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Contents

DISEASES

Etiology of birthwort yellow mosaic and grapevine yellow mosaic and decline
I. Tóbiás and J. Lázár 1

Production of polyclonal antibodies to grapevine leafroll associated virus isolated in Hungary and development of HRPO-based ELISA system
I. Tóbiás, J. Lázár, M. Kölber and E. Papp 5

Destruction of tobacco mosaic virus in developing local lesions in leaves of *Datura stramonium* L.
A. V. Reunov, G. D. Reunova, V. P. Nagorskaya, L. A. Lapshina and S. N. Lega 11

Reuse of ELISA plates: Development of a simple cleaning method
H. K. Manandhar, S. E. Albrechtsen and S. B. Mathur 19

Occurrence of tomato pith necrosis incited by *Pseudomonas corrugata* in Hungary
M. Hevesi, A. Zalka and K. Csizmár 31

Occurrence of *Septoria betulae* on birch (*Betula alba*) in Argentina
G. M. Dal Bello and A. E. Perello 35

A marker system for *Phytophthora parasitica* based on hybridization of PCR products with species-specific repetitive DNA
T. Érsek, J. E. Schoelz and J. T. English 37

INSECT PESTS

The winter mortality and developmental biology of *Pseudaulacaspis pentagona* Targioni-Tozzetti, 1886 (Homoptera: Coccoidea)
D. A. F. Sheble and F. Kozár 45

A preliminary study on the armoured scale insect (Homoptera, Coccoidea: Diaspididae) fauna of Antalya
F. Erler, F. Kozár and I. Tunç 53

Check-list of Coccoidea and Aleyrodoidea (Homoptera) of Afghanistan and Iran, with additional data to the scale insects of fruit trees in Iran
F. Kozár, M. A. Fowjhan and M. Zarrabi 61

Physical surface features of the grapevine leaf affecting the abundance of *Zetzellia mali* (Ewing) (Acari: Stigmaeidae)
G. Jenser and Rosica Koleva 75

The role of spiders as predators of insect pests with particular reference to orchards: A Review
S. Bogya and P. J. M. Mols 83

Acta Phytopathologica et Entomologica Hungarica 31 (1–2), pp. 1–4 (1996)

Etiology of Birthwort Yellow Mosaic and Grapevine Yellow Mosaic and Decline

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In a 20–25 years old vineyard patches of grapevine with yellow mosaic and decline were observed. Next to these diseased vines birthwort (*Aristolochia clematitis* L.) plants were present showing yellow spots and yellowing symptoms.

In diseased grapevine plants grapevine fanleaf virus, tomato black ring virus and grapevine chrome mosaic virus, grapevine vein necrosis and grapevine latent fleck were detected. Grapevine fanleaf virus was transmitted by mechanical inoculation from diseased birthwort to *Chenopodium quinoa* and identified serologically, and that it was probably the cause of the yellow mosaic symptom on this plant. In this way birthwort as a host plant may play a role in the infection cycle of grapevine fanleaf virus and grape.

Yellow mosaic, reduced growth and poor fruit setting were observed on cv. Olaszrizling in a 20–25 years old vineyard near Badacsonyőrs (Lake Balaton). The diseased plants were situated in a spot with a diameter of 8–10 m where some grapevines were dead and some new replanted ones were noticed presumably in place of dead grapes. The above-mentioned syndromes of grapevines could possibly be caused by grapevine fanleaf virus (GFLV), arabis mosaic virus (ArMV), tomato black ring virus (TBRV) or grapevine chrome mosaic virus (GCMV) (Bovey et al., 1980). In Hungary very similar symptoms were caused by grapevine chrome mosaic virus infection (Martelli et al., 1970).

Next to the diseased grapes birthwort plants (*Aristolochia clematitis* L.) were observed that showed yellow mosaic symptoms on the leaves similar to those of diseased grapevines. Birthwort is a common perennial weed in vineyards in Hungary, but hitherto there was no data about grapevine pathogen virus would infect it. It was supposed that some correlation might exist between the symptoms on grapevines and on birthwort plants.

To prove our hypothesis about this correlation additional studies were carried out to determine the causal agent(s) of diseased plants.

Materials and Methods

Three grapes showing different type of symptoms were designated for study. Code numbers: Bö-1-18 and Bö-2-15 grapes showed yellow mosaic symptoms and different degree of growth reduction; Bö-2-18 plant was characterized with yellow mosaic,

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reduced growth and poor fruit setting. Birthwort plants showing yellow mosaic symptoms were also collected (Aris).

Leaf materials were collected in August. To isolate the virus(es) transmission experiments were made by mechanical inoculation to *Chenopodium quinoa.*

In October canes were collected from the designated grapes to produce two buds cuttings and to use in chip-transmission tests (indexing) according to Lehoczky and Beczner (1980). The following woody indicator plants were used for chip-transmission: FS-4-201-39 (Siegfriedrebe), *Vitis rupestris* St. George, cv. Pinot Noir, cv. Chardonnay, cv. LN-33, cv. Red Velteliner, cv. Kékfrankos, *V. riparia* Gloire, *V. rupestris* × *V. berlandieri* 110R and *V. berlandieri* × *V. riparia* Kober 5 BB. The symptoms were evaluated in June and September over three years.

Serological tests

Ouchterlony's immunodiffusion tests were performed in 0.8% agarose gel (in 40 mM sodium barbital and 0.5 mM EDTA buffer, adjusted to pH 8.2 with hydrochloric acid). Antigens were obtained from leaves of *C. quinoa.* Antisera to the following virus isolates were used: ArMV-TYV1 (titer: 1:1024), GCMV-BYM-50 (titer: 1:1024), GCMV-SFL-2 (titer: 1:512), TBRV-MIRS-1 (titer: 1:1024) TBRV-MIRS-2 (titer: 1:256), GFVBBFL-1 (titer: 1:1024), GFV-PFL-16 (titer: 1:256).

The protein-A sandwich-ELISA (Edwards and Cooper, 1985) was used. For coating 1 μg/ml Protein-A (Pharmacia), for detecting antisera horseradish peroxidase enzyme (HRPO) conjugated Protein-A (prepared in Agricultural Biotechnology Center, Gödöllő) in dilution of 1:4000 were used. The above listed antisera were used in dilution 1:500 or 1:1000. The leaf extracts (grapes or *C. quinoa*) were used at dilution of 1:5, 1:20 and 1:50.

Results

Virus isolation from leaf samples of grapes collected in August were not successful. Virus(es) from birthwort and from greenhouse forced cuttings of donor grapes were transmitted and maintained on *C. quinoa* L. by mechanical inoculation.

The virus isolate from birthwort was tested on herbaceous plants and it gave symptoms very similar to those of GFLV except on *C. quinoa* which showed systemic mosaic with severe leaf deformation and stunting. Virus isolated from birthwort was seed transmitted on *C. quinoa* (3–18%) plants. In vitro properties of virus isolate were (TIP 60 °C, DEP 2.5×10^3, LIV 7 days) very similar to GFLV.

Indexing

Most of the woody indicators gave characteristic yellowing symptoms with growth reduction which may be caused by grapevine fanleaf virus yellow mosaic strain, grapevine chrome mosaic virus and tomato black ring virus, too. The observed symptoms

Table 1

Results of ELISA testing of donor grapes and *C. quinoa* plants, using antisera specific to GCMV-BYM-50, GCMV-SFL-2, TBRV-MIRS-1, TBRV-MIRS-2, GFV-BBFL-1 and GFV-PFL-16. (Data of ArMV-TYV-1 not shown)

Host plants	Grapevine fanleaf virus		Tomato black ring virus		Grapevine chrome mosaic virus	
	BBFL-1	PFL-16	MIRS-1	MIRS-2	BYM-50	SFL-2
Bö-1-18 grapevine	+	+	(+)	(+)	–	–
Bö-1-18 *C. quinoa*	+	+	+	+	–	–
Bö-2-15 grapevine	+	+	+	+	–	–
Bö-2-15 *C. quinoa*	+	+	+	+	–	–
Bö-2-18 grapevine	+	+	+	+	+	+
Bö-2-18 *C. quinoa*	+	+	+	+	+	+
Aris *C. quinoa*	+	+	–	–	–	–

+ – Absorbance values were three times higher than virus free control ones.

were slightly different from those caused by GFLV, GCMV or TBRV alone, probably because of the complex viral infection. Black discolouration and necrosis of main and auxiliary veins on *V. rupestris* × *V. berlandieri* 110 R indicator plants proved the occurrence of grapevine vein necrosis in all three donor grapes. On the upper leaves of *V. rupestris* St. George indicator plants developed characteristic transparent fleck, which verified the existence of grapevine fleck in the donor plants.

Serological tests

In Ouchterlony's immunodiffusion and ELISA tests mechanically transmissible grapevine fanleaf virus, tomato black ring virus and grapevine chrome mosaic virus were detected from leaf samples of *C. quinoa*. Leaf samples taken in June from donor cuttings and woody indicators were tested by ELISA and the same viruses were detected as from *C. quinoa* (Table 1).

Discussion

The tests carried out have demonstrated that grapevine yellow mosaic and decline syndrome was caused by a mixed virus infection. From diseased grapes sap transmissible GFLV, TBRV and GCMV were detected by test plants and by serological means. The symptomatological reactions of indicator vines showed the presence of vein necrosis and fleck. These two later mentioned viruses cause latent infection on *Vitis vinifera* L.

(Hewitt and Bovey, 1979) therefore in all likelihood the symptoms on grapes observed near Badacsonyőrs were caused by GFLV, TBRV and GCMV. In our experiments Bö-1-18 and Bö-2-15 donor grapes – showing yellow leaf and growth reduction symptoms – were infected by GFLV and TBRV. Bö-2-18 donor grape with yellow leaf, growth reduction and poor fruit setting had symptoms due to GFLV, TBRV and GCMV infection.

This type of mixed infection was detected for the first time in Hungary. Natural occurrence of TBRV in commercial vineyards is new because so far this virus was detected only in nursery (Lehoczky and Burgyán, 1986).

The results obtained show that GFLV naturally infects birthwort plants. These data confirm our preliminary report about the natural hosts of GFLV (Horváth et al., 1994). Infected plants showed yellow spots and yellow mosaic symptoms. Diseased birthwort plants were checked in 3 consecutive years and the GFLV was transmitted and serologically detected in all cases. In nature, GFLV has been recovered so far only from grapevine and not from weed species growing in vineyards in which GFLV was widespread (Martelli, 1978). Our data – the presence of GFLV in perennial weeds – compel us to be reconsider our knowledge about the ecological properties of GFLV. The birthwort plant my serve as reservoir of GFLV thus it may play a role in the infection cycle of this virus.

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Production of Polyclonal Antibodies to Grapevine Leafroll Associated Virus Isolated in Hungary and Development of HRPO-Based ELISA System

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Hungarian isolate of grapevine leafroll associated virus (GLRaV-III) was purified and antiserum was produced. The antiserum was conjugated with horseradish peroxidase (HRPO) and used in DAS-ELISA compared with BIOREBA kit. Out of 309 compared grape samples 287 gave the same results in both ELISA systems (93% agreement). Two samples were positive in Bioreba but negative in Hungarian HRPO system, while 20 samples were positive in HRPO system and negative in Bioreba kit. Further studies are needed to clear up whether these differences are due to serological differences of Swiss and Hungarian isolates of GLRaV-III or other reasons

Grapevine leafroll disease occurs worldwide and is of great economic importance (Goheen, 1970). Symptoms include downward rolling and interveinal reddening of leaves on varieties with dark coloured fruit. Varieties with light coloured fruit also show rolling of leaves but develop interveinal chlorosis. Fruit production is lowered in diseased vines and sugar content is reduced due to delayed ripening of fruit. Frost resistance also decreased (Goheen, 1970).

The causal viruses currently referred to as "leafroll associated" belong to two distinct genera: Closterovirus (particles 1400–2200 nm) and Trichovirus (particles less than 800 nm) (Martelli, 1993).

In Hungary grapevine leafroll disease (GLD) is one of the most widespread disease on grapevine. Nowadays sanitary selection is the only preventive strategy useful against this grapevine disease. Selection is usually based on indexing method by grafting canes on indicator varieties. An indexing is time consuming because the expression of leafroll symptoms needs 2 or 3 years. In order to develop a rapid detection procedure Gugerli et al. (1984) purified closterovirus like particles from GLR diseased grapevines and produced antiserum that could be used effectively in direct double sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Further characterization of GLR associated closteroviruses proved the existence of four distinct serotypes (Hu et al., 1990; Gugerli et al., 1984; Zimmerman et al., 1990; Gugerli and Ramel, 1993).

In this paper the production of polyclonal antibodies specific for closterovirus particles isolated from diseased Zweigelt (Hungary) showing leafroll symptoms and contained GLRaV-III antigens (Lehoczky et al., 1993) were described.

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Materials and Methods

Virus source

Leaves of GLRaV-III infected Zweigelt plants were collected (2200 g) near Kecskemét at the begining of September and kept frozen at –70 °C.

Virus purification

Virus purification was done according to Gugerli's procedure (Gugerli et al., 1984) for grapevine closteroviruses. Frozen leaf tissue (80 g) was grind to fine powder in liquid nitrogen than resuspended by stirring 40–60 min at 0 °C in 500 freshly prepared 0.5 M Tris/HCl buffer pH 8.2 containing 5% Triron X-100, 4% water insoluble PVP Polyclar AT and 0.2% 2-mercaptoethanol. Homogenate was filtered through a double layer of cheese-cloth and clarified by low speed centrifugation (20 min, 4000 rpm). The clarified supernatant was centrifuged (90 min, 41000 rpm, Beckman Ti 45 rotor) through 20% sucrose cushion (w/v in 0.1 M Tris/HCI pH 8.2). Sedimented virus particles were resuspended overnight in 0.02 M Tris/HCI pH 8.2 containing 0.001 M $MgCl_2$ on ice. To virus suspension 1% Triton X-100 was added and stirred on ice for 30 min than clarified by low speed centrifugation (10 min,1000 rpm) and the supernatant was purified by second centrifugation through 20% sucrose cushion (w/v in Tris/HCl pH 8.2, 0.001 $MgCL_2$ (120 min, 35000 rpm, Beckman AR 65 rotor). Resuspended pellet in 0.02 M Tris/HCI, 0.001 M $MgCl_2$ was further purified on Nycodenz gradient centrifugation (overnight, 40000 rpm). After centrifugation 0.25 ml fractions were taken and checked for virus content by ELISA (antibody and conjugate prepared by Gugerli). Fractions with high virus concentration were collected and used for immunization. Purified fractions were stored at –20 °C.

Antiserum preparation

Six months old New Zealand rabbit was injected intramuscularly with 6 sets of injections in two weeks intervals. Each set was consisting 2 ml emulsified virus suspension with Freund's complet (at first injection) or incomplete adjuvant in ratio 1:1. The rabbit was bled 3 weeks after the first injection and every 2 week thereafter.

Serology

The specificity of antiserum was determined with protein-A sandwich ELISA as it was described by Edwards and Cooper (1985). For coating 1 µg/ml Protein-A and for detecting antisera horseradish peroxidase enzyme (HRPO) conjugated Protein-A (prepared in Agricultural Biotechnology Center, Gödöllő) in dilution 1:4000 was used. Antisera under study were diluted for 1:500 and 1:1000 in the first layer and for 1:500,1:1000,1:3000 and 1:6000 in the second layer. Antiserum from the third bleeding

was conjugated with HRPO and compared with Bioreba GLRaV-III kit containing polyclonal antibody for coating and alkaline phosphatase-coupled monoclonal antibody conjugate. DAS-ELISA was done as described Clark and Adams (1977). ELISA plates were precoated with 0.5, 1, 1.5 µg/ml purified IgG and conjugate was diluted in 1:1000, 1:5000, 1:10000. Absorbance at 405 or 492 nm respectively were measured with Medilab ELISA Reader (Type OD-1) about 20 min following the addition of substrate. Extinction values twice higher than the average extinction of the healthy control plants were considered as positives (Cut off = $2 \times X_{healthy}$).

Plant material

Hungarian GLRaV isolates, identified previously on the base of woody indexing and of ELISA as type III as well as type isolate of GLRaV III, kindly provided by Gugerli and healthy plants of different grape varieties were applied for standardization of the experiments.

In order to check the reliability of the HRPO-ELISA system, simultaneous testing of symptom-showing Pinot Noir plants, inoculated with GLRaV III at PHSCS and about three hundred plants of different grape varieties, originating from various vine regions was carried out with BIOREBA GLRaV kit.

Autumn leaf veins, petioles and shavings from mature canes (phloem) were homogenized in ratio 1:5 with "TRIS extraction buffer" (pH 8.2) suggested by BIOREBA and than diluted further for 1:20 and 1:50.

Electron microscopy

Carbon coated grids were sprayed with semi-purified virus preparations and directly stained with 2% uranyl acetate.

Results

Virus purification

ELISA and electron microscopy were used to monitor virus particles in the purification procedures. Particles of semi-purified virus preparations were about 2000 nm (Fig. 1) and remained intact in all steps of purification procedure. After Nycodenz gradient centrifugation fractions with high virus content were used directly for immunization.

Antiserum production

Specificity of obtained antisera in different bleedings were tested with leaf samples and bark scrapings from cane of grapevine leafroll diseased plants and virus free grapes (Table 1). The average extinctions of healthy leaves (0.047–0.168) were rela-

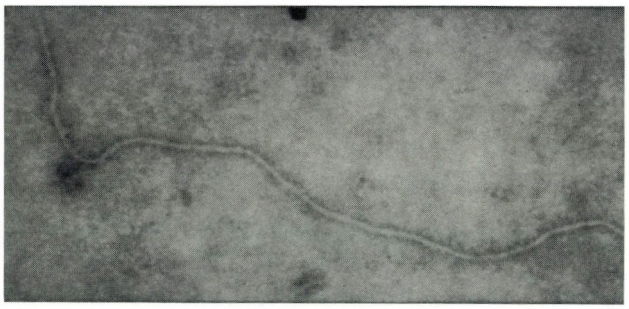

Fig. 1. Electron micrograph of GLRaV-III particle after sucrose cushion purification step

tively low and diseased leaves ranged between 0.221-0-704. It means that all bleedings except the 4. bleeding were able to produce useful antisera. Extinction values of infected samples reached 1.48–2.3× higher than the cut off ($2 \times X_{healthy}$).

The ELISA tests

Grapevine leafroll diseased plants kept in collection in Kecskemét were tested by GLRaV-III antisera produced in our experiments and compared with Bioreba kit. Results are shown in Table 2.

In the Hungarian polyclonal HRPO ELISA system the average extinctions of the blanks (0.046) and the healthy controls (0.049–0.121) were acceptable low. Extinctions of the infected samples ranged between 0.437 and 0.797 it means that they reacted 2.7–4.8× higher value than the cut off (0.164). Standard deviation at the blank and healthy controls did not exceed 0.010 and in case of the infected samples this was between 0.001 and 0.054.

Table 1

Comparison of antisera obtained from different bleeding in protein-A sandwich ELISA. Extinction values data representing of the means of three repetitions (leaf samples were diluted in 1:20)

Antiserum		Grapevine samples	
		healthy	infected
1. bleeding	1000×[a]/500×[b]	0.047+/–0.004	0.222+/–0.016
2. bleeding	500×/1000×	0.092+/–0.005	0.281+/–0.028
3. bleeding	500×/500×	0.054+/–0.004	0.248+/–0.026
4. bleeding	500×/500×	0.118+/–0.025	0.221+/–0.052
5. bleeding	500×/3000×	0.110+/–0.023	0.340+/–0.063
6. bleeding	500×/1000×	0.168+/–0.045	0.704+/–0.075

[a] – dilution of antisera in the first layer
[b] – dilution of antisera in the second layer

Table 2

Extinction values in DAS-ELISA obtained when comparing different grapevine plants showing severe leaf symptoms. Healthy control was virusfree grapevine

Samples	GLRaV-III/Hung	GLRaV-III/Bioreba	Symptoms
Healthy control 1	0.121+/–0.005	0.092+/–0.006	no
Healthy control 2	0.095+/–0.003	0.091+/–0.001	no
Healthy control 3	0.061+/–0.010	0.095+/–0.003	no
Healthy control 4	0.049+/–0.006	0.078+/–0.007	no
Pn. 25/1	0.560+/–0.021	2.203+/–0.272	severe
Pn. 25/2	0.609+/–0.015	2.618+/–0.074	severe
Pn. 25/3	0.530+/–0.011	2.016+/–0.269	severe
Pn. 25/4	0.580+/–0.010	2.357+/–0.069	severe
Pn. 25/5	0.437+/–0.001	1.261+/–0.073	severe
Pn. 25/7	0.498+/–0.034	1.688+/–0.030	severe
Pn. 25/8	0.477+/–0.001	1.596+/–0.130	severe
Pn. 25/12	0.498+/–0.037	1.783+/–0.060	severe
Pn. 25/14	0.488+/–0.031	1.826+/–0.177	severe
Pn. 25/18	0.631+/–0.026	2.595+/–0.023	severe
Pn. 25/19	0.489+/–0.004	2.142+/–0.016	severe
KelR-1	0.935+/–0.023	1.246+/–0.081	severe
ZolR-1	0.727+/–0.014	0.788+/–0.049	severe
1/37	0.714+/–0.027	1.246+/–0.081	severe
1/659	0.797+/–0.054	1.111+/–0.136	severe
Buffer control (blank)	0.046+/–0.004	0.055+/–0.006	–

GLRaV-III/Hung is horseradish peroxidase conjugate (1 µg/ml IgG for coating, 5000× dilution of conjugate) Substrate was incubated 10 min. GLRaV-III/Bioreba alkaline phosphatase conjugate (IgG 1:1000; conjugate 1:1000)

Data representing of average of two wells in one experiment.

Cut off = 2 $X_{\text{healthy controls}}$ (Hungarian HRPO: 0.164; BIOREBA: 0.178)

In the alkaline phosphatase-based polyclonal/monoclonal BIOREBA ELISA system blanks and healthy controls had low extinctions (0.055 and 0.078–0.095). In the infected plant saps much higher extinction values (1.111–2.618) were measured than at the HRPO system. The infected plants showed 6.2–14.7× higher extinctions than the cut off value (0.178). Low standard deviation values were obtained in case of blank (0.004) and healthy controls (0.001–0.007) as well as at infected samples (0.016–0.272).

Although with the monoclonal/polyclonal BIOREBA reagents the extinction values of infected plants were much higher than compared to the extinctions got in the HRPO-based polyclonal ELISA system, it was also able to detect all of the infections. The higher extinction values of the BIOREBA system most probably can be partly explained with combination of polyclonal and much more sensitive monoclonal antibodies.

Reliability of the HRPO system was further studied by comparative testing of about 300 grape samples using BIOREBA kit. Results of both system was the same in case of 287 samples out of 309 wich is 92.9% of agreement. At two samples BIOREBA gave positive reaction as those in HRPO system were negative, while at 20 samples GLRaV could not be detected with BIOREBA reagents but Hungarian HRPO ELISA system reacted positively. In spite the generally lower extinction values of the HRPO system than measured with BIOREBA kit more virus infected plants were recognized by the Hungarian antiserum. Therefore we may assume that the serological differences between Swiss and Hungarian GLRaV isolates resulted in less positive reaction with the BIOREBA kit. To final clear up of this question further serological experiments and woody indexing of the above-mentioned grapes are planned.

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Destruction of Tobacco Mosaic Virus in Developing Local Lesions in Leaves of *Datura stramonium* L.

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Growth of local lesions in *Datura stramonium* leaves infected with tobacco mosaic virus (TMV) was shown to accompany with destructive changes of virus particles. Infectivity in the lesions reached a maximum on the 5th day after inoculation of the leaves to fall thereafter. Quantity of the virus coat protein in the lesions assayed by ELISA and electrophoretic analysis reached a maximum on the 7th day after inoculation and then also decreased. Along with the normal virions, the abnormal (swollen and "thin") ones were observed in negatively stained with phosphotungstic acid virus preparations from the lesions. The immune electron microscopy showed that, unlike TMV particles with the normal diameter and swollen virions which became totally coated with TMV-specific antiserum, the thin virions lost ability to bind up with the antibodies. Possible mechanisrns of virus destruction are discussed.

There is evidence (Kassanis and Kenten, 1978; Reunov, 1980; Golinowski et al., 1981, 1986; Kassanis, 1981; Reunov and Lapshina, 1984; Roggero and Pennazio, 1984; Kolesnik, 1985; Ismail et al., 1987) testifying the possibility of plant virus destruction in vivo. It was shown (Roggero and Pennazio, 1984) that during the growth of the local lesions induced by tobacco necrosis virus in leaves of *Nicotiana tabacum* L. var. White Burley the amounts of viral antigen extracted from the necrotic centre linearly decreased with time suggesting virus degradation.

The aim of the present work was to investigate whether TMV particles undergo any destructive alterations during hypersensitive reaction in *Datura stramonium* leaves.

Materials and Methods

Assays were carried on plants of *Datura stramonium* L. (3-weeks old) grown in a greenhouse. Almost fully expanded leaves were dusted with Carborundum and mechanically inoculated with a sap of *Nicotiana tabacum* L. var. Samsun leaves infected with ordinary strain of TMV.

In experiments on determination of local lesion infectivity, 40 lesions were cut out from leaves 3, 4, 5, 7, 10 and 12 days after inoculation. Previously, diameters of the lesions were determined using MI-I (USSR) measuring microscope with a value of scale division of 0.01 mm. The lesions were homogenized in 1 ml of 0.1 M phosphate buffer,

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pH 7.0 and the virus containing in the homogenates obtained was titrated on 10 *Nicotiana tabacum* L. var. Xanthi-nc leaf halves in relation to the control preparation of TMV (10^{-5} mg/ml) purified according to Otsuki et al. (1977). Experiments were repeated five times.

In specially performed experiment, 200 local lesions were cut out from leaves 10 days after inoculation. The lesions were homogenized in 10 ml of 0.1 M phosphate buffer, pH 7.0. The homogenates were first clarified by centrifugation at 8000 g and then pelleted at 78 000 g. The supernatant obtained was mixed with purified TMV preparation (test). The TMV preparation dissolved with phosphate buffer was used as a control. The final TMV concentration was equal to 1 μg/ml in both cases. Comparison of the infectivities of the test and control samples was performed on 10 *N. tabacum* L. var. Xanthi-nc leaf halves.

The TMV content in the local lesions was measured with the double antibody sandwich ELISA using antibodies conjugated with peroxydase as described by Ogarkov et al. (1984). In every experiment, twenty local lesions were used. Lesion diameters were previously measured as above. Experiments were repeated three times.

For electrophoresis, twenty disks, every of which contained a single local lesion, were cut out at different times after inoculation using a punch of 3 mm in diameter. The disks obtained homogenized in 100 μl of the buffer system of Laemmli (1970). The homogenates were heated at 100 °C for 2 min and centrifuged for 5 min at 8000 g. The samples were subjected to electrophoresis in 12.5% SDS-polyacrylamide gel by Laemmli (1970).

For electron microscopy, virus suspensions were prepared by means of cutting local lesions by a razor blade into a drop of distilled water. In a series of experiments, the droplets of virus suspensions obtained were placed on Formvar-coated grids, desiccated and negatively stained with 2% phosphotungstic acid (PTA), pH 7.0. In separate experiments, virus suspensions designed to examination by negative-staining electron microscopy were obtained from local lesions after previous 3 h fixation of tissue pieces containing lesions in 6.5% solution of glutaraldehyde prepared on phosphate buffer, pH 7.4. In immuno-electron microscopic investigations, the grids mounted with virus suspension from lesions were placed into a drop of an antiserum and kept in a humid chamber for 15 min at 37 °C. Then the samples were carefully washed with distilled water, desiccated and stained with 2% PTA, pH 7.0. The prepared samples were investigated on an EMV-100 AK (USSR) electron microscope.

Results

The results show that the infectivity in local lesions induced by TMV in leaves of *D. stramonium* L. increased within 5 days after inoculation and then declined (Table 1). To evaluate if a decrease in lesion infectivity from 5 to 12 days after inoculation is caused by virus-induced inhibitory substances released in preparing the samples, the virus-free supernatant obtained by differential centrifugation of the homogenates from

Table 1

Alteration of relative infectivity and virus content in developing local lesions in *D. stramonium* leaves inoculated with TMV

Days after inoculation	Relative infectivity[a]	Virus content[b] (ng/mm^2)
3	25 ± 3.1	10 ± 1.1
4	48 ± 5.2	18 ± 1.7
5	62 ± 7.1	35 ± 4.3
7	28 ± 2.4	42 ± 5.3
10	15 ± 1.7	25 ± 2.9
12	11 ± 1.3	13 ± 1.5

[a] Number of local lesions per leaf half of *N. tabacum* L. var. Xanthi-nc expressed as a percentage of the control preparation of TMV (10^{-5} mg/ml). Each value is the average percentage obtained on 50 leaf halves of *N. tabacum* L. var Xanthi-nc. (±standard error). All relative infectivities in local lesions formed on *D. stramonium* leaves were calculated for equal lesion square.

[b] The TMV content in local lesions formed on *D. stramonium* leaves was measured by ELISA and calculated for lesion area of 1 mm^2. Each value is the average of three replicates (±standard error). The standard errors were calculated using Student's t-test, at P = 95%.

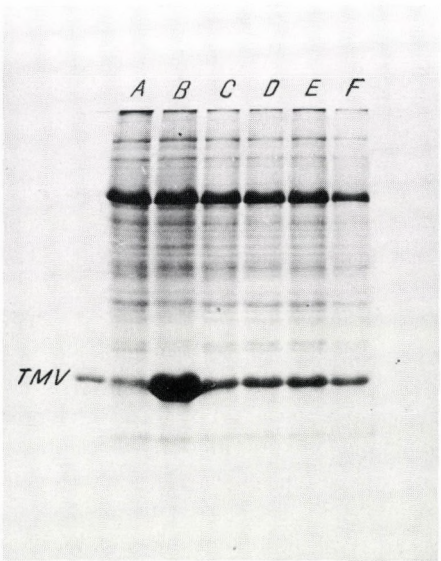


Fig. 1. SDS-polyacrylamide gel analysis of proteins of *D. stramonium* leaf tissues containing TMV-induced local lesions 5(A), 7(B), 11(C), 13(D), 15(E) and 18(F) days after inoculation

lesions cut out on the 10th day after inoculation was mixed with TMV and infectivity of the mixture was compared with that of the control (TMV + phosphate buffer). The marked difference in the infectivity of the test and control samples was not observed.

The amount of virus antigen detected in the local lesions by ELISA first increased reaching a maximum on the 7th day after inoculation and then decreased (Table 1). Similar data we have obtained by electrophoretic analysis (Fig. 1).

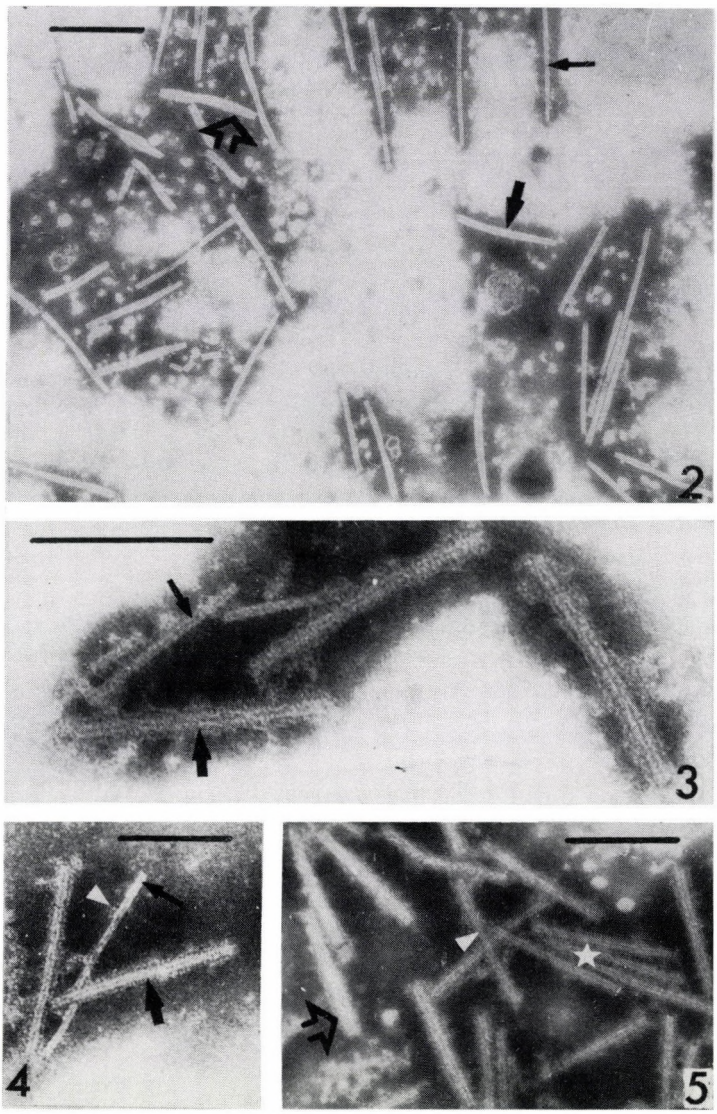
During the formation of the lesions, virus particles could undergo abnormal changes. In negatively stained with PTA suspensions obtained from the lesions, the swollen virions as soon as thin ones (with diameter from 10 to 17 nm) were observed along with TMV particles of the normal diameter (Fig. 2). Morphological characteristics of the virions in stained with PTA preparations obtained from the lesions previously fixed with glutaraldehyde did not markedly change: the normal, swollen and thin virions were observed as well.

The immuno-electron microscopic investigations showed that an antiserum prepared to TMV usually entirely decorated TMV particles having the normal diameter (Figs 3–4). Also, the antiserum covered the swollen virions (Fig. 5). The thin virions lost ability to bind up with the specific antibodies. It was difficult to find the antiserum decoration of the virus particles having diameter about 10 nm as in Fig. 3. The small antiserum bits bound up with similar virus particles were observed only sometimes (Fig. 4). The thin virions with diameter exceeding 10 nm might be in some degree covered with the antiserum (Fig. 5). In cases when virus particles had non-constant diameter, the antiserum decoration was preferably observed on the virion parts having comparatively larger diameter (Fig. 5). The control serum and antiserum to potato virus X did not form a complex with TMV particles.

Discussion

The above investigations show that the development of TMV-induced infection in *D. stramonium* leaves is accompanied by a decrease (after reaching of a maximum) in the infectivity as well as in quantity of the virus coat protein in lesions suggesting virus destruction.

In our experiments, the mixing of purified TMV preparation with virus-free supernatant obtained from the local lesions on the 10th day after inoculation did not affect the infectivity of TMV. Therefore, a decrease of infectivity in the local lesions after reaching of a maximum could not be caused by the compounds released in preparing the samples. We suggest that the reduction of infectivity in the lesions may be bound up with breakdown in stability of virion capsids resulting in inactivation of viral RNA with RNase. The decreased quantity of virus coat protein in the lesions revealed by electrophoretic analysis and ELISA testifies to its destruction that may be due to protease action. Earlier Golinowski et al. (1981) assumed the involvement of RNase and proteolytic enzymes in degradation of potato virus X in the intercellular spaces of potato leaves.



Figs 2–5. Preparations of TMV particles from the local lesions formed in *D. stramonium* leaves 4 days after inoculation. The middle, thick, and thin arrows indicate, respectively, the normal, swollen, and thin virions. Bar = 200 nm Fig. 2. Preparations negatively stained with PTA. Figs 3–5. Preparations treated with TMV-specific antiserum and stained with PTA. The arrowhead in Fig. 4. indicates the antiserum bit on thin TMV particle having diameter about 10 nm. In Fig. 5. the arrowhead indicates the partially decorated with antiserum TMV particle having non-constant diameter; the star indicates the group of thin TMV particles with diameter exceeding 10 nm

Destruction of coat protein of virions, in our opinion, may occur as follow. Under changed environmental conditions (for example pH), the conformation alterations of tertiary structure and partial loosening ("untwineing") of capsid protein seem to take place. As a result, virions swell that we observe in the preparations stained with PTA. In untwineing of polipeptides they can be subjected to proteolysis. This may cause the appearance of thin virions. Similarly, Everitt et al. (1988) accounted for proteolysis of proteins of adenovirus 2. It was established that hexons of adenovirus 2 change their conformation under the acid pH so that parts of hexsons sensitive to protease action expose outside and undergo proteolysis (Everitt et al., 1988).

It should be noted that swollen and thin virions along with normal ones were observed by us in negatively stained with PTA preparations from the local lesions both unfixed and previously fixed with glutaraldehyde. Therefore, the presence of abnormal virus particles in the preparations observed is not artefact.

The fact that thin TMV particles revealed, in contrast to those of normal diameter, lost ability to bind up with specific antiserum may be due to elimination of determinant groups responsible for boundering with antibodies during partial proteolysis of capsid protein.

The results presented are in accordance with the data on degradation of tobacco necrosis virus in the growing local lesions (Roggero and Pennazio, 1984) and supplement the other available evidence (Kassanis and Kenten, 1978; Reunov, 1980; Golinowski et al., 1981, 1986; Kassanis, 1981; Reunov and Lapshina, 1984; Kolesnik, 1985; Ismail et al., 1987) about destruction of plant viruses *in vivo*.

Acknowledgements

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Reuse of ELISA Plates: Development of a Simple Cleaning Method

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Biotex®, a commercial detergent for washing clothes, was effective in cleaning microtitre plates used for detecting plant viruses by the indirect enzyme-linked immunosorbent assay. Cleaning was achieved by shaking the plates in a solution of 5% Biotex® for 48 h followed by thorough washing with tap water and a final rinse in distilled water. Using this method plates could be reused at least three times for detection of cowpea mosaic, cowpea mild mottle, tobacco mosaic, bean common mosaic, cowpea aphid-borne mosaic, peanut stripe, and soybean mosaic viruses representing four plant virus groups. The method was also effective when applying the double antibody sandwich (DAS) ELISA for detection of pea seed-borne mosaic virus. By using Biotex® no adverse effect on the binding capacity of the plate was found. The efficacy of Biotex® in combination with other chemicals was also studied. Some general aspects of cleaning and reuse of microplates are discussed.

Enzyme-linked immunosorbent assay (ELISA) is the most widely used serological method for both the quantitative and qualitative detection of plant viruses. Since ELISA plates once used are disposed, the cost of the test is high, and it is considered an expensive method, particularly in developing countries.

Dissociation of double antibody sandwiches of citrus tristeza virus and carnation mottle virus from the antibody-coated microplates has been found (Bar-Joseph et al., 1979) when plates were treated with 0.2 M glycine-HCl buffer pH 2.2 for 60 minutes. Microplates used for potato viruses S, X and Y, and oat blue dwarf virus were cleaned (Banttari and Petersen, 1983) using concentrated sodium hydroxide in water and ethanol (1:1, v/v) or with Haemo-sol in water. Cleaning of the plates used for peach rosette mosaic virus was achieved (Stobbs and Van Schagen, 1986) when the plates were sonicated in a 0.5% solution of detergent at 60 °C for 30 minutes followed by rinsing several times in distilled water, and drying at 55 °C for 1 h. But there was a decline in plate efficacy, i.e. protein-binding capacity, with repeated cleaning and reuse of plates. However, when the wells were treated with 1% nitrocellulose after cleaning, a significant improvement in plate performance was achieved.

The objective of this work was to develop a simple and effective plate cleaning method which could be easily used also in developing countries. The study concentrated on indirect ELISA and eight viruses. Each experiment was repeated several times with similar results. Data of some experiments are not included, but discussed in brief.

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Materials and Methods

Viruses

Eight viruses belonging to different groups were used. They were: peanut stripe potyvirus (PStV), soybean mosaic potyvirus (SbMV), bean common mosaic potyvirus (BCMV), cowpea aphid-borne mosaic potyvirus (CABMV), pea seed-borne mosaic potyvirus (PSbMV), tobacco mosaic tobamovirus (TMV); cowpea mild mottle carlavirus (CPMMV), and cowpea mosaic comovirus (CPMV). Viruses maintained in their respective hosts were used as the infected material. Extracts of plants of the same species and cultivars were used as healthy material.

Antisera

Polyclonal antisera used in this study were kindly supplied by Dr. J. Dijkstra, Wageningen Agricultural University, The Netherlands (a-BCMV); Dr. S. Ghabrial, University of Kentucky, The USA (a-SbMV); Dr. R. Hampton, Oregon State University, The USA (aPSbMV); Dr. D. Reddy, ICRISAT, India (a-PStV & a-CPMMV); Dr. J. Vetten, BBA, Braunschweig, Germany (a-CABMV). Antisera of TMV and CPMV produced at the DGISP were also used.

ELISA and ELISA plates

An indirect ELISA procedure employing direct antigen-coating of plates (Hobbs et al., 1987) and the double antibody sandwich (DAS) ELISA (Clark and Adams, 1977) were followed.

For indirect ELISA crude plant extracts were diluted 1/100, antisera 1/500-1/1000 and anti-rabbit IgG enzyme conjugate 1/1000. For DAS-ELISA plant extracts were diluted 1/10, and both coating antibody and conjugate were diluted 1/200. Antisera were crossabsorbed with healthy host plant extracts according to Hobbs et al. (1987) prior to their use in indirect ELISA. Absorbances were measured in an ELISA reader at 405 nm using substrate buffer as the zero-reference. Readings were made three times for indirect ELISA, i.e. 30 min, 1 h (room temperature) and overnight (5 °C) after substrate loading. For DAS-ELISA readings were made after 30 min and 1 h of substrate incubation at room temperature.

Nunc-Immuno Plate MaxiSorp F96 microtiter plates (Danish trade mark) were employed in the present study.

Layout

For each virus four wells of a plate in two replications were loaded with the respective antigens of the healthy and infected materials (Fig. 1a). After completing the

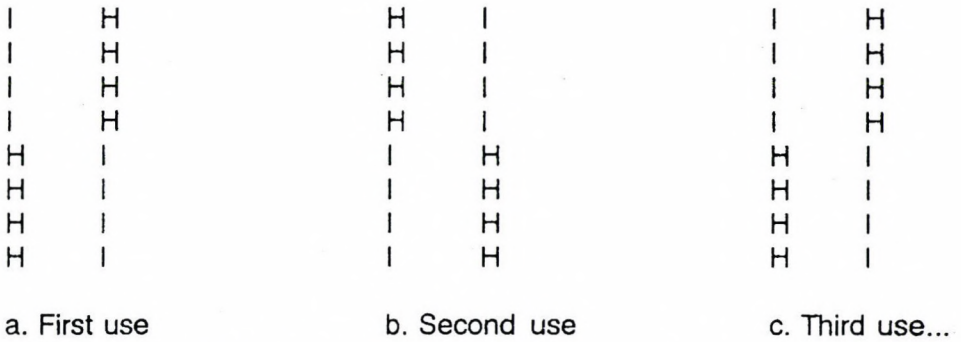

Fig. 1. Layout of the loading pattern for each virus (I) under test and the corresponding healthy (H) host extract (a). After each ELISA and cleaning, used wells were loaded reciprocally with the respective extracts (b and c)

ELISA and cleaning the plate, the same wells were used for the same antigens, but loaded reciprocally as shown in Fig. 1. For each reuse experiment new plates were run in parallel as controls.

Cleaning solutions

Various detergents such as SDS (sodium dodecyl sulfate), "RBS 35" (max. 2% sodium hypochlorite; max. 2% active chlorine; max. 0.9% sodium hydroxide and max. 2% sodium carbonate), Tween 20, and commercial detergents like Biotex®, Henkel® and Opvask® were used. Biotex® and Henkel® are washing powders containing protein degrading enzymes (used for washing clothes, pH 9.5 in solution), and Opvask® is a detergent used in dish washing. Concentrations of 1 to 5% (10 to 50 gram or ml of the product per liter) prepared in cold tap water were used. Biotex® was also used in combination with sodium hydroxide (NaOH), sodium chloride (NaCl), and hydrochloric acid (HCl).

As far as the composition of clothe washing powder is concerned Biotex® contains 15–30% of zeolites, 5–15% of anionic tensides and less than 5% of nonionic tensides, soap, polycarboxylates, phosphonates and enzymes. It also contains sodium bicarbonate, sodium sulfate, sodium carbonate and sodium silicate. This description is very brief and no further details are obtainable from the producer. However, a detailed composition given for a washing powder used as an international standard reference for testing washing machines is available (CEI/IEC, 1994). In that standard washing powder the content of proteolyte enzyme (activity 11 mAU/g) is 0.5%.

Cleaning procedures

Shortly after the last reading the used plates were first rinsed with tap water to remove hydrolysed substrate, and then immersed in the cleaning solution (well-side up) in a container. The container was shaken at about 60 rpm on a rotative shaker (Gerhardt

RO 10®, Germany) for a period of 24–48 h at room temperature. Care was taken to avoid overlapping of the plates during shaking. In between the shaking period the plates were agitated vigorously by hand in the solution, horizontally and vertically (upside down) to increase the washing efficiency. Before removal from the cleaning solution they were shaken once again vigorously in the solution as described above. The plates were washed thoroughly with a mild discharge of running tap water followed by several rinsings in distilled water and air dried.

Results

From the results of preliminary studies on the effectiveness of the detergents for cleaning the ELISA plates, only the type of detergent for washing clothes, such as Biotex® and Henkel® were found promising. The other detergents such as SDS, RBS 35, Tween 20, and Opvask® did not give satisfactory results and were thus discarded after preliminary tests. Biotex® and Henkel®-washed plates gave equally satisfactory results. The first detergent was chosen for further evaluations.

Cleaning of plates with 1% solution of Biotex® by shaking for 24 h was effective for the CPMV (comovirus) which has isometric particles, but not for other viruses which have elongated particles (Table 1; Fig. 2a). The cleaning efficiency was slightly improved by increasing the concentration of the detergent from 1 to 2% (data not shown).

When the 1% Biotex® containing 1 N NaOH was used as the cleaning solution, the cleaning efficiency was markedly increased and it was effective in cleaning plates used for TMV and CPMV but not for PStV (Fig. 2b). The binding capacity of the plates, however, was found to be reduced with this treatment compared to control plate. Cleaning was also tried with 1% Biotex® containing 1% NaCl, but it did not improve the cleaning efficacy (Fig. 2c).

Biotex® (2%) when used at pH 4 and 7 with HCl gave better results than previous treatments. The A_{405} value difference between "infected" and "healthy" wells was much larger for PStV, and it was satisfactory for two other potyviruses BCMV and CABMV (Fig. 2d–g). The cleaning was better with suspensions of pH 4 than of pH 7. The results, however, were still not satisfactory in the cases of SbMV and TMV.

Finally, when the concentration of Biotex® was increased from 2 to 5% without additives and the shaking time increased from 24 to 48 h, cleaning was successful for plates used in indirect ELISA for all viruses tested viz. PStV, TMV, BCMV, SbMV, CABMV, CPMMV (Table 2; Fig. 3) and CPMV (data not shown). With this method the plates were successfully reused for the same viruses three times (CPMMV tried only one time). Generally, with each reuse of cleaned plates the A_{405} value differences between "healthy" and "infected" wells were close to the differences recorded in corresponding wells of new plates run in parallel. The A_{405} values were 5 to 10 times higher for reused wells of BCMV, SbMV, and CPMMV as compared to "healthy" wells. For the other viruses A_{405} differences between "healthy" and "infected" of more than 10 times, and in a

Table 1

Effectiveness of 1% Biotex® (detergent for washing clothes) in cleaning plates used for indirect ELISA of plant viruses; 24-h wash by shaking. See also Fig. 2a

Plate	Incubation period before reading[3]	Absorbance (405 nm)[1]					
		PStV		CPMV		TMV	
		Healthy	Infected	Healthy	Infected	Healthy	Infected
First use	One h	0.004	0.142	0.003	0.235	0.013	0.306
	Overnight	0.055	0.926	0.025	1.382	0.068	1.667
Second use	One h	0.426	0.696	0.049	1.119	0.113	1.894
	Overnight	2.339	2.352	0.307	2.356	1.247	2.364
Control[2]	One h	0.069	0.798	0.041	1.521	0.474	2.046
	Overnight	0.595	2.347	0.219	2.395	0.579	2.349
Third use	One h	0.378	0.721	0.038	1.012	0.138	1.546
	Overnight	1.628	1.832	0.213	2.102	1.002	2.408
Control[2]	One h	0.075	0.824	0.047	1.321	0.187	1.742
	Overnight	0.389	2.042	0.241	2.213	0.417	2.402

[1] Mean value of eight wells
[2] Control: new, unused plate run in parallel
[3] One h substrate incubation at room temperature, and overnight incubation at 5 °C.

few cases up to 40 times, were maintained during the reuses (Table 2; Fig. 3). Generally the greatest A_{405} value differences were obtained after about 1 h substrate incubation. In this ELISA setup overnight incubation increased the absorbance values, but the differences between "healthy" and "infected" were still convincing (Table 2). The absolute A_{405} values from one reuse experiment to another showed some variation. However, since the control plates showed a similar trend in variation it must be attributed to variation in ELISA conditions, including antigen sources, between experiments.

The method was also successfully applied in reuse of plates employed for DAS-ELISA of PSbMV where A_{405} values for the second use of the plate were similar to those of the control plate (Table 3).

When new plates shaken in 5% Biotex® for 48 h were compared with non-treated new plates in testing for six different viruses, the binding capacity of the plates was unaffected (data not shown).

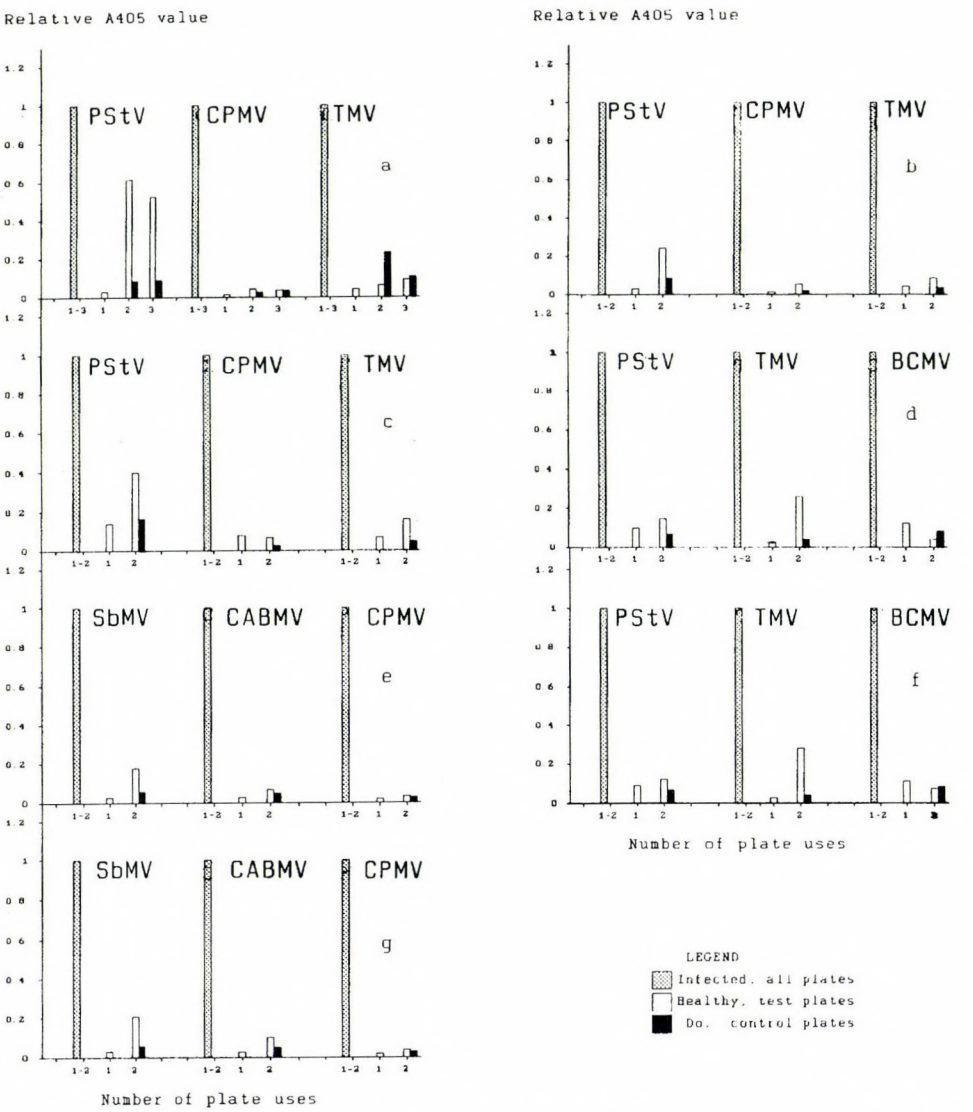

Fig. 2 Relative A_{405} values (one h substrate incubation) representing individual reactions of infected material both from test plates and control plates (infected all over = 1) compared with corresponding relative A_{405} values for reactions of healthy materia! when using the same ELISA plate up to three times. (a) to (g) represents effectiveness of different treatments in cleaning plates used for indirect ELISA of various plant viruses. a = 1% Biotex® (values from Table 1); b = 1% Biotex® containing 1 N NaOH; c =1% Biotex® containing 1% NaCl; d and e = 2% Biotex® + HCl, pH 7; f and g = 2% Biotex® + HCl, pH 4. Treatment period: 24 h under shaking.

Table 2

Effectiveness of 5% Biotex® in cleaning plates used for indirect ELISA of plant viruses; 8-h wash by shaking (continued). See also Fig. 3

Plate[2]	Incubation period before reading[3]	Absorbance (405 nm)[1]					
		PStV		BCMV		SbMV	
		Healthy	Infected	Healthy	Infected	Healthy	Infected
First use	One h	0.106	2.144	0.047	0.261	0.048	2.233
	Overnight	0.329	2.306	0.110	0.895	0.107	2.299
Second use	One h	0.016	0.270	0.008	0.090	0.008	0.049
	Overnight	0.166	1.969	0.087	0.879	0.020	0.218
Control[2]	One h	0.007	0.253	0.021	0.090	0.007	0.051
	Overnight	0.121	1.981	0.181	0.858	0.013	0.338
Third use	One h	0.042	1.742	0.030	1.398	0.023	0.207
	Overnight	0.319	2.275	0.280	2.268	0.249	1.570
Control[2]	One h	0.023	1.617	0.033	0.599	0.029	0.263
	Overnight	0.271	2.355	0.255	2.305	0.203	1.728
Fourth use	One h	0.067	0.766	0.049	0.312	0.042	0.396
	Overnight	0.337	2.304	0.098	1.102	0.307	2.114
Control[2]	One h	0.042	0.798	0.036	0.336	0.047	0.436
	Overnight	0.239	2.405	0.102	1.132	0.246	2.442
		TMV		CABMV		CPMMV	
		Healthy	Infected	Healthy	Infected	Healthy	Infected
First use	One h	0.174	1.717	0.081	1.948	0.022	0.243
	Overnight	0.583	2.301	0.216	2.314	0.072	1.526
Second use	One h	0.009	0.288	0.132	1.551	0.034	0.329
	Overnight	0.115	2.005	0.312	2.274	0.108	1.446
Control[2]	One h	0.012	0.248	0.109	1.612	0.026	0.301
	Overnight	0.082	1.939	0.287	2.323	0.098	1.357
Third use	One h	0.031	1.287	0.138	1.902	–	–
	Overnight	0.298	2.254	0.299	2.270	–	–
Control[2]	One h	0.037	1.341	0.117	1.837	–	–
	Overnight	0.307	2.353	0.239	2.305	–	–
Fourth use	One h	0.026	1.112	0.137	1.474	–	–
	Overnight	0.217	2.206	0.262	2.469	–	–
Control[2]	One h	0.015	1.019	0.118	1.512	–	–
	Overnight	0.202	2.254	0.243	2.604	–	–

[1] Mean value of eight wells

[2] Control: new, unused plate run in parallel

[3] One h substrate incubation at room temperature, and overnight incubation at 5 °C

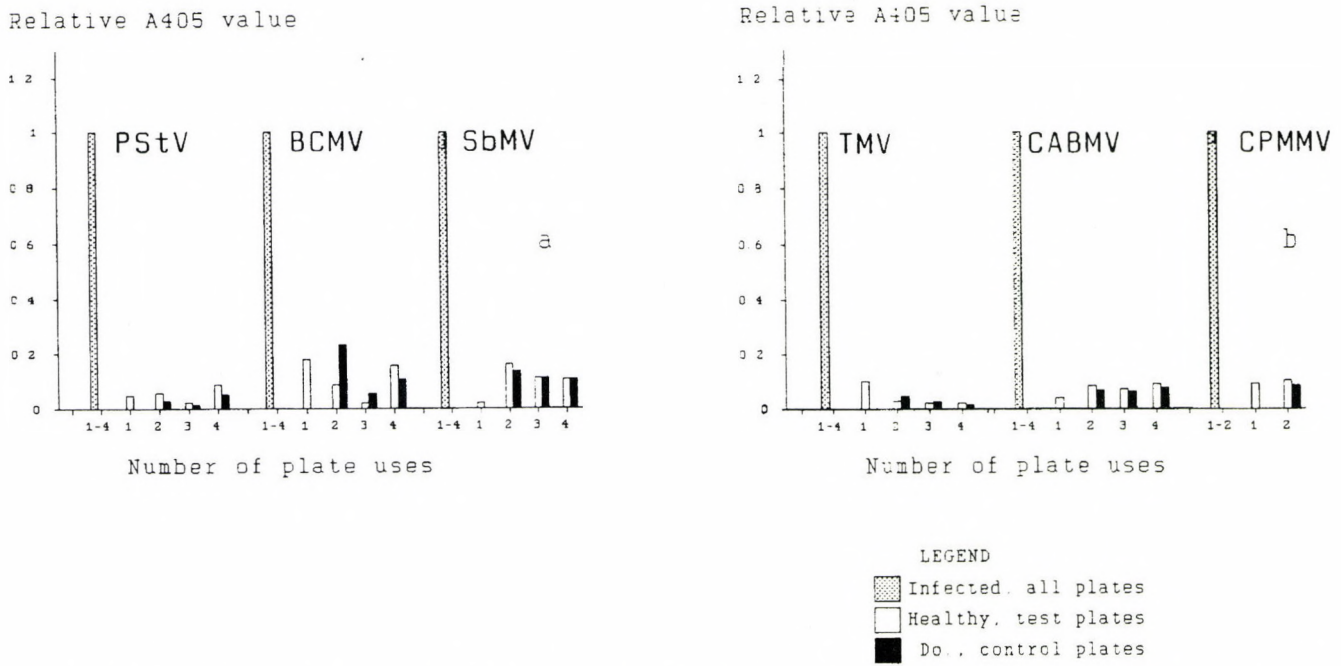

Fig. 3. Relative A_{405} values (one h substrate incubation) representing individual reactions of infected material both from test plates and from control plates (infected all over = 1) compared with corresponding relative A_{405} values for reactions of healthy material when using the same ELISA plate up to four times. a and b represent effectiveness of cleaning plates used for indirect ELISA of various plant viruses when applying 5% Biotex® for 48 h under shaking. (Values from Table 2)

Table 3

Effectiveness of 5% Biotex® in cleaning plates used for DAS-ELISA of pea seed-borne mosaic viruses; 48-h wash by shaking (continued). See also Fig. 3.

Plate	Incubation period before reading[3]	Absorbance(405 nm)[1]	
		Healthy	Infected
First use	0.5 h	0.129	2.374
	One h	0.346	2.381
Second use	0.5 h	0.123	2.349
	One h	0.247	2.353
Control[2]	0.5 h	0.117	2.353
	One h	0.239	2.354

[1] Mean value of eight wells
[2] Control: new, unused plate run in parallel
[3] One h substrate incubation at room temperature, and overnight incubation at 5 °C.

Discussion

Highly acidic or highly alkaline cleaning solutions have been reported to be effective in removing the proteins of some viruses from ELISA plates (Banttari and Petersen, 1983; Bar-Joseph et al., 1979). In this study a similar effect was found, but the protein binding capacity of the plates declined after cleaning in highly alkaline solution (Biotex® containing 1 N NaOH). It was also noted that when the pH 9.5 of Biotex® solution was adjusted to 12 with NaOH, the cleaning efficiency decreased. In another cleaning method (Stobbs and Van Schagen, 1986) sonication and high temperatures (60 and 55 °C) were used, but the plate efficacy was affected. With the use of Biotex® at its normal pH 9.5 and at room temperature we did not encounter such adverse effects on plates.

Reuse of plates is more critical for the indirect ELISA procedure used in this study than for DAS-ELISA. In the indirect ELISA, the wells are first coated with antigen and in DAS-ELISA with antibodies. It is believed that the first layer (bound to the polystyrene) is always the most difficult one to remove whether it is in case of indirect or DAS-ELISA. However, the complete removal of the first layer is crucial in the indirect ELISA but not that critical in DAS-ELISA if the plate is reused for the same virus. In this study the data suggest that Biotex® removes the first layer (antigen) efficiently from plates in case of indirect ELISA and the plates can be reused safely for the same virus. But in case of DAS-ELISA an additional experiment showed high A_{405} values when the

plate used for DAS-ELISA was cleaned and subsequently loaded only with antigen, conjugate and substrate. When only the conjugate and substrate, or only the substrate were loaded after cleaning no reaction took place. The high readings could be due to incomplete removal of the coating antibodies. According to Dr. M. Bar-Joseph, The Volcani Center, Israel (personal communication) it may not be necessary to re-coat with antibodies if plates are reused for the same virus.

If a plate is reused for another (not related) virus, any remnant of the first use, if in trace amount, will not disturb the ELISA reaction. If the remnant after the first use is substantial, it may, however, block for the new reagent. In one brief experiment when the plates were used interchangeably with other viruses (CPMMV followed by SbMV, and TMV followed by PStV) after cleaning by overnight shaking in 2% Biotex® (inefficient cleaning based on our data), the results were completely comparable to new control plates. It suggests that the plates can be reused safelyfor non-related viruses even when they are not properly cleaned.

It is recommended that the plates should be agitated vigorously in the cleaning solution by hand at least once in between the shaking period and once again at the end of shaking which, we believe, facilitates release of the virus particles. If the plates are placed vertically with necessary supports in the container having the cleaning solution, the extra agitation during the shaking period is not necessary, but agitation at the end of shaking is recommended. By vertical placement also more plates can be cleaned efficiently at a time than by horizontal placement.

It is important that the plates are thoroughly washed with running tap water followed by rinsing in distilled water several times. Remnants of the detergent and various contents of the tap water could otherwise affect the protein binding capacity of the plates. To avoid any interference in plate-transparency the back side of the plate must also be cleaned properly.

In one of the experiments, it seemed that there was no difference whether the plates were cleaned right after the substrate unloading or after storing for a few weeks after substrate unloading. In some cases cleaning of the stored plates was even easier than the cleaning of plates just unloaded.

We believe 5% Biotex® + 48 h shaking (despite the involvement of a shaking machine) is a simple method which can be applied in any laboratory, especially in developing countries. Besides of its simplicity, it is an inexpensive and non-toxic method.

In this work emphasis was laid on potyviruses since preliminary studies revealed that the viruses with elongated particles were difficult to eliminate from the polystyrene surface. Although the method is effective for cleaning the plates used for all viruses included in this study it may not be possible to conclude that the method is equally effective for other viruses or groups e.g. rhabdo, clostero, potex, tenuiviruses etc. Attention should also be paid to the fact that different batches of ELISA plates of the same brand may differ in binding conditions possibly influencing the effect of cleaning (M. Bar-Joseph, personal communication).

Acknowledgements

We gratefully acknowledge the valuable assistance of Ms. Lise Hasle, Mr. H. Jørskov Hansen, and Mrs. Subhadra Manandhar.

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Occurrence of Tomato Pith Necrosis Incited by *Pseudomonas corrugata* in Hungary

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Pith necrosis and hollowing of tomato stems was detected on greenhouse cultivated plants. Different varieties and lines of tomato showed large diversity in symptoms of the disease. The pathogenic bacterium was isolated and identified by biochemical, physiological and pathogenicity tests as *Pseudomonas corrugata* Roberts and Sclarlett. This is the first report of this bacterium on tomatoes in Hungary.

For five-six years pith necrosis like disease of tomato was observed in Hungary. From diseased plants *Erwinia carotovora* subsp. *carotovora, Pseudomonas fluorescens* and *P. putida* were isolated (7).

Similar disease appeared on tomato varieties and selected lines cultivated in unheated greenhouse in August of 1995. The wilted plants showed chlorosis on the top and a water-soaked brown discoloration extended on the surface of stems, leaf bases and nodes. Stems of the plants splitted and proliferation of adventitious roots emerged from the cracked tissues. Infected plants showed external browing and pith necrosis (Figs 1, 2, 3).

Syndrome of the disease was similar as reported first by Scarlett et al. in England in 1978 (8). This disease had been reported later nearly worldwide.

Materials and Methods

Isolation of strains

Infected plant tissues were soaked in 0.5% sodium hypoclorite and rinsed by sterile distilled water. Tissue homogenates were planted on Nutrient agar +1% glucose (NDA) and on King's B medium (3). Bacterial colonies that appeared were selected and incubated at 26 °C. For diagnostic and pathogenicity tests sixteen cultures were used.

Biochemical and physiological tests

The isolated bacteria were detected in the following test: presence of oxidase-, gelatinase-, arginin dihydrolase-, starch hydrolase enzymes, reduction of nitrate (5) and

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glucose metabolism (2). Pigment production and colony formation were tested on King's B (3) and a medium described by Starr et al. (9). The results of the tests were read after 3–7 days incubation time.

Hypersensitivity of tobacco

Leaves of *Nicotiana tabacum* L. cv. White Burley were infiltrated with bacterial suspension of 48-h old cultures, containing 10^8 ml^{-1} cells. Reaction was evaluated 24 h later (4). The appearing HR-like necroses indicated the pathogenicity of the isolated culture.

Soft rot of potato

7–8 mm thick small discs were cutted out from potato slices and holes were made in the tuber tissues. The holes were filled up with the bacterial suspensions. After 2 days incubation a positive result was recorded if the slice had rotted around the place of inoculation.

Inoculation experiment

Stems of five-week-old tomato plants were inoculated by pricking. The needle immersed in a 48 h culture of the isolates cultivated on NDA medium. Plants were kept in the greenhouse. One group of plants was irrigated daily with water (100 ml/pot), other group with 100 ml of 1.5% fertilizer solution ("Poly-feed", Biomark, Hungary). Reisolation of bacteria were carried out 6 weeks after inoculation.

Results and Discussion

As regards the biochemical and physiological tests there were no differences between isolates from naturally and artificially inoculated plants. Colonies isolated from plants 2 days after inoculation were cream to dirty color on NDA medium, round with slightly undulated margins and irregular surfaces. No fluorescent pigment production was observed on the King's B medium but a dark greyish – blue nearly black diffusible pigment developed around the colonies on 1% pepton-glucose agar. All isolates were Gram negative, positive in oxidase, lecitinase and gelatinase tests and for nitrate reduction. Glucose metabolism was oxidative, starch hydrolysis was negative. Bacteria induced hypersensitive raction on tobacco but not soft rotting on potato slices.

Bacterial isolates from pith necrosis of tomato caused typical disease symptoms on the inoculated plants. Intensive wilting and chlorosis were also observed in plants grown with extensive fertilization 3 weeks after inoculation. Characteristic browning and cavities of the piths appeared, but no adventitious rooting developed (Fig. 1).

Fig. 1. Tomato stem split with extreme pith necrosis and cavitation caused by *Pseudomonas corrugata*

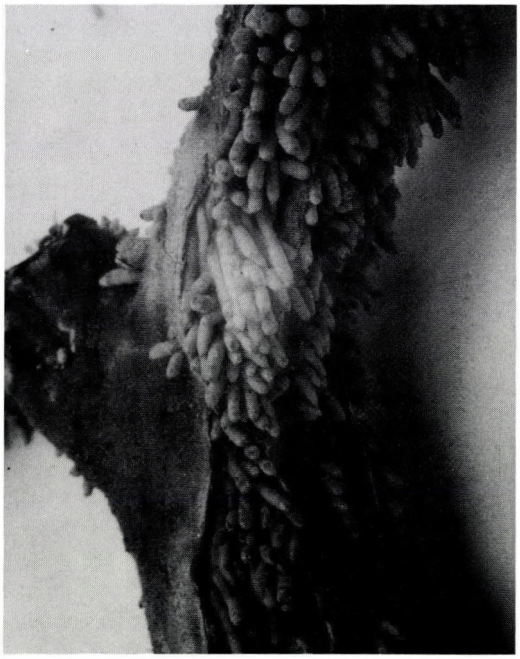

Fig. 2. Tomato stem of naturally infected tomato plant showing adventitious root formation

The disease developed on the watered plants after 5–6 weeks (Fig. 2) where the nutrients were not excessive. These results were in accordance with the observations of other authors (1, 6).

Because the disease severity in 20 out of 28 spontaneously infected tomato varieties and lines was about 3–12%, it is doubtful that the implementation of a breeding programme for resistance to *P. corrugata* would not be realizable easily.

Acknowledgement

We are grateful to I. Petrányi for the photos.

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Occurrence of *Septoria betulae* on Birch (*Betula alba*) in Argentina

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Necrotic spots were observed on leaves of birch plants grown in the suburbs of La Plata (Buenos Aires, Argentina). As the disease progressed, spots developed and spread out quickly resulting in death of the entire lef and in defolation. Laboratory tests proved that the causal organism of this disease was *Septoria batulae* Pass. Symptomatology and characteristics of the pathogen are described in this study. This disease was not previously reported in South America.

Betula alba L. is a forest and ornamental tree widely spread in Argentina. In April 1995, adults birch plants, in a forest nursery near La Plata city (Buenos Aires, Argentina), were severely affected by a foliar disease. Small isolated chlorotic spots were first observed on the surface of infected leaves. Chlorosis was followed by formation of amphigenous, isolated or confluent, polylangular to irregular, light-brown to redish-brown leasons (Fig. 1). Usually necrotic areas began to coalesce on the margins of leaves which become brittle, and dried. As the disease progressed spots developed and spread over the leaf surface resulting in death of the entire leaf and defoliation. Necrotic tissue was progressively covered with pycnidia.

Leaves with lesions were collected and disinfested with 0.5% NaOCl for 1 minute, rinsed twice with sterile distilled water, and incubated in Petri dishes containing 2% patato dextrose agar for 7 days. Isolates were cultured on 2% malt extract agar at 25 °C under continuous fluorescent light for 15 days.

Morphologically identical *Septoria* sp was isolated from necrotic leaf tissue. Colonies were velvety, pinkish at first and greyish later, with globose or subglobose black pycnidia 50–150 μm diameter, that produced conidia hyaline, obclavate to acicular, slightly curved, 2–7 (3–4) septate, base truncate, apex subacute to rounded, 21–56.5 (34.5) × 1.5–2 (1.6) μm. The pathogen was identified as *S. betulae* Pass. non (Lib.) Westend. ex Sacc. hom. illeg. by Dr. O. Constantinescu (Institute of Systematic Botany, Uppsala, Sweden, Herb. UPSC 3804).

Pathogenicity was determined by spraying a conidial suspension containing ca. 1×10^8 conidial/ml^{-1} onto leaves of 2 years old birch trees in the greenhouse at 20–25 °C and 85% relative humidity. The inoculated plants and controls were kept in a moist chamber for 24 hours. Plants were observed periodically from the third day after inoculation. Symptoms similar to those above described were evident within 3 weeks. The pathogen was reisolated and compared with the original fungus to fulfilling Koch's postulates. No symptoms appeared on any control plants.

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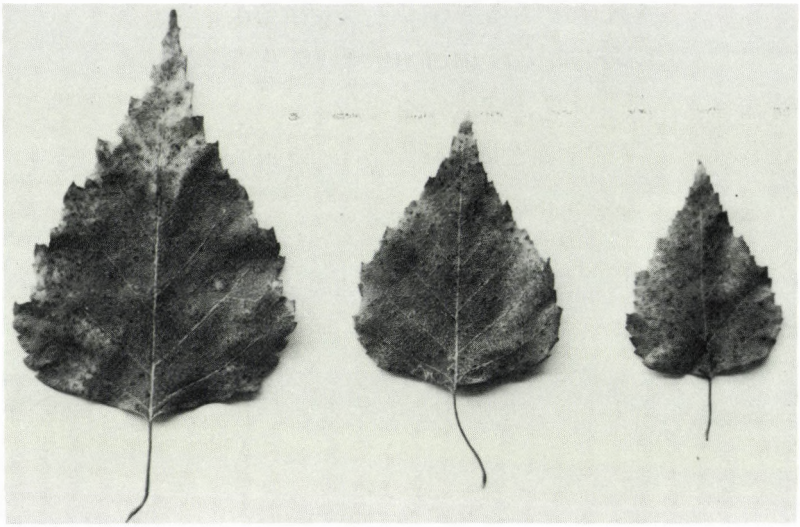

Fig. 1 Leaves of *Betula alba* naturally infected by *Septoria betulae*

S. betulae on *B. alba* have been reported in Czechoslovakia, Germany, Italy, Sweden, USSR (Constantinescu, 1984) and USA (Farr et aI., 1989).

This is the first record of *S. betulae* affecting birch plants in South America.

Acknowledgement

The assistance of Dr. O. Constantinescu who identified the fungus is gratefully acknowledged.

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SHORT COMMUNICATION

A Marker System for *Phytophthora parasitica* Based on Hybridization of PCR Products with Species-specific Repetitive DNA

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Polymerase chain reaction (PCR) products derived from amplification of species-specific, 24-mer oligonucleotide primers of *Phytophthora parasitica* hybridized with PCR products derived from the RAPD primer, OPG-02. No hybridization of products similarly generated for *P. citrophthora* was observed. Hybridization patterns were consistent among pathogen isolates collected from diseased tomato plants in California, and these differed from patterns of one isolate each collected from jojoba, pistachio, and tobacco. The variability that was detected among the DNA "fingerprints" contrasted with the relative uniformity of patterns of PCR products generated using either the species-specific or random primers. This method of fingerprinting may prove useful in characterizing a single isolate within a species whose populations are to be monitored against a background of related organisms in epidemiological studies.

The genus *Phytophthora* contains some of the world's most important soilborne pathogens. Among these are *Phytophthora parasitica* Dastur (syn. *P. nicotianae*) and *P. citrophthora* (Sm. & Sm.) Leonian, pathogens that cause severe economic losses in hundreds of crop species worldwide. It is not uncommon to find *P. parasitica* in the same field soil with other *Phytophthora* spp. The morphologies of many *Phytophthora* species are similar, and these similarities have made disease diagnosis and pathogen identification difficult. From a research perspective, morphological similarities have made it difficult to investigate species interactions at the field level.

The development of genetic markers would help to overcome difficulties of identification. Molecular probes based on species-specific DNA sequences, have been developed to distinguish *P. parasitica* and *P. citrophthora* from other *Phytophthora* spp. (Goodwin et al., 1989, 1990). Recently, we selected oligonucleotide sequences for *P. parasitica* and *P. citrophthora* that would distinguish each species on the basis of products derived from PCR (Érsek et al., 1994). Other investigators (Lee et al., 1993) have created species-specific markers for *P. capsici*, *P. cinnamomi*, *P. megakarya* and *P. palmivora*, based on variability in DNA sequences of the internal transcribed spacer (ITS) sequences of ribosomal DNA.

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Table 1

Isolates of *P. citrophtora* and *P. parasitica* tested and their sources

P. citrophtora		*P. parasitica*	
Isolate	Host and geographical source[1]	Isolate	Host and geographical source[1]
14A	Kiwi, California[a]	W1	Tomato, California[d]
35-3-3	Walnut, California[b]	5-3A	Tomato, California[d]
P776	Cacao, Brazil[c]	1-3A	Tomato, California[d]
P1839	Cacao, Brazil[c]	30-2DM	Tomato, California[d]
P1213	Cacao, Brazil[c]	37-4-1	Jojoba, California[d]
Wi 1	Citrus, California[d]	34-3-9	Pistachio, California[d]
P318	Citrus, Australia[e]	C-2CL	Citrus, Arizona[e]
P1323	Citrus, California[d]	1452	Tobacco, Kentucky[c]

[1] Obtained from the culture collection of [a] K. Conn, [b] S. M. Mircetich, [d] J. M. Duniway, Department of Plant Pathology, University of California, Davis; [c] M. D. Coffey, Department of Plant Pathology, University of California, Riverside; [e] M. E. Matheron, Yuma Mesa Agricultural Center, Somerton, AZ.

Up to this point, the markers that have been developed for *P. parasitica* exhibit considerable uniformity across most isolates within the species. Limited attention has been given to the development of genetic markers to identify specific isolates within the species. The availability of such markers for *P. parasitica*, *P. citrophthora* and other *Phytophthora* species would allow investigators with interests in population behaviors of these pathogens to monitor a specific pathogen genotype of interest without introducing mutations or other molecular manipulations that might disrupt the wild-type behavior of that isolate. In the course of examining intraspecific variability of *P. parasitica* and *P. citrophthora*, we encountered a method by which an isolate might be characterized by hybridization of its RAPD-PCR products with a species-specific probe. This report describes this fingerprinting methodology and the variability that was observed in resulting patterns among isolates within a single species of *Phytophthora.*

The isolates of *P. parasitica* and *P. citrophthora* that were used in these experiments were obtained from a variety of plant hosts and geographic locations in North and South America and Australia (Table 1). For each isolate, total genomic DNA was extracted from fungal mycelium by a "miniprep" procedure as described elsewhere (Érsek et al., 1994). A *P. parasitica*-specific 24-bp primer-pair (5'-CTGACGATCCAGATCC-TCTGCACG-3' and 5'-CTTGCGAGGCTTGACCGCTTCCTA-3'), was derived previously from the sequence of a cloned 1300-bp chromosomal DNA fragment of isolate 5-3A, isolated from a diseased tomato plant (Érsek et al., 1994). A *P. citrophthora*-specific, 24-bp primer-pair (5'-GTCGACGTCCTGCTTGGCACTCTG-3' and 5'-CGGT-GCTCCGCGACTGTTGTCCAC-3'), was derived previously from the sequence of a cloned 800-bp chromosomal DNA fragment of isolate P1323, isolated from citrus (Érsek et al., 1994).

