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1989

DISEASES

Proceedings of the 1st International Symposium on Maize Arthropods

August, 1987, Gödöllő, Hungary

Contents

PREFACE

The present issue of the *Acta Phytopathologica et Entomologica Hungarica* contains the *Proceedings of the 1st International Symposium on Maize Arthropods* held in Gödöllő, Hungary, in August 1987.

The idea of an international meeting of entomologists working on maize was raised by Drs Peter Duelli (Birmensdorf) and Ferenc Szentkirályi (Budapest) in 1984. Both were instrumental in realising the idea as well as the members of the Organising Committee, Drs B. Nagy, G. Jenser, Z. Mészáros, G. Bujáki, L. Szalay-Marzsó and W. W. M. Steiner. The time and setting was chosen to mark the tenth year of a complex maize agroecology research project at the Plant Protection Institute of the Hungarian Academy of Sciences, the host organisation for the Symposium.

The Symposium was attended by 57 scientists from 21 countries and the present Proceedings volume contains the majority of the lectures and posters presented.

Due to an unforeseen change in editorship and consequently, a rather uncomfortably short time limit for sending manuscripts to print, Dr Nigel E. Stork (Natural History Museum, London) was called in as a joint editor.

It was agreed that all presentations would be published in the Proceedings but a page limit was set. Our task as editors was to check the English, page limit and the clarity of expression. We have tried to keep modifications to a minimum. Where manuscripts were longer than required, we pruned them to the required length with great caution.

It follows from the above-mentioned practice that the authors retain sole responsibility for the scientific content of their contributions.

Finally, we would like to thank our colleagues for rapidly responding to our demands and keeping (most of them, at least) to the page limit.

We hope to see you all at the 2nd International Symposium on Maize Arthropods.

G. L. Lövei & N. E. Stork proc. Editors

Acta Phytopathologica et Entomologica Hungarica, 24 (1-2), pp. 5-12 (1989)

Composition and Density Fluctuations of the Invertebrate Fauna Occurring in a Maize Field at Melle (Belgium)

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In 1985 we started a detailed investigation on the spider fauna of maize fields. In order to follow as many aspects as possible of the ecology and dynamics of the spider populations present, several different sampling methods were used. The densities of spiders and their potential prey was estimated by means of quadrat sampling at two different sites: the centre of a maize field and its north-eastern edge.

In this contribution the composition of the invertebrate fauna occurring at both sites is discussed. The densities of some important groups are compared between the two sites. Seasonal density fluctuations of the most abundant invertebrate groups are discussed. Calculation of the patchiness showed that many groups were significantly aggregated. The influences of land management activities on densities are discussed.

In Belgium, as well as in many other countries, the area of agricultural land sown with maize has increased tremendously in the last decades. As a consequence, maize fields get more and more attention from agronomists and ecologists (Gay, 1987). The composition of the invertebrate fauna occuring on maize fields is, however, not very well known and density estimates are very scarce in the literature.

In 1985, we started an investigation on the spiders of cultivated fields, their ecology and dynamics. An important aspect of this kind of ecological investigation is the estimation of the densities of the spiders and their potential prey. This is of fundamental importance for further studies on the importance of spiders as biological control agents on cultivated fields (Nyffeler, 1982; Nyffeler & Benz, 1979; Riechert & Lockley, 1984). In this contribution the results obtained during the first year of sampling are presented.

Sites and Methods

Our study site was a field in the Experimental Farm of the State University of Ghent (Faculty of Agriculture) situated at Melle (near Ghent, Eastern Flanders, Belgium).

Since 1981, the field is cultivated in a three year crop rotation system. The crop sequence was maize, Italian ryegrass and finally experimental plots for all kinds of crops, then maize again, etc. In 1986, the field was ploughed on 9 and 10 April 1986, harrowed 20 April 1986, sown with maize on 8 May, treated with herbicide 10 May 1986 and harvested 29 September 1986. It was ploughed again on 6 October 1986, harrowed 8 October 1986 and sown with Italian ryegrass 11 October 1986. The field under study had an area of 45 200 square meter. The soil was a loamy sand.

From March 1986 till March 1987, monthly quadrat samples were taken at the centre of the field and at its north-eastern edge. The quadrats measured 12.5 x 12.5 cm (an area of 156.25 square centimeter). On each occasion, thirty quadrat samples were taken at random, taken to the laboratory, sorted out by hand and finally extracted by a Tullgren-Berlese extractor (Tulgren, 1917). All animals were stored in 4% formalin. Most groups were identified (if possible) to family, adult spiders and carabid beetles to species, and juvenile spiders (if possible) to genus level.

For each sampling of thirty quadrats the density per meter square is determined. As a measure for aggregation, Lloyd's patchiness formula was calculated (Southwood,

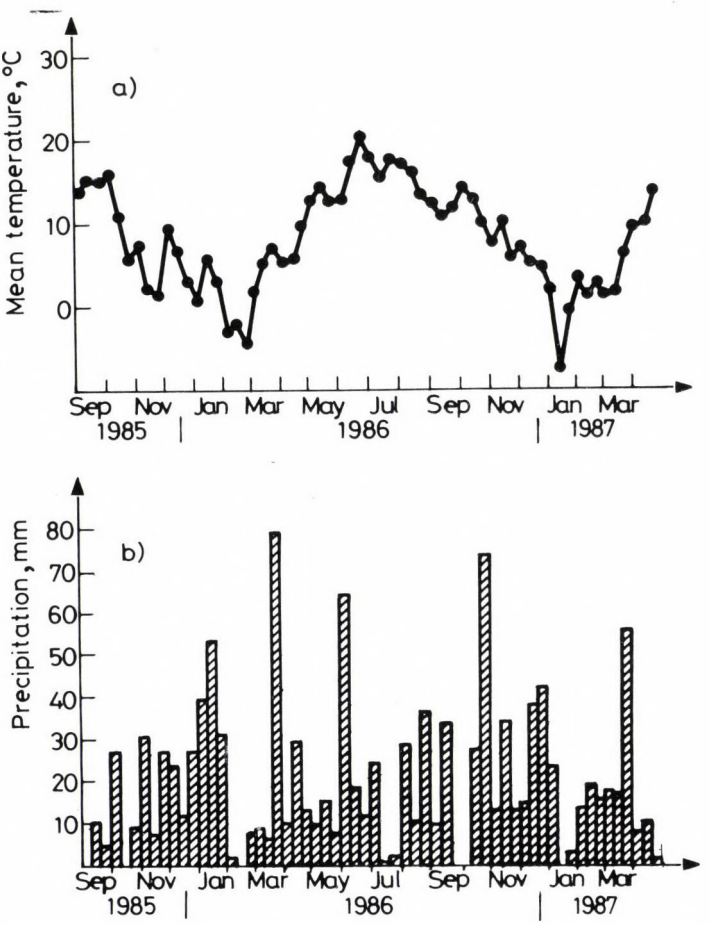

Fig. 1. (a) the mean temperature (°C) measured at 175 cm above ground level and (b) the amount of precipitation (mm) at ten days intervals, Melle September 1985 - April 1987

1978). Confidence limits were also calculated ($p=0.05$, always with 29 degrees of freedom).

We note that the densities of Collembola and Acari were estimated by much smaller quadrats, results of which are not yet available. These very abundant groups were not considered here.

Results and Discussion

Climate

From the several climatological data registrated by the meteorological station at Melle, we plotted (a) the mean temperature measured at 175 cm above ground level and (b) the amount of precipitation from September 1985 till April 1987 for ten days intervals (Fig 1).

Composition of the invertebrate faune and maximal densities

The composition of the invertebrate fauna obtained in the quadrats and the maximal density registrated for the two sites investigated are given on Table 1 (except for Collembola and Acari, see Methods).

The density estimates of the different invertebrate groups were obviously low in the centre of the maize field in comparison to other density estimates for other types of agricultural fields (e.g. Italian ryegrass fields, intensively grazed pastures). Spider densities, for instance, reached a maximum of 26 (± 20.43) individuals/m^{-2} in the centre of the field in July. The higher density of 38 (± 26.33) ind./m^2 (Table 1) was registrated in March when the maize was not yet sown (see Sites and Methods). In the centre of an Italian ryegrass field, densities of 43 (± 23) individuals/m^2 were found (Alderweireldt, 1987).

Density estimates of spiders occurring on cultivated fields are scarce in the literature. The densities Nyffeler (1982) reports from Switzerland vary from 13.38 to 35 individuals/m^2. Nyffeler (1982) also noted that the density of spiders of the vegetation layer itself was very low (less than 0.1 individuals/m^2), which is almost negligible. This is completely in agreement with our observations. From grasslands, most densities range between 50 and 200 individuals/m^2 (e.g. De Keer, pers. comm.; Dondale, 1971; Southwood & Van Emden 1967; Turnbull, 1973; Wolcott, 1937).

Beside spiders, carabid and staphylinid beetles were well represented in the centre of the maize field. They reached maxima of respectively 22 (± 18.11) and 37 (± 14.96) individuals/m^2. The larvae of both beetle families were also fairly abundant. Lumbricidae and Enchytraeidae are worth mentioning. It should, however, be noted that the method used probably underestimated several groups which can reach deeper soil-layers (e.g. Lumbricidae). For Lumbricidae, density estimates in literature are between 5 and 2000 individuals/m^2 in agricultural land (summarized by Lee, 1985).

The densities of most groups were higher at the edge (Table 1). Here spider densities reached 70 (± 38) individuals/m^2. Carabid beetles were more abundant with a

maximum of 175 (45.23) individuals/m^2 in early spring. Staphylinid beetles and their larvae were more abundant in the edge compared to the centre of the field.

Density fluctuations of some abundant invertebrate taxa

Several density fluctuation patterns, e.g. those of spiders and Staphylinidae, suggested that some invertebrate species performed a seasonal migration from the edge of the field to the centre and vice versa. The densities for those taxa were indeed high in the edge zone in early spring and autumn (and probably also in winter). On the other hand, the highest densities in the centre of the field were found during spring and summer (Table 1).

Table 1

Maximal densities (±95% confidence limits) for all the invertebrate taxa registrated in the field edge and field centre (except Collembola and Acari).

	Field Edge N/m^2±95% C.L.	Date	Field Centre N/m^2±95% C.L.	Date
PHYLUM ARTHROPODA				
CLASSIS ARACHNIDA				
ORDO ARANEAE				
TOTAL NUMBER ADULTS	29.86±18.54	05.11.86	38.40±26.33	12.03.86
TOTAL NUMBER JUVENILES	46.93±26.58	26.03.86	17.07±16.52	30.07.86
TOTAL NUMBER	70.40±37.84	26.03.86	49.07±25.63	12.03.86
ORDO OPILIONES				
Opiliones sp.	36.27±40.52	23.04.86		
CLASSIS INSECTA				
ORDO COLEOPTERA				
F. Carabidae				
TOTAL NUMBER ADULTS	174.9±45.23	26.03.86	21.33±18.11	04.06.86
Carabidae-larvae	49.00±21.40	21.05.86	76.80±36.30	04.06.86
F. Byrrhidae	14.93±12.04	10.09.86		
F. Cantharidae				
Cantharidae-larvae	106.7±49.95	05.11.86	4.26±8.72	22.10.86
F. Catopidae	4.26±8.72	03.12.86		
F. Chrysomelidae	10.67±11.02	10.09.86		
Chrysomelidae-larvae	42.67±61.60	21.05.86		
F. Coccinellidae	6.40±7.29	26.03.86	2.13±4.36	12.03.86
Coccinellidae-larvae			6.40±9.62	30.07.86
F. Crytophagidae	10.67±11.02	26.03.83	2.13±4.36	04.06.86
F. Curculionidae	17.07±18.76	26.03.86	2.13±4.36	27.08.86
Curculionidae-larvae	25.60±20.44	26.03.86	27.73±33.02	12.03.86
F. Elateridae	17.06±15.29	23.04.86	2.13±4.36	04.06.86
Elateridae-larvae	206.9±93.03	10.09.86	6.40±7.29	27.08.86

Table 1 continued

	Field Edge N/m^2±95% C.L.	Date	Field Centre N/m^2±95% C.L.	Date
F. Histeridae	2.13±4.26	26.03.86	2.13±4.36	12.03.86
F. Hydraenidae				
Helophorus sp.	4.27±6.06	18.06.86	2.13±4.36	30.07.86
F. Hydrophilidae	2.13±4.26	26.03.86		
F. Lagriidae	2.13±4.26	13.08.86		
F. Lathridiidae	12.80±11.57	08.10.86	2.13±4.36	30.07.86
F. Phalacridae	2.13±4.26	21.05.86		
F. Ptiliidae	8.53±13.66	23.04.86		
F. Scarabaiedae	6.40±7.29	03.12.86	8.53±10.37	11.02.87
Scarabaeidae-larvae	2.13±4.26	10.09.86		
F. Scydmaenidae	4.26±6.06	08.10.86		
F. Staphylinidae	257.2±152.4	08.10.86	59.73±41.12	12.03.86
Staphylinidae-larvae	234.0±73.38	21.05.86	23.46±18.11	04.06.86
F. Sylphidae				
Sylphidae-larvae	2.13±4.26	10.09.86		
Coleoptera sp.-larvae	59.73±28.71	10.09.86		
ORDO DIPTERA				
Subordo Nematocera				
F. Bibionidae-larvae	55.46±109.0	05.11.86		
F. Cecidomyidae	12.80±19.24	24.12.86	2.13±4.36	12.03.86
Cecidomyidae-larvae	151.5±275.0	21.05.86	6.40±7.29	12.03.86
Cecidomyidae-pupae	14.93±14.96	10.09.86		
F. Chironomidae	2.13±4.26	23.04.86	2.13±4.36	07.05.86
Chironomidae- larvae	8.53±10.38	26.03.86	8.53±8.26	09.04.86
F. Mycetophilidae	4.26±6.06	03.12.86		
Mycetophilidae-larvae	12.80±11.57	03.12.86	6.40±7.29	16.12.86
F. Sciaridae	104.5±45.04	23.04.86	23.47±23.88	12.03.86
Sciaridae-larvae	12.80±13.16	10.09.86		
Sciaridae-pupae	19.20±12.79	23.04.86	4.27±8.72	09.04.86
F. Thaumaleidae	2.13±4.26	13.08.86		
F. Tipulidae				
Tipulidae-larvae	4.26±6.06	03.12.86	4.27±6.06	12.03.86
F. Trichoceridae			2.13±4.36	24.09.86
Trichoceridae-larvae	2.13±4.26	23.04.86	76.80±93.60	12.03.86
Trichoceridae-pupae			8.53±7.26	24.09.86
Subordo Brachycera				
F. Calliphoridae	2.13±4.26	23.04.86		
F. Chloropidae	10.67±14.15	13.08.86		
F. Dolichopodidae	10.67±9.06	18.06.86		
Dolichopodidae-larvae	29.86±18.54	03.12.86	11.67±11.02	09.04.86
Dolichopodidae-pupae	4.27±6.06	18.06.86	2.13±4.36	28.06.86
F. Drosophilidae	4.27±6.06	21.05.86		
F. Empididae	29.86±25.67	18.06.86		
Empididae-larvae	10.67±11.02	16.07.86	8.53±8.26	04.06.86
Empididae-pupae			2.13±4.36	28.06.86
F. Lonchopteridae	2.13±4.26	26.03.86	4.27±6.06	12.03.86

Table 1 continued

	Field Edge $N/m^2 \pm 95\%$ C.L	Date	Field Centre $N/m^2 \pm 95\%$ C.L	Date
Lonchopteridae-larvae			2.13±4.36	12.03.86
F. Rhagionidae				
Rhagionidae-larvae	2.13±4.26	23.04.86		
F. Sepsidae	2.13±4.26	13.08.86		
F. Sphaeroceridae	2.13±4.26	23.04.86	4.27±6.06	09.04.86
F. Stratiomyidae				
Stratiomyidae-larvae	8.53±12.12	08.10.86	8.53±12.12	12.03.86
F. Syrphidae			2.13±4.36	07.05.86
Syrphidae-larvae	2.13±4.26	05.11.86		
Musciformes-larvae	49.06±29.22	10.09.86	4.27±6.06	12.03.86
Diptera sp.	4.27±6.06	16.07.86		
Diptera sp.-larvae	10.67±11.02	10.09.86		
ORDO HYMENOPTERA				
Subordo Apocrita				
Section Aculeata				
F. Formicidae	823±1283	26.03.86	23.46±48.00	09.04.86
Formicidaep-upae	8.53±13.65	16.07.86		
Section Parasitica	21.33±14.49	21.05.86	4.27±6.06	12.03.86
Hymenoptera-larvae	76.80±135.7	18.06.86	2.13±4.36	12.03.86
ORDO HEMIPTERA				
Subordo Heteroptera	6.40±7.29	13.08.86	4.27±6.06	12.03.86
Heteroptera- nymphs	4.26±6.06	23.04.86	2.13±4.36	27.08.86
Subordo Homoptera				
F. Aphididae	311.4±469.7	03.12.86	76.80±42.75	30.07.86
F. Cicadellidae	8.53±10.37	13.08.86		
Cicadellidae-nymphs	12.80±11.57	13.08.86		
F. Delphacidae	4.26±8.72	13.08.86		
Delphacidae-nymphs	19.20±22.76	10.09.86	6.40±7.29	12.03.86
ORDO LEPIDOPTERA			2.13±4.36	13.03.87
Lepidoptera-larvae	27.73±17.39	23.04.86	2.13±4.36	12.03.86
Lepidoptera-pupae	2.13±4.36	10.09.86		
ORDO ORTHOPTERA				
F. Acrididae				
Acrididae-nymphs	29.87±45.14	18.06.86		
ORDO NEUROPTERA				
Neuroptera-larvae	8.53±10.38	26.03.86		
ORDO SYPHONAPTERA	4.26±6.06	26.03.86		
ORDO MECOPTERA				
Mecoptera-larvae	10.67±9.05	10.09.86		
ORDO THYSANOPTERA	12.80±9.72	10.09.86		

Table 1 continued

	Field Edge $N/m^2 \pm 95\%$ C.L	Date	Field Centre $N/m^2 \pm 95\%$ C.L	Date
CLASSIS MYRIAPODA				
Subclassis Chilopoda	315.7±158.0	08.10.86	8.53±10.37	30.07.86
Subclassis Diplopoda	136.5±91.11	03.12.86	6.40±13.09	09.04.86
CLASSIS CRUSTACEA				
ORDO ISOPODA				
F. Onoscidae				
Philoscia muscorum	243.2±103.5	10.09.86		
F. Porcellionidae				
Porcellio scaber	23.47±15.98	21.05.86		
PHYLUM MOLLUSCA				
CLASSIS GASTROPODA	29.86±16.29	23.04.86		
PHYLUM ANNELIDA				
CLASSIS OLIGOCHAETA				
F. Enchytraeidae	328.5±91.33	08.10.86	53.33±19.93	09.04.86
F. Lumbricidae	81.06±25.83	23.04.86	125.8±25.48	09.04.86

This indicated that the edges served as hibernation refuges for certain invertebrate taxa. To prove this, one should consider other factors influencing densities like reproduction, life cycle patterns, dispersion, etc. For carabid and staphylinid beetles, it has already been proven that the edges of cultivated fields are very important for hibernation (Desender, 1982; D'Hulster & Desender, 1984). This is also true for certain spider species. For *Bathyphantes gracilis* (Blackwall, 1841) for instance, significant seasonal migration to the edge of the field was observed. These observations are mainly based on density fluctuation and activity distribution patterns registrated at increasing distances from the edge.

Aggregation and the influence of land management on the densities

For many invertebrate taxa (e.g. Staphylinidae, larvae of Staphylinidae, larvae of Elateridae, Sciaridae, Chilopoda, some Carabidae like *Agonum dorsale* the obtained values significantly exceeded one, showing that these groups were significantly aggregated.

In April 1986, the quadrat samples were taken just after ploughing and the densities of most invertebrate groups suddenly collapsed. However, some species, like *Clivina fossor* seemed to survive such a drastic disturbance. In the case of the *Clivina* species, this is probably due to their digging habit and subterranean way of life.

Harrowing seems to have no pronounced effect. In Belgium, the maize fields are treated with herbicides just after sowing. We did not detect any effect of this treatment on the densities of the invertebrate taxa present. No insecticides are used in Belgian maize fields.

It is also clear that recolonization of the field occurs rather quickly after ploughing and harrowing during spring. This is not the case after the same agricultural measures during autumn. In general, we conclude that most invertebrate species cannot survive on

intensively managed fields without the presence of edges or other less intensively managed zones.

Acknowledgements

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Susceptibility of Four Local Maize Varieties to Corn Borer Infestation under Natural Condition in Egypt

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Relative susceptibility of four Egyptian maize varieties (native Balady, Giza-1, D.C.101 and D.C.514) to *Sesamia cretica* and *Ostrinia nubilalis* infestation was evaluated. For *S. cretica*, Balady and D.C.202 rated as susceptible (S) and highly susceptible (HS), respectively, Giza-2 was relatively resistant (RR) and D.C.514 highly resistant (HR). These varieties reacted differently to infestation by the European corn borer, *O. nubilalis*. The most susceptible were Balady (HS) and D.C.514 (S). D.C. 202 was resistant (R), Giza-2 relatively resistant. Factors governing susceptibility or resistance of these varieties to corn borer infestations are discussed.

Maize plants are infested by a variety of insect pests, among which the Greater Sugar-cane borer, *Sesamia cretica* Led. (Issa, 1959; Ismail, 1968; El-Saadany and Hosny, 1976 and Simeada, 1985), and the European Corn Borer, *Ostrinia nubilalis* Hbn. (Patch and Bottger, 1937; El-Saadnay, 1969), are prominent in Egypt.

The present work evaluated the relative susceptibility of some local and introduced maize varieties to these two pests under natural field conditions.

Material and Methods

Four maize varieties: Balady, Giza-2, D.C.202, and D.C.514 were evaluated. A field of maize of about one feddan was divided into 32 plots, each consisting of eight rows (6 meters long by 70 cm wide with 30 cm distance between hills and one plant per hill). Each variety was sown in five plots in a complete randomized block design. All plantations received the same ordinary agricultural treatments; pest control measures were entirely avoided.

Relative susceptibility to infestation by *S. cretica* and *O. nubilalis* was based on symptoms of injury (Hosny and El-Saadany, 1967). Plants were sampled weekly and dissected. The infestation rate of each pest was established by the formula adapted by Simeada (1985).

Results and Discussion

1. The greater sugar-cane borer, Sesamia cretica *Led.:*

All four maize varieties were infested by *S. cretica* under natural conditions (Table 1). The initial infestation occurred during the third week of June and became evident when the plants were 30 days old. Although all cultivars were infested simultaneously, the first inspection showed that D.C.202 harboured the highest rate of infestation (IR), while D.C.514 had the lowest IR. This was found in both growing seasons studied. The highest percentages of infested plants were found in July 1984 and two weeks later in 1985 (Table 1).

When the total infestation rate (TIR) of both growing seasons were pooled, the highest susceptibility to *S. cretica* was shown by D.C.202, followed by Balady, Giza-2 and D.C.514. When the means of seasonal infestation rate (SMIR) were compared, D.C.514 proved to be highly resistant with the lowest IR (2.9). D.C.202 was highly susceptible (IR=6.5) while native Balady rated as susceptible (IR=5.6). These results correspond to the data obtained by Simeada (1985). The susceptibility of Balady was attributed to the

Table 1

Response of some local maize varieties to GSE, *Sesamia cretica* under natural infestation at El-Mahallah El-Kobra, Gharbia Governorate, Egypt.

Inspection date		Plant age (days)	Rates of infestation % 1st season:1984				2nd Season: 1985			
			Balady	Giza	2 D.C.202	D.C. 514	Balady	Giza	2 D.C.202	D.C. 514
June,	16	31	4.70	3.19	8.21	1.65	3.81	2.10	7.85	1.50
	23	38	4.49	1.02	4.81	1.45	4.10	2.10	7.85	1.25
	30	45	4.49	1.02	4.81	1.45	3.81	0.95	5.21	1.2
		MIR	4.56	1.74	5.94	1.52	3.91	1.72	6.97	1.33
July,	7	52	8.74	3.13	6.65	4.34	2.99	3.99	2.99	1.56
	14	59	13.23	5.96	12.85	4.58	6.33	6.33	6.66	1.65
	21	63	8.05	5.96	12.85	9.58	9.33	7.33	7.32	4.33
	28	70	9.22	4.40	11.27	5.85	14.66	4.99	17.33	5.66
		MIR	9.81	2.86	10.41	7.34	9.33	5.66	8.58	3.23
August,	4	77	8.05	3.95	6.42	7.78	14.66	4.99	17.33	5.50
	11	84	0.40	2.55	0.00	0.00	0.99	2.33	2.66	0.60
	18	91	0.40	2.35	0.00	0.00	0.00	0.66	0.00	0.66
	25	98	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		MIR	2.35	2.21	1.61	1.95	3.91	1.99	4.99	1.71
SMIR			5.7±3.9	3.0±1.9	6.2±4.6	3.8±2.6	5.5±2.01	3.3±1.3	6.8±1.5	2.2±1.7

MIR:Mean infestation rate
SMIR:Seasonal mean infestation rate

relative softness of plant stem (Issa, 1959). Another explanation was proposed by El-Saadany and Hosny (1976) who reported high percentage of hydrocyanic acid content in this cultivar.

2. The European corn borer, O. nubilalis:

Infestation by *O. nubilalis* was different in the two years studied: infestation rate was 0.32-39.27% in 1984, but 0.29-8.21% in 1985. The highest IR values occurred late in the season, the lowest rate was in July. This indicated that IR by ECB increased progressively from July to September and reached the maximum when plants aged 105-120 days. D.C.514 and Balady were more susceptible than Giza-2, while the least susceptible cultivar was D.C.202 (Table 2).

Susceptibility or resistance of maize cultivars to CB infestation varies according to physiological changes as the plant grows towards maturity (Neiswander and Huber, 1929). The higher susceptibility of Balady could be attributed to plant stem softness (Issa, 1959). Native Balady proved to be generally less tolerant than hybrids as stated by Patch (1937), Patch et al. (1942) and Issa (1959). Since D.C.514 is taller, its stem is thinner and flowers later than Giza-2 or D.C.202 (Soliman, 1986), it is expected to be more susceptible to ECB infestation. Ovipositing moths also tend to select taller plants. On the other

Table 2

Mean infestation rates of four local maize cultivars by *O.nubialis* under natural conditions at El-Mahallah El-Kobra, Gharbia Governorate.

Inspection date		Plant age (days)	Rates of infestation % 1st season:1984				2nd Season: 1985			
			Balady	Giza	2 D.C.202	D.C. 514	Balady	Giza	2 D.C.202	D.C. 514
July,	21	63	1.14	0.32	1.79	0.00	0.52	0.00	0.25	0.00
	28	70	2.63	0.32	0.00	0.00	0.66	0.00	0.33	0.00
monthly mean			1.86	0.32	0.40	0.00	0.59	0.00	0.29	0.00
August,	4	77	8.31	6.23	5.35	3.77	1.66	0.33	0.66	0.00
	11	84	8.31	6.23	5.35	3.77	3.33	0.99	0.33	0.00
	18	91	25.25	13.87	16.21	23.48	3.33	0.66	1.99	0.00
	25	98	25.25	13.87	16.21	23.48	4.99	3.33	2.66	2.33
monthly mean			16.78	10.05	10.78	13.53	3.33	1.32	1.41	0.58
Sept.	1	112	40.00	32.90	18.10	37.50	6.00	6.99	4.99	4.99
	8	112	40.00	32.90	18.10	37.50	6.00	6.99	4.99	4.90
	15	120	40.80	34.50	19.20	38.40	6.92	8.33	5.99	17.32
monthly mean			39.27	33.16	18.08	37.63	5.97	6.22	4.55	8.21
Seasonal mean			20.97	15.59	11.02	18.60	3.60	2.66	2.21	3.00

hand, the greater stem diameter, earlier flowering and silking in D.C.202 may explain the resistance of this cultivar to *O. nubilalis* infestation.

According to our results, Giza-2 can be recommended as the most favourable maize cultivar in Egypt since it proved to be resistant to both *S. cretica* and *O. nubilalis*, the main corn borers in Egypt. However, this evaluation should be complemented by more detailed studies of the factors influencing resistance such as sowing dates, plant spacing and rate of nitrogen fertilization.

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The Expression of Maize Resistance to the Spotted Stem Borer, *Chilo partellus* (Lepidoptera: Pyralidae) in Relation to Plant Age at Infestation.

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The colonization pattern of the spotted stem borer, *Chilo partellus* (Swinhoe) on maize plants shows two peaks of infestation. These coincide with early whorl and flowering stages of plant development. Varieties with good levels of resistance to the whorl stage infestation have been identified. Further investigations were designed to study and compare the resistance expressions in some of these varieties in relation to the time of infestation, and to use the results as a basis for developing a methodology for evaluating maize varieties for resistance to the flowering stage infestation. Some varieties that showed high levels of resistance to infestation at the whorl stage, showed susceptibility to infestation at the flowering stage in terms of: (i) larval establishment, growth and development; (ii) the severity of damage caused to plants and (iii) loss in grain yield. Infestation of plants near maturity resulted in minimal yield loss.

The expression of plant resistance to insect pests is usually age-specific: a variety that shows a high level of resistance to a pest at one growth stage may show susceptibility at another (Davis *et al.* 1972, Robinson *et al.* 1978). The reaction of a variety to infestation at different phenological stages is therefore essential to a full understanding of its resistance potential.

The colonization pattern of *Chilo partellus* (Swinhoe) on maize plants shows two peaks of infestation (Ampofo 1985, 1986) which coincide with mid-whorl and flowering stages of the plants. Varieties with good levels of resistance to the whorl stage infestation have been identified (Ampofo *et al.* 1986a,b). The present investigations were designed to study and compare the resistance expressions in some of these varieties in relation to the time of infestation, and to use the results as a basis for developing a methodology for evaluating maize varieties for resistance to flowering stage infestation by *C. partellus*.

Materials and Methods

Three maize varieties, Inbred A, ICZ2-CM and MP704 were planted at the International Centre of Insect Physiology and Ecology (ICIPE) Field Station at Mbita Point, western Kenya (1240 m above sea level, lat. 0.25°S). ICZ2-CM and MP704 had shown some good levels of resistance in earlier experiments, while Inbred A showed susceptibility.

Experimental design was split-plot with plant age at infestation as the main plot and varieties as the sub-plots. The main plots consisted of control (no infestation), and infestations at 4, 8, and 12 weeks after plant emergence (WAE). These periods (4, 8, and 12 WAE) represented the mid-whorl (stage 2), flowering (stage 4) and dough (stage 7)

stages of plant development, respectively (Hanway 1971). The infestation at 4 and 8 WAE simulated the 1st and 2nd brood *C. partellus* field infestations, respectively.

The sub-plots consisted of the individual maize varieties planted at 3-4 seeds per hole and thinned to a single uniform plant per hill after establishment. Planting distance was 30 cm within, and 75 cm between rows. This planting density is equivalent to a field plant population of 44,000 per ha. Each sub-plot consisted of 8 rows 5 m long and individual plots (varieties) were separated by 1 m bare gaps. The plots were fertilized at the rate of 60 kg N and 40 kg P and were hand weeded and irrigated as necessary.

A loose nylon mesh cage (16 m wide x 7 m long x 2.5 m high) was erected over each main plot to prevent contamination from the field population of *C. partellus* and other insects. The cage, however, caused a temperature buildup within the plots and the sides were raised each day between the hours of 0800 and 1700 to allow free circulation of air and reduce the temperature buildup.

The four central rows from each sub-plot were infested by placing 20 *C. partellus* neonate larvae in the whorls (4 wk old plants), in the axil of the leaf above the ear (8 wk old plants) or the axil of the 4th leaf below the tassel (12 wk old plants). The two outer rows were left as guard rows to prevent the migration of larvae from one sub-plot to the other. Three weeks after the infestation with larvae, five plants were randomly removed from the infested plots and examined for larval presence and damage. In addition, the effects of variety and plant age on larval establishment and growth were assessed. The effect of the infestation on such papameters as plant height at maturity and grain yield were also assessed. The experiment was conducted over the short rainy season (Oct-Jan) of 1985/86. Larval counts were transformed to square roots while percentages were transformed to arc sine. The data were then subjected to analysis of variance (Anova) using the general linear model procedure (Proc GLM) (SAS Institute 1985). Treatment means were sorted according to variety and separated by the Duncan's multiple range test.

Table 1

Analysis of variance table for larval survival, growth and development in relation to variety and time of infestation.

Source	df	Larval recov. (%)		HCW		Larval wt.		
		MS	Pr>			MS	PR>	MS
Replication	3	295.52	0.192	0.029	0.114	142.67	0.028	
Per	2	2788.78	0.000	0.020	0.079	291.69	0.004	
Error (a)	6	246.85	-	0.164	-	80.02	-	
Var	2	758.78	0.026	0.125	0.000	874.29	0.000	
Per x Var	4	1978.28	0.000	0.037	0.011	73.33	0.142	
Error (b)	18	168.52	-	0.008	-	37.20	-	

Per=Period of infestation, Var=Variety, MS=Mean squares, and Pr< indicates the level of significance.

Results and Discussion

The effects of variety and period of infestation, as well as their interactions, were significant for nearly all the variables monitored (Tables 1 & 2). Mean comparisons for periods of infestation were performed within varieties because of the high interactions.

Table 2

Analysis of variance table for plant damage, growth and yield characteristics in relation to maize

Source	df	Stem tunnels at harvest		Plant height at maturity		Cobs harvested		Grain weight per plot	
		MS	Pr>F	MS	Pr>F	MS	Pr>F	MS	Pr>F
Replication	3	19.14	0.667	53.63	0.767	0.001	0.727	226829.47	0.355
Per	3	2322.64	0.000	3915.54	0.000	0.037	0.000	4538852.71	0.000
Error (a)	9	34.40	-	487.76	-	0.006	-	248697.17	-
Var	2	910.60	0.000	3841.06	0.000	0.300	0.000	12620300.80	0.000
Per x Var	6	512.39	0.000	1503.48	0.000	0.030	0.000	333938.19	0.171
Error (b)	24	37.57	-	140.66	-	0.003	-	199753.37	-

Per=Period of infestation, Var= Variety, MS= Mean squares, and Pr>F indicates the level of significance.

After 3 weeks of infestation, foliar lesions were the main form of damage when plants were infested at 4 WAE. Nearly all the plants showed this form of damage. Stem tunneling was also observed but the intensity and extent (% of plants with stem tunnelled) were low (Table 3). Infestations at 8 and 12 WAE resulted in stem tunneling but caused no foliar lesions (Table 3). Damage (both foliar lesions and stem tunnels) was more severe on Inbred A than MP704. MP704 together with Inbred A, however, suffered more stem tunneling from infestations at 8 and 12 WAE than ICZ2-CM. The effect of stem tunneling on lodging could not be asessed because a storm caused the collapse of the cages and artificial lodging of several plants just before harvest.

The pattern of larval establishment in relation to plant age at infestation was similar for Inbred A and ICZ2-CM (Table 4). On both varieties there were no significant differences in larval establishment when plants were infested at 4 and 8 WAE. On MP704, however, larval establishment was very low (2.5%) for the infestation at 4 WAE but significantly higher (58.5%) for the infestation at 8 WAE. Larval establishment was low on all three varieties when plants were infested at 12 WAE.

Mean weights of larvae recovered from Inbred A and ICZ2-CM were similar (for each variety) for the infestations at 4 and 8 WAE but higher for the infestation at 12 WAE (Table 4). On MP704, however, larvae recovered from the infestation at 4 WAE were half as heavy as those recovered from the infestation at 8 WAE which were similar in weight. Mean instar of the recovered larvae (after 21 days of infestation) was generally higher for Inbred A then MP704 and ICZ2-CM.

Table 3

Levels of certain damage symptoms caused by *C. partellus* larvae in relation to time of infestation.

Variety	Infested at(WE)	Foliar damage	Stem tunneling (cm)at: 3 WAI	Harvest
ICZ2-CM	Control	1.0 b	0.0 a	1.4 b
	4	3.4 a	0.9 a	23.2 a
	8	1.0 b	0.9 a	23.0 a
	12	1.0 b	0.0 a	5.0 b
MP704	Control	1.0 b	0.0 b	0.1 b
	4	1.5 a	0.0 b	5.9 b
	8	1.0 b	7.3 a	26.2 a
	12	1.0 b	0.0 b	4.0 b
Inbred A	Control	1.0 b	0.0 c	1.5 d
	4	6.5 a	5.1 b	53.6 a
	8	1.0 b	10.9 a	30.8 b
	12	1.0 b	0.0 c	10.2 c

Control=no infestation.
Means for the different treatments (infestation) within a variety followed by the same letter(s) are not significantly different at the $P=0.05$ level according to the Duncan's multiple range test.

Table 4

C. partellus larval establishment and plant growth during 21 days after infestation on three maize varieties infested at different phenological stages.

Variety	Infested (at WAE)	% recovery (at 21 DAI)	Mean larval wt. (mg)	Mean HCW of larvae (instar)
ICZ2-CM	4	43.0 ab	14.7 ab	1.19 (4) a
	8	47.0 a	11.8 b	1.03 (4) b
	12	11.0 b	25.7 a	1.18 (4) a
Mp704	4	2.5 c	10.0 b	1.03 (4) a
	8	58.5 a	21.0 a	1-25 (4) a
	12	26.0 b	20.0 a	1.17 (4) a
Inbred A	4	67.0 a	29.1 a	1-26 (4) a
	8	44.5 b	29.9 a	1.33 (5) a
	12	22.0 c	37.0 a	1.37 (5) a

Means for the different treatments (infestation) within a variety followed by the same letter(s) are not significantly different at the P=0.05 level according to the Duncan's multiple range test.

Table 5

Effect of time of infestation on certain agronomic characteristics of the maize plants.

Variety	Infested at (WAE)	Plant ht. (cm) at maturity	Cobs harvested per plant	Grain wt. per plot (gm)
ICZ2-CM	Control	234.6 ab	1.3 a	3979.5 a
	4	214.9 b	1.3 a	2519.2 b
	8	225.8 ab	1.2 a	1998.4 b
	12	241.3 a	1.3 a	2825.2 ab
MP704	Control	206.7 a	1.5 a	1907.8 a
	4	193.6 a	1.5 a	1182.8 bc
	8	198.3 a	1.3 b	995.1 c
	12	206.9 a	1.3 b	1480.4 b
Inbred A	Control	209.7 a	1.2 a	2006.6 a
	4	145.8 b	1.0 b	520.5 c
	8	230.0 a	1.2 a	874.0 c
	12	227.9 a	1.2 a	1464.1 b

Control=no infestation.
Means for the different treatments (infestation) within a variety followed by the same letter(s) are not significantly different at the P=0.05 level according to the Duncan's multiple range test.

Infestation at 4 WAE resulted in stunted plant growth in Inbred A but did not significantly affect plant growth in the other varieties (Table 5). All the infestation treatments resulted in yield reduction over the control. There was, however, a treatment x variety interaction: infestation at 4 WAE caused the highest yield loss in Inbred A, while the infestation at 8 WAE caused the highest yield loss in MP704 and ICZ2-CM (Figure 1, Table 5). This indicates a differential response of the maize varieties to plant age at infestation; while Inbred A was more susceptible to whorl infestation, ICZ2-CM and MP704 were more susceptible to infestation at the reproductive stage. In all varieties, yield loss was the least when plants were infested at 12 WAE.

Previous studies at the ICIPE (Ampofo *et al.* 1986b, Ampofo and Kidiavai 1987) have indicated MP704 to be highly resistant to whorl stage (1st brood) infestation of *C. partellus*. The present study confirms this resistance but shows MP704 to be susceptible to the flowering stage (1nd brood) infestation of this pest.

In earlier evaluation experimetns (Chatterji *et al.* 1971, Dabrowski and Nyangiri 1983, Ampofo *et al.* 1986b) plants were infested at 4 WAE because this stage coincides with the major peak of infestation (1st brood) in the field. The second-brood infestation is usually low and generally does not cause severe damage. Results from this experiment indicate that, while a variety such as MP704 might be highly resistant to the whorl stage infestation, it may be susceptible to infestation at the flowering stage. This suggests that genes conditioning maize resistance to the whorl and flowering stage infestations by *C. partellus* may be different. Many other varieties may behave like MP704, in which case if severely infested during the flowering stage, they may succumb to the pressure. For a

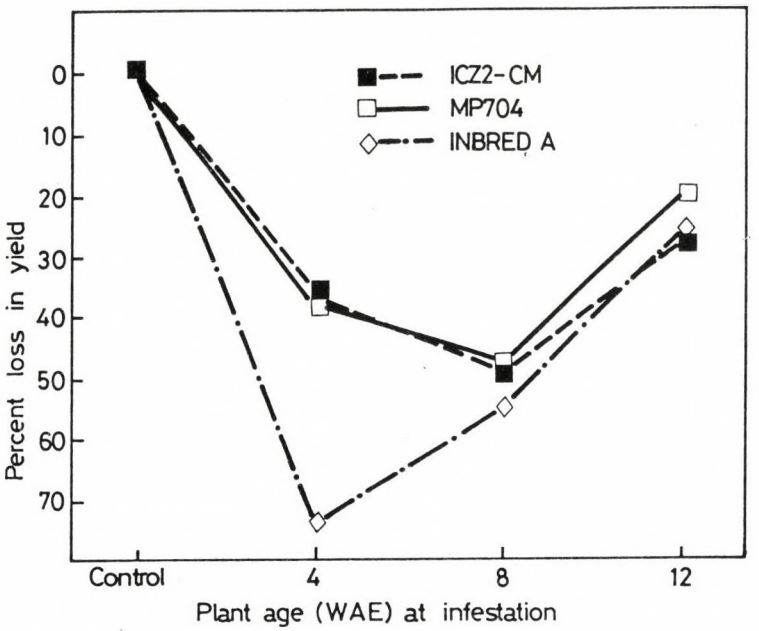

Fig. 1. Yield loss in relation to plant age at infestation

more stable resistance to infestation at all growth stages, it is proposed that in future evaluation experiments, selected plants from the whorl stage infestation should be reinfested during the flowering stage in order to select material with resistance to both the first and second brood attacks.

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The Effect of Benzoylphenyl Urea Moulting Inhibitors on Larvae and Eggs of the European Corn Borer, *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae)*

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The benzoylphenyl urea moulting inhibitors diflubenzuron (Dimilin), triflumuron (Alsystin), teflubenzuron (CME 134), chlorfluazuron (IKI-7899) and XRD-473 were assayed for their activity against larvae and eggs of European corn borer, *Ostrinia nubilalis* Hbn. Maize seedlings were sprayed and infested with 4-mg *O. nubilalis* larvae. After 3 days the larvae were transferred to an artificial diet. The number of dead and injured larvae and, ultimately, the percent pupation and percent adult emergence, were recorded. More than 90% mortality until emergence was obtained by teflubenzuron (S.C.) at a concentration of 0.1 ppm a.i.; XRD-473 (E.C.), 5 ppm; triflumuron (W.P.), 50 ppm; diflubenzuron (W.P.), 250 ppm; and chlorfluazuron (W.P.), 500 ppm. In the ovicidal tests only diflubenzuron (liquid formulation) and triflumuron (E.C.) were highly active, whereas chlorfluazuron (E.C.), XRD-473 (E.C.) and teflubenzuron (S.C.) were inactive at 100 ppm.

With the increase of the area planted to sweet corn in Israel, infestations of corn borers, especially *Sesamia nonagrioides* Lef., were observed (Melamed-Madjar and Tam 1980). An increase in populations of European corn borer (ECB), *Ostrinia nubilalis*Hbn., was noted in 1981 (Melamed-Madjar *et al.* 1985); since then the infestation increased, causing heavy damage and necessitating chemical control measures in sweet corn.

Since with many of the conventional insecticides, *e.g.* the OPs and carbamates, we face the hazards of toxic residues and mammalian toxicity and the possibility of the rapid development of resistance, it was decided to study the effect of the new, second-generation benzoylphenyl urea (BPU) chitin synthesis inhibitors as insecticides for the ECB. If we consider diflubenzuron, teflubenzuron and penfluron as the most prominent representatives of the first generation of BPUs, the best known second-generation compounds at present (developed in the 1980s) are teflubenzuron (CME 134, Celamerck) (Becher *et al.* 1983, Ascher and Nemny 1984); XRD-473 (Dow) (Sbragia *et al.* 1983); and chlorfluazuron ((IKI-7899, Ishihara) (Haga *et al.* 1982). Comparatively little work, mainly with diflubenzuron, has been done on the activity of BPUs against the ECB. Büchi (1978) reported on the larvicidal and ovicidal effects of several concentrations of diflubenzuron. Fragalla *et al.* (1980) have studied the ovicidal effects of diflubenzuron and their results were, in part, in contradiction with Brüdi's. In field experiments, maize trated with one application of diflubenzuron in either a 2% granular (G.) or a 25% wettable powder (W.P.) formulation had significantly fewer ECB larvae and less stalk damage than the untreated control, with the G. giving better results than the W. P. (Berry *et al.* 1980). Two applications of the W.P., but not of the G., were somewhat more effective than one.

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Of the second generation BPUs, teflubenzuron was tested in field trials in southern Germany against ECB. At 1.5 l/ha there was 83% control (Becher *et al.* 1983). Chlorfluazuron applied at rates of 100 and 50 ppm a.i. prevented any damage by *Ostrinia* to corn ears at the Nagano Station, Japan (ICI 1984).

Material and Methods

The insecticides

The BPUs used were: diflubenzuron as 25% Dimilin W.P. and as 5% liquid formulation (Philips-Duphar B.V., the Netherlands); triflumuron as 25% Alsystin W.P. and as 6.5% E.C. (Bayer AG, FRG); teflubenzuron (CME 134) [N-2,6-difluorobenzoyl-N'-2,4-difluoro-3,5-dichlorophenyl-urea] as 15% S.C. (suspension concentrate) (Celamerck GmbH, FRG); chlorfluazuron (IKI-7899, also called CGA 112,913 and PP 145) [N-2,6-difluorobenzoyl-N'-4-(3-chloro-5-trifluoromethylpyridin-2-yloxy)-3,5-dichlorophenyl-urea] as 25% W.P. and as 5% E.C. (Ishihara Sangyo Kaisha Ltd., Japan); and XRD-473 [N-2,6-difluorobenzoyl-N'-4-(1,1,2,2-tetrafluoroethoxy)-3,5- -dichlorophenyl-urea] as 5% E.C. (Dow Chemical Co., U.S.A. and U.K).

The insects

Adult moths of a laboratory ECB strain (Navon and Melamed-Madjar 1986) were kept in 30x20x22 cm height wooden frame, wooden base cages, having a cloth sleeve in front and being covered with Saran netting on the three other sides. On the top of the cage, covered by a coarse plastic net with 4x4 mm holes, a sheet of smooth white paper covered by a layer of wet foam rubber served as oviposition site. The adults were offered both a sacharose solution and water absorbed in cotton wool. The eggs were transferred to square (9x9x9 cm) plastic boxes, the walls of which were padded with wet filter paper. The boxes were covered with paper towelling and closed with plastic cover. On the covers, a large square was cut out and covered with fine netting to provide ventilation. Upon hatching, a cube of the semisynthetic diet described by Melamed-Madjar and Raccah (1979) was put into the boxes. The diet contained the following: corn meal, 330 g; dry yeast (NBCo), 50 g; ascorbic acid (technical), 6 g; methyl-*p*-benzoate (Busch, B.C.P.), 6 g; chloromycetin (technical), 2.5 g; and Bactoagar (Difco), 32 g in 1000 ml water. The culture was kept at 26°C and 70% R.H.

The Larvicidal Bioassay

Potted sweet corn seedlings (five seedlings per pot of cv. Giboli) were sprayed until runoff with a Desaga spray gun (Desaga Co., Heidelberg, FRG) with aqueous dilutions of the BPU insecticide formulations — the 25% W.P. formulations of chlorfluazuron, triflumuron and diflubenzuron, the 15% S.C. of teflubenzuron and the 5% E.C. of XRD-473 — to which one drop of Triton X-100 per 100 ml dilution had been added. Control plants were sprayed with water and Triton X-100 only. After the plants had been

allowed to dry, each seedling was inserted in a glass tube (diam 2 cm, length 14 cm) as follows: the lower edge of the tube was pushed into the soil and the leaves were folded to fit into the tube; ten larvae, 4-5 days old, with an average weight of 4 mg each, were put into the tube. The tubes were closed with a plug of cotton wool. After 3 days of feeding, during which time the larvae damaged the leaves (treated or untreated) badly, each group of larvae was transferred to a cube of artifical diet in a 1-oz. screw-cap jar (base diam and height, 4.5 cm); a 3-cm-diam hole had been cut in the plastic screw-caps of the jars and covered with a 200-mesh metal wire screen to prevent escape of larvae but nevertheless provide ventilation. The cubes of the diet were replaced with fresh ones and the number of dead and injured larvae was recorded every third day. Percentages of pupation and adult emergence were calculated. Each experiment was conducted with five replications of ten larvae each, per concentration and material. The experiments were conducted at 22-23°C under normal photoperiodic conditions.

The Ovicidal Bioassay

Egg batches 0-1 days old, were cut out from the oviposition-site paper mentioned above. The eggs were counted, and the paper with the eggs were dipped briefly into aqueous dilutions of the E.C.s of triflumuron, chlorfluazuron and XRD-473, the S.C. of teflubenzuron and the liquid formulation of diflubenzuron. 100- 300 eggs were used for each single treatment. The pieces of paper were put on filter paper discs (Whatman No. 1) in 9-cm Petri dish covers. The Petri dish halves were then placed (open) in an incubator at 27°C and 70% R.H. A ring of "Stikem Special" (Michel and Pelton Co., Emeryville, CA, U.S.A.) was applied to the circumference of the filter paper discs, to trap the neonate larvae. The number of unhatched eggs was counted after 4 days.

If there was mortality in a control, the results obtained with toxicants were corrected by Abbott's (1925) formula.

Results and Discussion

The various second-generation BPUs showed distinctly different toxicities to larvae of the ECB (Table 1). Teflubenzuron gave 90% criterion IV (cumulative up to the adult stage) mortality down to a spray concentration of 0.1 ppm and XRD-473 down to 5 ppm; chlorfluazuron was much less active against this insect (90% mortality limit at 500 ppm). The first generation BPUs were also of comparatively low activity: 90% mortality was obtained with diflubenzuron only at 250 ppm and with triflumuron, at 50 ppm.

Except at low concentrations, nearly all the mortality causing a final 50% mortality occurred in the larval stage before prepupation (criterion I, Table 1). However, several of the BPUs had practically unchanged larvicidal activity over a wide range of concentrations, e.g. diflubenzuron between 150 and 0.005 ppm caused criterion IV mortality rangig from 40-50%. A similar tendency was noted with teflubenzuron and chlorfluazuron, but not with XRD-473 or triflumuron.

As ovicids, only diflubenzuron (liquid formulation) and triflumuron (E.C.) were active (Table 2). Interestingly enough, the kill obtained by these formulations at 50, 10 and 1 ppm was not significantly different from that obtained with the analogous aqueous

Table 1

The toxic effects of different BPUs for larvae of *Ostrinia nubilalis*

Compound tested	Criterion*	ppm a.i. in the spraying liquid													
		1000	500	250	150	100	50	25	10	5.0	2.5	1.0	0.5	0.1	0.075
		Percentage of insects (±S.E.) affected according to criteria I-IV													
Teflubenzuron (S.C.)	I		100			100	100		100	89±5	96±2	98±2	89±6	91±4	21±5
	II		100			100	100		100	89±5	96±2	98±2	94±4	93±3	21±5
	III		100			100	100		100	89±5	98±2	98±2	96±3	93±3	28±4
	IV		*100*a			*100*a	*100*a		*100*a	*89*±5a	*100*a	*98*±2a	*96*±3a	*93*±3a	*40*±5a
XRD-473 (E.C.)	I		100	100		100	100	100	100	100	85±9	60±6	77±6	56±12	10±6
	II		100	100		100	100	100	100	100	85±9	65±4	79±4	61±9	15±5
	III		100	100		100	100	100	100	100	85±9	65±4	81±6	61±9	23±4
	IV		*100*a	*100*a		*100*a	*100*a	*100*a	*100*a	*100*a	*88*±9a	*70*±6b	*81*±6a	*61*±9b	*23*±4b
Triflumuron (W.P.)	I			100		93±5	97±2	74±8	39±8	17±7		9±9	12±5	10±4	
	II			100		98±2	97±2	74±8	41±8	22±10		11±9	14±6	10±4	
	III			100		98±2	97±2	74±8	44±6	29±12		11±9	14±6	12±6	
	IV			*100*a		*98*±2a	*97*±2a	*74*±2b	*53*±7b	*31*±13b		*14*±11c	*14*±6b	*16*±6c	
Diflubenzuron (W.P.)	I		100	100	37±4					32±10	38±15	40±6	23±9	26.5±9	
	II		100	100	40±4					39±7	38±15	40±6	25±9	32.5±8	
	III		100	100	50±5					43±5	38±15	40±6	43±6	37.1±8	
	IV		*100*a	*100*a	*51*±5					*43*±5b	*41*±13b	*40*±6c	*50*±4c	*40.3*±8d	
Chlorfluazuron (W.P.)	I	100	98±2	70±3		83±6	69±3		47±1	38±6	45±9	28±5	33±9	34±7	
	II	100	98±2	73±5		83±6	71±5		52±1	38±6	50±11	30±6	35±9	40±7	
	III	100	98±2	73±5		83±6	71±5		52±1	41±4	53±10	30±6	36±11	40±7	
	IV	*100*	*98*±2a	*75*±6		*88*±6a	*74*±5b		*67*±9b	*57*±11b	*58*±10b	*35*±6c	*38*±13bc	*40*±7d	

* I. Percentage of larvae dying before the prepupal stage; II. cumulative percentage of larva dying before and during the prepupal stage; III. cumulative percentage mortality up to pupation, plus percentage of abnormal pupae; IV. cumulative percentage mortality up to the adult stage (in italics). All percentages were corrected for injury and mortality among untreated insects.
Criterion IV means in the same column followed by different letters are significantly different at P=0.05 (Duncan's Multiple Range Test).

Table 2

The percent mortality of 0-1-day-old Ostrinia nubilalis eggs dipped in aqueous dilutions of five different BPUs. (T=27°C)

Concentration (ppm a.i.)	Diflubenzuron (liquid formulation)	Triflumuron (E.C.)
100	100	100
50	100	100
10	93	100
1.0	93	73
0.5	27	34
0.1	22	27.5

dilutions of the W.P.s. This is in contrast with the results against the eggs of *Spodoptera littoralis* with diflubenzuron, in which the Dimilin W.P. was much less active than the liquid formulation (Ascher and Nemny 1974). The other BPUs tested as E.C.s, XRD-473, chlorfluazuron and teflubenzuron, were practically inactive against ECB eggs at 100 ppm. In general, these latter findings are more or less in accordance with those obtained with *S. littoralis* eggs (Ascher *et al.* 1979; Ascher and Nemny 1984; Ascher and Nemny, unpublished results).

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Relative Susceptibility of Certain Sorghum Varieties to Infestation with the Corn Borer, *Sesamia cretica* Led. (Lepidoptera: Agrotidae)

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Sesamia cretica is a serious pest affecting Sorghum varieties. The relative susceptibility of 2 sorghum x sorghum hybrid (G 102 and NK 300) and 2 sorghum x sudangrass hybrid (Sordan 79 and FP 1) varieties to infestation with *Sesamia cretica* under field condition in Shebin El-Kom locality (Menoufia Governorate, Egyipt) was investigated during one sorghum growing season.

The population of the different developmental stages of this borer inside the stalks was significantly higher in sorghum x sorghum hybrid than in sorghum x sudangrass hybrid. Moreover, higher numbers of dead heart were observed on NK 300 than on G 102. NK 300 and FP 1 were the most and least susceptible varieties, respectively, while Sordan 79 was of intermediate susceptibility.

The greater sugar-cane borer, *Sesamia cretica* Led. is a major pest of corn, sorghum and sugar-cane in Egypt. Literature on the relative susceptibility of various maize varieties to infestation with the greater sugar-cane borer had been reported by several investigators (Willcocks, 1925; Issa, 1959; El-Sherif, 1962; El-Sawaf,1964; El-Saadany, 1965;Ismail, 1968; Singh, 1974; El-Saadany and Hosny, 1976; Anonymous, 1978; Mostafa, 1981).

The present work aims to study the relative susceptibility of four sorghum varieties to the infestation with the greater sugar-cane borer *S. cretica*.

Materials and Methods

Field experiments were carried out at Menoufia Province in Shebin El-Kom region during the sorghum growing season of 1986.

Four sorghum varieties: 2 sorghum x sorghum hybrid (G 102 and NK 300) and 2 sorghum x sudangrass hybrid (Sordan 79 and FP1), were tested. Grains were sown during the second week of April. An area of 8000 m^2 was divided into 16 plots, 500 m^2 each. Each variety was seeded in four plots distributed in a completely randomized block design.

Normal agricultural practices were followed and the plants were not treated with chemicals.

Starting from early May until the end of August, a sample of 100 plants of each variety (25 from each plot or replicate) was picked up randomly at weekly intervals. Stalks were examined for infestation by *S. cretica*. Numbers of larvae, pupae and dead heart plants were counted and recorded.

Table 1

Percentage of dead hearts during sorghum growing season of 1986.

Date of sampling	Week	Varieties			
		Sorghum x sorghum hybrid		Sorghum x sudangrass hybrid	
Month		G 102	NK 300	Sordan 79	FP 1
May	1st	0	0	0	0
	2nd	0	0	0	0
	3rd	9	11	7	0
	4th	36	42	18	8
June	1st	53	74	37	28
	2nd	34	57	24	18
	3rd	26	42	21	9
	4th	30	36	13	8
July	1st	31	38	12	9
	2nd	36	47	18	11
	3rd	29	32	13	6
	4th	27	34	11	7
August	1st	22	26	8	5
	2nd	10	16	7	3
	3rd	0	0	0	0
	4th	0	0	0	0
Grand mean		21.4	28.4	11.8	7

Results and Discussion

Percentage of dead hearts

The results presented in Table 1 summarize the relation of *S. cretica* infestation to the percentage of dead hearts of stalks. The periodical inspection revealed that the four tested sorghum varieties (G 102, NK 300, Sordan 79 and FP 1) were all liable to the infestation by the greater sugar-cane borer (Table 1). The initial infestation occurred by the third week of May. The sorghum x sorghum hybrid varieties had the highest level of infestation, while FP 1 received the lowest level of infestation. The degree of infestation by *S. cretica* was evidently high on the treated cultivars during June and July. The maximum infestation rate attained on the first week of June and second week of July. On the other hand, sorghum plants of 30-40 days suffered heavy infestation.

Remarkable differences in the degree of infestation was detected among the different sorghum varieties. Based on the grand mean percentage of dead hearts during the whole season, an extremely high infestation level with *S. cretica* was observed on NK 300 and G 102, while the lowest level was found on Sordan 79 and FP 1.

Larval population

Table 2 shows the population density of *S. cretica* larvae in the different sorghum varieties. Larvae began to occur about first week of May (3 weeks after seeding). Larval population was evidently high in all the tested varieties during May. The maximum number of larvae was in the second week of May. Throughout the first week of June, the

Table 2

Larval population of *S. cretica* during sorghum growing season of 1986.

Date of sampling	Week	Varieties			
		Sorghum x sorghum hybrid		Sorghum x sudangrass hybrid	
Months		G 102	NK 300	Sordan 79	FP 1
May	1st	242	243	112	98
	2nd	366	383	192	103
	3rd	336	362	204	96
	4th	248	288	136	94
June	1st	142	208	98	78
	2nd	157	195	82	62
	3rd	178	209	96	66
	4th	236	276	129	101
July	1st	172	236	109	76
	2nd	98	142	62	22
	3rd	73	111	55	26
	4th	108	152	62	33
August	1st	162	176	77	52
	2nd	42	46	16	11
	3rd	0	0	0	0
	4th	0	0	0	0
Grand mean		160.0	189.0	89.4	57.4

number of larvae tended to decrease then increased again and showed a peak by the fourth week of June. During August, the larval population decreased dramatically and the sorghum plants were completely free of infestation after 15 days.

The sorghum varieties tested showed different degrees of infestation under field conditions. The variety of NK 300 suffered the greatest infestation followed closely by G 102, while the lowest larval infestation was recorded in FP 1.

Pupal population

Data in Table 3 show that the mean number of pupae tended to increase gradually until the fourth week of May. Lower number of pupae was recorded in the different sorghum varieties throughout the period extending from June to the first week of July. Pupal populations showed a considerable peak by the second week of July. Results also showed that the pupal population of *S. cretica* was evidently high on the tested cultivars during August. The maximum population size was attained in the fourth week of August.

Careful view of the literature and data obtained by other investigators clearly indicates that the susceptibility or resistance of a certain maize cultivar to insect pests is governed by numerous factors such as environmental conditions, plant characters, plant chemical constituents, planting region and pest status. Issa (1959) and Willcocks (1925) maintained that the Balady cultivar is a susceptible variety and they attributed this to the

Table 3

Pupal population of *S. cretica* during sorghum growing season of 1986

Month	Date of sampling week	Varieties			
		Sorghum x sorghum hybrid		Sorghum x sudangrass hybrid	
		G 102	NK 300	Sordan 79	FP 1
May	1st	0	0	0	0
	2nd	56	42	27	13
	3rd	72	76	36	29
	4th	86	92	37	33
June	1st	42	67	23	18
	2nd	43	71	19	9
	3rd	57	82	27	9
	4th	66	97	31	19
July	1st	72	99	42	26
	2nd	81	109	47	33
	3rd	70	87	34	18
	4th	66	72	30	15
August	1st	76	83	30	16
	2nd	87	101	42	28
	3rd	88	109	47	29
	4th	93	111	56	37
Grand mean		65.9	81.1	33.0	20.8

relative softness of the plant stem. Another explanation, proposed by El-Saadany and Hosny (1976) is the presence of a high percentage of hydrocyanic acid (HCN) content.

On the other hand, cultivating sorghum plants which show some degree of resistance to borer attack is one of the recent trends in borer control.

According to our results, we recommend FP 1 as the most favourable sorghum variety for sowing in Menoufia province, since we have proved it to be resistant for *S. cretica* infestation under field conditions. However, this evaluation still requires more detailed studies about factors affectig resistance or susceptibility of sorghum cultivars.

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Flight of Moths of the European Corn Borer (*Ostrinia nubilalis*) in Zemun Polje in the Period 1966-1985

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Data on the flight of the European Corn Borer (*Ostrinia nubilalis* Hbn.) in Zemun Polje, Yugoslavia, during a 20 year period (1966-1985) are presented. These data are light trap catches.

A total of 22,309 specimens were captured (13,508 females and 8,801 males).

The flight period in this locality is characterized by two peaks. The occurence of the second peak is one of the indicators of the existence of the second brood.

In last 13 years the contribution of the 2nd brood of the total ECB population was between 52.2% and 84.4%.

In this paper, some other relevant data (dates, sexual indices and others) are also included.

The European Corn Borer (ECB) is the most important pest of maize in Yugoslavia. Therefore, it has been and continues to be an attractive object of research.

In this paper, data on the flight of ECB in Zemun Polje during the period 1966-1985 are presented.

In most papers published in Yugoslavia, ECB was described as univoltine species (Hergula, 1930; Vukasovic, 1932, 1953; Djurkic & Jovanic, 1959; Djulizibaric 1966; Hadzistevic, 1969). The occurence of the 2nd (summer) brood was mostly negligible: less than 3% (Hergula, 1930). An exception to this was the region of Bitola (near the Yugoslav-Greek frontier), where two broods of the ECB have been recorded (Arbuthnot, 1949).

The month of June was most often cited as the period of flight of ECB moths (Hergula, 1930; Vukasovic, 1953).

In recent years, the population of ECB in Zemun Polje has become more and more bivoltine (Baca & Hadzistevic, 1984).

In the past, in the countries bordering Yugoslavia to the North (Austria, Hungary, Romania), ECB was also generally described as an univoltine species (Zwoelfer, 1927; Kotlan, 1930; Arbuthnot, 1949). In the last two or three decades, the significant occurence of the 2nd brood of ECB was more often reported in Hungary (Nagy, 1961; Mészáros, 1971).

In the USA, where this pest was accidentally introduced, the occurence of two broods of ECB has been known for many years (Stuart & Caffrey, 1919; Vance, 1939; Wisfart, 1944; Neiswander, 1947).

Recently, even three and four generations per year were recorded along the southern part of the Atlantic coast and in Missouri, Arkansas, Kansas, Oklahoma and the Gulf states (Showers *et al.*, 1983).

Material and Methods

Zemjun Polje is located at 44°52'N, 20°21'E, and at an altitude of 88 m above sea level.

The flight of ECB in this locality was recorded by use of light traps. This was located at the border of the experimental field, near the buildings of the Maize Research Institute in Zemun Polje. The trap was in operation from May until September during the period 1966-1985. An exception to this were three seasons (1972-1974), when the light trap was in operation only until mid-August.

Two types of traps (similar in design) were used: the self-made type and Agrobecej model PZB-50. The principal parts of the trap are: lamp cover, bulb fitting, collecting funnel with small wings and collecting cage. The self-made trap fitted with a standard light bulb (tungsten spiral thread) of 200W was used during 1966-1972 and with a mercury bulb from 1972 till 1976. The Agrobecej PZB-50 trap fitted with a mercury bulb of 250W was used during 1977-1985. The light intensity of the 250W mercury bulb is four times the intensity of the standard bulb of 200W (11,000 lux/m: 2,800 lux/m). These light sources also differ in quality. It should be noted that the strong mercury bulbs which were installed in the surrounding suburban area in 1972 might have affected the number of specimens trapped.

A total of 22,309 specimens were captured (13,508 females and 8,801 males).

Results and Discussion

Data of the total number of moths of the ECB trapped per year, beginning and end of flight period and some other relevant data (sexual indices, relationship of the 1st and the 2nd brood in total population) are presented in Table 1 and Figure 1.

The number of trapped specimens varied greatly from year to year (60 to 3,625). The average number of trapped specimens per year was 1,105. There were three characteristic periods of flight during 1966-1985.

The first period of first years (1966-1969) was characteriz by a relatively small number of trapped moths (145-481) and relatively small participation of the 2nd brood moths in the total population (2.1%-28.5%). The second period of the next three years (1970-1972) was also characterized by a relatively small number of trapped moths (60-427), but without data on the occurrence of moths of the 2nd brood.

The third period covering the next 13 years (1973-1985) was characterized by significantly greater number of trapped moths (355-3,625) and an exceptionally high participation of the 2nd brood in the total ECB population (52.3-84.4).

The flight period of the 1st brood began in most years at the end of May or beginning of June and terminated in July. The earliest beginning of flight was recorded on the 4th of May and the latest on the 10th of June. The flight peak occured between 12th of June and 10th of July.

The flight period of the 2nd brood began in the second part of July or the first part of August and terminated at the end of August or in September. The flight peak occurred in August (at the earliest 8 August as in 1978, and the latest 30 August as in 1980).

Table 1

Total number of trapped moths of ECB, beginning and termination of flight, proportion of the 1st and 2nd brood and sexual index

Year	Number of trapped moths			Beginning of flight (date	Termination of flight (date)	Relationship		Sexual index		
	Male	Female	Total			1st brood (%)	2nd brood (%)	1st brood	2nd brood	for both broods
1966	216	265	481	24.05.	22.08.	90,0	10,0	0,52	0,90	0,55
1965	197	271	468	05.06.	09.09.	97,9	2,1	0,57	0,83	0,58
1968	177	216	393	02.06.	05.09.	84,7	15,3	0,53	0,65	0,55
1969	101	64	165	30.05.	05.09.	71,5	28,5	0,40	0,36	0,39
1970	144	145	289	10.06.	26.07.	na	na	na	na	0,50
1971	345	82	427	24.05.	14.07	na	na	na	na	0,19
1972	23	37	60	06.06.	29.06	na	na	na	na	0,62
1973	1.152	2.473	3.625	07.06.	29.08.	15,6	84,4	0,62	0,69	0,68
1974	542	1.084	1.626	07.06.	29.08	22,6	77,3	0,52	0,71	0,67
1975	377	1.155	1.532	23.05.	09.09.	28,7	71,3	0,68	0,78	0,75
1976	128	226	354	27.05.	01.09.	43,6	56,4	0,56	0,70	0,48
1977	692	1.103	1..795	04.05.	21.08.	44,7	55,3	0,50	0,70	0,61
1978	361	520	881	20.05.	19.09.	46,0	54,0	0,58	0,58	0,58
1979	502	735	1.237	26.05.	20.09.	21,7	78,3	0,54	0,61	0,59
1980	250	460	710	04.06.	01.10.	40,0	60,0	0,67	0,63	0,65
1981	613	979	1.592	25.05.	31.08.	23,7	76,3	0,49	0,64	0,61
1982	491	520	1.011	13.05.	24.09.	44,2	55,8	0,36	0,63	0,51
1983	1.289	1.522	2.811	18.05.	31.08.	47,7	52,3	0,47	0,60	,0,54
1984	394	475	869	22.05.	31.08.	21,6	78,4	0,40	0,59	0,55
1985	807	1.176	1.983	22.05.	29.09.	16,0	84,0	0,36	0,64	0,59
Total or average	8.801	13.508	22.309			44,7	55,3	0,52	0,66	0,56

These results are in agreement with the results obtained in Hungary (Nagy,1961; Mészáros,1969).

The share of the 2nd (summer) brood in the total population ranged between 2.1% and 84.4%.

The sexual index generally ranged from 0.5 to 0.9. It was lower in two cases only: 0.39 in 1969 and 0.19 in 1971.

The extremely low sexual index of 0.19 in 1971 was followed by both exceptionally small number of trapped moths and low ECB infestation in the next few years.

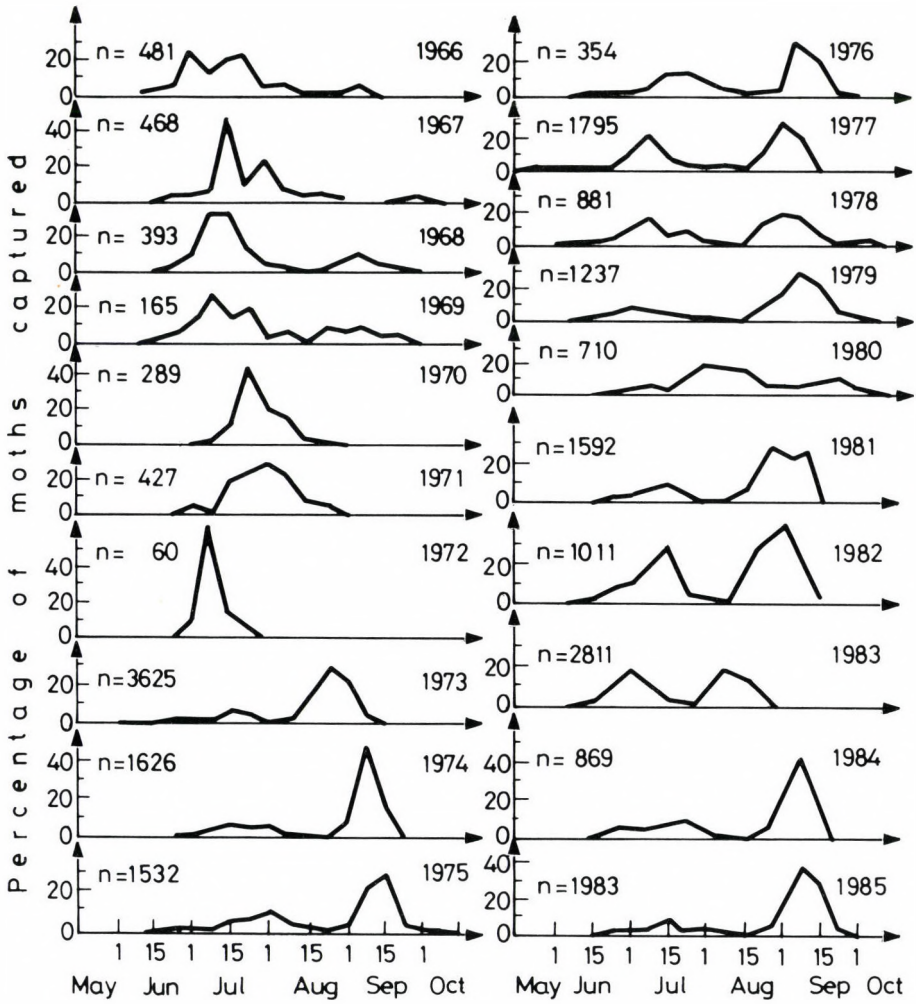

Fig. 1

It is also interesting to note that progenia was observed only in one year (1966) and it is considered as an exception. It also should be noted that the total number of attracted and trapped moths by the mercury bulb 250W was considerably higher than the number of moths trapped by standard bulb of 200W.

