.6	424	451	6.4
90	261	314	20.3	430	459	6.7
120	262	317	20.9	444	462	4.1
150	275	320	16.4	452	484	7.1
180	279	320	14.7	460	499	8.5

S = Susceptible to red rot
R = Resistant to red rot
RRT = Red rot toxin treatment
Optical density of 0.001 = 1 unit

Upon toxin treatment callus tissue of both the varieties exhibited higher absorbance representing an increase in enzyme activity. A gradual increase in enzyme activity was observed throughout the course of assay (from 0 to 180 sec). In the susceptible variety CoC 671 a maximum increase over control 108.6% was observed by 150 sec, and in the resistant variety BO 91 it was 141.8% increase measured at 120 sec. An early induction and spurt in activiy was observed within 2 minutes of red rot toxin treatment (Table 2).

Polyphenol oxidase activity was also induced by the red rot toxin treatment as in the previous studies but the degree of induction was not as rapid as that observed for peroxidase activity. The susceptible variety showed a maximum increase of activity over control i.e. 20.9% by 120 sec, whereas, the resistant variety recorded a maximum of 12.3% immediately after the toxin treatment i.e. within 30 seconds.

On comparison between the two varieties, it is quite obvious that the resistant var. BO 91 exhibited an overall increased activity than the susceptible variety throughout the course of study (Table 3).

Discussion

Peroxidase, an iron containing host enzyme is frequently correlated with disease resistance in many crops. It is an important enzyme in the synthesis of lignin and it catalyses the oxidation of phenolics into more toxic quinones and also the enzyme itself in considered inhibitory to some microorganisms (Vidhyasekaran, 1988). Peroxidase activ-

ity may contribute to induced resistance by helping to generate H_2O_2 as well as by increasing the concentration of free radicals and their polymerisation products.

Reports are available in plethora to prove the role of peroxidase and its induction in many host-pathogen interactions involving *Colletotrichum* spp. Hammerschmidt et al. (1982) observed a positive correlation between enhanced peroxidase activity in cucumber and systemic protection against *Colletotrichum lagenarium*. It was also reported in maize and *C. graminicola* interaction by Nicholson and Cadena Gomez (1987) that the induction of peroxidase was part of a non-specific host response to attempted fungal penetration. Harrison (1995) observed that specific host peroxidases were found to be induced in a forage legume *Stylosanthus humulis* in response to infection by *C. gloeosporioides*. An increase in soluble peroxidase activity in velvet leaf infected by *C. coccodes* was observed to have associated defense response (Nicholson et al., 1993). As in other hosts and *Colletotrichum* spp. interaction, sugarcane and *Colletotrichum falcatum* interaction clearly demonstrated the role of PO in defence related activities. This is evident from the fact that tissue samples either cane tissues or cell suspension of resistant genotype recorded higher PO activity (Table 1). In histopathological studies Mohanraj et al. (1997) found that in resistant variety BO 91, pathogen was not able to penetrate the host tissue and only in CoC 671 appressoria and infection pegs were formed in 24 h and profuse intercellular mycelium and acervuli formation within 72 h were observed. Viswanathan et al. (1996b) recently reported that pathogen infection in red rot resistant varieties BO 91 and CoS 767 higher accumulation of PO, whereas in susceptible varieties CoC 671 and COc 86062 after infection PO levels decreased considerably in stalk tissue and leaves.

Polyphenol oxidase is a copper containing enzyme, oxidises phenolics to highly toxic quinones and speculated to involve in the terminal oxidation in the diseased plant tissue which was attributed for its role in disease resistance (Kosuge, 1969). The increase in PPO activity might be due to the activation of the latent host enzyme, solubilization of host PPO which is normally particulate or even due to de novo synthesis (Manibushan Rao, 1988). Involvement of enzymes in phenyl propanoid pathway such as PAL and TAL was found to have specific activity in some of the resistant varieties (Madan, 1991). Earlier Srinivasan (1969) found correlation between the red rot resistance and polyphenoloxidase activity. In the present study also the resistant variety BO 91 recorded higher enzyme activity throughout the assay period. Upon toxin treatment cell lines of susceptible variety elicited more response than resistant variety for PPO activity. Though CoC 671 recorded higher induced PPO activity over BO 91, its PPO activity was always lower than BO 91. It indicates that RRT cannot induce more PPO enzyme in BO 91 as in CoC 671 over the inherent enzyme level (Table 3).

Mohanraj et al. (1995) reported that part of the red rot symptoms was induced by the partially purified toxin, which suggested the possible role of the toxin in red rot pathogenesis. Viswanathan (unpublished) observed that the pathogenic toxin was able to induce 3-deoxyanthocyanidin phytoalexins in sugarcane and demonstrated that the resistant variety BO 91 accumulated 2- to 3-fold higher phytoalexins than the susceptible variety CoC 671. The partially purified toxin also caused maximum electrolyte leakage in

cell suspension of susceptible variety CoC 671 when compared to that of the resistant variety BO 91 (Ramesh Sundar unpublished). These studies demonstrate that the role of pathogenic toxin in inducing certain symptoms and triggering the host resistant mechanism. Present studies have eventually proved that the toxic compounds also triggered the host defense enzymes. The induction of peroxidase activity was observed after toxin infusion, more pronounced in resistant variety, whereas in susceptible variety the enhancement occurs too late for activation of defense mechanism to prevent development of the disease. Earlier studies of Viswanathan et al. (1996b) revealed that PO activity may be a suitable biochemical marker for the disease resistance in sugarcane. Reports are also available on the use of PO as biochemical marker to index different genotypes for downymildew disease in cucumber (Reuveni et al., 1992). However present studies were carried out with callus tissue which is also giving similar results. Of the two enzymes studies PO exhibited higher induction upon toxin treatment. Even in the untreated cell PO activity was remarkably higher as compared to the PPO activity and the difference in the resistant and susceptible varieties for PO was multifold. Thus the present study suggested that the induction of PO activity can be utilised as a biochemical marker for red rot resistance in sugarcane.

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The Effect of *Macrophomina phaseolina* (Tassi) Goid. and Two Viruses on the Nutrient Content of Pepper (*Capsicum annuum* L.) Leaves

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The aim of our study was to compare the nutrient content (nitrogen, phosphorus, potassium and sodium) of the healty pepper plants and that of the pepper plants infected with U/246 strain of cucumber mosaic cucumovirus, NTN strain of potato Y *potyvirus* and *Macrophomina phaseolina* (Tassi) Goid. It is concluded that the infections has played an important role only in the early nutrient uptake of pepper. After flowering no significant reduction were observed in the nutrient content of the infected pepper leaves as compared to the healthy ones. In case of several mixed infection with viruses and fungus the reduction in the nutrient content of the pepper leaves was greater.

The different plants pathogens (viruses, phytoplasmas, bacteria and fungi) are believed to play important role in the pepper decline (Horváth, 1967, 1969, 1981, 1986; Kadlicskó et al., 1992; Dula, 1995; Csilléry et al., 1996; Kovács et al., 1996; Gáborjányi et al., 1997). In a previous study (Kazinczi et al., 1998) we have already examined the effect of mixed infection on the height, fresh and dry weight of pepper. It was concluded that in case of several mixed infections of viruses and *Macrophomina phaseolina* (Tassi) Goid. the injury of the pathogens has increased.

A lot of study have dealt with the effect of different nutrients on the development of pathogens and disease symptoms. The effect of different nutrients on the occurrence of disease symptoms and development of the pathogens may vary greatly, depending on the type and dosage of the fertilizers (Shanmugaru and Govindaswamy, 1973; Duvenhage et al., 1992; Reuveni et al., 1994; Cloud and Rupe, 1994), the ratio of the different elements (Standaert et al., 1978), host plant and pathogen species (Sivaprakasam et al., 1975; Sharma and Chaudhary, 1985; De and Chattopadhyay, 1992; Chung and Huang, 1993; Ziv et al., 1994; Reuveni et al., 1995) and the phase of the development of the pathogens (Hakeem and Ghaffar, 1977; Shaikh and Ghaffar, 1986).

The nutritional status of the soil greatly influences the severitiy of plant diseases in general and soil borne diseases in particular. Boron (B), copper (Cu), manganese (Mn) and zinc (Zn) inhibited the growth of *Macrophomina phaseolina* and combination of metals especially with Cu was effective in reducing the root rot of some species (Murugesan and Mahadevan, 1988). Garrett (1938) proposed that soil borne pathogens can be effectively controlled by management of the soil especially amendment with the trace elements and organic matter. Usually an increase in nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and microelements [especially zinc (Zn), iron (Fe), boron

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(B) and copper (Cu)] decreased susceptibility to *Macrophomina phaseolina* of the different host plant species (Sivaprakasam et al., 1975; Ji et al., 1977; Gamal et al., 1980; Murugesan and Mahadevan, 1988; Osunlaja, 1992). Viral infection may result in an increase of the exudation of nutrients from roots of infected hosts, which may enhance the potentiality of root fungal pathogens of those hosts (Beute and Lockwood, 1968).

In spite the fact that a lot of study have dealt with effect of nutrients on the development of the pathogens and plant disease, no data is available about the changing of the nutrient content of the host plants due to the different pathogens. Terbe (1996) reported that under glasshose conditions a pepper plant accumulates 2.7–5.4 g nitrogen (N), 0.3–0.8 g phosphorus (P) and 4.0–1.2 g potassium (K) depending on the duration of vegetation period, growing technology and crop yield (4–12 kg/m^2). 60–70% of the total N, P and K content accumulates in the crops, 10–20% in the leaves and only 3–15% in the roots. So far, no information is available about the nutrient content of the infected pepper plants.

Therefore the aim of our study was to compare the macroelement content of the healty and infected pepper plants.

Materials and Methods

Seeds of *Capsicum annuum* cv. Keszthelyi Rezisztens were sown in sterilized boxes in the virological glasshouse free of vectors. The seedlings were planted in plastic pots (28 cm in diameter), containing a soil mixture of sand (pH: 6.96, humus%: 0.27): peat (pH: 6.78, humus%: 9.98) 1:3. Pepper plants were inoculated at 6–8 leaf stage with the original Maradona isolate of PVY^{NTN} (Beczner et al., 1984) and CMV-U/246 (Schmidt and Horváth, 1982). Previously the viruses were propagated on *Nicotiana tabacum* L. cv. Xanthi-nc. The pepper plants were inoculated mechanically with tissue sap of *N. tabacum* cv. Xanthi-nc. Sörensen phosphate buffer (pH: 7.2) in the ratio 1:1 was used. To check the success of infection, back inoculation was also carried out to *N. tabacum* cv. Xanthi-nc and *Nicotiana glutinosa* L., as indicator plants. In order to make inoculation with fungus, pure culture of *M. phaseolina* was produced on potato-dextroze agar (PDA) culture medium. The suspension of ten-day-old microsclerotia culture (3×10^4 microsclerotia/cm^3 suspension) was poured on the surface of plastic pots at 12–14 leaf stage of pepper plants. Twenty ml suspension was applied for each pot and then the pots were covered with 1 cm thick layer of soil mixture. The pots were watered thoroughly and surface irrigation was used in future, too. In order to study the interactions among the pathogens, the following treatments were applied: 1. Control, 2. CMV-U/246, 3. PVY^{NTN}, 4. *M. phaseolina*, 5. CMV-U/246 + PVY^{NTN}, 6. CMV-U/246 + *M. phaseolina*, 7. PVY^{NTN} + *M. phaseolina*, 8. CMV-U/246+PVY^{NTN} + *M. phaseolina*. There were four plants in a pot and four replicates of each treatments. Leaf samples were collected twice; before flowering (at the end of June) and at the beginning of crop formation (in the middle of August). The nitrogen (N), phosphorus (P), potassium (K) and

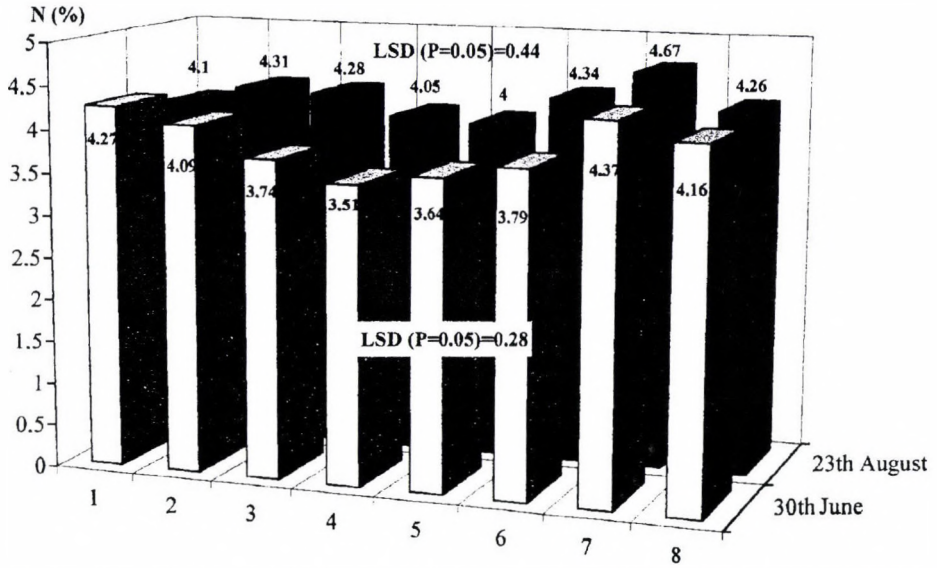

Fig. 1. The effect of the different infections on the nitrogen (N) content of pepper leaves. 1: Control; 2: CMV-U/246; 3: PVY^{NTN}; 4: *Macrophomina phaseolina*; 5: CMV-U/246 + PVY^{NTN}; 6: CMV-U/246 + *M. phaseolina*; 7: PVY^{NTN} + *M. phaseolina*; 8. CMV-U/246 + PVY^{NTN} + *M. phaseolina*

sodium (Na) content of pepper leaves was determined after destruction with sulphuric acid by a photometer. Flame photometer was used for the determination of K and Na content.

Result and Conclusions

The nitrogen content of pepper leaves varied between 3.51 and 4.67%, depending on the type of infection and vegetation time. The nitrogen and the phosphorus concentration of the pepper leaves was higher at generative, that at vegetative stage in case of all infection. Opposite effect was observed in case of the healthy plants, where the N content was higher at vegetative stage and P content did not change. The N content of the pepper leaves before flowering significantly reduced due to inoculation with PVY^{NTN}, *M. phaseolina*, CMV-U/246 + PVY^{NTN} and CMV-U/246 + *M. phaseolina*. Most severe (17.8%) reduction of N content of pepper leaves occurred in case of *M. phaseolina* infection as compared to healthy ones. The N content did not change significantly due to infection with CMV-U/246, PVY^{NTN} + *M. phaseolina*, CMV-U/246+PVY^{NTN} + *M. phaseolina*. Later (after flowering) – except PVY^{NTN} + *M. phaseolina* infection – there was no significant difference in N content of the leaves of the infected plants, as compared to the healthy ones (Fig. 1).

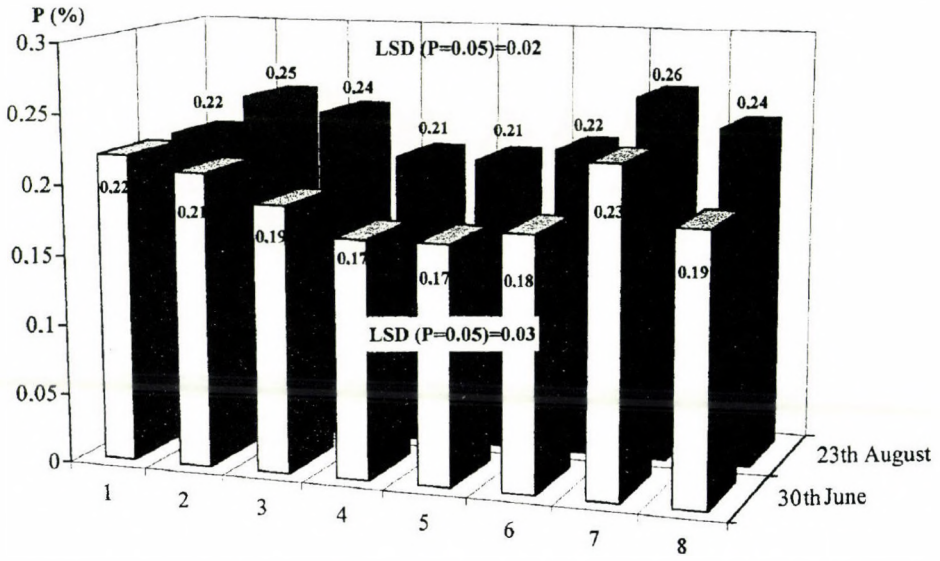

Fig. 2. The effect of the different infections on the phosphorus (P) content of pepper leaves. 1: Control; 2: CMV-U/246; 3: PVY^{NTN}; 4: *Macrophomina phaseolina*; 5: CMV-U/246 + PVY^{NTN}; 6: CMV-U/246 + *M. phaseolina*; 7: PVY^{NTN} + *M. phaseolina*; 8: CMV-U/246 + PVY^{NTN} + *M. phaseolina*

The P concentration in the pepper leaves was 0.17–0.26%. The P content was significantly reduced due to infections with PVY^{NTN}, *M. phaseolina*, CMV-U/246 + PVY^{NTN}, CMV-U/246 + *M. phaseolina* and CMV-U/246 + PVY^{NTN} + *M. phaseolina* at vegetative stage. Later (after flowering) infections did not reduce the P content of pepper leaves, but oppositely, the P content of plants infected with CMV-U/246 and PVY^{NTN} + *M. phaseolina* was higher, than that of the healthy ones (Fig. 2).

The K content of the leaves varied between 2.99 and 4.35%. It was the highest at vegetative period – suggesting the intensive growth stage of the plants – and reduced by the end of the vegetation period in all cases. The K content of leaves of the young pepper plants was reduced by 21.4, 17.2 and 15.9% due to infections with PVY^{NTN} + *M. phaseolina*, CMV-U/246 + *M. phaseolina* and CMV-U/246 + PVY^{NTN} + *M. phaseolina*, respectively. At the end of vegetation period there was no significant differences in potassium content of the leaves (Fig. 3).

The Na concentration of the pepper leaves has a comparatively constant value (0.01–0.03%). Either the different infections or the vegetation time did not influence significantly the Na content of the leaves (Fig. 4).

It is concluded that the infections has played an important role only in the early nutrient uptake of pepper and later no significant reduction were observed in the nutrient content of the infected pepper leaves as compared to the healthy ones. In case of several mixed infection with viruses and fungus the reduction in the nutrient content of the pep-

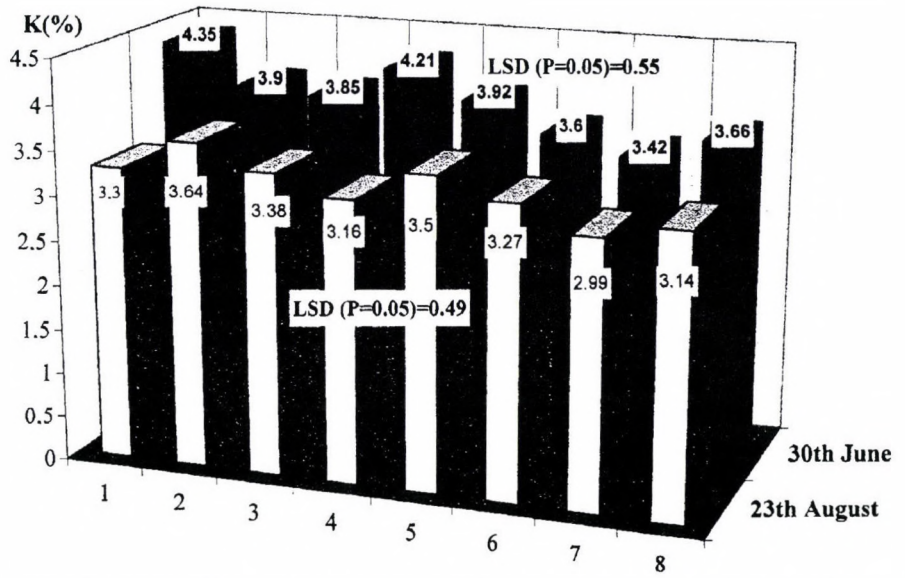

Fig. 3. The effect of the different infections on the potassium (K) content of pepper leaves. 1: Control; 2: CMV-U/246; 3: PVY^{NTN}; 4: *Macrophomina phaseolina*; 5: CMV-U/246 + PVY^{NTN}; 6: CMV-U/246 + *M. phaseolina*; 7: PVY^{NTN} + *M. phaseolina*; 8: CMV-U/246 + PVY^{NTN} + *M. phaseolina*

per leaves was greater. The different infections reduced not only the nutrient content but the fresh and dry weight of the pepper plants as well (Kazinczi et al., 1998).

The retarded nutrient uptake and development due to the infections may reduce the competitive ability and cause yield losses of the pepper plants as compared to the healthy ones.

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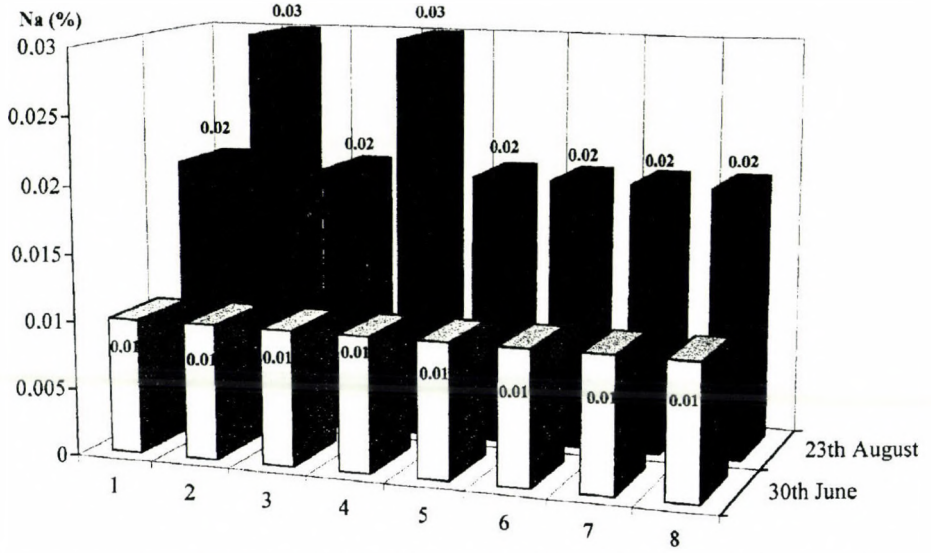

Fig. 4. The effect of the different infections on the sodium (Na) content of pepper leaves. 1: Control; 2: CMV-U/246; 3: PVY^{NTN}; 4: *Macrophomina phaseolina*; 5: CMV-U/246 + PVY^{NTN}; 6: CMV-U/246 + *M. phaseolina*; 7: PVY^{NTN} + *M. phaseolina*; 8: CMV-U/246 + PVY^{NTN} + *M. phaseolina*

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Occurrence of *Phomopsis longicolla* Hobbs on Soybean in Yugoslavia

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Several strains of *Phomopsis longicolla* have been isolated from soybean stems and seeds and their morphological and pathological characteristics studied. Morphological characteristics of *Phomopsis* strains are identical with those of *P. longicolla*, that were described by Hobbs et al. (1985), showing that *P. longicolla* is present in Yugoslavia as a new soybean pathogen.

Pathological characteristics of ten *P. longicolla* strains were studied, using the toothpick seedlings inoculation method and plant spraying inoculation with conidial suspension. Significant differences in virulence level were established among the fungus strains, especially after plaint infections with conidia. The symptoms caused by *P. longicolla* on soybean leaves, stems, pods and seeds were described.

Soybean is affected by several species from *Diaporthe/Phomopsis* genus which can cause numerous pathological changes on leaves, stems, pods and seeds. These diseases are named as the *Diaporthe/Phomopsis* disease complex of soybean (Kulik, 1983). Ploper (1989) cited the following fungi connected with soybean complex diseases: *Diaporthe phaseolorum* var. *caulivora*, agent of soybean stem canker, *Diaporthe phaseolorum* var. *sojae* and its anamorph *P. sojae*, the pod and stem blight pathogen, and *Phomopsis longicolla*, the agent of soybean seed decay. The species of *Diaporthe/Phomopsis* genus are economically important soybean pathogens, especially in the USA (Sinclair and Backman, 1989). However *Diaporthe phaseolorum* var. *caulivora* is economically important pathogen in Yugoslavia (Jasnić and Vidić, 1983, 1985), while *P. longicolla* is a new species, recently described in our country (Vidić and Jasnić, 1994). This species is most frequently and harmful pathogen of soybean seeds in the USA (McGee, 1992).

P. longicolla could be a potential threat to soybean in our and other European countries. For these reason a detailed comparatively study of the morphological characteristics and virulence level of *P. longicolla* strains from Yugoslavia and the USA could be of importance.

Materials and Methods

Fungi isolation

Using usual phytopathological methods numerous *P. longicolla* strains were isolated from soybean stems and seeds of infected plants.

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Morphological characteristics

Two groups of *P. longicolla* strains were chosen for morphological characteristics study, the first group originating from soybean stems, the second from soybean seeds. The fungal properties were studied on potato dextrose agar plates (acidifited to pH 4.5) incubated under continuous fluorescent light on laboratory bench at room temperature (20–25 °C). The development and shape of the colonies were monitored in the course of two weeks. The reproductive structures formation and their fructification were studied. Measurements of 100 alpha conidia and legth of picnidial necks were carried out from different strains. Conidiomata were cross-sectioned with a razor blade and the obtained fragments were colored and photographs taken on a light microscope.

Pathological characteristics

The pathological characteristics study included eight *P. longicolla* strains isolated from soybeans in Yugoslavia (Pl_1, Pl_8, Pl_{10}, Pl_{17} and Pl_{18} from soybean stems and Pl_3, Pl_{12}, Pl_{16} from soybean seeds) and two strains ($Pl_{5(A)}$ and $Pl_{RKV(A)}$) originating from the USA.

Two inoculation methods were used: a) the toothpick seedling inoculation (Keeling, 1982) and b) plants spraying inoculation with conidial suspension.

For the first method, soybean seeds (the line NS-L-2098) were sown in mid-May in plactic pots (16 cm in diameter) filled with a mixture of sterilized chernozem soil and peat at the ratio 3:1. The pots were kept in a vegetation shed. The experiment was established in three replications, each containing 10 seedling/pot (30 seedlings/variant). The seedlings were inoculated by inserting the tip of a toothpick overgrown with the mycelium of the *P. longicolla* strains into the hypocotyl. The seedlings in the control were inoculated by sterilized tootphicks. The final assessment of strains virulence were determinated by counting the number of wilted seedlings 10 days after inoculation.

The soybean plants (NS-L-2098) grown in Micherlich trays in a vegetation shed were used for the second method of inoculation. The sowing were done in late April, five plants/pot. The inoculation was performed in R6 stage. The inoculum was obtained from sterilized soybean stems inoculated with fungal strains according to the method of Frosheiser (1957). The experiment was established in four replicates, 20 plants/variant. The plants were sprayed with conidial suspension using portable sprayer and covered with wet polyethylene bags for five days. The plants in the control variant were treated with water. The occurrence and development of disease symptoms were followed through the vegetation period. After the plants matured, all plants in the experiment were assessed for disease intensity on the scale 0 to 9. Zero stood for plants having no visible simptoms, nine for the plants with stems completely covered with spots caused by *P. longicolla*. Disease index was calculated after Mc Kinney's formula, on the basis of disease intensity assessment. Reisolations were made from infected plants to verify the causal agent. The seeds from inoculated plants were visually observed to establish the percentual distribution of moldy seeds. Later, the presence of fungal mycelium in seed endosperm was analysed. One hundred seeds from each variant were isolated on PDA

medium. The isolated fungi were identified after the formation of mycelia and reproductive organs.

The data on the virulence of *P. longicolla* strains originating from Yugoslavia and USA were statistically processed by the analysis of variance for one – factorial experiment (each inoculation method separately). Differences among the strains were checked by multiple rang test (Duncan's test). Association between the inoculation methods and examined strains were determined by correlation coefficient.

Results

Morphological characteristics

PDA medium was favourable for the colony growth and reproductive organs formation of all *P. longicolla* strains. All strains studied exhibited no significant differences in the colony growth and development of fructification bodies.

Strains of *P. longicolla* produced a dense and compact white mycelium. Stromata occurred in masses and often extended over the entire bottom of culture dish (Fig. 1a). Pycnidial conidiomata formed in stromatic structures. These were solitary or often aggregated in groups (Fig. 1b). Conidiomata had very long prominent necks, mean length 337 μm (from 210 to 422 μm). All the strains studied had unilocular or multilocular conidiomata on transversal section (Fig. 1c). Multilocular conidiomata were dominant.

The *P. longicolla* strains formed only alpha conidia within the conidiomata. Alpha conidia were hyaline, non-septate, usually gutulate, and ellipsoid to fusiform (Fig. 1d). Mean alpha conidia dimension of the strains was 6.39 × 2.67 μm. *P. longicolla* did not form the perfect stage. On the basis of morphological characteristics of the studied strains we concluded that they belonged to *P. longicolla* species, the new soybean pathogen in Yugoslavia.

Pathological characteristics

The seedling toothpick inoculation method was very successful. The wilting of seedlings started only a few days (3–4) after inoculation and increased day by day. Most of *P. longicolla* strains exhibited high and uniform level of virulence, except strains Pl_1 and Pl_{17} which were significantly less virulent than the others (Table 1). Two strains originating from United States ($Pl_{5(A)}$ and $Pl_{(RKVA)}$) had a similar virulence degree, as they were more virulent than the strains from Yugoslavia. The seedlings wilting did not occur in control variant.

Plant spraying inoculation with *P. longicolla* conidial suspension caused the symptoms on all aboveground soybean parts. Large, sunken, circular or irregularly shaped lesions appeared as first symptom on the leaves. The lesions started to form at leaf margins and progressed inwards forming large necrotic areas. The spots were at first yellow-green to become dark brown with light brown center (Fig. 2a). The symptoms on

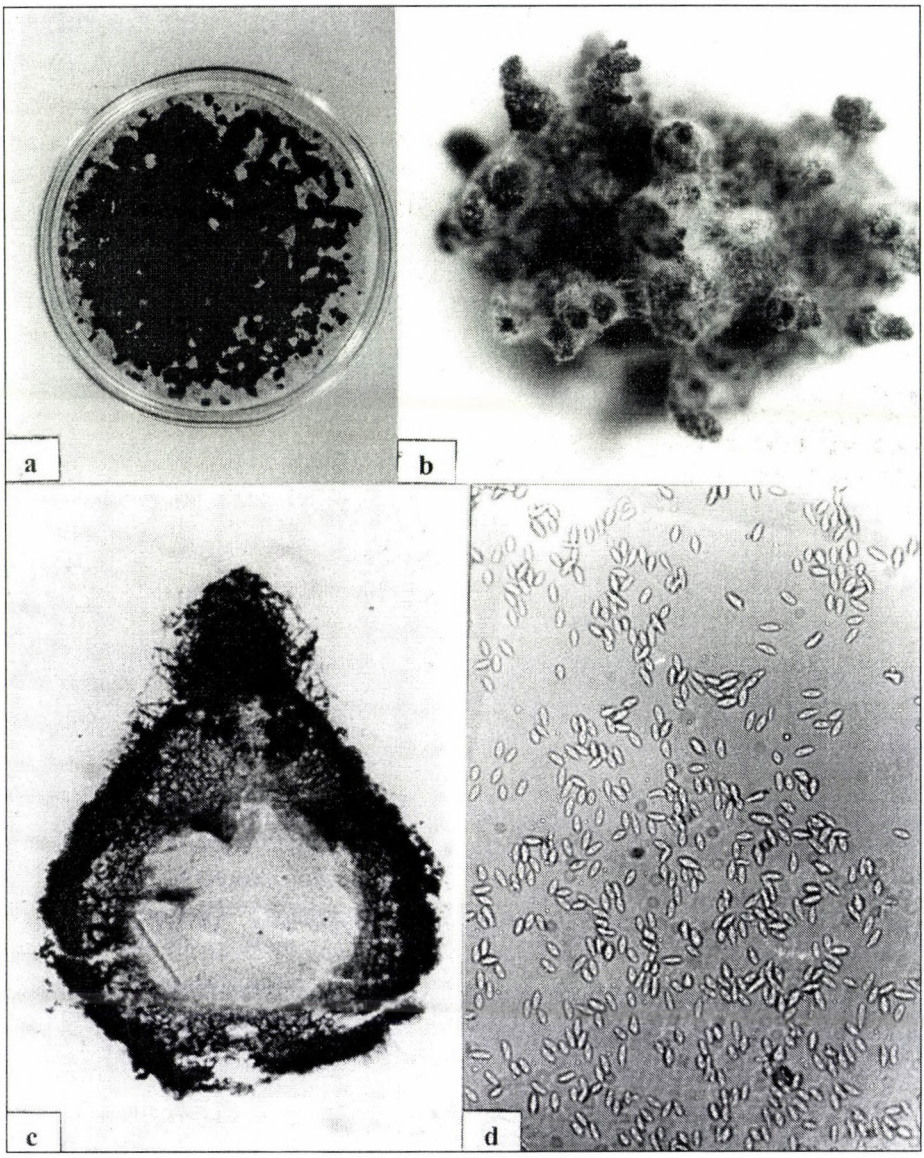

Fig. 1. *Phomopsis longicolla* – a) colonies appearance on potato dextrose agar (PDA) after two weeks development; b) conidiomata aggregated in stromatic structure with prominent necks formed on PDA; c) vertical section through the conidiomata with long neck; d) alpha conidia

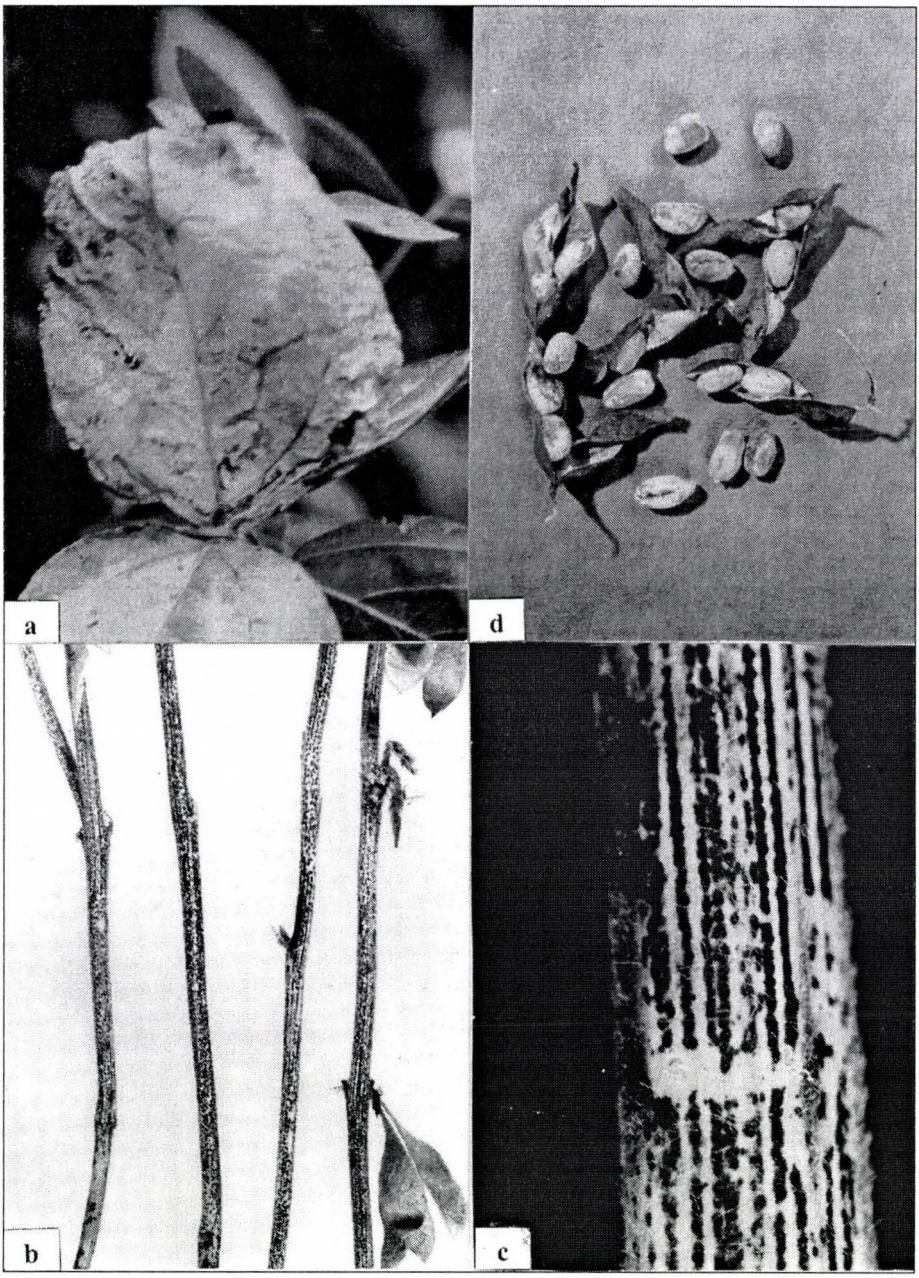

Fig. 2. Symptoms caused by *P. longicolla* – plant inoculation with conidial suspension a) large irregulary shaped lesions at the leaf margins; b) rooting of seeds; c) black patches on the soybean stems; d) picnidia arranged in vertical linear lines on stem (detail)

Table 1

Virulence of *P. longicolla* strains using two different inoculation methods

Strains	No. of witted seedlings*	Disease index in %**	% of moldy seeds**	% of infested seeds**
Pl_1	3.67 b	29.15 c	4.6	12.5
Pl_3	7.33 a	37.78 b	1.8	37.5
Pl_8	8.67 a	28.65 c	3.5	20.0
Pl_{10}	7.67 a	46.10 a	4.1	12.5
Pl_{12}	9.00 a	16.10 d	2.0	37.5
Pl_{16}	7.33 a	36.65 b	3.1	30.0
Pl_{17}	4.00 b	52.22 a	2.9	40.0
Pl_{18}	7.67 a	34.43 bc	6.3	55.0
$Pl_{5(A)}$	7.33 a	39.47 b	9.4	52.5
$Pl_{RKV(A)}$	8.33 a	30.00 c	2.4	15.0
Control	0.00 c	0.00 c	0.0	0.0
$LSD_{0.05}$	1.51	2.12	–	–

A – *P. longicolla* strains from USA
* – seedlings inoculation by toothpick metod
** – plants infection with conidia suspension

soybean stem, branches and pods started to develop when plants reached maturity. Numerous black pycnidia of the causal fungus developed on nodes of main stem spreading to adjacent internodes and forming black patches. The pycnidia were usually arranged in vertical linear lines (Fig. 2b, c). On the plants inoculated with certain strains the patches expanded to cover larger portions of the stem and lateral branches. The described symptoms are identical to those on soybean which cause the species *Phomopsis sojae* and it is impossible to distinguish them in the field.