In the course of evaluating a variety of 10-bp oligonucleotide primers for their abilities to differentiate among isolates within *P. parasitica* and *P. citrophthora* by RAPD-PCR (Williams et al., 1990), we selected three, including OPG-02, OPG-10, and OPG-16 (all purchased from Operon Technologies, Inc.) for use in these experiments. All reactions using 24-bp, species-specific or 10-bp random oligonucleotide primers were cycled with an automated thermal cycler (Hybaid, HB-TR1).

The species-specific primers (100 pmoles each) typically were added to a reaction mixture containing the commercial reaction buffer, 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphate (dNTP), 2.5 units of *Taq* DNA polymerase (all purchased from Promega Corp.) and 100 ng of fungal DNA in a total volume of 100 µl. The complete polymerization algorithm included 34 cycles of denaturation for 1 min at 94 °C, annealing for 2 min at 65 °C and 50 °C for *P. parasitica* and *P. citrophthora*, respectively; and an extension for 3 min at 72 °C. These cycles were preceded by one cycle with an extended denaturation time of 5 min at 94 °C and a final extension for 10 min at 72 °C.

Typically, RAPD reactions were conducted as follows. A primer (20 pmoles) was mixed with reaction buffer, $MgCl_2$ (2 mM), dNTPs (200 µM each), *Taq* DNA polymerase (2.5 units), fungal DNA (100 ng) and sterile, glass-distilled water in a total volume of 50 µl. The thermal cycler was programmed for 44 cycles of 94 °C for 1 min, 36 °C for 1 min and 72 °C for 2 min, preceded by one cycle with an extended, 5-min denaturation at 94 °C. The amplification products were resolved by electrophoresis in 1.2 or 1.5% agarose gels and stained with ethidium bromide, or were subjected to Southern transfer (Maniatis et al., 1982).

To label species-specific DNA with ^{32}P-CTP, species-specific sequences were first amplified from the respective vectors, pPP33A and pCIT15A of *P. parasitica* and *P. citrophthora*, respectively, using the corresponding 24-base oligonucleotide primers (Érsek et al., 1994). The amplification products, P1000 and CIT650, respectively, were electrophoresed, isolated from 0.8% low-melting agarose gel and then purified with the Magic™ DNA Clean-Up System (Promega Corp.) according to the instructions of the manufacturer. After labeling with [α-^{32}P]dCTP by the use of the Random Primed DNA Labeling Kit (U. S. Biochemicals Corp.), these probes were hybridized to nitrocellulose blots containing the RAPD-PCR products.

As in a previous study (Érsek et al., 1994), the 1000-bp *P. parasitica*-specific sequence was present in all isolates of this species tested (Fig. 1a). In addition, each RAPD pattern was fairly uniform over all isolates (Fig. 1b, c). Although the collection of isolates represented a limited number of host plants and geographic locations, the limited intraspecific variation agreed with previous reports of restricted variation within *P. parasitica*, based on molecular and biochemical markers (Förster and Coffey, 1991; Oudemans and Coffey, 1991).

In attempts to "fingerprint" the randomly amplified sequences with the species-specific probes, we observed that one of the RAPD sets that had been amplified with the OPG-02 primer (Fig. 2a) exhibited sequences with homology to the 1000-bp *P. parasitica*-specific sequence (Fig. 2b). Despite the low level, or the apparent lack of, DNA

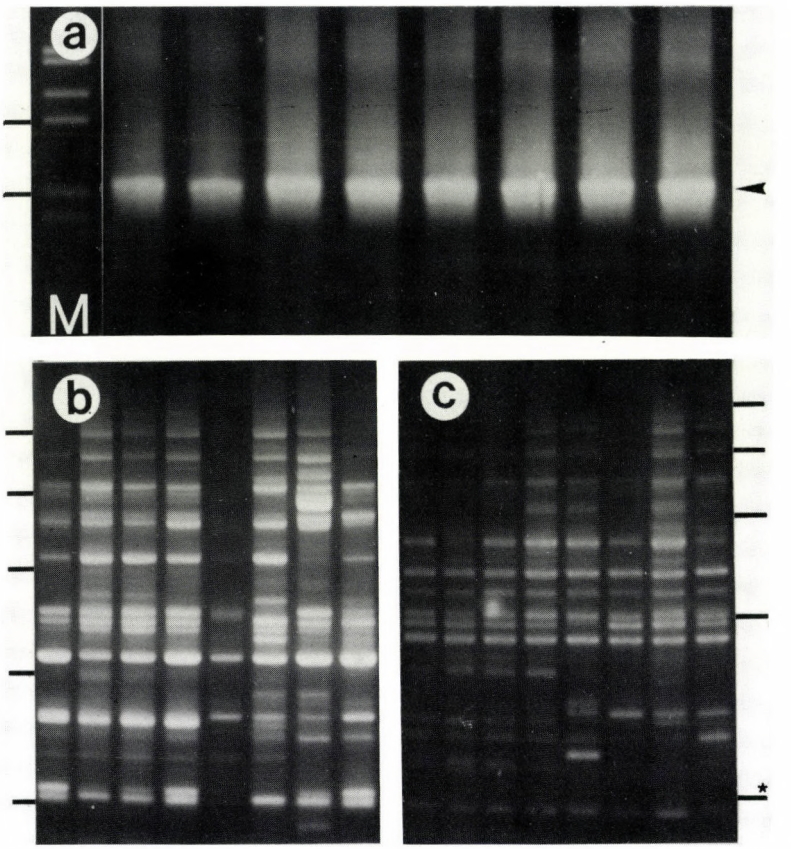

Fig. 1. Agarose gel image of DNA sequences from *P. parasitica* isolates following PCR with a *P. parasitica*-specific, 24-mer primer-pair (a) and, with single arbitrary primers OPG-16 (b) and OPG-10 (c). Lane M, molecular size markers of lambda *Eco*RI/*Hind*III digest (Promega Corp.) with marked position of the 1330-bp and the 983-bp fragments (a). Arrowhead indicates the 1000-bp species-specific fragment. DNA patterns amplified from total genomic DNA of 8 isolates of *P. parasitica*, are in the following order from left to right: 1, Wl (tomato); 2, 5-3A (tomato); 3, 1-3A (tomato); 4, 30-2DM (tomato); 5, 37-4-1 (jojoba); 6, 34-3-9 (pistachio); 7, C-2CL (citrus) and 8, var. *nicotianae* (tobacco) (a, b and c). Molecular sizes of 4277, 2027, 1330, 831 and 492 or 369 (*) are marked in b and c

polymorphisms among *P. parasitica* isolates on the agarose gel image following PCR with arbitrary primers (Fig. 2a), intraspecific variability was detected by hybridization of PCR products with the probe, P1000 (Fig. 2b). By this approach, the four tomato isolates were distinguished from the jojoba, pistachio, and tobacco isolates, particularly on the basis of the co-occurrence of the approximately 1230-bp and the 1750-bp hybridizing bands (appearing faintly in the leftmost lane of Fig. 2b). The degree of distinction of tomato isolates from that of citrus was less obvious.

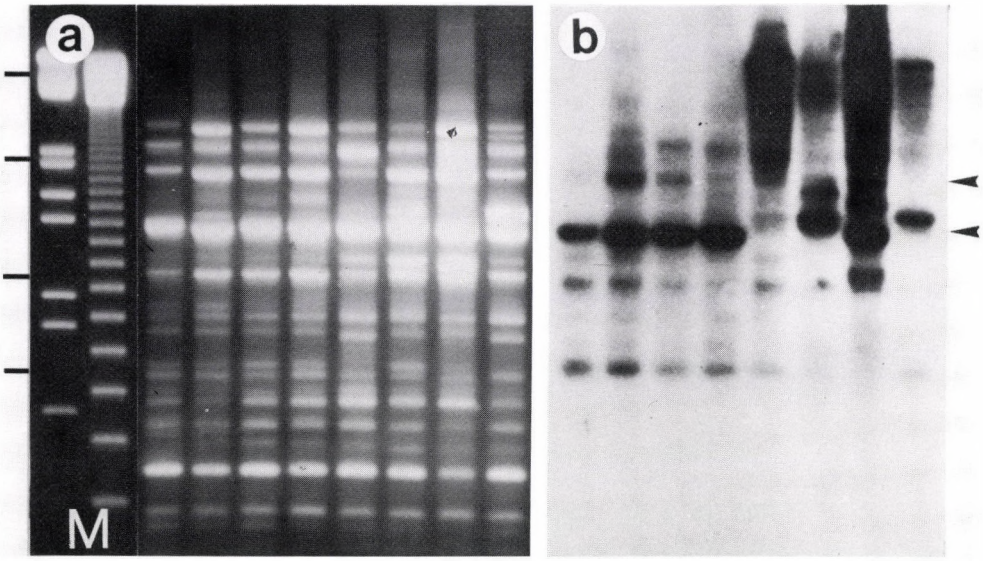

Fig. 2. DNA pattern of *P. parasitica* following PCR with OPG-02 (a) and the corresponding autoradiograph (b) after hybridization with the species-specific probe, P1000, showing fingerprint patterns of the representative isolates in an order as in Fig. 1. Arrowheads indicate the position of a ca. 1230-bp and a ca. 1750-bp band, the co-occurrence of which is characteristic of the tomato isolates in lanes 1 to 4. M, molecular size markers of lambda *Eco*RI/*Hind*III digest (left) and the 123-bp DNA ladder (Gibco BRL, Life Technologies, Inc.). Molecular sizes of 4277, 1722, 983 and 615 are marked on the left of the panel

Using the 24-base species-specific oligonucleotide primers, a 650-bp product was amplified by PCR in all isolates of *P. citrophthora*, regardless of host or geographic origin (Fig. 3a). Interestingly, the 5.1-kb sequence, from which the oligonucleotide primers were derived, failed to hybridize with DNA of these same isolates from cacao (Goodwin et al., 1990). Förster and Coffey (1991) also made a distinction between cacao and non-cacao isolates on the basis of RFLPs of mitochondrial DNA. Regardless of these inconsistencies, our finding regarding the molecular features of *P. citrophthora* is in concert with morphological data, in that all of the tested isolates, although differing from one another, have something in common that characterizes the species.

The RAPD profiles varied among the *P. citrophthora* isolates tested. Amplified DNA sequences of cacao isolates differed slightly from one another but were distinct from those of isolates from other hosts (Fig. 3b–d). A low degree of DNA polymorphisms was observed between the kiwi isolate and the walnut isolate as well as between the two isolates from citrus in California; these two groups (kiwi/walnut *vs.* citrus), however, were rather different from each other. Primers OPG-16 and OPG-02 clearly distinguished the citrus isolate from Australia from the California citrus isolates (Fig. 3b, d). These results are in agreement with earlier genetic groupings deduced from different

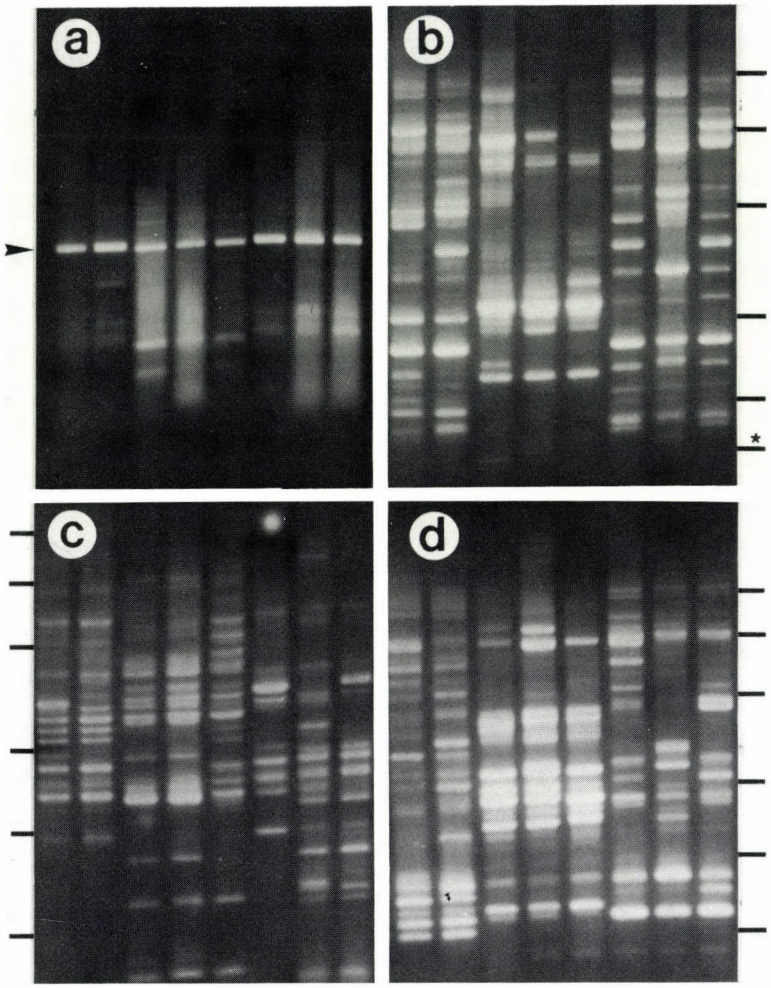

Fig. 3 Agarose gel separation of DNA sequences from *P. citrophthora* isolates following PCR with a *P. citrophthora*-specific, 24-mer primer-pair (a) and, with single arbitrary primers OPG-16 (b), OPG-10 (c) and OPG-02 (d). Arrowhead points to the 650-bp species-specific fragment. Molecular sizes of 4277, 2027, 1330, 831, 564 and 492 (*) or 369 base pairs are marked in b, c and d. Lanes 1 through 8, from left to right, contain DNA from isolates as follows. 1, 14A (kiwi); 2, 35-3-3 (walnut); 3, P776 (cacao); 4, P1813 (cacao); 5, P1213 (cacao); 6, Wi 1 (citrus); 7, P318 (citrus); and 8, P1323 (citrus).

approaches and confirm the notion of other investigators; there is no single isolate which is typical of *P. citrophthora*.

In contrast to the success of obtaining fingerprint patterns for *P. parasitica*, the 650-bp species-specific sequence of *P. citrophthora* did not hybridize with any of the RAPD-PCR products derived from OPG-02, OPG-10, or OPG-16.

In ecological studies with *Phytophthora* spp. and other soilborne pathogens, it is often necessary to identify and monitor the population behaviors of a specific isolate of interest against a potentially diverse background of other isolates. Often isolates for use in these types of studies are created or selected with specific antibiotic or other biochemical markers. Consequently, there are always questions concerning the possibility of anomalous behavior of such isolates relative to normal life cycle attributes and pathogenic behaviors.

The fingerprinting method which was developed may prove useful in population studies of *P. parasitica*, in particular. By this method we were able to characterize specific isolates or groups of isolates to a greater degree than had been reported previously for this pathogenic species. In contrast to *P. parasitica*, no fingerprint was detected for *P. citrophthora*. However, this may be less important for this species in which intraspecific variability can be detected to some extent by RAPD-PCR or other methods.

The described fingerprint method was based on hybridization of repetitive DNA. Further evaluations of the method are needed to assess the applicability of the approach over *Phytophthora* species, in general.

Acknowledgement

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The Winter Mortality and Developmental Biology of *Pseudaulacaspis pentagona* Targioni-Tozzetti, 1886 (Homoptera: Coccoidea)

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Diapausing females of *P. pentagona* started to lay eggs in laboratory after 15–22 days in winter, but after February this time decreased to 6–16 days. The hatching of crawlers started after 3–9 days in laboratory in winter. The condition of the females (diapausing and nondiapausing), and the host plants did not affect the hatching time. The temperature regulated reactivation can result an early egglaying in warm springs. In the second generation the highest number of males was observed in Budapest in 1991 (11460) with average winter temperature –1 °C. In 1993 (–3 °C) there was a tenfold decrease in the number of males as compared to 1991. In other places *P. pentagona* totally disappeared. On the other hand the number of males significantly increased at some other places during the same period. In the population dynamics of *P. pentagona* the winter temperature has very important role, causing very high mortality (up to 100%). The very high and dry summer temperature can cause decline of the population, too.

According to Jeszenszky (1971) and Varga (1971) under field conditions in Hungary the egglaying of the first generation starts in the second half of May. In South of France *P. pentagona* egglaying lasts from the end of April to the end of May (Benassy, 1961; Benassy and Milaire, 1970). According to Bennett and Brown (1958) the females begin oviposition in 14–16 days after mating in summer generations.

Hatching of crawlers starts 4–7 days after the beginning of the egg laying in Southern Europe (Tremblay, 1958). In the eastern USA *P. pentagona* larvae start hatching 3–4 days after egglaying (Yasuda, 1981; Yonce, and Jacklin, 1974). In Hungary the hatching of crawlers starts from end of May (Varga, 1971). In South of France the hatching of crawlers of the first generation starts from middle of May (Benassy and Milaire, 1970). In North of Greece the hatching of the crawlers starts from the second half of April (Paloukis, 1979).

The *P. pentagona* females spend the winter under the scale in a facultative diapause stage (Kozár, 1990), but the regulation of diapause and reactivation was not studied in detail. Scale insects as sedentary animals are heavily influenced by abiotic factors, especially by cold winter. The winter mortality of *P. pentagona* shows very large variations in different places and in different years. There was a heavy outbreak in Hungary in 1928–1929, but after a very cold winter the animals almost totally disappeared. New outbreaks were observed in 1935–1936, and in 1958 (Varga, 1971). Heavy infestation and quick spreading to new parts of the country was found after 1975 (Kozár and Nagy, 1986). There was more than 90% mortality during the cold winter of 1987

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(Kerényiné dr. Nemestóthy Klára, personal communication). From that time a new outbreak started in different parts of the country (Kozár et al., 1995). The natural winter mortality of *P. pentagona* females in France was only 15–22% in January, but it increased to 60–98% by the end of winter (Benassy, 1961), which shows the sensitivity of this species to the winter conditions. Similar results were found in Hungary in the case of *Quadraspidiotus perniciosus*, where the average winter mortality amounted to 55.5% (Kozár and Viktorin, 1980). The extreme fluctuations of winter temperature are especially dangerous for insects (Kozár, 1974), which sometimes can cause 100% mortality. In the case of the *Epidiaspis leperii*, which overwinters also as a female, Abd El-Kareim (1988) showed a mortality about 40% in Hungary.

The aim of this study was to analyse the effect of overwintering conditions on the beginning of egglaying, hatching of the larvae, the winter mortality, and population dynamics.

Materials and Methods

Winter mortality, egglaying and hatching of the larva of *P. pentagona* was studied in laboratory in 1991–1993. The experiment started from January and lasted till April. Samples were collected from parks of Budapest. Mortality % has been calculated on three different host plants. The natural mortality calculated from the number of healthy and dead females, which were counted under microscope. Mortality in different regions of Hungary has been observed in 1993. Branches from 20 places were collected (most of it was *Sophora japonica* except three places where we could find infestation only on *Morus sp.* and *Syringa vulgaris*). The density of *P. pentagona* was studied by pheromone traps in different parts of the country, and was related to winter temperatures. We used the winter temperature data (absolute minimum), and the average of the coldest month of the nearest station of the Hungarian Meteorological Service. For the statistical analyses we used the software package Statistical.

Results and Discussion

a. Beginning of egglaying

Diapausing females start to lay eggs in laboratory after 15–22 days in winter, which showed some variations in different years. But after February the egglaying started after 6–16 days (Table 1). The decrease of the time necessary for egglaying in different months was highly significant in each year ($p = 0.0000$). It shows that the winter diapause was finished by the end of February. After the females stay in a quiescence stage (in sense of Sáringer,1976), and can start egglaying more quickly, if the temperature is enough high. This is similar to results found in case of *Q. perniciosus* (Kozár, 1989) and *E. leperii* (Abd El-Kareim, 1988), where the winter diapause is terminated in middle of

Table 1

Time necessary for beginning of egglaying and larval hatching

Months	Host plants	Number of days					
		egglaying			hatching		
		1991	1992	1993	1991	1992	1993
January	Morus	18	15	15	3	5	8
	Syringa	18	15	16	3	5	7
	Sophora	18	15	15	3	5	8
February	Morus	10	15	20	5	5	7
	Syringa	10	15	22	6	5	7
	Sophora	10	15	20	5	5	8
March	Morus	11	12	16	7	6	9
	Syringa	11	12	16	7	6	9
	Sophora	11	12	16	7	6	9
April	Morus	9	14	10	6	5	7
	Syringa	8	13	10	7	6	7
	Sophora	6	13	10	8	6	7

winter (January–February). Afterwards the reactivation is regulated by temperature. The beginning of egglaying time was not affected by different host plants ($p = 0.1664$–0.4219). According to Jeszenszky (1971) and Varga (1971) in field conditions the egglaying starts in the second half of May. Our laboratory experiments suggest that this species can lay eggs much earlier in warm springs.

b. Beginning of hatching of crawlers

The time necessary for beginning of hatching of crawlers is 3–9 days under laboratory conditions. According to the results, the condition of the females (diapausing and nondiapausing), did not affect the hatching time. There was a small, but significant variability ($p = 0.0004$–0.0122) in different years and months, which was perhaps caused by the physiological conditon of the eggs. There was no significant difference between the different hosts ($p = 0.1664$–0.4219) (Table 1).

c. Winter mortality

The natural mortality of females of *P. pentagona* was determined at different places. We found that the mortality varied between 36.5–100%. In Baja it was the highest (100%) here the absolute minimum winter temperature was –20 °C. The smallest was in

Table 2

Mortality percentage of *P. pentagona* females in different places of Hungary and the winter temperature data (1993)

Places	Mortality in %	Temperature data from	Average minimum temperature (°C)	Average per coldest month (°C)
Százhalombatta	80.6	Martonvásár	–18	–2.0
Solt	96.6	Paks	–20	–2.4
Bátaszék (Sy. vulgaris)	97.3	Bátaszék	–14	–1.6
Pécs	75.8	Pécs	–14	–1.4
Szigetvár (Morus sp.)	96.2	Szigetvár	–18	–0.3
Budapest	66.6	Budapest	–10	–3.0
Szentendre (Morus sp.)	93.2	Szentendre	–15	–1.0
Baja	100	Baja	–20	–2.8
Bácsalmás	82.5	Bácsalmás	–17	–1.2
Kalocsa	77.3	Kalocsa	–16	–2.5
Kiskunfélegyháza	83.3	Kiskunfélegyháza	–15	–1.1
Kiskunhalas	94.8	Kiskunhalas	–18	–2.1
Makó	76.3	Makó	–17	–2.1
Csongrád	96.6	Szentes	–14	–1.7
Paks	74.5	Paks	–20	–2.4
Jánoshalma (Morus sp.)	82.5	Szeged	–17	–2.5
Szeged	46.6	Szeged	–17	–2.5
Hódmezővásárhely	88.9	Hódmezővásárhely	–17	–2.5
Szekszárd	89.7	Szekszárd	–14	–1.6
Dunaújváros	36.5	Dunaújváros	–20	–2.0
Mohács	78.1	Mohács	–14	–1.7

Budapest, Dunaújváros and Szeged (36.5–66,6%). The relationship between mortality and absolute minimum was not significant. It is clear that even –20 °C degree cannot stop totally the development and distribution of this species (Table 2). Winter mortality of females of *P. pentagona* on different host plants during three years in Budapest showed no significant difference (Table 3) and it was on a relatively low level (between 12.9–76.6%).

In the second generation we observed that the highest number of males were in Budapest in 1991 (11 460) with average temperature –1 °C, this number decreased to 1629 males in 1993 with average temperature –3 °C. On the other hand the number of males in Kecskemét increased from 201 in 1991 to 1375 in 1993 (Table 4). The sharp decrease in Budapest was studied in more detail. We found that this decrease happened in the Summer of 1992, where from first generation the traps collected 10617 males, but from second generation only 1033 (the ratio of first and second generation usually the opposite!). The only explanation what we could find for this, is the very high temperature in that Summer, which could cause high mortality of the crawlers.

Table 3

Winter mortality of *P. pentagona* in Budapest

Months	Host plants	Mortality in % Years 1991	1992	1993
January	Morus	31.37	25.9	29.2
	Syringa	32.60	12.9	23.9
	Sophora	31.57	32.1	30.7
February	Morus	21.00	17.1	30.7
	Syringa	23.67	26.6	16.7
	Sophora	27.92	23.6	16.1
March	Morus	34.63	76.6	23.4
	Syringa	21.77	70.6	27.5
	Sophora	18.93	23.3	31.3
April	Morus	22.72	16.4	35.3
	Syringa	19.89	29.9	31.7
	Sophora	21.94	23.3	32.8

According to these results in the population dynamics of *P. pentagona* the winter temperature has a very important role, causing high mortality. The species is especially endangered after February, when the diapausa stage terminated and the reactivation regulated by temperature. At this time fluctuations of the temperature could cause very high mortality in some places and years. Sometimes the very high and dry summer temperature can cause decline of the population. The temperature regulated reactivation (from February) can result very early egglaying in warm springs, as it is known in Southern Italy.

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Table 4

Number of males in pheromone traps in second generation, and the average temperature in the coldest month of the year

Places	Number of males			Average temperature (°C)		
	1991	1992	1993	1991	1992	1993
Nyíregyháza	1	0	0	–3.9	–2.7	–3.1
Eger	4	0	0	–3.4	–1	–1.9
Debrecen	99	15	0	–4.2	–2.2	–3.3
Miskolc	0	2	0	–3.8	–2.6	–2.5
Gyöngyös	0	51	–	–3.4	–1	–1.9
Hatvan	0	0	0	–3.4	–1	–1.9
Balatonakarattya	0	42	0	–3	0.8	–1.4
Székesfehérvár	384	–	–	–4.4	0	–1.9
Szolnok	463	–	–	–3.6	–1.1	–2.1
Szarvas	6	8	–	–3.3	–1.3	–2.5
Kecskemét	201	555	1375	–3.7	–0.4	–2.1
Békéscsaba	0	0	3	–3.9	–1.5	–3.4
Orosháza	68	–	–	–3	–1.5	–3.4
Veszprém	4	6	2	–1.6	0.4	–1.4
Tokod	–	444	–	–1.5	0	–2.1
Dunaalmás	–	4	12	–1.5	0	–2.1
Szőny	–	1	0	–1	0.2	–1.2
Tata	–	3	1	–1.5	0	–2.1
Lábatlan	–	–	5	–1.5	0	–2.1
Szentes	–	333	–	–1.8	0.8	–1.1
Kondoros	–	0	1	–3.3	–1.3	–2.5
Pápa	–	12	0	–2.2	0.9	–1.7
Hrkovce (Szlovákia)	–	–	1679	–2.4	0.3	–2
Komárno (Szlovákia)	–	–	557	–2.5	0.2	–1.2
Budapest	11460	1033	1629	–1	2	–3
Szántód	–	973	–	–2.4	1.3	0

– = No data

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A Preliminary Study on the Armoured Scale Insect (Homoptera, Coccoidea: Diaspididae) Fauna of Antalya

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The armoured scale insect fauna of Antalya, a West Mediterranean province of Turkey, was studied during 1992–1994. Totally 29 species were found, four of them being new for Turkish fauna namely, *Aonidia meditenanea* Ferris, *Neochionaspis asiatica* Borchsenius, *Lepidosaphes granati* Balachowsky and *Targionia nigra* Signoret. Host plant, phenology, and distribution data are given for all species.

Armoured scale insect fauna of Turkey is little known. The most comprehensive study was undertaken by Bodenheimer (1949) to date, and according to him 38 species was recorded till that time. Yasar (1995) recorded 84 species of armoured scale insects in Turkey. There are indications showing that there is a growing interest towards this group of insects in this country as exemplified by some recent studies on the fauna of certain provinces: Kozár et al. (1979); Çobanoglu and Düzgünes (1984), Ankara (Central Anatolia); Yasar (1990), Izmir (Aegean Region); Yasar et al. (1995), Van (Eastern Anatolia). It is expected that the interest in armoured scale insects will further grow with the realisation of their economic importance and the need for inventory studies. The information's related to the distribution of the species in Turkey are from works of Bodenheimer (1949) and Yasar (1995), and for the World are based on the works of Borchsenius (1966), Panis (1981), Kosztarab and Kozár (1988) and Danzig (1993).

Antalya province occupies the West Mediterranean part of Turkey (Fig. 1). Two major types of climates are found in the province. The continental climate which is characterised by cold and rainy and snowy winters, and hot and dry summers, dominates higher elevations over 800 m above sea level. The subtropical Mediterranean climate which is characterised by mild and rainy winters, and hot and dry summers, influences coastal areas up to 600–700 m above sea level. Therefore tropical and subtropical elements of any taxon of insects as well as temperate ones are encountered in the area of question and the armoured scale insects are no exception as the material discussed below indicates.

The divers conditions of the Region indicate presence of high number of scale insect species, among them important pests on different plants. The possible global warming also could cause a significant increase of the species number of insects in such Regions (Kozár, 1992). Therefore, the aim of our work was to study the present composition of the scale insect fauna under different conditions, on different plants, and to collect phenological information's.

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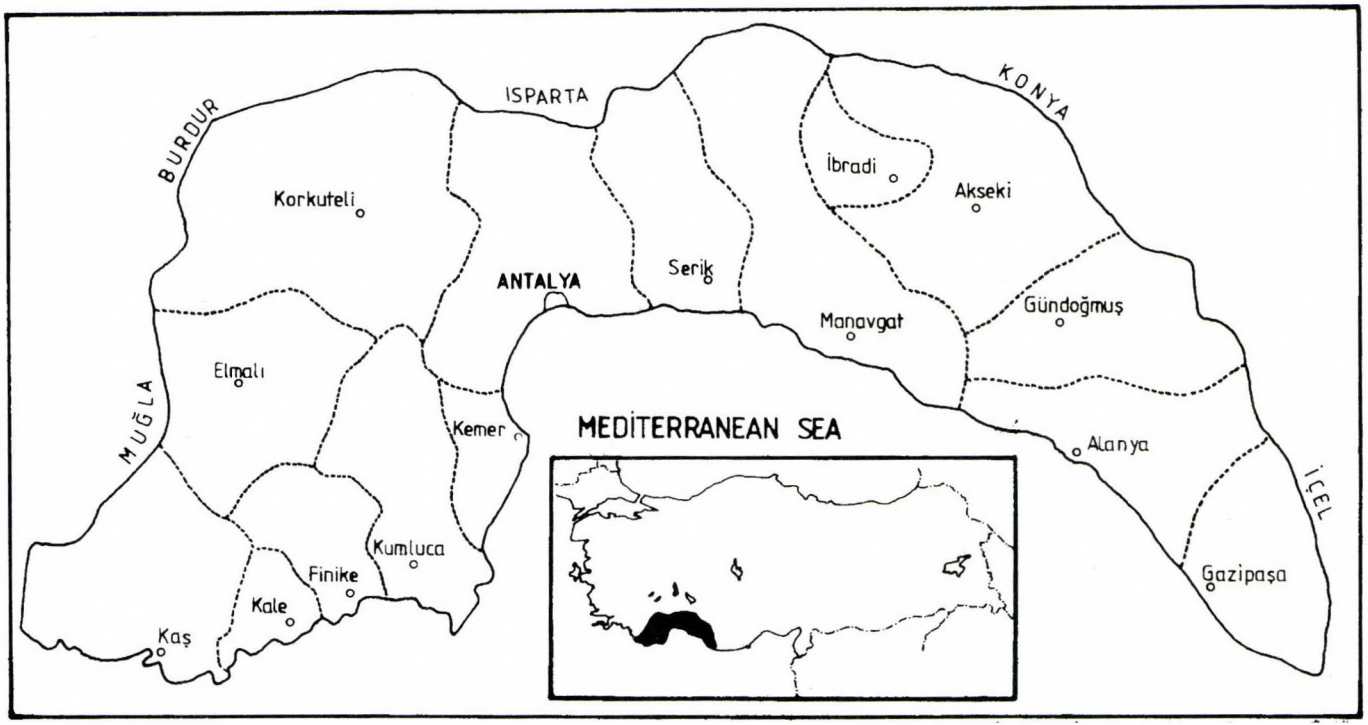

Fig. 1. Districts of Antalya Province. (Dashed lines represent the district boundaries. Words with lower-case letters are district names, words with capital letters (except Antalya, the central district) are the names of neighbouring provinces. Blackened area in the inserted map indicates the position of Antalya Province in Turkey)

Materials and Methods

Samples were collected in the years of 1992–1994. Sampling sites in Antalya (central district), Serik, Manavgat, Alanya, Kemer and Kas districts are in coastal areas whereas those in Elmalì, Korkuteli, Ibradı and Akseki districts are in higher elevations (Fig. 1). Specimens were taken from over 50 plant species belonging to 24 families.

Description of samples contain the names of identified scale insect species, place of collecting, host plant, date of collecting, found phenological stages, "D" denotes dead specimens and "L" those in larval stages in the text.

Parts of dry and mounted material are deposited at the Plant Protection Department of Akdeniz University, Antalya and Yüzüncü Yìl University (Van), Turkey, and on slides at the Plant Protection Institute, Hungarian Academy of Sciences (Budapest), Hungary.

Results and Discussion

Totally 29 species of armoured scale insects were found in Antalya province in this study. Four of them were new records for Turkey, namely *Aonidia mediterranea* Ferris, *Lepidosaphes granati* Balachowsky, *Neochionaspis asiatica* Borchsenius and *Targionia nigra* Signoret. With the results the number of species known from Turkey increased to 87. In our survey 13 species was new for the fauna of Antalya Region. The total number of species known from Antalya increased to 41. However, several species, recorded earlier, was not found in this study, namely: *Acanthomytilus cedricola* Balachowsky et Alkan, *Carulaspis juniperi* (Bouché), *Chrysomphalus pinnulifer* (Maskell) (Yasar, 1995); *Aspidiotus hedericola* Lindinger, *Chrysomphalus dictyospermi* Morgan, *Diaspis syriaca* Lindinger, *Gonaspidiotrs minimus* (Leonardi), *Leucaspis loewi* Colvée, *L. pusilla* Lőw, *L. pini* Hartig, *Oceanaspidiotus spinosus* (Comstock), *Parlatoria pergandii* (Comstock) (Bodenheimer, 1949). This may indicate the necessity of further studies on the scale insect fauna in this Region, too.

As to the zoogeographical distribution of the species involved, the majority are Palaearctic, some being introduced to U.S.A, too. The number of Palaearctic species is 19, which comprises of West Palaearctic (5), Mediterranean (4), Turano-Mediterranean (5), Ponto-Mediterranean (2), Turanian (2), European (1) species. Circum-Mediterranean species are in majority in the Palaearctic group. The rest of the species are Tropical–Subtropical (2), Cosmopolitan (4), Semi-Cosmopolitan (3), Oriental (1).

Several species found are considered as pests of fruit crops and are targeted in pest control programs in Turkey. The *Epidiaspis leperii* (Signoret), *Lepidosaphes ulmi* (Linnaeus), *Nilotaspis halli* (Green), *Parlatoria oleae* (Colvée), *Pseudaulacaspis pentagona* (Targioni-Tozzetti) and *Quadraspidiotus perniciosus* (Comstock) are pests of temperate fruits, whereas *Aonidiella aurantii* Maskell and *A. citrina* (Coquillet) are pests on citrus and other subtropical fruits.

List of collected species with host plant and phenological data

Adiscodiaspis tamaricicola Malenotti – Material examined: Antalya, *Tamarix* sp., 3. 10. 92; female and male. First record from Antalya. Previous records from Turkey: Central and Eastern Anatolia.

Aonidia lauri (Bouché) – Material examined: Antalya, *Laurus nobilis*, 31. 03. 93; D; female and male, Kemer, *L. nobilis*, 19. 06. 94; D; female and male, Termessos (Antalya), *L. nobilis*, 11. 08. 94; D; female and male. Previous records from Turkey: Marmara, Aegean, Mediterranean and Southeastern Anatolia Regions.

Aonidia mediterranea Ferris – Material examined: Antalya, *Cupressus sempervirens*, 9. 01. 93; female and male, Antalya, *Quercus* sp., 09. 01. 93; female and male. This species is new to Turkish fauna.

Aonidiella aurantii Maskell – Material examined: Antalya, *Rosa* sp., 13. 07. 92; female and male: Previous records from Turkey: Aegean, Mediterranean and Black Sea Regions.

A. citrina (Coquillet) – Material examined: Antalya, *Citrus aurantium*, 16. 01. 92; female and male, Antalya, *Citrus* sp., 15. 02. 92; female and male, Ornekköy (Antalya), *Ceratonia siliqua*, 18. 04. 93; D; female and male, Antalya, *Acacia cultriformis*, 10. 07. 93; female and male, Kemer, *Vitis vinifera*, 18. 07. 93; female and male, Kemer, *Laurus nobilis*, 18. 07. 93; female and male. Previous records from Turkey: Aegean, Mediterranean, Black Sea regions.

Aspidiotus nerii Bouché – Material examined: Antalya *Olea europaea*, 26. 06. 92; D; female and male, Antalya, *Nerium oleander*, 26. 06. 92; female and male, Antalya, *Sambucus nigra*, 13. 07. 92; female and male, Antalya, *Acacia cyanophylla*, 06. 09. 92, female and male, Antalya, *Melia azaderach*, 28. 09. 92; female and male, Antalya, *Catalpa bignonoides*, 17. 12. 92; female and male, Antalya, *C. bignonoides*, 09. 01. 93; female and male, Antalya, *Phillyrea* sp., 09. 01. 93; female and male, Antalya, *Smilax aspera*, 09. 01. 93; female and male, Ornekköy (Antalya), *Ceratonia siliqua*, 18. 06. 93; D; female, Antalya, *Jasminum officinalis*, 15. 05. 93; female and male, Gebiz (Serik), *O. europaea*, 01. 05. 93; D; female and male, Antalya, *Cercis siliquastrum*, 10. 07. 93; female and male, Kas, *Hedera helix*, 13. 11. 93; female and male. Previous records from Turkey: Aegean, Marmara, Mediterranean and Central Anatolia regions.

Carulaspis carueli (= **minima**) (Targioni-Tozzetti) – Material examined: Kas, *Cupressus sempervirens*, 13. 11. 93; male and female. Previous records from Turkey: Aegean and Mediterranean regions.

Cryptoparlatoreopsis longispina (Takahashi) – Material examined: Alanya, *Acacia cyanophylla*, 29. 09. 92; female and male, Antalya, *Acer negundo*, 10. 07. 93; D; female Antalya, *Acacia cultriformis*, 10. 07. 93; female and male, Antalya, *Celtis* sp., 30. 08. 93; female and male. Previous records from Turkey: Mediterranean region.

Dynaspidiotus britannicus (Newstead) – Material examined: Antalya, *Hedera helix*, 26. 06. 92; D; female and male. Previous records from Turkey: Aegean, Mediterranean and Marmara regions.

Epidiaspis leperii (Signoret) – Material examined: Elmalì, *Pyrus communis*, 09. 12. 92; D; female and male, Korkuteli, *P communis*, 10. 01. 93; female and male, Ibradì, *Pyrus elaeagrifoliae*, 15. 08. 93; female and male. First record from Antalya. Previous records from Turkey: Marmara, Central Anatolia, Aegean, Black Sea, Mediterranean and Eastern Anatolia regions.

Hemiberlesia lataniae (Signoret) – Material examined: Ornekköy (Antalya), *Ceratonia siliqua*, 18. 04. 93; D; female and male. First record from Antalya. Previous records from Turkey: Marmara and Aegean regions.

Lepidosaphes granati Balachowsky – Material examined: Antalya, *Ficus carica*, 10. 07. 93; D; female and male, *Acacia cultriformis*, 10. 07. 93; D; female and male, Manavgat, *Ulmus* sp., 01. 08. 93; D; female and male, Antalya, *Celtis* sp., 30. 08. 93; D; female and male. This species is new to Turkish fauna.

L. pistaciae Archangelskaya – Material examined: Antalya, Pistacia sp., 26. 06. 92; female and male. Previous records from Turkey: Aegean, Mediterranean, Southeastern Anatolia and Black Sea regions.

L. ulmi (Linnaeus) – Material examined: Antalya, Parthenosiscus sp., 17. 10. 92; female and male, Antalya, *Nerium oleander*, 10. 07. 93; D; female and male, Antalya, *Salix* sp., 10. 07. 93; D; female and male, Antalya, *Rubus* sp., 10. 07. 93; D; female and male, Kemer, *Vitis vinifera*, 18. 07. 93; female and male, Korkuteli, *Rosa* sp., 08. 08. 93; D; female and male, Korkuteli, *Cercis siliquastrum*, 08. 08. 93; D; female and male Korkuteli, *Parthenosiscus* sp., 08. 08. 93; female and male, Ibradì, *Styrax officinalis*, 15. 08. 93; female and male, Gebiz (Serik), *S. officinalis*, 15. 08. 93; female and male. Previous records from Turkey: Central Anatolia, Marmara, Mediterranean, Aegean, Black Sea and Eastern Anatolia regions.

Leucaspis riccae Targioni-Tozzetti – Material examined: Abdurrahmanlar (Serik), *Olea europaea*, 17. 03. 92; D; L, Antalya, *O. europaea*, 26. 06. 92; D; L. Previous records from Turkey: Aegean, Mediterranean, Southeastern Anatolia and Marmara regions.

Lineaspis striata (Newstead) – Material examined: Kemer, Cupressus sempervirens, 18. 07. 93; female and male. Previous records from Turkey: Mediterranean region.

Melanaspis inopinata (Leonardi) – Material examined: Antalya, Cydonia oblonga, 18. 04. 93; female and male, Elmalì, *Acer* sp., 09. 06. 94; D; male, Antalya, *Platanus orientalis*, 23. 07. 94; D; female. Previous records from Turkey: Aegean, Central Anatolia, Mediterranean and Marmara regions.

Neochionaspis asiatica (= N. prunorum) Borchsenius – Material examined: Elmalì, Pyrus communis, 04. 03. 92; D; female, Elmalì, *P. communis*, 09. 12. 92; D; female. This species is new to Turkish fauna.

Nilotaspis halli (Green) – Material examined: Antalya, *Prunus persicae*, 08. 08. 92; D; female, Çakìrlar (Antalya), *P. persicae*, 17. 09. 93; female and male. Previous records from Turkey: Central Anatolia, Aegean and Mediterranean regions.

Parlatoria oleae (Colvée) – Material examined: Aksu (Antalya), *Prunus domestica*, 11. 07. 92; female and male, Antalya, *Prunus armeniaca*, 02. 08. 92; D; female, Korkuteli, *Pyrus malus*, 18. 07. 92; female and male, Antalya, *P. domestica*, 11. 08. 92; female and male, Antalya, *Pyracantha* sp., 03. 10. 92; female and male, Alanya, *P. armeniaca*, 27. 09. 92; D; female, Antalya, *Catalpa bignonoides*, 03. 10. 92; D; female, Beldibi (Antalya), *Prunus amygdalus*, 27. 12. 92; female and male, Gebiz (Serik), *Olea europaea*, 01. 05. 93; D, female, Antalya, *P. domestica*, 15. 05. 93; female and male, Kemer, *P. persicae*, 18. 07. 93; female and male, Kemer, *P. malus*, 18. 07. 93; female and male, Korkuteli, *Acer rubrum*, 08. 08. 93; female and male, Akseki, *A. rubrum*, 16. 08. 93; D; female. Previous records from Turkey: Mediterranean, Aegean, Marmara, Central Anatolia and Black Sea regions.

Pseudaulacaspis pentagona (Targioni-Tozzetti) – Material examined: Antalya, *Morus nigra*, 02. 08. 92; female and male, Antalya, *M. alba*, 25. 01. 93; female, Antalya, *M. nigra*, 19. 12. 93; female, Alanya, *Prunus armeniaca*, 27. 05. 94; D; female. Previous records from Turkey: Marmara, Aegean, Black Sea and Mediterranean regions.

Quadraspidiotus marani Zahradnik – Material examined: Antalya, *Platanus orientalis*, 08. 08. 92; female, Manavgat, *Ulmus* sp., 01. 08. 93; female, Antalya, *P. orientalis*, 30. 08. 93; female. First record from Antalya. Previous records from Turkey: Central Anatolia, Black Sea, Mediterranean and Eastern Anatolia regions.

Q. perniciosus (Comstock) – Material examined: Korkuteli, *Pyrus malus*, 09. 07. 93; female and male. Previous records from Turkey: Marmara, Black Sea, Aegean, Eastern Anatolia, Central Anatolia and Mediterranean regions.

Salicicola archangelskyae Balachowsky – Material examined: Antalya, *Olea europaea*, 17. 07. 92; D; L. First record from Antalya. Previous records from Turkey: Black Sea and Mediterranean regions.

S. kermanensis (Lindinger) – Material examined: Antalya, *Salix* sp., 11. 07. 93; D; L, Antalya, *Populus* sp., 17. 09. 93; L. First record from Antalya. Previous records from Turkey: Mediterranean region.

S. pistaciae Balachowsky – Material examined: Kalkan (Kas), *Pistacia lentiscus*, 14. 11. 93; L. Previous records from Turkey: Mediterranean and Southeastern Anatolia regions.

Targionia nigra Signoret – Material examined: Manavgat, *Gleditschia* sp., 01. 08. 93; female and male. This species is new to Turkish fauna.

T. vitis (Signoret) – Material examined: Kemer, *Vitis vinifera*, 18. 07. 93; female and male, Manavgat, *V. vinifera*, 01. 08. 93; D; female. First record from Antalya. Previous records from Turkey: Central Anatolia, Marmara and Mediterranean regions.

Unaspis euonymi (Comstock) – Material examined: Antalya, *Euonymus japonicus*, 15. 02. 92; female and male. First record from Antalya. Previous records from Turkey: Central Anatolia, Marmara, Black Sea, Aegean and Mediterranean regions.

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Check-list of Coccoidea and Aleyrodoidea (Homoptera) of Afghanistan and Iran, with Additional Data to the Scale Insects of Fruit Trees in Iran

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A species list of scale insects and whiteflies of Afghanistan and Iran are given. In these two countries 202 species were recorded, 192 from Iran and only 37 from Afghanistan. Seven whitefly species are given for Iran and only one for Afghanistan. Fauna of both countries is still underexplored, comparing the given number with published 302 scale insect species for the whole Irano-Turanian subregion.

The scale insects detected on fruit tree samples in North-Iran were: *Aonidiella aurantii, Diaspidiotus prunorum, Nilotaspis halli, Parlatoria oleae, Parlatoria crypta, Pseudaulacaspis pentagona, Parthenolecanium corni* and *Sphaerolecanium prunastri*. The most important species were *P. oleae, P. corni* and *P. crypta* on apple, pear, plum and rose, *D. prunorum* on apricot and sweet cherry, *N. halli* on peach and *A. aurantii* on lemon. The most infested fruit plants were plum and peach, regarding both the number of scale insect species and rate of infestation. Rate of parasitization and predation is given scale insect species. A new whitefly species for Iran, *Dialeurodes citri*, was detected on the lemon.

The scale insect fauna of the Irano-Turanian Region only in some countries and in some families was studied in detail. According to the last analyses in this Region, 302 scale insect species were found (Kozár and Walter, 1985; Kozár and Drozdják, 1987). Lots of them are important pests. The intensive world trade cause a significant changes in the insect species composition of different countries. There were also several nomenclatural changes in species names. Therefore, it is important to have an up-to-date species list of the most important insect groups.

It was not until the latter half of the 19th century that the nations of the world became plant quarantine conscious. Earlier, very few plant pests had translocated from one part of the world to another, mainly because of restricted transportation possibilities. Until the steamship made its appearance, only hardy, stored product pests and others, such as San Jose scale (*Quadraspidiotus perniciosus*) and white peach scale (*Pseudaulacaspis pentagona*) that reached several parts of the world on imported nursery stock, managed to become established in new areas (Kárpáti, 1983).

The most important target of scale insects are the different fruit trees (Kozár 1990a). A survey and study of scale insects on fruit trees have been done by Konstantinova et al. (1981) in Middle Asia (Kazakhstan, Kirghiza, Tajikistan, Turkmania and Uzbekistan). Samples were also collected in several other regions of fruit growing areas

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like the island of Sachalin, Armenia, Azerbajdzhan and Georgia. They were analysed for parasitisation, as well as for predation according to a technique developed earlier by Kozár et al. (1982).

To the knowledge of scale insect fauna of Afghanistan several records were given by Archangelskaya (1937), Borchsenius (1966), Siddiqi (1966, 1981), Talhouk (1975), Danzig (1993) and Fowjhan and Kozár (1994). They mentioned the great importance of some species, as *Diaspidiotus prunorum*, *Suturaspis archangelskyae*, *Didesmococcus unifasciatus*, etc. in different parts of Asia. The scale insects in Turkey were investigated by Bodenheimer (1952–1953) in detail, and a survey have been done by Kozár et al. (1976) on fruit plants.

Survey and taxonomical classification of scale insects species in Iran, has been done by Akbarinoshad (1992), Albai (1984), Asadeh and Mossaddegh (1991), Babmorad (1993), Bodenheimer (1944), Dastgheibeheshti (1979), Davatchi and Tagizadeh (1954), Farahbakhach (1961, 1981), Habibian (1981, 1991), Habibian and Assadi (1989), Heidari (1993), Kaussari (1955), Kazemi (1985), Khalilmanesh (1969), Komeili (1983), Radjabi (1989), Sabzevari (1968), Seghatoleslami (1977), Takalloozadeh (1995), Zareh (1990), Zomorrodi (1969), etc. As they mentioned these studies did not include all parts of Iran, and all groups of scale insects.

Therefore, the aim of our work was to prepare a up-to-date list of the scale insect and whitefly species for Afghanistan and Iran, and also to study the species composition of the scale insects, the rate of parasitisation and predation especially related to different fruit cultures.

Materials and Methods

To collect the species names of scale insects and whiteflies recorded from these countries we used different monographs and papers published in different countries (Ben-Dov, 1993, 1994; Borchsenius, 1966; Danzig, 1993; Hoy, 1963; Mound and Halsey, 1978; Russell, 1941, etc.).

For studying the scale insects on fruit trees in N-Iran, twig, fruit and bark samples were collected from the 14th to 30th of September 1992, following the method of Kozár (1990b, c) and Kozár and Viktorin (1978). The samples were 10 cm long, taken from 2–3 years old branches. The 25 samples were collected on apple (3), on apricot (2), on pear (2), on peach (3), on plum (4), on sweet et cherry (4), on lemon (3), on rose (4), from 4 North regions of Iran (Fig. 1). Microscopic slides were prepared for determination of species, by standard method (Kosztarab and Kozár 1988).

The rate of parasitisation and predation was evaluated by examining emergence holes and also by examining the scale with preimaginal stages of parasitoids (dead pupae or their remainders, meconiums), or the holes made by predators (Kozár et al., 1982). Material was collected in the small scale backyard orchards.

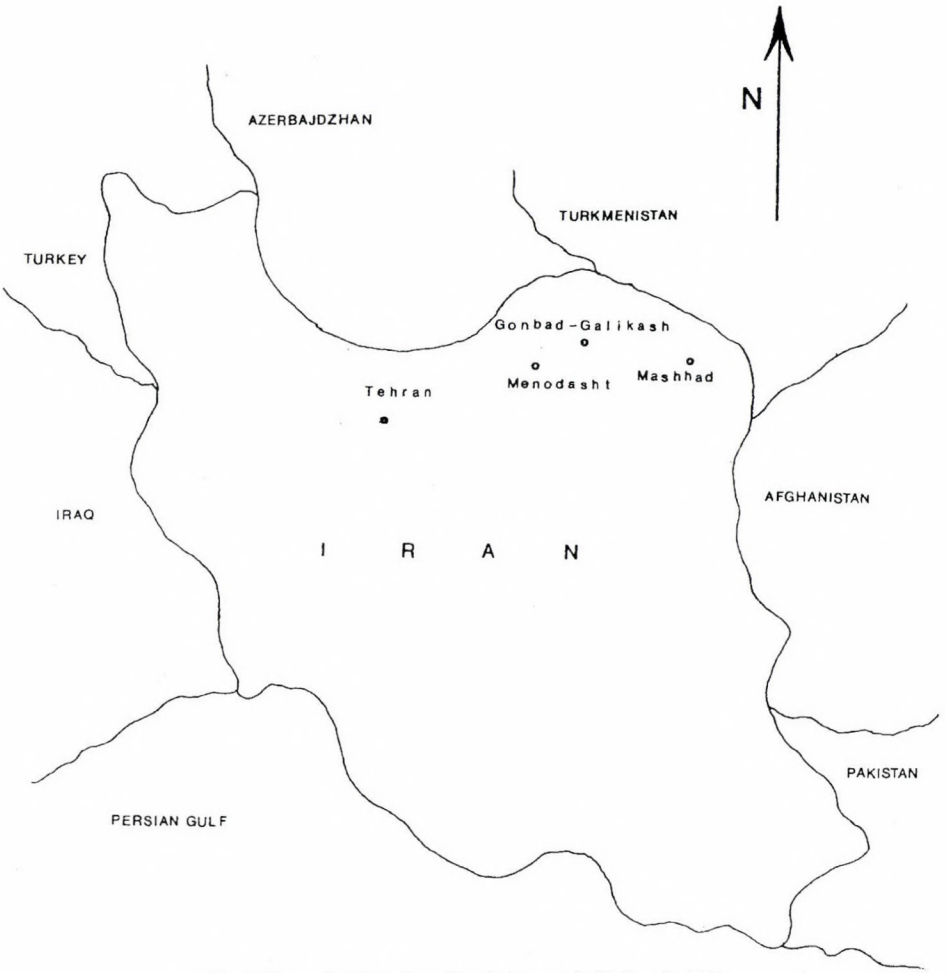

Fig.1. The collecting sites of scale insects in N.-Iran in 1992

Results and Discussion

1. Scale insect fauna of Afghanistan and Iran

Our literature survey resulted 202 scale insect species in 12 families for Afghanistan and Iran (Table 1). This number is very modest considering the 302 species recorded from the whole Irano-Turanian zoogeographic subregion. The scale insect fauna of Iran is relatively better known (192 scale insect species). The fauna of Afghanistan especially underexplored (only 37 recorded scale insect species). It is important to note, that until

Table 1

Species list of scale insects and white flies recorded from Afghanistan and Iran

Suborders, families, genera and species	Occurrence	
	Afghanistan	Iran
COCCOIDEA		
Ortheziidae		
Orthezia urticae (Linnaeus, 1758)	–	+
Margarodidae		
Icerya purchasi (Maskell, 1878)	–	+
Neomargarodes hamelii (Brandt, 1833)	–	+
Porphyrophora cynodontis (Archangelskaya, 1935)	–	+
Porphyrophora tritici (Bodenheimer, 1941)	–	+
Pseudaspidoproctus gramineus Jaschenko et Danzig 1992	+	–
Pseudococcidae		
Adelosoma phragmitidis (Borchsenius, 1948)	+	–
Antonia crawi Cockerell, 1900 (A. ?bambusae Khalid et Shafee, 1988)	–	+
Antonina graminis (Maskell, 1897)	–	+
Brevennia rehi (Lindinger, 1943)	–	+
Dysmicoccus boninsis (Kuwana, 1909)	–	+
Kiritshenkella stataria (Borchsensius, 1948)	+	–
Naiacoccus minor (Green, 1919)	+	+
Naiacoccus serpentinus Green, 1919	–	+
Nipaecoccus (Pseudococcus) filamentosus (Cockerell, 1893)	–	+
Nipaecoccus viridis (Newstead, 1894)	–	+
Peliococcus mesasiaticus (Borchsenius et Kozarzhevskaya, 1966)	+	–
Peliococcus sp. near talhouki Matile-Ferrero, 1984	–	+
Phenacoccus aceris (Signoret, 1875)	–	+
Phenacoccus sherbinovskyi (Bodenheimer, 1943)	+	+
Planococcus citri (Risso, 1813)	–	+
Planococcus ficus (Signoret, 1869) (vitis Nedzelskii, 1869)	–	+
Planococcus vovae (Nasonov, 1908)	–	+
Pseudococcus viburni Signoret, 1875 (affinis Maskell, 1894; obscurus Essig, 1909; maritimus Ehrhorn, 1900)	–	+
Pseudococcus comstocki (Kuwana, 1902)	–	+
Pseudococcus (Planococcus) cryptus (Hempel, 1918)	–	+
Pseudococcus longispinus (Targioni-Tozzetti, 1867) (adonidum Westwood, 1840)	–	+
Puto (Phenacoccus) euphorbiaefolius (Bodenheimer, 1943)	–	+
Spilococcus sp. near alhagii (Hall, 1926)	–	+
Spilococcus flavus (Borchsensius, 1949)	+	–
Trabutina crassispinosa (Borchsenius, 1936)	+	–
Vryburgia (Trionymus) amaryllidis (Bouché, 1837)	–	+
Eriococcidae		
Acanthococcus abaii Danzig, 1990	–	+
Gossyparia (Eriococcus) spuria (Modeer, 1778)	–	+
Pseudochermes fraxini (Kaltenbach, 1860)	–	+
Rhizococcus araucariae (Maskell, 1879)	–	+

Table 1 (cont.)

Suborders, families, genera and species	Occurrence	
	Afghanistan	Iran
Cryptococcidae		
Cryptococcus fagisuga Lindinger, 1936 (fagi, Baerensprung, 1849)	–	+
Coccidae		
Acanthopulvinaria orientalis (Nassonov, 1909)	+	+
Bodenheimera rachelae (Bodenheimer, 1924)	–	+
Ceroplastes floridensis Comstock, 1881	–	+
Ceroplastes rusci (Linneaus, 1758)	+	+
Ceroplastes sinensis Del Guerco, 1900	–	+
Chloropulvinaria (Pulvinaria) aurantii (Cockerell, 1896)	–	+
Chloropulvinaria (Pulvinaria) floccifera (Westwood, 1870)	–	+
Coccus hesperidum (Linneaus, 1758)	+	+
Coccus pseudomagnoliarum (Kuwana, 1914)	–	+
Didesmococcus unifasciatus (Archangelskaya, 1923)	+	+
Eriopeltis festucae (Fonscolombe, 1834)	–	+
Eulecanium ficiphilum (Borchsenius, 1955)	+	+
Eulecanium rugulosum (Archangelskaya, 1937)	–	+
Eulecanium (Lecanium) tiliae (Linnaeus, 1758) (coryli Linnaeus, 1758)	–	+
Palaeolacanium (Eulecanium, Lecanium) bituberculatum (Signoret, 1868)	+	–
Parthenolacanium (Eulecanium) corni (Bouché, 1844)	–	+
Parthenolacanium dickeri Komeili, 1977	–	+
Parthenolacanium (Eulecanium, Lecanium) persicae (Fabricius, 1776) (cecconii Leonardi, 1908)	+	+
Physokermes sp.	–	+
Pulvinaria (Filippia) gossypii (Bodenheimer, 1944)	–	+
Pulvinaria pistaciae (Bodenheimer, 1926)	+	+
Pulvinaria vitis (Linnaeus, 1758) (betulae Linnaeus, 1758)	–	+
Rhizopulvinaria (Pulvinaria) artemisiae (Signoret, 1873)	–	+
Rhizopulvinaria virgulata (Borchsenius, 1937)	–	+
Rhodococcus turanicus (Archangelskaya, 1923)	+	+
Saissetia coffeae (Walker, 1852) (hemisphaerica Targioni-Tozzetti, 1867)	–	+
Saissetia oleae (Olivier, 1791)	–	+
Sphaerolecanium (Eulecanium) prunastri (Fonscolombe, 1834)	–	+
Stozia ephedrae (Newstead, 1901)	–	+
Kermesiade		
Kermes (Kermococcus) quercus (Linnaeus, 1758)	–	+
Aclerdidae		
Aclerda sp.	–	+
Cerococcidae		
Cerococcus longipilosus Archangelskaya, 1930	–	+
Asterolecaniidae		
Asterodiapsis bella (Russell, 1941)	–	+
Asterodiapsis minus (Russell, 1941)	–	+
Palmaspis (Asterolecanium) phoenicis (Ramachandra Rao, 1921)	–	+
Russelaspis pustulans (Cockerell, 1892)	–	+

Table 1 (cont.)

Suborders, families, genera and species	Occurrence	
	Afghanistan	Iran
Phoenicococcidae		
Phoenicococcus marlatti (Cockerell, 1899)	–	+
Diaspididae		
?Aulacaspis phoenicis (Green, 1922)	–	+
Acanthomytilus farsianus (Balachowsky et Kaussari, 1955)	–	+
Acanthomytilus intermittens (Hall, 1924)	–	+
Acanthomytilus kurdicus (Bodenheimer, 1943)	–	+
Adiscodiaspis tamaricicola Malenotti, 1916	+	+
Aonidiella aurantii (Maskell, 1879)	–	+
Aonidiella citrina (Coquilett, 1891)	+	+
Aonidiella orientalis (Newstead, 1894)	–	+
Aspidaspis dentiloba (Kaussari et Balachowsky, 1953)	–	+
Aspidiotus destructor (Signoret, 1869)	–	+
Aspidiotus nerii (Bouché, 1833) (hederae Signoret, 1868)	–	+
Aulacaspis rosae (Bouché, 1834)	–	+
Balachowskyella salvadorae Kaussari, 1955	–	+
Carulaspis caruelii (Signoret, 1869) (minima Targioni-Tozzetti, 1868)	–	+
Carulaspis juniperi (Bouché, 1851)	–	+
Chionaspis etrusca Leonardi, 1908	+	+
Chionaspis lepineyi Balachowsky, 1928 (parastigma Balachowsky, 1954)	–	+
Chionaspis salicis (Linnaeus, 1758) (polypora Borchsenius, 1949)	–	+
Chortinaspis salavatiani (Balachowsky et Kaussari, 1951)	–	+
Chrysomphalus dictyospermi (Morgan, 1889)	–	+
Chrysomphalus pinnulifera (Maskell, 1891)	–	+
Contigaspis davatchi Kaussari, 1959	–	+
Contigaspis (Artemisaspis, Eremophallaspis) farsetiae (Hall, 1926)	–	+
Contigaspis zillae (Hall, 1923)	+	+
Contigaspis sarkissiani (Kaussari and Balachowsky, 1954)	–	+
Cryptoparlatoreopsis halli (Bodenheimer, 1929)	+	+
Cryptoparlatoreopsis tlaiae (Balachowsky, 1927)	–	+
Diaspidiotus (Quadraspidiotus) armenicus (Borchsenius, 1935)	–	+
Diaspidiotus (Quadraspidiotus) baiati (Kaussari, 1958)	–	+
Diaspidiotus caucasicus (Borchsenius, 1935)	–	+
Diaspidiotus cecconii (Leonardi, 1908)	–	+
Diaspidiotus elaeagni (Borchsenius, 1939)	–	+
Diaspidiotus farahbakhchi (Kaussari, 1955)	–	+
Diaspidiotus iranicus (Kaussari et Balachowsky, 1953)	–	+
Diaspidiotus kaussari (Balachowsky, 1950)	–	+
Diaspidiotus (Aspidaspis) lapperrinei (Balachowsky, 1929)	–	+
Diaspidiotus (Quadraspidiotus) ostreaeformis (Curtis, 1843)	–	+
Diaspidiotus (Aspidiotus, Quadraspidiotus) perniciosus (Comstock, 1881)	–	+
Diaspidiotus (Aspidiotus) prunorum (Laing, 1931)	+	+
Diaspidiotus(Aspidiotus, Quadraspidiotus) pyri (Lichtenstein, 1881)	–	+
Diaspidiotus (Quadraspidiotus) slavonicus (Green, 1934) (populi Bodenheimer, 1943)	+	+
Diaspidiotus spartii Kaussari, 1954	–	+

Table 1 (cont.)

Suborders, families, genera and species	Occurrence	
	Afghanistan	Iran
Diaspidiotus transcaspiensis (Marlatt, 1908)	–	+
Diaspidiotus turanicus (Borchsenius, 1935)	+	+
Diaspidiotus (Quadraspidiotus) zonatus (Frauenfeld, 1868)	–	+
Diaspis boisduvalii Signoret, 1869	–	+
Diaspis carmanicus (Davatchi et Balachowsky, 1956)	–	+
Diaspis syriaca (Lindinger, 1912)	–	+
Duplachionaspis graminella (Borchsenius, 1949)	+	+
Duplachionaspis stanotophri (Cooley, 1899)	–	+
Dynaspidiotus (Diaspidiotus) amygdalicola (Borchsenius, 1952)	–	+
Dynaspidiotus (Abgrallaspis, Archangelskaia, Ephedraspis) ephedrarum (Lindinger, 1912)	–	+
Dynaspidiotus medicus (Kaussari, 1956)	–	+
Dynaspidiotus spartii (Kaussari, 1954)	–	+
Epidiaspis gennadii (Leonardi, 1898)	–	+
Epidiaspis leperii (Signoret, 1869) (betulae)	–	+
Epidiaspis salicis (Bodenheimer, 1944)	–	+
Fiorinia (Aulacaspis) distinctissima (Newstead, 1896) (afchari)	–	+
Forggattiella (Odonaspis) penicillata (Green, 1896)	–	+
Gonaspidiotus (Abgrallaspis) kaussari (Balachowsky, 1959)	–	+
Hemiberlesia lataniae (Signoret, 1869)	–	+
Hemiberlesia rapax (Comstock, 1883) (camelliae Signoret, 1869)	–	+
Koroneaspis aegilopos (Koronéos, 1934)	–	+
Lepidosaphes ulmi (Linnaeus, 1758)	–	+
Lepidosaphes afghanensis (Borchsenius, 1962)	+	+
Lepidosaphes (Cornuaspis) beckii (Newman, 1869)	–	+
Lepidosaphes (Mytilaspis) belutchistanus (Balachowsky, 1954)	–	+
Lepidosaphes (Mytilaspis) conchyformis (Gmelin, 1789) (ficus Signoret, 1870; minima Newstead, 1897)	–	+
Lepidosaphes (Insulaspis) gloverii (Packard, 1869)	–	+
Lepidosaphes granati (Koronéos, 1934)	–	+
Lepidosaphes janaguai Balachowsky, 1954	–	+
Lepidosaphes (Insulaspis) juniperi (Lindinger, 1912)	–	+
Lepidosaphes malicola Borchsenius, 1947	–	+
Lepidosaphes pallida (Maskell, 1895) (maskelli Cockerell, 1897)	–	+
Lepidosaphes (Pistaciaspis) pistaciae (Archangelskaya, 1930)	–	+
Lepidosaphes (Pistaciaspis) pistacicola (Borchsenius, 1949)	–	+
Lepidosaphes rubri (Thiem, 1931)	+	–
Lepidosaphes turanica (Archangelskaya, 1937)	–	+
Leucaspsis loewi Colvée, 1882	–	+
Leucaspsis pusilla Löw, 1883	–	+
Leucaspsis riccae Targioni-Tozzetti, 1881	–	+
Lopholeucaspsis japonica (Cockerell, 1897)	+	+
Meleanaspis louristanus (Kaussari et Balachowsky, 1953)	–	+
Meleanaspis (Aonidiella) inopinata (Leonardi, 1913)	–	+
Mercetaspis (Nilostaspis) bicuspis (Hall, 1923)	–	+
Mercetaspis calligoni (Borchsenius, 1949)	–	+

Table 1 (cont.)

Suborders, families, genera and species	Occurrence	
	Afghanistan	Iran
Mercetaspis halli (Green, 1923)	+	+
Mercetaspis isis (Hall, 1923)	–	+
Mycetaspis personata (Comstock, 1883)	–	+
Neochionaspis (Childaspis, Chionaspis, Tecaspis, Voraspis) asiatica (Archangelskaya, 1930) (prunorum Borchsenius, 1939; adlei Balachowsky et Kaussari, 1955)	+	+
Odonaspis panici (Hall, 1926)	–	+
Odonaspis secreta (Cockerell, 1896)	–	+
Parlagena mckenziei (Balachowsky, 1950)	–	+
Parlagena remaudieri (Kaussari, 1955)	–	+
Parlangena buxi (Takahashi, 1936) (inops McKenzie, 1945)	–	+
Parlatoreopsis (Parlatoria) chinensis (Marlatt, 1908)	–	+
Parlatoreopsis longispina (Newstead, 1911)	–	+
Parlatoreopsis (Cryptoparlatoreopsis) meccae (Hall, 1927)	–	+
Parlatoria asiatica (Borchsenius, 1949)	–	+
Parlatoria blanchardi (Targioni-Tozzetti, 1892)	–	+
Parlatoria camelliae Comstock, 1883	–	+
Parlatoria crypta (McKenzie, 1943)	+	+
Parlatoria ephedrae (Lindinger, 1911)	–	+
Parlatoria oleae (Colvée, 1880)	+	+
Parlatoria pergandei Comstock, 1881	–	+
Parlatoria proteus (Curtis, 1843)	–	+
Parlatoria theae Cockerell, 1896	–	+
Parlatoria ziziphi (Lucas, 1853)	–	+
Pinnaspis aspidistrae (Signoret, 1869)	–	+
Pseudaulacaspis pentagona (Targioni-Tozzetti, 1886)	–	+
Pseudotargionia orientalis (Balachowsky et Kaussari, 1951)	–	+
Rhizaspidiotus canariensis (Lindinger, 1911)	–	+
Rhizaspidiotus secretus (Borchsenius, 1949)	+	+
Rungaspis macrolobis (Kaussari, 1958)	–	+
Rungaspis trabuti (Balachowsky, 1949)	–	+
Salicicola kermanensis (Lindinger, 1905)	+	+
Suturaspis (Leucaspis, Salicicola) archengelskyae (Lindinger, 1929)	+	+
Suturaspis (Salicicola) davatchi (Balachowsky et Kaussari, 1951)	+	+
Suturaspis (Salicicola) pistaciae (Lindinger, 1906)	–	+
Targionia anabasidis (Borchsenius, 1952)	–	+
Targionia arthrophyti (Archangelskaya, 1931)	–	+
Targionia balachowskyi (Kaussari, 1952)	–	+
Targionia haloxyloni (Hall, 1926)	–	+
Targionia (Pseudomelanaspis) minima (Borchsenius, 1952)	–	+
Targionia nigra (Signoret, 1868)	–	+
Targionia (Fisanotargionia) porifera (Borchsenius, 1949) (quadrilobata Balachowsky et Kaussari, 1953)	–	+
Targionia vitis (Signoret, 1876)	–	+
Unaspis euonymi (Comstock, 1881)	–	+

Table 1 (cont.)

Suborders, families, genera and species	Occurrence	
	Afghanistan	Iran
ALEYRODOIDEA		
Aleurocanthus woglumi Ashby, 1915	–	+
Aleyrodes crataegi Kiriukhin, 1947	–	+
Dialeurodes citri (Ashmead, 1885)	–	+
Bemisia tabaci (Gennadius, 1889)	–	+
Bulgarialeurodes cotesii (Maskell, 1895)	+	+
Trialeurodes ricini (Misra, 1924)	–	+
Trialeurodes vaporariorum (Westwood, 1856)	–	+

Remark: Several, often used in the literature, generic, and species name of scale insects are given in the table in parentheses.

now from Afghanistan even the most important pest species are missing from the list (Fowjhan and Kozár, 1994). There are 7 species of whiteflies known from Iran, but only one from Afghanistan.

2. Investigations on the occurrence of scale insects on fruit trees in Iran

Our investigation resulted 8 species of scale insects and 1 whitefly species for Iran, from which six species belong to the family of Diaspididae and two species to the family of Coccidae (Table 2). According to the literature (Kaussari, 1955; Davatchi and Taghizadeh, 1954; Seghatoleslami, 1977; Habibian, 1981) these species were known even earlier from some fruit species. These scale insects were reported earlier also by Konstantinova et al. (1981) and Kozár et al. (1982) from other countries of Central-Asia.

A. aurantii was found on apricot, lemon and rose in Mashhad province (NE-Iran) and in Minoodasht (N-Iran). *D. prunorum* was found on apricot, peach, sweet cherry and rose in Mashhad and Tehran. *N. halli* was detected on peach in Mashhad. *P. oleae* was found on apple, plum, pear and rose in Mashhad and Tehran. *P. crypta* was found on apple, plum and rose, and *P. pentagona* was found on pear in Tehran. *P. corni* was found on apricot, pear, plum, sweet cherry and rose in Mashhad and Tehran, and *S. prunastri* on peach in Masbhad.

Our investigations on the occurrence of scale insect species shows that the highest number of species was found on rose which could be a very dangerous source to the fruit trees, especially in small backyard orchards. Two-three species were occurred on pear, peach, plum, apricot, apple and sweet cherry. But only one species was found on lemon (Fig. 2). *P. corni* was found on most of the plants in question here, but *N. halli*, *P. pentagona* and *S. prunastri* were detected on one plant species, only.

Table 2

Number of infested host plant samples by scale insect species (N.-Iran, 1992)

Scale insect	Host plants								No. of infested samples
	Apple	Apricot	Lemon	Peach	Pear	Plum	Rose	Sweet cherry	
DIASPIDIDAE									
Aonidiella citrina (Coquillett, 1891)	–	1	3	–	–	–	1	–	5
Diaspidiotus prunorum (Laing, 1931)	–	1	–	1	–	–	1	3	6
Nilotaspis halli (Green, 1923)	–	–	–	3	–	–	–	–	3
Parlatoria oleae (Colvée, 1880)	3	–	–	–	2	2	2	–	9
Parlatoria crypta (McKenzie, 1943)	1	–	–	–	–	1	2	–	4
Pseudaulacaspis pentagona (Targioni-Tozzetti, 1886)	–	–	–	–	1	–	–	–	1
COCCIDAE									
Parthenolecanium corni (Bouché, 1844)	–	1	–	–	1	2	1	1	6
Sphaerolecanium prunastri (Fonscolombe, 1834)	–	–	–	1	–	–	–	–	1

The highest infestation rate occurred on plum and peach then it was followed by lemon, sweet cherry, pear, apricot, apple and rose. The density of *P. oleae* on apple, was the highest, while the *D. prunorum* was dominating on sweet cherry. The infestation by *A. aurantii* was approximately similar both on the branches and fruits of lemon. But, on other fruit trees mentioned here, the infestation on branches was higher, than that on trunks. Infestation by *Dialeurodes citri* (Ashmead, 1885) on lemon was also detected, this species was proved to be as new for the fauna of Iran.

3. The role of natural enemies (parasitoids, predators)

According to our investigations, the samples were infested by scale insect parasitoids between 0.0–100.0%, and between 0.0–100% by predators. The only one sample of *P. pentagona* was free both from parasitoids and predators, however, the rate of samples containing somewhat spures of parasitoids, were amounted in most cases as high as 50–60%, and even up to 100.0%, in the case of the only one sample with *S. prunastri*, which suffered damages both by parasitoids and by predators. The *N. halli* was heavily (100%) damaged by predators (Table 3).

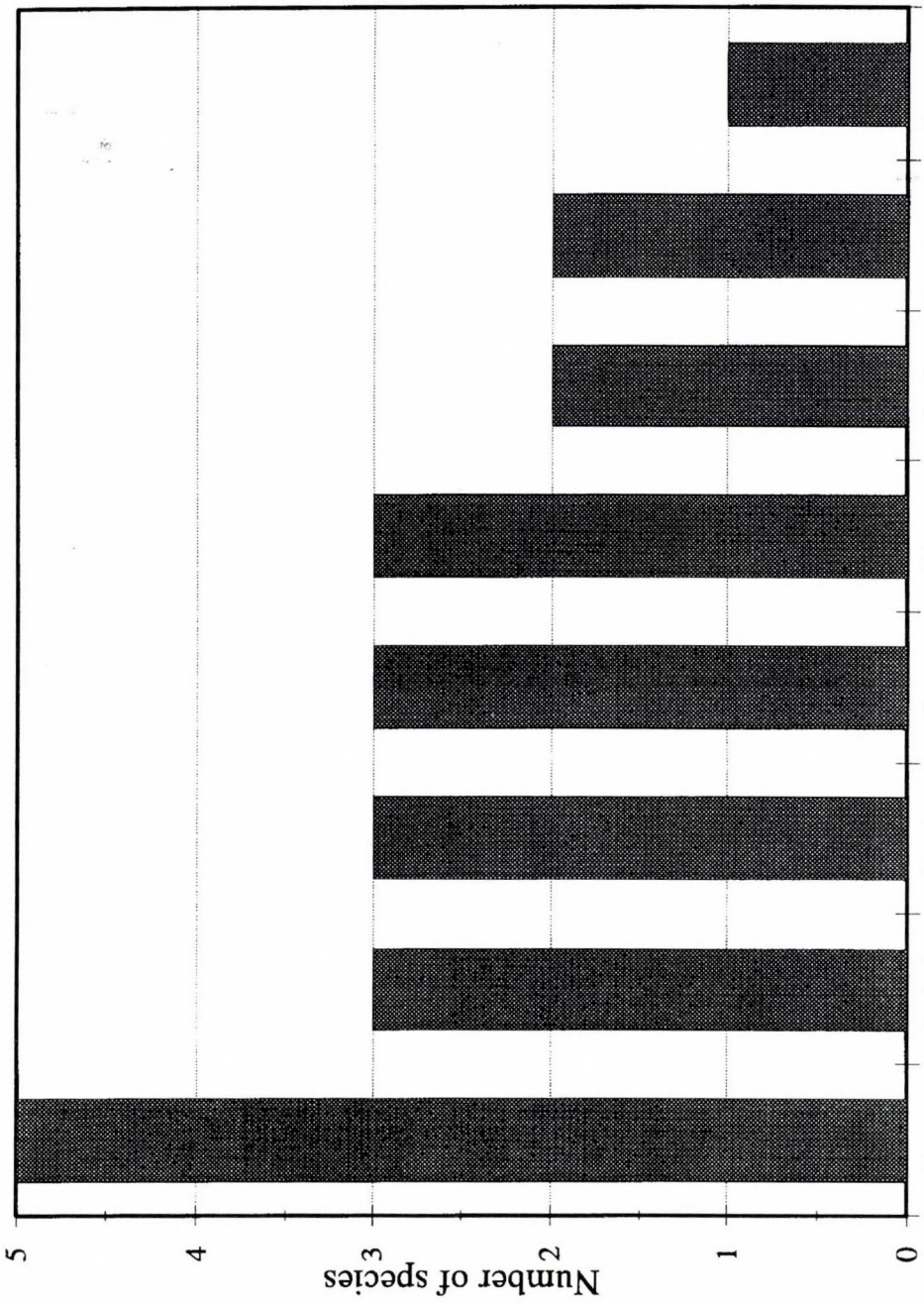

Fig. 2. Number of scale insect species detected on fruit trees in N.-Iran in 1992

Table 3

Infestation of scale insects caused by parasitoids and predators (N.-Iran, 1992)

Species	Infested samples in %		Infested scale insect specimens in %		Total biotic mortality in %
	by parasitoids	by predators	by parasitoids	by predators	
DIASPIDIDAE					
Aonidiella citrina	60.0	20.0	2.0	3.0	5.0
Diaspidiotus prunorum	66.6	66.6	5.0	3.3	8.3
Nilotaspis halli	66.6	100.0	3.3	6.6	9.9
Parlatoria oleae	55.5	22.2	3.9	1.7	5.6
Parlatoria crypta	50.0	50.0	3.75	3.75	7.5
Pseudaulacaspis pentagona	0.0	0.0	0.0	0.0	0.0
COCCIDAE					
Parthenolecanium corni	50.0	33.3	4.1	3.3	7.4
Sphaerolecanium prunastri	100.0	100.0	5.0	5.0	10.0

The highest parasitisation rate (% of infested scale insects) occurred on *S. prunastri,* and the highest predation rate occurred on *N. halli* and *S. prunastri*. The infestation of scale insects with parasitoids and predators were similar on *D. prunorum*, *P. crypta* and *S. prunastri*. The total mortality rates of scale insects caused by parasitoids and predators varied between 0.0 and 10.0% (Table 3).