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Host Plant Resistance: Maize Resistance to the European Corn Borer (Lepidoptera: Pyralidae)

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The European corn borer, *Ostrinia nubilalis* Hbn., is a primary economic pest of maize, *Zea mays* L., in the United States. At times it is an economic pest of peppers, sorghum, millet, soybeans and cotton. This insect has been found damaging plants as diverse as apple. ECB was introduced in the U.S. from Europe in 1917, where mug wort was the primary host.

Host plant resistance studies began in the U.S. in the 1920's. Considerable progress for resistance to the first generation ECB was made by the 1950's. Many techniques for rearing and identifying first generation ECB resistance have been developed and progress is very good, but resistance to the second generation is slow. However, several sources of resistance to second generation ECB have been identified from exotic germplasm.

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a significant economic pest in the United States, and annual losses of maize, *Zea mays* L. have been estimated from 192-400 million dollars in the Corn Belt.

Before the ECB arrived in the U.S. (1917) and before maize was taken to Europe (ca. 1500), mug wort, *Artemisia vulgaris* L. was the primary host in Europe. This insect is found in association with over 200 hosts including sorghum, millet, peppers, soybeans, cotton, and even apples. The ability of ECB to adjust to meet its dietary needs must be great.

Host plant resistance (HPR) investigations began in the 1920's. Patch and Pierce (1933) conducted early investigations demonstrating comparative reductions of yield and quality maize cultivars infested by ECB. Nearly all cultivars grown at that time were open-pollinated. They found it was necessary to artificially infest plants to insure an adequate, uniform level of infestation. They collected heavily infested stalks from a field in the fall and put them in a cage for moth emergence in the spring. In a laboratory, moths laid egg masses on wax paper, and the egg masses were then cut out on small discs and icubated on holding boards before pinning on the maize plant for hatching. During the early 1950's, artificial diets were studied for rearing insects in the laboratory. Artificial rearing of lepidopterous insects made a significant advance with the development of the Vanderzant-wheat-germ diet (Adkisson *et al.*, 1960). Laboratory rearing of the ECB (Guthrie *et al.*, 1965) was one of the more important advances in ECB HPR.

With the development of inbred and hybrid maize cultivars in the 1930s and 1940s, a resistance rating scale was developed (Guthrie *et al.*, 1960). Another recent development was the use of live larvae for infestation rather than egg masses (Mihm, 1983; Davis, 1976).

The maize plant is not attractive for oviposition or borer survival in the early development (less than 38 cm in height for a hybrid); but, as the plant reaches whorl stage, it may be very attractive. The term "first generation" is used to describe the infestation of ECB on maize when the plant is in the whorl-stage of development. "Second

generation" refers to the ECB infestation of maize plants during anthesis. The time between the two stages of plant development is about equal to the life cycle of ECB. Of course, what this means is that we talk in terms of plant development and insect infestation, rather than the actual generations of the insect during the season.

During the 1940's and 1950's, many sources of germplasm for first generation ECB resistance were identified from U. S. Corn Belt breeding materials. This was relatively easy since many of the resistance genes were available in cultivars already in existing breeding programs and the resistance was readily identified. Using the scale of Guthrie *et al.* (1960), one investigator can evaluate thousands of plants or cultivars in a relatively short time. Consequently, because of the ease of identification of a large number of cultivars, many inbred lines resistant to the first generation were developed and released for hybrid seed production (i.e., Oh43, Oh45, B49, CI31A).

Although it is not necessary to know what makes the plant resistant, such information may make it easier to develop a more efficient breeding program. Scott *et al.* (1966) showed that juice or extract from resistant plants added to susceptible plants reduced ECB larval survival by 50 %. Klun *et al.* (1967) determined that 2,4-dihydroxy-7-methoxy-benzoxazin-3-one (DIMBOA) was responsible for the resistance in maize to first generation of ECB. Later, Sullivan *et al.* (1974) found first generation resistance in some tropical maize germplasm that did not have the high levels of DIMBOA.

Resistance to the second generation of ECB is a different situation. A scale for easy identification of resistant germplasm in tropical maize is not available, but a scale is in limited use where very heavy infestations can be made on Corn Belt germplasm (Guthrie *et al.*, 1978). However, resistant germplasm sources are not available or at least they are not clearly identifiable in Corn Belt germplasm.

The genetic basis for first generation ECB resistance has received considerable attention, but less attention has been given to that for the second generation ECB. Penny and Dicke (1956, 1957) conducted two studies, in one they showed that resistance was controlled by three or more loci and in the other that a single gene-pair controlled segregation for resistance to first generation ECG. Guthrie and Stringfield (1961a,b) investigated the recovery of ECB resistance genes in a back crossing program and by using test crosses they decided that it was easy to recover resistance genes and that segregation for resistance persisted after five generations of selfing. This would suggest as many as 32 loci are involved. Later, Scott and Dicke (1965) and Scott *et al.* (1964, 1966) found that dominance of resistance to first generation ECB was low and that responsible genes were located on the short arm of chromosomes 1, 2 and 4 and the long arm of chromosomes 4 and 6. Second generation resistance genes have been located on the long arm of chromosomes 1, 2, 4 and 8 and the short arm of chromosomes 1, 3 and 5 (Onukogu *et al.*, 1978). These two traits are genetically different and they are quantitative traits.

In 1975, a program for HPR in maize for two maize stem borers was developed in Columbia, Missouri and second generation ECB was to receive primary emphasis. By 1976 it was determined that maize populations, PR-Mo2, PR-Mo2 x MoSQA and PR-Mo2 x MoSQB were more resistant than an intermediate-resistance hybrid, Pioneer Brand 3369a. A selection and breeding program was initiated in 1977 in these populations.

We began by planting 1000+ seeds of each population and infesting all plants with ECB egg masses during anthesis and selfing ca. 400 of these. From the 400 selfed plants,

Table 1

Stalk tunnelling by larvae of second-generation European corn borer in three maize populations at two locations during three cycles of recurrent selection for resistance and the least-squares estimates of gain due to selection.

Year of selection	Mean length of stalk tunnel (cm/plant)				
	Maize populations			Controls[b]	
	PR-Mo2	PR-Mo2 x Mo-SQA	PR-Mo2 x MoSQB	Intermediate	Resistant
1977	22.0	22.0	20.5	–	–
1978	22.6	25.3	20.3	22.6	15.3
1979[a]	22.5	32.5	21.3	30.1	20.3
1980	9.9	13.9	11.8	15.4	10.4

[a] The intermediate control was Pioneer Brand 3369a, except for 1980, when a susceptible single cross (WF9 x W182E) was used. The resistant control was Pioneer Brand 3184.
[b] Cycles of selection were conducted at two locations, Columbia and Portageville, MO., except in 1979, when drought destroyed the Portageville tests.

Type of mean[b]	Mean length of stalk tunnel (cm/plant) in the following maize populations[a]		
	PR-Mo2	PR-Mo2 x Mo-SQA	PR-Mo2 x Mo-SQB
C_0	12.99ab	16.4a	11.7b
C_1	12.3bc	13.9ab	10.2bc
C_2	11.4bc	14.4ab	8.6cd
C_3	10.0c	13.5b	7.3b
Resistance control	9.6c	9.6c	9.6bc
Susceptible control	15.2a	15.2ab	15.2ab
Gain/cycle ±SE	-0.96±41	-0.84±0.40	-1.48±0.34
% Gain cycle, based on predicted value of C_0	7.3	5.3	12.7

[a] Resistant control = Pioneer Brand 3184, susceptible control = single cross (WP9 x W182E); these checks were grown among all three populations.
[b] Means followed by the same letter within a column are not significantly different at the 5% level.

ca. 200 were selected (other agronomic traits considered), harvested, and dissected to measure ECB stem tunnelling. Ten percent of these with the least amount of stem tunnelling provided seed for genetic recombination in our Puerto Rican winter nursery. A row from each selected ear was planted in Puerto Rico as female rows and male rows for pollen were obtained by bulking 10 seeds from each selected ear. Selected ears (3 from ca. 8 pollinations) from each female row were used for insect selection in Missouri during next season. Plants were selfed and selected in Missouri during the growing season. This procedure amounts to two seasons per cycle or one cycle per year.

After five cycles of selection, Mo-2ECB (PR-Mo2 x MoSQB source) and after six cycles, Mo-2ECB-2 (PR-Mo2 source) were composite populations released in 1983 and 1984, respectively, as germplasm sources for second generation ECB resistance (Barry and Zuber, 1984; Barry *et al.*, 1985) (Table 1). About 10 selections from each of the two released populations are currently being studied for inbred line development for homozygous sources of second generation ECB resistance.

Table 2

Partial mean squares from the diallel analyses of variance for tunnel length (cm/plant).

Source of variation	df	Races x Pioneer Brand 3184					
		Columbia		Novelty		Combined	
		MS	*	MS	*	MS	*
Entries	98	17.41	–	27.73	**	29.76	**
Checks vs. diallel	1	262.54	**	222.52	**	484.22	**
Among checks	2	99.30	**	354.67	**	412.27	**
Among diallel	89	12.81	–	18.19	–	16.06	–
GCA	9	29.41	*	59.16	**	63.13	**
SCA	35	14.43	–	12.01	–	12.34	–
Reciprocal	45	8.23	–	14.80	–	9.53	–
Error	184	13.35		13.96			
CV%		65.5		35.0		5.9	

Source of variation	df	Races x (Wf9 x W182E)					
		Columbia		Novelty		Combined	
		MS	*	MS	*	MS	*
Entries	92	16.21	–	42.40	**	32.65	**
Checks vs. diallel	1	27.39	–	69.97	–	4.90	–
Among checks	2	76.47	**	73.38	*	94.11	**
Among diallel	89	4.73	–	41.39	**	31.58	**
GCA	9	28.98	–	59.39	**	56.73	**
SCA	35	10.64	–	27.44	–	16.39	–
Reciprocal	45	15.06	–	48.64	**	38.37	**
Error	184	16.15		23.03			
CV%		42.5		38.9		36.8	

* and ** = significant differences at the 5% and 1% levels, respectively.

We have also been screening materials from the Regional Maize Disease and Insect Resistance Evaluations which originated from the North Carolina State maize program. One of the early selections for second generation ECB resistance from these materials was designated NC 4-275. It came from Dr. M. M. Goodman's collection PAG VI-A, race Moroti Guapi, and this material had been crossed with Dr. C. W. Stuber's D-1 tester. This testcross was more resistant to second generation ECB than the original collection. However, the evaluations of the original collection should be intepreted with caution because this material was not adapted to the environment in which it was tested (Missouri).

Since then, several other resistant cultivars from these regional trials have been identified. Ten of these have been selected and inbred for five or more generations. Each of these lines has been crossed to a resistant (Pioneer Brand 3184) and a susceptible (WF x W182E) maize hybrid, and these testcrosses were evaluated for resistance. The resistant by resistant crosses were resistant and not significantly different from each other at $P=0.05$. The group of susceptible by resistant crosses was significantly different from each other at $P=0.05$ with five crosses being more susceptible than the other five. Each group, resistant by resistant and susceptible by resistant, was made into a set of diallel crosses.

In both cases there was significant general, but not specific, combining ability (Griffing, 1956) (Table 2).

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The Genetics of Morphological and Biochemical Markers in two *Heliothis* Species

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The study of insect control procedures, population dynamics and biology, and even the development of recombinant DNA technology can be greatly aided with the use of visible and biochemical genetic markers. Unfortunately, only a very small number of visible mutations, such as the eye color and body colour mutations, which are reviewed in this paper, have been reported in *Heliothis zea* (Boddie) or *Heliothis virescens* (F) (Lepidoptera: Noctuidae). Somewhat more work has been done on the estimation of allozyme variation in these species, but no extensive geographical comparisons have been reported. Suggestions are given for the development of multiple marker strains and for selection of useful detrimental traits in *H. zea*.

It is ironic that, while maize (*Zea mays* L), the crop plant with which we are concerned in this symposium, is one of the genetically best known of all economically important species (Coe and Neuffer 1977), the genetics of the insect pests of maize are rather poorly understood.

A number of pest control methods have been suggested to supplement or even to replace the use of insecticides. I suspect that ultimate control of maize insects will only be achieved using an intelligent and informed combination of several methods in an integrated control program. However, with the exception of the elegant use of hybrid sterility that is presently being developed for control of *H. virescens* (Proshold et al. 1983b), very little effort is being expended to develop genetic methods of control or to expand our knowledge of *Heliothis* genetic phenomena. My purpose in this paper is to show what kinds of genetic material are now available in *Heliothis* species and to specifically outline some approaches which should be taken to increase our genetic knowledge of the insects which attack maize.

With relation to insect control, I consider that insect genetics research has three primary objectives:

1) The discovery and isolation of simply-inherited mutations which could be useful in biological studies of pest species. 2) Artificial selection or genetic engineering of detrimental traits (either visible or hidden) which can be used in control procedures. 3) Modification of normal behavior through manipulation of genetic characters.

Simply Inherited Mutations

Only five visible mutations have been reported for *H. zea* and *H. virescens* (Table 1). Unfortunately, as far as I can determine, only two of these mutants are still in culture in *H. virescens* and only one is in culture for *H. zea* (as described by Widstrom et al. in this symposium).

Table 1

Visible mutations reported in the literature for *Heliothis zea* and *Heliothis virescens*

Heliothis zea	
yellow eye – autosomal recessive	Jones et al. (1977)
Heliothis virescens	
striped body – autosomal dominant	Raulston (pers. com.)
black body – autosomal dominant (with recessive lethal effects)	Proshold et al. (1983a)
ebony pupa – autosomal recessive	Whitten (1973)
yellow pupa – sex linked recessive	Proshold (1974)

A mutation of *H. virescens*, striped body (J. R. Raulston, personal communication), is an autosomal recessive character which produces a distinctive black coloration of the wings and an even more distinctive striped pattern on the abdomen. This mutation could be an excellent genetic marker because the striped body is so strikingly different from native individuals.

A second mutation in *H. virescens* is a dominant melanic body color mutation, black body (Proshold et al. 1983a), which is not only a good marker, but also shows recessive lethal effects. If black body individuals of either sex are crossed to wild-type (native) insects, half of the resulting progeny are black and half are wild-type. When two black body individuals are crossed, all of the homozygous black individuals die due to the recessive lethal effects of the black body locus. Of the 3/4 of the progeny left, 2/3 have the black body trait and 1/3 are wild-type. Further descriptions and discussions of the usefulness of this mutation can be found in Proshold et al.(1983a) and in Bartlett and Raulston (1982). A demonstration of the use of a similar mutation in *Trichoplusia ni* in a field release experiment can be found in Bartlett and Butler (1975). Since 1/2 to 2/3 of the progeny of black crosses are black, the gene acts as a marker which could be used in subsequent generations to follow the dispersal of both released insects and the progeny of the released insects.

The sex-linked recessive yellow pupae mutation in *H. virescens* (Proshold 1974) has unfortunately been lost, but if it could be re-isolated and crossed with the black body color strain, then one could easily sex pupae by color and release only males into a field population, thus avoiding adding any fertile females to the population and avoiding any increase in population size during the release period.

Electrophoretic variation

Electrophoretic analysis has been used to demonstrate genetic variation within a species and differentiation between species of *Heliothis*. For instance, eight enzyme loci show better than 98% discrimination between *zea* and *virescens* (Sluss et al. 1978a). Huettel (personal communication) found three loci that were diagnostic between *H. virescens* and *H. subflexa* which could be used to follow hybridization between these two

species. Sell et al. (1974a) analyzed 4 polymorphic enzyme systems in *H. zea* and Sell et al. (1974b) studied the inheritance of the esterase-II locus in *zea* and outlined the utility of such a system in studying the dispersal of this species.

Heliothis larvae are ideal subjects for isozyme analysis since their large size (compared to other economically important pests such as the pink bollworm) allows the taking of serum samples for analysis without killing the insect sampled. Sell et al. (1974b) demonstrated that hemolymph samples could be taken from living larvae with greater than 80% survival to adult emergence. The larval proleg can be snipped off and a micropipette used to draw off about 20 microliters of serum. The wound quickly heals and the larvae pupate and produce normal adults. Thus the genotypes of both parents and larvae can be identified and appropriate crosses made for the isolation of homozygous strains of particular electromorphs of interest. In addition, field surveys of isozyme variation can be made, using this technique, and the surviving adults can be checked for morphological variation, reproductive ability, pheromone response, or any other trait desired.

Field populations of *H. zea* and *virescens* show about 76% - 84% of the isozyme loci studied to be polymorphic. Laboratory strains are somewhat less variable (67%) than this (Sluss et al. 1978b). Daly and Gregg (1985) estimated 32% of the enzyme loci in *H. armigera* and *H. punctigera* field populations were polymorphic. An interesting isozyme comparison, that apparently has not yet been done, would be between *H. armigera* and *H. zea* since taxonomists do not seem sure whether these two are really separate species (Nye 1982).

Speculation

Since there is so little information available on the genetics of *H. zea*, I felt that it would be of interest to examine the literature and to see what kinds of characters one could find that might be selected to disrupt normal behavior or normal development to produce strains useful in a genetic control program. I was interested in characters that could be easily analyzed in most entomological research laboratories. Techniques useful for the isolation, use, and maintenance of genetic markers in economic insects have been given by Bartlett (1982) and Bartlett and Raulston (1982).

Neonate larvae of *H. zea* may exhibit positive geotaxis as they follow the corn silk into the ear, where they then cause damage to the kernels as they develop. Strains of *Drosophila pseudoobscura* have been successfully selected for increases in both positive and negative geotaxis (Woolf et al. 1978). Selection for negative geotaxis in *H. zea* would be extremely simple in the laboratory or field. One would infest eggs at a given point on a perpendicular surface, capture those larvae that climbed upward for a given distance on the surface, rear them to adults, and breed only from those that had met the selective criterion. If one observed an increase in the numbers of neonates that climbed upward as generations of selection increased, then additional selection should produce a strain homozygous for that character. The genetics of the trait could be determined and the strain used in release experiments to introduce the character into field populations, thus producing larvae that climb up the plant rather than down into the ear. Longer exposure to parasites, predators, and insecticide residues could lead to higher mortality in neonates

and thus reduce damage to the crop. If it is discovered that larval movement is chemotropic rather than geotropic, similar experiments could be run with the same goal in mind.

Certain corn cultivars produced high titers of a flavone glycoside (called maysin) in the corn silk and those cultivars showed increased resistance to *H. zea* (Waiss et al. 1979). Apparently it is difficult to get this character into commercial varieties of corn and levels of maysin decline as the corn ages (Waiss et al. 1982). Why doesn't an insect geneticist exploit this finding and select for increased susceptibility to maysin in the insect? The process will involve sib selection procedures which are well known to population geneticists (you can't breed from an insect you have just killed with maysin). But I am certain that variability in susceptibility to maysin exists (just as variability to susceptibility to insecticides exists) and that variability can be exploited in a selection program. When the susceptible strain has been obtained then release procedures which have already been developed can be used to drive the character into field populations.

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Biological Observations and Control of the Frit Fly (*Oscinella frit* L., Diptera, Chloropidae) on Maize Plantations in the Wielkopolska Region, Poland

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In the years 1980 -- 1987, field experiments were carried out to establish:

1) the dispersion of the frit fly from the edges of small- and medium-sized (10-30 ha) maize fields and the damage caused by larval feeding;

2) the dispersion range and egg laying by the frit fly at the edge of maize fields;

3) development of methods for treatments of maize plants at the edges of fields;

4) selection of the most effective insecticides for the control of frit fly adults.

The frit fly, a constant representative of the cereal crop fauna in Poland, lives on many grass species causing damage to all the cereal species and, more recently, also to maize. Larval damage caused by the larvae is estimated as 10-30%, with a yield reduction of about 4-10 q/ha (Kania 1962, Muller 1972, Lisowicz 1979, Bubniewicz 1984, 1986).

Materials and Methods

Experiments were performed in a random block design with 4 replicates. The area of one plot was 42.0 m^2. Six rows of maize 0.7 m apart were sown on each plot. Field trials were carried out on 10-30 ha. Granular insecticides were applied by a Horstine Farmery type applicator; for liquid insecticides, a tractor sprayer with a beam of 8 and 12 m wide was applied. Maize seeds were treated by the use of a hand drum seed-dresser.

Damage caused by the frit fly larvae was assessed at 6-8 leaf-stage. Five neighbouring plants in a row were taken from 10 places of each plot. Plant damage was estimated according to the scale based on the classification of Dolinka and Manninger (1962).

Studies were carried out on the varieties:

1980-1985 – KB-270 (Polish), Bukovinsky-3 (USSR), KB-270 and KB-310 (Jugoslavia), KB-DC-310, KB-DC-270 and Szegedi MTc-255 (Hungary).

1986 – KB-DC-270 and Szegedi MTc-255 (Hungary);

1987 – Mona, Dea and Scandia (USA).

Results

Frit fly adult captures showed their presence first at the edges; later they penetrated the field. Maize plant damage caused by the larvae at different distances from the field edge also showed spatial differences (Table 1):

In the centre of the field, damage was rarer. In small- and medium-sized (10-30 ha) maize fields frit fly adults concentrated in the marginal zone of the fields; they

Table 1

Frit fly damage to maize plants in relation to the distance from the field edge (Winnagóra 1981-1983).

Years	Field size (ha)	Distance from the field edge (m)	Plant damage %	index (Ipiff)
1981	12	0-24	13.8	0.171
		24-48	19.5	0.270
		48-72	12.6	0.169
		72-96	6.2	0.072
		96-120	5.9	0.075
1982	24	0-50	12.5	0.150
		50-100	5.4	0.060
1983	8	0-30	28.8	0.286
		30-45	24.6	0.227
		45-55	13.2	0.097

migrated about 100-150 m, max. 200 m into the field, depending on the surrounding cereal crops, meadows and pastures.

In 1981-1982, imagines of the first and second generations were trapped in the marginal 0-50 m of the field. The adults of the 2nd generation were found to carry red mites, *Microtrombidium demeijerei* Ouds. (Golebiowska, 1951), frequently located beween the abdominal segments and at the base of the elytron. It is suggested that this mite decreases the mobility of adults. As a consequence, protection measures against the frit fly can be restricted to edge zones, especially in years with low frit fly abundance.

The generally accepted damage threshold for the frit fly is 6 eggs per 10 plants or 4 eggs per 1 m^2 of maize. Treatment is limited to young plants. Application methods are: a) granular insecticide drilled into the soil during sowing; b) seed dressing; c) spraying of

Table 3

The effect of seed dressing against the frit fly, 1984-1986

Insecticides (active ingredient)	Dose per 1kg seed	Plant damage %	Index Ipiff
Furadan 35ST carbofuran	0.15kg	2.2	0.034
Marshal 25ST carbosulfan	40 g	2.0	0.012
PrometSCO666 furathiocarb	12 cm+6 g Zeolex	9.6	0.118
Control	–	40.1	0.666
Promet 300EW furathiocarb	0.12 l	4.0	0.040
Promet300EW	0.18 l	2.7	0.027
Control		41.3	0.565

Table 2

The effectiveness of insecticides against the frit fly on maize, 1980-85.

Insecticide		Doses	Plant damage %	Index Ipiff
Granulated insecticides (kg/ha)				
1.	Furadan 5G	30.00	4.9	0.052
2.	Dursban 5G	25.00	10.5	0.129
		20.00	15.0	0.225
3.	Temik 10G	9.25	2.0	0.026
		7.75	3.4	0.035
4.	Dyfonate 5G	20.00	10.0	0.075
5.	Dyfonate 10G	15.00	4.2	0.054
6.	Control		39.2	0.621
Liquid insecticide (l/ha)				
1.	Owadofos 50 fluid	1.50	18.2	0.495
		1.00	12.0	0.296
2.	Ekamet 50 EC	1.50	13.5	0.159
		1.00	13.0	0.256
3.	Deltanet 400 EC	0.60	17.5	0.195
4.	Decis 2.5 EC	0.40	5.7	0.124
		0.30	9.3	0.153
5.	Decis B	0.20	7.5	0.115
6.	Ambush 25 EC	0.40	13.5	0.099
7.	Ripcord 10 EC	0.30	5.4	0.108
		0.20	8.0	0.135
8.	Cymbush 10 EC	0.30	7.0	0.078
		0.20	10.5	0.159
9.	Fastac 10 EC	0.15	8.5	0.125
10.	Sumialfa 5 EC	0.10	12.5	0.225
11.	Control	-	41.9	0.701

Table 4

The effectiveness of the insecticide Promet 300EW in controlling the frit fly on maize in 1987 (precise experiments)

Variety	Chemical (insecticide)	Plant damage %	Index Ipiff	% decrease
MONA	Promet 300EW	3.6	0.029	83.9
FAO 250	Control	22.4	0.151	
DEA	Promet 300 EW	6.4	0.070	70.9
FAO300	Control	22.0	0.204	
SCANDIA	Promet 300 EW	9.6	0.082	60.0
FAO 160	Control	24.0	0.251	

plants at 1-3 leaf-stage. The effectiveness of the treatments was very high (Tables 2, 3), reaching 73-89% reduction of damage, with some variation by cultivars.

Discussion and Conclusions

The decision concerning the method of control depends on whether the pest is present before maize sowing. A prognosis can be based on the degree of the frit fly occurrence on winter crops in autumn or early spring in the neighbourhood of maize field. At predicted high rates of attack, seed dressing or soil treatment is recommended. These methods are effective also against soil pests.

In the case of a late appearance of the pest or weak infestation, the spraying of marginal bands or entire fields with insecticides should be used. This, however, is effective against egg-laying females at lower temperatures. They should be applied at 2-leaf-stage or earlier. Later, insecticides with contact and systemic effects are recommended, which kill young larvae, too. The best results were obtained with Deltanet 400 EC, Marshal 25 EC and Decis B. Seed dressing was most effective with Furadan 35 St, Marshal 25 ST and Promet 300 EW.

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Effect of Intercropping Maize-beans on Aphids and Aphidophagous Insects in Corn Fields of Southern Quebec, Canada

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Species composition and temporal distribution of aphids and their predators were studied in corn fields of southern Quebec, Canada. Effects of increased vegetation complexity (monoculture vs diculture) on these distributions were tested. A completed randomized block design was used consisting of two treatments, corn and corn with beans. Sixty corn plant were sampled weekly. Results show that the abundance at the two aphids *Rhopalosiphum maidis* (Fitch.) and *R. padi* (L.) did not differ in the two treatments. In contrast, the abundance of *Metopolophium dirhodum* was significantly higher in the monoculture than in the diculture. For the predators, the abundance of *Coleomegilla maculata lengi* Timb. and *Hippodamia tredecimpunctata* Say were significantly higher in the monoculture than in the diculture, but not those of *Coccinella septempunctata* L. and of spiders. The presence of bean plants in the diculture influenced primarily those aphids which exploit the lowest stratum of the corn plant. Results for predators contradict prevailing ecological theory, which predicts higher densities of predators in polycultures than monocultures. Differences in coccinellid population densities were caused by different aphid abundances in monoculture.

A mixture of corn and beans is the most prevalent polyculture in the Americas. Studies show that under a wide variety of climatic and soil conditions, corn produces as well or better when associated with a legume than it does in the monoculture (Faris *et al.* 1983). According to van Rheenan *et al.* (1980), maize-beans polycultures have been effective in the reduction of 22 pests and diseases. The resource concentration hypothesis (Root 1973) predicts that stenophage insect densities are lower in environments of higher plant diversity than in more simple habitats. Pimentel (1961) predicted that predation on herbivores should be higher in polycultures than in monocultures, but Andow and Risch (1981) showed that the density of aphidophagous coccinellids was higher on corn in monoculture than in maize-beans polycultures.

The purpose of this study is to determine the effects of diculture on abundances of corn aphids and their predators. We hypothesize that increased vegetation complexity reduces aphid densities while maximising coccinellid and spider densities in maize-beans diculture when compared to maize monoculture.

Methods

This study was conducted during the summer of 1985 at St-Hyacinthe Quebec, Canada (60 km east of Montreal). The experimental plots consisted of three replicates of maize monocultures and maize-beans dicultures treatments (completely randomized block design). Treatment areas were 15 m x 22 m and all blocks were separated by 7 m of weedy vegetation. Corn was grown in all plots at 76 cm spacing. One row of beans was sown between corn rows, leaving no space between beans and corn. Plots were sprayed

Table 1

Vertical distribution of aphids on maize monoculture.

	low leaves	ear	high leaves	tassel
R. maidis	9%	84%	2%	8%
R. padi	36%	55%	8%	1%
M. dirhodum	79%	0%	21%	0%

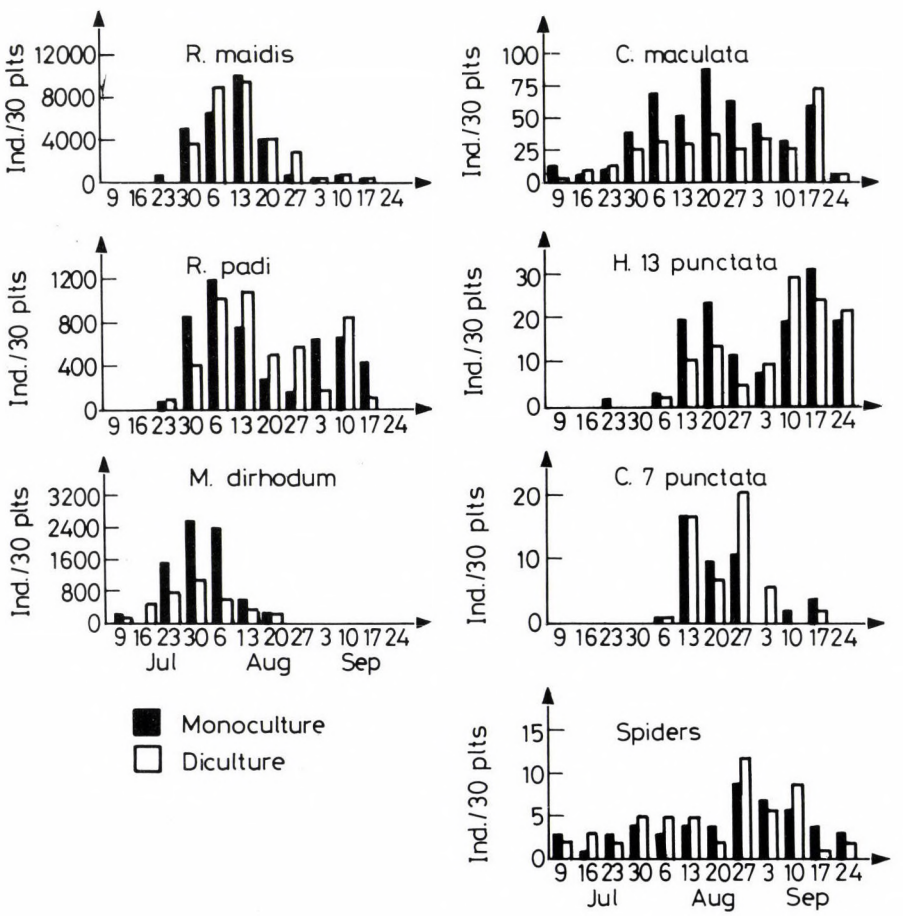

Fig. 1. Abundances of aphid and aphidophagous species in maize monoculture and beans diculture

with Round-up (TM) herbicide in mid-may, and weeding was done manually afterward. The plots had not been treated with insecticides in the previous years.

10 plants per treatment were sampled weekly from mid-May to the end of September. No individual corn plant was sampled more than once in the summer. The entire corn plant was inspected visually. Each plant was subdivided vertically into 14 levels corresponding to the leaves, the ear and the tassel.

Results

Aphids started to arrive on maize at the beginning of July. *R. maidis* dominated in the monoculture and the diculture. This species peaked in August when the plants were mature (Fig. 1) and was concentrated on the ear, under the spathes (Table 1). *R. padi* followed a bimodal distribution with a first peak abundance during pollination and a second in September when the plant was partially dry (Fig. 1). This species also preferred the ear (Table 1), but was found on leaves in July. *M. dirhodum* peaked at the end of July, before pollination (Fig. 1) and was found exclusively on the leaves closest to the ground (Table 1). *R. maidis* and *R. padi* abundances did not differ significantly in monoculture and diculture during all the period of aphid occurence (Fig. 1). In contrast, *M. dirhodum* abundance was significantly higher in the monoculture (two-way anova, $p<0.05$) than in diculture (Fig. 1). Populations were four times lower then beans were present, at the end of July.

The coccinellid *C. maculata* largely dominated the other aphidophagous insects in maize. This species peaked in August in the monoculture and in September in the diculture (Fig. 1). *H. tredecimpunctata* followed a bimodal distribution in both treatments and peaked in August and September (Fig. 1), whereas *C. septempunctata* obtained its highest abundance in August (Fig. 2). The spiders found on corn were diverse (19 species) but of very low abundance (Provencher et al. 1988). The commonest species were *Tretagnatha laboriosa* Hentz and *Clubiona pikei* Gertsh. The general trend for all spiders combined was an oscillation of densities around a plateau in July and August (Fig. 1). *C. maculata* and *H. tredecimpunctata* abundances were significantly higher in the monoculture than in the diculture (two-way anova, $p<0.05$). However *C. septempunctata* and spider abundances did not differ significantly in the two treatments.

Discussion

The resource concentration hypothesis (Root 1973) is supported by the results for *M. dirhodum* but not by the results observed for *R. maidis* and *R. padi. M. dirhodum* is the only species concentrated on the low leaves of corn and is easily in contact with beans foliage. *R. maidis* and *R. padi* preferred the ear and the tassel. Hence, the diculture will influence primarily aphids exploiting the lower stratum of the corn plant. The other aphids, hidden most of the time beneath the husk, cannot be influenced by the presence of bean plants.

The results for predators contradict prevailing ecological theory (Pimentel 1961), which predicts higher densities of predators in polycultures than in monocultures. Dif-

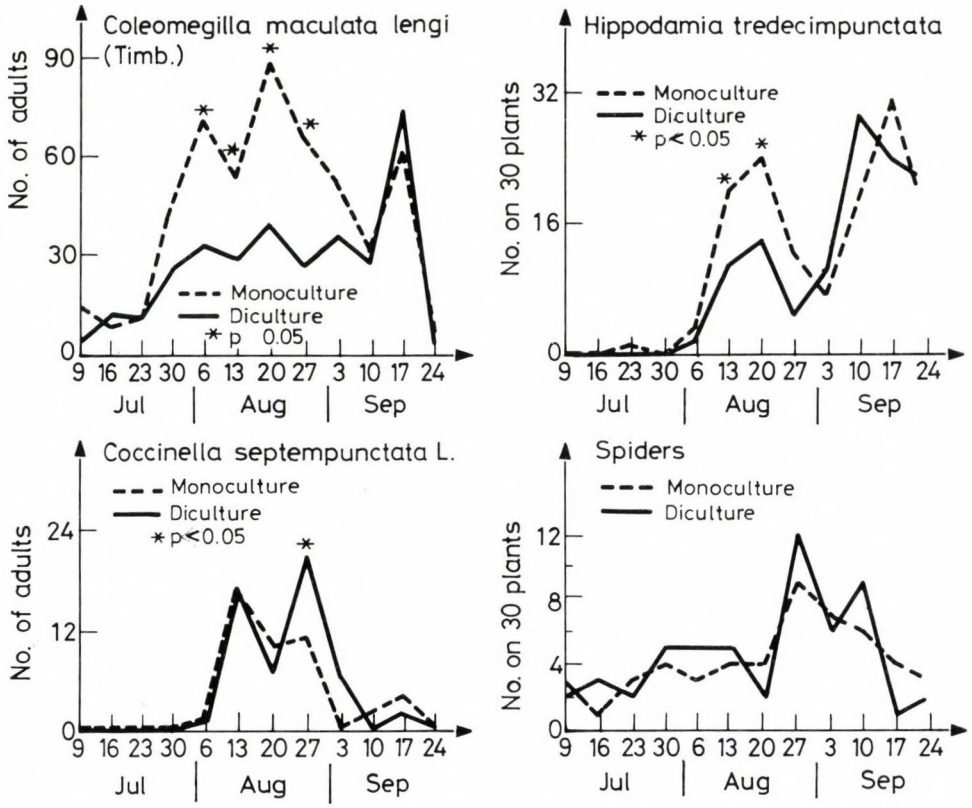

Fig. 2

ferences in coccinellid population densities were probably caused by higher aphid abundances in the monoculture. *C. maculata* and *H. tredecimpunctata* showed an aggregative numerical response to prey availability. We suggest that there were not insufficient alternative prey (e.g. aphids attacking beans) to maintain predators in a corn field supporting fewer aphids. In fact, the diculture increased the spatial heterogeneity of food resources, which could have reduced the predator population abundance and effectiveness (Andow and Risch 1985).

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Adaptive Dispersal in the European Corn Borer *Ostrinia nubilalis* (Lep.: Pyralidae) in Northwestern Switzerland

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To determine the dispersal of corn borer moths (one-generation, Z-strain), 12 black-light-traps were placed at emergence (wheat) and oviposition (first year maize) sites and at intermediary positions in grassland at a distance of at least 50 m from the emergence and oviposition sites.

At both the emergence and intermediary sites, up to 90% of trapped females were mated. Males out-numbered females significantly at the place of emergence (77%) but not in the maize fields (40%). Half of the females at the emergence sites were carrying eggs and a quarter of these were unmated. We therefore hypothesise that the dispersal flight for most females occurs after mating in the fields from which they had emerged and before the first oviposition. Males do not seem to perform such an adaptive dispersal.

Dissection revealed that 16-21% of the mated females contained more than one spermatophore. Females with one or two matings occured at all sites, but females with 3 spermatophores were found only in maize fields. Nomadism is characterised by intermittant oviposition among neighbouring fields, where additional matings take place.

Air temperatures below 13°C supress dispersal flights from maize or wheat fields, while appetitive flights for mating and oviposition within the vegetation layer are still possible because the higher temperature within the crop.

The distribution and abundance of an insect species depends heavily on its flight and oviposition strategies. To survive in unstable or spatially extreme habitats, many agricultural species have adapted to a very dispersive or even nomadic life style. Hence there is a need for area-wide and regional pest management studies for highly mobile, polyphagous insects such as the European Corn Borer (ECB) (Rabb & Kennedy 1979). Attainment of pest status by the ECB in Northern Switzerland and its expansion into new habitats is linked to the huge area of lush host crop provided by the increasing cultivation of grain corn. The annually changing distribution of overwintering and oviposition plots in areas employing crop rotation could have led to the selection of populations adapted to migration. Since 1972 there has been a Southward progression of ECB from the Rhine Valley, where the study area is situated, across the 600m high mountain chains and following tributaries of the Rhine (Bigler & Bosshart 1987). The distribution of ECB is estimated to be expanding at a rate of 3-5 km/year (Jäggi 1987, and pers. comm.). Only recently, have population movements begun to be assessed in Europe (e.g. Stockel *et al.* 1986). The objective of this contribution is to present information on the pattern of dispersal of ECB emerging from a non-host culture.

Material and Methods

Between 23 June and 15 August 1985 the flight of the ECB was monitored by 12 black-light traps (BLT). These were battery operated, using 20W light tubes of near-UV

and visible light (Sylvania F20T12/BL). Eight traps were of the usual omni-directional design and four of a directional type of monitoring migration rates into and out of a maize field (Cordillot & Duelli 1986).

The survey has been conducted in North-western Switzerland in an area where maize fields are spaced at a maximum of 250 m from each other (Cordillot 1988). The BLTs were located at kinds of sites: (1) 2 BLTs in wheat fields which in the previous year had been maize fields, i.e. emergence fields; (2) 4 BLTs in maize fields, i.e. sites for oviposition; (3) 3 BLTs in grassland with no previous maize, at intermediary positions, at 50-130 m away from the nearest emergence sites and at 80-150 m away from the nearest maize fields. Thermo-hyrographs were placed in maize, wheat and grassland (Fig. 1).

The catches were collected at least every 3 or 4 days and the sexes were determined. The reproductive condition of the females was noted: mature (carrying eggs in their ovarioles) and mated (containing spermatophores).

Results and Discussion

Sex-ratio in different habitats

Of the 2455 ECB moths caught, 1295 (52.7%) were males, 1160 (47.3%) females. A total of 65.6% were found in maize, 24.1% in wheat, and 10.3% at the intermediary positions. While males predominate(77.2%) in the catches at the emergence fields, females predominate (60.1%) at the oviposition sites (Yates corrected Chi-square for the sex-ratio differrent from 50%: $P_{(e)}<0.001$, resp. $P_{(o)}<0.05$). At intermediary positions the sex-ratio was more even ($P_{(i)}$ N.S.) (Fig. 2). In the same region, Büchi et al. (1981) also found 71.8% males in a wheat field (formerly maize) about 400 m far from the nearest maize plot. BLT catches during 1981-83 (Reh 1985) further North in the Rhine Valley, Germany, revealed also that in maize plots females, in general, out-numbered males by 1.3 to 5.3. The same was observed in France by Stockel *et al.* (1985, 1986).

Males are found concentrated around emergence sites. They might have accumulated there either because of calling virgin females after emergence or due to an innate sedentarism. Females out-number males in maize fields because these are preferred oviposition (Fiala et al. 1985) and feeding sites (dew containing sugars). Males may reach maize fields by following the tracks of female pheromones (Stockel *et al.* 1985) or maize kairomones. The even sex-ratio at the intermediary places between emergence and oviposition fields indicates an equal contribution of both sexes in dispersal flights.

Mating places and dispersal

Of a total of 1160 females caught, 88-94% were mated, even at the emergence sites, where a higher proportion of virgins might have been expected. On the other hand, virgins were also found in first year maize.

Checking the ovarioles of females we found both virgins and mated females with eggs. In wheat, 25% of the virgins (N=16) were carrying unfertilized eggs, in maize 20.7% (N=29), and at the intermediary positions 9.1% (N=11) (Fig. 3).

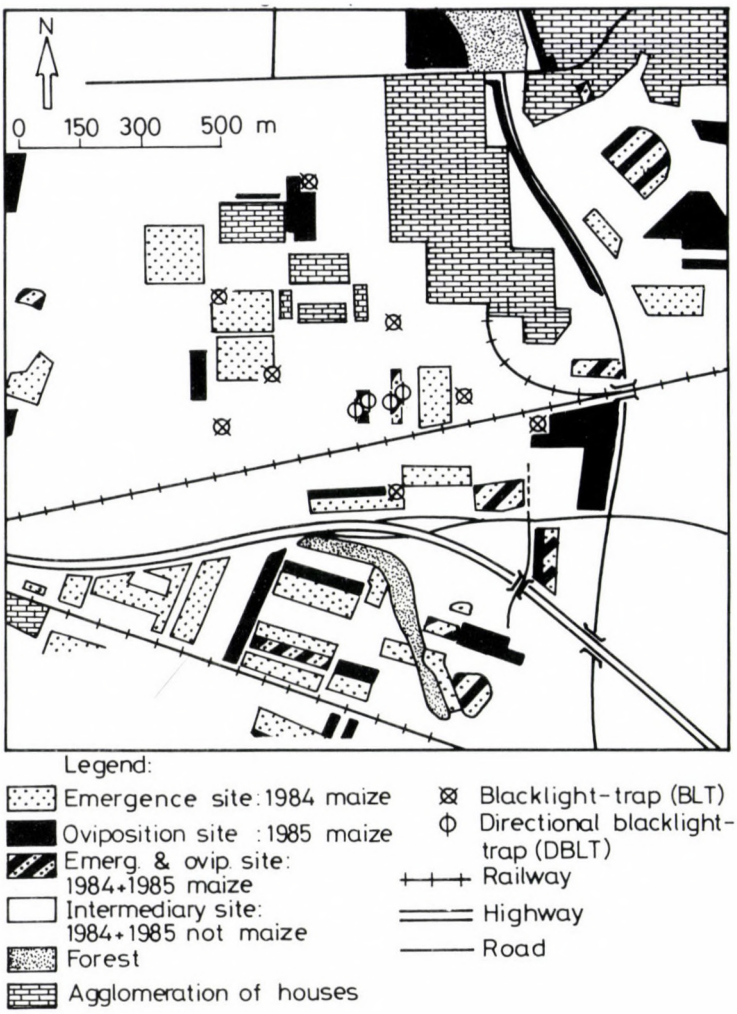


Fig. 1. The spatial distribution of the maize fields and the location of the 212 blacklight-traps

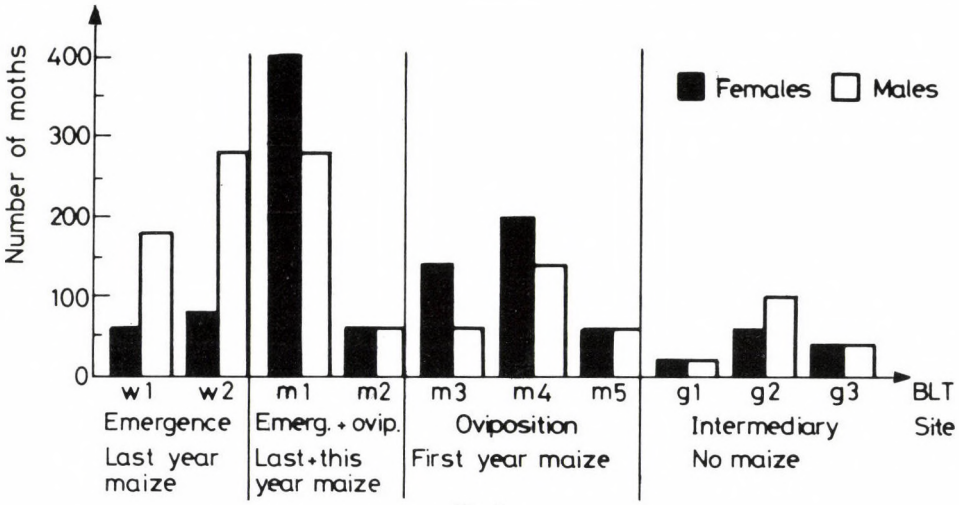
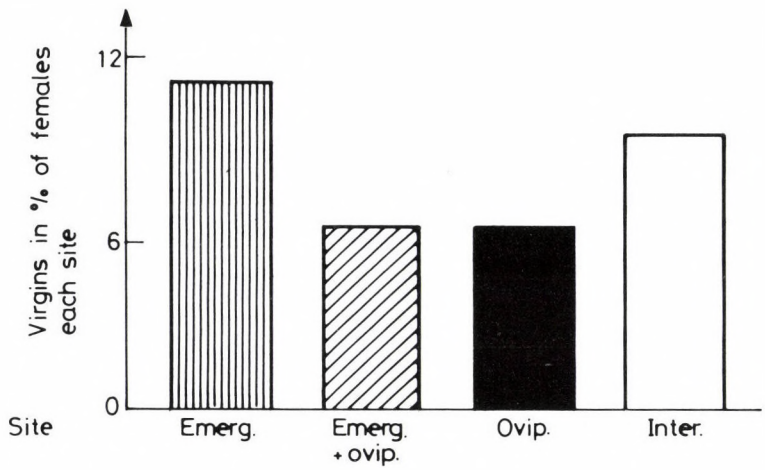


Fig. 2



Virgins/BLT	8	11	7	4
Females/BLT	68	160	109	37
Nb. of BLT	2	3	4	3

Fig. 3

The average number of spermatophores per mated female in each location was slightly more than 1. Multiple mating seems to be a rare event: 80.1% of the mated females (n=794) contained one spermatophore, the rest having two (n=188) or three (n=10). Females with 3 spermatophores were captured only in maize fields.

The exact location of the mating place appears quite controversial. Some investigators claim that aggregation and sexual activity occur as much as 100 m from the nearest corn field in so-called "aggregation/action sites" (Showers *et al.* 1976). Others found that mating takes place at the borders of maize plots, at so-called "rendez-vous" places (Stockel et al. 1985) or within the crop itself (Büchi *et al.* 1981; Stengel pers. comm.). Here we present evidence that first matings take place at the site of emergence, and that subsequent copulations are performed within or close to the ovipositon sites.

This is supported by the fact that most of the females caught at the overwintering sites had mated, and by the high concentration of males found there. Moreover, matings at emergence fields have been observed recently (Welling, 1987, pers. comm.). The observation that virgin females containing eggs were found at all collecting sites indicates that there are always some older virgins (carrying unfertilized eggs) which seem to be unable to mate. The homogeneous distribution of mated females within the whole area indicates a highly mobile flight behaviour of females mated once or twice. Similar proportion of mated females in maize and away from maize fields have already been observed by Büchi et al. (1981) near our study site and by Pesho (1961) in the USA. For mated females we therefore assume a nomadic flight behaviour after the first oviposition. The fact that the few females containing three spermatophores were only caught in maize, may indicate that they finally become sedentary there.

Air temperature for dispersal flights

At air temperatures just below the critical activity threshold of about 13°C (Cordillot 1988) as measured at open sites, no flights were recorded at the intermediary trap positions. But females might still emerge, mate or oviposit, if within the host crop the temperature remains above 15°C. While the air might be too cold for an extended dispersal flights, the moths can get accumulated within the crop vegetation.

General Discussion on the Dispersal Dynamics

We conclude that first matings take place at the emergence sites before dispersal. We suggest that extended dispersal flights from the place of emergence, take place after mating there. Additional matings may occur both away from and within maize fields during the nomadic period of the oviposotory stage. The post-copulatory flight behaviour, mainly attributed to the females, can be considered as "adaptive dispersal" (Fig. 4). Main vectors of spreading risks are the females mated once. This adaptive strategy of nomadism, together with the initial dispersal after emergence, is adapted to the yearly changing distribution and size of maize plots. It maximizes outbreeding, thus reducing the probability of local extinction. Some gravid females regularly fly beyond the breeding area, causing new outbreaks. However, uni-directional movements of EBC populations extending over 32 to 42 km, as reported in the United States by Schurr & Holdaway (1965) have

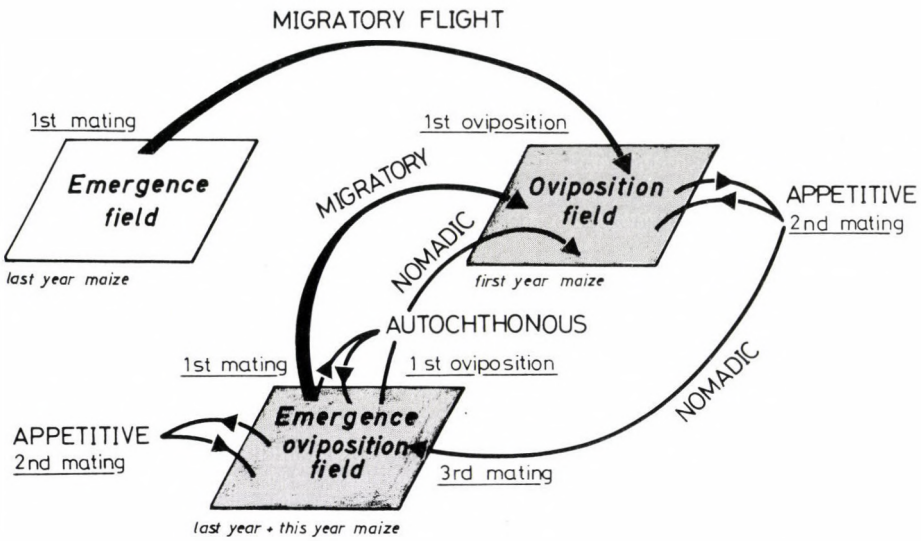


Fig. 4. Model of spatial dispersal dynamics of the European Corn Borer moths

never been observed in Europe. A better understanding of the basic strategies of dispersal and oviposition of EBC will improve predictions and explanation of outbreaks and will have implications for control practices of crop protection.

Acknowledgements

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The Influence of the Surroundings on Arthropod Diversity in Maize Fields

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Faunistic investigations in and around small sized (<2 ha) maize fields in a diverse mosaic landscape in Switzerland have shown that the arthropod fauna can be quite diverse, depending very much on the habitat quality of the surroundings.

Faunal exchange between maize and surrounding vegetation was monitored directly along the field edges using directional traps for surface dwelling arthropods.

The species richness of spiders and carabids was significantly higher in small fields and plots neighbouring semi-natural areas, even after excessive use of herbicides.

It is possible to maintain a high arthropod diversity even in intensely cultivated areas, as long as small sized plots of a variety of different crops are arranged in a mosaic pattern together with patches of natural or semi-natural areas.

In Central Europe, maize fields are considered the epitome of the cultural steppe, where the soil is bare of ground cover most of the year. Monoculture, heavy treatment with biocides, and an ever increasing area over used by industrial farming techniques have created an extremely bad image for maize fields, not only among naturalists.

However, faunistical investigations in and around small-sized maize fields in a diverse landscape in Switzerland have shown that the arthropod fauna, even in maize also can be quite diverse. In the course of a project extending over several years, we tested the hypothesis that the diversity of arthropods in crop fields depends very much on the habitat quality of the surrounding biotopes.

Material and Methods

In the Upper Rhine Valley in North-western Switzerland, where the densely populated landscape is characterized by a diverse pattern of land use, the arthropod fauna of crop fields and semi-natural areas was monitored by means of different trapping devices. Surface dwelling arthropods were collected in pitfall traps. The following results are based on catches of carabids and spiders from pitfall traps operated from 1983 to 1985.

Two types of pitfall traps were used. One consisted of a funnel of 15 cm diameter, placed inside a PVC tube. The second type was a directional pitfall trap, where two parallel troughs of 1.5 m length collected arthropods according to the direction of their approach (Duelli et al., unpublished).

Results

In two years of sampling in a maize field of 1.6 ha, a total of 78 species of spiders and 76 species of carabids were identified. How much of these astonishingly high numbers

for a heavily cultivated crop field can be attributed to intense collecting efforts, how much to the influx of faunal elements from surrounding biotopes in a diversified landscape? Two approaches were used to find evidence of faunal exchange: 1. Direct measurement of population movements over the field borders. 2. Comparing arthropod diversity in various maize fields with different surroundings.

Direct measurement of population movements over the field borders.

In a control experiment to check the consistency of the traps designed to measure directional movements, six directional pitfall traps were linked together by 25 cm high plastic barriers. All carabids and spiders attempting to cross this 20 m length of field border were collected either as "immigrants" or "emigrants". For carabids the ratio between immigration (in) and emigration (out) did not change considerably between the 6 traps. The fact that the traps at both ends of the row yielded the same degree of directionality as the traps in the centre of the barrier, where random movement around the trap was prevented, indicates that even a single trap of 1.5 m length gives a reliable measure of directionality. Four single traps were placed along the four sides of a 1.6 ha maize field. The results, which cannot be presented here in detail, can be summarized as follows: (1) The numbers of species and individuals were not significantly lower in the field centre than along the field border. (2) For carabids, all border trap catches showed more immigration than emigration. Irrespective of the surrounding crop or vegetation, on average 20% more carabids crawled in than out. The same was true in the experiment with the six linked barrier traps. A closer look revealed that the smaller the carabids, the higher the proportion of net immigrants, while the larger carabid species showed an even distribtion. A different picture emerged with spiders, where the numbers of species and individuals were always higher in the traps at the eastern side of each trap compound, even in the field centre. Westerly winds are prevalent during daytime in this area. As with the carabids the number of species and individuals in spiders were not significantly lower in the central traps than the traps at the field border.

Comparing arthropod diversity in various maize fields with different surroundings.

The influence of the surroundings can be shown by comparing maize fields on similar soil types, but with bordering biotopes of different faunal richness. Table 1 compares the carabid and spider fauna in various maize fields. For this comparison the catching method, number of traps and period of sampling have been standardized. No insecticide treatments were applied in any of the investigated maize fields, but herbicides were used in all fields. A double load (by mistake) in a 1.6 ha field in 1983 did not reduce diversity in that field considerably.

Maximum numbers of species and individuals were collected in a small strip of maize not far from semi-natural areas. All three fields in the Sisseln area had a much greater diversity of spiders than the field at Ins, in a typical area with intensive agriculture (Hanggi 1987). The size of the Ins field is comparable to the ones at Sisseln, but is surrounded by other crop fields of equally low arthropod diversity.

Comparison of faunal diversity in maize fields with different types of surroundings

Nine weeks of sampling in June - July	Spiders		Carabids	
	species	indiv.	species	indiv.
INS BE 1984 0.8 ha surrounded by intense agriculture	16	1161	n.a.	n.a.
SISSELN AG 1983 1.6 ha double load of herbicides, close to semi-natural area	40	745	31	868
SISSELN AG 1985 1.2 ha isolated maize fields, surrounded by industrial complex	41	755	33	1514
SISSELN AG 1986 0.2 ha 10 consecutive years of maize, small strip (20 by 100 m) adjacent to semi-natural area	45	2245	37	12'596

Discussion

When do we consider a fauna diverse? Species richness is a relative measure. A spider fauna of 16 species in a maize field of less than 1 ha is low, if we compare it to other biotope types in the same region (Hanggi, 1987). Our maize fields at Sisseln yielded 40 to 45 species of spiders and 31 to 37 species of carabids in 9 weeks of sampling with 5 funnel traps or 4 elements of directional pitfall traps per field. Combining the spider and carabid data from 12 trap elements over the whole growing period of maize for the 1.6 ha field, we reach a species total of 78 for spiders and 57 for carabids. Adding the data of a second season of maize, an overall species total of 76 is reached for carabids. So the number of species is very much a function of the searching effort. How can we compare data from published work? It requires exact information on the methods used and the effort given. Mészáros et al. (1984) collected Carabidae in two types of maize fields in Hungary. Within 5 years they collected with pitfall and light traps a total of 41 species in a maize monoculture and 35 species in a maize field under crop rotation system. These look like low numbers compared to our results, even if we assume differences in the collecting procedure. The most obvious difference is the size of the fields (20-400 ha in Hungary, 0.2-1.6 ha in Switzerland). In Hungary, the species richness in the monocultural field was higher, although it was at least four times larger, but neighboured by a "neglected park" (Mészáros et al. 1984). The result of our directional traps have shown that there indeed is a constant exchange of arthropod over the field borders.

Comparisons of sampling from different regions, and by different people, requires careful standardisation of the data. However, it is still clear that the species richness was high in all our maize fields in the Sisseln area.

So far it appears that the closer maize plot is situated to a semi-natural area, the higher the species richness of that plot. Moreover, the smaller the field, the stronger the influence of a neghbouring semi-natural area. Horvatovich and Szarukán (1986), in an

investigation on the carabid fauna of arable soils in Hungary, also found a negative correlation between size plot and number of species.

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Present Situation of Arthropod Pests in Maize in the Northeast of Spain

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Maize is a major crop in the Northeast of Spain (Catalonia, 30,000 ha and Aragon 52.000 ha). A significant number of arthropods reduce maize yield and growers are applying an increasing amount of insecticides and acaricides. Although crop loss assessments have not been made, the main arthropod pests are: soilworms (noctuids and elaterids), borers (*Sesamia nonagrioides* and *Ostrinia nubilalis*), virus vectors such as aphids (*Metopolophium dirhodum; Rhopalosiphum padi* and *Sitobion avenae*) and spider mites (*Tetranychus* sp.). Secondary arthropod pests are also referred to. Original and bibliographical data on pests are included, mainly on borers which are, probably, the key species.

The Maize Crop in Spain

Maize is a major crop in Spain, covering a surface area of 450,000 ha in 1984. Yield has increased considerably in the last few years and in 1984 it reached an average of 7.000 kg/ha. In Catalonia and Aragon (NE of the Iberian Peninsula) the maize crop surface area was 52,000 + 30,000 ha respectively, in 1984. Most of it is for grain production and small part is for silage. The former is sown during a period from mid April to late May and is harvested from mid October to mid November. Maize for silage is sown later (June and July) and harvested in October or November.

The Arthropod Pest Situation in Catalonia and Aragon

Arthropod pest incidence on maize is greater than it was some years ago, probably due to the increasing number of insecticide sprayings and other agricultural practices. Table 1 summarizes the arthropod pest situation for maize in our study area, according to Castañera (1986), Arias & Alvarado (1983) and our own observations.

Among root and collar feeders, Elateridae and Noctuidae are the most harmful insects and their damage seems to have been greater in the last few years, mainly due to maize monoculture. Seed treatment and the incorporation of insecticide granules into the soil at sowing time are frequent in our area, although their effectiveness against cutworms is low. *Oscinella frit* L. (Chloripidae) and *Tipula* spp (Tipulidae) are occasional pests in our area.

Stem borers present in Catalonia and Aragon are *O. nubilalis* and *S. nonagrioides*. *S. cretica* is only found in the southern half of the Iberian Peninsula. They are the most traditional maize pests in Spain. Formerly, organochlorine insecticides were applied and after their prohibition, they were replaced by organophosphates and more recently by

Table 1

Arthropod pest situation for maize in Catalonia and Aragon

Roots and collar	Elateridae	Several species
		Several species
		Several species
Stem	Noctuidae	*Sesamia nonagroides* Lef.
	Pyralidae	*Ostrinia nubilalis* (Hb)
Leaves	Cicadellidae	*Cicadella* sp.
		Macrosteles sp.
		Rhopalosiphum padi L.
		Sitobion avenae F.
		Metopolophium dirhodum Walk.
	Etranychidae	*Tetranychus* sp.
		Heliothis armigera (Hb)
	Pyralidae	*Ostrinia nubilalis* (Hb)

pyrethroids applied as an aerial spray. This highly unselective methodology has probably been the main cause of the proliferation of some other phytophages, such as spider mites, leafhoppers and aphids, formerly not considered as pests.

Stem borers are, undoubtedly, the key pests of maize in our area but more research effort is necessary in order to improve control strategies and tactics. In the South of Spain an economic threshold of 60% of infested plants by stem borer larvae has been proposed for the second generation (Alvarado *et al.* 1986). Use of resistant hybrids is one of the most promising tools for stem borer control. DIMBOA content of maize hybrids has been highly correlated with plant resistance to *S. nonagrioides* in Spain (Gutiérrez & Castañera, 1986), as had been previously proved for *O. nubilalis* (Russell *et al*, 1975) . We have devoted the last three years to studying the biology of *S. nonagrioides* in Lleida. In the last chapter of this communication we shall summarize the most significant results.

Leafhoppers are especially abundant in the lower leaves of maize but, presumably, they are not economically harmful in relation to their direct damage. However, they can play a major role as MRDV vectors. This virus has been recently detected in Lleida (López Abella, 1986).

In our area, Aphididae are represented on maize by several species: mainly *S. avenae, M. dirhodum* and *R. padi*. The latter is the most abundant in September and October but it rarely causes economic losses. However, aphids can be dangerous in the first stages of the crop as MDMV vectors, a virus repeatedly detected in Catalonia. Maize aphids will be the subject of another communication in this Symposium (Pons, Comas & Albajes).

Tetranychus sp. populations have increased in recent years and have become one of the most harmful pests for maize in Spain. Non-selective sprayings against stem borers have probably been the principal cause of their proliferation. Growers now treat once or

twice (or more) against spider mites (particularity in Aragon). In nontreated fields, several mite predators may be observed. Abundant chrysopids, anthocorids and phytoseiids have been recorded in nontreated maize fields in the South of Spain (Alvarado *et al.* 1986). This situation makes it especially necessary to find new stem borer control tactics.

Occasionally, *Mythimma* spp. larvae, feeding on leaves, have also been recorded in our area. Damage from *Oulema melanopus* L. (Chrysomelidae) is very rare.

Some Data on *S. nonagrioides* Biology in Catalonia and Aragon

In our area, adults developing from overwintering larvae are caught from the beginning of May until the end of June when light traps (Alfaro, 1972) or virgin females (Eizaguirre & Albajes, unpublished results) are used. Then, a second peak is recorded in July-August by the same authors and a third peak in late Setember or early October. By using virgin females, we have observed that this peak is frequenty higher than the others. The presence of second and third instar larvae in silage maize in October may confirm the occurrence of a third, probably incomplete generation. Some plant species other than maize may shelter this third generation when the grain crop is too dry for the development of *S. nonagrioides* larvae.

Three generations are referred to in Estremadura (SW of Spain) by Arias & Alvez (1973) and a fourth, incompletely developed one in Andalusia (South of Spain) (Alavarado *et al.* 1983).

Winter mortality is probably the main factor influencing *S. nonagrioides* abundance in spring and summer. Insectivorous birds, early after harvest, diseases and especially low temperatures act on overwintering larvae, decreasing their numbers drastically. In January 1985, when temperatures reached -14°C over a fortnight, a mortality rate of 99% was recorded. Presumably, young third generation larvae are more prone to winter mortality. Therefore, the population fraction of the second generation which develops into a third one may be an important point in determining the population density of the following year.

Our laboratory experiments show that photoperiod has a decisive influence on larvae "diapause" and temperature modifies the photoperiod action (i.e. a photoperiod-temperature interaction). The maximal photoperiod inducing development arrest in sixth instar larvae is lower at low temperatures than at higher ones. Thus, high temperatures in spring and summer favour the abundance of the third generation through double means: by accelerating development and decreasing the critical photoperiod which induces 2nd generation larvae "diapause". In short, in warm springs and summers, 2nd generation larvae developing into adults will be more abundant, as has been pointed out by Alfaro (1972).

Percentages of parasitism are very low throughout the year. Only a few *S. nonagrioides* parisitized by *Lydella thompsoni* Hert. have been recorded, mainly in the first generation. Overwintering larvae are very rarely parasitised.

Acknowledgements

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Insect Pests of Maize in Bulgaria and their Control

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The economically most important pests of maize in Bulgaria are: Elateridae, and Tenebrionidae (Coleoptera); Noctuidae (Lepidoptera); *Tanymecus dilaticollis* Gyll. (Coleoptera: Curculionidae); *Ostrinia nubilalis* Hbn. (Pyralidae); *Heliothis obsoleta* F., (Lepidoptera: Noctuidae); *Oulema melanopus* (Coleoptera: Chrysomelidae); and Aphididae (Homoptera). Their distribution and the type of their damage, as well as the applied integrated systems of their control, are pointed out.

From planting to harvest and during storage maize in Bulgaria is infested by about 85 insect pests. Among them of greatest importance are wireworms, Coleoptera: Elateridae; pseudowireworms, Coleoptera: Tenebrionidae; mole cricket, *Gryllotalpa gryllotalpa* L.; Orthoptera: Gryllotalpidae; cutworms, Lepidoptera: Noctuidae; gray corn weevil, *Tanymecus dilaticollis* Gyl., Curculionidae; European corn borer, Lepidoptera: Pyralidae: *Ostrinia nubilalis* Hbn.; farworm, Chlorideae; *Heliothis obsoleta* F., Lepidoptera: Noctuidae; aphids, Homoptera: Aphididae; cereal leaf beetle, *Oulema melanopus* L., Coleoptera: Chrysomelidae; agpoumois grain moth, *Sitotroga cerealella* Oliv., Lepidoptera: Gelechiidae.

Wireworms are distributed all over Bulgaria. 15 harmful species (Nikolova *et al.*, 1965) have been found. The assessed pests of maize are: *Agriotes ustulatus* Shall., *A. sputator* L., *A. lineatus* L., *A. obscurus* L., *A. brevis* Cand., *A. medvedevi* Dolin., *A. guargistanus* Fald., *A. litiglosus* Rossi, *Melanotus fusciceps* Gyll., *M. brunipes* Germ., *M. rufipes* Hebst, and *Selatosmus latus* F. (Popov, 1968; Gerginov & Hinkin, 1978; 1976; Hinkin, 1979).

Wireworms destroy the maize seeds sown, the young plants and later inflict injuries on the roots. They are more harmful during cool and rainy spring seasons. At the present technology of maize growing, chemical control is economically justified in case of densities higher than 1.5-3.0 larvae/m^2 (Hinkin, 1979). The following integrated system of Elateridae control has been accepted:

1. In crop rotation maize comes after leguminous crops which leave the fields relatively free of Elateridae.

2. The time of agrotechnical operations is crucial for a maximal effect, taking into account the biological characteristics of Elateridae.

High wireworm density is typical for meadows, alfalfa and other grass fields, therefore the main ploughing of the soil takes place in July or August – the summer's most dry and hot months, after preliminary cutting the roots necks by ploughing in the stubble. Thus larvae are deprived of fresh plant food. Moreover in loose soil they are more easily available to parasites and predators. The soil dries quickly and many larvae perish from dehydration, not being able to overcome the soil's "air cushion".

In case grass fields are in a proper state and are productive till late autumn, ploughing takes place after the temperature falls below -3°C. At that time the larvae are prepared for overwintering and are immobile. Ploughing at a depth above 23 cm displaces

the larvae to the soil's surface where low temperature kills them.

The same approach is applied on the stubbles after winter wheat in case of high wireworm density. With the following soil cultivation and the pre-planting cultivation, machines destroy a great number of larvae. Inter-row soil tilling of maize fields takes place at a time when the larvae languish and are immobile. Brought up on the soil's surface, they are an easy prey of parasites and predators.

3. Before sowing, the density of the larvae in every field is determined by soil sampling (Popov, 1967) and in case of densities of 1 larva/m^2 maize, seeds are sown without dressing, but the seeding rate is 15-20% higher. In cases of densities of 1-4 larvae/m^2, the seeds are treated with 10 kg heptachlor 40; 4 kg Faikam 80 or 6 kg ophtanol per 1 ton of seeds and a supplement of polymeric water dilutable adherents (Gerginov and Hinkin, 1981). If the density is higher than 4 larvae/m^2 the seeds are treated with systemic insecticides, such as furadan 35 ST or diafuran 35 ST, in a dose of 25 kg/ton of seeds (Hinkin *et al.*, 1983).

Pseudowireworms inhabit and are harmful in more water-permeable soils. *Pedinus femoralis* L. and *Opatrum sabulosum* L. are the species encountered in Bulgaria (Nikolova *et al.*, 1965). The kind of injuries inflicted and the means of control are the same as against wireworms.

The mole cricket is widely distributed along river courses and on irrigated areas. It destroys the seeds sown and the young plants. Seed treatment with fentiuram, furadan 35 ST and diafuran 35 ST ensures efficient control of *G. gryllotalpa*.

Cutworms are wide spread all over Bulgaria. They cause thinning of the crops. The species *Scotia* (*Agrotis*) *segetum* Schiff, *Euxoa temera* Hb. and *S.* (*A.*) *ypsilon* Hufn. Rott. are encountered. Their control relies on efficient weed control in all crops during the entire growth period both with herbicides and with mechanical soil cultivations. Carbofuran chemicals applied in seed treatment are an efficient control of Noctuidae, too.

Spraying with chemicals in the control of gray corn weevil is also a means of protection against cutworms. The accepted threshold damage is 0.4-0.6 destroyed plants per 1 m^2 (Nikolov, 1979). The chemicals Dursban 4E, Orten 75 WP or Selecron 50 EK in a 0.1% solution and Volaton 50 EK, Agrimex 50 EK Wofatox 50 EK in 0.3% solution are applied (Nikolov, 1979).

The gray corn weevil is distributed on carbonate, typical and leached chernozem soils. The larva is found mainly on maize roots while the adults feed on young plant leaves. It is very abundant on non-irrigated areas, when maize is sown after maize, sugar beets and sunflower. Protection against this pest is the following:

Maize is not sown after maize, sugar beets or sunflower. The new maize fields are at a distance of minimum 3 km from those of the previous year. In such a case, no chemical treatment is needed. However, maize fields neighbouring those of the previous year have to be sprayed peripherally, because the immigrating weevils. In cases where maize is sown after maize, the seeds are dressed with Furadan 35 ST or Diafuran 35 ST. If the seeds are not treated with these chemicals the fields are sprayed with Agria 1050 – 3 l., Lebeizide 50 – 2 l, Metation – 3.2 l, Thiocron extra – 3.2 l, Carbicron – 2 l, Tokution – 2.5 l or Selectron – 2.5 l/ha (Gerginov *et al.*, 1982). Spraying begins when the insect has destroyed about 30-50 % of the leaves (Straka *et al.*, 1983). This is the economic threshold for all leaf-chewing insects up to the phase of 5th-7th leaf, excepting Noctuidae.

The European corn borer develops one complete and a partial second generation

under the climatic conditions existing in Bulgaria (Gerginov, 1976; Zamfirov, 1967). Recent data show that the insect can develop two generations in the lower plains of South Bulgaria. The losses, amounting to 10 %, are due mainly to the first generation (Gerginov, 1976; Keita, 1987).

The control of European corn borer is carried out applying the following system:

Extension of practically resistant hybrids in maize production such as: H-708, Kn-611, Kn-530, Kn-510, Kn-430, Kn-556, Kn-557, Kn-614, Kn-673, etc.

Mechanized harvesting of the crop by which a great number of caterpillars are destroyed mechanically.

Ploughing in the plant residues which are previously cut into small pieces.