When soybean plants were inoculated with conidial suspension of *P. longicolla* strains, they exhibited a higher variability in virulence level than the plants inoculated by the toothpick seedling method. The stem and pod disease index varied significantly and ranged from 16 to 52% (Table 1). Four strains groups of different virulence level, checked by the multiple rang test, can be distinguished.

Strains Pl_{17} and Pl_{10} were most virulent, while strain Pl_{12} was least virulent. The strains originating from USA fall into the groups of medium ($Pl_{5(A)}$) and weak ($Pl_{RKV(A)}$) virulence. The plants in the control variant did not show the stem and pod blight symptoms. Significant differences in strains virulence existed with respect to the method of inoculation. Strain Pl_{12} was most virulent with the toothpick seedling inoculation method and least virulent with plant inoculation with conidial suspension. The other *P. longicolla* strains showed similar variations in virulence caused by the method of inoculation. Negative correlation existed between the toothpick method and the method of spraying

with a suspension of conidia (r = –0.469, statistically non-significant) in the virulence level of the strains studied.

The symptoms were present on the seeds of inoculated plants. The wrinkling and splitting of seed coat, covered with fungus mycelium and rotting of seeds were observed (Fig. 2d). Strain $Pl_{5(A)}$ originated from USA caused the highest degree ofs eed rotting while strains Pl_{18} and Pl_{1} from Yugoslavia exhibited somewhat lower virulence. When reisolated, a high percentage of seeds that appeared uninfected developed the mycelium on the nutritive medium. Some strains (Pl_{18} and $Pl_{RKV(A)}$) infected more than a half of soybean seeds studied (Table 1). Positive correlation existed between stem and pod blight intensity and soybean seeds infection (r = 0.467) but this correlation was not statistically significant.

Discussion

The fungi from the genus *Diaporthe/Phomopsis* have been known for a long period as soybean pathogens, particularly in USA. In early 1920s the species *Phomopsis sojae* was described and soon after that the perfect stage of the fungus was described and named *Diaporthe phaseolorum* var. *sojae* (Lehman, 1922; 1923). Later, numerous significant morphological and pathogenic differences between some strains of this pathogen were reported. Hildebrandt (1954) observed that beside common pycnidia of *P. sojae* without the neck, the pycnidia with prominent long necks in large stromata on same soybean stems has been formed. Peterson and Strelecki (1965) reported in addition the presence of a non-characteristic "undeterminated species" as *Phomopsis* sp. on soybean seeds.

Later on, Kmetz et al. (1978) pointed out that besides *D. phaseolorum* var. *sojae* and *D. phaseolorum* var. *caulivora*, there was an "undeterminated species" of *Phomopsis* sp. causing soybean seeds rot. They suggested "Phomopsis seed decay" as the name of the disease. Having comparatively studied the strains of *P. sojae* and the "undeterminated species" *Phomopsis* sp., Hobbs et al. (1985) concluded that the *Phomopsis* sp. strains belonged to a new species. They designated it as *Phomopsis longicolla* Hobbs. Numerous morphological differences served the authors to separate the new species. The shape of pycnidial conidiomata, dimension of alpha conidia and the absence of beta conidia and the perfect stage of *P. longicolla* are the basic differences. Morphological characteristics of *Phomopsis* strains studied in this paper are identical with those of *P. longicolla*, that were described by Hobbs et al. (1985), showing that *P. longicolla* in present in Yugoslavia as a new soybean pathogen.

The pathogenicity tests of the strains showed that all strains studied exhibited a certain level of virulence, with both methods of inoculations. There existed a significant variability in virulence within some strains, especially with the inoculation method of spraying plants with a suspension of conidia. The toothpick seedling inoculation method was not used for *P. longicolla* infection study. This method was often used to study virulence of other species from the genus *Diaporthe/Phomopsis – D. phaseolorum* var.

cualivora (Keeling, 1982; 1984; Kilen et al., 1985; Janić and Vidić, 1983; Higley and Tachibana, 1987; Vidić, 1991). Although the method showed to be successful, it is very drastic and quite different from natural infection in the field. Therefore, the inoculation of plants by spraying wiht *P. longicolla* conidia is a more reliable method than the toothpick method for virulence characteristics study of this fungus. When this method was used in our study, certain *P. longicolla* strains caused severe stem blight (Table 1). This disease occurs often in all soybean-growing regions, but its influence on yield is minor. *P. longicolla* is the most dangerous pathogen of soybean seed (McGee, 1986; Gleason et al., 1987). The fungus strains studied in this paper caused also seed decay but not in a high percentage, except for strain $P1_{5(A)}$ originating from USA. The intensity of seed rotting would probably have been more severe, had the inoculation been performed in R7 stage. According to McGee (1986) the soybean is the most susceptible to the pathogen is this stage.

These studies indicated that *Phomopsis longicolla* is present on soybean in Yugoslavia. This fungus could become an important pathogen, especially on soybean seeds, and therefore it should be studied further.

Acknowledgement

We are grateful to Mr. R. T. Hanlii for providing fungal strains of *P. longicolla* from the United States.

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Variability of *Tilletia indica*, the Causal Fungus of Karnal Bunt of Wheat

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The biochemical variability of 12 isolates of *Tilletia indica* was recorded. The isolates varied considerably in their cellular fatty acids (gas liquid chromatography), as no two isolates showed exactly the same pattern. The lipids (simple thin layer chromatography and autoradiobiography) analysis exhibited the overlapping pattern. The micro- and macroelemental composition of mycelium also showed the wide range of variation in their concentrations.

Tilletia indica Mitra [= *Neovossia indica* (Mitra) Mundkur], the incitant of Karnal bunt of wheat is a local lesion pathogen that infects individual ovaries of wheat. Holton (1949) made some observations on *N. indica* on the basis of cultural characteristics. In the past chemical composition, response to externally applied chemicals, biosynthetic pathways or structural organizations of chemicals present in different groups of plant pathogens have been used as a parameter for their chemotaxonomic studies (Abel et al., 1963; Morris et al., 1966; Tyrell, 1967). Since the first report of the Karnal bunt disease (Mitra, 1931), there has not been any attempt to distinguish the existing strains of the pathogen on the basis of chemical composition.

In the present investigation, fatty acid pattern, lipid pattern and elemental composition of several isolates of *T. indica* were compared to provide more criteria for accurate specialization of the fungus.

Materials and Methods

Fatty acid pattern

PREPARATION OF SAMPLE

Twelve isolates of *N. indica* were grown on glucose yeast extract both at 20 + 1 °C for 15 days. The growth was harvested on fat free Whatman No. 1, mycelium was dried in an oven at 60 °C for 24 h. One gram of dried mycelium was crushed with 20 ml of 2:1 chloroform–methanol in a mortar pestle and was shaken for 10 min. The extract was filtered through a fat free Whatman No.1 paper. The filtrate was washed 3–4 times with 0.09% saline solution in a separating funnel and the lower chloroform layer was

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collected and evaporated to dryness under vacuum. The dried mycelial residue was refluxed with 20 ml of methanol containing 1% H_2SO_4 (v/v) for 2 h. Refluxed sample was evaporated to dryness and then dissolved in 10 ml of chloroform. Unesterified fatty acids were removed by adding 10 ml of saturated aqueous solution of sodium bicarbonate. Esters of fatty acids were extracted with chloroform three times using 14 ml of chloroform in every extraction. The combined extract was washed twice with distilled water and 1 mg of BHT was added to it. Extract was dried for 30 min over anhydrous sodium sulfonate and was reduced to 0.5 ml under vacuum.

Gas liquid chromatography

Fatty acid methyl esters were analysed with a RLOy Toshniwal gas chromatograph. A glass colum (8′ ×) packed with DEGS (Diethylene glycol succinate) was operated isothermally at 18 °C with N_2 flow rate of 50 ml/min. Packs were identified by comparison of the relative retention time of the standards.

Lipid pattern

PREPARATION OF SAMPLE

The isolates were grown in 250 ml Erhlenmeyer flasks containing 50 ml broth of glucose yeast extract with 0.62 μCi/ml ^{14}C-acetate. However, ^{14}C-acetate was not used for simple thin layer chromatography (TLC). The inoculated flasks were incubated at 20 + 1 °C for 15 days. After incubation mycelial growth was harvested in a Buchner's funnel and washed twice with distilled water and dried for 24 h at 60 °C in the oven. One gram dried mycelium was crushed in mortar and pestle and extracted three times each with 20 ml of 2:1 chloroform–methanol and filtered through Whatman No. 1 paper. The extracts were pooled together and evaporated to dryness under reduced pressure after adding a few crystals of BHT, an antioxidant. Residue was dissolved in 1 ml of chloroform–methanol (2:1).

Thin layer chromatography

PREPARATION OF PLATES

Glass plates (20 × 20 cm) were coated with 0.25 mm layer of silica gel slurry made by uniformly blending 30 g silica gel-G, containing 13% calcium sulphate with 64 ml of glass distilled water. Addition of few drops of methanol in the slurry greatly aided in the uniform coating of the gel. The coated plates were first dried at room temperature, then activated in an oven at 110 °C for 30 min.

Solvents

All solvents used were of analytical grade and were used without further purification. The following two methods were used for development:

First method for non-radioactive plates – Ethyl acetate-hexane (15:85). Second method for radio-activie plates (Pernes et al., 1980)

Following three solvent systems were used.

I. Chloroform–methanol–distilled water (65:25:5 v/v)
II. Chloroform–n-hexane (3:1 v/v)
III. Carbon tetrachloride

Spotting

Prepared plates were spotted with 20 µl of samples.

Development procedure

Development of the chromatograms was carried out at room temperature in covered glass thanks internally liner with filter paper to saturate the environment. The tanks were filled with solvent to a level of 10 mm at least 4 h before use.

In the first method a single run was given in the same direction up to the upper edge of plate.

In the second method 3 runs each in a different solvent system were given as described by Pernes et al. (1980).

Detection of spots

Non-radioactive plates were sprayed with 5% H_2SO_4 in ethyl alcohol. Radioactive plates were incubated at room temperature in a tank saturated with iodine vapour.

Autoradiography

Developed TLC plates were autoradiographed by sand witching them with Indu Screen X-ray films. Exposure time was 20 days. Exposed X-ray films were developed in solidex X-ray developer for 5 min at 20 °C and fixed in Amfix high speed fixer for 8 min. Both developing and fixing procedures were carried out under red safe light in the dark room.

Measurement of radioactivity

Silica gel from the area corresponding to lipid spots on autoradiochromatogram was removed from TLC plates and dissolved in 5 ml methanol. After filtration, the methanolic extract was evaporated to dryness and subsequently dissolved again in 0.5 ml methanol to which 9.5 ml of scintillation mixture was added. The radioactivity was measured by liquid scintillation counting using chanell ratio method.

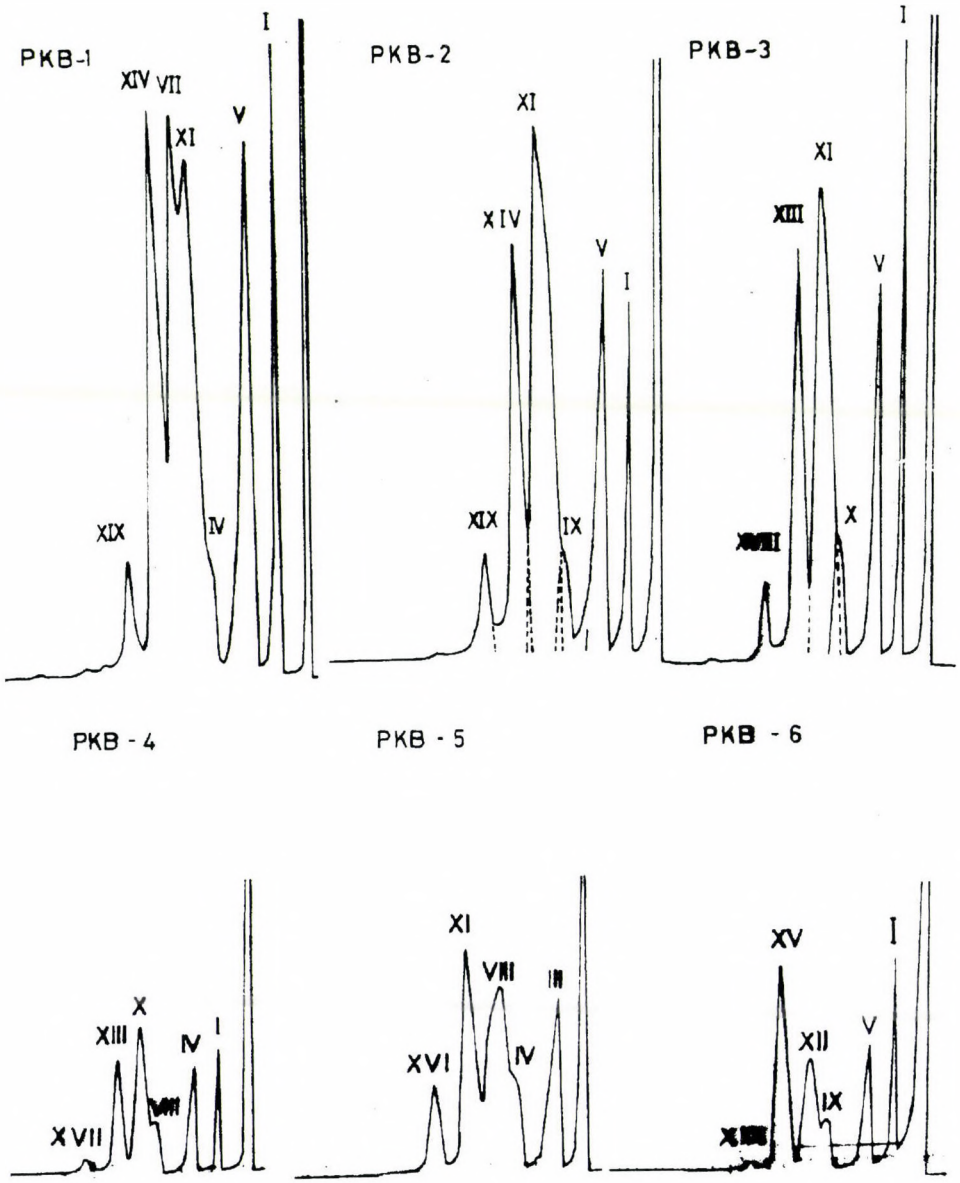

Fig. 1. Gas chromatographic pattern of fatty acids of *N. indica* isolates

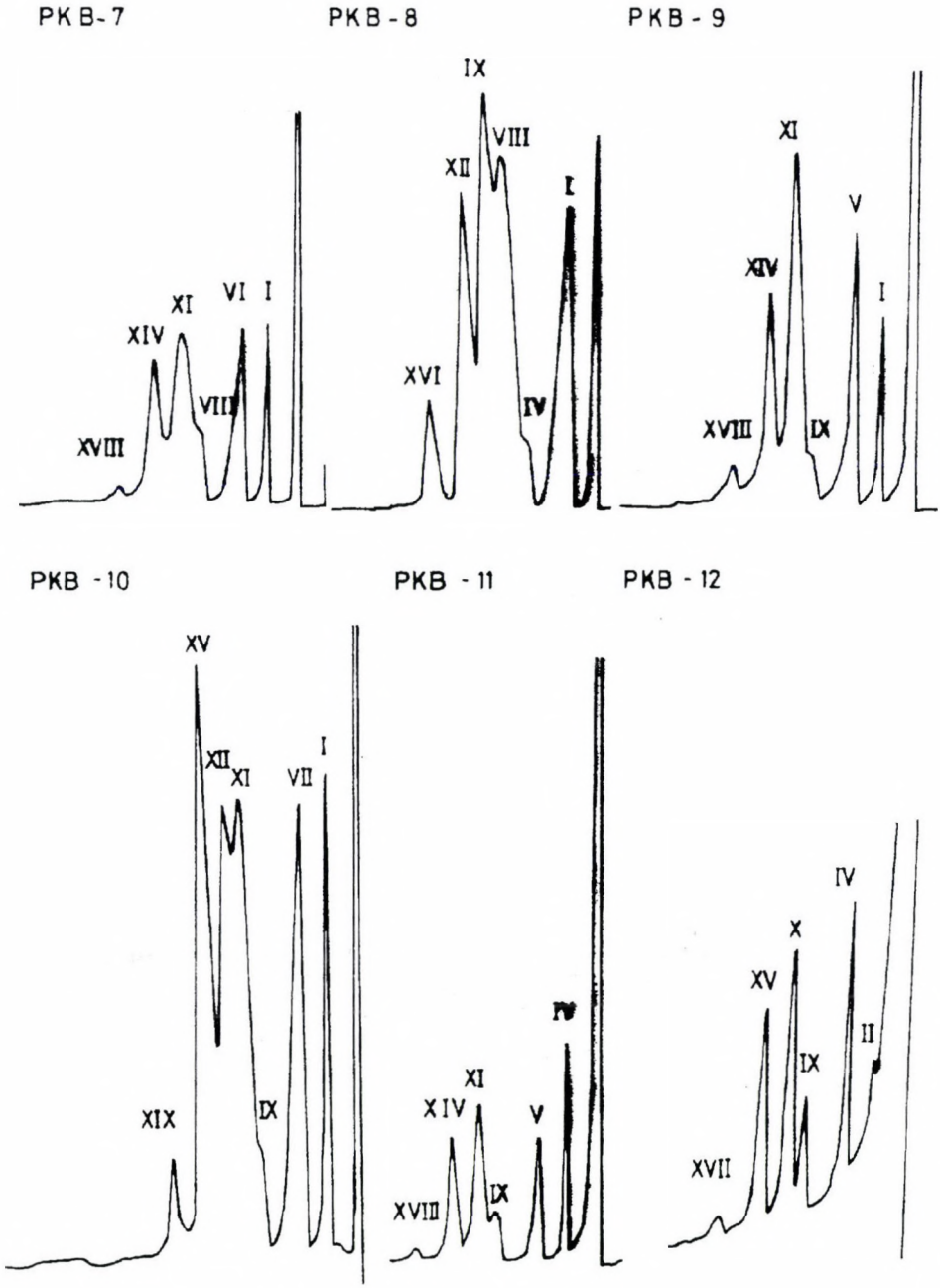
PKB-7
PKB-8
PKB-9
XVIII
XIV
XI
VIII
VI
I
XVI
IX
XII
VIII
IV
I
XVIII
IX
XIV
XI
V
I
PKB-10
PKB-11
PKB-12
XIX
XV
XII
XI
IX
VII
I
XVIII
XIV
XI
IX
V
IV
XVII
XV
X
IX
IV
II

Fig. 1. (cont.)

Table 1

Distribution and retention time (min) of fatty acid peaks from mycelial growth of twelve isolates of *N. indica* obtained in Gas Liquid Chromatographic analysis

Peak number	Isolate											
	PKB-1	PKB-2	PKB-3	PKB-4	PKB-5	PKB-6	PKB-7	PKB-8	PKB-9	PKB-10	PKB-11	PKB-12
I	2.8	2.8	2.8	2.6	–	2.6	2.6	2.8	2.8	2.6	–	–
II	–	–	–	–	–	–	–	–	–	–	–	3.2
III	–	–	–	–	3.8	–	–	–	–	–	–	–
IV	–	–	–	4.4	4.4	–	–	4.4	–	–	4.2	4.4
V	4.8	4.8	4.8	–	–	4.6	–	–	4.8	–	4.8	–
VI	–	–	–	–	–	–	5.0	–	–	–	–	–
VII	–	–	–	–	–	–	–	–	–	6.0	–	–
VIII	–	–	–	7.0	6.8	–	6.8	7.0	–	–	–	–
IX	7.6	7.6	–	–	–	7.6	–	7.4	7.6	7.6	7.6	7.4
X	–	–	8.2	8.2	–	–	–	–	–	–	–	8.4
XI	8.6	8.6	8.8	–	8.8	–	8.6	–	8.6	8.6	8.6	–
XII	9.0	–	–	–	–	9.8	–	9.2	–	9.2	–	–
XIII	–	–	10.0	10.0	–	–	–	–	–	–	–	–
XIV	–	10.6	–	–	–	–	10.4	–	10.4	–	10.4	–
XV	–	–	–	–	–	11.0	–	–	–	11.0	–	11.2
XVI	–	–	–	–	11.6	–	–	11.8	–	–	–	–
XVII	–	–	–	12.6	–	–	–	–	–	–	–	12.4
XVIII	–	–	12.8	–	–	–	13.0	–	13.0	–	13.0	–
XIX	13.6	13.4	–	–	–	13.6	–	–	–	13.6	–	–

Elemental analysis

Cultures were prepared as described in previous section. The dried mycelium mat was digested in 10 ml of concentrated HNO_3 till it was reduced to 0.5 ml. The residual amount was digested again with 10 ml of ternary acid (H_2SO_4–HNO_3–$HClO_4$, 0:10:4 v/v) until it was reduced to 0.5 ml to which 0.5 ml of 6 N HCl was added. All the samples were diluted with distilled water. All the elements were analysed by using atomic absorption spectrophotometer (Hitachi 207), except Na and K, which were analysed by using flame photometer.

Table 2

Relative area of fatty acids peaks on gas chromatogram of twelve isolates of *N. indica*

Peak number	Isolate											
	PKB-1	PKB-2	PKB-3	PKB-4	PKB-5	PKB-6	PKB-7	PKB-8	PKB-9	PKB-10	PKB-11	PKB-12
I	6.7	2.5	5.5	0.9	–	1.9	1.5	5.8	1.3	4.1	–	–
II	*	–	–	–	–	–	–	–	–	–	–	1.4
III	–	–	–	–	3.1	–	–	–	–	–	–	–
IV	–	–	–	1.1	1.5	–	–	1.0	–	–	1.8	3.4
V	9.4	6.1	5.3	–	–	1.3	–	–	3.3	–	1.2	–
VI	–	–	–	–	–	–	2.9	–	–	–	–	–
VII	–	–	–	–	–	–	–	–	–	7.7	–	–
VIII	–	–	–	0.63	5.3	–	1.1	10.3	–	–	–	–
IX	2.2	1.4	–	–	–	0.7	–	9.2	0.6	1.7	6.5	1.1
X	–	–	10.0	2.9	–	–	–	–	–	–	–	4.1
XI	18.2	13.1	1.4	–	4.7	–	4.0	–	6.1	16.3	2.7	–
XII	14.8	–	–	–	–	2.0	–	7.1	–	15.3	–	–
XIII	–	–	5.8	1.4	–	–	–	–	–	–	–	–
XIV	11.0	7.3	–	–	–	–	3.0	–	2.9	–	1.9	–
XV	–	–	–	–	–	3.2	–	–	–	13.1	–	2.9
XVI	–	–	–	–	1.2	–	–	1.5	–	–	–	–
XVII	–	–	–	0.2	–	–	–	–	–	–	–	9.2
XVIII	–	–	0.7	–	–	–	0.2	–	13.0	–	0.1	–
XIX	1.3	1.4	–	–	–	0.1	–	–	–	1.2	–	–

*Not detected

Results and Discussion

Variability of fatty acids

The gas liquid chromatographic analyses of cellular fatty acids extracted from mycelial growth of the twelve isolates of *N. indica* indicates that no two isolates have exactly the same pattern both qualitatively and quantitatively (Fig. 1). Total number of peaks obtained were 19. However, only peak number VII, VIII, IX and XI were identified by comparing standard fatty acids, which were eladiac, oleic, linoleic and linelenic acids, respectively. Retention time of peaks varied from 2.6 to 13.6 minutes (Table 1). Per cent peak area was also variable, in all the cases, the variation being highest in peak XVIII (Table 2). The above variation indicates that no group could be formed based on fatty acid pattern. Similar observations were also recorded by Panwar (1984) and Garg (1985) while attempting subspecific grouping in *Rhizoctonia solani* and *Neovossia horrida*, respectively. Attempts made by Abel et al. (1963), Dees and Moss (1975), Gunasekaran and Hunghes (1980) and Melhuish and Hacrkaylo (1980) using GLC pattern of fatty acids in classification have been successful only up to species level.

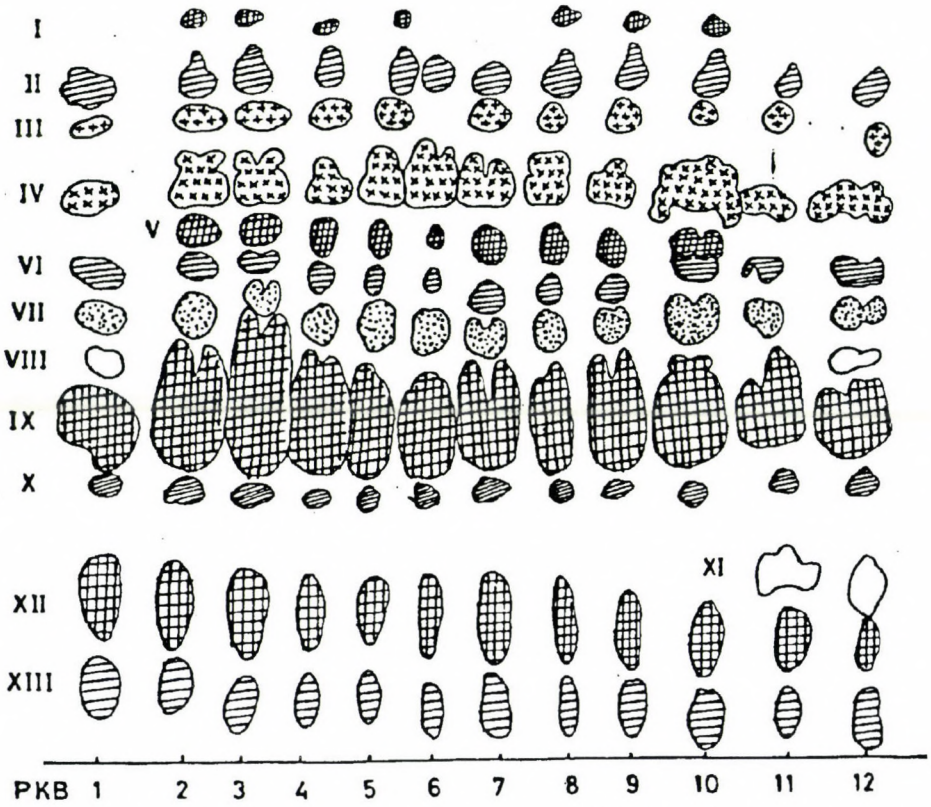

Fig. 2. Facsimile of thin layer autoradiochromatogram of lipids of *N. indica* isolates

Variability of lipids

The results obtained by TLC show that triple run in three different solvent systems was much better than single run in Ethyl acetate-hexane system. The maximum number of spots obtained by single run was 6 while the triple run gave maximum of 13 spots. Autoradiographic method of detection was for more sensitive than iodine vapours. The latter method developed only bigger spots with high lipid concentration. The spots which were too close showed as big blotches when developed in iodine vapours, while by autoradiographic method they could be distinguished clearly (Fig. 2). The concentration of each lipid measured as dpm g^{-1} dry weight by scintillation counting in individual isolates (Table 3) was found to be variable in all the twelve isolates. The variation in concentration and overlapping in spots does not permit to use lipid pattern alone for strain differentiation. In the past Tulloch and Ledingham (1960), Shaw (1966), Morris et al. (1966) and Garg (1985) have used lipid patterns for taxonomic purposes in Oomy-

Table 3

Relative concentration of (dpm g^{-1} dry weight) of lipids of twelve isolates of *N. indica* as obtained by scintillation counting

Peak number	Isolate											
	PKB-1	PKB-2	PKB-3	PKB-4	PKB-5	PKB-6	PKB-7	PKB-8	PKB-9	PKB-10	PKB-11	PKB-12
I	18.2	6.2	23.0	*	–	–	22.9	54.9	21.4	–	30.3	–
II	6.2	46.0	153.5	110.2	55.0	49.0	169.3	14.4	7.1	66.2	15.3	78.1
III	31.4	11.6	66.5	41.4	52.0	21.5	98.3	107.4	49.8	28.6	40.8	–
IV	300.1	229.1	940.6	468.3	747.3	222.7	525.4	606.8	1063.5	1479.6	259.7	416.7
V	10.4	29.2	18.3	–	22.7	–	35.1	22.4	8.3	–	24.9	44.6
VI	14.1	55.9	92.0	22.8	22.7	196.5	41.1	33.6	54.3	70.8	13.7	9.4
VII	22.7	101.5	222.5	69.4	59.7	32.3	61.3	27.5	20.8	166.2	25.5	10.8
VIII	–	–	–	–	–	76.0	–	–	–	–	–	
IX	965.4	1159.9	4456.0	282.7	1806.3	905.8	2732.4	1072.7	753.2	1262.0	1744.4	600.8
X	78.0	10.3	22.0	124.7	218.0	215.2	124.8	150.0	204.7	98.1	110.1	69.2
XI	–	–	–	26.6	–	–	–	–	–	18.9	–	–
XII	8.0	38.0	184.3	104.4	19.0	77.7	20.7	18.2	10.9	73.6	12.8	12.6
XIII	28.6	26.9	65.8	85.6	104.6	81.5	49.6	24.8	29.2	25.8	29.9	49.1

*Not detected

cetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti. But none of them could draw any definite conclusion. However, Panwar (1984) grouped *Rhizoctonia solani*, isolates based on their lipid pattern and two nonpathogenic isolates exhibited a common lipid, not found in other isolates.

Variability of elemental composition

The composition of the elements was found to be quite variable (Table 4). However, some isolates exhibited similar amounts of different micro- and macroelements. The variation shown by individual isolates makes it impossible to use this parameter in specific and subspecific classification of *N. indica*. Panwar (1984) also tried elemental analysis to group different isolates of *Rhizoctonia solani*, but she could not get success due to the variation of elements.

Since fatty acid pattern of different isolates exhibited too much variability to be used as a parameter for subspecific grouping, overall lipid pattern of different isolates was investigated. But, due to overlapping in spots and variation in concentration of individual lipids, this parameter cannot also be used as a sole criteria for strain differentiation. Same is true with elemental analysis also. However, other use of this can be made by adding of some microelements like Cr, Cd and Ni in totally synthetic medium in order to see their effect on growth. Because these elements were present in considerable

Table 4

Macro- and microelements composition (µg/g mycelium dry weight) in twelve isolates of *N. indica*

Isolate	Element µg/g dry wt									
	Cu	Fe	In	Mn	Cd	Ni	Cr	Pb	Na	K
PKB–1	51.0	163.1	120.0	60.0	1.8	76.4	1.2	16.75	600.0	30625.0
PKB–2	51.0	93.2	100.0	50.0	3.8	80.3	1.4	30.5	750.0	21875.0
PKB–3	85.0	116.5	116.0	90.0	1.8	36.0	0.8	33.4	987.5	28750.0
PKB–4	59.5	116.5	124.0	100.0	1.0	107.6	1.9	33.4	625.0	21875.0
PKB–5	102.0	116.5	126.0	60.0	1.8	37.5	1.2	33.4	687.5	15625.0
PKB–6	68.0	116.5	124.0	40.0	2.8	86.0	1.7	33.4	400.0	16875.0
PKB–7	51.0	116.5	132.0	70.0	2.5	73.5	0.6	30.0	675.0	21875.0
PKB–8	102.0	139.8	132.0	100.0	1.5	83.5	1.3	33.4	762.5	20000.0
PKB–9	59.5	139.8	134.0	90.0	2.5	26.6	0.6	26.7	737.5	24375.0
PKB–10	113.3	271.8	226.6	116.6	4.2	52.1	1.0	61.1	583.3	10416.2
PKB–11	68.0	139.8	140.0	90.0	2.8	18.8	0.6	36.7	575.0	18750.0
PKB–12	113.3	194.2	173.3	116.6	4.2	54.8	1.0	44.4	541.6	29165.5

amount in mycelium, the pathogen grown on such added medium can also be tried to inoculate wheat, in order to find out whether, these elements have any correlation with pathogenecity of the *T. indica.*

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Ortheziola saringeri sp. n. (Homoptera: Coccoidea, Ortheziidae) from Africa*

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A new species *Ortheziola saringeri* is described from Africa. The new data indicate that this group needs further study in different regions of Africa.

The genus was analysed in detail in the works of Morrison (1925, 1952), Kosztarab and Kozár (1988), and Kozár and Miller (1998). In these studies altogether 16 species were cited. The data show that the Ortheziola fauna of Africa is not poor, and needs further studies.

The aim of this work, is to continue the study of the Ortheziola genus in Africa, and to study the distribution pattern of the members of this genus.

Materials and Methods

This study present result of the analyses of 856 samples throughout Africa, with some additonal samples from Madagascar, Comoro, Reunion and Seychelles Islands. These insects were collected by Berlese funnel. The specimens studied are from the Collection of Arachnida of the Hungarian Natural History Museum, Budapest, Hungary.

All sizes of descriptions are given in microns. The insects studied are deposited as microscopic slides in the collection Plant Protection Institute, Hungarian Academy of Sciences, Budapest.

Result

A new species Ortheziola was found in our collection from Africa and described below.

*Dedicated to Professor Dr. Gy. Sáringer on the occasion of his 70th birthday.

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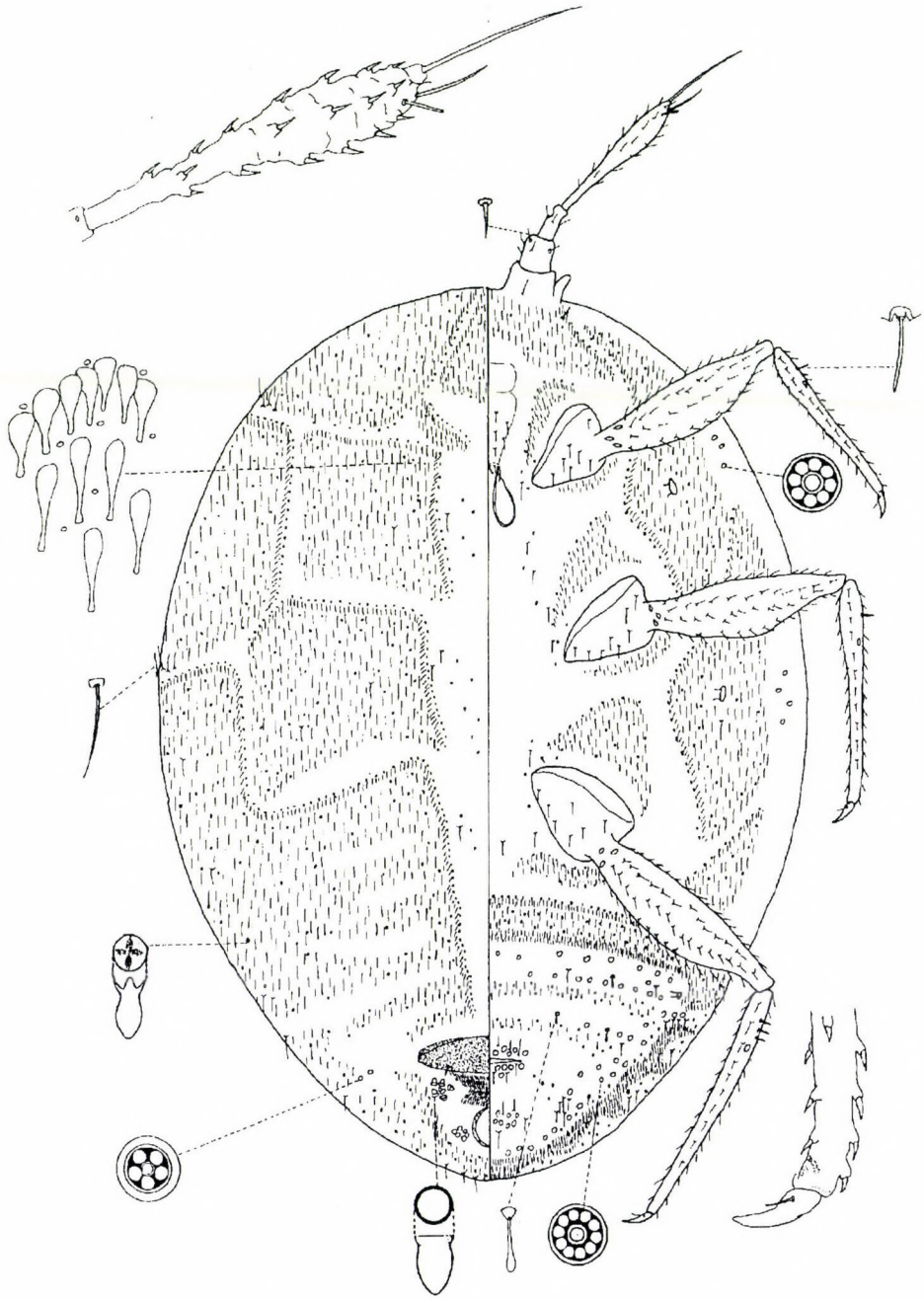

Fig. 1. *Ortheziola saringeri* sp. n., mounted female (holotype)

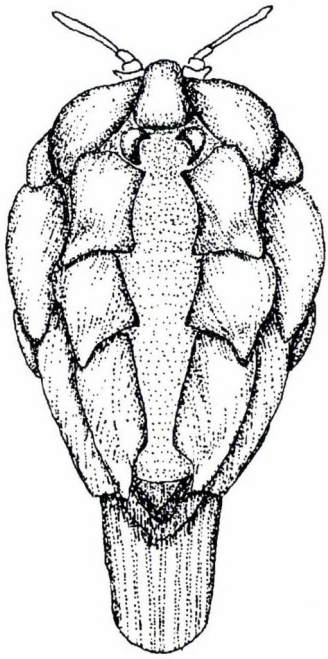

Fig. 2. *Ortheziola saringeri* sp. n., adult female

Ortheziola saringeri sp. n. (Figs 1, 2)

MATERIAL EXAMINED

Holotype, female, Angola, Dundo, forest, 7 iv. 1960 (A. de Barros Machado) (No. 14614-16) (No. 847 from the collection of S. *Mahunka*). Paratypes 4 female from the same collection as the holotype. One female, Angola, Cluango, 5. ix. 1961 (A. de Barros Machado) (No. 16805-23) (No. 846 from the collection of *S. Mahunka*).