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Physical Surface Features of the Grapevine Leaf Affecting the Abundance of *Zetzellia mali* (Ewing) (Acari: Stigmaeidae)

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The effect of the physical surface features of the grapevine leaf on the behaviour of *Zetzellia mali* has been examined under laboratory conditions. The specimens of *Z. mali* females show a characteristic thigmotaxis. They particularly favour the woolly, cobwebbed, or similarly structured leaf surfaces and abond the glabrous surfaces.

The occurrence and abundance of *Zetzellia mali* populations are definitely dissimilar on the leaves of the different fruit tree species. According to the data of a survey carried out in 40 orchards in the north-eastern part of Hungary, *Z. mali* were common in the apple orchards, but occurred very seldom and in low population densities in sour-cherry orchards (Molnárné and Kerényi-Nemestóthy, 1991). According to the data of our observations carried out in two neighbouring orchards for 3 years, the specimens of *Z. mali* occurred in the apple orchard and were not present among the acarophagous species in the sour-cherry orchard.

Owing to the cessation of the application of broad-spectrum insecticides and after the regular use of insect growth regulators e.g. diflubenzuron, fenoxycarb the *Z. mali* appears within a year and regulates the population dynamics of phytophagous mites populations in the apple orchards or in the vineyards. In accordance it is an important predator of the phytophagous mites at the introduction of integrated pest management (IPM) in certain orchards. Therefore it would be important to know the factors influencing the distribution and the survival of their populations.

According to Rasmy and El-Banhaway (1974), Rasmy (1977), Duso (1992) and Walter and O'Dowd (1992) the species composition of Phytoseiidae mites on the different varieties of grapevine is influenced by the physical surface features of the leaves. Accordingly we have supposed that the settlement and survival of *Z. mali* populations are determined by the same factors. The effect of the physical surface of the leaf on the behaviour of *Z. mali* has been examined by laboratory experiments.

Materials and Methods

The structure of the leaf surface of the grapevine varieties was characterized according to Németh (1966), Csepregi and Zilai (1976).

The surface of the grapevine leaf is

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– glabrous when it is entirely nude without any pubescence, excepting the setae in the vein axils. For preparing discs we used cutting from the leaf of the cultivar "Blaufrankisch",

– cobwebbed when it is covered with long, fine loosely interwined pubescence, much resembling the structure of spider's cobweb, and the surface is clearly visible. For preparing discs we used cuttings from the leaf of the cultivar "Riesling italien",

– woolly when it is densely covered by pubescence, yet the leaf surface is still visible. For preparing discs we used cuttings from the leaf discs of the cultivar "Steinschiller".

The behaviour of *Z. mali* females has been studied on 18 mm diameter leaf disc, cut from the middle of the leaf, so that the disc had both thick veins and vein axils. Discs were cut in two and halves of different cultivars were combined in the following manner:

(a)	glabrous	with	glabrous (cut from the same leaf)
(b)	glabrous	with	woolly
(c)	woolly	with	woolly (cut from the same leaf)
(d)	glabrous	with	cobwebbed
(e)	cobwebbed	with	cobwebbed (cut from the same leaf)
(f)	cobwebbed	with	woolly

Each combined disc was used in seven replicates.

The combined leaf discs were placed with their upper surface on a layer water soaked cotton-wool in Petri dishes. The two halves of the discs were in tight contact enabling unhindered locomotion of the mites on the whole combined disc.

Ten *Z. mali* females were placed on both halves of a disc. The effect of the leaf surface structure on their distribution was examined after two, five and eight days (combinations a, b, c). In an additional experiment it was assessed after one, two, and four days (combinations d, e, f).

The *Z. mali* females were transferred to the leaf discs from grapevine leaves collected at a vineyard near to Budapest. The experiment was carried out under laboratory condition at 25 °C, with a 16 h photoperiod (16 h of light/8 h of dark).

Statistical evolutions were carried out by applying the Student's t-test.

Results

The distribution of *Z. mali* specimens on the combined discs varied depending on the structure of the leaf surfaces. The final distribution of the specimens took place within 48 hours. Thereafter no significant change in their distribution was observed. The results of the experiments are summarized in Tables 1–2 and Figures 1–6.

In the case of combination of glabrous with woolly (b) (Fig. 1) a high ratio, 89% of the specimens settled down on the woolly half. Only a few specimens remained on the glabrous surface, finding shelter in the leaf vein axil. In the combination of cobwebbed with woolly (f) (Fig. 2) 71% of the specimens prefered the woolly half. In the combina-

Table 1

Distribution of *Zetzellia mali* adults on three (a, b, c) leafdiscs of different surface structures after 8 days

Mark of combination	Surface	Distribution of mites mean ± SD	%
a	glabrous	5.00±1.58	42.87
	glabrous	6.60±1.67	57.13
b	glabrous	2.14±2.48*	11.07
	woolly	17.14±2.73	88.93
c	woolly	9.57±1.81	53.96
	woolly	8.29±2.36	46.04

* significant at 0.1% probability level

tion of glabrous with cobwebbed (d) (Fig. 3) 65% of the specimens chose the cobwebbed half.

The specimens remained approximately in the same proportion (about fifty-fifty %) on both half discs in the cases of the following combination: glabrous with glabrous (a) (Fig. 4), woolly with woolly (c) (Fig. 5), cobwebbed with cobwebbed (e) (Fig. 6).

Table 2

Distribution of *Zetzellia mali* adults on three (d, e, f) leafdiscs of different surface structures after 4 days

Mark of combination	Surface	Distribution of mites mean ± SD	%
d	glabrous	6.86±1.21*	35.17
	cobwebbed	12.71±1.70	64.83
e	cobwebbed	9.14±2.04	49.13
	cobwebbed	9.43±1.81	50.87
f	cobwebbed	5.43±2.82*	29.23
	woolly	13.43±3.51	70.77

* significant at 0.1% probability level

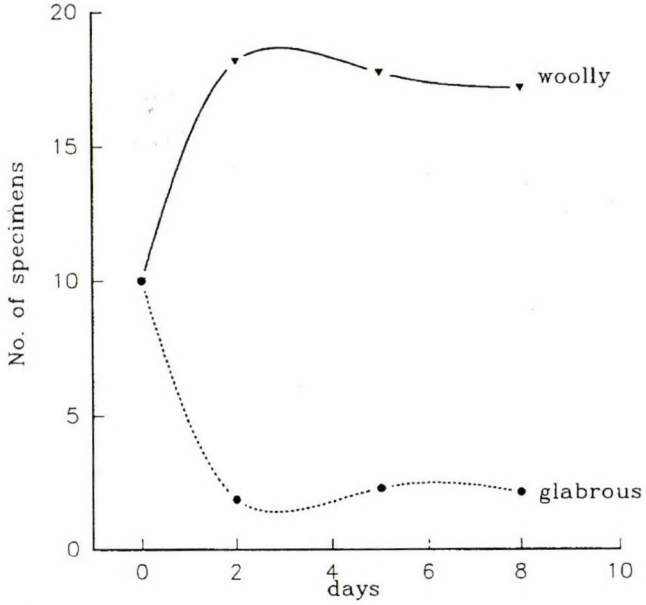

Fig. 1. Distribution of *Zetzellia mali* specimens on woolly with glabrous leaf surfaces

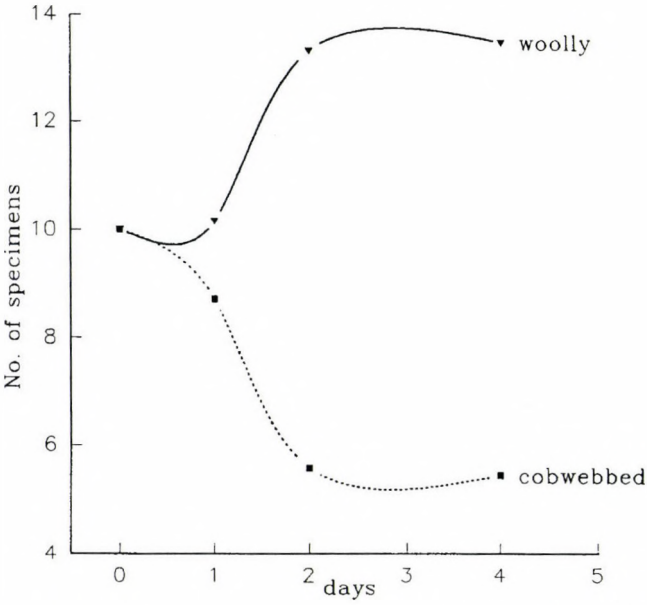

Fig. 2. Distribution of *Zetzellia mali* specimens on woolly with cobwebbed leaf surfaces

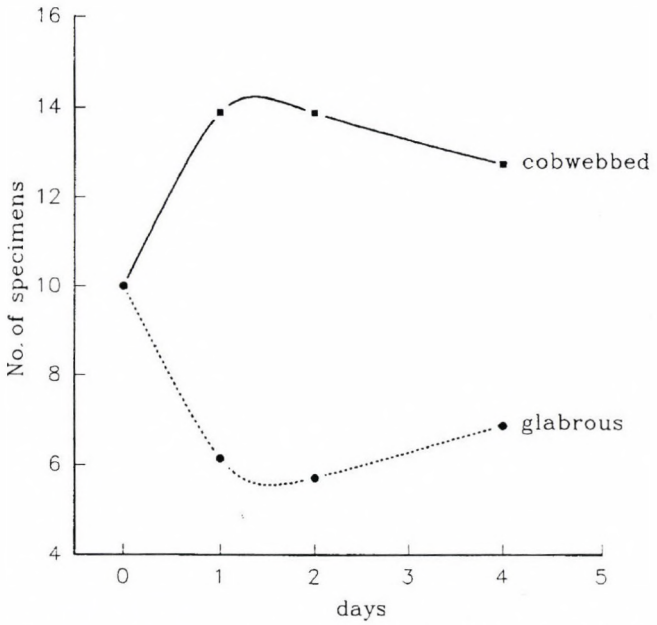

Fig. 3. Distribution of *Zetzellia mali* specimens on cobwebbed with glabrous leaf surfaces

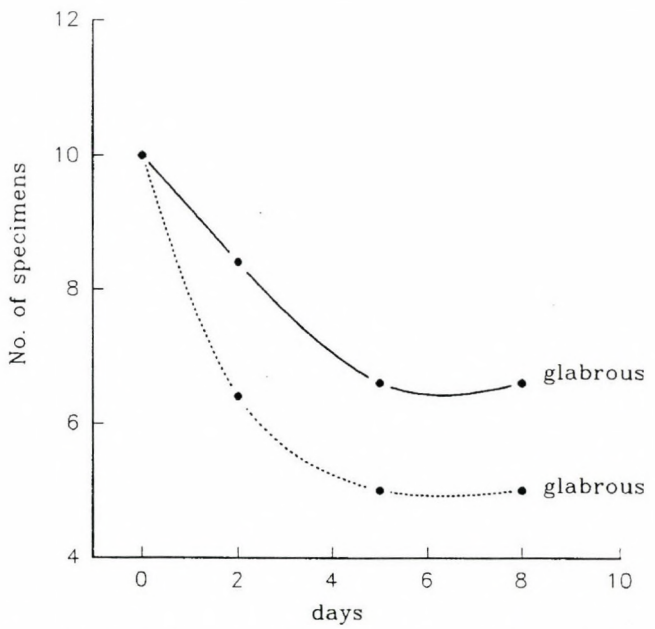

Fig. 4. Distribution of *Zetzellia mali* specimens on glabrous with glabrous leaf surfaces

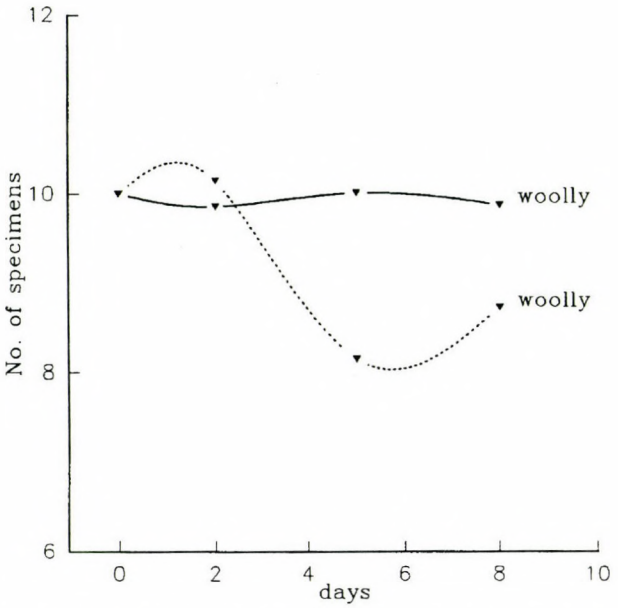

Fig. 5. Distribution of *Zetzellia mali* specimens on woolly with woolly leaf surfaces

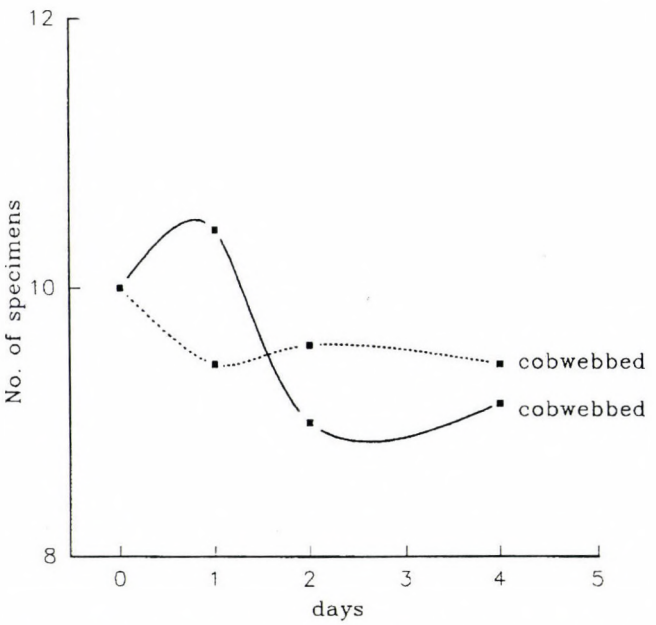

Fig. 6. Distribution of *Zetzellia mali* specimens on cobwebbed with cobwebbed leaf surfaces

While the number of the specimens decreased by about 10% in the combination of woolly with woolly (c) (Fig. 5), in the combination of glabrous with glabrous (a) (Fig. 4) the total number of mites was reduced, roughly by 35%. In the latter case *Z. mali* specimens tried to abandon the leaf and drowned in the water.

Discussion

The females of *Z. mali* show a characteristic thigmotaxis. The particularly favour the woolly, cobwebbed or similarly structured leaf surface and they abandon the glabrous surface. It is worthy to mention that even on the pubescent leaf surfaces (e.g. cobwebbed, woolly) they crowded at spots that were densely covered with pubescences. The preference of this predatory mite to the pubescent leaf surface explains its frequent occurrence on the apple and plum and its low density on sour-cherry trees. Most likely there are great differences in its population densities on the different grapevine varieties depending on the structure of the leaf surface.

Acknowledgement

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The Role of Spiders as Predators of Insect Pests with Particular Reference to Orchards: A Review

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Spiders are well known predators of insects (including insect pests) but about there role as biological control agents in agroecosystems (particularly in orchards) little is known. In the last decade new information (especially of the behaviour of spiders in different agroecosystems) has become available and this increased expectations about spiders as beneficial organisms. Spiders are a very heterogeneous group of animals with different hunting tactics and therefore, they play a different ecological role. At family level these tactics are rather similar and one species of the group can be used as representative example for ecological studies for the whole family. On the other hand properties and behaviour found in different species of one family can be seen as characteristic for the whole family. A comprehensive review of spiders as natural enemies of pest species of different crops is given offering information about the expected prey spectrum per family.

A qualitative evaluation of pest-spider relationships has been carried out for a whole range of agroecosystems and the results are transposed to spider groups inhabiting the orchard ecosystem.

The effect of pesticides on spiders, both from laboratory and field experiments is discussed and it has been shown to be the most important factor influencing spider occurrence and abundance in the field. Thus the pest management system (conventional or IPM or ecological) determines to a great extent the role of spiders can play in controlling pest organisms.

Only from a few species occurring in different ecosystems quantitative information of their searching and predatory potential is available resulting in functional response relationships to prey density. A list of methods for further quantitative evaluation of spider impact on pest in getting insight in predation processes is presented.

The last review of spiders as biological control agents was performed almost 10 years ago by Nyffeler and Benz, 1987. In the last decade an enormous amount of studies has been carried out especially on the behaviour of spiders in agroecosystems and nowadays we are gaining more sight on the role of this group of animals as predators of pests of economic importance. The aim of this review is to summarize the knowledge in this field, with particular reference to orchards and to indicate what can we expect from spiders as beneficial agents in IPM management systems.

Spiders as beneficial agents

Distribution and density

Spiders are one of the most common and ubiquitous group of animals. The species total has been estimated to be about 50 000 of which 30 000 species have been identified properly. They are found in all terrestrial ecosystems, including agroecosystems (Turnbull, 1973). All of them are predacious organisms and feed almost exclusively on

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insects (Riechert and Lockley, 1984). In agroecosystems, spiders are a part of the beneficial fauna. In the canopy of apple orchards the proportion spiders of the beneficial fauna varied between 40% and 95% (Olszak et al., 1994b; Specht and Dondale, 1960) and on the ground level varies between 10% and 13% (Loomans, 1978; Zhao et al., 1993).

However, concerning their usefulness there are some exceptions. In some tropical ecosystems (e.g. coffee, citrus and mango) the so-called colonial spiders tie green topical leaves of branches together and thus create a micro-climate to live in. When the leaves of these nests are dried-out, the colony moves to another green branch. Damage caused is such that sometimes insecticidal control is needed (Stejskal, 1976).

In pome and stone fruit orchards more than 10% of hunting spiders (together with other arthropods e.g. ladybirds and predatory bugs) contaminated with the propagules of cytospora cankers and wood-rotting pathogens. Laboratory investigations suggest that contaminated orchard arthropods play an important role in distribution of diseases (Helton et al., 1988a, b).

The species richness and spider density are very variable; respectively varying between 52 species on guar to 308 species on cotton (Young and Edwards, 1990) and the density from 1 individual per m^2 (Nyffeler et al., 1994a) to 1000 per m^2 (Nyffeler, 1982). The species richness of agroecosystems is generally smaller than of natural habitats (Nyffeler, 1984). Olszak et al. (1994a) found 51 species of spiders in apple orchards while, 72 species were found in its surroundings. Turnbull (1973) computed the average spiders density as 130.8 individuals per m^2 called "overall mean value" (based on 34 literature data from world-wide). Since this work several authors found even 100 times lower population density in agroecosystems (Nyffeler et al., 1994a). Investigations on the population density of foliage dwelling sac spiders (Clubionidae) by mark-recapture method in an IPM apple orchard in the Netherlands showed 6 individuals per m^2 (Bogya, 1995a).

Foraging behaviour

According to traditional foraging theory, spiders were considered to be predators of living, moving prey (Savory, 1928; Gertsch, 1949; Turnbull, 1973). More recent studies expanded this view since evidence was found that spiders utilize a much broader range of foraging strategies, including feeding on dead animals (Knost and Rovner, 1975; Williams et al., 1986), artificial diets (Peck and Whitcomb, 1968; Zhao and Zhao, 1983), plant components (Vité, 1953; Smith and Mommsen, 1984) and arthropod eggs (Whitcomb and Bell, 1964; Whitcomb, 1967; Nuessly, 1986). In most cases, the spiders were observed feeding on the eggs of Lepidoptera (families Noctuidae, Tortricidae, Lymantriidae, Pyralidae and Sphingidae), and to a lesser extent, on coleopteran eggs (family Curculionidae) (reviewed by Nyffeler et al., 1990).

Foraging strategies of spiders can be divided into two groups: web-building and wandering. Spiders are generalist predators (Riechert and Harp, 1987), this means they eat a wide variety of animals, and their sedentary foraging mode suggests that selection for habitat, not prey, should be the rule (Uetz, 1992). However, prey capture specialisation can be observed in bolas spiders, *Mastophora* spp. (Araneidae) which mimic the

odour of sex pheromones emitted by female moths (noctuids) and in this way prey only on male moths (Stowe et al., 1987).

According to Nentwig (1986) a part of the hunting spiders are more or less specialised to specific types of prey. He mentioned 4 types such as ants; termites; spiders and hymenopterans.

The most important factor determining success of prey capture is the size of the prey. If prey size is between 50–80% of the spider size this will result in the highest prey capture. However some spiders with strong poison can catch bigger prey (e.g. flower inhabiting crab spiders or social hunting spiders) can catch 3-times bigger prey than themselves (Nentwig and Wissel, 1986).

The "ideal" predator described by (Riechert and Lockley, 1984) is highly specialised to its prey. Spiders fit poor into that model, but several other investigations and computer simulations indicate that generalist predators, especially spiders just like specialists can play an important role in agroecosystems (Whitcomb, 1987; Riechert, 1974, 1990; Provencher and Riechert, 1994). However, pest species form only a fraction of the diet of spiders (Nyffeler, 1983; Nyffeler et al., 1987a; 1987b; Nyffeler and Benz, 1979; 1988a) (varying between 0–100%). They can survive periods of food shortage by decreasing their metabolic rate or by switching to alternative prey. Wasteful killing (like a fox in a chicken-house) is also an important property of valuable predators.

The spider web is a very efficient trap for insects. Web-builders normally catch as much prey as in *ad libitum* conditions in laboratory, but hunting spiders ingest much less in the field than in laboratory. This is very important if we want to estimate the predatory potential of these spiders in the field (Nyffeler and Breene, 1990).

Eggs, immatures and adult spiders can be found at the same time throughout the season (Schaefer, see in Nentwig, 1987), but most of them are mature in summer. A part of the spiders (so-called winter-active spiders) have no diapause, they are able to move, feed and even reproduce during winter (Schaefer, 1977 Aitchison, see in Nentwig, 1987). Down to –5 °C they can feed mainly on springtails and on dipterans. The winter active wolf and crab spiders prey on aphids, leafhoppers, bugs, orthopterans, lepidopterans and coleopterans (Aitchison, 1984). Investigations on winter-active clubionids indicate that the consumption of pest species in winter months is too low to be of economic importance, but in early spring when all other predators and parasitoids are still in diapause preying on larvae of leafrollers may be of importance (Bogya, 1995a, b).

Review of Spiders Occurring in Orchards and Other Ecosystems with Particular Reference to Their Role as Natural Enemies of Pests

The first author that did write about the role of spiders in controlling pest species was Bilsing (1920) who presented a list of observed victims of spiders (including orchards pest). Klein (1936) observed the first time that spiders prey on fruit tree red spi-

der mites in Palestine; Picket et al. (1946) mentioned the first time that spiders may be important predators in Canadian orchards; Chant (1956) presented a list of spiders preying on fruit tree red spider mites and bryobia mites in England and Le Roux (1960) concluded that spiders are the most important predators on apple in Canada.

Turnbull, 1973 summarized the ecology of true spiders (Araneomorphae), but he ignored their role in agroecosystems. Since his review, considerable progress has been made in the field, and we are better able to evaluate the predatory potential of spiders at this time. The first authors who summarized the role of spiders as biological control agents were Riechert and Lockley (1984). They reviewed 174 articles and concluded that one spider species alone was unable to control pest species, but the whole spider community could do it. In contradiction to them Spiller (1986) stated that one spider species alone can be used better for biological control than several species together because of the competition between the species. They also concluded that "usage" of spiders in pest control is most promising in orchards because this agroecosystem is the least disrupted. They recommended spraying at noon to save the spider populations because most of them are inactive during that time. Nyffeler and Benz (1987) also summarized the role of spiders in natural pest control (reviewed 300 articles) and concluded that the foliage dwelling spiders play a less important role than ground dwelling spiders because of their lower densities.

From literature it can be concluded that the following families of spiders occur in European apple orchards: Agelenidae, Anyphaenidae, Araneidae, Clubionidae, Dictynidae, Linyphiidae, Lycosidae, Oxyopidae, Philodromidae, Salticidae, Tetragnathidae, Theridiidae, Thomisidae.

They were recorded by Chant, 1956 (England); Loomans, 1978 and Langeslag, 1978 (Netherlands); Klein, 1988 (Germany) and Olszak et al., 1994b (Poland). Chant (1956) found 8 families of spiders in sprayed and 9 families of spiders in unsprayed orchards. The dominant families are Theridiidae and Linyphiidae. Loomans, (1978) and Langeslag, (1978) recorded 12 families of spiders from an experimental orchard. The dominant species in the canopy are *Theridion varians* Hahn (Theridiidae), *Araniella opistographa* Kulczynski (Araneidae), *Philodromus aureolus* (Philodromidae) and *Arelosimus vittatus*. In the ground level *Oedothorax fuscus, Centromerita bicolor, Centromerus sylvaticus, Lepthyphantes tenuis* and *Diplostyla concolor* (Linyphiidae) were dominant. Klein (1988) described 10 families. The dominant spiders are *Araniella opistographa* Kulczynski (Araneidae) and *Philodromus cespitum* Walckenaer (Philodromidae). Olszak (1994b) reported 11 families. The dominant species are *Araniella cucurbitina* Clerck (Araneidae) and *Theridion varians* Hahn (Theridiidae).

In the following sections these families are described shortly with their dominant characteristics.

A comparison with species of the same family occurring on crops of economic importance outside of Europe is included. Especially their role as natural enemies of pests and their predatory behaviour is emphasised.

Families of spiders inhabiting in European orchards

Agelenidae (Funnel-web spiders)

General description There are 29 species in 9 genera in Central Europe (Heimer and Nentwig, 1991). The majority of species have the posterior spinners clearly longer than the anteriors. Males resemble females in general appearance but have a slimmer abdomen and, in most cases, relatively longer legs. These spiders spin a tubular retreat from which extends either a small collar of silk, or a small to large sheet, which may be slightly funnel-shaped. Courtship varies between genera. It may involve tapping on the female's web, seizing her fairly quickly and mating on the sheet; other species may mate away from the retreat/web and there may be considerable stroking, with the female entering a torpid state. The egg sac is made within the retreat, and males often remain with their mates, eventually dying of old age. The size of these spiders varies between 3–20 mm (Roberts, 1995).

Hunting behaviour The spiders (diurnal hunters) run on the upper surface of the sheet to catch prey which has landed on it. Sometimes there is a superstructure of threads, and insects hitting this fall down on to the sheet. Prey is then dragged back into the retreat for consumption. (Intermediate behaviour between web-builders and hunting spiders.) According to Nyffeler et al. (1994b) the prey of these spiders are lepidopterans, bees, orthopterans and beetles.

Habitat and distribution They occur in built up areas; on bushes and plants or in low base vegetation; in, on or under grass; amongst stones and stone walls. Generally widespread and common in the region.

Importance in crop protection

– *Species occurring in orchards* Members of this family are reported from The Netherlands by Loomans (1978); from Poland by Olszak et al. (1994b); from Canada by Dondale (1956); from Japan by Hukusima (1961) and Okusima (1973); from USA by McÇaffrey and Horsburgh (1980) in apple orchards. This family represented by very few species with a small number of individuals in this habitat. It can thus be assumed that their presence on apple trees was accidental and was probably induced by wind movement from their habitats (Olszak et al., 1994b McCaffrey and Horsburgh, 1980).

– *Species occurring in other agroecosystems* Brignoli (1983) mentioned that egg sacs of *Agelena opulenta* L. were placed to mulberry trees infested by fall webworm (*Hyphantria cunea* Drury; Lep.: Arctiidae) in Japan and the spiders did manage to decrease the number of caterpillars under the economic threshold. *Agelenopsis emertoni* Chamberlin and Ivie and *A. pennsylvanica* C. L. Koch are commonly found in cotton fields in USA (Whitecomb et al., 1963). Members of this family preying on pest species are shown in Table 1.

– *Conclusion* This family of spiders is not abundant in orchards and their hunting behaviour suggest that they probably are of minor importance in controlling pest species.

Table

Agelenidae as predators of pests

Spiders	Common name	Scientific name
Agelena limbata Thorel	black pine bast scale	Matsucoccus thunbergianae Miller & Park
Agelena limbata Thorel	fall webworm	Hyphantria cunea Drury
Agelena limbata Thorel	fall webworm	Hyphantria cunea Drury
Agelena opulenta L.	fall webworm	Hyphantria cunea Drury
indet Ageleniadae	planthoppers	Magicicada spp.

Anyphaenidae (anyphaenids)

General description A single member of the family Anyphaenidae, *Anyphaena accentuata* Walckenaer occurs in Central Europe (Heimer and Nentwig, 1991). The spider is distinctively marked and the tracheal spiracles are easily visible halfway between the spinners and the epigastric fold. The species lives and hunts on the leaves of trees and bushes. Males vibrate the abdomen on the surface of a leaf in order to attract the female's attention prior to mating. The female attaches the egg sac to a curled leaf and remains on guard with it in a flimsy silk cell. By this time, the abdomen of the female has became rather slim and the colour darkened to an almost uniform grey-brown. The size of this species varies between 4.5–7.5 mm (Roberts, 1995).

Hunting behaviour Similar to clubionids see there.

Habitat and distribution This species occurs on the leaves of trees and bushes. Generally locally abundant.

Importance in crop protection

– *Species occurring in orchards* This species is reported from Germany by Klein (1988); from The Netherlands by Loomans (1978); and from Poland by Koslinka (1967) and Olszak et al. (1994b). Other members of this family are mentioned from Canada by Dondale (1956) and Specht and Dondale (1960); from USA by McCaffrey and Horsburgh (1980) in apple orchards and from USA by Mansour et al. (1982); from Mexico by Rodriguez Almaraz and Contreras Fernandez (1993) in citrus orchards. *Anyphaena pectorosa* L. Koch inhabiting on apple and prey on apple pests (aphids, planthoppers) (McCaffrey and Horsburgh, 1978) in USA. Other anyphaenids (*Aysha gracilis* Hentz) inhabiting citrus (Mansour et al., 1982) and pecan and prey on the blackmargined aphid (*Monellia caryella* Fitch.; Hom.: Aphididae) (Bumroongsook et al., 1992), the average daily consumption was 7.4 aphids in the field.

– *Species occurring in other agroecosystems* This species (*A. gracilis* Hentz) also inhabiting on cotton and preys on many cotton pests (see Table 2).

1

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Margarodidae	pine forest	Kim, 1993	Korea	
Lep.: Arctiidae	–	Kunimi, 1983	Japan	
Lep.: Arctiidae	–	Kayashima, 1967	Japan	
Lep.: Arctiidae	mullberry	Brignoli, 1983	Japan	
Hom.: Cicadelliadae	–	Smith et al., 1987	USA	

– *Conclusion* Only one and locally abundant species occurs in the region, but its hunting behaviour suggests that (where it is occurs) at least it contributes to reduction of pest species.

Araneidae (Araneids)

General description This family is represented by 46 species in Central Europe in 17 genera (Heimer and Nentwig, 1991). The small height of the clypeus, the lateral condyle on the chelicerae and the auxiliary foot claws are characteristics of this family. The males resemble females in patterns and markings, but have a much smaller abdomen. The carapace is sometimes rather narrow at the front and the front legs may be furnished with stout spines.

The species spin orb webs with a closed hub, the hole having been filled with a lattice of silk threads. A strong signal thread leads from the hub to a retreat amongst nearby vegetation or other structures, the spider waiting there and rushing down into the web in response to vibrations from ensnared prey.

Araneids generally have a number of strong teeth on the chelicerae and prey is chewed and mashed with digestive juices. The result is an unrecognisable pellet of insect remains as opposed to the near-perfect, sucked-out husks left by theridiids and thomisids. Size small to medium large 3–15 mm (Roberts, 1995).

Hunting behaviour The hunting strategy of these spiders is ambushing for prey in the web. They generally prey on a wide variety of insects such as orthopterans, dipterans, hemipterans, and are able to feed on hard cuticled (e.g. beetles) and chemically protected (bees) insects. The lepidopterans do generally avoid the orb-webs (Nyffeler et al., 1994b).

Habitat and distribution They occur in built up areas; in and up trees; in forests; in webs between trees; on bushes and plants or in low base vegetation; in meadows; in, on or under grass. Generally common and widespread throughout Europe.

Table

Anyphaenidae as predators of pests

Spiders	Common name	Scientific name
Anyphaena pacifica Banks	Douglas-fir tussock moth	Orygia pseudotsugata McDunnough
Anyphaena pacifica Banks	Douglas-fir tussock moth	Orygia pseudotsugata McDunnough
Anyphaena pacifica Banks	white fir sawfly	Neodiprion abietis Harris
Aysha gracilis Hentz	cotton aphid	Aphis gossypii Glov.
Aysha gracilis Hentz	cotton fleahopper	Pseudatomoscellis seriatus Reuter
Aysha gracilis Hentz	cotton fleahopper	Pseudatomoscellis seriatus Reuter
Aysha gracilis Hentz	cotton leafworm	Alabama argillacea Hubner
Aysha gracilis Hentz	tobacco budworm	Heliothis virescens F.
Aysha gracilis Hentz	fall webworm	Hyphantria cunea Drury
Aysha velox Becker	sugarcane rootstalk borer	Diaprepes abbreviatus L.

Importance in crop protection

– *Species occurring in orchards* Members of this family are recorded from England by Chant (1956); from the Netherlands by Loomans (1978); from Poland by Koslinka (1967) and Olszak et al. (1994b); from Australia by Dondale (1966); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley (1974); Dondale et al. (1979) and Bostanian et al. (1984); from Japan by Hukusima (1961) and Okuma (1973); from USA by McCaffrey and Horsburgh (1980) in apple orchards and from Japan by Nakao and Okuma (1958); from USA by Mansour et al. (1982) and from Mexico by Rodriguez Almaraz and Contreras Fernandez (1993) in citrus orchards. *Araniella cucurbitina* Clerck is widespread in this ecosystem in USSR (Anchipanova and Shternbergs, 1987; Tarabaev and Sheykin, 1990) in France (Naton, 1974) in England (Chant, 1956) and in Poland (Olszak et al., 1994b). Together with theridiids and micryphantids the main food source of these spiders in this habitat are the apple sucker (*Psylla mali* Schmidberger; Hom.: Psyllidae), the green apple aphid (*Aphis pomi* Deg.; Hom.: Aphididae) (Anchipanova and Shternbergs, 1987; Tretyakov, 1984), apple blossom weevil (*Anthonomus pomorum* L.; Col.: Curculionidae) (Tretyakov, 1984), fruit tree red spider mite (*Panonychus ulmi* Koch) and the bryobia mite (*Bryobia praetiosa* Koch) (Chant, 1956). (The biology of this species is described by Bakken (1978) in Norway.) An other closely related species *Araniella opistographa* Kulczynski was found as one of the most common species on apple in Germany (Klein, 1988; Sengonca et al., 1986), and investigated the prey spectrum of this species in the field by Sengonca and Klein (1988) (Tortricidae, Geometridae, Aphididae, Psyllidae, Curculionidae). *Araniella displicata* Hentz is one of the dominant foliage dwelling species on apple in Canada (Dondale, 1958; Dondale et al., 1979) and reported as a predator of the mites *Tetranychus urticae* Koch and *Panonychus ulmi* Koch (Parent, 1967). *Araneus transmarinus* Keyserling was

2

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Lep.: Limantriidae	pine forest	Swezey et al., 1991	USA	
Lep.: Limantriidae	pine forest	Mason & Torgersen, 1983	USA	
Hym.: Diprionidae	pine forest	Swezey et al., 1991	USA	
Hom.: Aphididae	cotton	Whitecomb et al., 1963	USA	
Hem.: Miridae	cotton	Kagan, 1943	USA	
Hem.: Miridae	woolly cotton	Breene et al., 1988	USA	
Lep.: Noctuidae	cotton	Gravena & Sterling, 1983	USA	
Lep.: Noctuidae	cotton	McDaniel et al., 1981	USA	
Lep.: Arctiidae	–	Warren et al., 1967	USA	
Col.: Curculionidae	citrus	Richman et al., 1983	USA	

mentioned as natural enemy of the light brown apple moth (*Epiphyas postwittana* Walker; Lep.: Tortricidae) in Australia Dondale (1966; Danthanarayana, 1983) and another 8 araneids preying on this pest was reported by Dondale (1966). *Neoscona* sp. was the most frequently observed spider that preys on citrus psylla (*Trioza erytreae* Del Guercio; Hom.: Triozidae) in South Africa (Berg et al., 1987; Berg et al., 1992). *Neoscona arabesca* Walckenaer preys on pecan aphids (*Monellia caryella*) in USA (Liao et al., 1984; Bumroongsook et al., 1992). The aphid consumption was an average of 7.72 per day. *Argiope trifasciata* Forskäl occurs in citrus orchards (Muma, 1975) and takes adults of citrus weevil (*Diaprepes abbreviatus* L.) as prey (Mansour et al., 1982).

– *Species occurring in other agroecosystems* Members of this family occurring in many agroecosystems e.g. cotton (Nyffeler et al., 1989), soybean (Culin and Yeargan, 1982) and rice (Kamal et al., 1992) and prey on many insect pests (see Table 3). The prey spectrum of *Argiope aurantia* Lucas is well investigated in cotton in USA by (Kagan, 1943; Nyffeler et al., 1987b). They obtained as a result that approx. 50% of the diet belonged to pest species (30% aphids and 17.9% orthopterans).

– *Conclusion* The smaller species of this family requlary spin their webs in higher vegetation and their prey are smaller (<4 mm) mainly dipterans and homopterans (Pasguet, 1984). Some of them are common and widely distributed in orchards (Klein, 1988; Olszak et al., 1994b). Their early appearance in spring and long activity period to late autumn makes these spiders probably an important group of natural enemies in orchards (Klein, 1988; Wyss, 1995). The large members of this family (e.g. *Argiope bruennichi* Scop.; *Araneus quadratus* Clerck; *A. diadematus* Clerck) spin strong orb-webs in lower vegetation (0–50) (Brown, 1981; Pasguet, 1984) and prey mainly on large insects (e.g. orthopterans), but aphids (Nyffeler and Benz, 1989) (especially the cereal aphid,

Table

Araneidae as predators of pests

Spiders	Common name	Scientific name
Araneus bituberculatus Walckenaer	fall webworm	Hyphantria cunea Dury
Araneus cucurbitinus Clerck	buckthorn – potato aphid	Aphis nasturtii Kalt.
Araneus diadematus Clerck	cherry blackfly	Myzus cerasi F.
Araneus diadematus Clerck		Myzus lythri Schr.
Araneus diadematus Clerck	Bird cherry – Oat aphid	Rhopalosiphum padi L.
Araneus diadematus Clerck	Bird cherry – Oat aphid	Rhopalosiphum padi L.
Araneus diadematus Clerck	thistle aphid	Brachycaudus cardui L.
Araneus diadematus Clerck	mealy cabbige aphid	Brevicoryne brassicae L.
Araneus diadematus Clerck	black bean aphid	Aphis fabae Scop.
Araneus diadematus Clerck	ceanothus leafminer	Tischeria immaculata Braun
Araneus diadematus Clerck	pine moth	Dendrolimus pini L.
Araneus quadratus Clerck	orthopterans	
Araneus quadratus Clerck	orthopterans	
Araneus quadratus Clerck	Bird cherry – Oat aphid	Rhopalosiphum padi L.
Araneus quadratus Clerck	black bean aphid	Aphis fabae Scop.
Araneus quadratus Clerck	green peach aphid	Myzus persicae Sulz.
Araneus sclopetarius Clerck	red cotton bug	Dysdercus cingulatus F.
Araneus sclopetarius Clerck	spotted bollworm	Earias vitella F.
Araneus sclopetarius Clerck	spiny bollworm	Earias insulana Boisd.
Araneus sinhagadensis Tikader	mango jassid	Idioscopus clypealis Lethierry
Araneus sinhagadensis Tikader	spotted stalk borer	Chilo partellus Swinhoe
Araneus sinhagadensis Tikader	spotted stalk borer	Chilo partellus Swinhoe
Araneus sinhagadensis Tikader	mango shot borer	Chlumetia transversa Wlk.
Araneus sp.	spotted stalk borer	Chilo partellus Swinhoe
Araneus sp.	spotted stalk borer	Chilo partellus Swinhoe
Araneus sp.	spotted stalk borer	Chilo partellus Swinhoe
Araneus sp.	spotted stalk borer	Chilo partellus Swinhoe
Araneus sp.	rice leaffolder	Cnaphalocrocis medinalis Guenee
Araneus sp.	gypsy moth	Lymantria dispar L.
Araneus sp.		Cletus signatus Walker
Argiope aemula Walckenaer	rice leaffolder	Cnaphalocrocis medinalis Guenee
Argiope argentata Fabricius	rice brown planthopper	Nilaparvata lugens Stal.
Argiope bruennichi Scopoli	slugs	Agriolimax sp.
Argiope bruennichi Scopoli	Bird cherry – Oat aphid	Rhopalosiphum padi L.
Argiope bruennichi Scopoli	mealy plum aphid	Hyalopterus pruni Geoffr.
Argiope catenulata Doleschall	white-backed planthopper	Sogatella furcifera Horvath
Argiope pulchella Thorell	rice brown planthopper	Nilaparvata lugens Stal.
Argiope pulchella Thorell	rice brown planthopper	Nilaparvata lugens Stal.
Argiope sp.	small rice grasshopper	Oxya nitidula W.
Argiope sp.	sugarcane stalk borer	Eldana saccharina Walker
Argiope sp.	sorghum mite	Oligonychus indicus Hirst
Cyclosa insulana Costa	spotted stalk borer	Chilo partellus Swinhoe
Cyrthophora sp.	sugarcane leaf hopper	Pyrilla perpusilla Walker
Neosconia arabesca Walckenaer	velvet bean caterpillar	Anticarsia gemmatalis Hubner
Neosconia arabesca Walckenaer	cotton leafworm	Alabama argillacea Hubner
Neosconia arabesca Walckenaer	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnogh
Neoscona nautica L. Koch	rose aphid	Macrosiphum rose L.
Neosconia theisi Walckenaer	hibiscus jassid	Amrasca biguttula biguttula Shir.
Neosconia sp.	spotted stalk borer	Chilo partellus Swinhoe

3

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Lep.: Arctiidae	Morus alba L.	Groppali et al., 1993	Italy	
Hom.: Aphididae	potato	Galecka, 1966	Poland	
Hom.: Aphididae	–	Nyffeler, 1983	Switzerland	
Hom.: Aphididae	–	Nyffeler, 1983	Switzerland	
Hom.: Aphididae	–	Nyffeler, 1983	Switzerland	
Hom.: Aphididae	grassland	Nyffeler & Benz, 1982	Switzerland	
Hom.: Aphididae	–	Nyffeler, 1983	Switzerland	
Hom.: Aphididae	–	Nyffeler, 1983	Switzerland	
Hom.: Aphididae	grassland	Nyffeler & Benz, 1982	Switzerland	
Lep.: Tischeridae	Ceanothus griseus Yankee Point	Fasoranti, 1984	USA	
Lep.: Lasiocampidae	pine forest	Csoka et al., 1989	Hungary	
Orthoptera	meadows	Kajak et al., 1968	Poland	
Orthoptera	meadows	Nyffeler & Breene, 1991	Switzerland	
Hom.: Aphididae	grassland	Nyffeler & Benz, 1982	Switzerland	
Hom.: Aphididae	grassland	Nyffeler & Benz, 1982	Switzerland	
Hom.: Aphididae	grassland	Nyffeler & Benz, 1982	Switzerland	
Hem.: Pyrrhocoridae	cotton	Battu, 1990	India	
Lep.: Noctuidae	cotton	Battu, 1990	India	
Lep.: Noctuidae	cotton	Battu, 1990	India	
Hom.: Cicadellidae	mango	Tandon & Lal, 1983	India	
Lep.: Pyralidae	Maize and sorghum	Sharma & Sarup, 1979	India	
Lep.: Pyralidae	Maize and sorghum	Singh & Sandhu, 1976	India	
Lep.: Noctuidae	mango	Tandon & Lal, 1983	India	
Lep.: Pyralidae	Maize and sorghum	Mohan, 1991	India	
Lep.: Pyralidae	Maize and sorghum	Sharma & Sarup, 1979	India	
Lep.: Pyralidae	Maize and sorghum	Singh et al., 1975	India	
Lep.: Pyralidae	Maize and sorghum	Singh & Sandu, 1976	India	
Lep.: Pyralidae	rice	Mun, 1982	Malaysia	
Lep.: Lymantriidae	forest	Schaefer et al., 1984	China	
Hem.: Coreidae	–	Agarwal & Dhiman, 1989	India	
Lep.: Pyralidae	rice	Barrion et al., 1979	Philippine	
Hom.: Delphacidae	rice	Bastidas et al., 1994	Colombia	4.1
Mollusca: Limacidae	–	Quicke, 1987	France	
Hom.: Aphididae	grassland	Nyffeler & Benz, 1982	Switzerland	
Hom.: Aphididae	grassland	Nyffeler & Benz, 1982	Switzerland	
Hom.: Delphacidae	rice	Kamal & Dyck, 1994	Bangladesh	1–2
Hom.: Delphacidae	rice	Rao et al., 1978a	India	
Hom.: Delphacidae	rice	Rao et al., 1978b	India	16
Orth.: Acrididae	rice	Mohan & Manoharan, 1987	India	2
Lep.: Pyralidae	sugarcane	Leslie & Boreham, 1981	South Africa	
Acarina: Tetranychidae	maize	Manjunatha, 1989	India	
Lep.: Pyralidae	Maize and sorghum	Sharma & Sarup, 1979	India	
Hom.: Lophopidae	sugarcane	Miah, 1986	Bangladesh	
Lep.: Noctuidae	soybean	Gregory et al., 1989	USA	
Lep.: Noctuidae	cotton	Gravena & Sterling, 1983	USA	
Lep.: Lymantriidae	pine forest	Mason & Torgersen, 1983	USA	
Hom.: Aphididae	rose	Raychaudhuri et al., 1979	India	
Hom.: Cicadellidae	hibiscus	Rao et al., 1981	India	
Lep.: Pyralidae	Maize and sorghum	Mohan, 1991	India	

Rhopalosiphum padi L.) are also an important part of the diet (Nyffeler and Benz, 1982; Nyffeler, 1983; Nyffeler Benz, 1989). Sometimes these species catch honeybees (*Apis mellifera* L.; Hym.: Apiidae) too (Thakur and Sharma, 1984), but the rate of predation on this beneficial insect is rather low (approx. 1% of the diet) (Nyffeler and Breene, 1991). These spiders are sensitive to grazing, mowing (Gibson et al., 1992) and also spraying, because the spider web is an efficient collector of pesticides (Samu et al., 1992) and the orb-weavers are recycling their web every day.

Clubionidae (Sac spiders)

General description There are 37 species in Central Europe in two genera (Heimer and Nentwig, 1991): *Clubiona* (leaf-curling sac spiders) and *Cheiracanthium* (long-legged sac spiders). Their special characteristic is their 'black face' appearance. Clubionids have long legs with scopulae on the tarsi and tarsal claws (with help of this organ these spiders are able to run on the foliage very easily). The chelicerae are long and rather stout and black. Some species have chevron markings on the abdomen. The eyes are small almost of the same size, and situated in two transverse rows. In *Clubiona* usually the fourth pair of legs is the longest, while in *Cheiracanthium* the first pair of legs the longest. The carapace of *Clubiona* has fovea and *Cheiracanthium* has not.

Most members of this family construct tubular or flat sacs of dense white silk, either open at the end or closed, to be used as retreat. *Clubiona* makes a sac in rolled-up leaves, in folded blades of grass or under loose bark. *Cheiracanthium*, which is often found inside houses makes a flattened, disc-shaped sac in the folds of curtains, behind and under the objects. The sacs are papery and shiny in appearance and very tough. The egg sac is similar but smaller. Size: small to medium large 3–15 mm (Roberts, 1995).

Hunting behaviour Clubionids are typical wandering spiders, rapid runners for short-distances with poor eyesight and hunt at night.

Some wander on the soil surface and others (most of them) range over vegetation. Sac spiders are free-roaming, aggressive hunters, they catch their prey with great speed and agility, leaping on it and grabbing it with outstretched front legs.

Habitat These spiders occur under bark and stones; amongst low vegetation and leaf-litter; on bushes and trees; in marshy habitats and on sand dunes; in built up areas. Generally common and widespread in the region.

Importance in crop protection

– *Species occurring in orchards* Members of this family are recorded from England by Chant (1956); from The Netherlands by Loomans (1978); from Poland by Koslinka (1967) and Olszak et al. (1994b); from USSR by Selivanov (1991); from Australia by Dondale (1966); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley (1974) and Bostanian et al. (1984); from Japan by Hukusima (1961) and Okuma (1973); Takeda et al. (1978); from USA by McCaffrey and Horsburgh (1980); in apple orchards and from USA by Mansour et al. (1982); from Japan by Nakao and Okuma (1958); from Mexico by Rodriguez Almaraz and Contreras Fernandez (1993) and from

China by Yan and Wang (1987) in citrus orchards. One of the most important and widely distributed species of this family is *Cheiracanthium mildei* L. Koch. This spider preys upon a wide range of insect pest of several crops. Its prey are spotted tentiform leafminer (*Phylonorycter blancardella* F.; Lep.: Gracillariidae) in Canada (Corrigan and Bennett, 1987) and in Israel (Mansour et al., 1980a), codling moth (*Cydia pomonella* L. Lep.: Tortricidae), red and two spotted spidermites *Tetranychus cinnabarinus* Boisd. and *T. urticae* Koch (Acarina: Tetranychidae). Mediterranean fruit fly (*Ceratitis capitata* Wied.; Dip.: Trypetidae), aphids (Hom.: Aphididae), leopard moth (*Zeuzera pyrina* L.; Lep.: Cossidae) (Mansour et al., 1980a), Egyptian cotton leafworm (*Spodoptera littoralis* Boisduval; Lep.: Noctuidae) (Mansour et al., 1977; 1980b; 1980c; 1980d) and the giant-looper (*Boarmia (Ascotis) selenaria* Denis and Schiffermuller; Lep.: Geometridae) (Wysoki and Izhar, 1980) in Israel. In addition to predation the "disturbing effect" may be mentioned (Mansour et al., 1981a) (young caterpillars fall down because of the movement of spiders and then are unable to walk back) being sometimes much more important than predation (Nakasuji et al., 1973a; 1973b). Young spiders cause lower predation and a higher "disturbing effect" than mature spiders (Mansour et al., 1981a).

The effect of pesticides on this spider was carefully investigated by Mansour, (1987a) and Hassan et al. (1994) in the standard of IOBC/WPRS and they found that the diflubenzuron caused 95–99% mortality. Mansour et al. (1981b) investigated the toxicity of traditionally used insecticides on this species in Israel and they found that this spider is very sensitive to endosulfan and less sensitive to azinphosmethyl and cyhexatin. Mansour (1984) collected a malathion tolerant strain of this spider from citrus orchards (resistant factor 3.3), but this strain was sensitive to chlorpyrifos.

Sac spiders are predators of the polyphagous leafroller (*Epiphyas postvittana* Walker; Lep.: Tortricidae) on apple in Australia (Dondale, 1966; MacLellan, 1973). These spiders contain 20% of the spider fauna of the foliage of avocado in Israel and prey on the geometrid *Boarmia selenaria* (Mansour et al., 1985).

Clubiona johnsoni Gentseh and *Clubiona moesta* Banks reported as predators of the mites *Tetranychus urticae* and *Panonychus ulmi* in Canada (Parent, 1967). *Clubiona pallidula* and *Clubiona phragmitis* recorded as predators of leafrollers (Lep.: Tortricidae) (daily consumption 4.5 L_2 larvae in laboratory and 3.1 larvae in the field), pear suckers (*Cacopsylla pyricola* Förster and *C. pyri* L. Hom.: Psyllidae) (daily consumption 10–12 adults in laboratory) in The Netherlands (Bogya, 1995a, 1995b) and the pear lace bug (*Stephanitis pyri* F.; Hem.: Tingidae) in Hungary (Bogya and Marko, 1995a; 1995b). Sac spiders were thought to be the most important natural enemies of arthropod citrus pest too in USA (Carrol, 1980). *Clubiona* sp. was seen actively preying upon hairy-caterpillars of *Euproctis lunata* Wlk. and *Porthesia scintillans* Wlk. (Lep.: Noctuidae) in damaging leaves and even fruits of *Zizyphus jujuba* L. in India (Battu, 1990). *Cheiracanthium lawrencei* Roewer reported as predator of citrus psylla (*T. erytreae*) in South Africa (Berg et al., 1992). *Trachelas volutus* Gertsch has been observed feeding on black-margined aphid (*M. caryella*) on pecan (Liao et al., 1984; Bumroongsook et al., 1992). *Cheiracanthium inclusum* Hentz and *Clubiona reichlini* reported from citrus orchards by (Mansour et al., 1982; Yan and Wang, 1987; Yan, 1988). Sac spiders (*Clubiona*

corrugata and *Cl. japonicola*) are dominant in tea plantations too (Zhang, 1993) in China.

– *Species occurring in other agroecosystems* This family of spiders occurring in many agroecosystems and prey on a wide variety of insect pests as shown in Table 4.

– *Conclusion* Mansour and Whitecomb (1986) and Mansour (1987b) performed experiments to evaluate the predatory role of spiders (mainly clubionids) in different ecosystems (citrus and cotton). After removing spiders, the pests (*Ceroplastes floridensis* Comstock (Hom.: Coccidae) on citrus and *Spodoptera littoralis* Boisd. (Lep.: Noctuidae) on cotton) caused significantly higher damage compared to the control. In conventional apple orchards (treated with nonselective insecticides) the number specimens belonging to this family was reduced the smallest (25%) compared with the control (Olszak et al., 1994b). It can be concluded that these spiders potentially play a major role in orchards as nocturnal hunters of lepidopteran pests (see Fig. 1.).

Dictynidae (Hackled-web spiders)

General description 21 species in 8 genera occur in Central Europe (Heimer and Nentwig, 1991). The cribellate spiders in this family are less than 4 mm in length and have a calamistrum on metatarsus IV comprising a single row of bristles. The male vibrates his legs on the web and approaches to touch the female before mating; this takes place in the summer. Males have the inner margins of the chelicerae bowed outwards slightly and this allows grasping of the female chelicerae during mating. The size of these spiders are 2–4 mm (Roberts, 1995).

Hunting behaviour They spin a cribellate web in the heads of plants and on gorse bushes and heather and seem to prefer dry, dead vegetation or hard-leaved bushes. The dense weave of the cribellate web might well trap too much moisture if spun on rapidly transpiring leaves; this would encourage mould growth on the considerable number of prey remains and be a risk to the egg sacs.

The web is a permanent structure which is added to daily and it becomes dense near the centre, where the retreat is made. The prey of these spiders are generally small insects mainly aphids and bugs (Nyffeler et al., 1994b).

Habitat and distribution They occur often on dead plants and on foliage of low vegetation; on leaves of bushes and trees; in built up areas. Generally widespread throughout the region.

Importance in crop protection

– *Species occurring in orchards* Members of this family are reported from England by Chant (1956); from The Netherlands by Loomans (1978); from Poland by Koslinka (1967) and Olszak et al. (1994b); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley 1974; Dondale et al. 1979; Bostanian et al. 1984; from Japan by Hukusima (1961) and Okuma (1973); Takeda et al., (1978); from USA by McCaffrey and Horsburgh (1980) in apple orchards. But Specht and Dondale (1960) and Olszak et al. (1994b) mentioned that these spiders are probably not characteristic of

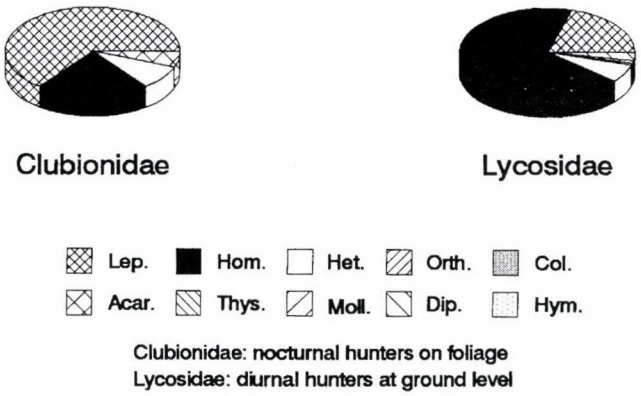

Fig. 1. Prey composition of other hunters

orchards and their presence there was rather fortuitous. Hagley and Allen (1989) found that *Dictyna annulipes* Blackwall preys on the white apple leafhopper (*Typhlocyba pomaria* McAtee; Hom.: Cicadellidae), apple maggot (*Rhagoletis pomonella* Walsh), the green apple aphid *(Aphis pomi* DeGeer) and the spotted tentiform leafminer (*Phyllonorycter blancardella* Fabr.) on apple in Canada and an other species of this genus (*D. sublata* Hentz) feeds on apple inhabiting aphids (*Aphis* sp., *Dysaphis plantaginea* Passerini) (both alate and apterous forms were accepted) and thysanopterans (*Leptothrips mali* Fitch) in USA (McCaffrey and Horsburgh, 1978). Putman (1967) investigated the predators of fruit tree red spider mites *Panonychus ulmi* and 92% of the collected *Dictyna sublata* showed positive reactions by paper chromatography; Parent (1967) also mentioned that *Dictyna* sp. is a predator of *P. ulmi* and *T. urticae*. Dictynids are common in citrus orchards too (Mansour et al., 1982; Muma, 1975). Muma (1975) recorded that unidentified *Dictyna* spp. are natural enemies of whiteflies on citrus in USA. Temerak (1981) investigated the prey spectrum of the most common spiders (*Dictyna* sp.) on pomegranate in Egypt, and found that 54% of the diet was aphids and whiteflies.

– *Species occurring in other agroecosystems* This family of spiders are not abundant in agroecosystems as shown in Table 5. Nyffeler et al. (1988) found that 71.6% of the diet of *Dictyna segregata* is aphids on cotton and potential predator of the bug (*Pseudatomoscelis seriatus* Reuter; Hem.: Miridae) too.

– *Conclusion* Dictynids prefer other areas (e.g. dry vegetations) than agroecosystems. These spiders were observed feeding on many orchard pests but their size are too small to play an important role in controlling them.

Nuessly and Goeden, (1983) observed that the spider *Dictyna reticulata* Gertsch and Ivie did feed on the larvae of *Coleophora parthenica* Meyrick (Lep.: Coleophoridae) which is an important biological control agent of the weed Russian thistle (*Salsola australis* R. Brown; Chenopodiaceae). This beneficial insect contained ±71% of the diet of the spider in USA.

Table

Clubionidae as predators of pests

Spiders	Common name	Scientific name
Cheiracanthium danieli Tikader	mango jassid	Idioscopus clypealis Lethiery
Cheiracanthium danieli Tikader	tobacco cutworm	Spodoptera litura F.
Cheiracanthium danieli Tikader	tobacco cutworm	Spodoptera litura F.
Cheiracanthium diversum L. Koch	noctuids	Heliothis spp.
Cheiracanthium diversum L. Koch	American bollworm	Heliothis armigera Hubner
Cheiracanthium diversum L. Koch		Heliothis punctigera Wallengren
Cheiracanthium inclusum Hentz	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Cheiracanthium inclusum Hentz	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Cheiracanthium inclusum Hentz	soybean looper	Pseudoplusia includens Walker
Cheiracanthium inclusum Hentz	cotton leafworm	Alabama argillacea Hubner
Cheiracanthium inclusum Hentz	tobacco cutworm	Heliothis virescens F.
Cheiracanthium inclusum Hentz	fall webworm	Hyphantria cunea Drury
Cheiracanthium melanostomum Thorel	hibiscus jassid	Amrasca biguttula biguttula Shir.
Cheiracanthium mildei L. Koch	sycamore lace bug	Corythucha ciliata Say
Cheiracanthium mildei L. Koch	fall webworm	Hyphantria cunea Drury
Cheiracanthium mildei L. Koch	carmine spider mite	Tetranychus cinnabarinus Boisduval
Cheiracanthium sp.	coconut black headed caterpillar	Opisina arenosella Wlk.
Cheiracanthium sp.	spotted stalk borer	Chilo partellus Swinhoe
Cheiracanthium sp.	spotted stalk borer	Chilo partellus Swinhoe
Cheiracanthium sp.	spotted stalk borer	Chilo partellus Swinhoe
Cheiracanthium sp.	sorghum mite	Oligonychus indicus Hirst.
Clubiona abottii L. Koch	bean butterfly	Lampides boeticus L.
Clubiona drassodes	sugarcane leafhopper	Pyrilla perpusilla Walker
Clubiona japonicola Boes. et Str.	white-backed planthopper	Sogatella furcifera Horvath
Clubiona japonicola Boes. et Str.	brown planthopper	Nilaparvata lugens Stal.
Clubiona japonicola Boes. et Str.	green leaf bug	Lygocoris lucorum Meyer
Clubiona japonicola Boes. et Str.		Adelphocoris suturalis Jakovlev
Clubiona pickei Gertsh	corn leaf aphid	Rhopalosiphum maidis Fitch
Clubiona pickei Gertsh	Bird cherry – Oat aphid	Rhopalosiphum padi L.
Clubiona saraswatii Tikader	spotted stalk borer	Chilo partellus Swinhoe
Clubiona sp.	grain aphid	Sitobion avenae F.
Clubiona sp.	spotted stalk borer	Chilo partellus Swinhoe
Clubiona sp.	spotted stalk borer	Chilo partellus Swinhoe
Clubiona sp.	rice leaffolder	Cnaphalocrocis medinalis Guenee
Clubiona sp.	fall webworm	Hyphantria cunea Drury
Clubiona sp.	tobacco cutworm	Spodoptera litura F.

4

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Cicadellidae	mango	Tandon & Lal, 1983	India	
Lep.: Noctuidae	tobacco	Sitaramaiah et al., 1980	India	
Lep.: Noctuidae	–	Rao et al., 1993	India	
Lep.: Noctuidae	cotton	Bishop & Blood, 1981	USA	
Lep.: Noctuidae	–	Room, 1979	Australia	
Lep.: Noctuidae	–	Room, 1979	Australia	
Lep.: Noctuidae	soybean	Buschman et al., 1977	USA	
Lep.: Noctuidae	soybean	O'Neil & Stimac, 1988	USA	
Lep.: Noctuidae	soybean	Richman et al., 1980	USA	9.16 eggs
Lep.: Noctuidae	cotton	Gravena & Sterling, 1983		
Lep.: Noctuidae	cotton	McDaniel et al., 1981	USA	
Lep.: Arctiidae	–	Warren et al., 1967		
Hom.: Cicadellidae	hibiscus	Rao et al., 1981	India	
Hem.: Tingidae	Platanus sp.	Balarin & Polonec, 1984	Yugoslavia	8.2
Lep.: Arctiidae	Morus alba L.	Groppali et al., 1993	Italy	
Acarina: Tetranychidae	–	Mansour et al., 1995	Israel	27.5
Lep.: Xyloryctidae	coconut	Sathiamma et al., 1987	India	
Lep.: Pyralidae	sorghum and maize	Mohan, 1991	India	2.84–3.04
Lep.: Pyralidae	sorghum and maize	Sharma & Sarup, 1979	India	
Lep.: Pyralidae	sorghum and maize	Singh et al., 1975	India	
Acarina: Tetranychidae	sorghum	Manjunatha, 1989	India	
Lep.: Lycaenidae	leguminosae	Singh & Mavi, 1984	India	
Hom.: Lophopiidae	sugarcane	Dhaliwal & Bains, 1983	India	
Hom.: Delphacidae	rice	Wu et al., 1990	China	
Hom.: Delphacidae	rice	Wu et al., 1993	China	
Hem.: Miridae	cotton	Cao, 1986	China	
Hem.: Miridae	cotton	Cao, 1986	China	
Hom.: Aphididae	cereals	Provencher & Coderre, 1987	Canada	
Hom.: Aphididae	cereals	Provencher & Coderre, 1987	Canada	
Lep.: Pyralidae	sorghum and maize	Singh et al., 1975	India	
Hom.: Aphididae	wheat and barley	Bhagat et al., 1990	India	
Lep.: Pyralidae	sorghum and maize	Mohan, 1991	India	
Lep.: Pyralidae	sorghum and maize	Singh et al., 1975	India	
Lep.: Pyralidae	rice	Mun, 1982	Malaysia	
Lep.: Arctiidae	–	Sharov et al., 1984	USSR	
Lep.: Noctuidae	tobacco	Sitaramaiah et al., 1980	India	

Table

Dictynidae as predators of pests

Spiders	Common name	Scientific name
Dictyna felis Boes. & Str.	corn leaf aphid	Rhopalosiphum maidis Fitch
Dictyna flavescens Walck.	oleander scale	Aspidiotus nerii Bouche
Dictyna flavescens Walck.	soft brown scale	Coccus hesperidum L.
Dictyna foliicola Boes. & Str.	fall webworm	Hyphantria cunea Drury
Dictyna pusilla Thorell	fall webworm	Hyphantria cunea Drury
Dictyna pusilla Thorell	fall webworm	Hyphantria cunea Drury
Dictyna volucripes Keyserling	guar bud midge	Contarinia texana Felt

Linyphiidae (Linyphiids, Money spiders)

General description This is the largest family of European spiders and contains well over four hundred species in over one hundred and twenty genera (Heimer and Nentwig, 1991). The majority are known as 'money spiders', this is undoubtedly the best known and most frequently used common name for a group of spiders. The name applies to fairly small, grey or black-bodied spiders with no pattern. The name does not apply to all members of the Linyphiidae; those with patterns and markings are definitely excluded. The males of some species have ridges on the outer surface of the chelicerae and an opposing tooth on the inner side of the palpal femur. This is used in stridulation during courtship. Relatively little is known of the biology of most of these species. These spiders are rather small, the size varies between 2–7 mm (Roberts, 1995).

Hunting behaviour Most species make sheet webs, with no retreat, and run upsidedown on the underside of the sheet. Generally abundant in fields and meadows. The sheet web catch selectively from the potential (available) food sources (Nentwig, 1980); the main victims are cereal aphids (12–40%), springtails, dipterans. Beetles and lepidopterans escape easily from the web (Nyffeler et al., 1994b), most of the predators avoid the web because of their sharper vision (Nentwig, 1980).

Habitat and distribution They occur on the bark of trees; on bushes and low vegetation; amongst adjacent leaf-litter and grass; under stones; on open ground. Generally widespread and fairly common in the region.

Importance in crop protection

– *Species occurring in orchards* Members of these family are reported from England by Chant (1956); from The Netherlands by Loomans (1978); from Poland by Koslinka (1967) and Olszak et al. (1994b); from USSR by Selivanov (1991); from Canada by Dondale (1956); Specht and Dondale (1960); Dondale et al. (1979); Bostanian et al.

5

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Aphididae	cereals	Cong, 1992	China	
Hom.: Diaspididae	–	Schmutterer, 1953	Germany	
Hom.: Coccidae	–	Schmutterer, 1953	Germany	
Lep.: Arctiidae	–	Kayashima, 1967	Japan	
Lep.: Arctiidae	Acer negundo L.	Groppali et al., 1994	Italy	
Lep.: Arctiidae	Morus alba L.	Groppali et al., 1993	Italy	
Dip.: Cecydomiidae	guar	Rogers & Horner, 1977	USA	

(1984); from Japan by Okuma (1973) and from USA by McCaffrey and Horsburgh (1980) in apple orchards and by (Mansour et al., 1982; Muma, 1975; Nakao and Okuma, 1958; Rodriguez Almaraz and Contreras Fernandez, 1993 and Yan and Wang, 1987) from citrus orchards. Selivanov (1991) found that 60.5% of the collected spiders belong to this family in apple orchards in USSR and the main food source the apple psyllid (*Cacopsylla mali*). Chant (1956) observed that *Entelecara acuminata* Wider, *Moebelia penicillata* Westring, *Erigonidium graminicolum* Sundevall, *Erigone dentipalpis* Wider and *Bathyphantes gracilis* Blackwall prey on the mites *P. ulmi* and *B. praetiosa*. *Ceraticelus* sp., *Ceratinopsis anglicana* Hentz and *Tennesseellum formicum* Emerton were observed preying on pecan aphid (*M. caryella*) in USA (Liao et al., 1984; Bumroongsook et al., 1992). Mansour et al. (1985) found that linyphiids are abundant (19% of the all spiders) on the ground level of avocado orchards in Israel and McMurtry and Johnson (1966) observed that indet. linyphiids fed on the avocado brown mite *Oligonychus punicae* Hirst in USA.

– *Species occurring in other agroecosystems* Linyphiids are the most common spiders in wheat fields (Carter et al., 1982; Nyffeler and Benz, 1979; 1988c). Their webs covered 0.3% in April and 30% in July of the surface of the soil (Carter et al., 1982). Considerable part of the diet (according to Sunderland et al., (1986) ±12%) are the cereal aphids *Sitobion avenae* F. (Carter et al., 1982; Nyffeler and Benz, 1988c; Sunderland, 1987) and *Rhopaloshiphum padi* L. (De Barro, 1992; Mansour and Heimbach, 1993; Nyffeler and Benz, 1988c; Sunderland, 1987); furthermore collembolans (Nyffeler and Benz, 1979; 1988c). Janssens and Clercq (1990) analysed the gut content of the aphid predators in the field by ELISA, and found that the most important predators are *Erigone atra* Blackwall and *Oedothorax apicatus* in Belgium. If the money spiders are removed from the field, the population of *R. padi* increased 2–6 times (Chiverton, 1986). Alderweireld (1994) was able to increase the number of linyphiids by making holes in the field.

According to Zhao (1984; 1993); Zhou and Xiang (1987); and Li et al. (1983) the spider, *Erigonidium graminicolum* Sund. is one of the dominant spiders on cotton and peanut fields in China and preys on many cotton pests (see Table 6). Successfully mass-rearing of this spider was developed on artificial diet (Zhao and Zhao, 1983) against pests.

– *Conclusion* It can be concluded that these spiders are very important in crop protection, but rather in arable fields are not in orchards (Fig. 3).

Lycosidae (Wolf spiders)

General description The family Lycosidae is represented by 84 species in Central Europe in 10 genera (Heimer and Nentwig, 1991). The eye arrangement is very characteristic: front face of carapace with a row of four small, equal-sized anterior eyes and behind these a larger pair of posterior median and further back a pair of posterior lateral eyes of the same size. Although most species are brownish in overall coloration, many are attractively marked. Some parts of the markings and patterns are in the cuticle, this is frequently reinforced or modified by the dense, coloured hairs with which these spiders are clothed. In some cases, the pattern may be almost entirely due to light and dark hairs and this effect is largely lost when the spider is immersed in alcohol for preservation. The males of many species have their palps furnished with dense black hairs, and others have the first pair of legs conspicuously modified. Lycosids have good eyesight and having first located a female by her pheromones, the male waves the modified legs and palps about in front of her in a courtship display, prior to mating. The females of some genera excavate small burrows where they remain, with their egg sacs, until the spiderlings emerge. In the majority of these spiders the egg sac is attached to the spinners and carried around by the female. The egg sac in some species is spherical and white or being in colour; in other species it is lenticular, with a pale seam, and brownish or green-blue in colour. The egg sac is periodically removed from the spinners, turned around and then reattached. Females frequently open the sac, introduce fluid from their mouthparts on to the developing eggs, and then reseal it with silk. Some species periodically dip the sac in water and most will orient themselves so that the bundle of developing eggs gets the optimum exposure to the warmth of the sun. Those living in burrows will periodically expose the egg sac near the entrance. When the spiderlings are ready to emerge, they rely on the female to open the egg sac for them. Once out, the spiderlings climb on to their mother's abdomen and are carried around by her for the first week or so (Roberts, 1995). The young spiders disperse by 'ballooning' (Greenstone, 1990) to prevent cannibalism. The size of these spiders is 4–20 mm.

Hunting behaviour They are all hunting spiders, mostly at ground level but occasionally on low vegetation. Some make silk-lined burrows in which they spend part of their time and *Aulonia* makes a flimsy sheet web with a tubular retreat. On warm sunny days, large number of lycosids may be seen running rapidly on the ground. This, together with their brown, furry appearance, has given rise to the common name of 'wolf spiders'. Many species, particularly of *Trochosa*, are also active at night (during the day they are

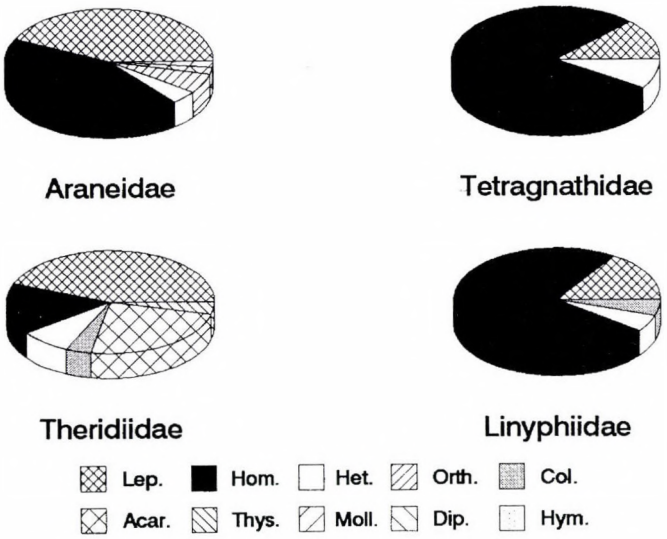

Fig. 3. Prey composition of web-building spiders

sheltered in undergrowth) and some of them are typical 'sit-and-wait' predators (Uetz, 1992; Stratton, 1985; Nyffeler et al., 1994b). In addition to their ability to run at speed, most species can also jump; this is most noticeably in the species such as *Pardosa nigriceps*, which hunts on low vegetation and is adapted to leaping from leaf to leaf (Roberts, 1995). The main prey of these spiders are collenbolans, aphids, orthopterans (Cherril and Begon, 1989), noctuids, other spiders and dipterans (Nyffeler et al., 1994b).

Habitat and distribution They occur on dry, sandy or stony ground and grassland; on low vegetation and bushes; in woodland; on mountains and in cultivated land. Generally common and widespread in the region.

Importance in crop protection

– *Species occurring in orchards* Members of this family mentioned from England by Chant (1956); from The Netherlands by Loomans (1978); from Poland by Olszak et al. (1994b); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley (1974); Bostanian et al. (1984) and from Japan by Okuma (1973); Takeda et al. (1978) in apple orchards and by Mansour et al. (1982); Muma (1975); Rodriguez Almaraz and Contreras Fernandez (1993) from citrus orchards. The spider *Trochosa terricola* Thorell preys on the apple maggot (*Rhagoletis pomonella* Walsh.; Dip.: Tephritidae) (Allen and Hagley, 1990), and on *Aphis pomi* (7.7% of the collected spider was serologically positive) (Hagley and Allen, 1990) in Canada.