Application of the egg parasite *Trichogramma maidis* on irrigated areas and along the course of large rivers.

The infestation level in Bulgaria is low and application of chemicals is economically unjustified.

Earworms are spread all over this country. It proliferates most massively during periods of drought and gnaws the tops of maize ears. Its control consists in sowing practically resistant hybrids and growing seed-production areas under irrigation.

Infestation of maize by adult cereal leaf beetle occurs after winter crops have ripened, if its control on fields with such crops has not been successful. In such cases cereal leaf beetle control is achieved mainly by chemical means as in the remaining cereals (Pavlov, 1986).

The main leaf aphid species affecting maize are *Rhopalosiphum maidis* Fitch., *R. padi* L., *Sipha maidis* Pass, etc. (Grigorov, 1982).

The basic means of aphid control is extension of practically resistant hybrids.

Angoumois grain moth infests maize ears of fields in the neighbourhood of warehouse premises where no protection against stored product pests was carried out before maize plants ripened. This infestation is limited and the main means of control is timely disinfection of the warehouses.

Other maize pests are of no great importance in Bulgaria. Many of the world-known maize pest species are not encountered in this country, a fact requiring strict quarantine.

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The Australian Species of *Heliothis*: Identification, Genetic Variation and Migration

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The Australian *Heliothis* complex consists of five described species of which two, *H. armigera* (Hübner) and *H. punctigera* Wallengren, are serious agricultural pests. Both are highly polyphagous but only *H. armigera* attacks cereal crops, including maize. *H. armigera* has developed resistance to insecticides but *H. punctigera* has not.

The two pest species are very similar but can be separated on morphological criteria in the adult, pupal and late larval stages. The eggs and small larvae are indistinguishable except through electrophoretic variation, being fixed or almost so for different alleles at seven loci. Differences in the ICD and PGD bands can be used to indicate when the potentially resistant species *H. armigera* is present.

The genetic distance between *H. armigera* and *H. punctigera* is 0.34, lower than the comparable value for the two American pest species. The percentage of loci which are polymorphic and the average heterozygosity are also relatively low.

Genetic distances between widely separated populations are less than 0.01 in both species, and the same rare alleles are present in most populations. These results can be explained by extensive migration. Both species are long distance migrants and can be found in areas remote from cultivated hosts. The rapid spread of resistance in *H. armigera* is probably due to migration.

Maize is a relatively minor crop in Australia but the area planted to it is increasing. Australian maize is free of many of the serious northern hemisphere pests. In most regions where it is grown the major pest is *Heliothis armigera* (Hübner). High population densities are tolerated and most maize is not sprayed, but it can serve as a source for damaging infestations in other crops such as cotton.

The Australian *Heliothis* complex consists of five species (Common, 1985). Of these, only *H. armigera* and *H. punctigera* Walengren are significant as agricultural pests. *H. armigera* attacks maize and other grain crops including sorghum and wheat, as well as many broad-leaved crops. Most broad-leaved crops are also attacked by *H. punctigera* but that species is rarely found on grain crops. Both species have an extensive host range among introduced weeds and native plants. Zalucki et al. (1987) list a total of 159 plant species in 49 families on which one or both *Heliothis* species have been recorded.

H. armigera has a long history of insecticide resistance in Australia. Resistance to DDT first occurred in the Ord River area of Western Australia in 1972 (Wilson, 1974). In the cropping areas of eastern Australia, resistance to DDT and other insecticides was recorded in 1972 (Twine and Kay, 1973). In 1983 resistance to the synthetic pyrethroids resulted in significant yield losses in cotton in the Emerald area of central Queensland and there is now widespread resistance throughout eastern Australia (Gunning et al., 1984). The frequency of resistant individuals in most areas is not high enough to result in control failures but there is risk of this in the future. In order to manage pyrethroid

resistance a strategy which confines the use of these insecticides to a single generation per season has been adopted (Forrester et al., 1985).

In contrast, *H. punctigera* has not developed resistance to either DDT or the pyrethroids, even though it is frequently subjected to spraying on crops. This difference highlights a number of questions in ecological genetics of both theoretical and practical interest. Firstly, there is a need for identification of the two species as eggs and small larvae, because this is the stage at which insecticides must be applied. Secondly, an understanding of gene flow, migration and genetic population structure would help in predicting the spread of resistance from one crop to another, and from one croping region to another. It would improve our understanding of the role of unsprayed crops and non-cropping areas as refugia, allowing us to better evaluate both the existing pyrethroid management strategy and possible alternatives.

Materials and Methods

Twelve populations of *H. armigera* and seven of *H. punctigera* were sampled during the period July-October, 1983 in the regions shown in Fig. 1. Details of the sampling methods and the sample size for each population are given by Daly and Gregg (1985). Most populations were sampled either for adults by using species specific pheromone traps (Rothschild, 1978) or for larvae by hand sampling with identification based on morphological criteria (Stanley, 1978). For some populations both adults and larvae were sampled. Sample sizes usually ranged from 20-50.

Electrophoretic variation was examined in a total of 24 enzymes representing 28 presumptive loci. The enzymes studied and the electrophoretic techniques used for them are given by Daly and Gregg (1985). Those which were intensively studied and form the basis for much of the discussion here were ICD, PGD, GC, HBDH, PGM, PGI, GDA and MPI.

Genetic distances were calculated using Nei's (1978) formulae. Intra-specific comparisons were made between populations with at least 17 loci in *H. armigera* and 8 in *H. punctigera*, while inter-specific comparisons were made between two populations which had 27 loci in common.

Results and Discussion

Species Identification

The two species were fixed, or almost so, for different alleles at seven loci (*Icd-1, Pgd, Gd, Pep-2, Got-2, Ldh and Ak-2*). This enabled a comparison to be made between electrophoretic identification and the identification based on morphological criteria.

Adults can be distinguished externally by the presence of a pale patch on the dark terminal fascia of the hindwing in *H. armigera* and internally by differences in the genitalia (Common, 1953), as well as by their responses to specific pheromones. Electrophoretic comparisons confirmed that these criteria effectively separate the two species. In the

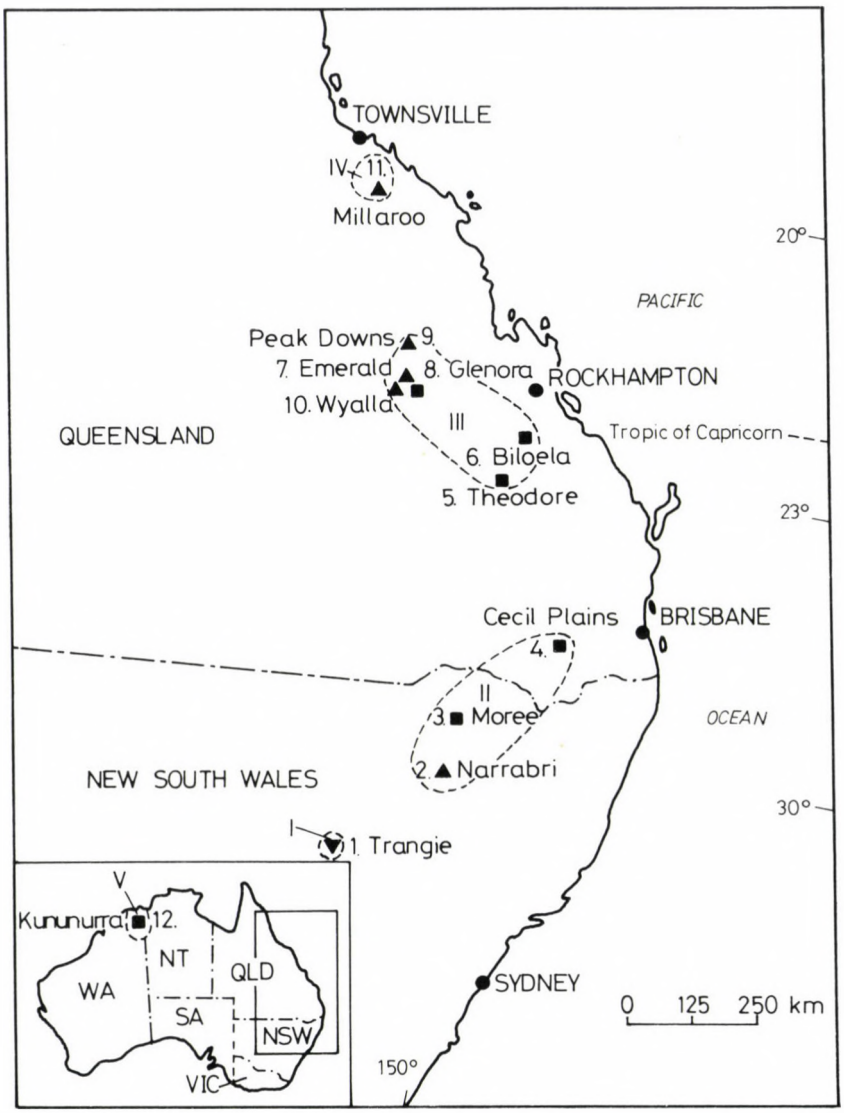

Fig. 1. Areas where samples were collected. Samples (1-12) were grouped into five regions (I-IV) denoted by - - -. ▲ *H. armigera* only, ▼ *H. punctigera* only, ■ both species.

pupal stage *H. armigera* can be separated from *H. punctigera* by differences in the spacing of the cremaster spines (Kirkpatrick, 1961), and this is also a reliable method.

The existing method for distinguishing large larvae was shown to be unreliable. Stanley (1978) used dark "saddle" markings on the first abdominal segment and dark pigmentation of the legs to separate the species in laboratory populations. In our field material, specimens which showed these characters were nearly always *H.* armigera may lose these marking. In such cases, the species can be distinguished by the colour of the dorsal cervical shield hairs (Forrester, pers. comm. 1987).

Despite extensive studies using light and scanning electron microscopy, it has not proved possible to distinguish the eggs and small larvae. Although significant differences exist in certain quantitative characters, no qualitative differences have been found (Gregg and Cahill, unpublished). In these life stages, electrophoretic methods presently represent the only alternative to tedious and costly rearing procedures. Identification at the egg or small larval stage is important because it is at this time that insecticides are applied. For routine electrophoretic separation the Icd-1 and Pgd loci are used (Daly and Gregg, 1985). In both species the same Icd-1 allele is common, so this band serves as a convenient marker. The method has a theoretical accuracy of better than 98 %.

Genetic variation

In *H. armigera* 9/28 loci (32%) were polymorphic at the 95% level while, for *H. punctigera*, the figure was 6/28 (21%). The mean heterozygosity for *H. armigera* was 11.3% and for *H. punctigera* it was 10.8%. These values are lower than the corresponding ones for the American *Heliothis* species (Sluss *et al.*, 1978) and for many other insect pests. It has been suggested that high levels of variability should be characteristic of pests (Sluss *et al.*, 1978), but the Australian *Heliothis* appear to be an exception.

The genetic distance between the two species was 0.34 ± 0.03, which is also lower than that calculated for the American species (Sluss *et al.*, 1978). This supports the taxonomic classification proposed by Hardwick (1965) which placed the two Australian species in the same subgenus but the two American species in different subgenera. For different populations of *H. armigera* the mean genetic distance was 0.004 ± 0.002 while,

Table 1

Genetic distances between populations of *H. armigera* (above the diagonal) and *H. punctigera*(below the diagonal).

	Trangie	Moree	Cecil Plains	Biloela	Emerald	Glenora	Kununurra
Trangie		na	na	na	na	na	na
Moree	.006		.004	.004	.003	.006	.009
Cecil Plains	.010	.001		.001	.003	.002	.007
Biolea	na	na	na		.001-.003	.006	
Emerald	na	na	na	na		.004	.008
Glenora	.007	.010	.014	na	na		.006
Kununurra	.007	.007	.008	na	na	.001	

na=no data available

for *H. punctigera* it was 0.007±0.004. There was no evidence of any geographic pattern in genetic distance (Table 1) except that for *H. armigera*, the Kununurra population (Region V in Western Australia) appeared to be more different from the eastern populations that the latter were from each other. Approximately 2500 km of mostly arid land separates Kununura from the eastern locations.

Gene Flow and Migration

Large genetic distances may result from lack of gene flow but small ones do not necessarily reflect extensive gene flow. However, the widespread distribution of rare alleles is a strong indication of considerable gene flow (Slatkin, 1981). In the Australian *Heliothis* genetic distances are low and the same rare alleles were found in many widely-spaced populations. Fig. 2 shows an example of this for alleles of Pgm in each species.

Extensive gene flow implies that significant migration occurs between widely-spaced populations in at least some generations. Farrow and Daly (1987) reviewed the evidence for this in *H. armigera* and *H. punctigera*. The latter is known to disperse over 400 km from mainland Australia to Tasmania. In the cropping areas of eastern Australia, it frequently occurs at times or in numbers which are difficult to explain by local population dynamics. *H. armigera* is also known to cover considerable distance to islands. On the other hand, it appears to be sedentary in the presence of flowering hosts (Roome, 1975; Wardhaugh *et al.*, 1980). Farrow and Daly (1987) concluded that migration was less important in *H. armigera* than in other *Heliothis* species, but the widespread distribution of rare alleles suggests that an understading of migratory patterns is needed for both Australian pest species.

Recent work, using an upward facing light trap mounted on a tower in an area where no breeding populations of either species exist, has provided some indication of the frequency of migration in the New England region of northern N.S.W. (Gregg *et al* ., 987). Large catches of both *H. armigera* and *H. punctigera* were obtained on several occasions in each spring and summer when the passage of cold fronts produced strong north-westerly winds. Moths could have been transported over distances of 300-500 km by these systems. In the summer and autumn, catches were associated with easterly winds produced by anticyclones and sea breezes. These systems might have transported moths 100-300 km westwards to the main cropping areas. Together with the observed distribution of rare alleles this suggests that, for the purposes of managing resistance, moths of both species should be regarded as single populations at least within the area of southern Queensland and all of New South Wales. The rapid spread of resistance in *H. armigera* between cropping areas supports this view, as does the recent observation of high frequencies of resistant individuals in populations remote from sprayed areas (R. Gunning, pers. comm.).

It has been suggested that the failure of *H. punctigera* to develop resistance is due to greater migration from unsprayed areas to sprayed crops which might reduce the frequency of resistance genes (Zalucki *et al.*, 1986). However, the results presented here indicate that long distance migration is also common in *H. armigera*. It seems likely that, if there are differences between the species in the role of unsprayed refugia, they involve movement at the local rather than the regional level.

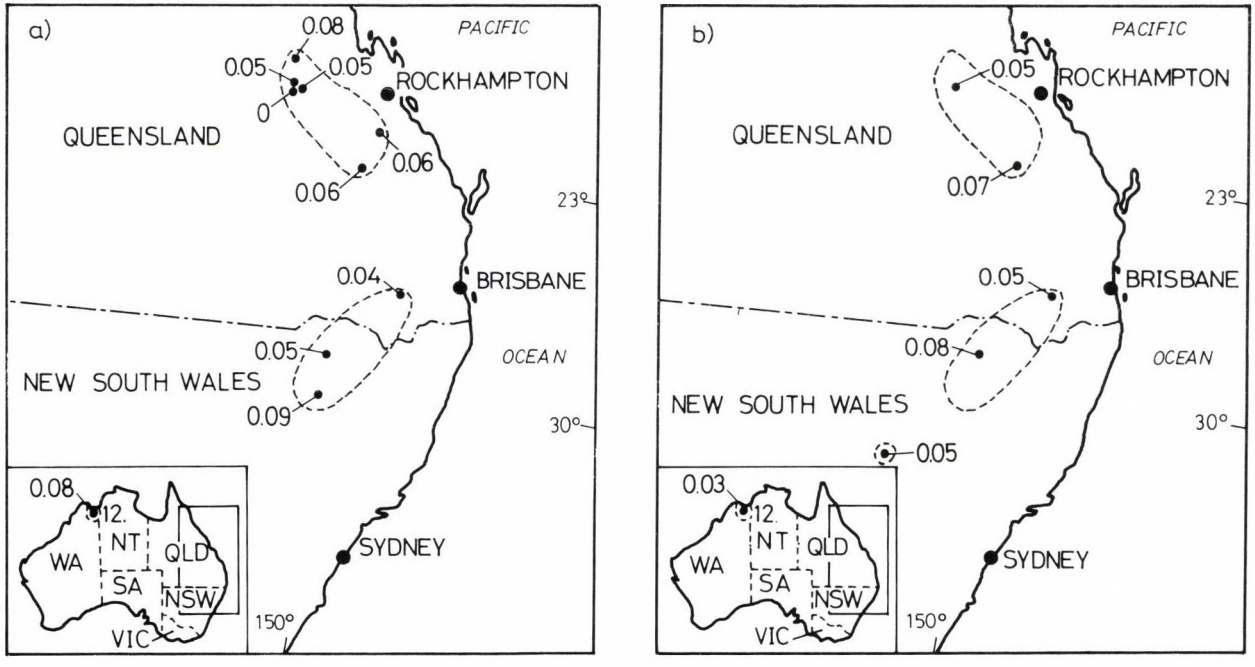

Fig. 2. Distribution and frequency of rare alleles at the *Pqm* locus. a. *H. armigera* (Pqm^e), and b. *H. punctigera* (Pqm^l)

Literature

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Some Aspects of Biology and Population Dynamics of *Diabrotica balteata* Lec. and *Systena basalis* Duval. (Coleoptera, Chrysomelidae), Maize and Bean Pests in Cuba

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In Cuba *D. balteata* and *S. basalis* are most important pests of beans (*Phaseolus vulgaris* L.) and maize plants (*Zea mays* L.) as well. By their interaction both host plants exert a clear influence on the harmful occurrence of the mentioned chrysomelids. An increased infestation of bean plants becomes possible only when preceding maize cultivation promoted the appearance a large population of *S. basalis*. Although *D. balteata* may propagate also in beans, the above-said applies to this species as well, if presuming a normal cropping period of beans. In bean crops, attention must be paid to adults feeding on the leaves, whereas in maize more serious harm is caused by larvae feeding on the roots.

In Cuba, beans (*Phaseolus vulgaris* L.) are a priority staple food. For this reason it was decided to extend the cultivation area and to raise the yields. This requires, however, the application of more effective plant protection measures, especially pest control. Besides the leafhopper, *Empoasca kraemeri* (Ross and Moore), and the whitefly *Bemisia tabaci* Genn., *Diabrotica balteata* Lec. and *Systena basalis* Duval. also play an important role as likewise polyphagous chrysomelids. Therefore it became necessary to gather more detailed information on their life cycle and in doing so, special emphasize had to be placed on the developmental time and population dynamics of the pests as well as on the two host plants maize (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.).

Methods

In order to study the duration of development of both beetles, eggs of defined age were put into Petri-dishes and kept under observation until the larvae hatched. These larvae were put into glass tubes 2 cm wide and 7 cm in height. The tubes had been lined with moist cotton before planting into them one maize or bean seedling (*P. vulgaris* or *Vigna sinensis* Savi.) which had already developed a strong root system. Then the tubes were closed with cotton plugs and put into boxes with drills to prevent the larvae from intense light exposure. The duration of development was studied at 7 temperature treatments. During this time the larvae were measured for body lenght and head capsule size (28 individuals/measurement).

Data of the population dynamics of the chrysomelids were collected on 9 bean plots at different times of the growth period. For this purpose, sweepings were made (3x100 sweeeps) on large plots of 1300 m^2 and the number of chrysomelids in the sweep samples were counted in the laboratory.

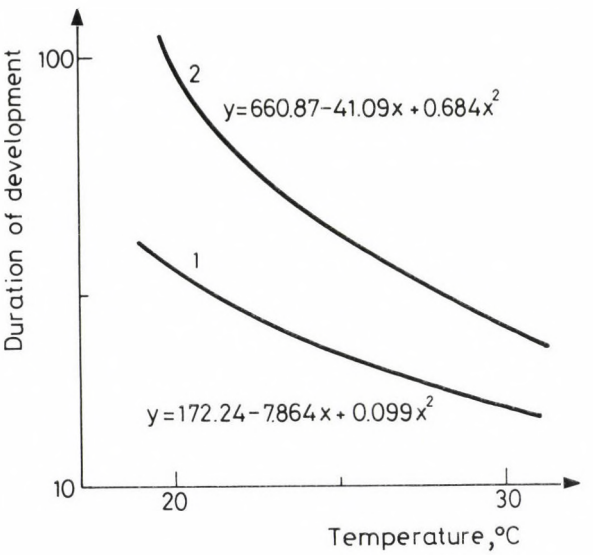

Fig. 1. Dependence of the life cycle of *Diabrotica balteata* Lec. (1) and *Systena basalis* Duval. (2) on temperatures

Results

The recorded total time of development of *D. balteata* and *S. basalis* is demostrated in Fig. 1 and it becomes clear that the developmental cycle of *S. basalis* takes essentially longer. For comparable temperatures this difference amounts to 15-22 days. Threshold values calculated according to the duration of development (Blunck, 1923) and effective total temperatures as well produced different values for both species.

Covering all developmental stages they come to 9.1°C and 588.3 d° for *D. balteata* and 15.0°C and 645.8 d° for *S. basalis*. According to this, *S. basalis* requires significantly higher temperatures than *D. balteata*. Varying temperature needs are revealed also for larval development (s. Fig. 2). Here, threshold temperatures for the development are 12°C (*D. balteata*) and 16.7°C (*S. basalis*). The necessary effective total temperatures until pupation amounted to 333 d° and 350 d° for *D. balteata* and *S. basalis*, respectively.

It is important that, comparing the host plants maize and beans, no significant differences were recorded for the developmental period of the pests. Differences exist, however, in the body lenght of the larvae. On maize both species show greater mass increases than on beans, which is expressed also by lower mortality rates on that host.

Differences were recorded also for the population dynamics of both chrysomelids in beans. They are demonstrated in Fig. 3 taking two bean populations as examples (16.8 1983, 8.11 1983). The results confirm those obtained in the laboratory, because the flea beetle *S. basalis* did not propagate in the bean crops, as was proved by the fact that only

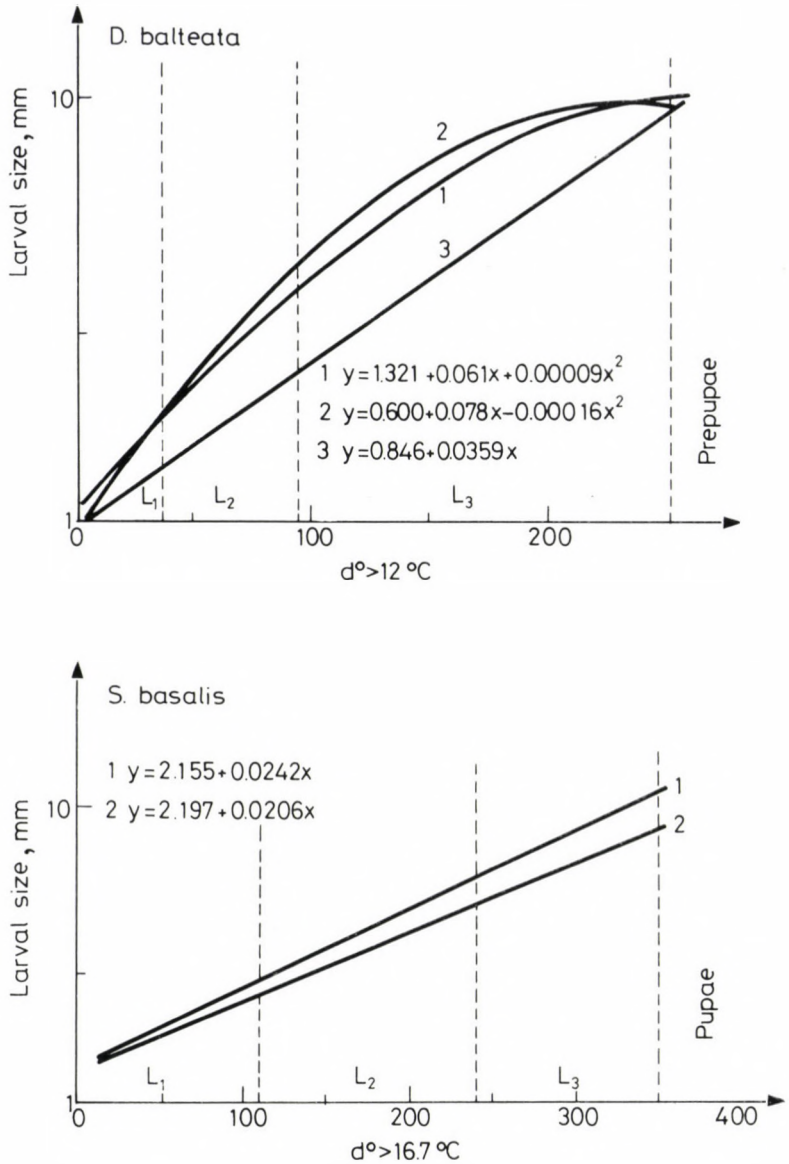

Fig. 2. The influence of the host on larval development of *D. balteata* and *S. basalis* (1 - maize, 2 - beans (*Phaseolus vulgaris*), 3 - beans (*Vigna sinensis*))

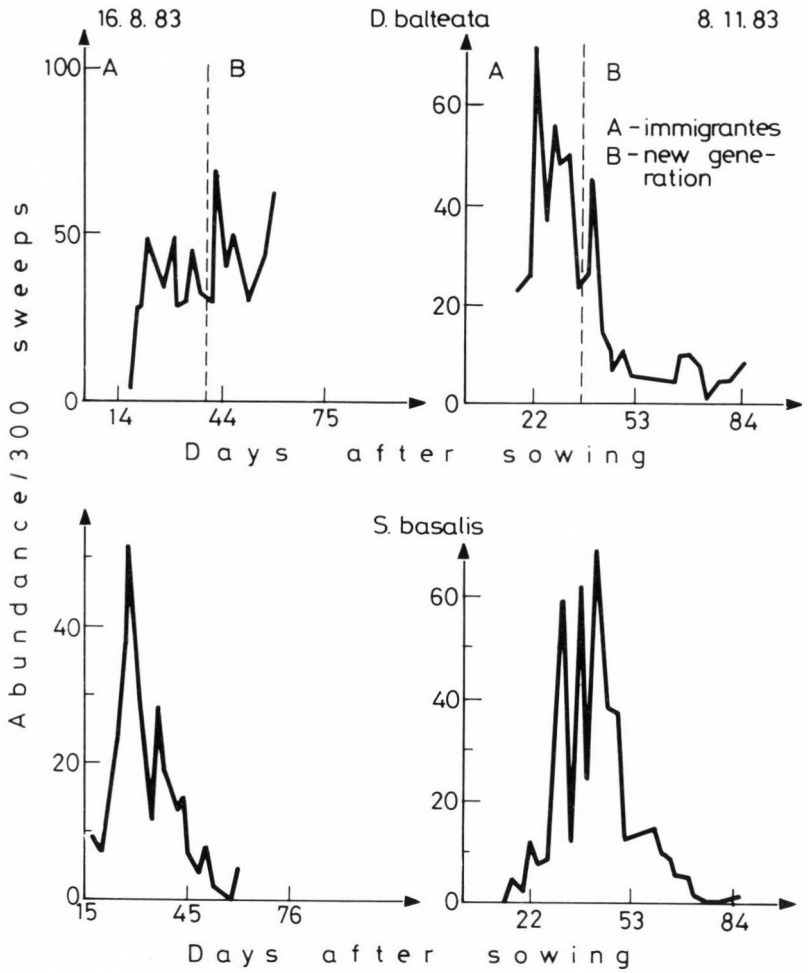

Fig. 3. Abundance dynamics of *D. balteata* and *S. basalis* in bean crops

immigrants could be identified in the field, irrespective of the date of sowing. *D. balteata*, however, may propagate in bean populations. Each time a new generation appeared. In the transitional period from rainy to dry seasons (September - November, March - May) its abundance persistently exceeded the number of immigrated beetles. In the colder month as well as during the rainy season the new generation turned out to be smaller.

Conclusions

Regarding the degree of incidence of the leaf beetles *D. balteata* and *S. basalis*, a clear relationship between maize and beans in their capacity as host plants was recorded. In beans, *S. basalis* will develop abundantly only when a previous growing of other host plants - here maize is of great importance due to its cropping period - provided for a high initial population of the pests. Beans help the adults of this species to outlive. Although temperatures and growth period of beans (about 90 days) allow *D. balteata* to propagate on these plants during the main cropping period (November - February), a gradation can be expected only when beyond this growth period other host plants are also available in the territory. In this connection the crop rotation maize - beans which is not so seldom promotes the propagation of the pests. Beside that, it should be noted that the bean crops great harm is caused by the adults' feeding on bean larvae, whereas in maize the main damage is caused by the leaves feeding on roots.

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No-tillage and Legume Cover Cropping in Corn Agroecosystems: Effects on Soil Arthropods

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Winter legume and grain cover crops preceding corn, *Zea mais* L., grown using conventional and no-tillage methods were investigated for their effect on soil arthropod population dynamics and community structure.

Hairy vetch, *Vicia villosa* Roth, supported higher below-ground arthropod population densities and a more taxonomically diverse fauna than either crimson clover, *Trifolium incarnatum* L., or wheat, *Triticum aestivum* L.

Soil arthropods, both pest and beneficial, were most abundant in no-tillage corn preceded by hairy vetch. Arthropod predators were more numerous in no-tillage than conventional-tillage systems regardless of previous cover crops. No-tillage practices promoted a more trophically balanced soil arthropod community than conventional tillage during early- and mid-season. Soil arthropod species diversity was also higher under no-tillage than conventional tillage.

Divergences in soil arthropod community structure among cover crop species, evident early in the season, dissipated by mid-season.

In 1987 seedling corn plants in no-tillage vetch treatments sustained significantly higher ($P<0.05$) damage from the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, than other treatments.

Tillage system preference was shown by herbivores: seed corn maggot, *Delia platura* (Meigen), occurred in large numbers in conventional tillage, and southern corn rootworm populations were high in no-tillage, especially following legume cover crops.

Conservation tillage practices, especially continuous no-tillage, generate complex soil biotic interactions in addition to changes in soil physical and chemical properties (Doran 1980, Blevins *et al.* 1983, Hendrix *et al.*, 1986). The large amount of organic matter (e.g., up to 12,000 kg/ha for corn or sorghum residues) left on the soil surface by no-tillage practices provides a favorable habitat for soil arthropods and other invertebrates through reducing moisture loss, ameliorating temperature extremes, and providing a continuous substrate for many decomposer organisms (Crossley *et al.*, 1984). Although crop damage by some pest insects such as corn billbugs, *Sphenophorus callosus* (Olivier), increase under no tillage (All *et al.*, 1984), damage from other pests such as lesser cornstalk borers, *Elasmopalpus lignosellus* (Zeller), are reduced by eliminating tillage (Cheshire and All, 1979). Recent work strongly indicates that positive interactions such as biological control of lepidopteran pests by ground beetles occur more frequently under no-tillage than conventional tillage conditions (Brust *et al.*, 1985). However, uncertainties remain concerning the combined impact of no-tillage practices and legume and grain cover crops on the population dynamics of below-ground arthropods.

The objective of the present study was to quantify and compare the effects of conventional and no-tillage cropping practices in combination with a winter legume or wheat cover crop on soil arthropod population dynamics, community structure, and damage to corn.

Field research was conducted from 1984 through 1987 at the Central Crops Research Station, Clayton, N.C., a site representative of the coastal plain of the southeastern United States.

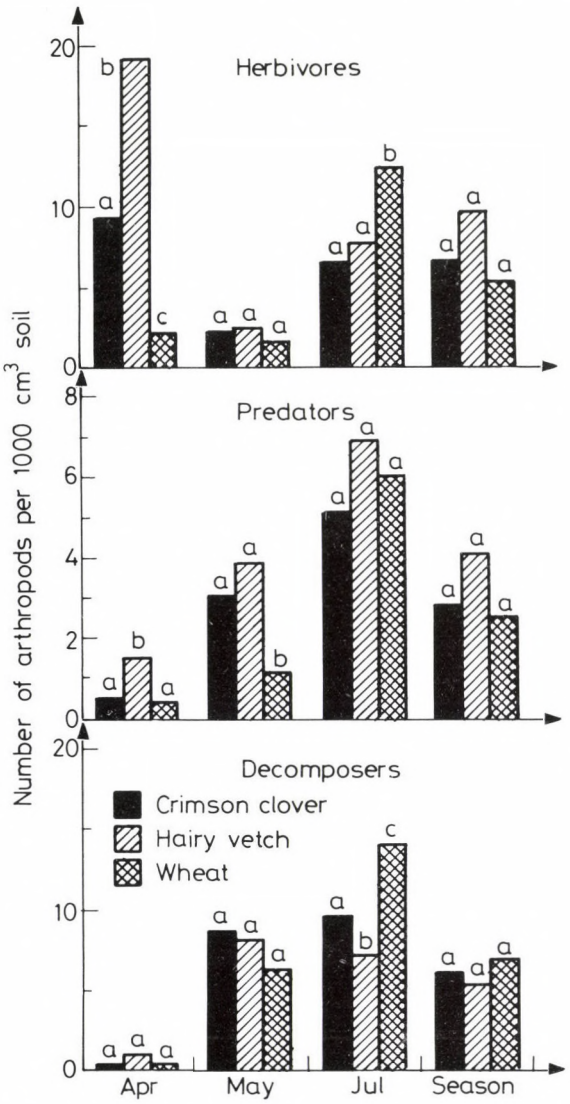

Fig. 1. Seasonal dynamics of soil arthropods in each of three trophic groups (Herbivores, predators, decomposers). Mean number of soil arthropods per 1000 cubic centimeters of soil from crimson clover, hairy vetch, and wheat treatments. Dissimiliar letters above treatment columns indicate significant difference ($P<0.05$)

Soil arthropods were extracted from soil cores using elutriation techniques from corn preceded by three cover crops of wheat, *Triticum aestivum* L., crimson clover, *Trifolium incarnatum* L., and hairy vetch, *Vicia villosa* Roth.

Results and Discussion

Cover Cropping Effects

In 1985 the effect of cover crop species on soil arthropod population number was most pronounced early in the season, decreasing thereafter (Fig. 1). Prior to cover crop destruction by tillage or herbicide, vetch supported larger arthropod population densities than crimson clover or wheat (April in Fig. 1). By July, however, this pattern was reversed, and wheat supported the highest soil arthropod densities among the three cover crops.

Each soil arthropod trophic group (i.e., herbivores, predators, and decomposers) showed a different population response to cover crop type. In all three cover crop types, arthropod predator numbers increased throughout the season, while herbivorous arthropod numbers paralleled crop phenology and biomass (Fig. 1). The destruction of cover crops late in April substantially reduced herbivore populations in May (Fig. 1); soil arthropod herbivore numbers were depressed similarly by tillage in conventional systems or by herbicides in no-tillage. In all cover crops, soil arthropod decomposer numbers increased rapidly in May (Fig. 1). Decomposer arthropod population dynamics reflect their response to differences in the decomposition rate of each crop residue. Wheat straw with its slow decomposition rate promoted a robust decomposer soil arthropod fauna by July (Fig. 1). We suspect these decomposer arthropods (fly larvae, collembolans, mites, etc.) were an important alternate host for no-tillage predators and probably enhanced predator numbers. Decomposers comprised nearly one-half of the total fauna from wheat and clover, but only about one-fourth of the total from vetch, which decomposes rapidly.

Similarity of arthropod taxa among cover crops was lowest in April with 55% shared taxa, increasing to 90% by July, indicating a continuous dissipation of cover crop influence over soil arthropod composition throughout the season. However, differences in soil arthropod composition among cover crops became more apparent by increasing taxonomic resolution.

Tillage System Effects

In both 1985 and 1987 tillage method had a significant effect on specific soil arthropod taxa as well as on the larger trophic groupings. Soil arthropod species diversity (Simpson & Shannon-Wiener indices) was higher ($P<0.05$) under no-tillage during early- and mid-season (1985) than conventional tillage (Fig. 2).

In 1985, the seedcorn maggot, *Delia platura* (Meigen), was the dominant herbivore and occurred in larger numbers ($P<0.05$) in conventional than no-tillage. The saphrophytic adult female *D. platura*, was attracted to, and oviposited in the decaying crop residue mixed into the soil in conventional tillage.

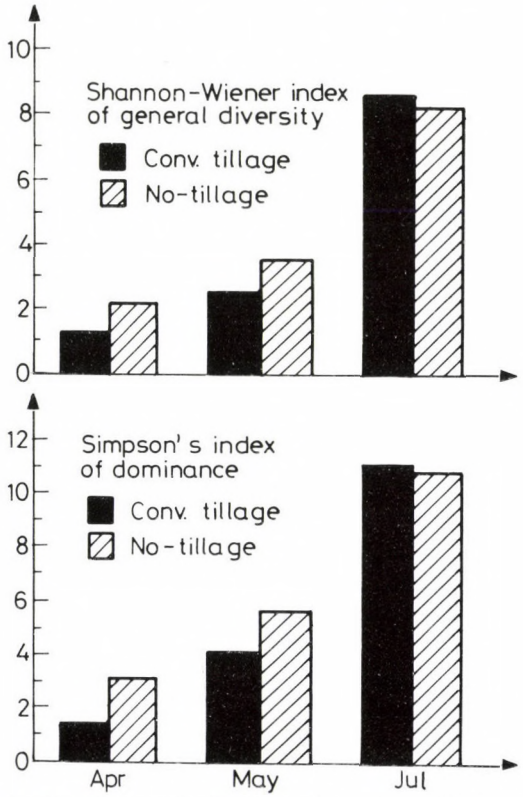

Fig. 2. Soil arthropod species diversity occurring in conventional- and no-tillage systems. Simpson's index of dominance = (ni/N2), where ni = each species, and N = total number of species. Shannon-Wiener indes of general diversity = (ni/N) log (ni/N)

Trophic composition analysis revealed that during the critical early season months of April and May, no-till systems supported a higher number of predators than conventional tillage systems. Biological control of pest insects occurred during this period. For example, southern corn rootworm egg predation by beneficial arthropods, including mites, carabids, and staphylinids, occurred earlier and was consistently higher ($P<0.01$) in no-till plots compared with conventional-tillage conditions (Brust & House, unpublished data).

Herbivorous soil arthropods were higher in conventional tillage plots compared to no-till plots during these same periods. However, by July herbivore, predator, and decomposer percentages were similar in both tillage systems; again, indicating the dissipation of tillage method effects through time.

In 1987, however, the soil arthropod herbivore numbers were higher in no-tillage than in conventional systems, except for Japanese beetle larvae (*Popillia japonica* Newman, Scarabaeidae) (Fig. 3). Curculionids, especially the southern corn billbug (*S. callous*) and several other minor groups of Coleoptera, primarily decomposers, were present

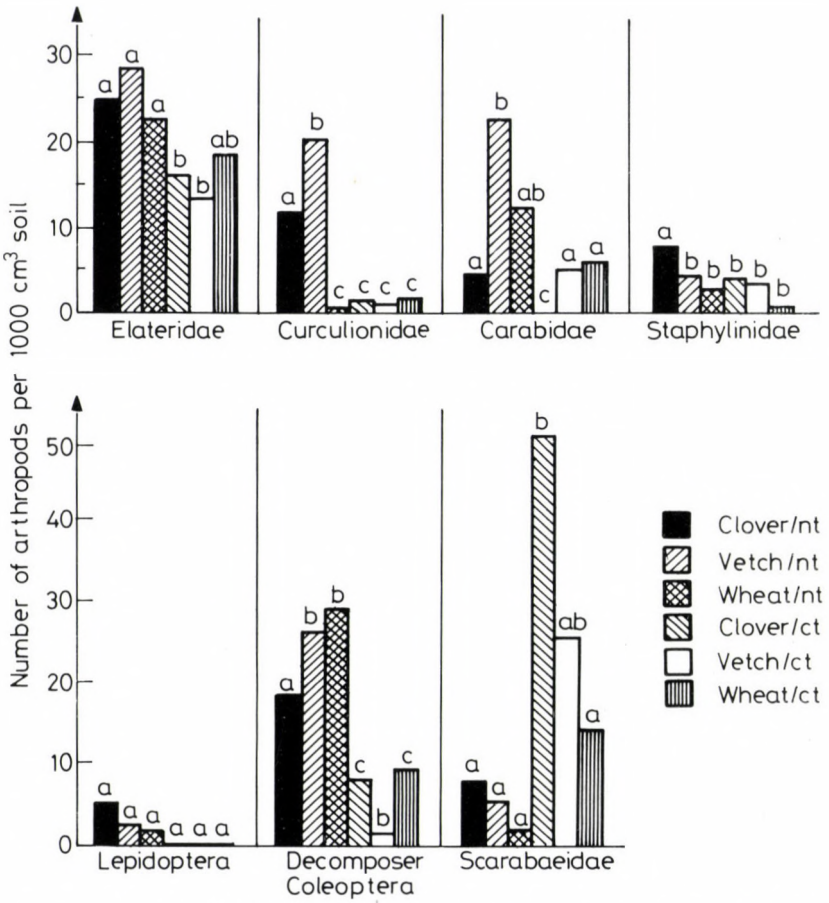

Fig. 3. Taxonomic composition of soil arthropods from six tillage and cover crop combinations. Mean number per 1000 cubic centimeters of soil is shown. Dissimiliar letters above treatment columns indicate significant difference ($p<0.05$)

predominantly in no-tillage treatments. In 1987 the single exception to this trend was scarab beetle larvae, which occurred in higher number in conventional than in no-tillage systems. Over 90% of the scarab larvae collected belonged to the genus *Ataenius*, which are almost exclusively decomposers. *Ataenius* species were probably attracted to the decaying crop residue in conventionally tilled systems. Elaterid (*Conoderus vespertinus* (F.), *C. falli* Lane, *Melanotus communis* Gyllenhal) larvae were the dominant herbivores in no-tillage systems in both 1985 and 1987.

In 1987, seedling corn plants in no-tillage in combination with legume treatments sustained higher ($P<0.05$) damage from the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber (Chrysomelidae), than conventional tillage. Root, shoot, and

whole plant biomass were lower in no-tillage than conventional-tillage treatments, reflecting a combination of rootworm damage and the slower growth pattern common to no-tillage crops. We suspect that in 1987 a convergence of climatic and cultural factors aggravated the southern corn rootworm infestation in no-tillage legume systems. These included a lack of rotation (three years of continuous corn on the same plots), wet soil conditions, and planting corn immediately following the destruction of cover crops with herbicides.

In conclusion, conventional tillage has a more consistent impact on soil arthropod community composition than no-till systems. In 1985 no-till promoted and maintained a more trophically balanced soil arthropod community composition than no-till systems. In 1985 no-till promoted and maintained a more trophically balanced soil arthropod community structure than the conventional-tillage system during early and mid-season, possibly providing some measure of biological control when crops were sensitive to insect damage. However, in 1987, a combination of continuous corn, moist soil, and planting immediately after herbiciding cover crops increased southern corn rootworm infestations in no-tillage systems, especially those following legume cover crops. Thus no-tillage requires more intensive management than conventional cropping methods, largely because information is lacking on the response of arthropods to varying cultural and climatic factors in these complex systems.

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Relationship of Maize Fertilization to the Damage Caused by *Ostrinia nubilalis* (Hbn.) (Lepidoptera, Pyraustidae)

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Experiments during 1985 and 1986 were carried out to study the relationship of maize fertilization to the damage caused by the European corn borer (ECB) *Ostrinia nubilalis* (Hbn.). Four levels of K with four hybrids of maize were tested. The results showed that levels of potassium (K) fertilizer tested did not influence the life characteristics of *O. nubilalis* or lead to significant damage. On the other hand, the highest K level significantly decreased the yield in all studied hybrids in some years.

In recent years when intense application of commercial fertilizers in agricultural systems has been used, rather high levels of fertilizers have accumulated in the soil. Consequently, this study is focussed on the influence of the high level of fertilizers on the population dynamics of the pest *O. nubilalis*. Chiang (1975) reported that the population of second generation corn borer was greater on maize grown in fertilezed soil compared to that on maize in fields where commercial fertilizers were not used. Taylor *et al.* (1952) studied the response of certain insects to plants grown on soils varying in fertility. They found, in greenhouse tests, that European corn borer (ECB) larvae showed slightly faster growth rates on plants having a balanced diet than on plants with distinct deficiences in nitrogen, potassium (K), or potassium and phosphorus. In field tests, corn borer survival was better on vigorous plants than on small nutrient deficient plants of the same age. Cannon and Ortega (1966) observed that the nutrition of the host plants plays some role in larval survival. Addition of nitrogenous fertilizers increased borer survival. The greatest number of corn borer larvae was found on plants that recieved 200 mg of nitrogen per kg of soil. However, no differences in survival were found in plants given different levels (5-80 mg) of phosphorus per kg of soil.

Mengel and Kirkby (1982) reported that potassium is highly important in raising disease resistance of many crop species. In maize, stalk rot and lodging are usually more severe when the level of K is low in relation to other nutrients. The nature of the action of K in controlling the severity of plant disease is still not understood. It may relate, in part, to the effect of K in promoting the development of thicker outer walls in epidermal cells thus preventing disease attack. Plant metabolism is very much influenced by K, therefore it is possible that plant diseases may be favoured by changes in metabolism associated with low plant K content.

Kralovic (1984) and Kralovic and Uher (1986) stated that K is a determining agent for daily photosynthetic production consequently influencing the yield forming processes. The optimum level of water-soluble K is considered to be 90-110 $mg.kg^{-1}$ of soil, depending on soil type. A higher level of K in the soil negatively influences the yield and increases

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the nitrate contents in plant products as, has been observed in sugar-beet, barley and carrot.

The purpose of our work was to study the influence of different potassium levels on the harmfulness of ECB and on the maize yield.

Material and Methods

In the two seasons of 1985 and 1986, experiments were conducted on an experimental area of the Institute of Experimental Phytopathology and Entomology at Ivanka pri Dunaji (CSSR) using hybrids supplied by the Maize Research Institute in Trnava. The soil type was Fulvi-calcaris Phaeozem with good supply of Ca (pH-7.62) (silty-loam). In 1985 four hybrids: TAC 138-1/75 (sweet maize), CE 200, Ta Mv 310 and DEA were planted on 6 May at four planned levels of potassium: 500 mg (plot A), 250 mg (B), 150 mg (C) and 100 mg.kg^{-1} of soil (D). To reach the above mentioned levels, the following amounts of potassium sulphate were applied: 3.7 kg/plot A (3960 kg.ha^{-1}), 1.8 kg/plot B (2000 kg.ha^{1}), 1.1 kg/plot C (1200 kg.ha^{-1}). On plot D, potassium sulphate was not applied. Approximately one month before planting, manure was applied to the field at a rate of 30 t.ha^{-1}. Each hybrid was planted on the four plots. Each plot consisted of four rows with 10 plants in each row. The distance between rows was 70 cm, and 20 cm between the plants in the row. Nitrogen, in the form of urea, was applied uniformly on all plots at a rate of 200 kg.ha^{-1} (i.e. 112 g/plot) in three applications. One third of the total dose was dissolved in 10 l of water and sprayed on the surface of the plots, on three occasions, first on sowing, and the other two monthly.

An artificial infestation with ECB egg masses at their black head stage was made when the plants reached the late whorl stage of development using four egg masses per plant.

During harvest, 20 plants per plot were cut randomly above the ground and, after counting the entrance holes, the plants were dissected, the number of surviving larvae counted and the length of tunnels measured. Individual ears were dried up to 14% of kernel moisture, weighed and after hand shelled the kernels were also weighed. In the autumn 1985, soil analysis revealed the following levels of K: plot A – 751 mg, plot B – 456 mg, plot C – 323 mg and plot D – 147 mg.kg^{-1} of soil.

In 1986, the same hybrids on the same plots were used as in the previous year. Since the residual K from the previous year was sufficient for our experiment, we did not add more potassium. Nitrogen was applied as in the previous year on 4 May, 5 June and 7 July. The hybrids were planted on 4 May. The artificial infestation and the evaluation of damage and yield were similar to that in 1985.

Soil analysis made in autumn 1986 from individual plots showed the following residual K levels: plot A – 329 mg, B – 210 mg, C – 168 mg and D – 110 mg.kg^{-1} of soil.

The data were analysed by analysis of variance for factorial experimental design with individual plants serving as the experimental units. The means were separated by the new Duncan's multiple range test (Steel and Torrie 1980). Moreover, to study the influence of different levels of K on the quality of kernels, the weight of 1000 kernels from each plot was taken. Data of kernel's weight were analysed by two way analysis of variance without replicates and with Tukey's test for nonaditivity to separate the means.

Results

The influence of different levels of potassium on the damage caused by ECB and the average yield of maize in 1985 and 1986 is in Table 1. In general, lower yield of kernels was recorded in the dry and hot season of 1986 than in the humid season of 1985. The average temperature from April to September in 1985 was 16.4°C while in 1986 it was 17.3°C. The amount of precipitation in 1985 was 304.9 mm compared to 198.8 mm in 1986. The highest level of K decreased the yield significantly ($P<0.05$) compared to the other levels of K over the two years.

Table 1

Influence of different levels of potassium on damage of the corn borer and the yield of maize, Ivanka pri Dunaji, 1985 and 1986. Averaged over hybrids.

Year	Level of K $mg.kg^{-1}$ of soil	Mean kernel weight per plant (in gm)	Mean No. of entrance holes/plant	Mean No. of survived larvae/plant	Mean length of tunnels/ plant (in cm)	Mean weight of 1,000 kernels (in gm)
1985	A 751	92.66 b	12.59 a	4.78 a	65.94 a	
	B 456	107.36 a	13.44 a	5.81 b	72.69 a	
	C 323	114.16 a	12.64 a	6.23 bc	71.30 a	
	D 147	107.06 a	13.19 a	7.13 c	78.55 a	
1986	A 329	58.60 b	8.31 a	3.50 a	41.94 a	186 b
	B 210	74.21 a	8.13 a	3.39 a	41.65 a	196 ab
	C 168	77.59 a	8.24 a	4.01 a	44.45 a	209 ab
	D 110	82.80 a	8.46 a	3.56 a	43.73 a	215 a

Means followed by the same letter in each column for individual year are not significantly different at 5 % level.

With the exception of the mean number of surviving larvae in 1985, no significant differences were found in measured parameters of damage in either year. Significantly less mean number of surviving larvae per plant was recorded in treatment A than in other treatments. Since the mean number of entrance holes and the mean length of tunnels were not different in all treatments, we suppose that the higher mortality of mature larvae due to a higher level of K occurred in the humid season of 1985. The highest level of K in 1986 significantly decreased the weight of 1000 kernels compared with treatment D.

Yield of the maize hybrids studied, mean number of entrance holes, mean number of surviving larve, mean length of tunnels and mean weight of 1000 kernels averaged over all K levels are presented on Table 2. In 1985, the highest yield was in hybrid DEA and the lowest in TAC 138-1/75. Significant differences at 5% level between all hybrids were recorded, while in 1986 (dry season) the hybrid TA Mv 310 gave a significant difference compared to CE 200 and DEA. In the other parameters studied over the two years, significant differences were recorded between the hybrid TAC 138-1/75 and the other three hybrids, i.e. higher mean number of entrance holes and surviving larvae and higher mean length of tunnels. Concerning the above mentioned parameters, the differences

between the three hybrids were not consistent and varied slightly in individual parameters.

Table 2

Yield of maize hybrids, mean No. of entrance holes, survived larvae and length of tunnels averaged over different levels of potassium, Ivanka pri Dunaji, 1985 and 1986.

Year	Hybrid	Mean kernel weight in gm per plant	Mean No. of entrance holes/plant	Mean No. of survived larvae/plant	Mean length of tunnels in cm/plant	Mean weight of 1,00 kernels (in gm)
1985	TAC 138-1/75	79.13 d	19.18 b	8.41 d	100.66 c	
	CE 200	102.80 c	11.56 a	6.16 c	68.73 b	
	TA Mv 310	114.49 b	10.49 a	4.20 a	56.08 a	
	DEA	124.84 a	10.63 a	5.16 b	63.00 a	
1986	TAC 138-1/75	39.21 c	11.78 c	4.31 b	53.19 c	128 c
	CE 200	75.33 b	6.51 a	3.04 a	34.98 a	208 b
	TA Mv 310	97.53 a	8.13 b	3.59 a	42.09 b	231 a
	DEA	81.13 b	6.74 a	3.53 a	41.51 b	239 a

Means followed by the same letter in each column for individual year are not significantly different at 5 % level.

Discussion

The results obtained in two years revealed that potassium plays an important role in maize nutrition. Mengel and Kirkby (1982) reported that the level of available K in soil depends on the crop as well as climate and soil conditions. Concerning the influence of tested levels of potassium on harmfulness of corn borer, no significant differences were recorded in spite of the highest level of K in 1985 being 751 mg $K.kg^{-1}$ of soil. Taylor *et al.* (1952), in an experiment with sweet corn, employed two fertility levels: high and low with phosphorus and potassium. No significant difference was detected in survival of corn borer on experimental plots. In another experiment the larvae tended to develop more satisfactorily on plants grown on a complete nutrient. On the other hand, significant negative influence of the highest K levels on yield was noted in both years of our observations. The plants grown at the highest K level gave a significantly lower yield. A similar phenomenon was observed by Forbes *et al.* (1984) in experiments with K fertilization for maximizing yield of cabbage, sweet corn and soybeans. They found some yield depression where the highest rate (400 $kg.ha^{-1}$) of K was applied to cabbage.

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Field Observations on the Oviposition Behaviour of Different Corn Borers and Estimation of Economic Thresholds

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Corn borers are the most serious pest attacking maize in Egypt. The moths of *Sesamia cretica* start to lay their eggs when the corn plants reach 20 cms in height, and continue to lay until the plants reach 100 cms. The moths prefer to lay eggs on plants 40 cms in height. The corn plants are attractive to female moths of *Chilo agamemnon* which lay their eggs when the height of the plants ranges from 90 to 230 cms. These plants of 175 cms had the highes number of egg-masses. Oviposition of *Ostrinia nubilalis* reaches its peak when the corn plants reached about 178 cms in height. Eggs of this species are not encountered on plants of more than 260 cms or less than 65 cms in height.

The economic threshold for these borers was determined statistically as 8 egg-masses/100 plants for *S. cretica* and 20 egg-masses/100 plants for both *C. agamemnon* and *O. nubilalis*. Above these levels control measures should be applied before serious damage takes place.

The corn borers, *Sesamia cretica* L., *Chilo agamemnon* Bles. and *Ostrinia nubilalis* (Hbn.) are major pests on maize plants in Egypt. They have attracted the attention of many workers because of the damage, they cause, their general distribution, multitude of generations and wide range of hosts. However, the oviposition behaviour of these pests and the distribution of egg-masses on maize plants have been studied only partially. Little work has been done on the relationship between the egg-laying of corn borers and plant height (Neiswander & Hubner, 1929; Marston & Dibble, 1930; Ficht, 1931 & 1936; Patch, 1942 and Ahmed & Kira, 1960).Accordingly, additional information is needed to investigate the behaviour of egg-laying of these pests on maize plants so that some benefit may accrue from the view of controlling these pests. The present paper describes information on the oviposition behaviour of corn borers and on levels of economic threshold.

Material and Methods

To study the oviposition behaviour and economic threshold of the three species of corn borer, an experiment was carried out at the Experimental Farm, Faculty of Agriculture, Cairo University, Egypt. Corn was planted on 14 successive dates at 15-day intervals. A randomized plot design was used. Each plot was replicated four times and all the usual agricultural processes were undertaken. Once the plants reached 10-15 cm extended height (The extended height of the plant from the surface of the ground to the tip of the longest leafblade when extended), the procedure on 14 successive days consisted of counting egg-masses laid upon the different leaves of 100 plants (25 plants per plot) and dissection of plants to provide larval survival. At 7 day intervals, corn extended height, the number of egg-masses, number of larvae, and the level of infestation were recorded.

Table 1

Distribution of corn borer egg-masses on the different leaves of the corn plant

Leaf No.	*S. cretica*	*C. agamemnon*	*O. nubilalis*
1	13.36	0	0
2	57.87	0	0
3	23.17	0	0
4	3.01	0.85	0
5	1.20	2.17	1.78
6	0.60	9.52	13.49
7	0.50	12.16	12.26
8	0.22	17.93	29.60
9	0.07	17.32	10.09
10	0	15.27	11.90
11	0	12.27	9.57
12	0	5.73	3.94
13	0	4.76	3.34
14	0	1.55	1.63
15	0	0.34	1.27
16	0	0.09	0.11
17	0	0.03	0.02

Results and Discussion

a) The pink borer, S. cretica *L.*
1. – Oviposition site:

The present field observations showed that the female moths of *S. cretica* laid their egg-masses in one or more rows on the inner surface of the 1st to 9th leaf sheaths. Data presented in Table 1 and Fig. 1 indicate the distribution of *S. cretica* egg-masses on the first nine leaves of the corn plant. It is clear that out of hundred egg-masses, 97.41 were found on the first leaves. Also, within these first four leaves, there appeared to be a preference for the second and third leaves.

2. – Relationship of extended height of corn plants and the number of S. cretica *egg-masses:*

The data presented in Fig. 2 indicate that female *S. cretica* tend to lay their egg-masses on plants of extended height ranging between 20-100 cm. Furthermore, the same data indicate that the maximum number of egg-masses was found on corn plants of about 40 cm extended height. These results are more or less agreement with the findings of Achmed and Kira (1960). The possibility of laying egg-masses on plants shorter or longer than the previously mentioned length range (20-100) cannot be eliminated. However, this is based upon the fact that *S. cretica* egg were observed in few cases on plants less than 20 cm or more than 185 cm extended height.

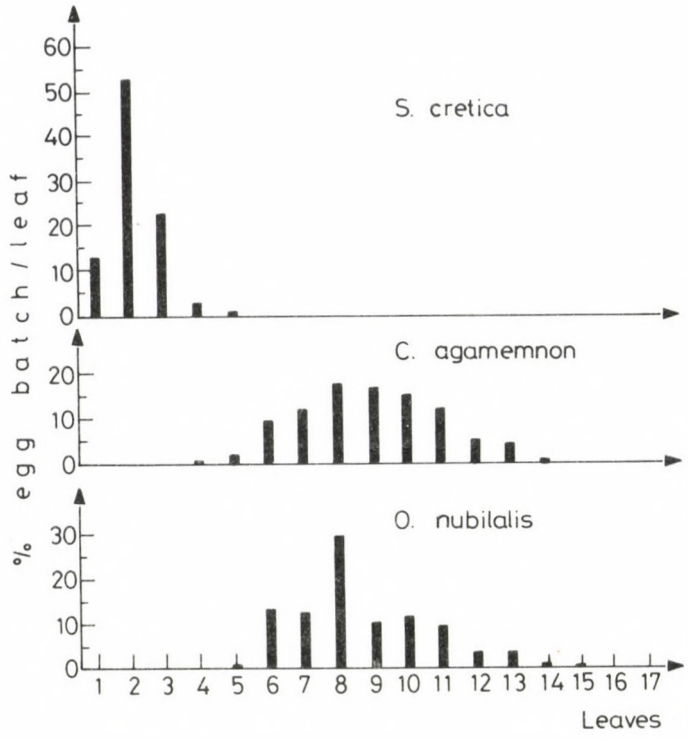

Fig. 1

b) The purple lined borer, C. agamemnon *Bles.*
1. – Oviposition site:

Under field conditions, female *C. agamemnon* lay their egg-masses on either the upper or the lower surfaces of the 4th to the 17th leaves of corn plants of different ages. Table 1 and Fig. 1 show the distribution of *C. agamemnon* egg-masses among the different leaves of the corn plant of different ages. These data indicate that more than 50% of the egg-masses were laid on the 8ht, 9th and 10th leaves in almost equal numbers, whereas 7th and 11th leaves received less egg-masses. The average number of egg-masses laid on leaves located below the 7th and above the 11th tended to decrease rapidly. These data suggested that intermediate leaves of the corn plant are preferred for egg deposition. It is clear from the field observations that the number of egg-masses laid on the upper surface significantly exceed those on the lower surface of the leaves. The average numbers of the egg-masses per 100 plants on the upper and lower surfaces of the leaves were 87.85 and 12.15, respectively.

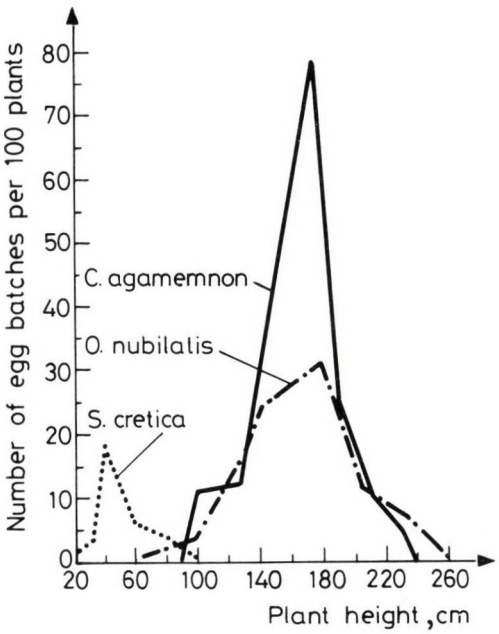

Fig. 2

2 – Relationship of extended height of corn plants and the number of C. agamemnon *egg-masses:*

Female *C. agamemnon* started to lay egg-masses on the corn plants when their extended height reached about 90 cm, and continued until the plants reached 230 cm extended height (Fig. 2). The most preferable plants for *C. agamemnon* egg deposition were those of between 128 and 190 cm extended height with an optimim height about 175 cm. These results are in disagreement with those obtained by Ahmed and Kira (1960) who indicated that the female *C. suppressalis* begin to lay their eggs on plants of 125 cm extended height and an optimum height of 145.8 cm.

c) The European Corn Borer, O. nubilalis *Hbn.*
1. – Oviposition site:

The field observations obtained showed that the moths of the European corn borer lay the majority of their eggs on both surfaces of the 5th to 17th leaves of corn plants. It appears from Table 1 and Fig. 1 that the majority of egg-masses (86.91%) were laid on the 6th to 10th leaves, whereas the rest of the leaves received relatively few egg-masses.In

contrast to the egg-deposition behaviour of *C. agamemnon*, the majority of the European corn borer masses (79.1%) were found on the lower surface of the leaves. The average number of egg-masses per hundred plants emphasizes that the lower surfaces of corn plant leaves are more preferable for egg deposition.

2 – Relationship of extended height of corn plants and the number O. nubilalis egg-masses:

The present field observation confirmed the previously known relationship between abudance of the European corn borer egg-masses and extended height of the corn plants at the time of the moth flight. This relationship is represented graphically in Fig. 2. Female moths did not lay their eggs on the plants until they reached an extended height of about 65 cm. Oviposition reached its peak when the extended height of the plants reached about 178 cm. Eggs were not encountered on plants of more than 260 cm, or less than 65 cm extended height. These results are in disagreement with those of Marston and Dibble (1930) and Ficht (1930 & 1936) in Michigan, U. S. A..

Economic threshold:

Analysis of the data indicated that the economic threshold could be determined as 8 egg-masses per 100 plants for *S. cretica* and 20 egg-masses per 100 plants for both *C. agamemnon* and *O. nubilalis* at which levels control measures should be applied before serious damage takes place.According to this, an experiment was conducted to determine the appropriate timing of insecticide treatment of corn plants and the minimum number of applications economically sufficient to reduce *C. agamemnon* and *O. nubilalis* infestation. To achieve better establishment among the experimental plots, the corn was planted later in the season. The plants were divided into three groups; the first was given one, the second two, while the third was given three applications. In each experimental plot the first application was about 35 days after planting and the plant had reached 145 cm extended height,harbouring about 20 egg-masses per 100 plants. Twelve day intervals were allowed between the other two applications. The results indicate that spraying the corn plants two times, twelve days apart (starting when the plants were 35 days old and reached 145 cm) gave better control and a more economic crop than that from untreated plants.

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EAC and Single Cell Responses of the European Corn Borer, *Ostrinia nubilalis*, Z-strain (Hbn) to the Sex Pheromone Components *z*11-14:Ac, *e*11-14:Ac, Structural Analogues and some of their Mixtures

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Efficacy values were determined electrophysiologically for the sex-pheromone components *z* and *e*-tetradecenyl acetate (*z*11-14:Ac and *e*11-14:Ac) and about 60 structural analogues and some of their binary mixtures in both sexes of laboratory reared forms of the Z-strain of the European Corn Borer, *Ostrinia nubilalis* (Hbn). The efficacy values were defined, on the basis of EAG- dose response relations, as the ratios of source loads required for the different compounds to elicit a standard EAG-response. The compounds induced no EAG-responses in the females, but, with only small inter- and intra-individual variations, strongly differentiated responses in the males. The efficacy values varied between 1 (for the most effective compound *z*11-14:Ac) and about 300. Binary mixtures of the highly effective compounds induced EAG-responses which were up to nearly 100% higher than those ones which were induced by merely doubling the individual source loads (EAG enlargement about 30%). Supported by preliminary recordings from single receptor cells within morphologically different types of sensilla trichodea, there are thus indicated at least 5 different types of receptor cells. They respond either to the *z*- or to the *e*- pheromone component (both types were found within one type of sensillum) or to some of the analogues e.g. *z*11-14:Ac, and a (97:3) mixture of *e*9- and *e*9-14:Ac. Responses were also elicited by *y*11-14:Ac (*y* stands for acetylenic C-C bonds), but it is still open if *e*11-14:Ac and *y*11-14:Ac excite one and the same type of receptor cell.

The European corn borer *Ostrinia nubilalis* (Hbn), one of the most important insect pests in maize fields, is classified into three pheromonal phenotypes. They are named "Z-" (97:3), "E-" (3:97) and hybrid (50:50) strains on the basis of (i) the ratios of the Z- and *e* isomers of 11-tetradecenyl acetate (*e*- and *z*-11-14:Ac) found in the female pheromone glands, (ii) the relevant behavioral and (iii) electrophysiological effects on males (1, 4, 5, 8, 12, 14, 19, 20, 21, 22). Important differences in behavior are even found within different voltinic Z-populations (7).

In spite of this the application of pheromones, however, does not play an important role in the control of *O. nubilalis*. If the pheromones are used at all they are restricted to monitoring systems. But even here, they scarcely satisfy the conditions for integrated pest control because of their relatively small trapping rates (20).

Encouraged by successful applications of odorants in other insects (e.g. the wine yard pests *Lobesia botrana* and *Eupoecilia ambiguella* (3, 15)) we started the search for more effective odorants also for *Ostrinia* by means of electrophysiological and behavioral methods (3, 10). As a first step we compared the effect of the pheromone components and about 60 structural analogues, and determined the factors of source loads (=reciprocal efficacy values, 11) which are required to elicit standard EAG-responses equivalent to the responses to 10-2 μg of the pheromone component *z*11-14:Ac. On the base of the idea that mixtures of compounds (which are selectively perceived by different types

of receptor cells) induce higher EAC-responses than the single compounds at doubled source loads, we asked in a second step for number and specialisation of the underlying types of receptor cells and recorded the EAG-responses to binary mixtures composed of highly effective compounds as revealed from step 1. In a third step we tried to characterize the relevant receptor cell types and their reaction spectra. Studies concerning the quantification of the succeeding orientational effects (9) will be published elsewhere.

Materials and Methods

The males and females of the Z-strain of *Ostrinia nubilalis* (Hbn) originated from wild types caught in the surroundings of Limburgerhof (FRG) and Leningrad (USSR). Sorted according to sex at the pupal stage, they emerged in Seewiesen in a 16-hr photophase at 22°C and 65% relative humidity.

Test Substances and Stimulation

The stimulus sources consisted of filter papers (1x1 cm^2 each loaded with 10 μl of hexane solutions containing either 10-3, 10-2, or 10-1 μg of the test compound (GC-purity >98%). For stimulation they were put into a glass cartridge and overblown by a water-saturated air stream (20°C, 800 ms, 30 cm/s) directed onto the antenna of the insect. Binary mixtures were tested at source loads composed of 10 μl hexane solutions containing 10-2 μg of the pheromone components (*z*11-14:Ac, *e*11-14:Ac) and 10-1 μg of the other compounds. The intervals between stimuli ranged from 2 min (for small responses) up to 30 min (for high responses).

Recording and Evaluation

For EAG-recordings micro glass-capillary electrodes (2 μm at tip) were inserted at the thorax and the tip of the antenna. The signals (0.1 to 3 mV were fed into a 12 MΩ impedance conventer and after (8-bit) AD-convertion (sampling rates: 220 Hz for EAG, 10 kHz for nerve impulse recording) stored in a disc database (11). To account for life time and adaptation processes the responses were each referred to those adjacent to the time of stimulation. Single receptor cell responses were recorded from at least two types of sensilla trichodea, predestinated for intersexual communication on account of their sexually dimorph structures (Fig.1) (6).

Results

Within the set of the highly effective compounds (reciprocal efficacy values between 1 and 10, Table 1) most of the acetates are more effective than the corresponding alcohols. None of the compounds, however, induces significant EAG-responses in *O. nubilalis* females. No obvious response differences are found between the animals originating from Limburgerhof or Leningrad. The effect of some binary mixtures of is demonstrated in Table 2. It shows that the the percentage enhancement of the EAG-response

Table 1

Ostrinia nubilalis (Hbn, Z-strain), males: EAG-reciprocal efficacy values of the pheromone components z11-14:Ac, *e*11-14:Ac and 70 structural analogues determined from dose-response relations; Ac: acetates, Al: aldehydes, Fo: formates, Ol: alcohols (s. text)

Ac	10:Ac	100		*z*8-12:Ac	30	*z*9	*e*12-14:Ac	30
				*e*9-12:Ac	100		*e*11-14:Ac	3
Ol	*y*5-12:Ol	100		*y*9-12:Ac	100		*z*11-14:Ac	1
	*z*5-12:Ol	300		*z*9-12:Ac	100			
	*y*7-12:Ol	100		*e*10-12:Ac	100	Ol	*y*5-16:Ol	100
	*z*7-12:Ol	10	*e*8	*e*10-12:Ac	100		*z*5-16:Ol	30
	*y*8-12:Ol	300					*y*7-16:Ol	100
	*z*8-12:Ol	300	Ol	*z*4-14:Ol	100		*z*7-17Ol	100
				*y*5-14:Ol	100		*y*9-16:Ol	100
	*y*9-12:Ol	100		*y*7-14:Ol	100		*z*9-16:Ol	100
	*z*9-12:Ol	30		*y*9-14:Ol	30		*y*11-16:Ol	30
*e*7	*z*9-12:Ol	100		*z*9-14:Ol	30		*z*11-16:Ol	30
				*y*11-14:Ol	100			
Al	*z*5-12:Al	100		*z*11-14:Ol	100	Al	*z*11-16:Al	100
	*z*7-12:Al	100				Fo	*z*11-16:Fo	100
	*y*9-12:Al	300	Al	*z*7-14:Al	100			
				*z*9-14:Al	300	Ac	16:Ac	100
Ac	12:Ac	30	Ac	14:Ac	30		*z*5-16:Ac	100
	*y*5-12:Ac	30		*y*5-14:Ac	30		*y*7-16:Ac	100
	*z*5-12:Ac	30		*z*5-14:Ac	100		*z*7-16:Ac	100
	*e*7-12:Ac	300		*y*7-14:Ac	30		*z*9-16:Ac	100
	*y*7-12:Ac	300		*y*9-14:Ac	100		*y*11-16:Ac	100
	*z*7-12:Ac	100		*y*11-14:Ac	10		*z*11-16:Ac	10

Table 2

Mean (n=10) EAG-responses to binary mixtures. Numbers indicate percentual enhancement of the EAG-responses by adding the compound indicated in the first line. A large EAG-enhancement indicates the response of at least two different types of receptor cells (numbers in brackets: +/- sd) (s. text)

	*z*11-14:Ac		*e*11-14:Ac		*y*11-14:Ac		*z*11-16Ac		*z*9/*e*9-14Ac	
*z*11-14:Ac	28	(9)	92	(28)	82	(22)	85	(25)	65	(18)
*e*11-14:Ac	98	(23)	35	(12)	40	(15)	70	(19)	72	(22)
*y*11-14:Ac	86	(37)	38	(15)	22	(7)	–		48	(14)
*z*11-14:Ac	95	(32)	63	(22)	75	(22)	22	(11)	54	(9)
*z*9/*e*9-14:Ac	73	(4)	55	(12)	45	(10)	60	(8)	24	(7)

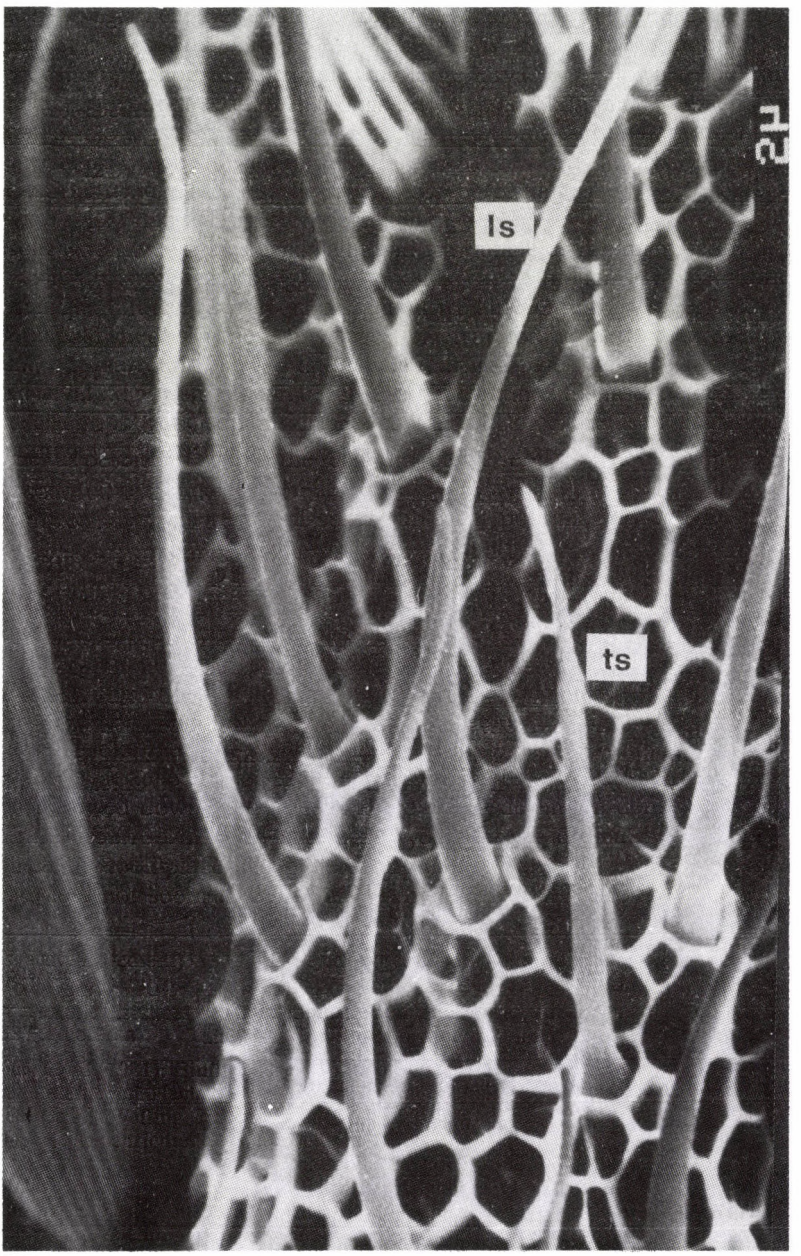

Fig. 1. *Ostrinia nubilalis* (Hbn, Z-strain), male: different types of sense organs (sensilla) on the 10th segment of antenna; ts = short sensilla trichodea, ls:long sensilla trichodea

remains within the range of 22% to 35% when the binary stimulus composes of the doubled source load of one and the same compound. Aside from mixtures of *y*11-14:Ac and *e*11-14:Ac or *y*11-14:Ac and *z*9/*e*9-14:Ac the enhancements for heterogeneous composed stimuli, however, range at significantly higher values from 63% to 98%.