DESCRIPTION OF ADULT FEMALE

Most of the dorsum covered with wax plates. Only a narrow band of the dorsum bare in the midline, whitest, the segmentation not visible (Fig. 2). Mounted specimen (Fig. 1) 1.6 mm long and 0.9 mm wide. Antenna 3 segmented, eye stalks fused with pseudobasal antennal segment, the size of the segments: 1st – 60 micrometer (in further μm) long and 55 μm wide, 2nd – 54 μm and 31 μm, 3rd – 230 μm and 40 μm. There is one sensory pore on the 2nd segment of the antenna. The 3rd segments are club-shaped. Apical seta of antenna slender 157 μm long, subapical seta 48 μm. Near to apical seta a flagellate sensory seta 24 μm long, and one microseta situated. The segments of the antenna are covered with small number of robust setae, mostly situated near to apex, 7 μm long Eye stalks elongate, thumblike.

VENTER

Labium one segmented, 118 μm long. Stylet loop shorter than labium. Legs robust: coxa of anterior legs 134 μm, trochanter-femur 294 μm, tibia and tarsus 307 μm, and claw 29 μm, claw digitules 6 μm long. Coxa of middle legs 134 μm, trochanter-femur 320 μm, tibia and tarsus 326 μm, and claw 29 μm, claw digitules 6 μm long. Coxa of posterior legs 141 μm, torchanter-femur 365 μm, tibia and tarsus 398 μm, and claw 36 μm, claw with hairlike digitules, 8 μm long. Claw without denticle. Legs with rows of robust setae 8 μm long, and with one sensory pore and one flagellate sensory seta 17 μm long on tibia of 1st and second legs and three on the third. Thoracic spiracle openings with a wide marginal band of wax plates, with additional triangular shaped bands around of the coxae, and with an additional interrupted band in front of the eggsack band. The margin in front of the spiracles a mall group of multilocular pores present, containing 3–4, 8-locular pores, 7 μm in diameter, and with 5–6 setae on dorsal side. The diameter of anterior spiracles 18 μm. Venter of thorax with a small number of scattered setae. Venter of abdomen with 2 interrupted in the middle line bands of multilocular pores 10 μm in diameter (10-locular in center with one pores) in front of the vulva. With two spine bands inside of the ovisac band. There are some hairlike and capitate seta on venter of abdomen. Abdominal spiracles visible posterior of the eggsack band.

DORSUM

The dorsal wax plate bands cover most of the dorsum. The wax plates situated at the margin of the bands 13 μm long, in the middle of the bands they are 17 μm long Some slender setae situated in dorsal plate bands and in bare segments. Some multilocular pores with 5 loculi and 5 μm in diameter are present at the margin of the abdomen. The sclerotized plate situated in front of the anal ring 61 μm wide and 198 μm long. Anal ring destroyed. At both sides of anal ring a group of thumblike pores visible. There are some five-locular pores on the dorsum of abdomen. The microtubular ducts (2 μm long) scattered on the dorsum around the wax plate bands and between plates and scattered on venter.

The species is named in honour of Professor Dr. Gyula Sáringer, member of the Hungarian Academy of Sciences, acknowledging his kind help in my entomological studies, and dedicated for his 70th year birthday.

COMMENTS

This species is distinct from other species of Ortheziola, especially by additional interrupted wax plate band in front of the eggsack band, knobbed shape, with a small number of robust seta of the third segment of the antenna.

Acknowledgements

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Data to the Spider Fauna (Araneae) of Kőrös-Maros National Park (Hungary)*

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In the first year of its arachnological investigations a total of 69 of spider species have been found in the Kőrös-Maros National Park, including some rarities (*Metopobactrus deserticola, Bathyphantes similis, Crustulina sticta*). One species, *Hahnia mierophthalma* is new to the Hungarian fauna.

This study presents the first arachnological results from a series of investigations of the fauna and flora of the Kőrös-Maros National Park (KMNP), SE Hungary. This region was declared as a national park in 1996. This part of the country is a mosaic of agricultural areas, old fields, the original loess steppe vegetation and secondary alkaline grasses. Floristical studies were made earlier (Molnár, 1992), but faunistical research started only in 1997 as a joint project of the Plant Protection Institute, Hungarian Academy of Sciences and the Hungarian Natural History Museum, Budapest.

Compared to other regions of Hungary our knowledge of the spider fauna of the Great Hungarian Plain is rather poor (Chyzer and Kulczynski, 1891, 1894, 1897; Kolosváry, 1928, 1932). Arachnological data is especially sparse from the region of the rivers Kőrös and Maros Loksa (1969) mentions the occurrence of *Lycosa singoriensis* and *Pardosa agrestis*. The only place from the region mentioned in the list of over 600 localities of Chyzer and Kulczynski (1897) was Kunágota.

Since investigations on the spider fauna of the Great Hungarian Plain was mentioned as a priority for future research by Prof. Gyula Sáringer (Szelényi et al., 1974), therefore we would like dedicate this work to him.

Materials and Methods

The material was collected between 21/05/1997 and 22/10/1997 in different types of habitats of KMNP. 1997 was devoted to identifying adequate sampling places for the studies of the next few years.

Three kind of sampling methods were applied: sweepneting (100 sweeps/sample),

* Dedicated to Professor Dr. Gy. Sáringer on the occassion of his 70th birthday.

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hand-held suction sampling, this technique was described by Samu (1995), and limb beating. For the reasons mentioned above the present contribution is restricted to reporting faunistical and taxonomica data only.

Figures 3–5 are K. Bleicher's works, Figs 1, 2 and 6–8 were made by É. Szita.

Results and Discussion

Faunistical part

The total catch in the area was 2039 spiders. The 567 adult specimens belonged to 69 species. In the following years this species list will probably increase significantly because of the large number of juvenile spiders which cannot be determined to species level. Table 1 reports all species we have found and the respective number of individuals collected.

Taxonomical part

One of the species, *Hahnia microphthalma* Snazell and Duffey, 1980 is new to the Hungarian fauna. *Metopobactrus deserticola* Loksa, 1981, *Bathyphantes similis* Kulczynski, 1894 and *Crustulina sticta* (O. P.-Cambridge, 1861) are rarely found in Hungary (Samu and Szinetár, in press).

Hahnia microphthalma Snazell and Duffey, 1980 *Figs 1, 2*

One female specimen was taken by sweepnet at Blaskovits heath in an old field which was an agricultural area until 1985. This spider is the third collected of this species in the world. The previous two ones were found in Great Britain by pitfall traps on a steep south-facing slope of chalk grassland which is grazed by cattle during some of the winter months (Snazell and Duffey, 1980). Snazell and Duffey found that the posterior median eyes (PME) of the specimen were reduced. In contrast to their findings in our specimen the PME were asymmetrical, one of normal size (nearly the same size as AME), the other somewhat reduced. This fact may probably mean that the reduction of PME is a newly acquired and not quite fixed character in *H. microphthalma*.

Metopobactrus deserticola Loksa, 1981 *Figs 3–8*

This species was described from the Hortobágy National Park by Loksa. Since that time it was not caught at all. We caught these spiders on saline steppes in a relatively large number (50 specimens). This habitat is similar to Kunmadaras from which Loksa originally reported this species. Although the two collecting sites belong to two national parks, the overall similarities and relative commonn characteres of the habitats and the short distance (c. 70 km) between them suggest that this species might be after all not so

rare in SE Hungary. In his 1981 paper Loksa gave drawings of the male palp and vulva. Here we would like to give a complete set of diagnostic drawings including the habitus of the animal.

Bathyphanthes similis Kulczynski, 1894

This species is rarely found in Europe. It was only mentioned in one case from Őrség (Western Hungary) (Szinetár, 1995). According to Miller (1971) it occurres in the undergrowth of woods.

Crustulina sticta (O. P.-Cambridge, 1861)

Locally distributed in Western and Central Europe. We have only few and old record under the name *C. rugosa* (Thorell, 1875) from Sátoraljaújhely and Kőszeg in Hungary (Chyzer, 1896; Kolosváry, 1943).

Table 1

The list of the species and respective number of individuals collected from the KMNP

Species/family	Total No. caught
Agelenidae	
Agelena gracilens C. L. Koch, 1841	2
Anyphaenidae	
Anyphaenidae juv.	7
Araneidae	
Aculepeira juv.	1
Araneidae juv.	28
Araneus juv.	30
Araneus quadratus Clerck, 1757	1
Argiope juv.	1
Argiope bruennichi (Scopoli, 1772)	13
Cercidia prominens (Westring, 1851)	1
Gibbaranea bituberculata (Walckenaer, 1802)	1
Hypsosinga juv.	172
Hypsosinga pygmaea (Sundevall, 1832	1
Hypsosinga sanguinea (C. L. Koch, 1844)	1
Larinioides juv.	9
Larinioides cornutus (Clerck, 1757)	2
Larinioides folium (Schrank, 1803)	3
Larinioides patagiatus (Clerck, 1757)	2
Singa juv.	29

Table 1 (cont.)

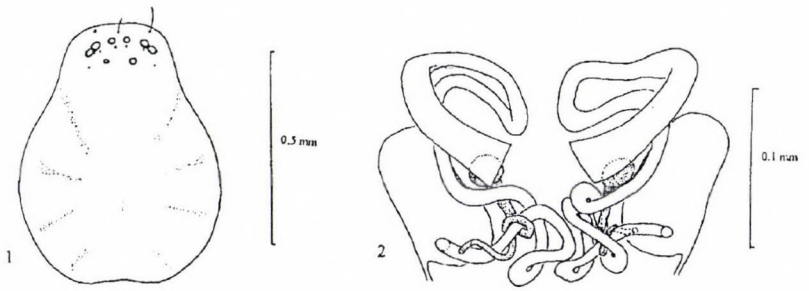
Species/family	Total No. caught
Clubionidae	
Cheiracanthium juv.	11
Cheiracanthium pennyi O. P.-Cambridge, 1873	6
Clubiona pallidula (Clerck, 1757)	1
Clubiona subtilis L. Koch, 1867	1
Clubiona juv.	29
Dictynidae	
Cicurina cicur (Fabricius, 1793)	1
Dictyna arundinacea (Linnaeus, 1758)	2
Dictynidae juv.	16
Gnaphosidae	
Gnaphosidae juv.	11
Zelotes juv.	12
Zelotes electus (C. L. Koch, 1839)	1
Hahniidae	
Hahnia juv.	4
Hahnia microphthalma Snazell and Duffey, 1980	1
Hahnia nava (Blackwall, 1841)	6
Heteropodidae	
Micrommata virescens (Clerck, 1757)	2
Linyphiidae	
Araeoncus humilis (Blackwall, 1841)	17
Bathyphantes gracilis (Blackwall, 1841)	15
Bathyphantes similis (Kulczynski, 1894)	1
Diplostyla concolor (Wider, 1834)	1
Erigonoplus globipes (L. Koch, 1872)	1
Gnathonarium dentatum (Wider, 1834)	15
Lepthyphantes quadrimaculatus Kulczynski, 1896	2
Lepthyphantes tenuis (Blackwall, 1852)	4
Linyphia triangularis (Clerck, 1757)	5
Linyphiidae juv.	463
Meioneta rurestris (C. L. Koch, 1836)	43
Meioneta saxatilis (Blackwall, 1844)	1
Meioneta simplicitarsis (Simon, 1884)	4
Metopobactrus deserticola Loksa, 1981	50
Microlinyphia impigra (O. P.-Cambridge, 1871)	1
Neriene clathrata (Sundevall, 1830)	4
Neriene furtiva (O. P.-Cambridge, 1870)	3
Oedothorax apicatus (Blackwall, 1850)	47
Oedothorax retusus (Westring, 1851)	1
Porrhomma microphthalmum (O. P.-Cambridge, 1871)	2
Silometopus reussi (Thorell, 1871)	4

Table 1 (cont.)

Species/family	Total No. caught
Stemonyphantes lineatus (Linneaeus, 1758)	1
Syedra gracilis (Menge, 1869)	4
Tallusia vindobonensis (Kulczynski, 1898)	11
Trichoncoides piscator (Simon, 1884)	10
Trichoncus hackmani Millidge, 1956	7
Trichopterna cito (O. P.-Cambridge, 1872)	46
Walckenaeria capito (Westring, 1861)	1
Liocranidae	
Agroeca brunnea (Blackwall, 1833)	2
Liocranidae juv.	1
Lycosidae	
Alopecosa juv.	1
Aulonia albimana (Walckenaer, 1805)	2
Lycosidae juv.	146
Trochosa robusta (Simon, 1876)	2
Mimetidae	
Ero cambridgei Kulczynski, 1911	1
Philodromidae	
Philodromidae juv.	6
Philodromus juv.	84
Philodromus cespitum (Walckenaer, 1802)	2
Thanatus juv.	12
Tibellus juv.	95
Tibellus oblongus (Walckenaer, 1802)	117
Pisauridae	
Pisaura mirabilis (Clerck, 1757)	65
Salticidae	
Euophrys juv.	7
Evarcha juv.	5
Evarcha arcuata (Clerck, 1757)	7
Heliophanus juv.	38
Heliophanus flavipes Hahn, 1832	2
Marpissa nivoyi (Lucas, 1846)	1
Salticidae juv.	17
Tetragnathidae	
Metellina segmentata (Clerck, 1757)	1
Pachygnatha juv.	16
Pachygnatha clercki Sundevall, 1823	1
Pachygnatha degeeri Sundevall, 1830	11
Tetragnatha juv.	4

Table 1 (cont.)

Species/family	Total No. caught
Theridiidae	
Crustulina juv.	1
Crustulina sticta (O. P.-Cambridge, 1861)	1
Enoplognatha juv.	3
Enoplognatha thoracica (Hahn, 1833)	7
Theridiidae juv.	59
Theridion impressum L. Koch, 1881	2
Thomisidae	
Heriaeus graminicola (Doleschall, 1852)	2
Misumenops tricuspidatus (Fabricius, 1775)	7
Ozyptila juv.	3
Runcinia juv.	5
Runcinia grammica (C. L. Koch, 1837)	6
Thomisidae juv.	7
Thomisus onustus Walckenaer, 1806	2
Xysticus juv.	73
Xysticus kochi Thorell, 1872	6
Xysticus striatipes L. Koch, 1870	32
Xysticus ulmi (Hahn, 1831)	3
Zoridae	
Zora juv.	12
Zora spinimana (Sundevall, 1833)	2



Figs 1–2. *Hahnia microphthalma*. 1 – prosoma; 2 – vulva ventral view

Acknowledgements

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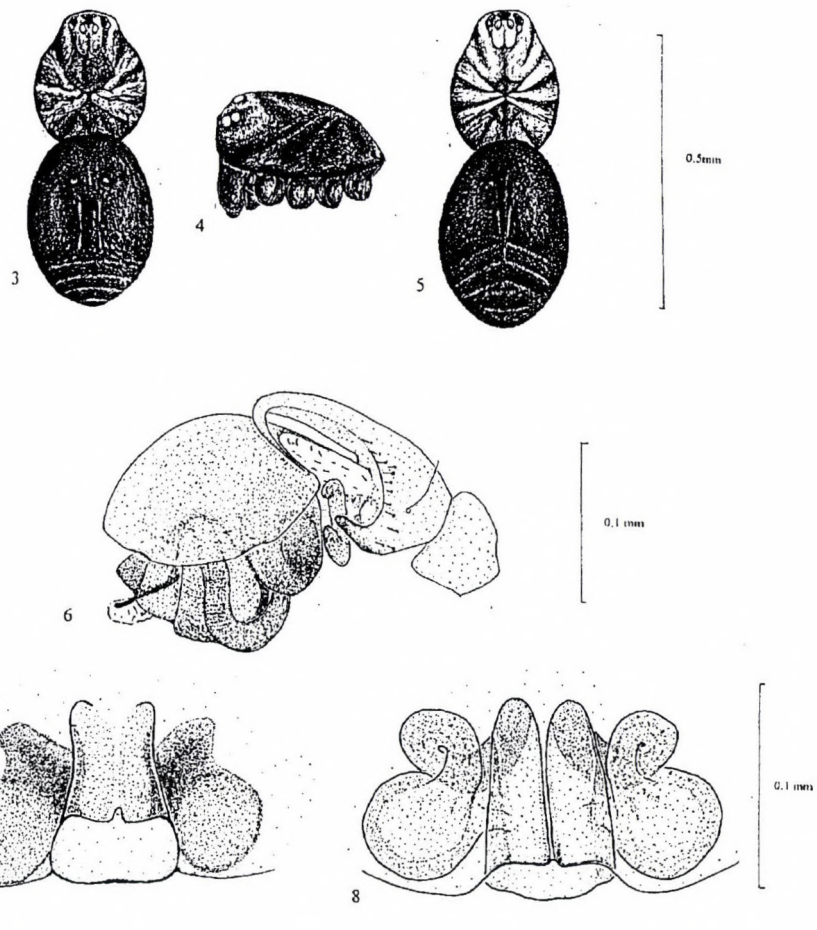

Figs 3–8. *Metopobactrus deserticola*. 3 – body colouration of male; 4 – male prosoma, lateral view; 5 – body colouration of female; 6 – male palpus, retrolateral view; 7 – epigyne; 8 – vulva, ventral view

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Preliminary Observations on Species Specificity of *Sitona lineatus* (L.) Pheromone Traps in Hungary (Coleoptera: Curculionidae)*

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In field tests in Hungary, traps baited with 4-methyl-3,5-heptanedione, the aggregation pheromone of the pea and bean weevil *Sitona lineatus*, caught significant numbers of several other species including *S. crinitus* (syn. *S. macularius*) and *S. humeralis*. Lower numbers of *S. suturalis, S. hispidulus* and *S. inops* were also caught. Of the species captured in higher numbers in this study, *S. crinitus* is one of the most important pests of peas in Hungary, and *S. humeralis* causes damage to alfalfa. The other species are usually regarded as pests of secondary importance. The fact that the *S. lineatus* pheromone trap caught other *Sitona* species is very useful, since crop damage usually results from attack by a complex of pests rather than a single species. A trap capable of catching several of the pest *Sitona* species will be able to detect and predict overall pest pressure more effectively than a trap highly selective for a single species.

Sitona species are important pests of various field crops in Central Europe. Adults migrate from overwintering sites to cultivated fields in early spring, and can cause considerable damage to seedlings of peas, beans, and many other fabaceous crops. Timely detection of migrating adult weevils would indicate if control measures are necessary, and ensure accurate timing of pesticide application.

Males of the pea and bean weevil (*Sitona lineatus* L.) (Coleoptera: Curculionidae), produce 4-methyl-3,5-heptanedione as an aggregation pheromone (Blight et al., 1984, 1991; Blight and Wadhams, 1987), and traps baited with this compound have caught significant numbers of *S. lineatus* in field trials in Britain (Blight et al., 1991; Biddle et al., 1996) and in Denmark (Nielsen and Jensen, 1993). The present, preliminary experiments were undertaken to study the performance of aggregation pheromone-baited traps in Hungary, and particularly, to determine whether the traps would catch *Sitona* species other than *S. lineatus*. As a second objective, we intended to test the suitability of a readily available sticky trap design as compared to the more sophisticated cone trap specifically developed for capturing *S. lineatus*.

* Dedicated to Professor Dr. Gy. Sáringer on the occasion of his 70th birthday.

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Materials and Methods

Baits

A sample of 4-methyl-3,5-heptanedione was purchased from Boris G. Kovalev (Kishinev, Moldavia) and was >95% pure by gas chromatography.

The following dispenser types were used:

RUBBER: pieces of rubber tubing (Taurus, Budapest, Hungary; No. MSZ 9691/6) extracted 3 times in boiling ethanol for 10 min, and then 3 times in methylene chloride and left overnight, prior to usage.

PE Kartell: 0.7 ml polyethylene vials with lid (No. 730, Kartell Co., Italy)

PE IACR: 2.5 ml polyethylene vials with lid (Just Plastics Ltd., London, UK), containing 25 µl (approximately 20 mg) of 4-methyl-3,5-heptanedione, giving a release rate of ca 250 µg per day. (These were prepared at IACR Rothamsted, and were sent by post to the Budapest Laboratory.)

For the other baits, the required amounts of the pheromone, in hexane solution, were applied either to the surface or the rubber or into PE Kartell vials. When the hexane had evaporated, the lids of the PE Kartell vials were closed and each vial was wrapped in aluminium foil. All dispensers were stored at –30 °C until use. For dosages tested see Table 1. No information on respective release rates was available for the rubber and PE Kartell dispenser types.

Traps

The CONE trap was the modified "Foundation" or "Scout" boll weevil trap, developed previously for the capture of *S. lineatus*, as described by Glinwood et al. (1993).

The STICKY traps were the "Delta" design normally used in Hungary for trapping moth species (Szőcs, 1993; Tóth and Szőcs, 1993). The trap body consisted of a transparent plastic sheet (23 × 36 cm), folded into a triangular prism (length 23 cm, all three sides 12 cm) with the two ends open. The pheromone bait was suspended centrally inside the trap. Insects entering the trap were captured on a replaceable sticky insert (16 × 10 cm) which was placed on the floor of the trap body. Traps were set up on the soil surface, and held in place with soil at the opening, to encourage crawling insects to enter.

The STICKY (yel.) trap was a Delta trap as described above, but had yellow plastic sheets (10 × 16 cm) placed on the soil in front of both openings to try to enhance the trap's attractiveness. Nielsen and Jensen, (1993) caught flying *S. lineatus* on pheromone-baited yellow sticky traps.

An unbaited STICKY Delta trap was used as the conrol.

Different trap/bait combinations were set up in a rectangle-shaped group. One group of traps contained one of each trap/bait combination in a randomized order. The distance between traps within a group was 10–15 m. The distance between groups ranged from 100–1000 m. Traps were moved one position forward within a block each time the

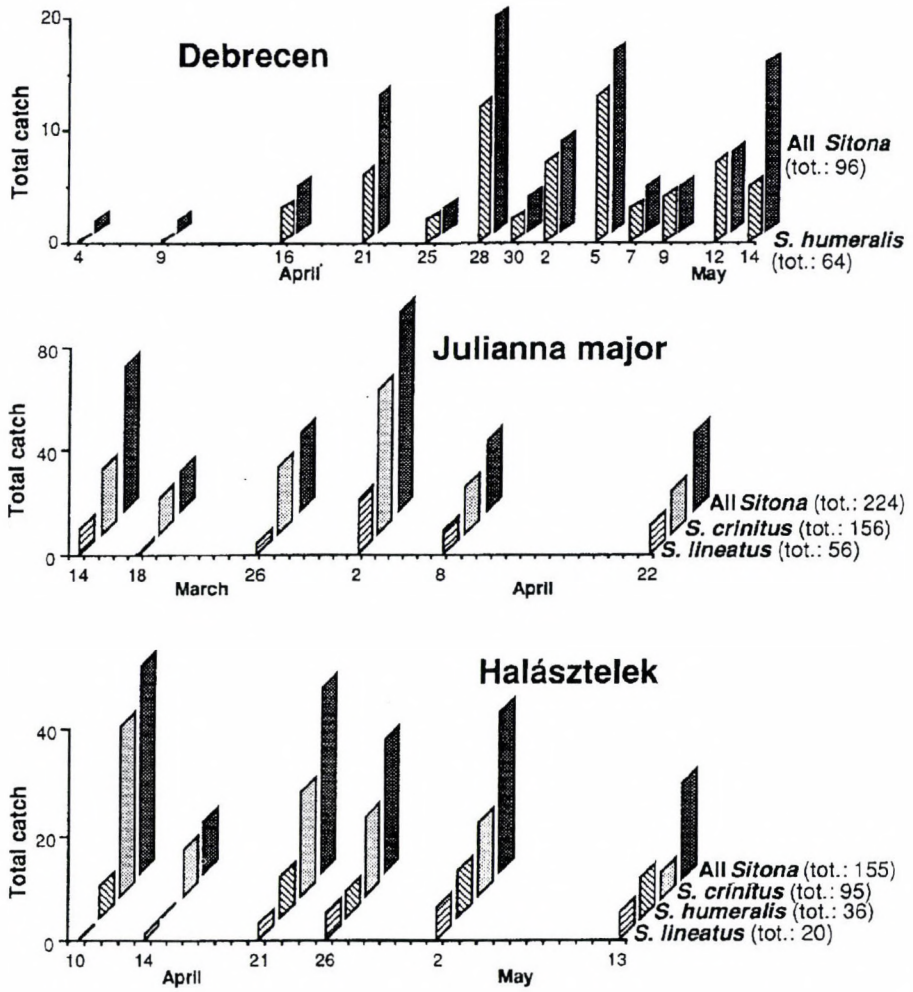

Fig. 1. Catches of *Sitona* spp. on single inspections in traps baited with 4-methyl-3,5-heptanedione at several sites in Hungary

traps were inspected (for inspection dates please refer to Fig. 1). At the same time, captured weevils were recorded and sticky inserts replaced, if necessary. Baits were replaced at 3 to 4 week intervals.

Sites

The trapping experiments were deployed at the following sites:

Debrecen: an alfalfa field near Debrecen, Hajdú-Bihar county, April 2–May 14, 1997.

Halásztelek: a weedy edge of an alfalfa field and young cherry orchard near Halásztelek, Pest county, April 4–May 13, 1997.

Julianna major: a weedy edge of an alfalfa field at the Julianna major Experimental Station of the Plant Protection Institute, Hungarian Academy of Sciences, Budapest, March 10–April 22, 1997.

Two parallel replicates (groups of traps containing one of each trap/bait combination) were tested at each experimental site.

Statistics

For statistical analyses, catches recorded at an inspection were regarded as replicates. Catch data were transformed to $(x + 0.5)^{1/2}$ and differences between means were tested for significance by ANOVA followed by Duncan's New Multiple Range Test (DNMRT) (Duncan, 1955). In case of a treatment catching nil (frequently so in unbaited traps) zero catch cannot be analysed by ANOVA, as it has no variation. In such cases differences between catches in individual treatments compared to the unbaited control were tested by a one-sample t-test. Statistical analyses were performed by the software packages StatView® v.4.01 and SuperANOVA® v1.11 (Abacus Concepts, Inc., Berkeley, USA).

Result and Discussion

Several *Sitona* species were captured in the trials (Table 1). In descending order of overall catch numbers, they were as follows:

S. crinitus Herbst (syn. *S. macularius* Marsham) – Most *S. crinitus* were captured at Julianna major and Halásztelek, and several trap/bait combinations caught significantly more weevils, than the unbaited control. Few were recorded at Debrecen.

S. humeralis Steph. – Most *S. humeralis* were recorded in the traps deployed at Debrecen, where all trap/bait combinations caught significantly more than the unbaited traps, which caught nothing. At Halásztelek, where moderate catches were observed, only the CONE trap/IACR PE bait combination was statistically different from unbaited traps, although several weevils were caught in the other baited combinations.

S. lineatus L. – Like *S. crinitus, S. lineatus* were caught in relatively large numbers at both Julianna major and Halásztelek, but very few were caught at Debrecen. Several of the trap/bait combinations at Julianna major, and the CONE trap/IACR PE bait combination at Halásztelek caught significantly more weevils than the unbaited traps, confirming the effectiveness of traps baited with the aggregation pheromone (Blight et al., 1991; Nielsen and Jensen, 1993).

Table 1

Catches of *Sitona* spp. in traps baited with 4-methyl-3,5-heptanedione at several sites in Hungary

Treatments			Catches by species and sites (totals, followed by mean/trap/inspection in parentheses)												
			S. crinitus			*S. humeralis*			*S. lineatus*			*S. suturalis*	*S. hispidulus*		*S. inops*
Trap type	Dispenser	Dosage (µl)	Julianna m.	Halásztelek	Debrecen	Debrecen	Halásztelek	Julianna m.	Julianna m.	Halásztelek	Debrecen	Julianna m.	Debrecen	Halásztelek	Debrecen
CONE	PE IACR	25	27 (2.25a)	14 (1.17abc)	6	28* (1.27b)	15 (1.25b)	0	30* (2.50a)	12* (1.00b)	0	4* (0.33a)	0	1	1
STICKY	PE IACR	25	29 (2.42ab)	0	0	11* (050a)	1 (0.08a)	2	10* (0.83a)	0	1	1 (0.17a)	1	0	1
STICKY	PE Kartell	20	27 (2.25a)	18 (1.50bc)	0	7* (032a)	2 (0.17a)	0	6* (0.50a)	0	2	4* (0.33a)	2	0	0
STICKY	rubber	20	70 (5.83b)	14 (1.17abc)	1	7* (0.32a)	5 (0.42ab)	0	10* (0.83a)	1 (0.08a)	1	0	1	2	1
STICKY (yel.)	rubber	20	not tested	25 (2.08c)	not tested	not tested	3 (0.25a)	not tested	not tested	3 (0.25a)	not tested	0	not tested	0	not tested
STICKY	rubber	5	not tested	23 (1.92c)	not tested	not tested	8 (0.67ab)	not tested	not tested	4 (0.33a)	not tested	1 (0.17a)	not tested	1	not tested
STICKY	unbaited	0	3 (0.25a)	1 (0.08a)	0	0	2 (0.17a)	0	0	0	0	0	0	0	0

* A means followed by same letter within one column are not significantly different at P = 0.05 by ANOVA followed by DNMRT. Catches followed by an asterisk are significantly different from zero catch of the unbaited traps at P = 0.05 by one-sample *t*-test

S. suturalis Steph. – This species was only caught at Julianna major. Although the catch of the CONE trap/IACR PE and STICKY trap/PE Kartell variations was shown to be significantly different from that of the unbaited traps, due to the overall low numbers caught this may only be a preliminary indication of possible attraction, which needs to be confirmed in future tests.

Other *Sitona* species. – Specimens of *S. hispidulus* Fabr. and *S. inops* Gyll. were also captured in some of the trap/bait combinations, but the catches were too low for statistical analysis. It is notable, however, that no catches were recorded in the unbaited traps.

Far-reaching conclusions cannot be drawn when comparing the performance of the different trap/bait combinations, due to the low numbers caught (Table 1). The CONE trap performed somewhat better than the STICKY trap types for capture of *S. lineatus* and *S. humeralis*, but the STICKY traps were equally as good at catching *S. crinitus*. Further minor modifications and improvements to this trap in future trials could lead to the development of a monitoring system made from a locally available, cheap trap design.

There was no striking difference between rubber and polythene as dispenser substrates (Table 1).

The trend of catches on the respective sites (Fig. 1) indicates that pheromone-baited traps can be useful indicators of *Sitona* weevil activity at or near to overwintering sites in Hungary. At Julianna major, where traps were deployed earlier, weevil activity was detected in March despite unusually low temperatures in this period in 1997. Evidently at Halásztelek, weevils were already active when trapping started, hence the large catches at the first inspection date. At Debrecen, highest numbers were recorded in the second half on April, when *S. humeralis* accounted for approximately two-thirds of all *Sitona* caught. Catches at all sites began to decline after the end of April, and trapping was not continued beyond the middle of May. This was partly due to lack of funds, and partly because Biddle et al. (1996) reported a decrease in catches of *S. lineatus* in pheromone-baited traps at the end of spring.

The results suggest that 4-methyl-3,5-heptanedione is attractive to *S. crinitus*, and *S. humeralis* as well as to *S. lineatus*. Further investigation is required, but this compound may also be present as a component of the natural pheromone of the respective species.

Among Coleoptera, it is not uncommon for the same compound to be present in the pheromone of several species. A remarkable example was reported by Williams et al. (1995) for *Carpophilus* spp. (Coleoptera: Nitidulidae), where 9 species share different combinations of similar aggregation pheromone components, resulting in cross-attraction of synthetic baits within selected groups of species. Several components of the pheromone of the boll weevil, *Anthonomus grandis* Boheman (Tumlinson et al., 1969), were found to be present in the pheromone of other curculionid species, e.g. *Curculio caryae* Horn (Hedin et al., 1979), *Pissodes* spp. (Booth et al., 1983) and, more recently is *Anthonomus eugenii* Cano (Eller et al., 1994). Such nonspecificity for the *S. lineatus* pheromone would be highly advantageous, as damage on a given site and in a given crop is usually caused by a *Sitona* species complex rather than by a single species alone. A

pheromone trap capable of catching several pest *Sitona* species would be more accurate and sensitive in detecting the threat of infestation for a range of crops, and therefore more practical, than a trap highly selective for a single species.

Of the species captured in pheromone traps in this study, *S. crinitus* is one of the most important pest *Sitona* species in Hungary, causing damage predominantly to peas and, to some extent, alfalfa (Sáringer, 1990). *S. humeralis* is mainly a pest of alfalfa, while *S. suturalis* is usually regarded as a pest of secondary importance (Sáringer, 1990).

Although no quantitative comparison with actual population densities was attempted in the present study, it was felt that capture rates were somewhat low and highly variable between traps and inspection dates. To smooth out the variability in trap catch associated with trap position, the deployment of at least three traps per site was suggested by Biddle et al. (1996) when monitoring for *S. lineatus*. The addition of attractive host-plant volatiles may increase the attraction of traps baited with 4-methyl-3,5-heptanedione for other *Sitona* species. Some plant volatiles attractive to *Sitona* species have already been identified (Blight et al., 1984; Landon et al., 1997), however, further investigation of the pheromones produced by these species may be more productive, and ultimately lead to a more effective trap.

Acknowledgements

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A Simple Method for Separating Beneficial Parasitoids of the Alfalfa Seed Chalcid (*Bruchophagus roddi*) from Alfalfa Chaff*

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A simple method has been established to separate diapausing larvae of beneficial hymenopterous species from alfalfa chaff by a pneumatic separator, so that these parasitoids can later be recycled as biocontrol agents against the alfalfa seed chalcid, *Bruchophagus roddi* Gussakovsky (Hymenoptera: Eurytomidae). *Tetrastichus (Aprostocetus) bruchophagi* Gahan (Hymenoptera: Eulophidae), an abundant parasitoid of *B. roddi* in North America and in Europe, can be obtained from chaff collected during normal seed harvest, by precisely adjusted floating rate cleaning. A chaff fraction collected between floating rates 25–27.5 m^3/h (in terms of air speed: 2.77–3.05 m/s) by a Petkus K-293 pneumatic separator (Wutha, former East-Germany) was found to be rich in alfalfa seeds with a diapausing *T. bruchophagi* larva inside, but practically free from seeds infested by non-parasitized *B. roddi*. The distribution of two other beneficial parasitoids, *Liodontomerus perplexus* Gahan and *Habrocytus medicaginis* Gahan (=*Pteromalus sequester* Walker) (Hymenoptera: Torymidae and Pteromalidae, respectively), as well as of their host, *B. roddi* in fractions collected at stepwise increased floating speeds was also recorded.