– *Species occurring in other agroecosystems* One of the most important species of this family occurs in paddy fields (Reddy, 1991) is *Lycosa pseudoannulata* Boesenberg and Strand (Zhu and Zheng, 1984). This spider preys on a wide range of insect pests

Table

Linyphiidae as predators of pests

Spiders	Common name	Scientific name
Erigone atra Blackwall	cereal aphids	Rhopalosiphum sp.
Erigone atra Blackwall	grain aphid	Sitobion avenae F.
Erigone atra Blackwall	buckthorn – potato aphid	Aphis nasturtii Kalt.
Erigonidium graminocolum Sund.	cotton aphid	Aphis gossypii Glov.
Erigonidium graminocolum Sund.	cotton aphid	Aphis gossypii Glov.
Erigonidium graminocolum Sund.	cotton aphid	Aphis gossypii Glov.
Erigonidium graminocolum Sund.	cotton aphid	Aphis gossypii Glov.
Erigonidium graminocolum Sund.	cotton aphid	Aphis gossypii Glov.
Erigonidium graminocolum Sund.	brown planthopper	Nillaparvata lugens Stal.
Erigonidium graminocolum Sund.	brown planthopper	Nillaparvata lugens Stal.
Erigonidium graminocolum Sund.	green leaf bug	Lygocoris lucorum Meyer
Erigonidium graminocolum Sund.		Adelphocoris suturalis Jakovlev
Erigonidium graminocolum Sund.	American bollworm	Heliothis armigera Hubner
Erigonidium graminocolum Sund.	American bollworm	Heliothis armigera Hubner
Frontinella communis Hentz	European pine shoot moth	Rhyacionia buoliana Schiff.
Linyphia triangularis Clerck	green oak tortix	Tortrix viridana L.
Oedothorax apicatus Blackwall	cereal aphids	Rhopalosiphum sp.
Oedothorax insecticeps Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Oedothorax insecticeps Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Oedothorax insecticeps Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Oedothorax insecticeps Boesenberg & Strand	zigzag leafhopper	Recilia dorsalis Motsch.
Oedothorax insecticeps Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Oedothorax insecticeps Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Oedothorax insecticeps Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Oedothorax insecticeps Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Oedothorax insecticeps Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Oedothorax insecticeps Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Oedothorax insecticeps Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Oedothorax insecticeps Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Oedothorax insecticeps Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Oedothorax insecticeps Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Oedothorax insecticeps Boesenberg & Strand	tobacco cutworm	Spodoptera litura F.
Oedothorax insecticeps Boesenberg & Strand	tobacco cutworm	Spodoptera litura F.
Oedothorax formosana	brown planthopper	Nilaparvata lugens Stal.
Oedothorax formosana	white-backed planthopper	Sogatella furcifera Horvath
Troxochorus nasutus Schenkel	bark beetle	Hylurgops palliatus Gylh.
Troxochorus nasutus Schenkel	bark beetle	Pityogenes chalcographus L.
indet. Linyphiidae	grain aphid	Sitibion avenae F.
indet. Linyphiidae	Southwestern corn borer	Diatraea grandiosella

6

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Aphididae	winter wheat	Janssens et al., 1990	Belgium	
Hom.: Aphididae	cereals	Sopp et al., 1992	Great Britain	
Hom.: Aphididae	potato	Galecka, 1966	Poland	
Hom.: Aphididae	cotton	Dong & Xu, 1984	China	
Hom.: Aphididae	cotton	Mao & Xia, 1983	China	
Hom.: Aphididae	cotton	Zhang, 1985	China	48
Hom.: Aphididae	cotton	Zhang, 1992	China	
Hom.: Aphididae	cotton	Zhou & Xiang, 1987	China	42.8
Hom.: Delphacidae	rice	Cheng, 1989	China	4.2–7.8
Hom.: Delphacidae	rice	Yan & Wu, 1989	China	
Hem.: Miridae	cotton	Cao, 1986	China	
Hem.: Miridae	cotton	Cao, 1986	China	
Lep.: Noctuidae	cotton	Dong & Xu, 1984	China	
Lep.: Noctuidae	cotton	Wu et al., 1981	China	
Lep.: Olethreutidae	pine forest	Pointing, 1966	Canada	
Lep.: Tortricidae	oak forest	Joly, 1956	Germany	
Hom.: Aphididae	winter wheat	Janssens et al., 1990	Belgium	
Hom.: Cicadellidae	rice	Kang & Kiritani, 1978	Japan	
Hom.: Cicadellidae	rice	Nyffeler et al., 1994b	USA	
Hom.: Cicadellidae	rice	Chang & Oka, 1984	Taiwan	
Hom.: Cicadellidae	rice	Chang & Oka, 1984	Taiwan	
Hom.: Delphacidae	rice	Kang & Kiritani, 1978	Japan	
Hom.: Delphacidae	rice	Nyffeler et al., 1994b	USA	
Hom.: Delphacidae	rice	Cheng, 1989	China	4.2–7.8
Hom.: Delphacidae	rice	Lin & Liu, 1984	Taiwan	
Hom.: Delphacidae	rice	Chen & Chiu, 1979	Taiwan	
Hom.: Delphacidae	rice	Chen & Chiu, 1981	Taiwan	
Hom.: Delphacidae	rice	Chang & Oka, 1984	Taiwan	
Hom.: Delphacidae	rice	Chiu & Chen, 1981	Taiwan	
Hom.: Delphacidae	rice	Chang & Oka, 1984	Taiwan	
Hom.: Delphacidae	rice	Wu et al., 1990	China	
Lep.: Noctuidae	taro	Nakasuji et al., 1973a	Japan	
Lep.: Noctuidae	taro	Yamanaka et al., 1972	Japan	
Hom.: Delphacidae	rice	Heong et al., 1992	Philippine	
Hom.: Delphacidae	rice	Heong et al., 1992	Philippine	
Col.: Scolytidae	forest	Moor & Nyffeler, 1983	Switzerland	
Col.: Scolytidae	forest	Moor & Nyffeler, 1983	Switzerland	
Hom.: Aphididae	cereals	Carter et al., 1982	Great Britain	
Lep.: Pyralidae	corn	Knutson & Gilstrap, 1989	USA	

(Chen and Gao, 1992) (see Table 7). But this species also preys on beneficial insects such as the predatory bug (*Cyrtorhinus lividipennis* Reuter; Hem.: Miridae) (Heong et al., 1989) in Philippine; the daily consumption in laboratory was 22 specimen of the prey. *Pardosa t-insignita* Boesenberg and Strand is the dominant spider (41% of the collected spiders) on ground level of ground nut (Li et al., 1983) and on cotton (Zhao, 1984) in China. *P. agrestis* Westring is one of the dominant spiders occurring in winter wheat fields in Europe (Nyffeler and Benz, 1982) and is able to cause 34–58% population reductions of the cereal aphid (*Rhopalosiphum padi* L.) (Mansour and Heimbach, 1993). Although half of its diet contains springtails (Nyffeler and Benz, 1979; 1988a). Another species, *P. ramulosa* is able to decrease by 84–96% of the population of the aster leafhopper (*Macrosteles fascifrons* Stal.; Hem.: Deltocephalidae) in paddy fields, compared with the control (Oraze and Grigarick, 1989).

– *Conclusion* Wolf spiders are abundant in many agroecosystems (including orchards), but they are hunting only on the ground level and not much information exist about their predatory role in orchards. In other ecosystems they are one of the most important predators.

Oxyopidae (Lynx spiders)

General description Only one genus, *Oxyopes*, with 4 species occurs in the region (Heimer and Nentwig, 1991). The hexagonal arrangement of the eyes, and the long leg spines, are the majority of this family. Courtship is visual recognition being followed by the male waving his palps and legs as he approaches, first to touch, and then tomate. Females place their rather flat-looking, discoid egg sacs near the top of low vegetation and stand guard over them. The size of these spiders is 4–10 mm (Roberts, 1995).

Hunting behaviour They are long legged, diurnal, hunting spiders, capable of running very rapidly on low vegetation and also jumping on their prey. Although their eyes are much smaller than those of the Salticidae and Lycosidae, their vision is obviously accurate enough to enable them to recognize potential prey.

Habitat and distribution They occur on low vegetation, bushes and the lower branches of trees. Generally widespread in the region.

Importance in crop protection

– *Species occurring in orchards* Members of this family are recorded from The Netherlands by Loomans (1978); from Canada by Specht and Dondale (1960); from Japan by Hukusima (1961); Okuma (1973); and Takeda et al. (1978); from USA by McCaffrey and Horsburgh (1980); in apple orchards and by Mansour et al. (1982); Muma (1975); Nakao and Okuma (1958); Rodriguez Almaraz and Contreras Fernandez (1993) from citrus orchards. One of the most important species in this family the striped lynx spider, *Oxyopes salticus* Hentz occurs on several crops in USA (Whitecomb et al., 1963; Nyffeler et al., 1987a; Young and Edwards, 1990). This spider was common and represented 1.2–10.1% of the total spiders collected from apple orchards (McCaffrey

and Horsburgh, 1980). This species together with the green lynx spider, *Peucetia viridans* Hentz as observed preying on pecan aphid (*M. caryella*) in USA (Bumroongsook et al., 1992). *Oxyopes elegans* showed positive reaction to anti-*Epiphyas postvittana* serum in Australia (Danthanarayana, 1983).

– *Species occurring in other agroecosystems* Lynx spiders are abundant in many ecosystems and prey on a wide variety of insect pests (Kamal et al., 1992) as shown in Table 8.

– *Conclusions* Only 4 species of lynx spiders occur in the region, but it can be concluded that their hunting behaviour (diurnal wandering spiders on vegetation) suggest that they at least contribute to reducing pest species in orchards (Fig. 2).

Philodromidae (Philodromids)

General description 24 species occur in the region in three genera (Heimer and Nentwig, 1991). Formerly this family belonged to the family Thomisidae as subfamily Philodrominae. The appearance of these spiders is not crab-like, but the legs are fairly long. The abdomen is usually oval, quite elongate in some males. Claw tufts are present. The eyes are almost of the same size and positioned in two recurved rows. Courtship and mating appears to be very brief in this family. Egg sacs usually have a woolly or gauze-like exterior and females stand guard directly over them in foliage or on bark. The size of the species ranged between 3–10 mm (Roberts, 1995).

Hunting behaviour Most species are tree- or grass inhabiting and are wanderers. Philodromids do actively pursue their prey on vegetation, without making a web.

Habitat and distribution They occur on low vegetation, bushes and the lower branches of trees; on long grasses and at the ground level, sometimes in drier, sandy habitats.

Importance in crop protection

– *Species occurring in orchards* Members of this family are recorded from The Netherlands by Loomans (1978); from Poland by Koslinka (1967) and Olszak et al. (1994b); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley (1974); Dondale et al. (1979); Bostanian et al. (1984); from Japan by Hukusima (1961) and Okuma (1973); from USA by McCaffrey and Horsburgh (1980) in apple orchards and by Rodriguez Almaraz and Contreras Fernandez (1993) from citrus orchards. Philodromids are common in apple orchards (representing 7.5–29.6% of the total spiders collected (McCaffrey and Horsburgh, 1980)); *P. cespiticolis* Walckenaer, *P. praelustris* Keyserling and *P. rufus* Walckenaer recorded from Canada and USA (Dondale, 1958; Dondale et al., 1979; Specht and Dondale, 1960; Legner and Oatman, 1964; Bostanian et al., 1984; Arnoldi et al., 1991), *P. cespitum* (Klein, 1988; Klein and Sengonca, 1988) and *P. aureolus* (Sengonca et al., 1986) from Germany, *P. placidus* Banks from USA (McCaffrey and Horsburgh, 1978). These species prey on many apple pests *Aphis sp.*, *Dysaphis plantaginea, Platynota flavedana* Clements (Hom.: Cicadellidae), *Tetranychus urticae, Panonychus ulmi, Lygus lineolaris*, and *Lygoris communis* (McCaffrey and

Table

Lycosidae as predators of pests

Spiders	Common name	Scientific name
Hippasa holmerae Thorell	white-backed planthopper	Sogatella furcifera Horvath
Hippasa holmerae Thorell	brown planthopper	Nilaparvata lugens Stal.
Hippasa holmerae Thorell	hibiscus jassid	Amrasca biguttula biguttula Shir.
Hippasa holmerae Thorell	stem fly	Ophiomyia phaesoli Tryon
Lycosa anteculana Montgomery	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Lycosa anteculana Montgomery	tobacco budworm	Heliothis virescens F.
Lycosa chaperi Simon	brown planthopper	Nilaparvata lugens Stal.
Lycosa helluo Walckenaer	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Lycosa helluo Walckenaer	maize armyworm	Pseudaletia unipunctata Haworth
Lycosa helluo Walckenaer	tobacco budworm	Heliothis virescens F.
Lycosa pseudoannulata Boesenberg & Strand	rice leaffolder	Cnaphalocrocis medinalis Guenee
Lycosa pseudoannulata Boesenberg & Strand	rice leaffolder	Cnaphalocrocis medinalis Guenee
Lycosa pseudoannulata Boesenberg & Strand	rice leaffolder	Cnaphalocrocis medinalis Guenee
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.

7

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Delphacidae	rice	Barrion & Litsinger, 1981	Philippine	
Hom.: Delphacidae	rice	Barrion & Litsinger, 1981	Philippine	
Hom.: Cicadellidae	leguminosae	Barrion & Litsinger, 1981	Philippine	
Dip.: Agromyzidae	leguminosae	Barrion & Litsinger, 1981	Philippine	
Hem.: Miridae	cotton	Hayes & Lockley, 1990	USA	
Lep.: Noctuidae	cotton	Hayes & Lockley, 1990	USA	
Hom.: Delphacidae	rice	Samal & Misra, 1985	India	
Hem.: Miridae	cotton	Hayes & Lockley, 1990	USA	
Lep.: Noctuidae	maize	Clark et al., 1994	USA	
Lep.: Noctuidae	cotton	Hayes & Lockley, 1990	USA	
Lep.: Pyralidae	rice	Joshi et al., 1987	Philippine	
Lep.: Pyralidae	rice	Pang et al., 1988	China	
Lep.: Pyralidae	rice	Tang et al., 1987	China	
Hom.: Delphacidae	rice	Kamal & Dyck, 1994	Bangladesh	1–2
Hom.: Delphacidae	rice	Zhou, 1986	China	
Hom.: Delphacidae	rice	Nakamura, 1977	Japan	
Hom.: Delphacidae	rice	Kaushik et al., 1986	India	
Hom.: Delphacidae	rice	Salim & Heinrichs, 1986	Philippine	
Hom.: Delphacidae	rice	Salim & Heinrichs, 1986	Philippine	5.9
Hom.: Delphacidae	rice	Cruz & Litsinger, 1986	Philippine	
Hom.: Delphacidae	rice	Chang & Oka, 1984	Taiwan	
Hom.: Delphacidae	rice	Heong et al., 1992	Philippine	
Hom.: Delphacidae	rice	Heinrichs et al., 1984	Philippine	
Hom.: Delphacidae	rice	Luong, 1987	Vietnam	
Hom.: Delphacidae	rice	Samal & Misra, 1975	India	
Hom.: Delphacidae	rice	Murugesan & Chelliah, 1982	India	6
Hom.: Delphacidae	rice	Reissing et al., 1982	Philippine	
Hom.: Delphacidae	rice	Gavarra & Raros, 1973	Philippine	
Hom.: Delphacidae	rice	Thang et al., 1987	Philippine	
Hom.: Delphacidae	rice	Thang et al., 1988	Philippine	
Hom.: Delphacidae	rice	Kaushik et al., 1986	India	
Hom.: Delphacidae	rice	Cruz & Litsinger, 1986	Philippine	
Hom.: Delphacidae	rice	Kuno & Dick, 1984	Philippine	
Hom.: Delphacidae	rice	Heong et al., 1991	Philippine	
Hom.: Delphacidae	rice	Sawada et al., 1993	Indonesia	
Hom.: Delphacidae	rice	Chen & Chiu, 1979	Taiwan	
Hom.: Delphacidae	rice	Chen & Chiu, 1981	Taiwan	
Hom.: Delphacidae	rice	Chang & Oka, 1984	Taiwan	
Hom.: Delphacidae	rice	Kobayashi & Shibata, 1973	Japan	
Hom.: Delphacidae	rice	Sasaba et al., 1973	Japan	
Hom.: Delphacidae	rice	Ooi, 1988	Malaysia	
Hom.: Delphacidae	rice	Heong et al., 1992	Philippine	
Hom.: Delphacidae	rice	Baskaran et al., 1979	India	
Hom.: Delphacidae	rice	Chiu & Chen, 1981	Taiwan	

Table

Spiders	Common name	Scientific name
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix virescens Dist.
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix virescens Dist.
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix virescens Dist.
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix virescens Dist.
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix virescens Dist.
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix virescens Dist.
Lycosa pseudoannulata Boesenberg & Strand	green rice leafhopper	Nephotettix virescens Dist.
Lycosa pseudoannulata Boesenberg & Strand	zigzag leafhopper	Recilia dorsalis Motsch.
Lycosa pseudoannulata Boesenberg & Strand	rice stem borer	Chilo supressalis Walk
Lycosa pseudoannulata Boesenberg & Strand	yellow stem borer	Scirpophaga incertulas Wlk.
Lycosa pseudoannulata Boesenberg & Strand	yellow stem borer	Scirpophaga incertulas Wlk.
Lycosa pseudoannulata Boesenberg & Strand	diamond-back moth	Plutella xylostella L.
Lycosa pseudoannulata Boesenberg & Strand	rice gall midge	Orseolia oryzae Wood-Mason
Pardosa agrestis Westring	Bird cherry – Oat aphid	Rhopalosiphium padi L.
Pardosa agrestis Westring	rose-grain aphid	Metopolophium dirhodum Walker
Pardosa amentata Cl.	rose-grain aphid	Metopolophium dirhodum Walker
Pardosa astrigera L. Koch	cotton aphid	Aphis gossypii Glov.
Pardosa astrigera L. Koch	cotton aphid	Aphis gossypii Glov.
Pardosa astrigera L. Koch	cotton aphid	Aphis gossypii Glov.
Pardosa astrigera L. Koch	black pine bast scale	Matsucoccus thunbergianae M. & P.
Pardosa astrigera L. Koch	noctuids	
Pardosa astrigera L. Koch	oriental tobacco budmoth	Helicoverpa assulta Guenee
Pardosa astrigera L. Koch	American bollworm	Heliothis amigera Hubner
Pardosa astrigera L. Koch	oriental corn borer	Ostrinia furnacalis M. & M.
Pardosa astrigera L. Koch	diamond-back moth	Plutella xylostella L.
Pardosa crassipalpis Purcell	red spider mite	Tetranychus cinnabarinus Boisduval
Pardosa laura Karsch	paddy armyworm	Pseudaletia separata Walker
Pardosa lugubris	pine needle gall midge	Thecodiplosis japonensis U. & I.
Pardosa milvina Hentz	pink bollworm	Pectinophora gossypella Saund.
Pardosa milvina Hentz	diamond-back moth	Plutella xylostella L.
Pardosa monticola Cl.	cereal bug	Eurygaster integriceps Put.
Pardosa palustris L.	rose-grain aphid	Metopolophium dirhodum Walker

7 (cont.)

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Delphacidae	rice	Ku & Wang, 1981	Taiwan	
Hom.: Delphacidae	rice	Zhou & Chen, 1986	China	
Hom.: Delphacidae	rice	Kartohardjono & Heinrichs, 1984	Philippine	
Hom.: Delphacidae	rice	Heinrichs et al., 1984	Philippine	
Hom.: Cicadellidae	rice	Heong et al., 1992	Philippine	
Hom.: Cicadellidae	rice	Kiritani et al., 1972	Japan	
Hom.: Cicadellidae	rice	Kang & Kiritani, 1978	Japan	
Hom.: Cicadellidae	rice	Kiritani et al., 1977	Japan	
Hom.: Cicadellidae	rice	Suzuki & Kiritani, 1974	Japan	
Hom.: Cicadellidae	rice	Sasaba & Kiritani, 1974	Japan	
Hom.: Cicadellidae	rice	Nakamura, 1977	Japan	
Hom.: Cicadellidae	rice	Xie & Liu, 1992	China	
Hom.: Cicadellidae	rice	Chang & Oka, 1984	Taiwan	
Hom.: Cicadellidae	rice	Barrion & Litsinger, 1984	Philippine	
Hom.: Cicadellidae	rice	Hsieh & Dyck, 1975	Philippine	
Hom.: Cicadellidae	rice	Kaushik et al., 1986	India	
Hom.: Cicadellidae	rice	Cruz & Litsinger, 1986	Philippine	
Hom.: Cicadellidae	rice	Heong et al., 1992	Philippine	
Hom.: Cicadellidae	rice	Myint et al., 1986	Philippine	
Hom.: Cicadellidae	rice	Heinrichs et al., 1984	Philippine	
Hom.: Cicadellidae	rice	Chang & Oka, 1984	Taiwan	
Lep.: Pyralidae	rice	Barrion & Litsinger, 1984	Philippine	
Lep.: Pyralidae	rice	Barrion & Litsinger, 1984	Philippine	
Lep.: Pyralidae	rice	Rubia et al., 1990	Philippine	
Lep.: Plutellidae	cabbige	Yamada & Yamaguchi, 1985	Japan	
Dip.: Cecydomiidae	rice	Barrion & Litsinger, 1984	Philippine	
Hom.: Aphididae	cereals	Mansour & Heimbach, 1993	Germany	
Hom.: Aphididae	cereals	Nyffeler & Benz, 1982	Switzerland	
Hom.: Aphididae	cereals	Nyffeler & Benz, 1982	Switzerland	
Hom.: Aphididae	cotton	Zhang, 1992	China	17.3
Hom.: Aphididae	cotton	Dong & Xu, 1984	China	
Hom.: Aphididae	cotton	Zhao et al., 1989	China	4–8
Hom.: Margarodidae	pine forest	Kim, 1993	Korea	
Lep.: Noctuidae	cotton	Dong & Xu, 1984	China	
Lep.: Noctuidae	cotton	Zhao et al., 1989	China	20–25 eggs
Lep.: Noctuidae	cotton	Zhao et al., 1989	China	0.5–3 larvae
Lep.: Pyralidae	corn	Zhao et al., 1989	China	
Lep.: Plutellidae	cabbige	Nemoto, 1993	Japan	
Acarina: Tetranychidae	strawberry	Dippenar Schoeman, 1977	Pretoria	
Lep.: Noctuidae	maize	Kanda, 1987	Japan	
Dip.: Cecidomiidae	pine forest	Kim & Kim, 1975	Korea	
Lep.: Noctuidae	cotton	Clark & Glick, 1961	USA	
Lep.: Plutellidae	collard	Muckenfuss & Shepard, 1994	USA	
Hem.: Scutelleridae	cereals	Titova & Egorova, 1978	USSR	
Hom.: Aphididae	cereals	Nyffeler & Benz, 1982	Switzerland	
Lep.: Noctuidae	peanut	Agnew & Smith, 1989	USA	

Table

Spiders	Common name	Scientific name
Pardosa pauxilla Montgomery	noctuids	Heliothis spp.
Pardosa ramulosa McCook	aster leafhopper	Macrosteles fascifrons Stal.
Pardosa ramulosa McCook	pea aphid	Acyrthosiphon pisum Harris
Pardosa tikaderi Tikader	turpod bug	Clavigralla sp.
Pardosa tikaderi Tikader	turpod fly	Melanagromyza obtusa Malloch
Pardosa t-insignita Boes. & Str.	green leaf bug	Lygocoris lucorum Meyer
Pardosa t-insignita Boes. & Str.		Adelphocoris suturalis Jakovlev
Pardosa t-insignita Boes. & Str.	fall webworm	Hyphantria cunea Drury
Pardosa sp.	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Pardosa sp.	Colorado potato beetle	Leptinotarsa decemlineata Say
Pirata japonicus	cotton aphid	Aphis gossypii Glov.
Pirata subpiraticus Boesenberg & Strand	small brown planthopper	Laodelphax striatelle Fall.
Pirata subpiraticus Boesenberg & Strand	green rice leafhopper	Nephotettix cinctipes Uhler
Pirata subpiraticus Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Pirata subpiraticus Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Pirata subpiraticus Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Pirata subpiraticus Boesenberg & Strand	brown planthopper	Nilaparvata lugens Stal.
Pirata subpiraticus Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Pirata subpiraticus Boesenberg & Strand	white-backed planthopper	Sogatella furcifera Horvath
Trochosa terricola Thorell	cereal bug	Eurygaster integriceps Put.
indet. Lycosidae	green peach aphid	Myzus persicae Sulzer
indet. Lycosidae	glasshouse-potato aphid	Aulacorthum solani Kaltenbach
indet. Lycosidae	large sugarcane borer	Sesamia cretica Led.

Horsburgh, 1978; Parent, 1967; Putman, 1967; Putman and Herne, 1966; Sengonca and Klein, 988; Arnoldi et al., 1991) as shown in Table 1, too.

– *Species occurring in other agroecosystems* Philodromids occur in other ecosystems too and prey on pest species as shown in Table 9. *P. aureolus* (Polesnyi, 1990) and *Philodromus* sp. (Mansour and Nentwig, 1988) are tolerant for 30 pesticides in Europe.

– *Conclusion* Not much information exist on the predatory role of these spiders, but their predatory behaviour is suitable for crop protection. The pesticide tolerance is very valuable property too.

Salticidae (Jumping spiders)

General description Around 80 salticid species are recorded from Central Europe, in 23 genera (Heimer and Nentwig, 1991). The square-fronted carapace, with four large, forward-facing eyes, makes members of this family easily recognisable in the field, even though some are quite small spiders. Although popularly called 'jumping spiders' they are not alone in having this ability members of the Lycosidae, Clubionidae, Oxyopidae

7 (cont.)

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Deltocaphalidae	rice	Oraze & Grigarick, 1989	USA	
Hom.: Aphididae	alfalfa	Yeargan, 1975	USA	
Hem.: Coreidae	pigeonpea	Arora & Monga, 1993	India	
Dip.: Agromyzidae	pigeonpea	Arora & Monga, 1993	India	
Hem.: Miridae	cotton	Cao, 1986	China	
Hem.: Miridae	cotton	Cao, 1986	China	
Lep.: Arctiidae	–	Kayashima, 1967	Japan	
Lep.: Noctuidae	soybean	Reed et al., 1984	USA	3.3
Col.: Chrysomelidae	potato	Heimpel& Hough-Goldstein, 1992	USA	
Hom.: Aphididae	cotton	Zhou & Xiang, 1987	China	42.8
Hom.: Delphacidae	rice	Okuma et al., 1978	Korea	
Hom.: Cicadellidae	rice	Okuma et al., 1978	Korea	
Hom.: Delphacidae	rice	Okuma et al., 1978	Korea	
Hom.: Delphacidae	rice	Cheng, 1989	China	4.2–7.8
Hom.: Delphacidae	rice	Wu et al., 1993	China	
Hom.: Delphacidae	rice	Kim & Lee, 1994	Korea	17.4
Hom.: Delphacidae	rice	Okuma et al., 1978	Korea	
Hom.: Delphacidae	rice	Wu et al., 1990	China	
Hem.: Scutelleridae	cereals	Titova & Egorova, 1978	USSR	
Hom.: Aphididae	sugarbeet	Wratten & Pearson, 1982	New Zealand	
Hom.: Aphididae	sugarbeet	Wratten & Pearson, 1982	New Zealand	
Lep.: Noctuidae	sorghum	Temerak, 1978	Egypt	

and Agelenidae can also jump, and frequently do so in order to avoid capture or to get from one leaf to the next. Salticids use the third and/or fourth pairs of legs for jumping. Before leaping, the spider attaches a silk thread to the substrate and draws in the hind legs. Compared with fleas and grasshoppers, the salticids are very poor jumpers but some small species can achieve distances of over twenty times their own length. The eyes of salticids have a greater range of movement than our own, elaborate focusing, binocular vision and are probably sensitive to colour as well as to polarised light. The smaller eyes, further back on the carapace, are able to detect movement, but less detail; if something enters the rear or side field of vision the spider jumps around to focus the large front eyes upon it. Many species are clothed with coloured, shining or iridescent hairs, with the eyes attractively fringed, and males frequently have enlarged, coloured front legs and decorated palps. These find use, in conjunction with the great visual acuity, in elaborate courtship displays when legs and palps are waved semaphore-style as the male moves rhythmically about in front of the female. Females remain guarding their egg sacs within a silken cell, which the young spiderlings leave as soon as they are capable of an independent existence. The size of these spiders is 2–10 mm (Roberts, 1995).

Table

Oxyopidae as predators of pests

Spiders	Common name	Scientific name
Oxyopes badius Yaginuma	gipsy moth	Lymantria dispar L.
Oxyopes badius Yaginuma	pine moth	Dendrolimus spectabilis Butler.
Oxyopes elegans Koch	American bollworm	Heliothis armigera Hubner
Oxyopes elegans Koch		Heliothis puntigera Wallengren
Oxyopes javanus Thorell	hibiscus jassid	Amrasca biguttula biguttula Shir.
Oxyopes javanus Thorell	white backed planthopper	Sogatella furcifera Horvath
Oxyopes javanus Thorell	brown planthopper	Nilaparvata lugens Stal.
Oxyopes javanus Thorell	Malayan black bug	Scotinophora coarctata F.
Oxyopes javanus Thorell	rice leaffolder	Cnaphalocrocis medinalis Guenee
Oxyopes mundulus L. Koch	noctuids	Heliothis spp.
Oxyopes pandae Tikader	maize jassid	Ziginidia manaliensis Singh
Oxyopes pandae Tikader	tobacco cutworm	Spodoptera litura F.
Oxyopes pandae Tikader	maize borer	Chilo partelus Swinhoe
Oxyopes pandae Tikader	maize borer	Chilo partelus Swinhoe
Oxyopes pandae Tikader	maize borer	Chilo partelus Swinhoe
Oxyopes salticus Hentz	pea aphid	Acyrthosyphon pisum Harris
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Oxyopes salticus Hentz	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Oxyopes salticus Hentz	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Oxyopes salticus Hentz	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Oxyopes salticus Hentz	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Oxyopes salticus Hentz	rapid plant bug	Adelphocoris rapidus Say
Oxyopes salticus Hentz	Southern green stink bug	Nezara viridula L.
Oxyopes salticus Hentz	cotton leafworm	Alabama argillacea Hubner
Oxyopes salticus Hentz	cotton leafworm	Alabama argillacea Hubner
Oxyopes salticus Hentz	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Oxyopes salticus Hentz	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Oxyopes salticus Hentz	soybean looper	Pseudoplusia includens Walker
Oxyopes salticus Hentz	soybean looper	Pseudoplusia includens Walker
Oxyopes salticus Hentz	soybean looper	Pseudoplusia includens Walker
Oxyopes salticus Hentz	cabbage looper	Trichoplusia ni Hubner
Oxyopes salticus Hentz	cabbage looper	Trichoplusia ni Hubner
Oxyopes salticus Hentz	tobacco budworm	Heliothis virescens F.
Oxyopes salticus Hentz	tobacco budworm	Heliothis virescens F.
Oxyopes salticus Hentz	noctuids	Heliothis spp.

8

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Lep.: Lymantriidae	pine forest	Furuta, 1977	Japan	
Lep.: Lasiocampidae	pine forest	Furuta, 1977	Japan	
Lep.: Noctuidae	–	Room, 1979	Australia	
Lep.: Noctuidae	–	Room, 1979	Australia	
Hom.: Cicadellidae	hibiscus	Rao et al., 1981	India	
Hom.: Delphacidae	rice	Kamal & Dyck, 1994	Bangladesh	2–3
Hom.: Delphacidae	rice	Sawada et al., 1993	Indonesia	
Hem.: Pentatomidae	rice	Perez et al., 1989	Philippine	
Lep.: Pyralidae	rice	Barrion et al., 1979	Philippine	
Lep.: Noctuidae	cotton	Bishop & Blood, 1981	USA	
Hom.: Cicadellidae	maize and sorghum	Singh & Sandhu, 1976	India	
Lep.: Noctuidae	tobacco	Sitaramaiah et al., 1980	India	
Lep.: Pyralidae	maize and sorghum	Sharma & Sharup, 1979	India	
Lep.: Pyralidae	maize and sorghum	Singh et al., 1975	India	
Lep.: Pyralidae	maize and sorghum	Singh & Sandhu, 1976	India	
Hom.: Aphididae	alfalfa	Howell & Pienkowski, 1971	USA	
Hem.: Miridae	cotton	Kagan, 1943	USA	
Hem.: Miridae	cotton	Almand, 1974	USA	
Hem.: Miridae	cotton	Nyffeler et al., 1992a	USA	
Hem.: Miridae	cotton	Nyffeler et al., 1992b	USA	
Hem.: Miridae	cotton	Nyffeler et al., 1994b	USA	
Hem.: Miridae	cotton	Breene et al., 1988	USA	
Hem.: Miridae	cotton	Breene et al., 1989	USA	
Hem.: Miridae	cotton	Breene et al., 1990	USA	
Hem.: Miridae	cotton	Breene & Sterling, 1988	USA	
Hem.: Miridae	cotton	Lockley & Young, 1988	USA	
Hem.: Miridae	cotton	Whitcomb & Bell, 1964	USA	
Hem.: Miridae	–	Young & Lockley, 1986	USA	
Hem.: Miridae	cotton	Scott et al., 1988	USA	
Hem.: Miridae	cotton	Lockley & Young, 1988	USA	
Hem.: Miridae	cotton	Kagan, 1943	USA	
Hem.: Pentatomidae	soybean	Ragsdale et al., 1981	USA	
Lep.: Noctuidae	cotton	Gravena & Pazetto, 1987	Brazil	
Lep.: Noctuidae	cotton	Gravena & Da-Cuhna, 1991	Brazil	
Lep.: Noctuidae	soybean	McCarty et al., 1980	USA	
Lep.: Noctuidae	soybean	Reed et al., 1984	USA	7.4
Lep.: Noctuidae	soybean	McCarty et al., 1980	USA	
Lep.: Noctuidae	soybean	Richman et al., 1980	USA	1.14 larva
Lep.: Noctuidae	soybean	Reed et al., 1984	USA	7.4
Lep.: Noctuidae	soybean	Reed et al., 1984	USA	7.4
Lep.: Noctuidae	cotton	Lockley & Young, 1988	USA	
Lep.: Noctuidae	cotton	McDaniel & Sterling, 1979	USA	
Lep.: Noctuidae	cotton	McDaniel et al., 1981	USA	
Lep.: Noctuidae	cotton	Smith et al., 1978	USA	

Table

Spiders	Common name	Scientific name
Oxyopes salticus Hentz	noctuids	Heliothis spp.
Oxyopes salticus Hentz	lesser corn stalk borer	Elasmopalpus lignosellus Zeller
Oxyopes salticus Hentz	thrips	Frankliniella spp.
Oxyopes scalaris Hentz	bark beetle	Ips pini Say
Oxyopes sertatus L. Koch	diamond back moth	Plutella xylostella L.
Oxyopes sertatus L. Koch	gipsy moth	Lymantria dispar L.
Oxyopes sertatus L. Koch	pine moth	Dendrolimus spectabilis Butler.
Oxyopes sp.	apple blossom thrips	Thrips flavus Schrank
Oxyopes sp.	banana thrips	Thrips hawaiiensis Morgan
Oxyopes sp.	European corn borer	Ostrinia nubilalis Hubner
Oxyopes sp.	pink borer	Chilo partellus Swin.
Oxyopes sp.		Dichomeris ianthes Meyr.
Peucetia viridans Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Peucetia viridans Hentz	cotton leafworm	Alabama argillacea Hubner
Peucetia viridans Hentz	cotton leafworm	Alabama argillacea Hubner
Peucetia viridans Hentz	cotton leafworm	Alabama argillacea Hubner
Peucetia viridans Hentz	tobacco budworm	Heliothis virescens F.
Peucetia viridans Hentz	corn earworm	Heliothis zea Boddie
Peucetia viridans Hentz	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Peucetia viridans Hentz	Nantucket pine tip moth	Rhyacionia frustrana Comstock
indet. Oxyopidae	greenhouse leafminer	Liriomyza trifolii Burgess
indet. Oxyopidae	carmine spider mite	Tetranychus cinnabarinus Boisduval

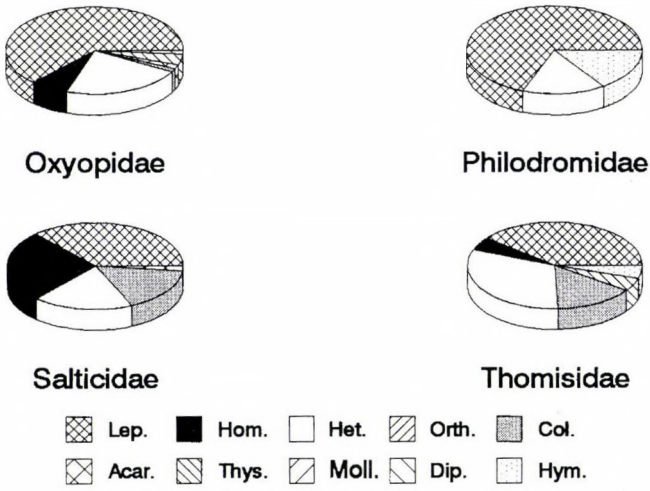

Fig. 2. Prey composition of diurnal hunters on foliage

8 (cont.)

Taxon	Crops	Authors	Countries	Daily consumption
Lep.: Noctuidae	soybean	McCarty et al., 1980	USA	
Lep.: Pyralidae	peanut	Mack et al., 1988	USA	
Thys.: Thripidae	peanut	Agnew & Smith, 1989	USA	
Col.: Scolytidae	forest	Jennings & Pase, 1975	USA	
Lep.: Plutellidae	cabbige	Yamada & Yamaguchi, 1985	Japan	
Lep.: Lymantriidae	pine forest	Furuta, 1977	Japan	
Lep.: Lasiocampidae	pine forest	Furuta, 1977	Japan	
Thys.: Thripidae	–	Veer, 1984	India	
Thys.: Thripidae	–	Veer, 1984	India	
Lep.: Pyralidae	corn	Godfrey et al., 1991	USA	
Lep.: Pyralidae	sorghum	Mohan, 1991	India	
Lep.: Gelechiidae	indigo plant	Gope, 1981	India	2–3
Hem.: Miridae	cotton	Nyffeler et al., 1987	USA	1
Lep.: Noctuidae	cotton	Gravena & Sterling, 1983	USA	
Lep.: Noctuidae	cotton	Nyffeler et al., 1987c	USA	
Lep.: Noctuidae	cotton	Weems & Whitcomb, 1977	USA	
Lep.: Noctuidae	cotton	McDaniel & Sterling, 1979	USA	
Lep.: Noctuidae	cotton	Nyffeler et al., 1987c	USA	
Lep.: Noctuidae	soybean	Gregory et al., 1989	USA	
Lep.: Tortricidae	pine forest	Eikenbary & Fox, 1968	USA	
Dip.: Agromyzidae	chrysanthenum	Prieto et al., 1980	Colombia	
Acarina: Tetranychidae	–	Mansour et al., 1995	Israel	16.8

Hunting behaviour The Salticidae are diurnal, wandering spiders, stalking prey which comes within their vision and finally leaping on to it (Forster, 1977). In warm, sunny weather they are extremely active creatures on vegetations. Rather polyphagous, but some species mimic ants and are specialised to prey on them (Nentwig, 1986).

Habitat and distribution They occur on the branches and trunks of trees; on low vegetation and in undergrowth, in built-up areas mainly on the walls and fences. Generally common and widespread throughout the region.

Importance in crop protection

– *Species occurring in orchards* Members of this family mentioned from England by Chant, 1956; from The Netherlands by Loomans (1978); from Poland by Koslinka (1967) and Olszak et al. (1994b); from Australia by Dondale (1966); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley (1974); Dondale et al. (1979); Bostanian et al. (1984); from Japan by Hukusima (1961) and Okuma (1973); from USA by McCaffrey and Horsburgh (1980) in apple orchards and by Mansour et al. (1982); Muma (1975); Nakao and Okuma (1958); Rodriguez Almaraz and Contreras Fernandez (1993) from citrus orchards. *Phidippus audax* Hentz is one of the dominant spider in

Table

Philodromidae as predators of pests

Spiders	Common name	Scientific name
Appolophanes margareta Lowrie & Gertsch	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnogh
Appolophanes margareta Lowrie & Gertsch	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnogh
Appolophanes margareta Lowrie & Gertsch	white fir sawfly	Neodiprion abietis Harris
Philodromus aureolus Clerck	fall webworm	Hyphantria cunea Drury
Philodromus aureolus Clerck	fall webworm	Hyphantria cunea Drury
Philodromus dispar Walckenaer	ceanothus leafminer	Tischeria immaculata Braun
Philodromus rufus Walckenaer	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnogh
Philodromus rufus Walckenaer	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnogh
Philodromus rufus Walckenaer	white fir sawfly	Neodiprion abietis Harris
Philodromus speciosus Gertsch	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnogh
Philodromus spectabilis Keyserling	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnogh
Tibellus sp.	black grass bug	Labops hesperius Uhler
Tibellus sp.	black grass bug	Labops hesperius Uhler

several crops in USA (Young and Edwards, 1990; Bumroongsook et al., 1992) including apple orchards (McCaffrey and Horsburgh, 1978). This species preys on *Aphis* sp. and on the rosy apple aphid (*Dysaphis plantaginea*) (McCaffrey and Horsburgh, 1978) in that habitat and on pecan aphid (Bumroongsook et al., 1992). *Paraphidipus marginatus* Walckenaer and *Metaphidipus profercus* Walckenaer were recorded as one of the dominant species from the foliage of apple trees from Canada (Dondale, 1958; Legner and Oatman, 1964) and these species prey on the mites, *T. urticae* and *P. ulmi* (Parent, 1967). *Metaphidippus galathea* Walckenaer is preying on the orchard pests eye-spotted bud worm (*Spilonota ocellana* Schiff.), fall webworm (*Hyphantria cunea*) (Horner, 1972), *Aphis* sp., *Dysaphis plantaginea, Leptothrips mali, Platynota flavidana* (Hom.: Cicadellidae) (McCaffrey and Horsburgh, 1978) blackmargined aphid (*M. caryella*) (Bumroongsook et al., 1992). *Salticus zebraneus* C. L. Koch was found as preying on pear psyllids (*Cacopsylla* spp.) (Angeli et al., 1994). 41% of the collected salticids showed positive precipitin reactions (fed on) the polyphagous leafroller (*Epiphyas postvittana*; Lep.: Tortricidae) in apple orchards in Australia (MacLellan, 1973). *Hentzia palmarum* Hentz common and abundant in apple orchards in Canada (Specht and Dondale, 1960) and preys on apple inhabiting aphids, *Aphis* sp. and *Dysaphis plantaginea* (McCaffrey and Horsburgh, 1978) and on pecan aphid (Bumroongsook et al., 1992).

– *Species occurring in other agroecosystems Phidippus audax* is one of the most dominant spider in cotton fields (Whitecomb et al., 1987; Heiss et al., 1988) and it was reported to prey on cotton pests by many authors (see Table 10). But also preying on the curculionid (*Rhinocyllus conicus* Froelich; Col.: Curculionidae) which is a biological control agents against musk thistle (*Carduus nutans* L.; Asteraceae) (Dowd and Kok, 1981).

9

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Lep.: Lymantriidae	pine forest	Swezey et al., 1991	USA	
Lep.: Lymantriidae	pine forest	Mason & Torgersen, 1983	USA	
Hym.: Diprionidae	pine forest	Swezey et al., 1991	USA	
Lep.: Arctiidae	Acer negundo L.	Groppali et al., 1994	Italy	
Lep.: Arctiidae	Morus alba L.	Groppali et al., 1993	Italy	
Lep.: Tischeriidae	Ceanothus griseus	Fasoranti, 1984	USA	
Lep.: Lymantriidae	pine forest	Swezey et al., 1991	USA	
Lep.: Lymantriidae	pine forest	Mason & Torgersen, 1983	USA	
Hym.: Diprionidae	pine forest	Swezey et al., 1991	USA	
Lep.: Lymantriidae	pine forest	Swezey et al., 1991	USA	
Lep.: Lymantriidae	pine forest	Swezey et al., 1991	USA	
Hem.: Miridae	grassland	Araya & Haws, 1988	Chile	
Hem.: Miridae	grassland	Araya & Haws, 1991	Chile	

Other salticids abundant in paddy fields (Togashi and Taka, 1988). *Paraphidipus marginatus* and *Metaphidipus profercus* were recorded from foliage of soybean from USA (Ferguson et al., 1984).

– *Conclusion* It can be concluded that jumping spiders are very important in crop protection (mainly outside of Europe), but the size of the European species are too small to play major role (Fig. 2).

Tetragnathidae (Tetragnathids)

. *General description* This family is represented in Central Europe by 11 species in two genera (Heimer and Nentwig, 1991). Species of *Tetragnatha* are elongate spiders with long chelicerae and legs. *Pachygnatha* species are of more 'normal' proportions but have large chelicerae which are elongate in males. All have relatively simple epigynes and male palpal organs which are very similar in design and function. The maxillae are longer than broad in all species. The size of these spiders is varies between 3–11 mm (Roberts, 1995).

Hunting behaviour Most species spin orb webs with a small hole in the hub on vegetation, but older spiderlings and adults of *Pachygnatha* abandon web spinning and hunt at ground level. Generally preying on small soft-bodied insects such as aphids, planthoppers, dipterans (Nyffeler et al., 1994b).

Habitat and distribution They occur on low vegetation, bushes and trees sometimes on grasses and leaf-litter in damp habitats. Generally common and widespread in the Europe.

Table

Salticidae as predators of pests

Spiders	Common name	Scientific name
Chrysilla versicolor	tea leafhopper	Empoasca pirisuga
Chrysilla versicolor	tea leafhopper	Empoasca pirisuga
Hentzia palmarum Hentz	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Hentzia palmarum Hentz	soybean looper	Pseudoplusia includens Walker
Hentzia sp.	Royal palm bug	Xylastodoris luteolus
Marpissa tigrina Tikader	citrus psylla	Diaphorina citri Kuwayama
Marpissa ludhianaensis		Brahmaloka sp.
Marpissa sp.	mango jassid	Idioscopus clypealis Lethiery
Marpissa sp.	mango mealybug	Drosicha mangiferae Green
Marpissa sp.	tobacco cutworm	Spodoptera litura F.
Marpissa sp.	khapra beetle	Trogoderma granarium Everts
Marpissa sp.	rice weevil	Sitophilus oryzae L.
Marpissa sp.	lesser grain borer	Rhyzopertha dominica F.
Marpissa sp.	red flour beetle	Tribolium castaneum Herbst.
Marpissa sp.	lesser mealworms	Alphitobius sp.
Metaphidippus aeneolus	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnough
Metaphidippus galathea Walckenaer	pea aphid	Acyrtosiphon pisum Harris
Metaphidippus galathea Walckenaer	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Metaphidippus galathea Walckenaer	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Metaphidippus galathea Walckenaer	corn earworm	Heliothis zea Boddie
Metaphidippus galathea Walckenaer	tobacco budworm	Heliothis virescens F.
Metaphidippus galathea Walckenaer	soybean looper	Pseudoplusia includens Walker
Metaphidippus galathea Walckenaer	Nantucket pine tip moth	Rhacionia frustrana Comstock
Metaphidippus galathea Walckenaer	tobacco budworm	Heliothis virescens F.
Metaphidippus harfordi Peckham	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnough
Myrmaranchne plataleodis Cambridge	spotted stalk borer	Chilo partellus Swinhoe
Phidippus audax Hentz	sweetpotato whitefly	Bemisia tabaci Gennadius
Phidippus audax Hentz	green cereal aphid	Schizaphis graminum Rond.
Phidippus audax Hentz	pea aphid	Acyrthosiphon pisum Harris
Phidippus audax Hentz	jassids	Magicicada spp.
Phidippus audax Hentz	buffalo treehopper	Ceresa bubalus F.
Phidippus audax Hentz	threecornered alfalfa hopper	Spissistilus festinus Say
Phidippus audax Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Phidippus audax Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Phidippus audax Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Phidippus audax Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Phidippus audax Hentz	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Phidippus audax Hentz	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Phidippus audax Hentz	Southern green stink bug	Nezara viridula L.
Phidippus audax Hentz	tobacco budworm	Heliothis virescens F.
Phidippus audax Hentz	fall webworm	Hyphantria cunea Drury
Phidippus audax Hentz	fall webworm	Hyphantria cunea Drury
Phidippus audax Hentz	spotted cucumber beetle	Diabrotica undecimpunctata Barber
Phidippus audax Hentz	spotted cucumber beetle	Diabrotica undecimpunctata Barber

10

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Cicadellidae	tea	Xie, 1993	China	47.6 adults
Hom.: Cicadellidae	tea	Xie, 1993	China	80.5 nymphs
Lep.: Noctuidae	soybean	O'Neil & Stimac, 1988	USA	
Lep.: Noctuidae	soybean	Richman et al., 1980	USA	3.71 larva
	palm trees	Reinert, 1975	USA	
Hom.: Aphalaridae	citrus	Sanda, 1991	India	
Hom.: Fulgoridae	grapevine	Sandana & Sandhu, 1977	India	14.3
Hom.: Cicadellidae	mango	Tandon & Lal, 1983	India	
Hom.: Margarodidae	mango	Tandon & Lal, 1983	India	
Lep.: Noctuidae	–	Rao et al., 1993	India	
Col.: Dermestidae	stored products	Battu et al., 1975	India	
Col.: Curculionidae	stored products	Battu & Dhaliwal, 1975	India	
Col.: Bostrychidae	stored products	Battu & Dhaliwal, 1975	India	
Col.: Tenebrionidae	stored products	Battu & Dhaliwal, 1975	India	
Col.: Tenebrionidae	stored products	Battu & Dhaliwal, 1975	India	
Lep.: Lymantriidae	pine forest	Mason, 1988	USA	
Hom.: Aphididae	–	Horner, 1972	USA	
Hem.: Miridae	cotton	Dean et al., 1987	USA	
Hem.: Miridae	cotton	Breene et al., 1988	USA	
Lep.: Noctuidae	–	Horner, 1972	USA	
Lep.: Noctuidae	–	Horner, 1972	USA	
Lep.: Noctuidae	soybean	Richman, 1980	USA	3.36 eggs
Lep.: Tortricidae	pine forest	Eikenbary & Fox, 1968	USA	
Lep.: Noctuidae	cotton	Lincoln et al., 1967	USA	
Lep.: Lymantriidae	pine forest	Swezey et al., 1991	USA	
Lep.: Pyralidae	maize and sorghum	Sharma & Sarup, 1979	India	
Hom.: Aleurodidae	–	Roach, 1987	USA	
Hom.: Aphididae	barley	Muniappan & Chada, 1970	USA	
Hom.: Aphididae	alfalfa	Howell & Pienkowski, 1971	USA	
Hom.: Cicadellidae	–	Smith et al., 1987	USA	
Hom.: Membracidae	–	Bilsing, 1920	USA	
Hem.: Miridae	alfalfa	Young, 1989a	USA	
Hem.: Miridae	cotton	Dean et al., 1987	USA	
Hem.: Miridae	–	Roach, 1987	USA	
Hem.: Miridae	cotton	Breene et al., 1988	USA	
Hem.: Miridae	cotton	Breene et al., 1990	USA	
Hem.: Miridae	cotton	Young, 1989a	USA	
Hem.: Miridae	cotton	Young, 1989b	USA	
Hem.: Pentatomidae	cotton	Young, 1989b	USA	
Lep.: Noctuidae	cotton	McDaniel et al., 1981	USA	
Lep.: Arctiidae	–	Oliver, 1964	USA	
Lep.: Arctiidae	–	Warren et al., 1967	USA	
Col.: Chrysomelidae	cotton	Young, 1989a	USA	
Col.: Chrysomelidae	–	Roach, 1987	USA	

Table

Spiders	Common name	Scientific name
Phidippus audax Hentz	spotted cucumber beetle	Diabrotica undecimpunctata Barber
Phidippus punjabensis Tikader	spotted stalk borer	Chilo partellus Swinhoe
Phidippus punjabensis Tikader	tobacco cutworm	Spodoptera litura F.
Phidippus punjabensis Tikader	tobacco cutworm	Spodoptera litura F.
Phidippus punjabensis Tikader	jasminum leaf webworm	Nausinoe geometralis Gn.
Phidippus regius Koch	citrus weevil	Diaprepes abbreviatus L.
Phidippus sp.	mango mealybug	Drosicha mangiferae Green
Phidippus sp.	mango jassid	Idioscopus clypealis Leth.
Phidippus sp.	European pine shoot moth	Rhyacionia buoliana Schiff.
Phidippus sp.	walnut caterpillar	Datana integerrima G. & R.
Phidippus sp.	spotted stalk borer	Chilo partellus Swinhoe
Platycryptus undatus DeGeer	Southern pine beetle	Dendroctonus frontalis Zimmermann
Plexippus paykullii Audouin	sugarcane leafhopper	Pyrilla perpusilla Walker
Plexippus paykullii Audouin	hibiscus jassid	Amrasca biguttula biguttula Shir.
Plexippus paykullii Audouin	diamond-back moth	Plutella xylostella L.
Plexippus paykullii Audouin	fall webworm	Hyphantria cunea Drury
Salticus ranjitus Tikader	spotted stalk borer	Chilo partellus Swinhoe
Salticus zebraneus C. L. Koch	fall webworm	Hyphantria cunea Drury
Salticus sp.	pine bark bug	Aradus cinnamomeus Panz.
indet. Salticiade	American sugarcane borer	Diatraea saccharalis F.
indet. Salticiade	carmine spider mite	Tetranychus cinnabarinus Boisduval

Importance in crop protection

– *Species occurring in orchards* Members of this family are reported from England by Chant (1956); from Poland by Olszak et al. (1994b); from Australia by Dondale (1966); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley (1974); Dondale et al. (1979); Bostanian et al. (1984); from Japan by Hukusima (1961); Okuma (1973) and Takeda et al. (1978); from USA by McCaffrey and Horsburgh (1980) in apple orchards and by Mansour et al. (1982); Muma (1975); Nakao and Okuma (1958); Rodriguez Almaraz and Contreras Fernandez (1993); Yan and Wang (1987) from citrus orchards. *Tetragnatha squamata* Karsch is recorded from the foliage of apple trees in Japan (Hukusima, 1961) and constituted 10% of the foliage-dwelling spider fauna. This species mentioned as predator of fall webworm (*Hyphantria cunea*) (Kunimi, 1983). *Tetragnatha versicolor* Walckenaer reported from apple orchards in USA by (Legner and Oatman, 1964) and this species preys on *T. urticae* and *P. ulmi* (Parent, 1967). *Tetragnatha extensa* L. reported as a predator of the mites, *P. ulmi* and *B.*

10 (cont.)

Taxon	Crops	Authors	Countries	Daily consumption
Col.: Chrysomelidae	–	Roach, 1987	USA	
Lep.: Pyralidae	maize and sorghum	Sing & Sandhu, 1976	India	
Lep.: Noctuidae	tobacco	Sitaramaiah et al., 1980	India	
Lep.: Noctuidae	–	Rao et al., 1993	India	
Lep.: Pyralidae	Arabian jasmine	Shukla & Sandhu, 1983	India	
Col.: Curculionidae	citrus, sugarcane	Edwards, 1981	USA	
Hom.: Margarodidae	mango	Tandon & Lal, 1983	India	
Hom.: Cicadellidae	mango	Tandon & Lal, 1983	India	
Lep.: Olethreutidae	pine forest	Juillet, 1961	Canada	
Lep.: Notodontidae	black valnut	Farris & Appleby, 1979	USA	
Lep.: Pyralidae	maize and sorghum	Singh et al., 1975	India	
Col.: Scolytidae	forest	Jennings & Pase, 1986	USA	
Hom.: Lophopiidae	sugarcane	Miah, 1986	Bangladesh	
Hom.: Cicadellidae	hibiscus	Rao et al., 1981	India	
Lep.: Plutellidae	cabbige	Yamada & Yamaguchi, 1985	Japan	
Lep.: Arctiidae	–	Kayashima, 1967	Japan	
Lep.: Pyralidae	maize and sorghum	Sharma & Sarup, 1979	India	
Lep.: Arctiidae	Acer negundo L.	Groppali et al., 1994	Italy	
Hem.: Aradidae	pine forest	Doom, 1981	Netherlands	
Lep.: Pyralidae	sugarcane	Sousa-Silva et al., 1992	Brazil	
Acarina: Tetranychidae	–	Mansour et al., 1995	Israel	10.1

praetiosa (Chant, 1956). *Tetragnatha quadridens* feeds on the light brown apple moth (*Epiphyas postvittana*) in Australia (Dondale, 1966). *T. laboriosa* Hentz preys on pecan aphid (Liao et al., 1984; Bumroongsook et al., 1992), the daily consumption in average of 19.35 aphids a day in the field.

– *Species occurring in other agroecosystems* One of the most important species which is occurring on several crops in USA is *Tetragnatha laboriosa* (Young and Edwards, 1990; McIver and Belnavis, 1986; Provencher et al., 1988; Bumroongsook et al., 1992; Nyffeler et al., 1989; Heiss et al., 1988). This a species is preying mainly on homopterans, hemipterans (Culin and Yeargan, 1982; Nyffeler et al., 1989 LeSar and Unzicker, 1978) including pests (see Table 11). *Tetragnatha mandibulata* (Kamal et al., 1992) and *T. japonica* Boes et Str. (Kamal and Dyck, 1994) are recorded from paddy fields in Bangladesh. This family of spiders extremely sensitive to pesticides (Whitford et al., 1987)

– *Conclusion* This family of spiders mainly feed on homopteran pests (Fig. 3), but their importance because of the high sensitivity to pesticides are rather minor.

Table

Tetragnathidae as predators of pests

Spiders	Common name	Scientific name
Pachygnatha clerck Sundevall	green rice leafhopper	Nephotettix cinctipes Uhler
Pachygnatha clerck Sundevall	small brown planthopper	Laodelphax striatella Fall.
Pachygnatha clerck Sundevall	brown planthopper	Nilaparvata lugens Stal.
Pachygnatha clerck Sundevall	white-backed planthopper	Sogatella furcifera Horvath
Pachygnatha clerck Sundevall	black pine bast scale	Matsucoccus thunbergianae Miller & Park.
Tetragnatha japonica Boes. & Str.	white-backed planthopper	Sogatella furcifera Horvath
Tetragnatha japonica Boes. & Str.	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha javana Thorell	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha javana Thorell	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha laboriosa Hentz	Bird cherry – Oat aphid	Rhopalosiphum padi L.
Tetragnatha laboriosa Hentz	corn leaf aphid	Rhopalosiphum maidis Fitch
Tetragnatha laboriosa Hentz	cotton aphid	Aphis gossypii Glov.
Tetragnatha laboriosa Hentz	cotton aphid	Aphis gossypii Glov.
Tetragnatha laboriosa Hentz	pea aphid	Acyrthosyphon pisum Harris
Tetragnatha laboriosa Hentz	pine cinaran aphids	Cinara spp.
Tetragnatha laboriosa Hentz	cotton fleahopper	Pseudatomoscellis seriatus Reuter
Tetragnatha laboriosa Hentz	cotton fleahopper	Pseudatomoscellis seriatus Reuter
Tetragnatha laboriosa Hentz	cotton leaf worm	Alabama argillacea Hubner
Tetragnatha nitens Audouin	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha nitens Audouin	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha praedonia L. Koch	tea leafhopper	Empoasca pirisuga
Tetragnatha praedonia L. Koch	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha sutherlandi Gravely	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha sutherlandi Gravely	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha versicolor Walckenaer	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnogh
Tetragnatha virescens	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha virescens	brown planthopper	Nilaparvata lugens Stal.
Tetragnatha virescens	Malayan black bug	Scotinophara coarctata F.
Tetragnatha sp.	brown planthopper	Nilaparvata lugens Stal.
indet. Tetragnathidae	sugarcane leaf hopper	Pyrilla perpusilla Walker
indet. Tetragnathidae	European corn borer	Ostrinia nubilalis Hubner
indet. Tetragnathidae	rice leaffolder	Cnaphalocrocis medinalis Guenee

Theridiidae (Comb-footed or cobweb spiders)

General description seventy species represented in the region in 16 genera (Heimer and Nentwig, 1991). One of the majority the tarsal 'comb'of serrated bristles is not visible with a lens, and sometimes not even with a microscope and reduced or absent in males and small species. Another characteristic has an abdominal pattern, but some of the species are uniform greyish or black and resemble small members of the Linyphiidae.

11

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Hom.: Cicadellidae	rice	Okuma et al., 1978	Korea	
Hom.: Delphacidae	rice	Okuma et al., 1978	Korea	
Hom.: Delphacidae	rice	Okuma et al., 1978	Korea	
Hom.: Delphacidae	rice	Okuma et al., 1978	Korea	
Hom.: Margarodidae	pine forest	Kim, 1993	China	
Hom.: Delphacidae	rice	Kamal & Dyck, 1994	Bangladesh	1–2
Hom.: Delphacidae	rice	Cheng, 1989	China	4.2–7.8
Hom.: Delphacidae	rice	Luong, 1987	Vietnam	
Hom.: Delphacidae	rice	Vungsilabutr, 1988	Thailand	
Hom.: Aphididae	cereals	Provencher & Coderre, 1987	Canada	
Hom.: Aphididae	cereals	Provencher & Coderre, 1987	Canada	
Hom.: Aphididae	cotton	Kagan, 1943	USA	
Hom.: Aphididae	cotton	Nyffeler et al., 1989	USA	
Hom.: Aphididae	alfalfa	Howell & Pienkowski, 1971	USA	
Hom.: Lachnidae	pine forest	Fox & Griffith, 1976	USA	
Hem.: Miridae	cotton	Kagan, 1943	USA	
Hem.: Miridae	cotton	Nyffeller et al., 1989	USA	
Lep.: Noctuidae	cotton	Gravena & Sterling, 1983	USA	
Hom.: Delphacidae	rice	Luong, 1987	Vietnam	
Hom.: Delphacidae	rice	Vungsilabutr, 1988	Thailand	
Hom.: Cicadellidae	tea	Chen, 1992	China	
Hom.: Delphacidae	rice	Wu et al., 1993	China	
Hom.: Delphacidae	rice	Rao et al., 1978a	India	
Hom.: Delphacidae	rice	Rao et al., 1978b	India	14
Lep.: Lymantriidae	pine forest	Mason & Torgersen, 1983	USA	
Hom.: Delphacidae	rice	Luong, 1987	Vietnam	
Hom.: Delphacidae	rice	Vungsilabutr, 1988	Thailand	
Hem.: Pentatomidae	rice	Perez et al., 1989	Philippine	
Hom.: Delphacidae	rice	Bastidas et al., 1994	Colombia	0.9–3.5
Hom.: Lophopidae	sugarcane	Miah, 1986	Bangladesh	
Lep.: Pyralidae	corn	Godfrey et al., 1991	USA	
Lep.: Pyralidae	rice	Mun, 1982	Malaysia	

Members of this family exhibit great variety in shape and coloration. The legs have very few spines, and this is a useful character for separating theridiids with an abdominal pattern from the families Tetragnathidae, Araneidae and Linyphiidae. The size of these spiders varies between 1.5–14 mm (Roberts, 1995).

Hunting behaviour Many species of theridiids spin a considerable tangle of criss-cross threads higher up on vegetation which, with use, may develop into quite a dense structure centrally and usually incorporates a retreat for egg laying. The individual

catching threads consist of a strand of silk loosely attached to the substrate (a leaf surface, bark etc.). The loosely attached end has a number of sticky droplets along it. Insects sticking to the droplets struggle, break the attachment of the thread, and find themselves hanging helplessly in the air.

They are very polyphagous predators, but on places where ants are numerous, these form a large part of the diet. They often prey on aphids (Nyffeler et al., 1988) and beetles or cleptoparasites (Nyffeler et al., 1994b).

Habitat and distribution They occur on the foliage of shrubs and trees and on low vegetation, but frequently at ground level under stones. Generally common and widespread in the region.

Importance in crop protection

– *Species occurring in orchards* Members of this family are mentioned from England by Chant (1956); from The Netherlands by Loomans (1978); from Poland by Koslinka (1967) and Olszak et al. (1994b); from USSR by Selivanov (1991); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley (1974); Dondale et al. (1979); Bostanian et al. (1984); from Japan by Hukusima (1961); Okuma (1973) and Takeda et al. (1978); from USA by McCaffrey and Horsburgh (1980) in apple orchards. Theridiids are abundant in apple (Olszak et al., 1994b; Anchipanova and Shternbergs, 1987; Dondale et al., 1979; Bostanian et al., 1984; McCaffrey and Horsburgh, 1978; Selivanov, 1991), in citrus (Carrol, 1980; Mansour et al., 1982; Muma, 1975; Berg et al., 1987; Berg et al., 1992; Nakao and Okuma, 1958; Rodriguez Almaraz and Contreras Fernandez, 1993; Yan and Wang, 1987) in pecan (Liao et al., 1984; Mansour, 1993; Bumroongsook et al., 1992) and in avocado (Mansour et al., 1985) orchards too and feed on *Psylla mali, Aphis pomi* (Anchipanova and Shternbergs, 1987; Selivanov, 1991), *Dysaphis plantaginea, Leptothrips mali* Fitch (McCaffrey and Horsburgh, 1978) and *Epiphyas postvittana* (Lep.: Tortricidae) (MacLellan, 1973; Dondale, 1966), but very sensitive to pesticides (Olszak et al., 1994b).

Theridion octomaculatum (*Coleosoma maculatum*) is an important species occurring on several crops (Dong and Xu, 1984) including apple (Hukusima and Kondo, 1962a; Takeda et al., 1978) and preying on insect pests such as *Aphis gossypii* (Dong and Xu, 1984; Mao and Xia, 1983; Zhang, 1992) (it can consume an average of 21 aphids per day (Zhang, 1992)), pear aphids (*Toxoptera piricola* Matsumura) (Hukusima and Kondo, 1962a), apple leaf-curling aphids (*Myzus malisuctus* Matsumura) (Hukusima and Kondo, 1962a), larvae of noctuids (Dong and Xu, 1984). *Theridion pallens* Blackwall, *Theridion ovatum* Clerck and *Theridion varians* Hahn were reported as predators of the spidermites *P. ulmi* and *B. praetiosa* (Chant, 1956). *T. crispulum* Simon and *T. murarium* Emerton feed on pecan aphid (Bumroongsook et al., 1992).

– *Species occurring in other agroecosystems* The cobweb spiders are abundantly represented in cotton fields in USA (Whitecomb et al., 1963; Heiss et al., 1988). The winter wheat field inhabiting cobweb spiders, particularly the *Theridion bimaculatum* L. (Sunderland et al., 1987) and *Achaearanea riparia* Blackw. are prey on cereal aphids and orthopterans (Nyffeler and Benz, 1988b) (Table 12).

– *Conclusion* This family of spiders is abundant in orchards and they are very polyphagous (Fig. 3), but because of the sensitivity to pesticides their importance is rather minor.

Thomisidae (Crab spiders)

General description Forty-two pecies of the family Thomisidae are known from the region, in 12 genera (Heimer and Nentwig, 1991). The majority of species are rather crablike in appearance, have the first two pairs of legs longer than the rest, and can walk sideways, as well as forwards and backwards. Thomisids have small chelicerae with no large teeth, and prey is sucked dry, rather than mashed up, leaving a perfectly formed husk. Some species have little ceremony before mating and the females usually stand guard over their egg sacs, but frequently die before the spiderlings emerge. The egg sacs themselves may be rather flat, silk structures fastened to vegetation, or may take the form of a woolly ball or papery sac which is guarded on vegetation, on bark or at ground level under stones. The size of these spiders is 2–11 mm (Roberts, 1995).

Hunting behaviour Part of the species are typical 'sit-and-wait' predators; camouflaged in flowers, and ambush visiting insects, have venom which is highly toxic to insects such as bumble bees, which are much larger than the spiders themselves. When an insect approaches the flower, the spider opens wide the first two pairs of legs, and may also subtly realign itself with the prey. Only when the victim is definitely within grasp do the legs fold around, although there may be some almost imperceptible movement as it gets close and perhaps wanders away again. Once gripped, the prey is bitten and quickly dies from the poison.

Others (*Xysticus*) are more active hunters, occurring on low vegetation or at ground level.

Generally they prey on aphids (Pisarenko and Sumarokov, 1983), thysanopterans, beetles, hymenopterans and dipterans (Nyffeler et al., 1994b).

Habitat and distribution They occur in woodland; on bushes, lower branches of trees; on grasses and on flowers, especially white and yellow blooms. Generally common and widespread in the region.

Importance in crop protection

– *Species occurring in orchards* Members of this family are recorded from England by Chant (1956); from The Netherlands by Loomans (1978); from Poland by Olszak et al. (1994b); from Australia by Dondale (1966); from Canada by Dondale (1956); Specht and Dondale (1960); Hagley (1974); Dondale et al. (1979); Bostanian et al. (1984); from Japan by Hukusima (1961) and Okuma (1973); Takeda et al. (1978); from USA by McCaffrey and Horsburgh (1980); in apple orchards and by Mansour et al. (1982); Nakao and Okuma (1958); Rodriguez Almaraz and Contreras Fernandez (1993) from citrus orchards. *Misumenops tricuspidata* F. occurring on apple (11% of the foliage-dwelling spider fauna) (Hukusima, 1961; Takeda et al., 1978) and cotton (Wu et al., 1981; Zhou and Xiang, 1987; Zhang, 1992) and recorded preying on pear psylla (Angeli

Table

Theridiidae as predators of pests

Spiders	Common name	Scientific name
Achaearanea tepidariorum C. Koch	fall webworm	Hyphantria cunea Drury
Achaearanea tepidariorum C. Koch	Kanzawa spider mite	Tetranychus kanzawai Kishida
Achaearanea tepidariorum C. Koch	Kanzawa spider mite	Tetranychus kanzawai Kishida
Coleosoma blandum	thrips	
Euryopsis episinoides Walckenaer	giant looper	Boarmia selenaria Schiff.
Theridion adamsoni Berland	American palm planthopper	Myndus crudus Van Duzee
Theridion goodnightorum Levi	bark beetle	Ips pini Say
Theridion lunatum Oliv.	sycamore lace bug	Corythucha ciliata Say
Theridion lunatum Oliv.	sycamore lace bug	Corythucha ciliata Say
Theridion melanosticum	American palm planthopper	Myndus crudus Van Duzee
Theridion octomaculatum Boes. & Str.	brown planthopper	Nilaparvata lugens Stal.
Theridion octomaculatum Boes. & Str.	brown planthopper	Nilaparvata lugens Stal.
Theridion octomaculatum Boes. & Str.	pink mite	Acaphylla theae Watt.
Theridion redimitum L.	sycamore lace bug	Corythucha ciliata Say
Theridion takayense Saito	fall webworm	Hyphantria cunea Drury
Theridion volubile Keyserling	cotton leafworm	Alabama argillacea Hubner
Theridion volubile Keyserling	cotton leafworm	Alabama argillacea Hubner
Theridion sp.	giant looper	Boarmia selenaria Schiff.
Theridion sp.	cotton leafperforator	Bucculatrix thurberiella Busck
Theridion sp.	sorghum mite	Oligonychus indicus Hirst
Theridula gonygaster Simon	cotton leafworm	Alabama argillacea Hubner
Theridula gonygaster Simon	cotton leafworm	Alabama argillacea Hubner
Theridula sp.	cotton leafperforator	Bucculatrix thurberiella Busck
Theridula sp.	sorghum mite	Oligonychus indicus Hirst
indet. Theridiidae	spruce budworm	Choristoneura fumiferana Clem.
indet. Theridiidae	carmine spider mite	Tetranychus cinnabarinus Boisduval

et al., 1994), apple-inhabiting aphids such as pear aphids (*Toxoptera piricola*) and apple leaf-curling aphids (*Myzus malisuctus*) (Hukusima and Kondo, 1962a), fall webworm (*H. cunea*) (Kayashima, 1967; Kunimi, 1983) cotton aphid (*A. gossypii*) (Zhang, 1992) and on the American bollworm (*H. armigera*) (Wu et al., 1981). In the laboratory one *M. tricuspidatus* could consume 23–44 aphids a day (Zhang, 1992). Thomisids are abundant on apple in Australia too and 53% of the collected crab spiders fed on the light brown apple moth (*Epiphyas postvittana*) (MacLellan, 1973; Dondale, 1966). *Misumenops asperatus* Hentz, *Misumena vatia* Clerck and *Xysticus emertoni* Keyserling were reported as predators of red and two spotted spidermites (Parent, 1967). *Misumena vatia* was recorded by (Chant, 1956) as predator of the bryobia mite (*Bryobia praetiosa*) too. McCaffrey and Horsburgh (1978) mentioned that *Misumenops oblongus* Keyserling preys on apple aphids such as *Aphis* sp. and *Dysaphis plantaginea* and on pecan aphids

12

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Lep.: Arctiidae	–	Kunimi, 1983	Japan	
Acarina: Tetranychidae	grapevine	Ashihara et al., 1987	Japan	
Acarina: Tetranychidae	grapevine	Ashihara et al., 1992	Japan	
Thys.: Thripidae	potato	Amalin & Barrion, 1990	Philippine	
Lep.: Geometridae	avocado	Wysoki & Izhar, 1980	Israel	
Hom.: Cixiidae	coconut	Howard & Edwards, 1984	USA	
Col.: Scolytidae	forest	Jennings & Pase, 1975	USA	
Hem.: Tingidae	Platanus sp.	Tavella & Arzone, 1987	Italy	
Hem.: Tingidae	Platanus sp.	Balarin & Polonec, 1984	Yugoslavia	3.1
Hom.: Cixiidae	coconut	Howard & Edwards, 1984	USA	
Hom.: Delphacidae	rice	Cheng, 1989	China	4.2–7.8
Hom.: Delphacidae	rice	Ge & Chen, 1989	China	0.25–1.88
Acarina: Eriophyidae	tea	Zhao & Hou, 1993	China	
Hem.: Tingidae	Platanus sp.	Howard & Edwards, 1984	Italy	
Lep.: Arctiidae	–	Kunimi, 1983	Japan	
Lep.: Noctuidae	cotton	Gravena & Pazetto, 1987	Brazil	
Lep.: Noctuidae	cotton	Gravena & Da-Cuhna, 1991	Brazil	
Lep.: Geometridae	avocado	Wysoki & Izhar, 1980	Israel	
Lep.: Lyonettidae	cotton	Herrera & Alvarez, 1979	Peru	
Acarina: Tetranychidae	sorghum	Manjunatha, 1989	India	
Lep.: Noctuidae	cotton	Gravena & Pazetto, 1987	Brazil	
Lep.: Noctuidae	cotton	Gravena & Da-Cuhna, 1991	Brazil	
Lep.: Lyonettidae	cotton	Herrera & Alvarez, 1979	Peru	
Acarina: Tetranychidae	sorghum	Manjunatha, 1989	India	
Lep.: Tortricidae	pine forest	Loughton et al., 1963	USA	
Acarina: Tetranychidae	–	Mansour et al., 1995	Israel	9.5

(*M. caryella*) (Liao et al., 1984; Bumroongsook et al., 1992) in USA. An unidentified crab spider is mentioned as natural enemy of the green apple aphid (*Aphis pomi*) in USSR (Melnyik et al., 1976). *Diaea* sp. is recorded as natural enemy of apple-inhabiting leafrollers in New Zealand (Baker, 1983). The crab spider *Xysticus punctatus* Keyserling was observed feeding on the mirids, *Lygus lineolaris* Palisot de Beauvois and *Lygocoris communis* Knight on apple in Canada (Arnoldi et al., 1991). *Misumenops rubrodecorata* Millot was observed as predator of citrus psylla (*Trioza erytreae*) in citrus orchards managed under integrated control programmes in South Africa (Berg et al., 1987; 1992).

– *Species occurring in other agroecosystems* Members of this family are abundant in many agroecosystems, and feed on many insect pests as shown in Table 13.

Table

Thomisidae as predators of pests

Spiders	Common name	Scientific name
Misumena vatia Clerck	cabbage looper	Trichoplusia ni Hubner
Misumena vatia Clerck	spotted cucumber beetle	Diabrotica undecimpunctata Barber
Misumena sp.	colorado potato beetle	Leptinotarsa decemlineata Say
Misumenoides formocipes Walck.	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Misumenops celer Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Misumenops celer Hentz	cotton fleahopper	Pseudatomoscelis seriatus Reuter
Misumenops celer Hentz		Lygus sp.
Misumenops celer Hentz		Lopidea nigridea Uhler
Misumenops celer Hentz	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Misumenops celer Hentz	velvetbean caterpillar	Anticarsia gemmatalis Hubner
Misumenops celer Hentz	American bollworm	Heliothis armigera Hubner
Misumenops celer Hentz	Southwestern corn borer	Diatraea grandiosella Dyar
Misumenops lepidus Thorell	black grass bug	Labops hesperius Uhler
Misumenops lepidus Thorell	black grass bug	Labops hesperius Uhler
Misumenops lepidus Thorell	Douglas-fir tussock moth	Orgyia pseudotsugata McDunnough
Misumenops lepidus Thorell	white fir sawfly	Neodiprion abietis Harris
Misumenops tricuspidata F.	flower thrips	Frankliniella intonsa Trybom
Misumenops tricuspidata F.	cotton aphid	Aphis gossypii Glov.
Misumenops tricuspidata F.	green leaf bug	Lygocoris lucorum Meyer
Misumenops tricuspidata F.		Adelphocoris suturalis Jakovlev
Misumenops tricuspidata F.		Adelphocoris suturalis Jakovlev
Misumenops tricuspidata F.	diamond-back moth	Plutella xylostella L.
Misumenops sp.	tarnished plant bug	Lygus lineolaris Palisot de Beauvois
Misumenops sp.	cotton leafworm	Alabama argillacea Hubner
Misumenops sp.	cotton leafworm	Alabama argillacea Hubner
Misumenops sp.	walnut caterpillar	Datana integerrima G. & R.
Thomisus cherapunjeus Tikader	spotted stalk borer	Chilo partellus Swinhoe
Thomisus lobodus Tikader	tobacco cutworm	Spodoptera litura F.
Thomisus onustus Walckenaer	giant looper	Boarmia selenaria Schiff.
Thomisus projectus Tikader	tobacco cutworm	Spodoptera litura F.
Thomisus shivajiensis Tikader	bean butterfly	Lampides boeticus L.
Thomisus sp.	thrips	
Thomisus sp.	rose aphid	Macrosiphum rosae L.
Xysticus cunctator Thorell	black grass bug	Labops hesperius Uhler
Xysticus cunctator Thorell	black grass bug	Labops hesperius Uhler
Xysticus cunctator Thorell	Douglas-fir tussock moth	Orgyia pseudotsagata McDunnough
Xysticus cunctator Thorell	white fir sawfly	Neodiprion abietis Harris
Xysticus kochii Thorell	colorado potato beetle	Leptinotarsa decemlineata Say
Xysticus kochii Thorell	colorado potato beetle	Leptinotarsa decemlineata Say
Xysticus kochii Thorell	cereal leaf beetles	Oulema spp.
Xysticus sp.	pine bark bug	Aradus cinnamomeus Panz.
Xysticus sp.	alfalfa weevil	Hypera postica Gylh.