Nerve impulse recordings are shown in Fig. 2. On base of the selective responses there might exist at least 5 differently specialized types of receptor cells. Two types are obviously spectalized for the interaction with either the *e*- (small nerve impulses) or the *z*- (large nerve impulses) isomer of the pheromone blend. They occur simultaneously in one sensillum. A third type of receptor cell responds to *z*-11-16:Ac and, slightly, also to *e*11-14:Ac. It is so far open if the response to *y*-14:Ac (*y* stands for acetylenic C-C bond) is in overlap to *e*11-14:Ac or if it arises from a potential fourth type of receptor cell. Two further receptor cells respond either to one or both compounds of the 97:3 mixture of *z*9- and *e*9-14:Ac.

Discussion

Electrophysiological studies of the olfactory system of *O. nubilalis* so far have been more concerned with the characterisation of the pheromonal polymorphism than with the existence and function of further behaviour modifying odorants: Büchi *et al.* (4) showed that males of the Z-strain gave higher EAG-voltages when stimulated with the *z*- than with equivalent amounts of the corresponding *e*-isomer. In the E-strain they found just reverse EAC-relations. By single receptor cell recordings, Hansson *et al.* (8) characterize the Z-strain by a large spiking receptor cell type responding to the *z*11- and a small spiking type to the *e*11-14:Ac. In the E-strain they found a small spiking "*z*"- and a large spiking "*e*-cell". The relevant cell types in the so-called hybrid strain respond both with small spike amplitudes.

Some of the compounds found to be active in our measurements, however, now make it possible to direct the search for a more effective application of odorants in the control of *O. nubilalis*.

In combination with flight track analysis (9, Kafka and Kafka, jr. unpubl.) in qualitatively, quantitatively and geometrically varying odor fields (9, 10) experiments are now running to provide insight into the synergistics and roles played by the different types of receptor cells during the sequence of intersexual orientation behavior.

For this, however, it seems interesting to note, that there is no unique relation between EAG-response, behavioral response and the fact that a compound is a constituent of the pheromone bouquet: Tetradecanyl acetate (14:Ac; (16)) which synergistically enhances trapping rates (24) does not induce a large EAC-response. The gland constituents like *z*11-14:O1 (2) and *z*11-16:Ac (22) induce clear cut EAG- and, the latter case, single receptor cell responses (Fig. 2), but do not cause evident behavioral reactions (19).

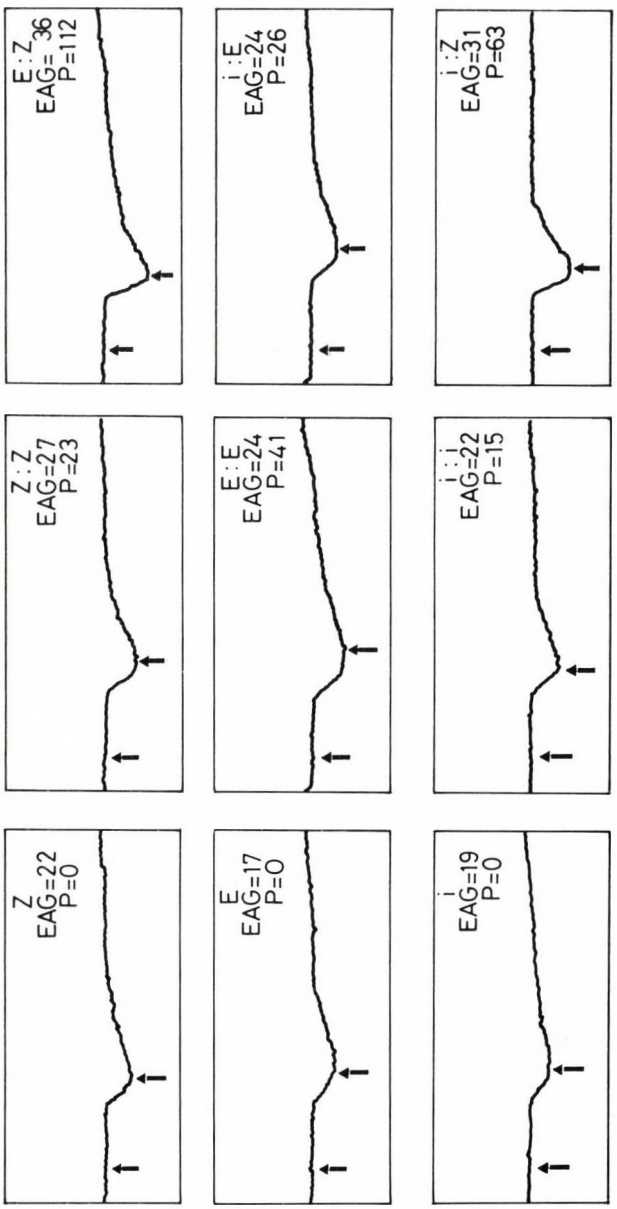

Fig. 2. *Ostrinia nubilalis* (Hbn, Z-strain), male: a-d) Selective responses of at least five different types of receptor cells of low spontanous activity; each line represents recording from a different s. trichodeum; blacks bars: vertical = 1 mV, horizontal = stimulus lenght 800 ms; source loads for the *z*- and *e*-isomer of 14-Ac 10-2 μg else 10-1 μg

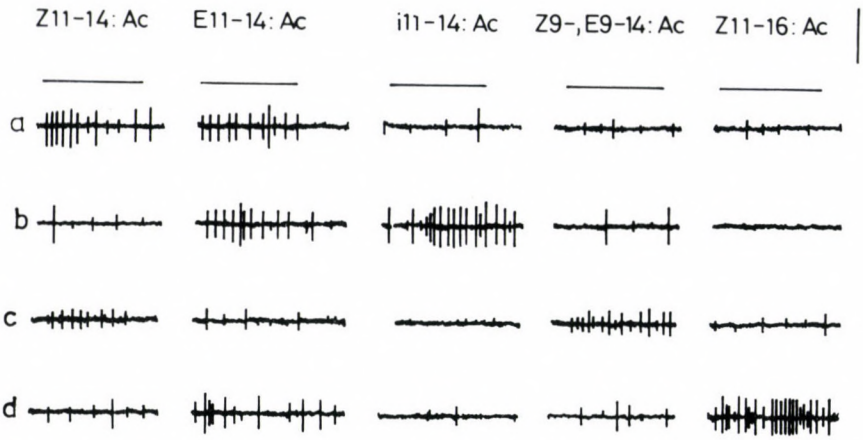

Fig. 3.

Acknowledgement

We thank Ms Carola Schmid for her expert preparation of the stimulus sources and, together with Ms Cornelia Bock - for the SEM - preparations. We are very indepted to Ms Dr. Blanka Kalinova from the Institute of Biochemistry Praha, CCSR for providing pupae and for valuable discussions, and to Dr Les Williams for reading the manuscript.

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The use of *Trichogramma* Species against the European Corn Borer (*Ostrinia nubilalis* Hbn.) in Bulgaria

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Maize is a major grain crop in Bulgaria, second in importance after wheat.

The maize fields in southern Bulgaria are particularly heavily infested by the European corn borer. The pest has two generations per year, damaging up to 98 % of the plants, with a peak density of 5-6 larvae/plant.

In Bulgaria, both the univoltine and bivoltine type of the European corn borer is present. The univoltine form occurs both in northern and southern Bulgaria. In the north only one generation, rarely a partial second generation develops.

However, 25% larval survival is sufficient to damage more than 80% of the plants (Karadjov 1982).

The initial feeding of the corn borer on leaves has no economic significance. Our investigations showed that when 3 larvae bore into the stalk under the corn ear, the growth rate was reduced by 7-12 cm per plant, and grain yield decreased by 35%. In the region of Plovdiv infestation of maize plants reached 98% with 18% of the ears infested. The density of egg masses averaged 40-60 per 100 plants, but we have registered a maximum of 12 egg masses per plant (Karadjov, Keita, 1986).

Application of *Trichogramma* against the European Corn Borer

In a number of countries where maize is grown on a large scale (USA, Canada, France, FRG and others) control of the corn borer is achieved by chemical treatments (Angland, 1977; Hassan 1984; Neuffer, 1982; Maedgen, Mathis, 1984).

In Bulgaria, irrespective of the high corn borer density the control is conducted completely by *Trichogramma*. The control by *Trichogramma* began 10 years ago today, parasites are used on 140,000 ha of maize. In recent years, investigations have been carried out examining the best conditions for *Trichogramma* application.

Field investigations revealed four species as corn borer parasites: *Trichogramma maidis* (Pintureau et Voegelé), *T. euproctidis* (Pintoi), *T. prenana* (Voegelé et Russo) and *T. dendrolimi* (Matsumara). Predominant in southern Bulgaria is *T. maidis*, which, under natural conditions, parasitizes up to 78% of the eggs of the second generation of the corn borer.

Experiments carried out on maize plots with *T. maidis* and *T. euproctidis* demonstrated the low dispersal and searching ability of these species (Fig. 1). During its 5 day of life, both species dispersed about 20 m from the point of release southwards; 15 m – northwards; 17 m – eastwards and 12 m westwards; 80% of the eggs were laid during the first 3 days. Vertically *Trichogramma* spp. parasitized more eggs on the 1st and 2nd level of the plants and less at the top.

Table 1

Effectiveness of *T. maidis* against the European corn borer, depending on the rate of release

Number of parasites released/ha	Number of eggs/100 plants	Parazitised %	Stems infested %	Larvae per plant	Cobs infested %	Effective - ness (protected plants) %
Control	361	-	73.0	2.18	15.0	-
100 000	220	47.7	45.0	1.14	11.0	38.3
150 000	252	64.2	31.0	0.82	7.0	57.5
200 000	260	70.3	21.0	0.62	3.0	71.2

Comparative field studies of 4 *Trichogramma* species reared in Bulgaria, showed *T. maidis* (72%) to be the most effective followed by *T. cacoeciae* and *T. embriophagum* (51-43%) and *T. euproctidis* (Pintoi) whose effectiveness was 42%. Field experiments with *T. maidis* showed 70% effectiveness at the release rate of 200,000 parasites/ha (Table 1). The first release was carried out when the first eggs were laid, the second one at the maximum flight activity of the moths (6-8 days after the first), and the third release was after 8-10 days.

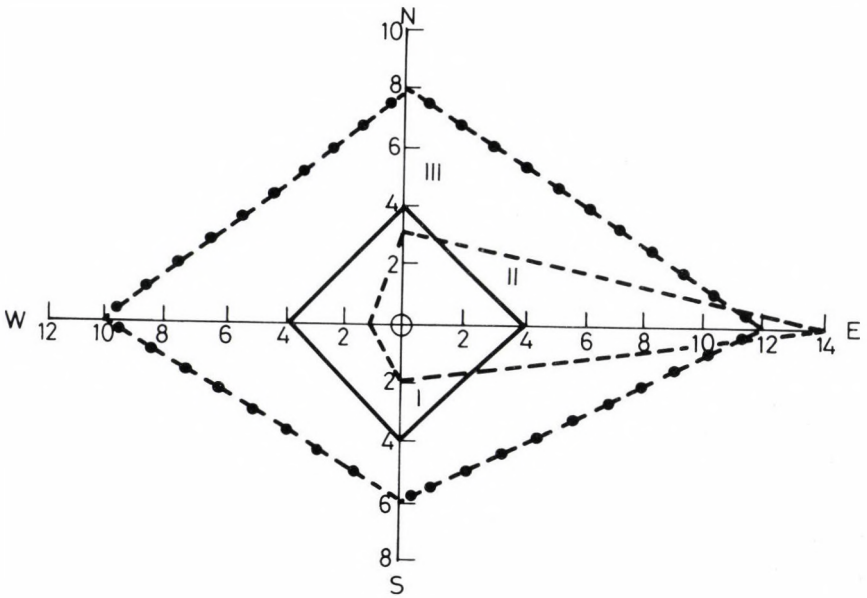

Fig. 1. Dynamics of flight and oviposition of *O. nubilalis*
flight, oviposition (Kostinbrod 1984)

Table 2

Effectiveness of *T. maidis* against corn borer, depending on release density

Number of parasites released/ha	Number of eggs/100 plants	Parazitised %	Stems infested %	Larvae per plant	Cobs infested %	Effective - ness (protected plants) %
Control*	467	8.2	67.0	2.21	18.0	-
100	386	33.3	42.0	1.15	14.0	37.3
200	228	39.8	37.0	0.63	12.0	45.0
300	221	43.8	28.0	0.49	13.0	58.2
400	210	48.5	21.0	0.32	11.0	69.0
500	336	64.4	17.0	0.27	6.0	75.0

*Release points per hectar

In relation to the number of release points, the highest effectiveness was at 500 points/ha (4 x 5 m) with 64% parasitism and 75% effectiveness. Stalk infestation was reduced by 70-80% and that of the corn ears by 70% (Table 2).

Good results were obtained throught the release by hand of parasites as pupae in host eggs, fixed on small cards, or as imagos placed on the maize plants. This method proved effective as a method of seasonal colonization for small maize fields (Karadjov, 1982). The mass application of parasites on large fields is possible by mechanical release. In the USSR, new methods of release from airplane and tractor are being developed (Andreev, Abashkin, 1986).

Recently, the capsule method for airplane release was developed and applied in Bulgaria. For that purpose, a cardboard – 1 mm thick, waterproof on one side, is used. This technology needs three pieces of equipment – one for preparing the capsules (6 mm in diameter 4 mm thick), one for filling the capsules with *Trichogramma* (at the rate of 300-500 eggs and pupae/caps) and a third for sealing the capsules. In the latest version, the 2nd and 3rd phases are combined.

The product is prepared -- several days before its release, placed in polythene bags and delivered to the farms. The release is conducted by spraying from an airplane "AN-2" flying at 15 m over the plants. Capsules are dropped 2-4 m from each other over a width of 60 m. Capsule density is 20 capsules/100 m^2, falling on plants and soil. The plane can cover 3.8 ha/hr. The airplane needs to be re-loaded with capsules once or twice daily.

Conclusions

As a result of 15 years of research work, an effective technology for *Trichogramma* application for the control of the European corn borer has been developed and utilized on large areas in Bulgaria (in the next few years – 200,000 ha) providing good results (up to 87% parasitized eggs 89% protected plants and at a quarter of the cost of chemical control. Two treatments with chemicals are completely avoided; this protects the environment and crop production from pesticide residues.

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Comparison of Insect Biomass in Maize Crops with Biomass in other Agricultural Ecosystems

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The insect biomass in crops in the neighbourhood of Turew (midwest Poland) has been estimated for many years. These investigations are part of a wide-ranging of the research programme which aims at qualifying the principles of agroecosystem functioning.[1]

It was possible to define faunal impoverishment of agroecosystems by estimating the biomass structure of different animal groups (Ryszkowski 1985). In crops, especially annual crops (in comparison to other land agroecosystems), faunal impoverishment takes place both in terms of biomass and species composition. The fact that maize cultivation is a vital element in the mosaic of the studied ecosystems (about 15% of the area), means that this may significantly influence the insect fauna of the whole landscape.

In this paper, the results are presented of research carried out in the period 1975-1980 on the biomass of insect fauna of crops in the neighbourhood if Turew.

Study Area and Methods

The area investigated, about 40 km from Turew, was on a typical landscape of mid-west Poland, characterized by different fields, forest plantations and small woodlands.

The average size of cultivated fields is not high (not higher than tens of hectares). The research area is in the Atlantic climate zone. The region is warm for Poland. The average annual temperature is 8°C and the vegetation period is long (average 225 days) while the annual precipitation does not exceed 527 mm.

Cereal crops cover about 50% and root crops about 20%, of the whole area given to agriculture. The total arable area amounts to about 87% and woods and afforestations (shelterbelts) about 10% of the whole landscape.

Quick-traps were used to estimate above-ground insects; samples were collected (20 samples in each series) at 10-15 day intervals from March to October (Ryszkowski and Karg 1977). To evaluate the density and biomass of flying insects a motor-net was used (Karg 1980), with insects caught in three nets tied to a pole fixed in turn to a motorcycle with a side- car. The nets were placed at 0.5, 1.5 and 2.5 m above the ground. Sampling consisted of a trip accross a given ecosystem (with an average velocity of 35 km/h) along a specified transect. Calculations per 100 m^3 (or 1 m^3) of air were made using the formula for volume of the cylinder ($\pi r^2 h$). Samples were taken with an average frequency of 20 days during the vegetation season (March to October). A total of 1161

1 Studies carried out within the project CPBP 04.10.03.

samples were collected from each of the three air layers. The material included 479,087 specimens representing 173 families and 18 orders of insects.

Results

Above-ground insects

The average biomass of the above-ground insects, in the agroecosystems and meadows studied, amounted to 23.0 mg dw m^{-2}. The lowest biomass was found in maize and barley (about 11.0 mg dw m^{-2}) while the highest in meadows (52.0 mg dw m^{-2}) and potato fields (about 45.0 mg dw m^{-2}) (Table 1). Diptera represented as much as 70% of the total insects (oats), and was the dominant group in the majority of the ecosystems.

Table 1

Taxonomic structure of above-ground insect biomass in ecosystems of agricultural landscape (mg dw m^{-2}, percent)*

Order	Maize	Barley	Rye	Oat	Wheat	Sugar beet	Rape	Potato	Alfa-lfa	Meadow	Mean
Diptera	4.0	5.1	7.5	13.8	10.9	7.4	14.0	8.5	6.0	15.7	9.3
	(33.9)	(45.1)	(41.2)	(69.0)	(47.8)	(48.1)	(66.4)	(19.1)	(46.2)	(30.2)	(40.4)
Coleoptera	4.9	3.9	3.1	3.6	4.2	3.0	5.0	31.3	3.7	1.0	6.3
	(41.6)	(34.5)	(17.0)	(18.0)	(18.5)	(19.5)	(23.7)	(70.2)	(28.5)	(1.9)	(27.4)
Heteroptera	1.8	1.0	4.6	0.8	2.4	2.1	0.6	3.8	2.0	0.9	2.0
	(3.4)	(2.7)	(12.1)	(2.0)	(18.9)	(5.8)	(2.8)	(1.1)	(3.8)	(6.9)	(6.1)
Hymenoptera	0.3	0.4	0.7	0.7	0.9	0.9	0.9	0.4	0.2	0.6	0.6
	(2.5)	(3.5)	(3.8)	(3.5)	(3.9)	(5.8)	(4.3)	(0.9)	(1.5)	(1.1)	(2.6)
Orthoptera	0.1	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.4	30.1	3.1
	(0.8)	(0.9)	(0.0)	(0.0)	(0.0)	(1.3)	(0.0)	(0.0)	(3.1)	(57.9)	(13.5)
Other	0.3	0.5	0.1	0.7	0.1	0.9	0.0	0.1	0.2	0.1	0.3
	(2.5)	(4.5)	(0.6)	(3.5)	(0.4)	(5.8)	(0.0)	(0.2)	(1.5)	(0.2)	(1.3)
Total	11.8	11.3	18.2	20.1	22.8	15.4	21.1	44.6	13.0	52.0	23.0

* Without *Aphidae* and *Thysanoptera*. In the brackets is the percentage share.

Other groups of insects are dominant in biomass only in three ranges; in meadows Orthoptera (57.9% mainly species of *Chorthippus*), in potato fields Colorado beetle (Coleoptera) (as much as 70.2%), and on maize fields Coleoptera were also dominant (41.6%) (Table 1). Large predatory species (Coccinellidae), comprise as much as 30% the total insect biomass in maize cultivations. Coccinellidae also occur in relatively large numbers in other crops, especially barley and potato. Their occurence is connected with the occurence of aphids. Heteroptera, with Miridae dominant, also have a vital share of the insect biomass in maize. Other orders are less well represented in terms of total insect biomass in maize while Homoptera (excluding Aphidae), are more imprtant in other

crops (wheat, rye). Maize, then, is characterized by the lowest or one of lowest biomass of Diptera (4.0 mg dw m^{-2}), Hymenoptera (0.3 mg dw^{-2}) and Homoptera (Table 1).

In the trophic structure of the above-ground insects, phytophagous and saprophagous forms (on the average 85%) are prevalent in the majority of the crops studied. Only in maize and rye were predators dominant. Here their biomass was lower than all other trophic groups taken together but higher than in all other ecosystems studied. The predators share in the total above-ground insect biomass reached on the average 23% while in maize it was as much as 45.9% due to the presence of large species of Coccinellidae (Table 2).

Table 2

Trophic structure of above-ground insect biomass in different ecosystems of agricultural landscape (percentage share)

Trophic group	Maize	Barley	Rye	Oat	Sugar beet	Rape	Potato	Alfalfa	Meadow
Saprophages	27.6	26.4	23.3	37.3	36.9	44.5	44.9	43.0	12.5
Phytophages	25.9	33.3	34.6	33.2	28.8	30.5	43.5	38.8	82.5
Predators	45.9	38.5	40.5	26.3	31.5	21.9	8.5	16.7	4.3
Parasites	0.6	1.8	1.6	3.2	2.8	3.1	3.1	1.5	0.7

Flying insects

The average biomass of flying insects for all ecosystems is 0.22 mg dw m^{-3} while in successive ecosystem it ranges from 0.10 to 0.29 mg dw m^{-3} (Table 3). The lowest biomass values were observed in the environment of the villages, roads and spring crops (maize), while highest biomass values were recorded in and around winter crops, perennial crops and meadows as well as ecotones and forests; in other words in more natural ecosystems under least human influence. Diptera (particularly Anthomyiidae, Muscidae, Syrphidae, Calliphoridae) contribute to the high biomass as well as Hymenoptera (Ichneumonidae) and, on meadows, Orthoptera (Acridiidae) (Table 3).

Maize is one of the most impoverished crops as far as flying insect biomass values are concerned, with only 0.11 mg dw m^{-3}, with fewer recorded families (only 104 families, while 173 in the whole landscape). Similarly to other ecosystems, maize is dominated by Diptera. Quite a vital biomass is characteristic for species of Scarabaeidae and Apidae. Species of Coccinellidae, have a noticeable share of the above-ground insect biomass but a low share in the flying insect biomass of maize (only about 1%).

In maize as well as in other crops, the majority of flying insects were caught in the lowest air layer (0.5 m). The ratio of flying insect biomass in air layers from the lowest to the highest (2.5 m) is as follows: 1:0.38:0.29.

In maize a lower share of saprophagous insects in the flying insect biomass was observed than in other spring crops, while a higher share of phytophagous and predatory forms was noticed (Table 3).

Table 3

Mean biomass of flying insects family dominating in different types of ecosystems (mg dw 100 m^{-3} and trophic structure, %)

Dominating family	Maize	Villages	Roads	Spring crops	Winter crops	Perennial crops	Meadow	Ecotons	Forests
Anthomyiidae	1.31	1.23	1.52	2.23	5.95	2.37	3.20	6.52	5.69
Calliphoridae	1.16	0.48	0.52	0.59	0.59	2.81	3.15	1.97	2.95
Muscidae	0.25	0.72	0.50	0.49	2.07	1.02	1.52	1.40	2.60
Syrphidae	0.44	0.46	0.55	0.76	1.12	0.97	2.66	1.35	2.26
Chironomidae	0.81	0.37	0.75	1.29	1.51	2.20	1.74	0.84	0.34
Sphaeroceridae	0.76	1.49	1.05	0.72	1.26	2.33	0.57	0.81	0.47
Scarabaeidae	0.79	0.66	0.62	0.99	0.95	1.45	1.04	1.40	0.62
Empididae	0.12	0.25	0.21	0.21	1.74	0.29	1.81	0.90	1.42
Staphylinidae	0.46	0.54	0.85	0.66	1.00	1.24	0.65	1.18	0.66
Ichneumonidae	0.08	0.31	0.39	0.22	0.50	0.37	0.33	0.88	1.52
Scatophagidae	0.87	0.33	0.07	0.46	0.42	0.67	0.43	0.63	1.00
Bibionidae	0.64	0.10	0.31	1.03	0.45	0.47	0.85	0.55	0.33
Apidae	0.67	0.15	0.43	0.54	1.13	0.86	0.25	0.42	0.12
Nymphalidae	0.07	0.03	0.02	0.07	0.39	0.06	1.19	0.63	0.84
Acridiidae	0.00	0.08	0.11	0.05	0.00	0.23	2.66	0.00	0.04
Chloropidae	0.30	0.19	0.26	0.34	0.59	0.46	0.47	0.44	0.35
Sciaridae	0.20	0.20	0.23	0.23	0.37	0.30	0.41	0.39	0.36
Nitidulidae	0.04	0.05	0.11	0.09	0.31	0.10	0.86	0.26	0.06
Coccinellidae	0.13	0.02	0.11	0.10	0.05	0.13	0.36	0.44	0.28
Tenthredinidae	0.09	0.09	0.10	0.06	0.38	0.03	0.36	0.11	0.19
Other (153)	2.46	1.79	2.58	2.14	3.99	3.05	1.79	4.37	6.73
Total	11.65	9.88	11.47	13.40	25.22	21.58	29.53	25.79	29.04
Saprophages	56.0	70.7	60.9	67.2	61.5	73.6	47.5	62.0	53.4
Phytophages	26.5	15.2	21.7	19.4	19.8	13.4	28.8	16.7	19.0
Predators	9.2	10.1	12.2	10.4	15.5	10.2	21.7	16.3	20.7
Parasites	7.9	4.0	5.2	3.0	3.2	2.8	2.0	5.0	6.9

Summary and Conclusions

The above-ground insect biomass and the flying insect biomass is lower in maize than in other ecosystems. The taxonomic composition of insects of maize is also more impoverished than in other agroecosystems. The trophic structure of the above-ground insect biomass of maize is different from the other ecosystems in its higher share of predators (mainly Coccinellidae). The interdependence was not observed in the case of flying insects while the higher share of phytophagous forms was noticed. Analysis of the flying insect biomass particularly points to an impoverishment of the spring crops fauna which is probably due to their short duration and the frequent use of herbicides (especially in the case of maize).

The share in agricultural landscapes, of defined types of ecosystems has a direct influence over the changes taking place in the fauna of the landscape. Enriching the landscape in forest ecosystems which have a rich fauna influences the increase both the quantitative and qualitative variability.

The large amount of agriculture ecosystems such as spring crops and paricularly maize leads to quantitative and qualitative impoverishment of the insect fauna and also leads to an increase the phytophagous species biomass. It may eventually lead to a higher threat to cultivation from phytophagous pest species.

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Characteristics of the Macrolepidopteran Assemblage of a Maize Stand in Hungary

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For capturing adults of Macrolepidoptera a light trap was operated in a maize field monoculture during the growing season from 1976 to 1984. During the 8 year's survey 8567 individuals of 236 species of macrolepidoptera were caught by the light trap. The continuous maize growing itself did not cause an outbreak of macrolepidopteran pests. Only 15 of the 236 were present every year, but these represented 55% of the total catch. The food plants of these dominant species may be maize and the weeds and grasses in or around the field. The presence of phytophagous macrolepidoptera means also the presence of their parasites and predators, which are important in the zoocoenosis of maize.

On the suggestion of Academician T. Jermy, investigations were initiated in 1976 in agro-ecosystems. Surveys have been carried out in different types of apple and maize stands, as representing the changes in their production technologies. The aim of these studies was to establish the ecological foundation of an integrated plant protection system. This was only possible by becoming acquainted with the structure of their animal communities, factors that influence the population dynamics of important phytophagous species and types and significance of the controlling mechanisms (Jermy 1977, 1979).

Our work was done in the framework of this research program. In Hungary investigations on the structure of the macrolepidopteran assemblages were conducted in forests, in natural reserves, but no data are available from agricultural areas, or crops.

In the relevant literature on maize pests, few data refer to the macrolepidopteran assemblages. Camprag (1971) described more than 120 macrolepidoptera species feeding or causing damage to maize in Yugoslavia. Tawfik, Kira & Metwally (1974) list phytophagous and predatory species from maize in Egypt. Wedberg, Campbell & Helme (1975) pointed to the differences of webworm (*Crambus* species, etc.) damage in the first year of the production and in the consequent years of the production in the USA. They also found differences in the diversity of carabids, elaterids, tenebrionids and scarabeids in maize and in the neighbouring pasture land. Chambon (1982) sampled 800 species or group of species in a crop rotation system of wheat-maize and wheat-dicotyledon in France by using pitfall traps and yellow pan traps.

Material and Methods

In our investigation, sampling was carried out in a maize field of about 400 ha, located in Fejér county in Hungary. Maize had been grown since 1965 on the field studied. The cultivation of maize was carried out with maximal mechanisation and accompained with intensive chemical treatments (fertilisers, herbicides, fungicides and soil

Table 1

Feeding categories of Macrolepidoptera regarding the food plants of larvae

1. Species of tree crown and bush level	
	1.1. Polyphagous spp.
	1.2. Mono- and Oligophagous spp.
	1.3. Spp. on Populus and Salix (occasionally on Betula and Alnus)
	1.4. Spp. on Pinus
2. Species feeding on aerial parts of low bushes and herbaceous undergrowth	
	2.1. Spp. mostly on graminaceous plants
	2.2. Spp. mostly on dicotyledonous plants
	2.3. Mono- and Oligophagous spp. on dicotyledonous plants
	2.4. Spp. on flowers and fruits
3. Species feeding on ground level (on roots and on lower leaves, stem parts of herbaceous plants)	
	3.1. Spp. mostly on graminaceous plants
	3.2. Spp. mostly on dicotyledonous plants
	3.3. Spp. on bulbs and rhizomes
4. Endophagous species	
	4.1. Spp. in grass stem and roots
	4.2. Spp. in reed stalks and in other marshland Graminaceae
	4.4. Spp. in bulbs (Liliaceae) and rhizomes (Iridaceae)
	4.5. Spp. in tree stems
5. Species feeding on lichens and mosses	
6. Saprophagous species	
7. Predatory species	
	7.1. Obligate predators
	7.2. Facultative predators

disinfectans). In the area surrounding the maize stand, different crop plants were grown each year such as sunflower and wheat, and the maize plot was bordered on one side by a neglected park.

For sampling the adult lepidoptera a light trap of Jermy-type with a 100W normal bulb was used from 1976 to 1984 with the exception of 1981. The light trap was located 80-100 m from the field border, partially covered by the maize from middle July, and operated from the beginning of the maize growing season until harvest (from May to October).

Adult macrolepidoptera caught by the light trap were identified and categorized according to the food plants of their larvae (Tables 1, 2).

Results and Discussion

During the 8 years, 8567 individuals of 236 macrolepidopteran species were caught by the light trap. We are well aware of the possible errors of using light trapping, as a sampling method, but a long-term survey supported by larval sampling may reduce the errors.

Table 2

Percentages of macrolepidoptera individuals, according to the food plants of their larvae

		1976	1977	1978	1979	1980	1982	1983	1984
1.	1.1.	8.1	4.0	4.6	10.1	3.3	5.5	7.0	3.4
	1.2.	0.6	1.0	-	0.1	-	0.1	0.2	0.2
	1.3.	1.5	0.8	0.9	0.7	0.8	0.8	1.2	1.2
	1.4.	-	-	-	-	-	-	-	-
2.	2.1	12.0	8.5	6.3	2.3	7.4	17.0	10.2	7.5
	2.2.	48.1	64.5	37.9	73.4	63.9	66.6	67.4	64.4
	2.3.	-	-	0.3	0.1	-	-	0.1	0.1
	2.4.	0.6	0.5	0.9	0.2	-	0.8	0.7	1.3
3.	3.1.	-	12.5	3.4	1.4	7.4	0.9	1.0	4.1
	3.2.	16.3	5.6	43.9	8.9	10.7	5.1	8.3	9.0
	3.3.	-	-	-	-	-	0.1	-	0.1
4.	4.1.	-	-	-	-	-	-	-	-
	4.2.	11.1	2.1	1.4	0.9	4.9	1.4	1.8	0.4
	4.3.	-	-	-	0.1	-	0.1	-	0.1
	4.4.	-	-	-	-	-	-	-	-
	4.5.	0.3	-	-	0.1	-	-	-	0.1
5.	0.3	0.3	0.6	1.0	1.6	1.0	1.8	7.2	
6.	-	0.3	-	0.1	-	0.7	0.5	1.0	
7.	7.1.	-	-	-	-	-	-	-	-
	7.2.	-	-	-	-	-	-	-	-

Despite of the intensive maize growing in a relatively large area, a great number of macrolepidopteran species could be found, representing about 25% of the whole Hungarian macrolepidopteran fauna. The yearly catches (species and individuals) varied greatly. From the 236 species, only 10% were really maize pests. This result is similar to that of Chambon (1982), where only 5% of the species caught by traps in a wheat rotation system lived on cereals. Maize can also occasionally serve as food plant for other species. Regarding the number of individuals, 8-20% of the total catches were maize pests. It was established, that the long-term continuous maize growing did not cause an outbreak of macrolepidopteran pests. The percentages of individuals categorised according to the food plants of their larvae are presented on Table 2. Individuals feeding on tree crown and bush levels occurred every year in 4-11%. Their habitats could be the trees and bushes of the neighbouring park and of the field edges. Individuals feeding on higher parts of low bushes and herbaceous undergrowth were 45-82% of the total catch. As a consequence of the relatively dry climate, 6-47% of the individuals were ground-level feeding species. The endophagous species (mostly feeding in reed stalks and in other marshland Gramineae) were 1-12% of the catch. Individuals feeding on lichens and mosses (as plants of undisturbed areas) were caught every year in small percentage (1%). They could have also lived in the park and in the field border.

Regarding the frequency of occurence of species (Figure 1) and their total number of individuals (Figure 2), from the 23 species caught during the 8 years survey only 15

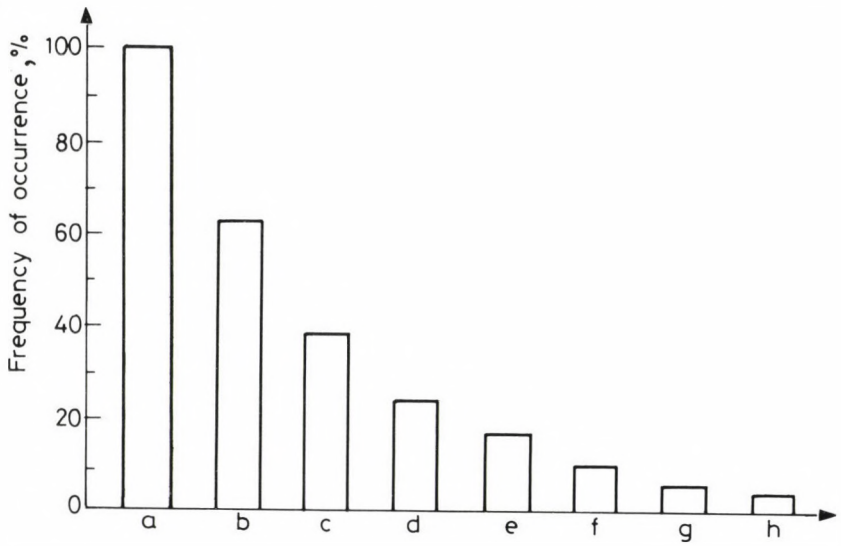

Fig. 1. Species occured at least in a=1; b=2; c=3; d=4; e=5; f=6; g=7; h=8 year

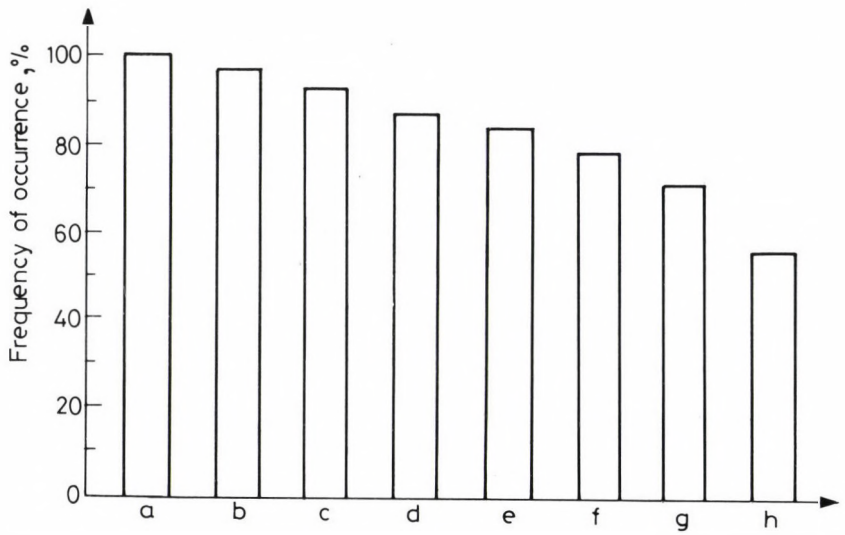

Fig. 2. Individuals of species occured at least in a=1; b=2; c=3; d=4; e=5; f=6;g=7; h=8 year

species were present every year, but their numbers represented 55% of the total catch. This supports the findings of Chambon (1982) who found that only 180 were present every year of the 800 species captured and that these represented 97% of the total catch.

By summarizing the results, we can establish that the macrolepidopteran assemblage of the maize field examined consists of a great number of species with consistent dynamic changes of the fauna of the area. The food plants of these species are the maize itself and the weeds and grasses in or around the field. Regarding the zoocoenosis of the maize, the field border and its weeds provide food and habitat of the Macrolepidoptera. Therefore the saving of the field border (omission of any chemical treatment) may be beneficial for the macrolepidoptera, for their predators and parasites and so for the whole zoocoenosis of the maize.

Acknowledgements

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Preliminary Investigation of the Maize Entomofauna in the Warsaw Region

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The literature on the occurrence of insects on maize in Poland is very scarce. The first systematic studies of maize pests were performed in 1956-1959 in South Poland in the vicinity of Wroclaw (Kania 1962b). Later investigations dealt with registration of pests in maize plantations in various regions of Poland (Walczak 1977, Studzinski 1968, Studzinski and Juszczak 1972). Between 1961-1978, studies were made of some more important maize pests, e.g. *Ostrinia nubilalis* Hbn. (Lepidoptera) and flies – Chloropidae (Kania 1961, 1966, Napiorkowska-Kowalik and Ziarkiewicz 1978).

In Poland, the importance of maize has markedly increased during the past decade. In 1979 (1st year of the present study) the area of maize plantations in Poland amounted to about 1 million ha, i.e. it was 6 times greater than that in 1970.

The aim of the present study was to gain knowledge of the qualitative composition and relative abundance of the entomofauna on maize cultivated in the vicinity of Warsaw. This region is the most northern part of Poland in which maize still attains full grain ripeness.

Study Area, Methods and Material

Studies were carried out in 1979 and 1980 in the maize plantation (about 12 ha) grown for fodder in Lomna (Warsaw voivodeship). Maize was sown mid-May and harvested in the first days September.

Sweep-netting, direct counting of insects on maize plants, and inspection of collected isolated plants were made. 10 x 25 sweeps were taken with a sweep-net at 7-day intervals. Observations were made on 50 plants.

All main groups of phyto- and zoophagous insects occurring on maize, except for Diptera, were studied. Our material of Diptera was not identified precisely enough to sort them into trophic groups.

During two seasons of investigations a total of 2062 phytophages and 171 zoophages were caught by the sweep-net method.

Results

The present results are based on data obtained with the use of a sweep-net. They concern insects inhabiting young maize plants or the lower and middle parts of older plants. In general, data obtained by sweep-netting and direct counting were consistent.

Table 1

Percentages of main phytophagous insect groups (sweep-netting) on maize fields in 1979 and 1980.

Group	Percentage	
	1979	1980
Thysanoptera	37.6	32.8
Hom., Auchenorrhyncha	33.4	41.8
Hom., Aphidodea	15.8	15.1
Hom., Psyllodea	0	2.5
Heteroptera	10.6	6.3
Coleoptera	0.8	1.0
Lepidoptera	1.7	0.5

The qualitative structure of the maize entomocoenosis was characterized by a much higher proportion of phytophages than of zoophages. In 1979 and 1980, the number of phytophages was 6 and 10 times, respectively, higher than that of zoophages. Thysanoptera and Homoptera (Auchenorrhyncha and Aphidodea) accounted for the highest percentages of the total numbers of phytophages. In 1979 and 1980, Thysanoptera represented 32 and 37%, Auchenorrhyncha 33 and 41%, respectively, and Aphidodea – 15%. The percentages of Heteroptera were lower (6 and 10%, respectively). Lepidoptera and Coleoptera accounted for no more than 2% (Table 1.).

Among zoophages, in 1979 and 1980 parasitic Hymenoptera (71 and 59%) and Coleoptera (14 and 26%, among which Coccinellidae were dominant) reached the highest percentages. The proportion of Neuroptera and Syrphidae were lower (Table 2.).

In 1979 and 1980 the mean number of phytophages per 250 sweeps was not very high; it amounted for Thysanoptera to 63 and 71 individuals, for Aphidodea to 23 and 32 individuals, and for Heteroptera to 16 and 13 individuals, respectively.

Among zoophages, in 1979 and 1980 the mean number per 250 sweeps in the season was highest for Hymenoptera (8 and 11 individuals) and for Coccinellidae (5 individuals).

Table 2

Percentages of main zoophagous insect groups (sweep-netting) on maize fields in 1979 and 1980.

Group	Percentage	
	1979	1980
Coleoptera (predaceous)	14.2	26.9
Neuroptera	12.5	6.9
Hymenoptera (parasitic)	71.4	59.1
Dipt., Syrphidae	1.9	6.9

The number dynamics of the different groups of phytophages were synchronized with the phenology of maize. At the beginning of June, when the height of maize plants was about 20 cm, Auchenorrhyncha occurred in greatest numbers. *Macrosteles laevis* (Rib.) was the dominant species; its numbers were maximal in mid-June, then in the end of August, when the corn-cobs were fully formed.

In the end of June, prior to florescence, maize plants varied between 50 cm - 1 m in height and then the mirid *Trigonotylus coelestialium* (Kirk.) was relatively abundant. In mid-July, during florescence, when the plants reached 2 m, aphids were the most numerous phytophages. In this period *Metopolophium dirhodum* Walk. was the dominant aphid species.

The numbers of Thysanoptera increased in the beginning of August when the corn-cobs were fully formed. *Haplothrips acleatus* Fabr. was the dominant species. Late August and early September, when the grain was not yet fully ripe, mirids (among which *Lygus rugulipennis* Popp. was dominant) were abundant. Moreover, in this period, outbreak of the aphid species *Rhopalosiphum padi* L. was also noted.

In the investigated area near Warsaw, rye is one of the most frequently grown cereals. The numbers of insect groups mostly represented on rye were compared with their numbers on maize. It was found that the number of Heteroptera and of Thysanoptera was 20 to 14 times lower on maize than on rye. The seasonal dynamics of various groups or species on rye and maize was similar.

In conclusion, in the region of Poland investigated, the qualitative structure of the entomocoenosis of maize resembles that of other cereals. However, numbers of phytophages and zoophages were much lower on maize than on other cereals. It seems that the conditions prevailing on maize, as compared with other cereals, were less favourable for the insects. This is, among others, due to the fact that the developmental cycles of the main phytophages not always coincide with the phenology of maize. The process of migration of insects from cereals to maize and their adaptation to the new conditions in central part of Poland are not yet fully investigated.

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Xylena vetusta Hb. (Lep., Noctuidae, Cucullianae), A New Pest of Maize in Poland

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In 1980, on maize plantations belonging to the Plant Breeding Station at Mikulice (province of Przemysl), some plants damaged by larvae of *Xylena vetusta* Hb. were observed. Later this pest was found damaging maize not only in South-East Poland but also in a distant Zulawy region and in Lower Silesia. This insect appears to occur more numerously in areas with high rainfall, on maize plantations localized on heavy soils. Should the density of this insect increase on the plantations – then, because of the voracity of its larvae, it would become one of the most dangerous maize pests in Poland.

In 1980 caterpillars of the moth *Xylena vetusta* Hb. were found on single plants in a maize plantation belonging to the Plant Breeding Station in Mikulice (province of Przemysl). During the next few years infestation of maize by this pest was observed not only in South-eastern Poland (province of Przemysl and Rzeszow) but also in Zulawy region (northern Poland) and in Lower Silesia (south-western Poland).

Plant damage varied according to the density of the noctuid populations on the maize plantations. The main parts of the plant damaged are the leaves (mostly the fifth up to tenth ones), and sporadically, also the panicles. The most frequently observed symptoms of noctuid feeding are some loss of leaf surface at their edges or tips, and round or irregular holes. Sometimes as a result of feeding on leaf just above its base, extending from both edges to the central vein, a fracture occurs and some parts of leaves dry and even drop off. In some plants, the leaves are nearly totally eaten and their lower fragments remain in the form of stumps. Caterpillars mostly damage the top parts of the panicles. Damaged plants are stunted as their growth and development is delayed and frequently form additional stalks. Severly damaged plants did not produce cobs.

The life cycle of *X. vetusta* on maize in South-eastern Poland is as follows. In April, after the stabilization of warmer weather, the overwintered adults start their flights. Females lay their eggs on maize leaves in the second and third week of May up to the first days of June. Egg-laying takes place in the period when the maize forms leaves from the fourth to the seventh one. The caterpillars feed on plants in June and in the first and second week of July. In the second half of July, they come down into the soil. Here, underneath the withered plants or in cracks in the soil, they spin their cocoons in which they stay for about 30 days. At the end of that period they pupate and, some days later, adults emerge. The most intensive period of the adult flight takes place in the third week of August. Flight continues also in September and October until temperature drops which causes the moths to look for overwintering sites.

As early as in 1907, Lampert stated, that *X. vetusta* caterpillars feed on *Polygonum* spp., *Iris* spp. and some swamp-grasses. Heintze (1978) stated, that this species occurs throughout the whole Poland in meadows, fields, forest edges, swamps, peatbogs and gardens. According to him, the caterpillars of this noctuid feed on perennials and on willows. Novak (1980) noted, that this moth is distributed in Europe, Asia and North

America in forests and meadows, and that caterpillars frequently occur on cultivated plants.

In Poland, maize has become, a plant that the caterpillars of *X. vetusta* are willing to attack. This insect occurs more numerously in the regions with high rainfall, on maize plantations situated on heavy soils, rich in humus. If in the future the density of this species increases, then, because of the great voracity of the caterpillars, it may become one of the most dangerous of maize pests. Probably such a situation will not occur, because populations of *X. vetusta* are diminished by birds which willingly feed on the caterpillars.

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Chemical Control of the European Corn Borer (*Ostrinia nubilalis*) in Czechoslovakia

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In Czechoslovakia, maize is grown on an area of about 600,000 ha, with maize on about 200,000 ha. Maize is grown mainly in south-western Slovakia and it is this region where European corn borer causes the greatest damage. No chemical control had been used in the past because only Thiodan 35 EC (endosuplphan), an especially toxic compound, was registered. Since the control against the European corn borer in Czechoslovakia is by aerial spraying, this preparation could not be used.

In 1984, experiments with different chemical and biological preparations were initiated, when new chemicals were registered: the synthetic pyrethroids, Decis 2.5 EC (deltamothrin) 0.5 l/ha, Cymbush 10 EC (cypermethrin) 0.5 l/ha, Karate 5 EC (lambdacyhalothrin) 0.25 l/ha, Vaztak 10 EC (alphacypermethrin) 0.3 l/ha. Insect growth regulators, such as Nomolt 15 SC (teflubenzuron) 1 l/ha, Alsystin 480 SC (triflumuron) and biological preparations, such as Bathurin 82 (*Bacillus thuringiensis*) and Boverol Spofa (*Beauveria bassiana*) were also tested.

Experiments with chemical or biological control against European corn borer were carried out in two ways: 1. aerial sprays; in agricultural farms on larger areas. These experiments were organized by the Central Agricultural Control and Test Institute in Brno and Bratislava, and they were carried out in southern Slovakia and southern Moravia. These regions are usually infested by the European corn borer every year. Two small experimental plots were tested with a knapsack sprayer at the Maize Research Institute in Trnava.

In the experimental ground of the Maize Research Institute there is a twenty plus year old monoculture of maize and the rate of infestation by the European corn borer is relatively high. No chemical control is practised. The 1984 and 1985 experiments were on a small scale, (60 plants evaluated). The study was expanded (200 plants, i.e. 4 replications with 50 plants in each) in 1986 and 1987. The plants were evaluated in autumn those infested and broken under the ear were marked. Eventually, the plants were cut and caterpillars were counted.

In 1986, 11 preparations were tested (Table 1). In 1987, only 5 preparations were included in the test. No biological preparations were tested because Boverol was withdrawn by its producer from testing with European corn borer (Table 2).

Although synthetic pyrethroids gave very good control, they will be applied only until experiments with biological preparations are succesfully finished. Experiments are being carried out to test the effectiveness of the parasitoid *Trichogramma evanescens* against the European corn borer.

Table 1

The effectiveness of selected chemicals against the European corn borer, 1986

Preparation	Infected plants			Caterpillar number/plant			Broken plants under ear	
	%	Number	BE %	S		BE %	Number	%
Untreated control	80	160	-	134	0.9	-	23	11.5
Dursban 4 E	67	134	16.5	138	1.0	0	30	15.0
Decemthion EK 20	75.5	151	5.6	135	0.9	0	40	24.0
Alsystin 480 SC	55.5	111	30.6	88	0.8	34.3	15	7.5
Nomolt	34.5	69	56.9	59	0.9	56.0	37	18.5
WL 115 110	129.5	59	70.5	40	0.7	70.1	14	7.0
Karate	28.0	56	65.0	42	0.8	68.6	14	7.0
Vaztak	14.0	28	82.5	9	0.3	93.3	7	3.5
Vaztak	33.5	67	58.1	47	0.7	64.9	23	11.5
Decis 2.5 EC st.	52.0	104	35.8	72	0.7	46.3	35	17.6
Boverol Spofa	50.0	100	37.5	76	0.8	43.3	45	22.5
Bathurin 82	74.0	148	7.5	146	1.0	0	39	19.5
Dipel	48.0	96	40.0	73	0.8	45.5	29	14.5

Table 2

The effectiveness of selected chemicals against the European corn borer, 1987

Preparation	Infected plants			Caterpillar number/plant			Broken plants under ear	
	%	Number	BE %	S		BE %	Number	%
Karate	7.5	15	89.4	22	1.5	85.9	1	6.7
Decis	25.0	50	64.5	53	1.1	66.0	15	30.0
Kaskade	38.5	77	45.4	77	1.0	50.6	11	14.3
Alsystin	22.5	45	68.1	50	1.1	67.9	13	28.9
Nomolt	13.5	27	80.9	28	1.0	82.1	4	14.8
Untreated control	70.5	141	-	156	1.1	-	8	5.7

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Some Problems with Arthropods on Maize in Czechoslovakia

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The aim of this paper is to review the occurrence of maize pests in agroecological conditions of maize growing in Czechoslovakia. Owing to the fact that Czechoslovakia can be considered a border region of maize growing, the variety of pests is not great although their occurrence is sometimes calamitous. In South-west Slovakia maize is damaged mainly by larvae of Elateridae - wireworms, frit fly (*Oscinella frit* L.), European corn borer (*Ostrinia nubilalis* Hbn.), various Aphididae and Thysanoptera and caterpillars of Noctuidae.

Maize is an important crop in Czechoslovakia, whether grown for grain or silage, and owing to its economic importance it is often called "the queen of fields". In Czechoslovakia 630,000 ha maize is grown, 310,000 ha being silage maize and 320,000 ha grain maize.

On the map of Czechoslovakia (Fig. 1) the maize-growing regions can be seen. These areas are approximately similar in size, but there is a difference in the kind of maize grown; in Bohemia 260,000 ha of silage maize and 50,000 ha of grain maize, in

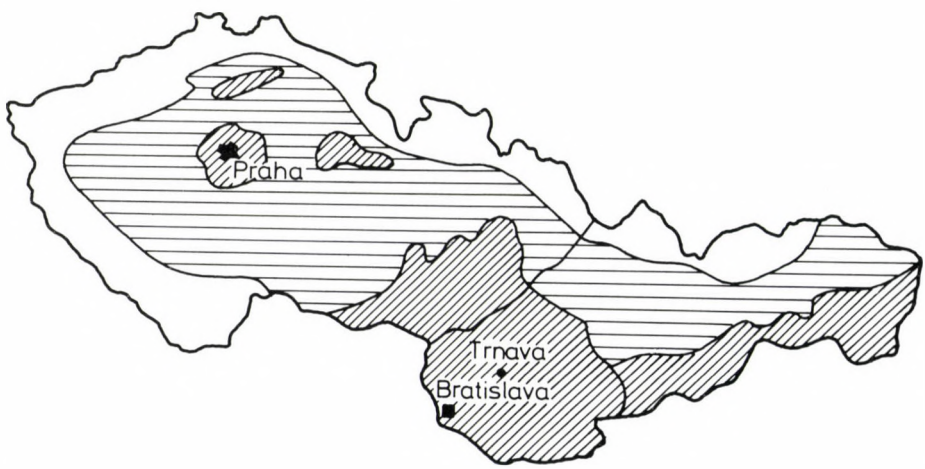

Fig. 1. Maize growing regions in Czechoslovakia
grain maize, silage maize

Slovakia 120,000 ha of silage maize and 200,000 ha of grain maize is grown. Such division corresponds to the agroecological conditions in Czechoslovakia, which is the border region of maize growing.

In this report attention will be paid to maize pests in Slovakia since about 80% of grain maize areas are concentrated in South-western Slovakia.

In our agroecological conditions maize is damaged by larvae of click beetles (Elateridae) - wireworms, frit fly (*Oscinella frit* L.), European corn borer (*Ostrinia nubilalis* Hbn.), different Aphididae and Thysanoptera, caterpillars of Noctuidae. In 1987 *Tanymecus dilaticollis* Gyll. appeared as a major pest in some localities of Southern Slovakia for the first time.

At the beginning of maize emergence the greatest damage is caused by wireworms. In 1964-1972, our soil research worker, M. Masler found the following genera of Elateridae: *Agriotes, Corymbytes, Athous, Melonothus, Lacon.* The dominant genus is *Agriotes* with species *A. brevis* Cand., then *A. sputator* L., *A. lineatus* L., *A. ustulatus* Schall., *A. obscurus* L. The occurrence of click-beetles is greatest in wheat, the second place in lucerne. A uniform scale to express the intensity of infestation by wireworms has been proposed:

weak infestation – less than 10 individuals per m^2
medium infestation – 10-20 individuals per m^2
heavy infestation – 21-50 individuals per m^2
very heavy infestation – over 50 individuals per m^2.

In maize 7-10 individuals per m^2, on light soils in dry regions – 7 individuals are considered critical.

The choice of plots to be treated is based upon soil sampling, the number of soil cores depending on the size of plots, i.e. up to 5 ha – 8 samples, over 5 ha – min 12 samples. If maize is to follow wheat or lucerne, it is recommended to do soil samples already in autumn and to complete them with controls in spring. Protection against wireworms should be done only above critical levels. Insurance treatment is quite common in practice, because soil temperature is usually low in spring and wireworms are impossible to find. They appear later in May or June and around this time critical densities may be reached.

For treatment against European corn borer a methodology has been worked out which allows to observe the flight of imagos into the light traps. For grain maize and for the seed plots the time of treatment is signalled by the maximum flight of moths. A second treatment should be made 7-10 days after the first one.

The evaluation of occurrence of the European corn borer is done about 14 days before harvest. 10 places 5 plants are examined. The occurrence is evaluated as weak when less than 40% of the plants are attacked, medium when 40-70% of the plants are attacked and heavy when more than 70% of the plants are attacked.

Phyllotreta, Haltica (Chrysomelidae) species are only sporadically pests on maize.

In this report we want to refer to the occurrence of some pests on maize in the most productive area of its growing, South-western Slovakia, in 1980-1983. In 1980-1982 April was a cold to very cold month and only in 1983 was it normal, even warm. During cold Aprils few or no wireworms were found. Later in May and June their occurrence was quite heavy, even very heavy, over a large area (Fig. 2).

Fig. 2. The occurrence of wireworms in the years 1980-1983
○ weak occurrence, ◑ heavy occurrence, ● medium occurrence

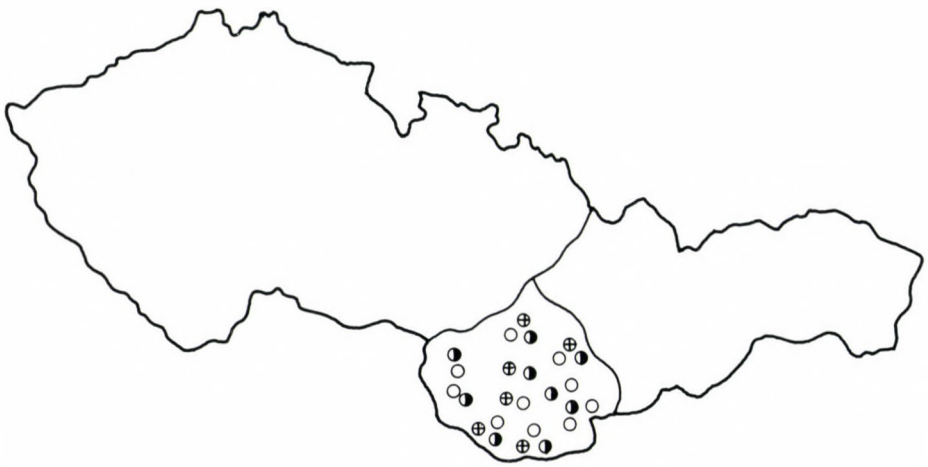

Fig. 3. The occurrence of European corn borer in the years 1980-1983
○ weak occurrence, ◑ heavy occurrence, ⊕ medium occurrence

On Fig. 3 the occurrence of *Ostrinia nubilalis* can be seen. The occurrence of this pest can be observed over the whole territory of South-western Slovakia. In 1980-1981, its attack was of medium intensity because conditions for the development of this pest in June and July were not favourable. In 1982, this pest occurred again in all regions in weak intensity. But during the long and warm autumn, the attack increased and in October it was very heavy in many places. The vegetation period in 1983 was normal, even warm, mostly dry – very dry (precipitation 200-300 mm). June was characterized as extraordinarily warm month (e.g. in Bratislava 27 days were registered as tropical). In spite of this, *Ostrinia nubilalis* occurred at medium density in 3 localities only. Dry and hot weather negatively influenced the development of the pest in our country, although, on the other side, the 2nd generation of this pest was observed for the first time in the Southern districts of Slovakia.

In all the years studied maize plants were slightly attacked by frit fly. This pest occurs also in the Northern maize growing regions (especially in silage maize) (Fig. 4).

Here we have referred only to those pests of maize which occur to such extent that chemical treatment is necessary.

Data presented here were from observations recorded in the Central Agricultural Control and Test Office in Bratislava.

Fig. 4. The occurrence of some other pests on maize in the years 1980-1983
○ Aphidoidea, ● Oscinella frit, ◑ Phyllotreta, ⊕ *Scotia segetum*

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Sesamia nonagrioides Sex Pheromone: Field Attractiveness Use of Pheromone Traps for Monitoring and Control

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The synthetic sex pheromone blend of the *Sesamia nonagrioides* (Lef.), (Lepidoptera:Noctuidae) was tested for male attractiveness under field conditions in 1985 and 1986. Funnel traps baited with rubber septa loaded with pheromone blend were as effective as the blacklight traps. Pilot tests were initiated in 1986 to test the efficiency of pheromone traps in suppressing the insect population by the male mass trapping method. First year results indicated that ten traps per hectare loaded with 200 μg of the pheromone blend, suppressed the insect population and crop infestation remained at a low level, in early sown corn. In late sown corn, infestation in the mass-trapped field was as high as in the control.

The corn stalk borer *Sesamia nonagrioides* (Lef.) (Lepidoptera: Noctuidae) is considered one of the most serious pests of corn. Losses due to this pest in the late corn crop (maize sown in the beginning of July) are very high (Prota, 1965, Stayrakis, 1967). The sex pheromone produced by females was identified recently. Sreng *et al*, (1985), Rotundo *et al*, (1985) reported that the insect utilizes a sex pheromone system composed of two components, Z-11-hexadecenyl acetate (Z-11-16:OAc) and Z-11-hexadecenol (Z-11-16:OH). They also indicated that the synthesized two components blend (95:5) attracted high numbers of *Pheudaletia unipuncta* males. Mazomenos (1985, 1987), identified two additional components, from diethyl ether washes of virgin females and head space volatiles, namely, Z-11-hexadecenal (Z-11-16:Ald) and dodecyl acetate (12:OAc), which improve male captures in field tests and also reduced significantly *P. unipuncta* male attraction. This paper presents the results of field studies aimed at testing the efficiency of pheromone traps in monitoring or suppressing *S. nonagrioides* population.

Materials and Methods

Field tests

Tests were conducted in Kopais, Biotia county and Servota Trikala county in 1985 and 1986. Funnel traps, (Phytophyl, Athens, Greece), baited with pheromone, and black light traps were used. The pheromone traps were suspended 1.5 m above the ground 100 m apart and were baited with rubber septa loaded with the pheromone blend. A slow release formulation of OVP was used as killing agent.

Mass trapping

An experiment was initiated in 1986, to study the effectiveness of the mass trapping method with pheromone traps in suppressing insect populations. A 30 ha field, partially isolated from others, was selected for the experiment. Corn was sown early in May. Another 4 ha field about 2 km away and sown on the same date was used as control. Near the two fields, 1.5 ha of corn was sown late in July. Ten traps per hectare were installed in the experimental field on May 12. The traps were baited with 200 μg of the pheromone blend with the pheromone dispensers being replaced once during the experiment. Corn plants were sampled regularly and the number of egg masses and level of larval infestation recorded.

Chemicals

Z-ll-l6:OAc, Z-11-,16:OH, Z-11-16:Ald, (Sigma Chem Co.) 12:OAc was synthesized in our laboratory. The components were found to be 96-98% pure when analyzed by capillary GC.

Results and Discussion

Evaluation of the Pheromone Trap

Pheromone traps baited with 100, 150, 200, 250 μg of the pheromone (constituents mixed in a ratio of 69:8:8:15 for Z-11-16:OAc:Z-11-16:OH:Z-11-16:Ald:12:OAC) were

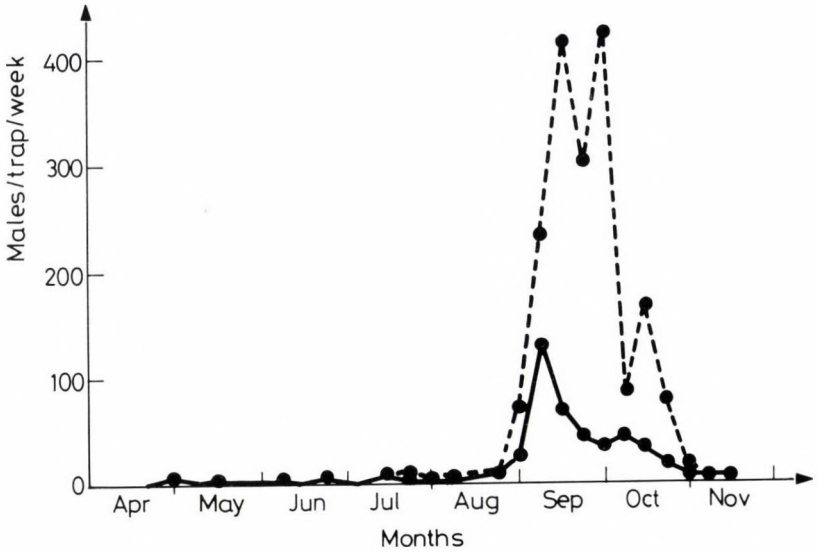

Fig. 1.

Table 1

Captures of *S. nonagrioides* males in traps baited with four concentration of the synthetic sex pheromone blend, and at black light traps, at Kopais, Biotia county, Greece, 1985

Conc. (μg)	Date						Total	Mean[1]
	17/3	18/3	19/3	20/3	21/3	22/3		
100	2	15	26	4	36	12	95	15.8
150	15	32	35	5	40	24	151	25.2
200	5	42	42	16	52	25	182	30.3
250	8	13	14	12	40	40	127	21.2
Light trap	17	18	40	29	24	17	145	24.2

[1]Means are not significantly different from each other (Duncan's multiple-range test P=0.05).

very effective in capturing males (Table 1). The mean number of males captured in the pheromone traps were not significantly different from the mean number of males captured in black light traps.

Mass trapping – Early sown corn

The total number of males captured per hectare in the experimental field during the insect flying period was 833 males/ha. Traps that were suspended across the borders of the field captured more males than traps suspended inside the field. The number of males captured in the control and the experimental field, were not significantly different until mid-August. Late in August and during September, the male catches in the control field increased and were higher than those in the experimental field (Fig. 2A).

The plant infestation in the experimental field was lower than in the control field. The level of infestation is shown in Fig. 2B, 51% of the stems and 10% of the ears in the control field were found to be infested, while 17% of the stems and 5% of the ears were infested in the experimental field.

Late sown corn field

The insect population density remained low in the experimental field compared to that of the control field. However the number of egg masses recorded during the regular sampling of 100 plants was high, particularly at the perimeter of the field. In August egg masses were found on 31% of the inspected plants fields and 12% of those in the experimental field. In September-October the number of plants with egg-masses was approximately the same in both fields. Three insecticide treatments were applied on the control field (27 Aug., 6 Sept. and 1 Oct.) and the last two treatments were also applied in the experimental field. On the last examination of the fields infestation was found to be approximately 70% both fields. The density of larvae varied among the plants, the majority of plants being infested with one to five larvae.

The main objective of the mass trapping method was to reduce the population of early season *S. nonagrioides* so that successful and multiple mating also is reduced, and

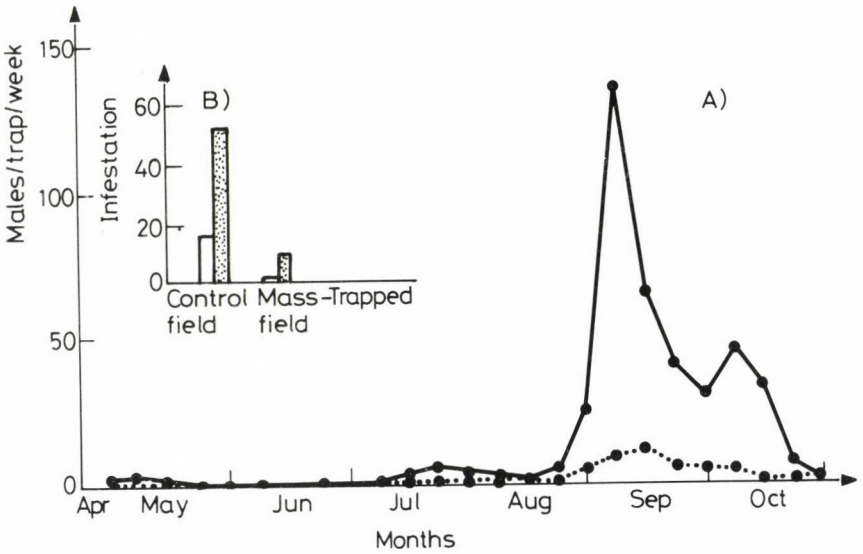

Fig. 2

insect population is suppressed or delayed. The results indicate that a reduction in the early season population occurred and infestation remained low in the early sown corn. In late sown corn, although the onset of infestation was delayed by two weeks the infestation level was high, due to the lack of adequate isolation of the experimental field. Much work remains to be done in order to develop and evaluate the mass trapping method, which seem to have the potential of being a major component in an integrated program aimed at controlling *S. nonagrioides*.

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Systematics of *Diabrotica* Pests of Corn (Coleoptera: Chrysomelidae)

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The principal *Diabrotica* pests of corn in the United States are *D. virgifera virgifera* LeConte, *D. v. zeae* Krysan and Smith, and *D. barberi* Smith and Lawrence. All three taxa are classed in the *virgifera* group of the genus. Our understanding of the evolutionary and taxonomic relations of the *virgifera* group members is the result of a progressive accumulation of biological, ecological and genetic information. Data of this nature are discussed in this paper as they relate to the historical development and delineation of corn rootworm species complexes in the United States corn growing regions.

The chrysomelid genus *Diabrotica* evolved in the neotropics. *Diabrotica* larvae are root feeders and those species which feed on corn are called corn rootworms. The major rootworm pests of corn in the United States are classed in the *virgifera* group and include *D. virgifera virgifera* LeConte, *D. v. zeae* Krysan and Smith, and *D. barberi* Smith and Lawrence, i.e., the western, Mexican and northern corn rootworms, respectively. Historically, rootworm control has involved crop rotation, the use of soil insecticides for larval control, or the use of insecticides for adult control. The control procedures are often inadequate and there has been an ongoing effort to find improved management methods. Biosystematic studies have represented an essential part of the research effort. This paper provides a broad overview of data emerging from the systematics work, particularly as such pertains to evolutionary patterns and delineations of variation among intra- or interspecific populations.