The yield of alfalfa seed production is often drastically reduced by the preharvest attack of seed boring insects. The alfalfa seed chalcid, *Bruchophagus roddi* Gussakovsky (Hymenoptera: Eurytomidae), which is one of the most destructive pests throughout the cultivation area of alfalfa, was found to cause up to 47% seed-damage in Hungary (Erdélyi et al., 1979), and up to 80% seed-damage in the U. S. A. (Urbahns, cit. App and Manglitz, 1972). In Russia, *B. roddi* and other seed-boring insects caused 53% seed-damage (Ivanov, 1980). Apart from any possibility of controlling larvae of the seed chalcid which lives under the protection of pod and seed shell, pesticide applications against adults have also been hampered by the concidence in the flight period of *B. roddi* and alfalfa pollinators. Agrotechnical recommendations, such as retaining chaff at harvest in the threshing mashine and then eliminating it (Manninger et al., 1958), or burning chaff (Pedersen et al., 1972) have not been adopted into the technology.

B. roddi hosts a number of parasitoids (Butler et al., 1968; Erdélyi, 1994; Kolobova, 1959; Nikol'skaya, 1932). Although it has been suggested that some of these parasitoids can be promising biological control agents against *B. roddi* (Aeschlimann and Vitou, 1989), no method of their practical application has been developed till now. These beneficial parasitoids, just as *B. roddi*, belong to the long-day type of insects with facul-

*Dedicated to Professor Dr. Gy. Sáringer of the occasion of his 70th birthday.

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tative diapause (cf. Sáringer, 1976). This enable us to keep the beneficial parasitoids, which are already in the stage of diapause at the time when commercial seed cleaning is performed, in prolonged diapause, and later, at a desired time, recycle them into the alfalfa field to be protected and let them emerge.

Here we report on a simple method which allows efficient collection of diapausing parasitoids from chaff at normal alfalfa-seed harvest. These parasitoids can then, at the desired time, be used as biocontrol agents against *B. roddi.*

Materials and Methods

The alfalfa chaff sample was collected at the cleaning barn of the Kompolt Agricultural Research Institute of the Gödöllő Agricultural University (Kompolt, Hungary) in December, 1992. The chaff was from freshly air-cleaned seed harvested in the the previous months in a nearby region, and, according to the commercial technology, was waiting under ambient temperature for transportation to the site of elimination. Such chaffs contain lot's of alfalfa seeds, which do not reach the weight settled by international regulations for sowing-seed. Many of these seeds are infested by *B. roddi*, which usually hosts beneficial parasitoids. Seeds containing a wasp larva inside are always lighter than sound seeds of the same geometrical size; at commercial air cleaning all alfalfa seeds infested by wasps go to the chaff. From this commercial cleaning barn the chaff was taken into the laboratory of the Plant Protection Institute and stored at +5 °C for more than four months. Out of several other similar samples this one was selected for the experiment because according to a preliminary check it contained the greatest variety and highest density of parasitoid species.

Floating separation of ca. 4 kg of the sample was carried out on a precisely variable floating rate pneumatic separator (Petkus K-293, Wutha, Thüringen, former East Germany) in the National Inspectorate for seed and Vegetative Reproduction Material. The fractionating protocoll was as follows: The whole samples was separated into two at the lowest chosen floating rate (15 m^3/h). The resulting "lighter fraction" was taken away as the first ready-made fraction, while the "heavier fraction" was separated again into two at the next floating rate (20 m^3/h). The resulting "lighter fraction" (15–20 m^3/h) served the second ready-made fraction, while the heavier fraction was separated again at the next floating rate, etc. The sequence of stepwise increased floating rates, used in this protocoll is as follows: Values are given in m^3/h, as the control flow-meter of the Petkus pneumatic separator used in this study was calibrated in that units. They correspond with the following airspeeds in m/s, according to calculation based on the geometrical parameters of the separating flight tunnel of this instrument: 15 m^3/h to 1.66 m/s, 20 m^3/h to 2.22 m/s, 25 m^3/h to 2.77 m/s, 27.5 m^3/h to 3.05 m/s, 30 m^3/h to 3.33 m/s, 32.5 m^3/h to 3.61 m/s, 35 m^3/h to 3.88 m/s, 37.5 m^3/h to 4.16 m/s, 40 m^3/h to 4.44 m/s, 42.5 m^3/h to 4.72 m/s and 45 m^3/h to 4.99 m/s.

The following seed cleaning parameters were measured in each fraction, according to international protocols (International Rules, ISTA, 1976): 1000 seed weight (the

average weight of 1000 alfalfa seeds; calculated from mean weights of four samples, each consisting of 500 randomly chosen alfalfa seeds), the percentage of the weight of all alfalfa seeds (from a part of a fraction which contained 2000 alfalfa seeds the rest, which consisted of alfalfa pod and stem debries and seeds of weed species, was separated, both the 2000 alfalfa seeds and the rest part were weighed, then the percentage was calculated). The total weights of the whole fractions were also measured. Fractions then were taken back to the laboratory of the Plant Protection Institute and 100 g aliquots (or the total fraction, if it weighed less than 100 g) were taken out and placed in the bottom of glass jars (vol 4 l) in a layer of 1 cm thickness. Jars were covered by linen sheets and kept under constant + 26 °C temperature and 18/6 light/dark fotoregime. Emerging adult wasps were collected daily, their species identity and sex were determined.

To determine the weight of wasps, dead insects, which were allowed to dry out at room temperature for more than a year, were measured by hundreds. Insects were emerged in the laboratory from the above-mentioned, as well as from similar alfalfa chaff samples.

Results

Fractions in order of increasing floating rate contained alfalfa seed populations of gradually increasing 1000 seed weight (Fig. 1A). No seeds were found in the fraction below 15 m^3/h floating rate. Then, in the next fraction (between 15 and 20 m^3/h floating rates) the 1000 seed weight was 0.225 gram. Heaviest seeds occurred in the fraction above 45 m^3/h floating rate, their 1000 seed weight was 1.92 gram. The percentage of the weight of all alfalfa seeds in subsequent fractions showed an increasing trend, with a slight fluctuation: from 5.8% (15–20 m^3/h) to 93.2% (above 45 m^3/h) (Fig. 1B). The total weight of the whole fractions increased also with a slight fluctuation: from 12.3 gram (15–20 m^3/h) to 814.4 gram (above 45 m^3/h) (Fig. 1C).

B. roddi, as well as its three beneficial parasitoids, *Habrocytus medicaginis* Gahan (recently synonymized with *Pteromalus sequester* Walker) (Hymenoptera: Pteromalidae), *Liodontomerus perplexus* Gaham (Hymenoptera: Torymidae), and *Tetrastichus* (recently synonymized with *Aprostocetus* genus) *bruchophagi* Gaham (Hymenoptera: Eulophidae) emerged from the chaff fractions. The summarized abundance in all fractions of these species were found as follows (*B. roddi* was taken as 100%; the total No. of emerging specimens are in parentheses): *B. roddi* 100% (1161), *L. perplexus* 32.1% (373), *H. medicaginis* 13.6% (158), and *T. bruchophagi* 1.3% (16).

The distribution of males and females of each of these species within fractions were found as follows (the sum of emerging adults of a given sex within a species in each fraction was taken as 100%).

B. roddi females were most abundant in fractions at 35–37.5 and 37.5–40 m^3/h (28.9% and 24.7%, respectively) (Fig. 2, *B. roddi*). The lowest fraction they appeared in was at 27.5–30 m^3/h (1.0%), the highest one at above 45 m^3/h (1.3%). *B. roddi* males were most abundant in the fraction at 35–37.5 m^3/h (30.9%), their range of occurrence grasps fractions from 25–27.5 m^3/h (0.1%) to above 45 m^3/h (0.3%).

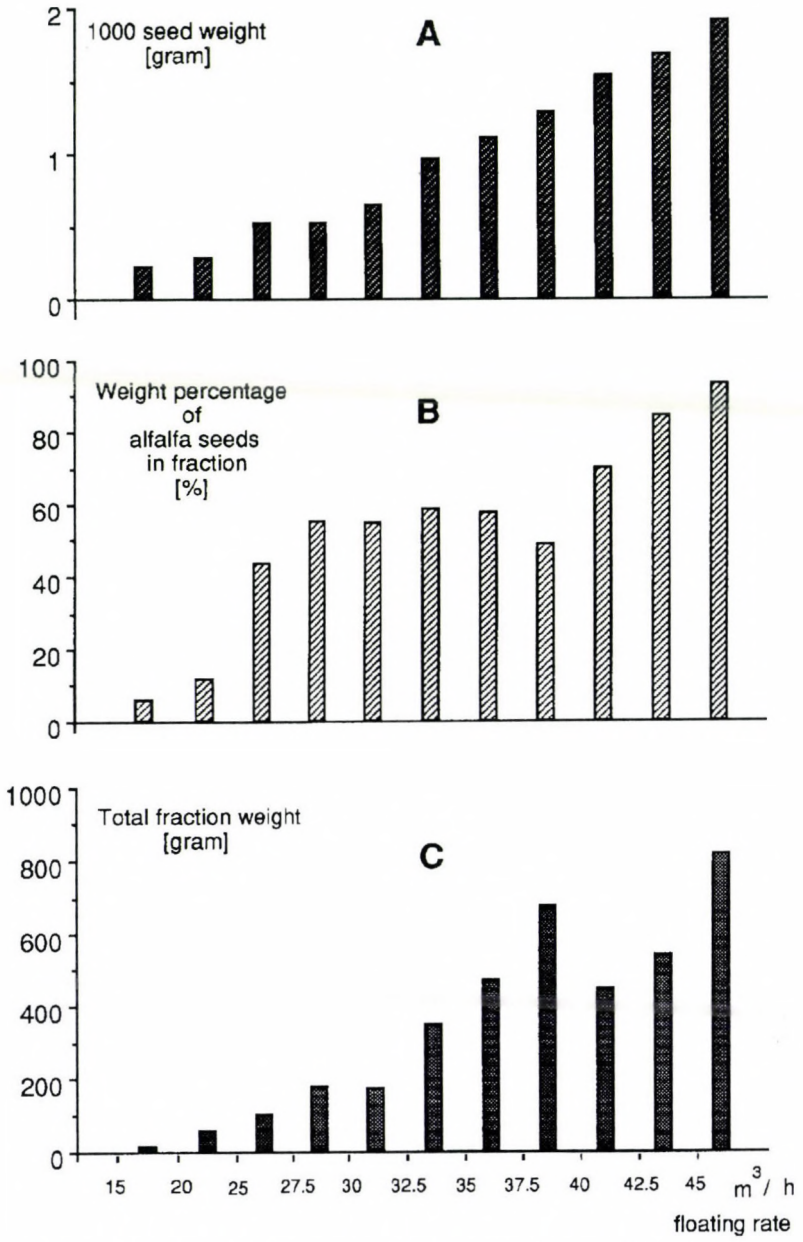

Fig. 1. The distribution of (**A**) 1000 seed weights of alfalfa seed populations, (**B**) weight percentages of all alfalfa seeds in a given fraction, and (**C**) total fraction weights, in fractions of seed-harvested alfalfa chaff, fractionated by a pneumatic separator using stepwise elevated floating rates

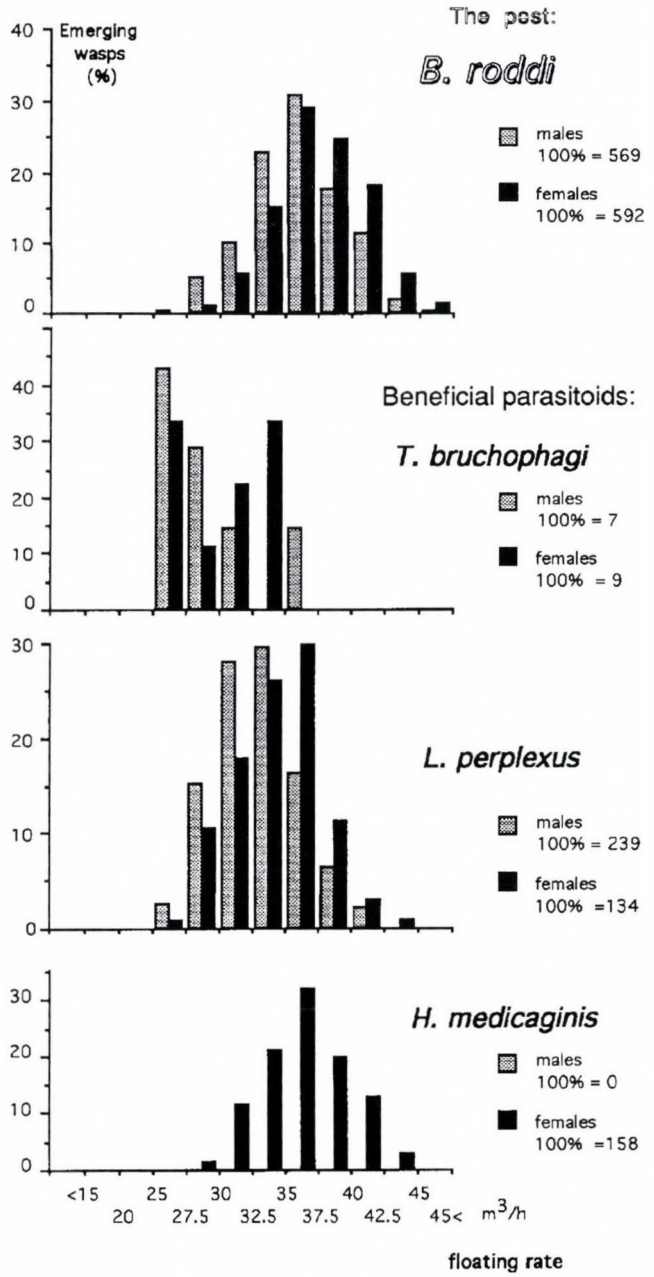

Fig. 2. The distribution of alfalfa seed chalcid (*Bruchophagus roddi*) and its 3 parasitoids, emerging from fractions of seed-harvested alfalfa chaff, fractionated by a pneumatic separator using stepwise elevated foating rates

Table 1

The dry weight of *Bruchophagus roddi* and its 3 parasitoids

Species	Sex	Weight (mg/100 wasps)	Numbers weighted
Tetrastichus bruchophagi	male	2.10	50
Tetrastichus bruchophagi	female	4.18	60
Liodontomerus perplexus	male	5.72	100
Liodontomerus perplexus	female	7.58	100
Habrocytus medicaginis	female	7.97	100
Bruchophagus roddi	male	8.60	100
Bruchophagus roddi	female	12.33	100

The distribution of *H. medicaginis* females (only females were found in the sample) in fractions showed a maximum at 35–37.5 m^3/h with a range from 27.5–30 m^3/h (1.2%) to 42.5–45 m^3/h (2.5%) (Fig. 2, *H. medicaginis*). Female *L. perplexus* was most abundant at 35–37.5 m^3/h (29.9%), its occurrence ranged from 25–27.5 m^3/h (0.7%) to 42.5–45 m^3/h (0.7%) (Fig. 2, *L. perplexus*). Highest abundance of *L. perplexus* males was found at 32.5–35 m^3/h (29.7%), marginal values were found at 25–27.5 m^3/h (2.5%) and 40–42.5 m^3/h (2.0). The greatest number of *T. bruchophagi* females were found in fractions at 25–27.5 m^3/h and 32.5–35 m^3/h (33.3% in both case) with smaller numbers in the intermediate fraction (Fig. 2, *T. bruchophagi*). The maximal abundance of *T. bruchophagi* males was at 25–27.5 m^3/h (42.9%), and this fraction represented the lowest margin of occurrence. The highest fraction where *T. bruchophagi* males occurred was at 35–37.5 m^3/h (14.3%).

The dry weight of *B. roddi* and its 3 parasitoids are listed in Table 1. *B. roddi* was found to be the heaviest species, while *T. bruchophagi* proved to be the lightest out of the four species measured. Females were heavier than males within a species in all three species where both sexes were available.

Discussion

The gradually increasing 1000 seed weights of alfalfa seed populations in fractions in order of increasing floating rates shows that heavier seeds remain in fractions at higher floating rates. This is in a line of general experiences in pneumatic seed-cleaning processes. The slight differences in 1000 seed weight of alfalfa seed populations which occurred sometimes between subsequent fractions show how fine separation can be achieved by the Petkus pneumatic separator operating even at increaments of as great as 2.5 m^3/h, which is far not the top resolution of this instrument. The increasing trend in the percentages of the weight of all alfalfa seeds in fractions reflects to the decreasing por-

tions of alfalfa pod and stem debries, while the slight fluctuation may be a sign of minor amounts of plant particules and/or seeds of another plant species. The increase of the weight of the whole fraction is assigned to the increasing portion of heavier alfalfa seeds, while the slight fluctuation may reflect again to the presence of a minor amout of non-alfalfa particules.

The chaff material of the conventionally seed-harvested alfalfa, investigated by us was infested by *B. roddi* in large numbers (from a ca. 4 kg aliquot over 1100 adults emerged). However, 3 beneficial parasitoid wasp species, each of which develops in *B. roddi* and causes the death of its host, was also found in the chaff. The total number of these three beneficial parasitoids was nearly half (47%) of the total number of *B. roddi* (i.e. the parasitization was 32%).

The chaff was fractionated with a pneumatic separator operated at stepwise increased floating speeds. The relative abundance of wasps emerging from these fractions showed various distributions according to species identity and sex. The general shape of these distributions appeared more or less symmetric with a maximum around the middle, with the only exception of *T. bruchophagi*. The reason for this exception is not known, and low numbers obtained from this species makes any speculation ambiguous. The floating range for seeds infested by *B. roddi* found in the present study (from 25 to 45 m^3/h = from 2.77 m/s to 4.99 m/s; above 45 m^3/h only 2.3% of *B. roddi* population occurred) correspond well with data reported by Manninger et al., (1958) (from 1.6 to 5 m/s), and with data reported by Berlage et al., (1986) saying that pneumatic cleaning at an airspeed at 4.62 m/s removed 99% of *B. roddi* infested alfalfa seeds.

In comparing distributions of *B. roddi* and its parasitoids, it turned out that the fraction between 25–27.5 m^3/h floating rates was rich in beneficial parasitoids, but practically free from non-parasitized *B. roddi*. In this fraction occurred 42.9% of males and 33.3% of females of *T. bruchophagi*, and also 2% of males and 0.75% of females of *L. perplexus*, both parasitoids of *B. roddi*. This fraction was perfectly free from non-parasitized *B. roddi* females, and the relative abundance of non-parasitized *B. roddi* males was only as low as 0.18%. Even in the next fraction the relative abundance of these two parasitoids drastically outnumbered that of the non-parasitized *B. roddi*. In the subsequent fractions (some of) the parasitoid species and non-parasitized *B. roddi* were present together. The distribution of *H. medicaginis* females coincided with that of non-parasitized *B. roddi* females.

Differences were found not only in the floating properties of alfalfa seeds containing inside a *T. bruchophagi*, or a *L. perplexus* larva versus a non-parasitized *B. roddi* larva, but also between dry weights of adults of these species.

To our knowledge this is the first case that diapausing beneficial parasitoids could be separated from *B. roddi* in seed-harvested alfalfa chaff. Pneumatic spearation of viable alfalfa seeds from *B. roddi* infested seeds has been reported (Manninger et al., 1958, Berlage et al., 1986). Unfortunately, this latter method has not come through into practice. Our findings indicate that a fraction rich in diapausing larvae of *T. bruchophagi* and *L. perplexus* can be gained from conventionally seed harvested alfalfa chaff. The overwintering, diapausing larvae of the benefical parasitoids can be storted for a long period

in this chaff fraction under appropriate low temperature regime, and later, at the desired time, can be let emerge. This way our findings point to a new possibility in biocontrol against *B. roddi*.

However, there are a number of questions which should be clarified to establish the basis of a biocontrol method based on our recent findings. Selecting chaff materials in which *T. bruchophagi* and *L. perplexus* are abundant enough for the aim of a practical application is the first problem. Although both of these parasitoids have a wide range of occurrence over the world, and are abundant in Europe just as well in North America, the natural population density of these parasitoids greatly varies in various localities and years (see e.g. for Russia: Nikol'skaya, 1932; Kolobova, 1959; for North America: Thoenes and Moffett, 1990; and for Hungary: Erdélyi, 1994). Further study would be needed to more finely tune the fractionation procedure and to determine the possible differences in critical separating floating rates between alfalfa cultivars. Also, further studies would be needed to clean the selected fraction from incidentally occurring weed seeds, to clarify storage and transport conditions, and to specify when and how parasitoids should be released. Solving these problems could lead to a new biocontrol method. This new method could open a wide, yet unexploited possibility to control *B. roddi*, which would significantly enhance the amount of sound alfalfa seeds at harvest. Furthermore, this new method could serve as a model for developing similar methods against other *Bruchophagus* species which host the same benefical parasitoids, while themselves infesting hostplant specifically other small-seed papilionaceous fodder plants.

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Seasonal Flight Pattern of *Harpalus rufipes* (De Geer) Captured by Light Traps in Hungary (Coleoptera: Carabidae)*

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Seasonal flight activity patterns of adult *Harpalus rufipes* (De Geer) was studied in Hungary by light trapping. For the analyses, data of 16 seasonal flights were collected from five trapping sites scattered in highland and lowland regions during the period 1981–1989.

Seasonal length of adult flights was longer (12–14 weeks) in lowland region with warmer climate than in cooler mountainous areas (6–10 weeks). Generally, the mass flights lasted from early July to first week of August and peaked during second half of July. The patterns were more synchronous independently of the sites or years. The mean distribution of seasonal flights was unimodal, but in some cases a smaller peak was detected in first half of June. The sporadic catches over seasons were probably due to rarity of nights with optimal meteorological conditions for flying. The flight activity of *H. rufipes* increased above 18 °C nightly minimum temperature, and the catches were highest between 21–23 °C.

Harpalus rufipes is a widespread Palearctic species, which is common and abundant in cultivated fields of many agricultural and horticultural crops (Briggs, 1965; Luff, 1980). The life cycle of *H. rufipes* is biennial in most areas of Europe, though more authors found that it might be annual, too (Luff, 1980). Recently, Matalin's detailed study (Matalin, 1997) on this species indicates, that in the Southwestern steppe zone of Eastern Europe the annual life cycle is characterised by a spring-summer reproduction with overwintering imagoes and a late-summer-autumn reproduction with overwintering larval stages.

In Hungary, *H. rufipes* is also common in arable fields, but its biology, seasonality is largely unknown. Because our previous results showed that this carabid species can be easily collected by light traps (Kádár and Szentkirályi, 1984) we decided to analyse the seasonal flight activity pattern in Hungary, using a great number of flights. The aims of our investigation were as follows:

a) to describe the general flight pattern of *H. rufipes*

b) to indicate the variability of flight pattern characteristics (length of seasonal activity, number of peaks, modality of flight distribution, etc.)

c) to analyse the rate of synchrony between patterns

d) to study the influence of minimum air temperature on night-flight activity.

* Dedicated to Professor Dr. Gy. Sáringer on the occasion of his 70th birthday.

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Table 1

Localities and characteristics of the light trappings of *H. rufipes*

Locality	Year of trappings	Type of bulb	Habitat type	Region	Geographical coordinates
Makó	1981	125 W, MV	maize field	lowland	46°13′ [a]
	1982–83	100 W, NW			20°28′ [b]
Agárd	1981	125 W, MV	maize field	lowland	47°12′
	1982–83	100 W, NW			18°37′
Nagykovácsi	1981–83	125 W, MV	apple orchard	mountainous	47°33′
	1984	100 W, NW			18°58′
	1985–86	160 W, BL			
Pilismarót	1983–84	125 W, MV	mixed forest	mountainous	47°47′
					18°52′
Tahitótfalu	1988–89	125 W, MV	garden	mountainous	47°40′
					19°04′

Abbreviations: MV = mercury vapour; NW = normal white light; BL = blended light; a = latitude N, b = longitude E

Materials and Methods

Sites of light trapping

Information about sampling sites and light traps are shown in Table 1. The light traps were settled at five localities to collect *H. rufipes*. Two light traps operated in continuous monoculture maize field. These large-sized fields (Makó, 400 ha; Agárd, 529 ha) were selected within the Hungarian corn belt where the density of *H. rufipes* was great, as indicated by pitfall trapping. In lowland cornfields the light traps worked for three years. There were three light traps in mountainous region. One of them was set up in an apple orchard (Nagykovácsi, 5.8 ha), bordered by oak forest and other orchard. This trap operated over six years. The remaining two light traps were in a mixed deciduous forest (Pilismarót) and in a garden (Tahitótfalu) and they operated for only two years.

Characteristics of the light trap

The type of light traps (Minnesota-type) was identical at each site, and they were supplied with three buffles. The light source was two meter above the ground level. Three kinds of bulbs were applied during investigating years: mercury vapour (125 W), normal white (100 W) and blended (160 W) source of light. The traps were emptied daily and they worked from April 1 to October 30. Meteorological observations (temperature, precipitation) were in process near two trapping stations (Pilismarót, Tahitótfalu).

Data processing and statistical analyses

From the localities and years, data of 16 flights could be collected totally for the analyses. Daily catches were summed using standard weeks (Szentkirályi, 1997), and the percentage distributions of weekly catches were displayed for the seasonal flight pattern analyses (Fig. 1).

The mean flight activity pattern was gained by averaging the weekly relative catches of yearly local patterns (Fig. 2). To analyse the similarity and synchrony level between each seasonal flights, the second author's methods (Szentkirályi, 1997) were used. Cross-correlation function was applied to show the rate of synchrony between patterns. The seasonal time-series lagged by weekly intervals were considered synchronised if positive significant correlation was detected at both 0 and 1 week lag. The other method to measure the similarity between the flights was the Renkonen-index, which gives the percentage value of the overlap.

To analyse the effect of nightly minimum air temperature near the traps on daily flight activity of *H. rufipes*, the recorded values were arranged by increasing order. For each temperature intervals increasing 1 °C, the average catch percent and standard error was calculated (Fig. 4). MANOVA (post hoc LSD test) was applied to determine the differences between catch means. The analyses were run by the Statistica program package (StatSoft, 1994).

Results

Characteristics of seasonal flight activity patterns

Figure 1 shows the percentage seasonal distribution of local yearly flights of *H. rufipes* detected by light traps. The flight patterns found in the two maize fields, the apple orchard, the mixed deciduous forest and the garden are illustrated by Fig. 1a, b and c, respectively.

Considering the total flights, the earliest and latest time of *H. rufipes* catch was the 22nd and the 35th week, respectively. This means the seasonal activity lasted from early June to early September. The length of seasonality, measured by number of weeks between the first and last day with catches, was significantly varied depending on year and locality. The shortest flight lengths (6–10 weeks) were registered in mountainous regions (forest: 9–10, garden: 8–9, apple orchard: 6–10 weeks). Longer (8–10 weeks) seasonality was indicated in maize field of mid-western part of the country (Agárd), while the longest one (12–14 weeks) was in maize field of south-eastern region.

According to the varied flight lengths, the start and end of flights were greatly variable depending on localities and years. The flights started from early June to first decade of July (22–28 standard weeks), while the end of flights varied throughout August (31–35 standard weeks). In most cases flight distribution was discontinuous with sporadic catches. Some weekly catches were exceptionally great, many times exceeding

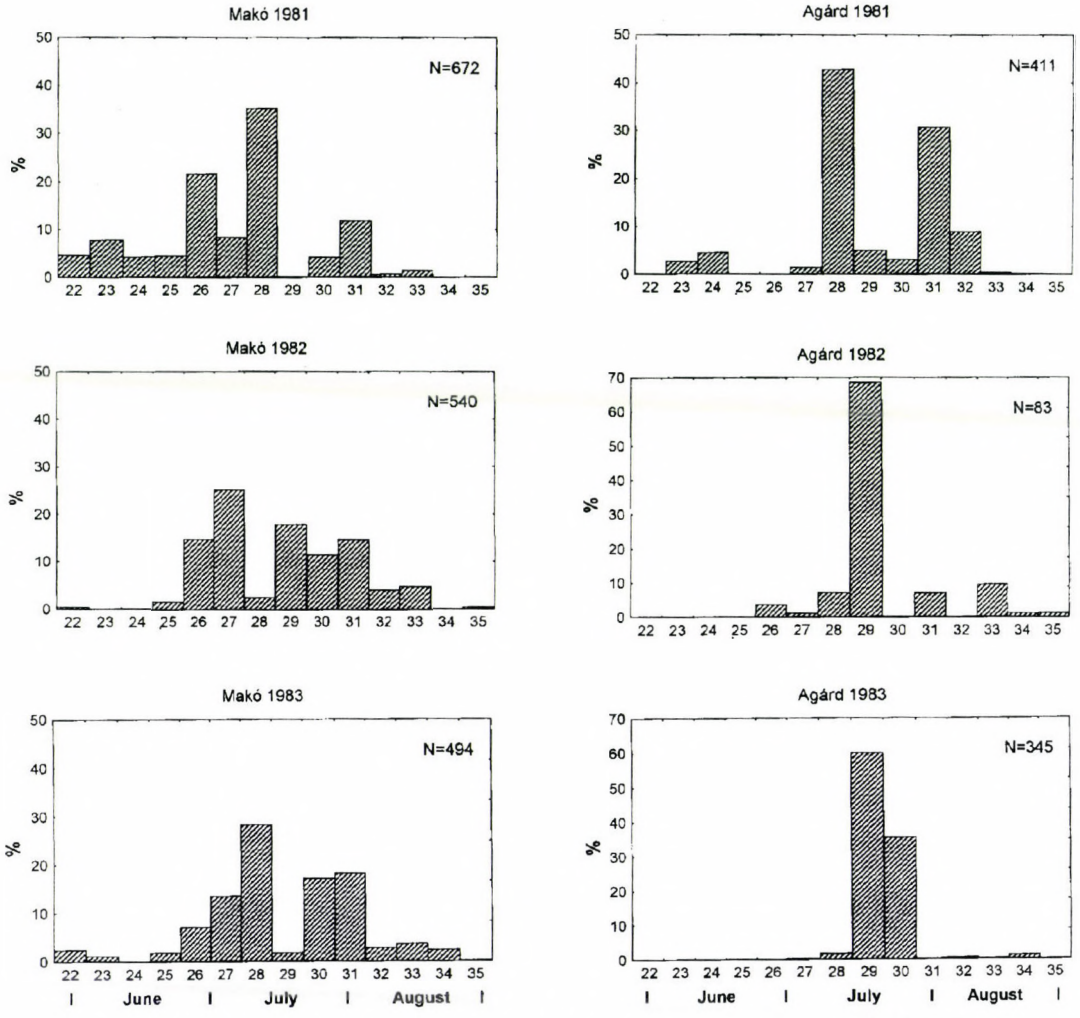

Fig. 1a. Seasonal flight patterns of *H. rufipes* collected by light trapping in two maize fields (numbers of x-axis mean standard weeks; N = total number of adults caught during the season)

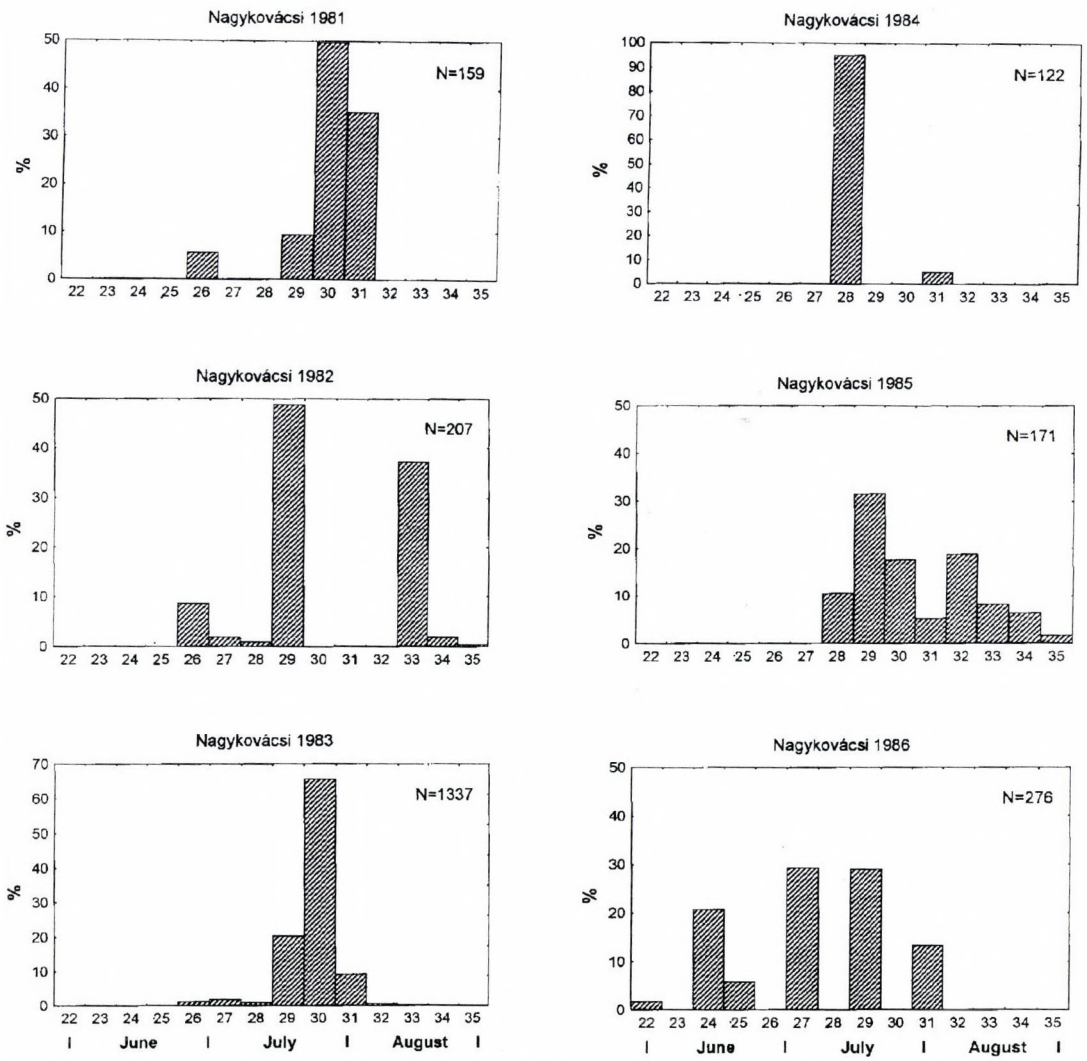

Fig. 1b. Seasonal flight patterns of *H. rufipes* collected by light trapping in an apple orchard (numbers of x-axis mean standard weeks; N = total number of adults caught during the season)

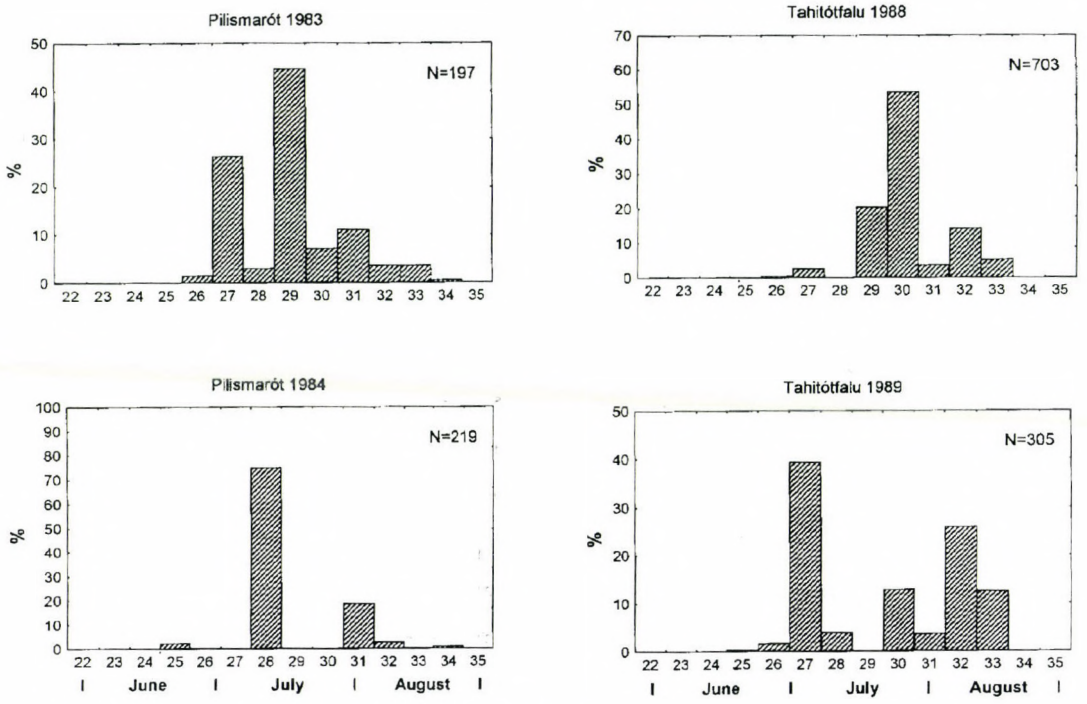

Fig. 1c. Seasonal flight patterns of *H. rufipes* collected by light trapping in the mixed forest (Pilismarót) and the garden (Tahitótfalu) (numbers of x-axis mean standard weeks; N = total number of adults caught during the season)

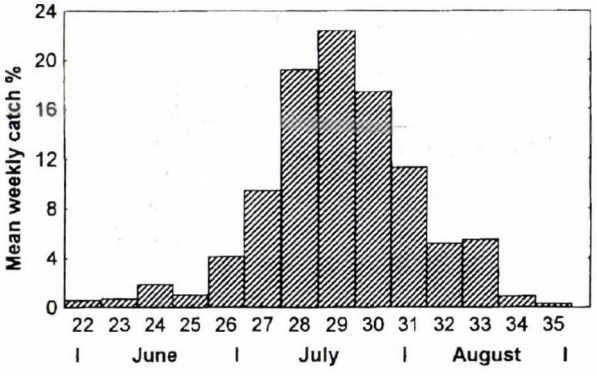

Fig. 2. Mean percentage distribution of the seasonal flight activity of *H. rufipes* based on local light trappings

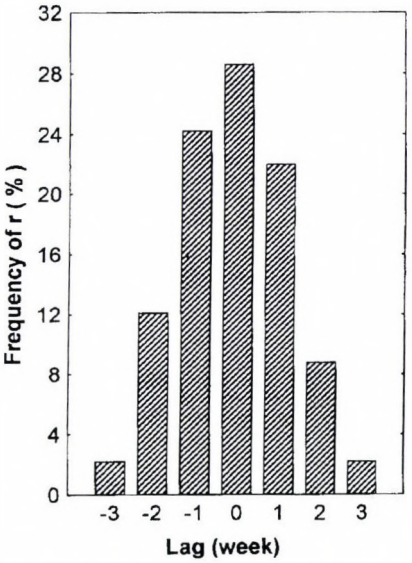

Fig. 3. Frequency distribution of significant positive cross-correlation coefficient values (r, $p < 0.05$) found around 0 lag between the local yearly flight patterns of *H. rufipes*

40–50 percent of total yearly catch. The seasonal number of these weekly peak catches was 1, 2 and rarely 3. The lack of continuous flight activity and the temporal variation of peak catches makes very hard to determine any real modality based on yearly, local patterns. Peak catches were mostly in a 27–31 standard week interval, namely during July to first week of August. It suggests that pattern distributions are rather unimodal.