13

occurring in other ecosystems

Taxon	Crops	Authors	Countries	Daily consumption
Lep.: Noctuidae	crimson clover	Lockley et al., 1989	USA	
Col.: Chrysomelidae	crimson clover	Lockley et al., 1989	USA	
Col.: Chrysomelidae	potato	Cappaert et al., 1991	USA	
Lep.: Noctuidae	soybean	Gregory et al., 1989	USA	
Hem.: Miridae	cotton	Kagan, 1943	USA	
Hem.: Miridae	cotton	Breene et al., 1990	USA	
Hem.: Miridae	cotton	Plagens, 1983	USA	
Hem.: Miridae	lupin	McIver & Lattin, 1990	USA	
Lep.: Noctuidae	soybean	Gregory et al., 1989	USA	
Lep.: Noctuidae	soybean	O'Neil & Stimac, 1988	USA	
Lep.: Noctuidae	cotton	Kagan, 1943	USA	
Lep.: Pyralidae	corn	Knutson & Gilstrap, 1989	USA	
Hem.: Miridae	grassland	Araya & Haws, 1988	Chile	
Hem.: Miridae	grassland	Araya & Haws, 1991	Chile	
Lep.: Lymantriidae	pine forest	Swezey et al., 1991	USA	
Hym.: Diprionidae	pine forest	Swezey et al., 1991	USA	
Thys.: Thripidae	white clover	Murai, 1988	Japan	
Hom.: Aphididae	cotton	Zhou & Xiang, 1987	China	42.8
Hem.: Miridae	cotton	Cao, 1986	China	
Hem.: Miridae	cotton	Liu & Gu, 1990	China	2.88–4.2
Hem.: Miridae	cotton	Cao, 1986	China	
Lep.: Plutellidae	cabbige	Yamada & Yamaguchi, 1985	Japan	
Hem.: Miridae	cotton	Young, 1989	USA	
Lep.: Noctuidae	cotton	Gravena & Sterling, 1983	USA	
Lep.: Noctuidae	cotton	Gravena & Pazetto, 1987	Brazil	
Lep.: Notodontidae	black walnut	Farris & Appleby, 1979	USA	
Lep.: Pyralidae	maize and sorghum	Singh & Sandhu, 1976	India	
Lep.: Noctuidae	tobacco	Sitaramaiah et al., 1980	India	
Lep.: Geometridae	avocado	Wysoki & Izhar, 1980	Israel	
Lep.: Noctuidae	tobacco	Sitaramaiah et al., 1980	India	
Lep.: Lycaenidae	leguminosae	Singh & Mavi, 1984	India	
Thys.: Thripidae	–	Veer, 1984	India	
Hom.: Aphididae	rose	Raychaudhuri et al., 1979	India	
Hem.: Miridae	grassland	Araya & Haws, 1988	Chile	
Hem.: Miridae	grassland	Araya & Haws, 1991	Chile	
Lep.: Lymantriidae	pine forest	Swezey et al., 1991	USA	
Hym.: Diprionidae	pine forest	Swezey et al., 1991	USA	
Col.: Chrysomelidae	potato	Koval, 1976	USSR	
Col.: Chrysomelidae	potato	Sorokin, 1982	USSR	
Col.: Chrysomelidae	cereals	Szabolcs & Horváth, 1991	Hungary	
Hem.: Aradidae	pine forest	Doom, 1981	Netherlands	
Col.: Curculionidae	alfalfa	Ouayogode & Davis, 1981	USA	

– *Conclusion* Crab spiders are abundant in orchards and prey on many orchard pests. It can be concluded that this family of spiders probably are able to play an important role in this habitat (see Fig. 2 too).

Conclusions

It can be concluded that:

– According to our criteria for measuring the usefulness of spiders (abundance; hunting tactics; diet) the following 10 families of spiders have importance in agriculture (Araneidae; Clubionidae; Linyphiidae; Lycosidae; Oxyopidae; Philodromidae; Salticidae; Tetragnathidae; Theridiidae; Thomisidae). Members of all of these occur in European orchards.

– Spiders prey on all kind of pest species (homopterans, heteropterans, orthopterans, thysanopterans, lepidopterans, coleopterans, hymenopterans, dipterans and mites).

– The orchard inhabiting spiders belonging to 4 different groups, but theoretically only 3 will possible play a role as predators of orchard pests (see Fig. 4 too):

– foliage dwelling wandering spiders
(Clubionidae; Oxyopidae; Philodromidae; Salticidae; Thomisidae)
– foliage dwelling web-building spiders
(Araneidae; Linyphiidae; Tetragnathidae; Theridiidae)
– ground dwelling wandering spiders
(Lycosidae)
– [ground dwelling web-building spiders]

– The foliage dwelling wandering spiders feed mainly on caterpillars (larvae of lepidopteran pests), but also on homopterans, heteropterans and especially the young spiders on mites (Figs 1, 2, 4).

– The foliage dwelling web-building spiders prey mainly on homopterans and lepidopterans (Figs 3, 4).

– Almost no experimental data exist about the importance of ground dwelling spiders in orchards, but in other agroecosystems they are one of the most important predators (Fig. 4).

The Effect of Chemical Treatments on Performance of Spider Communities

The pesticide application is the most important factor influencing spider assemblages in the field. This effect on performance of spider communities under different regimes of pesticides in different management systems (conventional versus IPM) is discussed.

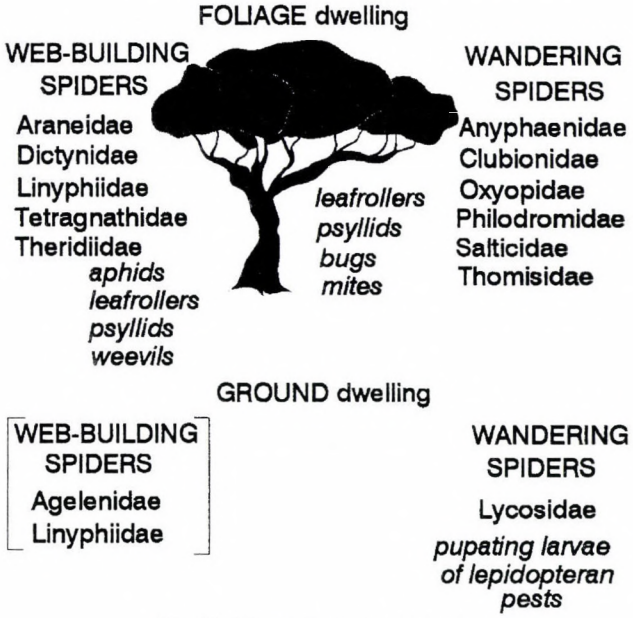

Fig. 4 Spider-orchard pest interactions

Pesticide effect on spiders

Nowadays it is well known that the spider fauna of sprayed and unsprayed fields differ completely (Chant, 1956; Hukusima and Kondo, 1962b). Insecticide treatments disturb (Basedow et al., 1985) and prevent normal build up of the population peaks (Mansour, 1987). Olszak et al. (1994b) found that some species, probably the most sensitive ones, disappeared from the treated orchard. The different spider groups react differently to pesticide treatments (e.g. the ratio of web-building and hunting spiders changed after the treatments). Many authors stated that hunting spiders are more sensitive to pesticides (Chant, 1956; Specht and Dondale, 1960; Legner and Oatman, 1964; Bostanian et al., 1984), others (McCaffrey and Horsburgh, 1980; Olszak et al., 1994b) found opposite results. It can be concluded that in the time when the first group of authors investigated the effect of pesticides on spiders the pesticide usage was completely different from the second group of authors. Because the pesticide usage has been changed significantly since the 1970s. Earlier the chlorinated hydrocarbons (HCH), lime sulphur, lead arsenate, nicotine dominated which changed to organophosphates (OP) and synthetic pyrethroids (SP) in conventional systems and to insect growth regulators (IGR) and "natural" pesticides (compounds of biological origin) in IPM systems.

The spider web is an efficient collector of the agrochemicals (Samu et al., 1992). The collecting rate depends on the droplet size (smaller droplets can easier be adsorbed). This observation agrees with Olszak et al. (1994b) who found that in sprayed orchards

the family Theridiidae was most affected by chemicals. Especially the orb-weavers who recycle their web every day are generally very susceptible to insecticides (Whitford et al., 1987).

The effect of pesticides used in IPM on spiders has been investigated by laboratory and field spraying. Laboratory investigations on pesticide effect on spiders were performed by many authors. Mansour (Hassan et al., 1994) tested the effect of diflubenzuron (IGR) on *Cheiracanthium mildei* and it was found that this insecticide caused 95–99% mortality in this species. Field applications of *Bacillus thuringiensis* preparates showed that the concentration of a normal application (2%) was harmless to spiders although caused 71–72% mortality on larvae of *Heliothis armigera* (Umarov et al., 1975; Sklyarov, 1983; Shiryaeva and Savin, 1988) to which it was applied. IGRs especially diflubenzuron in case of foliage application appeared to be harmless to the ground dwelling spider fauna (Winter, 1979; Martinat et al., 1993), but was harmful to the foliage dwelling spiders (Pan and Zhao, 1990; Wolfenbarger and Nemec, 1991).

The effect of three commercial pesticides fenvalerate (SP), endosulfan (HCH) and pyrazophos (OP), in Europe commonly used against cereal pests, on two dominant cereal field inhabiting spider species *Pardosa agrestis* and *Erigone atra* were evaluated by Mansour et al. (1992).

The effect of the pesticides has influenced by the substrate on which it was sprayed. Fenvalerate was more toxic than the other two pesticides and had a longer residual effect on all spiders when tested on moistened sand than on filter paper. Endosulfan had a high initial toxicity on sand, but was more toxic on filter paper for *Pardosa*. Pyrazophos was non-toxic to the spiders tested, regardless of the substrate. The some general conclusions can be drawn from the experiment of Mansour and Nentwig (1988) who determined the susceptibility of 4 spider species to 30 pesticides (16 insecticides, 4 acaricides, 1 herbicide and 9 fungicides). *Philodromus aureolus* (a hunting spider) from Germany was completely resistant to all the compounds tested (a similar result was found by Polesnyi, 1990 in Austria). While *Argyope argentata* (from Panama), *Linyphia triangularis* (from Germany) (both web-building spiders) and *Cheiracanthium mildei* (from Israel) (a wandering spider) showed medium to high susceptibility. The effects of insecticides varied widely from no mortality (mostly compounds of biological origin) and medium mortality (pyrethrins and organophosphorus and carbamate compounds) to high mortality (cyclo compounds). Most acaricides were highly toxic to spiders, whereas the herbicides and fungicides were not. These are probably the basic effects of pesticides which will be modified by many factors in the field. From observations in the lab and field it can be concluded that the different factors which can modify the basic effect of pesticides in the field are:

The *daily activity* of spiders that influenced their reaction to pesticide treatments. Some of the pyrethroids and OP's (e.g. deltamethrin, DDVP) which are generally highly toxic to spiders have short (within 8 hours) contact toxicity. In case of normal pesticide application (in the morning) the nocturnal hunting spiders (e.g. Clubionidae) are sheltered (spending the daytime in silken chamber or under the loose bark etc.), and protected from insecticides. When the spiders become active again the insecticide is not

toxic anymore. Olszak et al. (1994b) found that clubionid spiders were the least affected by pesticides, although the laboratory observations indicate that they are susceptible to these pesticides.

The *habitat*. The active ingredients of herbicides are generally non-toxic to spiders, but they destroy the habitat of spiders which can cause an indirect population decrease (Krause, 1987). On the other hand by diversification of the habitat by multicropping or mulching the number of ground dwelling spiders is augmented. It more or less protects spiders from the pesticides (Koslinka, 1967; Altieri et al., 1985: Altieri and Schmidt, 1986; Nurindah, 1988; Riechert and Bishop, 1990).

The *lack of prey*. After insecticide treatments prey is dead or decreased in number which also influences the spider abundance (Krause, 1987).

Different soil types. The higher percentage of clay, silt or organic matter can decrease the effects of the pesticides on spiders (Heimbach et al., 1992). This effect is very obvious with organochlorines, less distinct with organophosphates and not present with pyrethroids. This might be due to the lipophilic character of the pesticides (Heimbach et al., 1995).

Temperature and *humidity*. High temperature and low air humidity can cause higher mortality (Everts, 1990; Everts et al., 1991).

Neurological disturbance. The walking speed of spiders decreases by exposure to deltamethrin (Jagers op Akkerhuis, 1993) and this is followed by a higher predation by carabids (Everts et al., 1991).

Luczak (1979) and Mansour et al. (1983) stated that spiders are generally more tolerant to pesticides that most of the predators.

Conclusions

It can be concluded that:

– The effect of pesticides on the different groups of spiders has changed in the course of years by the change of pesticides.

– The spiders are generally more tolerant to pesticides than most of the other predators.

– Chlorinated hydrocarbons (esp. used before the 70s) are highly toxic to spiders.

– Both organophosphates and pyrethroids are toxic to spiders but this effect in some extends can be modified in the field.

– IGRs are also moderately toxic to spiders.

– Natural insecticides (eg. B. t. preparates) are non-toxic to spiders.

The Predatory Potential of Orchard-Inhabiting Spiders

It can be concluded that most of the literature mentioned in Chapter 2 concernes qualitative observations or laboratory investigations on prey consumption by spiders. These data cannot be applied directly to field situations (Nyffeler, 1982). Hunting spiders feed much less in the field than in the laboratory (Nyffeler and Breene, 1990). In this chapter the possibilities for evaluation of usefulness of spiders is discussed.

Theoretical background

A predator has the potential to regulate a prey population only if the predator responds to increases in prey density by inflicting a higher mortality percentage (Wise, 1993). Whether or not a population of predators causes such density dependent mortality depends upon the nature of the functional and numerical responses, which concept was introduced by Solomon (1949) and developed further by Holling (1959a, 1959b, 1961, 1965, 1966). The functional response is defined as the change in the rate at which an individual predator captures prey as prey density changes. The numerical response is the change in population density of predators as a function of changing prey density. Together these components of a predator's response to changes in prey density comprise the total response, which is expressed as a fraction of the prey population consumed.

Holling (1959a, b) defined three basic types of functional responses:

Type I shows a linear rise in the number of prey captured until a plateau is reached. (Animals having an automatic prey capture mechanism e.g. filter feeders, web-building spiders.) (Nakamura, 1977)

Type II is a saturation curve. This is the most common pattern of functional response seen in invertebrate predators (Nakamura, 1977).

Type III is an S-shaped or sigmoid curve, observed mainly in vertebrate predators (Nakamura, 1977).

Most of the spiders have Type II response, but Nakamura (1977) found Type III responses by wolf spiders Haynes and Sisojevic (1966) by a crab spider.

A predator has a potential to regulate its prey even in the absence of a Type III functional response if it exhibits a numerical response. Spiders show both aggregational and reproductive numerical responses to increases in prey densities in nature. Spiders have been shown to aggregate in habitats with higher prey densities, and temporal increases in prey density within a particular habitat can be correlated with increases in rates of spider reproduction (Reddy, 1991).

How spiders can be evaluated?

An overview of the methods which can be useful for evaluation of spiders is given, divided into field and laboratory methods.

Methods in the field

1. Introduction and augmentation

The introduction of new spider species to the field has not been performed extensively until now, because they are too generalist predators. The existing species can be mass-reared and released to control pest species. Wang and Zhou (1984) in China and Thang et al. (1990) in the Philippines developed a method to mass-rear the wolf spider, *Lycosa pseudoannulata* to control rice pests. In China 200 000 spiders were released to paddy fields. Zhao and Zhao (1983) did manage to rear the spider, *Erigonidium graminicolum* on artificial diet.

Several methods exist to increase the number of spiders. They can be divided into indirect and direct methods. The most important indirect methods are habitat management, intercropping, improving edge effect, mulching and using corrugated fiberboard belts as overwintering place described (Mansour et al., 1983; Whitcomb, 1987; Altieri et al., 1985; Altieri and Schmidt, 1986; Desender et al., 1989; Fye, 1985; Makarov and Tarabaev, 1990; Mangan and Byers, 1989; Mizell and Schiffhauer, 1987; Riechert and Bishop, 1990). Direct methods are releasing alternative foods (e.g. *Drosophila flies*) (Kobayashi, 1975), placing egg sacs of spiders into crops (Brignoli, 1983) or releasing mass-rear spiders. Some of these techniques can be useful in orchards too.

2. Removal of natural enemies

A.) Specific small-scale inclusion and exclusion techniques (cages, barriers or hand removal)

These techniques in small-scale level based on specific prey-predator relationships. Clark and Grant (1968) were the first to demonstrate experimentally that spiders can have a strong stabilising influence on prey. They located four 13 m^2 areas 'chosen for structural uniformity' in a beech-maple forest. They removed as many spiders as possible from one area, which was enclosed with a sheet-metal fence, by sieving litter over a one week period. Another fenced area in which litter was sieved but spiders were not removed served as a control. Two open areas served as controls to assess the effect of enclosing the plots. One of these open controls was undisturbed and litter was sieved in the other. Plots were sampled by taking ten 0.09 m^2 samples from each area on several sampling days over a 10-week period. Each plot had been sampled once before the week of the perturbation. Over the course of the study the average number of spiders per sample in the removal plot was approximately half the nunber in the three control plots. Numbers of springtails, a major prey of spiders, were highest in the removal plot.

Mansour et al. (1985) and Mansour and Whitecomb (1986) performed experiments to evaluate the role of spiders in controlling pest species (based also on removal) and they obtained as result that on branches where spiders were removed the pests caused significantly higher damage compared with the control.

B.) Non-specific large-scale removal of natural enemies (by insecticides)

The controlling effect of natural enemies can be investigated by removal of them with insecticides or acaricides (spiders). These techniques are not very specific, but can be used to get indications on the total role of natural enemies. See fruit tree red spider mite problem (Chant, 1966; Rabbinge, 1976).

3. Prey enrichment

Kobayashi (1975) increased the number of spiders in paddy fields by releasing fruit flies (Drosophila) and the number of rice pests decreased. But the correlation between the number of spiders and the number of released fruit flies was not strong.

4. Direct observation

One of the best examples that spiders are able to influence pest densities comes from studies of rice paddies. The wolf spider *Lycosa pseudoannulata* is the dominant spider in rice fields and has been studied by many authors (see Table 7). The diet of this lycosid consists primarily of two major pests of rice, the green rice leafhopper (GRL) and the brown planthopper (BPH). Estimated rates of predation by *L. pseudoannulata* upon these pests ranged from a few percent to 100%. The high mortality rates make it reasonable to predict that wolf spiders depress homopteran populations. Kenmore et al. (1984) sprayed one rice field with insecticide and left another field 500 m away, as a control. Densities of BPH were 800-times higher on the sprayed field; densities of spiders and veliid bugs (also predators of BPH) were lower in the treated field. Jones (1981) reported that Chinese have used straw bundles as shelters for spiders to conserve their numbers during irrigation of rice paddies. This approach to spider conservation was associated with a 50–60 decline in pesticide use in 1977 over a 3000 ha region of Hunan Province.

5. Determination of prey predator relationships

A.) Gut analysis of predators (electrophoresis, monoclonal antibodies, ELISA)

The gut analysis of spiders by serological methods has been performed by many authors (e.g. Angeli et al., 1994; Chen and Gao, 1992; Cherril and Begon, 1989; Sunderland et al., 1987). They got evidence that pest species constitute a part of the spiders' diet.

B.) P^{32}-radiolabelling technique

The predator complex of a given pest species can be investigated by this method. The existing results show that spiders constituted a large part of the predators occurring in agroecosystems (e.g. Clark and Glick, 1961; McDaniel et al., 1981; Gravena and Sterling, 1983).

6. Interaction with other beneficial agents

Generalist predators especially spiders prey on other beneficial organisms such as ladybirds, lacewings, other spiders etc. Most of the existing literature about spider predation on beneficial insects are laboratory observations (e.g. McCaffrey and Horsburgh, 1978; Sengonca and Klein, 1988; Heong et al., 1989), but some other field observations definitely indicate that spider sometimes feed on beneficials too (Krämer, 1961; Temerak, 1981; Nuessly and Goeden, 1983; Nyffeler and Benz, 1988b). According to Nyffeler et al. (1994b) this may help to survive periods of food shortage. On the other hand, Ghorpade (1979) reported that ladybirds (*Menochilus sexmaculatus* F., *Micraspis cardoni* Weise, *Jauravia dorsalis* Weise) preyed on the spider *Sparassus lamarcki* Latr. too.

Green lacewings have a special escape strategy (described by Masters and Eisner, 1990) from orb webs (escaping rate 90%).

Methods in the laboratory

1. Determination of prey acceptance

These experiments have been carried out by many authors (see Tables) to determine which pest species are acceptable as food by spiders in given agroecosystems. In most of the cases the daily consumption in laboratory at constant temperatures are also given. But these data cannot be applied directly to the field situation. Success ratio experiments in relation to hunger give more information about the real situation.

2. Assessment of potential feeding capacity (max. gut content, ingestion and digestion rates etc.)

To assess the potential role of spiders information is needed about the potential food consumption. This may be assessed by measuring the mealsize, the relative rate of gut emptying, assimilation and respiration rate (Bogya and Mols, 1995).

3. Reproduction experiments with a specific pest as prey

To assess the nutritional value of a given pest species for spiders or in other words to determine whether the given pest species is essential food for spiders may be difficult to investigate. Many investigations show that spiders on monodiet did not reach adulthood (e.g. Uetz et al., 1992).

4. Prey preference experiments

The aim of these experiments to rank potential prey types in order. It offers information on the chance a pest has to be killed by a predator when other prey is also available (Provencher and Coderre, 1987; Heong et al., 1990; Toft, 1995).

5. Searching and predatory behaviour

It gives information on the performance of a spider to different densities and distribution of the prey (leads to assess most of functional response curves).

The complete searching and predatory behaviour as has been described by Mols, (1987, 1988, 1993) for a carabid is lacking for spiders.

6. Simulation models can show potentials for biological control

Simulation models as a combined results of the laboratory and field experiments may be used to evaluate theoretically the role of spiders. Good models are lacking.

Conclusions

It can be concluded that:

– The correct evaluation of spiders as biological control agents can be obtained by following the above-mentioned list. Some of the mentioned experiments alone (e.g. laboratory prey consumption experiments) are not enough to take a decision.

– The number of spiders in the field can be augmented by several methods (e.g. intercropping, mulching, habitat management).

– Experiments and observations indicate that spiders are a part of the predator complex of pest species on many crops.

– But quantitative data (about searching and predatory behaviour; potential and actual feeding capacity) concerning their predatory potential are hardly available.

Discussion

Spiders are polyphagous predators, but most of the cases they show high preference to types of prey. There are many advantages of this hunting behaviour. They are preying on a wide variety of insect pests and in case of low level of pest densities they can switch to alternative prey. The disadvantage of this hunting behaviour is that only a fraction of their diet consist of pest species which is very variable. Other beneficial organisms seems to be less important for spiders as prey than phytophagous insects. In orchard ecosystems probably the foliage dwelling wandering spiders are the most important in crop protectional point of view. Their hunting behaviour suggest that maybe they are important as predators of many pest species. The web-building spiders mainly specialized to catch flying insects, this behaviour is also suitable for plant protection. The ground dwelling spiders play less important role in controlling pest species in orchards, because most of the orchard pests are living in the canopy of fruit trees.

It can be concluded that spiders because of their high abundance, their constant presence and their predatory capacity belong to the most important predators of many pest species. Although their impact on pest insects are strongly depends on the pesticide usage. In addition to the pesticide usage, other cultural methods (e.g. intercropping,

irrigation) can augment the number of spiders in agroecosystems. The establishment of suitable overwintering places (e.g. treebands, hedge around the orchards) are also very important. In IPM (or organic) management systems, where the pesticide use is low (together with the cultural methods), spiders have a considerable impact on the reduction of number of pest organisms. However still many more carefully controlled field experiments are needed to test this hypothesis.

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Contents

DISEASES

Simple and rapid detection of carnation etched ring virus by polymerase chain reaction
L. Palkovics and E. Balázs 161

Reaction of unknown *Solanum stoloniferum* Schlechtd. et bche and *Solanum demissum* Lindl. accessions to the tuber necrosis strain of potato Y *Potyvirus* (PVY^{NTN})
Z. Bősze, G. Kazinczi and J. Horváth 169

The effect of virus infection on the growth and photosynthetic pigment content of the virophilous Jimson weed (*Datura stramonium* L.)
G. Kazinczi, J. Horváth and M. Pogány 175

Comparison of ultrastructural changes of *Nicotiana benthamiana* infected with three different viruses
A. Almási, M. Ekés and R. Gáborjányi 181

Changes in total phenols and some oxidative enzymes in cowpea leaves infected with yellow mosaic virus
R. S. Sangwan, G. P. Lodhi and Y. P. Luthra 191

Induction of resistance to tomato spotted wilt virus in mungbean (*Vigna radiata* (L.) Wilczek) by antiviral principles
K. Manickam and P. Narayanasamy 199

Fusarium solani as a cause of vessel discolouration in declining oak
Gy. Bohár 205

Ceratocystis erinaceus: A new endophyte in the heartwood of oak
Gy. Bohár 213

A spatio-temporal analysis of fungal pathogens on reed in natural habitats
Rita Bán, G. Fischl and F. Virányi 219

Synthesis of phytoalexins in sugarcane in response to infection by *Colletotrichum falcatum* Went
R. Viswanathan, D. Mohanraj, P. Padmanaban and K. C. Alexander 229

INSECT PESTS

Feeding and survival of the European corn borer larvae on seventeen inbred lines of maize
Ľ. Cagaň and P. Kmec 239

Resistance in wild *Cucumis* species to twospotted spider mite (*Tetranychus urticae*)
A. Lebeda 247

Diversity and community structure of spiders of alfalfa fields and grassy field margins in South Hungary
F. Samu, G. Vörös and Erika Botos 253

After introduction of *Frankliniella occidentalis* in Europe: Prevention of establishment of *Thrips palmi* (Thysanoptera: Thripidae)
G. Vierbergen 267

Egyptian Aleyrodidae
S. Abd-Rabou 275

Psocoptera (Insects) fauna of stored maize
Irma Kalinović and Marija Ivezić 287

PESTICIDE CHEMISTRY

Comparative studies on enzyme-immunoassays for triazole fungicides tetraconazole and myclobutanil
A. Székács, S. Cairoli, H. M. Le and S. Pagani 293

Book Review 303

Acta Phytopathologica et Entomologica Hungarica 31 (3–4), pp. 161–168 (1996)

Simple and Rapid Detection of Carnation Etched Ring Virus by Polymerase Chain Reaction

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Polymerase chain reaction was used to identify carnation etched ring virus (CERV) in leaves of infected carnations. Two 20-mer oligonucleotide primers were designed to the conserved intergenic sequence of carnation etched ring 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 labeling to prove the viral origin of the PCR products amplified from nucleic acid samples originating from plant extracts. Different DNA extraction methods were compared and the alkaline extraction method was chosen, due to its simplicity to screen large numbers of samples.

Infection of carnations with carnation etched ring virus (CERV) can occur without visible symptoms. Typical symptoms are induced on certain carnation varieties, or in some varieties pronounced symptoms develop only with complex infections with carnation mottle virus. CERV alone does not have as great an effect on the foliage as other carnation viruses, but it causes severe damage to the flowers, which often show color break, calyx splitting and distortion (Smookler and Loebenstein, 1975).

Detection of carnation etched ring virus is quite difficult because of the lack of a good herbaceous differential host and the difficulties of obtaning high quality monoclonal and polyclonal antibody (Lawson and Civerolo, 1976, 1978; Hsu and Lawson, 1985). Recent development in the polymerase chain reaction technology have led to the introduction of this very efficient and sensitive technique to detect various plant viruses. Recent applications of this technique include the use of degenerate primers for detection of potyviruses (Langeveld et al., 1991), and the PCR membrane spot assay used for detection of plum pox virus RNA in bark of infected trees (Korschineck et al., 1991). Reverse transcription polymerase chain reaction method was adopted for the detection and analysis of zucchini yellow mosaic potyvirus sequence variability (Thomson et al., 1995) and also for differentiation between different potato virus Y isolates (Glaiss et al., 1996). PCR is often applied when closely related plant viruses need to be identified (Barbara et al., 1995). This technique was also used for detection of tomato infecting single stranded DNA containing geminiviruses, in viruliferous whiteflys (Mehta et al., 1994). Carnation etched ring virus has a double stranded DNA genome and belongs to the caulimovirus group, a simple PCR technique could be developed for virus detection. The primary structure of the carnation etched ring virus DNA has been determined (Hull et al., 1986) and the sequence can be used for designing virus specific primers for the polymerase

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chain reactions. Here we report on the isolation and characterization of a virus-specific sequence which was amplified with polymerase chain reaction. We also provide a comparison of three different sample preparation techniques.

Materials and Methods

Virus-specific sequences

Two primers were designed and synthesized for the polymerase chain reaction. Sequences were obtained from the EMBL databank. The following two primers were synthesized and were homologous at he positions 7031–7050 and 7861–7880, respectively, to the published sequence:

5'-GCCAGGGAGGATCTGGATAC-3' and 5'-GTTTCCGCCGTCGCAGCTCT-3'

Crude nucleic acid extractions

Carnation samples showing typical symptoms of carnation etched ring virus were used for nucleic acid preparations. *Phenol extraction*: Hundred mg of carnation leaf tissue was homogenized in 0.4 ml extraction buffer (50 mM Tris, pH 7.6, 100 mM NaCl, 1 mM EDTA, 0.5% SDS) with mortar and pestle. The ground tissue was extracted with an equal volume of phenol followed by phenol-chloroform (1:1) and chloroform extraction. Total nucleic acids were precipitated with two volumes of ethanol. Pelleted nucleic acids were dissolved in 0.1 ml sterile water (Sambrook et al., 1989). *Isopropanol extraction:* Hundred mg of carnation leaf tissue was homogenized in 0.4 ml extraction buffer (200 mM Tris pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) with mortar and pestle (Edwards et al., 1991). After a brief spin in an Eppendorf centrifuge the nucleic acids were precipitated from the supernatant by adding an equal volume of isopropanol. The precipitated nucleic acids were dissolved in 0.1 ml sterile water. *Alkaline extraction*: Carnation leaf samples (100 mg) were homogenized in 1 ml 0.5 N NaOH with mortar and pestle. To 10 µl of homogenate 990 µl of 100 mM Tris (pH 8.0) was added (Wang et al., 1993).

PCR

Amplification of DNA was carried out using 2 µl plant samples and 50 µl PCR reaction buffer (supplied by Amersham) containing 100–100 ng primers, 0.2 mM dNTP and 2.5 U Taq polymerase (Amersham). The amplification involved 35 cycles denaturation at 94 °C, 30 sec. primer annealing at 55 °C and 1.5 min. primer extension at 72 °C (Innis et al., 1990). Ten microliter PCR products were separated on 1% agarose gel and visualised by UV light after EtBr staining. Fragments corresponding to the expected 850 nt were isolated from the agarose gel and cloned into pBSK + plasmids in the EcoRV site (Sambrook et al., 1989).

Sequencing and radioactive labelling of the cloned fragment

The sequence of the amplified and cloned DNA was determined after Sänger et al. (1977). Using the T7 Quick primer kit (Pharmacia), a labelled probe was made according to manufacturer's instructions. Using this labelled probe the viral origin of the PCR products was confirmed with Southern hybridization (Southern, 1975).

Results

Isolation and characterization of a CERV specific clone

Based on the nucleotide sequence of carnation etched ring virus DNA (Hull et al., 1986), a viral sequence was amplified. Two primers were designed for the conserved non-coding region of the caulimovirus group between the genes VI and I using the carnation etched ring virus sequence data. The amplified 850 nt long DNA fragment was cloned into the EcoRV site of Bluescript plasmid and its primary structure was determined with the dideoxy chain termination method (Sänger et al., 1977). The amplified and cloned fragment had 95.6% homology with the known sequence (Fig. 1).

Comparison of the different extraction methods

All three extraction methods for the isolation of nucleic acids from carnation leaf tissue proved to be useful and gave almost identical results (Fig. 2). Phenol extraction gave the highest purity of nucleic acid samples; however, it proved to be the most time consuming and took several hours. Isopropanol extraction resulted in less pure nucleic acid samples but the preparation of the samplex took less time than using the previous method. Using alkaline maceration extraction, sample preparation takes only one minute. Another advantage of using this latter technique is that it needs only one Eppendorf tube per sample. For testing the sensitivity of the PCR detection of this virus, the alkaline extracted samples were diluted (the first dilution contains 2 µg plant material). Dilution of the samples up to hundred thousand fold still gave positive result in the PCR amplification (Fig. 3). Radioactive labelled cloned virus fragment (see Fig. 1) was used for proving the fragments viral origin (data not shown).

Detection of CERV in different carnation samples

Different symptomless and diseased carnation samples were analysed using the alkaline extraction method and the polymerase chain reaction with the designed primers. Amplified nucleic acid were separated on 1% agarose gel and visualised with EtBr. To prove their viral origin a radioactive probe of the amplified viral fragment was used (Southern, 1975). Samples from varieties like White SIM, Red Lena, Gigi, a Bulgarian breeding line and Iuri were positive while two samples of Tanga, Madonna and Lighting were negative. Controlling these carnations samples 50% of the tested tissue were positive although most of the plants were symptomless (Fig. 4).

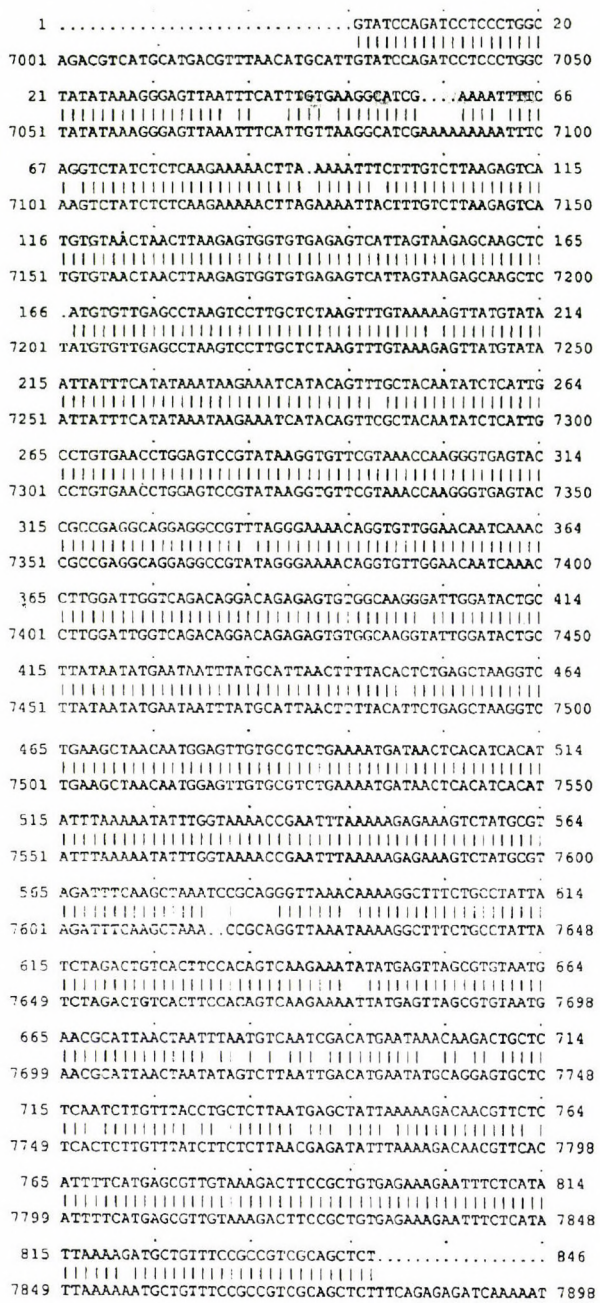

Fig. 1. Sequence comparison of PCR amplified and cloned fragment (upper line) (EMBL accession no.: Z71511) with the published (Hull et al., 1986) CERV nucleotide sequence (lower lines)

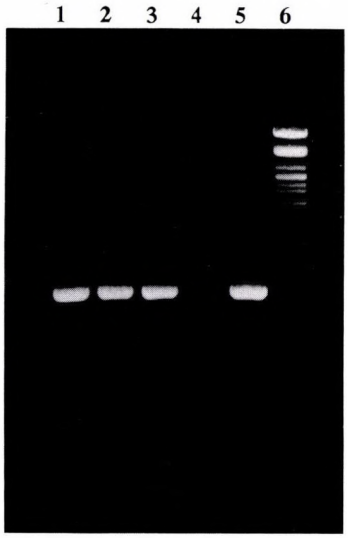

Fig. 2. Comparison of different extraction methods for CERV detection by PCR from infected carnation samples. Lane 1, PCR product of phenol extracted; lane 2, isopropanol extracted; lane 3 NaOH extracted carnation samples. Lane 4, PCR product of phenol extracted healthy plant. Lane 5, PCR product of cloned viral fragment. Lane 6, molecular weight marker (λ/PstI)

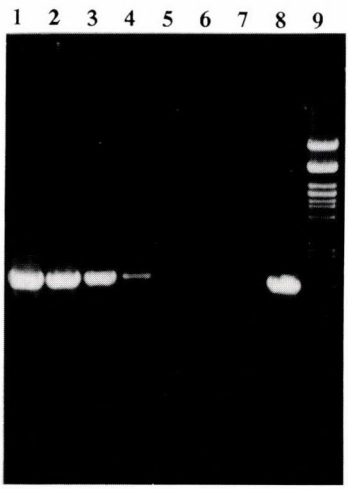

Fig. 3. Sensitivity of CERV detection by PCR. Dilution series of NaOH extracts of plant samples, Lane 1 diluted 10-, 100-, 1000-, 10 000-, 100 000-fold, lanes 2, 3, 4, 5, and 6, respectively. Lane 7, healthy plant. Lane 8, cloned virus fragment. Lane 9, molecular weight marker (λ/PstI)

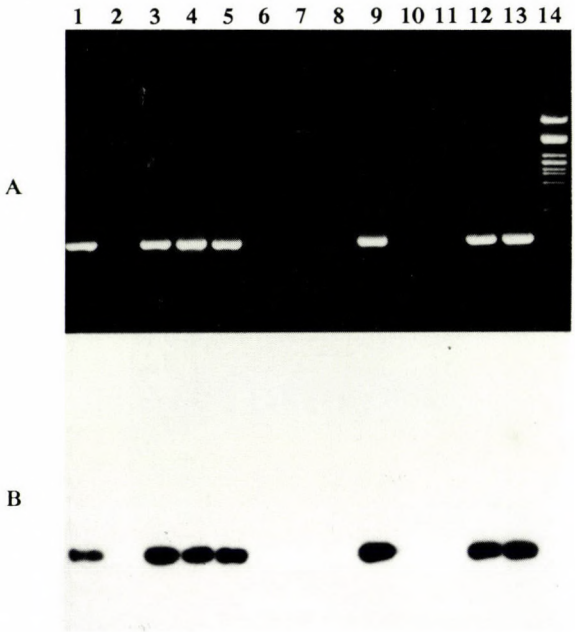

Fig. 4. Agarose gel analysis of PCR products of different carnation varieties. Lane 1, Tanga; lane 2, Salome; lane 3, White SIM; lane 4, Red Lena; lane 5, Gigi; lane 6, Madonna; lane 7, Tanga; lane 8, Lighting; lane 9, a Bulgarian breeding line; lane 10, Tanga; lane 11, Newton as the negative carnation control; lane 12, IURI as the positive carnation control; lane 13, cloned viral fragment; lane 14, molecular weight marker (λ/PstI). (A) Southern blot of the agarose gel analysed PCR products. Hybridization was performed with radioactive labelled cloned viral fragment (B)

Discussion

Nowadays different PCR techniques are widely used in plant quarantine systems. The reproducibility, rapidity and sensitivity of this method make it very attractive and useful. Regular virus indexing of valuable genetic materials and stock plants could relay on this technology expecially when plants could be infected with several different viruses and the presence of a given virus is not distinguishable phenologically.

The rapid and simple nucleic acid extraction along with the use of the virus specific primers in the amplification of the viral sequences lead to a very simple and sensitive PCR for identification of carnation etched ring virus. The sequence of the cloned PCR fragment demonstrate that the amplified fragment derived from CERV. The sequence showed high similarity with the only known sequence (Hull et al., 1986), and the sequence identity was highest in the central third of this clone. Earlier polyclonal and monoclonal antibodies were used for detection of CERV. The sensitivity of monoclonal antibodies was greater than that of the polyclonal antibodies (Hsu and Lawson, 1985).

Nevertheless, the sensitivity of this PCR techniques is much higher then even the triple antibody sandwich ELISA using monoclonal antibodies, because the ELISA was able to detect the virus in 1:1280 dilution (the first dilution contained 5 mg leaf material per well) of the infected plant sap, while we could detect the CERV 1:10 000 dilution (the first dilution contained 2 μg leaf material per PCR reaction). Consequently this PCR method is 20 000-fold more sensitive than the previously published ELISA method. This technique can be adopted to any other DNA viruses due to its 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.

Acknowledgements

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Reaction of Unknown *Solanum stoloniferum* Schlechtd. et Bche and *Solanum demissum* Lindl. Accessions to the Tuber Necrosis Strain of Potato Y *Potyvirus* (PVY^{NTN})[1]

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Reactions of fifteen accessions both of *Solanum stoloniferum* and *Solanum demissum* to NTN strain of potato Y *Potyvirus* (PVY^{NTN}) have been studied. Out of *S. stoloniferum* accessions PI. 160225, 545800 and 558465 were immune, while PI. 161152, 161160, 243458, 255534, 283109, 545805 and 558453 showed hypersensitive reaction. Other accessions were systemically susceptible to PVY^{NTN}. Out of *S. demissum* accessions no resistant were found.

Potato Y *Potyvirus* (PVY) is the type member of the family Potyviridae, which constitutes the largest known and economically most important family of plant viruses (Shukla et al., 1994). Tuber necrotic ringspot disease of potato is caused by a new strain of PVY, which produces severe necrotic ring symptoms on the tubers and berries, too. This strain belongs to the tobacco veinal necrosis strain group, named PVY^{NTN}.

The disease was first described in Hungary by Beczner et al. (1984). During the past twelve years the virus became distributed throughout Europe and was reported from Germany (Radtke, 1984; Weidemann, 1985), the former Czechoslovakia (Dedic et al., 1988), Austria (Schiessendoppler, 1990), the former Yugoslavia (Buturovic and Kus, 1989, 1990), Lebanon (Le Romancer and Kerlan, 1990), France (Le Romancer and Kerlan, 1991), Great Britain (Wright, 1992), Denmark (Nielsen, 1992) and from Belgium (Le Romancer et al., 1994).

Currently, potato necrotic ringspot disease is a major problem to potato growers and breeders, because it affects cultivars that are either immune (Weidemann, 1990; Le Romancer and Kerlan, 1992; Van den Heuvel et al., 1994) or posses high levels of field resistance to PVY (Beczner et al., 1984; Buturovic and Kus, 1989; Steinbach and Hamann, 1989; Le Romancer and Kerlan, 1991). There is a growing frequency of occurrence of PVY^{NTN} in European countries. Due to an epidemy almost the entire seed potato production in Slovenia has been destroyed since 1988 (Kus, 1990, 1995a). This was the consequence of the sensitivity of the current potato cultivars to PVY^{NTN} which are grown

[1] Dedicated to Prof. Dr. Z. Klement academician on the occasion of his 70th birthday.

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in former Yugoslavia (Kus, 1995b; Milosevic, 1995; Pepelnjak, 1995). A number of potato cultivars in other European countries are also frequently affected with PVY^{NTN} (Wright, 1992; Weidemann, 1993).

Due to the destructive nature of potato necrotic ringspot disease, the identification of sources or resistance is of great importance. The objective of this study was to identify potential resistance sources among the wild *Solanum* species that could be used in a potato breeding program.

Materials and Methods

Fifteen accessions both of *Solanum stoloniferum* and *Solanum demissum* were inoculated at 8–10 leaf stage with the original Maradona isolate of PVY^{NTN} (Beczner et al., 1984) five weeks after sowing. Previously the virus was propagated on *Nicotiana tabacum* cv. Xanthi-nc. The *Solanum* accessions were inoculated by carborundum-spatula technique with tissue sap of *Nicotiana tabacum* cv. Xanthi-nc. Sörensen phosphate buffer (pH 7.2) in the ratio 1:1 was used. Seven plants of each accessions were inoculated. The inoculated plants were symptomatologically checked for infection every seven days. Five weeks after mechanical inoculation the accessions were tested using direct double-antibody sandwich ELISA (DAS-ELISA) method, after 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 twenty minutes after adding the substrate, at 405 nm wavelength on Dynatech ELISA reader. Of the seven plants of each accessions the highest absorbance value was recorded. Test samples were considered positive if their absorbance values exceeded twice that of the healthy control samples. In latent host-virus relations back inoculation was also carried out to *N. tabacum* cv. Xanthi, as indicator plant.

Results and Conclusions

Fifteen accessions both of *S. stoloniferum* and *S. demissum* were studied for their reactions to PVY^{NTN}. Ten *S. stoloniferum* accessions were found to be resistant to PVY^{NTN} (Table 1). Three of them (PI. 160225, 545800 and 558465) showed the highest resistance (immunity). Neither the inoculated nor the non-inoculated leaves showed symptoms and the virus could not be detected in them by serological and biological tests. Seven accessions of *S. stoloniferum* (PI. 161152, 161160, 243458, 255534, 283109, 545805, 558453) were hypersensitive to PVY^{NTN} (see Table 1). Most severe symptoms were obtained in accessions of *S. stoloniferum* PI. 545737 and 558471. Vein necrosis were observed in some accessions (e.g. PI. 547740). Back inoculation of tissue sap of the symptomless plants failed to give rise to infection. Of the fifteen *S. demissum* accessions no resistant were found (Table 2).

Table 1

Reaction of *Solanum stoloniferum* accessions to PVY^{NTN}

Accession or PI number	Symptoms*		Absorbance
	IL	NIL	
160225	–	–	0.005
161152	Chl, Nl	–	0.009
161160	Chl, Nl	–	0.007
243458	Chl	–	0.007
255534	Chl, Nl	–	0.005
283109	Nl, Ld	–	0.006
545737	Nl, Ld	Vn, Tn	0.051
547740	–	Vn, Vc	0.077
545792	Chl	Vn	0.080
545800	–	–	0.005
545805	Chl, Nl	–	0.005
558453	Chl	–	0.005
558465	–	–	0.005
558466	Chl, Nl	Vn	0.065
558471	–	Ton, D	0.090
Negative control	–	–	0.005

*IL, inoculated leaves; NIL, non-inoculated leaves; Chl, chlorotic lesions; Ld, leaf drop; Nl, necrotic lesions; Vn, vein necrosis; Vc, vein clearing; Tn, top necrosis; Ton, total necrosis; D, death of the plant; –, no reaction

There are several known sources of resistance to viruses among the cultivated and wild *Solanum* species (Webb and Schultz, 1961; Bagnall, 1972; Ross, 1986; Horváth, 1988; Horváth et al., 1988; Horváth and Hoekstra, 1989; Horváth and Wolf, 1991; Harrison, 1992; Valkonen, 1994). The *R* genes are inherited in a monogenic dominant way (Cockerham, 1970) and they code for extreme resistance against a broad spectrum of virus strains. The *N* genes are also inherited in monogenic dominant way, but they code for a strain specific hypersensitive reaction to viruses (Cadman, 1942; Cockerham, 1970; Jones, 1990). The *Ry* gene derived from *S. stoloniferum* (Barker and Harrison, 1984) and the *Ny* gene from *S. demissum* (Cockerham, 1970) is of potential use in potato breeding programs against PVY. In search for sources of resistance against a presumably resistance-breaking strain of PVY we found ten *S. stoloniferum* accessions to be resistant, however, all the examined *S. demissum* accessions were susceptible to PVY^{NTN}.

Our results underpin that *Ry* gene derived from *S. stoloniferum* can confer resistance against a wide range of strains, and *Ny* gene derived from *S. demissum* is strain specific.

Table 2

Reaction of *Solanum demissum* accessions to PVY^{NTN}

Accession of PI number	Symptoms*		Absorbance
	IL	NIL	
160208	Chl, Nl	Nsp	0.063
161149	–	Nsp	0.066
161366	–	Nsp	0.077
161715	–	Nsp, Vn, Mo	0.085
205514	–	Nsp, Mo	0.044
205516	–	Nsp	0.037
275211	–	Nsp	0.054
498012	–	Nsp	0.066
558052	Chl	Nsp	0.066
558386	Nl, Ld	Nsp, Mo	0.084
558387	Chl, Nl	Mo	0.095
558389	–	Nsp	0.075
558390	–	Nsp, Mo	0.067
558391	Chl, Nl	Nsp, Vn, Mo	0.089
558463	Chl	Nsp	0.067
Negative contol	–	–	0.005

*IL, inoculated leaves; NIL, non-inoculated leaves; Chl, chlorotic lesions; Ld, leaf drop; Nl, necrotic lesions; Nsp, necrotic spots; Vn, vein necrosis; Mo, mosaic; –, no reaction

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The Effect of Virus Infection on the Growth and Photosynthetic Pigment Content of the Virophilous Jimson Weed (*Datura stramonium* L.)

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Investigations were carried out to examine the growth and photosynthetic pigment content of jimson weed (*Datura stramonium* L.) infected with the cucumber mosaic *Cucumovirus* (CMV) and henbane mosaic *Potyvirus* (HeMV). Both CMV and HeMV retarded the growth of jimson weed. The reduction of fresh and dry weight of shoots and roots was stronger when the plants were infected with HeMV. The enhanced dry matter content suggests the disturbance of water uptake of roots due to the virus infection. The chlorophyl-a, chlorophyl-b and the carotinoid content of the leaves showing systemic symptoms was significantly reduced, when the plants were infected with CMV.

The weeds influence the quality and quantity of crops not only in direct way (e.g. nutrient and water uptake) but are very 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 weed species play important role in the epidemiology and overwintering of viruses, therefore the weeds may be primary infection sources at the beginning of the vegetation period. *D. stramonium* is one of the ten most serious weeds in Hungary. In the first weed survey made in Hungary it occurred with a cover of average 0.0054%. Twenty years later (in 1970) the cover percentage was ten times larger (Tóth et al., 1989). The virophilous *D. stramonium* is host of 70 viruses and good test plant of 26 viruses (Horváth, 1967; 1993). Among others *D. stramonium* is natural host of the polyphagous – possessing more then 1000 hosts – CMV as well (Horváth, 1979; Edwardson, 1991). Host range of HeMV is limited mainly to the species of family of *Solanaceae* (Govier and Plumb, 1972). *D. stramonium* plays the most important role in the spreading and epidemiology of HeMV in Hungary (Horváth et al., 1988).

The aim of our study was to compare the development and photosynthetic pigment content of the healthy and the diseased *D. stramonium* plants infected by CMV and HeMV. Considering that formerly the physiological examinations of the virus diseased plants were connected mainly with the cultivated plants, therefore our present report supplies new data to the knowledge of the physiological changes of the virus diseased weed plants.

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Materials and Methods

The seeds of *D. stramonium* were sown in sterilized boxes in our virological glasshouse free of vectors. The seedlings were planted in pots (12 cm in diameter), containing standard soil mixture (humus: 55%, pH: 7.2). The plants were inoculated at 4–6 leaves phenological stages using carborundum-spatula technique with plant tissue sap containing viruses diluted with distilled water in the ratio 1:1. The U-246 strain of CMV and W/H strain of HeMV was used (Schmidt and Horváth, 1982; Horváth et al., 1988). Three weeks before inoculation the viruses were propagated on *Nicotiana tabacum* 'Xanthi-nc'. Four weeks after inoculation samples were prepared to determine the chlorophyl-a, chlorophyl-b and carotinoid content of the leaves showing systemic symptoms. With the knowledge of the adsorption maximums of the different pigments the extinction values were measured with a spectrophotometer 'Beckman DU-65'. The chlorophyl-a, chlorophyl-b and the carotinoid content were determined after Allaga et al. (1992). The fresh and dry weight both of the shoots and the roots were measured five weeks after inoculation. Dry matter content was counted as well [dry matter content (%) = dry weight * 100 * fresh weight^{-1}].

Analysis of variance was used for statistical evaluation of the result.

Results and Discussion

In consequence of virus infection the leaves of *D. stramonium* showed typical local and systemic symptoms (Table 1). Besides that both CMV and HeMV infection strongly retarded the growth of the plants, wilting is a characteristics symptom for plants infected with HeMV.

The fresh weight both of the shoots and roots was significantly reduced due to the virus inoculation. HeMV reduced the fresh weight of the roots and shoots of *D. stramonium* by 73 and 62%, respectively. The most expressive reduction of dry weight was due to HeMV infection, too. Dry matter content was significantly influenced only in case of HeMV infection, too. Dry matter content was significantly influenced only in case of HeMV infection (Table 2). It is well known that growth reduction is a general symptom for a lot of host-virus relation (Bailiss, 1974; Sing et al., 1977; Cordrey and Bergman, 1979; Singh and Singh, 1980; Singh et al., 1991; Jones, 1992). Physiological background of the growth reduction is related with the retarded water and nutrient uptake due to the changing of the permeability of the root membrane (Ghabrial and Pirone, 1964; Matthews, 1981; Goodman et al., 1991), inhibited translocation of assimilates due to the phloem degeneration (Goodman et al., 1991), changing of quantity and activity of enzymes responsible for the transformation of carbonhydrates (Faccioli et al., 1971) and growth regulators (Kavanagh et al., 1969; Kuriger and Agrios, 1977; Goodman et al., 1991). The considerable wilting, strong inhibition both of the shoots and roots and the

Table 1

The symptoms of CMV and HeMV infection on the leaves of *D. stramonium*

Viruses*	Symptoms**	
	local	systemic
CMV	Chl, ClRi	ClRi, Mo
HeMV	Led, W	Vc, W

* CMV, cucumber mosaic *Cucumovirus*; HeMV, henbane mosaic *Potyvirus*

** Chl, chlorotic lesions, ClRi, chlorotic rings; Mo, mosaic; Led, leaf drop; Vc, vein clearing; W, wilting

Table 2

The effect of virus infection on the growth of *D. stramonium*

Viruses*	Fresh weight (g)		Dry weight (g)		Dry matter content (%)
	shoot	root	shoot	root	
CMV	6.9	4.1	1.0	0.2	13.5
HeMV	4.4	1.3	0.6	0.2	14.4
Control	11.6	5.8	1.6	0.6	12.5
LSD (P=0.05)	2.0	0.3	0.3	0.2	1.5

* CMV, cucumber mosaic *Cucumovirus*; HeMV, henbane mosaic *Potyvirus*

enhanced dry matter content of *D. stramonium* infected with HeMV suggests the disturbance of water relation which can be explained with the degenerative effect of HeMV on the roots.

Both CMV and HeMV reduced the pohotosynthetic pigment content of the systemically infected leaves, but the reduction was statistically significant only in case of CMV infection. The most considerable (33%) reduction of chlorophyl-b content was detected when the plants were infected with CMV (Table 3). It is concluded that physiological changes due to the HeMV probably are not related with the photosynthetic pigment content.

A lot of virus infection can be characterized with the reduction of chlorophyl content in the tissue of the virus infected plant (Hampton et al., 1966; Tu et al., 1968; Gates and Gudauskas, 1969; Bailiss, 1970; Hunter and Peat, 1973; Mohamed, 1973; Crosbie and Matthews, 1974; Kato and Misawa, 1974; Roberts and Wood, 1982; Singh and Singh, 1991), which is occasionally accompanies the degradation of carotinoids

Table 3

The effect of virus infection on the photosynthetic pigment content of *D. stramonium*

Viruses*	Chlorophyl-a (mg/g)	Chlorophyl-b (mg/g)	Carotinoid (mg/g)
CMV	1.863	0.305	0.504
HeMV	2.367	0.405	0.675
Control	2.696	0.456	0.685
LSD (P=0.05)	0.684	0.130	0.154

* CMV, cucumber mosaic Cucumovirus;
HeMV, henbane mosaic *Potyvirus*

(Crosbie and Matthews, 1974; Goel and Varma, 1977). As a contrast Kato and Misawa (1974) reported enhanced carotinoid content, with special regard to the plant tissues showing chlorotic symptoms.

Our results call attention to the fact that viruses unfavourably influence physiological processes not only of the cultivated but the weed plants as well, therefore – in indirect way – they contribute to the reduction of their competitive ability in the intra- and intraspecific competition.

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Comparison of Ultrastructural Changes of *Nicotiana benthamiana* Infected with Three Different Viruses

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Fine structural changes of *Nicotiana benthamiana* infected with three different viruses were investigated. Plum pox virus (PPV) infection manifested mild systemic mosaic symptoms and did not cause significant differences in chloroplast structure. Tomato spotted wilt virus (TSWV) infection caused severe chlorosis and systemic necrosis. Parallel with symptom production the chloroplasts showed degenerative changes: swelling, more osmiophilic plastoglobuli, and loosened thylakoid structure. In the acute phase chloroplasts were completely deformed, large cytoplasmic invaginations were frequently observed. Alterations connected with systemic chlorosis proceeded step by step resembled to the process of senescence of healthy leaves. However, tobacco necrosis virus (TNV) infection caused local necrotic lesions within few days. In hypersensitive reaction the cell structures rapidly collapsed and the chloroplasts disintegrated. During the symptom expression the coat protein level of PPV was relatively higher than the amount of coat protein of TSWV, indicating that not the concentration of virus-related proteins, but their toxicity was essential in symptom development. Cytopathological changes provoked by TSWV were different both from PPV induced mild reactions and the rapid cell death caused by TNV.

Shortly after viral infection start the recognition of the pathogen and the early events of host metabolism leading up to systemic infection in a susceptible host (compatibility) or to local infection in resistant plant (incompatibility). Much research emphasis has been given to analyse and elucidate the metabolic and ultrastructural changes of hypersensitive reaction (HR) to unravel the phenomenon of plant resistance to viruses. However, few attention has been made to investigate the development of symptoms in systemically infected susceptible plants. In the latter cases replication of the pathogen was unhindered. In advanced infections different symptoms, slight or severe chlorosis, mosaic, leaf deformations and reduced growth are the most common consequences of the systemic spread of the pathogen, but the host plant survives the virus infection. In contrast in few cases such as brome mosaic virus infected maize plants or tomato spotted wilt virus infected *Nicotiana benthamiana* seedlings no, or mild symptoms develop in inoculated leaves, but later the plants suddenly wilt and die of. This type of systemic necrosis is completely different from the hypersensitive necrosis of resistant plants, where the lesions are able to localize the infection and the uninfected parts remain intact. The physiological and ultrastructural changes of the systemic necrosis was never studied in details.

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The aim of this work was to follow the ultrastructural changes in two, different types of compatible host-virus combinations, in which the virus infection elicited only mild mosaic symptoms and another, where the infected plants were died in systemic necrosis. We tried to compare the cytological effect of systemic necrosis in susceptible plants with the hypersensitive reaction in resistant ones. Since infected plants produced chlorotic spots before systemic necrosis, our experiments were focused mainly to the structural disorganization of chloroplasts.

Materials and Methods

Viruses and plants

A group of *Nicotiana benthamiana* plants were infected with plum pox potyvirus (PPV) to get mild mosaic symptoms. The second group of plants were inoculated with tomato spotted wilt tospovirus (TSWV) to obtain systemic necrosis and the third-one with tobacco necrosis necrovirus (TNV) which produced local necrotic lesions. Plants were grown under normal greenhouse conditions (16 h in the light, and 8 h in the dark without supplemental illumination at about 22 °C) and were mechanically inoculated with TSWV isolate T35 previously isolated from tobacco (Gáborjányi et al., 1995), PPV originated from almond trees (Gáborjányi, 1994, unpublished) and TNV from tobacco roots (Szirmai, 1939).

Electron microscopy

Small samples of leaf tissues were cut from healthy and infected leaves in different stages of symptom development. Samples were fixed in 3% glutaraldehyde in 0.025 M K-Na phosphate buffer (pH 7.4) for 3–4 h at 4 °C. After washing with this buffer the samples were postfixed in 1% osmium tetroxide dissolved in the same buffer for 1.5 h at 4 °C. After rinsing with this buffer the leaf strips were dehydrated in graded ethanol series and propylene oxide, and inflitrated and embedded in Durcupan ACM. Ultrathin sections left unstained or poststained with uranyl acetate and lead citrate were examined in TESLA BS 500 transmission electron microscope.

Immunoassay

Accumulation of viral coat protein in the leaves of systemically infected by PPV or TSWV was analysed by DAS-ELISA (Clark and Adams, 1970). Leaf samples were taken in 3–4 day-intervals, and diluted 2.5-folds with extraction buffer. Antisera of PPV and TSWV were 1:1000 and 1:500, respectively.

Results

PPV infected plants showed no visual symptoms on the inoculated leaves. Mild mosaic with green and yellow areas appeared about two weeks after inoculation, and the systemically infected plants were slightly stunted. Samples for electron microscopy were taken both from yellow and green parts of the mesophylls. In both cases the cells from the infected tissues did not differ from those of the healthy plants. No significant differences were found in the ultrastructure of the cells forming either the green or yellow areas. Chloroplasts did not show any conspicuous or discernible change in structure in comparison to the chloroplast of healthy plants (Fig. 1A and B). The chloroplasts slightly deformed occasionally containing few (1–2) starch grains (Fig. 1C). Very rarely bundle-like aggregates of particles associated with the cytoplasmic inclusions were found (Fig. 1D). However, no virions could be seen neither in the chloroplasts nor in the cytoplasm. Cells usually contained pinwheel shaped cytoplasmic inclusion bodies (Fig. 2A) as well as crystalline nuclear inclusions, which confirms of the former observations (Edwardson, 1992; Martin, 1992). More inclusions were found in the older, lower leaves than in the upper, younger ones.

TSWV infection induced more severe symptoms. About ten days after inoculation chlorotic spots developed both in inoculated lower and in non-inoculated, upper leaves. Two weeks post-inoculation the chlorosis became systemic and later the plants suddenly wilted and died of systemic necrosis. The systemic chlorosis and complete necrosis corresponded to the cytopathological changes detected by electron microscopy in distinctive phases of the disease. Early in infection, about 8 days after inoculation clusters of spherical glycoprotein enveloped viral particles within membrane-bound cavities of endoplasmic reticulum accumulated near the chloroplast membranes (Fig. 2B and C). Fibrillar inclusions and nucleocapsid aggregations also occurred in the cytoplasm. The chloroplasts showed degenerative changes: they often were swollen and contained numerous osmiophilic plastoglobuli. In few cases plastoglobuli of less electron opacity could be seen (Fig. 2D). Virions were never observed inside chloroplasts.

In the last phase, before the development of systemic necrosis (Fig. 3A) the chloroplasts were swollen, deformed, and the thylakoidal structure were loosened. Frequently cytoplasmic invaginations into the chloroplasts were observed (Fig. 3B). Rarely these cytoplasm invaginations contained mature virions. These pathological alterations of chloroplasts have not been described before in TSWV infected cells. The thylakoid structure appeared to be disrupted. Vesiculation of chloroplasts were also occurred. In the last, necrotic stage only collapsed cell structures could be observed, the chloroplast membranes were completely disintegrated (Fig. 3C).

TNV infection caused formation of necrotic local lesions, but the infection never became systemic. The hypersensitive reaction accomplished within three days. Rapid collapse of cell structures, deformation and disruption of chloroplasts, destruction of lamellar structure, disintegration of membranes, accumulation of osmiophilic globuli and masses of amorphous aggregates were observed (Fig. 3D).

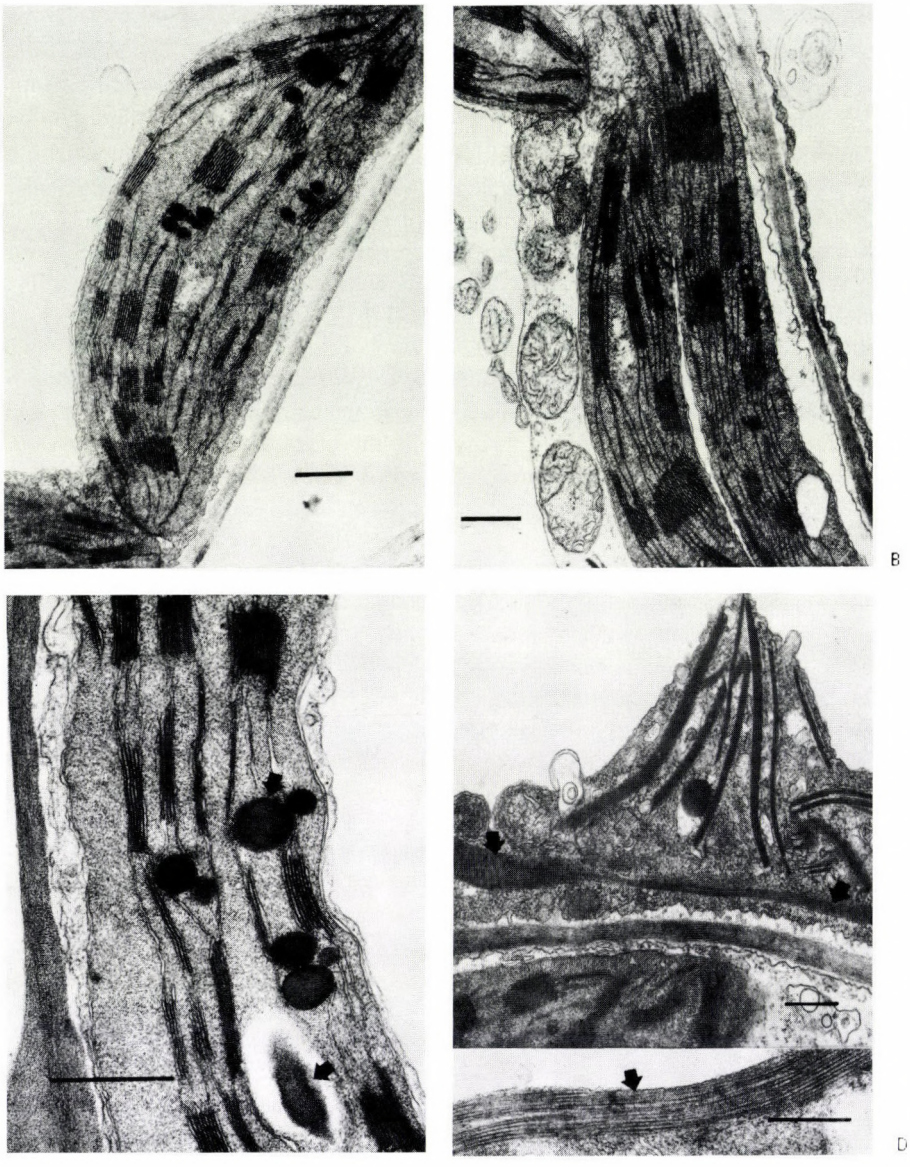

Fig. 1. (A) Transmission electron micrograph of a chloroplast of healthy *Nicotiana benthamiana* mesophyll cell. Scale = 500 nm (B) Chloroplast of plum pox virus (PPV) infected *N. benthamiana* leaves shows slight deformation and normal granal structure in the early stage of infection. Scale = 500 nm (C) Starch grains and osmiophylic plastoglobuli (arrows) in (PPV) infedted leaves. Scale = 500 nm (D) Plum pox virion aggregates in the cytoplasm near to cylindrical inclusion bodies (arrows). Scale = 500 nm. Bottom: insert of an aggregation. Scale = 500 nm

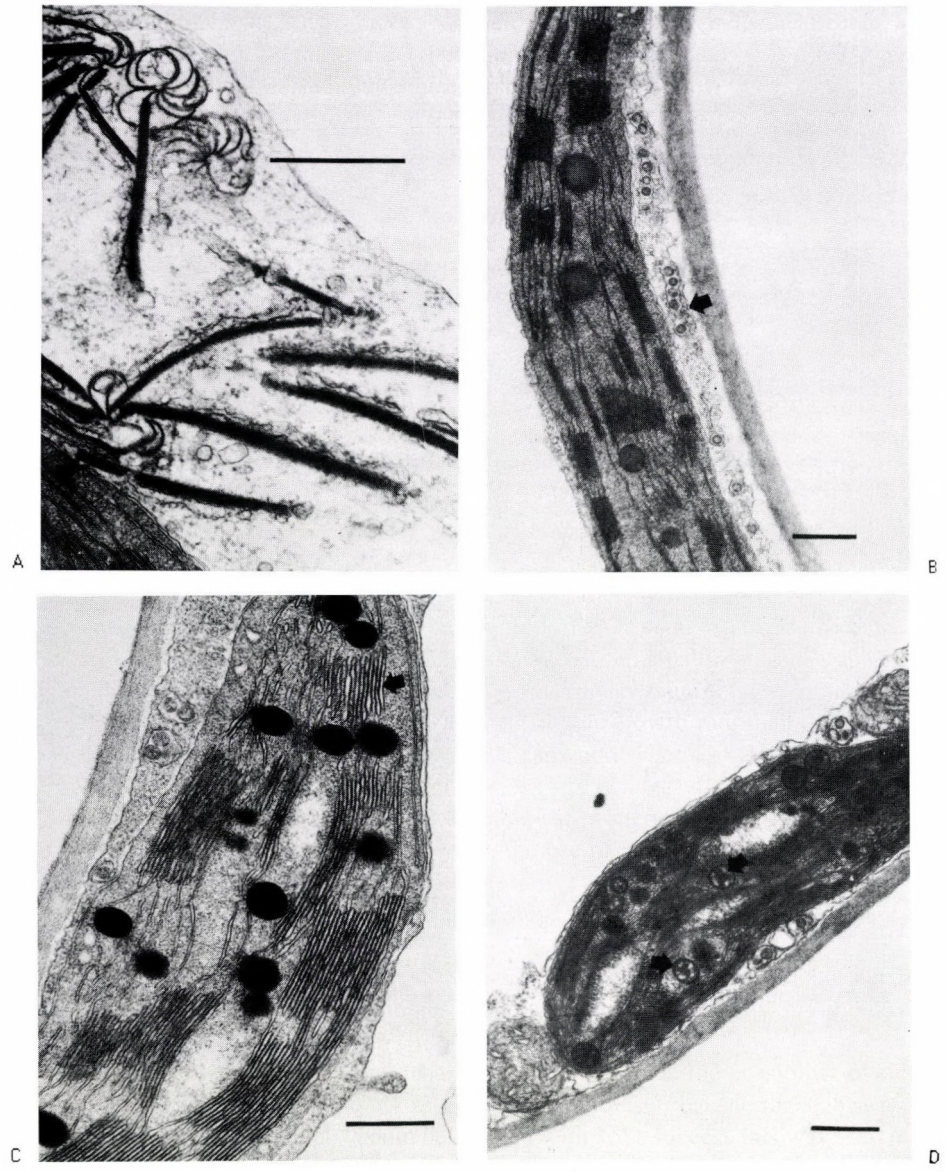

Fig. 2. (A) Pinwheel cytoplasmic inclusion bodies in PPV infected *N. benthamiana* leaf cell. Scale = 500 nm (B) Chloroplast of tomato spotted wilt virus (TSWV) infected *N. benthamiana* leaves in early stage of the infection. Clusters of spherical glycoprotein enveloped virus particles (arrow) accumulated near to the chloroplast. Scale = 500 nm (C) Chloroplast showing numerous osmiophylic plastoglobuli and slightly loosened granal structures (arrow). Scale = 500 nm (D) Less electron-opaque plastoglobuli (arrows) with unusual structure in the stroma of chloroplast early after TSWV infection. Scale = 500 nm

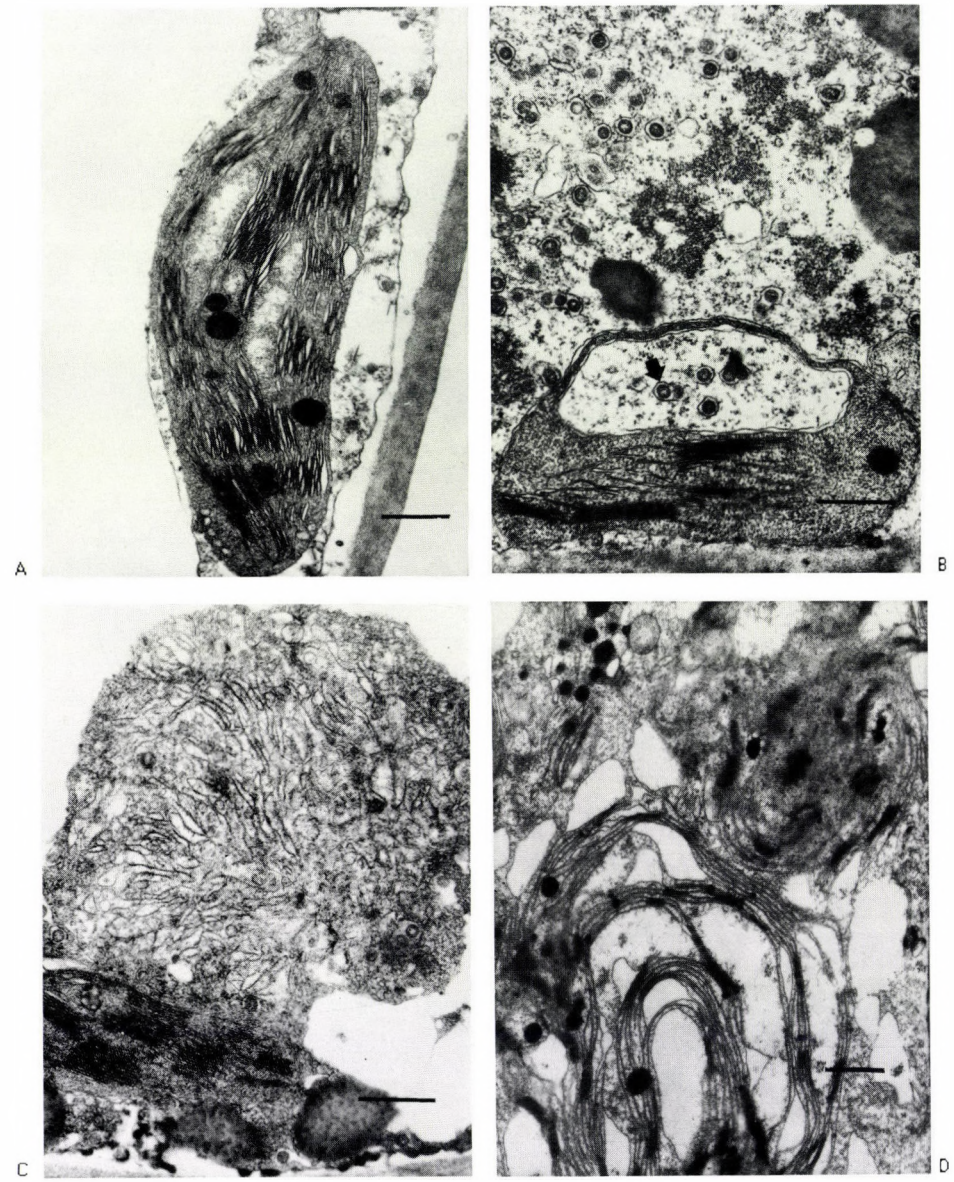

Fig. 3. (A) Loosened thylakoid membranes of *Nicotiana benthamiana* chloroplast in the acute phase of TSWV infection. Scale = 500 nm (B) Unusual cup-shaped cytoplasmic invagination of the chloroplast in the acute phase of infection. Note the mature virions (arrow) inside of cytoplasmic depression. Thylakoid structure is completely disrupted. Scale = 500 nm (C) Collapsed cells in the last, necrotic stage of TSWV infection. No individual cell compartments can be observed. Scale = 500 nm (D) Tobacco necrosis virus infected mesophyll cell dying of a rapid cell disorganization. Scale = 500 nm

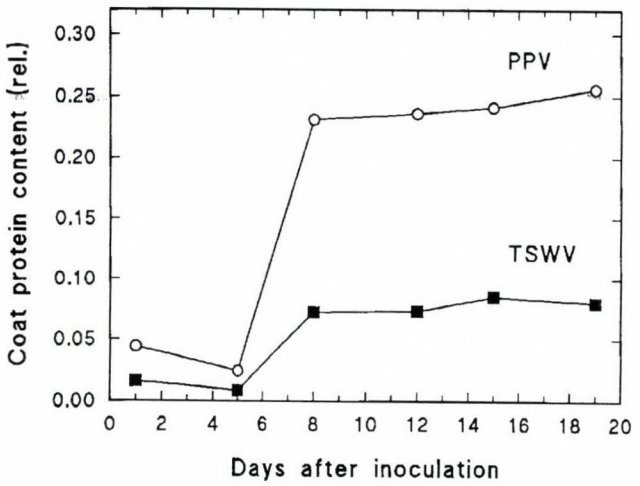

Fig. 4. Accumulation of coat proteins in *Nicotiana benthamiana* after the infection of plum pox virus or tomato spotted wilt virus

To make it clear, what kind of correlation exists between the observed ultrastructural changes and virus replication, accumulation of virus coat protein (CP) was measured at stated intervals in both compatible host-virus relationships. In PPV infected tissues more CP accumulatled than in TSWV infected ones (Fig. 4). No correlation was found between the accumulation of CPs and the severity of symptoms caused by the two distinct pathogens.

Discussion

Chlorosis and necrosis are the most frequent responses to viral infections in plants. However, no comparison has been made on the ultrastructural level between these two, different types of symptoms. Ultrastructural studies of infected plants, especially have been focused on the degradation of chloroplasts in systemically infected plants to explain the decreased capacity of photosynthesis. Most of the changes associated with infections are: The reduced number of chloroplasts, the decreased chlorophyll content, the occurrence of large starch grains in the swollen and deformed plastids, the accumulation of osmiophilic plastoglobuli and the disorganization of lamellar structures of chloroplasts (Esau, 1968; Goodman et al., 1986; Fraser, 1987).

The formation of local necrosis and the development of an "active zone" around the lesions have been discussed in details (Weintraub and Ragetli, 1964, Israel and Ross, 1967; Loebenstein, 1972). According to these studies the most characteristic changes in the formation of local lesions were the rapid collapse of cellular homeostasis and the

specific changes responsible for the eventual blocking of virus spread. The ultrastructure of the local lesions were different from that of the surrounding zone (Israel and Ross, 1967). Cytoplasmic and chloroplast membranes were disrupted and the thylakoidal elements dispersed. The cell death proceeded within few hours and the discrete phases of necrobiosis could be followed only by the observation of cells neighbouring the dead ones. On the other hand, cells of the active zone surrounding the mature lesions showed enhanced metabolic activity, containing large vacuoles, increased amount of cytoplasm and ribosomes. The fine structure of these cells resembled the effect of induced juvenility. Chloroplasts of this area were intact and has a lens or amoeboid-like shape, sometimes dividing chloroplasts were also seen (Israel and Ross, 1967).

Ultrastructural changes were noticed in the cell during the development of TSWV infection (Ie, 1971; Mohamed, 1973, Kormelink et al., 1991, Kitajima et al., 1992). The studies were focused on the question where the membrane-bounded virions were formed in the cell and which genom products were found in the viroplasm and in the fibrillar structures. Virus particles were detected only in the cytoplasm, and the different ways of virion maturation were also described. It was established that chloroplast ribosomes were not necessary for virus synthesis but cytoplasmic ones were essential. As a secondary effect of virus infection peripheral amoeboid extensions of chloroplast membranes were found in infected tissues. Chloroplasts were slightly deformed.

In order to study the development of complete necroses, we haven taken samples of TSWV systemically infected leaves at different intervals. Three stages of chloroplast destruction were detected. In the first, early stage, when only slight chlorotic leaf spots were developed, the thylakoid structure seemed to be relatively intact. In the second, late stage all the leaf surface became chlorotic. Disorganized granal structures were observed mainly in this late stage of infection. Similar structure of chloroplasts could be observed in the healthy, but senescent leaves. Cup-shaped chloroplasts were frequently seen in the acute phase, with mature virions in their invaginations. This pathological alteration of chloroplast was not published before. All the alterations in the fine structure of the TSWV infected cells gradually led to necrosis, and were completely different from the rapid, hypersensitive necrosis induced by TNV.