History of the *Virgifera* Group Taxa as Pests

Only six taxa of *Diabrotica* overwinter in the United States where freezing occurs annually. The six taxa are in the *virgifera* group and include the rootworm pests along with three noneconomic species. All six forms are univoltine and share an egg dormancy mechanism which probably evolved in the tropics or subtropics as an adaptation to wet and dry periods. It has been postulated that the ancestor of the *virgifera* group emerged as a specialist on the roots of grasses somewhere in Mexico (Branson & Krysan 1981). Feeding specialization, coupled with the preadaptive egg dormancy trait, then allowed group members to radiate into temperate regions of North America. *D. barberi* seems to have occupied much of its range in the United States in association with native hosts. However, corn became the preferred host when farmers began to cultivate this crop in the midwest during the 1860's. *D. barberi* has since become a pest on corn across the United States Corn Belt. Evidence suggests that *D. virgifera* and corn evolved in the same general region of subtropical America. The larvae of *D. v. virgifera* are monophagous on

corn and it is probable that this species entered the U. S. as corn was introduced less than 1,000 years ago. Economic damage to corn by western corn rootworms was first noted during 1909 in Colorado, but serious infestations did not occur until the 1940's when growers began to cultivate corn continuously in the Great Plains. *D. v. virgifera* has since extended its range to the Atlantic coast and is regarded as the most important pest attacking corn. The entry of *D. v. zeae* into the United States may have paralleled that of *D. v. virgifera*. *D. v. zeae* is found from Oklahoma, south into Central America. The subspecies is a pest in Central Mexico and areas of Texas.

Taxonomy of *D. barberi* and *D. longicornis* (Say)

D. barberi and *D. longicornis* are classed as sibling species (Krysan, Smith & Guss 1983). *D. barberi* occurs from the Atlantic coast west to the Great Plains. *D. longicornis* is found from Mexico and Arizona east to Texas and hence north into Kansas and Nebraska where its range overlaps with that of *D. barberi*. Beetles from the range of either species were classed as *D. longicornis* until the late 1960's. Thereupon, the taxa were recognized as subspecies of *D. longicornis* because of geographic variation of adult structures which were termed piceous in *longicornis* vs. testaceous in *barberi*. Studies of museum specimens further led to the distinction of dark "eastern" vs. light "western" forms of *barberi*. Extensive analyses of geographic samples were then performed and *barberi* was elevated to the species rank when the data revealed a bimodal distribution of adult color forms in the Great Plains region along with biological differences between the two beetle types. The latter work also demonstrated clinal variation in beetle coloration in the northeast, thus indicating that recognition of an "eastern" population was unjustified. Additional research on *D. barberi* has included examinations of both enzymatic and chromatic variation in geographic samples (McDonald, Krysan & Johnson 1985). Genetic distance measures computed from the enzyme date were characteristic of conspecific populations and there were no significant differences in population heterozygosities. Conversely, there were correlations between enzyme heterozygosities and color variation scores of adults. These findings supported the idea that chromatically variable populations were geographic races despite the occurrence of complex color patterns in some areas.

Various data suggest the close affinity of *D. barberi* with *D. longicornis*. Thus, some beetles from areas of sympatry are not easily identified based solely on their color. Also, reproductive structures which distinguish other *Diabrotica* are not diagnostic for the siblings. Morphometric analyses have indicated some divergence between the taxa, but are likewise inadequate for identification. The species have been hybridized in the laboratory and electrophoresis has demonstrated identical electromorphs at various loci; the only diagnostic enzymes known segregate against null alleles at an autosomal, sex-limited, esterase locus. Finally, enzyme variation has been compared among geographic populations of the siblings occurring within and distal to the area of range overlap. There were no statistical differences between the average within-vs. the average between-species genetic distance values derived from the enzyme date (I. McDonald & J. Krysan unpublished).

In summary, there is ample evidence of divergence between *D. barberi* and *D. longicornis*, but a recent evolutionary history is suggested by the enzyme data and by the fact that isolation results mainly from premating barriers. One can speculate that speciation occurred in the present geographic juxtaposition of the taxa and that an important step in the process involved a shift in adult feeding which allowed the ancestors of *D. barberi* to enter the midwest before corn was introduced. The range of *D. longicornis* is limited to the distribution of wild cucurbits which adults feed upon. Curcurbit feeding seems to be a vestigial trait in *D. barberi*. However, the species is a more general feeder and may leave old corn to feed on a variety of flowering hosts. The inference is that *D. barberi* used similar hosts for food before the advent of intensive corn cropping.

Relationship of *D. v. virgifera* and *D. v. zeae*

D. v. virgifera and *D. v. zeae* are classed as subspecies (Krysan *et al.* 1980), but they differ in color and *zeae* may sometimes be confused with *D. longicornis* on that basis. However, *zeae* is usually distinguishable from *longicornis* by color and separation is afforded by differences in genitalia or egg chorion traits which characterize both *zeae* and *virgifera*. Studies of pheromone response, mating behavior, and female insemination have indicated a lack of premating isolation between the subspecies. Also, progenies from crosses of *virgifera* ♀ x *zeae* ♂ have included beetles of intermediate color; similar intermediates occur in an intergradation zone in the Texas panhandle. Lastly, isozyme studies have revealed nearly identical electromorph profiles for the taxa and both possess an identical and diagnostic male-limited esterase not found in *D. longicornis* or *D. barberi*.

Studies of *D. virgifera* and *D. barberi*

It was suggested that *D. virgifera* and *D. barberi* were hybridizing as areas of sympatry between the species expanded during the 1960's and 1970's. Supportive data included observations of beetles with atypical markings along with sightings of interspecific mating attempts. Also, fertile hybrids were reported from laboratory crosses. Other work demonstrated that males of both species respond to the same sex pheromone and are promiscuous in their mating attempts. Nevertheless, more recent studies have shown that hybridization is precluded by a number of pre- and post-mating factors. Also, genetic distance indices derived from isozyme data are typical of well-defined species and diagnostic enzymes have been reported for both larvae and adults.

D. virgifera is less variable locally than *D. barberi* as suggested by enzyme studies. It may be relevant that *D. virgifera* is more vagile than *D. barberi* or the variation differences may reflect the longer and closer association of *D. virgifera* with corn. The importance of vagility is suggested by patterns of aldrin resistance in the species complexes. *D. barberi* exhibited a variable geographic pattern of resistance to this now discontinued pesticide, while resistance occurred universally in *D. virgifera* populations. Geographic variation in *D. barberi* is further indicated by reports on dispause. Crop rotation has long been used for control because rootworm larvae are not mobile and require corn as food. Rotation patterns once covered several years, but present methods often involve planting corn

followed by a non-host crop in succeeding years. *D. barberi* damage to corn grown by such a cycle has been a sporadic, but increasing problem. It now appears that the rotation system may sometimes select for eggs having a prolonged or two year diapause. Studies are presently needed to determine the genetic bases of diapause and to examine the extent of diapause variation.

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Genetic Studies of Resistance to Baculovirus Infection in *Heliothis*

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The study of genetic resistance of caterpillar pests of agriculture and forestry has far reaching implications with regards to their successful control by baculoviruses. Relatively few studies have been conducted on the genetics of resistance of these pests to baculoviruses and most of these studies have been done in the laboratory.

The study of resistance of insects to entomopathogens is of paramount importance because of their potential application for the control of insect pests of agriculture and forestry. However, the narrow range of interspecific susceptibility of pest insects to baculoviruses has made genetic studies of resistance difficult (Briese 1981).

Among the insect viruses infecting Lepidoptera, baculoviruses (Wildy 1971) are the agents of choice for control because of their specificity, virulence and safety features, i.e. they do not infect vertebrates. Four baculoviruses have been licensed by the EPA in the U.S. for the control of insect pests. These include: the nuclear polyhedrosis virus (NPV) of the cotton bollworm *Heliothis zea*; the NPV of the gypsy moth *Lymantria dispar*; the NPV of the Douglas-fir tussock moth *Orgyia pseudotsugata*; and the NPV of the European pine sawfly *Neodiprion sertifer*.

Both resistance (Glaser 1915, Bergold 1951, Martignoni 1957) and failure to develop resistance (Franz and Niklas 1954, Ignoffo and Allen 1972, Whitlock 1977) to baculoviruses have been reported.

Genetic Studies on Resistance to Baculoviruses

Total resistance to baculoviruses has not been observed in *Heliothis* species or in other Lepidoptera. Currently, seven species of *Heliothis* are reported susceptible to isolates of HzSNPV (Ignoffo and Couch 1981). Significant differences in susceptibility between larvae of a non-agronomic pest, *H. subflexa* and *H. zea* to HzSNPV have been demonstrated. Exposure of the same species to the multiple enveloped virus of *H. armigera* (HaMNPV) showed negligible differences in susceptibility (Ignoffo et al. 1983).

Backcross sterile-male hybrids derived from hybridization of *H. subflexa* females to *H. virescens* males have been proposed for suppression of *H. virescens* (Laster 1972). These two species were used as models to study the genetic resistance of hybrids and F1 backcrosses to HzSNPV (Ignoffo et al. 1985). The results (Table 1) indicated that resistance of *H. subflexa* to HzSNPV appears to be controlled by a single non sex-linked gene. Specific genes have also been implicated in the resistance to an NPV and a granulosis virus (GV) (Reichelderfer and Benton 1974, Briese 1982, 1985).

Table 1

Relative resistance based on LC_{50} values, of larvae of *Heliothis subflexa*, *H. virescens*, hybrids and backcrosses exposed to the single-enveloped nuclear polyhedrosis virus of *H. zea*

Genotype	LC_{50}	Relative resistance
H. virescens (Hv)	9.2	1
H. subflexa (Hs)	9159	996
Hs♀ x Hv♂	4150	451
Hs♀ x Hv♂	5243	570
Bc♀ x Hv♂ 64-66	7.8	0.8

Doses 100,000 to 100 PIB per cm^2 inclusive for *H.subflexa, H.subflexa* hybrids and their backcrosses. Doses 500 to I PIB per cm^2 inclusive for *H. virescens*. Relative resistance compared with *H.virescens*. Backcrosses are of the Hs♀x Hv♂ hybrid. From Ignoffo et al. 1985.

Selection for Resistance to Baculoviruses

The variable response of field populations of larvae to baculoviruses suggest the possibility of a gene(s) regulating susceptibility. Several studies (Ignoffo and Allen 1972, Whitlock 1977) have been conducted in the laboratory to experimentally induce genetic resistance of *Heliothis* larvae to baculoviruses without success through 25 generations (ca. 10 years).

Both Ignoffo and Allen (1972) and Whitlock (1977) concluded that if resistance to baculoviruses of *Heliothis* develops, it will take considerably longer than reported for methyl parathion. It would take about 4 years for *H. zea* to develop resistance to methyl parathion (Carter and Phillips 1968).

Significance of Genetic Resistance to Baculoviruses

None of the major, large scale field applications of baculoviruses (*N. certifer, O. pseudotsugata, H. zea, H. virescens, H. armigera, L. dispar, Spodoptera littoralis, Laspeyresia pomonella*, and *Oryctes rhinoceros*) has resulted an increase in resistance of the target pest (Briese 1985). However, more studies, to monitor possible occurrence of resistance to baculoviruses in field populations of insect pests, need to be conducted. When these studies are contemplated (laboratory or field populations) two parameters should be seriously considered. First, the challenge-virus should be well characterized and demonstrated to be clean of other contaminating microbes and viruses. Second, insect populations should be free of secondary contaminants or latent-occult microbes that could interfere with replication of the challenge virus. Tests to demonstrate both the first and second parameter are now possible using restriction endonuclease analysis and specific probes.

Chemical insecticides have a distinct advantage over baculoviruses because of their faster action giving rapid control of expanding field populations. However, the major

disadvantage of employing chemicals insecticides is the development of resistant populations over a relatively short time period. Ignoffo and Allen (1972) suggested that the problem with chemical resistance might be alleviated and forestalled with the judicious alternate use of a chemical insecticide and a microbial insecticide. Since chemical and microbials have different modes of activity and chemically-resistant populations are as susceptible to microbials as non chemically resistant populations (Ignoffo and Rousch 1986), the use of a microbial could inhibit the rapid emergence of chemically resistant populations of insects.

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Investigations on the Population Dynamics of *Ostrinia nubilalis* (Lep., Pyralidae) in South-western Germany

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Ostrinia nubilalis (Hbn.) has only one generation per year in Central Europe. Flight occurs usually from the end of June to the beginning of August. The outbreak area in Western Germany was originally confined to the Upper Rhine Valley, which has a warm climate and where maize is grown mainly for grain and seed production. According to the observations and records of the plant protection service, the population dynamics of the pest follow a somewhat cyclic pattern. Periods with serious infestations, during which the outbreak area expands, are followed by periods, during which the pest occurs in lower density and the outbreak area shrinks (Brod, pers. comm.). During the last increase, which started in 1979 (Fig. 1), economic damage occurred in areas, which had never seen corn borer damage before (e.g., some localities near Recklinghausen/Westphalia–Langenbruch *et al* 1985).

In one of these areas – the Filder Plain south of Stuttgart, which has a cooler climate than the Rhine Valley – the dynamics of the corn borer population were followed using an extensive, semi-quantitative method. The edges of 18 - 30 maize fields were searched for entrance holes and the number of larvae found in them were counted. The

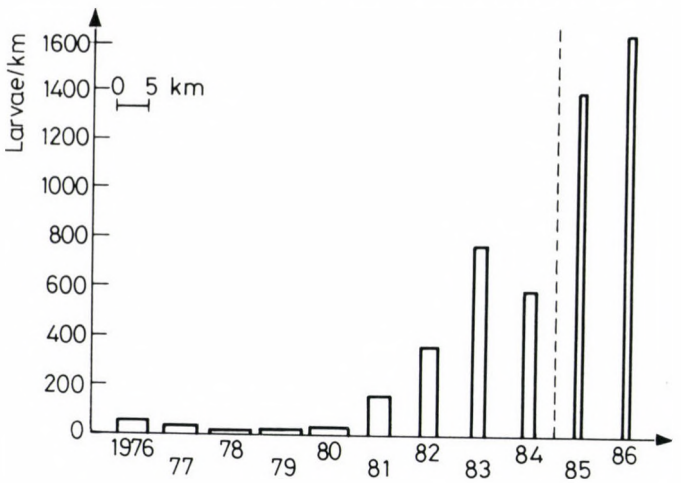

Fig. 1. Population dynamics of *O. nubilalis* in the Filder Plain. Height of columns: numer of larvae found per 100 m of field edge. Width of columns: Length of field edges which had been searched upon in the resp. year. After 1984 a different method of searching was adopted by which less larvae were missed

Table 1

Corn borer density in different districts of the Filder Plain relative to the mean density of the whole area, and proportion of maize area devoted to grain production.5 years combined.

district	% grain production	relative larval density %
Neuhausen	89	67-619
Sielmingen	41	31-450
Wolfschlugen	23	50-159
Harthausen	22	74-176
Bonlanden + Plattenhardt	10	11-38
Scharnhausen	0	10-21

results are shown in Fig. 1. After a decline from 1976 to 1978 the average corn borer density increased until 1983; thereafter it remained at a rather high level.

The population was unevenly distributed throughout the Filder Plain. In districts where maize was mainly grown for fodder or silage and harvested early, the population density was low. In districts where maize was grown mainly for grain and harvested late in the year, the density was high (Table 1). These findings indicate that by early harvest the majority of corn borer larvae are removed from the field and the population density is largely reduced.

During the last years, many growers have switched from fodder or silage production to the production of corn cob mix, which is harvested late in the year. It may be assumed that this change is one major cause of the spread of the outbreak area since 1980.

In addition, there are indications that the local populations of *O. nubilalis* in the new outbreak areas have become adapted to cooler climate. The post-diapause larvae from the Filder Plain require less day-degrees for completing their development than those from the Rhine Yalley (Ohnesorge & Reh 1987) and larvae from Recklinghausen require still less.

Life table studies were performed by Reh (1985) in 1983 and 1984 in the Upper Rhine Valley. They covered the period between oviposition in July until the end of hibernation in the following March. They revealed a low, egg mortality (5-14%), a severe mortality of the first larval instars, which had not yet entered the stems (57-74%), a lower mortality of the old larvae (0-38%) and important losses during hibernation: 28-47% in the Rhine Valley and 49-64% in the Filder Plain.

Total mortality amounted to about 84%. Since an average corn borer female produces more than 200 eggs (see below), there must be additional mortality of more than 90% acting upon either the post-diapause larvae, the pupae or the moths to remove the surplus progeny. Langenbruch (1981) estimated the total mortality from the start of hibernation until eclosion of the moths to be from 95 to 99%, depending on the type of soil cultivation.

These figures show that there are two stages in the life cycle of *O. nubilalis*, which are especially vulnerable to mortality factors, the young larva, and the post-diapause larva and/or pupa.

In order to estimate the fertility of *O. nubilalis*, larvae were collected from maize stubble in the fall, kept at 5°C during the winter and reared from mid-March at three different temperatures. The female moths were kept singly - together with one male - in oviposition cages at 25°C and dissected after death. The deposited eggs and the completely developed eggs in the abdomen were counted. Fig. 2 shows that the temperatures at which the post-diapause larvae had been reared exerted a major influence on the fertility: Individuals reared at 15°C produced less than half as many eggs as those individuals which had been reared at 25°C. We may expect, therefore, that low temperatures in spring and early summer reduce the fertility of female corn borers.

In addition, they delay moth flight and oviposition. In this way, they can also increase larval mortality. The results of artificial infestation experiments in 1979 and 1981 indicate that larvae, which hatch late in the year have less chances for survival than the early hatching ones (Table 2).

In order to investigate whether larvae hatching late suffer from malnutrition and yield less fertile moths, larvae were collected in fall 1986 in 2 maize fields. Field D had received a Deltamethrine treatment at an early growth stage and it can be assumed that mainly larvae hatching late had escaped the residues of the insecticide. In field T, *Trichogramma evanescens* had been released at such a late date, that probably the first egg batches in this field had been left unparasitized. After the larvae had been reared in the

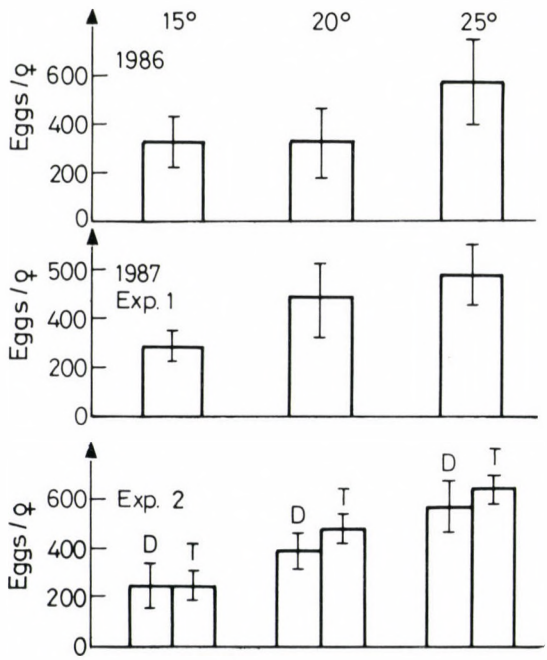

Fig. 2. Mean fecundity of *O. nubilalis* females which had been reared at 3 different temperatures after hibernation. D: Individuals from a Deltamethrine-treated field; T: Individuals from a *Trichogramma*-treated field (see text)

Table 2

Recovery rate (%) of corn borer larvae in relation to infestation date. Maize plants were artificially infested with 60 eggs/plant. Larvae were recovered later by dissecting the infested plant and 8 neighbouring plants. (REH 1985) 1979:10 replicates. 1981:5 replicates.

	Date of infestation	Date of dissection	Growth stage of maize at infestation	Mean recovery rate %
1979	3. 7.	1. 8.	end of shooting	17.1
	18. 7.	5. 9.	start of flowering	5.2
	1. 8.	5. 9.	end of flowering	1.4
1981	1. 7.	31. 7.	end of shooting	38.1
	16. 7.	17. 8.	start of flowering	13.1
	30. 7.	30. 8.	end of flowering	25.0
	13. 8.	14. 9.	milk stage	8.7

same manner as described above, the fecundity of the females was determined. As can be seen from Fig. 2, the females from field D had only a slightly reduced fecundity, which might have been also the result of some sublethal poisoning by the Deltamethrine residues.

The moths from field D did not hatch later than the moths from field T. Application of insecticide had apparently not exerted a selection pressure in favour of late hatching.

From the afore-mentioned results we might expect that temperature during the first half of the year influences the population dynamics of *O. nubilalis* by determining female fecundity, flight period and thereby larval survival. Indeed, some details of the population curve (Fig. 1) point in this direction. Below-average temperatures during post-diapause development of the pest occurred in 1978, 1980 and 1984. In 1978, the corn borer density reached its lowest level: in 1980 the recovery of the population seems to have been slowed down: and in 1984 there was a decline in density. Temperatures above average occurred in 1981, and in this year an extraordinarily steep increase in population density took place. The general population trend, however, which is assumed to be cyclic, does not seem to be disturbed in an essential way by these influences of weather.

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Aphids and Aphidophages on Maize in Central Poland

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Aphids (Hom., Aphidodea) are potential pests of maize in Poland. The aim of the present studies was to determine the species composition of aphids feeding on maize and describe the communities of their natural enemies in central Poland. This type of study has so far been performed mainly in southern Poland (Kania 1962a, b).

Study Area and Methods

Studies were carried out in 1979 in the 12-ha maize plantation of the State Farm in Lomna (Warsaw voivodeship). The species composition and density of aphids were determined once a week by searching 50 randomly selected maize plants. The numbers and dynamics of the natural enemies of aphids (aphidophages) were estimated by direct counts during ten 5-minute observation periods. The degree of aphid parasitization by parasitic wasps (Hymenoptera) was evaluated by dissection of aphids. For the determination of the species composition of parasitoids, mummified aphids containing cocoons of hymenopterans were collected from the plantation and kept in the laboratory until the parasitoids emerged.

Results

Three aphid species: *Metopolophium dirhodum* Walk., *Sitobion avenae* F. and *Rhopalosiphum padi* L. were found on maize. There were two peaks of aphid numbers: one in mid-July (on average more than 50 aphids per plant), caused mainly by *M. dirhodum*, and a second one in the end of August, mostly caused by *R. padi* (Table 1). Colonies of

Table 1

Seasonal dynamics of aphids (all individuals/100 plants) on maize central Poland. Lomna State Farm 1979.

Aphid developmental stage	June				July				August				
	6	13	20	27	4	11	18	25	1	8	15	22	29
alate forms	0	2	11	55	52	99	48	44	39	43	32	5	8
wingless mature stages	0	0	6	22	97	255	143	116	86	20	41	24	50
larval stages	0	5	5	177	498	2146	1305	651	554	234	448	245	755
total	0	7	22	254	674	2500	1496	811	679	297	521	274	813

R. padi, numbering thousands of individuals, were mainly on the cover leaves of maize cobs. During an aphid outbreak, 93% of plants were infested, 35 % of them very seriously.

Aphidophages

Observations of the aphids showed ladybirds (Col., Coccinellidae) and green lacewings (Neur., Chrysopidae) to be the most numerous (54.5 and 11.6 individuals, respectively); syrphids (Dipt., Syrphidae) and predatory bugs (Het., Nabidae) least numerous (6.3 and 0.3 individuals, respectively) of the aphidophages (Table 2).

Table 2

Aphidophagous predators on maize in 1979 (total of adults and larvae per 50 minutes of inspection)

group	June			July			August			
	6	20	27	11	18	1	8	15	22	29
Coccinellidae	17	9	13	4	10	66	157	98	78	93
Chrysopidae	-	-	-	32	52	18	3	9	-	2
Syrphidae	3	-	2	7	7	15	12	7	8	2
Nabidae	-	-	-	-	-	-	-	1	-	2

Four species of Coccinellidae were found. *Coccinella septempunctata* L. (131 imagines), *C. quinquepunctata* L. (91 imagines) and *Propylaea quatuordecimpunctata* L. (45 imagines) were the most common. For the fourth species (*Adalia bipunctata* L.), only 5 imagines were recorded. The seasonal dynamics of these species are shown on Table 3.

Three species of predatory Neuroptera occurred on maize. *Chrysopa carnea* Steph. was overwhelmingly dominant, being accompanied by *C. phyllochroma* Wesm. and *C. perla* L.

Syrphids were not very numerous. Adults were present throughout the period studied, with a peak abundance at the beginning of August; larvae were observed from 11 July until 19 September.

The species composition of parasitic hymenopterans was determined by breeding 540 mummified aphids collected from maize plants. Six species of primary parasitoids of

Table 3

Ladybird species (*Col., Coccinellidae*) occuring on maize in 1979 (adults per 50 minutes of inspection)

Species	June			July		August				
	6	20	27	11	18	1	8	15	22	29
Adalia bipunctata	-	-	-	-	1	2	-	-	-	2
Coccinella septempunctata	13	6	2	-	-	-	15	14	23	58
Coccinella quinquepunctata	4	-	1	-	1	5	5	15	30	30
Propylaea quatuordecimpunctata	-	3	10	3	2	2	10	11	3	1

the family *Aphidiidae* were found. The relative abundance of the different species in the communities were as follows: *Praon volucre* Nees — 36.9 %, *Aphidius picipes* Nees — 25.0 %, *A. uzbekistanicus* Luzh. — 20.2 %, *Ephedrus plagiator* Nees — 10.7 %, *A. rhopalosiphi* De Stefani-Perez — 4.8 % and *A. ervi* Haliday — 2.4 %.

The degree of aphid parasitization was determined only in July, when *M. dirhodum* dominated. Maximum parasitism (12 %) was observed just after the aphid peak; later the infestation dropped to 7%.

Conclusions

In central Poland, maize is infested by a number of phytophages which are widespread in central Europe. The great increase in numbers of aphids such as *R. padi* in late summer has been reported from other regions of Europe (Kania 1962b, Stepanovicova and Beláková 1960). Species of predators and parasitoids attacking aphids on maize near Warsaw, are widely distributed on all cereals (Stary 1976, 1978, Pankanin-Franczyk 1978). However, there are some quantitative differences in the structure of the communities of aphids and aphidophages on maize and on other cereals. These differences can be attributed, to a great extent, to the phenology of maize, considerably retarded as compared with other cereals (particularly with winter crops). In early summer, the numbers of phytophages on maize is relatively low, since they infest other cereals, available earlier in spring. In late summer, the situation is reversed. Maize still remains an attractive host plant for phytophages such as aphids, while the remaining cereals are already ripe and the number of aphids infesting them abruptly decreases.

Phytophages finding favourable developmental conditions on maize and occurring on this crop in a greater number than on other cereals, comprised predominantly of the aphid *M. dirhodum*, where as zoophages predominantly consist of the ladybird *C. septempunctata*.

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Maize Aphids in the North-east of Spain

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The population dynamics of maize aphids in Lleida (NE of Spain) have been studied since 1983. The most important species were *Rhopalosiphum padi, Sitobion avenae* and *Mtopolophim dirhodum*. Aphids probably from winter cereals arrive shortly after the crop has emerged. Aphid numbers increase rapidly but populations remain at low levels in July and August. In September, aphid numbers are again high, *R. padi* being the most common species. Maize fields are probably a source of aphids that infest early sown winter cereals. Direct and Sooty-mould damage appear to be low. The importance of aphids in maize is as vectors of viruses, mainly MDMV and BYDV.

Catalonia grows more than 10% of Spanish irrigated maize, Lleida being the most important Catalan area with about 57% of the surface area and 65% of the production.Aphids are one of the main potential maize pests, being the source of direct damage and vectors of viruses such as MDMV and BYDV (Pons & Albajes 1987 a).Studies, begun in Lleida in 1983, have enabled us to describe the biology of the main aphid species and evaluate their importance.

Materials and Methods

Aphid populations were monitored in different maize fields (cv. 3183 Pioneer) from 1983 to 1987. The sampling method was a visual count "in situ". Sampling lasted from the end of May or first week in June until harvesting. In each field a variable number of plants, equidistant on the field diagonal, was examined; this number ranged from a maximum of 140 plants to a minimum of 25, maintaining a standard error of about 20%. Aphid species were identified in the field and classified as first-third instar nymphs (N I-III), apteriform (N IV) or alatiform (N IV) fourth instar nymphs, and apterous (A) or alate (A) adults. The position of each individual on the plant was also recorded.The crop development stage was recorded on each sampling date following Hanway (1966).In order to relate the infestation average to the mean number of aphids per plant, a linear regression was used.

Results

The aphid species identified on maize were *Rhopalosiphum padi* (Linnaeus), *Sitobion avenae* (Fabricius), *Metopolophium dirhodum* (Walker), *M. festucae* (Theobald), *R. maidis* (Fitch), *Schizaphis graminum* (Rondani), *Sipha maydis* (Passerini), *Macrosiphum euphorbiae* (Thomas) and *Aphis fabae* (Scopoli). The first three species were the most abundant and in this paper only the results for these are presented.

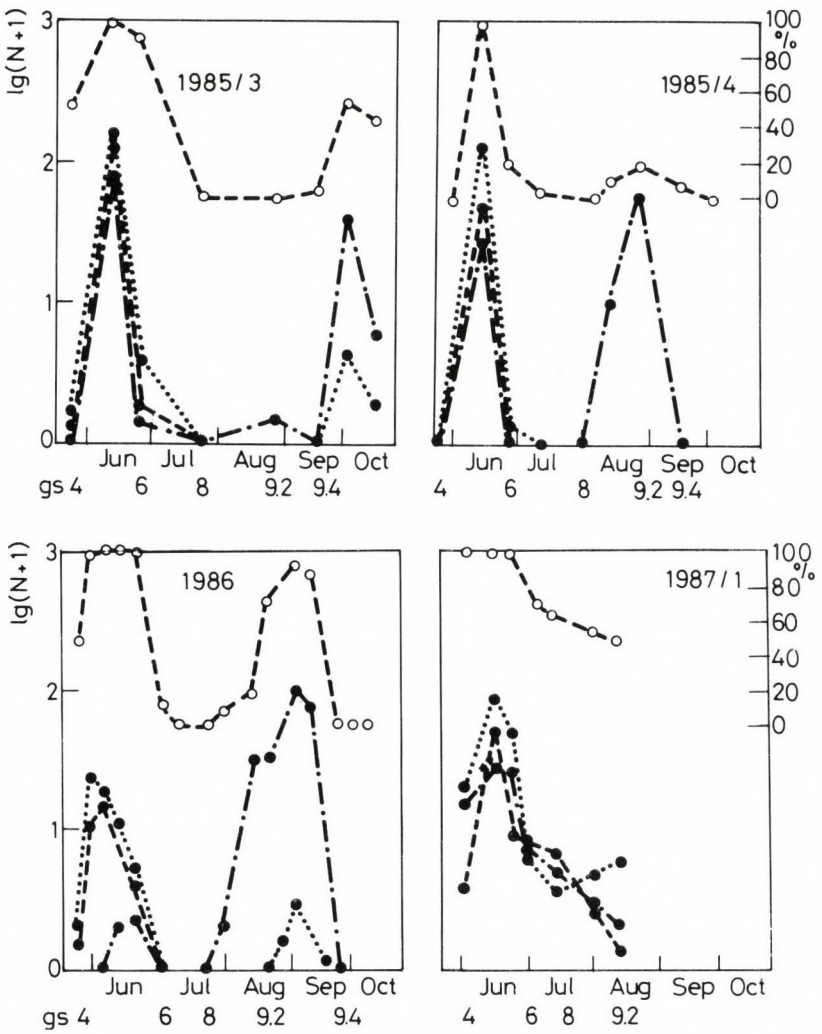

Fig. 1. Population dynamics of *S. avenae* (•·····•), *R. padi* (•–·–·•) and *M. dirhodum* (•– – –•) and percentage of infested plants (•– –•) in some sampled fields. Growth stage is expressed below the month according to Hanway (1966)

Colonization starts from mid-May to beginning of June. When alate aphids arrive, nymphs are produced. Aphid population levels increase very rapidly and the proportion of infested plants reaches 100% (Fig. 1). At this time the population is almost entirely alate and first-third instar nymphs. This situation remains until the maximum population (first fortnight of June), after which the population level decreases to very small values.

Until then most aphids are located below the 60 cm level of the plant, on the stem and leaves. When the aphid population level is higher, it is possible to find alate aphids and small colonies of *S. avenae*. and sometimes of *R. padi*, on the top leaves and on the tassel. At the end of August, the aphid population starts to grow again, but at this time *R. padi* is the most frequent species. *S. avenae* being less important and *M. dirhodum* practically nil. In October the population level reaches a new maximum, aphids still being located on the plant's old leaves although they can reach up to 150 cm on the plant. Honeydew and sooty-mould are important neither in the early growth nor late stages of maize.

A high correlation ($r = 0{,}8$, $p < 0.001$) between average number of aphids/plant and the percentage of occupied plants, has been found in 83 samples taken in the years 1984-87. Regression line was: probit $y = 3.6 + 1.66x$.

Discussion

The species found in the course of the study were basically those stated as being predominant in Lleida's winter cereals (Pons & Albajes 1987b). Emergence of maize frequently coincides with significant aphid levels in winter cereals. Aphid populations in corn increase in parallel with the decrease in aphid numbers in winter cereals. This fact, together with the age structure of the aphid populations at early growth stages of maize lends us to suggest that these aphids come from winter cereals.

A sudden decrease in aphid populations in maize in July and August has been pointed out by several authors (Hand & Carrillo 1982; Pons & Albajes 1987a). High temperature may be an important cause of this population decrease in mid- summer, but other reasons must be looked for, as has been pointed out by Pons and Albajes (1986) and Hand and Carrillo (1982).

Aphid levels recorded by us are lower than the economic thresholds quoted by Foot and Timmins (1973). Moreover, most recorded aphids have been found on the oldest leaves, which contribute less to grain yield (Palmer *et al.* 1973). Sooty-mould on the plant was always below the ear level. The most harmful damage of aphids in our area is the transmission of MDMV. This was considered to be responsible for severe losses in maize three and four years ago in Catalonia. The coincidence of three main factors during early maize growth stages contributes to the severity of the virus: high vector populations, high level of primary inocculum (strain A overwinters in *Sorghum halepense*), and use of susceptible hybrids. Insecticides are not effective in preventing the spread of the disease (Albajes *et al.* 1985). However, the use of tolerant hybrids has partially solved the problem.

BYDV has also been detected in maize in Catalonia (Alfaro, pers. com.). In our country, it is mainly harmful for winter cereals, but maize plays a major role in the maintenance of a high BYDV incoculum level in summer (Pons & Albajes 1987a).

Direct counts of aphids on maize are very time-consuming, particulary when plants are fully grown. Counting occupied and nonoccupied plants may be a time-saving method, although more experimental work is necessary.

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Influence of Mineral Nutrition on the Resistance of Maize to *Ostrinia nubilalis* Hbn.

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The life history of *Ostrinia nubilalis* in maize is influenced by the physiological state of the plant. Thus, the level of nutrients in plant tissues becomes very important, as the number of ears with larvae and losses of harvest demonstrates, which is strongly related to the level of N, Ca, Mn and Zn in plant tissue.

Recently, the influence of herbivorous nutrition on the development of the plant has been emphasized (Fowler & Lawton, 1985) because it can synthetize or motivate chemical defenses and leads the nutrients towards the damaged area (Harris, 1974, cited by Whittaker 1984). This has been established with documentary evidence in the last few years (Rhodes & Cates, 1979; Haukioja & Neuvonen, 1985). It is known that it depends on the physiological state of plant (Visser & Minks, 1982). In some cases, the influence of a certain compound on the mortality, larval development and fertility upon the larvae of *Sesamia nonagroides* (Gutierrez & Castanera, 1986) and *Ostrinia nubilalis* (Robinson*et al.*, 1982) has been observed.

However, the level and the type of secondary chemistry are related to the content of nutrients on the plant. In this way, the natural or acquired fertility of the soil is fundamental (Lamb, 1985).

Nitrogen is, of course, the most studied element and, according to general agreement, that of the greatest importance (Mattson, 1980; West, 1985). Nitrogen controls the level of phytophages, their density (Room & Thomas, 1986) and also their fertility (Rodriguez *et al.*, 1970). As it was demonstrated by Zuber & Dicke (1964), the maize fertilized with a high level of nitrogen has a softer tissue and a thinner cuticule. For this reason it is more easily damaged by the corn borer.

In this paper it is intended to clarify the relationship between the fertilisation, level of nutrients in the plant and occurency of *O. nubilalis* in maize, analysing it through different parameters.

Material and Methods

The maize planting took place in April on a free alkaline-neutral soil. The plots were 50 m^2 and the density of sowed field 50.000 plants/ha. There have been four treatments with fertilizers and one (T0) unfertilized; T1, T2, T3 and T4 have the same fertilisation (100 kg/ha of N), but the three latter were amended with 375, 750 and 1125 kg/ha of a complex 4-8-12. The basic nitrogen fertilizer was applied in three stages, as an ammonia compound. The complex fertilizer was applied before the sowing, and contained 15% of organic matter. Each treatment was repeated three times.

The control of the nutrition has been made through foliar analysis, always taking the central third of the first ripe leaf of the plant.

Table 1

Average levels of nutrients on the plant. Each item derives from eight samples. N, 10P, K, Ca and Mg are expressed as a percentage of dry matter. Fe, Mn and Zn in ppm.

	N	10P	K	Ca	Mg	Fe	Mn	Zn
T0	1.88	1.80	2.34	0.23	0.16	114	76	35
T1	2.39	1.84	2.20	0.27	0.22	138	122	42
T2	2.38	1.88	2.45	0.29	0.22	152	119	41
T3	2.57	1.83	2.29	0.26	0.21	128	121	43
T4	2.54	2.02	2.45	0.25	0.21	132	114	43
mds (0.05)	0.47	0.28	0.24	0.04	0.57	17	34	7

The nitrogen has been determined by a microkjeldahl method; the P by colorimetric ascorbic acid reduction method; the K by flame emission and the other elements (Ca, Mg, Fe, Mn and Zn) by atomic absorption spectrophotometry.

The incidence of *O. nubilalis* was determined in the ears, taking a sample of each plot and considering the percentage of ears with larvae (% M) and the damage which has been caused (D); it is applied accordig to a scale in which the coefficient 1 corresponded to a consumption less than 10%, in terms of weight; 2 indicated that the consumption was between 10 and 25%, and 3 represented 25-50%, etc. The percentage of estimated losses at harvest (P%) is also based on the two former parameters.

Results and Discussion

The average levels of nutrients are shown on Table 1. Each data was taken from eight samples made during the vegetative cycle.

The nitrogen showed a very low level in T0, while it was higher in the other four treatments. The nitrogen supplied was very similar (100, 115, 130 and 145 kg/ha of N). The differences between T0 and the other treatments were significant ($F= 23.8$; $p<0.05$). However, N levels were low compared to the optimum values given by other authors (Loue, 1984)

The percentage of P (expressed as 10P so that they can be more illustrative) and those of K did not change significantly, while the Ca ($F=10.49$; $p<0.01$) between T0 and the rest of the treatments, and the Mg did ($F=7.30$; $p<0.05$). These results (Ca and Mg) were below the critical level (Loue, 1984).

T0 showed a lower level than other treatments for Fe ($F=6.34$; $p<0.05$) and Mn ($F=22.15$; $p<0.01$) while for Zn the differences were not significant. The average value of several nutrients were closely related: for N and Mn ($r=0.8302$; $p<0.001$; $n=15$); Ca and Mn ($r=0.6792$; $p< 0.01$) and, of course, Mn-Zn ($r=0.8991$; $p<0.001$).

Therefore, the fertilization involved an increase of the macro- and micro-nutrients on the tissues and the average level of fertilized plants was superior to that of the nonfertilized.

Table 2

Average levels of occurance of *Ostrinia nubilalis* in ears (%M), coefficient of estimated losses of harvest (%P) and harvest obtained (H, in kg/plot).

	% M	D	% P	H
T0	96	13.78	13.05	7.09
T1	89	8.16	7.48	16.43
T2	80	9.04	7.32	18.45
T3	80	8.80	7.10	15.66
T4	84	8.12	7.44	17.95
mds (0.05)	14	5.04	4.79	11.23

In Table 2, incidence values of *O. nubilalis* and yields are shown. It should be observed that all the treatments suffered a strong attack by *O. nubilalis*. There was no significant difference among treatments in relation to this parameter; but, in the case of coefficient D, the difference between T0 and the other treatments was significant ($F=9.85$; $p<0.01$), as was %P ($F=5.2$; $p<0.05$). On the figure, the relationships between each two parameters can be observed.

The physiological state clearly affected the incidence of *O. nubilalis* on the maize since it resembled the distribution of nutrients. The four treatments involving fertilization gave, in general, similar results with regard to the unfertilized treatment. So, the percentage of ears with *O. nubilalis* is lower than in T0 (Table 2); the mean damage was also lower; so was the percentage of losses.

However, all the nutrients did not seem to have the same influence. The most significant were Mn, Zn and Ca. In fact, as shown on the figure, a major level of Mn losses and a minor percentage of losses and a minor damage was caused in each year.

The level of Ca seemed to influence the percentage of ears which are attacked by *Ostrinia* ($r=-0.7036$; $p<0.001$) perhaps because it hardened the tissues.

Curiously, nitrogen did not show the most significant correlations. Though, it directly influenced the levels of Mn and Zn and also the percentages of losses ($r=-0.5473$; $p<0.05$) and the damage caused ($r=-.549$; $p<0.05$).

%P and D were logically related ($r=0.9663$; $p<0.001$) since the first comes from the second. %P and %M were not ($r=0.4026$; NS). This fact implies that the losses were influenced more by the damage caused by each larva than the size of the attack.

The importance of the action of the borer diminishes when the harvest is bigger and for this reason the relative losses are also less (see figure). So, the correlation between H and D is significant ($r=-0.7647$; $p<0.01$). The same happened between %P and harvest (H) ($r=-0.7939$; $p<0.01$).

In conclusion, the action of *O. nubilalis* is influenced by the level of nutrients in plant tissues; particularly Mn and Zn, which are strongly related to the N level; and Ca, which influences the distribution of larvae introducing a greater structural resistance of tissues to the larvae.

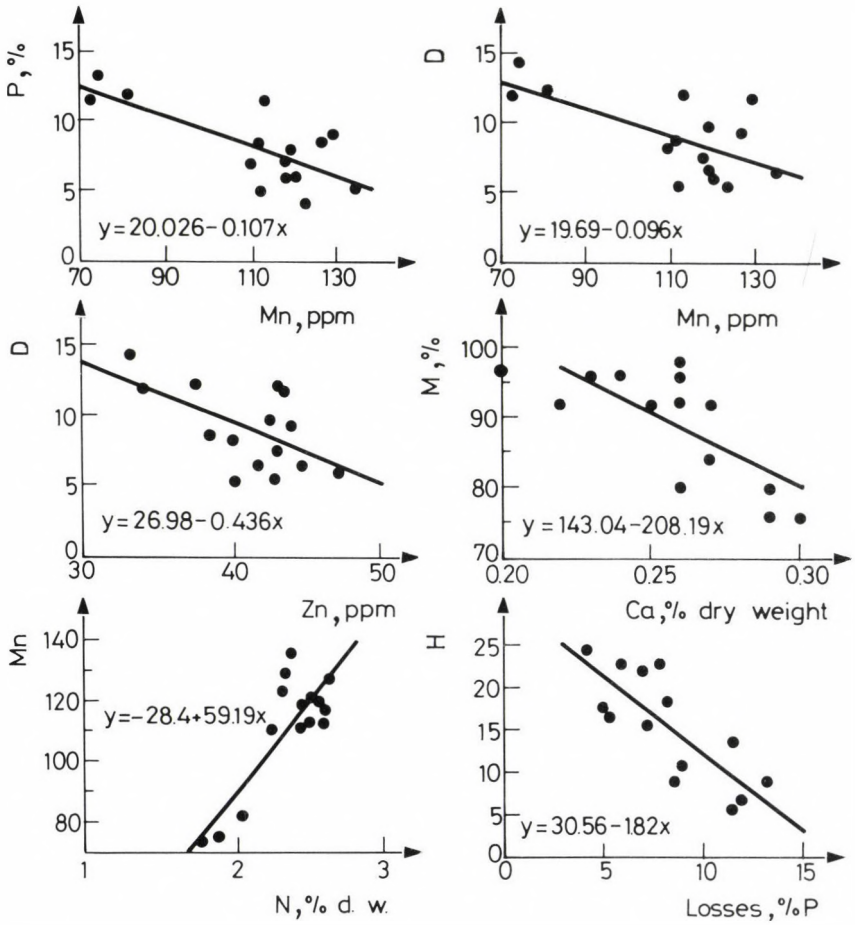

Fig. 1

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Population Dynamics of Aphids in Unsprayed Corn Fields

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The population dynamics of aphids and their natural enemies were observed in the fields of dent corn. No insecticides were used, but the density of aphids was not more than ten per plant. Several species of natural enemies were observed and inoculation experiments showed that these natural enemies were very active. Most natural enemies occurred in proportion to the aphid density, and may have kept the aphids at a low level of density by preying on them before aphid colonies developed. Predation of these natural enemies may have been promoted by the existence of weeds growing together with the corn plants.

Rhopalosiphum padi (Linnaeus) and *R. maidis* (Fitch) are major pests of corn in Japan (*Macrosiphum akebiae* (Shinji) is minor). However, in our corn fields, the corn plants have not been injured by aphids or other insect pests for more than fifteen years, although no insecticides have ever been used there. It seems that populations of aphids are kept at low levels by their natural enemies (Sakuratani 1977b). Furuta (1976) has pointed out that analysis of factors that operate at low density levels rather than at high ones may be helpful for integrated control. We studied the population dynamics of aphids and also evaluated the effects of their natural enemies.

Methods

Several plots, each consisting of twenty plants of dent corn, were chosen from unsprayed and weedy fields with areas of 0.6-1.2 ha at the Livestock Farm, College of Agriculture, Kyoto University. The numbers of aphids and their natural enemies were counted once or twice weekly in 1973-1975. In 1974, two cages that could not be entered by the natural enemies were placed in the corn field for investigation of the aphid population and evaluation of the effects of their natural enemies. In 1975, potted corn plants with many aphids were introduced and the activities of natural enemies evaluated on them. In 1974, some of the plots were kept weeded in a study of the effect of the weeds upon the populations of aphids and their natural enemies.

Results

Aphid densities were below ten aphids per plant even at the peak (Sakuratani 1977a and Fig. 2). Serious injury by aphids or other insect pests to the corn plants was not found. The aphid population grew exponentially in both cages, unlike in the open field, but after removal of Cage B, the growth slowed and then declined rapidly (Fig. 1a). In each inoculation experiment, the aphid density decreased rapidly and often became zero (Fig. 1b-d). Many natural enemies attacked the aphids after removal of the cage or in the inoculation experiments. These natural enemies tended to be found in proportion

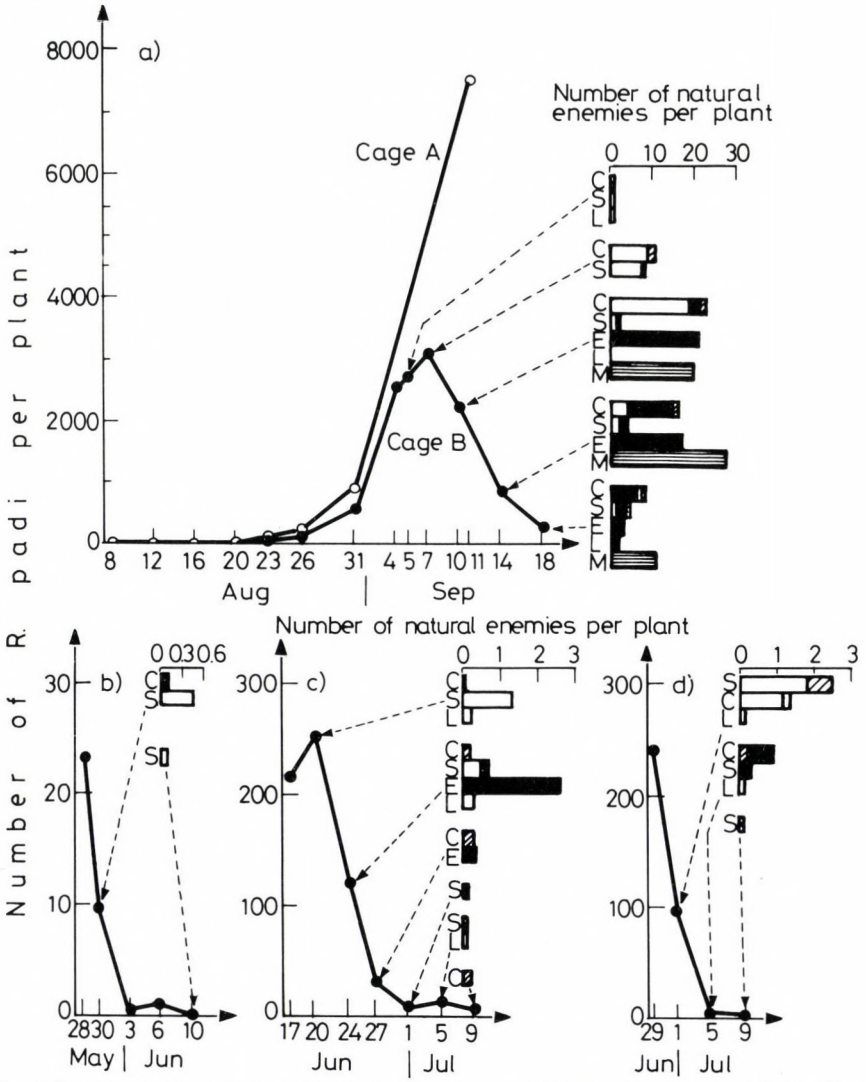

Fig. 1. Inoculation experiments for evaluation of the growth of aphid populations and the effect of natural enemies in the corn fields.

a) Population growth of *Rhopalosiphum padi* in a cage (1.3x1.3x1.7 m high) made of fine mesh net that excluded natural enemies. Each cage enclosed 10 plants of dent corn and two alata nymphs were released onto each plant on 8 August 1974. One cage (B) was removed on 4 September 1974 and the aphids were exposed to attack by their natural enemies (bar graphs). C: coccinellids, S: syrphids, E: cecidomyiids, L: lacewings, M: mummies. □: egg, ■ :larva, ⁞⁞⁞: pupa, ▨: adult.

b)-d) Other inoculation experiments. Nine pots of with dent corn bearing many aphids (*R. padi*) were put into the corn field on 28 May, 17 June, and 29 June 1975, and the numbers of aphids and natural enemies were counted. Symbols for natural enemies as in a)

Table 1

Indices of correlation, ω, for the overlapping of the distributions of aphids and their natural enemies based on different two spatial units of plots (10 or 20 plants of corn neighbouring each other) and plants, and calculated from data from inoculation experiments. Indices of ω vary from 1 in complete overlapping through 0 in independent occurence and to -1 in complete exclusion (Iwao 1977).

Natural enemies		Date	ω	
			Plot	Plant
Propylea japonica	eggs	10 Sept. '74	0.969	0.286
	larvae	18 Sept. '74	0.995	0.482
	adults	1 July '75	0.939	-
Scymnus hoffmanni	larvae	18 Sept.'74	0.961	-
	adults	7 Sept.'74	0.423	-
Syrphids	eggs	7 Sept.'74	0.980	0.567
	eggs	1 July '75	0.705	-
	larvae	18 Sept.'74	0.986	0.857
Cecidomyiids	larvae	10 Sept.'74	0.999	0.418
Lacewings	eggs	20 June'75	0.737	-
Mummies		14 Sept.'74	0.999	0.844
Spiders		10 Sept.'74	-0.007	0.366
		24 June'75	0.113	-

to the aphid density; more natural enemies were found in plots or on plants with more aphids. In general, the indices of correlation, ω (Iwao 1977) for overlapping of the distribution of aphids and their natural enemies were high except for spiders (Table 1). The aphid population tended to have a higher density in the weeded plots than the weedy ones (Fig. 2). However, most populations of natural enemies (except lacewings) tended to be higher in the weedy plots.

Discussion

In our corn fields, the aphid density was much lower than in other reports (e.g., Foot & Timmins 1973). Evaluation of predation by natural enemies is not easy when the densities of both aphids and natural enemies are low. In such circumstances, the inoculation method may be useful for evaluation of the factors that keep the insect pests at a low density (Furuta 1976). Use of the cage method in this corn field showed that the aphids had a high potential to increase. Use of the inoculation method showed that the response of natural enemies to changes in the aphid densities was rapid, and their predation was active. In corn fields, aphids are distributed contagiously in plots, on plants, and on leaves (Sakuratani 1977a). The natural enemies aggregated in areas with high aphid density and may prey on the aphids before a colony develops. Thus, the aphids

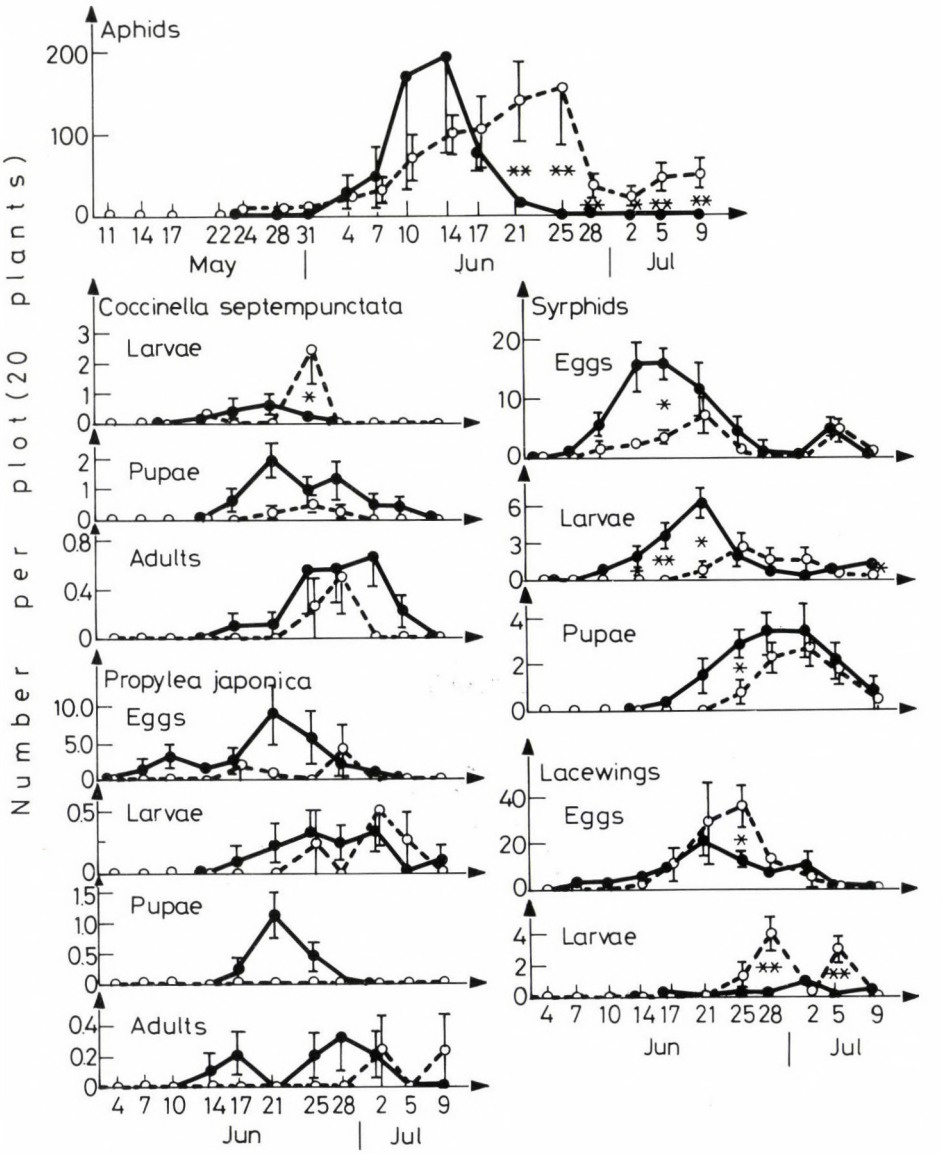

Fig. 2. Numbers of aphids (sum of three species) and natural enemies in weedy plots (●) and weeded plots (○). In May, the number of natural enemies was zero or very few.
Means ±S.E.. *:$p<0.05$; **: $p<0.01$

may be kept at a low density by their natural enemies, except for spiders, in our corn fields. The activities of most of the natural enemies seemed to be promoted both by insecticides not being used and by the existence of weeds, which may provide substitute prey and a favourable habitat.

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A Contribution to the Knowledge of Some Species of Coleoptera in Corn Fields in Yugoslavia

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Corn is the most important and the most widely grown crop in Yugoslavia. Fifty percent of the total corn area is located in Vojvodina Province, the North-eastern part of Yugoslavia. Considering the importance of this crop, it is no wonder that considerable efforts are invested in gaining knowledge of the insects attacking the crop and their role and importance for the crop. This report reviews the results of a long-term study on the species of Coleoptera in corn fields in Vojvodina Province. This problem has been dealt with by several researchers from the Faculty of Agriculture in Novi Sad (Camprag, Djurkic and Sekulic, 1975; Sekulic, 1976; Camprag, Sekulic and Zabel, 1987); therefore, some results presented here have been presented elsewhere.

Methods

Barber traps and soil sampling were used for the collecting of Coleoptera in corn fields on chernozem soils in Vojvodina Province. In the course of three years, 59 corn fields totalling about 800 hectares were checked in the period September - October, i.e., after the corn season. We collected larvae, pupae, and adult coleoptera stages from 954 soil samples each 50 x 50 x50 cms.

Barber pitfall traps were used in 100 hectare fields for four years. The traps were kept in the field from March 1 to November 30. There were four series of seven traps in each field, a total of 28 traps per field. The distance among the traps within a single series was 15 m, 400 m among the series. Four % formaline and a few drops of detergent were used to kill and preserve the trapped insects.

Results

Table 1 shows the results of inspection of soil after a corn season. We found a total of 4.3 specimens/m^2, representing seven families of beetles. *Carabidae* and *Scarabaeidae* were dominant. *Elateridae* and *Curculionidae* were close behind; other families were less numerous. The soil inspections indicated that corn is attacked by more than 30 harmful species whose number averaged 3.1 larvae and imagos/m^2.

The indentified species of *Carabidae* have a wide distribution. The predominant species were *H. distinguendus* Duft. and *H. pubescens* Müll.. Still numerous but less so than the previous two species were: *Poecilus cupreus* L., *Pterostichus melanarius* L., *Ani-*

Table 1

Frequency of some families of coleoptera order in maize fields in Vojvodina province

Family	3-year average	
	N° per m^2	Dominance %
Carabidae	1.189	27.6
Scarabaeidae	1.131	26.3
Elateridae	0.713	16.6
Curculionidae	0.689	16.0
Alleculidae	0.317	7.3
Tenebrionidae	0.254	5.9
Cerambycidae	0.012	0.3
Total	4.305	100.0

sodactylus signatus Panz., *Amara ingenua* Duft., *Calathus ambiguus* Payk., *Agonum dorsale* Pont., and *Calosoma auropunctatum* Hrbst. Herbivores were represented by two species (*Zabrus tenebrioides* Goeze. and *Z. spinipines* F.), omnivores made up to 50 % of the total population. Of the *Scarabaeidae, Anisoplia austriaca* Hrbst. was the commonest species, making up to 50 % of the specimens of that family. *A. segetum* Hrbst. was in the second place with 16 %, and *Rhizotrogus aequinoctialis* Hrbst. in the third with 14%. Other species were, *Amphimallon solstitialis* L., *Anisoplia agricola* Poda, *A. lata* Er., *Lethrus apterus* Laxm., *Maladera holosericea* Scop., *Melolontha melolontha* L., *Pentodon idiota* Hrbst., and *Rhizotrogus vernus* Germ. Including other soil types and other localities, eight genera and eleven species of that family were recorded. *Polyphylla fullo* Fabr. larvae were found in corn fields on sandy soils.

Elateridae was represented by eight genera. *Agriotes* was dominant making up 78% of the total specimens from that family. Other genera, in decreasing order are: *Adrastus* (21%), *Melanotus, Selatosomus*, and *Athous. Agriotes ustulatus* Schall. was the commonest species comprising 66% percent of the total Elateridae. *A. sputator* L. was in the second place with 9%. These two are at the same time the most economically important species for corn grown in Vojvodina Province.

The entomofauna of the soils under corn was found to include 20 species of Elateridae including the two mentioned above and *Adrastus nitidulus* Marsh., *A. rachifer* Geoffr., *Agriotes brevis* Cand., *A. incognitus* Schw., *A. lineatis* L., *A. litigiosus* Rossi., *A. obscurus* L., *A. pilosus* Panz., *A. sordidus* Ill., *Athous hirtus* Hrbst., *Lacon murinus* L., *Limonius pilosus* Leske. *Melanotus brunnipes* Germ., *M. cinerascens* Küst., *M. crassicollis* Er., *M. punctolineatus* Peler., *Slatosomus latus* F., and *Synaptus filiformis* F.

The results of our long-term observations (1961-1985) indicate that the number of the *Elateridae* larvae is increasing in spite of an intensive application of inseciticides aimed at their control. In the 1960s, their average density was 1.8/m^2, in the seventies it was 3.9 and in last five years (1981-1985) it was 6.1. This appears to be due, among other factors, to the decrease in the average density of carabids (their major predators), from 0.9/m^2 in the 1970s to 0.6 in the 1980s. A negative correlation was found between the densities of *Agriotes* larvae and of carabids (Camprag *et al.*, 1987).

Curculionidae was represented in the soil under corn by four species from three genera (*Psalidium, Otiorrhynchus*, and *Tanymecus*). *Tanymecus dilaticollis* Gyll. was the commonest species. Its numbers increase particularly rapidly in corn monoculture.

Opatrum sabulosum L. was the dominant herbivore representing the *Tenebrionidae*. This species comprised about 50% of the total number of tenebrionids collected. *Pedinus femoralis* L. also appeared in considerable numbers. In total, five genera and seven species of tenebrionids were recorded including two mentioned above and *Blaps halophila* Fisch., *B. lethifera* Marsch., *B. mortisaga* L., *Crypticus quisquilius* Pk., and *Gonocephalum pusillum* F.

Alleculidae was also found in an increased number 7.3 %. Two species were found: *Omophlus lepturoides* F. and *O. proteus* Kirsch. Few *Cerambycidae* were found including species of *Dorcadion*.

The two-year study of the surface entomofauna of corn fields using Barber traps indicated that species from 13 different families were present, totalling about 14.000 specimens, 6.200 of these belonging to the five most common families. Considering the collecting method used, it is not surprising that the commonest group was the *Carabidae* the species of which typify the surface fauna. There followed the families *Elateridae, Curculionidae, Scarabaeidae* and *Tenebrionidae*. The seasonal dynamics of the families was quite variable and specific for each family (Fig. 1.).

Species of *Curculionidae* and *Tenebrionidae* were dominant in early spring, during March and April. Later on, their numbers decreased so that only individual specimens were found during the summer. The number of species of *Elateridae* increased gradually during spring to reach a maximum in June when they represented 45% of the total specimens collected. Representatives of *Scarabaeidae* were most frequent in the second half of spring. Their number increased also at the end of the season. The activity of

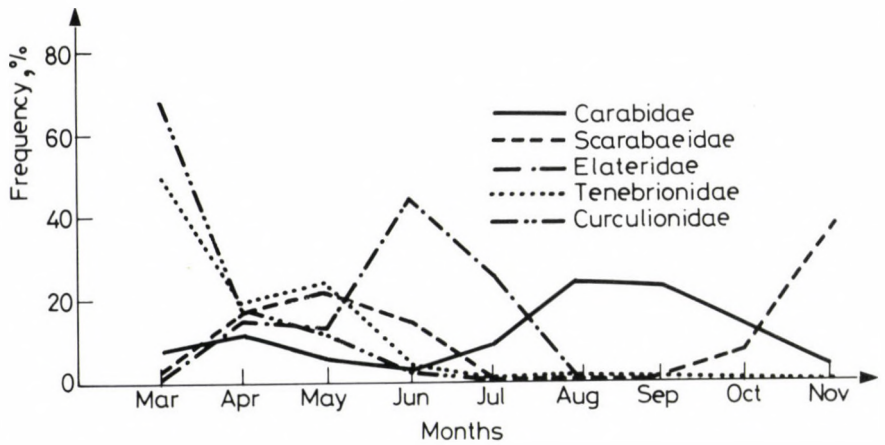

Fig. 1. Occurrence and frequency of major families of Coleoptera order in maize fields (pitfall trap)

carabids showed the usual two maxima, one in April and the other in August and September, the former being lower than the latter.

About 30 species Coleoptera have been found attacking the corn in Yugoslavia. The most important ones, which attack the roots, are larvae of *Agriotes ustulatus, A. sputator, Anisoplia austriaca, Amphimallon solstitialis, Rhizotrogus aequinoctialis*, and *Melolontha melolontha*. The most important species which attack the parts of the plant above ground are *T. dilaticollis* and *Phyllotreta vittula*. The most harmful of the species listed above are *A. ustulatus* and *T. dilaticollis*.

Clivina fossor L. (*Carabidae*) was recorded attacking corn in Yugoslavia for the first time in 1986. The degree of damage was up to 90%. This pest attacked corn seedlings several days after sowing. Although this species has been described as an omnivore which prefers food of animal origin (Desender and Pollet, 1985), more attention should be paid to this species in the future.

Conclusions

The following conclusions were drawn on the basis of a longterm study of the *Coleoptera* fauna occuring in corn fields on the chernozem soil in the North-eastern part of Yugoslavia (Vojvodina Province):

1. About 120 *Coleoptera* species from fourteen families were found in corn fields. Fourty-five of them were *Carabidae* followed by *Elateridae* and Scarabaeidae.

2. The average distribution of *Coleoptera* in the soil was over 4.3 specimens/m^2. The dominant species came from the families *Carabidae, Scarabeidae, Elateridae* and *Curculionidae*, which made for 86 percent of the total specimens collected. Corn plots were attacked by over 30 species of *Coleoptera*, with an average number of 3.1 larvae and imagos/m^2.

3. On the soil surface, *Coleoptera* were present throughout the corn growing season. Species from the families *Curculionidae, Tenebrionidae*, and *Carabidae* were dominant in the spring. Species from the families Elateridae and *Staphylinidae* were very active in late spring and early summer. Species from the families *Carabidae* and *Staphylinidae* were dominant in late summer and fall.

4. Regarding nutrition carnivores, herbivores, and omnivores were present. Distinct herbivores were present at a low percentage and omnivores with carnivorous preferences were dominant.

5. The most important pests of young corn plants were *A. ustulatus* which attacks the roots and *T. dilaticollis* which attacks above-ground parts of the plant. *A. sputator, A. austriaca, A. solstitialis, R. aequinoctialis, M. melolontha* and *P.vittula* should be counted among the major pests of corn.

6. *C. fossor* was recorded for the first time in corn in Yugoslavia. It attacks corn seedlings several days after sowing. The degree of damage was up to 90% in some regions.

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The Potential Importance of Insect Migration to Management Strategies of Maize Arthropods

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Zea mays (L.) is the third most important cereal crop common to agricultural communities around the world. In 1985, about 25% of the land used for production of the three major cereal crops (wheat, rice, maize) was used for maize production (Anon. 1985). Numerous species of insects attack maize, and in most instances, the economic value of the crop is not sufficient to warrant chemical control. Thus, maize becomes an efficient insectary for the production of insects, many of which are migratory and utilize numerous other agricultural crops as hosts. When these maize-produced, migratory species leave their decaying habitat and move into high economic value agricultural crops (vegetables) or crops which are vulnerable to the insect's injury (cotton), then chemical control is mandatory. At that time, the importance of insect migration to management strategies of maize arthropods is expressed. Generally, efforts to control these migratory species are conducted on a field-by-field basis that is inefficient, ineffective, and may become prohibitive by the cost/benefit ratio.

The family of insects, Noctuidae, contains at least two genera, *Heliothis* and *Spodoptera*, that are exemplary of the above scenario for several locations around the world. This paper uses the case history of the production of *Heliothis zea* (Boddie) on and subsequent movement from 200,000 ha of maize in the lower Rio Grande valley of south Texas, USA, and northeast Tamaulipas, Mexico, to extrapolate the potential importance of insects produced on maize to other agricultural crops.

Williams (1958) reviewed historical documents and cited records of massive flights of butterflies in Japan in 1248, near Cuba in 1494, and in the British Isles in 1508. In the last decade, research on insect migration has surged tremendously as evidenced by the contributed chapters to books by authors from around the world and edited by Rabb and Kennedy (1979), MacKenzie *et al.* (1985) and Danthanarayana (1986).

Man has fallen victim to insect migrations that have severely damaged and sometimes devastated his crops from the time he first turned his efforts to producing his food and fiber from soil. Initially and continuing even today, insect control is undertaken on a field-by-field basis. In the last quarter century, entomologists, farmers, and administrators of public research funds have begun collectively to concede that the control of highly mobile insect species on a field-by-field basis is not the most economically sound, efficient, or safe method for the farmer, the consumer of farm products, or the environment.

Theories dealing with the basic principles of supressing or managing insect populations on an area-wide basis were first published by Knipling (1955, 1979). Those theories advocated the use of autocidal techniques (sterile-male technique, pathogens, parasites, pheromones, etc.) used independently or in combination. One of the eight major criteria listed as a technical requirement for area-wide management of insects was knowledge of the migratory habits and the degree of impact of the migratory pest on the insect populations to be managed.

The larvae of at least four species within the family Noctuidae and genera *Heliothis* [*Heliothis zea* (Boddie), *H. virescens* (Fabricius), *H. armigera* (Hübner), *H. punctigera* Wallengren] are agricultural pests throughout the world. The moths of these species have the capacity for long-range migratory flight which magnifies their status as agricultural pests. *H. zea* (Boddie), the corn earworm, is a prominent pest of maize, cotton, sorghum, tobacco, and numerous vegetable crops in the United States. This species and a closely related species, *H. virescens* (F.), the tobacco budworm, are known as the *Heliothis* complex in the United States. Collectively, the complex reduces American fermers' income by more than $ 1.5 billion/year due to costs of control plus damages incurred.

Callahan *et al.* (1972), Snow, Cantelo & Bowman (1969), Sparks (1972), Sparks *et al.* (1975, 1986a, b), and Hendrix *et al.* (1985) have concluded that *H. zea* regularly fly at altitudes of at least 322 m throughout the growing season: *H. zea* emerging from a small, centralized plot of maize will disperse themselves over a 217.5 km^2 island; *H. zea* utilize cool weather fronts to fly at least 169 km into the Gulf of Mexico; and *H. zea* disperse as far as 880 km from a feeding source.

Widmer and Schofield (1983) compiled a bibliography of *Heliothis* dispersal and migration. Farrow and Daly (1987) reviewed the occurrence and detection of *H. zea* and *H. virescens* of the New World as well as *H. armigera* (Hübner) and *H. punctigera* Wallengren of Australia. We report results of research to examine the movement of massive numbers of *H. zea* produced annually on 200,000 ha of maize in the lower Rio Grande valley of Texas and Mexico and the potential result of the mass movement of that population from the maize as it matures and is harvested.

Materials and Methods

An ecological description of the region of the study area was provided by Raulston and Houghtaling (1986). Briefly, the study area (Fig. 1) is in the lower Rio Grande valley of Texas, USA and Tamaulipas, Mexico. The Ciudad Camargo-Rio Grande City area (98°50'W) and the Matamoros-Brownsville area (97°15'W) along the Rio Grande River are the western and eastern boundaries, respectively. The southern boundary is just north of Valle Hermoso, Mexico (25°35'N), and the norther boundary (26°10'N) is located just south of Edinburg, Texas. Annual precipitation reaches 680 to 760 mm per year with the major peak in September and lesser peaks in May and June. Minimum mean monthly temperature ranges from 15° -19°C in January through February and maximum means range from 27°-32°C between May and August. A large portion of the study area is irrigated from the Rio Grande River and reservoirs along the river. The major crops are maize, cotton and sorghum. Maize is by far the most important crop for production of massive numbers of *H. zea* populations. Annually, about 200,000 ha of maize are produced in this area. The crop is planted in February and matures in late May and June. About 200,000 ha of sorghum are generally planted in late February and are mostly produced on non-irrigated land. In late February and early March, about 120,000 ha of cotton are planted for harvest in August. This area was selected for study of *H. zea* migration for several reasons.