Nevertheless at some sites and years a weak peak catch period could be observed in the first half of June (between the 22nd and 25th weeks), which was more or less separated from the later flight activity (Agárd, 1981; Makó, 1981–83; Nagykovácsi, 1986). The mean seasonal flight pattern of *H. rufipes* is shown in Fig. 2. The shape of generalised distribution, calculated from local yearly patterns, clearly supports the unimodality of flight. The peak of seasonal activity of *H. rufipes* was allocated in the second half of July (29th week). By the generalised frequency distribution, mass flight occurred in July and first half of August (between 27th and 31st weeks).

Rate of synchrony between flight patterns

The result of the time series analytical method, used for detecting the synchrony between yearly local flight patterns, is presented in Fig. 3. The frequency distribution of significant positive correlation at different lags (number of lagged weeks = 0–3) suggest that in 75% of comparisons, the flights were in synchrony (see the frequency of r at 0 and ±1 week lag). An overlap of some degree between flight patterns was indicated with an

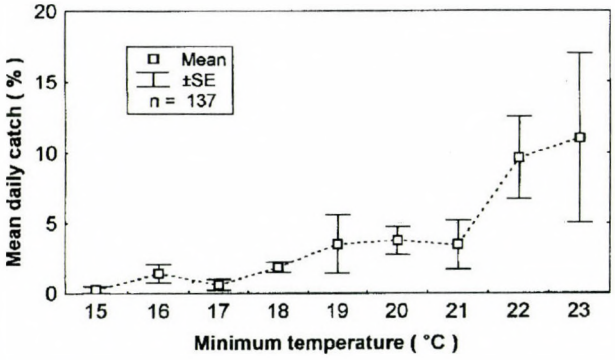

Fig. 4. Changes in mean daily light trap catches of *H. rufipes* depending on increasing nightly minimum air temperature values (data derived from sites: Pilismarót, 1983–84; Tahitótfalu, 1988–89) (n = total number of catching days)

analysis of percentage similarity (Renkonen) index. The value of index indicated a medium or strong similarity in 53% of comparisons. In certain cases the identical site (Makó 1981–83; Nagykovácsi 1981–83) or identical year (1981, between Makó and Agárd; 1982, between Nagykovácsi and Agárd; 1983, between Nagykovácsi and Agárd; 1984, between Nagykovácsi and Pilismarót) was the basis of this similarity. However, flights, belonging to different localities and years, were often strongly similar (similarity above 70%). This tendency of similarity also suggests that the flight of *H. rufipes*, mainly its mass flight, happens at the same time at different places and years in Hungary.

Influence of minimum air temperature on flight activity

According to MANOVA, the minimum air temperature during nights had an influence on the flight activity of *H. rufipes* and thus on light trap catches as well. The results are shown in Fig. 4, where the mean daily relative catches ± SE are arranged in order of increasing temperature classes (the limits of categories are at half degree, for instance 18 °C means temperature between 17.5–18.5 °C). The mean catches are significantly separated ($F = 8.61$; $df = 8, 128$; $p < 0.001$) depending on three different increasing temperature intervals. The highest mean catches were between 21.5–23.5 °C of minimum temperature at night. These catches significantly differed ($p < 0.01$; LSD-test) from all the others. The mean catches in a temperature interval of 17.5–21.5 °C were significantly ($p < 0.05$; LSD-test) greater than the values under 17.5 °C.

From these facts it can be seen that the flight activity of *H. rufipes* raises as minimum temperature at night increases. This increase in activity was moderated above 18 °C and high above 21 °C.

Discussion

Analysing the 16 flight patterns, it is obvious that the length of seasonality of *H. rufipes* adults was significantly varied by localities and years. Light trap catches suggest that the adult flight activity starts no earlier than early June and it finished no later than late August–early September. There is a noticeable tendency in local flight lengths, depending on sites in mountainous regions with cooler climate, shorter (6–10 weeks), while warmer southeastern lowland having earlier spring (Hungarian Great Plain), longer (Makó: 12–14 weeks) activity periods were indicated. All of these refer of the fact that the favourable conditions necessary for flights are rather guaranteed for longer periods in regions of warmer climate.

Other authors also presented similar flight lengths based on their light trap catches. For example, the flight lasted in Azerbaijan during mid-June to early September (Belousov, 1986), in Moldova (Matalin, 1994, 1997), in Czech Republic (Honěk and Pulpán, 1983), in Germany (Basedow and Dickler, 1981) during mid-June to 3rd decade of August, in. U. S. A. during second half of June to early August (Zhang et al., 1997). Taking these into consideration, it is likely that the flight of *H. rufipes* lasts from mid-June to 3rd decade of August. The situation is similarly uniform in case of mass flights and flight peaks of *H. rufipes*. According to analyses, mass flight was mainly during early July to second week of August, and flight peak was generally during 2nd half of July in Hungary. The previously mentioned authors also reported that mass flight takes place in July and in certain cases it may continue in early August (Moldova, Czech Republic). In these countries the catch peaks were in second half of July, too.

Contrary to geographically uniform phenology, both in present work and other investigations (Briggs, 1965; Honěk and Pulpán, 1983; Zhang et al., 1997) the temporal process of *H. rufipes* flights was discontinuous, often even 1–2 weeks passed between consecutive catches, without any activity. Conspicuously, the sporadic occurrence of catches with greater number of adults, was typical. The reason may be the infrequent occurrence of favourable weather conditions for flying. From the meteorological elements influencing flight activities of ground beetles, the temperature has an outstanding role (Pausch, 1979; Van Huizen, 1979; Honěk and Pulpán, 1983). Kádár and Erdélyi (1992) analysing light trap catches of carabids found that flight activity was significantly increased with daily minimum temperature. By meteorological observations daily minimum temperatures occur at night mainly in dawning hours. The nightly flight activity of *H. rufipes* was recorded between 21–04 h by Basedow et al. (1990). Most flying adults were caught by their traps between 22 and 2 hours. Consequently, the activity of this species may be strongly influenced by nightly minimum air temperature. Our results indicate that flight activity of *H. rufipes* begins to increase above 18 °C minimum temperature, but it is significantly greater only above 21 °C. Other authors presented similar results, regarding light trap catches of carabid beetles. Briggs (1965) and Pausch (1979) recorded high light trap catches on those nights, when at 22 h the air temperature was above 18 °C. Honěk and Pulpán (1983) reported that, on days of peak catches, the average of measured temperatures (19.1 °C) was 2.2 °C higher than on preceding days. Base-

dow and Dickler (1981) recorded 22–23 °C air temperature at 21.30, when maximal flight occurred. Belousov (1986) also stated that mass flights were between 20–25 °C. Both high number of individuals suited for flight and an optimal nightly temperature are required to be peak flight. High nightly temperature values are rare during the season, occurring mainly in July and first half of August in Europe.

It is clear, that in June in Hungary, the optimal temperature for flights is rare, because nights are cooler then, and consequently the light trap catches of *H. rufipes* are low level. Why the favourable temperature has a major role in forcing greater light trap catches, such peaks occurring during season, were not considered as population maxima of patterns. A general flight was averaged from 16 unique patterns, to find the modality of patterns. It was found, that flights, despite discontinuity, were in synchrony with each other to a high degree. As a consequence of the present study, the seasonal flight of *H. rufipes* is mainly unimodal. However, in some cases, there was a smaller activity period in first half of June. In connection with *H. rufipes* light trap catches, a similar phenomenon was discovered by Belousov (1986) and Zhang et al. (1997), who found a smaller distinct flight period at the end of June. Zhang et al. (1997) hypothesised, this first peak in June is caused by flight of overwintering beetles, while the second mid-July mass flight represented by newly emerged adults, flying before oviposition. The hypothesis was supported by the fact that 97% of females caught by light trap had no matured eggs, as it turned out after dissection. Though Matalin (1997) found two separate, a "spring" and an "autumn", reproduction periods during pitfall trapping, still, the light trappings did not indicate any distinct flight peak in June. He proved that a predominant part of the analysed individuals of the July mass flight was immature (Matalin, 1994, 1997). Our investigations also indicate that *H. rufipes* adults flying in June were predominantly immature, while the population light trapped in July had a teneral and a greater immature part (Kádár and Szentkirályi, unpublished). In our opinion also, the mass flight in July is caused by the pre-reproductive dispersion of newly emerged adults. Matalin's (1994, 1997) detailed analyses also support the above statement, reporting, that during intensive flight in July, the imagoes of new generation, overwintered in larval stage, migrate. According to light and pitfall trapping analyses conducted by several authors, this pre-reproductive dispersal flight precedes the ground surface activity of adults (Honěk and Pulpán, 1983; Belousov, 1986; Matalin, 1994, 1997; Zhang et al., 1997).

The life cycle of *H. rufipes* may be varied depending on the climatic conditions of different regions (see e.g. Luff, 1980; Matalin, 1997), therefore further investigations are required to clear the origin of the two distinct flight peaks.

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Edge Effect on Carabids in an Oak-Hornbeam Forest at the Aggtelek National Park (Hungary)

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We have tested the edge effect hypothesis along a transect for carabid assemblages (*Coleoptera: Carabidae*) in a *Querco-Carpinetum* forest using pitfall traps, in a hilly region of Northern Hungary at the Aggtelek National Park. The transect can be divided into four sections. A forest without shrubs and herbaceuos vegetation, a forest with moderate ground layer vegetation and two types of edges: a forest edge similar to the forest interior but increased cover of ground vegetation and shrubs, and shrubby edge without canopy trees. The dominance structures of the assemblages of forest habitats were similar, while the edges' assemblages were more species rich. We have demonstrated by multidimensional scaling that the species composition of the shrubby edge was different from the other assemblages and the seasonal dynamics of the assemblage was also strikingly different. Diversity increased along the transect, and it was the highest in the shrubby edge. The number of trapped individuals was relatively stable in the forest with a slight increment towards the forest edge; however, it was much lower in the shrubby edge than in the forest or in the forest edge. Scale-dependent diversity profiles of the forest habitats were similar, while the shrubby edge was significantly more diverse. Six of 24 species were present exclusively in the edges. Our result suggests that edges may have a crucial role in the maintenance of the diversity of carabid assemblages.

Nowadays, there is a fairly large interest in the ecology of landscape boundaries (Holland et al., 1991). Edge zones may differ both structurally and compositionally from the patch interior. In the edge might happen abrupt changes in light regime, substrate, water conditons, species composition and other abiotic and biotic factors that generally characterise the edge. These factors are highly significant for insects, especially of surface-dwelling carabids (Hansen and di Castri, 1992).

Carabids are a suitable group for bioindication of the changes of these factors (Refseth, 1980; Müller-Motzfeld, 1989) and are favourable subjects for comparative ecological studies, because their reproductive cycles, habitat choice, body size, and physiology and behaviour vary considerably among species (Thiele, 1977; Den Boer, 1986).

As the edges ma be associated with greater species richness, heterogeneity of habitats, and establishment "stepping-stones" (Den Boer, 1970), their study is essential. Several authors have described the distribution and movement of carabid beetles in such edge-zones (Burel, 1989; Petit and Burel, 1993; Gruttke, 1994; Petit, 1994; Šustek, 1994). Few of them, however, tried to measure the increase in species richness at edges (Báldi and Kisbenedek, 1994; Bedford and Usher, 1994; Butovsky, 1994; Magura and Tóthmérész, 1997).

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Classical edge effect hypothesis states that species richness and diversity will be higher in edges than in adjacent areas (Leopold, 1933; Samways, 1994). The goal of our study was to test this hypothesis for carabid (*Coleoptera: Carabidae*) assemblages of an oak-hornbeam (*Querco-Carpinetum*) forest and two types of forest edges, and to characterise the diversity along a transect using diversity indices and scale-dependent diversity characterisations (diversity orderings).

Material and Methods

Study area and sampling

The study area was located in the North Hungarian Mountain at the Aggtelek National Park, in the buffer zone of the central part of a Biosphere Reserve Area. In this region the zonal forest association is oak-hornbeam (*Querco-Carpinetum*), which is the most extensive forest type in this region.

The research area was divided into 4 parts: (1) oak-hornbeam forest, almost without herbaceous vegetation; F-NV. (2) oak-hornbeam forest, with moderate herbaceous vegetation; F-MV. (3) forest edge, with shrubs and large cover of herbaceous vegetation; F-E. (4) shrubby edge, without trees in the canopy layer, and characterised by a dense shrub stand with *Carpinus betulus*, and *Prunus spinosa*; Sh-E.

Samples were collected using pitfall traps consisting of plastic cups (diameter 100 mm, volume 500 ml) containing ethylene-glycol as a killing-preserving solution. A transect method, currently widespread for the study of carabid assemblages of adjacent habitats (Usher et al., 1993; Bedford and Usher, 1994), was used in this study. Overall, there were 112 pitfall traps; 28 traps in each of the habitat areas. They were placed every 0.5 m along the transect. Trapped individuals were collected monthly from April to October. The trapping period was 2 weeks in each months (Niemelä et al., 1990). For the numerical analysis pooled samples were used, based on the same pitfall number and arrangement and with the same total catching perimeter (Baars, 1979/a). All carabid beetles taken in pitfall traps were identified to species using standard keys (Freude et al., 1976).

Data analyses

The traditional diversity index of Shannon was used (e.g. Pielou, 1975). Species richness was also calculated for 100 sampled individuals using the rare faction or $ES(m)$ diversity (Hurlbert, 1971). We compared Shannon diversities using the t-test described by Hutcheson (1970). Diversity profiles (diversity ordering) were used for scale-dependent diversity characterisation. It can be performed with a family of diversity functions, which has a scale parameter. At the different values of the scale parameter the sensitivity of the diversity index family shifts from the rare towards the frequent species. Thus, we can compare the assemblages by means of this parameter. One of the assemblages is more diverse than the other if it is more diverse throughout the range of the

scale parameter. When the diversity profiles cross each other, the assemblages cannot be ordered by diversity, because one of them is more diverse for the rare species while the other is more diverse to the abundant ones. Rény diversity is sensitive to the rare species for small values of the scale parameter, whereas it is sensitive to the abundant species for large values of the scale parameter. We used the Rényi diversity index family because it was reported to provide good result for broad sample sizes and research situations (Tóthmérész, 1995). It is defined as ($\alpha \geq 0$, $\alpha \neq 1$):

$$HR(\alpha) = \frac{\log \sum_{i=1}^{ST} p_i^{\alpha}}{1-\alpha}$$

where p_i is the abundance of the i-th species of the assemblage. It was the first published family of diversity indices (Rényi, 1961). In the original definition the base number of the logarithm was 2; in ecological applications the natural logarithm is the most frequently used. It used to be mentioned as Rényi diverstiy index family, because it includes classical diversity indices as special cases. $HR(\alpha=0)$ is the logarithm of the total number of species; $HR(\alpha \rightarrow 1)$ is the Shannon index; $HR(\alpha=2)$ is the logarithm of the reciprocal of Simpson index; $HR(\alpha=\infty)$ is the logarithm of the reciprocal of the relative abundance of the commonest species. This is the logarithm of the reciprocal of Berger-Parker index (Berger and Parker, 1970). α may be interpreted as a scale parameter; when $\alpha=0$ it is the most sensitive to the rare species, because the rarest species have the same contribution to $HR(\alpha)$ as the most frequent. As α increases the influence of the frequent species also increases. When $\alpha=\infty$, the $HR(\alpha)$ diversity depends only on the dominance of the most frequent species. According to this interpretation α scales along an abundance scale. Sometimes these techniques are mentioned as diversity ordering, because they produce a partial ranking of the assemblages (Tóthmérész, 1993/a, 1995).

Diversity profiles were calculated by the DivOrd package (Tóthmérész, 1993/a). Tests of the differences of the diversity profiles were based on the normal approximations published by Tong (1983). Similarity of the species composition was calculated by Bray-Curtis similarity (percentage similarity), which is a generally used measure of compositional similarity (Ludwig and Reynods, 1988; Niemelä et al., 1993). The similarity structure was displayed by nonmetric multidimensional scaling (Gordon, 1981), using the NuCoSA package (Tóthmérész, 1993/b).

Results

The carabid assemblage of habitat Sh-E was the most diverse followed by F-E, F-NV, F-MV (Table 1). This ordering is the same for the number of species and for the rare faction diversity except that F-MV is more species rich than F-NV. The assemblage Sh-E was significantly more diverse than the other assemblages by the Shannon diversity index (Hutcheson's t-test, $df > 100$, $p < 0.001$), while the diversity measure of the other assemblages were not different significantly.

Table 1

Diversity of the carabid assemblages along the transect. *ES(100)* is the expected number of species in a random sample of 100 individuals

	Habitat F-NV	Habitat F-MV	Habitat F-E	Habitat Sh-E
Total number of species	15	18	19	20
ES(100)	9.6	10.0	11.2	17.1
Number of individuals	557	713	746	205
Shannon diversity	1.4370	1.2674	1.5670	2.3741

It is clear by the Rényi diversity index family that assemblage Sh-E is more diverse than the other assemblages throughout the range of the scale parameter (Fig. 1). Diversity profiles of the other communities are similar to each other. There are just minor differences. The assemblage of habitat F-E is more diverse than the assemblages of habitat F-NV and F-MV. Diversity profiles of the carabid assemblages of the F-NV and F-MV cross each other (denoted by <>), which means that F-MV is more diverse for the rare species while F-NV is more diverse for the frequent ones. Therefore, Sh-E, > F-E > F-MV <> F-NV. Diversity profiles of the assemblages of habitat F-NV and F-E were not different significantly, while the differences between all the other profiles are significant (t-test, df > 100, $p < 0.01$).

Composition of the carabid assemblages F-NV, F-MV, and F-E was similar across months, while the assemblage of the shrubby edge (Sh-E) was separated from them by nonmetric scaling. A seasonal dynamics can be recognised clearly on the ordination diagram. The F-NV, F-MV and F-E assemblages were more similar to each other within a month than across months. The only exception is the edge where the difference from the assemblages F-NV, F-MV, and F-E in the composition is larger than the influence of the seasonal dynamics (Fig. 2).

Discussion

The carabid assemblage of habitat Sh-E was the most species rich as well as the most diverse as we have demonstrated by scale-dependent diversity characterisations. The total number of species was slightly higher for the shrubby edge (Sh-E) than for the other assemblages. The difference was large by the Shannon diversity index and was highly significant.

Comparing F-NV and F-MV assemblages we received that species richness was higher for the assemblage F-MV while all the Shannon diversity was higher for the assemblage F-NV. Therefore, the ordering is different for these assemblages based on the number of species and on the Shannon index. The solution of this ambiguity is clearly

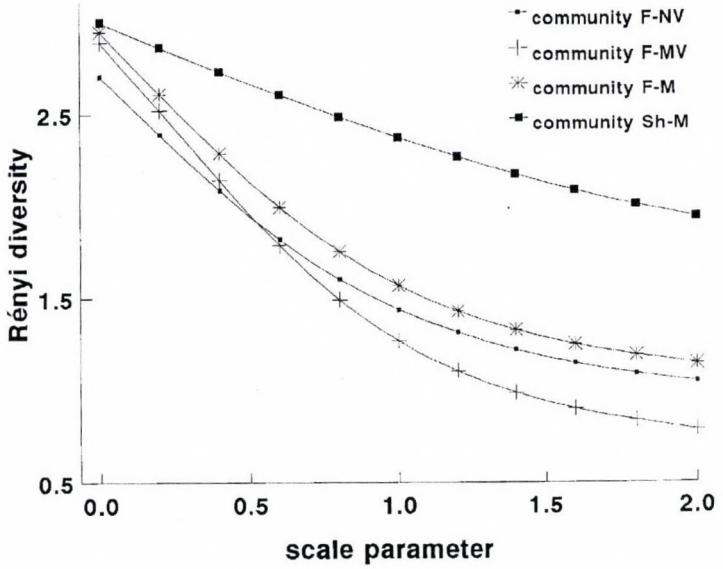

Fig. 1. Diversity profiles of the carabid assemblages with the Rényi diversity index family

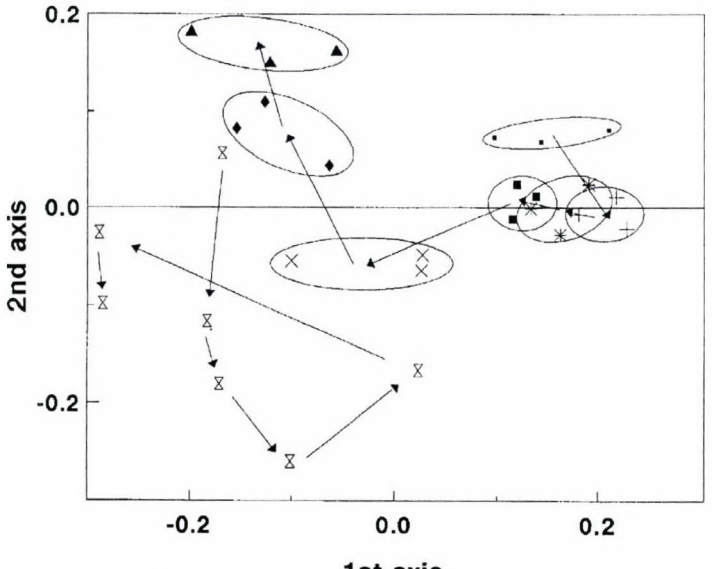

Fig. 2. Ordination of the studied carabid assemblages by nonmetric multidimensional scaling using Bray-Curtis dissimilarity. It is based on monthly samples. Because of the strong seasonal dynamics, assemblages F-NV, F-MV and F-E are marked by the same sign in each month, while in the case of the edge (Sh-E) the same sign is used for each month. Arrows are showing the direction of seasonal dynamics from April to October

shown by the scale dependent diversity characterization in Fig. 1. For the $\alpha=0$ scale parameter value the F-MV assemblage was more diverse and for the $\alpha \rightarrow 1$ scale parameter value the F-NV assemblage was more diverse. It is clear from the diversity profiles that F-MV is more diverse for the rare species while the assemblage F-NV is more diverse for the frequent ones.

The carabid assemblage of habitat Sh-E is the most diverse. The assemblage of habitat F-E is more diverse than the assemblages of habitat F-NV and F-MV. The observed difference between the diversity profiles of the assemblages of habitat F-NV and F-E is not significant, while the differences found between the other profiles are significant.

Our study proved that the carabid assemblage of shrubby edge was the most diverse. Báldi and Kisbenedek (1994) and Bedford and Usher (1994) also came to the conclusion that the carabid assemblage of the edge was the most diverse. Liebherr and Mahon (1979), Refseth (1980), Parmenter and MacMahon (1984), Niemelä et al. (1988), Báldi (1990) and Magura et al. (1997) also pointed out that the diversity of carabid assemblages was related to the heterogeneity of the habitat. In the case of habitat Sh-E, it can be stated that the herbaceous vegetation from the nearby grass (*Polygalo-Brachypodietum pinnati*) and the shrubs and seedlings from the forest significantly contribute to the heterogeneity of the habitat and support the development of microhabitats (cf. favourable microclimatic conditions, diversity of the habitat, wider range of food resources etc.). The same can be said of habitat F-E, where besides forest plants, species from the shrubby edge can be found as well. The increased habitat heterogeneity produces favourable microclimatic conditions. Both of them may increase the number of prey abundance and the increased niche-specialisation and the smaller resource-overlap may explain the high diversity value, we observed of the edges (MacMahon, 1980; Parmenter and MacMahon, 1984).

Small scale dispersal between habitat patches considerably influences species composition and structure of carabid assemblages (Niemelä, 1988). The high diversity value of the assemblage in shrubby edge, and the less pronounced seasonal dynamics observed there (Figs 1 and 2) may be caused by dispersal processes, as carabids are able to cover large distances (Baars, 1979/b; Magura, 1995) and exhibit density-dependent migration (Grüm, 1971) and aggregation (Bryan and Wratten, 1984).

The initial states of the carabid beetles' development (eggs and larvae) are very sensitive to environmental factors (Thiele, 1977; Müller-Motzfeld, 1989); therefore, the adult beetles lay their eggs in habitats with suitable environmental conditions and overwinter in such habitats. Thus, the smaller number of individuals in the shrubby edge may be explained by the newly hatched and adult beetles prefer oak-hornbeam forest after overwintering as a habitat with more suitable environmental factors.

Six of 24 species were present exclusively in the edges (Sh-E and F-E). These were: *Amara aenea* (De Geer, 1774); *Carabus arcensis* Herbst, 1784; *Harpalus latus* (Linnaeus, 1758); *Panagaeus cruxmajor* (Linnaeus, 1758); *Pseudophonus rufipes* (De Geer, 1774); *Pterostichus strenuus* (Panzer, 1797). These account for 25% of the total species pool, which is a rather high figure. These species had a high density in habitat

Sh-E and a much smaller density in F-E. In F-E the composition, density and cover of the vegetation, the most important factors influencing the migration of carabid beetles (Burel, 1989; Šustek, 1994) are similar to the condition of the edge zone Sh-E, which makes possible the migration from Sh-E to F-E.

Our research pointed out that the edges may have a crucial role in the maintenance and preservation of biodiversity; therefore, their conservation is essential. The edges may also serve as a corridor for the migration of individuals, making them important elements in preventing the isolation of the populations (Den Boer, 1970; Burel, 1989; Petit and Burel, 1993; Petit, 1994). Biomonitoring of the edge areas is essential as this includes early observation of structural changes, degradation and succession. Such early observations may also contribute to the success of operative nature conservation.

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Inundative Releases of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) for the Control of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) on Outdoor Crops

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The cotton whitefly, *Bemisa tabaci* (Genn.) is one of the most important pests in Egypt. The parasitoid *Encarsia formosa* Gahan was introduced from Italy and United Kingdom, mass reared and colonized. Weekly inundative releases of *E. formosa* began from August to November, 1996 to control *B. tabaci* on eight crops in five locations, in Egypt. Rate of release was 5–8 adult females/plant. The parasitoid *E. formosa* established rapidly and spread over the released locations. Maximum parasitism rate of 83% observed in the 11th week on *Lantana camara* in Giza.

The cotton whitefly, *Bemisa tabaci* (Genn.) is a polyphagous species that attacks crops found in Mediterranean, desert tropical and subtropical regions of the world and on glasshouse vegetables crops in temperate regions. Recently, molecular, behavioural and morphological evidence points to the occurrence of difference biotypes (so-called strains or races) of *B. tabaci* and this may account for increased crop losses to this pest (Polaszek et al., 1992; Brown et al., 1992 and Bellows et al., 1994).

In Egypt during 1992–1993, *B. tabaci* has become a serious pest transmitting viral diseases to cotton and tomato and causing feeding damage to tomatoes (Abd-Rabou, 1994). *B. tabaci* is parasitized by species of *Encarsia* and *Eretmocerus* (Gerling, 1974; Lopez-Avila, 1987; Polaszek et al., 1992 and Myartseva and Yasnosh, 1992). One of these parasitoids, *Encarsia formosa* Gahan is a well-known biological control agent of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera-Aleyrodidae). Recent work has focussed on assessing the efficacy of inundative releases of *E. formosa* for control of *B. tabaci* strain B on greenhouse crops (Parrella et al., 1991 and Hoddle and Van Driesche, 1996).

The efficacy of inundative releases of *E. formosa* for control of *B. tabaci* on field crops has not been extensively studied. We assessed the impact of weekly inundative releases of *E. formosa* on *B. tabaci* population growth on eight host plants (cabbage, cucumber, cotton, seet potato, yellow sage, tomato, common bean and eggplant).

We monitored numbers of live and parasitized larvae each week over a 15 week cropping season.

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Materials and Methods

Shipments of the parasitoid *E. formosa* were sent to Egypt from Ciba Bunting company (UK) and Biolab Company (Italy) during March, 1996 (personal communication, S. Abd-Rabou). In the greenhouse (5 × 3 × 3 m), the parasitoid *E. formosa* was successfully mass reared on infestation of the greenhouse whitefly, *T. vaporariorum* that were feeding on *Lantana camara* plant. Parasitized pupal case of whitefly were collected from the culture by brush them off leaves and put it in vials after counting for releasing. Vials containing adult parasitoids, were tied to an infested portion of the central plant and then the vials were opened at both ends to allow the parasitoids to crawl out slowly.

E. formosa were released weekly on eight plants, *Brassica oleracea* var. capitata (290 plant) (cabbage), *Cucumis sativus* (1150 plant) (cucumber), *Gossypium barbadense* (850 plant) (cotton), *Ipomoea batatas* (1120 plant) (sweet potato), *Lantana camara* (50 plant) (yellow sage), *Lycopersicum esculentum* (2600 plant) (tomato), *Phaseolus vulgaris* (1600 plant) (common bean), and *Solanum melongena* (340 plant) (eggplant), in five governorates, Demyaat, fayoum, Giza, Qalyubiya and Minufiya. The study conducted in 1/4 feddan located in each of the 8 localities listed above. Weekly releases of 5–8 parasitoid pupae/plant were began from August to November, 1996. Remove (*B. tabaci* second, third and fourth larval instars) and replace with five leaves from (5 plants) cabbage, (5 plants) eggplant and (5 plants) cucumber, ten from cotton (10 plants), 20 from sweet potato (20 plants) and 30 from each tomato (30 plants), yellow sage (6 plants) and common beans (30 plants). Leaves were then transferred to the laboratory. *B. tabaci* eggs and first larval stage were eliminated as well as any other insects under stereoscopic microscope. The second, third and fourth larval instars were recorded per leaf. Each leaf was stored in well-ventilated emergence glass tubes and monitored daily for parasitoids emergence. Percentage parasitism = no. parasitized/no. parasitized + no. unparasitized. The same procedures of treatment experiment were conducted on the control experiment. For distinguishing the parasitoids of *Encarsia*, *E. lutea* has transparent pupal skins but *E. formosa* black skin. Where *Eretmocerus mundus* has yellow skin.

Results and Discussion

Per cent parasitism of *B. tabaci* by *E. formosan* on certain plants varied according to the region in Egypt. Qalyubiya region is located in Lower Egypt and is characterized by mild temperatures and high humidity. Environmental conditions may have affected levels of parasitism in this area. Per cent parasitism of *B. tabaci* by *E. formosa* was highest on cabbage at 81% in the 14th week. Parasitism reaches 80% and 65% on beans and eggplants in the 12th week of the trial, the lowest level of parasitism were observed on cucumber (33% at week 14) (Fig. 1). Work by Hulspas-Jordan and Van Lenteren (1978) and Li et al. (1987) found hair density on cucumber plant leads to a proportional decrease in walking speed of the parasitoid *E. formosa* and their affects parasitism levels. Trichomes on cucumber may have resulted in lower parasitism levels. Also, Fayoum

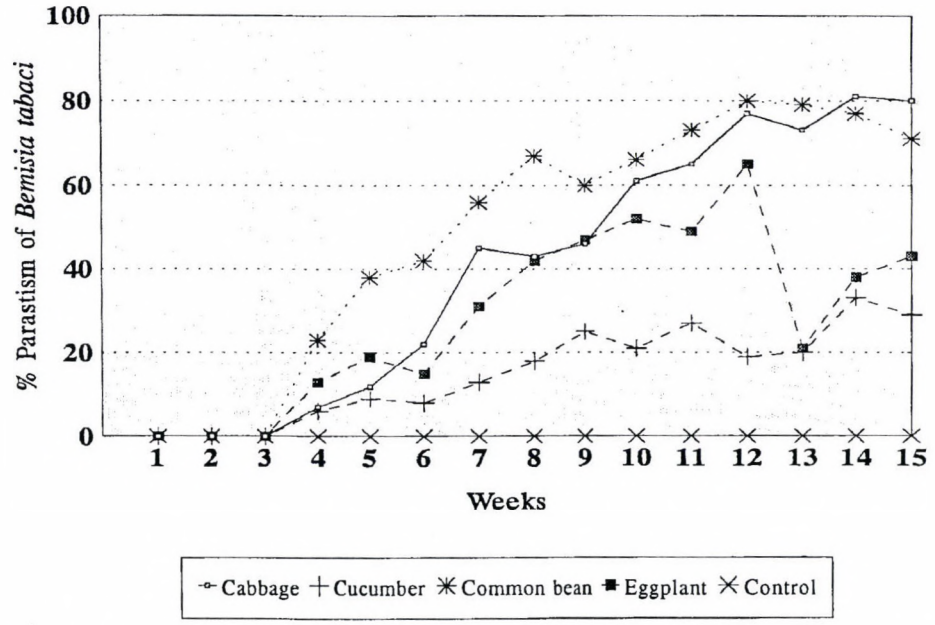

Fig. 1. Per cent parasitism of *Bemisia tabaci* on some plant by *Encarsia formosa* in Qalyubiya Governorate

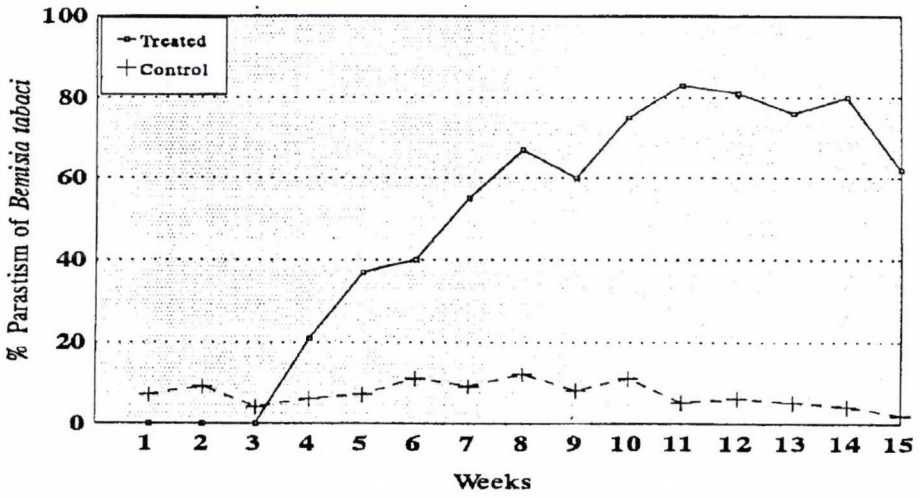

Fig. 2. Per cent parasitism of *Bemisia tabaci* on yellow sage plant by *Encarsia formosa* in Giza Governorate

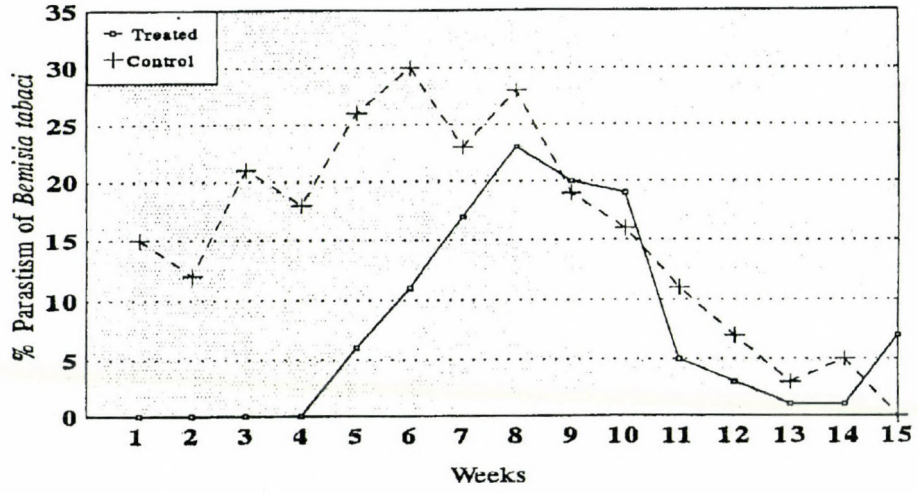

Fig. 3. Per cent parasitism of *Bemisia tabaci* on cotton plant by *Encarsia formosa* in Demyaat Governorate

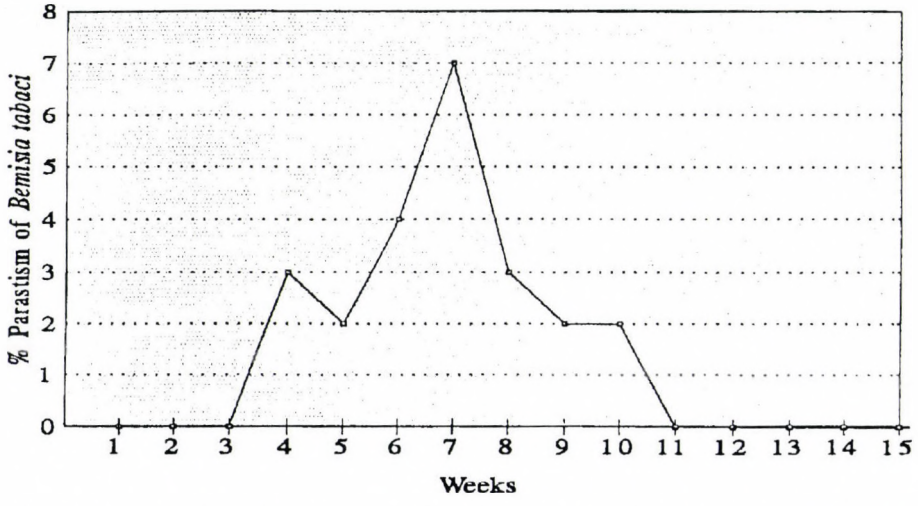

Fig. 4. Per cent parasitism of *Bemisia tabaci* on tomato plant by *Encarsia formosa* in Fayoum Governorate

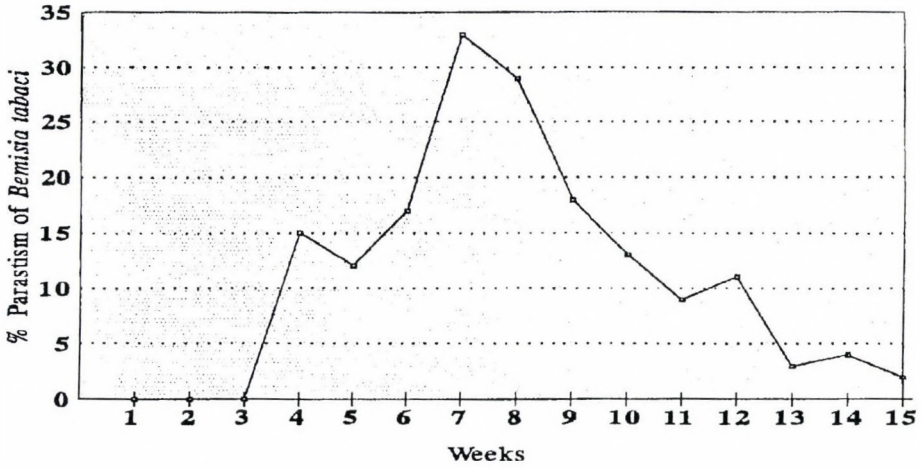

Fig. 5. Per cent parasitism of *B. tabaci* on sweet potato by *Encarsia formosa* in Minufiya Governorate

region is isolated area in Upper Egypt and can be characterized by special environmental condition. This may have reflected on lower per cent parasitism on *B. tabaci* by *E. formosa* of this area. Per cent parasitism of *E. formosa* reached 7% in the 7th week (Fig. 4) on tomato plant.