It was evident that chloroplast degradation or decreased synthesis of chloroplast-associated proteins occurred in plants infected by a virus which replicated in the chloroplasts, as in the case of barley stripe mosaic virus (Brakke et al., 1988). However, abnormalities in chloroplast structure and function were reported for tobacco mosaic virus (TMV) infected plants. This virus do not replicate in the chloroplasts but in the cytoplasm. Although TMV virion-like structures aggregated in the stroma of chloroplast (Esau, 1968). Later studies demonstrated that these pseudo virus-like structures were encapsidated forms of chloroplast DNA transcripts (Rochon and Siegel, 1984).

According to Reneiro and Beachy (1986, 1989) the TMV coat protein (CP) was directly associated to chloroplast membranes and could cause their instability and lead to chlorosis. Different levels of TMV CP were reported in connection with the reduced photosystem II (PSII) activity. In contrary, Lindbeck et al. (1991, 1992) demonstrated that CP-related proteins of CP deletion mutants did not accumulate in the chloroplast, but

inclusion bodies containing coat protein were formed in the cytoplasm. It was supposed that the CP outside of the chloroplast interfered with chloroplast protein synthesis and were transported in the cytoplasm. Hodgson et al. (1989) suggested that not the CP, but the replicase proteins of TMV caused the attenuation of mosaic symptoms. Most probably the TMV CP was synthesized in the cytoplasm. According to the suggestions of Banerjee and Zaitlin (1992) TMV CP was rapidly imported into isolated chloroplasts and was found almost entirely in the thylakoid membrane fraction. Independently from the source of CP, it seems plausible that either CP or other TMV translation products were responsible for chloroplast destructions.

Although it is hard to draw parallel conclusions between changes caused by TMV and TSWV infections, however, it is believed that accumulation of CP can influence the symptom severity, independently from the site of virus replication. In PPV infected tissues the CP was accumulated in a relatively higher degree, than in TSWV infected cells. Similarly to Kormelink et al. (1991) we suppose that not the amount, but the toxicity of CP or related polypeptides can modify the symptom severity. In our experiments TSWV particles have never been found in the chloroplasts. Among the gene products of TSWV only the non-structural (NS_s) protein, the product of the smaller RNA proved to be pathogenic for the host plant (Kormelink et al., 1991; Kitajima et al., 1992). Ns_s protein formed fibrous structures in the cytoplasm, but which do not occur in the chloroplasts. Its function has not been discovered. The presence of TSWV CP or of other TSWV encoded polypeptides in the plastids is still an open question. To answer it by using isolated chloroplast is the topic of our following study.

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Changes in Total Phenols and Some Oxidative Enzymes in Cowpea Leaves Infected with Yellow Mosaic Virus

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The levels of total phenols and specific activities of polyphenol oxidase (PPO), peroxidase (PO) and catalase in leaves of cowpea yellow mosaic virus (CYMV) resistant (CS 39 and CS 55), tolerant (ARL 25) and susceptible genotypes (HFC 42-1 and GC 2) of cowpea were measured at 40, 50 and 60 days after sowing (DAS). Resistant genotypes had higher activity of PPO and PO alongwith higher phenolic content in comparison to healthy leaves of susceptible ones at 40 and 50 DAS. PO activity was several times more as compared to PPO activity and increased markedly in response to infection in susceptible genotypes. Total phenols in resistant genotypes remained more or less same at 40 and 50 DAS but increased in susceptible ones, while its level in tolerant genotype decreased continuously throughout the growth period. Catalase activity was generally higher in resistant genotypes in comparison to both healthy and diseased leaves of susceptible genotypes. There was proportional reduction in catalase activity with respect to disease severity. The role of phenolics and oxidative enzymes in determining resistance in cowpea against CYMV disease has been highlighted.

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume particularly in the third world, used as grain pulse, green pod, green seeds and tender leaves. The productivity of cowpea is very low (250–500 kg/ha, Singh et al., 1991) because of the prevalence of biotic stresses such as fungi, bacteria, virus and nematodes. Viral diseases particularly cowpea yellow mosaic virus (CYMV) alone accounts for 35–100 per cent losses in grain yield (Williams, 1977).

Phenolic compounds are generally believed to be important in plant resistance mechanisms and their accumulation is much higher is resistant genotypes than susceptible ones (Luthra et al., 1988; Sohal and Bajaj, 1993; Sarma et al., 1995). Oxidative enzymes viz. polyphenol oxidase (PPO) and peroxidase (PO) confer resistance by restricting the infection of disease as they produce phenol oxidation products which are more toxic than phenol themselves. A positive association between the activities of these enzymes and disease resistance has also been observed in different field crops (Loebenstein and Linsey, 1966; Barbara and Wood, 1972). The information on various biochemical parameters importing resistance to CYMV is scanty. Therefore, the present investigation was carried out to quantify the changes in total phenols and specific activities of PPO, PO and catalase in resistant, tolerant and susceptible genotypes of cowpea during infection of yellow mosaic virus disease at different stages of plant growth.

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Materials and Methods

Healthy (having no visible symptoms of CYMV) and diseased cowpea leaves (having 50 and 100% infected leaf area) of two resistant (CS 39 and CS 55), one tolerant (ARL 25) and two susceptible genotypes (HFC 42-1 and GC 2) were collected at 40, 50 and 60 days after sowing. These leaves were freed of foreign mateiral by washing thoroughly with running tap water followed by distilled water and water drops from the surface were removed by filter paper.

To 1.0 g fresh weight of finely chopped leaves was added 4–5 ml of 0.01 M phosphate buffer, pH 7.0 and extract prepared by hand homogenization at 0–4 °C (Arora and Bajaj, 1981). It was strained through four layers of cheese cloth and then centrifuged for 20 min. at 10,000 × g (0–4 °C). The resulting supernatant was used as enzyme extract. In each case, three separate extractions were made. PPO and PO were assayed according to the modified methods of Taneja and Sachar (1974) and Shannon et al. (1966), respectively. Enzyme units were expressed as change in 0.01 absorbance min^{-1} mg^{-1} protein. Catalase activity was measured by adopting the procedure of Sinha (1972) and expressed as µmoles of H_2O_2 utilized min^{-1} mg^{-1} protein. Suitable blanks in each case were also run simultaneously. Soluble protein in the enzyme extract was precipitated by TCA and determined according to Lowry et al. (1951).

Leaf samples left after extraction of enzymes, were dried at 60 °C in a hot air oven and ground in a micro-Willey mill. Total phenols were then extracted and estimated as per the method of Swain and Hillis (1959).

Results

Total phenols

Phenol content was higher in resistant genotypes throughout the growth period in comparison to healthy leaves of susceptible genotypes and its leves increased after infection of CYMV in susceptible genotypes (Table 1). However, the differences in phenol content with respect to 50 and 100 per cent disease severity were non significant. Total phenols in resistant genotypes remained more or less same at 40 and 50 DAS but declined at 60 DAS. In tolerant genotype, phenolic content decreased continuously throughout the growth period, whereas its level in susceptible genotypes increased initially and then decreased with age of the plant.

Polyphenol oxidase, peroxidase and catalase activity

Resistant and tolerant genotypes exhibited high PPO activity as compared to healthy leaves of susceptible genotypes at 40 and 50 DAS (Table 2). The enzyme activity in resistant genotypes decreased with the advancement of the age of crop, whereas a

Table 1

Total phenols (mg g^{-1}) in Healthy (H) and Diseased (D) cowpea leaves of Resistant, Tolerant and Susceptible genotypes at different stages of growth on dry weight basis

Genotypes	Days after sowing (DAS) 40	50	60
Resistant			
CS 55 (H)	18.20 ± 0.17	18.60 ± 0.50	16.10 ± 0.65
CS 39 (H)	21.50 ± 0.30	21.10 ± 0.28	17.20 ± 0.16
Tolerant			
ARL 25 (H)	18.20 ± 0.17	15.90 ± 0.35	12.90 ± 0.23
Susceptible			
HFC 42-1 (H)	16.00 ± 0.48	17.10 ± 0.14	12.70 ± 0.31
HFC 42-1 (50% D)	16.70 ± 0.19	18.90 ± 0.46	17.20 ± 0.33
HFC 42-1 (100% D)	16.70 ± 0.22	19.90 ± 0.17	17.50 ± 0.46
GC2 (H)	15.10 ± 0.31	19.20 ± 0.60	14.90 ± 0.15
GC2 (50% D)	20.10 ± 0.58	22.90 ± 0.20	19.80 ± 0.17
GC2 (100% D)	20.10 ± 0.65	23.00 ± 0.31	19.90 ± 0.36
CD at 5%	1.43	0.98	1.52

Mean values of triplicate determinations.

Table 2

Specific activity of polyphenol oxidase* in Healthy (H) and Diseased (D) cowpea leaves of Resistant, Tolerant and Susceptible genotypes at different stages of growth

Genotypes	Days after sowing (DAS) 40	50	60
Resistant			
CS 55 (H)	2.38 ± 0.16	2.36 ± 0.02	1.79 ± 0.03
Cs 39 (H)	3.15 ± 0.12	2.52 ± 0.10	2.21 ± 0.06
Tolerant			
ARL 25 (H)	2.14 ± 0.11	2.41 ± 0.13	2.73 ± 0.16
Susceptible			
HFC 42-1 (H)	1.70 ± 0.15	1.69 ± 0.04	2.03 ± 0.16
HFC 42-1 (50% D)	2.12 ± 0.02	2.54 ± 0.12	2.86 ± 0.17
HFC 42-1 (100% D)	1.89 ± 0.09	2.12 ± 0.22	2.75 ± 0.24
GC 2 (H)	1.93 ± 0.05	2.28 ± 0.05	2.47 ± 0.08
GC 2 (50% D)	2.38 ± 0.09	2.95 ± 0.21	3.54 ± 0.18
GC 2 (100% D)	2.37 ± 0.09	2.72 ± 0.13	3.08 ± 0.05
CD at 5%	0.24	0.29	0.42

* Enzyme units – Change in 0.01 absorbance min^{-1} mg^{-1} protein.

Table 3

Specific activity of Peroxidase* in Healthy (H) and Diseased (D) cowpea leaves of Resistant, Tolerant and Susceptible genotypes at different stages of growth

Genotypes	Days after sowing (DAS) 40	50	60
Resistant			
CS 55 (H)	$2.28 \times 10^3 \pm 0.37 \times 10^1$	$3.18 \times 10^3 \pm 0.24 \times 10^3$	$2.01 \times 10^3 \pm 0.07 \times 10^3$
CS 39 (H)	$3.22 \times 10^3 \pm 0.15 \times 10^1$	$4.46 \times 10^3 \pm 0.23 \times 10^1$	$3.27 \times 10^3 \pm 0.37 \times 10^3$
Tolerant			
ARL 25 (H)	$2.71 \times 10^3 \pm 0.21 \times 10^1$	$5.63 \times 10^3 \pm 0.22 \times 10^3$	$6.01 \times 10^3 \pm 0.41 \times 10^3$
Susceptible			
HFC 42-1 (H)	$1.68 \times 10^3 \pm 0.09 \times 10^3$	$2.88 \times 10^3 \pm 0.02 \times 10^3$	$3.47 \times 10^3 \pm 0.10 \times 10^3$
HFC 42-1 (50% D)	$3.07 \times 10^3 \pm 0.46 \times 10^1$	$4.33 \times 10^3 \pm 0.22 \times 10^3$	$5.28 \times 10^3 \pm 0.29 \times 10^3$
HFC 42-1 (100% D)	$3.41 \times 10^3 \pm 0.51 \times 10^3$	$4.60 \times 10^3 \pm 0.03 \times 10^3$	$5.71 \times 10^3 \pm 0.33 \times 10^3$
GC 2 (H)	$1.31 \times 10^3 \pm 0.06 \times 10^3$	$3.08 \times 10^3 \pm 0.11 \times 10^3$	$7.47 \times 10^3 \pm 0.41 \times 10^3$
GC 2 (50% D)	$4.61 \times 10^3 \pm 0.06 \times 10^3$	$7.25 \times 10^3 \pm 0.26 \times 10^3$	$8.27 \times 10^3 \pm 0.30 \times 10^3$
GC 2 (100% D)	$4.66 \times 10^3 \pm 0.17 \times 10^3$	$6.90 \times 10^3 \pm 0.29 \times 10^3$	$7.86 \times 10^3 \pm 0.54 \times 10^3$
CD at 5%	1.24×10^3	1.41×10^3	0.51×10^3

* Enzyme Units – change in 0.01 absorbance min^{-1} mg^{-1} protein.

Table 4

Specific activity of Catalase* in Healthy (H) and Diseased (D) cowpea leaves of Resistant, Tolerant and Susceptible genotypes at different stages of growth

Genotypes	Days after sowing (DAS) 40	50	60
Resistant			
CS 55 (H)	$3.03 \times 10^3 \pm 0.15 \times 10^1$	$4.53 \times 10^3 \pm 0.26 \times 10^3$	$3.20 \times 10^3 \pm 0.26 \times 10^3$
CS 39 (H)	$2.95 \times 10^3 \pm 0.15 \times 10^1$	$3.65 \times 10^3 \pm 0.04 \times 10^3$	$3.56 \times 10^3 \pm 0.04 \times 10^3$
Tolerant			
ARL 24 (H)	$3.61 \times 10^3 \pm 0.18 \times 10^3$	$4.35 \times 10^3 \pm 0.28 \times 10^3$	$3.24 \times 10^3 \pm 0.29 \times 10^3$
Susceptible			
HFC 42-1 (H)	$2.73 \times 10^3 \pm 0.18 \times 10^3$	$3.78 \times 10^3 \pm 0.20 \times 10^3$	$3.32 \times 10^3 \pm 0.18 \times 10^3$
HFC 42-1 (50% D)	$2.63 \times 10^3 \pm 0.19 \times 10^3$	$3.44 \times 10^3 \pm 0.04 \times 10^3$	$3.42 \times 10^3 \pm 0.20 \times 10^3$
HFC 42-1 (100% D)	$2.16 \times 10^3 \pm 0.09 \times 10^3$	$2.73 \times 10^3 \pm 0.21 \times 10^3$	$1.60 \times 10^3 \pm 0.13 \times 10^3$
GC 2 (H)	$2.67 \times 10^3 \pm 0.17 \times 10^3$	$3.53 \times 10^3 \pm 0.35 \times 10^3$	$3.03 \times 10^3 \pm 0.05 \times 10^3$
GC 2 (50% D)	$2.31 \times 10^3 \pm 0.11 \times 10^3$	$2.54 \times 10^3 \pm 0.15 \times 10^3$	$1.19 \times 10^3 \pm 0.07 \times 10^3$
CG 3 (100% D)	$1.78 \times 10^3 \pm 0.22 \times 10^3$	$2.01 \times 10^3 \pm 0.08 \times 10^3$	$0.98 \times 10^3 \pm 0.32 \times 10^3$
CD at 5%	0.24×10^3	0.57×10^3	0.39×10^3

* Enzyme units – μmoles of H_2O_2 utilized min^{-1} mg^{-1} protein.

reverse trend was observed in tolerant genotype. In susceptible genotypes, PPO activity increased gradually in response to both infection and growth stage. But enzyme activity in 50 per cent infected leaves of susceptible genotypes was considerably more than the corresponding activity in 100% infected leaves. The specific activity of PO in all the genotypes was many folds than that of PPO at different stages of growth (Table 3). As PPO, the specific activity of PO was also higher in resistant and tolerant genotypes both at 40 and 50 DAS in comparison to healthy leaves of susceptible ones and its activity in resistant genotypes increased gradually from 40 to 50 DAS and then decreased at 60 DAS. However, in tolerant and susceptible genotypes, its activity increased markedly with the advancement of growth stage. The leaves of susceptible genotype HFC 42-1 was found to have maximum PO activity at 100 per cent disease severity, whereas its activity in other susceptible genotype GC2 was maximum at 50% disease intensity. Infected leaves of susceptible genotypes had exceptionally higher enzyme activity than the corresponding healthy leaves.

Resistant and tolerant genotypes, in general, had higher catalase activity in their leaves in comparison to both healthy and diseased leaves of susceptible one at all stage of plant growth (Table 4). In all the three sets of genotypes, the catalase activity was maximum at 50 DAS, in comparison to its activity at 40 and 60 DAS. A general decline in catalase activity was obsereved with varying infection level at all stages of growth.

Discussion

Phenolic compounds and oxidative enzymes act as performed resistance factors and generally considered as most responsible parameteres for disease resistance (Sathiyanathan and Vidhyasekaran, 1981). Also phenolic accumulation is usually higher in esistant genotypes than susceptible ones (Luthra et al., 1988; Sohal and Bajaj, 1993; Sarma et al., 1995). In the present study too, the presence of more phenolics in leaves of resistant genotypes and their increase in response to infection suggests a positive role of phenolics in resistance of cowpea plants to CYMV disease. The post-infectional increase in total phenols is in agreement with the findings of Luthra et al. (1988). However, in-some instances, phenolic content has been shown to decrease after infection (Gupta et al., 1995).

It was observed during the present investigation that specific activities of both PPO and PO were relatively more in resistant and tolerant genotypes as compared to susceptible ones. Moreover, the specific activity of PO was many folds than that of PPO in all the genotypes at all stages of plant growth. This fact indicates their possible involvement in disease resistance mechanism. The importance of PPO and PO in host parasite interaction is that they contribute towards resistance by oxidation of phenolic compounds to quinones, which are more toxic to micro-organisms. Moreover, PO is also involved in lignin biosynthesis viz polymerisation of polypropane compounds by an oxidative H_2O_2 dependent system (Vance et al., 1980). High PPO and PO activity in

resistant genotypes has also been observed in other host pathogen interactions (Luthra et al., 1988; Gupta et al., 1995). The considerable increase in PPO and PO activities following infection in both resistant and susceptible plants has also been reported (Tripathi et al., 1975; Luthra et al., 1988; Sohal and Bajaj, 1993; Gupta et al., 1995). The presence of high activity of PPO and PO in healthy leaves of susceptible genotypes at 60 DAS in the present case may be due to the fact that theese leaves might have become infected without the development of visible symptoms.

The role of catalase in disease resistance still remains obscure. The presence of this enzyme in higher concentration is resistant genotypes in comparison to susceptible ones indicates its possible role towards resistance. Almost similar observations have been made in clusterbean (Chaudhary and Lodhi, 1981). The observed decrease in catalase activity following infection is contrary to the findings of Singh and Mall (1974) who observed increased catalase activity in urdbean infected with urdbean mosaic virus. The increased catalase activity probably reduces the efficacy of natural defences of plant through suppression of peroxidase activity by destroying its substrate H_2O_2 (Rudolph and Stahmann, 1964).

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Induction of Resistance to Tomato Spotted Wilt Virus in Mungbean (*Vigna radiata* (L.) Wilczek) by Antiviral Principles

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Antiviral principles (AVP) from aqueous leaf extracts of non-host plants like *Cocos nucifera*. L. *Sorghum Vulgare*. L., *Prosopis chilensis* (M) Stuntz. and *Croton sparsiflorus*. L., when applied before challenge inoculation, induced systemic acquired resistance and altered the physiology of test host. Changes in levels of total sugars, total soluble proteins and enzymes such as catalase, peroxidase and phenylalanine ammonia lyase (PAL) have been recorded. Application of AVPs did not alter the level of total sugars when compared to healthy controls. The reducing and nonreducing sugar contents increased marginally in plants treated with AVPs wile the contents declined in virus inoculated plants. In AVP-treated plants protein content increased marginally over healthy plants. The activites of three enzymes were stimulated to a maximum extent in plants treated by *C. sparsiflorus* AVP.

Resistance to plant viruses can be induced in susceptible plants by the application of antiviral principles (AVPs) (Allard, 1914) and chemicals (Gicherman and Loebenstein, 1968). AVP present in partially clarified aqueous leaf extracts of some plant species such as *Cocos nucifera*. L., *Sorghum vulgare*. L., *Croton sparsiflorus* L. and *Prosopis chilensis* (M) Stuntz induced systemic resistance against challenge inoculation of tomato spotted wilt virus (TSWV) on mungbean. With a view to understanding the role of sugars and proteins in the development of resistance induced by AVP, the present study was conducted to determine changes in mungbean plants following the application of AVP and inoculation with TSWV, in total sugars, total soluble proteins and the activity of enzymes which are known to be involved in the development of resistance to the virus infection.

Materials and Methods

Seeds of test host mungbean variety Co 5 were sown in pots filled with sterillized soil mixture, one-week-old plants were used for different experiments. The culture of tomato spotted wilt virus (TSWV) was maintained in local lesion assay host cowpea (Cv152) (*Vigna unguiculata* L.). The virus inoculum was prepared and inoculated on test plant by ice tray technique as described by Subramanian and Narayanasamy (1973). The AVPs from leaves of *C. nucifera*, *P. chilensis*, *S. vulgare* and *C. sparsiflorus* were tested.

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One gram of leaf tissues from above-mentioned plant species with 10 ml of distilled water was homogenised using a pestle and mortar and squeezed through cheese cloth, the extracted plant juice contains AVP. One ml of AVP was sprayed with the help of holmsprayer on the primary leaves of mungbean after one hour of inoculation of TSWV was done. The treatments were plants treated with AVP alone, plants treated with A and inoculated, uninoculated healthy and untreated inoculated controls. The samples were drawn at 1, 5 and 10 days after inoculation and the average is presented in Tables 1 and 2. Each treatment was replicated five times.

Total sugars and reducing sugars were estimated by Somogyi method (1952). The difference between total sugars and reducing sugars estimated corresponds to non-reducing sugars. Protein estimation was carried out by microkjeldahl method (Humphries, 1956). Enzyme activity was calculated on the basis of change in the absorbance per minute/mg of fresh sample (Mahadevan and Sridhar, 1986).

Results

Total sugars contents both in healthy and inoculated plants were increased with increase in plant age. The total sugar increased significantly in plants treated with AVP (23.93, 24.06, 24.3, 24.4 mg/g) while the contents showed a decline in inoculated plants (22.15 mg/g). Inoculation of plants treated with AVP showed very slight increase in total sugar contents (23.8, 23.9, 24.1, 24.3 mg/g) when compared to uninoculated healthy plants (23.6 mg/g).

The reducing sugar content increased marginally in the plants treated with AVP (12.66, 12.7, 12.86, 12.93 mg/g) while the contents declined in inoculated plants (11.8 mg/g). Inoculation of mungbean plants treated with AVP showed marginal decrease in reducing sugar contents (12.6, 12.6, 12.73, 12.83 mg/g), which however were more than that of inoculated plants (11.8 mg/g). However, the observed differences were not statistically significant. The non-reducing sugar content also exhibited similar changes as in the case of reducing sugar in healthy and inoculated mungbean plants following treatment with AVP (Table 1).

The total soluble protein contents increased significantly in inoculated plants reacting the maximum at 10 days after inoculation (141.41 mg/g). In AVP-treated plants, the protein contents increased marginally over healthy plants (138.65, 138.71, 138.73, 138.73 mg/g). Inoculation of mungbean plants treated with AVP showed marginal increase in protein content (138.7, 138.76, 138.76, 138.73 mg/g) when compared to AVP-treated plants, but it was significantly less than that of inoculated plants (141.41 mg/g), indicating that AVP might prevent the increase in protein content following inoculation as in untreated plants (138.33 mg/g) (Table 1).

AVP-treated plants stimulated the enzyme activity remarkably. The catalase activity was greater in inoculated plants (0.272 OD/min/mg) compared to healthy plants (0.256 OD/min/mg). The catalase activity was maximum in plants treated with AVP from

Table 1

Effect of AVPs on the total sugar, reducing sugar and non-reducing sugar and total soluble protein contents of mungbean

Treatments	Total sugar content (mg/g)	Reducing sugar content (mg/g)	Non-reducing sugar content (mg/g)	total soluble protein (mg/g)
Healthy	23.6*^{ab}	12.53*	11.13*	138.33^{b}*
Control inoculated	22.15^{b} (–6.14)**	11.8 (–5.82)	10.35 (–7.00)	141.41^{a} (2.22)
C. nucifera AVP treated	23.93^{ab} (1.39)	12.66 (1.03)	11.26 (1.69)	138.65^{b} (0.231)
Treated + inoculated	28.8^{ab} (0.84)	12.6 (0.55)	11.2 (0.62)	138.7^{b} (0.267)
S. vulgare AVP treated	24.06^{a} (1.94)	12.7 (1.59)	11.33 (1.779)	138.71^{b} (0.274)
Treated + inoculated	23.9^{ab} (1.27)	12.6 (0.79)	11.26 (1.16)	138.76^{b} (0.310)
P. chilensis AVP treated	24.3^{a} (2.96)	12.86 (2.63)	11.43 (2.6)	138.73^{b} (0.289)
Treated + inoculated	24.1^{a} (2.11)	12.73 (3.19)	11.3 (1.52)	138.76^{b} (0.310)
C. sparsiflorus AVP treated	24.4^{a} (0.38)	12.93 (3.19)	11.5 (3.32)	138.73^{b} (0.289)
Treated + inoculated	24.3^{a} (2.96)	12.83 (2.39)	11.43 (2.69)	138.73^{b} (0.289)
CD	1.80	Ns	Ns	2.68

*Mean of 5 replicates

**Data in parentheses are percentage increase or decrease over healthy.

Figures in a columns followed by the same letter are significantly different (P = 0.05) from each other according to DMRT.

C. sparsiflorus (0.279 OD/min/mg) followed by plants treated with *P. chilensis* (0.278 OD/min/mg) and *S. vulgare* AVP. The peroxidase and phenylalanine ammonia lyase (PAL) activities were augmented in plants treated with *C. sparsiflorus* AVP (0.037 and 0.134 OD/min/mg) followed by *P. chilensis* and *S. vulgare* AVP.

The activities of these enzymes were stimulated to the maximum extent by the AVP from *C. sparsiflorus* followed by *P. chilensis, S. vulgare* and *C. nucifera* AVP. Though the activities of these enzymes were less in plants treated with AVP following inoculation, the level of activity was greater than that of inoculated and healthy plants (Table 2).

Table 2

Effect of AVPs on the activities of catalase, peroxidase and phenyl alanine ammonia – lyase (PAL) in mungbean

Treatments	Catalase activity, OD/min/mg	Peroxidase activity, OD/min/mg	Phenyl alanine ammonia-lyase, OD/min/mg
Healthy	0.256^{c}*	0.029^{c}*	0.123^{d}*
Control (inoculated)	0.272^{d} (6.25)**	0.035^{b} (20.68)	0.129^{c} (4.87)
C. nucifera AVP treated	0.277^{bc} (8.20)	0.036^{ab} (24.13)	0.132^{b} (7.31)
Treated + inoculated	0.276^{c} (7.81)	0.036^{ab} (24.13)	0.132^{b} (7.31)
S. vulgare AVP treated	0.278^{ab} (8.59)	0.037^{a} (27.38)	0.133^{ab} (8.13)
Treated + inoculated	0.277^{bc} (8.20)	0.037^{a} (27.38)	0.132^{b} (7.31)
P. chilensis AVP treated	0.278^{ab} (8.59)	0.037^{a} (27.38)	0.133^{ab} (8.13)
Treated + inoculated	0.277^{bc} (8.20)	0.037^{a} (27.38)	0.133^{ab} (8.13)
C. sparsiflorus AVP treated	0.279^{a} (8.98)	0.037^{a} (27.38)	0.134^{a} (8.94)
Treated + inoculated	0.279^{a} (8.98)	0.037^{a} (27.38)	0.134^{a} (8.94)
CD	0.00135	0.00137	0.00186

* Mean of 5 replicates

** Data in parentheses are percentage increase (or) decrease over healthy.

Figures in a columns followed by the same letter are not significantly different (P=0.05) from each other according to DMRT.

Discussion

Plant viruses induce characteristic changes in the physiology of infected plants to enable them to have required proximate constituents for their replication. Attempts have been made to manipulate the defense genes using AVP and chemicals that can activate defense system (Srinivasulu and Narayanasamy, 1990). The changes in host physiology to increased host resistance following the application of AVP are discussed.

There was a marginal increase in the sugars in AVP-treated mungbean plants. It is possible that AVP might prevent depletion of the total sugar contents. The decrease in total sugar contents in TSWV-infected leaves might be due to a shift from production of sugars to aminoacids and organic acid was observed by Magyarosy et al. (1973) in squash plants infected with squash mosaic virus and young chinese cabbage infected with TYMV and Bedrock and Matthews (1973). Rao et al. (1989) reported that carbohydrates contents decreased in raddish plants infected by raddish mosaic virus and suggested that it might be due to assimilation of carbon towards protein synthesis resulting in the decreased level of carbohydrates.

Mungbean plants treated with AVP, showed marginal increase in protein contents, but TSWV infected plants had significantly greater amount of protein than AVP-treated plants (Table 1). The increase in protein contents in infected mungbean plants might be probably due to synthesis of virus coat protein, PR-proteins and other associated nitrogenous substances synthesised in response to infection. The synthesis of soluble protein was induced due to hypersensitive reaction in petunia inoculated with TMV suggesting a role for them in hypersensitive reaction leading to development of resistance (Dumas et al., 1988). Rao et al. (1989) reported that higher protein content in raddish plants infected by raddish mosaic virus, was due to increased activity of RNA synthetase/RNA polymerase which stimulated the synthesis of viral proteins and treatment with AVP resulted in inhibition of virus replication and formation of virus-related protein.

The AVPs might induce the mungbean plants to synthesis new proteins which might be responsible for increasing the level of resistance of mungbean plants to TSWV as suggested by Naseer et al. (1990) and Piper Point et al. (1990).

The higher activities of enzymes were noticed in AVP-treated plants when compared to inoculated and healthy plants. The AVP might switch of free expression of repressed genetic elements to yield higher quantities of existing enzymes or to synthesise new enzyme components which may be involved in the development of resistance. Verma and Prasad, (1987) reported AVP from *Clerodendrum aculeatum* induced resistance to sunnhemp rosette virus in *Cyamopsis tetragonoloba* and this resistance was due to increased activity of catalase, peroxidase and polyphenol oxidase following treatment with AVP. Similar changes in *Nicotiana tabacum* cv. Samsun NN inoculated with TNV resulting in systemic acquired resistance were reported by Verma and Prasad (1987). Systemic acquired resistance in hypersensitive tobacco to TMV infection was attributed to higher activities of catalase, peroxidase and PPO (Van Kammen and Brouwer, 1964; Simons and Ross, 1971).

Higher activities of oxidative enzymes might lead to accumulation of oxidised phenolics and quinones leading to development to resistance in treated plants Verma and Prasad (1987). The changes in the activities of oxidative enzymes indicate a possible activation of defense mechanism of the host by the AVP. Studies to determine the role of other enzymes involved in protein synthesis may help to understand the mechanism of action of antiviral principles in inducing resistance.

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Fusarium solani as a Cause of Vessel Discolouration in Declining Oak

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Vessel discolouration was found in oaks affected by oak decline. The discoloured vessels originated from short tunnels of an *Agrilus* species. The host reacted to the vessel infection by compartmentalizing discoloured vessels. From the discoloured vessel, *Fusarium solani* (Mart.) Sacc. could be isolated most frequently. In artificial inoculation experiments, *F. solani* produced the inner symptom of the vessel discolouration, and the fungus could be reisolated readily.

This disease may contribute to the oak decline syndrome. The effect of the disease on the health stage of oaks depends on the scale of *Agrilus* infestation.

By analyzing earlier reports on "vascular mycosis of oak" by mostly Eastern and Central European authors, it seems possible that the symptom described in those studies are the same as the vascular mycosis-like symptom examined in this paper.

The forest pathological literature of Eastern and Central Europe has repeatedly identified vascular mycosis of oak brought about mainly by various *Ophiostoma* species as a cause of oak mortality (Kryukova and Plotnikova, 1979/a, b; Kuzmichev, 19983/b; Urosevic, 1983; Guseinov, 1984; Capek, 1985). Although critical evidence for the role of these fungi is lacking, the threat of their spead has been taken seriously by researchers in Western Europe (Gibbs, 1981; Delatour, 1983, 1986). During an investigation of declining oaks in Hungary, vascular mycosis-like symptoms were found. This paper describes the characteristic features of this condition, its causal agent and its role in oak decline.

Materials and Methods

Sixty declining oaks, mostly *Quercus petraea* (Matt.) Liebl. and *Q. robur* L., but also including some *Q. cerris* L., were investigated in 1992 and 1993 in eleven plots in mid- and Western Hungary. The condition of each tree was assessed on a scale ranging from 0 (healthy) to 4 (dying). Most of the sample trees were classified as 1 (slightly damaged) and 2 (moderately damaged), since early symptoms of the decline were searched. Some asymptomatic control trees were investigated, as well. Fifteen trees were felled and the bark was partially removed from the butt to the twigs. On the remaining trees, only the lower 2 m of the trunk were examined.

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Samples of outer xylem showing discoloured vessels were removed with a chisel from twelve *Q. robur*, ten *Q. petraea* and three *Q. cerris*. Samples were taken randomly along discoloured vessels, but in a class 1 pedunculate oak, samples were arranged as originating in right next to the infection port (at 1–2 cm distance), near the infection port (at 5–15 cm distance) and far from the infection port (at several meters distance). Xylem chips, ca. 0.1–0.2 cm^3, were removed aseptically from the samples, placed on 2% malt or tap-water agar plates and incubated at room temperature. Development of microorganisms was checked daily from the second to seventh day after isolation. In order to identify the *Fusarium* spp., culturing was carried out on potato sucrose agar according to the procedures of Booth (1971).

Histological studies of wood segments were carried out with light microscope. Segments of outer xylem were removed by blade. Samples were not stained before examination.

An inoculation experiment was carried out on five *Q. robur* trees, including one control, with a diameter of 10–20 cm from 7th to 28th of August in 1992 in a pure oak wood ath the village Telki in mid-Hungary. Another inoculation experiment was carried out on four *Q. petraea* trees with a diameter of 3.5–4.5 cm from 6th of May to 15th of November in 1993 in a pure oak wood close to the previous location. One control and one comparative inoculation with *Ophiostoma piceae* (Münch) H. et P. (isolates derived from oak) were included. *O. piceae* was chosen because *Ophiostoma roboris* Georgescu et Teodoru, one of the specified causal agents of oak vascular mycosis, is probably synonymous with *O. piceae* (Przybyl and De Hoog, 1989). For the preparation of inoculum, 2- and 3-week-old malt agar cultures of *Fusarium solani* and *O. piceae*, respectively, were used. A suspension containing ca. 500,000 spores per ml was prepared by washing conidia from the plates with sterile water. For inoculation, eight holes were drilled in each tree at 1.2 m height. Inoculation holes were evenly distributed around the trunk. The diameter of the holes was 6 or 12 mm, the depth about 10–20 mm according to the tree's diameter. Approximately 0.5 or 2.5 ml of conidia suspension were applied to each hole according to the hole's dimension. After inoculation, the holes were sealed with adhesive tape. Control inoculations were conducted in the same way using sterile water.

Results

Discoloured vessels were observed in various extent in the wood of all the 32 investigated declining oak trees in 1992 but the symptom was much rarely found in 1993 and it has never appeared in healthy trees. Declining trees showed a typical decrease of the annual ring width in the last 2–7 years. After the bark had been removed, long bluish or brown streaks were visible on the xylem surface (Fig. 1), these forming small dots when viewed in cross section. The discoloured vessels were clearly linked to the larval tunnels of an *Agrilus* sp., probably *A. angustulus* Ill. These tunnels were unbranched, approximately 1 mm wide, 5–15 mm long, and usually horizontally positioned. Dark

Fig. 1. Vascular mycosis-like vessel discolouration in a young sessile oak (after bark removal)

spot could be observed in several cases on the bark surface above the larval tunnel as a result of slime flux in the spring. The tunnels were excavated in the xylem surface and it appeared that large, early-wood vessels of the current year that were damaged by tunnel construction very often formed the infection ports.

Detailed histological study provided some evidence for host defence reactions. In particular, vessels next those that were infected were often blocked with tylosis. Tylosis and gum-like substances appeared scattered along the infected vessels. Discolouration extended, in some cases, several meters up and down from an insect tunnel, but in most cases the infection ceased after a distance of only a few centimeters.

Where ray parenchyma was present next to the infected vessels, it seemed to substitute tylosis formation. Thus, parenchyma tissue may act as a barrier. If a tree in decline was able to continue its life, new annual rings were formed and discoloured vessels were buried in the xylem. A "barrier zone" lacking broad vessels was formed in succeeding one or two annual rings near the infected vessels. The cambium becomes dead around the *Agrilus* tunnel and eventually a vertically elongated, rhomboid wound (5 to 20 mm wide and 15 to 150 mm long) is formed in the wood tissue that appears as a vertical gap on the outer bark.

Depending on the particular year of infection, discoloured vessels were located in one of the outer annual rings of the xylem. They were found mainly in the bole and only in a few twigs. Regarding a particular tree, the seriousness of the vessel discolouration was associated with the scale of *Agrilus* infestation. In heavily infested trees considerable portions of transporting vessels were blocked as the result of infection.

The vessel discolouration symptom could be found on each three *Quercus* species examined and almost on each plots.

F. solani was the fungus most frequently isolated from the samples taken from discoloured vessels. The freqency of isolation was 33% from pedunculate oaks, 30% from sessile oaks and the fungus was present in turkey oaks, as well. A *Leptographium* sp. was also isolated both from sessile and pedunculate oaks (17% and 11%, respectively), and a *Cephalosporium* sp. was isolated from pedunculate oaks (11%). About 25% of the sampled xylem chips were sterile. *F. solani* was also the most widespread species when isolations were made from trees at different geographical locations. The closer to an *Agrilus* sp. tunnel the wood chips were taken, the higher was the occurrence of *F. solani*. In a particular class 1 pendunculate oak, the rate of occurrence of *F. solani* was 56% right next to the *Agrilus* tunnel, 31% near the tunnel and 19% far from the tunnel. The isolation of *F. solani* was less successful and sparse from discoloured vessels buried in the xylem.

In the pathogenicity test in 1992, the control inoculations gave negative results, only a very pale discolouration could be observed extending 1 to 5 cm below and above the inoculation holes. Inoculation with *F. solani* caused vessel discolouration that appeared very similar, both in macroscopic and in microscopic views, to the symptoms observed on declining oaks. The average of length of discoloured vessels extending below the individual inoculation points was 132 mm, with a range of 30 mm to 235 mm maximum. Above the inoculation holes, the average of the maximum lengths of discoloured vessels originating from the individual inoculation points was 326 mm, the minimum and the maximum was 170 mm and 900 mm, respectively. *F. solani* was reisolated from 100% of discoloured vessels.

In the pathogenicity test in 1993 the control inoculations resulted in a pale brown discolouration extending maximum 10 cm below and above the inoculation holes. Infection with *F. solani* caused a vessel discolouration ranging from a maximum of 110 cm below and maximum 100 cm above the inoculation point. Inoculations with *O. piceae* showed a vessel discolouration spreading maximum 60 cm below and maximum 150 cm above the inoculation hole. *F. solani* could be reisolated in all but every cases. *O. piceae* was reisolated from 50% of the samples, and it was contaminated with *F. solani* in a few cases.

Description of *Fusarium solani*: Linear growth rate of the cultures was 4.0 to 4.2 mm/day, so the diameter of colonies was about 32 mm after 4 days at room temperature. Aerial mycelia with micro condiophore were white and sporodochia were blue. The agar surface under the culture was lilac and the agar reverse was pink. Micro conidiophore were developed and formed conidia after 1 to 2 days of inoculation. Micro conidiophore were *Cephalosporium* – like, 45 to 110 μm long, 3 to 4.5 μm wide at the base and 1.6 to

2.2 µm wide at the cylindrical phialid neck, more ore less branched, producing conidia in heads. Macro conidiophore were developed after a week of incubation or later, aggregating in sporodochia. Micro conidia were usually one-, rarely two-celled, 6.5–11 (–16.5) × × 2–4.5 µm. Macro conidia were curved, (2–)3–5(–8) septate, 30–50 × 5 µm. Septa were often hardly visible, the foot cell was only slightly expressed and was recognizable only on a part of the macroconidia.

Discussion

The host reaction succeeding *F. solani* infection partially blocks water transport. Regarding the narrow outer annual rings of declining trees, effect of *F. solani* infection on water transport capacity can be significant. The harmful effect of phytotoxins produced by *F. solani* may also decrease the host's vitality (Dellavalle et al., 1993; Ragazzi et al., 1993). Consequently, *A. angustulus* infestation followed by *F. solani* infection may contribute to the oak decline process.

F. solani is frequently reported to cause cankers in woody plants (Booth, 1971). This fungus is associated also with an annual canker of oaks (Gibbs, 1982). Brasier (1990) described limited vessel discolouration caused by *F. solani* in elms that was associated with tunnels produced by *Scolytus multistriatus* Marsh. where *Ophiostoma ulmi* (Buism.) Nanf. was apparently absent. In an experiment on banana, Beckman and Halmos (in: Garrett, 1970) found that *F. solani* and *F. roseum* (Link) initially developed well when inoculated directly into vessels but was stopped later by the host's defense mechanisms. Considering all of this, the presence and the function of *F. solani* is not surprising in oaks.

The symptom of *F. solani* infection is similar to a vascular mycosis, at first sight. As in a vascular disease, the pathogen is confined to the vessels. However, unlike real vascular pathogens, *F. solani* is not able to break out from the infected vessel. Because of the compartmentalization, this symptom cannot be considered as a vascular mycosis as defined by Beckman (1987). This phenomenon is a vascular mycosis-like vascular infection. Only high numbers of individual vessel infections can cause serious damage to a tree.

There are many reports in the forest pathological literature of Eastern and Central Europe about vascular mycosis of oak. The term "vascular mycosis" is used in several reports for obviously different symptoms like local canker or sapstain (Urosevic and Jancarík, 1959; Urosevic, 1983; Capek et al., 1985). Vascular mycosis of oak is usually attributed to different *Ophiostoma* or *Ceratocystis* species. The name *Ceratocystis* is generally used as the synonym of *Ophiostoma*, not regarding the taxonomic difference between the two genus (Harrington, 1981; De Hoog, 1984; Brasier, 1993).

Similarities can be found between the symptoms of the so-called vascular mycosis of oak and the vascular mycosis-like symptom caused by *F. solani*. For instance, dark dots can be seen in cross-section view of affected tissues, usually in one particular annual ring. The early wood's vessels are affected. Vessel discolouration is not continuous, it

has a definite length ranging from some centimeters to a few meters (Kryukova and Plotnikova, 1979/a, 19779/b, 1982; Kuzmichev, 1983/a, 1983/b; Guseinov, 1984; Capek et al., 1985; Hesko, 1985; Selochnik and Kondrashova, 1989; Kryukova, 1991). The last feature does not follow the definition of vascular mycosis. On the other hand, real vascular mycosis would affect the tree from the root tips up to the leaves, whereas the symptoms of vascular mycosis of oak can be found mostly in the trunk and in thick branches (Kuzmichev, 1983/a; Capek et al., 1985; Selochnik and Kondrashova, 1989).

Varying origins of infection are described in the literature, as broken twigs and feeding wounds of *Scolytus* species (Kuzmichev, 1983/a; Selochnik and Kondrashova, 1989). Nevertheless, the infection is brought into connection also with larvae of an *Agrilus* species (Capek et al., 1985; Hesko, 1985).

Kuzmichev (1983/a) mentioned also two *Fusarium* species that cause vascular mycosis, including *F. solani*. Kuzmichev (1983/a) stated that these *Fusarium* species are more dangerous causal agents of vascular mycosis than *Ophiostoma* species.

A workshop on the role of *Ophiostoma* species in oak decline was held in 1990 in Berlin. Author of this paper could get there some *Ophiostoma kubanicum* Scherbin-Parf. cultures from Dr. E. A. Kryukova. This species is considered to be the most harmful vascular pathogen on oak by Kryukova and Plotnikova (1979/a, 1979/b, 1982, 1991). The fungus in Kryukova's cultures was identified as *F. solani*. Dr. C. M. Brasier has made similar observations (Brasier, personal communication in 1992).

Considering all of the above, the disease called "vascular mycosis of oak" may be a limited vascular infection whose causal agents include *F. solani*. This pathogen is surely adapted to tree species, but it is not a specialized pathogen on oak. As it could be seen in the inoculation experiment, *O. piceae* is also able to produce the same symptom.

Conclusion

In most cases *Fusarium solani* seemed to be the causal agent of the vessel discolouration in pedunculate, sessile and turkey oak. This disease may contribute to the oak decline syndrome. The disease can be serious only at a high level of *Agrilus angustulus* infestation that makes it possible for the fungus to get directly into the vessels.

In Eastern and Central Europe, efforts to explain the vessel discolouration gave birth to the forest pathological "phantom" called "vascular mycosis of oak" that can rather be a compartmentalized vascular infection than a true vascular mycosis.

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Ceratocystis erinaceus: A New Endophyte in the Heartwood of Oak

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A probably very common, new *Ceratocystis* species was found in *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. Presence and mycelial growth of the fungus is restricted to the heartwood. The new *Ceratocystis* species may be an endophyte, mostly found in the butt and in the lower bole. Description of the new species is given.

Oak decline is a widespread complex disease occurring in Europe and North America (Luisi et al., 1993). However, reports have been published in the East and Central European forest pathological literature about a vascular mycosis of oak, a single factor disease, that is attributed usually to various *Ophiostoma* or *Ceratocystis* species (Kryukova and Plotnikova, 1979; Urosevic, 1983). These *Ophiostoma* or *Ceratocystis* species have been poorly documented (Przybyl and De Hoog, 1989). The name *Ceratocystis* is generally used in these reports as a synonym of *Ophiostoma*, not distinguishing the difference between the two genus according to Harrington (1981) and De Hoog (1984).

In relation to oak decline in Hungary, considering the above reports, it seemed to be necessary to examine what particular *Ophiostoma* or *Ceratocystis* species occur in oaks.

While this study was going on, a *Ceratocystis* species was frequently found in damp chamber experiments which fungus could not be identified using the available monographs and the later descriptions of new species (Hunt, 1956; Upadhyay, 1981; Butin and Aquilar, 1984; Bridges and Perry, 1987; Kile and Walker, 1987; Redfern et al., 1987; Hutchison and Reid, 1988; Wingfield et al., 1988; Kowalski and Butin, 1989). Since this fungus did not correspond to any of the reported species, it was described as a new one.

This paper concentrates on the description of the new species and its known ecological properties, but deals only briefly with the results of the study which are interesting mainly from a forest pathological point of view.

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Materials and Methods

Since the original aim of this study was surveying *Ophiostoma* and *Ceratocystis* species present in healthy and declining *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L., it seemed to be an adequate method to mainly use damp chambers.

The samples were collected from altogether 47 *Q. petraea* and 27. *Q. robur* trees in oak forests in 7 locations in West Hungary in 1989 and 1990. One declining *Q. petraea* tree was examined in Gloucestershire in Great Britain in 1991. Eighty percent of the sample trees were in various levels of decline, the remaining ones were healthy.

Three to six disks were taken from trees starting at the base of the trees and going as high as 10 cm thick branches. These disks of *Q. petraea* and *Q. robur* were 5 cm thick, and were cut by chainsaw and put into nylon bags. The bags were sealed and after 21 days of incubation in normal changes of light at room temperature the disks were examined.

Isolations were made aseptically, as well, using surface sterilized wood pieces of 30–40 cm^3, in order to exclude surface contamination. Surface sterilization was made by flaming.

Cultures were grown on 2% malt agar at room temperature and at 22 °C in darkness. Morphological investigations were conducted on freshly made tap water preparations, using monospore cultures derived from ascospores.

Results and Discussion

Immediately after the collection there was no significant discoloration on the cutting surfaces of the disks from healthy and even from declining trees.

Two *Ophiostoma* species appeared after incubation in the sapwood on the cut surface of the sample wood disks. These species were identified as *O. piceae* (Münch) H. and P. Syd. and *O. stenoceras* (Robak) Melin and Nannf. Their occurrence did not have any relation to the health stage of the trees, and they might be present in the sapwood as endophytes. *O. piceae* was the more frequent. It was found in almost all of the trees in the sample. Frequency of the occurrence of *O. stenoceras* was 70% and 55% in *Q. petraea* and *Q. robur,* respectively.

The cut surface of the heartwood was colonized in many cases by an unknown *Ceratocystis* species. On the one hand, heartwood colonizers are usually not too important from a plant pathology point of view, on the other hand, it was remarkable to find a *Ceratocystis* species in the heartwood because all the known species of *Ceratocystis* are sapwood colonizers and some of them are causal agent of vascular mycosis.

Description of the fungus:

Ceratocystis erinaceus Bohár sp. nov. Status conidialis: *Chalara* sp.

Ascomata nigra, basis elliptica vel globosa, 45–80 (120) µm in diam., ornata longis, rigidis, septis hyphis, 3–4 × –550 µm; collum 540–1425 µm longum, ad basim 17–26 µm, ad apicem 6–15 µm latum; fimbriae ostiolares 4–10, hyalinae, convergentes ad apicem hypharum ostiolarium, 18–60 µm longae, ad basim 1 µm latae. Asci saccati, evanescentes. Ascosporae hyalinae, in vagina gelatinosa, extendae ellipticae, leniter curvatae 3.7–5.5 × 1.3–1.7 µm.

Conidiophorae singulares, non ramificatae, septae, 14–160 (300) µm longae, ad basim 2–5 µm in diam.; cellulae conidiogenae phialides, ad latitudinem maximam 3–7 µm, ad apicem 1–2.5 (3) µm latae. Conidiae hyalinae, cylindricae, in extremo plus minusive rotundatae, 4–14 × 1–2 (3) µm. formatae singulariter vel in catena.

Habitat: In duramine *Quercus petraea* (Matt.) Liebl.

Holotypus: Sitke, in Hungaria, 6. IX. 1989. BP FN 677

Colonies 45 mm in diameter in 12 days at 22 °C, velvety, grey with a green shade of colour at first, becoming whitish-grey with dark-greyish, forming blackish spots or zones and later sectors as a result of copious production of conidia (white in mass) and ascocarps (black); reverse dark with some mm wide light skirt.

Mycelium superficial and immersed; superficial hyphae pale brown, thick-walled, 1.3–3.9 µm wide; submerged hyphae hyaline to pale brown, thin- to thick-walled, 0.7–5.5 µm wide.

Conidiophores (Fig. 1C) single, simple, septate, pale brown, 14–160 (300) µm long including terminal conidiogenous cell, 2–5 µm wide at the base. Conidiogenous cells phialidic, cylindrical, pale brown turning to hyaline toward the tip, at the broadening 3–7 µm, at the apex 1–2.5 (3) µm wide. Conidia hyaline, cylindrical, more or less rounded at the ends, 4–14 × 1–2 (3) µm, formed singly or in chains.

Ascocarps (Fig. 1A, D) black, superficial but in the middle part of culture the bases mostly embedded in the dense tangle of aerial mycelia, conidiophores and ornamentation of bases. Bases elliptic or globose, 45–80 (120) µm in diameter, ornamented with brown, septate, long, rigid hyphae up to 550 × 3–4 µm. Outer peridium composed of dark, thick-walled cells up to 12 µm in diameter. Necks black, straight, 540–1425 µm long including ostiolar hyphae, 17–26 µm wide at the base, 6–15 µm wide just below the ostiolar hyphae. Ostiolar hyphae (Fig. 1B) 4–10 in number, hyaline, nonseptate, convergent toward the tip, 18–60 µm long, 1 µm wide at the base. Asci saccate, evanescent, can be seen only in the young, matured ascocarps. Ascospores (Fig. 1E) hyaline, slightly bent elongated elliptical in side view, elongated elliptical in face view, circular in end view, 3.7–5.5 × 1.3–1.7 µm, surrounded by a thin gelatinous sheath, collecting in a white coloured drop at the tip of the neck.

Habitat: In heartwood of *Quercus petraea*.

Holotype: Dried culture BP FN 677 isolated from *Quercus petraea* near Sitke, Hungary; 6 September 1989.

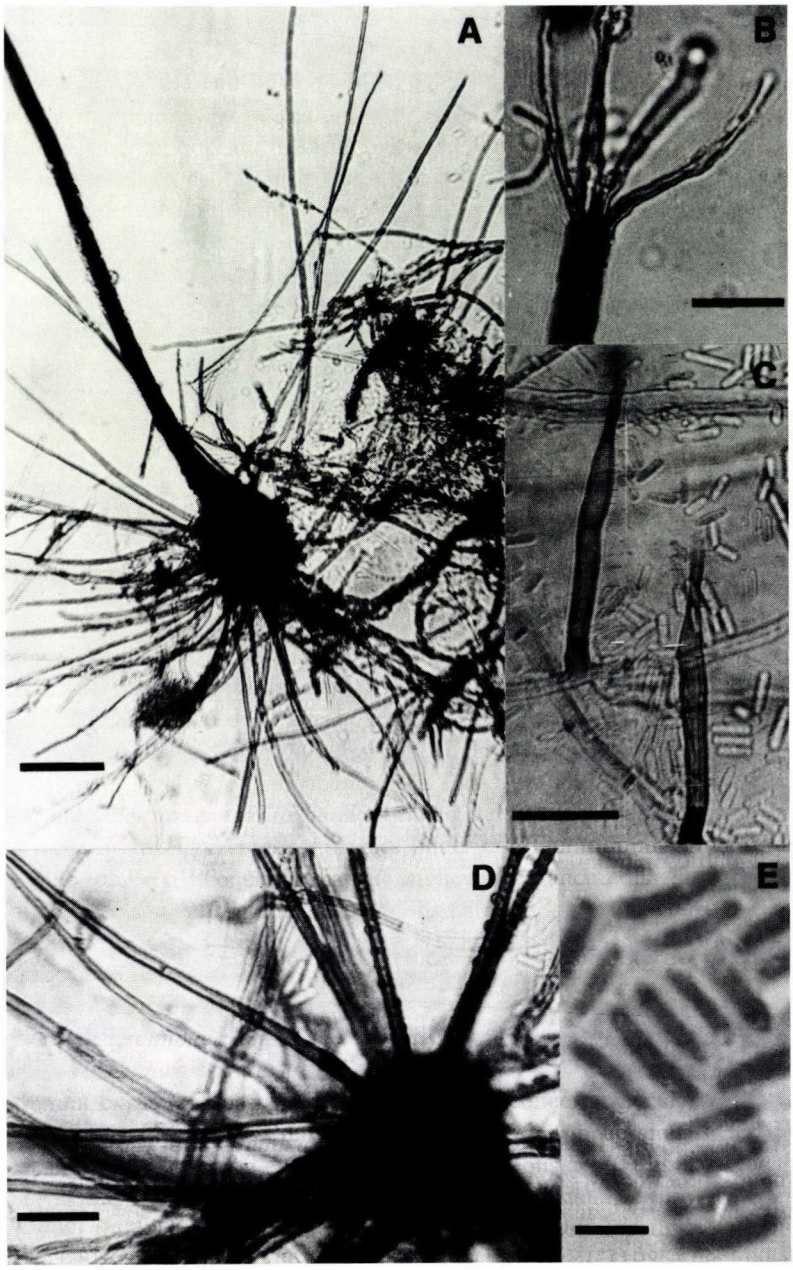

Fig. 1. *Ceratocystis erinaceus*. A: ascocarp with long, rigid ornaments stemming from the base (Bar = 50 μm); B: ostiolar hyphae (Bar = 20 μm); C: *Chalara* state with conidia (Bar = 20 μm); D: forming ascocarp base with rigid ornaments (Bar = 20 μm); E: Ascospores (Bar = 3 μm)

Living cultures: NCAIM F 00885 isolated from *Quercus petraea* near Sitke, Hungary; NCAIM F 00886 isolated from *Q. petraea* near Bejcgyertyános, Hungary; NCAIM F 00887 isolated from *Q. petraea* in Gloucestershire, Great Britain.

There are some similarities between *C. erinaceus* and *Ceratocystis coerulescens* (Münch) Bakshi in the shape and ornamentation of ascocarps. Comparing the descriptions of *C. coerulescens* (Hunt, 1956; Upadhyay, 1981) with *C. erinaceus*, however the growth rate and colours of cultures, as well as almost all the characteristic measures are very different.

One strain of *C. erinaceus* isolated in Hungary and one isolated in Great Britain were compared with one strain of *C. coerulescens* isolated by Gibbs in Great Britain from *Pinus sylvestris*. Growing at room temperature, the colony diameters were 31 mm and 59 mm of *C. erinaceus* and *C. coerulescens* cultures after 8 days, respectively. Colonies of *C. erinaceus* were velvety with light grey colour while colonies of *C. coerulescens* were fluffy and dark grey. Diameters of the bases of ascocarps were 63–93 (–100) µm and 150–225 (–275) µm, respectively. Therefore, the two species can be distinguished readily.

Wingfield has suggested that *C. erinaceus* may be identical with *Ceratocystis virescens* Davidson (personal communication), a species considered to be synonymous (Hunt, 1956) with *C. coerulescens*. Even if we accept the existence of *C. virescens* as a separate species, comparing the original description of Davidson (1944) with data of *C. erinaceus*, we can find profound differences. Main measurements of the ascocarp and the ascospores are very different. *C. erinaceus* does not produce barell-shape conidia like *C. virescens* and *C. coerulescens*, and it has a much slower growth rate than the latter two species. Furthermore, *C. virescens* colonizes the sapwood whereas *C. erinaceus* can be found in the heartwood only.

Based on morphological studies and ecological properties of the fungus, we consider *C. erinaceus* a seperate species. However, the use of molecular taxonomy seems to be inevitable, to corroborate our present findings.

C. erinaceus is a homothallic species, because it is also able to develop ascocarps in monospore culture.

C. erinaceus could be found in all the studied locations, even is Gloucestershire. Frequency of the occurrence of the fungus in the investigated trees was 50% both in *Q. petraea* and in *Q. robur*.

The use of the method of damp chamber cannot preclude the possibility of subsequent contamination, so some isolations were done with surface sterilized heartwood pieces of 30–40 cm^3. *C. erinaceus* could be isolated with 15% success this way.

The occurrence of *C. erinaceus* did not have any relation to the health stage of the trees, the fungus was present equally both in healthy and in declining trees. *C. erinaceus* was found exclusively in the samples originating from the lower part of the trunks.

In damp chambers, *C. erinaceus* colonized only the cut surface of the heartwood. The fungus never appeared on the cut surface of the sapwood or on the bark. When ascospores of *C. erinaceus* were placed onto both the sapwood and heartwood surface of freshly cut disks originated from *Q. robur*, the fungus colonized the surface of the heart-

wood, but was not able to grow on the sapwood surface. However, *C. erinaceus* grew well on autoclaved sapwood pieces.

C. erinaceus was found on the incubated wood surface with ascocarps and in the *Chalara* state. Beneath the colonies the heartwood surface turned greyish.

Considering the data above, *C. erinaceus* seems to be an ubiquitous latent colonizer present in the heartwood of oak that has a peculiar metabolism adapted to the heartwood and may cause some discolouration of it.

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A Spatio-temporal Analysis of Fungal Pathogens on Reed in Natural Habitats

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Two different habitats at Lake Balaton (Hungary) were compared for temporal and spatial spread of pathogenic fungi on reed (Phragmites communis Trin.) in 1995. Since *Puccinia phragmitis, Puccinia magnusiana, Polythrinciopsis phragmitis, Deightoniella arundinacea* and a *Phoma*-like fungus are among the most common pathogens on reed leaves and stems (Fischl et al., 1993) these species were the subject of our investigation.

Seasonal surveys have shown differences in disease development between the two habitats. Higher infection rates were found for *Puccinia phragmitis, Polythrinciopsis phragmitis* and the *Phoma*-like fungus at Fenékpuszta, whereas *Deightoniella arundinacea* followed by *Puccinia magnusiana* caused the heaviest infection at Balatonszentgyörgy. Differences in the environmental factors between the two habitats are considered to be affecting disease development differently.

Common reed (Phragmites communis Trin.) plays a significant role in natural waters. As a common uliginal plant it produces a great amount of biomass, cleans the water of lakes and ponds and also provides shelter for animals (Kovács et al., 1979).

Some of the pathogens known from common reed (eg.: *Ustilago grandis, Deightoniella arundinacea*) can seriously damage the stem and leaves thus making industrial use impossible (Ellis, 1957; Ruttkay et al., 1964). The extent to which the causal agents of leaf spot diseases (e.g.: *Puccinia phragmitis, Puccinia magnusiana, Polythrinciopsis phragmitis, Deightoniella arundinacea* and the *Phoma*-like fungus) are associated with losses in reed stem quality is still unknown.

Reed decline (or dieback) is a common phenomenon in lakes in Hungary but the possible involvement of pathogenic fungi in this process is not yet clear (Kovács et al., 1979; Fischl, 1993).

Recent observation on fungal diseases in wetlands suggested that those areas may act as reservoir sites for field crop pathogens, since some of them can infect both agriculturally and horticulturally important plants [e.g.: Maize dwarf mosaic virus (Horváth et al., 1987), rusts of Carex spp. (Bánhegyi et al., 1985–87), *Fusarium* sp., *Pseudoperonospora cubensis* (Fischl, 1996), etc.].

Although many fungi have been described and isolated from reed so far (Moesz, 1930, 1933; Apinis et al., 1972; etc.), epidemiological and ecological studies are lacking.

The aim of the present study was to investigate the spatial and temporal distribution of the most common fungal pathogens on reed and to explore any variation in the development of diseases at the two different habitats.

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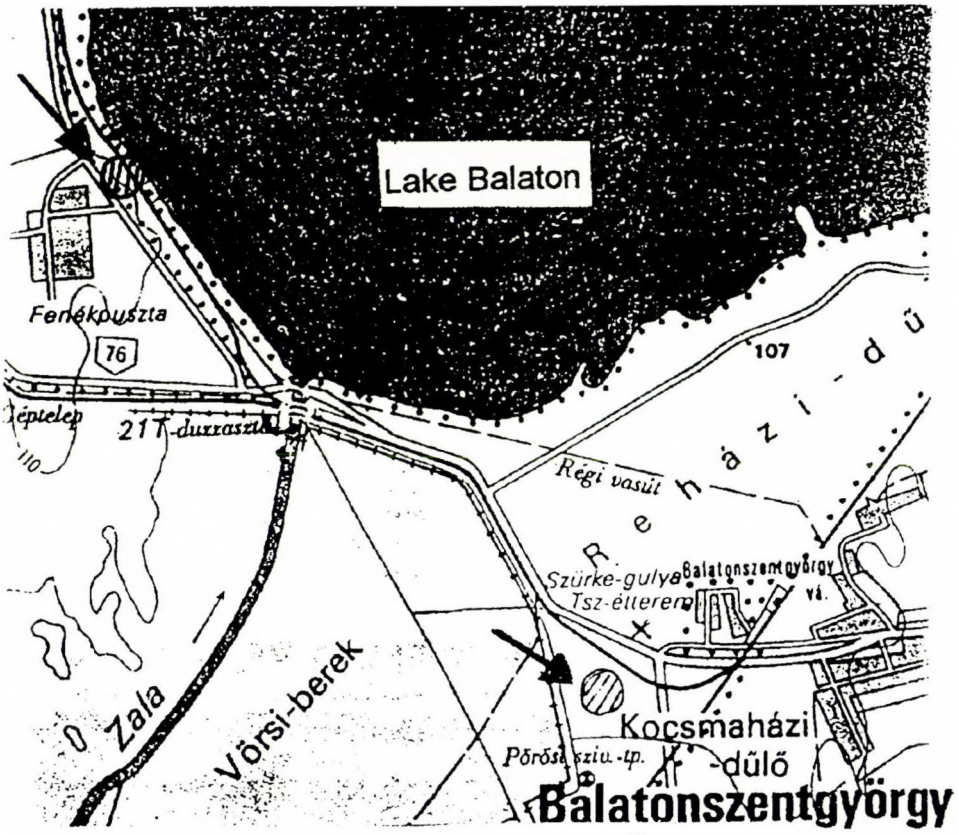

Fig. 1. Survey sites at Lake Balaton (▨)

Materials and Methods

Sites of survey

Investigations were carried out in two locations both situated on the west coast of Lake Balaton (Fig. 1). One site at Balatonszentgyörgy consisted of an extended reed stand in a semi-arid area which is temporarily flooded only in spring and in autumn and is 2–3 km from the lake.

Another site at Fenékpuszta, however, forms a reedbelt along the cost and is cut by landing stages in many sites. In both locations reed is partly harvested from year to year.

Pathogenic fungi

Five common fungal pathogens of reed were involved in the experiment.

Puccinia phragmitis (Schumacher) Koernicke is the most widespread rust fungus on reed causing redish brown and black, elongated pustules (uredo- and teleutosori) on leaves and stems. The fungus is heteroecious completing its life cycle on reed and Rumex sp.

Puccinia magnusiana Koernicke another rust fungus can frequently be found on reed but to less extent than the previous one. The latter forms small, orange teleutosori on leaves and it is also heteroecious (reed-Ranunculus sp.).

Polythrinciopsis phragmitis (Walker, 1966) is a mitosporic fungus. Infection of *P. phragmitis* is followed by the formation of large, brown spots on leaves which seem to involve two concentric circles. During heavy infections spots can fully cover the area of leaves and reduce the assimilative surface.

Phoma-like fungus. A Phoma-like fungus often causes leaf spot on reed. Exact identification of this fungus is needed in the future.

Deightoniella arundinacea (Corda) Hughes, unlike the previous pathogens, exhibits different symptoms. These are characterized either by diamond-shaped spots or dead leaves on whick black masses of conidia occur. Infection by *D. arundinacea* often results in a decrease in plant growth.

Disease assessment

Disease assessment was carried out by random sampling of 50 plants in July and October at both sites. The number of diseased plants were considered and the disease severity calculated by determining the percentage of diseased leaves within each single plant. Then plants were categorized (disease categories) by their disease severity rates according to the scale:

0: 0–1%; 1: 1,1–10%; 2: 10,1–20% ... 10: 90,1–100%.

Finally, infection indices were calculated.

Results

Frequency distributions of plants with different disease levels (or disease categories) by different pathogens were compared at two locations in two assessment dates (Figs 2, 3). There were marked differences in the disease development between the locations. Both of the rusts (*Puccinia phragmitis* and *P. magnusiana*) and *Polythrinciopsis phragmitis* developed much earlier at Fenékpuszta than at Balatonszentgyörgy (Fig. 2). Comparing the incidence of the two rusts in July it can be stated that *Puccinia magnusiana* was more scattered than *Puccinia phragmitis* at Balatonszentgyörgy. Furthermore, in the case of *Polythrinciopsis phragmitis* there were disease free plants at Balatonszent-

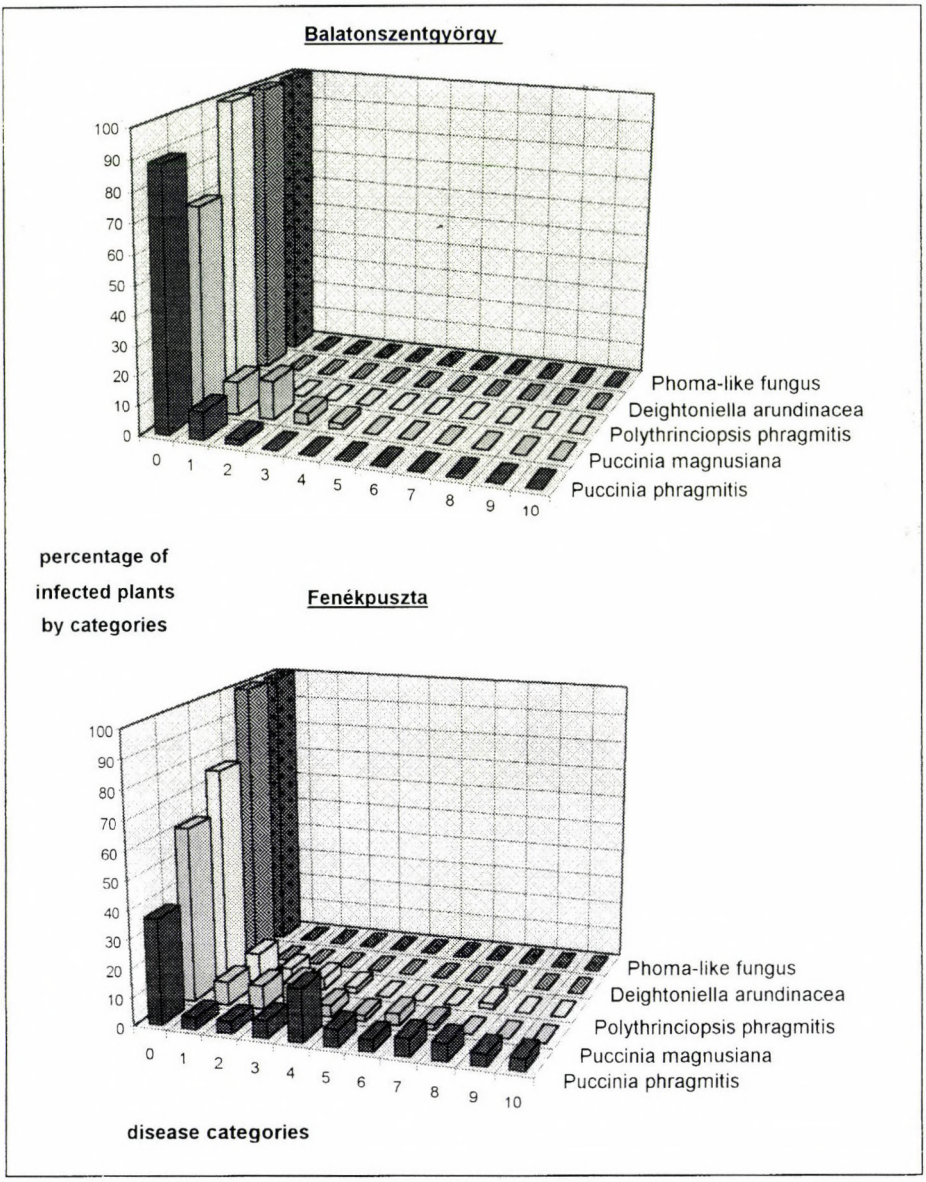

Fig. 2. Frequency distributions of reed plants with different % disease levels by different pathogens in July 1995

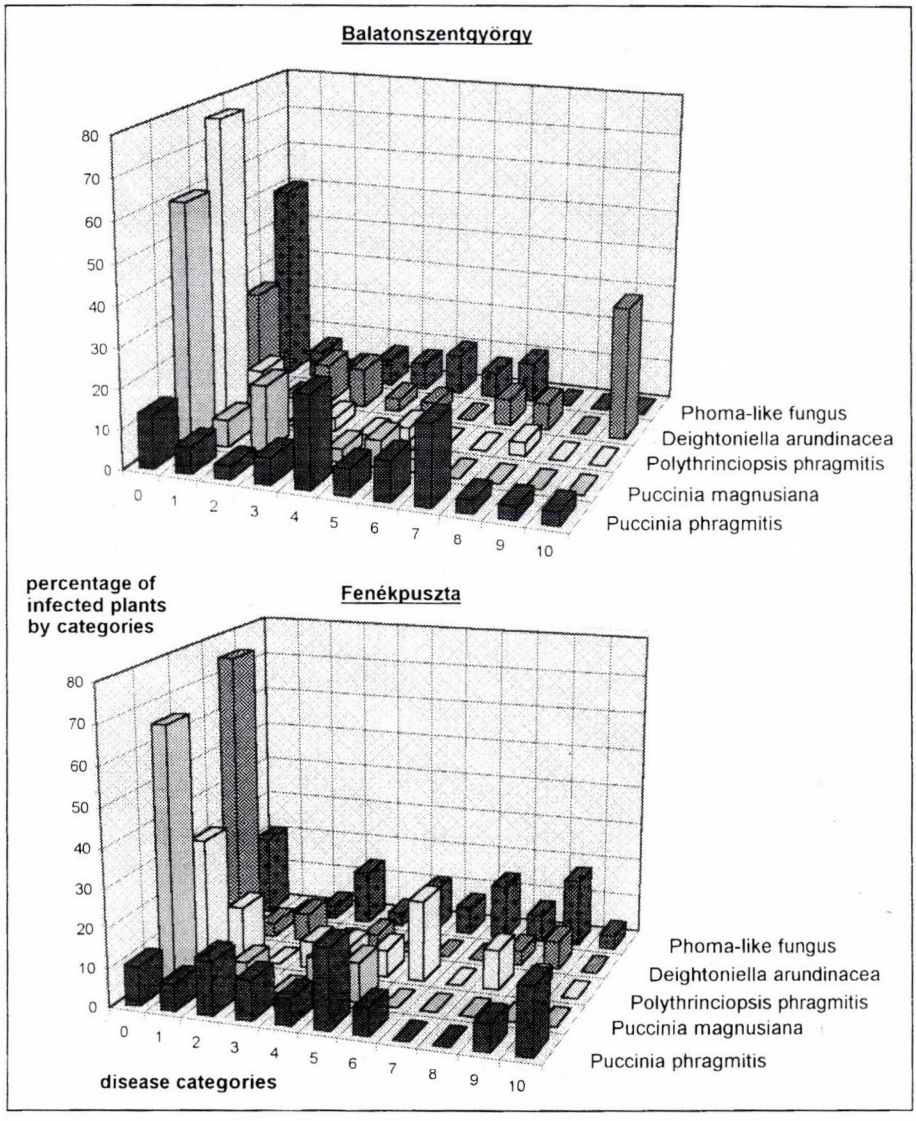

Fig. 3. Frequency distributions of reed plants with different % disease levels by different pathogens in October 1995

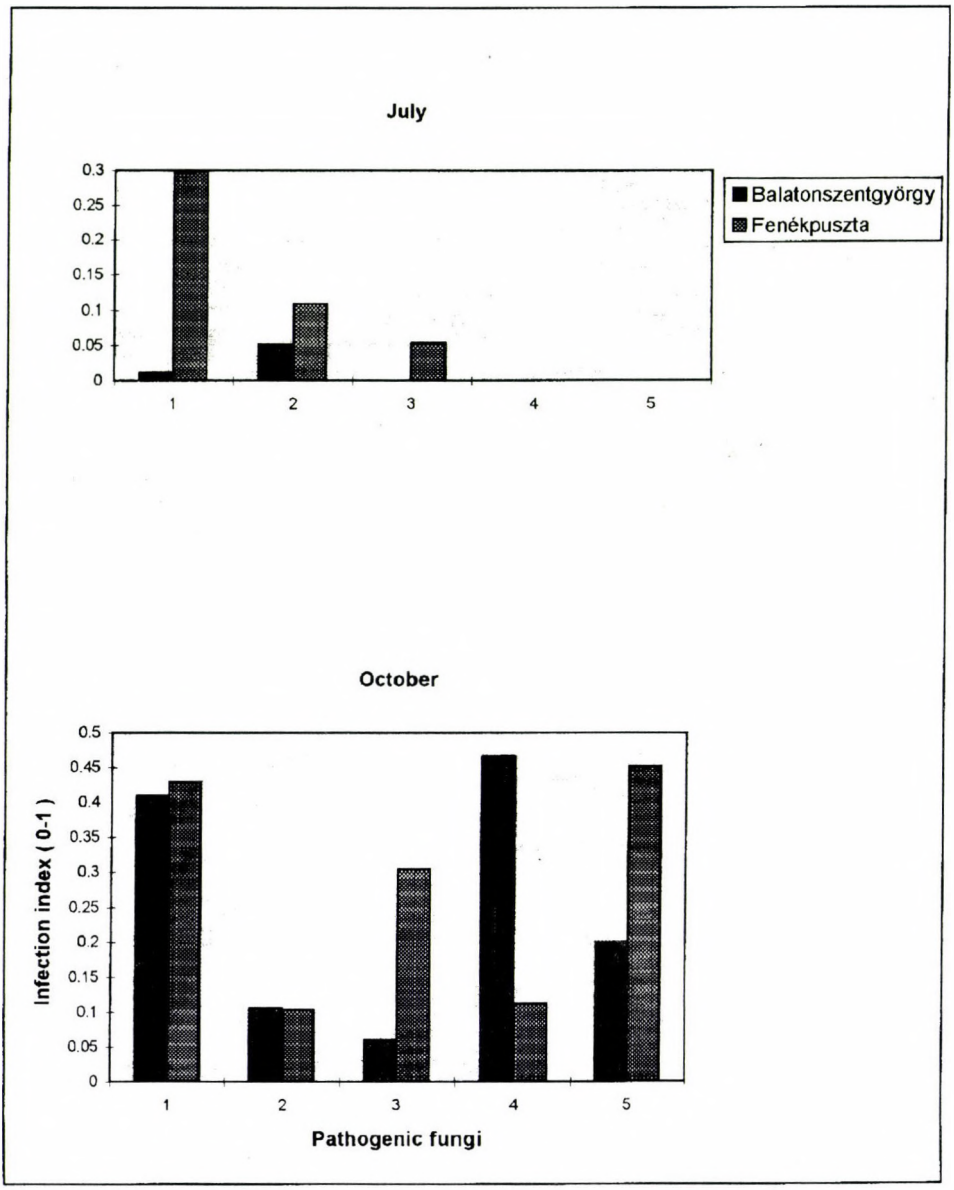

Fig. 4. Infection indices of five pathogenic fungi (1. *Puccinia phragmitis* 2. *Puccinia magnusiana* 3. *Polythrinciopsis phragmitis* 4. *Deightoniella arundinacea* 5. *Phoma*-like fungus) at two locations and in two dates

györgy in July only, and *Deightoniella arundinacea* and the *Phoma*-like fungus developed much later in the season at both locations.

By October reed plants were scattered into various levels of diseases in both locations. Frequency distributions of plants with different disease levels in October are shown in Fig. 3 and the results are also expressed in the form of infection indices (Fig. 4). In spite of differences in the initial disease occurrence (in July), *Puccinia phragmitis* and *Puccinia magnusiana* have reached the same level in both locations. Increament in the infection index of *Puccinia phragmitis* was much more significant than that of *Puccinia magnusiana* in both locations.

In comparison, by the assessment date in October *Polythrinciopsis phragmitis* and the *Phoma*-like species were much more frequent at Fenékpuszta than at Balatonszentgyörgy and in contrast *Deightoniella arundinacea* was the only fungus exhibiting higher infection index at Balatonszentgyörgy.

Discussion

Seasonal surveys have shown considerable differences in disease developments caused by common reed pathogens in two locations. Except *Deightoniella arundinacea*, infection indices of the investigated pathogens were higher at Fenékpuszta than at Balatonszentgyörgy. Furthermore, the most frequent pathogens at Balatonszentgyörgy were *Deightoniella arundinacea* and *Puccinia phragmitis*, while the *Phoma*-like fungus, *Puccinia phragmitis* and *Polythrinciopsis phragmitis* caused heavy infections at Fenékpuszta on reed.