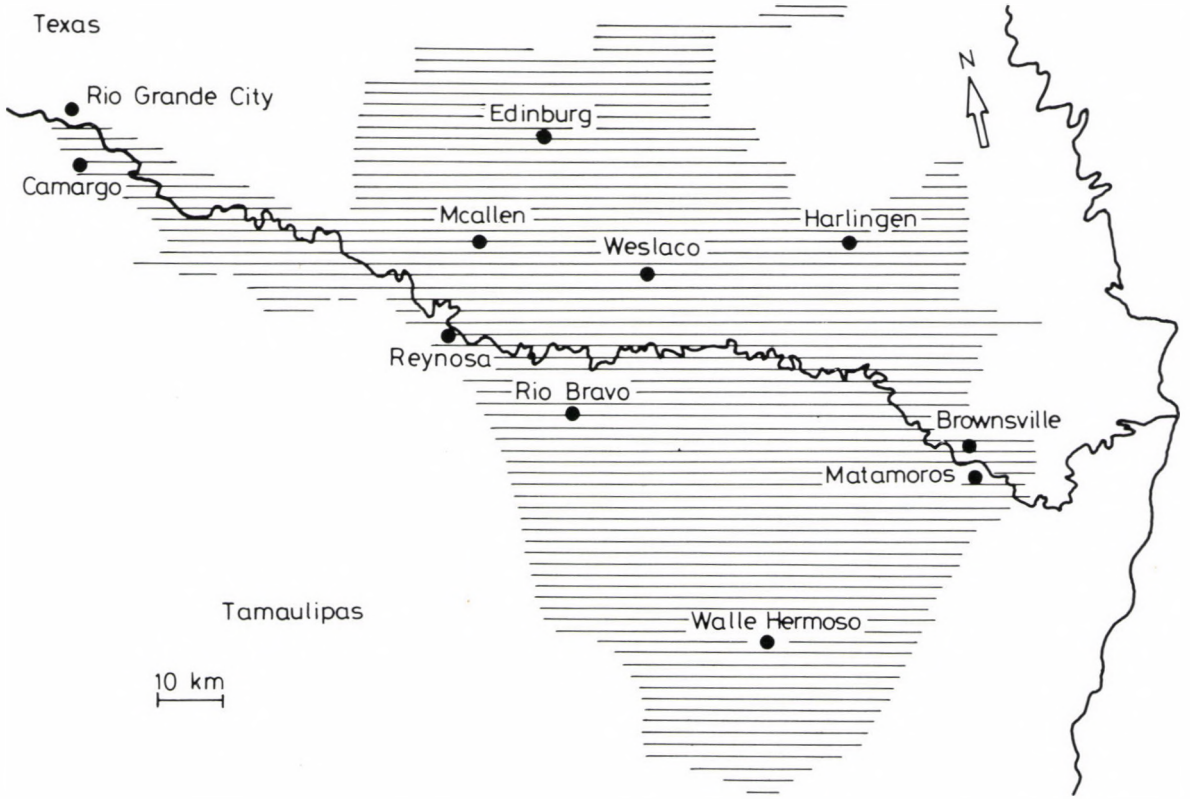

Fig. 1. Irrigated region of the lower Rio Grande Valley of Texas, USA and Tamaulipas, Mexico

(1) The total production of *H. zea* on a large aea of hosts (primarily maize) is sufficiently uniform in development to provide large populations of adults for radar observations over a 10-14 day period.

(2) An extensive body of knowledge of the biology-ecology-behavior of *H. zea* is documented for the area.

(3) Frequent wind jets have been documented (Bonner 1968) at approximately 500-600 m altitude in the south-central United States capable of displacing *H. zea* north-northwest from the lower Rio Grande valley at speeds up to 100 km per hour.

(4) The study area is an area suitable for area-wide management of *H. zea*, if such a phenomenon proves feasible.

An interdisciplinary team approach is necessary to research insect migration as it occurs in nature. Our team includes entomologists, meteorologists and engineers. Equipment and techniques will be discussed by discipline.

Entomology

The primary objective is the characterization of the population of *H. zea* produced on the 200,000 ha of maize. The goal is to estimate the number of adults emerging from the soil over a short span of time in June. These adults are available for transport by atmospheric circulations to crop areas to the North and could be observed by radar.

Data on biological factors affecting the *H. zea* population throughout the season were collected; however, those data will be the source of another paper. Here, our main interest is the total number of adults available for transport. Thus, immediately after the majority of the *H. zea* larvae had "cut-out" of the maturing ears, two 1 m^2 x 15 cm deep areas of soil were examined for pupae in each of about 100 fields. The samples were taken in fields selected at regular intervals along pre-chosen, designated roads in Mexico and Texas. The routes were chosen to assure sampling of most of the maize grown in the study area. Alle pupae and exuviae were collected to provide information on diseased insects, pupae dead for unknown reasons, and live pupae. The total exuviae and live pupae/m^2 was used in combination with the estimated ha of maize to extrapolate the total imagos produced int the lower Rio Grande valley. We assumed a 1:1 sex ratio and that 10 % of the females survived to the reproductive stage. We then assumed each female oviposited 1000 eggs and than 25,000 eggs per ha represented an economic infestation level. With these assumptions, the potential total acres of crops infested from the adults produced on the maize crop in the lower Rio Grande valley were estimated.

Meteorology

In the United States of America, synoptic-scale meteorological data to support insect dispersal are generally available from the National Weather Service (NWS). The date are not as specific as we would like for our field investigations because the location of meteorological stations is biased toward population centers rather than agricultural production regions. Meteorological weather data are reported hourly at principal airports

and daily at cooperative weather stations. Twice-daly NWS upper-air meteorological observations are taken at 0000 and 1200 Coordinated Universal Time (UTC). These data are not sufficiently frequent to document phenomena for transient features such as sea breeze circulations, thunderstorm updraft/outflow, and low-level jets (Bonner 1968). Thus, local atmospheric measurements are required to complete the NWS synoptic-scale meteorological data with respect to *H. zea* dispersal research.

Helium-filled balloons (pibals) were launched at approximately 1-hour intervals to reveal wind velocity profiles. Every third pibal carried instrumentation to measure additional atmospheric variables. An observer optically tracked the pibal ascents with a theodolite to measure the elevation and azimuth angles of the pibal displacements. Pibal elevation and azimuth angle data were transmitted (0.16 Hz) via a theodolite transducer cable to an Apple IIe microcomputer. An Apple IIe Basic computer program analyzed theodolite-tracked, non-instrumented pibal azimuth and elevation angles to calculate wind velocities based upon a constant (known) rate of ascent.

An A.I.R., Inc., model TS-1A-1 403.5 MHz ground receiver and radiosondes measured in-situ vertical profiles of atmospheric properties. The airsonde housed two thermistors (one bare and one covered with a moist wick) and an aneroid barometer. The airsonde was attached to a pibal and during ascent transmitted (0.16 Hz) wet-bulb temperature, dry-bulb temperature, and atmospheric pressure to a ground receiver. An Apple IIe microcomputer recorded telemetered data from the airsonde, elevation and azimuth angles from the theodolite, and elapsed time. The microcomputer calculated vertical profiles of dry-bulb temperature, relative humidity, wind speed and wind direction.

NWS upper-air data for the south-central United States (1984-86) and Agricultural Research Service (ARS) upper-air data (1984-87) for the lower Rio Grande valley were summarized to disclose atmospheric transport potential which would enhance the northward dispersal of noctuid moths in the late spring and early summer. Vertical profiles of ARS upper-air data were summarized to display the interannual climatic variability within the planetary boundary layer (PBL) and to reveal altitudes of enhanced atmospheric transport potential.

Radar

An entomological radar (Wolf, Westbrook & Sparks 1986) was used to measure insect density and orientation at selected altitudes. The radar wavelength was 3.2 cm with peak power of 25 kilowatts and pulse length of 250 nanoseconds. A 1.22-m diameter parabolic antenna produced a pencil-shaped beam which was scanned in azimuth and elevation. The data were displayed on a conventional Plan Position Indicator (PPI), which indicated the distance and direction of individual targets from the radar.

Radar observation periods varied with respect to peak *H. zea* emergence due to logistics and lack of funds. The 1984-86 observations occurred near and after peak emergence. In 1987, with adverse weather conditions, observations were initiated before significant emergence began and continued through peak emergence.

Quantitative data were obtained by visually assessing the PPI display. This quantitative information was converted into insect density and orientation versus altitude and time of night using techniques similar to those described by Drake (1981), Riley (1978),

and Schaefer (1976). Insect flux (number of insects that passed the radar per unit area per unit time) is the product of insect density and displacement velocity. The data were sorted into three time categories: dusk = sunset to 1.5 hours after sunset; early = 1.5-3.5 hours after sunset; late = greater than 3.5 hours after sunset. The radar did not provide displacement veliocities when densities were large nor at high altitudes; therefore, displacement velocity was approximated by using wind velocity. The insect flux was treated as a vector, and the east-west and north-south component of each observation was used to integrate over altitude and time. The resultant flux vectors were then reconstructed.

Results

The total number of live *H. zea* pupae produced on 200,000 ha of maize in the years 1984-87 is shown in Table 1. In that four-year period of time, the maximum variability of the total production of *H. zea* was about five-fold between 1987 and 1985. The variability was caused by the environment and biological phenomena associated with the life cycle of the insect.

The most pronounced environmental effect occurred in 1987 when a cold front passed through the lower Rio Grande valley in late March and subjected most of the irrigated cropping area to -12.5°C (25°F) temperatures for several hours. The maize throughout the study area suffered moderate to severe damage with about 25 % of the area having to be replanted. At the time of the freeze, the pre-whorl and whorl-stage maize was about 30 % infested with *H. zea* larvae. The freeze caused the surviving maize crop to develop in a sporadic manner, reduced larval survival, and extended the period of emergence. Only 1.21×10^9 total live pupae were produced on the first planted maize and 1.26×10^8 were produced on the replanted maize for a total production of 1.33×10^9 *H. zea* in 1987, the lowest production of *H. zea* during the study.

At the same time of approximate peak larval "cut-out" from the maize ears in 1986 and 1987, an extended period of rainy weather occurred. The wet soil facilitated the parasitic nematode, *Neoplectana carpocapsi* (Filipjeb), infections and reduced the *H. zea*

Table 1

Production of H. zea on 200.000 ha of maize in the lower Rio Grande valley of Texas and Mexico in early summer, 1984-87, and potential number of ha with 25,000 eggs/ha infestation

Year	No. of samples	Total forms x 10^9	Surviving[1] females x 10^6	Potential infested[2] ha x 10^6
1984	200	2.22	111	4.44
1985	200	6.54	327	13.08
1986	220	5.62	281	11.24
1987	210	1.33*	66	2.66

[1] Estimate 10% of live pupae found during survey reach reproductive maturity and each surviving female oviposits 1000 eggs.
[2] Estimate infestation rate of 25,000 eggs/ha.
* Thirty samples taken of replanted maize indicated that late-planted maize produced less than 10% of population.

populations by 30 % in 1986 and about 22 % in both first planting and second planting of maize in 1987. The nematode parasitism was not observed in samples taken in 1984 or 1985 under relatively dry weather conditions.

The potential problems resulting from migration of *H. zea* from the mature maize are shown in Table 1. Assuming that one-half of the live pupae found in the soil sampling survey are female, that 10 % of those females reach reproductive maturity and that those females oviposit 1000 eggs each, then one can calculate the potential for infesting a given number of ha after migration has occurred. In this case, we assumed that 25,000 *H. zea* eggs per ha would be an economic infestation. The range of numbers for potential infested ha was 2.66×10^6 to 13.08×10^6 . These data suggested that the 200,000 ha of maize grown in the lower Rio Grande valley provides sufficient *H. zea* to infest 13 to 65 times the original area of maize.

Significant interannual climatic variation was apparent across the south-central United States for 15 May through 30 June 1984-87. The meridional (i.e., north-south) wind speed component at 500 m above ground level (AGL) was analyzed for 12 NWS stations in the south-central United States and two cooperating stations in Mexico. The percent frequency and mean speed of northward wind was computed for each station. A northward atmospheric transport potential value was calculated as the product of the percent frequency and mean speed of northward wind. The maps in Fig. 2 reveal a

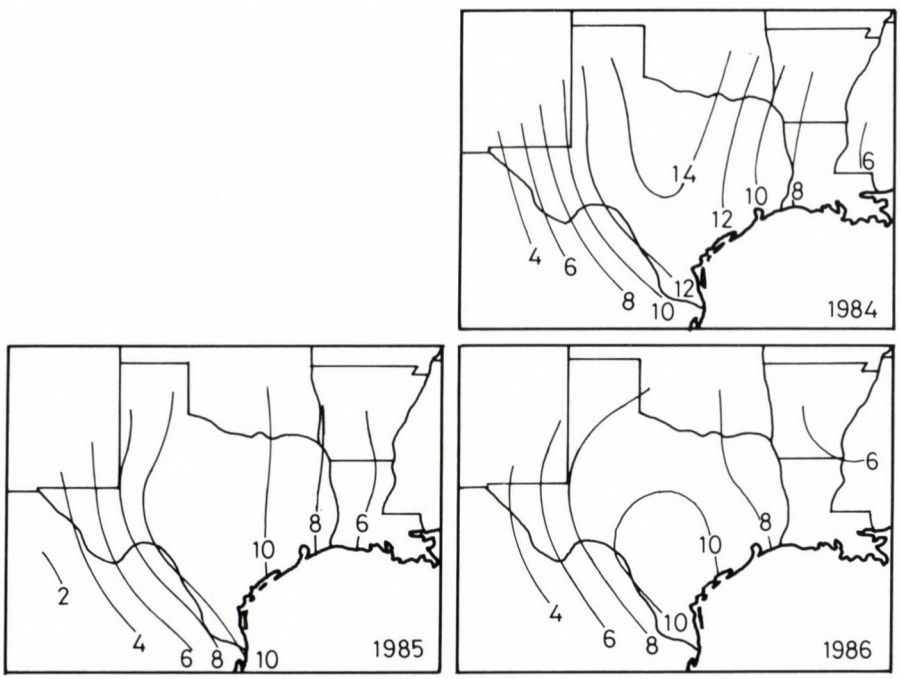

Fig. 2. Northward atmospheric transport potential (m/sec) at 500 m above ground level for Brownsville, Texas, from 15 May - 30 June (southward wind speed set to zero)

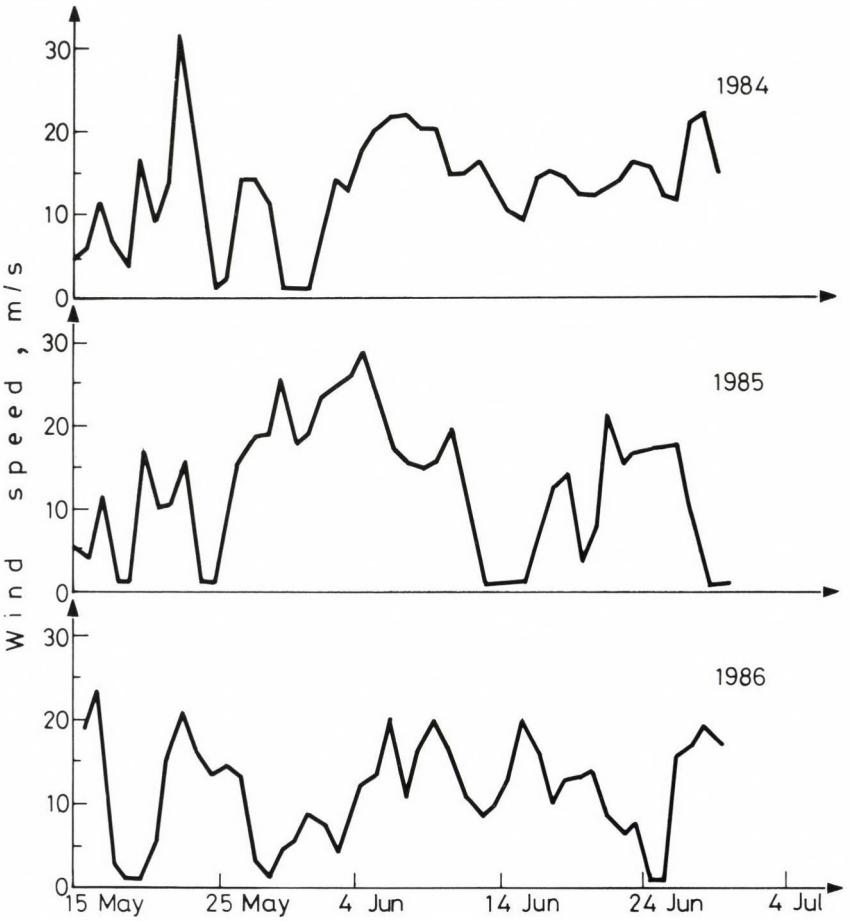

Fig. 3. Northward atmospheric transport potential (m/sec) at 500 m above ground level for Brownsville, Texas (southward wind speed set to zero)

maximum northward transport potential of 14 m/s, 10 m/s, and 10 m/s for 1984, 1985 and 1986, respectively. Data for 1987 have not been completed. The center line of maximum northward transport potential extended north-northwestward from Corpus Christi, Texas, to Stephenville, Texas, and northward to Oklahoma City, Oklahoma. Thus, an excellent atmospheric circulation was available each year for long-distance dispersal from the lower Rio Grande valley to cropping regions in northern Texas and central Oklahoma.

Time series plots (Fig. 3) of the daily mean (i.e., average of the 0000 and 1200 UTC observations) 500-m AGL northward wind speed at Brownsville, Texas, revealed significant interannual variation of atmospheric transport potential. The maximum daily

Table 2

Mean wind velocity profiles for the lower Rio Grande valley calculated from balloons tracked by radar on site, 1984-87

Altitude (m)	June 8-22, 1984* Wind		June 7-25, 1985 Wind		June 3-12, 1986 Wind		May 27-June 12, 1987 Wind	
	Speed m/s	Direction (deg.)	Speed m/s	Direction (deg.)	Speed m/s	Direction (deg.)	Speed m/s	Direction (deg.)
100	6.6	12	7.1	118	8.9	133	6.6	121
200	7.9	127	8.7	122	9.9	135	8.6	123
300	9.0	131	9.8	123	10.5	137	9.3	142
400	9.7	133	10.4	126	10.8	139	9.6	143
500	9.9	131	10.7	125	10.6	142	9.3	152
600	9.9	131	11.0	126	10.3	143	9.6	155
700	10.0	132	10.8	125	9.8	143	9.0	158
800	9.9	133	10.4	124	9.4	141	9.0	175
900	9.9	141	9.9	125	8.9	137	7.3	167
1000	9.5	136	9.4	125	8.4	134	5.7	75
1100	9.2	136	8.9	123	7.7	132	5.4	162
1200	9.8	136	8.9	123	7.7	132	5.4	162
1200	9.8	135	8.7	121	7.1	137	5.2	168

*Abasolo, Mexico

mean northward wind speed decreased from 30 m/s (1984) to 28 m/s (1985) and 23 m/s (1986). Only eight days of minor (i.e., speed 5 m/s) transport potential occurred in 1984, while 12 and 11 days of minor transport potential occurred in 1985 and 1986, respectively. The maximum number of consecutive days with major (i.e., speed 5 m/s) transport potential ranged from 29 days in 1984 to 18 and 19 days in 1985 and 1986, respectively.

A summary of mean ARS-derived wind velocity profiles documented substantial interannual (1984-87) variability of wind speed and wind direction. Although a mid-PBL wind speed maximum occurred each year, the altitude and value of the wind speed maximum differed from year to year. The annual mean wind speed maximum ranged from 9.6 m/s (1987) to 11.0 m/s (1985) and occurred at an altitude between 400 m (1986) and 700 m (1984). The wind direction veered from the east-southeast (1984 and 1985) to southeast (1986) and south (above 800 m AGL in 1987). Intra-annual variability of wind speed and wind direction based on NWS data differed from year to year and often conflicted with the mean profiles measured at the radar site (Table 2).

The radar data showed large night-to-night fluctuations in the number of airborne insects. These fluctuations occurred as a result of insects' behavioral response to meteorological conditions, time of night, crop phenology, population density and time of year. Since the radar was located near the northern edge of the maize-growing area, winds from any direction other than southerly resulted in low densities of airborne insects observed by radar. Our primary interest was the number of insects leaving the maize-growing area and moving northward.

The data in Table 3 show radar-derived insect flux data for the early summer when wind direction was southerly. These date are estimates of the relative numbers of dispersing insects as they left the decaying habitat in the early summers of 1984-87. Insect flight began about 30 minutes after sunset. During dusk, the first 1.5 hours following sunset,

Table 3

Relative insect flux passing the radar during periods when the wind had a southerly component near Weslaco, Texas, 1984-87.

Year	Dusk 0-1.5 hrs after sunset			Early 1.5-3.5 hrs after sunset			Late >3.5 hrs after sunset			Annual		
	Radar hrs	Flux	Dir	Radar hrs	Flux	Dir	Radar hrs	Flux	Dir	Radar hrs	Flux	Dir
1984	8.2	2300	124°	20.7	460	126°	23.0	8	135°	51.9	550	125°
1985	12.7	2400	137°	20.6	980	134°	5.0	56	140°	38.4	1300	136
1986	4.6	980	138°	9.8	570	139°	3.6	117	137°	18.0	580	138°
1987	6.9	28	136°	13.2	32	145°	19.9	16	157°	39.9	23	147°

Radar hrs = Accumulative hours of radar data during southerly wind and time classification.
Flux = Insects per hour passing through a 1-m wide window extending from 100 to 1200 m above the ground. Insect flux vectors derived from radar and wind data were integrated over observed altitude and time.
Dir = Direction from which targets approached the radar.

the greatest flux of insects was observed. The flux rate during early evening, 1.5-3.5 hours after sunset, varied from a slight increase (1987) to an 80 % decrease (1984). Radar-observed insect flight occurring more than 3.5 hours after sunset was severely reduced. Extrapolation of the seasonal measured fluxes over the approximate 50 km-wide source area suggests that 1.4×10^9, 2.9×10^9, and 0.046×10^9 insects moved northward from the source area during the periods of radar operation in 1984 to 1987, respectively. Note that the duration of radar operation varied from season to season and that peak emergence did not always occur during our radar operating period.

Discussion

Numerous known/unknown factors govern an insect species' propensity and capability to migrate. The mechanism that triggers a portion of an insect species' population to enter into high altitude flight about 30 minutes after sundown while the remainder of the population of the same species remains in the crop is unknown. However, that particular daily activity is the key to the success of the survival of a migratory species. When a portion of the population enters into an early-evening flight activity and moves with the wind in a local dispersion manner while a portion of the population remains in the field, the species has efficiently "explored" the available niches. Then, when an atmospheric circulation occurs with capabilities for long-range transport, the species becomes a long-range migrant. In case of *H. zea* production on maize in the lower Rio Grande valley and subsequent movement from the decaying habitat, literally millions of moths per night could be available for long-range transport on favorable atmospheric circulatory systems and into crops north of the area.

Pair *et al.* (1987) and Sparks *et al.* (1986b) surveyed *H. zea* production from the maize-growing area in the lower Rio Grande valley in 1984 and 1985. Also, they surveyed the High Plains of Texas maize area for *H. zea* production in those years. In 1984, 2.2×10^9 *H. zea* produced in the lower Rio Grande valley were subjected to favorable atmos-

pheric circulation for transport to the High Plains of Texas where 110,000 ha of maize produced 87,333 adult *H. zea* per ha. However, in 1985, 6.5 x 10^9 *H. zea* were produced in the maize-growing area of the lower Rio Grande valley; no favorable atmospheric long-range transport circulatory systems developed during the adult emergence cycle; and *H. zea* production on the High Plains dropped to 36,200 per ha. Thus, there was a three-times greater production of *H. zea* in the lower Rio Grande valley in 1985 and a 60 % reduction in *H. zea* production on the High Plains. We suggest that the lower infestation on the High Plains in 1985 was strongly influenced by less favorable transport from the lower Rio Grande valley.

Stadelbacher and Pfrimmer (1972) observed a 33-day hiatus between initial capture of *H. zea* in light traps and emergence from diapause at Stoneville, Mississippi. Hartstack *et al.* (1982) reported a 19-day difference in initial peak trap capture and mean emergence from diapause in central Texas. Thus, slowly but surely, more and more strongly circumstantial evidence indicates that *H. zea* migration is a major concern to be reckoned with in any program designed to manage *H. zea* populations by any method other than insecticides and on a field-by-field basis – the current standard management technique that is expensive, inefficient, and results in environmental contamination.

However, before extensive changes in management strategies can be implemented, research on migration must be intensified. Researchers must become capable of identifying the source area from which a migrant moth originated and the fall-out area of moths that have been transported by atmospheric circulations. Radar specialists must develop techniques to measure duration of flight and separate migrant insects into groups initially and species of close proximity eventually. Entomologists must develop capabilities to assess the monetary impact of migrants on the crops within a given large area. Then, we will be able to evaluate the technical and economic feasibilities of controlling a migrant species such as *H. zea* in a large area such as the lower Rio Grande valley prior to the time the species migrates.

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Aphidophagous Chrysopids and Hemerobiids (Neuropteroidea) Subguilds in Different Maize Fields: Influence of Vegetational Diversity on Subguild Structure*

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(1) The structure of earlier recognized aphidophagous Chrysopid and Hemerobiid subguilds (Szentkirályi, 1986) were studied in maize stands under continuous monocultural and rotation management in two agricultural areas characterized by diverse (area-"D") and simple (area-"S") surrounding non-crop vegetation for 6 years.

(2) The sampling methods (light trapping, pitfall trapping, sweep netting, soil sampling, visual survey of maize and non-crop plants) were conducted at weekly intervals from May (sowing) to mid-November.

(3) In the maize fields bordered by diverse shrubby and woody vegetation, the subguilds had more additional species associated with the shrub and crown layers. The dominant members of both subguilds were extremely ubiquist or herb-layer inhabitants.

(4) The higher structural diversity of the vegetation increased the species richness and diversity of subguilds considerably. The greater species richness of aphids and their host-plants promoted also the lacewing egg-laying in bordering vegetation.

(5) The similarity of species composition of the subguilds was considerably higher for within area than for within cultivation type comparisons. Consequently, there was no essential impact of maize production technologies on the structural characteristics of subguilds.

The enemies hypothesis (Root, 1973) predicts that more stable populations of arthropod predators can persist in more diverse vegetational environments. The more complex vegetation has more diverse habitat patterns providing more suitable sites for shelter, oviposition, overwintering and more essential and alternative food for predators. One of the important questions is how can the crop and surrounding non-crop vegetation influence the predatory arthropod coalitions. Van Emden (1965) dealt with the role of uncultivated land in the colonization of insect predators into crop fields. Altieri *et al* (1977) suggested that the predators of crop fields may depend on the weed species of their surroundings. Altieri & Whitcomb (1979) collected 43 percent of predatory arthropod spp. living on a weed sp. from nearby maize fields, too. Altieri & Whitcomb (1980) and Altieri & Todd (1981) pointed out that the complex neighbouring vegetation, compared to the simple one increased both density and diversity of predators in maize plots and soybean field. So it seems to be important that a crop field is surrounded by weedy borders, forest edges, shelterbelts, shrub patches etc., because of these reservoir habitats connect the entomophages into complex foodwebs (Zwölfer, 1981) and affect the arthropod colonization and distribution within crop stands (Lewis, 1965, Ryszkowski & Karg, 1976).

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During the investigation of the arthropod community of maize field with different production systems (Mészáros *et al.*, 1983), I found (Szentkirályi, 1986) that the Chrysopid and Hemerobiid lacewings formed two subguilds within the aphidophagous guild they showed spatio-temporal and food separation. The question dealt with here is whether the different maize production systems and the diversity of adjacent vegetation can influence the structural characteristics of the two subguilds or not.

Material and Methods

All life stages of Chrysopid and Hemerobiid subguild members and their potential aphid prey were investigated on maize stands and on non-crop plants of adjacent vegetation during 1978-83. Two maize growing areas were selected: area-"D" had diverse vegetation bordering the arable fields formed by shelterbelts, species-rich herbaceous, shrubby and forest patches. The area-"S" was characterized by simple non-crop vegetation without considerable shrub and tree-foliage levels among the large-sized crop fields. Within area-"D" we investigated: (1) a continuous maize monoculture (CD) of 410 ha. (2) six crop rotation fields (RD), between 18-123 ha. The fields were within 10 km from each other. Within area-"S" a single 339 ha continuous maize monoculture (CS) was studied. Agrotechnical and pest management practices were almost identical in all fields. Insecticide treatment was applied once at sowing against the soil-inhabiting pests. The main difference among the stands was the preceding crops and the bordering vegetation (diverse vs simple).

The collecting methods were: light-trapping, sweep-netting, stalk-trapping, soil sampling, pitfall-trapping, and visual inspection of maize and non-crop plants. The insect material was collected weekly (light-trapping daily) from sowing until harvest, in the bordering vegetation until mid-November.

The structural characteristics were the species richness, the mean dominance, diversity (Shannon-Wiener index) and similarity (Renkonen index, Jaccard index) based on all methods and years.

Results

From the Chrysopid subguild (Table 1) the most dominant and frequent member was *Chrysoperla carnea* followed by *Chrysopa phyllochroma* and *Ch. formosa* on the maize fields. Other spp occured sporadically at low density. Within the Hemerobiid subguild there were 4 dominant spp: the most one was *Micromus angulatus* in all stands, followed by *M. variegatus, Hemerobius humulinus, Wesmaelius subnebulosus.* The other Hemerobiids were sporadical.

In the CS field with nondiverse surrounding vegetation only the ubiquitous, eurytopic and the herb-layer species could survive. On the contrary, in the CD and RD maize stands other additional subguild members occured, too, which have a habitat preference for shrubs and trees (Table 1).

The species richness and consequently the mean species diversities of both subguilds were higher in area-"D" (Table 2). Between years the most similar subguild com-

Table 1

Species composition and the mean percent of dominance values (based on different collecting methods) of neuropteroid subguilds on maize fields

Species	CS	CD	RD	HP
Chrysopidae				
Chrysoperla carnea (Stephens)	93.1	46.9	56.4	W, S, H
Chrysopa phyllochroma Wesmael	+	41.8	23.2	H
Chrysopa formosa Brauer	+	+	15.1	S, H
Chrysopa abbreviata Curtis		+	+	H
Chrysopa perla (Linnaeus)		+	+	W, S, H
Chrysopa septempunctata Wesmael	+	+	+	W, S
Anisochrysa ventralis (Curtis)		+	+	W, S
Chrysotropia ciliata (Wesmael)		+		W, S
Nineta flava (Scopoli)		+	+	W, S
Cunctochrysa albolineata (Killington)			+	W, S
Hemerobiidae				
Micromus angulatus (Stephens)	93.7	46.0	50.3	H
Micromus variegatus (Fabricius)		22.9	+	H
Hemerobius humulinus Linnaeus	+	16.9	22.5	W, S, H
Wesmaelius subnebulosus (Stephens)		14.1	11.3	W, S
Wesmaelius nervosus (Fabricius)		+	+	W, S
Wesmaelius quadrifasciatus (Reuter)		+		W
Hemerobius nitidulus Fabricius			+	W
Hemerobius atrifrons McLachlan			+	W
Hemerobius micans Olivier			+	W, S
Sympherobius elegans (Stephens)		+	+	W
Sympherobius pygmaeus (Rambur)		+	+	W
Psectra diptera (Burmeister)		+		H

CS=continuous monoculture with simple, CD=continuous monoculture with diverse and RD=crop rotation with diverse adjacent vegetations; HP=habitat preference; H=herbaceous, S=shrubby and W=woody habitats; +=species with <10% dominance

Table 2

Structural characteristics of Chrysopid and Hemerobiid subguilds of various maize fields

Characteristics	Chrysopidae			Hemerobiidae		
	CS	CD	RD	CS	CD	RD
Number of species	4	9	9	2	9	10
Mean species diversity[1]	0.12	0.45	0.46	0.10	0.33	0.49
Mean between year similarity[2]	0.89	0.66	0.61	1.00	0.65	0.59

Abbreviations: see Table 1;
Notes: 1 = Shannon-Wiener index, 2 = Renkonen index

Table 3

Species similarity (Jaccard index) of Chrysopid and Hemerobiid subguilds between various maize fields

Comparison	Chrysopidae	Hemerobiidae
CD-RD	0.80	0.58
CD-CS	0.44	0.22
RD-CS	0.44	0.20

positions were in CS field due to the low species richness, while in area-"D" the similarities ranged between narrow limits (0.50-0.66) (Table 2). The subguild similarity between CD and RD fields (Table 3) was high (0.80) in the case of Chrysopids and medium (0.58) in Hemerobiids. In comparisons between area-"D" and -"S" both subguild similarities were low, about half of the CD-RD values. The adjacent vegetation of CD and RD fields was characterized by much higher species richness of shrubs and trees, with higher levels of vertical stratification than in the CS stand (Table 4). Because of the higher diversity of bordering vegetation, the species richness of aphids and their host plants was 4-7 times higher. Due to this, Chrysopid and Hemerobiid eggs were found on more plant species in area-"D" than in area-"S" (Table 4).

The above-mentioned differences between areas existed also when considering only the most frequent and abundant plant spp (Table 4).

Discussion

The maize fields studied differed mainly in species richness of their bordering noncultivated vegetation caused by the presence or absence of shrubby and tree-crown levels. Within area-D the diverse surrounding vegetation supported more aphid species via higher number of host-plant species. This greater habitat complexity and trophic

Table 4

Relations among diversity of adjacent vegetation, the potential food sources and the neuropteroid egg-laying

No. of species	Areas		
	CS	CD	RD
Dicotyledonous herb	25(14)	29(14)	70(19)
Shrub	1(0)	6(3)	7(3)
Tree	2(2)	12(6)	10(5)
Aphid-infested plants	4(1)	20(13)	29(16)
Aphids	4	16	14
Plants with Chrysopid eggs	2(0)	22(11)	31(15)
Plants with Hemerobiid eggs	0(0)	12(7)	5(4)

Abbreviations: see Table 1; In brackets: No.of common plant species

diversity of adjacent vegetation had a strong impact on the structure of lacewing subguilds. The lacewing species composition reflected the preference for vertical habitat stratification: within area-"D" the complex habitat patterns allowed the buildup of such subguilds whose members were associated mainly with shrub- and crown-layers. These species absent in area-"S", could visit or colonize the maize stands temporally. The dominant and constant members were identical in both areas: either extremely ubiquist (e.g. *Chp. carnea*) or typical herb-layer inhabitants (*Micromus* spp).

The higher diversity of adjacent vegetation within area-"D" increased considerably the species richness, the species diversity, and frequency of egg-laying in both subguilds. Because of the low species richness, the mean subguild similarity in within stand between year comparisons in area-"S" was very high, while in area-"D" it was medium. Subguild similarities between field-types were much higher in area-"D" than for the same field-types between two areas. Based on this latter fact and the similar values of species richness and diversity within area-"D", it can be concluded that there was no impact of maize production systems on the structure of green and brown lacewing subguilds.

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The Corn Stalk Borer, *Sesamia nonagroides* (Lepidoptera, Noctuidae): Population Fluctuation and Plant Infestation Relationships

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The corn stalk borer, *Sesamia nonagroides* (Lef.) (Lepidoptera, Noctuidae), is the most important pest of corn in the Mediterranean countries. In Greece, it is most important in the crop sown after the harvest of small cereals. The population fluctuations of this insect has been studied by light traps. The adult flight pattern is similar from year to year. The first adults appear in the end of April- early May and the last ones in the end of October- early November. Until mid-August, the population density is very low, then it increases reaching peak levels in September, declining sharply in October, sometimes showing a second peak. Three to four generations occur in a year. The corn infestation levels vary from year to year, from area to area, and between the early and the late crop. In the latter case, stalks and ears can be completely devoured. By artifically infesting plants at four different developmental stages (6-7 leaves, before flowering, seeds at milk stage and seed drying with green top leaves) with ready-to-hatch eggs, the highest stalk and ear infestation was observed in the second and third stages of development, while yield reduction was highest at the first two stages.

The corn stalk borer, *Sesamia nonagroides* (Lef.) (Lepidoptera, Noctuidae), is an important pest of corn in Greece (Stavrakis, 1967; Tsitsipis *et al.*, 1983) and in other countries of the Mediterranean Basin (Lespes & Jourdan, 1940; Nucifora, 1966; Prota and Delrio, 1968; Alfaro, 1972; Melamed-Madjar & Tam, 1980).

The adults appear from April-May to October-early November completing 3-4 generations. In Greece, during 1983, the population was very low until mid-August in the area of Kopais in central Greece. Then, it increased abruptly giving two peaks at the beginning and the end of September, declinig sharply thereafter (Tstitsipis *et al.*, 1984).

In Greece, extensive damage is done to the late corn crop, sown early in July, although considerable damage has been also reported in the early one. In 1982, a 80% yield loss was recorded in Larissa (Gliatis, 1983). In early corn crop at harvest, 20-97% of the plants and 4-29% of the ears were infested. The infestation intensity ranged from 1.5-27.3% and 0-35% for the stems and the ears, respectively (Tsitsipis et al., 1985). In a spray program experiment, a treatment with three sprays suffered 23% ear infestation compared to 62.5% in the control (Tsitsipis, 1988).

The present work reports on the seasonal appearance and the population of the corn stalk borer in the area of Kopais, during 1983-1986 as well as the relationship between plant stages of development and infestation intensity.

Materials and Methods

Adults of *S. nonagroides* were caught with Pennsylvannia type light traps using fluorescent 5 (1983) or 15 Watt BL lamp (1984-86). Ethyl acetate or dichlorvos impreg-

nated polyurethane plaquettes (Vapona, Shell Chemical Co.) were used as killing agents. Traps were checked three times per week and they functioned from April to November.

Insect host plant infestation relationships were studied, in a field experiment, by artificially infesting corn plants at 4 different growth stages with 1-2 ready-to-hatch egg masses of 80-100 eggs each. Eggs came from a colony, originating from the same area, and kept on artificial larval diet (Tsitsipis, 1984). Egg masses were placed in internodes between the leaf sheath and the stem. The stages were: 6-7 leaf plants, before inflorescence, milk stage ears and hardened seeds with green ear leaves and above. Egg masses were placed in the 2nd-3rd internode in the first case and in the 5th-6th in the rest. Infestations were made every 21 days. The experiment was set up in a completely randomized block design on a regular corn field with rows 80 cm apart and distances between the plants 18-20 cm. Twenty one blocks, of 8-treated plants, were placed every 6th line. The treatments of each block were done on plants found every 3m (5-17 plants). Evaluation was made at harvest. Stem and ear infestation as well as dry seed production were recorded. Infestation was expressed as percent of the total infested by the larvae.

Temperature values were recorded with a thermo-hygrograph. The experiment was done in the Agricultureal Research Station of Aliartos in Kopais (latitude 38°14' N).

Results and Discussion

The seasonal appearance and the population fluctuations of the corn stalk borer during the years 1983-86 is shown on Fig.1. The first adults were caught in the end of April early May and the last ones in the end of October-early November. The population during all years remained at very low levels until mid-August. In the years 1983, 1985, 1986 it increased very rapidly, reaching peak numbers in the beginning of September, then declined slightly and rose again showing a second peak at the end of September or the beginning of October declining sharply afterwards. In 1984, the population started rising in the beginning of September and reached its peak at the beginning of October. In 1984, highest weekly captures were lower (126 insects) than in other years, when 2.5 to 4 times more insects were caught. A third generation seems to be represented by the second peak in the end of September. A partial 4th generation occurs. The last 2-3 generations appear extensively overlapping. The larvae of the 3rd or 4th generation enter diapause over-wintering in the last larval instar, giving rise to adults next April-May. More or less similar results have been reported by Prota (1965), Nucifora (1966), Alfaro (1972), Galichet (1982). The number of generations per year, however, according to different publications, varied from three in Sardinia (prota 1965), to four in Sicily (Nucifora, 1966), two to three in Spain (Alfaro 1972), and four in Morocco (Lespes & Jourdan, 1940). The most characteristic of the present data is the very low occurence of adults until mid-August in comparison with the data in Italy (Prota & Delrio, 1968) and France (Galichet 1982) where relatively high numbers of the insect appear in May, June, July.

The results of the effect of infestation intensity on corn plants found at four different stages are shown in Table 1. The infestation of the stems was most severe when two egg masses infested the second and the third stages of development tested or the third stage with one egg mass. The respective degree of infestation values were 38.67, 33.67 and 25.86%. Then, infestation with one egg mass in the second stage of develop-

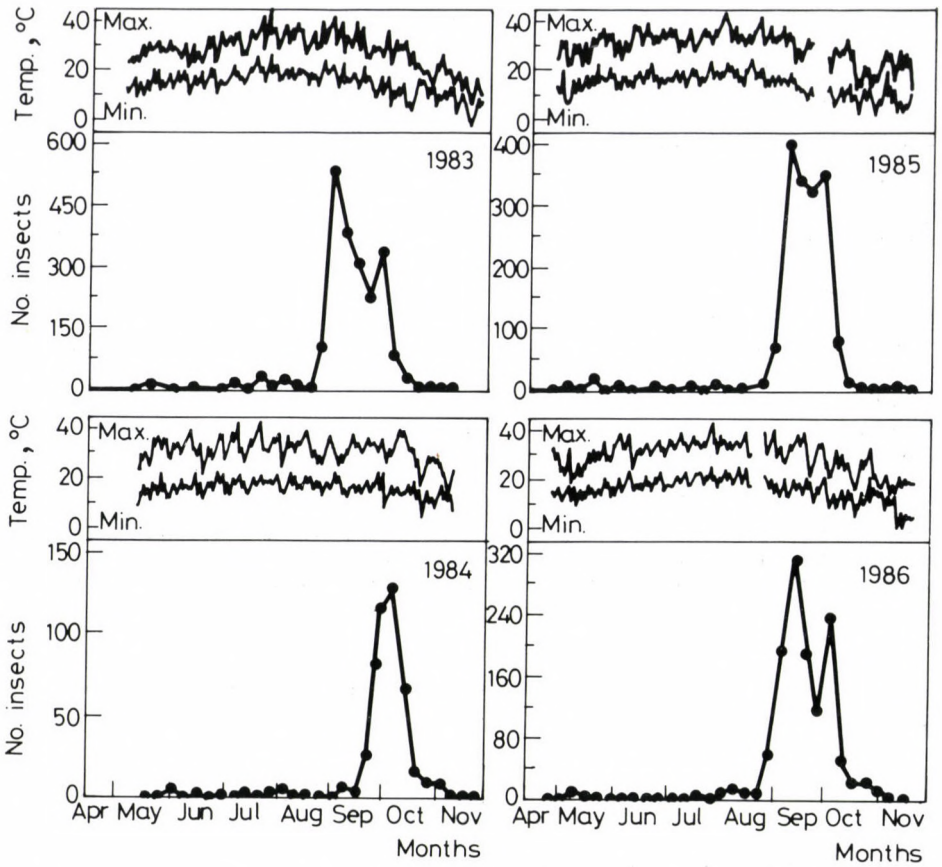

Fig. 1. Seasonal appearance and population fluctuation of *Sesamia nonagroides* adults caught in light traps and daily maximum and minimum temperature values in Kopais, Central Greece, during the years 1983-86

ment followed. One or two egg mass infestation at the first or the last stage of development did not differ among them. The lowest infestation was observed in the control (natural infestation), although there was no statistical difference with the previous category. A similar pattern was observed in the ear infestation with the first and last stages having the lowest infestation and the two intermediate ones the highest. The mean yield was, however, more adversely affected at both the densities of the insect that infested the first two stages of plant development. The lowest yield was recorded in plants infested with egg masses at the milk stage (175.81g). The mean seed yield of infested plants, collected in the corn field, was not different from the infested ones in the two last stages of plant development. This is an indication that the physiology of the plants was more adversely affected by lighter insect infestation brought about early in the plant develop-

Table 1

Effect of artificial infestation of four different stages of development (*) of corn by one ortwo egg masses, 80-100 eggs each, of *Sesamia nonagrioides*, in a completely randomized block design with 21 blocks.

Treatments	Infestation intensity (%)		Mean seed yield per ear (g)
	Stems	Ears	
First stage-one egg mass	16.95cde**	0.11c	209.81bcd
First stage-two egg masses	17.14cde	0.70c	197.55bcd
Second stage-one egg mass	19.19cd	5.98b	189.10cd
Second stage-two egg masses	38.67a	11.71a	175.81d
Third stage-one egg mass	25.86bc	5.95b	231.55ab
Third stage-two egg masses	33.67ab	7.90ab	227.17abc
Fourth stage-one egg mass	12.76de	0.30c	252.25a
Fourth stage-two egg masses	14.29de	0.94c	234.00ab
Natural infestation (control)	7.14e	0.02c	227.88abc
Without any infestation	-	-	230.07ab

(*)First stage: 6-8 leaves, Second stage: Just before flowering, Third stage: seed milk stage, Fourth stage: Leaves below ears dry. Consecutive stages were 21 days apart from each other.
(**)Mean separation by Duncan's multiple range test. Values followed by same letter do not differ significantly ($P < 0.05$).

ment. The heaviest crop loss in dry seed was at least 24%. These data show that late attack of the plants does not affect yield levels.

Acknowledgements

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Investigation of Syrphids in Maize Stands

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The authors studied the occurence and the composition of species of aphidophagous syrphids and their phenology in connection with the population dynamics of aphids in maize stands in 1980-1985, by studying 50 plants in weekly surveys. A close relationship was established between the number of syrphids and aphid densities. *Episyrphus balteatus* Deg. and *Sphaerophoria scripta* L. were found to be the dominant species.

A Malaise trap was also operated in maize stands during the vegetation period in 1983, 1984 and 1985. In the material collected by the trap 26 aphidophagous syrphid species were found among which *Metasyrphus corollae* F., *Sphaerophoria scripta* L. and *Episyrphus balteatus* Deg. were dominant.

Regular surveys in maize ecosystems have been carried out in Hungary since 1976 (Jermy, 1977, 1979). Our studies aimed to discover and describe step by step the faunal elements living in maize stands with different cultivation methods (Mészáros *et al.*, 1984) and second, to establish the role of these elements played in the ecosystem. Within the framework of these investigations, special studies were undertaken to determine the densities and phenology of aphidophagous predators in connection with the population dynamics of aphids and other prey animals. In the present paper we would like to discuss some results and observations gained from the study of syrphids.

Methods

The investigations presented here were made in Agárd (about 70 kilometers southwest of Budapest) in a continuous (more than 10 years) maize monoculture and in the same area in other maize stands, grown in a 3-year rotation system.

From among the 12 different methods used in weekly surveys, individual collections on 50 plants and the rearing of syrphid larvae collected were found the most reliable. In the period of 1983-85 also a Malaise trap was operated in the maize stand during the vegetative season in order to follow the flight of syrphid adults. Important data were collected also with the help of the Szentkirályi-type stem traps applied between 1979 and 1981.

In the research area, besides a soil disinfestation and a pre-emergent weed control, no other chemical treatment was applied.

Results and Discussion

The data gained by the individual collections on 50 plants showed, that syrphids reacted very sensitively to the increases in aphid density (Rácz and Visnyovszky, 1985).

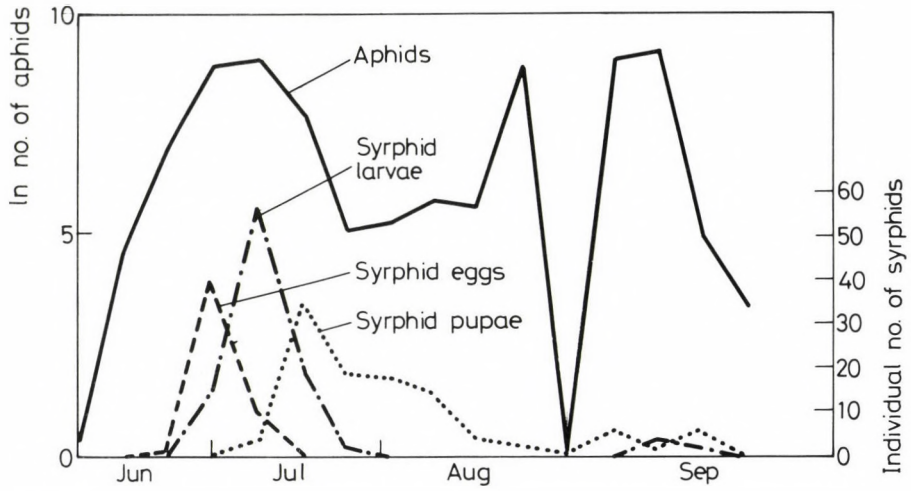

Fig. 1. Individual densities of syrphids (eggs, larvae and pupae) and in maize grown in continuous monoculture in 1985

Soon after their appearance, syrphid flies lay eggs in aphid colonies, followed some days later, by the appearance of larvae, and later still by the characteristic pupae. (Fig. 1., 2.). Their phenologies show considerable coincidence. In years with high aphid densities a

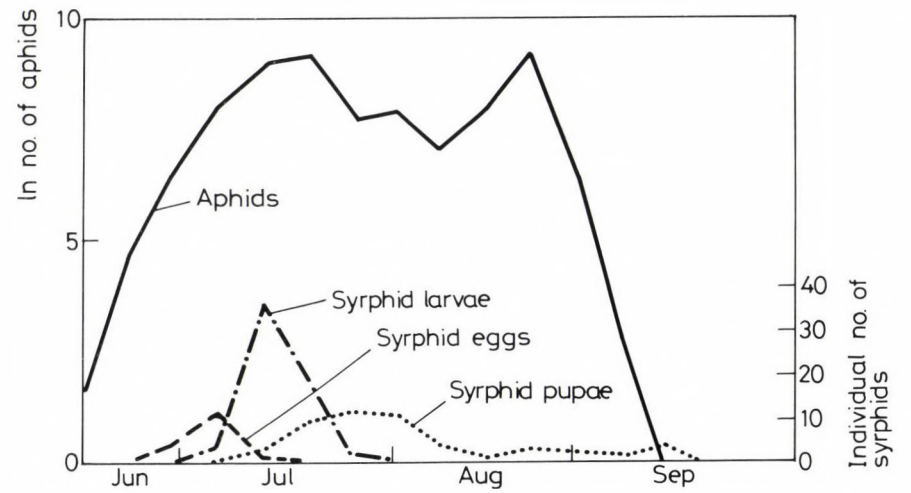

Fig. 2. Individual densities of syrphids (eggs, larvae and pupae) and maize grown in crop sequence in 1985

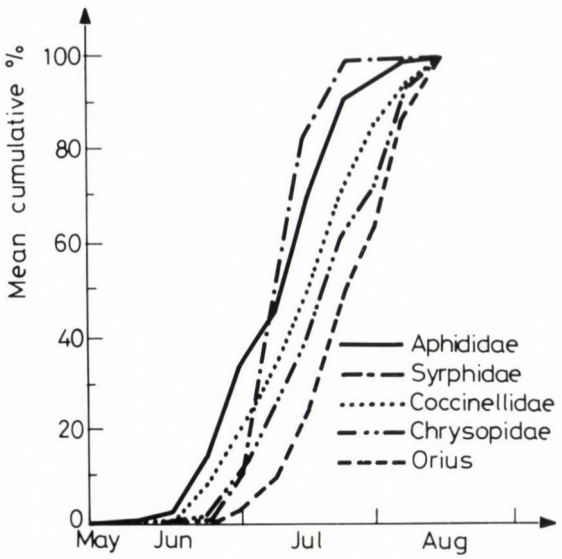

Fig. 3. Seasonal changes in the egg-laying of aphidophages and the number of aphids in maize grown in continuous monoculture (1989-1985)

second generation may develop. These data were well established in 1985 both in the maize monoculture and in the rotation system.

It has to be mentioned that one part of the syrphid pupae found either on the maize plants themselves or reared until pupation were parasitized, especially towards the end of the swarming period. In 1985 parasites belonging to *Ichneumonidae* or *Chalcididae* emerged from 14.95% of the pupae.

If the numerical response of syrphid adults to the changes in aphid densities are considered (Fig. 3., 4.) it is remarkable that, compared to other predators, the egg-laying of syrphids begins slowly and females deposit their eggs at a stage of intensive aphid propagation (Rácz *et al.*, 1986). This means that syrphids may play an important role in the early stages of aphid outbreaks. On the other hand, as mentioned earlier, the syrphids react very sensitively to changes in the prey (i.e. aphid) populations. Their developmental period is the shortest from among the natural enemies of aphids. Although by egg numbers, the syrphids stand quite low on the list of aphidophages, there were, however, no cases of egg parasitization observed. It is also remarkable that syrphid females lay their eggs almost only on plants infested by aphids, the larvae are very voracious, so they may contribute with high efficiency to the decimation of aphid populations.

With collections on individual plants, and by using stem traps, the following aphidophagous syrphids were found on maize (Table 1). The highest dominance values were shown by *Episyrphus balteatus* Degeer, followed by *Sphaerophoria scripta* L. The other species observed were found in much lower individual densities.

The Malaise trap collected 39 syrphid species between 1983 and 1985, 26 of which were aphidophages. These, however, may have flown also from longer distances as shown

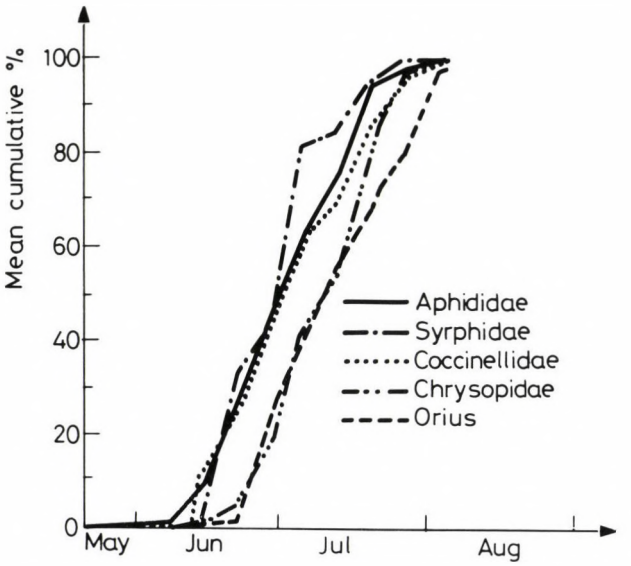

Fig. 4. Seasonal changes in the egg-laying of aphidophages and the number of aphids in maize grown in crop rotation (1980-1985)

on Table 2. The material collected by the Malaise trap, however, emphasizes the importance of the environment of the cultivated plant. The strip of weeds bordering the plant stand, a forest belt or other crop plant may present both food and shelter to benefical insects during periods which are unfavourable for them within the maize stand. This enables them to survive adverse conditions and to re- colonize the maize after the aphid hosts had reappeared.

Table 1

Syrphids collected in maize stands

by plant survey in 1983, 1984, 1985	by stem survey in 1979, 1980, 1981
Episyrphus balteatus Deg.	*Episyrphus balteatus* Deg.
Spaeroropgoria scripta L.	*Metasyrphus corollae* F.
Metasyrphus corollae F.	*Syrphus vitripennis* Mg.
Scaeva pyrastri L.	*Scaeva pyrastri* L.
Syrphus vitripennis Mg.	
Syrphus ribesii L.	

Table 2

Syphids collected in maize stands by Malaise trap in 1983, 1984, 1985

Species	No.
Metasyrphus corollae F.	1832
Episyrphus balteatus Deg.	1007
Melanostoma mellinum L.	323
Melanostoma scalare F.	250
Scaeva pyrastri L.	182

It may be stated as a concluding remark that aphidophagous syrphids play an important role in controlling the numbers of aphids damaging maize. Their aphidophagous activity is especially important in the early period of the first wave of aphid outbreak.

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The Appearance of Different Strains of the European Corn Borer in Germany

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The European corn borer, an important pest of maize in Southern Germany, was known in Northern Germany only feeding on mugwort (*Artemisia vulgaris*). In the northern part of the Ruhr-district, however, it also occurs on maize. In 1983 it was found there for the first time. The appearance in maize seems to be correlated with the degree of infestation in nearby mugwort plants. Most of the larvae occurred in the border rows of the fields.

The corn borers of Northern Germany have adapted to the climate there and have formed a special temperature-race. Compared with the Southern German populations, the emergence of the moths starts earlier, the development of the larvae is faster in the laboratory and they gain a higher weight for hibernation under the same conditions. Therefore, a complete development of the larvae in maize before harvesting is possible in warmer years.

In Southern Germany nearly exclusively moths of the Z- pheromone-strain (97:3 Z:E 11-tda) were trapped, whereas the borers in North Germany belong to the E-strain (3:97 Z:E 11- tda).

The southern corn borer population seems to spread northward along the warm Rhine-valley so that both Z-strain and E- strain may cause damage to maize in Northern Germany in the near future.

Already for decades, the European corn borer (*Ostrinia nubilalis* Hübn., Lep., Pyralidae) has caused damage to maize in great parts of Europe and in North America (McLaine 1922, Flacke 1982). It occurs in different temperature- and pheromone-races and has a wide feeding range. Although maize is the most important host plant, the polyphagous borer feeds on more than 200 other plants (Burgstaller 1974). In warmer regions, mostly the second or third generation, it infests alternative plants: cotton in North Carolina (USA) (Savinelli *et al.* 1986) or pepper in Alabama (USA) (Eden 1956). In Canada, the borer was found on several weeds like *Chenopodium album, Amaranthus retroflexus* and *Echinochloa crus-galli* (Crawford & Spencer 1922) as well as on sweet pepper (McLeod 1981). The larvae can even occur in the fruits and in young shoots of apple trees (Straub *et al.* 1986). In Germany the borer also caused damage to hops (*Humulus lupulus*) and hemp (*Cannabis sativa*) in former times (Schlumberger 1941, Andersen 1943, Lagenbruch *et al.* 1985). Another common host plant seems to be mugwort (*Artemisia vulgaris*).

The traditional maize cultivating areas in the Federal Republic of Germany are in the south, in Baden-Württemberg and South-Hessia. Here the corn borer has been an important pest since the beginning of the century. In the last 20 years, however, the cultivated area has increased threefold with the greatest expansions in Niedersachsen and Nordrhein- Westfalen, North Germany. From this region *O. nubilalis* was only recorded on mugwort (Heddergott 1977), but in 1983 it was found on maize for the first time (Welling & Langenbruch 1984). To estimate the potential danger for the North

German stands of maize, special studies on the spread and bionomics of the corn borer have been carried out since then (Welling 1984, 1986; Hosang 1985, Pomikalko 1986).

Material and Methods

In North Germany, we examined maize fields in the northern Ruhr-district near Recklinghausen and 100 km to the north at Osnabrück. Comparative investigations were conducted in South Germany on fields in the Rhine-Main-area at Darmstadt and Mainz. Growth and weight of the larvae from North and South Germany as well as from mugwort and maize was recorded. In autumn, 200 larvae of the northern and southern populations were put into field-cages in Recklinghausen and Darmstadt in order to record possible differences in the emergence of the moths in the next summer. Both the northern and the southern strain was reared in the laboratory on artificial medium under same conditions (16 h light, 29 °C) to compare the developmental period and larval weight. For tests on feeding preference, young larvae were put into boxes with leaves of maize and mugwort. The evaluation was made by counting the larvae sitting on the different leaves. In addition, the spread of the southern corn borer population was recorded every year.

Results

During the warm summer of 1983 we observed heavy infestations of mugwort by the European corn borer in the northern Ruhr-district. Here infestations were also found in several maize fields near Recklinghausen. The presence in maize was correlated with infested mugwort standing at the field banks or nearby within the range of 50-100 m. Edge rows of the maize stands were preferably attacked, while infestation in the middle of the fields was minimal. Deposition of egg masses on maize leaves revealed that infestation of maize is not only due to migration of larvae from mugwort to maize. Damage to the cobs was low in 1983, but considerable in 1984. In Osnabrück, the corn borer occurred at a low population level on mugwort, but not on maize.

Although in North Germany the climatic conditions are less favourable, compared to the Rhine-Main-area, the moths in Recklinghausen emerge nearly at the same time as in Darmstadt. The results of the field-cage experiments may explain this fact: In the cages in Recklinghausen as well as in Darmstadt, moths derived from North Germany emerged 2 weeks earlier than those of South German origin. Development of larvae was slower in North Germany. In warmer years, such as 1983, most of the larvae can probably reach adequate weight for hibernation in maize, but if there are cold summers, such as in 1984 or 1987, only larvae in non- harvested weeds, such as mugwort, have a chance to survive. Whereas in North Germany no significant difference in the weight of larvae feeding on mugwort or on maize could be confirmed in 1983, the maize feeding larvae were considerably heavier in 1984.

In the laboratory rearing, the North German strain developed faster than samples from South Germany. Developmental time was defined as the number of days from oviposition to pupation. After 29 days, half of the northern larval population (195 larvae examined) had pupated, the corresponding period of the southern population (68 larvae

examined) was 34 days. The weight of the pupae was significantly higher in the northern population.

In the feeding tests the young North German larvae, as well as larvae from South Germany, preferred mugwort.

The maize-feeding *Ostrinia*-population in South Germany is advancing northward from the Rhine-Main-area. Climatic maps disclose the relatively warm Rhinevalley with its 15-16°C isotherm to be the most promising route for northern progression. In fact, we found larvae in maize up to the town Boppard (50°13' latitude) in 1983.

Discussion

Laboratory tests and field investigations have proved the existence of the different temperature-strains of the European corn borer in Germany. The North German borer populations, occurring in the Ruhr-district, in Münster, Osnabrück, and probably further northward, have fairly adapted to the less favourable climate there: in laboratory rearings the moths emerged earlier, and the larvae developed faster and became heavier, than South German borers under the same conditions. These abilities, however, cannot compensate for the climatic conditions. In the field they need longer time to develop and even then they do not gain the same weight for hibernation as compared to larvae of the Rhine-Main-area. The temperature is the limiting factor for the northern populations. Adaptation to the climate and formation of temperature-strains also took place in the USA (Chiang et al., 1968) and in France (Stengel & Schubert 1982).

The North German strain mainly feeds on mugwort. Maize can act as an alternative host plant in areas with a high infestation level on mugwort (Welling 1986). Adaptation on maize may certainly be favoured by several warm years in succession.

Surprisingly, in 1984 we proved that the North Geman borers belong to the E-pheromone-strain (3:97 Z:E 11-tda) (Hosang 1985, Langenbruch et al. 1985). Up to that time, only the Z-strain (97:3 Z:E 11-tda) was known in Germany, as ascertained by pheromone trap catches in different parts of the South (Klun *et al.* 1975, Anglade et al. 1984), The E- strain also occurs in the Netherlands and (sympatric) south of Paris (Klun *et al.* 1975, Anglade 1977). Obviously this strain seems to dominate in the northern part of Central Europe.

Both pheromone-strains also occur in North America. They were introduced from Europe prior to 1920 at 2 or 3 different localities (Carde *et al.* 1975). Whereas the Z-strain occupied extended parts of the maize cultivating region there, the spread of the E-strain was confined to certain areas.

The appearance of the E-strain on mugwort in North Germany supports the hypothesis of Anglade (1977), who assumes the Z-strain to be the best adapted to maize, and the E-strain to be more polyphagous. Descriptions of the life history of the corn borer in the older literature can lead to the conclusion that in Central Europe the distribution of the E- strain was greater in former times than it is today. The increase of maize cultivation favours the Z-strain, so that this strain now is dominant in most of the traditional maize-cultivating areas.

The Ruhr-district is one of the northernmost regions in Europe where the corn borer was observed feeding in maize. On the same latitude, the borer causes damage to maize only in Poland: in the region of Wroclaw and Glogow (50°40') (Kania 1968).

Although the North German stands of maize presently do not suffer from corn borer damage yet, they may be endangered by two possible future events: adaptation of the endemic E- strain (as happened, for example, in Switzerland (Büchi *et al.* 1982) and immigration of the Z-strain from southern Germany. Recent investigations in the Rhine Valley show the northernmost occurence of the maize-feeding borer near Koblenz, but it could also be found on mugwort a bit more northward near Andernach and Sinzig (Lorenz 1987). Here the pheromonestrain is not yet known.

The investigations in North Germany are continuing.

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Morphological Mutants of the Corn Earworm and their Potential Usefulness in Selection and Biological Control Programs

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Morphological mutants of insect pests have potential as tools for research and biological control systems. Our objectives were to describe the inheritance of a rose coloration mutant of corn earworm, *Heliothis zea* (Boddie), and outline procedures for use of mutant forms. The rose mutant was crossed to the wild type. The F_1 was sib-mated to obtain an F_2, and also backcrossed to rose individuals. Chi-square tests indicated 3:1 and 1:1 wild type: rose segregation in the F_2 and backcross, respectively. Rose is, therefore, conditioned by a single recessive gene. Suggested uses of such mutants were: (i) as tools to identify individuals recaptured after release, (ii) to estimate wild population size from frequencies of mutant types in samples of recaptured individuals, and (iii) to estimate efficiency of successive matings by identifying marker types among the progeny. Formulae were developed for estimating population size from frequencies of captured marker types.

Mutant forms of plant pests are of interest because of their potential use in survey and control programs. Morphological variants of corn earworm (CEW), *Heliothis zea* (Boddie), have been used for species identification (Garman, 1920), but some color variation was found to be sex-related (Smith & Allen, 1928). Sell *et al.* (1974) described a four-allele polymorphic esterase system in CEW, suggesting its use for studyiny long-range insect dispersal. These genotypes, however, require chemical analysis, delaying identification of alleles being monitored in populations. Sluss *et al.* (1979) demonstrated that allozymes for several enzymes could be used to differentiate CEW from tobacco budworm, *Heliothis virescens* Fab.

Use of sub-sterilizing doses of radiation to suppress reproduction of CEW was suggested by Carpenter *et al.* (1987). Chromosome aberrations are common when lepidopterous moths are subjected to sub-lethal radiation (North & Snow, 1978). Mutants appear in radiation experiments, such as the yellow pupal body type (Proshold, 1974) in tobacco budworm but also occur naturally: black pupae in tobacco budworm (Whitten, 1973), yelloweye in CEW (Jones *et al.*, 1977), and other eye-color mutants (Hagstrum, 1974).

Our objectives were to report inheritance of the rose mutant in CEW and to suggest uses for mutant forms in population survey and control programs.

Materials and Methods

Moths of CEW with rose coloration were obtained in 1983 from a laboratory colony of normal buff color. Rose x normal crosses resulted in normal progeny that were

Table 1

Chi-square tests of individuals in the first backcross and F_2 (sib-mated F_1) generations segregating for the rose mutant of corn earworm

Cross ♀ x ♂	No. observed		x^2
	Normal	Rose	
Backcross generation tested for 1:1 segregation ratio			
rose (rc/rc) x F_1 #	45	53	0.50 ns
rose (rc/rc) x F_1	49	55	0.24 ns
F_1 x rose (rc/rc)	36	50	1.96 ns
F_1 x rose (rc/rc)	59	46	1.37 ns
Pooled	189	204	0.50 ns
F_2 (sib-mated F_1) generation tested for 3:1 segregation ratio			
F_1 x F_1	286	70	5.13*
F_1 x F_1	238	62	2.78 ns
F_1 x F_1	173	72	2.29 ns
F_1 x F_1	242	83	0.02 ns
F_1 x F_1	101	42	1.23 ns
Pooled	1040	329	0.65 ns

– F_1 progeny (rc/+) resulting from crosses between rose and normal
ns – probability >0.05
* – probability <0.05

sib-mated and backcrossed to rose moths for progeny data. Classified individuals were tested for fit to genetic ratios by chi-square procedures. Data were analyzed separately, then pooled over F_2's and backcrosses.

Procedures are proposed for use of morphological markers as tools in population survey and control programs. Formulae for estimating population size are developed.

Results and Discussion

The rose phenotype appeared as dark rose coloration of body and wings in the CEW adult. Pigmentation was most prominent on the forewings. Rose individuals had low vigor and fecundity with wing and body deformities. Wild types produced few deformed individuals: therefore, non-classifiable deformed individuals were grouped in the rose category. Matings between rose individuals produced only rose progeny. Backcross and F_2 progenies clearly segregated 1:1 and 3:1, respectively, for normal and rose phenotypes, indicating that rose is conditioned by a single recessive gene (Table 1). Progeny from one sib-mated F_1 pair deviated significantly from the expected ratio. Pooled values gave a good fit to expected ratios. Inheritance of this CEW morphology gene, designated rose coloration (*rc*), is the second reported, yelloweye (*y*) (Jones *et al.*, 1977) being the first.

Uses of morphological mutants include tracing of released individuals, estimation of population size from numbers released and captured, and estimation of mating efficiency in polyandrous species like CEW by using males with different markers. We emphasized the first two uses, but additional uses are easily imagined.

Few assumptions are needed about vigor, viability, or competitiveness of mutant types when used to identify recaptured individuals. Assumptions become critical, however, when capture results are used to determine frequencies of individuals or genes in the natural population. When the frequency of a recessive marker allele in the wild population is not zero, mating of released marker individuals with the wild population is an effective way of estimating the frequency of the allele.

Estimation of population size requires assumptions. The following seem most important: (i) equal viability and fecundity of marker and wild population individuals, (ii) equal vigor for mating, flight, and avoidance of predation in the marker and wild population, (iii) uniform dispersion of released individuals, (iv) frequency of the mutant allele in the wild population is known. These assumptions are seldom completely satisfied.

When N_r insects of mutant phenotype are released into a test area, the number (N_w) in the wild population can be estimated from frequencies of mutant and wild types in a captured representative sample from the test area. If f_r and f_w are the frequencies of released and wild individuals in the population, then:

$$\frac{f_r}{f_w} = \frac{N_r}{N_w} \quad \text{and} \quad \hat{N}_w = \frac{\hat{f}_w \, N_r}{\hat{f}_r} \tag{1}$$

where $\hat{N}_w$ estimates wild population size, and $\hat{f}_r$ and $\hat{f}_w$ are estimates from the captured sample. The release must be homozygous recessive or a homozygous or heterozygous dominant. The allele for the released mutant is usually rare in the wild population so that the frequency of mutant phenotypes (f_m) in the wild population is negligible; otherwise a correction is needed when calculating $\hat{N}_w$. Mutant individuals in the captured sample from the wild population will bias f_r upward and f_w downward, giving an estimate of N_w that is biased downward. When $\hat{f}_m$ is known, it is subtracted from $\hat{f}_r$ and added to $\hat{f}_w$ in equation (1) giving an unbiased $\hat{N}_w$. The frequency $\hat{f}_m$ will be assumed negligible in the discussion of procedures for various release situations.

Let f_{r1} and f_{w1} be the frequency of mutant and wild phenotypes, respectively, in the progeny generation (F_1). These frequencies may be written in terms of f_r and f_w (previously defined) for each release situation. The estimates $\hat{f}_{r1}$ and $\hat{f}_{w1}$, obtained from a sample captured from the F_1, are used to obtain $\hat{f}_r$ and $\hat{f}_w$, providing the estimate N_w in (1), since N_r is always known.

When the release is heterozygous recessive

Matings of frequency f_r^2 between released individuals yield progeny in the F_1, 1/4 of which will be the mutant phenotype.

$$\therefore \hat{f}_{r1} = \tfrac{1}{4} f_r^2, \quad 4 \hat{f}_{r1} = \hat{f}_r^2, \quad \text{and} \quad \hat{f}_r = \sqrt{4\hat{f}_{r1}} = 2\sqrt{\hat{f}_{r1}}$$

Since $f_r = 1 - f_w$, $\hat{f}_w = 1 - \hat{f}_r$, and $\hat{N}_w$ is calculated using equation (1)

When the release is homozygous recessive

Matings between released individuals will yield all mutant progeny in the F_1.

$\therefore f_{r1} = f_r^2$, $\hat{f}_r = \sqrt{\hat{f}_{r1}}$ $\hat{f}_w = 1 - \hat{f}_r$, and $\hat{N}_w$ is obtained using (1).

When the release is homozygous dominant

Matings involving at least one released individual will yield all mutant progeny in the F_1 and matings between wild population individuals will yield only non-mutant progeny. The frequency of mating between wild population individuals is f_w^2

$$\therefore f_{w1} = f_w^2 \ , \quad \hat{f}_w = \sqrt{\hat{f}_{w1}} \ , \quad f_r = 1 - \hat{f}_w \text{ and } \hat{N}_w \text{ is obtained using (1).}$$

When the release is heterozygous dominant

Matings of frequency f_r^2 between released individuals will yield 3/4 mutant and 1/4 non-mutant progeny in the F_1. Matings between released and wild population individuals will yield 1/2 mutant and 1/2 non-mutant progeny, where the mating frequency is $2f_rf_w$. Matings of wild population individuals, of course, will yield all non-mutant progeny.

$$\therefore f_{r1} = \tfrac{3}{4} f_r^2 + f_r f_w = f_r (\tfrac{3}{4} f_r + f_w) \qquad (2)$$

$$\text{and, } f_{w1} = \tfrac{1}{4} f_r^2 + f_r f_w + f_{w_2} = (\tfrac{1}{2} f_r + f_w)^2,$$

$$\text{also, } \sqrt{f_{w1}} = \tfrac{1}{2} f_2 + f_w \quad \text{or} \quad f_w = \sqrt{f_{w1}} - \tfrac{1}{2} f_r$$

$$\text{substituting in (2): } f_{r1} = f_r (\tfrac{3}{4} f_r + \sqrt{f_{w1}} - \tfrac{1}{2} f_r) = f_r (\tfrac{1}{4} f_r + \sqrt{f_{w1}})$$

solving: $\tfrac{1}{4} f_r^2 + \sqrt{f_{w1}}\, f_r - f_{r1} = 0$, where f_{r1} and f_{w1} are estimated from the F_1.