In Giza region is located in Upper Egypt, the per cent parasitism on *B. tabaci* by *E. formosa* on *Lantana camara* reached 83% in the 11th week (Fig. 2). *Lantana camara* is an ornamental plant and is not sprayed with insecticides, parasitism reached 12% in the 8th week in the control experiment. This host plant acts as a reservoir for indigenous parasitoids of *B. tabaci* (e.g. *Encarsia lutea* and *Eretmocerus mundus*) where parasitism reached 12% in 8th week of the experiment.

In Minufiya and Demyaat regions are located in lower Egypt, the per cent parasitism on *B. tabaci* by *E. formosa* on cotton and sweet potato plants reached 23 and 33% in the 8th and 7th week, respectively. On cotton there which was not sprayed, parasitism reached 28% in the 8th week in the control experiment (Figs 3, 5). The result of examining the control experiment in Qalyubiya, Fayoum and Minufiya indicated nell parasitism by local parasitoids.

The parasitoids attacking *B. tabaci* in Egypt are obviously inadequate. The dominant species *E. lutea* and *Er. mundus* are ineffective parasitoids (Abd-Rabou, 1994). The present work agree with this statement by the result of the control experiment. So, the importation of additional natural enemies is, therefore, highly recommended. Hoddle and Van Driesche (1996) confirmed the important biological control agent of *E. formosa* for controlling *B. tabaci* on greenhouse crops. The present work deals with the importation, colonization and releasing of *E. formosa* on *B. tabaci* on certain economic plants in different various bioclimatic regions.

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New Data to the Knowledge on the Phytoseiid Fauna in Hungary (Acari: Mesostigmata)

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Author gives a report on the results of the survey made between 1990 and 1997 on street trees, in parks, green spaces of housing estates, arboreta and home gardens. Phytoseiid mites were found on 84 woody plant species out of 301 studied. A total of 29 phytoseiid mite species were found. The following 6 species were new for the Hungarian fauna: *Amblydromella intercalaris* (Livshitz et Kuznetzov, 1972), *A. rhenana* (Oudemans, 1905), *Amblyseius versutus* Begljarov (1981), *Neoseiulus huron* (Chant et Hansell, 1971), *Typhlodromus ernesti* Ragusa et Swirski (1978) and *Typhlodromus repens* (Begljarov, 1981).

Euseius finlandicus (Oudemans, 1915) and *Kampimodromus aberrans* (Oudemans, 1930) were the two most frequent phytoseiid species.

Phytoseiid mites are worldwide groups of predatory mites. They are predaceous upon phytophagous mites, and play role in the integrated pest management in orchard (McMurtry, 1983; Chant, 1985). Representatives of Phytoseiidae are the most widespread predatory mite on foliage in temperate zone (Eickwort, 1983). Most members of Phytoseiidae are generalist predators in contrast to a few specialist phytoseiids (McMurtry, 1992).

In the Hungarian research on phytoseiid mites, after Kropczynska and Jenser (1968), Komlovszky (1980, 1984), Komlovszky and Jenser (1987), the work of Bozai (1980, 1987, 1996) was significant. He confirmed the occurrence of 45 species in Hungary. In the last two decades several papers were published on phytoseiid mites on fruit trees and grapevine (Molnár and Boldog, 1989; Sárospataki et al., 1992; Bognár and Jenser, 1996; Molnár, 1996; Ripka, 1997).

Materials and Methods

Between 1990 and 1997, a mite survey was made on ornamental trees and shrubs of traffic roads, squares, parks, botanical gardens, home gardens, and green areas of housing estates in all districts of Budapest (in some other localities e.g. in Hungary and Croatia, also herbaceous plants were occasionally examined). Plant samples from 301 woody plant species (minimum 5 shoots, branches or bark/plant or 25 leaves/plant) were taken in plastic bags from January to late November. During plant examination with

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binocular microscope (upper and lower surfaces of the leaves, petioles, buds, bark, flowers, galls, etc.), all the mites found on the plant samples were put into AGA solution or directly into lactic acid. After the clearing of phytoseiid mites in the lactic acid, they were placed into Keifer's medium 2 (Keifer, 1952) following the Keifer's mounting method for eriophyoid mites. The cleaned and dyed mites were then placed into Keifer's medium 3. The Hoyer's medium with sorbitol (Keifer, 1975), and Heinze's polyvinylalcohol (PVA) medium (Schmutterer, 1959) were used alternatively for mounting the specimens. The slide preparations were dried in thermostat at 32 °C and then sealed with nail varnish. Specimens were examined with a phase contrast microscope. For mite determination the keys of Chant (1959), Karg (1971, 1993), Livshitz and Kuznetzov (1972), Kolodochka (1978) and Bozai (1987), as well as several original species descriptions and other available reports were used.

The species and genus names of catalog of Moraes et al. (1986) were used.

Results

Phytoseiid mites were collected on 84 species of 301 woody plant species belonging to 28 families. Out of the total 958 plant samples phytoseiids were present in 227 samples. A total of 29 phytoseiid species were found (see Table 1 for the exact host/locality data).

Most phytoseiid species: 13 on Salicaceae, 8 on Tiliaceae, 7 on Aceraceae, and 6 on Oleaceae occurred, respectively (see Table 2). Phytoseiids occurred on 14 plant species of family Rosaceae, on 13 plant species of Salicaceae, on 7 plant species of Aceraceae, Oleaceae and Tiliaceae.

Euseius finlandicus was the most frequent dendrophil predatory mite. It was found on 56 woody plants. Among the arboreal mites *E. finlandicus* was the second most frequent species behind *Tydeus californicus* (Banks, 1904) (Acari: Prostigmata: Tydeidae). *Kampimodromus aberrans* was the second most frequent phytoseiid mite. It was present on 33 tree and shrub species. *Typhloctonus tiliarum* was the third, collected from 7 woody plant species. The following 6 species are recorded for the first time for Hungarian fauna: *Amblydromella intercalaris, Amblydromella rhenana, Amblyseius versutus, Neoseiulus huron, Typhlodromus ernesti, Typhlodromus repens.*

Several species of Phytoseiidae overwintered under colonies of scale insects. Overwintering adults of *T. repens, Amblydromella recki, K. aberrans, E. finlandicus* were found under the scales of *Pseudaulacaspis pentagona* (Targioni-Tozzetti), *Epidiaspis leperii* (Signoret) and *Parthenolecanium corni* (Bouché) (for more data on Coccoidea see Ripka et al. 1996).

In winter the females of *E. finlandicus* and *Amblyseius versutus* were found in the twig and bud galls of *Aceria populi* (Nalepa, 1980) on *Populus* × *berolinensis.* Females of *Neoseiulus huron* overwintered also in the galls of *A. populi* on *Populus alba.*

Table 1

Phytoseiid mite species collected from ornamental trees and shrubs

Mite species	Plant species	Sampling place	Sampling date
**Amblydromella intercalaris* (Livshitz et Kuznetzov, 1972)			
	Quercus cerris	Széchenyi-hegy	11. 03. 1995
	Quercus ilex	Lanterna (Croatia)	05. 07. 1996
Amblydromella recki (Wainstein, 1958)			
	Escallonia × *langleyensis*	Gellérthegy	28. 01. 1995
	Pyrus betulifolia	Gellérthegy	18. 01. 1995
	Rhus typhina	Kőbánya	04. 01. 1995
**Amblydromella rhenana* (Oudemans, 1905)			
	Euonymus europaeus	Gellérthegy	15. 06. 1997
	Juglans nigra	Kőbánya	07. 07. 1995
Amblyseius andersoni (Chant, 1957)			
	Acer campestre	Szabadsághegy	22. 08. 1991
	Acer saccharinum	Óbuda	30. 04. 1991
		Mesteri (Vas c.)	15. 08. 1997
	Geum urbanum	Mesteri (Vas c.)	15. 08. 1997
	Phyllostachys viridi-glaucescens	Törökvész	23. 07. 1997
	Salix elaeagnos	Mesteri (Vas c.)	15. 08. 1997
**Amblyseius versutus* Begljarov, 1981			
	Populus × *berolinensis*	Kispest	09. 04. 1993
Bawus talbii (Athias-Henriot, 1960)			
	Acer campestre	Keszthely (Zala c.)	25. 07. 1994
	Alnus glutinosa	Baskó (Borsod-A.-Z. c.)	12. 08. 1994
	Prunus dulcis	Széchenyi-hegy	02. 07. 1995
	Salix viminalis	Sasad	11. 06. 1993
Euseius finlandicus (Oudemans, 1915)			
	Acer campestre	Józsefváros	09. 06. 1992
		Németvölgy	20. 07. 1993
		Keszthely (Zala c.)	25. 07. 1994
	Acer negundo	Belváros	02. 05. 1992
		Zugló	25. 08. 1992
		Csepel	18. 08. 1993
	Acer platanoides	Alsórákos	04. 08. 1993
		Zirc (Veszprém c.)	26. 05. 1997
	Acer pseudoplatanus	Pestszentlőrinc	22. 09. 1992
		Pestszentlőrinc	05. 08. 1993
		Újlak	13. 10. 1993
		Törökvész	07. 05. 1994
		Sárvár (Vas c.)	26. 08. 1994
		Zirc (Veszprém c.)	21. 05. 1997
	Acer saccharinum	Óbuda	30. 04. 1991
		Kőbánya	16. 05. 1991
		Zugló	04. 08. 1993
		Mesteri (Vas c.)	15. 08. 1997
	Acer tataricum	Németvölgy	23. 05. 1993
		Rézmál	10. 06. 1994

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Aesculus hippocastanum	Németvölgy	09. 09. 1992
		Budafok	20. 07. 1993
		Németvölgy	17. 08. 1993
	Aesculus pavia	Zirc (Veszprém c.)	26. 05. 1997
	Ailanthus altissima	Kispest	25. 06. 1991
	Alnus glutinosa	Tabán	16. 05. 1992
	Carpinus betulus	Tabán	26. 05. 1992
		Gellérthegy	23. 08. 1994
	Clematis vitalba	Törökvész	29. 05. 1993
	Cornus sanguinea	Krisztinaváros	20. 06. 1991
		Törökvész	15. 05. 1993
		Margitsziget	30. 05. 1993
		Törökvész	29. 07. 1994
	Corylus avellana	Gazdagrét	04. 08. 1993
	Corylus colurna	Józsefváros	18. 07. 1993
		Budafok	20. 07. 1993
	Crataegus laevigata	Pasarét	02. 05. 1992
	Deutzia × *magnifica*	Törökvész	12. 06. 1993
	Fraxinus americana	Gellérthegy	01. 10. 1992
	Fraxinus angustifolia	Óbuda	23. 05. 1991
		Újpalota	28. 08. 1991
		Belváros	29. 06. 1992
		Belváros	17. 07. 1992
	Fraxinus excelsior	Felhévíz	24. 08. 1991
		Rákosszentmihály	28. 08. 1991
		Belváros	30. 08. 1991
		Újpalota	04. 08. 1993
	Fraxinus ornus	Rákosszentmihály	11. 09. 1992
		Vérmező	22. 08. 1994
	Fraxinus pennsylvanica	Rákoskeresztúr	27. 05. 1991
		Újpalota	04. 08. 1993
	Gleditsia triacanthos	Angyalföld	12. 06. 1993
	Juglans nigra	Kőbánya	07. 07. 1995
		Kőbánya	10. 08. 1996
	Juglans regia	Mesteri (Vas c.)	28. 07. 1993
	Juglans sp.	Gellérthegy	11. 08. 1995
	Koelreuteria paniculata	Pasarét	12. 06. 1993
		Budatétény	03. 08. 1994
	Ligustrum vulgare	Törökvész	30. 05. 1992
	Magnolia kobus	Víziváros	05. 06. 1994
	Malus × *purpurea*	Vérhalom	21. 05. 1993
	Platanus × *hispanica*	Vérmező	23. 08. 1994
	Populus alba	Kispest	22. 09. 1992
	Populus × *berolinensis*	Kispest	03. 04. 1992
		Kispest	11. 07. 1993
	Prunus avium	Országút (Bp. II. d.)	03. 06. 1993

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Prunus cerasifera	Törökvész	30. 04. 1995
	Prunus domestica	Újpalota	12. 02. 1991
		Pestújhely	01. 06. 1992
	Prunus padus	Pasarét	10. 07. 1992
		Rákoskeresztúr	06. 08. 1993
	Prunus serrulata	Vérmező	26. 05. 1991
	Quercus robur	Rákoskeresztúr	25. 07. 1997
	Rhamnus utilis	Gellérthegy	09. 06. 1994
		Gellérthegy	17. 07. 1997
	Rhus typhina	Rákoskeresztúr	04. 08. 1997
	Salix alba	Rákospalota	30. 08. 1993
	Salix matsudana 'Tortousa'	Törökvész	17. 08. 1994
	Sambucus nigra	Pestszenterzsébet	05. 08. 1993
	Staphylea pinnata	Gellérthegy	02. 06. 1994
	Tilia americana	Józsefváros	29. 06. 1994
	Tilia cordata	Józsefváros	22. 07. 1991
		Ferencváros	09. 06. 1992
		Józsefváros	18. 07. 1993
		Budafok	20. 07. 1993
		Törökvész	25. 07. 1993
		Pasarét	16. 08. 1994
		Mesteri (Vas c.)	21. 08. 1994
	Tilia × *euchlora*	Józsefváros	29. 06. 1994
	Tilia × *europaea*	Józsefváros	29. 06. 1994
	Tilia miqueliana	Józsefváros	29. 06. 1994
	Tilia platyphyllos	Keszthely (Zala c.)	02. 07. 1993
		Józsefváros	18. 07. 1993
		Kelenföld	30. 08. 1994
		Zirc (Veszprém c.)	21. 05. 1997
	Tilia tomentosa	Józsefváros	18. 07. 1991
		Tabán	26. 05. 1992
		Erzsébetváros	04. 08. 1993
		Alsórákos	04. 08. 1993
		Széphalom (Borsod-A.-Z. c.)	09. 08. 1994
		Törökvész	08. 06. 1995
	Ulmus minor	Törökvész	01. 05. 1995
	Ulmus scabra	Törökvész	11. 06. 1992
		Törökvész	08. 06. 1993
	Xanthoceras sorbifolium	Józsefváros	01. 05. 1994
	Zelkova serrata	Felhévíz	14. 10. 1996
Galendromus longipilus (Nesbitt, 1951)			
	Corylus avellana	Pestszenterzsébet	05. 08. 1993
	Quercus robur	Rákoskeresztúr	25. 07. 1997
	Salix aegyptiaca	Pestszenterzsébet	19. 06. 1992

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Tilia platyphyllos	Terézváros	06. 07. 1991
		Csepel	26. 08. 1991
Kampimodromus aberrans (Oudemans, 1930)			
	Acer campestre	Józsefváros	09. 06. 1992
	Acer negundo	Csepel	18. 08. 1993
	Acer opalus	Gellérthegy	07. 06. 1994
	Acer pseudoplatanus	Pestszentlőrinc	22. 09. 1992
		Újlak	13. 10. 1993
		Törökvész	07. 05. 1994
	Acer saccharinum	Mesteri (Vas c.)	15. 08. 1997
	Aesculus hippocastanum	Németvölgy	09. 09. 1992
		Budafok	20. 07. 1993
		Németvölgy	17. 08. 1993
	Carpinus betulus	Gellérthegy	23. 08. 1994
	Celtis australis	Novigrad (Croatia)	03. 07. 1996
	Celtis occidentalis	Pestszenterzsébet	28. 05. 1993
	Corylus avellana	Pestszenterzsébet	05. 08. 1993
		Törökvész	01. 08. 1994
	Corylus colurna	Budafok	20. 07. 1993
	Crataegus × *lavallei*	Törökvész	30. 05. 1992
	Fraxinus angustifolia	Pasarét	17. 09. 1992
	Fraxinus excelsior	Felhévíz	24. 08. 1991
	Fraxinus ornus	Rákosszentmihály	11. 09. 1992
	Fraxinus pennsylvanica	Zugló	17. 09. 1992
	Juglans nigra	Kőbánya	29. 03. 1995
		Kőbánya	07. 07. 1995
	Malux × *purpurea*	Vérhalom	21. 05. 1993
	Malus sp.	Kőbánya	19. 07. 1992
	Platanus × *hispanica*	Pestszentlőrinc	24. 09. 1992
	Populus alba	Budafok	07. 02. 1995
		Őrmező	12. 05. 1995
	Populus × *canadensis*	Törtel (Pest c.)	17. 08. 1995
	Prunus domestica	Újpalota	12. 02. 1991
	Prunus spinosa	Törökvész	26. 04. 1993
	Pseudocydonia sinensis	Gellérthegy	13. 06. 1994
	Quercus cerris	Széchenyi-hegy	01. 03. 1995
	Rhus typhina	Kőbánya	04. 01. 1995
		Rákoskeresztúr	14. 05. 1995
		Rákoskeresztúr	04. 08. 1997
	Salix aegyptiaca	Gellérthegy	13. 06. 1994
	Tilia cordata	Ferencváros	09. 06. 1992
		Erzsébetváros	26. 08. 1992
		Margitsziget	16. 08. 1993
	Tilia tomentosa	Erzsébetváros	04. 08. 1993
	Ulmus laevis	Józsefváros	29. 06. 1994

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Viburnum carlesii	Gellérthegy	07. 06. 1994
	Viburnum opulus	Törökvész	30. 05. 1992
Neoseiulus agrestis (Karg, 1960)			
	Juniperus virginiana	Soroksár	03. 07. 1994
Neoseiulus astutus (Begljarov, 1960)			
	Populus × *berolinensis*	Kőbánya	07. 01. 1991
		Kispest	19. 11. 1992
		Kispest	11. 07. 1993
	Populus nigra	Pestszentlőrinc	24. 09. 1992
	Populus simonii	Kispest	09. 04. 1993
	Salix caprea	Rákoskeresztúr	12. 04. 1993
**Neoseiulus huron* (Chant et Hansell, 1971)			
	Populus alba	Budafok	07. 02. 1995
		Őrmező	12. 05. 1995
Paraseiulus incognitus (Wainstein et Arutunjan, 1967)			
	Fraxinus angustifolia	Újpalota	04. 08. 1993
	Fraxinus excelsior	Kelenföld	24. 09. 1991
		Újpalota	04. 08. 1993
	Fraxinus pennsylvanica	Újpalota	04. 08. 1993
	Phyllostachys viridi-glaucescens	Törökvész	05. 08. 1996
	Tilia cordata	Erzsébetváros	26. 08. 1992
	Tilia miqueliana	Józsefváros	29. 06. 1994
Paraseiulus soleiger (Ribaga, 1902)			
	Fraxinus angustifolia	Újpalota	28. 08. 1991
		Belváros	29. 06. 1992
		Víziváros	17. 07. 1992
		Újpalota	04. 08. 1993
	Fraxinus excelsior	Felhévíz	24. 08. 1991
		Kelenföld	24. 09. 1991
		Újpalota	04. 08. 1993
	Fraxinus pennsylvanica	Újpalota	04. 08. 1993
	Phyllostachys viridi-glaucescens	Törökvész	05. 08. 1996
	Tilia cordata	Erzsébetváros	26. 08. 1992
	Tilia miqueliana	Józsefváros	29. 06. 1994
Phytoseius echinus Wainstein et Arutunjan, 1970			
	Acer pseudoplatanus	Kőbánya	18. 07. 1993
	Salix caprea	Rákospalota	30. 08. 1993
	Salix elaeagnos	Rákoskeresztúr	18. 07. 1993
		Rákoskeresztúr	23. 05. 1996
Phytoseius juvenis Wainstein et Arutunjan, 1970			
	Salix caprea	Rákospalota	08. 09. 1993
Phytoseius macropilis (Banks, 1909)			
	Salix elaeagnos	Rákoskeresztúr	25. 07. 1997
Phytoseius plumifer (Canestrini et Fanzago, 1876)			
	Salix 'Mesuneco'	Gellérthegy	20. 05. 1996

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
Typhloctonus aceri (Collyer, 1957)			
	Acer campestre	Törökvész	01. 05. 1995
Typhloctonus formosus (Wainstein, 1958)			
	Acer campestre	Keszthely (Zala c.)	25. 07. 1994
	Corylus avellana	Törökvész	01. 08. 1994
	Quercus cerris	Széchenyi-hegy	01. 03. 1995
Typhloctonus squamiger (Wainstein, 1960)			
	Acer platanoides	Alsórákos	22. 09. 1992
		Kispest	11. 07. 1993
		Zugló	04. 08. 1993
		Pünkösdfürdő	18. 08. 1993
Typhloctonus tiliarum (Oudemans, 1930)			
	Aesculus hippocastanum	Budafok	20. 07. 1993
		Németvölgy	17. 08. 1993
	Corylus colurna	Józsefváros	18. 07. 1993
		Törökvész	21. 05. 1995
	Fraxinus angustifolia	Belváros	30. 08. 1991
		Pestszenterzsébet	24. 09. 1991
	Tilia americana	Józsefváros	29. 06. 1994
	Tilia cordata	Rákosszentmihály	29. 08. 1991
		Budafok	21. 07. 1992
		Törökvész	16. 08. 1994
		Mesteri (Vas c.)	21. 08. 1994
	Tilia miqueliana	Józsefváros	29. 06. 1994
	Tilia platyphyllos	Terézváros	06. 07. 1991
		Csepel	26. 08. 1991
		Budatétény	04. 09. 1992
		Kelenföld	30. 08. 1994
Typhlodromus cotoneastri Wainstein, 1961			
	Salix babylonica	Gellérthegy	08. 06. 1994
**Typhlodromus ernesti* Ragusa et Swirski, 1978			
	Pinus sylvestris	Óbuda	17. 03. 1994
Typhlodromus perbibus Wainstein et Arutunjan, 1968			
	Tilia miqueliana	Józsefváros	29. 06. 1994
Typhlodromus phialatus Athias–Henriot, 1960			
	Taxus baccata	Gellérthegy	13. 02. 1995
	Thuja occidentalis	Kőbánya	23. 01. 1995
Typhlodromus pyri Scheuten, 1857			
	Taxus baccata	Pasarét	03. 08. 1995
		Gellérthegy	11. 08. 1995
	Ulmus minor	Törökvész	01. 05. 1995
**Typhlodromus repens* (Begljarov, 1981)			
	Juglans nigra	Kőbánya	29. 03. 1995
Typhlodromus tiliae Oudemans, 1929			
	Fraxinus excelsior	Pasarét	17. 09. 1992
	Tilia platyphyllos	Zirc (Veszprém c.)	21. 05. 1997

* = new species for the Hungarian fauna

Table 2

Number of phytoseiid species on plant families

Class	Series	Family	Number of mite species
Coniferopsida	Coniferales	Cupressaceae	1
		Taxaceae	2
Dicotyledonopsida	Magnoliales	Magnoliaceae	1
	Ranunculales	Ranunculaceae	1
	Hamamelidales	Platanaceae	2
	Saxifragales	Escalloniaceae	1
		Hydrangeaceae	1
	Rosales	Rosaceae	5
	Fabales	Caesalpiniaceae	1
	Rutales	Simaroubaceae	1
	Sapindales	Anacardiaceae	3
		Aceraceae	7
		Hippocastanaceae	3
		Sapindaceae	1
	Celastrales	Celastraceae	1
		Staphyleaceae	1
	Rhamnales	Rhamnaceae	1
	Cornales	Cornaceae	1
	Dipsacales	Caprifoliaceae	2
	Oleales	Oleaceae	6
	Malvales	Tiliaceae	8
	Salicales	Salicaceae	13
	Urticales	Ulmaceae	3
	Fagales	Betulaceae	2
		Corylaceae	4
		Fagaceae	5
	Junglandales	Junglandaceae	4
Monocotyledonopsida	Poales	Poaceae	3

During the vegetation period phytoseiids were usually collected from the underside of the leaves. During plant examitation with binocular microscope they often escaped to the dark side of the leaves.

Phytoseiids prefer the pubescent veinangles, and midribs. From May to September phytoseiid populations of 4–10 specimens were present on the leaves of *Acer campestre, A. pseudoplatanus, A. negundo, Fraxinus angustifolia, F. ornus, Aesculus hippocastanum* and *Tilia cordata.* Phytoseiid mites were found considerably on the leaves without phytophagous mites (so-called on "clean leaves") and together with tydeids e.g. *E. finlandicus* and *K. aberrans*. Both species are generalist predators. These species consume also plant origin foods such as pollen.

Discussion

The Hungarian fauna of species Phytoseiidae has been investigated by several specialists. The papers published up to present report species on wild and cultivated plants and in soil. Bozai (1996) reported 45 species that belonged to the subfamily of Phytoseiinae from herbaceous and woody plants and from soil, too. According to Bozai (1996) *E. finlandicus* proved to be the most common. It is the most frequent predatory mite on ornamental trees and shrubs and the second among all dendrophil mites. It was very common on the leaves of *Acer pseudoplatanus*, *Fraxinus angustifolia*, *F. excelsior*, *Tilia cordata, T. tomentosa. K. aberrans, Amblyseius andersoni, T. tiliarum, Paraseiulus soleiger* and *Phytoseius macropilis* were reported as frequent species (Bozai, 1996). *K. aberrans* was the second most frequent phytoseiid mite. It prefers *A. pseudoplatanus*, *A. hippocastanum* and *T. cordata. Paraseiulus soleiger* was frequently found on *F angustifolia* and *F. excelsior* while *T. tiliarum* on *T. platyphyllos* and *T. cordata*. Species of *Phytoseius* seems to be correlated to *Salix* spp., whereas *Neoseiulus* spp. to *Populus* spp.

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New Data to the Knowledge on the Tydeid Fauna in Hungary (Acari: Prostigmata)

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Authors give a report on the results of collection made between 1990 on 1997 and street trees, in parks, green spaces of housing estates and arboreta. Tydeid mites were found on 104 woody plant species out of 301 studied. A total of 21 tydeid mite species were found. Three species of *Lorryia* and two of *Tydeus* could not be identified with certainty. Two other species belong to new genera. The following 8 species were new for the Hungarian fauna: *Lorryia pulchra* (Oudemans, 1929); *L. mali* (Oudemans, 1929); *L. obliqua* (Kuznetzov, 1973); *Tydeus calabrus* (Castagnoli, 1984); *T. longisetosus* Kuznetzov et Zapletina, 1972; *T. praeditus* Livshitz et Zapletina, 1972; *Triophtydeus triophthalmus* (Oudemans, 1929); *Homeopronematus staerki* (Schruft, 1972). *Tydeus californicus* (Banks, 1904) was the most frequent dendrophil mite.

Tydeids are common in soil, in mosses and lichens, on plants, rarelier in stored grain, in nests or on insects – probably as a phoretic form. According to Momen (1988), representatives of Tydeidae are the most widespread plant-inhabiting mites. The role of the tydeid mites play in relation to plants and other arthropods is rather unclear. Generally they are poorly specialised feeders (Krantz and Lindquist, 1979). A few species were recorded as plant feeders, the majority of species are rather omnivorous: saprophagous, fungivorous, and predators of the smallest arthropods and their eggs. Among other so-called indifferent mites the tydeids serve as secondary prey of predatory mites (Karg, 1992).

In Spain (Garcia Mari et al., 1986) and in Italy (Corino and Duverney, 1989), the tydeids were the most frequent mites on citrus species and grapevines. Tydeids frequently occur in leaf domatia on the underside of the leaves (Walter and Denmark, 1991; Willson, 1991). In Belgium André (1986) identified 22 species of Tydeidae collected on bark of different tree species. He reported that *Triophtydeus lebruni* was the most frequent corticolous epiphyte dweller arthropod.

The tydeid fauna of Hungary is only fragmentarily known. In the Hungarian research on tydeid mites, after the works of Komlovszky (1980, 1984), the work of Bozai (1997) was significant. He confirmed the occurrence of 20 species. Further information on tydeids living on trees and grapevines are available from studies of Dellei and Szendrey (1989, 1991), and Molnár (1990).

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Materials and Methods

Between 1990 and 1997, mite survey was made on ornamental trees and shrubs of traffic roads, squares, parks, botanical gardens, home gardens, and green areas of housing estates in all districts of Budapest (in some other locatlities e.g. in Croatia and Yugoslavia, also herbaceous plants were occasionally examined). Plant samples from 301 woody plant species (minimum 5 shoots, branches or bark/plant or 25 leaves/plant) were taken in plastic bags from January to late November. During plant examination with binocular microscope (upper and lower surfaces of the leaves, petioles, buds, bark, flowers, galls, etc.), all the mites found on the plant samples were put into AGA solution or directly into lactic acid. After the clearing of tydeid mites in the lactic acid, they were placed into Keifer's medium 2 (Keifer, 1952) following the Keifer's mounting method for eriophyoid mites. The cleaned and dyed mites were then placed into Keifer's medium 3. The Hoyer's medium with sorbitol (Keifer, 1975), and Heinze's polyvinylalcohol (PVA) medium (Schmutterer, 1959) were used alternatively for mounting the specimens. The slide preparations were dried in thermostat at 32 °C and then sealed with nail varnish. Specimens were examined with a phase contrast microscope. For mite determination the keys of Baker (1965, 1970), André (1979, 1980), and Kaźmierski (1989), as well as original species descriptions by Castagnoli (1984) and Kaźmierski (1997) were used.

Results

Tydeids were present in 229 plant samples out of total 958 samples. Tydeid mites were collected on 104 species of 301 woody plant species belonging to 34 families. A total of 21 tydeid species were found (see Table 1 for the exact host/locality data).

Three species of *Lorryia* and two of *Tydeus* could not be identified with certainty; they may appear to be newly discovered. The following 8 species were new for the Hungarian fauna: *Lorryia pulchra, L. mali, L. obliqua, Tydeus calabrus, T. longisetosus, T. praeditus, Triophtydeus triophthalmus, Homeopronematus staerki.* Two other species belong to new genera. No physical damage that could be associated with tydeid mites with certainty was observed on infested plant species. Tydeid mites occurred on 20 plant species of family Rosaceae, on 10 species of Oleaceae, and on 9 species of Salicaceae.

Tydeus californicus and *Triophtydeus immanis* were the two most frequent species found on 60 and 23 host plant species, respectively.