Searching for the reason of such differences, environmental conditions of the habitats surveyed were considered and summerized in Table 1.

Table 1

Characteristics of the two habitats investigated for reed pathogens

Environmental conditions	Location	
	Fenékpuszta	Balatonszentgyörgy
Connection to water	flooded	semi-arid
Shape and size of the stand	narrow, coastal reedbelt	extended stand
Effects of climatic and other factors	wave, wind and human effects (landing stages, beach, etc.)	less effect (extended, deserted area)

Rusts (*Puccinia phragmitis* and *P. magnusiana*) cause infection at both locations, but according to the assessment period investigated it is not clear whether they occurred much earlier at Fenékpuszta and developed later but much faster at Balatonszentgyörgy or the infections appeared at the same time in both locations, but developed much faster at Fenékpuszta. According to observations this year rusts occurred in mid-June at Fenékpuszta, while no infection had yet been observed at Balatonszentgyörgy, thus rusts seem to distribute later but faster in the latter site. Consequently, a rather closed and homogeneous stand, such as at Balatonszentgyörgy, seems to be more favourable for the fast distribution of these rusts than a coastal reedbelt. Their spread from Fenékpuszta towards Balatonszentgyörgy is also possible. Interestingly, the two rusts were not found at Lake Velencei in the last two years (Agárdiné Bán, R. 1995), although these pathogens have been detected at the lake before.

In the case of *Polythrinciopsis phragmitis* infection was significant at Fenékpuszta and this year in June it appeared at Lake Velencei, too, thus the vicinity of water seems to favour for this fungus.

Our observation on the distribution of *Deightoniella arundinacea* is similar to Ellis's founding (Ellis, 1957), who pointed out that this species is usually found on reed plants where these grow on hard ground and neighbouring reeds growing on softer ground do not seem to be attacked. Our investigations showed significant occurrence of *D. arundinacea* at Balatonszentgyörgy and around Lake Velencei in dry areas. This pathogen was also found in the last two years on reeds around the islands of Lake Velencei (Szúnyog Island, Lul Island).

Finally, higher infection rates for nearly all of the pathogens at Fenékpuszta can be explained by the increased disposition of reed plants to human and climatic factors.

Involvement of some of these factors in studying pathogenic fungi on common reed is of increasing importance. On one hand no information exists on the epidemics of reed diseases although they can affect field or horticultural crops, too. On the other hand reed decline should be investigated to understand and maintain the balance between natural and agroecosystems with the involvement of environmental and biological factors (pathogens, pests) which affect reeds.

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Synthesis of Phytoalexins in Sugarcane in Response to Infection by *Colletotrichum falcatum* Went

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Involvement of 3-deoxyanthocyanidins, luteolinidin, apigeninidin and the caffeic acid ester of 5–0-apigeninidin in the pathogenic interaction between sugarcane and the red rot pathogen *Colletotrichum falcatum* Went (Teleomorph; *Glomerelia tucumanensis* (Speg.) *Arx and Mueller*) was identified. There was higher accumulation of these compounds in disease resistant genotypes than in susceptible genotypes. Of these compounds, luteolinidin accumulation was highest. Synthesis of these phytoalexins was also triggered by mechanical injury, but this had no relation to resistance of the genotypes. Studies on differential host interactions revealed that incompatible combinations accumulated greater amounts of the phytoalexins. In compatible interactions either there was less phytoalexin accumulation or no induction occurred. Partially perified red rot pathogen toxin also triggered more phytoalexins in BO 91 (resistant genotype) than in CoC 671 (susceptible genotype).

Red rot caused by *C. falcatum* is a serious disease of sugarcane occurring in most parts of the sugarcane growing tracts in the Indian subcontinent. The pathogen also causes loss in yield and quality of the cane as a result of sucrose inversion (Singh and Waraitch, 1977). The disease in the field is being contained by release of resistant varieties developed through breeding programmes. The newly released varieties succumb to the pathogen quickly in the field due to the devolopment of new pathogenic races (Anonymous., 1983). The presence of differential interactions between sugarcane and isolates of the red rot pathogen is well established (Alexander et al., 1993). The factors that contribute to biochemical resistance to red rot of sugarcane are not clearly elucidated. Though phenolics have been reported to play a role in resistance (Rao et al., 1968) later it was found that they have no correlation with disease resistance (Singh et al., 1976).

In infected sugarcane stalks, a red substance is released in cells and intercellular spaces near invading hyphae. The resistance of the genotype is correlated with the intensity of pigment production (Edgerton and Carvajal, 1944). Fractionation of the pigment showed several compounds (Godshall and Loneregan, 1987). The red rot pigments (RRP) were found to inhibit red rot spore germination and mycelial growth. Genotype variation in the amount of the pigments and the antifungal activity of the RRP was also demonstrated (Viswanathan et al., 1994). Our initial fractionation of RRP showed the presence of 3-deoxyanthocyanidins. Accumulation of 3-deoxyanthocyanidins was reported in the sorghum/*Colletotrichum graminicola* (Ces) Wils. disease interaction

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(Hipskind et al., 1990). Similarly, studies were carried out to find relationships between these phytoalexins and disease resistance, the role of phytoalexins in differential interactions, and the role of pathogen toxin in triggering phytoalexins.

Materials and Methods

Sugarcane genotypes and pathogen isolates

Sugarcane genotypes in host differential collections maintained at the Sugarcane Breeding Institute were selected for the experiments. Pathogen isolates of Cf 1148, Cf 7717 and Cf 671 were obtained from the red rot type-culture-collection maintained at the Plant Pathology laboratory. The *C. falcatum* isolates were grown at 25 °C under constant fluorescent light on oat meal agar to induce sporulation. Conidia were obtained from 2-week-old cultures and the inoculum concentration was adjusted to one million conidia/ml in distilled water containing Tween 20 (1 µl/1 ml) as a wetting agent.

Red rot inoculation and phytoalexin extraction

Eight month old sugarcane stalks (4 feet long) of varieties CoC 671, CoC 8001 and CoC 86062 (all susceptible), Co 7704, BO 91 and Baragua (all resistant) were inoculated with *C. falcatum* spore suspension through an 8 mm cork borer at the centre of alternate internodes. Another set of stalks from these genotypes were similarly treated with sterile water. The bore holes were sealed with plastic clay. The third set of canes served as uninjured controls. The cut ends of the canes were sealed with wax and the stalks were incubated at room temperature (28 °C). After 72 h incubation 500 mg of internode tissue adjoining the pathogen inoculated/injury site and intact uninjured canes were drawn and immediately immersed in 1 ml of HPLC grade methanol. The compounds were allowed to leach into the methanol overnight at 4 °C. The extract was centrifuged at 16,000 g for 10 min. and the supernatant was analysed by HPLC. Subsequently, genotypes known to express a differential host pathogen reaction (Table 1) were inoculated with conidial suspensions of the *C. falcatum* isolates Cf 1148, Cf 7717 and Cf 671. Phytoalexin extraction was carried out as in the previous experiment.

C. falcatum *toxin preparation and inoculation*

The pathogen CoC 671 isolate was cultured on Czepek's liquid medium in which sucrose was substituted with a host extract as the carbon source (30 ml cane extract/1). The host extract was prepared by homogenization and filtration. After 10 days of growth, the mycelial mats and culture filtrates were pooled, homogenized in a blendor, filtered, and the filtrate used as a source of combined exo- and endo-toxins of the pathogen. The toxin metabolite(s) in the filtrate were fractionated by a modified method of Nair and Ramakrishnan (1973). The filtrate was reduced to 1/10 volume under reduced pressure,

Table 1

Phytoalexin content in sugarcane differential hosts inoculated with different *C. falcatum* pathotypes

S. No.	Genotype	Pathotype	Red rot reaction	Phytoalexin content µg gfw^{-1}		
				Luteolinidin	Apigeninidin	Caffeic acid ester
1.	Co 62175	1	MR	0.23 ± .21	0.00	0.00
		2	HS	0.00	0.00	0.00
		3	HS	0.00	0.00	0.00
2.	CoC 671	1	MS	0.73 ± .03	0.73 ± .06	0.11 ± .03
		2	MS	0.73 ± .03	0.24 ± .01	0.00
		3	HS	0.00	0.00	0.00
3.	94069	1	MR	1.54 ± .11	0.20 ± .01	0.06 ± .01
		2	MR	2.85 ± .16	0.86 ± .03	0.22 ± .02
		3	MR	3.11 ± .09	0.29 ± .01	0.00
4.	Co 7717	1	MR	0.50 ± .02	0.20 ± .02	0.00
		2	HS	0.00	0.00	0.00
5.	Co 1148	1	HS	0.00	0.00	0.00
		2	MR	0.96 ± .05	0.43 ± .02	0.17 ± .02
6.	Co 6806	1	S	0.00	0.00	0.00
		2	MR	0.31 ± .01	0.26 ± .01	0.00
7.	Co 997	1	HS	0.46 ± .02	0.13 ± .01	0.00
		2	MR	6.61 ± .27	2.61 ± .18	0.44 ± .03
8.	BO 91	3	MR	4.23 ± .32	0.93 ± .07	0.00

Pathotypes 1, 2 and 3 are cultures of Cf 1148, Cf 7717 and Cf 671, respectively.

Red rot reaction: MR: Moderately Resistant; MS: Moderately susceptible; S: Susceptible; HS: Highly susceptible.

mixed with an equal volume of methanol, retained overnight and filtered. The methanol was removed by vacuum evaporation at 40 °C, the pH of the aqueous phase adjusted to 3.5 with HCl and the mixture shaken with an equal volume of diethylether. The ether phase was separated and mixed and then shaken with an equal volume of 5% Na_2CO_3. The aqueous phase was discarded. The ether phase was evaporated to dryness. The purified toxin fraction was dissolved in sterile distilled water (1 mg toxin/ml) and 1 ml of the dissolved toxin fraction was injected into 2 genotypes, BO 91 and CoC 671, to determine if the toxin induced phytoalexin synthesis. Phytoalexin extraction was performed as described above.

Chromatographic analysis

Samples were injected into the HPLC system through a 100 µl/loop. Separation was performed on a Beckmann (Fullerton, CA) reversed-phase C_{18} ultrasphere column (250 by 4.6 mm). Solvent A was mixture of water/acetic acid/methanol in the ratio

71:10:9 (V/V/V) and solvent B was 9:1 (V/V methanol/acetic acid) (Hipskind et al., 1990). These solvents were delivered through a Beckmann Model 110A pump ensuring a constant flow rate. The phytoalexin pigments were eluted with a linear gradient from 0 to 25% B in 13 min. followed by constant 25% B for 5 min. after which %B was linearly decreased to 0 in 2 min. The column was reequilibrated for 10 min. between samples. Compounds were detected at 480 nm with a Beckmann System Gold Programmable Detection Module, Model 166. Standard solutions of known concentrations of the three phytoalexins were chromatographed, and their retention times and peak areas were determined with a Hewlett-Packard 3396 A integrator. Retention times served to identify the phytoalexins in test samples and the amount of each pigment in a sample was obtained by comparison of the compound peak area to the peak area of the known standard (Hipskind et al., 1990).

Phytoalexin content was expressed as micromoles per gram of fresh tissue based on molecular weights of 271.3, 255.5 and 549.5 for luteolinidin, apigeninidin and the caffeic acid ester of arabinosyl 5–0-apigeninidin, respectively.

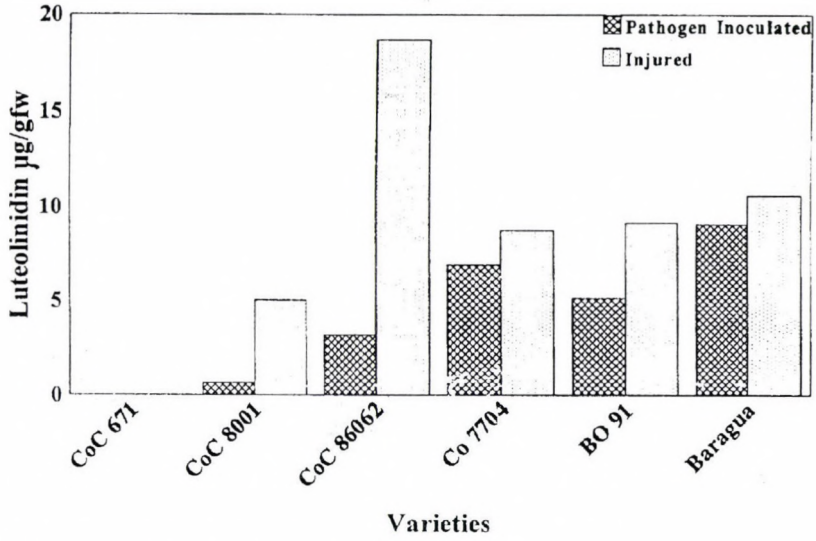
Results

Phytoalexin accumulation in sugarcane in relation to red rot resistance

The results of the first experiment showed the presence of the 3-deoxyanthocyanidins flavonoids luteolinidin and apigeninidin in inoculated and injured canes. In uninjured canes, none of these compounds were detected. The quantity of luteolinidin was more than that of apigeninidin in all samples. No attempt was made in this set of treatments to detect the caffeic acid ester of 5–0-apigeninidin. In response to pathogen inoculation all resistant varieties accumulated more of these compounds than susceptible varieties by 72 h after inoculation. Susceptible varieties had 6 to 10 times increase in 3-deoxyanthocyanidins in injured canes over inoculated ones, however, in resistant genotypes this increase was less and the content remained same or with little increase in injured canes (Figs 1 and 2).

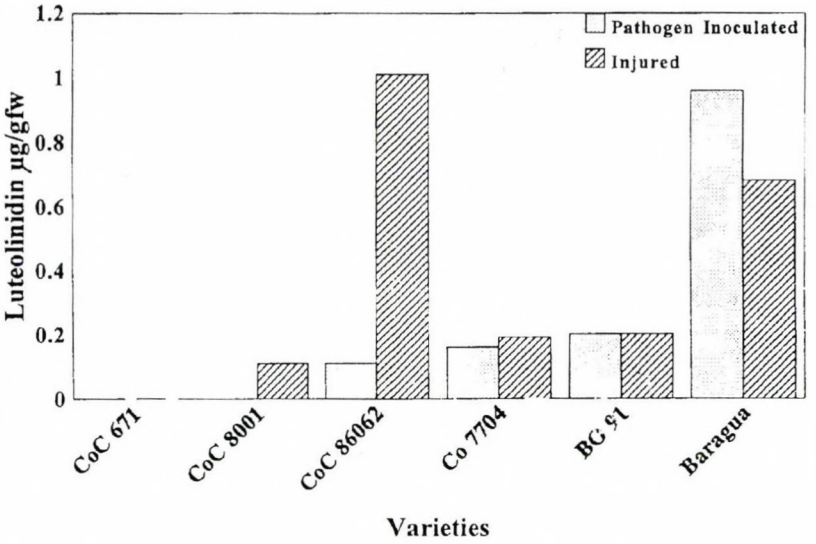
Phytoalexin accumulation as a function of differential interaction

Results of studies with 8 genotypes against 3 pathotypes tested showed a clear variation in pigment accumulation. The caffeic acid ester of 5–0-apigeninidin was identified in some treatment combinations in addition to the luteolinidin and apigeninidin compounds found in the other experiment. When phytoalexin accumulation in different genotype and pathotype interactions was compared with field disease ratings for resistant combinations there was higher amount of phytoalexin synthesis. In susceptible combinations there was nos synthesis of these phytoalexins except in the genotype Co 997. In this case, as in the susceptible interaction with pathotype 1, there was a significantly lower amount of luteolinidin and apigeninidin and the caffeic acid ester of apigeninidin was



CoC 671,CoC 8001 and CoC 86062 are susceptible to the disease.
Co 7704,BO 91 and Baragua are resistant to the disease.

Fig. 1. Accumulation of luteolinidin in sugarcane in response to pathogen infection/injury



CoC 671,CoC 8001 and CoC 86062 are susceptible to the disease.
Co 7704,BO 91 and Baragua are resistant to the disease.

Fig. 2. Accumulation of apigeninidin in sugarcane in response to pathogen infection/injury

Table 2

Effect of *C. falcatum* toxin on phytoalexin accumulation in sugarcane genotypes

Genotype	Inoculum	Phytoalexin content $\mu g\ g^{-1}$ tissue		
		Luteolinidin	Apigeninidin	Caffeic acid ester
1. BO 91	Conidial suspension	4.23 ± .23	0.93 ± .04	0.00
2. CoC 671	Conidial suspension	0.00	0.00	0.00
3. BO 91	Red rot toxin	5.76 ± .28	0.13 ± .01	0.00
4. CoC 671	Red rot toxin	0.61 ± .04	0.12 ± .01	0.00

absent when compared to the resistant interactions with pathotype 2. In all these treatments, the caffeic acid ester was observed only in incompatible interactions. The genotype 94069 which showed resistance to all the 3 pathotypes, had higher pigment accumulation irrespective of pathotype. The genotype Co 997 showing resistance against pathotype 2 accumulated the highest quantity of phytoalexins. The other genotype, BO 91 which is the resistant standard in differenctial interaction studies, also showed a higher accumulation of luteolinidin and apigeninidin against pathotype 3 (Table 1).

Role of C. falcatum *toxin in phytoalexin synthesis*

When partially purified red rot toxin at a concentration of 1000 ppm was inoculated into sugarcane internodes it triggered phytoalexin production as in the case of inoculation with the pathogen. The results also showed clear distinction between the clones BO 91 and CoC 671 which express extreme variation in red rot reaction among the differential tests for resistance and susceptibility, respectively. A comparison of phytoalexin synthesis induced by toxin treatment with that induced by the pathogen showed that the toxin tiggered more phytoalexin accumulation than the pathogen itself. In the compatible host pathogen combination of clone CoC 671 with the pathotype 3 (CoC 671) no phytoalexins were produced. However, the same genotype expressed pigment synthesis when treated with the toxin (Table 2).

Discussion

The results clearly indicate the involvement of anthocyanin pigments in red rot resistance. In general the luteolinidin fraction was always higher than the apigeninidin fraction in the treated canes. The results from the first experiment (Figs 1 and 2) clearly show that in response to pathogen inoculation all resistant varieties accumulated more of pigments than the susceptible varieties by 72 h after inoculation. Less accumulation of these flavonoids in some susceptible canes may be due to the rapid destruction of the

parenchymatous tissues by the pathogen and the resulting lack of infected tissue to synthesize the phytoalexins. In the susceptible hosts the pathogen completed its life cycle within 72 h (Mohanraj et al., 1994) whereas in resistant genotypes the pathogen had only sparse hyphal growth restricted to the inoculation site. So, in the compatible combinations host tissues were not able to defend themselves against the progressive colonisation by the pathogen. Apigeninidin content was below detectable levels in the clone CoC 8001 inoculated with the pathogen, whereas, in the other susceptible variety CoC 671 both luteolinidin and apigeninidin were not detectable in pathogen inoculated and injured cane samples. These phytoalexin fractions were completely absent in samples from intact canes of all varieties implying that induction of 3-deoxyanthocyanidin phytoalexins upon injury or encounter with the pathogen is a specific reaction of the resistant clones. The present study shows a positive relation between the amount of pigment accumulation and resistance to red rot. Although upon injury there was phytoalexin induction it was found to have no relation with resistance. Mechanical injury, exposure to other abiotic elicitors like heavy metal salts, detergents, oligoglucans, ethylene, fatty acids, chitosan oligomers, cold and UV-light are known to induce phytoalexins in several other hosts (Kuc, 1995).

The existence of a differential interaction between sugarcane and the red rot pathogen has been studied earlier (Alexander et al., 1993). About 30 genotypes, including those tested in this experiment, have been identified as host differentials and 20 pathotypes collected from different agroclimatic zones in India are currently maintained at the National red rot type culture collection facility at the Sugarcane Breeding Institute, Coimbatore. Serological differences among the pathotypes have been studies by immuno-double diffusion (Jothi, 1989) and direct antigen coating-enzyme linked immunosorbent assays (Viswanathan, unpublished results). The results of studies on phytoalexin accumulation in differential host-pathogen interactions clearly demonstrate a biochemical basis of red rot resistance in sugarcane against *C. falcatum* pathotypes. Presence of all the three pigment fractions was noticed only in incompatible interactions. In compatible combinations (except with the clone Co 997) no phytoalexin was detected (Table 1). The biochemical basis of resistance in sugarcane to red rot is still unresolved. The current findings suggest the possible role played by 3-deoxyanthocyanidin phytoalexins in conferring resistance. Accumulation of 3-deoxyanthocyanidin pigments could be possibly used as a marker to identify clones resistant to red rot after further standardisation as in the case of sorghum anthracnose (Tenkouano et al., 1993). Similarly, following infection with an incompatible race of a pathogen and phytoalexin accumulation and no induction in compatible interaction in other host-pathogen interaction have been reported (Keen and Horsch, 1972; Staskawicz et al., 1994).

The partially purified phytotoxin obtained from the pathogen culture was a crystalline brownish-yellow powder. It is readily soluble in water, methanol and other organic solvents. Spot application of solutions of the toxin on leaf segments produced brown necrotic spots with a yellow-brown margin and a yellow halo which elongated along the veins within 48 to 72 h. On inoculated cane stalks restricted non-spreading reddish lesions in the internal tissues were obtained after toxin treatment of 1 mg/1 ml (Mohanraj et al., 1992). It is clear that the partially purified toxin is able to elicit part of the disease

symptoms in the inoculated canes as reddish discolouration. The present study confirms the role of toxin in triggering phytoalexin synthesis. Further, phytoalexin induction was comparatively higher in the resistant variety than in the susceptible variety (Table 2). A similar mechanism in phytoalexin accumulation after pathogen inoculation as well as its toxin infusion was also observed. Here the phytoalexin may function as elicitor in triggering phytoalexins. Involvement of elicitors from fungal origin such as enzymes, toxins, cell wall compounds and metabolic products are well established in other host-pathogen systems (Ebel, 1986).

Evidence is presented here which suggests that 3-deoxyanthocyanidin compounds function as phytoalexins against attempted infection by *C. falcatum* in sugarcane. These compounds have been reported as sorghum phytoalexins (Hipskind et al., 1990; Tenkouano et al., 1993),and shown to accumulate to fungitoxic levels against *C. graminicola* in sorghum (Snyder et al., 1991). The fungitoxicity of these compounds against *C. falcatum* is not known. However, the red rot pigments and the anthocyanin extracts from diseased canes were found to be inhibitory to the red rot pathogen (Viswanathan et al., 1994). Accumulation of these phytoalexins after pathogen inoculation was found to be correlated with red rot resistance and the phytoalexins have a major role in differential interaction among sugarcane clones and *C. falcatum*. We propose that changes in phytoalexin compounds accumulation may be useful indicators for evaluating resistance and susceptibility to red rot in sugarcane.

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Feeding and Survival of the European Corn Borer Larvae on Seventeen Inbred Lines of Maize

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In 1991 and 1992 the damage of plants caused by the European corn borer (ECB) and number of surviving larvae were observed in Nitra in southwestern Slovakia. Artificial infestation with two egg mases of the ECB caused longer stalk tunnels and more larvae per infested plant than natural infestation. Also the differences among the genotypes were more distinct than under natural infestation. In both years, only six inbred lines (To558, B85, Oh43, F574, B86, DE811) showed the same result as the most resistant genotype in the experiment with artificial infestation of plants. Inbreds A619 and B75 have been found susceptible to borer tunnelling, and the number of the ECB larvae in the stalks of these inbreds was very high. Very high resistance (under natural, and also under artificial infestation) was shown by Slovakian inbred To558. This inbred was comparable to highly resistant lines B85, B86, and DE811.

Numerous studies have shown that maize genotypes with a higher resistance to the European corn borer can influence the bionomy of the pest. Larvae developed faster and weighed more when fed on plants of susceptible corn than when fed on plants of resistant corn (Patch, 1943). More than 95% larval mortality occurred on resistant lines such as B52 within 3 days after egg hatch (Guthrie et al., 1970). The mortality of the ECB larvae feeding on resistant maize was higher than mortality of larvae feeding on susceptible maize (Shapiro and Pereverzev, 1975; Hudon and Chiang, 1977; Dharmalingan et al., 1984; Davis et al., 1989). Fewer egg masses were found on resistant inbreds (CI31A, F574, and Oh43) than on susceptible inbreds (Shapiro et al., 1979).

Many maize inbred lines were recorded as resistant to the European corn borer. CI31A, B75, A619 and Oh43 belong to inbreds that are resistant to the first-generation of the pest in the Corn Belt states (Guthrie et al., 1970; Guthrie and Dicke, 1972; Russell, 1979; Russell and Guthrie, 1979; Reid et al., 1991; Barry et al., 1994). Inbred B52 has a high degree of resistance to second-generation ECBs (Barry et al., 1983, 1985; Barry and Zuber, 1984). Other genotypes such as BS9CBC4, B86, DE811, and SC213 have been developed with resistance throughout the life of the maize plant (Russel and Guthrie, 1982; Hawk, 1985; Klenke et al., 1986; Guthrie et al., 1989). The inbred line A619 had the greatest resistance to the ECB damage in Canada (Chiang and Hudon, 1976; Hudon et al., 1991), in France (Anglade and Molot, 1967), and in Slovakia (Masler, 1977). Inbreds F574 (Shapiro et al., 1979) and A632 (Panouille et al., 1984) also showed resistance to the ECB.

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Inbred lines with high resistance to ECB were found in Romania (Mustea et al., 1975; Barbulescu et al., 1982) and Yugoslavia (Hadžistević, 1968). The inbreds A619, NR1065, PLS14, 72–75–61, T393, and A295 had a high degree of resistance to ECB in Slovakia (Masler, 1977). Over 500 inbreds on maize have been exchanged among the members of International Working Group on *Ostrinia nubilalis* (IWGO), from these many hybrids and synthetics with borer resistance and local adaptivity have been developed (Anglade et al., 1984; Chiang, 1984).

The objective of this study was to determine the ECB resistance of 17 inbred lines in Slovakia and evaluate their potential as a sources of resistance to the ECB. The study included inbreds with defined resistance from the world collection and inbreds used in the breeding of maize in Slovakia.

Materials and Methods

In 1991 and 1992, damage caused by the European corn borer (ECB) and number of surviving larvae were observed in Nitra in southwestern Slovakia. The location was at the altitude 190 m, 12 km eastern of the city of Nitra.

Seventeen maize inbred lines were examined for the length of the tunnels caused by the ECB larvae and number of surviving larvae. Eight of the inbreds (indicated by To-) were used in the breeding of maize in Slovakia (FAO numbers from 350 to 450). Their resistance to the ECB was not known. Inbred WF9 is known to be susceptible to the ECB (Guthrie et al., 1982). In experiments of other authors the inbred lines B85, Oh43, A632, F574, B75, A619, B86, DE811 were resistant to the ECB (see Introduction).

Two experiments were organized in the study:

Inbred lines in the first experiment were grown in a randomized block with each genotype replicated 4 times. Seeds were sown at the end of April. Each replication consisted of 25 seeds planted in one row. Plants were infested only by naturally occurring ECB.

The second experiment was isolated from the first to prevent larval migration between the experiments. About 15 plants of each inbred line were infested with two egg masses per plant. The egg masses were produced in the laboratory using moths caught in action sites near the maize fields (Showers et al., 1989).

In both experiments the plants were dissected at the end of September to determine the length of the corn borer tunnels and number of surviving larvae.

Data obtained in these experiments were subjected to an analysis of variance to determine the differences among the inbred lines in each year and location.

Table 1

Stalk tunnel lengths caused by the ECB larvae und number of ECB larvae survived in seventeen inbred lines of maize at location Nitra, under natural infestation, in 1991

Inbred line	N	PD	Stalk tunnel length, cm, per plant	Stalk tunnel length, cm per damaged plant	Larvae per plant	Larvae per damaged plant
To549	81	29	2.94abc	8.21abcde	0.333abcd	0.9311abcd
To337	80	49	8.50e	13.87cg	0.913e	1.490ceg
To548	77	37	3.88abcd	8.08abcdef	0.494abcd	1.027abcdefg
To565–6	78	29	3.37abcd	9.07abcdefg	0.564acde	1.517abcdefg
To565–4	75	27	3.96abcd	11.00abcdefg	0.547acde	1.519abcdefg
To566–6	83	37	6.34de	14.22abcdefg	0.446abcd	1.000abcdefg
To566–4	85	36	5.48bcde	12.94bcdfg	0.471abcd	1.083abcdefg
To558	70	17	1.11a	4.60a	0.157ab	0.647ab
B85	73	15	1.37a	5.88abcd	0.151ab	0.733abc
Oh43	78	43	5.29bcde	9.60abcdefg	0.487abcd	0.884abcdef
A632	80	39	6.00cde	12.31bcdefg	0.513abcd	1.051abcdefg
WF9	71	39	5.32bcde	9.69abcdefg	0.563acde	1.026abcdefg
F574	27	10	3.00abcd	8.10abcdefg	0.333abcd	0.900abcdefg
B75	61	22	3.18abcd	8.82abcdefg	0.393abcd	1.091abcdefg
A619	37	10	1.68ab	6.20abc	0.135a	0.500a
B86	63	15	1.44a	6.07ab	0.190abc	0.800abcde
DE811	77	21	2.78abc	10.19abcdefg	0.325abc	1.190abcdefg

Means followed by the same letter in the same column are not significantly different at $P > 0.05$ (Tuckey's multiple range test). N = number of plants observed, PD = number of damaged plants

Results

All 17 inbred lines were infested naturally by the European corn borer (Tables 1 and 3). The degree of natural infestation was higher in 1991 (20.55–61.25%) than in 1992 (6.06–26.98%). Differences among the inbred lines in stalk tunnel length per damaged plant and number of larvae per damaged plant were usually not significant. Significant differences were found only between the most resistant and the most susceptible genotypes. Inbred lines with the shortest stalk tunnel lengths per damaged plant under natural ECB infestation were To558, B85, B86, A619 in 1991, and A619, To565–6, To549, B75 in 1992. The lowest number of larvae in stalks was in inbreds A619, To558, B85, B86 (1991), and To549, To558, Oh43, F574 (1992), respectively.

Artificial infestation with two egg masses of the ECB caused longer stalk tunnels and higher number of the pest larvae per infested plant than under natural infestation. Also the differences among the genotypes were more distinct than under the natural infestation (Tables 2 and 4). Inbred lines B86, To558, B85, and Oh43 had the shortest stalk

Table 2

Stalk tunnel lengths caused by the ECB larvae and number of ECB larvae survived in seventeen inbred lines of maize at location Nitra, under artificial infestation with two egg mases, in 1991

Inbred line	N	PD	Stalk tunnel length, cm, per plant	Stalk tunnel lenght, cm per damaged plant	Larvae per plant	Larvae per damaged plant
To549	15	15	30.40efgh	–	3.067cde	–
To377	13	13	35.38fgh	–	4.308def	–
To548	14	14	26.50cdefg	–	3.643cde	–
To565–6	14	14	41.57h	–	6.286f	–
To565–4	15	15	27.67defg	–	4.133de	–
To566–6	14	12	22.29bcdef	26.01	2.357abcd	2.750
To566–4	15	15	38.13gh	–	3.333cde	–
To558	15	11	9.40ab	12.82	0.733a	1.000
B85	12	12	13.25abc	–	2.000abc	–
Oh43	13	13	15.08abcd	–	1.769abc	–
A632	12	12	28.17defgh	–	3.083bcde	–
WF9	12	12	31.25efgh	–	4.583ef	–
F574	11	11	15.27abcd	–	2.909bcde	–
B75	12	12	28.58defgh	–	3.083bcde	–
A619	7	7	32.71efgh	–	5.429ef	–
B86	12	11	7.67a	8.36	0.917ab	1.000
DE811	15	15	18.07abcde	–	1.800abc	–

Means followed by the same letter in the same column are not significantly different at $P > 0.05$ (Tuckey's multiple range test). N = number of plants observed, PD = number of damaged plants

tunnel lengths in 1991, and inbred lines B85, To558, F574, and DE811 had the shortest stalk tunnel lengths in 1992. In 1991, inbreds To558, B86, Oh43, and B85 had the lowest number of ECB larvae. In 1992, inbreds B86, B85, To566–4, and To558 had the lowest number of ECB larvae.

In both years, only six inbred lines (To558, B85, Oh43, F574, B86, DE811) showed the same result as the most resistant genotype in the experiment with artificial infestation of plants.

Discussion

The most resistant inbred lines found in our trials were B86 and DE811. From the lines tested these two were recorded to be resistant to both generations of the pest in the U. S. A. (Russell and Guthrie, 1979; Hawk, 1985).

Table 3

Stalk tunnel lengths caused by the ECB larvae and number of ECB larvae survived in seventeen inbred lines of maize at location Nitra, under artificial infestation, in 1992

Inbred line	N	PD	Stalk tunnel length, cm, per plant	Stalk tunnel length, cm per damaged plant	Larvae per plant	Larvae per damaged plant
To549	56	5	0.59ab	6.60a	0.036a	0.400a
To337	63	17	7.25f	26.88bd	0.635ab	2.352bd
To548	59	8	1.31abcde	9.63abc	0.186a	1.375abcd
To565–6	63	5	0.38a	4.80a	0.063a	0.800ab
To565–4	58	5	1.40abcde	16.20abcd	0.155a	1.800abcd
To566–6	61	11	3.20abcde	17.73abcd	0.131a	0.727a
To566–4	61	15	4.00bcdef	16.27abcd	0.230a	0.933abc
To558	58	5	0.71ab	8.20a	0.034a	0.400a
B85	66	6	0.86abcd	9.50abc	0.076a	0.833ab
Oh43	43	4	0.84abc	9.00ab	0.047a	0.500a
A632	57	4	1.28abcde	18.25abcd	0.175a	2.500abcd
WF9	56	14	4.55cef	18.21abcd	0.321a	1.286abcd
F574	53	6	1.13abcde	10.00abc	0.057a	0.500a
B75	61	13	1.44abcde	6.76a	0.180a	0.846abc
A619	51	5	0.39ab	4.00a	0.078a	0.800ab
B86	66	4	1.03abcd	17.00abcd	0.045a	0.750ab
DE811	40	7	2.35abcde	13.43abc	0.200a	1.143abc

Means followed by the same letter in the same column are not significantly different at $P > 0.05$ (Tuckey's multiple range test). N = number of plants observed, PD = number of damaged plants

Inbred B85 is highly resistant to leaf feeding by the first brood of the ECB (Russell and Guthrie, 1979) and it showed the same resistance as B86. The advantage of this inbred in Slovakia is its earlier maturation than B86.

Oh43 was among the most resistant inbreds in the experiment under artificial infestation. However, under natural infestation in 1991 it was one of the most damaged genotypes. Oh43 is recorded as resistant to first-brood infestation of the ECB but highly susceptible to second-brood infestation (Guthrie and Dicke, 1972; Guthrie et al., 1970). Reid et al. (1991) defined Oh43 as resistant to leef feeding and only intermediate to stalk tunelling. Its use in the breeding of maize for resistance to the ECB in Slovakia will be probably connected with higher natural infestation. Results similar to Oh43 were achieved also with the inbred F574.

Inbred To558 appears to have very high resistance under both natural and artificial infestation. We strongly recommend its use in resistance breeding programmes in countries with one generation of the ECB because its maturity classification is 420. This inbred was comparable with B85, B86 and DE811. At the date of infestation the plants of

Table 4

Stalk tunnel lengths caused by the ECB larvae and number of ECB larvae survived in seventeen inbred lines of maize at location Nitra, under artificial infestation with two egg masses, in 1991

Inbred line	N	PD	Stalk tunnel length, cm, per plant	Stalk tunnel length, cm per damaged plant	Larvae per plant	Larvae per damaged plant
To549	15	15	23.27aabcd	–	1.400abcd	–
To337	16	16	30.75cde	–	1.875abcdef	–
To548	15	15	37.13e	–	2.867f	–
To565–6	15	15	23.47abcd	–	2.600def	–
To565–4	15	15	26.33bcde	–	2.133cdef	–
To566–6	15	15	33.67de	–	1.533abcde	–
To566–4	15	15	27.07bcde	–	1.133abc	–
To558	14	13	15.21ab	16.38	1.286abcd	1.385
B85	15	12	11.53a	14.42	1.067abc	1.334
Oh43	14	14	19.86abc	–	1.571abcdef	–
A632	14	14	26.29bcde	–	1.714abcdef	–
WF9	15	15	27.20bcde	–	1.933abcdef	–
F574	15	13	18.60abc	21.46	1.533abcdef	1.769
B75	13	13	35.85de	–	2.077abcdef	–
A619	10	10	33.10cde	–	2.900ef	–
B86	15	14	20.47abc	21.92	0.800ab	0.857
DE811	15	15	20.27abc	–	1.667abcedf	–

Means followed by the same letter in the same column are not significantly different at $P > 0.05$ (Tuckey's multiple range test). N = number of plants observed, PD = number of damaged plants

this genotype were more mature than the plants of B85, B86 and DE811 and thus were more susceptible to the pest.

Inbred A619 had the greatest resistance-tolerance when six inbred lines were evaluated at four locations across Ontario and Québec (Hudon et al., 1991). It is resistant to leaf feeding (Reid et al., 1991) and intermediate in resistance to stalk tunelling (Reid et al., 1991). This inbred also had the greatest resistance to the ECB in Slovakia (Masler, 1977). Our observations indicate that under natural infestation this inbred was very resistant to the ECB. However, in experiments with artificial infestation it has been found to be susceptible to borer tunnelling. In addition the number of ECB larvae in the stalks of this inbred was very high.

Inbred B75 was reported as highly resistant to leaf feeding by first-brood ECB but moderately susceptible to leaf-sheath and collar feeding by the second-brood (Russell, 1979). In our experiments it was as susceptible to borer tunnelling as the inbred A619.

High resistance to the ECB was also shown by the inbred A632 (Panouille et al., 1984), but we did not confirmed this in our experiments.

We found that the length of the tunnels in the stalks in closely connected with the number of surviving larvae. Thus, in agreement with many authors (Patch, 1943; Shapiro and Pereverzev, 1975; Hudon and Chiang, 1977; Davis et al., 1989; Barry and Mends-Cole, 1991), larval mortality on resistant inbred lines was higher. However, the most resistant genotypes were highly damaged by the larvae. As an example we can use our infestation experiment in 1992. Average length of the tunnels in the stalks of B85 (the most resistant) was 11.53 cm. In stalks of To558 it was 15.21 cm, and in stalks of B86 it was 20.47 cm. Stalk tunnels length per plant of the most suceptible inbred To566–6 achieved 33.67 cm under the same conditions.

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Resistance in Wild *Cucumis* Species to Twospotted Spider Mite (*Tetranychus urticae*)

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Fifty-three accessions of 13 *Cucumis* species and varieties were screened in the glasshouse for resistance to the twospotted spider mite (*Tetranychus urticae*). Resistance was evaluated by expression of absolute number of live mites per leaf area (detached leaf disk) naturally affected. The highest level of resistance was found in accessions of *C. africanus* (PI 274036, PI 203974, PI 299569) and *C. zeyheri* (PI 299572, PIVT 0181, PIVVT 0330). No fully resistant species or accessions were found among the plants tested.

Spider mites are major problems in the production of cucumbers, melons, squash, pumpkins, and other cucurbits (Robinson, 1992). The twospotted spider mite, *Tetranychus urticae*, is a widespread and an important pest of cucumbers and Cucurbitaceae in glasshouse (De Ponti, 1977a) but also in the field (Knipping et al., 1975).

Resistance to spider mites has been reported in many crops (De Ponti, 1977a), including cucumbers (De Ponti, 1978; Kooistra, 1971; Tulisalo, 1972). Screening of more than 800 cultivars of cucumbers indicated that there are some accessions or lines with a high level of resistance (De Ponti, 1977b). These were subsequently used in plant breeding (De Ponti, 1980, 1985).

Information concerning occurrence of resistance in other Cucurbitaceae is rather limited (De Ponti, 1978; Kiessling et al., 1985; Knipping et al., 1975; Lebeda, 1992; Tulisalo, 1972). The objective of the present investigation was to assess wild *Cucumis* species for differences in resistance to *T. urticae*.

Materials and Methods

Fifty-three accessions have been studied representing 13 wild species and varieties of the genus *Cucumis*. Seeds were kindly provided by D. L. Visser (Institute for Horticultural Plant Breeding; now Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, The Netherlands); H. R. Hanes (Germplasm Resources Laboratory, U. S. D. A., A. R. S., Beltsville, USA) and Ch. Lehmann (Central Institute for Genetic and Research of Crop Plants, Gatersleben, Germany).

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The plants were grown in the glasshouse in plastic pots filled with garden soil. The temperature was 22–28 °C, air humidity 60–80%. The growing period was from the beginning of May till the end of September. Four plants per accession were grown for evaluation. Single species and accessions were randomized in the glasshouse. No chemical treatment was made, only fertilization by water solution of Cucumin.

The following procedure was used for resistance evaluation. Two leaves were removed (5 September) from the middle part of each naturally affected plant. One leaf disk (20 mm in diameter) was cut out of the central part of each leaf. Eight leaf disks were evaluated per accession to get an absolute number of live mites. The degree of resistance was expressed as a mean value of eight evaluations.

Cucumis sativus line VURV 1/85 (originating from Dr. E. Troníčkova-Pekárková, Research Institute for Plant Production, Praha) served as a susceptible control. Results were evaluated statistically using the analysis of variance and the Scheffe test for multiple comparisons.

Results

Table 1 summarizes results on the mean number of mites recorded in the accessions under study. There were highly significant differences between some species and accessions (Table 2). There was also a remarkable difference between the *C. sativus* control line and the whole set of wild *Cucumis* species. The highest level of resistance was found in the accessions of *C. africanus* (PI 274036, PI 203974, PI 299569) and *C. zeyheri* (PI 299572, PIVT 0181, PIVT 0330). In contrast, little resistance was observed in *C. leptodermis* (PI 374152), *C. africanus* (PI 374151), *C. myriocarpus* (PIVT 0182, PIVT 0202) and *C. dipsaceus* (PI 236468).

Differences among respective species under consideration are given in Table 3. From this table, one may conclude that the highest resistance could be expected in *C. figarei, C. zeyheri, C. meeusii, C. africanus*. Most susceptible are *C. leptodermis* and *C. myriocarpus*.

Discussion

Only four experimental papers were discovered concerning resistance of wild *Cucumis* species to *T. urticae*. De Ponti (1978) screened eight accessions, Kiessling et al. (1985) 14 accessions, Knipping et al. (1975) ten accessions and Tulisalo (1972) 12 wild *Cucumis* accessions. Knipping et al. (1975) reported high resistance in *C. africanus*. Also De Ponti (1978) found significantly lower acceptance and net reproduction of *T. urticae* on one accession of *C. africanus*. According to Kiessling et al. (1985) the highest resistance could be expected in *C. anguria* var. *longipes* and *C. metuliferus*. In the present experiment, three accessions of *C. africanus* were highly resistant (Table 1) and one was susceptible (PI 374151). Staub et al. (1987) mentioned that PI 374151 is not *C. africanus*

Table 1

Differences in resistance of accessions of wild *Cucumis* species to *Tetranychus urticae*

Serial number	*Cucumis* species (accession)	Mean live mites (per 8 leaf disk)
1	*C. africanus* (PI 274036)	2.6
2	*C. zeyheri* (PIVT 0181)	3.1
3	*C. zeyheri* (PI 299572)	3.1
4	*C. africanus* (PI 203974)	3.3
5	*C. zeyheri* (PIVT 0330)	4.3
6	*C. africanus* (PI 299569)	4.9
7	*C. zeyheri* (PIVT 1750)	5.0
8	*C. figarei* (PIVT 1706)	5.1
9	*C. anguria* (PIVT 0307)	5.3
10	*C. ficifolius* (PIVT 1729)	7.8
11	*C. zeyheri* (PI 364473)	8.4
12	*C. ficifolius* (PIVT 1801)	8.6
13	*C. metuliferus* (PI 202681)	8.8
14	*C. anguria* (PI 390449)	11.0
15	*C. anguria* var. *longipes* (PI 282442)	11.3
16	*C. zeyheri* (PI 282450)	11.6
17	*C. dipsaceus* (PIVT 1728)	11.8
18	*C. zeyheri* (PIVT 1053)	11.9
19	*C. meeusei* (PIVT 1800)	12.1
20	*C. ficifolius* (PI 273648)	13.1
21	*C. metuliferus* (PI 292190)	13.5
22	*C. anguria* var. *longipes* (PIVT 0198)	13.8
23	*C. anguria* (PI 386031)	15.5
24	*C. melo* subsp. *agrestis* (PIVT 1165)	17.0
25	*C. melo* subsp. *agrestis* (Taschkent" (CUM 257/1976)	18.0
26	*C. melo* subsp. *agrestis* (PIVT 0309)	18.5
27	*C. dipsaceus* (PI 390450)	18.9
28	*C. anguria* (PI 233646)	19.3
29	*C. zeyheri* (PI 299570)	19.9
30	*C. prophetarum* (PIVT 1752)	20.9
31	*C. metuliferus* (PIVT 1747)	21.3
32	*C. myriocarpus* (PI 203977)	21.5
33	*C. myriocarpus* (PI 374153)	22.6
34	*C. dipsaceus* (PIVT 0163)	23.4
35	*C. anguria* var. *longipes* (PIVT 1736)	24.1
36	*C. melo* subsp. *dudaim* "Afghanistan" (CUM 254/1975)	24.1
37	*C. zeyheri* (PI 299571)	25.0
38	*C. metuliferus* (PIVT 0164)	26.5
39	*C. anguria* var. *anguria* (CUC 9/1974)	27.1
40	*C. ficifolius* (PI 196844)	27.4
41	*C. myriocarpus* (PI 282449)	28.3
42	*C. anguria* (PI 147065)	28.8
43	*C. melo* subsp. *flexuosus* "Adzur" (CUM 234/1973)	30.0

Table 1 (cont.)

Serial number	*Cucumis* species (accession)	Mean live mites (per 8 leaf disk)
44	*C. anguria* var. *longipes* (PIVT 1751)	30.9
45	*C. myriocarpus* (PI 282447)	31.5
46	*C. ficifolius* (PI 280231)	32.1
47	*C. melo* subsp. *conomon* "Baj-Gua" (CUM 238/1974)	36.8
48	*C. anguria* var. *longipes* (CUC 28/1974)	39.0
49	*C. dipsaceus* (PI 236468)	40.5
50	*C. myriocarpus* (PIVT 0202)	43.8
51	*C. africanus* (PI 374151)	47.0
52	*C. myriocarpus* (PIVT 0182)	47.4
53	*C. leptodermis* (PI 374152)	50.0
54	*C. sativus* (line VURV 1/85, control)	83.4

Origin of seed:
PI = Germplasm Resources Laboratory, U. S. D. A., A. R. S., Beltsville, USA
PIVT = Centre for Plant Breeding and Reproduction Research (CPRO-DLO/ (formerly Institute for Horticultural Plant Breeding), Wageningen, The Netherlands
CUM = Central Institute for Genetics and Research of Crop Plants, Gatersleben, Germany

but probably *C. metuliferus*. Limited data Tulisalo (1972), Knipping et al. (1975) and De Ponti (1978) concerning resistance and moderate resistance in *C. anguria* and *C. ficifolius* are in good agreement with our present results.

The accessions of *C. zeyheri* were rated as more resistant. There is a small variation among different accessions. Based on recent and published data it is concluded that *C. africanus, C. anguria, C. ficifolius, C. figarei, C. metuliferus* and *C. zeyheri* are

Table 2

Results of Scheffe multiple comparison test to compare the reaction of *Cucumis* accessions to *Tetranychus urticae*

Serial number of *Cucumis* accession	52	53	54
4, 10–13	*		
1–3, 5–9	**		
4, 7, 13–19		*	
1–3, 5, 6, 8–12		**	
1–48			**

* statistically significant at P = 0.05
** statistically significant at P = 0.01

Table 3

The mean susceptibility of *Cucumis* species to *Tetranychus urticae*

Cucumis spp.	Number of accessions tested	Overall mean number of live mites
C. figarei	1	5.1
C. zeyheri	9	10.2
C. meeusei	1	12.1
C. africanus	4	14.4
C. metuliferus	4	17.5
C. ficifolius	5	17.8
C. anguria	11	20.5
C. prophetarum	1	20.9
C. dipsaceus	4	23.6
C. melo	6	24.1
C. myriocarpus	6	32.5
C. leptodermis	1	50.0
C. sativus (control)	1	83.4

probably not nutritionally suitable for growth and development of mites (Table 3). *C. ficifolius, C. zeyheri* and *C. anguria* were also more resistant to glasshouse whitefly (*Trialeurodes vaporariorum*) (Láska and Lebeda, 1989). It is possible that these species have the same biochemical mechanism or factors conferring for resistance to the above-mentioned pests.

It is concluded that some wild *Cucumis* species can be good sources of resistance to *T. urticae*. Applications for practical breeding will depend on overcoming crossability barriers between *C. sativus* and *Cucumis* species originating from African gene centre (Den Nijs and Custers, 1990). Some recent results are very enthusiastic in this respect (Lebeda et al., 1996).

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Diversity and Community Structure of Spiders of Alfalfa Fields and Grassy Field Margins in South Hungary

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This paper presents the results of a 3 years' survey of the spider fauna of alfalfa fields and their field margins in South Hungary. During the study samples were taken by pitfall trap, sweep net and suction sampler (hand-held D-vac). The studied alfalfa fields and their margins had a diverse spider community. The overall community structures at both sites and at both habitat types were rather similar, which, we suggest, reflected the moderately disturbed state of these habitats. The communities were dominated by few super abundant species. *Pardosa agrestis*, the most common species, together with few other common species were more abundant in alfalfa, but occurred frequently in the margins, as well. Many of the moderately common species, on the other hand, were either associated with alfalfa or with field margin. Spider densities as assessed from suction sampling were high throughout the study period in both habitats. Average density of spiders in the alfalfa was 85 indiv./m^2, and 184 indiv./m^2 in the margin.

Agricultural areas cover c. 80% of the land surface in Central Europe. However, our understanding of the significance of diversity and community process in agricultural land is still very limited. In Hungary spiders has been relatively well studied in natural habitats (e.g. Chyzer and Kulczynski, 1891–97; Balogh, 1935; Loksa, 1966, 1987; Szinetár 1993), but this important group of predators has been underrepresented in the studies of agrobiocoenoses. There is only one previous study from alfalfa (Balogh and Loksa, 1956), and in other agricultural habitat types there are only two recent studies (in orchards: Samu and Lövei, 1995; in wheat: Tóth et al., 1996).

The significance of studying agricultural spider communities is, in our opinion, two-fold. Firstly, there has alwasys been a hope to use spiders as biocontrol agents against insect pests. Bein generalist predators and showing a moderately effective functional response (Samu and Bíró, 1993), spiders are likely to exert only a limited effect on prey population. However, this effect can still be significant especially in the early stages of pest population growth (Riechert and Lockley, 1984). Attempts to show effective pest control by spiders has been successful only in a number of cases (in rice: Kiritani et al., 1972; in citrus: Mansour et al., 1981; in greenhouse: Klein et al., 1994), in other instances the role of spiders, although present in large densities, was harder to assess (Luczak, 1979; Sunderland et al., 1986; Nyffeler and Breene, 1992).

The second aspect why spiders can be important in agroecosystems, is that not only certain spider species interact with certain insect pests but hey also comprise an

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important part of these animal communities. By increasing the stability of agroecosystems the likelihood of pest outbreaks can be reduced. Diversification within field (Nentwig, 1988) and the use of hedges, field margins (Alderweireldt, 1989; Kromp and Steinberger, 1992) increases the diversity and abundance of spiders and other predators and increases stability. In the present study we investigated agricultural spider communities mainly from this second viewpoint. Spiders in two Hungarian alfalfa fields and the nearby field edges were sampled by pitfalls, sweep net and suction sampler. In this study the ecological characteristics of the dominant species and the community structures of these habitats are described.

Materials and Methods

The study fields were situated close to the town of Szekszárd, Tolna county, in the South Transdanubian part of Hungary, in a hilly area (c. 200 m above sea level). At the Kisdorog site (KD) the alfalfa field was 5 years old, 16 ha in area. The neighbouring areas were wheat and maize fields, a pasture and a small forest close to the corner of the field. The Felsőnána site (FN) was 9 km far from the KD site. At FN the alfalfa field was 3 years old, 43 ha in area. The neighbouring areas were further alfalfa fields and a forest strip. Management on both fields consisted of regular mowing (3–4 times per year). Small blocks of unmowed alfalfa were present at both sites, these were created for other experimental purposes.

Survey of the spider community at the FN site lasted for two years (1993–94). Sampling methods were pitfall trapping (3 traps, 5 m apart, plastic cups, 7.5 cm diam., containing solution of ethylene glycol and detergent as preservative, weekly emptied between April and September), and sweep net samples (3 × 10 regular sweeps, weekly). Field margin was sampled by 3 pitfalls in 1994. At the KD site a more intensive sampling procedure was applied. Nine pitfalls (identical to the ones used in FN) were operated in the field and three in the margin between April and September 1995; the traps were emptied weekly. At KD, instead of sweep netting, suction sampling was applied to obtain information about the plant dwelling spiders. The device was a hand-held D-vac modified from a leaf blower, as described in Samu and Sárospataki (1995). With this device 23 transect samples totalling 0.1 m^2 each were taken in the alfalfa field at two weeks' periods. In the field margin five 0.1 m^2 samples were taken by the hand-held D-vac on the same days as in the field.

Spiders sorted out from the samples were identified in the case of adults to the species level, in the case of juveniles to the furthest possible (usually generic) level. Nomenclature in Heimer and Nentwig (1991) was used. In the species list all effective taxa are listed, i.e. all species plus data on those juveniles which were identified to genus but no adult from that genus was represented. Data on adults were used in calculating diversity and other community characteristics.

Results

In the study 5033 spider specimens, representing minimum 99 species, were collected (Table 1). While in the pitfall catches the majority (c. 80%) of the animals were adults, in the D-vac and sweep net samples most of the spiders (75–95%) represented a juvenile stage (Fig. 1).

Since D-vac samples were taken from a definite area in the field, the results can be considered as relative indicators of spider density. Average spider density in the KD alfalfa field was 85.6 ± 75.46 (mean $\pm$ SD), while average density in the margin of the field (184.3 ± 115.79) was significantly higher (t-test for dependent samples: d.f. $= 7$, $t = 3.40$, $P < 0.05$). There was one definite peak at the beginning of August, where spider density in the alfalfa and the margin reached 190 and 418 spiders/m^2, respectively (Fig. 2).

Species richness was the highest in the FN field (61 species). If species richness data rarefracted to the smallest sample size (Hurlbert, 1971) are considered no such difference can be found between the two alfalfa fields, but higher values were obtained for the margin habitats (Table 2). Shannon diversity and Shannon evenness were also higher in the margins as compared to the respective fields. However, if Q diversity figures, which are the least sensitive to sample size differences (Magurran, 1988), are compared such a trend is not observable (Table 2). Rank abundance plots of the respective communities showed a very similar structure in both fields and field margins (Fig. 3).

Blocks (i.e. blocks of data, as distinguished by habitat, site, year and collecting method) were ordinated on species caught in them by PCA. The ordination result (Fig. 4a) indicated that the largest difference between the species composition of the blocks were due to the respective sampling method applied. Blocks where collection was made by pitfalls clustered close to each other and were clearly separated from blocks distinguished by the application of D-vac and sweep net sampling. D-vac samples from the alfalfa at the KD site were somewhat apart from other blocks. No other separation by site, habitat or year was observable.

The most common species were similarly ordinated by blocks (in the above sense), using PCA. This resulted in three clearly separable groups of species (Fig. 4b) These groups contain species that are similar to each other in many of their ecological characteristics (Table 3). The most common species was the lycosid *Pardosa agrestis*. A total of 989 adult *P. agrestis* specimens were caught, and 231 *Pardosa* sp. juveniles, most of them also likely to belong to this species. This species is a typical agrobiont, found in largest numbers in arable fields in Central Europe. *P. agrestis* occurred in all study sites and was not among the three most abundant species in the field margin at KD only. *Xysticus kochi* was typically found in larger numbers in the alfalfa only, while *Zelotes pusillus* and *Zelotes praeficus* were occurring together in large numbers in the alfalfa field at FN, but were less frequent at the KD site. Common property of these four species is their strong association to agricultural areas, secondary habitats and the epigeic life mode. They are almost exclusively trappable by pitfall (Table 3). Species in the second group can often be found in forest and were catchable only by pitfall. They prefer

Table 1

Number of individuals caught during the different collecting sessions in alfalfa and the field margins. Only taxa that represent a distinct species are included (i.e. juveniles that are otherwise represented are not presented in this table)

Species	Alfalfa						Field margin		
	FN93		FN94		KD95		FN94	KD95	
	PF	SN	PF	SN	PF	DV	PF	PF	DV
Agelenidae									
Coelotes longispina Kulcz..	1	0	0	0	0	0	0	0	0
Araneidae									
Araniella sp. juv.	0	0	0	0	0	0	0	0	1
Agriope bruennichi (Scopoli)	0	0	0	0	0	0	0	0	1
Gibbaranea sp. juv.	0	0	0	0	0	0	0	0	1
Hypsosinga pygmaea (Sundevall)	0	2	0	9	0	0	0	0	0
Mangora acalypha (Walckenaer)	0	1	0	8	0	14	0	0	2
Clubionidae									
Cheiracanthium sp. juv.	0	0	0	1	0	0	0	0	0
Clubiona diversa O. P.-Cambr.	0	0	0	0	0	1	0	0	0
Ctenizidae									
Nemesia pannonica (Herman)	0	0	0	0	1	0	0	0	1
Dictynidae									
Argenna subnigra (O. P.-Cambr.)	0	0	1	0	4	3	0	0	11
Dictyna latens (Fabricius)	0	1	0	0	0	0	0	0	0
Dysderidae									
Harpactea rubicunda C. L. Koch	0	0	1	0	0	0	0	0	0
Linyphiidae									
Bathyphantes gracilis (Blackw.)	0	0	0	0	0	1	0	0	0
Diplostyla concolor (Wider)	0	0	0	0	0	0	0	0	3
Lepthyphantes flavipes (Blackw.)	0	0	1	3	0	5	0	0	2
Lepthyphantes pillichi Kulcz.	0	0	0	0	0	0	1	0	0
Lepthyphantes tenuis (Blackw.)	0	0	0	1	0	6	0	0	16
Linyphia triangularis (Clerck)	0	0	0	0	0	0	0	0	1
Meioneta beata (O. P.-Cambr.)	0	0	0	0	0	3	0	0	0
Meioneta rurestris (C. L. Koch)	4	0	1	10	2	265	1	1	11
Meioneta simplicitarsis (Simon)	0	0	0	0	0	3	0	0	1
Microlinyphia pusilla (Sundevall)	0	0	0	7	0	0	0	0	1
Porrhomma microphthalmum O. P.-C.	0	0	0	0	0	0	1	0	0
Araeoncus humilis (Blackw.)	0	0	0	0	0	3	0	0	0
Erigone dentipalpis (Wider)	0	0	1	1	9	15	0	2	0
Oedothorax apicaatus (Blackw.)	2	0	0	1	0	4	0	0	0

Table 1 (cont.)

Species	Alfalfa						Field margin		
	FN93		FN94		KD95		FN94	KD95	
	PF	SN	PF	SN	PF	DV	PF	PF	DV
Pocadicnemis pumila (Blackw.)	0	0	0	0	0	0	0	0	1
Silometopus reussi (Thorell)	0	0	0	0	0	1	0	0	0
Trichopterna cito (O. P.-Cambr.)	0	0	0	0	0	0	0	0	1
Gnaphosidae									
Drassodes lapidosus (Walckenaer)	1	0	0	0	0	0	0	0	0
Haplodrassus dalmatensis (L. Koch)	1	0	15	0	1	0	0	0	0
Haplodrassus signifer (C. L. Koch)	0	0	8	0	2	0	0	2	0
Micaria formicaria (Sundevall)	0	0	0	1	0	0	0	0	0
Zelotes aeneus (Simon)	0	0	1	0	0	0	0	0	0
Zelotes apricorum (L. Koch)	0	0	0	0	0	0	5	0	0
Zelotes aurantiacus Miller	0	0	1	0	0	0	6	1	1
Zelotes gracilis Canestrini	0	0	3	0	0	0	0	0	0
Zelotes latreillei Simon	0	0	1	0	0	0	0	0	0
Zelotes mundus (Kulcz.)	1	0	0	0	0	0	0	0	0
Zelotes pedestris (C. L. Koch)	1	0	3	0	0	0	10	4	0
Zelotes praeficus (L. Koch)	1	0	39	0	3	0	17	1	0
Zelotes pumilus (C. L. Koch)	0	0	0	0	0	0	1	0	0
Zelotes pusillus (C. L. Koch)	0	0	65	0	9	0	15	0	0
Zelotes pygmaeus Miller	1	0	0	0	0	0	0	0	0
Zelotes subterraneus (C. L. Koch)	0	0	0	0	0	0	1	0	0
Zelotes villicus (Thorell)	2	0	4	0	2	0	100	0	0
Hahnidae									
Hahnia nava (Blackw.)	0	0	2	0	3	0	0	2	13
Liocranidae									
Agroeca pullata Thorell	0	0	0	0	0	0	3	0	3
Phrurolithus festivus (C. L. Koch)	0	0	1	0	0	0	9	1	2
Lycosidae									
Alopecosa accentuata (Latreille)	0	0	2	0	3	0	0	0	0
Alopecosa aculeata (Clerck)	0	0	0	0	1	0	0	0	0
Alopecosa cuneata (Clerk)	0	0	1	0	5	0	1	5	0
Alopecosa pulverulenta (Clerck)	0	0	0	1	2	0	0	0	0
Alopecosa trabalis (Clerck)	0	0	4	0	0	0	3	0	0
Aulonia albimana (Walckenaer)	0	0	3	0	1	0	5	21	10
Lycosa radiata (Latreille)	1	0	0	0	0	0	0	0	0
Pardosa agrestis (Westring)	208	3	498	10	144	3	70	29	0
Pardosa hortensis (Thorell)	0	0	15	1	5	0	4	1	0
Pardosa lugurbris (Walckenaer)	0	0	5	1	2	0	21	1	0
Pardosa palustris (Linnaeus)	5	0	14	1	1	0	4	0	0
Pardosa proxima (C. L. Koch)	0	0	2	1	0	0	1	0	0

Table 1 (cont.)

Species	Alfalfa						Field margin		
	FN93		FN94		KD95		FN94	KD95	
	PF	SN	PF	SN	PF	DV	PF	PF	DV
Pardosa pullata (Clerck)	0	0	0	0	2	0	0	0	0
Trochosa terricola Thorell	0	0	1	0	2	0	0	0	0
Xerolycosa miniata (C. L. Koch)	0	0	0	0	3	0	0	2	0
Mimetidae									
Ero sp. juv.	0	0	0	0	0	1	0	0	6
Oxyopidae									
Oxyopes lineatus (Latreille)	0	0	0	1	0	0	0	0	0
Philodromidae									
Philodromus aureolus (Clerck)	0	1	0	0	0	0	0	0	0
Thanatus arenarius Thorell	0	0	1	0	0	0	0	0	0
Tibellus oblongus (Walckenaer)	0	0	0	12	0	7	0	0	1
Pisauridae									
Pisaura mirabilis (Clerck)	0	6	0	87	0	9	1	0	75
Salticidae									
Bianor aurocinctus (Ohlert)	0	0	0	0	0	0	0	0	1
Euophrys frontalis (Walckenaer)	0	0	0	0	0	0	3	1	1
Euophrys obsoleta (Simon)	0	0	0	0	0	1	0	0	0
Evarcha arcuata (Clerck)	0	0	0	0	0	1	0	0	0
Evarcha laetabunda (C. L. Koch)	0	0	0	1	0	0	0	0	0
Heliophanus cupreus (Walckenaer)	0	0	0	0	1	1	1	0	0
Heliophanus flavipes Hahn	0	0	0	0	0	2	0	0	0
Tetragnathidae									
Pachygnatha degeeri Sundevall	6	0	5	3	51	239	0	4	27
Tetragnatha extensa (Linnaeus)	0	0	0	3	0	0	0	0	0
Theridiidae									
Enoplognatha lineata (Blackw.)	0	4	0	0	0	0	0	0	0
Enoplognatha thoracica (Hahn)	0	0	10	1	3	2	0	0	0
Episinus truncatus Latreille	0	0	0	0	0	0	0	0	1
Neottiura bimaculata (Linnaeus)	0	0	0	0	0	1	0	0	0
Neottiura suaveolens (Simon)	0	0	0	0	0	1	0	0	1
Robertus arundineti (O. P.-Cambr.)	1	0	1	0	1	0	0	0	0
Steatoda phalerata (Panzer)	0	0	2	0	0	0	0	0	0
Theridion impressum L. Koch	0	0	0	1	0	0	0	0	0
Titanoecidae									
Titanoeca schineri (L. Koch)	0	0	0	0	0	0	2	0	0

Table 1 (cont.)

Species	Alfalfa						Field margin		
	FN93		FN94		KD95		FN94	KD95	
	PF	SN	PF	SN	PF	DV	PF	PF	DV
Thomisidae									
Heriaeus graminicola (Doleschall)	0	0	0	0	0	0	0	0	1
Misumena vatia (Clerck)	0	1	0	5	0	0	0	0	0
Oxyptila nigrita (Thorell)	0	0	0	0	0	0	0	1	2
Runcinia lateralis (C. L. Koch)	0	0	0	1	0	0	0	0	0
Thomisus onustus Walckenaer	0	0	0	1	0	0	0	0	0
Xysticus acerbus Thorell	0	0	0	1	0	0	0	0	0
Xysticus cristatus (Clerck)	0	0	3	0	0	0	1	0	0
Xysticus kochi Thorell	1	0	40	13	10	0	2	0	0
Xysticus ulmi (Hahn)	0	4	0	0	0	0	0	0	0
Zodariidae									
Zodarion germanicum (C. L. Koch)	0	0	0	0	0	0	2	0	0
Zoridae									
Zora sp. juv.	0	0	1	0	0	0	0	0	6

Sampling methods: PF = pitfall trapping, SN = sweep net sampling, DV = D-vac sampling

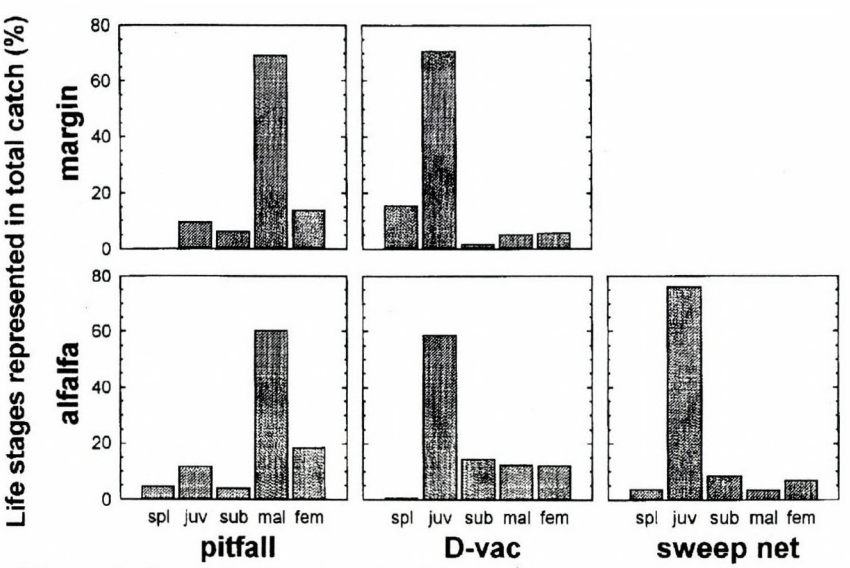

Fig. 1. Life stage distribution of spiders collected by different methods in the alfalfa fields and in the margins. spl = spiderling, juv = juvenile, sub = subadult, mal = male, fem = female

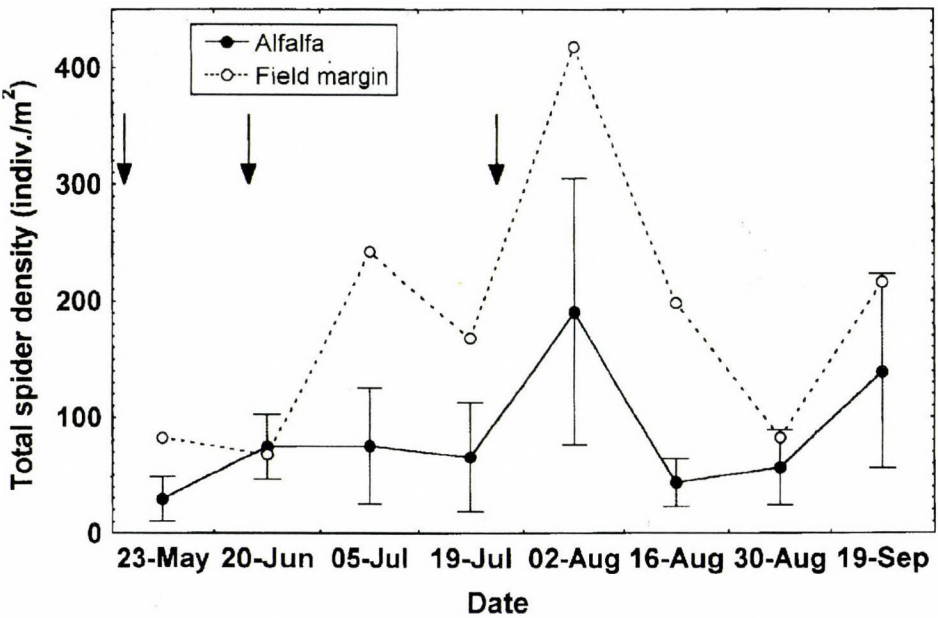

Fig. 2. Seasonal dynamics of the total spider density at the Kisdorog site, estimated from D-vac samples. Arrows indicate mowing, bars are S.D.

Table 2

Species richness and diversity measures of the spider fauna by site, method, habitat and year. Rarefraction was made to the smallest sample size (number of individuals = 23); the Shannon index and the respective equitability are ln based

Habitat	Alfalfa						Field margin		
Site	Felsőnána				Kisdorog		Felsőnána	Kisdorog	
Year	1993		1994		1995		1994	1995	
Method	sweep net	pitfall	sweep net	pitfall	D-vac	pitfall	pitfall	D-vac	pitfall
No. of species	9	17	27	29	22	21	23	23	15
No. of specimens	23	238	185	744	585	257	278	166	53
Species richness, rarefraction	9.00	3.63	9.45	6.13	4.80	6.60	8.21	7.79	9.02
Berger-Parker	0.26	0.87	0.47	0.66	0.45	0.56	0.35	0.45	0.54
Shannon diversity	1.98	0.69	2.16	1.43	1.35	1.62	2.06	1.94	1.81
Shannon equitability	0.90	0.24	0.65	0.42	0.43	0.53	0.65	0.62	0.66
Q	4.57	4.65	8.19	6.76	5.02	5.99	6.05	6.61	6.05
alfa	5.44	4.18	8.70	6.00	4.51	5.41	5.94	7.24	6.96

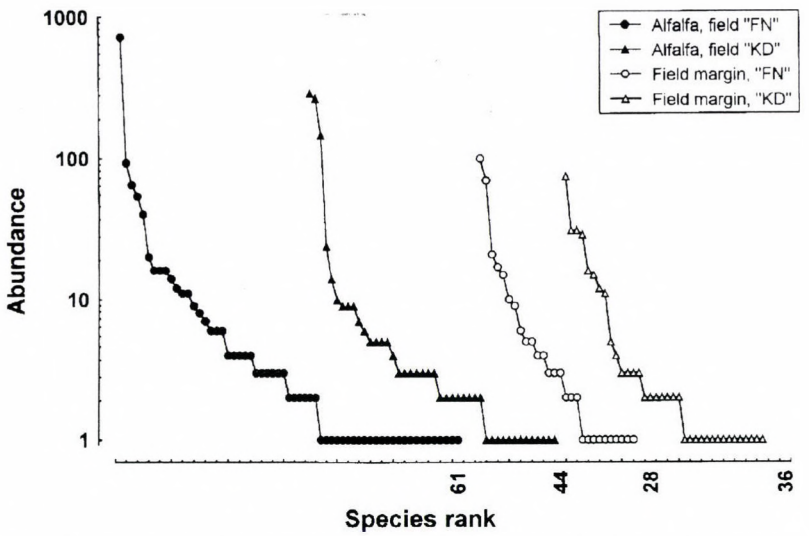

Fig. 3. Rank-abundance curves of the spider communities in the field and in the margin at both experimental sites. The curves represent the combined result of sweep net plus pitfall sampling and D-vac plus pitfall sampling applied at the FN and KD sites

Table 3

Ecological characteristics of the most abundant species

Species	After Buchar, 1992					After Hänggi et al., 1995				In the present study				
	C	T	H_1	F	S	M	S	H_2	PCA	H_3	DV	SN	PF
Pardosa agrestis	I	m	s	n	e	p	e	cereal fields	1	x	0.3	1.3	98.4
Xysticus kochi	I	m	s	n	x	p	e	disturbed areas	1	–	0	18.8	81.2
Zelotes pussilus	I	n	s	n	e	–	–	–	1	a	0	0	100
Zelotes praeficus	II	t	n	n	e	–	–	–	1	–	0	0	100
Pachygnatha degeeri	I	n	s	x	e	p	e	cereal fields	3	x	78.5	0.9	20.6
Meioneta rurestris	I	n	s	x	e	p	e	cereal fields	3	a	93.7	3.3	3
Pisaura mirabilis	II	n	s	x	p	p	x	dry meadows	3	x	48.6	50.8	0.5
Pardosa lugubris	II	n	n	x	e	p	e	forest edges	2	m	0	2	98
Zelotes villicus	III	t	n	x	e	–	–	–	2	m	0	0	100

C: commonness of the species (I = common, II = moderately common, III = rare), T: thermophilia (t = thermophil, m = mesotherm, n = no specific thermal requirement), H_1: habitat type (s = secondary, man influenced, n = natural), F: association to forest (n = non-forest living, x = occurs inside and outside of forsts), S: preferred stratum (e = epigeic, p = on plants, x = both on ground and plants), M: method most often caught by (p = pitfall), H_2: habitat type most abundantly occurs, PCA:PCA group (Fig. 4b), H_3: habitat, where among the first three most abundant species (a = alfalfa, m = margin, x = both), DV: % of individuals caught by D-vac, SN: % caught by sweep net, PF: % caught by pitfall

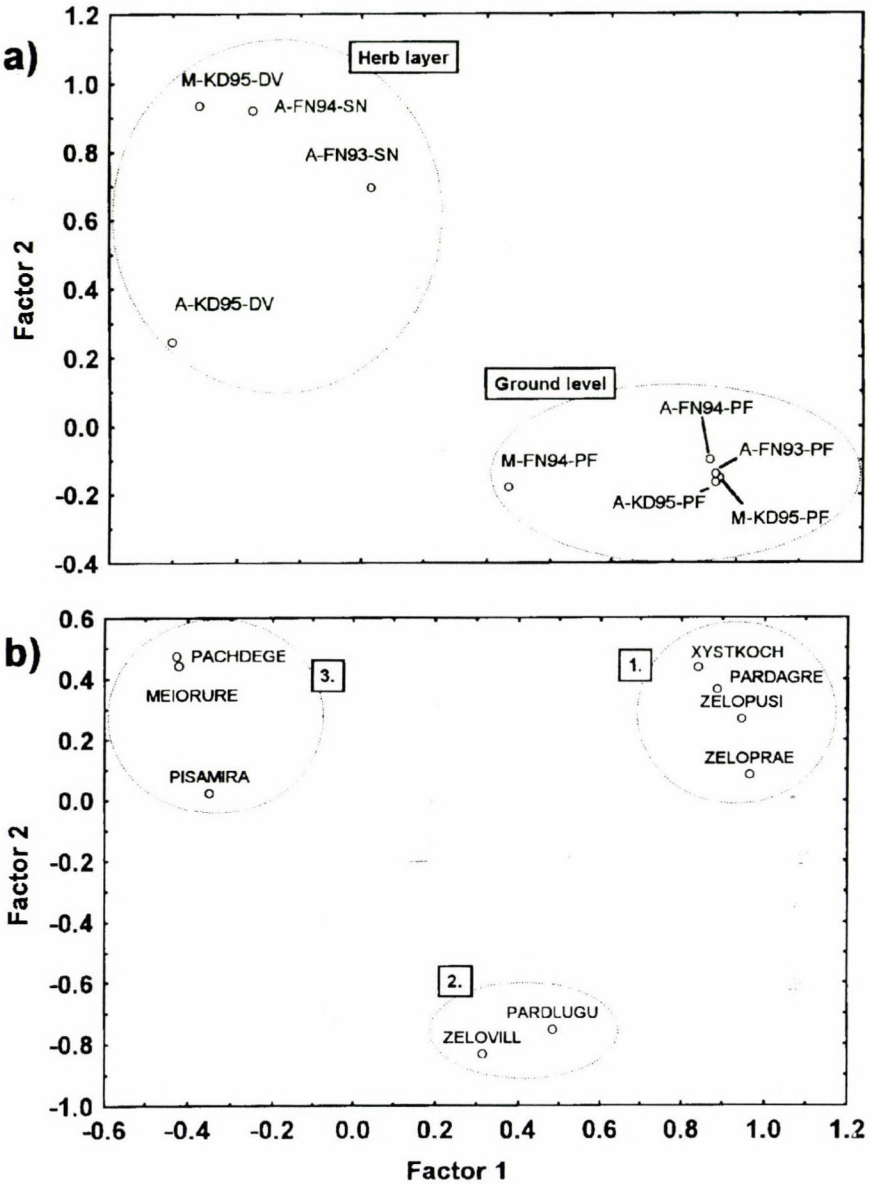

Fig. 4. a) PCA ordination of blocks of data (as distinguisheld by habitat, site, year and collecting method) on species caught in them. First part of block code: A = alfalfa, M = margin, second part indicate site and year, third part gives method as in Table 1. b) PCA ordination of species on blocks

natural habitats to secondary ones, and both were found in the margin of the FN field, where there was a forest strip nearby. The third PCA group of species can be characterised by an association to agricultural areas, too, although they had a higher dominance in the margins than that of the species belonging to the previous group. A second characteristics of this group is, that these species are more often caught by D-vac or sweep net than by pitfalls. This different trappability reflects difference in the microhabitat preference: juveniles *Pachygnatha degeeri* build small webs. These animals are vagrant as adults, moving both on the ground and on in the herb layer. *Pisaura mirabilis* is also a vagrant spider which has a preference for staying on low vegetation, while the linyphiid spider *Meioneta rurestris* builds small web attached to low vegetation, as well.