This solution reveals the familiar quadratic form, $ax^2 + bx + c = 0$ in which $a = 1/4$, $b = \sqrt{\hat{f}_{w1}}$, $c = -\hat{f}_{r1}$, and $\hat{x} = f_r$.

$$\text{solving: } \hat{f}_r = \frac{-\sqrt{\hat{f}_{w1}} \pm \sqrt{\hat{f}_{w1} - (4)(\tfrac{1}{2})(-\hat{f}_{r1})}}{-2(\tfrac{1}{2})} = -\frac{\sqrt{\hat{f}_{w1}} \pm f_{w1} + f_{r1}}{\tfrac{1}{2}} \text{ which reduces}$$

$$\text{to } \hat{f}_r = -\sqrt{\hat{f}_{w1}} \pm \sqrt{1} = -2\sqrt{\hat{f}_{w1}} \pm 2$$

for which the positive root is f_r, and $0 < \hat{f}_r < 1$.

Since $\hat{f}_w = 1 - \hat{f}_r$, $\hat{N}_w$ is easily found using (1).

The uses described for morphological mutants have potential as tools for research and biological control for CEW and other insects. Dominant markers have the greatest potential for the purposes described.

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Sex Pheromones of two Species of Corn Borers Living in China

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The corn borer which is spreading widely in China is a serious pest of corn, sorghum and other crops. From external morphology, this species is almost indistinguishable from the European corn borer (*Ostrinia nubilalis* Hubner). Therefore, some authors (Cai, 1973) described the major species of Chinese corn borer as the European corn borer. But Mutuura *et al.* (1970) identified it as Asian corn borer (*Ostrinia furnacalis* Guenee). It has been known for several years that the European corn borer uses Z- and E-11-tetradecenyl acetates as its sex pheromone (Klun, 1977). However, the sex pheromone of *O. furnacalis* was not identified until 1978.

From August 1974 to 1975, we carried out field screening tests with synthetic unsaturated and saturated straight chain acetates in corn fields in southern China. Neither pure Z- and E-11-tetradecenyl acetate nor their mixtures in different ratios attracted male borer moths in the experiment. Xi, 1978 pointed out the difference in the retention time between 11-tetradecenyl acetates and pheromonal compounds of corn borer present in the suburb of Tianjin, northern China. These results implied that the major species of Chinese corn borer may not be identical with the European corn borer.

In 1977, we identified the sex pheromone of the major species of Chinese corn borer with tetradecenyl acetates by means of microchemical reactions and GC-MS measurements. In 1978, we finally detected and determined the chemical structure of three long-chain acetates from the pheromonally active fractions. These were tetradecyl- and 12-tetradecenyl acetates. The bioassays on the synthetic samples of these compounds were carried out in 1979, and its attractive activity was quite satisfactory. In 1980, a co-operation group working on corn borer began to use the synthetic sex pheromone of Asian and European corn borers to attract male borer moths in more than ten provinces and municipalities in China in order to identify the major species of the Chinese corn borer. From three years of field tests, we concluded that the major species of Chinese corn borer is a species of Asian corn borer. This conclusion is just the same as that drawn from morphotaxonomy and reproductive isolation.

Meanwhile, we found out that the sex pheromone of the Asian corn borer did not attract male borers in some regions in Xingjiang, Inner Mongolia and Gansu provinces, North-western China, while the sex pheromone of the European corn borer did attract male borers in the same region. In order to make further research on the Chinese corn borer, we did some work on the sex pheromonal compounds in the extract from the corn borer in Yili region, Xingjing. The experiment showed that the sex pheromone of the corn borer in this region were Z- and E-11-tetradecenyl acetates. The isomer ratio of Z to E is 97 to 3, which was identical that of the sex pheromone of the European corn borer in Iowa state, USA.

Material and Methods

Collection and Extraction

All corn borers used in the study were collected in corn fields and reared at room conditions. The pupae were sexed and the females were allowed to emerge under room conditions. The moths were anaesthetized with diethyl ether vapor at 3-5 AM and the abdominal tips were excised into methylene chloride. After filtration, the crude extracts were obtained.

Thin Layer and Column Chromatography

Thin-layer chromatography was performed on Silica gel G using petroleum ether and diethyl ether (95:5, v/v) as a solvent system.

A glass column (1 cm ID) packed with 30 g of 100-140 mesh silica gel was used to purify the crude extracts utilizing the same solution system as above. Extracts from 2000 females were loaded onto a column each time. Fractions were collected every 6 ml. The active fraction was monitored by field trapping tests or by comparing with the authentic samples.

Microchemical Reactions

The oxidation reaction of crude or purified extract was carried out at room temperature with $KMnO_4$ in acetone, acidified by H_2SO_4. Addition reaction of pheromone-active fractions eluted from a silica gel column was performed with bromine solution in CCl_4. Elimination of bromic from brominated product was accomplished by grinding the dibromides with zinc powder in ethanol.

Saponification of pheromone-active fractions was carried out in a 4 % KOH-ethanol solution under reflux for an hour. Reacethylation was performed by addition of acetyl chloride to the alcoholic constituents, which were obtained by extraction of the saponified mixture with petroleum ether or diethyl ether.

Gas Chromatography

All chromatographic data in the present work were obtained on a Varian Aerograph 2740 GC-MAT311A MS system using the single ion detection (SID) technique at 70 eV. The advantages of this technique over the conventional total ion current monitoring method are its good selectivity and high sensitivity.

It is most important to choose the proper ions for the SID technique. The ion at m/z 61 $(CH_3COOH_2)^+$ is suitable for the detection of straight-chain acetates. Its intensity is always high, and in most cases the interference from impurities is negligible. On the other hand, in some cases the intensity of the molecular ion M is too weak to be detected, but fortunately the intense ion $(M- CH_3COOH)^+$ may be used to calculate the molecular weight of the original compound.

Field Trapping Tests

All steps in purification and identification were monitored for pheromonal activity by field trapping tests. The synthetic pheromone components were also tested by the same technique. A simple water trap was used for this purpose. This was a 30 cm-diameter vessel filled with water, with detergent added to reduce the surface tension of water. A paper roll impregnated with pheromonal solution and supported 1-1.5 cm above the water surface was used as bait. The lures in the traps were changed every night.

Results and Discussion

Identification of Pheromone Components

Ostrinia furnacalis Guenee: The pheromone-active components from female corn borers in Southern China were completly destroyed by bromination or oxidation by $KMnO_4$ and restored in the first case by debromination. The activity was also destroyed by saponification and could be restored by reacetylation. The results suggested that the pheromone of *O. furnacalis* consists of acetates of unsaturated alcohols. The eluted volume of the active fraction was coincident with that of synthetic long straight-chain acetates. When the ion at m/z 61 was monitored by SID technique, three well-separated GC peaks were found in the pheromone-active fraction. The retention time of these peaks were 20.43, 26.70 and 28.85 minutes.

According to the retention time and mass fragmentation patterns, the compounds represented by these peaks were identified as tetradecyl acetate (14:AC), E-12-tetradecenyl acetate (E-12-14:AC) and Z-12-tetradecenyl acetate (Z-12- 14:AC). The E to Z ratio for 12-tetradecenyl acetates determined by GC peak areas was 53:47. At the same time, the quantity of 14:AC was about 1:8 times the sum of the other compounds.

Ostrinia nubilalis Hubner: Using the m/z 61 and 194 as a characteristic ions, the active fractions from the corn borer in Yili rgion, Xingjiang, were detected and the retention time of these was compared with that of the authentic compounds. Two components had the same characteristic ions and retention times as Z- and E-11- tetradecnyl acetate did. It shows that the Z- and E-11-14:AC are really present in this pheromone system. According to the calculation of peak areas, the Z to E ratio is 97 to 3, which is identical with that of the sex pheromone of the European corn borer in Iowa state, USA (Klun, 1979).

Field Trapping Tests

Mixtures of E-12-14:AC and Z-12-14:AC in nearly all possible ratios were tested, and the mean catches reached their maximum at the natural E/Z ratio. The preparation in this ratio was used to study the relationship between doses used and mean catches. Within a quite broad range ($5x10^{-9}$- $2x10^{-5}$ g/trap) the traps were attractive to the males in the field. The maximal mean catches were found in a dosage range from $1x10^{-7}$ to $1x10^{-5}$ g/trap, within which no dependence of mean catches on doses used could be found.

The mean catches, however, diminished rapidly, when doses used deviated from this range.

The addition of 14:AC to mixtures of E-12-14:AC and Z-12- 14:AC in natural ratio resulted in a decrease of trap catches. Even a 5 % of 14:AC added caused significant decrease in mean catches. So, the 14:AC plays a role of inhibitor in the sex pheromone communication system regardless of its presence in the extract from the female corn borer.

The mean trap catches of the synthetic mixture of Z-11-14:AC and E-11-14:AC, the isomer ratio of which is 97 to 3, were more than that of the virgin females which were held 48 hours after emergence and that of the crude extracts of these females. Such a phenomenon may be caused by the existence of an inhibitor in the sex pheromone system of the corn borer in Yili region. However, whether there is a possibility of the existence of inhibitor in the European corn borer is still an open question.

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Closing Remarks: Faunistic Studies on Maize Arthropods

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Faunistic studies can either be concerned with the whole fauna of a maize growing area, the fauna of maize fields in general, or the fauna of a single maize plot at a particular time. Since maize monocultures are the epitome the "cultural steppe", information on faunal richness - or depletion - can be used as some kind of a bio-indicator, a measure how far a crop field is from what we consider a "natural biotope". Howewver, most work on the fauna of maize is focused on a specific group of arthropods, such as a pest complex, the Aphidophaga, carabids or spiders. There usually is a clear goal to describe those arthropods of agricultural importance.

In lectures and posters of various contributors at the ISMA, ther was an outspoken or underlying aim to quantfy or qualify information on the fauna of maize fields. As soon as we try to use abundance, species composition or indices of deiversity in order to produce comparable results, we run into the problem of standardization. If we want to evaluate a biotope, or a specific treatment, a cultivation technique, or the impact of enviromental distruption on the fauna, we have to be able to compare data sets.

For any attempt at comparing faunistic information, standardization is a must at different stages of an investigation: collecting methods, effort, level of taxonomis identification. Raw data sets are produced, which can be evaluated and interpreted at different and individual levels of sophistication.

On the first day which focussed on faunistics, we heard contributions on the influence of large scale population movements,and on the arthropod fauna of maize fields in Hungary, Poland, Czechoslovakia and Yugoslavia.

From other countries, Like Bulgaria, Spain and Egypt, we were provided with information mainly on the pest fauna in maize. In several posters, the carabid and spider faunas of Hungary and Belgium were treated.

This was the first time that people from so many different maize growing areas exchanged their information on the arthropod fauna in maize, and it became clear that many more should join this forum to overcome the first stage of scattered and preliminary information. To me, the faunistic contents of all these contributions appear like pieces of rock, which somehow have to be trimmes into square bicks before they can be fitted together and become a support for each other our "castle of knowledge".

The problem with raw data in faunistic studies, in general, is that they are usually not published in full in international journals. Often they shortened and interpreted in a way that makes it impossible to assess hoiw they were originally gathered.

As a recommendation for the near future, e.g. the next ISMA, I propose that the specialists of important groups of arthropods in maize, such as the aphids, Aphidophaga, Lepidoptera, carabids, staphylinids, spiders and Heteroptera, collect the presently available information on "their" group, based on published records and recent contacts among

colleagues at the ISMA. They should develop recommendations for faunistic investigations on "their" group, which will hopefully provide a commonly accepted standard for collecting methods, storage of material and information, and to a certain extent even for the evaluation of data. In this way, we may reach a "common language of faunistics", which, in spite of a wealth of dialects, will allow for comparative interpretations.

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László Beczner
1938—1988

László Beczner (Fig. 1), Head of the Virology Group, at the Plant Protection Institute, Budapest passed away of cancer on 10th of November 1988. He was born in 14 August, 1938 in Debrecen, Hungary. After finishing the Agricultural University at Gödöllő he served for one year in the agricultural practice in a plant protection laboratory. He joined to the Plant Protection Institute in 1963 as research assistant and earned the University doctoral degree at the Agricultural University, Gödöllő in 1966.

During his Ph. D. thesis research on virus diseases of lucerne with Dr János Szirmai, Dr Beczner obtained experimental data on virus disease of lucerne with special respect to the alfalfa mosaic virus. The several papers resulting from his doctoral thesis were among the first detailed study of alfalfa mosaic virus in Hungary. After obtaining the candidate degree (equivalent to Ph. D.) he started to work on virus identification and virus serology. In 1971 he spent six months at the Institute for Plant Pathology in Aschersleben, GDR, and later four months in Wageningen in the Institute for Phytopathological Research. In 1974 he was promoted as a principal plant virologist and became the head of the Virology Group. He visited the Genetic Institute of the Polish Academy of Sciences at Poznań and organized the research work there on the serology of bean yellow mosaic virus group. He was a member of the Hungarian Society of Agricultural Sciences, and the International Working Group on Legume Viruses. For a period

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he was elected as a member of the steering committee of the IWGLV and took over the task of the executive secretary. He was the driving force on the coordination of the Hungarian plant virus research behind the "virus-free propagation material program". He did excellent work on the introduction of modern technology in the plant virus identification. In 1985 he spent ten months at the Agricultural Research Station Canada, Vancouver working on serology of plant viruses using monoclonal antibodies. One of his last effort was to set up a monoclonal laboratory in our Institute. His results has been published in more than 100 papers mostly written in English or German. His untimely death is an immesurable loss for the future development of our plant virology group.

His memory will remain in all of us as an enthusiastic scientist, an experienced plant virologist and a distinguished colleague.

Ervin Balázs

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Effect of Rust Infection on Levels of Superoxide Dismutases, Catalase and Peroxidase Activities in Different Fractions of Bean Leaf Homogenate

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Three crude fractions were obtained by differential centrifugation of healthy and rust-infected bean leaf homogenate from susceptible and hypersensitive plants. The three fractions corresponded to the 2,500 g and 15,000 g sediments and the 15,000 g supernatant. After treatment with 0.1% Triton X-100 and precipitation with acetone or ammonium sulfate, the solubilized protein solutions were assayed for superoxide dismutases (SODs), catalase and peroxidase activities.

As a consequence of the infection, there was an increase in cyanide-insensitive SOD (Mn-SOD) in susceptible cultivars (Pinto 650 and Golden Gate Wax) only, starting early after infection (2 days after inoculation) and this was mainly localized in the subcellular fraction sedimented at 15,000 g, thereby indicating that particle-associated or membrane-bound cyanide-insensitive SOD activity was stimulated during the early stage of the infection. An increase in the same enzymic activity was also detected in the 15,000 g supernatant of the cultivar Golden Gate Wax from 6 days after inoculation (beginning mycelial stroma differentiation in the mesophyll).

Catalase activity was raised in the 15,000 g sediment, and sometimes also in the 2,500 g sediment, both in susceptible and hypersensitive cultivars, from 2–3 days after inoculation, whereas it was decreased in the 15,000 g supernatant fraction 3–4 days after inoculation (necrotic lesion differentiation) in the hypersensitive cultivar and 9 days after inoculation (uredospores differentiation) in the susceptible cultivar.

A general increase in peroxidase activity in all fractions of rusted bean leaf homogenate was observed in the hypersensitive cultivar 2 days after inoculation, but the highest rises were detected 3–5 days after inoculation (necrotic lesion differentiation). There was also a general increase in peroxidase activity in the susceptible cultivars starting 3–4 days after inoculation but the percentage increase were always lower than those recorded in the hypersensitive rust-infected cultivar.

It is suggested that changes in the activity of these enzymes may be involved in determining the compatibility or the incompatibility between *Phaseolus vulgaris* and *Uromyces phaseoli*.

The occurrence of superoxide (O_2^-) generation, mediated by an unknown membrane bound oxidase, is associated with hypersensitive cell death in potato-*Phytophthora infestans* and tobacco-TMV incompatible host-pathogen interactions (Doke, 1983, 1985; Doke and Ohashi, 1988; Chai and Doke, 1987). The compatible interactions of the same host-pathogen combinations are also characterized by O_2^- generation, but to a lesser extent. Experiments carried out on potato tuber protoplast (Doke, 1983b) demonstrated that O_2^- production

induced by incompatible races of *P. infestans* was inhibited by a mycelium extract from compatible races of the same fungus, thereby indicating that a hypersensitivity-inhibiting factor or suppressor (warer-soluble β-glucan) minght effectively inhibit O_2^- generation. However, there is at present, no explanation for the low O_2^- generation observed in the compatible host-pathogen interaction.

Since no parallel determinations of superoxide dismutases (SODs) activity levels were done in these experiments, we do not know whether the large amount of superoxide generated during hypersensitivity was related to a lack of activation or to an inactivation of these enzymes, which are scavengers of the superoxide anion.

A relationship between activation of the cyanide-insensitive SOD enzyme and biotrophic growth of the rust fungus was clearly established in previous investigations on rust-infected bean leaves (Buonaurio *et al.*, 1987; Montalbini, 1987). Cyanide-insensitive SOD started to increase about three days after inoculation in compatible combination but not in the incompatible one, suggesting that, in rust-infected bean leaves, O_2^- generation during the hypersensitive response is related to lack of SOD stimulation.

The present study re-examined the SOD increase in rusted bean leaves by investigating the enzyme activity in different fractions of leaf homogenate and simultaneously determining peroxidase and catalase activity. The three enzymes studied are synergistic in several way. Together they protect cell structure from oxygen toxicity and each other from inactivation by O_2^- and H_2O_2 (for pertinent literature see: Rabinowitch and Fridovich, 1983). The elimination of O^2 and H_2O_2 by these enzymes prevents the production of the hydroxyl radical ($OH\cdot$) that may be formed in the Haber–Weiss reaction (Haber and Weiss, 1934). $OH\cdot$, the most potent oxidant known to chemistry, is involved in the abiotic elicitation of phytoalexins (Epperlein *et al.*, 1986). Furthermore, certain reactions that involve peroxidase activity also generate O_2^- (Halliwell, 1977, 1978).

Materials and Methods

Biological material, inoculation and samplings

Plants of *Phaseolus vulgaris* L. cvs. Golden Gate Wax and Pinto 650 (susceptible) and K. W. 765 (hypersensitive), grown in soil in a greenhouse at 18–22 °C under natural conditions, supplemented during the winter with artificial illumination for 12 h photoperiod, were inoculated on the primary leaves by spraying with a dense suspension of uredospores of *Uromyces phaseoli* (Pers.) Wint. Samples of uninoculated (control) and inoculated leaves were collected as shown in Tables 2–4. After infection, the bean cultivars used displayed characteristics similar to those reported for susceptible cultivar Pinto 111 and the hypersensitive K. W. 765 (Marte and Montalbini, 1972; Buonaurio *et al.*, 1987).

Preparation and fractionation of crude leaf homogenate

Crude homogenates were prepared from fully expanded healthy and infected bean leaves using a grinding medium similar to that used for isolating chloroplasts from *Vicia faba* leaves (Montalbini and Buchanan, 1974). Twenty g of leaf tissue were homogenized for 5 s with a Waring blender in 150 ml of homogenizing medium containing 0.35 M sucrose, 50 mM Tricine buffer, pH 7.5, 5 mM NaCl, 5 mM $MgCl_2$, 2 mM Na-isoascorbate and 0.1% bovine serum albumine. The homogenate was filtered through nylon cloth and the filtrate fractionated by differential centrifugation. It was first centrifuged for 2 min at 2,500 g and then the resulting supernatant was further centrifuged for 20 min at 15,000 g. The 2,500 g and 15,000 g sediments were resuspended in 10 ml of a solution containing 50 mM Tris-HCl buffer, pH 7.5, 0.1% Triton X-100 and shaken for 30 min. After centrifugation at 20,000 g for 30 min, the supernatant was precipitated by 80% acetone (−20 °C) and dialyzed against 5 mM phosphate buffer, pH 7.8, containing 0.1 mM EDTA. The 15,000 g supernatant obtained by differential centrifugation of the crude leaf homogenate was dialyzed at 5 °C against two changes of 5 mM phosphate buffer, pH 7.8, containing 2 mM Na-isoascorbate and 0.1 mM EDTA and further fractionated by ammonium sulfate (35–90% saturation) and again dialyzed against 5 mM phosphate buffer, pH 7.8, containing 0.1 mM EDTA. All steps, except for acetone precipitation, were performed at 5 °C. The dialyzed solutions after acetone precipitation that corresponded to 2,500 g and 15,000 g sediments, and the dialyzed solution after ammonium sulfate fractionation that corresponded to the 15,000 g supernatant, were clarified by centrifugation and assayed for peroxidase and catalase activities. Aliquots of clarified solutions were precipitated by protamine sulfate as previously described (Montalbini and Buonaurio, 1976; Buonaurio *et al.*, 1977), and assayed for SODs activities.

Analytical methods

Superoxide dismutases activities were determined spectrophotometrically as inhibition of xanthine-xanthine oxidase mediated reduction of cytochrome *c* (McCord and Fridovich, 1969) as previously described (Montalbini and Buonaurio, 1986). Peroxidase activity was determined using o-dianisidine as the electron donor according to the method described in the Worthington manual (1963), as previously described (Montalbini and Marte, 1972). Catalase was determined by the decrease in A at 240 nm (Aebi, 1971) due to the disappearance of H_2O_2. Malate and succinate oxidation were assayed polarographically (Bonner, 1967). Chlorophyll was estimated by the method of Arnon (1949). Protein measured with Folin-phenol reagent (Sutherland *et al.*, 1949) using bovine serum albumine as standard.

Results

Relative concentration of SODs, catalase and peroxidase in different fractions isolated after differential centrifugation

The average specific activities of the enzymes in each fraction of healthy leaf homogenate are reported in Table 1. There was little difference in the specific Cu, Zn-SOD activity in the three fractions obtained by differential centrifugation.

Table 1

Average of specific activities of Cu, Zn-SOD, Mn-SOD, catalase and peroxidase in different fractions of healthy bean leaf homogenate, during the period of the experiments

Bean cultivar	Homogenate fraction	Cu, Zn-SOD	Mn-SOD	Catalase	Peroxidase
Golden Gate Wax	2,500 g sediment	1.59 AB	0.59 BC	0.030 AB	0.093 A
	15,500 g sediment	1.85 BD	1.86 E	0.064 C	0.447 C
	15,000 g supernatant	1.62 AC	0.17 A	0.017 A	0.202 A
Pinto 650	2,500 g sediment	1.57 AB	1.09 D	0.054 BC	0.067 A
	15,500 g sediment	1.05 A	0.57 BC	0.034 AB	0.432 BC
	15,000 g supernatant	1.86 BD	0.35 AC	0.023 A	0.180 A
K. W. 765	2,500 g sediment	2.53 D	0.67 C	0.054 BC	0.078 A
	15,500 sediment	2.25 BD	0.67 C	0.060 C	0.514 C
	15,000 g supernatant	2.45 CD	0.33 AB	0.048 BC	0.237 AB

Data are the means of eight replications. Numbers in a column followed by the same letter/s indicate that there is no significant difference at $P = 0.01$ (Duncan's test).

However, it was higher in the cv. K. W. 765. Mn-SOD was more concentrated in the two sediments than in the supernatant, but there were variations among the cultivars. Catalase activity followed a similar trend. Peroxidase activity was very low in 2,500 g sediment and particularly high in the 15,000 g sediment. The value for the 15,000 g supernatant was almost half that recorded in 15,000 g sediment.

Approximate characterization of the fractions

The three fractions of the leaf homogenate obtained after differential centrifugation can be characterized to a certain degree by considering chlorophyll distribution, catalase distribution and the relative concentration of the enzymes tested.

The chlorophyll content (data not shown) was almost completely concentrated in the 2,500 g sediment that contained and was mainly composed of

the bulk of chloroplasts. The 15,000 g sediment contained chloroplast fragments (for about 10% of total chlorophyll extracted) and mitochondria, as demonstrated by the malate and succinate oxidation activity. Since fractionation of leaf subcellular particles by differential centrifugation neither successfully isolates peroxisome nor removes contaminating peroxisomes from other particles such as chloroplasts and mitochondria, catalase may be considered an enzyme marker for peroxisomes (Tolbert, 1971) and used as a measure of the degree of contamination in chloroplasts and mitochondria. Therefore, the data of Tables 1 and 3 may indicate that both 2,500 g and 15,000 g sediments contained peroxisomes. It is more difficult to establish the intercellular source of peroxidase activity in the two sediments and the supernatant of fractionated bean leaf homogenate. Peroxidases occur in the intercellular spaces of the cell wall (Hepler *et al.*, 1972; Mäder *et al.*, 1975), where they participate in lignin synthesis. Electron microscopy studies and differential centrifugation of cell-free extracts reveal an association between peroxidase and complexes or membranes of different cytoplasmic origin (for pertinent literature see: Stafford, 1974). The activity in particulate fractions has been reported to be associated with mitochondria and ribosomes (Penon *et al.*, 1970; Plesnicar *et al.*, 1967). Furthermore, after purification of nuclei,

Table 2

Superoxide dismutase activities in three fraction of healthy and rust-infected bean leaf homogenate

Cultivar	Days after inoculations	Crude 2500 g sediment			
		Cu, Zn-SOD		Mn-SOD	
		H	I	H	I
Golden Gate Wax: (susceptible)	2	1.53±0.12	1.59±0.12	0.38±0.00	0.38±0.02
	3	1.79±0.11	1.91±0.22	0.58±0.04	0.73±0.12
	4	1.62±0.10	2.04±0.41	0.53±0.02	0.68±0.09
	6	1.33±0.12	1.01±0.05	0.57±0.05	1.17±0.05** 205%
	9	1.11±0.38	0.79±0.14	0.77±0.00	1.72±0.20** 223%
Pinto 650: (susceptible)	2	0.80±0.18	0.75±0.09	0.60±0.07	0.34±0.12
	3	0.80±0.18	0.97±0.14	0.60±0.06	0.30±0.07* 50%
	7	2.34±0.46	1.19±0.02	1.59±0.39	1.47±0.07
K. W. 765: (hypersensitive)	2	1.37±0.03	2.09±0.09** 152%	0.63±0.03	0.48±0.00** 76%
	3	1.40±0.04	1.60±0.12	0.58±0.05	0.48±0.02
	4	3.67±1.01	3.03±0.59	0.75±0.12	0.75±0.06
	5	3.70±1.00	4.00±0.72	0.72±0.12	0.62±0.02

(Table 2 continued)

Cultivar	Days after inoculations	Crude 15000 g sediment			
		Cu, Zn-SOD		Mn-SOD	
		H	I	H	I
Golden Gate Wax: (susceptible)	2	1.97±0.20	1.76±0.12	1.26±0.13	1.18±0.13
	3	1.89±0.21	2.08±0.44	1.51±0.08	2.33±0.22* 154%
	4	1.74±0.37	1.33±0.32	1.48±0.07	2.21±0.03*** 149%
	6	1.17±0.14	0.74±0.00* 63%	0.94±0.08	1.46±0.01** 155%
	9	0.67±0.47	0.91±0.20	2.24±0.09	3.63±0.17** 162%
Pinto 650: (susceptible)	2	0.52±0.00	0.70±0.22	0.48±0.06	1.02±0.14* 212%
	3	0.52±0.00	0.92±0.01*** 177%	0.48±0.06	1.52±0.23* 317%
	7	1.58±0.10	1.52±0.02	0.66±0.01	1.81±0.18** 274%
K. W. 765: (hypersensitive)	2	1.87±0.59	1.59±0.27	0.76±0.01	0.65±0.05
	3	1.71±0.25	1.77±0.18	0.58±0.11	1.08±0.25
	4	2.95±0.33	2.61±0.21	0.67±0.12	0.78±0.05
	5	2.85±0.33	2.67±0.29	0.67±0.12	1.38±0.00** 206%

amyloplasts, mitochondria and microsomes from cultured peanut cells most peroxidases were found to be present in the microsomal pellet, probably in association with the Golgi apparatus (Chibbar and Huystee, 1986).

Effect of rust infection on the levels of SODs, catalase and peroxidase in the different fractions

Changes in cyanid-sensitive SOD (Cu, Zn-SOD) and cyanide-insensitive SOD (Mn-SOD), catalase and peroxidase activities following rust infection in the three bean leaf homogenate fractions of both susceptible and hypersensitive cultivars, are reported in Tables 2–4. As shown in Table 2, alterations in cyanide--sensitive SOD (Cu, Zn-SOD) were negligible both in the susceptible and hypersensitive cultivars.

Mn-SOD (Table 2) was significantly stimulated in susceptible cultivars throughout the whole incubation period of the disease, starting 2 days after inoculation and reaching levels 2–3 times higher than those observed in control leaves. The increase in Mn-SOD in the cv. Pinto 650 (susceptible) started 2 days

(Table 2 continued)

Cultivar	Days after inoculations	15000 g supernatant			
		Cu, Zn-SOD		Mn-SOD	
		H	I	H	I
Golden Gate Wax: (susceptible)	2	1.23±0.07	1.29±0.03	0.22±0.01	0.20±0.02
	3	1.91±0.28	2.05±0.30	0.18±0.01	0.19±0.02
	4	0.72±0.12	1.34±0.18* 186%	0.16±0.00	0.14±0.02
	6	2.00±0.20	1.84±0.11	0.17±0.01	0.49±0.05** 288%
	9	1.96±0.14	1.81±0.13	0.11±0.00	0.34±0.04** 309%
Pinto 650:	2	1.37±0.06	1.39±0.15	0.26±0.00	0.22±0.02
	3	1.37±0.06	1.56±0.19	0.26±0.00	0.19±0.00** 73%
	7	2.33±0.26	3.90±0.72	0.43±0.09	0.57±0.03
K. W. 765: (hypersensitive)	2	2.72±0.07	2.77±0.12	0.39±0.00	0.51±0.03* 131%
	3	2.32±0.24	2.36±0.39	0.34±0.03	0.35±0.06
	4	2.18±0.17	2.87±0.20	0.27±0.03	0.33±0.01
	5	2.18±0.17	3.03±0.18	0.27±0.03	0.24±0.01

Values expressed as units mg-1 protein are the mean ± S.E. of 3–4 independent experiments. According to the Student's t-test:*, differences are significant at P = 0,05; **, P = 0.01;***, P = 0.001. When there are significant difference between healthy and infected, the percentage increase or decrease is reported.

after inoculation and was detected only in the 15,000 g sediment; in the cv. Golden Gate Wax (also susceptible) the rise began 3 days post-inoculation in the 15,000 g sediment and, in contrast to cv. Pinto 650, it was also present in both the 2,500 g sediment and the 15,000 g supernatant from 6 days after inoculation. Mn-SOD decreased in the 2,500 g fraction from Pinto 650 at 3 days after inoculation. There were no significant changes in this enzyme activity in the hypersensitive cv. K. W. 765 during the necrotic lesions differentiation 2 to 4 days after inoculation. However, a significant rise (206% with respect to controls) was documented on the 5th post-inoculation day when lesions were dry and brown and probably derived from region of leaf area unaffected by infection.

Catalase (Table 3) was significantly stimulated starting 2–3 days after inoculation in the susceptible cultivars and 3 days after inoculation in the hypersensitive cultivar. The increased activity was mainly localized in the 15,000 g sediment and reached 153–216% of the control values in the susceptible cultivar and 167–239% in the hypersensitive one. There was a rise in catalase activity

Table 3

Catalase activity in three fractions of healthy and rust-infected bean leaf homogenate

Cultivar	Days after inocu-lations	Crude 2500 g sediment		Crude 15000 g sediment		15000 g supernatant	
		H	I	H	I	H	I
Golden Gate Wax: (susceptible)	2	0.037± 0.000	0.035± 0.002	0.099± 0.006	0.086± 0.005	0.012± 0.001	0.012± 0.001
	3	0.019± 0.003	0.025± 0.001	0.036± 0.005	0.055± 0.001* 153%	0.022± 0.000	0.020± 0.002
	4	0.019± 0.003	0.032± 0.001* 168%	0.039± 0.006	0.074± 0.010* 190%	0.022± 0.000	0.018± 0.003
	6	0.030± 0.005	0.038± 0.001	0.062± 0.015	0.080± 0.004	0.018± 0.004	0.015± 0.002
	9	0.034± 0.003	0.019± 0.004* 56%	0.060± 0.010	0.100± 0.005* 167%	0.017± 0.000	0.015± 0.001
Pinto 650: (susceptible)	2	0.039± 0.000	0.029± 0.003	0.030± 0.003	0.054± 0.003** 180%	0.020± 0.000	0.024± 0.003
	3	0.039± 0.000	0.035± 0.002	0.034± 0.003	0.059± 0.005* 174%	0.021± 0.000	0.021± 0.001
	7	0.105± 0.032	0.105± 0.002	0.045± 0.001	0.097± 0.013* 216%	0.026± 0.002	0.032± 0.000* 123%
K. W. 765: (hypersensitive)	2	0.045± 0.014	0.065± 0.003	0.058± 0.010	0.057± 0.004	0.047± 0.003	0.048± 0.001
	3	0.045± 0.014	0.065± 0.002	0.060± 0.010	0.100± 0.001* 167%	0.044± 0.003	0.057± 0.002* 130%
	4	0.057± 0.003	0.103± 0.012* 181%	0.073± 0.015	0.162± 0.016* 222%	0.056± 0.003	0.039± 0.003* 70%
	5	0.056± 0.003	0.108± 0.002*** 193%	0.070± 0.014	0.167± 0.015** 239%	0.052± 0.003	0.023± 0.002* 44%

Values expressed as the rate constant for the overall reaction on mg protein basis (K mg^{-1} protein) are the mean ± S.E. of 3–4 independent experiments. According to the Studient's t-test: *, differences are significant at P = 0.05; **, P = 0.01; ***, P = 0.001. When there are significant differences between healthy and infected the percentage increase or decrease is reported.

Table 4

Peroxidase activity in three fractions of healthy and rust-infected bean leaf homogenate

Cultivar	Days after inoculations	Crude 2500 g sediment		Crude 15000 g sediment		15000 g supernatant	
		H	I	H	I	H	I
Golden Gate Wax: (susceptible)	2	0.086± 0.007	0.097± 0.009	0.420± 0.010	0.387± 0.035	0.087± 0.005	0.138± 0.006** 159%
	3	0.075± 0.005	0.086± 0.012	0.370± 0.026	0.372± 0.004	0.185± 0.014	0.180± 0.006
	4	0.075± 0.005	0.101± 0.004* 135%	0.370± 0.026	0.312± 0.022	0.185± 0.014	0.188± 0.003
	6	0.121± 0.011	0.166± 0.007* 137%	0.410± 0.044	0.554± 0.020* 135%	0.280± 0.008	0.399± 0.017** 142%
	9	0.110± 0.011	0.338± 0.003*** 307%	0.633± 0.007	1.012± 0.087* 160%	0.331± 0.004	0.779± 0.049*** 235%
Pinto 650: (susceptible)	2	0.060± 0.007	0.064± 0.000	0.208± 0.007	0.311± 0.016** 150%	0.130± 0.005	0.200± 0.015* 154%
	3	0.055± 0.001	0.082± 0 006** 149%	0.202± 0.006	0.297± 0.016** 147%	0.127± 0.002	0.198± 0.001*** 156%
	7	0.098± 0.006	0.203± 0.023* 207%	1.005± 0.018	0.950± 0.100	0.247± 0.015	0.500± 0.006*** 202%
K. W. 765: (hypersensitive)	2	0.060± 0.011	0.097± 0.008* 162%	0.451± 0.029	0.625± 0.022** 139%	0.258± 0.007	0.320± 0.007** 124%
	3	0.057± 0.010	0.152± 0.008** 267%	0.462± 0.040	0.828± 0.070* 179%	0.252± 0.010	0.502± 0.011*** 199%
	4	0.100± 0.018	0.270± 0.001*** 270%	0.575± 0.021	1.315± 0.058*** 229%	0.222± 0.005	0.581± 0.017*** 262%
	5	0.096± 0.014	0.334± 0.008*** 348%	0.567± 0.016	1.266± 0.160* 223%	0.215± 0.002	0.748± 0.043*** 348%

Values expressed as μmol H_2O_2 reduced min mg protein are the mean ± S.E. of 3–4 independent experiments. According to the Student's t-test: *, differences are significant at P = 0.05; **, P ± 0.01; ***, P ± 0.001. When there are significant differences between healthy and infected percentage increase or decrease is reported.

in the 2,500 sediment (181–193% of the control), which was correlated with marked decrease in 15,000 g supernatant (70–44% of the control), in the hypersensitive cv. K. W. 765, starting 4 days after inoculation, when local necrotic lesions were well established.

Peroxidase (Table 4) was significantly stimulated in the hypersensitive cv. K. W. 765 at 2 days after inoculation before the appearance of necrotis lesions. All fractions of the leaf extract were involved but the highest levels were observed in the 2,500 g sediment and the 15,000 g supernatant fractions 3–5 days after inoculation, i.e. during the differentiation of necrotic lesions (348% of the control at 5 days after inoculation). Considerable increases in peroxidase activity were also documented in the susceptible cultivars but mainly at a later stage of the incubation cycle, that is during the maximum fleck differentiation and the beginning of uredospore production (7–9 days after inoculation). All the fractions of the leaf extract were involved but, as in hypersensitive cultivar, the highest increases were recorded in the 2,500 g sediment and 15,000 g supernatant (with maximum values 307 and 235% that of the control respectively). Peroxidase activity did not increase in the susceptible cv. Golden Gate Wax 2–3 days post-inoculation, but it did in the susceptible cv. Pinto 650, where the percent rise was similar to that observed in the cv. K. W. 765 on the 2nd day, but lower on the 3rd.

Discussion

The data on alterations in superoxide dismutases in different fractions of leaf homogenate following rust infection presented in this communication largely confirm those reported for total-leaf extracts in previous papers (Buonaurio *et al.*, 1987; Montalbini, 1987). An increase in cyanide-insensitive SOD seems to be characteristic of compatible, but not incompatible host-pathogen interaction between *Phaseolus vulgaris* and *Uromyces phaseoli*. Furthermore, the present data shows that the increase in Mn-SOD, which was detected very early (from the 2nd post-inoculation day) at least in cv. Pinto 650, was mainly localized in subcellular fraction sedimented at 15,000 g, suggesting that a particle-associated or membrane-bound Mn-SOD activity, probably belonging to peroxisomes or mitochondria, is involved. The cv. Golden Gate Wax also displayed rises in Mn-SOD in the 2,500 g sediment and 15,000 g supernatant from 6 days after inoculation. This may indicate that the sedimentation properties of the organelles in this cultivar vary during the later stages of the disease (stroma differentiation), or that, owing to variations in intrinsic properties, the two susceptible cultivars have diverse metabolic responses that involve different subcellular compartments.

Higher levels of catalase activity were documented not only in the 15,000 g sediment, but also sometimes in the 2,500 g sediment of both susceptible and hypersensitive cultivars (Table 3). Peroxisomes are characterized by H_2O_2 generation derived from the activity of different peroxide-producing enzymes, for

example during glycolic and uric acid oxidation (Tolbert, 1982). Peroxisomes are also rich in catalase that destroys the H_2O_2 produced (Tolbert, 1971, 1982). The increase of catalase activity in the 15,000 g sediment of infected leaves may indicate enhanced metabolism of peroxisomes, irrespective of whether the compatible or incompatible combination is involved. Catalase may behave differently in other fractions of the leaf extract i.e. there was a considerable decrease in the 15,000 g supernatant of the hypersensitive cv. K. W. 765 when necrotic lesions were well-differentiated (4–5 days after inoculation). Therefore, catalase inhibition connected with the protective role of this enzyme may be imputed during the necrotic process. Moreover, the drop in catalase activity in the 2,500 g sediment during uredospore production (9 days after inoculation) in the susceptible cv. Golden Gate Wax may be related to the incipient cell collapse occurring in the susceptible cultivars during the later stages of infection. The decline in catalase activity agrees with the previous data obtained in total-leaf extracts of rusted bean leaves (Montalbini and Marte, 1972).

The general rise seen in the peroxidase activity of all fractions of rusted hypersensitive bean leaf homogenate 2 days after inoculation reached higher values 3–5 days post-inoculation, the time when necrotic lesions appeared. There was a similar trend in susceptible cultivars, but the increase was lower at 3–5 days and marked rises were only documented in the 2,500 g sediment and 15,000 g supernatant during the late stages of infection (fleck and uredospores differentiation 7–9 days after inoculation).

Changes of peroxidase activity does not differentiate compatible and incompatible combinations and alterations occurring during the necrotic process are probably mainly responsible for the markedly higher peroxidase activity recorded in the cv. K. W. 765 at 4–5 days after inoculation.

The increase of Mn-SOD (mainly localized in the 15,000 g sediment of the leaf homogenate) in the susceptible leaves would seem to be the only feature that distinguishes compatible from incompatible combinations. Such an increase, which may be associated with enhanced superoxide anion production, could represent a protective function against oxygen toxicity. On the other hand, the absence of any increase of SOD activity in the incompatible combination may be related to a lack of protection against oxygen toxicity and connected, at least in part, to a damage of invaded cells and then to hypersensitivity.

The absence of Mn-SOD stimulation in this case of incompatibility between host and pathogen should kept in mind when considering a possible role for peroxidase in defence against pathogenic invasion. Since certain reactions peroxidase catalyzed *in vitro* (Halliwell, 1977, 1978) are known to produce superoxide anion, peroxidase stimulation may indicate production of superoxide over the physiological levels in both compatible and incompatible combinations. However, the lack of a concomitant enhancement in SOD activity only in the incompatible combination may provoke peroxidase-induced unscavenged superoxide generation, which could account for both host damage (cell death) and pathogen inhibition (host resistance).

Recently has been reported a glyoxysomes-linked xanthine oxidase activity that results in production of superoxide in these organelles (Sandalio *et al.*, 1988). A possible involvement of superoxide anion production in peroxisomes, associated with xanthine oxidation which are the first steps of purine catabolism and ureide biosynthesis, could be hypothesized during rust infection of bean leaves. Many reports also indicate that the Mn-SOD is localized in peroxisomes (Del Rio *et al.*, 1983; Sandalio and Del Rio, 1987; Sandalio *et al.*, 1987). Recent experiments in our Institute seems to indicate that uricase activity, a marker peroxisome enzyme, is enhanced during rust infection of bean leaves.

In summary, the present findings demonstrate that a Mn-SOD activity which is mainly linked to a particulate or membrane fraction of leaf extract, is specifically enhanced in the compatible combination during active growth of rust mycelium in bean leaf mesophyll. On the basis of quantitative data, among the three-enzymes tested, changes of Mn-SOD activity seems the only that differentiate compatible from incompatible *P. vulgaris–U. phaseoli* combinations. The results of this investigation only give an approximative idea on the subcellular localization of the alterations observed. The purification of subcellular organelles by isopycnic sucrose gradient centrifugation may provide a more reliable picture of the subcellular localization of the alterations of these enzymes in rust-infected bean leaves.

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Quantification of the Activity of Superoxide Dismutase by Densitometric Analysis of Acrylamide Gels: Application to Mildewed Barley

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A novel method was developed for the quantification of superoxide dismutase (SOD) activity on polyacrylamide gel slabs after electrophoresis. By the use of negative gel staining method of Beauchamp and Fridovich (1971) various amounts of horseradish SOD were determined densitometrically on the basis of peak areas. Activity, i.e. peak areas, expressed as a function of increasing amount of enzyme protein was found to be linear up to 250 ng protein. The ng enzyme amounts were converted to enzyme units (EU). One SOD EU was equivalent to the amount of SOD that inhibits 50% of basic reaction rate under the conditions detailed in text. With standard curve obtained it is possible to determine activity of SOD in various plant extracts.

In the present paper this method as applied to powdery mildewed barley plants is described.

This new method seems to overcome certain disadvantages of widely used spectrophotometric assays.

The oxygen consumption process by cells is fraught with danger. Besides its classical role as electron acceptor to form a molecule of water, oxygen can be partially reduced, and generate several toxic intermediates such as superoxide ($O_2^{\cdot -}$) and hydroxyl ($OH^{\cdot}$) free radicals. Superoxide dismutase (SOD, E. C. 1.15.1.1.) removes $O_2^{\cdot -}$ produced by the aerobic metabolic processes of many types of cellular and subcellular systems including neutrophils, monocytes and macrophages (Halliwell and Gutteridge, 1985), mitochondria (Nohl and Jordan, 1986), chloroplast (Asada, 1984), microsomes (Kuthan and Ulrich, 1982), nuclei (Patton et al., 1980), glyoxysomes (Sandalio et al., 1988) and plasmalemma (Pilloud et al., 1989). SOD catalyzes the disproportionation of $O_2^{\cdot -}$ to H_2O_2 and dioxygen. If there is sufficient $O_2^{\cdot -}$ production or relatively low level of SOD activity, reactions, such as the iron-catalyzed Haber–Weiss reaction may take place leading to the generation of $OH^{\cdot}$ radicals. Hydroxyl radicals then react indiscriminately with membrane lipid compounds, and are capable of abstracting hydrogen from polyunsaturated fatty acids of phospholipid bilayer. The abstraction of hydrogen triggers the chain reaction of lipid peroxidation. If uncontrolled, this process may affect membrane integrity, producing a decrease in electrical resistance and membrane fluidity (Pauls and Thompson, 1980).

The above processes, involving changes in SOD activity, are undoubtedly of great importance in hypersensitive response (HR) of plants to pathogens

(Doke, 1983; Doke and Ohashi, 1988; Ádám et al., 1989). Consequently, the measurement of changes in activity or in isoenzyme pattern of SOD are especially interesting in some host-parasite interactions.

The photochemical method based on the use of the riboflavin/*p*-nitroblue tetrazolium (NBT) system has been stated to be the most reliable and reproducible spectrophotometric SOD assay (Giannapolitis and Ries, 1977). However, some problems may arise if not, at least partially purified, enzyme extract is used (Shaaltier and Gressel, 1986). Phenolics form complexes with proteins and are readily oxidized to quinones. Quinones, in turn, oxidize functional groups of proteins or form covalent bonds with proteins and, in addition may serve as $O_2^{\cdot -}$ scavengers (Rafat et al., 1987). Application of 1–3% polyvinylpirrolidone (PVP, both the soluble and insoluble forms) is recommended in many SOD extraction procedures for scavenging phenolics (Dhindsa et al., 1981; Lee and Bennett, 1982; Monk et al., 1987). Besides phenolic compounds some other substances in a crude enzyme extract may disturb the measurement of SOD activity. Substances other than SOD may influence both the rate of photochemical $O_2^{\cdot -}$ generation in the assay solution and the rate of formation of diformazan (which is a reduction product of NBT).

To overcome these difficulties, SOD activity was quantified in slabs of polyacrylamide gel by densitometry after electrophoresis and specific staining.

Materials and Methods

Polyacrylamide gel electrophoresis (PAGE) and staining for SOD activity

Electrophoresis was carried out in 0.8 mm thick, 80 mm long gel slabs in a vertical instrument at 4 °C. The slabs were prepared at the concentration of 12.5%. Electrophoresis was conducted in 0.2 M Tris-glycine buffer, pH 8.3 (Laemmli, 1970). After pre-run electrophoresis at 30 mA for 30 min, 10 μl samples containing various amounts of horseradish SOD (Sigma, USA) dissolved in 50 mM K-phosphate buffer (pH 7.8) were applied to the gel. A bromophenol blue crystal was added to the samples to permit visualization of the front. The samples were concentrated in the gel at a current of 15 mA for about 10 min, then further run was continued at 25 mA for 70–90 min until the bromphenol blue marker riched the bottom of the gel. Subsequent to PAGE, SOD bands were located on the gel by the negatively stained photochemical procedure described by Beauchamp and Fridovich (1971). First, the gels were rinsed in 0.05 M K-phosphate buffer (pH 7.8) containing 0.6×10^{-3} M NBT for 20 min. Then the gels were transferred to 0.05 M K-phosphate buffer (pH 7.8) containing 3×10^{-3} M EDTA and 3×10^{-5} M riboflavin. After staining, the gels were washed in the same buffer and placed 30 cm below a 200 W lamp for about 10 min. The whole gel stained bluish-lilac except in the zones containing SOD. Photographs were then taken. Within several hours after staining, gels were scanned with a Shimadzu CS-930 scanner (Japan) at 550 nm by transmission operation mode.

Determination of one SOD enzyme unit: a spectrophotometric assay

In order to convert ng enzyme amounts to SOD EU, the amount of horseradish SOD (Sigma, USA) that inhibits 50% of basic reaction rate was determined. One SOD EU was defined as 50% inhibition of basic reaction rate (McCord and Fridovich, 1969). The reaction conditions were similar to that used by Dhindsa et al. (1981) with some modifications. To 1.5 ml 0.05 M K-phosphate buffer (pH 7.8) containing 150 μM NBT (Aldrich, Germany) 0.2 mM EDTA and 26 mM methionine (Sigma, USA) 1.5 ml of 8 μM riboflavin and 2–50 μl enzyme solution (0.05 mg ml^{-1}), both dissolved in the same buffer were added. Test tubes containing the mixture were placed 10 cm below a 2×6 W fluorescent light tube for 6 min. During this time the reaction was found to be linear. The photochemical reduction of NBT (i.e. formation of diformazan) was measured at 560 nm against a reagent blank kept in the dark.

Extraction of SOD from barley leaf tissue

Leaf material (1 g) was firstly frozen in liquid nitrogen, then homogenized in 5 ml extraction buffer (50 mM K-phosphate, pH 7.8) containing 0.1 mM EDTA and 1% PVP (Polyclar AT, Serva, Germany). The samples were centrifuged at 16,000 g for 20 min and the supernatant was used as crude enzyme extract.

Protein assay

Soluble protein content of the extracts was taken as a reference volume for SOD activity and measured by the binding of Coomassie Brilliant Blue G-250 (Ferak, Germany) to proteins according to Bradford (1976). We used BSA as a standard.

Results and Discussion

Using polyacrylamide slab gels we found a linear relationship between the densitometrically determined peak area and the amount of horseradish SOD applied to the gel (Figs 1, 2 and 4). Application of SOD above 250 ng led to 'saturation' of the line (Figs 2 and 4). The ng enzyme amounts on the standard curve (Fig. 2) were converted to SOD EU. Fifty % inhibition of the basic reaction rate (indicated by dotted line in Fig. 3) served as one SOD EU (McCord and Fridovich, 1969).

The measurement of SOD activity in slab gel (Fig. 2) showed that this method is at least as sensitive as the spectrophotometric determination (Fig. 3).

The linear standard curve was used for the determination of SOD activity in powdery mildewed barley leaves. Infection with incompatible races of *Erysiphe graminis* did not result in changes of soluble SOD activity (Fig. 5a–d). Infection

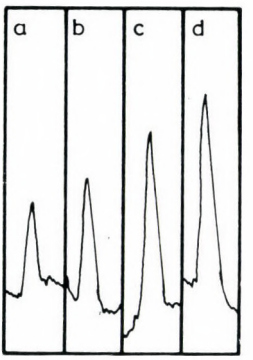

Fig. 1. Densitometric traces of different amounts of horseradish SOD after PAGE and specific staining. In a–d, 31.25, 62.5, 125 and 250 ng enzyme protein, respectively, was applied to the gel. The gel was scanned at 550 nm. For further details of experimental conditions see Materials and Methods

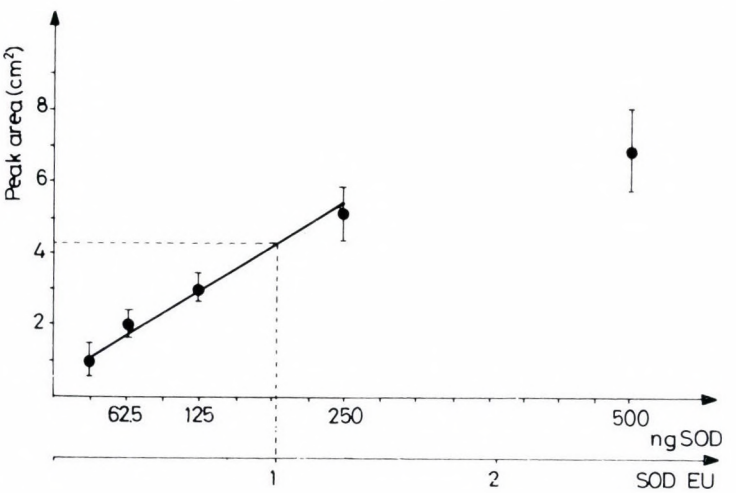

Fig. 2. Relationship between the densitometrically determined peak area and the amount of SOD applied to the gel. The ng enzyme values were converted to SOD EU using the amount of SOD (191.5 ng) which inhibited 50% the basic reaction rate (see Fig. 3). The dotted line indicates that one SOD EU is equal to 4.2 cm^2 peak area in the gel. Vertical bars are mean $\pm$ SE of 3 independent experiments

with compatible races of *E. graminis* resulted in appearance of two new bands (indicated by open arrows in Fig. 5e). Since in the incompatible host-parasite interaction powdery mildew fungus can not develop the mycelial network on the surface of epidermal cells (Brushnell and Gay, 1978), the two minor bands in the compatible interaction are probably of fungal origin. However, in *Phaseolus vulgaris–Uromyces phaseoli* host-parasite system (as Buonaurio et al. [1987] and

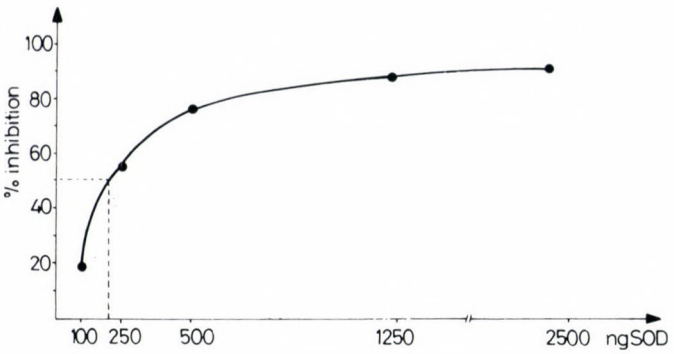

Fig. 3. Inhibition of photochemical reduction of *p*-nitro blue tetrazolium (NBT) to diformazan by various amounts of horseradish SOD. One unit of SOD was defined as 50% inhibition of basic reaction rate (indicated by dotted line). Further increase of the enzyme amount did not enhance the percentage of inhibition considerably

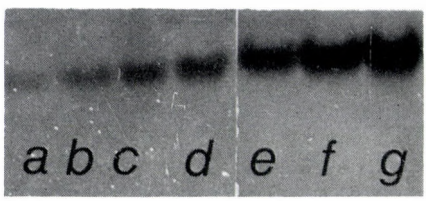

Fig. 4. Various amounts of SOD (a–g, 31.25, 62.5, 125, 250, 500, 1000 and 2000 ng enzyme protein, respectively) after PAGE and specific staining

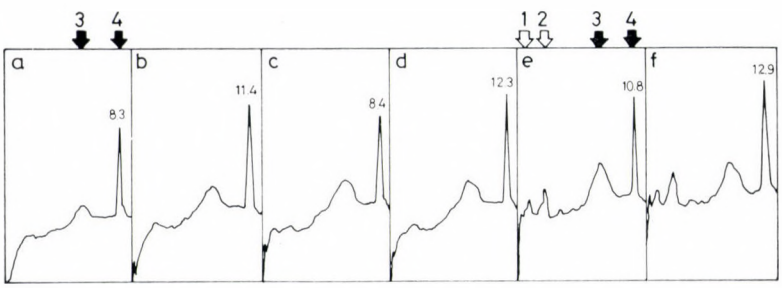

Fig. 5. Application of the method to powdery mildewed barley plants. The soluble SOD activity was extracted from whole leaf, 7 days after infection with *Erysiphe graminis*. *a*, *c* and *e*, *Hordeum vulgare* cv. *Emir* plants; *b*, *d* and *f*, *H. vulgare* cv. *Amsel* plants; *a* and *b*, uninfected, control plants; *c* and *d*, infected with incompatible races of powdery mildew; *e* and *f*, infected with compatible races of powdery mildew. The same amount of samples on a protein basis (13.8 μg protein) was loaded on the gel. The numbers indicate the SOD activity (EU mg^{-1} protein) of main bands

Montalbini and Buonaurio [1989] reported) changes in the activity of SOD could be involved in the determination of compatibility or incompatibility. To get more evidence in connection with *E. graminis* infection, it is necessary to measure SOD activity in epidermal skin of mildewed tissue since, contrary to rust infection, only epidermal cells are infected, whereas mesophyll cells, at least directly, are not influenced. The fungal origin of the two new bands are also supported by the fact that two days after infection, when mycelial network is slightly developed, these bands are not detectable (data not shown). It should also be considered that as a result of infection of tobacco with *E. cichoracearum* (Lupu et al., 1980), the increased activity of lipoxygenase in the compatible host could be related to the activation of SOD.

The above example gives a clear evidence for the main advantage of estimation of SOD activity in gels. That is, the different isoenzymes and/or enzymes of different origin can be distinguished from one another. Application of gradient gel can enhance the effectiveness of separation. The further advantages of this method over spectrophotometric assays could be characterized as follows: (i) electrophoresis itself serves as a purification of the sample. Consequently, no other time-consuming purification procedures are required, (ii) dilution and loss of enzyme activity which may occur during usual purification procedures (desalting on Sephadex G-25 column, ammonium sulphate precipitation, DEAE-cellulose separation, acetone powder extraction), could be prevented. For example Monk et al. (1987) suggested passing the extract through molecular filter with pore size of 10,000 to remove naturally occurring small molecular antioxidants (Lee et al., 1986). We suppose that these molecules can run off the gel during electrophoresis, consequently do not interfere with SOD assay.

Acknowledgement

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A Method for Quantitative Determination of the Hypersensitive Discoloration of Potato Tubers

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A method is described for measuring the degree of the hypersensitive tissue response of potato tuber discs to treatment with cell-free sonicate of *Phytophthora infestans* mycelium. The measurement is based upon densitometric scanning of color prints of tuber discs with a chromato-scanner. The method seems to be applicable to several sorts of plant tissues and to their responses to phytotoxic agents that cause surface discoloration.

The hypersensitive reaction of potato tuber tissues is a multiple phenomenon in appearance since it includes browning and necrotization. Nevertheless, the necrotized area may consist of necroses varying in size and shape from pin-point lesions to large coalescent spots. Cultivars, furthermore, have different capacity for the hypersensitive response which is associated with measurable biochemical changes. It is desirable to precisely reveal the sequence and the correlations of these events (Deverall, 1982). However, the estimation of tissue response by visual observation, as having been made so far (e.g. Varns and Kuć, 1971, Érsek et al., 1977, Kurantz and Zacharius, 1981), cannot give an appropriate, thus satisfactory, solution of the problem. We have developed a method for densitometrically measuring the degree of the hypersensitive tissue response with a chromato-scanner.

Materials and Methods

Test organisms and treatment

Tubers of potato cultivars Gülbaba (r) and Desirée (r) and line Ke. 6 (R_1R_3) were stored at 4 to 6 °C for 5 to 6 months. Before use they were surface-sterilized and a core of tissue (1.5 cm diam.) was removed from the central parenchymatous tissue with a cylindrical cutter then sliced into discs of uniform thickness (2 mm). Discs were washed three times with distilled water and distributed in plastic boxes lined with moistened filter paper. Prior to treatment with distilled water (control) and with crude elicitor preparation tuber discs were aged for 3 h at room temperature. Twenty-five μl of water or elicitor was added

to each disc (25 discs per cultivar) and distributed evenly on the surface with a blunt glass rod. Crude elicitor preparation was obtained from the mycelium of *Phytophthora infestans* (Mont.) de Bary race 2.3.4 kindly supplied by Dr. H. Lyr, Kleinmachnow, GDR (Érsek et al., 1977). Two g frozen mycelial mat was homogenized with mortar and pestle in 20 ml distilled water. The homogenate was then sonicated with a MSE sonifier for 10 min at maximum intensity. Sonicate was centrifuged at 6000 g for 20 min and finally autoclaved.

Densitometry

Selected tuber discs 48 and 72 h after treatment were placed onto a carton, as background, with color similar to, but lighter than, the color of control discs and then photographed on color negative film. Prints were made so that the diameter of a disc was 5 mm. The surface discoloration of discs on the photoprint was densitometrically quantified with Shimadzu dual-wavelength thin-layer chromato-scanner (model CS-930). The photometric system used was in the reflection-absorption mode with a light source of tungsten lamp. Zigzag swing width, correspondingly to the diameter of discs on print, was adjusted to 5 mm. Before evaluation of tissue response, the reflection-absorption spectrum of control discs was recorded, the absorption of the background was zeroed at the absorption maximum of 440 nm. Scanning was then performed at this wavelength.

Results and Discussion

All three cultivars developed necrosis and browning typical of the hypersensitive response by the second day after the application of the crude elicitor preparation. The discoloration (browning and/or necrosis) became stronger by the third day. Twenty-four h after treatment only slight browning on disc surfaces could be observed. These data are consistent with previous ones (e.g. Sato et al., 1968, Varns and Kuć, 1971 and Érsek et al., 1977) that indicate that the elicitor preparation is capable of inducing hypersensitive response in any potato cultivar, regardless of the race it derived from. The degree of tissue response, however, differed with the cultivars we used, which contradicts to previous findings (Varns et al., 1971). Difference in response of discs within one cultivar was also observed (Fig. 1). The 5 elicitor-treated discs per cultivar that were selected for photography, as shown in Fig. 1, represent the variation found in the set of 25 discs used in one experiment. Control (water-treated) discs exhibited some slight browning that also differed with cultivars. Neither visual nor densitometric estimation referred to detectable variation in discoloration of control discs within one cultivar.

Visual observation, when apparently sufficient, corresponds to recorded densitometric profile (Fig. 1), i.e. to automatically calculated peak areas. However, visualization by itself proves insufficient when one tries to make distinction be-

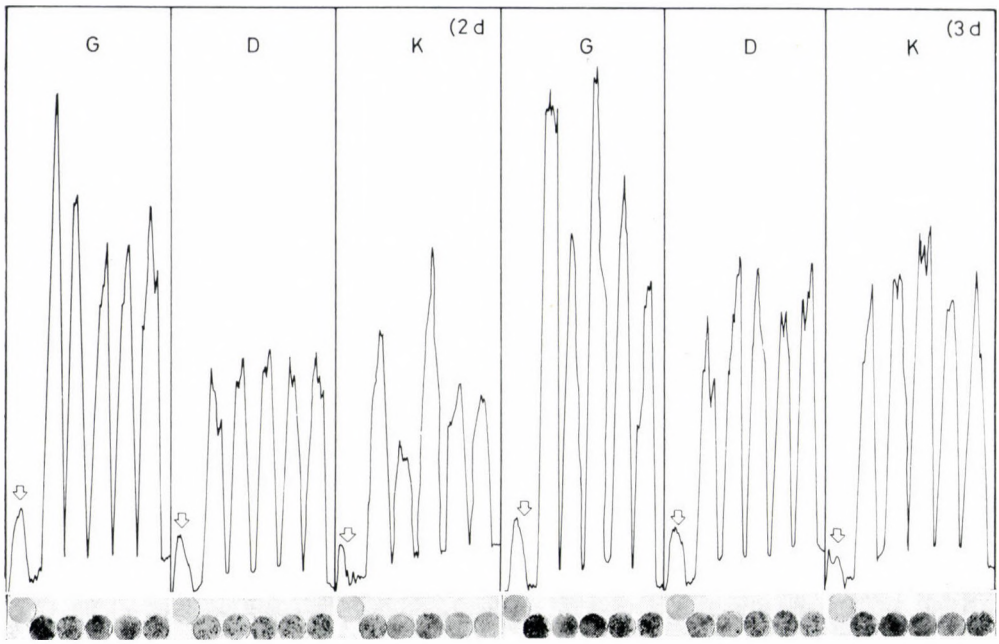

Fig. 1. Densitograms of the color copies of attached photoprints of potato tuber discs, 2 (2d) and 3 (3d) days after treatment with crude elicitor preparation from Phytophthora infestans, as compared to control (indicated by arrow). The same discs were photographed at both times but the order of discs may differ. For abbreviation of cultivars see Table 1

tween the degree of discoloration of cv. Desirée and that of Ke.6, 3 days after treatment, because the appearance of discoloration in these cultivars considerably differs from one another. On the basis of quantitative determination, discoloration is obviously stronger in discs of Ke.6. It is worth noting that the response of elicitor-treated tissue was evaluated as a function of that of control (Table 1).

Table 1

Degree of the hypersensitive tissue response of potato tuber discs as calculated on the basis of densitometric measurements shown in Fig. 1

Potato	Degree of response* after	
	2 days	3 days
Gülbaba (G)	3.05± 0.21	3.45± 0.42
Desirée (D)	1.90± 0.08	2.69± 0.21
Ke. 6 (K)	2.03± 0.38	3.14± 0.17

* Values in cm^2 (± SE) were obtained by the subtraction of control peak from the mean peak area of 5 elicitor-treated replicate discs within one experiment.

Color prints, rather than black-and-white ones with which the resolving power is far less (unpublished), are definitely suitable for slight distinctions to be made by densitometry.

Heretofore we have outlined a method for the quantitative determination of the hypersensitive response of potato tuber tissues. Beyond this scope, the method seems to be applicable to the estimation of any tissue's reaction to any phytotoxic agent that cause surface discoloration.

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Biological Control of Fusarium Root Rot of Broad Bean

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An isolate of a soil-inhabiting *Bacillus subtilis* which was parasitic to *Fusarium solani* (Mart.) Sacc. in culture, was also an effective biological control agent to this pathogen of broad bean in greenhouse studies. Bacterium applied as seed treatment significantly reduced seed colonization by the pathogen and root rot disease of broad bean.

B. subtilis inhibited the growth of the pathogenic fungus on potato dextrose agar. The culture filtrate of bacterium caused significant reduction in percentage germination of spores of the fungus. These indicate that *B. subtilis* may produce some metabolites which inhibit the fungus. The significaance of this antagonistic bacterium is discussed in relation to the control of root rot disease of broad bean.

Root rot disease of broad bean (*Vicia laba* L.) caused by the soil borne fungus *Fusarium solani* (Mart.) Sacc. may destroy entire fields of broad bean in areas highly infested with the pathogen. No field treatment has been devised that will eradicate the fungus from soil. Several biological control systems have been described for soil borne plant pathogens (Bacman and Kabana, 1975; Hadar et al., 1979; Papavizas and Lumsden, 1980; Scardaci, 1981). At present this pathogen can not be controlled successfully with chemicals. Hence attention has focused on biological control, in particular the use of bacterial antagonists (Utkhede and Rahe, 1980).

Many micro-organisms were held responsible for adversely affecting fungal growth. The major bacterial antagonists to pathogenic fungi described by many workers (Utkhede, 1983; Wong and Hughes, 1986; Capper and Campell, 1986) was *Bacillus* spp.

This paper describes the investigation of an isolate of *Bacillus subtilis* as a biological control agent of *Fusarium solani* caused the root rot disease of broad bean in culture and in greenhouse experiments.

Materials and Methods

Experiments were conducted in the laboratory and greenhouse. Broad bean (*Vicia faba* L.) seeds were used.

Isolation of the Micro-organisms

Fusarium solani (Mart.) Sacc. was isolated from broad bean seed, purification and identification of the fungus were as reported previously (Sharif et al., 1987).

For isolation of *Bacillus subtilis*, soil samples were collected from different fields. A 10 g sample was suspended in 100 ml of sterile distilled water and shaken gently for 10 min with an orbital shaker. Soil suspension was diluted in a tenfold series and 0.1 ml amount from each dilution were plated on nutrient agar using the spread-plate method and incubated for 5 days.

Screening Bacteria for Antagonism

Colonies of bacteria were sub-cultured and screened for antagonism. Each test colony was streaked across side of a potato dextrose agar plate. A 0.5 cm disc of 5-day-culture of *Fusarium solani* on PDA was placed 2 cm from the centre on the opposite side to the test bacterium. The plates were incubated at 25 °C for 7–10 days and then examined for inhibition of mycelial growth considered indicative of bacterial antagonism. Strain of *Bacillus subtilis* antagonistic to *Fusarium solani* was retained.

Effect of Culture Filtrate

Culture filtrate of bacterium was obtained from nutrient broth medium. For this purpose nutrient broth was used for growing the bacteria. Similar nutrient broth without the bacterium was used as control. The cultured and the control broth were kept al 25 °C for one week then filtered through bacterial proof filter. The culture filtrates were tested by placing drops of it on a glass slide then seeding them with spores of *F. solani* and keeping the slides in a moist conditions inside petri dishes. The spores were examined after 16 hours to observe the germination.

Seed Coating Experiments in the Greenhouse

Broad bean seeds were coated by dipping them into bacteria inoculum preparation. The coated seeds were dried for at least 12 hours prior to use. Bean seeds were also treated with preparation of bacteria or with the systemic fungicide benomyl which was also used with conventional treatment (0.1 %) to compare the application of bacteria in controlling root disease, or seeds were not treated. Soil was left noninfested or infested with inoculum of *F. solani*. Four replications of each treatment were done in plastic dishes ($30 \times 20 \times 10$) containing sterile soil and 10 treated or nontreated seeds were plated in the soil of each dish immediately after infestation with the fungus. After 2–3 weeks from planting percentage of seed germination were recorded and seedling were uprooted and examined. All results were subjected to statistical analyzation using complete randomized design and Duncan test.

Results and Discussion

Inhibition of Fungal Growth and Spore Germination

The growth of *F. solani* was adversely affected by *Bacillus subtilis* when grown next to each other on PDA. The expansion of the growth was suddenly checked at a distance at about 3–4 cm from the bacterial colony which prevented the advancement of the fungal hyphae and the sporulation. Examination of the

Fig. 1. Effect of *B. subtilis* colony on the hyphal growth of *F. solani* on PDA

hyphal touching the inhibition zone showed lysis of the hyphae (Fig. 1). It was shown that *B. subtilis* also gave similar effects against *Curvnlaria verruculosa* (Damirdagh, 1980).

The culture filtrate of *B. subtilis* reduced significantly percentage germination of the spores of *F. solani* (Table 1). This is in accordance with previous work (Chinn and Ledingham, 1961) that *B. subtilis* reduced spore germination of *Helminthosporium sativum* and this contribute in eradication of the fungal spores from amended soil.

Devay et al. (1968) have demonstrated the production of some metabolites by pathogenic bacteria which inhibited the growth of many pathogenic fungi.

Bacterium Used as a Seed Coating

In greenhouse experiments, broad bean seeds coated with *B. subtilis* inoculum produced significantly greater percentage of healthy seedlings of bean after 2–3 weeks from planting in soil infested with *F. solani* than did nontreated seeds (Table 2) and the healthy seedlings were comparable to those achieved with benomyl-treated seeds. Reduction in seedling infection was associated with significant reduction of seed colonization by the fungus. Utkhede (1983) and Utkhede and Rahe (1983) showed that *B. subtilis* applied as seed treatments

Table 1

Effect of culture filtrate of *B. subtilis* and benomyl on spore germination of *F. silani*

Treatment	Spore germination %
Water control	87.33a*
Medium control	80.25ab
B. subtilis	29.72e
Benomyl	15.69f

* Values followed by the same letter are not significantly different, P = 0.05, according to Duncan's test.

Table 2

Comparison between *B. subtilis* and benomyl as seed protection against *F. solani* in soil

Seed treatment	*F. solani* infestation (+ or −)	Seed germination %	Seed Colonization %	Diseased* seedlings %
Nontreated	−	88.9a	0e	0e**
B. subtilis	−	85.8a	0e	0e
Benomyl	−	90.5a	0e	0e
Nontreated	+	68.2c	48.8a	69.3a
B. subtilis	+	81.9av	13.7c	25.8c
Benomyl	+	86.3a	5.5d	15.9d

* Average percentage of diseased seedlings from four replicates of 10 plants each.
** Values followed by the same letter are not significantly different, P = 0.05, according to Duncan's test.

significantly reduced white rot of onion. Paparizas and Lumsden (1980) reported that *Bacillus* spp. were the commonest group of soil inhabitant that produce antibiotics for controlling plant diseases.

The results presented here may indicate that *B. subtilis* produce some metabolites which affected the pathogenic fungus. The evaluation under laboratory and greenhouse situations of selected antagonist for biological control of root rot of broad bean is currently under investigation.

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Occurrence and Pathogenicity of Damping-off Fungi of Pine Seedlings in Northern Iraq

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The occurrence of damping-off fungi in diseased pine seedlings were studied. Isolation and pathogenicity tests showed that damping-off diseases of seedlings in northern Iraq nurseries are mainly caused by *Rhizoctonia solani* and *Fusarium oxysporum*. The seed isolations showed the *Fusarium oxysporum* is a seed borne fungus.