Out of 21 tydeid species the following 11 species (52.4%) were found solely on foliage: *Lorryia pulchra, L. mali, L.* cf. *elinguis* sp. n. III., *Tydeus calabrus, T. longisetosus, T. praeditus, T. inclutus, T.* cf. *maculatus* sp. n. IV., *Tydeus* cf. *linarocatus* sp. n. V., Tydaeolinae gen. nov., sp. n. VI., Pronematinae gen. nov., sp. n. VII. While 2 species (9.5%) were found solely on bark, viz. *Lorryia reticulata* and *L. obliqua.* The remaining 8 species (38.1%) were collected both on foliage and bark: *Lorryia ferula, L.* cf. *wooleyi* sp. n. I., *L.* cf. *woolleyi* sp. n. II., *Tydeus caudatus, T. californicus,*

Table 1

Tydeid mite species collected from ornamental trees and shrubs

Mite species	Plant species	Sampling place	Sampling date
Tydeinae André, 1979			
Lorryia Oudemans, 1925 sensu Kaźmierski, 1989			
Lorryia reticulata (Oudemans, 1928)			
	Escallonia × *langleyensis*	Gellérthegy	28. 01. 1995
	Euonymus europaeus	Gellérthegy	15. 06. 1997
		Gellérthegy	09. 09. 1997
	Morus alba	Széchenyi-hegy	04. 02. 1991
**Lorryia pulchra* (Oudemans, 1929)			
	Acer campestre	Törökvész	01. 05. 1995
	Acer pseudoplatanus	Sárvár (Vas c.)	26. 08. 1994
		Zirc (Veszprém c.)	21. 05. 1997
	Alnus glutinosa	Baskó (Borsod-A.-Z. c.)	12. 08. 1994
	Cornus sanguinea	Törökvész	29. 07. 1994
	Tilia platyphyllos	Keszthely (Zala c.)	02. 07. 1993
Lorryia ferula Baker, 1944	*Salix alba*	Sashalom	13. 03. 1996
**Lorryia mali* (Oudemans, 1929)			
	Acer campestre	Keszthely (Zala c.)	25. 07. 1994
	Cornus sanguinea	Törökvész	29. 07. 1994
	Prunus cerasifera	Törökvész	30. 04. 1995
	Tilia tomentosa	Széphalom (Borsod-A.-Z. c.)	09. 08. 1994
**Lorryia obliqua* (Kuznetzov, 1973)			
	Prunus domestica	Újpalota	12. 02. 1991
Lorryia cf. *woolleyi* sp. n. I.	*Acer platanoides*	Alsórákos	04. 08. 1993
	Fraxinus excelsior	Vár	05. 01. 1995
	Tilia cordata	Törökvész	16. 08. 1994
Lorryia cf. *woolleyi* sp. n. II.	*Cornus stolonifera*	Gazdagrét	10. 02. 1995
	Forsythia suspensa	Kispest	13. 06. 1993
	Phellodendron amurense	Margitsziget	21. 02. 1991
	Populus alba	Budafok	07. 02. 1995
	Prunus domestica	Újpalota	12. 02. 1991
	Prunus spinosa	Vérmező	17. 08. 1993
	Rosa sp.	Kispest	09. 03. 1992
	Thuja occidentalis	Budaörs (Pest c.)	03. 03. 1993
		Őrmező	13. 02. 1995
Lorryia cf. *elinguis* sp. n. III.	*Acer campestre*	Keszthely (Zala c.)	25. 08. 1994
	Tilia cordata	Keszthely (Zala c.)	25. 07. 1994
	Tilia platyphyllos	Keszthely (Zala c.)	02. 07. 1993
Tydeus Koch, 1835 sensu Kaźmierski, 1989			
Tydeus caudatus (Dugés, 1834)			
	Acer pseudoplatanus	Zirc (Veszprém c.)	21. 05. 1997
	Actinidia chinensis	Józsefváros	15. 05. 1996
	Celtis australis	Novigrad (Croatia)	03. 07. 1996
	Cornus sanguinea	Vérmező	22. 05. 1991
		Vérmező	20. 06. 1991
		Törökvész	15. 05. 1993

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
		Margitsziget	30. 05. 1993
		Törökvész	29. 07. 1994
	Fraxinus angustifolia	Óbuda	23. 05. 1991
		Pasarét	17. 09. 1992
	Prunus spinosa	Vérmező	26. 04. 1993
		Vérmező	27. 04. 1993
		Vérmező	17. 08. 1993
	Rubus idaeus	Törökvész	20. 03. 1994
	Salix babylonica	Gellérthegy	08. 06. 1994
	Tilia cordata	Törökvész	25. 07. 1993
	Tilia × euchlora	Józsefváros	29. 06. 1994
	Ulmus scabra	Törökvész	11. 06. 1992
		Törökvész	08. 06. 1993
		Törökvész	11. 06. 1993
	Ulmus laevis	Józsefváros	29. 06. 1994
		Józsefváros	15. 05. 1996
Tydeus californicus (Banks, 1904)			
	Acer negundo	Zugló	25. 08. 1992
		Csepel	18. 08. 1993
	Acer opalus	Gellérthegy	07. 06. 1994
	Acer platanoides	Alsórákos	22. 09. 1992
		Alsórákos	04. 08. 1993
		Pünkösdfürdő	18. 08. 1993
	Acer pseudoplatanus	Kőbánya	07. 05. 1991
		Pestszentlőrinc	22. 09. 1992
		Pestszentlőrinc	05. 08. 1993
		Újlak	08. 10. 1993
		Újlak	13. 10. 1993
	Acer saccharinum	Pestszentlőrinc	22. 09. 1992
		Pestszentlőrinc	05. 08. 1993
		Zugló	04. 08. 1993
		Mesteri (Vas c.)	15. 08. 1997
	Ailanthus altissima	Újpalota	11. 09. 1992
	Amelanchier canadensis	Gödöllő (Pest c.)	03. 06. 1994
	Carpinus betulus	Gellérthegy	23. 08. 1994
	Catalpa bignonioides	Ferencváros	09. 06. 1992
	Cornus alba	Budaörs (Pest c.)	01. 03. 1995
	Cornus stolonifera	Gazdagrét	10. 02. 1995
	Corylus avellana	Gazdagrét	04. 08. 1993
	Corylus colurna	Józsefváros	18. 07. 1993
		Budafok	20. 07. 1993
		Rákoskeresztúr	21. 05. 1995
	Crataegus laevigata	Pestszentlőrinc	22. 09. 1992
	Crataegus succullenta	Gellérthegy	24. 01. 1995
	Diospyros sp.	Zimony (Yugoslavia)	31. 07. 1997
	Deutzia × magnifica	Törökvész	12. 06. 1993

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Elaeagnus commutata	Rákoskeresztúr	29. 03. 1995
	Elaeagnus umbellata	Gellérthegy	24. 08. 1994
	Euodia hupehensis	Rákoskeresztúr	27. 06. 1993
	Euonymus europaeus	Gellérthegy	08. 06. 1997
		Gellérthegy	15. 06. 1997
	Fontanesia fortunei	Zugló	04. 08. 1993
	Fraxinus americana	Gellérthegy	01. 10. 1992
	Fraxinus excelsior	Margitsziget	29. 11. 1990
		Kelenföld	24. 09. 1991
		Vár	05. 01. 1995
	Fraxinus ornus	Rákosszentmihály	11. 09. 1992
	Fraxinus pennsylvanica	Újpalota	11. 09. 1992
		Újpalota	04. 08. 1993
	Hippophaë rhamnoides	Pestszentlőrinc	25. 07. 1994
	Juglans nigra	Kőbánya	07. 07. 1995
		Kőbánya	11. 08. 1996
	Juglans sp.	Gellérthegy	24. 05. 1995
		Gellérthegy	11. 08. 1995
	Koelreuteria paniculata	Budatétény	04. 09. 1992
		Budatétény	03. 08. 1994
	Ligustrum vulgare	Tabán	06. 02. 1991
		Zugló	17. 07. 1992
		Vár	10. 03. 1993
	Magnolia kobus	Víziváros	05. 06. 1994
	Malus × *purpurea*	Vérhalom	21. 05. 1993
	Osmanthus × *borkwoodi*	Józsefváros	04. 01. 1995
	Phyllostachys viridi-glaucescens	Törökvész	05. 08. 1996
	Platanus × *hispanica*	Pestszentlőrinc	24. 09. 1992
	Populus alba	Óbuda	10. 06. 1993
		Budafok	07. 02. 1995
	Populus simonii	Kispest	09. 04. 1993
	Prunus domestica	Újpalota	12. 02. 1991
		Szentendre (Pest c.)	23. 01. 1995
	Prunus dulcis	Széchenyi-hegy	02. 07. 1995
	Prunus padus	Rákoskeresztúr	06. 08. 1993
	Prunus spinosa	Vérmező	17. 08. 1993
	Pyrus pyraster	Józsefváros	04. 01. 1995
	Quercus robur	Rákoskeresztúr	25. 07. 1997
	Quercus turneri	Kőbánya	04. 01. 1995
	Rhamnus utilis	Gellérthegy	17. 07. 1997
	Rhus typhina	Rákoskeresztúr	04. 08. 1997
	Ribes nigrum	Mátyásföld	14. 07. 1993
	Ribes sp.	Józsefváros	23. 06. 1995
	Salix aegyptiaca	Gellérthegy	13. 06. 1994
	Salix alba	Rákoskeresztúr	31. 05. 1993
	Sambucus nigra	Gazdagrét	15. 07. 1993

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Solanum dulcamara	Törökvész	16. 08. 1994
	Sorbus borbásii	Békásmegyer	18. 08. 1992
	Symphoricarpos orbiculatus	Gellérthegy	23. 08. 1994
	Tilia cordata	Csepel	20. 07. 1993
	Tilia × *europaea*	Józsefváros	29. 06. 1994
	Tilia platyphyllos	Erzsébetváros	26. 08. 1992
		Józsefváros	18. 07. 1993
	Tilia tomentosa	Erzsébetváros	04. 08. 1993
		Alsórákos	04. 08. 1993
		Széphalom (Borsod-A.-Z. c.)	09. 08. 1994
	Ulmus laevis	Józsefváros	29. 06. 1994
**Tydeus calabrus* (Castagnoli, 1984)			
	Acer campestre	Zirc (Veszprém c.)	26. 05. 1997
	Acer pseudoplatanus	Zirc (Veszprém c.)	21. 05. 1997
	Aesculus pavia	Zirc (Veszprém c.)	26. 05. 1997
	Tilia platyphyllos	Zirc (Veszprém c.)	21. 05. 1997
**Tydeus longisetosus* Kuznetzov et Zapletina, 1972			
	Fraxinus excelsior	Kelenföld	24. 09. 1991
	Salix elaeagnos	Rákoskeresztúr	23. 05. 1996
**Tydeus praeditus* Livshitz et Zapletina, 1972			
	Setaria verticillata	Maglód (Pest c.)	10. 09. 1995
Tydeus inclutus Livshitz, 1973	*Geum urbanum*	Mesteri (Vas c.)	16. 08. 1997
	Phyllostachys viridi-glaucaescens	Törökvész	23. 07. 1997
Tydeus cf. *maculatus* sp. n. IV.	*Acer campestre*	Zirc (Veszprém c.)	26. 05. 1997
	Aesculus pavia	Zirc (Veszprém c.)	26. 05. 1997
Tydeus cf. *linarocatus* sp. n. V.	*Acer saccharinum*	Mesteri (Vas c.)	15. 08. 1997
Tydaeolinae André, 1979			
Gen. nov., sp. n. VI.	*Salix aegyptiaca*	Pasarét	07. 06. 1997
Triophtydeinae André, 1979			
Triophtydeus Thor, 1932			
**Triophtydeus triophthalmus* (Oudemans, 1929)			
	Acer campestre	Törökvész	01. 05. 1995
		Zirc (Veszprém c.)	26. 05. 1997
	Acer pseudoplatanus	Zirc (Veszprém c.)	21. 05. 1997
	Celastrus scandens	Józsefváros	04. 01. 1995
	Escallonia × *langleyensis*	Gellérthegy	28. 01. 1995
	Fraxinus angustifolia	Pasarét	17. 09. 1992
	Juglans nigra	Kőbánya	07. 07. 1995
		Kőbánya	11. 08. 1996
	Ligustrum vulgare	Vár	10. 03. 1993
	Maclura pomifera	Törökvész	01. 06. 1993
	Quercus turneri	Kőbánya	04. 01. 1995
	Rhamnus utilis	Gellérthegy	17. 07. 1997
	Ribes silvestre	Józsefváros	04. 01. 1995
	Salix aegyptiaca	Gellérthegy	13. 06. 1994

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Tilia platyphyllos	Zirc (Veszprém c.)	21. 05. 1997
	Ulmus laevis	Józsefváros	29. 06. 1994
Triophtydeus immanis Kuznetzov, 1973			
	Celastrus orbiculatus	Gellérthegy	19. 04. 1994
	Crataegus monogyna	Gellérthegy	26. 01. 1995
	Elaeagnus commutata	Rákoskeresztúr	29. 03. 1995
	Escallonia × *langleyensis*	Gellérthegy	28. 01. 1995
	Euonymus europaeus	Rákoskeresztúr	17. 08. 1997
	Fraxinus pennsylvanica	Víziváros	08. 02. 1991
	Jasminum beesianum	Gellérthegy	27. 01. 1995
	Malus baccata	Gellérthegy	24. 01. 1995
	Malus pumila var. *niedzwetzkyana*	Gellérthegy	26. 01. 1995
	Osmanthus × *borkwoodi*	Józsefváros	04. 01. 1995
	Phyllostachys viridi-glaucescens	Törökvész	05. 08. 1994
		Törökvész	05. 08. 1996
		Törökvész	23. 07. 1997
	Prunus dulcis	Újpalota	12. 02. 1991
	Prunus spinosa	Józsefváros	31. 01. 1995
	Pyrus pashia	Gellérthegy	18. 01. 1995
	Pyrus pyraster	Józsefváros	04. 01. 1995
	Quercus ilex	Lanterna (Croatia)	05. 07. 1996
	Quercus turneri	Kőbánya	04. 01. 1995
	Ribes silvestre	Józsefváros	04. 01. 1995
	Salix aegyptiaca	Gellérthegy	13. 06. 1994
	Salix alba	Devecser (Vas c.)	08. 04. 1993
		Rákoskeresztúr	31. 05. 1993
		Sashalom	31. 05. 1993
		Rákospalota	30. 08. 1993
		Rákospalota	08. 09. 1993
	Salix elaeagnos	Rákoskeresztúr	18. 07. 1993
	Salix sp.	Rákospalota	15. 12. 1992
	Spartium junceum	Törökvész	23. 03. 1993
Pronematinae André, 1979			
**Homeopronematus staerki* (Schruft, 1972)			
	Elaeagnus angustifolia	Vérmező	26. 09. 1994
	Juglans nigra	Kőbánya	11. 08. 1996
	Koelreuteria paniculata	Budatétény	04. 09. 1992
	Pyrus communis	Pestszenterzsébet	14. 10. 1993
	Ribes silvestre	Józsefváros	04. 01. 1995
	Rosa canina	Törökvész	25. 03. 1993
	Salix caprea	Rákospalota	30. 08. 1993
		Rákospalota	08. 09. 1993
	Sambucus nigra	Törökvész	13. 08. 1992
	Solanum dulcamara	Törökvész	16. 08. 1994
	Ulmus laevis	Józsefváros	29. 06. 1994

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
Pronematinae			
Gen. nov., sp. n. VII.	*Acer campestre*	Németvölgy	18. 09. 1992
		Németvölgy	20. 07. 1993
	Acer negundo	Csepel	18. 08. 1993
	Aesculus hippocastanum	Németvölgy	09. 09. 1992
	Ailanthus altissima	Újpalota	11. 09. 1992
	Celtis occidentalis	Pestszenterzsébet	28. 05. 1993
		Pestszenterzsébet	05. 08. 1993
	Corylus colurna	Budafok	20. 07. 1993
	Fraxinus angustifolia	Pasarét	17. 09. 1992
	Fraxinus ornus	Rákosszentmihály	11. 09. 1992
	Fraxinus pennsylvanica	Újpalota	11. 09. 1992
	Koelreuteria paniculata	Budatétény	04. 09. 1992
	Ligustrum vulgare	Vár	22. 08. 1993
	Robinia pseudoacacia	Csepel	25. 09. 1992
	Sambucus nigra	Gazdagrét	13. 08. 1993
	Solanum dulcamara	Törökvész	16. 08. 1994
	Sophora japonica	Zugló	22. 09. 1992
	Symphoricarpos albus	Gazdagrét	04. 08. 1993
	Symphoricarpos × *chenaultii*	Gazdagrét	14. 07. 1993
	Symphoricarpos orbiculatus	Gellérthegy	23. 08. 1994

* = new species for the Hungarian fauna

Triophtydeus triophthalmus, T. immanis, Homeopronematus staerki. The following 8 species were present on bark in association with scale insects (Homoptera: Coccoidea): *Lorryia reticulata, L. obliqua, L.* cf. *wolleyi* sp. n. II., *Tydeus caudatus, T. californicus, Triophtydeus triophthalmus, T. immanis, Homeopronematus staerki.*

Out of 229 samples tydeid mites were found on foliage in 173 samples (75.5%). *Tydeus californicus* (72 samples), *T. caudatus* (21) and Pronematinae gen. nov., sp. n. VII (20) were the dominant foliage-inhabiting species. During the vegetation period the majority of tydeids were collected from the underside of the leaves. They prefer the leaf domatia e.g. pubescent midribs and veinangles, also leaf galls and erinea of eriophyoid mites. Usually they co-existed with vagrant eriphyoids, also tarsonemids, phytoseiids, and stigmaeids on *Acer platanoides, A. pseudoplatanus, Aesculus hippocastanum, Koelreuteria paniculata* and *Juglans nigra.*

Tydeid species were collected on bark in 56 samples (24.5%). *Triophtydeus immanis* (19), *T. californicus* (17), *Lorryia* cf. *woolley* sp. n. II. (5) were the dominant cortex dwellers. Tydeids co-occurred with scale insects in 39 samples (17%). *Triophtydeus immanis* (17), *Tydeus californicus* (7) and *Lorryia* cf. *woolley* sp. n. II. (4) were frequently present together with the following scale insects: *Pseudaulacaspis pentagona* (Targioni-Tozzetti), *Unaspis euonymi* (Comstock), *Epidiaspis leperii* (Signoret), *Aula-*

caspis rosae (Bouché), *Pseudochermes fraxini* (Kaltenbach), *Quadraspidiotus perniciosus* (Comstock), etc. (for more data on Coccoidea see Ripka et al., 1996).

The numbers of tydeid species of single plant families developed as follows: 11 on Aceraceae, 9 on Salicaceae, and 8 on Rosaceae, Oleaceae and Tiliaceae.

Discussion

The Hungarian fauna of species of Tydeidae has not been so far investigated extensively. The papers published up to present report species in soil and on raised vegetation. The studies of André (1986), Kaźmierski (1989) also Momen and Lundqvist (1996) testify, that the expected diversity of tydeid forms living in a relatively small area can appear surprisingly rich. Thus, it would not be possible to form a general view of Hungarian fauna before completing detailed studies of various habitats and geographic areas for the presence and diversity of tydeid mites. Among the tydeids found only on bark, *Lorryia reticulata* was collected from three woody plant species. It prefers xerothermic slopes, straw, hay, stored grain, the nests of birds and mammals, as well as the synanthropic habitats. It was also recorded from the "ears" (= tympana) of noctuid moths (Lepidoptera: Noctuidae) (Treat, 1975). André (1986) reported this species as *"Tydeus bedfordiensis"* from tree bark.

Lorryia obliqua was described from leaves of *Spiraea* sp. in Yalta (Crimea). Kaźmierski also found this species in B. Stojnić's collection from Yugoslavia (unpublished up to date).

Lorryia ferula is a very broadly distributed species, found mainly on leaves of different plants. This species probably can prey the eggs of coccid scale insects and spider mites (Baker, 1965). It was found only in the preceding year's eriophyoid catkin galls of *Salix alba* associated with *Tarsonemus lobosus* Suski, 1965 and *T. nodosus* Schaarschmidt, 1959.

Baker (1970) stated that *Tydeus caudatus* is replaced in the semitropical regions by *T. californicus*, and it is a closely related "sister" species. This point of view seems to be incorrect now, regarding the new records of appearance of *T. californicus*: probably both mentioned species are cosmopolitan ones, although it is true, that they exclude reciprocally each other. *T. caudatus* in undoubtedly a microphytophagous mite, whereas *T. californicus* was considered as phytophagous (Fleschner and Arakawa, 1953; Zaher and Shehata, 1963), predaceous (Baker and Wharton, 1952). According to present opinion, *T. californicus* is microphytophagous. *T. caudatus* and *T. californicus* were more frequent on leaves than on bark. *T. californicus* was collected in co-occurrence with 16 aphid species (Table 2). *T. caudatus* also co-existed with the following aphid species: *Aphis fabae* Scopoli, *A. gossypii* Glover, *Drepanosiphum oregonensis* Granovsky, *Drepanosiphum platanoidis* (Schrank), and *Periphyllus testudinaceus* (Fernie). Both tydeid species probably feed on honeydew of aphids.

Table 2

Co-occurrence of *Tydeus californicus* with some aphid species on woody plants

Host plant	Aphid species
Amelanchier canadensis	*Aphis pomi* De Geer (2 localities)
Corylus colurna	*Myzocallis coryli* (Goetze)
Euodia hupehensis	*Aphis fabae* Scopoli (2 localities)
Fraxinus angustifolia	*Prociphylus fraxini* (Fabricius)
Juglans nigra	*Aphis craccivora* Koch, *Chromaphis juglandicola* (Kaltenbach), *Monelliopsis caryae* (Monell)
Juglans sp.	*Chromaphis juglandicola* (2 localities)
Laburnum anagyroides	*Aphis cytisorum* Hartig
Magnolia kobus	*Aphis fabae*
Malux × *purpurea*	*Aphis pomi*
Populus alba	*Chaitophorus populeti* (Panzer)
Prunus cerasifera	*Phorodon humuli* (Schrank) (2 localities)
Prunus spinosa	*Phorodon humuli*
Ribes sp.	*Cryptomyzus korschelti* Börner, *Hyperomyzus picridis* (Börner et Blunck)
Salix aegyptiaca	*Aphis farinosa* Gmelin, *Chaitophorus ramicola* (Börner), *Pterocomma salicis* (Linnaeus)
Tilia × *europaea*	*Eucallipterus tiliae* (Linnaeus) (2 localities)
Tilia platyphyllos	*Eucallipterus tiliae*

Homeopronematus staerki was found on the leaves infested with eriophyoid mites from *Juglans nigra, Koelreuteria paniculata, Salix caprea, Sambucus nigra, Solanum dulcamara* and *Ulmus laevis*. According to Schruft (1972) this species probably preys upon eriophyoid mites.

Triophtydeus immanis was more frequent on bark with scale insects whereas *T. triophthalmus* on leaves.

Tydeus inclutus was described from litter under *Phyllostachys bambusoides niger* in Crimea. Kaźmierski also found this species in Iran. The present Hungarian localities concern *Phyllostachys viridi-glaucescens* and *Geum urbanum*.

Tydeus cf. *maculatus* sp. n. IV. has a very singular type of ornamentation (Kaźmierski, 1997). It is tempting to analyze relationships between tydeid mites and other microarthropods dwelling in the vegetation habitats. The nature of the association is most likely indirect, i.e. relying on effects induced onto the host plant and associated fungi by other microarthropod components of the subsystem.

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Acta Phytopathologica et Entomologica Hungarica 33 (3–4), pp. 419–424 (1998)

New Data to the Knowledge on the Stigmaeid Fauna in Hungary (Acari: Prostigmata)

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Authors give a report on the results of the regular collections made between 1990 and 1997 on street trees, in parks, green spaces of housing estates, arboreta and home gardens. Stigmaeid mites were found on 44 woody plant species out of 301 studied. A total of 4 stigmaeid mite species were found. One species of *Mediolata* could not be identified with certainty. *Mediolata mariaefrancae* André, 1977 is recorded for the first time for the Hungarian fauna. *Zetzellia mali* (Ewing, 1917) was the most frequent stigmaeid species.

Stigmaeid mites may be either associated with plants or live in soil and organic material. Majority of plant-inhabiting stigmaeids live on woody plants and are predators. Few species of *Eustigmaeus* are known as phytophagous (Krantz and Lindquist, 1979) and some *Stigmaeus* as parasitic (Swift, 1987).

Stigmaeid mites from the genera *Zetzellia* Oudemans (1927) and *Mediolata* G. Canestrini (1889) are apparently predaceous upon several species of phytophagous mites and insect pests of agricultural crops (Gonzalez-Rodriguez, 1965). Among them *Zetzellia mali* (Ewing, 1917) is the most important and frequent species. It plays role in integrated pest management of orchard (Laing and Knop, 1983).

In Belgium André (1986) found 6 stigmaeid mites on bark of different tree species, of which *Mediolata mariaefrancae* André (1977) was the most frequent and abundant. In Ireland Momen (1987) reported 6 species of *Mediolata* and one of *Eryngiopus* from an unsprayed apple orchard. In Italy Vacante and Gerson (1987) found and described three species of *Eryngiopus* in association with scale insects.

The stigmaeid fauna of Hungary is only fragmentarily known. In the Hungarian research on stigmaeid mites after the works of Komlovszky (1980, 1984) Komlovszky and Jenser (1992) reported 7 dendrophil stigmaeid species. On fruit trees and grapevines *Z. mali* was the dominant stigmaeid mite (Molnár, 1987, 1988; Dellei and Szendrey, 1989, 1991; Molnár and Nemestóthy, 1991). Jenser and Koleva (1994) reported that *Z. mali* prefers the pubescent leaf surfaces.

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Materials and Methods

Between 1990 and 1997, mite survey was made on ornamental trees and shrubs of traffic roads, squares, parks, botanical gardens, home gardens, and green areas of housing estates in all districts of Budapest (in some other localities e.g. in Croatia, also herbaceous plants were occasionally examined). Plant samples from 301 woody plant species (minimum 5 shoots, branches or bark/plant or 25 leaves/plant) were taken in plastic bags from January to late November. During plant examination with binocular microscope (upper and lower surfaces of the leaves, petioles, buds, barks, flowers, galls, etc.), all the mites found on the plant samples were put into AGA solution or directly into lactic acid. After the clearing of mites in the lactic acid, they were placed into Keifer's medium 2 (Keifer, 1952) following the Keifer's mounting method for eriophyoid mites. The cleaned and dyed mites were then placed into Keifer's medium 3. The Hoyer's medium with sorbitol (Keifer, 1975), and Heinze's polyvinylalcohol (PVA) medium (Schmutterer, 1959) were used alternatively for mounting the specimens. The slide preprations were dried in thermostat at 32 °C and then sealed with nail varnish. Specimens were examined with a phase contrast microscope. For mite determination the keys of Gonzalez-Rodriguez (1965) as well as original species descriptions were used.

Results

Stigmaeid mites were collected on 44 species of 301 woody plant species belonging to 18 families. Out of the total 958 plant samples stigmaeid mites were present in 72 samples. A total of 4 stigmaeid mites were found (see Table 1 for the exact host/locality data).

One species of *Mediolata* could not be identified with certainty, it may appear to be newly discovered. *Mediolata mariaefrancae* was new for the Hungarian fauna. Stigmaeids occurred on 7 plant species of family Oleaceae, on 6 species of Salicaceae and on 5 species of Aceraceae and Tiliaceae, respectively.

Zetzellia mali was the most frequent arboreal stigmaeid mite. It was found on 40 woody plants in 66 samples. During the vegetation period most stigmaeids were collected from the underside of the leaves. *Z. mali* prefers the pubescent (hairy) veinangles and midribs. It was frequently found in this sturcture on *Corylus colurna, Acer campestre, A. negundo, A. pseudoplatanus, Fraxinus angustifolia, F. excelsior, Salix caprea* and *Aesculus hippocastanum.*

Z. mali usually co-occurred with vagrant eriophyoids, also phytoseiids, tydeids, tetranychids and tarsonemids. In winter and early spring *Z. mali* was collected in bark crevices and on rose's twig together with *Aulacaspis rosae* (Bouché).

Z. mali was observed that on the leaf of horse chestnut the stigmaeids prey on eriophyoid mite. They perforate the vagrant mites with their stylets.

Specimens of *M. mariaefrancae* were found on bark infested with the following

Table 1

Stigmaeid mite species collected from ornamental trees and shrubs

Mite species	Plant species	Sampling place	Sampling date
Mediolata G. Canestrini, 1889			
**Mediolata mariaefrancae* André, 1977			
	Euonymus europaeus	Gellérthegy	15. 06. 1997
	Jasminum beesianum	Gellérthegy	27. 01. 1995
	Rhamnus catharticus	Józsefváros	04. 01. 1995
	Tilia × *euchlora*	Józsefváros	29. 06. 1994
Mediolata sp. nov.	*Tilia tomentosa*	Széphalom (Borsod-A.-Z. c.)	09. 08. 1994
Zetzellia Oudemans, 1927			
Zetzellia mali (Ewing, 1917)	*Acer campestre*	Németvölgy	18. 09. 1992
		Pasarét	06. 10. 1992
		Németvölgy	20. 07. 1993
		Keszthely (Zala c.)	25. 08. 1994
		Törökvész	01. 05. 1995
		Törökvész	02. 06. 1996
	Acer platanoides	Alsórákos	04. 08. 1993
	Acer pseudoplatanus	Pestszentlőrinc	22. 09. 1992
		Pestszentlőrinc	05. 08. 1993
		Újlak	13. 10. 1993
	Acer negundo	Csepel	26. 08. 1990
		Zugló	25. 08. 1992
		Csepel	18. 08. 1993
	Acer saccharinum	Mesteri (Vas c.)	15. 08. 1997
	Aesculus hippocastanum	Németvölgy	09. 09. 1992
		Budafok	20. 07. 1993
		Németvölgy	17. 08. 1993
	Alnus glutinosa	Pesterzsébet	28. 05. 1993
	Carpinus betulus	Gellérthegy	23. 08. 1994
	Celtis occidentalis	Pesterzsébet	28. 05. 1993
	Cornus sanguinea	Margitsziget	30. 05. 1993
	Corylus avellana	Gazdagrét	04. 08. 1993
		Pesterzsébet	05. 08. 1993
	Carpinus betulus	Gellérthegy	23. 08. 1994
	Corylus colurna	Józsefváros	18. 07. 1993
		Budafok	20. 07. 1993
		Rákoskeresztúr	21. 05. 1995
		Törökvész	21. 05. 1995
	Elaeagnus angustifolia	Vérmező	26. 09. 1994
	Fraxinus angustifolia	Pasarét	14. 08. 1990
		Pasarét	17. 09. 1992
		Pasarét	23. 07. 1993
		Újpalota	04. 08. 1993
	Fraxinus excelsior	Újlak	24. 08. 1991
		Rákosszentmihály	28. 08. 1991
		Pasarét	17. 09. 1992

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Fraxinus ornus	Rákosszentmihály	11. 09. 1992
		Széchenyi-hegy	13. 09. 1992
	Koelreuteria paniculata	Budatétény	03. 08. 1994
	Ligustrum vulgare	Vár	22. 08. 1993
	Ligustrum ovalifolium	Törökvész	21. 09. 1992
	Malus × *purpurea*	Vérhalom	21. 05. 1993
	Populus simonii	Kispest	09. 04. 1993
	Platanus × *hispanica*	Vérmező	23. 08. 1994
	Prunus spinosa	Vérmező	26. 04. 1993
		Vérmező	17. 08. 1993
	Pyrus communis	Pesterzsébet	14. 10. 1993
	Rosa canina	Törökvész	25. 03. 1993
	Salix aegyptiaca	Gellérthegy	13. 06. 1994
	Salix elaeagnos	Rákoskeresztúr	18. 07. 1993
		Rákoskeresztúr	15. 08. 1997
	Salix caprea	Rákospalota	30. 08. 1993
		Rákospalota	08. 09. 1993
		Rákoskeresztúr	12. 14. 1993
	Salix 'Mesuneco'	Gellérthegy	24. 05. 1995
	Salix viminalis	Sasad	16. 07. 1993
	Solanum dulcamara	Törökvész	16. 08. 1994
	Symphoricarpos orbiculatus	Gellérthegy	23. 08. 1994
	Tilia cordata	Keszthely (Zala c.)	25. 07. 1994
	Tilia miqueliana	Józsefváros	29. 06. 1994
	Tilia platyphyllos	Erzsébetváros	26. 08. 1992
		Józsefváros	18. 07. 1993
	Tilia tomentosa	Erzsébetváros	04. 08. 1993
		Törökvész	08. 06. 1995
	Vitis vinifera	Szigetszentmárton (Pest c.)	05. 08. 1994
	Ulmus pumila	Országút (Bp. II. d.)	24. 07. 1993
	Ulmus scabra	Törökvész	08. 06. 1993
Zetzellia crassirostris (Leonardi, 1899)			
	Quercus ilex	Lanterna (Croatia)	05. 07. 1996

* = new species for the Hungarian fauna

scale insects: *Pseudaulacaspis pentagona* (Targioni-Tozzetti), *Quadraspidiotus ostreaeformis* (Curtis), and *Unaspis euonymi* (Comstock) (for more data on Cocoidea see Ripka et al., 1996).

Among the arboreal mites *Z. mali* was the second most frequent predatory species behind *Euseius finlandicus* (Oudemans, 1915) (Acari: Mesostigmata: Phytoseiidae).

Discussion

The Hungarian fauna of species Stigmaeidae has not been so far investigated extensively. Two species of *Mediolata* and two species of *Zetzellia* were found on ornamental trees and shrubs in this survey. According to Molnár (1987), Dellei and Szendrey (1989, 1991), Molnár and Nemestóthy (1991) *Z. mali* was the most frequent stigmaeid on foliage. This species has a definite preference for the leaves with hairy lower sides. It is in accordance with opinion of Jenser and Koleva (1994). *Z. mali* was frequently present on leaves of *Corylus colurna, Acer campestre, A. negundo, Fraxinus angustifolia* and *Salix caprea,* etc. These plants have hairy veins and veinangles on the underside of the leaves. *Z. mali* was rare on glabrous leaves e.g. *Acer platanoides* and *Ligustrum ovalifolium.*

M. mariaefrancae was collected on bark in association with 3 armored scale insects. It is probably predaceous upon diaspidids.

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New Data to the Knowledge on the Tetranychid and Tenuipalpid Fauna in Hungary (Acari: Prostigmata)

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Author gives a report on the results of the regular collections made between 1990 and 1997 from ornamental trees and shrubs on streets, in green areas of housing estates, in home gardens and in arboreta. Spider mites were found on 67 woody ornamental species out of 301 studied. A total of 19 tetranychid species were identified. Flat mites were found on 10 woody ornamentals. A total of 3 tenuipalpid species were identified. *Tetranychus urticae* Koch, 1836 was the most frequent species found on most (26) host plant species. Severe damages were caused by *Tetranychus urticae, Eotetranychus tiliarium* (Hermann, 1804), *Eotetranychus populi* (Koch, 1838), *Schizotetranychus schizopus* (Zacher, 1913) and *Schizotetranychus garmani* Pritchard et Baker, 1955.

Oligonychus brevipodes (Targioni-Tozzetti, 1887) is recorded for the first time for the Hungarian fauna.

Among plant-inhabiting mites several phytophagous mites are potentially serious pests of agricultural crops and ornamentals. Spider mites comprise a large group of plant-feeding mites. Several representatives of Tetranychidae are agricultural pest species e.g. *Panonychus ulmi* (Koch), *Tetranychus urticae, Tetranychus turkestani* (Ugarov et Nikolski), *Oligonychus ununguis* (Jacobi). They can cause heavy yield losses. Members of the Tenuipalpidae attack various agricultural crops as well as ornamental plants. Many flat (false spider) mite species are economically important especially in the subtropics and tropics.

In Poland Kropczynska et al. (1985) studied the spider mite fauna of ornamental trees. They found *E. tiliarium* on linden, *Oligonychus pritchardi* and *Schizotetranychus garmani* on *Quercus robur*, *Eotetranychus carpini* on *Carpinus betulus*, *Eotetranychus uncatus* on *Acer saccharinum* and *Alnus glutinosa*. In the former Soviet Union Mitrofanov (1967) identified six species of *Oligonychus*, one species of *Eurytetranychus* and also one species of *Eurytetranychoides* from conifers. In Georgia Zajtseva et al. (1983) besides *Panonychus ulmi*, *Tetranychus telarius* and *Eurytetranychus thujae* reported 9 species of *Schizotetranychus,* 6 species of *Oligonychus*, 2 species of *Bryobia* in urban environment. In the former Soviet Union Livshitz and Mitrofanov (1967) reported many tenuipalpid species out of total 40, from ornamental trees and shrubs. In Italy Castagnoli (1987) described *Cenopalpus halperini* from *Pinus* spp. and *Cenopalpus pegazzanoae* from *Abies alba*. In France five tenuipalpid species were found on conifers (Gutierrez et al., 1989).

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In the Hungarian research on tetranychid and tenuipalpid mites, after the works of Bognár (1965, 1979) that of Bozai (1969, 1970a, b, c; 1971a, b; 1975; 1976) was significant.

He surveyed the distribution and dominance of spider mites and flat mites especially in orchards and gave a key (Bozai, 1970c).In the last two decades several papers were published on tetranychid and tenuipalpid mites attacking woody ornamentals (Komlovszky, 1980, 1984; Nemestóthy and Molnár, 1988; Ripka et al., 1993; Bozai and Bream, 1995; Bream and Bozai, 1995; Bognár and Jenser, 1996; Ripka, 1997).

Materials and Methods

Between 1990 and 1997, a mite survey was made on ornamental trees and shrubs of traffic roads, squares, parks, botanical gardens, home gardens, and green areas of housing estates in all districts of Budapest (in some other localities in Hungary, also herbaceous plants were occasionally examined). Plant samples from 301 woody plant species (minimum 5 shoots, branches or bark/plant or 25 leaves/plant) were taken in plastic bags from January to late November. During plant examination with binocular microscope (upper and lower surfaces of the leaves, petioles, buds, bark, flowers, galls, etc.), all the mites found on the plant samples were put into AGA solution or directly into lactic acid. After the clearing of mites in the lactic acid, they were placed into Keifer's medium 2 (Keifer, 1952) following the Keifer's mounting method for eriophyoid mites. The cleaned and dyed mites were then placed into Keifer's medium 3. The Hoyer's medium with sorbitol (Keifer, 1975), and Heinze's polyvinylalcohol (PVA) medium (Schmutterer, 1959) were used alternatively for mounting the specimens. The slide preparations were dried in thermostat at 32 °C and then sealed with nail varnish. Specimens were examined with a phase contrast microscope. For mite determination the keys of Pritchard and Baker (1955), Bozai (1969, 1970c), Livshitz and Mitrofanov (1971), Mitrofanov et al. (1987), Mitrofanov and Strunkova (1979), Meyer (1979), Baker and Tuttle (1994), as well as several original species descriptions and other available reports were used.

Results

Tetranychid mites were collected on 67 woody plant species out of 301 studied belonging to 23 families. Tenuipalpid mites were found on 10 woody plant species belonging to 4 families. A total of 19 tetranychid and 3 tenuipalpid species were identified (see Table 1 and Table 2 for the exact host/locality data).

Out of the total 958 plant samples, tetranychid mites were present in 120 samples, while tenuipalpid mites in 15 samples. Most tetranychid species: 9 on family Rosaceae, 6 on Salicaceae, 4 on Aceraceae, Corylaceae and Fagaceae occurred, respectively. *Tetranychus urticae* was the most frequent spider mite. It was found on 26 host plants.