There were spider species that were typically abundant in the alfalfa fields at both sites, but were seldom found in the margins: *Xysticus kochi, Enoplognatha thoracica, Mangora acalypha, Tibellus oblongus*. Although these animals were strongly associated with alfalfa, they were not among the "super abundant" species. Other similarly moderately abundant species were found in the field margins nearly exclusively: *Aulonia albimana, Phrurolithus festivus, Zelotes aurantiacus, Agroeca pullata.*

Several rarities were caught at both sites (Table 1). *Nemesia pannonica*, a ctenizid trap-door spider, is a distinghuished rare element of the Hungarian fauna. This is the first demonstrated occurrence in the region, and also in an agricultural area. *Meioneta beata* and *Trichopterna cito* are rare linyphiid species, the previous is new for the Hungarian fauna. Among the *Zelotes* species *Z. aurantiacus* and *Z. pygmaeus* are new for the Hungarian fauna. *Zelotes mundus* is a previously little known East European species.

Discussion

The traditional notion of agricultural areas has been that they are impoverished in biodiversity. Recently our view on this issue became more subtle. Toft (1989) reported 85 spider species from two barley fields in Denmark, which figure was as large as species richness in the richest natural habitats. Howell and Pienkowski (1971) identified 112 species in an American alfalfa field, Kromp and Steinberger (1992) found 80 spider species in a wheat in Austria. Species richness figures from the present study are much higher than those reported by Balogh and Loksa (1956) from alfalfa (22 species), in Hungary or by Gajdos (1992) from pea (21 species), in Slovakia. One reason for this newly found diversity in agricultural areas can be the turn of research interest towards these habitats. Although differences in diversity figures, that were reported in the above case studies, can partly be explained by the variety of sampling methods and sampling efforts applied (Sunderland et al., 1995; Samu and Sárospataki, 1995), perhaps there is also a bias towards the faunistically more interesting and nowadays popular "biologically" cultivated fields. In many annual crops, as Nyffeler et al. (1994) described after harvest the system becomes an "ecological desert", where a high spider diversity is unlikely to build up.

Measuring and expressing diversity in agricultural areas is important because a condensed measure about the quality of the habitat can be provided. However, to gain real understanding of the processes that take place in an arable field, more detailed studies of the animal communities and an understanding of the ecological characteristics and role of at least the most dominant species is needed. Comparison ot the species abundance curves for alfalfa fields and the respective margins in the present study reveals very little difference between the community structures. This is perhaps because margins are also secondary, disturbed areas, and in a several years old alfalfa crop there is not a great contrast between successional age of the community in the field and in the margin. It can be supposed that this picture is different in cereals, where harvest and subsequent ploughing resets the successional age of the field to zero in every year (Luczak, 1979), while margins can stay in a more advanced stage. Species abundance curves of spiders in wheat and adjacent margins were much more distinct in the study of Kromp and Steinberger (1992).

Figures about the density of spiders in crop fields vary a lot in the literature. Nyffeler et al. (1994) in their review of 37 studies on spiders in agricultural areas in the USA, found that the mean number of plant-dwelling spiders was only c. 1 individual/m^2, the maximum value reported being 3.4 spiders/m^2. From European studies values one order of magnitude higher are known. In wheat Sunderland and Topping (1994) reported the peak of spiders to be 78–123 spiders/m^2, similarly c. 120 spiders/m^2 was found in wheat by Dinter (1995), and even higher values of 300–600 spiders/m^2 by Toft et al. (1995). Balogh and Loksa (1956) using quadrate method estimated the spider density in a Hungarian alfalfa field to be c. 80 individuals/m^2. Estimates from the present study fall within the range reported from other European crop fields. High densities of spiders, even if many of the animals are juvenile, show that spiders comprise a very significant part of the predatory assemblages that are present in Hungarian agroecosystems, and indicate that the spider community is likely to exert a considerable predatory pressure in these habitats.

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After Introduction of *Frankliniella occidentalis* in Europe: Prevention of Establishment of *Thrips palmi* (Thysanoptera: Thripidae)

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In The Netherlands the first introduction of *Thrips palmi* was prevented by destroying infested crops in a greenhouse in 1988. This first quarantine measure aimed at the elimination of a thrips species was followed by elimination actions on three infested *Ficus* nurseries in 1992. In 1994 and 1995 imidacloprid was used to clear several infested nurseries from the thrips. The decrease of the populations was monitored with blue and yellow sticky boards. In Dutch greenhouses the active dispersal of *T. palmi* was observed to be lower than that of the earlier introduced *Frankliniella occidentalis* (Pergande). Because of this low active dispersal in combination with a low introduction pressure, preventing the establishment of *T. palmi* is still a viable and realistic option.

Over the past 18 years, *Thrips palmi* Karny has spread to Japan, several Indian and Pacific islands, the Caribbean, the USA (Hawaii, Florida), Australia, Venezuela and Brazil. In the original distribution area (Southeast Asia) damage to tobacco was reported in the thirties, but until an outbreak on watermelon in the Philippines in 1977, the incidence of *T. palmi* was not recorded (Hirose, 1991). Since 1977 damage to especially cucurbits, but also eggplant, cotton and several other crops was recorded in different countries. In 1978 the spread of *T. palmi* started with the introduction in Japan, New Caledonia and Wallis Islands (Sakimura et al., 1986).

The knowledge of the impact exotic thrips species (*Frankliniella occidentalis* (Pergande)) may have and the threat of introduction of *T. palmi* into Europe, urged in 1988 the then European Community to regard the latter as a quarantine organism. As a result of the seventh Directive of the EC Commission of July 1st (88/430/EEC), *T. palmi* appeared on the list of quarantine organisms, whose introduction must be prohibited in all member states. Since then, not only for *T. palmi*, but for thrips as a whole inspections at ports of entry in The Netherlands were intensified. In this paper an explanation is given for the increased significance of thrips concerning quarantine and an overview of measures to prevent the introduction of *Thrips palmi* in The Netherlands is presented.

Thysanoptera and quarantine

In the midst of the nineteenth century the first thrips species were found outside their original distribution area (*Heliothrips haemorrhoidalis* (Bouché) and *Parthenothrips dracaenae* (Heeger)). The economic impact of these species was very low and

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they were merely regarded as a curiosity. One of the most noxious species dispersed by commerce in the beginning of this century is *Thrips simplex* Morison, the gladiolus thrips (Morison, 1957). This species was transported with corms of *Gladiolus*, but did not really arise the interest of quarantine inspectors. Thysanoptera as a whole were not in the picture untill *Frankliniella occidentalis* (Pergande), the western flower thrips, started to disperse in the seventies from Western North America, its natural distribution area. *F. occidentalis* was introduced in Europe in 1983 and spread rapidly from country to country (Tommasini and Maini, 1995). The early discovery of its occurrence in the Western European countries suggests that the spread was a result of the flight activity of the thrips. There are, however, indications that the spread was caused by international trade in plant products. For example, in Russia the species was first observed on chrysanthemum cuttings in St. Petersburg, introduced from Western Europe (Ivanova et al., 1991). The rapid dispersal of *F. occidentalis* surprised plant inspectors. The species was not identified in Europe until 1986, which precluded regulatory quarantine actions. The rapid dispersal of *Thrips palmi* out of its distribution area and increasing number of interceptions at ports of entry incited European plant protectors to add this thrips to the list of quarantine organisms. Soon it became clear that costly quarantine measures would be the result of this 'addition'.

In 1993 the names of three *Scirtothrips* species (*S. aurantii* Faure, *S. citri* Moulton and *S. dorsalis* Hood) were added to the EC list of quarantine organisms in order to protect the *Citrus* cultures in Mediterranean member states against introduction of these major pests. The impact of quarantine measures necessary to prevent these species from introduction was low until now (Vierbergen, 1993).

Biology and dispersal

Synanthropic phytophagous Thysanoptera such as *Frankliniella occidentalis* and *Thrips palmi* have some favourable characteristics in common from a biological standpoint, which are:

- feeding on different host plants (polyphagous),
- arrhenotokous reproduction,
- high reproduction rate,
- development on commercial greenhouse crops,
- resistance against most commonly used insecticides (Robb et al., 1988; Kawai, 1990; Martin and Workman, 1994).

Most of the economical significant synanthropics are opportunists, which are highly adaptable, feeding on leaf and flower tissue as well as being predators on other arthropods (Mound and Teulon, 1995).

Differences in biology, however, result in different establishment potential of *F. occidentalis* and *T. palmi*. *F. occidentalis* can feed on both pollen and liquid derived from leaf cells, but *T. palmi* only feeds on cell liquid. The search for pollen urges adult *F. occidentalis* to find flowers in a certain stage of development. The dispersal of *F. occidentalis* consequently will thus be greater than of *T. palmi*. This is in favour of the former

concerning chances of settlement. The host plant range of both species is very wide, but insufficiently known. On many plants thrips are only incidental visitors and not able to reproduce on them. Comparison of lists of plants from which both species have been collected, is therefore insufficient to evaluate differences in host plant range.

'Preventive' quarantine actions

In Japan the observed damage to economically important crops and the rapid dispersal to the North (greenhouses) stimulated Japanese plant inspectors to notify European plant protection organizations of the risk of introduction of *Thrips palmi*. Moreover, the appearence of a new, highly noxious strain was suggested (Bournier, 1987). Interceptions during import inspections in France and The Netherlands (Table 1) and the occurrence in a greenhouse in The Netherlands underlined the need to treat *T. palmi* as a quarantine organism and as a result in 1988 it was added to the quarantine list by the European Community. Following the introduction of *Frankliniella occidentalis*, surveys on imported cut flowers at the large flower auctions had already started, but were intensified to prevent the introduction of *T. palmi* (Vierbergen, 1994). The risks of numerous interceptions of *T. palmi* in Europe on orchids very probably are of minor importance, because there have never been introductions into the extensive culture of orchids in Europe. Furthermore, can be assumed that it does not concern (the) noxious strain(s), because the orchids originate from countries within the area of original distribution of *T. palmi* and not from newly colonized areas. Possibly biochemical identification methods can be useful for the typification of strains.

'Eliminating' quarantine actions

There are no reports of eliminations of populations of Thysanoptera after introduction, except from The Netherlands. For almost every introduction the discovery of the thrips was late and the identification was completed long after the first local establishment of the thrips. Recently, as a result of more intensive inspections for thrips and quicker identification service, elimination perspectives of introduced populations are increased. In The Netherlands *Thrips palmi* was subjected to quarantine measures aimed at its elimination from 1988 onwards. At the time of detection of *T. palmi* in Florida measures aimed at elimination were suggested, but not carried out (Mellinger and Mellinger, in litt.). In both Australia and the USA authorities concentrated on control rather than on elimination (Layland et al., 1994).

In contrast with the Australian and American situation, in The Netherlands only introduction in greenhouses may be expected. The first introduction, probably originating from Japan, was in a greenhouse in 1988. *Thrips palmi* occurred there in low numbers in flowers of Cactaceae together with *Frankliniella occidentalis, F. schultzei* (Trybom) and *Thrips tabaci* Lindeman. *T. palmi* was eliminated from this nursery by destroying the infested flowers (Vierbergen, 1989). In 1992 inspectors of the Dutch Plant Protection Service collected *T. palmi* in three nurseries on *Ficus benjamina*. The infested plants

Table 1

Interceptions of *Thrips palmi* in Europe (partly derived from a list prepared by R. Baker, Central Science Laboratory, U. K., 1994)

Year	Country	Plant	Origin	Interceptions
1987	Netherlands	*Dendranthema*	Japan	1
	United Kingdom	*Basella alba*	Mauritius	1
1988	France	*Solanum melongena*	Guadeloupe	1
	Netherlands	Orchidaceae	Thailand	2
1991	Finland	Orchidaceae	Thailand	3
	Germany	Orchidaceae	Thailand	1
1992	Finland	Orchidaceae	Thailand	4
	Norway	Orchidaceae	Thailand	1
1993	Finland	Orchidaceae	Thailand	3
1995	Denmark	Orchidaceae	Thailand	3
	Finland	Orchidaceae	Thailand	1
	Netherlands	Orchidaceae	Singapore	1
		Orchidaceae	Thailand	4
	Sweden	Orchidaceae	Thailand	1
1996	Denmark	Orchidaceae	Thailand	1
	France	*Capsicum*	Mauritius	1
		Cucurbita maxima	Mauritius	3
		Dendrobium	Thailand	9
		Momordica	Dominican Republic	2
		Orchidaceae	Singapore	3
		Orchidaceae	Thailand	9
		Solanum melongena	Dominican Republic	2
		Solanum melongena	Mauritius	5
	Netherlands	*Ficus benjamina*	USA (Florida)	1
	Netherlands	*Dendrobium*	Thailand	1

were destroyed, because, at that time, no effective chemicals for eradication were available (Schmidt, 1994).

In 1994, the systemic insecticide imidacloprid became available for eradication purposes and was used for quarantine actions in order to eliminate populations of *T. palmi*. Most of the 21 infestations in 1994 and 10 infestation in 1995 resulted from exchange of infested *Ficus benjamina*-plants between the growers. Significant sucking, damage to *Ficus* has not been observed. With a combination of insecticides (carbofuran, dichloorvos, imidacloprid and methiocarb) elimination of *T. palmi* from the nurseries was achieved. The decrease of the populations was monitored with yellow and blue sticky boards in equal numbers, which were changed by inspectors of the Dutch Plant

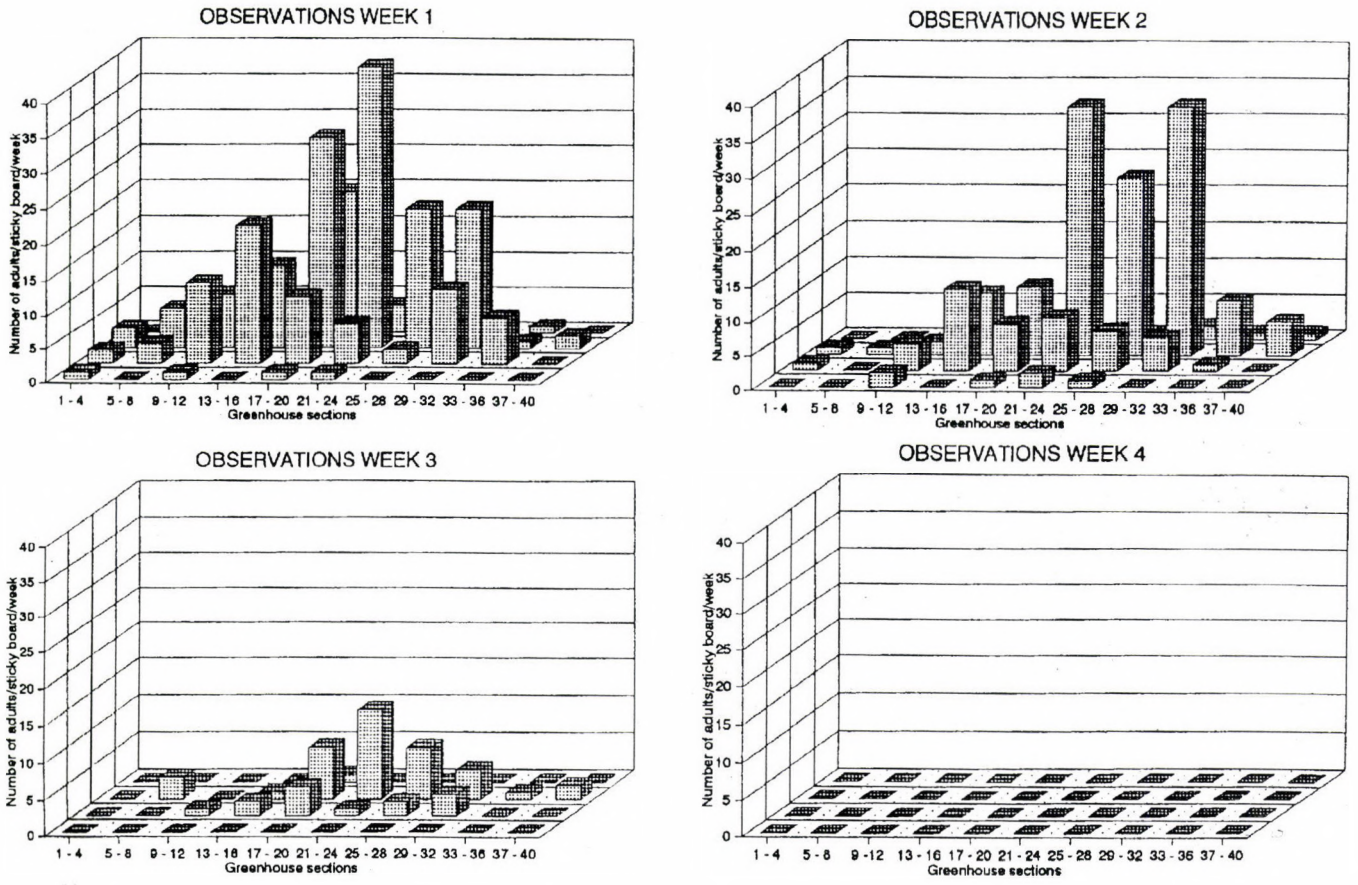

Fig. 1. Decreas of *Thrips palmi* population at Maasland. Detection on sticky boards; application imidacloprid at beginning of week 1

Protection Service every week. The number of boards per nursery varied from 10 to 58 according to the number of greenhouse compartments. During the quarantine period observations of the boards were checked for the presence of *T. palmi* by the section of Entomology of the Dutch Plant Protection Service. The duration of the quarantine period was based on the temperature and life cycle calculated as follows:

$T_q = 2 \times T_{pupa} + 2 \times T_{egg}$, in which
T_q = quarantine period
T_{pupa} = lifetime pupa + 3 × standard deviation
T_{egg} = lifetime egg + 3 × standard deviation
T_{pupa} and T_{egg} were derived from Kawai (1985) and extrapolated according to the mean daily temperature.

Based on the observations, the active dispersal of *Thrips palmi* in Dutch greenhouses was concluded to be very low. Kawai (1986) found lower dispersal activity in *T. palmi* if populations were covered by vinyl film absorbing the ultra violet spectrum. The glass cover used in Dutch greenhouses also absorbs ultraviolet light, possibly the main factor inhibiting dispersal activities of the thrips. Most of the dispersal within and between greenhouses can therefore be ascribed to the transfer of plant material (passive dispersal). During the observations in none of the nurseries significant dispersal was observed and even at high population densities the level of dispersal was limited (Fig. 1). On yellow and blue sticky boards outside and close to heavely infested greenhouses *Frankliniella occidentalis* was commonly observed, but *T. palmi* was absent, even when boards were operated close to greenhouses with large populations of *T. palmi* (Vierbergen, 1995).

Outlook

In The Netherlands measures aiming at the elimination of *T. palmi* are meaningful, because interceptions at ports of entry in Europe give no indication of a high introduction pressure and active dispersal of the thrips within greenhouses is negligible. Preventing the introduction of *T. palmi* is still a quarantine topic in Europe. However, it is not certain if *T. palmi* can be prevented from introduction in Europe in the long run, especially if more parts of the world are invaded and the introduction pressure increases. The extent of the pressure can be assessed for the greater part from the interceptions at port of entry.

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Egyptian Aleyrodidae

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The present work deals with the taxonomy of twenty species belonging to fifteen genera from family Aleyrodidae as known to occurre in Egypt. Each species is listed under its currently accepted name followed by author and reference in which it was first described and the date of first record, distribution, parasites in Egypt. Six species were recorded for the first time in Egypt during this work. Keys are provided for genera of whiteflies in Egypt and to species.

Whiteflies are minute, usually inconspicuous insects. They may be extremely injurious to vegetables, citrus, cucurbits, cotton and ornamental plants Byrne et al. (1990). In Egypt during the last few years *Bemisia tabaci* (Gennadius) has become a serious pest that acts as a transmitter of virus disseases to cotton and tomato, causing serious damage to tomatoes Abd-Rabou (1994). Many taxinomic work had been done in Egypt on i.e. Priesner and Hosny (1934), identified ten new species belonging to eight different genera.

Habib and Farag (1970) redescribed and classified the above, same species Zahradnik (1970) recorded *Ramsesseus follioti* as a new species. El-Helaly et al. (1972) recorded the another species *Aleyrodes proletella* (L.) and Bink-Moenen (1983) collected and described *Aleuroplatus acaciae* and *Tetraleurodes leguminicola* for the first time as a new species.

Six species were recored for the first time during this work from, Egypt, these are *Aleurolobus olivinus* (Silvestri), *Aleuromarginatus tephrosiae* Corbett, *Aleurovigginus adrianae* laccarino, *Dialeurodes citri* (Ashmead), *Parabemisia myricae* (Kuwana), and *Trialeurodes vaporariorum* (Westwood).

Each species is listed under its currently valid name followed by the first record, distribution, parasites and the type species.

Keys based mainly on the main taxonomic characters of the pupal stage to facilitate the accurate identification of both genera and species.

Key to the genera of Aleyrodidae in Egypt

1. Pupal case black.....2
   Pupal case pale or yellowish.....6

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2.(1) Operculum filling about one quarter of vasiform orifice .... *Acaudaleyrodes* Takahashi
Operculum almost filling vasiform orifice 3
3.(2) Median moulting suture reaching margin or submargin, caudal setae usually absent 4
Median moulting suture reaching margin, caudal setae present 5
4.(3) Margin strongly reflexed, most of reflexed part broad .... *Aleuroviggianus* Laccarino
Margin not reflexed ..... *Tetraleurodes* Cockerell
5.(3) Tracheal pore areas mostly differentiated from margin by a comb .... *Aleurolobus* Quaintance and Baker.
Tracheal pore areas not differentiated from margin .... *Aleuroplatus* Quaintance and Baker
6.(1) Vasiform orifice relatively small, subcircular posterior margin often with small median tubercle. Operculum usually concealing lingula. Inner margins toothed or smooth ..... *Dialeurodes* Cockerell
Vasiform orifice not as such 7
7.(6) Abdominal tracheal fold indicated by row of spinules 8
Abdominal tracheal fold absent or different 9
8.(7) Anterior marginal setae probably absent, caudal furrow partly developed ..... *Ramsesseus* Zahradnik
Anterior marginal setae present, caudal furrow absent .... *Aleurocanthus* Quaintance and Baker
9.(7) First abdominal setae present 10
First abdominal setae absent 11
10.(9) Abdominal tracheal pore area differentiated by a comb from margin .... *Siphoninus* Silvestri
Abdominal tracheal pore area not differentiated from margin .... *Aleuromarginatus* Corbett.
11.(9) Vasiform orifice open, operculum subequal in shape, about three-fourth as long as and not quite half the length of the orifice, lingula about five-sixth of the orifice, the distal two-fifths enlarged and arrow shaped, thickly setose and terminating setae Floor with transverse ridges distinct, teeth of inner margin present ..... *Parabemisia* Takahashi
Vasiform orifice not as such 12
12.(11) Spiracles usually small and subequal in size, sometimes the anterior ..... abdominal spiracles are reduced or absent 13
Spiracles not as such 14
13.(12) Margin crenulate ..... *Aleurotuberculatus* Takahashi
Margin slightly crenate or smooth ..... *Trialeurodes* Cockerell
14.(12) Median parts of abdominal segments 6 and 7 subequal in length .... *Aleyrodes* Latreille

Median part of abdominal segment 7 apparently shorter than that of segment 6 ..... *Bemisia* Quaintance and Baker.

Genus *Acaudaleyrodes* Takahashi, 1951

Type-species – *Acaudaleyrodes pauliani* Takahashi, 1952 (by monotypy)

1. *Acaudaleyrodes citri* (Prienser and Hosny, 1934) (Fig. 1–1)

Aleurotrachelus citri Priesner and Hosny, 1934: 7

Acaudaleyrodes citri Russell, 1962: 64; Mound 1965: 119; Cohic, 1969: 4; Habib and Farag, 1970: 32.

Aleurotrachelus alhagi Priesner and Hosny, 1934: 9, synonymized by Mound, 1965: 119.

Acaudalyrodes alhagi Russell, 1962: 64; Habib and Farag, 1970: 37.

The date of first record: By Priesner and Hosny (1934)

Distribution: Asyut (N11), Aswan (Q5), Beni Suef (M15), Cairo (N17), Dakhla Oasis (17), Damanhur (M19), Eastern Desert (P11), El Khatatba (M17), El Minya (M13), El Qantara (P18), Isna (Q7), Qena (Q9) and Tanta (M18).

Parasites: *Encarsia davidi* Viggiani
E. galilea Rivny
E. mineoi Viggiani
Eretmocerus diversicilatus Silvestri
E. mundus (Mercet)

Genus *Aleurocanthus* Quaintance and Baker, 1914

Type-species – *Aleurodes spinifera* Quaintance and Baker, 1903 (by original designation)

1. *Aleurocanthus zizyphi* Priesner and Hosny, 1934. (Fig. 1–2)

Aleurocanthus zizyphi Priesner and Hosny, 1934: 2; Habib and Farag, 1970: 28.

Aleurocanthus hansfordi Corbett, 1935: 240; Cohic, 1969: 16. Syn. nov.

Aleurocanthus aberrans Cohic, 1968: 10.

The date of first record: By Priesner and Hosny (1934)

Distribution: Asyut (N11), Aswan (Q5), Dakhla Oasis (17) and El Minya (M13).

Prasites: No parasites

Genus *Aleurolobus* Quaintance and Baker, 1914

Type-species – *Aleurodes marlatti* Quaintance, 1903 (by original designation)

This genus is represented in Egypt by two species.

Key to species

1.(2) Pup al case subcircular. Tracheal combs with about six teeth. Cadual setae absent ..... *olivinus* (Silvestri)

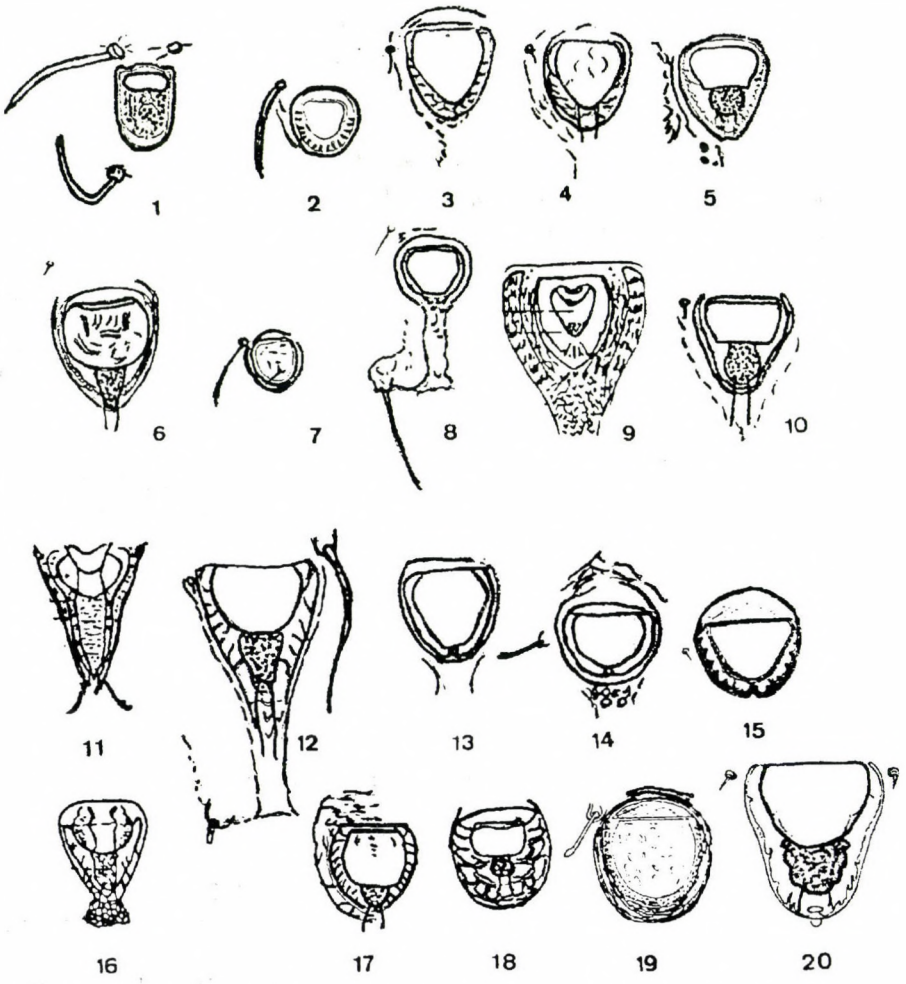

Fig. 1. 1–20. Aleyrodidae. Vasiform orifice: 1. *Acaudaleyrodes citri*; 2. *Aleurocanthus zizyphi*; 3. *Aleurolobus niloticus*; 4. *A. olivinus*; 5. *Aleuromarginatus tephrosiae*; 6. *Aleuroplatus acaciae*; 7. *A. cadabae*; 8. *Aleurotuberculatus porosus*; 9. *Aleuroviggianus adrianae*; 10. *Aleyrodes proletella*; 11. *Bemisia afer*; 12. *B. tabaci*; 13. *Dialeurodes citri*; 14. *D. elbaensis*; 15. *D. kirkaldyi*; 16. *Parabemisia myricae*; 17. *Ramsesseus follioti*; 18. *Siphoninus phillyreae*; 19. *Tetraleurodes leguminicola*; 20. *Trialeurodes vaporariorum*

2.(1) Pupal case suboval. Tracheal combs with tree teeth. Caudal setae conspicuous ..... *niloticus* Priesner and Hosny

1. *Aleurolobus niloticus* Priesner and Hosny, 1934. (Fig. 1–3)

Aleurolobus niloticus Priesner and Hosny, 1934 5: 1; Cohic, 1969: 50; Habib and Farag, 1970: 21.

The date of first record: By Willcocks (1922)

Distribution: Asyut (N11), Aswan (Q5), Cairo (N17), Dakhla Oasis (17), Eastern Desert (P11), El Kharga (L7), El Minya (M13), Qena (Q9), Saqqara (N16) and Sinai (S16)

Parasites: *Encarsia davidi* Viggiani
E. elegans Masi
E. lutea (Masi)

2. *Aleurolobus olivinus* (Silvestri, 1911) (Fig. 1–4)
Aleurodes olivinus Silvestri, 1911: 214
Aleurolobus olivinus Quaintance and Baker 1915: XI

Genus *Aleuromarginatus* Corbett, 1935

Type-species – *Aleuromarginatus tephrosiae* Corbett, 1935 (by monotypy)

1. *Aleuromarginatus tephrosiae* Corbett, 1935. (Fig. 1–5)
Aleuromarginatus tephrosiae Corbett, 1935, 247; Mound, 1965: 131; Cohic, 1968: 31; 1969: 61

The date of first record: In the present work

Distribution: Eastern Desert (P11)

Parasites: No parasites

Genus *Aleuroplatus* Quaintance and Baker, 1914

Type-species – *Aleurodes qyercus-aquaticae* Quaintance, 1900 (by original designation).

This genus is represented in Egypt by two species

Key to species

1.(2) Marginal crenations connected with a membraneous area. Dorsal simple pores absent. Lingula tip lartly exposed ..... *acaciae* Bink-Moenen

2.(1) Marginal crenations not connected with a membraneous area. Dorsal surface with paired row of simple pores. Lingula tip concealed .... *cadabae* Priesner and Hosny

1. *Aleuroplatus acaciae* Bink-Moenen, 1983. (Fig. 1–6)
Aleuroplatus acaciae Bink-Moenen, 1983: 63–65

The date of first record: By Bink-Moenen (1983)

Distribution: Aswan (Q5) and Qena (Q9)

Parasites: *Encarsia* sp.
Eretmocerus sp.

2. *Aleuroplatus cadabae* Priesner and Hosny, 1934. (Fig. 1–7)
Aleuroplatus cadabae Priesner and Hosny, 1934, 5; Gameel, 1969: 68

The date of first record: By Priesner and Hosny (1934)

Distribution: Eastern Desert (P11)

Parasites: *Eretmocerus cadabae* Viggiani

Genus *Aleurotuberculatus* Takahashi, 1932

Type-species – *Aleurotuberculatus gordoniae* Takahashi, 1932 (by original designation)

1. *Aleurotuberculatus porosus* (Priesner and Hosny, 1937). (Fig. 1–8)

Trialeurodes porosus Priesner and Hosny, 1937: 45

Aleurotuberculatus porosus Mound and Halsey, 1978: 87

The date of first record: By Priesner and Hosny (1937)

Distribution: Asyut (N11), Aswan (Q5), Cairo (N17), El Minya (M13) and El Qantara (P18)

Parasites: No parasites

Genus *Aleuroviggianus* laccarino, 1982

Type-species: *Aleuroviggianus adrianae* laccarino, 1982 (by monotypy)

1. *Aleuroviggianus adrianae* laccarino, 1982. (Fig. 1–9)

Aleuroviggianus adrianae laccariono, 1982: 38

The date of first record: In the present work

Distribution: Alexandria (L18)

Parasites: No parasites

Genus *Aleyrodes* Latreille, 1796

Type-species - *Phalaena (Tinea) proletella*, Linnaeus, 1758 (by subsequent designation and monotypy)

1. *Aleyrodes proletella* (Linnaeus, 1758). (Fig. 1–10)

Phalaena (Tinea) proletella Linnaeus, 1758: 537

Aleyrodes proletella Latreille, 1801–2: 264; Mound, 1966: 404; Cohic, 1969: 100

The date of first record: By El-Helaly et al. (1972)

Distribution: Alexandria (L18), Cairo (N17), El Qantara (P18) and Saqqara (N16)

Parasites: *Encarsia davidi* Viggiani

E. inaron (Walker)

Eretmocerus mundus (Mercet)

Euderomphale sp

Genus *Bemisia* Quaintance and Baker, 1914

Type-species - *Aleyrodes inconspicua* Quaintance, 1900 (a junior synonym of *Aleurodes tabaci* Gennadius, 1889) (by original designation)

This genus is represented in Egypt by two species

Key to species

1.(2) Vasiform orifice posteriorly with some transverse ridges. Antennae with basal spines ..... *afer* Priesner and Hosny

2.(1) Vasiform arifice posteriorly with tubercles. Antennae without basa spines ..... *tabaci* (Gennadius)

1. *Bemisia afer* (Priesner and Hosny, 1934) (Fig. 1–11)

Dialeurodoides afer Priesner and Hosny, 1934: 6

Bemisia afer Gameel, 1968: 151; Habib and Farag, 1970: 8

Bemisia hancocki Corbett, 1936: 20; Mound, 1966: 140; Cohic, 1968: 38; 1969: 112 syn. nov.

The date of first record: By Priesner and Hosny (1934)

Distribution: Aswan (Q5), Cairo (N17), Damanhur (M19), Eastern Desert (P11), El Minya (M13), El Qantara (P18), Qena (Q9) and Zagazig (N18)

Parasites: *Eretmocerus* sp.

2. *Bemisia tabaci* (Gennadius, 1889) (Fig. 1–12)

Bemisia tabaci Gennadius, 1889: 1.

Bemisia tabaci Takahashi, 1936: 110; Mound, 1963: 171

Bemisia gossypiperda var. *mosaicivecture*, 1934 in Mayné and Ghesquiére, 1934: 30, synonymized by Russell, 1957: 122

Bemisia longispina Priesner and Hosny, 1934: 6 synonymized by Russell, 1957: 122

Bemisia nigeriensis Corbett, 1935: 250, synonymized by Russell. 1958: 123

Bemisia rhodesiaensis Corbett, 1936: 22, synonymized by Russell, 1957: 123

The date of first record: By Priesner and Hosny, (1934)

Distribution: Alexandria (L18), Asyut (N11), Aswan (Q5), Beni Suef (M15), Dakhla Oasis (17), Damanhur (M19), Eastern Desert (P11), El Faiyum (M15), Ismailiya (P18), Isna (Q7), Saqqara (N16), Sinai (S16), Suez (P17), Tanta (M18), Western Desert (C12) and Zagazig (N18)

Parasites: *Encarsia davidi* Viggiani

E. elegans Masi

E. formosa Gahan

E. inaron (Walker)

E. lutea (Masi)

E. mineoi Viggiani

Eretmocerus corni Haldeman

E. diversicilatus Silvestri

E. mundus (Mercet)

Genus *Dialeurodes* Cockerell, 1902

Type-species - *Aleyrodes citri* Riley and Howard, 1893 (by original designation), a junior synonym and homonym of *Aleyrodes citri* Ashmead, 1885

This genus is represented in Egypt by three species.

Key to species:

1. Subdorsal setae not extending beyond margin of pupal case. Anterior rim short, length about one-eighth of length of vasiform orifice .... *elbaensis* Priesner and Hosny
Subdorsal setae different. Anterior rim long 2

2.(1) 1st abdominal setae present, there is a brown area, in the median area of the dorsum, the margin of vasiform orifice without median tubercle posteriorly ..... *kirkaldyi* (Kotinsky)
1st abdominal setae absent, no brown area, in the median area of the dorsum, the margin of vasiform orifice with median tubercle posteriorly ..... *citri* (Ashmead)

1. *Dialeurodes citri* (Ashmead, 1885) (Fig. 1–13)

Aleurodes citri Ashmead, 1885: 274
Dialeurodes citri (Ashmead) var. *Kinyana*, Takahashi, 1934
Dialeurodes citri (Ashmead) var. *hederae* Takahashi, 1936

The date of first record: In the present work

Distribution: Alexanderia (L18), Damanhur (M19), El Qantara (P18) and Tanta (M18)

Parasites: *Encarisa* sp.

2. *Dialeurodes elbaensis* Priesner and Hosny (1934). (Fig. 1–14)

Dialeurodes (Gialeurodes) elbaensis Priesner and Hosny, 1934: 10; Cohic, 1969: 121

The date of first record: By Priesner and Hosny (1934)

Distribution: Eastern Desert (P11)

Parasites: No parasites.

3. *Dialeurodes kirkaldyi* (Kotinsky, 1907) (Fig. 1–15)

Aleyrodes kirkaldyi Kotinsky, 1907: 95
Dialeurodes kirkaldyi Quaintance and Baker, 1914: 98

The date of first record: By Priesner and Hosny (1934)

Distribution: Alexanderia (L18), Aswan (Q5), Cairo (N17), El Minya (M13), Ismailiya (P18), Saqqara (N16) and Tanta (M18)

Parasites: *Eretmocerus* sp.

Genus *Parabemisia* Takahashi, 1952

Type-species - *Parabemisia maculata* Takahashi, 1952 by original designation

1. *Parabemisia myricae* (Kuwana, 1927) (Fig. 1–16)

Bemisia myricae Kuwana, 1927: 249
Parabemisia myricae (Kuwana) Takahashi, 1952: 24

The date of first record: In the present work

Distribution: Benha (N17), El Khatatba (M17), and Tanta (M18)

Parasites: *Amitus* sp.
Encarsia lutea (Masi)

Genus *Ramsesseus* Zahradnik, 1970

Type-species-*Ramsesseus follioti* Zahradnik, 1970 (by monotypy)

1. *Ramsesseus follioti* Zahradnik, 1970 (Fig. 1–17)

Ramsesseus follioti Zahradnik, 1970: 48

The date of first record: By Zahradnik (1970)

Distribution: Aswan (Q5), El Kharga Oasis (L7) and Qena (Q9).

Parasites: *Encarsia* sp.

Eretmocerus sp.

Genus *Siphoninus* Silvestri, 1915

Type-species - *Siphoninus finitimus* Silvestri, 1915 a synonym of *Aleyrodes phillyreae* Haliday, 1835 (by original designation)

1. *Siphoninus phillyreae* (Haliday, 1835) (Fig. 1–18)

Aleyrodes phillyreae Haliday, 1835: 119

Asterochiton phillyreae Quaintance and Baker, 1914: 105

Trialeurodes phillyreae Quaintance and Baker, 1915: xi

Siphoninus phillyreae Silvestri, 1915: 247

Siphoninus finitimus Silvestri, 1915: 247, synonymized by Goux, 1949: 10

Siphoninus granati Priesner and Hosny, 1932: 1; Habib and Farag, 1970: 13, synonymized by Mound and Halsey, 1978: 192

The date of first record: Priesner and Hosny (1934)

Distribution: Asyut (N11), Aswan (Q5), Cairo (N17), Damanhur (M19), El Faiyum, El Kharga (L7), El Minya (M13), El Qantara (P18), Qena (Q9), Saqqara (N16), Sinai (S16), Sohag (O10), Western Desert (C12) and Zagazig (N18)

Parasites: *Encarsia davidi* Viggiani

E. inaron (Walker)

E. lutea (Masi)

E. mineoi Viggiani

Eretmocerus corni Haldeman

E. diversicilatus Silvestri

E. munus (Mercet)

Genus *Tetraleurodes* Cockerell, 1902

Type-species - *Aleyrodes (Tetraleurodes) perileuca* Cockerell, 1902 (by original designation)

1. *Tetraleurodes leguminicola* Bink-Moenen, 1983 (Fig. 1–19)

Tetraleurodes leguminicola Bink-Moenen, 1983: 172

The date of first record: By Bink-Moenen (1983)

Distribution: Aswan (Q5) and Qena (Q9)

Parasites: *Encarsia* sp.

Eretmocerus sp.

Genus *Trialeurodes* Cockerell, 1902

Type-species - *Aleurodes pergandei* Quaintance, 1900 (by original designation)

1. *Trialeurodes vaporariorum* (Westwood, 1856) (Fig. 1–20)

Aleyrodes vaporariorum Westwood, 1856: 852

Trialeurodes vaporariorum Quaintance and Baker, 1915: 11

Trialeurodes mosoppi Corbett, 1935: 9, synonymized by Russell, 1948: 43

Trialeurodes natalensis Corbett, 1936: 18, synonymized by Russell, 1948: 44

The date of first record: In the present work

Distribution: Beni Suef (M15), Benha (N17), Cairo (N17) and Tanta (M18)

Parasites: *Encarsia inaron* (Walker)

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Psocoptera (Insects) Fauna of Stored Maize

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The paper is a contribution to the investigation of Psocoptera genus, on maize cobs, stored in "basket"* by private farmers in the eastern part of Slavonija and Baranja districts (Croatia), 4 species of Psocoptera have been found.

Agricultural crops and products manufactured and stored are attacked by many insect pests, including representatives of the Psocoptera genus. The insects have been intensively studied (Kalinović, 1976; Kalinović and Günther, 1982, 1985) and 21 species of Psocoptera have been reported in large store houses.

Insects, especially representatives of Psocoptera that appear in cases of bulk storages in small store houses of private manufacturers have rarely been investigated so far (Kalinović and Ivezić, 1993; Pivar et al., 1977).

Within the total storing capacities of agricultural products of the Republic of Croatia the share of maize storage in baskets amounts 1.9% (Par and Tratnik, 1993) and they are mainly located at private sector husbandries.

Fungi and molds grow on maize cobs having a relative humidity of 20–22% are that fed upon by representatives of Psocoptera genus (Kalinović et al., 1978, 1981). Recent data (Kalinović and Ivezić, 1993) show that Psocoptera species reached 44.10% of the total insect and mite fauna on stored maize cobs. The aim of this paper was to show fauna Psocoptera found on maize cobs stored in baskets.

Materials and Methods

Maize cob samples were taken from 5 maize storing baskets of private farmers in Valpolvo area (Osijek-Baranja county) during storage season (from October 1992 to August 1993). Each sample contained 5–7 maize cobs and weighed 550–1000 g. A total of 275 samples has been investigated. In the laboratory after cheking the cobs' surface, they were shaked, husked and the maize cob sifted by means of automatic sieve having a

* Basket made of wire to store crop

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mesh size of 0.5–2.5 mm. Psocopterans were collected by fine brushes and put into 70% alcohol. Following preparing of permanent preparations by maceration method (Günther, 1974) for wingless Psocoptera forms, determination of all representatives have been performed by available keys (Günther, 1974; Smithers, 1990) by the help of stereoscopic and phase contrasting microscopes. Moisture content of husked maize corbs was measured by a hygrometer (type "Dicky John").

Results and Discussion

During the maize cob storage season in the maize stored in baskets by private manufacturers, a total of four species of Psocoptera have been identified, such as:

Suborder: TROGIOMORPHA;
Family: Atropetae, Trogiidae;
Species: Lepinotus reticulatus ENDERLEIN, 1905.
Family: Psocathropetae, Psyllipsocidae;
Species: Psyllipsocus ramburi SELYS-SONGCHAMPS, 1872 var. destructor;
Suborder: TROCTOMORPHA;
Family: Nanopsocetae, Liposcelidae;
Species: Liposcelis corrodens HEYMONS, 1909.
Suborder: PSOCOMORPHA
Family: Homilopsocidae, Lachesillidae;
Species: Lachesilla pedicularia LINNEAUS, 1758.

Seasonal Psocoptera fluctuation on maize cobs stored in baskets is shown in Fig. 1. Psocopteras less numerous at the beginning of maize cob storage during October 1992, and they were not present in samples during winter (November, December 1992, January, February and March 1993). The first Psocopteras were noticed in April 1993. Their numbers were gradually increasing with temperature increased in spring and summer. The maximal number was recorded in July and their number decreased in August, by the end of maize cob storage season.

Distribution of established Psocoptera species in presented in Fig. 2. *P. ramburi* var. destructor appeared to be most numerous (144 adults were recorded). Variety destructor (=Nymphosocus destructor) is widespread in Central Europe and it is an exclusively domestic Psocoptera species, that lives in shedded places, as grain store houses. Earlier investigations (Kalinović, 1976; Kalinović and Günther, 1990) found this species on wheat stored in silo bins and small sized store houses for small holders. It is worth paying attention to the presence of *P. ramburi* var. destructor in maize cobs stored in baskets by small farmers since we came to the conclusion that this species having been observed in large store houses are able to adapt natural storing conditions at small holders.

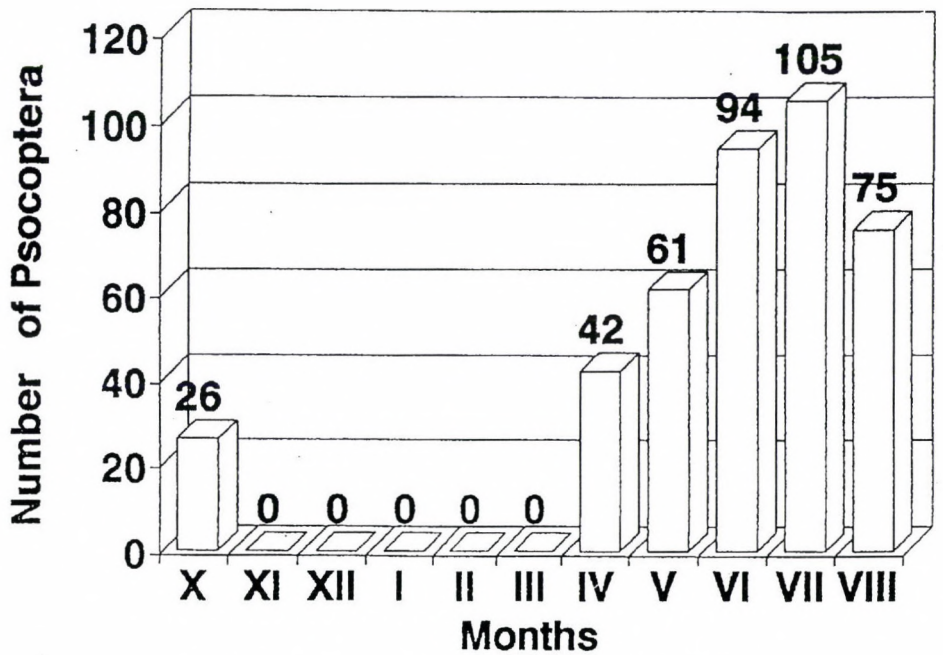

Fig. 1. The seasonal fluctuation of Psocoptera during 1992/93

The next most numerous species, a wingless species, Liposcelis corrodens (109 adults) is widespread in warehouses. In Croatia it was present in significant numbers on wheat and maize, stored bulk in storage barns of small holders or in silos (Kalinović, 1979; Kalinović and Günther, 1990) and it was less frequent on food products (rice, pasta, wheat flour, dry meat) (Kalinović et al., 1981). This Psocoptera species also appears in nature, in humid biotopes, in bird's nests (nidicole) or under tree bark

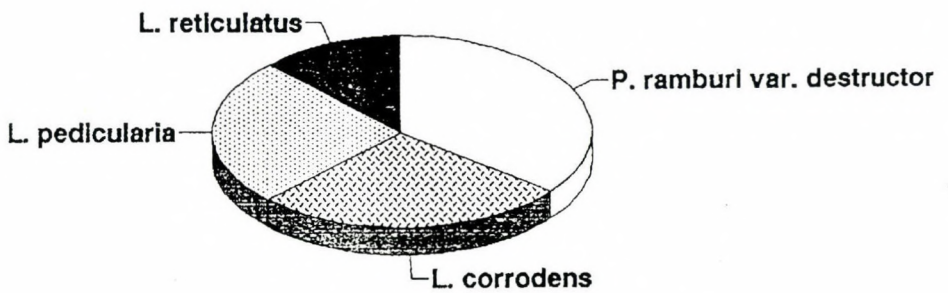

Fig. 2. The distribution of Psocoptera species

(corticole), (Günther, 1974). We found *L. corrodens* on the internal parts of maize cob husk, also attacked by fungi and moulds.

Lachesilla pedicularia, a Psocoptera species with completely developed wings, was also found (100 adults). It is a cosmopolitan species occurring in nature but seldom indoors. In Croatia, it was found as an indoor species in silos, and in both large and small bulk storage of wheat, maize, sugar beet seed, as well as in groats packages (Kalinović and Günther, 1990). Finding of this Psocoptera species on maize cobs stored by small holders confirmed previous data (Günther, 1974; Kalinović and Günther, 1985) and conclusion that it was transferred from fields, during maize picking, to maize baskets where it would spend winter.

L. reticulatus, a species with rudimentary wings has been found in small numbers (50 adults) on maize cobs stored in baskets. As an indoor species it is widespread in whole Europe but it can also be found in nature, in bird's nests, hives and in wasp nests. Our previous investigations also reported it from stored wheat in large scale silos as well as from bulk storage, and sporadically on stored maize grain (Kalinović et al., 1976; Kalinović and Günther, 1990). *L. reticulatus* was found by means of checking maize grains of husked maize cobs attacked by moulds.

Psocopteras are insect pests for stored products and foodstuffs. These provide favourable ecological conditions for their growth (relative humidity 55–90%, moisture content of the product 14–20% and temperature 15–30 °C). Among such circumstances microorganisms (fungi and bacteria) develop and Psocopteras feed on them. For their good migration ability, they transport microorganisms directly from infested places to other parts of store houses, by means of their bodies (hairs). They are also transported by their faces from which the lasting forms, spores can be regenerated again (Kalinović et al., 1978). Especially dangerous are fungi of the genera Aspergillus and Fusarium, which produce a great number of mycotoxins, of which aflatoxin is the most important. This toxin is very dangerous for both human and domestic health.

Chemical control of Psocoptera is bakets for maize storage at small holders in Croatia is not carried out. Some baskets were treated preventively (floor cleaning and pest control sprayings containing Dichlorovos and Pirimiphos-methyl-active ingredients).

Conclusion

For the first time in private sector store houses of east Croatian region (Valpovo, Osijek – Baranja county), while the maize cobs were stored in baskets, throughout the storage season, representatives of four species of Psocoptera (*Lepinotus reticulatus* ENDERLEIN, *Psyllipsocus ramburi* SELYS-LONGCHAMPS var. destructor, *Liposcelis corrodens* HEYMONS and *Lachesilla pedicularia* LINNEAUS) have been found. They were recorded immidiately after picking (October). Later in the storage season (April, May, June, July, August) they were found in large numbers, but in winter (November, December, January, February, March) they were not observed in maize cob samples. The most numerous species was *P. ramburi* var. destructor.

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Comparative Studies on Enzyme-Immunoassays for Triazole Fungicides Tetraconazole and Myclobutanil

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Enzyme-linked immunosorbent assay (ELISA) systems have recently been accepted as validated methods for environmental monitoring. An immunoassay for the detection of tetraconazole, penconazole and their metabolytic derivative (DTP) and a similar assay for the triazole fungicide, myclobutanil have been developed. The present paper describes a comparative study of the two ELISA systems, in regards of sensitivities and cross-reactivities, taking into account two different strategies for the synthesis of the two immunogens. The two sera have been utilized in comparable immobilized antigen formats, and the resultant IC_{50} values were found to be 1.3 ± 0.4 ppm for tetraconazole in one assay, and 4.9 ± 0.5 ppm for myclobutanil in the other. The antibodies against tetraconazole have also displayed higher selectivity than those raised against the myclobutanil conjugate. Modulation of the assay sensitivity by the level of hapten loading in the antigen coating was also investigated.

A permanent challenge in immunoassay development against low-molecular weight compounds is the approach to hapten and immunogen synthesis: preparation of protein conjugates that present most of the epitopes of the target analyte (Harrison et al., 1991; Hammock et al., 1990; Székács et al., 1995) is an important step for the ultimate sensitivity of ELISAs. Conjugation to proteins means modification of the structure of the parent compound, and therefore appropriate haptens are needed to be designed, providing optimal similarity to the target analyte in their conjugated form.

Retaining as many immunogenic features as possible is particularly crucial when dealing with molecules of low natural immunogenicity e.g., in the case of triazole compounds. In contras to triazines that are known to be immunogenic (Goodrow et al., 1990; Karu et al., 1991; Giersch et al., 1993; Muldoon et al., 1994; Fránek et al., 1995; Weil et al., 1995; Del Valle et al., 1996), triazoles do not appear to induce strong immunogenic reaction (antibody production) in vertebrates. This was clearly seen in previous immunoassays developed against various triazole fungicides [e.g., triadimefon (Newsome, 1986), tetraconazole (Forlani et al., 1992), amitrole (Jung et al., 1991) and myclobutanil (Székács and Hammock, 1995)], where either low antibody affinity or no cross-reactivity with 1, 2,4-triazole was seen.

High affinity antibodies are desired not only for high assay signals, but also for proper serum dilutions. High affinity antibodies allow high serum dilution or low hapten density in the coating antigen that, in turn, strongly affects assay sensitivity (detectable analyte concentration range). As a consequence, in developing ELISA systems for detec-

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tion of small molecules (e.g., pesticides), first of all, the strategy of hapten derivatization for conjugation to the carrier protein has to be considered.

ELISA systems for the detection of two fungicides of the triazole family (tetraconazole and myclobutanil) were previously optimized (Forlani et al., 1992; Székács and Hammock, 1995). In the approach to develop an ELISA system against myclobutanil (Fig. 1), the parent compound was hydrolyzed on the nitrile group, and the resultant carboxylic acid derivative (NKI39168) was directly conjugated to carrier proteins CONA and BSA (Székács and Hammock, 1995). In the development of the tetraconazole immunoassay, a hydrolytic derivative 2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl) propanol (DTP) (Fig. 1) was utilized as the hapten (Forlani et al., 1992). Here a hemiester of succinic acid was formed on the hydroxyl group of DTP, and the hemisuccinate (FF18) was then conjugated to carrier proteins CONA and BSA. The antibodies raised against FF18-BSA showed higher affinity to two related triazole fungicides, tetraconazole and penconazole, that to the parent immunogen, DTP.

In the present study, we analyzed the antibodies raised against CONA-FF18 (serum Rab2) and against CONA-NKI39168 (serum #1034). The two antibodies were raised with comparable procedures and with the same carrier protein, but different hapten design strategies were used for producing the immunogens. Comparison of these ELISA assays has been possible because parameters, like hapten loading of immobilized antigen coating, which are considered effective in the ultimate sensitivity and specificity of the assay (Morris, 1985) were identical.

Materials and Methods

Peroxidase-conjugated anti-rabbit IgG, bovine serum albumin (BSA), hen ovotransferrin (conalbumin, CONA) and *o*-phenylenediamine were purchased from Sigma Chemical Co. (St. Louis, MO). All of the chemicals used were reagent grade from Aldrich, Carlo Erba and Fluka. Tetraconazole and DTP were supplied by Agrimont. Myclobutanil was kindly provided by Rohm and Haas Co. through Nitrokémia Ipartelepek Company (Fűzfőgyártelep, Hungary). All other fungicides were supplied by Labservice (Bologna, Italy).

Hapten coupling

The procedure used for conjugation of FF18 to CONA or to BSA is reported in Forlani et al. 1992). Myclobutanil was conjugated to carrier proteins as described in Székács and Hammock (1995). The two immunogens (Fig. 1) contained 21 and 14 mol hapten/mol CONA, respectively, as judged from the colorimetric determination of free amino groups of the proteins by trinitrobenzenesulfonic acid (Habeeb, 1966).

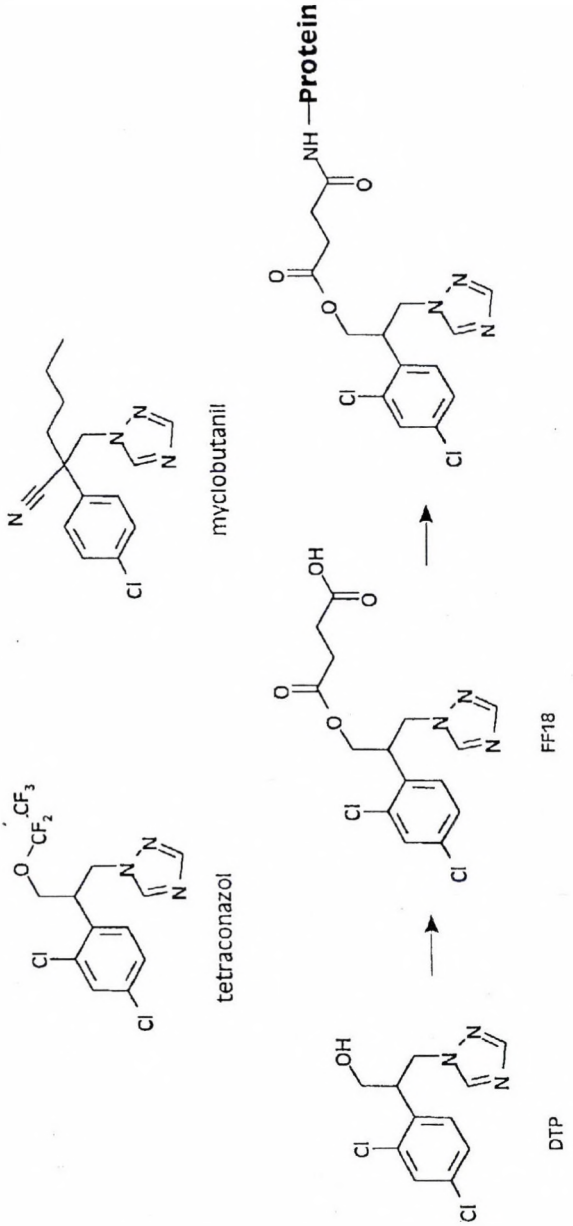

Fig. 1. Chemical structures of the principal analytes tetraconazole and myclobutanil, and the scheme of hapten conjugation of each analyte

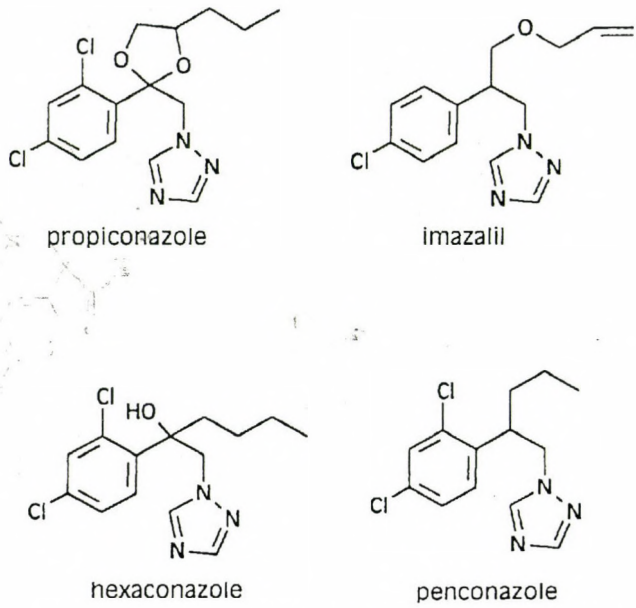

Fig. 2. Chemical structures of related triazole fungicides studied

Immunization, ELISA

Antibodies either to CONA-FF18 or CONA-NKI39168 were raised following the immunization procedure described previously (Forlani et al., 1992; Székács and Hammock, 1995). Enzyme-linked immunosorbent assays (ELISAs) were carried out based on the principles described by Voller et al. (1976). Antibody specificity was evaluated by cross-reactivities of the assays determined with various related triazole fungicide active ingredients (Fig. 2).

Results and Discussion

Titration of antisera

The titer of both antibodies were determined by using different dilutions of either antiserum against CONA-FF18 (serum Rab2) or CONA-NKI39168 (serum #1034) in non-competitive solid-phase immunoassays in which the polystyrene plates were coated with BSA-FF18 and BSA-NKI39168, respectively.

The antiserum after the third immunization against the tetraconazole conjugate gave a titer (defined as the serum dilution corresponding to 50% binding to the coating

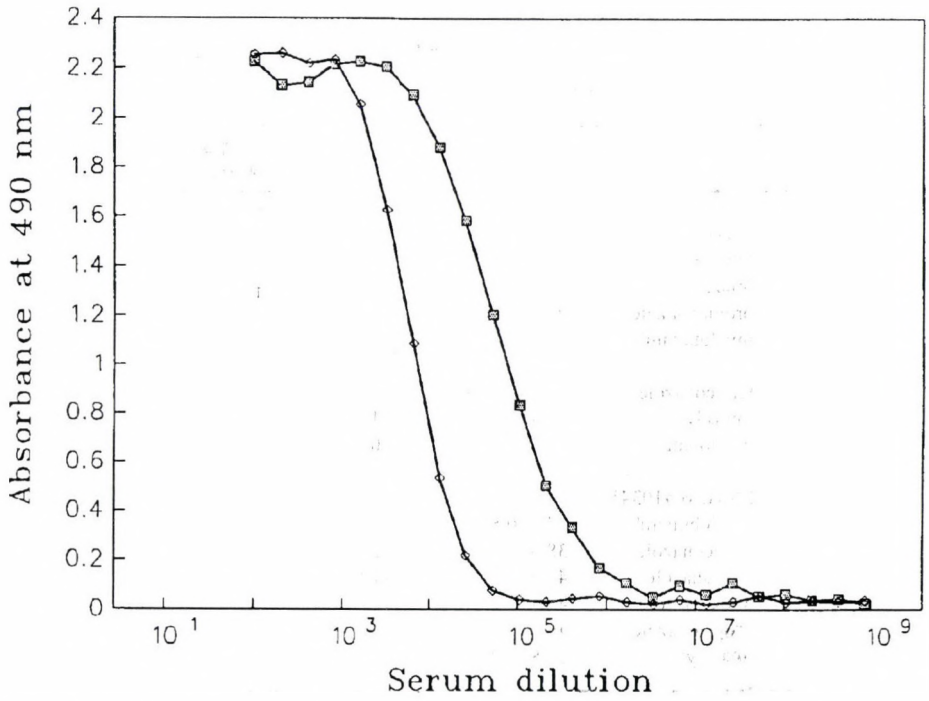

Fig. 3. Two-dimensional titration curves of antisera obtained against tetraconazole (▩) and against myclobutanil (◇). ELISA was used to evaluate the binding of the antibodies raised against CONA conjugates to the hapten-homologous BSA conjugates immobilized on the well surface of 96-well microplates

antigen and thus, providing a 50% signal as compared to the total absorbance) of 1:60 000. The antiserum against the myclobutanil conjugate gave 50% absorbance at antiserum dilution of 1:2700 (Fig. 3).

ELISA performance and sensitivity

In the case of the ELISA systems in the immobilized antigen format, homologous BSA conjugates (the same hapten conjugated to different carrier proteins) were used as coating antigens. Table 1 shows the results obtained under optimized conditions using tetraconazole for serum Rab2 and myclobutanil for serum #1034 as principal analytes. Direct comparison of these results is possible since the hapten-coating antigen molar ratios were similar. The IC_{50} values (inhibitor concentrations that inhibit the assay by 50%) for the principal analyte indicated that the sensitivity of serum Rab2 was about four times higher than that of serum #1034. The selectivity of serum Rab2 appeared to be higher than that of serum #1034, this latter showing cross-reactivity with tetraconazole and other triazole derivatives.

Table 1

IC_{50} values and cross-reactivities detected by ELISA

Analyte	IC_{50} (ppm)	%CR[a]	Molar ratio (hapten/carrier protein)
SERUM Rab2[b]			
tetraconazole[c]	1.292±0.434	100	
imazalyl	29.7	4.4	15
propiconazole	73.4	1.8	
myclobutanil	>85.9	<1.5	
tetraconazole	0.085±0.019	100	
imazalyl	5.1	1.7	1
myclobutanil	38.75 + 18.70	0.2	
SERUM #1034[d]			
myclobutanil[c]	4.9±0.5	100	
hexaconazole	38.5	12.7	
penconazole	41.4	11.8	16
tetraconazole	45.2	10.8	
propiconazole	49.3	9.9	
imazalyl	81.8±2.7	6.0	

[a] Percent cross-reactivity based on the IC_{50} value of the given compound compared to that of the principal analytes, tetraconazole or myclobutanil, in the assay using a homologous coating antigen.

[b] Rabbits were immunized with a CONA based antigen of 21 mol/mol (hapten/carrier) epitope density.

[c] Principal analyte.

[d] Rabbits were immunized with a CONA based antigen of 14 mol/mol (hapten/carrier) epitope density.

These evidences can be explained considering the immunogen synthesis protocols. Modification of the existing (nitrile) functional group in the myclobutanil assay apparently modulated the antibody sensitivity away from the target analyte. In contrast, restoration of the polarized side chain of tetraconazole by esterification of DTP with succinic acid resulted in better antigen similarity and therefore in higher antibody sensitivity to tetraconazole. In addition, the hemisuccinate moiety in the latter approach not only helped to regenerate – to some extent – the parent structure of tetraconazole, but it also served as a spacer group, placing the haptenic moiety in a more distinct position on the protein, and therefore, potentially better presented to the immune system. As a result, the antiserum against myclobutanil was found more than one order of magnitude lower in titer and significantly less sensitive in the concentration range of detection (based on the IC_{50} values) than the serum against tetraconazole. High serum titer is an important feature

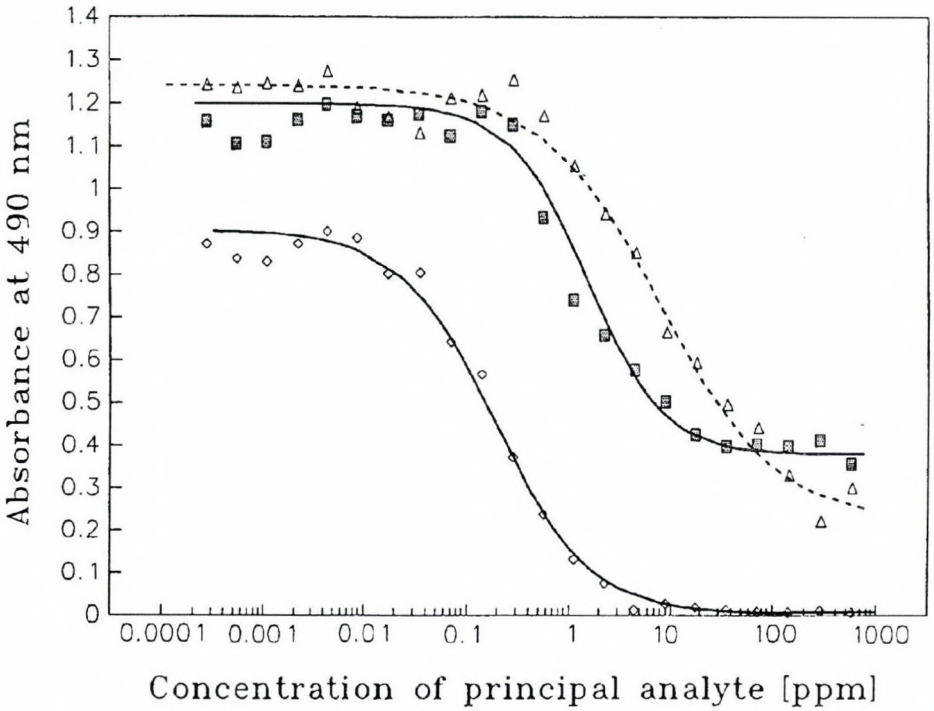

Fig. 4. Standard curves for the determination of tetraconazole (serum Rab2) and myclobutanil (serum #1034) obtained with two different ELISA formats
Serum Rab2: immobilized antigen (▩) and immobilized antibody (◇) format
Serum #1034: immobilized antigen (△) format

for both assay sensitivity and specificity. Increasing serum dilutions mean lowering possible interferences by contaminants when the ELISA system is used for complex matrix analysis.

The better performance of serum Rab2 allowed an investigation on the effect of hapten-loading in the coating antigen in modulating the sensitivity and specificity of the ELISA system. It is expected, however, that the concentration range of the analyte detection decreases with the decrease of the hapten density on the solid surface: less analyte in the sample should therefore be enough to compete with the haptenic epitopes on the coating antigen for the combining site of a given antibody. The effect of epitope density of the coating antigen on assay sensivity is shown for the tetraconazole immunoassay in Table 1. Hapten-loading on the coating antigen of 1 mol/mol BSA, as compared to the assay with coating antigen containing 15 mol hapten/mol BSA, increased assay sensitivity about 15 times (based on IC_{50} values for tetraconazole). The figures of cross-reactivity were too low for any speculation about the influence of hapten loading on

assay specificity. The increase of assay sensitivity did not significantly affect other assay parameters (signal range, slope of the standard curve).

When the hapten density in the myclobutanil immunoassay was decreased from 16 mol hapten/mol BSA to 3 mol hapten/mol BSA, the signal magnitude dropped from 1200 to 100–150 mOD, even in the case of extended incubation with the substrate. No evaluation of the detection range of myclobutanil was, therefore, possible.

These results indicated that the modulation of assay sensitivity by hapten density of the coating antigen can be investigated only when antibodies of sufficient affinity are used.

In the hope of increasing assay sensitivity, the ELISA format with immobilized antibody was exploited for both antisera. For this purpose, conjugates of the homologous haptens with horseradish peroxidase (HRP) as a reporter enzyme were prepared as described by Cairoli et al. (1996). In Fig. 4. the standard curves for determination of tetraconazole and myclobutanil obtained by using immobilized antigen or antibody formats are shown. The immunoassay against tetraconazole in the immobilized antibody format provided a significantly sharper standard curve, and a considerably improved signal/noise ratio (the signal due to non-specific binding decreased to 3–8 mOD from 370–450 mOD in the former format). Unfortunately no relevant improvement in sensitivity was found for serum Rab2 by using the immobilized antibody ELISA format. Similarly, an improved background signal (non-specific binding) was seen for the assay format with immobilized antibody for myclobutanil, but the maximal assay signal (B_0) was inappropriate for workable assay conditions.

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Acta Phytopathologica et Entomologica Hungarica 31 (3–4), p. 303 (1996)

BOOK REVIEW

Kosztarab, M.: Scale insects of Northeastern North America. Identification, Biology and Distribution

Virginia Museum of Natural History, Special Publication Number 3. Martinsville, 650 p. (1996)

This book will make an excellent resource for coccidologists, entomologists, plant protection and extension experts. Similar books, treating all families of the group, appeared only in Central Europe (1988), and in the Pacific Region (1988–1990). There are also several monographic works in Russia (1949–1993), Ukraine (1975–1986), China (1977–1992), Japan (1980), but in these cases the linguistic barrier inhibits their wide use. In North-America the Atlas of Ferris (1937–1955) was widely used, but because of the nomenclatural changes and new distribution records it became partly outdated.

The book treats 254 species (of the 856 North-American species) belonging to 12 families, and 93 genera. The Author utilized collections of 13 largest Museums. One new genus and 11 new species are described here for the first time. There are 32 colour photos and 263 microscopic drawings of excellent quality. The nomenclature in some cases is not entirely consequent (e.g. Pulvinaria), what is due to discrepancies between Ben-Dov (1993) Coccidae Catalogue and Hodgson (1994) Coccidae Monograph.

The detailed list of references (943 items) gives an opportunity for the reader to find a deeper insight into the questions of his/her special interest. Some more books, well-known to Author, could have been included, as the monograph of Danzig (1993), the book of Saakjan-Baranova et al. (1971) about *Parthenolecanium corni*, or some current American and Hungarian ecological publications on scale insect-hostplant relationships.

The different indices (taxonomic, beneficial insects, host plant, distribution, common names) allow readers easily to use the book.

This book is an excellent and comprehensive guide to several pests with world-wide distribution, and of quarantine importance and will be a key reference for pest managers. This book will remain valid and useful for long time.

Ferenc Kozár

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INDEX

NUMBERS 1–2

DISEASES

Etiology of birthwort yellow mosaic and grapevine yellow mosaic and decline
I. Tóbiás and J. Lázár 1

Production of polyclonal antibodies to grapevine leafroll associated virus isolated in Hungary and development of HRPO-based ELISA system
I. Tóbiás, J. Lázár, M. Kölber and E. Papp 5

Destruction of tobacco mosaic virus in developing local lesions in leaves of *Datura stramonium* L.
A. V. Reunov, G. D. Reunova, V. P. Nagorskaya, L. A. Lapshina and S. N. Lega 11

Reuse of ELISA plates: Development of a simple cleaning method
H. K. Manandhar, S. E. Albrechtsen and S. B. Mathur 19

Occurrence of tomato pith necrosis incited by *Pseudomonas corrugata* in Hungary
M. Hevesi, A. Zalka and K. Csizmár 31

Occurrence of *Septoria betulae* on birch (*Betula alba*) in Argentina
G. M. Dal Bello and A. E. Perello 35

A marker system for *Phytophthora parasitica* based on hybridization of PCR products with species-specific repetitive DNA
T. Érsek, J. E. Schoelz and J. T. English 37

INSECT PESTS

The winter mortality and developmental biology of *Pseudaulacaspis pentagona* Targioni-Tozzetti, 1886 (Homoptera: Coccoidea)
D. A. F. Sheble and F. Kozár 45

A preliminary study on the armoured scale insect (Homoptera, Coccoidea: Diaspididae) fauna of Antalya
F. Erler, F. Kozár and I. Tunç 53

Check-list of Coccoidea and Aleyrodoidea (Homoptera) of Afghanistan and Iran, with additional data to the scale insects of fruit trees in Iran
F. Kozár, M. A. Fowjhan and M. Zarrabi 61

Physical surface features of the grapevine leaf affecting the abundance of *Zetzellia mali* (Ewing) (Acari: Stigmaeidae)
G. Jenser and Rosica Koleva 75

The role of spiders as predators of insect pests with particular reference to orchards: A Review
S. Bogya and P. J. M. Mols 83

NUMBER 3–4

DISEASES

Simple and rapid detection of carnation etched ring virus by polymerase chain reaction
L. Palkovics and E. Balázs 161

Reaction of unknown *Solanum stoloniferum* Schlechtd. et bche and *Solanum demissum* Lindl. accessions to the tuber necrosis strain of potato Y *Potyvirus* (PVY^{NTN})
Z. Bősze, G. Kazinczi and J. Horváth 169

The effect of virus infection on the growth and photosynthetic pigment content of the virophilous Jimson weed (*Datura stramonium* L.)
G. Kazinczi, J. Horváth and M. Pogány 175

Comparison of ultrastructural changes of *Nicotiana benthamiana* infected with three different viruses
A. Almási, M. Ekés and R. Gáborjányi 181

Changes in total phenols and some oxidative enzymes in cowpea leaves infected with yellow mosaic virus
R. S. Sangwan, G. P. Lodhi and Y. P. Luthra 191

Induction of resistance to tomato spotted wilt virus in mungbean (*Vigna radiata* (L.) Wilczek) by antiviral principles
K. Manickam and P. Narayanasamy 199

Fusarium solani as a cause of vessel discolouration in declining oak
Gy. Bohár 205

Ceratocystis erinaceus: A new endophyte in the heartwood of oak
Gy. Bohár 213

A spatio-temporal analysis of fungal pathogens on reed in natural habitats
Rita Bán, G. Fischl and F. Virányi 219

Synthesis of phytoalexins in sugarcane in response to infection by *Colletotrichum falcatum* Went
R. Viswanathan, D. Mohanraj, P. Padmanaban and K. C. Alexander 229

INSECT PESTS

Feeding and survival of the European corn borer larvae on seventeen inbred lines of maize
Ľ. Cagaň and P. Kmec 239

Resistance in wild *Cucumis* species to twospotted spider mite (*Tetranychus urticae*)
A. Lebeda 247

Diversity and community structure of spiders of alfalfa fields and grassy field margins in South Hungary
F. Samu, G. Vörös and Erika Botos 253

After introduction of *Frankliniella occidentalis* in Europe: Prevention of establishment of *Thrips palmi* (Thysanoptera: Thripidae)
G. Vierbergen 267

Egyptian Aleyrodidae
S. Abd-Rabou 275

Psocoptera (Insects) fauna of stored maize
Irma Kalinović and Marija Ivezić 287

PESTICIDE CHEMISTRY

Comparative studies on enzyme-immunoassays for triazole fungicides tetraconazole and myclobutanil
A. Székács, S. Cairoli, H. M. Le and S. Pagani 293

Book Review 303

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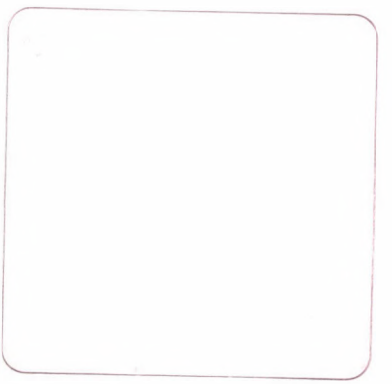

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