Table 1

Tetranychid mite species collected from ornamental trees and shrubs

Mite species	Plant species	Sampling place	Sampling date
Bryobia graminum (Schrank, 1781)			
	Pinus strobus	Vérmező	29. 04. 1992
Bryobia lagodechiana Reck, 1953			
	Platanus × *hispanica*	Józsefváros	16. 05. 1991
Bryobia rubrioculus (Scheuten, 1857)			
	Corylus avellana	Pesterzsébet	05. 08. 1993
	Crataegus laevigata	Pasarét	02. 05. 1992
	Malus × *purpurea*	Vérhalom	21. 05. 1993
	Prunus avium	Kispest	02. 04. 1992
	Prunus cerasifera	Pasarét	10. 07. 1992
		Kurucles	23. 07. 1993
	Prunus dulcis	Zöldmál	21. 05. 1993
		Széchenyi-hegy	02. 07. 1995
	Prunus spinosa	Vérmező	29. 04. 1993
Bryobia tiliae (Oudemans, 1928)			
	Tilia tomentosa	Erzsébetváros	04. 08. 1993
Eotetranychus carpini (Oudemans, 1905)			
	Acer campestre	Pasarét	06. 10. 1992
		Németvölgy	20. 07. 1993
	Aesculus hippocastanum	Budafok	20. 07. 1993
	Carpinus betulus	Tabán	26. 05. 1992
		Gellérthegy	23. 08. 1994
	Corylus avellana	Gazdagrét	04. 08. 1993
		Pesterzsébet	05. 08. 1993
	Corylus colurna	Sasad	14. 06. 1994
	Fraxinus ornus	Csepel	28. 08. 1991
	Malus × *purpurea*	Békásmegyer	11. 06. 1992
		Törökvész	21. 05. 1993
	Quercus robur	Rákoskeresztúr	25. 07. 1997
	Salix viminalis	Sasad	16. 07. 1993
Eotetranychus coryli (Reck, 1950)			
	Corylus avellana	Törökvész	01. 08. 1994
	Corylus colurna	Budafok	30. 08. 1991
		Budafok	20. 07. 1993
Eotetranychus populi (C. L. Koch, 1838)			
	Acer negundo	Zugló	25. 08. 1992
	Populus alba	Kispest	22. 09. 1992
	Populus × *berolinensis*	Kispest	03. 04. 1992
		Kispest	09. 04. 1993
		Vérmező	21. 06. 1994
	Populus × *canadensis*	Törtel (Pest c.)	17. 08. 1995
	Populus × *canescens*	Törökőr	12. 09. 1990
		Óbuda	03. 04. 1991
		Kispest	17. 08. 1991

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
		Gazdagrét	08. 06. 1993
	Populus nigra	Budatétény	30. 08. 1991
	Populus tremula	Füzér (Borsod-A.-Z. c.)	13. 08. 1994
	Prunus persica	Budafok	29. 01. 1991
	Salix alba	Budafok	19. 01. 1991
	Salix daphnoides	Törökvész	12. 08. 1993
	Syringa vulgaris	Tabán	11. 02. 1991
Eotetranychus pruni (Oudemans, 1931)			
	Malus × *purpurea*	Vérhalom	21. 05. 1993
	Prunus spinosa	Vérmező	17. 08. 1993
Eotetranychus tiliarium (Hermann, 1804)			
	Acer campestre	Törökvész	01. 05. 1995
	Acer pseudoplatanus	Kőbánya	18. 07. 1993
	Malus × *purpurea*	Pünkösdfürdő	11. 06. 1992
	Rosa rugosa	Kispest	24. 06. 1992
	Tilia americana	Józsefváros	29. 06. 1994
	Tilia cordata	Józsefváros	22. 08. 1991
		Rákosszentmihály	29. 08. 1991
		Erzsébetváros	26. 08. 1992
		Budafok	20. 07. 1993
		Margitsziget	16. 08. 1993
		Törökvész	16. 08. 1994
	Tilia × *euchlora*	Józsefváros	29. 06. 1994
	Tilia × *europaea*	Józsefváros	29. 06. 1994
	Tilia platyphyllos	Józsefváros	17. 07. 1991
		Tabán	09. 06. 1992
		Budafok	21. 07. 1992
		Erzsébetváros	26. 08. 1992
		Budatétény	04. 09. 1992
		Keszthely (Zala c.)	02. 07. 1993
		Kelenföld	30. 08. 1994
		Zirc (Veszprém c.)	21. 05. 1997
	Tilia tomentosa	Törökőr	12. 09. 1990
		Józsefváros	18. 07. 1991
		Sasad	29. 08. 1991
		Tabán	26. 05. 1992
		Terézváros	26. 08. 1992
		Erzsébetváros	04. 08. 1993
		Széphalom (Borsod-A.-Z. c.)	09. 08. 1994
**Oligonychus brevipodes* (Targioni-Tozzetti, 1887)			
	Quercus robur	Pesterzsébet	19. 06. 1992
Oligonychus ununguis (Jacobi, 1905)			
	Chamaecyparis lawsoniana	Zugliget	05. 05. 1995
Panonychus ulmi (C. L. Koch, 1836)			
	Malus domestica	Velence (Fejér c.)	25. 07. 1996
	Prunus cerasifera	Pasarét	10. 07. 1992

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
		Kurucles	23. 07. 1993
	Prunus domestica	Pestújhely	01. 06. 1992
	Prunus spinosa	Vérmező	17. 08. 1993
	Robinia pseudoacacia	Belváros	17. 07. 1992
	Salix elaeagnos	Rákoskeresztúr	25. 07. 1997
	Sophora japonica	Zugló	22. 09. 1992
	Sorbus aucuparia	Budafok	21. 07. 1992
	Tilia tomentosa	Tabán	26. 05. 1992
Schizotetranychus garmani Pritchard et Baker, 1955			
	Quercus robur	Hűvösvölgy	22. 09. 1993
		Rákoskeresztúr	14. 05. 1995
	Salix aegyptiaca	Pesterzsébet	19. 06. 1992
	Salix matsudana 'Tortuosa'	Rákoskeresztúr	11. 07. 1993
	Salix 'Mesuneco'	Gellérthegy	20. 05. 1996
	Salix viminalis	Sasad	11. 06. 1993
Schizotetranychus schizopus (Zacher, 1913)			
	Salix alba	Rákospalota	30. 08. 1993
	Salix caprea	Rákospalota	08. 09. 1993
	Salix elaeagnos	Rákoskeresztúr	23. 05. 1996
	Salix purpurea	Gellérthegy	22. 08. 1994
Schizotetranychus ugarovi Wainstein, 1960			
	Prunus spinosa	Törökvész	26. 04. 1993
Tetranychus atlanticus McGregor, 1941			
	Parthenocissus tricuspidata	Lágymányos	09. 07. 1993
Tetranychus urticae Koch, 1836			
	Acer campestre	Törökvész	02. 06. 1996
	Actinidia chinensis	Rákospalota	30. 06. 1994
	Caragana arborescens	Németvölgy	28. 06. 1992
	Celtis occidentalis	Józsefváros	22. 08. 1991
		Országút	17. 07. 1992
	Crataegus laevigata	Pestszentlőrinc	22. 09. 1992
	Datura arborea	Budaörs (Pest c.)	05. 08. 1994
	Deutzia × *hybrida*	Gellérthegy	08. 06. 1994
	Elaeagnus umbellata	Gellérthegy	22. 07. 1997
	Euonymus europaeus	Rákoskeresztúr	11. 10. 1997
	Forsythia suspensa	Gazdagrét	24. 07. 1992
	Fraxinus excelsior	Rákosszentmihály	28. 08. 1991
		Víziváros	17. 07. 1992
	Fraxinus ornus	Széchenyi-hegy	13. 09. 1992
	Lonicera tatarica	Törökvész	13. 06. 1992
	Morus alba	Chania (Crete, Greece)	13. 06. 1995
	Nerium oleander	Virányos	05. 07. 1994
	Parthenocissus tricuspidata	Lágymányos	09. 07. 1993
	Poncirus trifoliata	Józsefváros	23. 03. 1995
	Populus canadensis	Törtel (Pest c.)	17. 08. 1995
	Potentilla fruticosa	Széchenyi-hegy	05. 07. 1993

Table 1 (cont.)

Mite species	Plant species	Sampling place	Sampling date
	Rhamnus imeretinus	Józsefváros	01. 05. 1994
	Rosa sp.	Szigetszentmárton (Pest c.)	05. 08. 1994
	Salix alba	Devecser (Veszprém c.)	01. 07. 1993
		Rákospalota	30. 08. 1993
		Tornyosnémeti (Borsod-A.-Z. c.)	11. 08. 1994
	Salix caprea	Rákospalota	08. 09. 1993
	Sambucus nigra	Pasarét	25. 07. 1992
	Sorbus aucuparia	Budafok	21. 07. 1992
	Syringa vulgaris	Budafok	29. 01. 1991
Tetranychus viennensis Zacher, 1920			
	Crataegus laevigata	Pasarét	02. 05. 1992
		Pestszentlőrinc	22. 09. 1992
	Populus alba	Kispest	22. 09. 1992
	Prunus cerasifera	Pasarét	10. 07. 1992
	Prunus domestica	Pestújhely	01. 06. 1992
	Prunus dulcis	Széchenyi-hegy	12. 02. 1991
	Prunus mahaleb	Ferencváros	09. 06. 1992
	Prunus padus	Pasarét	10. 07. 1992
		Rákoskeresztúr	06. 08. 1993
	Prunus spinosa	Vérmező	26. 04. 1993
	Pyrus communis	Pesterzsébet	14. 10. 1993
	Sorbus aucuparia	Budafok	21. 07. 1992
	Sorbus borbasii	Békásmegyer	18. 08. 1992
Tetranycopsis horridus (Canestrini et Fanzago, 1876)			
	Corylus avellana	Pesterzsébet	05. 08. 1993

* = new species for the Hungarian fauna

Eotetranychus populi and *Tetranychus viennensis* were present on 11 woody plants. *E. tiliarium* was collected from 10 food plants. *T. urticae* caused severe damage (leaf yellowing, bronzing, defoliation) on *Celtis occidentalis*, *Fraxinus excelsior* and *Nerium oleander*. On *Tilia* spp. *E. tiliarium* is the most important phytophagous mite causing also severe infestations. *E. populi* on *Populus* × *canescens, Eotetranychus coryli* on *Corylus avellana* and *C. colurna, Schizotetranychus garmani* on *Salix matsudana* 'Tortuosa' were found in dense colonies. The numbers of plant samples of frequent tetranychid species developed as follows: 31 of *T. urticae*, 27 of *E. tiliarium,* 16 of *E. populi*, 13 of *E. carpini* and *T. viennensis*, respectively.

Brevipalpus pulcher was collected on 2 and 6 plant species of family Cornaceae and Rosaceae, respectively. This species caused no physical damage on infested plants. *Pentamerismus oregonensis* occurred in high density on *Thuja occidentalis*, as well as *P. taxi* on *Taxus baccata*. Both tenuipalpids caused visible damage (yellowing and defoliation) on infested plants.

Table 2

Tenuipalpid mite species collected from ornamental trees and shrubs

Mite species	Plant species	Sampling place	Sampling date
Brevipalpus pulcher (Canestrini et Fanzago, 1876)			
	Cornus alba	Őrmező	28. 03. 1995
	Cornus sanguinea	Törökvész	15. 05. 1993
		Törökvész	29. 07. 1994
	Cotoneaster microphylla var. *thymifolia*	Őrmező	25. 05. 1993
	Cotoneaster tomentosa	Békásmegyer	18. 08. 1991
	Crataegus laevigata	Pasarét	16. 05. 1992
		Gellérthegy	24. 01. 1995
	Crataegus monogyna	Gellérthegy	26. 01. 1995
	Crataegus succulenta	Gellérthegy	24. 01. 1995
	Sorbus borbasii	Békásmegyer	18. 08. 1992
Pentamerismus oregonensis McGregor, 1949			
	Thuja occidentalis	Surány (Pest c.)	09. 11. 1993
		Óbuda	24. 10. 1994
Pentamerismus taxi (Haller, 1877)			
	Taxus baccata	Mártonhegy	25. 08. 1994
		Pasarét	03. 08. 1995
		Gellérthegy	11. 08. 1995

Overwintering specimens of spider mites and flat mites were found in bark crevices and under the scales of died coccids e.g. *E. populi* under the scales of *Pseudaulacaspis pentagona* and *Quadraspidiotus gigas; T. urticae – P. pentagona; T. viennensis – P. pentagona, Epidiaspis leperii, Parthenolecanium corni, Quadraspidiotus ostreaeformis; B. pulcher – E. leperii,* respectively (for more data on Coccoidea see Ripka et al., 1996). *E. populi* overwintered also in twig and bud galls of *Aceria populi* (Nalepa) (Acari: Eriophyoidea).

Bryobia graminum overwintered in the bark crevices of *Pinus strobus*. During the vegetation period this mite lives on grasses.

Discussion

The Hungarian fauna of species Tetranychidae and Tenuipalpidae has been considerably explored. Since the sixties several papers have been published on these mites. Komlovszky (1980) reported 9 tetranychid and 8 tenuipalpid species from ornamental trees and shrubs.

Ripka et al. (1993) found 8 spider mite species on woody ornamentals. Bozai and Bream (1995) reported the presence of *Brevipalpus tiliae* (De Leon) in Hungary. Bream and Bozai (1995) identified 3 tetranychid species, one bryobiid and one tenuipalpid mites from linden trees. According to Bozai (1975), *E. tiliarium* is the most important plant parasitic mite of linden. This spider mite is very common on *Tilia platyphyllos*, *T. cordata* and *T. tomentosa*.

T. urticae is a real polyphagous species. It was found on plants of 17 families.

P. ulmi and *T. viennensis* were found especially on species of Rosaceae while *E. populi* on plants of Salicaceae.

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Sinophorus turionus Ratz, the Parasitoid of the European Corn Borer, *Ostrinia nubilalis* Hbn. in Slovakia, Czech Republic and Southwestern Poland

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Sinophorus turionus, the parasitoid of the European corn borer, *O. nubilalis*, larvae was studied at four locations of Central Europe during three years. Parasitism achieved 0.76–2.04% in Nitra, southwestern Slovakia, 0.24–2.72% at Král'ovský Chlmec, eastern Slovakia, and 0.88–2.78% at Blatnice in Czech Republic. *S. turionus* was not found at Wroclaw in Poland during study. Parasitoids did not develop when the host larvae were collected until the end of July. The first parasitized host larvae were found on July 31. Parasitoid pupae developed usually in October but not before September 29. Parasitoid adults emerged during March 17–31, 1994, March 27–April 11, 1995, and April 21–27, 1996. Development threshold temperature for 50% of adult emergence was determined to be 0 °C, the respective thermal constant was 358.2–390.3 °C degree-days. Results show that there are minimum two generations of the parasitoid a year in Central Europe. Paper summarise many European records about the *S. turionus*, the parasitoid which was not naturalised in North America after its introduction in 1920–1940.

This paper reports on the insect which was named *Eulimneria crassifemur* Thoms. (Babcock and Vance, 1929, Thompson and Parker, 1928), *Limnerium alkae* Ell. and Sacht. (Babcock and Vance, 1929, Thompson and Parker, 1928, Jones, 1929), *Limnerium (Eulimneria) alkae* Ell. and Sacht. (Goidanich, 1931), *Eulimneria alkae* (Clark, 1934), *Campoplex alkae* Ell. and Sacht. (Baker et al., 1949, Blickenstaff et al., 1953, Manojlovic, 1984a, b, 1989), *Sinophorus alkae* Ell. and Sacht. (Maini, 1974, Platia and Maini, 1975), and *S. turionus* (Ratz.) (Barbatini, 1976, Maini and Burgio, 1990). In following text will be used the name *Sinophorus turionus* Ratz.

According to Thompson and Parker (1930), *S. turionus* Ratz (*Hymenoptera, Ichneumonidae*) is an important parasite of the European corn borer, *Ostrinia nubilalis*, in Europe where distribution records have been obtained from Spain, France, Belgium, Germany, Sweden, Switzerland, Italy and the central European plains. In Germany two the most important *O. nubilalis* parasitoids were *Microgaster tibialis* Nees and *S. turionus* (Zwölfer, 1929; Kunike, 1930). In 1961 *S. turionus* parasitized 1.6% *O. nubilalis* larvae at Hartheim/Rh. in Germany (Adlung, 1963). Later on, this parasitoid was reported form Poland (Kania, 1962), cold region of former Soviet Union (Tkalich, 1967), eastern Hungary (Bánk, 1972), Romania (Rosca and Barbulescu, 1983), former soviet Georgia and south of Russia (as *Limneria rufifemur*) (Chao You Shin, 1960), Yugoslavia (Manojlovic et al., 1994) and Italy (Maini, 1974; Barbatini, 1986).

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Baker et al. (1949) stated that *S. turionus* was not naturalised in North America after its introduction during 1920–1940. Blickenstaff et al. (1953) postulated that no cocoons were found in Iowa in the fall of 1950. It was also not found in Connecticut (Arbuthnot, 1955), Ontario (Wressel, 1973), Nebraska (Hill et al., 1978), and the East Central United States (Mason et al., 1994).

On the basis of studies in late 1920s Thompson and Parker (1930) summarised that the adults of *S. turionus* emerge from the ovewintering cocoons form March to June and lay their eggs in the earliest appearing corn borer larvae in July and August. The larval growth takes place in summer, and overwintering cocoons are spun in September or October. After 1930, the information about the bionomy of this parasitoid are very scarce. It was supposed that *S. turionus* probably has secondary host during spring in Yugoslavia (Manojlovic, 1984a).

The purpose of this work was a detailed investigation of *S. turionus* occurrence and bionomy in Slovakia, Czech Republic and Poland. In these countries the maize is grown in colder regions than in Yugoslavia or Italy and information about the parasitoid is almost non exist.

Materials and Methods

During 1993–1995 the larvae of the European corn borer, *Ostrinia nubilalis,* were systematically collected at four locations in Central Europe, Kráľovský Chlmec in eastern Slovakia (48° 26′ N, 21° 59′ E), Nitra in southwestern Slovakia (48° 20′ N, 18° 05′ E), Blatnice in Moravia (eastern Czech Republic, district Uherské Hradiště, 48° 57′ N, 17° 27′ E), and Wroclaw in southwestern Poland (51° 03′ N, 16° 57′ E).

The larvae of *O. nubilalis* and parasitoid cocoons were collected at the different sample sites at the end of maize growing season in September and October.

After the dissection of maize stems the host larvae and parasitoid pupae were removed and placed in 200 ml glass containers. In each container 20 larvae were kept together with two pieces of corrugated paper (3 × 5 cm), which were covered by transparent plastic foil. Larvae would move to the tunnels and crawl in between the corrugated paper and the plastic foil. This allowed us to observe the development of the larvae and of the parasitoid pupae. Containers were covered by 2 layers of cloth and placed in wooden cages. Cages with the larvae were placed outside and exposed to natural wather conditions, but were protected against rainfall. Larvae from all locations were brought to Nitra and observed under the same conditions in order to compare the development of parasitoids that originated from different locations. Cubes containing water bound by 1.5% agar were added to the glass containers every 2 weeks in autumn and spring for humidity and allwo for possible water intake by *O. nubilalis* larvae.

To monitor the development of *S. turionus*, containers with the *O. nubilalis* larvae were usually first checked at three days interval and then daily thereafter the first parasitoid adult was recovered. The time of appearance of parasitoid pupae and of emergence of adult were recorded.

After parasitoid adults had emerged, they were kept in the laboratory at 18 °C to determine their longevity.

Overall parasitism was estimated from the number of *S. turionus* pupae. The number of *S. turionus* pupae plus the coccons of the other parasitoids collected in autumn was added to the number of collected *O. nubilalis* larvae to yield the 100% value for the calculation of percent of parasitism.

An estimation of developmental thresholds and thermal constans for *S. turionus* was made based on field assessments. The phenology of the parasitoid was monitored in Nitra during three successive years and related to temperature date obtained from Agrometeorology Station at the Slovak University of Agriculture in Nitra.

The calculation of the degree-days accumulation was based on the rectangle method, taking the average of the daily minimal and maximal temperatures and subtracting from this value the estimated development thresholds. To gain such threshold values for spring development of adults under the local conditions, the following approach was used: 1) The potential thermal constants for the event "50% adult emergence" were calculated for a large number of base temperatures, rangign from 0.1, 0.2 etc. °C up to 5 °C. 2) The standard deviations of comparable values for the three years of observation were determined. 3) The base temperatures yielding a minimal standard deviation for each of the thermal constant for adult development were then taken as estimated threshold temperatures.

In autumn of 1994, 1995 and 1996. *O. nubilalis* larvae were also collected from July until the first week of December at Nitra. Collected larvae were placed to containers and the number of newly developed parasitoid cocoons were observed. The aim was to find the period of parazitisation and parasitoid pupation.

Results

Table 1 shows the parasitization of *O. nubilalis* larvae by *S. turionus* in autumn 1993–1995. In Nitra, southwestern Slovakia the parasitization was 0.76–2.04%. At Kráľovský Chlmec, eastern Slovakia it was 0.24–2.72% and at Blatnice in Czech Republic the parasitization achieved 0.63–2.78%. *S. turionus* was not found at Wroclaw in Poland during autumn 1993–1995.

The first *S. turionus* cocoons developed at the end of September (September 29; 1996), or at the beginning of October (October 19, 1994; October 6, 1995). In 1995 and 1996 all parasitoids finish their pupation during October. In 1994 new coccons were found until the beginning of December (Fig. 1).

Parasitoids did not develop when the host larvae were collected until the end of July during 1995 and until the beginning of August in 1994, or 1996. The first parasitized host larvae were found on August 3, 1994, July 31, 1995, or August 6, 1996.

Figure 2 shows the flight periods of *S. turionus* during spring in 1994–1996. The first *S. turionus* adults emerged on March 17 in 1994. In the same year, more than half number of *S. turionus* adults developed until March 30. During the spring of 1995 the

Table 1

Parasitization of the Eurpean corn borer (ECB) larvae by *Sinophorus turionus* Ratz (ST) in 1993–1995 at four locations in central Europe

	Location			
	Nitra, southwestern Slovakia	K. Chlmec, eastern Slovakia	Blatnice, Moravia, Czech Republic	Wroclaw, wouthwestern Poland
		1993–1994		
ECB larvae collected	2434	176	172	413
ST cocoons	51	5	5	0
Cocoons of other prasitoids	20	3	3	3
% parasitization by ST	2.04	2.72	2.78	0
		1994–1995		
ECB larvae collected	5020	1138	1653	1265
ST cocoons	62	8	15	0
Cocoons of other prasitoids	46	111	36	2
% parasitization by ST	1.21	0.64	0.88	0
		1995–1996		
ECB larvae collected	3737	837	1707	940
ST cocoons	29	2	11	0
Cocoons of other prasitoids	31	7	33	3
% parasitization by ST	0.76	0.24	0.63	0

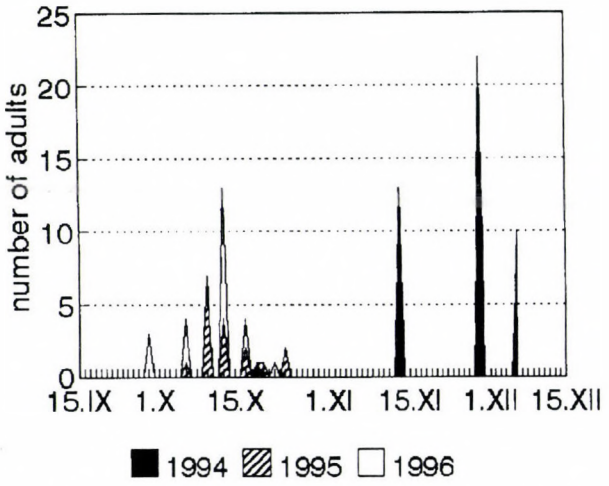

Fig. 1. Number of *Sinophorus turionus* adults originating from *Ostrinia nubilalis* larvae collected at Nitra (southwestern Slovakia) emerged in autumn 1994–1996

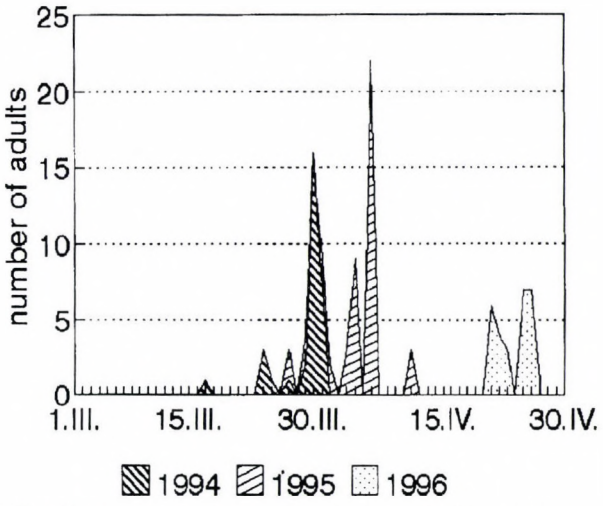

Fig. 2. Number of *Sinophorus turionus* adults originating from *Ostrinia nubilalis* larvae collected at Nitra (southwestern Slovakia) emerged in spring 1994–1996

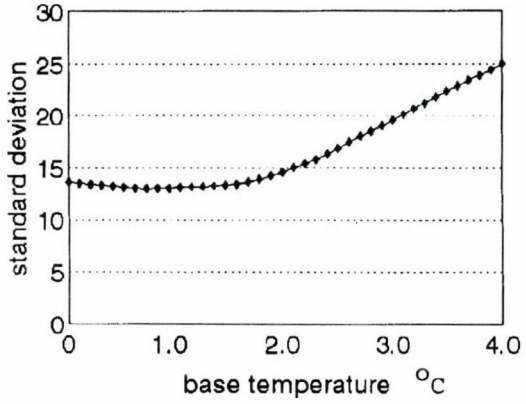

Fig. 3. Estimation of threshold temperatures for the event "adult emergence" for *Sinophorus turionus* by minimizing the standard deviation of the pontential thermal constants for three years of observation (for details see "Material and Methods")

Table 2

Development of *Sinophorus turionus* Ratz emerging from the European corn borer larvae collected in Nitra, southwestern Slovakia during 1994–1996

	Year		
	1994	1995	1996
Date of the first adults	17. III	27. III	21. IV
DD_0	299.5	288.1	295.3
Date of >50% imagoes	30. III	6. IV	25. IV
DD_0	390.3	358.2	373.4
Date of the last imagoes	31. III	11. IV	27. IV
DD_0	403.7	388.5	404.1

DD_0 = sums of degree days (base = O °C)

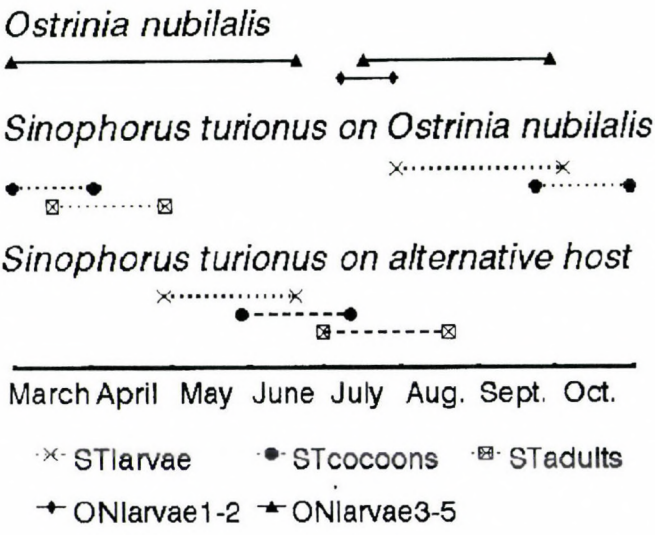

Fig. 4. Synopsis of the phenologies of the parasitoid *Sinophorus turionus* (ST) and its known primary host *Ostrinia nubilalis* (ON), at Nitra including the parasitoid generation not found on this host. The phenology of *Ostrinia nubilalis* was adapted according to Cagáň (1993) and Cagáň and Barabás (1996a, b). Probable development fo the parasitoid on an alternative host is marked by spotted and dashed lines

Table 3

Level of parasitism of the European corn borer larvae by *Sinophorus turionus* Ratz reported from different regions of Europe

Location	Geographic latitude	% parasitism	Reference
Briansk (Russia)	53° N	recorded	Tkalich, 1967
Sumy (Ukraine)	51° N	recorded	Tkalich, 1967
Wroclaw (Poland)	51° N	recorded	Kania, 1962
Wroclaw (Poland)	51° N	0.0	Cagáň, Bokor, 1998
Hartheim/Rh. (Germany)	50° N	1.6	Adlung, 1963
Poltava (Ukraine)	50° N	recorded	Ellinger and Sachtleben, 1929
Blatnice (Czech Republic	49° N	0.88–2.78	Cagáň, Bokor, 1998
Baden (Germany)	49° N	0.79	Zwölfer, 1929
Rastatt (Germany)	49° N	0.36–1.60	Kunike, 1930
Rastatt (Germany)	49° N	0.2–1.0	Zwölfer, 1928
Paris (France)	49° N	1.10	Thompson and Parker, 1930
Southern Czechoslovakia	48° N	1.3–4.3	Babcock and Vance, 1929
Kráľovský Chlmec (Slovakia)	48° N	0.24–2.72	Cagáň, Bokor, 1998
Nitra, (Slovakia)	48° N	0.76–2.04	Cagáň, Bokor, 1998
Colmar, Strassbourg (France)	48° N	6.40	Thompson and Parker, 1930
Western France	48° N	1.20	Thompson and Parker, 1930
Jura (France)	47° N	5.35	Thompson and Parker, 1930
Hungary	46–48° N	0.7–4.5	Babcock and Vance, 1929
Hungary	46–48° N	1.58	Thompson and Parker, 1930
Hungary	46–48° N	3.0–6.0	Kotlán, 1929
Fejér, Veszprém, Tolna (Hungary)	46–47° N	recorded	Dudich, 1928
District Hajdú-Bihar (Hungary)	47° N	recorded	Bánk, 1972
Keszthely (Hungary)	47° N	3.96	Sachtleben, 1930
Romania	46° N	11.7	Rosca and Barbulescu, 1983
Zagreb (Croatia)	46° N	0.8	Hergula, 1930
Friuli Region (Italy)	46° N	recorded	Barbatini, 1986
Armavir (Russia)	45° N	recorded	Ellinger and Sachtleben, 1929
Stavropol (Russia)	45° N	recorded	Chao You Shin, 1960
Kuban (Russia)	45° N	17.0	Ellinger, 1928
Merisan, Stirbei Voda (Romania)	45° N	recorded	Sachtleben, 1930
Novi Sad (Yugoslavia)	45° N	5.8	Hergula, 1929
Yugoslavia	45° N	7.20	Thompson and Parker, 1930
Northern Yugoslavia	45° N	1.2–13.2	Babcock and Vance, 1929
Zemun (Yugoslavia)	45° N	recorded	Bjegovic and Lazarevic, 1963
Yugoslavia	45° N	4.83–20.67	Manojlovic, 1984a
Mionica (Yugoslavia)	45° N	1.75–3.05	Manojlovic, 1989
Suvobor (Yugoslavia)	45° N	0.88–4.20	Manojlovic, 1989
Plain near Bologna (Italy)	44° N	0.13	Maini, 1974
Mountain near Bologna (Italy)	44° N	0.65	Maini, 1974
Southwestern France	44° N	9.87	Thompson and Parker, 1930
Vladikavkaz (Russia)	43° N	recorded	Ellinger and Sachtleben, 1929
Trojan (Bulgaria)	43° N	recorded	Sachtleben, 1930
Northwestern Spain	43° N	16.60	Thompson and Parker, 1930
Batumi (Georgia)	42° N	recorded	Chao You Shin, 1960

adults of the parasite emerged in March 27–April 11 with the maximum in the first week of April. In 1996, the parasitoid adults started to flight on April 21, and until April 27 all adults abandoned the cocoons.

Calculation for base temperatures, ranging from 0.1, 0.2 etc. °C up to 5 °C (see Materials and Methods and Fig. 3) showed, that estimated threshold temperature for adult development of *S. turionus* was 0 °C. The respective thermal constant for the event "50% adult emergence" was found to be 358.2–390.3 °C. Table 2 shows the calculations for events "first adult emergence" and "last adult emergence".

Breeding of insects in the laboratory during 1996 indicated, that parasitoid produced two new generations until the end of August at 18 °C (one generation per two months).

Figure 4 shows synopsis of the phenologies of the parasitoid *S. turionus* and its known primary host *O. nubilalis*, at Nitra including the parasitoid generarion not found on this host. Synopsis shows that there are minimum two generations of the parasitoid a year in Central Europe.

Discussion

During our study we found that parasitization of *O. nubilalis* larvae caused by *S. turionus* never exceeded 3% in central European countries. Weather conditons probably influenced the degree of parasitization which was the highest in 1993, and the lowest in 1995 at three locations. Our findings suggest that *S. turionus* would not be the crucial parasite of *O. nubilalis* in central Europe and its potential significance in the biological control of the pest is little. Our former observations (Bokor, 1998) showed that the parasitism of *O. nubilalis* by tachinid parasitoid *L. thompsoni* is higher under warm and dry climatic conditions. This was not such distinctive in the case of *S. turionus*. The degree of parasitism in Czech Republic and in Slovakia was almost the same. In some warmer regions of maize growing in Europe levels of parasitism achieved higher value compared to those from Central Europe but it was not the rule (Table 3).

Usual time of the parasitoid flight in Slovakia is the end of March and the beginning of April, and similar results were reported from studies in other European countries. The parasite emerged in April in Germany (Zwölfer, 1928, 1929), eastern France (Paillot, 1928), and Yugoslavia (Manojlovic, 1984a). Imagoes of *S. turionus* emerged on May 1 and May 6 in Poland (Kania, 1962). Our results show that spring emergence of *S. turionus* imagoes depends on the weather and the development continues at very low temperatures (over 0 °C). Thompson and Parker (1930) supposed that this feature is the reason for the absence of the parasite in the southern Mediterranean zone where it emerges in winter. Maini (1974) found that adults of *S. turionus* emerged during the winter since the cocoons containing the mature larvae were stored at a temperature of 18–20 °C. In our experiments the adults of the parasitoid emerged approximately when degree-days accumulation at threshold 0 °C (DD_0) achieved 300–400 °C. Similarly, Thompson and Parker (1930) described that when cocoons were kept at 18 °C, they did not produce the

adults until the 22nd day (DD_0 = 396 °C), whereas those kept at 27 °C produced the adults in 11 days (DD_0 = 279 °C).

Zwölfer (1929) and Manojlovic et al. (1994) reported that parasitoids leave *O. nubilalis* larvae and spin their cocoons from late September to the end of November. Our results show that the first cocoons of the parasite developed in Slovakia in October, or, in 1996, on September 29. In addition, during the summer and in September we did not observed any empty *S. turionus* cocoon in the maize stems. Where was the parasitoid from April to October? Laboratory experiment indicate that *S. turionus* is capable of living for a long period (70–75 days) at moderate temperatures, and even longer (100–110 days) at low temperatures (Thompson and Parker, 1928). This led to idea that there is but one generation of the parasitoid a year (Thompson and Parker, 1930). However, it seems probable that most of the parasites would have any host because of the gap between their emergence and the development of the *O. nubilalis* larvae. Hergula (1929) suggested that it is still unknown how the parasite spends the time from the emergence of the adult in early spring to the appearance of the young. *O. nubilalis* larvae in July. Zwölfer (1929) submitted overwintered *O. nubilalis* larvae to emerged *S. turionus* imagoes, but the larvae were not accepted by the parasitoid. Zwölfer (1928) and Paillot (1928) believed that the parasite must produce two generations a year, and the first must develop on another host. According to Paillot (1924 cit. Thompson and Parker, 1928) *S. turionus* was recorded as a parasite of the larvae of several *Microlepidoptera*, such as *Clysia (Conchylis) ambiquella* Hübn. and *Polychrosis botrana* Schiff. In the laboratory at 18 °C the second generation of the parasitoid emerged at the end of June in our experiments. This probably corresponded to the first generation of the parasitoid developing on the spring host. We found that larvae of *O. nubilalis* could be parasitized by the second generation of *S. turionus* at the end of July, and the cocoons developed in October. The only question is why the cocoons of the parasitoid did not develop sooner in the season.

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BOOK REVIEWS

Waller, J. M., Ritchie, B. J. and Holderness, M.: Plant Clinic Handbook. CAB International Wallingford 1998. 104 pp.

Since its creation in 1920, The International Mycological Institute (IMI) has been concerned with the identification of plant-pathogenic fungi and baceria. The Plant Clinic Handbook is complementary to the previous numbers in the series which deal with Biochemical Techniques for Filamentous Fungi (1994) and The Preservation and Maintance of Living Fungi (second edition, 1994). Plant Clinic Handbook provides information on all aspects of the technical procedures, material requirements and organisation for the effective operation of a plant clinic. It describes the diagnostic process, from field observation and collection through to methods of culture, microscopy, keeping records and reference material, general laboratory practice and equipment maintenance. Essential reading for those involved in setting up and running plant clinics and plant quarantine facilities. This handbook will also be of interest to all practitioners and advanced students of plant pathology, particularly those concerned with diagnostics and to crop protection specialists working in an advisory capacity. This book consists of five chapters: 1. The Plant Clinic, 2. Plant Disease Diagnosis, 3. Microscopy, 4. Culture Methods, 5. Appendices. Its price: £ 15.00 (USD 28.00). It can be ordered: CAB International Wallingford, Oxon OX10 8DE, UK. Tel: 344(0)1491 832111 Fax: +44(0)1491 826090 E-mail: cabi@cabi.org

G. Kazinczi

Bridge, P., Jeffries, P., Morse, D. R. and Scott, P. R. (eds): Information Technology, Plant Pathology and Biodiversity. CAB International Wallingford 1998. 478 pp.

Information technology is revolutionising the handling of biological information. The British Society for Plant Pathology (BSPP) has been at the forefront of several initiatives in handling information electronically, while the Systematics Association has a long-standing involvement in computer-based species identification. BSPP and the Systematics Association recognised the opportunity ot join forces and develop a combined

programme for a conference on these themes, which was held in December 1996 at the University of Kent at Canterbury. This book presents forty edited and revised papers from that conference. The topics covered are wide-ranging and focus on several themes. There are papers on subjects as diverse as biological databases, geographic information systems, probabilistic identification systems and electronic teaching aids. This book was written by 57 authors from Europe, North and Central America, China, India and New Zealand. The book consists of nine main chapters, included further 40 minor chapters. The main chapters are: 1. Setting the Scene, 2. Handling Facts to Produce Information, 3. Interpreting Information to Produce Knowledge, 4. Using Knowledge to Support Decision Making, 5. Computer-Based Species Identification, 6. Applications of Computer-Based Species Identification, 7. Passing on Knowledge in Education and Training, 8. Storing and Disseminating Knowledge, 9. Biology and Information Technology: The Road Ahead. The book provides an essential review for plant pathologists and taxonomists as well as other biologists wishing to keep up with the information revolution.

J. Horváth

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