Pathogenicity of these fungi revealed that *R. solani* was the most virulent on pine seedlings. Some control trials against damping-off in *Pinus brutia* seedlings were carried out by chemical treatments. The efficacy of captan and lime on controlling the disease was studied under greenhouse conditions. The chemicals were showing satisfactory results in controlling damping-off fungi and the best results were obtained by captan treatment.

Forest nursery production in northern Iraq was increased to provide tree seedlings needed for the expanding reforestation program, and this is based primarily on pine plantations. Therefore, it is particularly important to have reliable seedlings production. Damping-off diseases were a serious problem in most nurseries (Vaartaja, 1967).

The literature indicates that *Rhizoctonia* and *Fusarium* are universally important pathogens and cause the damping-off diseases of pine seedlings (Saksena and Vaartaja, 1961; Vaartaja and Salisbury, 1961; Smith, 1967 and Beach 1974). Sterilization or fumigation of soils prior to planting is recommended in forest nurseries where serious diseases exist. Peterson (1970) reported that damping-off diseases of pine was reduced in seedbeds treated with methyl bromide. It was found that the application of fungicides into the soil were able to give a significant control of damping-off disease of pine (Carlson and Belcher, 1969) and of other crop species (Cetas, 1961). Also, the application of lime to the soil in order to control *Fusarium* diseases is one of the oldest methods of diseases control and is still widely practised (Woltz and Engelhard, 1970; Fletcher et al., 1982 and Sarhan, 1982).

In this study, experiments were done to isolate and identify the causal organisms from diseased pine seedlings and to test the pathogenicity of the isolates, and the control of the disease by using captan and lime.

Materials and Methods

One thousand pine seedlings (five samples, 200 seedling each) were collected from the plantation center in the northen Iraq (Arbil region) during damping-off epidemics and the associated fungi were isolated and identified. For isolatiou purpose, pieces of diseased seedlings were washed thoroughly in tap water, surface sterilized in 1% sodium hypochlorite solution for 3 minntes, rinsed in sterile distilled water two times, dried on sterile filter paper, aud plated on PDA and incubated at 25 °C for one week. Pine seeds were also taken for fungi isolation. Two hundred seeds were washed in sterile distilled water, dried on sterile filter paper, plated on petri dishes containing PDA and incubated at 25 °C for one week.

The isolated fungi were identified by the Commonwealth Mycological Institute as *Rhizoctonia solani* and *Fusarium oxysporum*.

The potential pathogenicity of the isolated fungi was tested. Plastic dishes ($30 \times 20 \times 10$ cm) containing sterile sandy soil. Twenty five sterile seeds were sown in each dish, 4 replicates were used. Then the dishes were inoculated with the spore suspension (1×10^5 spore/ml) of 7 days old culture of the isolated fungi at the rate of 50 ml/dish. The dishes were kept in the greenhouse at 25 ± 5 °C. After 6 weeks of inoculation the data were recorded, the incidence of the disease was estimated every second week.

Washed, surface sterilized pine seed were immersed in spore suspension (1×10^5 spore/ml) of each fungus or in sterile distilled water (as control) for 1 min, then transferred to sterile petri dishes (4 replicates, 10 seed each) lined with moistured filter paper. After one week from inoculation, the percentage of germination was recorded.

For chemical control of this disease, captan (N-trichloromethyl thiotetrahydrophthalimide) and hydrated lime ($Ca(OH)_2$) were used. Pine seeds were sown in sterile sandy soil in plastic dishes and after 2 weeks the seedlings were transplanted into 10 cm pots containing sterile sandy soil previously amended with the various amount of calcium hydroxide (0.5, 1.0 and 2.0 g $Ca(OH)_2$/Kg soil)

Table 1

Potential pathogenicity of isolated fungi on percentage of seed germination and infection of pine seedlings

Fungi	Seed germination* %	Seedling infection* %
Fusarium oxysporum	60.5b	53.3b
Rhizoctonia solani	48.3c	79.1a
Check (no fungus)	88.6a	0.0c

* Numbers within a column followed by the same letter not significantly different at $p = 0.05$ using Duncan's multiple range test.

to establish different soil pH. The pH values of soil were determined, and the soil samples for determination of pH were prepared with 50–50 (v/v) soil-distilled water which equilibrated one hour. The fungicide, captan, was used at the rate of 0.1% (a typical concentration used in practice). Pots were drenched with captan solution one day before inoculation, each pot received 60 ml. Pine seedlings were inoculated by removing them from the dishes, immersing the roots in the spore suspension for 2 minutes and planting them in the pots. In each experiment 10 plants, 4 replicates per treatment were used. Pots were kept in the greenhouse at 25 ± 5 °C. Results were recorded after 6 weeks of inoculation. Data were analysed for variance by Duncan's Multiple Range test.

Results and Discussion

From the isolation test the following fungi were frequently isolated from damped-off seedlings collected from the pine nurseries: *Rhizoctonia solani* and *Fusarium oxysporum*. The incidences of *R. solani* were high in all epidemics, and the incidence of the other fungus varied greatly. The seed isolations showed that *F. oxysporum* is a seed borne fungus. This suggests that this fungus can be disseminated by pine seed. Our results in agreement with that of Pawuk (1978). Also, it was found by Vaartaja et al. (1961) and Smith (1973) that *Fusarium* spp. were commonly associated with certain damping-off epidemics of forest nurseries. The results of pathogenicity tests of the isolated fungi against *Pinus brutia* are presented in Table 1. Data showed that *Rhizoctonia solani* was highly pathogenic, and caused around 50% pre-emergence damping-off of pine seeds. This is in accord with the results obtained by Robert and William (1973).

Table 2

Effect of captan and hydrated lime on infection of pine seedlings with *Rhizoctonia solani*

Treatment	pH values of soil	Number of infected seedlings at different intervals from infection (weeks)[1]			Seedling survival %[2]
		2	4	6	
Nontreated-noninfected	6.8	0c[3]	0c	0c	100a
Nontreated-infected	6.8	6a	7a	9a	10d
Captan, 0.1%	6.9	2b	3b	3b	70b
$Ca(OH)_2$, g/Kg soil	7.2	5a	6a	8b	20d
$Ca(OH)_2$, 1.0 g/Kg soil	7.5	2b	4ab	5ab	50c
$Ca(OH)_2$, 2.0 g/Kg soil	8.1	2b	3b	4b	60bc

[1] Number of seedlings was 10.

[2] Percentage of seedling survival was recorded after 42 days from infection.

[3] Number within a column followed by the same letter not significantly different at 0.05 level.

Table 3

Effect of captan and hydrated lime on infection of pine seedlings with *Fusarium oxysporum*

Treatment	pH values of soil	Number of infected seedlings at different intervals from infection (weeks)[1]			Seedling survival %
		2	4	6	
Nontreated-noninfected	6.8	0b[3]	0d	0d	100a
Nontreated-infected	6.8	2a	7a	7a	30d
Captan, 0.1%	6.9	1ab	3ab	4ab	60bc
$Ca(OH)_2$, 0.5 g/Kg soil	7.2	0b	4b	6ab	40d
$Ca(OH)_2$, 1.0 g/Kg soil	7.5	1ab	4b	4b	60bc
$Ca(OH)_2$, 2.0 g/Kg soil	8.1	0b	3bc	3bc	70c

[1] Number of seedlings was 10.
[2] Percentage of seedling survival was recorded after 42 days from infection.
[3] Number within a column followed by the same letter not significantly different at 0.05 level.

Soil treatments with the fungicide, captan, have given a good control of damping-off of pine (Tables 2 and 3). This is in line with Bassett (1964).

The application of hydrated lime increased the soil reaction of pH to 8.1. The incidence of *R. solani* and *F. oxysporum* decreased as the soil pH increased after liming. The pH values of the soil were determined for each treatment because of the significance of this parameter of soil environment in the development of *Fusarium* spp. (Sarhan, 1982). Our results showed that *Fusarium* and *Rhizoctonia* were affected by lime application and this was as effective as application of captan.

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Inoculation Experiment on Trellised Grapevines with *Agrobacterium tumefaciens* for Study the Process of Crown Gall Disease

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Inoculation experiment was carried out on trellised 4-year-old vines in a cv Green Veltliner vineyard. Inoculation was made by putting of 0.2 ml suspension of A. tumefaciens biovar 3 on sterile wound of the arms at 1.40 height. The wound was tied with plastic band. In the first year, tumour tissue appeared only on the wounds. Small gall formation in the following three years occurred in decreasing rate on, and around the wounds. Galls were not observed in any other place of the inoculated plants. Even more, during the next three years, the inoculated vines together the wounds, became totally symptomless. Typical gall formation was found first in the 8th year after inoculation on the basal part of the trunks of three vines from 8 treated ones (37.5%). The process of systemic spreading of the tumour inducing bacteria in the whole vine could not have been followed by the symptoms. Presumedly, the crown gall disease in such instances becomes serious if the rootstock is invaded and totally flooded in systemic way by the tumour inducing bacteria. For this reason a non host rootstock would be a promising tool to decrease the harmfulness of crown gall disease on grapevine.

The crown or trunk gall is a serious disease of the grapevine in several grape growing regions that are in the cooler part of temperate zone of the world causing *Agrobacterium tumefaciens* (Smith and Townsend) Conn. Many experimental data substantiate that the bacteria spread in the vines systemically and are detectable in springtime in the bleeding fluids of vascular system of aerial parts on diseased vines (Lehoczky, 1968a; Malenin, 1970; Burr and Katz, 1983; Tarbah and Goodman, 1987; Goodman et al., 1987), in vessel fluids collecting after wash through it by sterile distilled water (Tarbah and Goodman, 1986; Goodman et al., 1987), as well as in the tissue of roots (Lehoczky, 1971; Süle, 1986; Burr et al., 1987). Therefore the bacteria can be disseminated by the propagating materials (e.g. bench grafts, green-wood grafting, ownrooted cuttings) as concomitant for the aboves (Lehoczky, 1968b; Burr and Katz, 1984; Tarbah and Goodman, 1986; Goodman et al., 1987). It is remarkable, that in the canes of symptomless propagating materials derived from some nursuries of different grape growing regions in U.S.A., tumour inducing bacteria could be detected using a special tool, demonstrating that the infection might be latent for a various long term (Tarbah and Goodman, 1986; Goodman et al., 1987). This fact gives further

evidence about the practically important way of distribution of the crown gall disease.

It is verified that the population of a *A. tumefaciens* is habitant in the soil of the vineyard or nonvineyard and with it a reinfection potential arises for a bacterium-free, clean propagating materials (Schroth et al., 1965; Burr et al., 1987) first of all on the root-system or on the woody parts above the soil level. For this reason it was decided to clarify the phases of crown gall disease if the bacterial infection takes place in the vineyard, in situ. This paper describes the long-term experiment on the trellised vines inoculating them with *A. tumefaciens* in order to study the disease process following up it on the way of visible tumour production.

Materials and Methods

Location of the experiment and the inoculated vines

The experiment was carried out in a vineyard ($\approx$ 7 hectares) consisted of 4-year-old trellised vines of cv. *Green Veltliner/T 5C*, near Pusztaszentgyörgy village (Somogy county). The vines were in a good condition (Fig. 1) and did

Fig. 1. Experimental trellised cv. Green Veltliner vines. The arrows show the sites of inoculations on the arms

not show any symptoms of neither crown and trunk galls after natural infections nor of virus infections or others.

Some hard-wood canes were gathered randomly from the experimental vines for detection a possible latent infection of *A. tumefaciens* in the tips of fresh roots of forced cuttings with method applied and described formerly (Lehoczky, 1971).

Time of inoculation

Inoculation was made on 4th May, 1978 in bleeding phaenological stage, just before bud burst (03 stage: according to Eichorn and Lorenz, 1977).

Bacterium isolate used for inoculation

A. tumefaciens isolate (AT-1) was obtained from fresh tumour tissue of cv. *Olimpia grape* (1968) and determined to be belonged to the group of biovar 3 (Süle, 1978). The pathogen was grown on *Oxoid bouillon* completed with 10 g glucose and 15 g agar. Slant cultures were kept at 28 °C for 48 hs and then a suspension was made with sterile distilled water in $2–4 \times 10^8$ cells/ml concentration checked by dilution technique.

Inoculation of the arms

In the vineyard in two places, rather far to each other, 4–4 vines were chosen one after another for inoculation and just behind them 4–4 vines served as control ones. On the arms of these vines from the trunk after the first nodal region was the site of the inoculation at 1.40 m in height above the soil level (Fig. 1). From the surface of arm the rhytidome was removed then washed with 70% ethanol. On the upper surface of the arm a 25–30 mm long and 15–20 mm width tongue-shaped tissue consisted of phloem + cambial zone + a rather thick xylem was incised using of a sterile scalpel. After lift the separated tissues, four 50 μl drops with $6–7 \times 10^6$ cells were put onto the surface of the wounds (Fig. 2a). Then the tissue let down again and the wound was tied with plastic bands (Fig. 2b) and covered with white filter paper protecting it against the direct sunshine for 30 days. The control vines were treated similarly, but sterile distilled water was used instead of bacterial suspension. The plastic bands were removed in the first year's August.

Evaluation of the experimental vines

The inoculated vines were surveyed symptomatically in every year in June and October and in the latter cases the place of produced gall tissues was registered and were cut at their basic line for measurement.

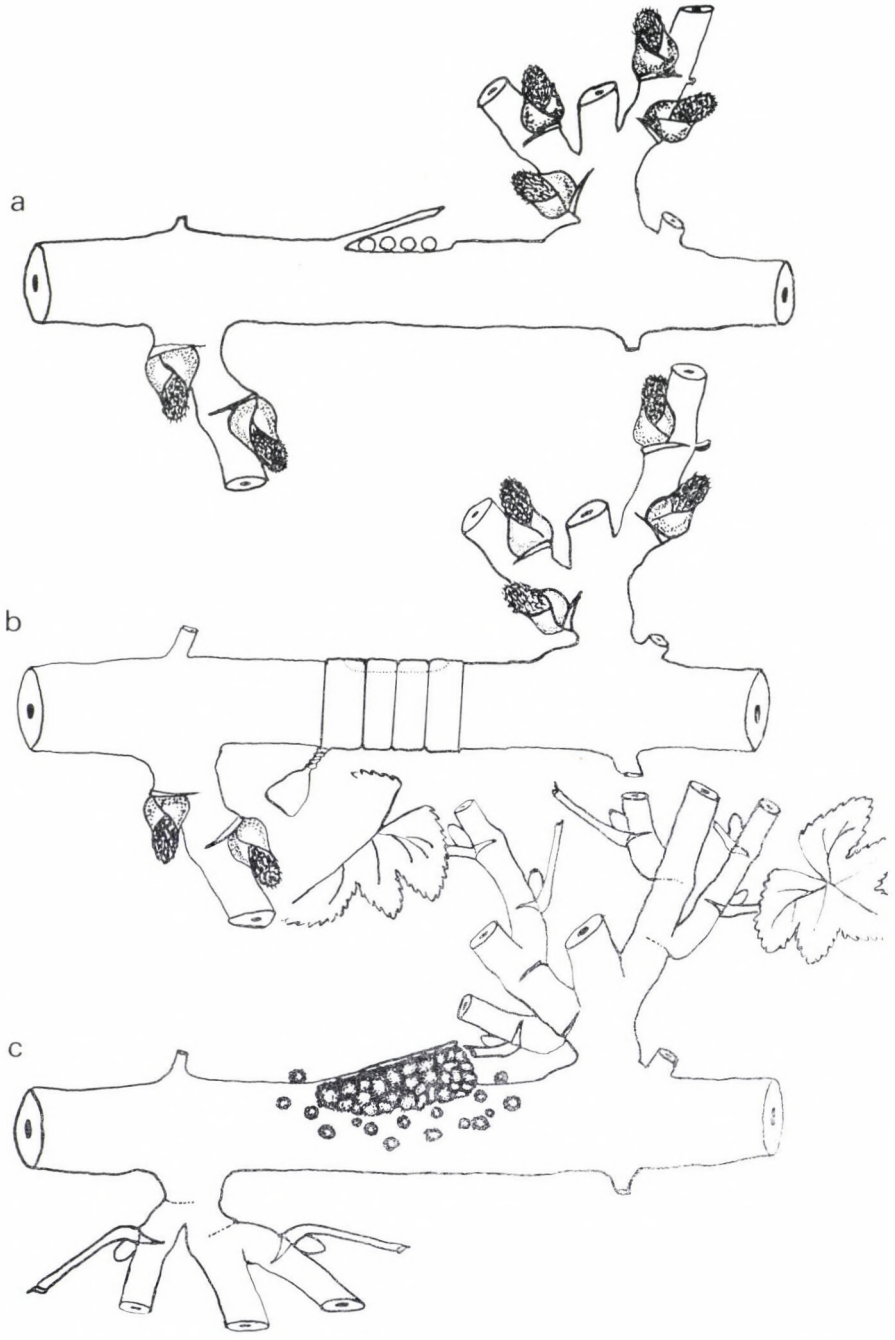

Fig. 2. Method and symptoms of inoculation: a. incised and lifted living tissue with four droplets of bacterial suspension under it; b. inoculated wound with binding of plastic band; c. gall formation on the site of the wound and the appearance of single small ones around it

Fig. 3. Tumourous tissue formation on the wound pushing up the living tissue in the first year's fall

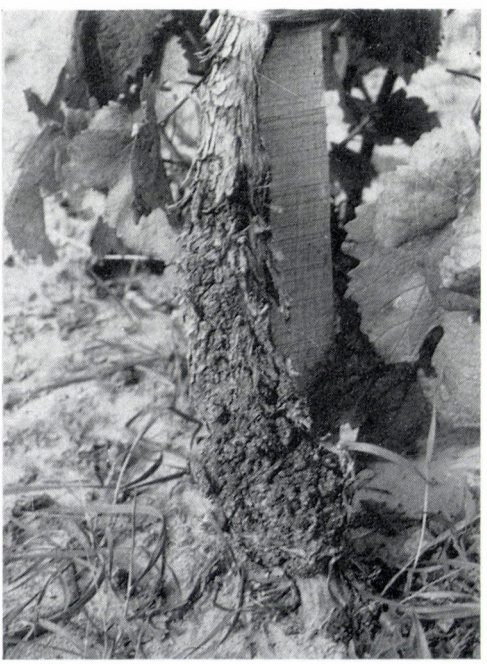

Fig. 4. Girdling serious gall formation on the basal part of the trunk just above the graft-union in the eighth year after inoculation

Meteorological conditions during the winter seasons

Every year in the experimental period, fell the temperature in the winter months below −10–18 °C for a shorter or longer term, except the winter of 1983–84, and it was favourable to the development of the symptoms.

Results

Cheking isolation to prove the occurrence of cells of *A. tumefaciens* biovars latently in the canes were gathered randomly from the experimental vines before treatments, was unsuccessful. Results verified that the vines used for inoculation and control were not infected latently.

Results of the inoculation experiment show (Table 1) the mass of gall tissue grew first only on the wound along the cut-line pushing up the living bark tissue (Fig. 3). From the second year small single tumourous protuberances appeared sporadically but closely around the inoculated wounds (Fig. 2c) until 1981, in slowly decreasing rate. Later, neither on the wound, nor closely around it, gall did not grow any more and on the places of the inoculations remained apparently a 'non-active' pillow-like outgrowth. During the folowing three years (1982–1984) gall formation nowhere was found on the woody parts of the experimental vines. Typical sectorial or girdling gall formation on the basal part of the trunks was observed first in 1985 (Fig. 4), on three treated vines fom 8 ones (37.5%).

The control vines did not show any symptoms on the woody parts during the experimental period, even more, other vines of the ≈7 hectares cv. *Green Veltliner* vineyard were also free of gall formation.

Discussion

Unfortunately, the experimental vines seriously suffered from the hard winter frost began in 1984–1985 and continued over the next two winter seasons (1985–86, 1986–87) causing injuries again and the trunks must have been amputated repeatedly and this catastrophe broke further observation of the disease process.

As it has been already mentioned in the part of introduction the crown gall disease can distribute dangerously by propagating materials (grafts, own-rooted cuttings) derived from infected mother vines. Whereas the disease process little is known when the infection takes place in situ in the vineyard. The inoculation experiment described above, gave some unexpected results. It was presumed that around the wound where the bacteria invaded slowly spread in the tissue apically or basipetally and it was detectable visually more or less by the expanding gall formation, depending on the winter frost injury. Opposite to the expectation on or around the wounds decreasing gall formation was found in the first four years. Later this local activity stopped and symptoms were found nowhere on

Table 1

Tumour-tissue formation and their weights on and around or far from the sites of inoculations on the arms of the trellised vines

	No. of vines inoculated and controls cv. *Green Veltliner*	Weight of tumour tissues in											
		1978		1979		1980		1981		1982–1984		1985	
		on or around	far from	on or around	far from	on or around	far from	on or around	far from	on or around	far from	on or around	far from
		the sites of the inoculations in mg											
	35–VII–1	78.1	·	101.0	·	65.3	·	·	·	·	·	·	·
	35–VII–2	97.8	·	81.2	·	25.2	·	·	·	·	·	·	>1× 10^5s
	35–VII–3	224.3	·	98.9	·	155.6	·	55.0	·	·	·	·	·
	35–VII–4	246.7	·	30.9	·	50.5	·	·	·	·	·	·	>1× 10^5s
Controls	35–VII–5	·	·	·	·	·	·	·	·	·	·	·	·
Controls	35–VII–6	·	·	·	·	·	·	·	·	·	·	·	·
Controls	35–VII–7	·	·	·	·	·	·	·	·	·	·	·	·
Controls	35–VII–8	·	·	·	·	·	·	·	·	·	·	·	·
	55–VII–1	254.5	·	346.5	·	76.0	·	96.7	·	·	·	·	·
	55–VII–2	779.3	·	562.7	·	61.7	·	·	·	·	·	·	·
	55–VII–3	306.0	·	276.5	·	102.6	·	·	·	·	·	·	>2× 10^5g
	55–VII–4	125.7	·	95.6	·	18.7	·	·	·	·	·	·	·
Controls	55–VII–5	·	·	·	·	·	·	·	·	·	·	·	·
Controls	55–VII–6	·	·	·	·	·	·	·	·	·	·	·	·
Controls	55–VII–7	·	·	·	·	·	·	·	·	·	·	·	·
Controls	55–VII–8	·	·	·	·	·	·	·	·	·	·	·	·

s = sectorial gall formation
g = girdling gall formation

any woody parts above the soil level over the subsequent three years. Finally in the 8th year after inoculation typical gall formation appeared on the basal part of the trunks in various extension.

Looking over the experimental results it can be carefully assumed that after inoculation/natural infection the disease doesn't become serious until the root system is flooded totally by the bacteria on a systemic way and for the future it serves as a reservoir (Lehoczky, 1978), similarly as in the case of tobacco mosaic virus in the plant (Samuel, 1934, cit.: Matthews, 1970). In springtime when the function of the root system becomes active the vascular fluid transports bacteria up towards the aerial woody parts (Lehoczky, 1978), causing induced cell division where the cambial zone or pith ray and xylem parenchyma cells, on the effect of the winter frost or mechanical injuries (Tarbah and Goodman, 1988). Tarbah and Goodman's (1986) findings that the number of *A. tumefaciens* cells in the vascular system of canes upwards from below is decreasing and probably in the trunks, too, supports the probability that the root-system plays an important role every year in the cycle of the gall formation. Süle (1986) attributed also a great importance to the bacteria survived in the tissue of the root-system. If it is true then the sensitivity of the rootstock is an important factor in the process of the disease and those vines suffer seriously, that have such of rootstocks which are host of *A. tumefaciens* cells, carrying them. Tarbah and Goodman (1986) and Goodman et al. (1987) reported that in the canes of some well known and generally used rootstocks as *Vitis* berlandieri X V. riparia T 5C; TK 5BB and SO4; V. riparia X V. rupestris 101–14 and V. riparia X V. rupestris 3309 as well as, at the same time, Süle (1986) in the root tissue from phloem of the T 5C rootstock of the diseased cv. Merlot, found the cells of the tumour inducing bacteria.

If there would be some *Vitis* spp. or various hybrids which are non-host, namely are resistant to gall formation and even more to be unfavourable 'habitation' for *A. tumefaciens* biovars it gave a promising possibility for the practical control. Taking the aboves into consideration, the rootstock V. riparia Michx. cv. Portalis (= Gloire de Montpellier) is resistant to the gall formation (Szegedi, 1983), as well as V. amurensis and its hybrids can serve as sources of crown gall resistance using them in the rootstock hybridization (Szegedi et al., 1984; Szegedi and Kozma 1984). Probably, the aerial crown gall formation, namely the tissue disorders in the canes and trunks can be avoid using of non-host and crown gall resistant rootstocks. It can be theoretically expected, if the scion parts visually or latently infected after grafting onto some 'non-host' rootstocks the vines can be maintained in a commercial vineyard with acceptable performances, without serious tissue disorders and economical disadvantages.

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The Isolation and Characteristics of *Roesleria hypogaea* Thüm. *et* Pass

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The fungus, *Roesleria hypogaea*, is living on the roots of fruit-trees, damaging root hair formation; consequently, the trees may die. To examine this fungus, we made inoculations on Biomalz-agar medium with the suspension of spores obtained from apothecia formed on quince roots. The culture started to grow after 2 weeks at 18–20 °C. It grew throughout the whole surface of the medium in a Petri-dish (95 mm diameter) in 3 months. The apothecia developed after 7–9 months.

We investigated with SEM the apothecia and ascospores developing under both natural conditions on incubated roots, and on agar plates and we found that they were identical.

The *R. hypogaea* strain isolated from quince root in Érd is preserved in the Department of Plant Protection of GYDFV as comparative material aiding future identification.

In Hungary, *Roesleria hypogaea* occurs not only on the roots of vine but of fruit-trees as well and it kills mainly the young trees (Véghelyi, 1984a). Its mycelium lives under the epidermis of roots, its fruiting-body, the apothecium, developes in large numbers on the roots, mostly in autumn (Fig. 1). As a result of this, few or no root hairs are formed on the fibrous roots. The trees grow poorly because of reduced nutrient uptake and during a period of drought they may die (Véghelyi, 1985a).

The pathogen spends its entire life cycle 5–35 cm deep in the soil which makes it difficult to study its biology, pathogenesis and the influence of fungicides. To carry out in *vitro* tests a pure culture of the pathogen was needed.

Review of the literature

R. hypogaea was isolated and first cultured by Beckwith (1924). He obtained ascospores from the fruiting bodies of *R. hypogaea* from roots of apple trees, on corn-meal agar and oat-meal agar. On these media he obtained colonies with a whitish shine, later blackish green appearance. In five to seven months, whitish grey apothecia developed. The heads contained asci and paraphyses which were slender and extended beyond the asci. The fruiting bodies (4 to 4.5 mm × 1 mm) were somewhat larger than those on the roots.

Fig. 1. The fruiting bodies of *Roesleria hypogaea* on quince root (foto: L. Migend)

Beatrix Hanf at Forschungsanstalt Geisenheim, Institut für Phytomedizin und Pflanzenschutz also succeeded in isolating and establishing cultures of *R. hypogaea* (unpublished). She kindly sent me a sample culture, isolated from vine root on 10 May, 1984. This isolate was registered in the plant disease collection of GYDFV. The culture was light green and no apothecium developed even after several subcultures.

Materials and Methods

In the autumn of 1977, 152 'Bosc kobakja' pear-trees on quince rootstock (with Hardy intergrafting) were planted in the experimental orchard of the GYDFV in Érd. The trees were bought from the nursery garden of the "Szikrai" State Farm, Kecskemét. We identified *R. hypogaea* infection on the trees already at the date of purchase, and 46% of the infected trees died in the first year (Véghelyi, 1985b). Due to the death of 60% of the trees within 3 years, all the trees were uprooted in June, 1980. We confirmed that the cause of death was infection by *R. hypogaea*.

In spring, 1981 the plot was replanted, without sterilizing the soil, by pear/quince trees from the nursery-garden of GYDFV in Érd. We took samples from the roots of the graftings before planting and foun that it was free from *R. hypogaea*. A number of these trees were removed on 15 March 1983 to verify whether the originally healthy roots had been infected in the soil by *R. hypogaea*. We took samples from the roots of 30 randomly chosen uprooted trees. The samples, after soaking and washing the roots in running water, were numbered and incubated. They were regularly checked every month.

On 12 June, 1984, spore suspension obtained from the developing fruiting bodies of the samples was poured onto Biomalz-agar according to the Koch-method. Monospore cultures were established from the germinating spores after one week and further subcultures were made on 17 August and 13 September, 1984.

The apothecia and ascospores, developing on the roots and on the plates, were examined by a JEOL JSM 25 S II scanning electron microscope to prove that the culture growing on the Biomalz-agar medium was identical with the pathogen *R. hypogaea.*

Results

From the 30 pear/quince trees uprooted 15 March 1983, 6 had already died by the time of uprooting, while 6 were poorly developed and partly dead and 18 was weakly developed. On two of the dead trees, old fruiting bodies of *R.hypogaea* were visible. After incubation, mass development offfruiting bodies was found on 4 samples by January 1984, on 2 samples by February, on 2 samples by March and on 19 by September. No apothecium was found on one sample.

On the roots of the poorly developed but still living tree No. 757/1983, there was no identifiable pathogen when uprooting. We found developing apothecia on 17 January 1984 and fully developed apothecia on 12 March 1984. The roots continued to develop and to form calli while being incubated. The fully developed apothecium opened so we poured on agar plate the ripe ascospores gathered in large numbers from the apothecium head. The germinating spores from isolate 1395/1984. 03. 12. were individual subcultured on 7 May 1984 and from the

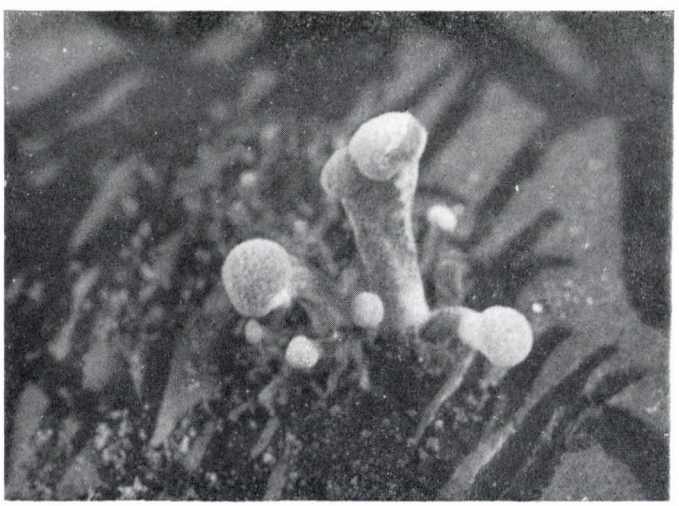

Fig. 2. The fruiting bodies of *Roesleria hypogaea* on Biomalz-agar medium (foto: L. Migend)

monospore-cultures obtained this way, we made further transfers on 17 August and 13 Sept. 1984.

The monospore-cultures developed extremely slowly. Two weeks after the transfer, the diameter of the cultures developing in an ordinary appearance was 6–7 mm and after 4 weeks it was 35–37 mm. It covered the whole surface of Biomalz-agar in the Petri-dish during 3–4 months.

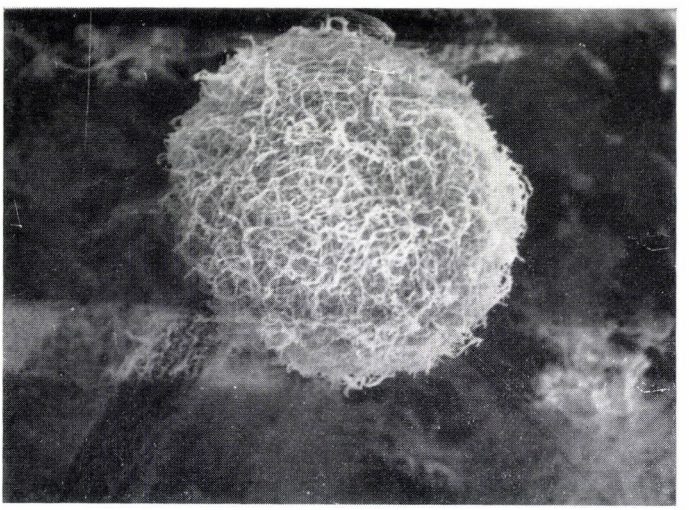

Fig. 3. An apothecium of *Roesleria hypogaea* from quince root (foto: E. Eiler)

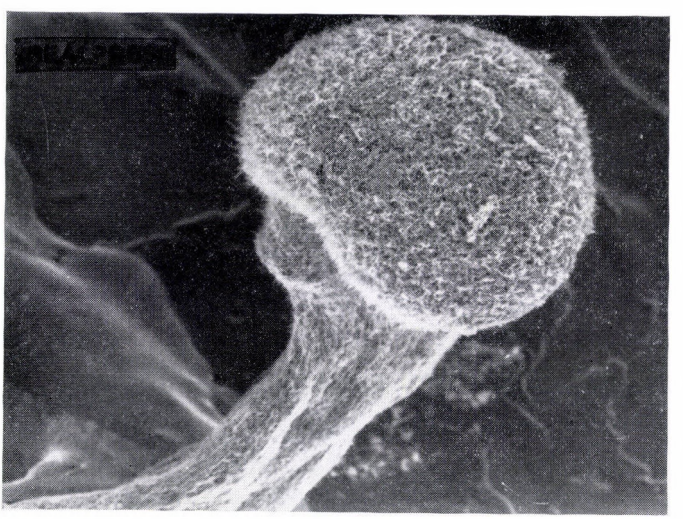

Fig. 4. An apothecium of *Roesleria hypogaea* from Biomalz-agar medium (foto: E. Eiler)

The colour of the young colony was white, later pale moss-green and consisted of non-sporulating, loose and downy mycelium. The darkest was the centre of the colony and it was surrounded by ring-shaped zones gradually lighter towards the edge. The outermost ring was nearly white. After half a year, the culture darkened, turned into moss-green and the zonality disappeared. The medium under the culture darkened, became black and compact.

Fig. 5. The ascospores of *Roesleria hypogaea* from a quince root. Magnification: 1500× Bar equals 10 μm (foto: E. Eiler)

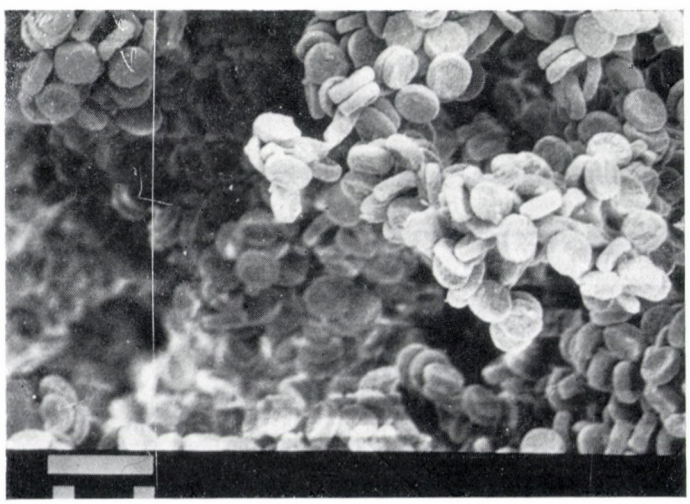

Fig. 6. The ascospores of *Roesleria hypogaea* from Biomalz-agar medium. Magnification: 1500× Bar: 10 μm (foto: E. Eiler)

On the cultures, kept in either daylight or in darkness, fruiting bodies developed by 20 March 1985. The apothecia grown in the darkness were white while those cultures under natural photoperiodic conditions produced fruiting bodies with pale-grey heads and light-grey stalks. The apothecia grew in abundant groups, but only 8–12 in one culture became fully developed (Fig. 2). The other fruiting body initials could not fully develop and did not yield spores because the medium had dried up.

The shape of the apothecia developing on the medium differed from those formed under natural conditions or on roots in the laboratory (Fig. 1). For this reason, the identification was carried out with a scanning electron microscope. Fruiting bodies developing on a single root are of different size even under natural conditions. The apothecia developing on cultured mycelia differed in size, too, but all the apothecia which were fully developed and yielded ascospores, were in size between the two extremes measured on the root.

The length of the stalks of the fruiting bodies produced on the medium was 1–6 mm, with a mean of 4 mm (10 measurements). This was equal to the 1.5–7 mm range, mean 4.05 mm (N = 100), measured on vine-roots, but greater than the length of the stalks on the roots of fruit-trees, which, for example, on quince hosts was 1–4 mm with a mean of 2.15 mm (Véghelyi, 1984b).

The diameter of the head was also similar to those on vine but larger than those on fruit-trees. The important difference was the ratio of the diameters of the head to the stalk. While the diameter of the fruiting bodies developed in natural conditions 4–5 times exceeding that of the stalks, the diameter of the fruiting bodies developed on medium was only 2–3 times larger than that of the stalks. This difference is clearly visible on the SEM pictures (Figs 3 and 4). By means of the electron microscopic examinations, we found the formation of the ascospores, of the paraphyses and the structure of the fruiting bodies to be identical.

The shape and size of the ascospores from apothecia growing either on the medium or on the root were quite similar. The ascospores were disk-shaped, measuring 4.5–5 μm in diameter with a disk thickness of 1.5–2 μm. They were formed in groups of 8 laying on each other in the ascus (Figs 5 and 6).

The isolate No. 1395/1984, derived from the isolation of *R. hypogaea* ascospores on roots of a quince tree coded Érd, 757/1983, and examined with SEM was found to be identical with the fungus *Roesleria hypogaea*. The strain identified in this way is being maintaned in the Pathology Laboratory of the Department of Plant Protection, GYDFV. This strain can serve as a comparative material for the identification of pathogens obtained from symptom-free fruit-tree roots. The culture gives the opportunity of examining the fungicide sensitivity of *R. hypogaea*, as well.

Acknowledgements

I wish to thank Emil Eiler, the leader of Realpress ÁT. Laboratory, for giving me access to the SEM and for taking the photographs.

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Cross Protection of Watermelon and Cucumber Plants Against Wilt by Prior Inoculation With an Irrespective Forma Specialis of *Fusarium oxysporum*

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Fusarium oxysporum f. sp. *niveum* and *F. o.* f. sp. *cucumerinum* were found to be externally and internally seed-borne in watermelon and cucumber, respectively. The seed-borne nature of the two formae speciales of *F. oxysporum* might be regarded as the first record on the two hosts in Egypt. Each forma specialis was found in plant debris mixed with seeds of the respective host. Each forma specialis caused severe wilt symptoms to its respective host. Prior inoculation of watermelon and cucumber plants with the irrespective then, five days later, with the respective isolates, resulted in no apparent wilt symptoms. In vitro tests, both formae speciales showed antagonistic effect on PDA and Czapek Dox agar media when one forma specialis was grown on Czapek Dox liquid medium incorporated with crude filtrate of the other forma specialis. The dry weight decreased with increase in crude filtrate concentration. The crude filtrate of one forma specialis substantially reduced the percentage of conidial germination and germ tube elongation of the other forma specialis.

Watermelon (*Citrullus lanatus* L.) and cucumber (*Cucumis sativus* L.) are popular cucurbits in Egypt. Cucurbits seeds were reported to bear important phytopathogenic mycoflora. *Fusarium oxysporum* Schl. had been reported to be seed-borne in tinda (*Citrullus vulgaris* Schad. var. *fistulosus* Stock) in India (Suryananryana and Nath, 1963). Protection against *F. oxysporum* f. sp. *niveum* was induced by inoculation with *Helminthosporium carbonum* then with *F. oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen or *Verticillium albo-atrum* Reinke and Berth. (Scimotsuma et al., 1972). Ishiba et al. (1981) have shown cross protection of cucumber scedlings against anthracnose by using a hypovirulent strain of *F. oxysporum* f. sp. *cucumerinum* Owen. They found that the number and area of lesions on the first leaf were reduced on placing microconidia on hypocotyl cut ends two to four weeks before challange inoculation with *Colletotrichum lagenarium* (Pass) Ellis and Halst.

The present work was carried out with respect to (1) test health of seed samples of watermelon and cucumber for *F. oxysporum*, (2) to test the effect of prior inoculation of watermelon and cucumber plants with irrespective isolate before challenge inoculation with the respective one.

Table 1

Comparison between Agar and Blotter plate methods in seed health testing for (200 seeds on agar and 200 seeds

Plant species	Fungus	Localities					
		Alexandria					
		1		2		3	
		A	B	A	B	A	B
Watermelon	*F. oxysporum*	—	—	6	11	17	18
Cucumber	*F. oxysporum*	35	10	16	4	14	6

A: Agar method, B: Blotter method, — : not detected.

Materials and Methods

Seed health testing

Six seed samples, each of watermelon and cucumber were collected from seed dealers in Alexandria and Behera Governorates during the seasons of 1984 and 1985. Two hundred seeds of each sample were tested using the standard blotter and Agar methods (ISTA, 1976). In the Blotter method, ten seeds were plated on three moistened blotters in the petri dish. In the Agar method, seeds were pretreated with 1% sodium hypochlorite solution for five minutes, then placed on agar plates at the rate of 10 seeds/dish. Dishes were incubated at 20 °C under 12 hours alternating cycles of NUV light and darkness for 7 days. Developing fungal cultures were examined and recorded. Pute cultures were maintained on PDA slants at 5 °C.

Location of fungi in the different seed parts

One hundred seeds each of watermelon and cucumber were used. The seeds were first soaked in tap water for 60 minutes in order to facilitate separation of the different seed parts. Seed parts consisted of the testa, cotyledons and embryo axis. Seed parts of each seed were incubated at the same conditions as mentioned before.

Isolation from plant debris mixed with seed samples

Isolations were carried out from plant debris mixed with watermelon and cucumber seed samples. One hundred small pieces each from four seed samples of watermelon and two seed samples of cucumber were surface-sterilized for five minuts in 1% sodium hypochlorite solution, then plated at the rate of 5 pieces/dish. Dishes were incubated at the same conditions as indicated earlier.

Fusarium oxysporum of watermelon (cv. Giza 1) and Cucumber (cv. Beta Alfa) on blotters for each sample)

and Sample number									
Beheira						Giza			
4		5		6		8		9	
A	B	A	B	A	B	A	B	A	B
12	8	19	10	—	—	21	14	30	21
25	15	13	8	15	8	—	—	—	—

Prior inoculation tests

Isolates of a certain fungus obtained both from watermelon and cucumber seeds were grown each on 50 ml Czapeh Dox liquid medium contained in 250 ml conical flasks and incubated at 30 °C for five days. Fungal mats were blended and filtered through two layers of muslin cloth. Conidial concentration in each filtrate was determined and adjusted to 10^6 conidial/ml using a haemocytometer. Inoculation tests were carried out using Bugbee and Sappenfield's method (1968). One ml of the spore suspension was injected into the hypocotyl of 4-week-old watermelon and cucumber plants 23 gauge hypodermic needle and 5 ml syringe. A bead of conidial suspension was formed at the needle was injected into the lower stem at 45° to the stem until the bevel of the point was just visible. The drop of inoculum that formed in the axis of the stem and needle disappeared into the plant, giving visual evidence of inoculation.

Injection was carried out, firstly by the irrespective isolate, then followed by the respective isolate after five days, i.e. injection was carried out to watermelon plants by the cucumber isolate of the fungus under test, then followed by the watermelon isolate of the same fungal species after five days from the first inoculation. The reverse was carried out with cucumber plants. Check treatments were carried out either by injecting watermelon and cucumber plants with their respective fungal isolates, and with sterile Czapek Dox solution, or by keeping the plants uninoculated.

Results

Seed health testing

Seed health testing of watermelon and cucumber (Table 1) show that *Fusarium oxysporum* is present in seed samples of both hosts. The fungus was more frequently found on agar than on blotter, reaching up to 30% in sample no. 9 of watermelon and up to 35% sample no. 1 of cucumber.

Location of the fungus in seed parts

Isolations from seed parts of watermelon and cucumber (Table 2) show the presence of *F. oxysporum* in the testa, cotyledons and embryo axis, with higher frequencies on agar than on blotters. The fungus was located more in the testa.

Table 2

Location of *Fusarium oxysporum* in seeds of watermelon (cv. Giza 1) and cucumber (cv. Beta Alfa) (100 seeds on agar and 100 seeds on blotters for each sample)

Plant Species	Sample no	Infection Percentage					
		Agar			Blotters		
		Testa	Coty-ledons	Em-bryo axis	Testa	Coty-ledon	Em-bryo axis
Watermelon 3	*F. oxysporum*	12	2	1	14	4	1
Cucumber 1	*F. oxysporum*	18	9	6	10	2	1

Isolations from plant debris

Isolations from plant debris mixed with watermelon and cucumber seeds showed the presence of *F. oxysporum* at the rate of 7–18% in plant debris of four seed samples of watermelon and at the rate of 5 and 23% in plant debris of two samples of cucumber.

Prior inoculation tests

The watermelon and cucumber seed-borne *F. oxysporum* isolates caused severe wilt symptoms (Figs 1 and 2 middle) and vascular necrosis to their respective host plants. Hence the present seed-borne watermelon and cucumber isolates of *F. oxysporum* could be identified as *F. oxysporum* schl. f. sp. *niveum* (E. F. Smith) Snyder of Hansen and *F. oxysporum* schl. f. sp. *cucumerinum* Owen, respectively. However, when watermelon and cucumber plants were pre-inoculated with irrespective formae speciales of *F. oxysporum*, then inoculated, five days later, with their respective formae speciales, no wilt symptoms appeared (Fig. 1 & 2 right) and on microscopical examination no distinct, vascular necrosis occurred.

In Vitro interaction between F. oxysporum f. sp. niveum and F. oxysporum f. sp. cucumerinum

Growing *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *cucumerinum* against each other on Gzapek Dox agar and PDA media showed antagonism between them as expressed by a marked inhibition zone (Fig. 3).

Growing *F. oxysporum* f. sp. *niveum* or *F. oxysporum* f. sp. *cucumerinum* each on different concentrations (0, 10, 20 and 50%) of crude sterile Gzapek Dox culture filtrate of the other forma specialis showed decrease in dry weight

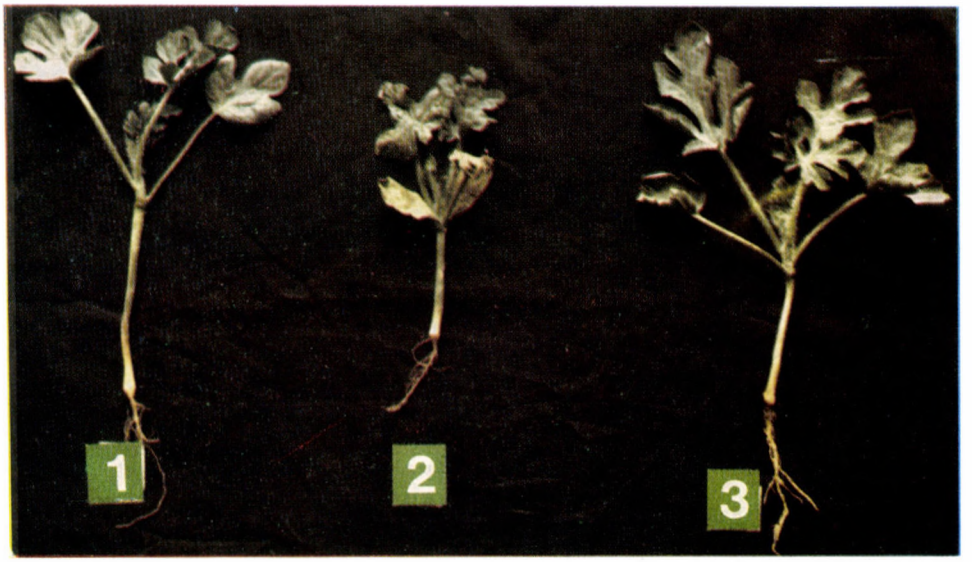

Fig. 1. Watermelon plants non-inoculated check (1), inoculated with *F. oxysporum* f. sp. *niveum* (2), inoculated first with *F. oxysporum* f. sp. *cucumerinum* then 5 days later with *F. oxysporum* f. sp. *niveum* (3)

Fig. 2. Cucumber plants, non-inoculated check (1), inoculated with *F. oxysporum* f. sp. *cucumerinum* (2), inoculated first with *F. oxysporum* f. sp. *niveum* then 5 days later with *F. oxysporum* f. sp. *cucumerinum* (3)

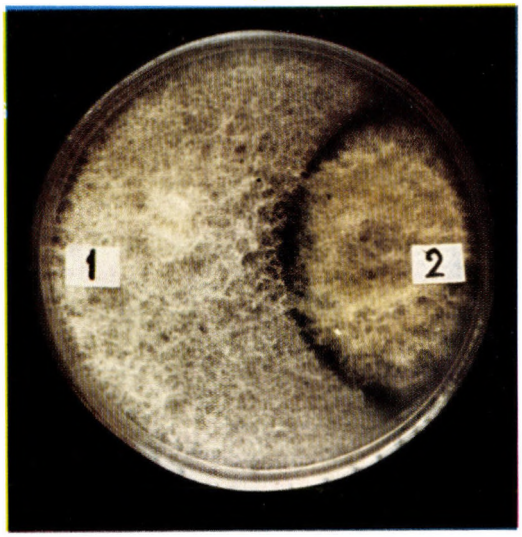

Fig. 3. Antagonism between *F. oxysporum* f. sp. *cucumerinum* (1) and *F. oxysporum* f. sp. *niveum* (2) on PDA after 10 days at 30 °C

of the mycelial growth, and the decrease was proportional with the increase in the concentration of the filtrate (Table 3).

Determining the precentage of conidial germination and germ tube elongation of *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *cucumerinum* in the crude sterile Gzapek Dox culture filtrate of the respective and irrespective forma

Table 4

Effect of crude filtrates of *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp.

Time (hours)	Germination Percentage					
	Conidia of					
	F. oxysporum f. sp. *niveum* in filtrate of				Water	
	F. oxysporum f. sp. *niveum*		*F. oxysporum* f. sp. *cucumerinum*			
	% germination	germ tube length (μ)	% germination	germ tube length (μ)	% germination	germ tube length (μ)
2	2.8	16.5	0.8	6.6	6.6	25.6
4	28.0	81.1	1.2	23.8	16.0	53.8
8	87.0	126.4	3.2	28.6	61.0	88.1

* Mean of 5 replicates, each replicate 100 spores.

Table 3

Effect of different concentrations of crude culture filtrates of *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *cucumerinum* on mycelial growth of the other fungus after 17 days of incubation at 30 °C

Filtrate of	Fungus	Dry weight (mg)			
		Filtrate concentration %*			
		0	10	20	50
F. oxysporum f. sp. *niveum*	*F. oxysporum* f. sp. *cucumerinum*	2870	2540	1940	500
F. oxysporum f. sp. *cucumerinum*	*F. oxysporum* f. sp. *niveum*	6150	5430	2580	2060

* Means of four replicates

specialis in Van Tieghem cells (Table 4) showed that the culture filtrate of each forma specialis had a strong inhibitory effect on conidial germination and germ tube elongation of the other forma specialis. At the meantine, however, the crude filtrate of each forma specialis promoted the germination of its conidia.

Discussion

The seed-borne mature of *F. oxysporium* f. sp. *niveum* and *F. oxysporum* f. sp. *cucumerinum* on watermelon and cucumber is regarded as first record on these hosts in Egypt (El-Helaly et al., 1966 and Aly et al., 1972). Each forma specialis caused severe wilt and vascular necrosis to its respective host, however,

cucumerinum conidial germination and germ tube length of the two formae speciales

and germ tube length*

Conidia of					
F. oxysporum f. sp. *cucumerinum* in filtrate of				Water	
F. oxysporum f. sp. *niveum*		*F. oxysporum* f. sp. *cucumerinum*			
% germination	germ tube length (μ)	% germination	germ tube length (μ)	% germination	germ tube length (μ)
0.0	0.0	2.6	13.2	10.0	21.1
1.2	13.2	34.4	75.1	16.6	45.1
1.2	17.1	78.0	114.2	31.6	87.8

prior inoculation of watermelon and cucumber with irrespective forma specialis, then five days later, with the respective isolate, resulted in no wilt symptoms. *In vitro* tests showed antagonism between both formae speciales when grown opposite to each other on PDA. Such antagonistic effect appeared also when one forma specialis was grown in Gzapek Dox liquid medium incorporated with crude filtrate of the other forma specialis. The dry weight of the growing fungus was adversely affected. The crude filtrate of one forma specialis adversely affected also the conidial germination percentage and germ tube elongation of the other forma specialis. This phenomenon supports the work of Long (1963) who found that development of cabbage wilt incited by *F. bulbigenum* var. *tracheiphilum* was inhibited by pre-inoculation with *Cephalosporium* spp. Roy and Patel (1963) found that when one-week old cotton seedlings were preinoculated with isolates of *Cephalosporium* sp. then, inoculated with a virulent isolate of *F. o.* f. sp. *vasinfectum* within 48 hours the prior inoculation could inhibit cotton wilt. Davis (1964) obtained cross protection of *Fusarium* wilt with eight formae speciales of *F. oxysporum*. He inoculated seedlings of wilt suscepts in test tubes with forms non-pathogenic to the host species concerned. He found that the non-pathogenic forms readily invaded the root a few millimeters from the point of inoculation. Ishiba et al. (1981) obtained cross protection of cucumber seed lings against anthracnose by preinoculating a hypovirulent strain of *F. oxysporum* f. sp. *cucumerinum*. The number and size of lesions on the first true leaf were reduced by placing microconidia on hypocotyl cut ends two to four weeks before challenge inoculation with *Colletotrichum lagnarium*. Ogawa (1985) obtained cross protection of sweet potato against wilt incited by *F. oxysporum* f. sp. *batatas* by prior inoculation with non-pathogenic *F. oxysporium* isolate. Garibaldi et al. (1986) could reduce Fusarium wilt of carnation by artificial infestation of steam disinfested soil or root dipping with saprophytic *F. oxysporum* and *F. solani*.

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Characteristics of Isolates of *Sporidesmium sclerotivorum* Uecker, Ayers and Adams in Hungary

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In the spring of 1987 *Sporidesmium sclerotivorum* was isolated from sclerotia of *Sclerotinia sclerotiorum* overwintered in the field. Sclerotia had been collected in the vicinity of Tac, Fejér county, Hungary. The morphological characteristics of these isolates are the same as those of the species described at Beltsville in 1978. The only difference is the number of transversal septa in the conidia. In our isolates conidia with 9 transversal septa are common.

Our isolates could not be satisfactorily cultured in the media suggested by American researchers.

On the overwintered sclerotia, beside *Sporidesmium sclerotivorum*, other mycoparasitic fungi such as *Coniothyrium minitans*, *Trichoderma viride* and *Gliocladium catenulatum* could be found, too.

Sporidesmium sclerotivorum is a mycoparasitic fungus belonging to the Dematiaceae family of the Hyphomycetes subclass. It was first found at Beltsville, USA, in 1978 (Uecker et al., 1978). All mycoparysitic organisms that decrease significantly the quantity of *Sclerotinia sclerotiorum* inoculum in nature, may potentially be suitable for the biological control of the pathogens. Among the so far known mycoparasitic fungi of *Sclerotinia sclerotiorum*, *Sporidesmium sclerotivorum* has proved to be one of the most promising antagonist organism (Ayers et Adams, 1981). *Sporidesmium sclerotivorum* has special mycoparasitic characteristics: 1. living sclerotia in the soil emit compounds that stimulate the germination of conidia of *S. sclerotivorum;* 2. *S. sclerotivorum* colonizes living sclerotia more actively than dead ones; 3. Mycelia of the mycoparasite grow from one sclerotium to the other, in case if the distance between the two sclerotia is not more than 9 mm; 4. *S. sclerotivorum* tolerates environmental factors (Papavizas, 1984).

According to the American investigations, the mycoparasite at a soil concentration of 100–1000 conidium/kg is capable of destroying the sclerotia of *Sclerotinia spp.*, *Sclerotium cepivorum* and *Botrytis cinerea* (but not those of *Sclerotinia rolfsii)* at a rate of 95% in 10 weeks (Papavizas, 1984). Papavizas also reported (1984) that a private enterprise in the USA had started manufacturing Sporidesmium biopreparatum for the purpose of biological control of Sclerotinia species.

In Hungary regular investigations have been carried out since 1983 in order to establish the occurrence of the antagonists and mycoparasites of *Sclerotinia sclerotiorum*. During these investigations we have established the occurrence of *Sporidesmium sclerotivorum*.

Materials and Methods

In the spring of 1987 we collected overwintered sclerotia on the plot I/15 of the "Kinizsi" collective farm near Tac. The collective farm had cultivated corn on this plot. The predecessor of corn was wheat. The soil of this plot is of chernozem type. The pH value of the soil suspension was 6.25. 50 kg of soil sample was collected from 100 sampling spots in the plot and of the plot we gained 135 sclerotia through bolting. Sclerotia were put to laboratory examination.

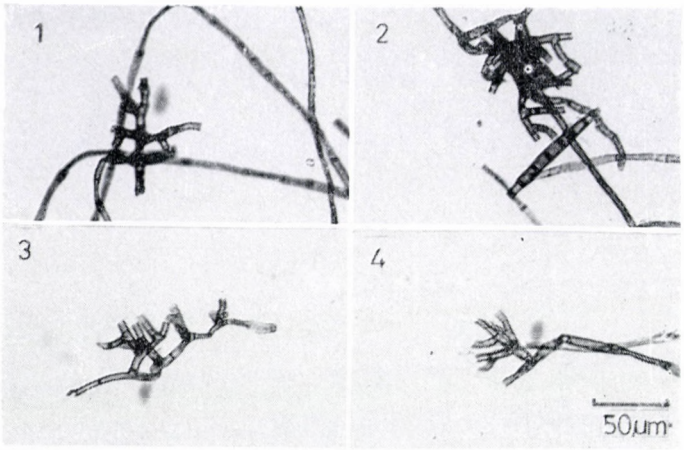
Sclerotia were surface sterilized in 75% ethanol for 30 seconds, then sclerotia were put in Petri dishes with diameter of 10 cm, containing non-sterile chernozem-type soil. Incubation took 5 weeks. The temperature of incubation was $20 \pm 2\,^{\circ}C$. The development of fungus colonies appearing on the surface of sclerotia was observed daily by means of stereomicroscope. Fungus was identified on the basis of the morphological characteristics. Choosing these methods we took into account and made use of Vajna's data (1987) concerning the growing of mycoparasitic fungi.

Results

According to the examination made after the expiration of the incubation time, 61 sclerotia out of 135 were infected by *Sporidesmium sclerotivorum*.

The colony of *Sporidesmium sclerotivorum* on the sclerotia of *Sclerotinia sclerotiorum* as well as the morphology of the fungus can be characterized as follows:

The loose, medium brown mycelium of *Sporidesmium sclerotivorum* completely enlaces the surface of the sclerotia, and several thousands of conidia can be observed on the conidiophores formed on the hypha. The mycelium of *Sporidesmium sclerotivorum* enlaces also the soil particle that are near the sclerotia. The conidiophore is brown, forming separately or in groups (Figures 1, 2, 3, 4). It has a smooth surface and consists of two or three cells. The last cell of conidiophore is the conidiogenous cell. After separating of conidia, the conidiogenous cells always remain without a vertical wall, they are "empty" (Figure 5). Conidia are forming one by one in a holoblastic way (Figure 6). The young conidium is of light brown colour (Figure 6). The young conidium is of light brown colour (Figure 6) straight or slightly bended. The septa in the conidium are slightly darker than the



Figs 1.2.3.4. Branched conidiophores of *Sporidesmium sclerotivorum*

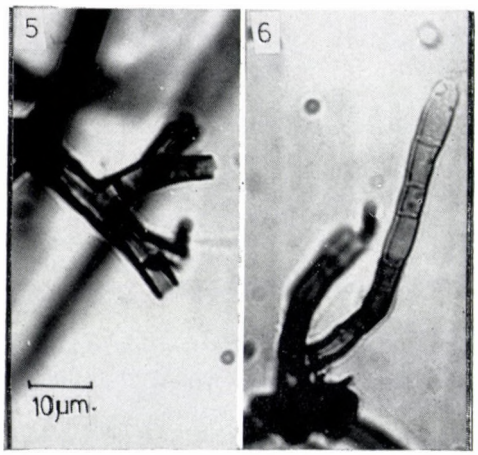
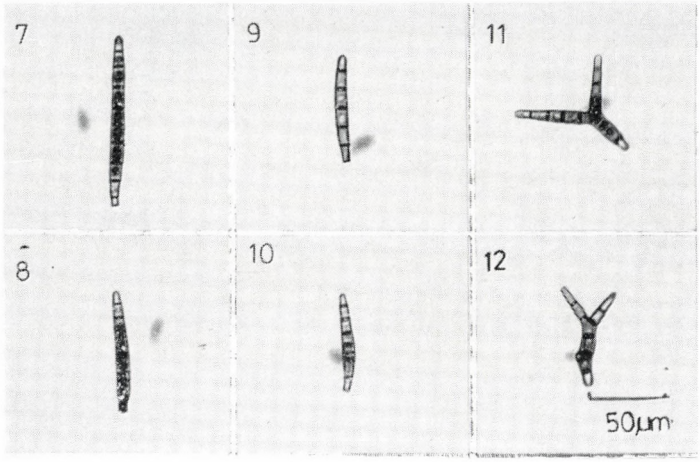


Fig. 5. Conidiogenous cell always remain without a vertical wall, "empty"
Fig. 6. Conidia are forming one by one

cell wall of conidium. Their size is 60–125 × 6–8 µm. The number of vertical walls in the conidia is (5)–6–7–8–(9) (Figures 7, 8, 9, 10). Conidia of the shape of Y often can be observed (Figures 11, 12).

S. sclerotivorum can safely be identified on the basis of the conidia, conidiophores and the way of conidium development. Conidiophores of the Selenosporella type, whose appearance had also been stated by American authors (Uecker et al., 1978) were rarely found in our isolates.



Figs 7.8.9.10. Different septedated conidia of *Sporidesmium sclerotivorum*
Fig. 11.12. Y shaped conidia

At the examination of the 135 sclerotia overwintered in nature beside *Sporidesmium sclerotivorum* we also found the mycoparasitic fungi *Coniothyrium minitans*, *Gliocladium catenulatum*, *G. roseum* and *Trichoderma viride*.

Discussion

Sporidesmium sclerotivorum was first described by Uecker, Ayers and Adams in 1978. The morphological characteristics of the Hungarian isolates were exactly the same as those described by the above mentioned authors. In the colonies of Sporidesmium isolated in Hungary often could be found conidia with 9 vertical septa, while the American description marks (5)–6–7–8 vertical septa inside the conidium. Conidia of Hungarian isolates are 60–125 μm in length, Uecker and his co-workers (1978) mark this length to be 60–92 μm.

This mycoparasite could not be cultured artificially for longer than a year. In the soil our isolates grew badly.

The only difference stated by us was in the number of the transversal septa in the conidia.

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Application of Multivariate Analysis for Characterizing the Relationships Between Wild *Lactuca* spp. and *Bremia lactucae*

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In studies on host-parasite specificity infection types and patterns of interaction sometimes need to be described quantitatively. However the application of classical statistical methods sometimes results in the loss of information. Data relating to host-parasite interaction can be examined using methods of multivariate analysis such as cluster analysis and principal component analysis. These procedures have been applied to data on the interaction between genotypes of *Lactuca* spp. and isolates of *Bremia lactucae*. On the basis of localization in dendrograms or graphs of principal components, host genotypes and species or parasite isolates can be grouped according to their similarity. The relationships allow interpretations to be made about the characteristics of resistance and the relationship (genetic or taxonomic) between host and parasite phenotypes.

Types of host-parasite specificity can be categorized in several ways (Lebeda, 1984a) and are likely to result from ieveral different mechanisms. Race-specific resistance, regulated by gene-for-gene relationships, has been investigated intensively. Phenotypes are often described by a binary notation: "+" (susceptible response) or "−" (resistant response). When examining the outcome of any host parasite interaction the aegricorpus phenotype is what is actually assessed (Loegering, 1966). The aegricorpus phenotype can be described in several ways, but infection type (sensu Browder, 1985) is the most usual. Infection type is often seen to be quantitative and continuous when a sufficiently large number of different interactions are examined.

The study of host plant resistance is an important activity (Harris and Frederiksen, 1984), but the application of mathematical theory to the solution of practical problems has received little attention. Van Der Plank (1968) suggested the use of two-way analysis of variance (ANOVA) to study interaction and later described the application of ranking-tests (Van Der Plank, 1978). Winer (1984) introduced the idea of using additive and multiplicative models to distinguish between different types of resistance. Jenns and Leonard (1985) used analysis of variance and regression to estimate the relative host specificity of quantitatively expressed resistance.

Interpretation of large numbers of interactions is difficult even when viewed qualitatively (Lebeda and Jendrulek, 1987a; Priestly et al., 1984). When quantitative data are considered, interpretation is more difficult. Methods of multivariate analysis (e.g. cluster analysis, principal component analysis, factor analysis) may therefore be helpful in the interpretation of these types of data. In studies on isolate pathogenicity determining similarity between isolates is important for theoretical and practical reasons. Cluster analysis and graphical representation of data differentiates quantitative pathogenicity characters. Kennedy et al. (1986) used cluster analysis successfully to express differences in virulence and aggressiveness of *Phytophthora fragariae* isolates. Multivariate analysis of quantitative data could also be exploited for studies on changes in pathogen populations conducted over time and space and for observing evolutionary trends in host-pathogen association.

Materials and Methods

The data used resulted from a study on the interactions between 36 *Lactuca* accessions plus one *L. serriola* × *L. sativa* hybrid and 7 *Bremia lactucae* isolates originating from *L. serriola* (Lebeda, 1986). Interactions were examined in seedlings and adult plants. The parameter assessed was sporulation intensity over the range 0 to 100%. More information on methodology has been reported previously (Lebeda, 1986).

Mathematical methods of analysis were based on hierarchical clustering techniques. To determine the dissimilarity and distance apart of individual interaction patterns, calculation of the Euclidean distance was involved. The initial matrix of dissimilarity coefficients was analyzed using a "Ward-Wishart" clustering method (Lukasova and Sarmanova, 1985).

Principal component analysis was conducted to assess variability in the data set. It was achieved in the usual way using a covariance matrix. Principal components are newly calculated, compound parameters, based on all original characters. Each principal component consists of an eigenvalue indicating the represented amount of total variation and an eigenvector with factor loads indicating the relation between the principal component and the original characters. The principal components were ranged in decreasing order, the first one being the most important. The calculations were made using AVIDAT, a set of programmes for multivariate data analysis (Jendrulek, unpublished; Lebeda and Jendrulek, 1987a, c, 1988).

Results

Determination of similarity among Lactuca accessions

Cluster analysis of sporulation intensity on seedlings of 35 *Lactuca* species and accessions is plotted in Fig. 1. The set can be subdivided into seven more or

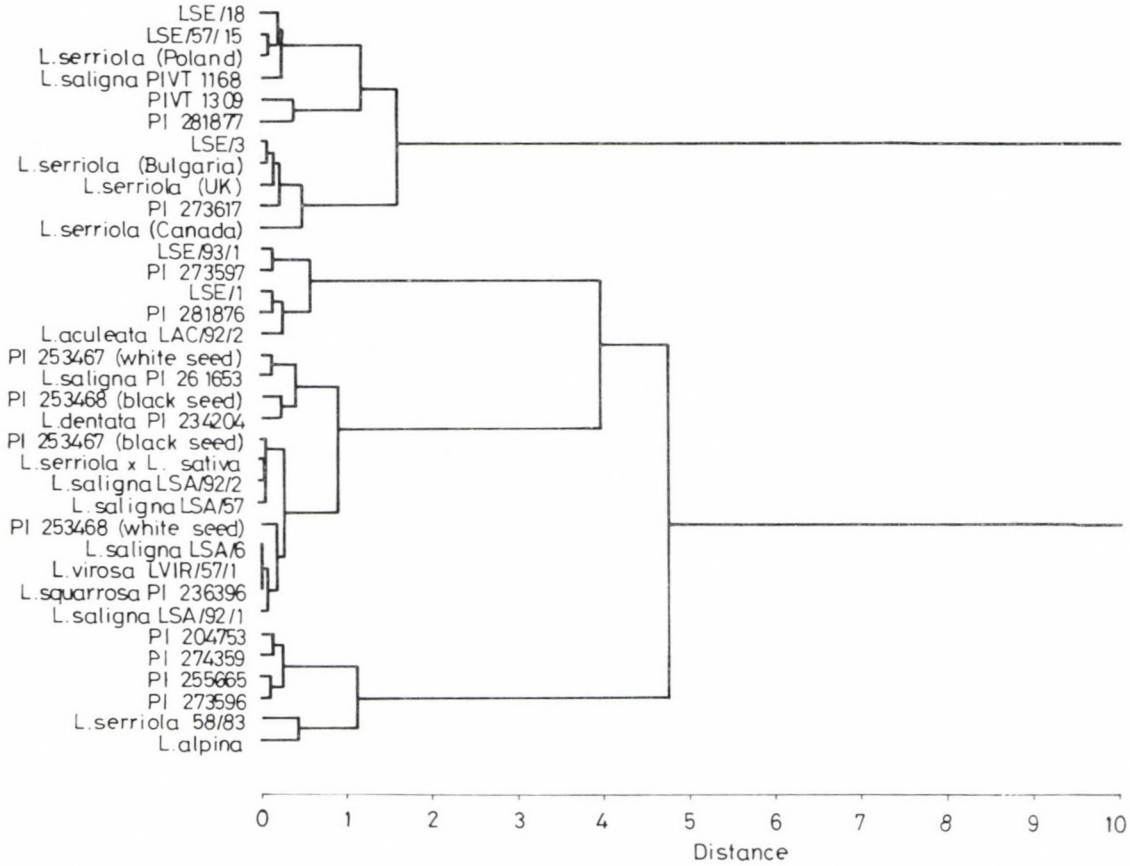

Fig. 1. A dendrogram (Ward-Wishart method) showing similarity and successive clustering of 35 *Lactuca* species and accessions (seedling stage)

less clear-cut clusters on the distance level 1. In the two clusters at the top of Fig. 1 *L. serriola* is predominant and these are independent from the remainder. Similarity is not related to geographical origin. PIVT 1168 (received as *L. saligna)* is an accession of *L. serriola* (Lebeda, 1986). High susceptibility to most or all isolates and a high level of sporulation intensity are typical of accessions in these two clusters. *L. serriola* also predominantly comprises the fourth cluster from the top of Fig. 1 (the only exception being *L. aculeata*). These four clusters represent most of the *L. serriola* accessions studied, and diversity is due to the presence or absence of a particular differential response. A further cluster is formed especially by species such as *L. saligna*, *L. dentata*, *L. virosa*, *L. squarrosa* and by the *L. serriola* × *L. sativa* hybrid. *L. saligna* and *L. virosa*, in particular, form a compact cluster noted for great similarity of interaction patterns, and probably reflecting a "non-host" resistance (Lebeda, 1986). The last two clusters are formed by accessions of *L. serriola* (and one of *L. alpina*). Generally a low sporulation intensity in all accession/isolate interactions is characteristic of these clusters. PI 204753 represents an exception in which no sporulation was observed after inoculation with the isolate 3/82. These clusters are interpreted as accessions possessing a high level of race non-specific resistance.

In adult plants the responses appear to be more clear-cut and this was also reflected in the clustering. The set broke up into six conspicuous clusters (on the distance level 2.5) (Fig. 2). The first five clusters contain all accessions of *L. serriola*, together with *L. alpina*, *L. dentata*, and PIVT 1168. In the first cluster accessions express a high level of universal susceptibility. In other cluster groups clear-cut differential responses (e.g. PI 204753, PI 274359) are apparent or high levels of race non-specific resistance (e.g. LSE/93/1, PI 253467, PI 253468) are expressed. Species taxonomically distant from *L. serriola* are exclusively represented in the last isolated cluster. The majority of these species can be considered to possess "non-host" resistance.

Using principal components analysis we sought to answer the following questions: which are the most important sources of variability for resistance or susceptibility and have the accessions behaving similarity been grouped together? Data from seedling and adult plant tests were analyzed separately.

Figure 3 shows results of seedlings considering the first two principal components with clusters clearly marked. The first principal component was the virulence of isolate 12/81, 26/81 , 27/81 and 2/82. On the right-hand side of the Fig. 3 (clusters A and B) there are accessions (largely *L. serriola*) characterized by a strong susceptibility to these isolates. On the left, resistance to these isolates is increasing, and included in clusters D and E are accessions possessing a high level of resistance to these isolates, especially "non-host" resistance. The second principal component represents response to isolates 1/82 and 4/82. In general in the lower part of Fig. 3 are located accessions resistant to these isolates (e.g. LSE/1). Accessions exhibiting high susceptibility (e.g. PI 274359) are located in the upper part of the Fig. 3. The extreme cases represent conspicuous differential responses.

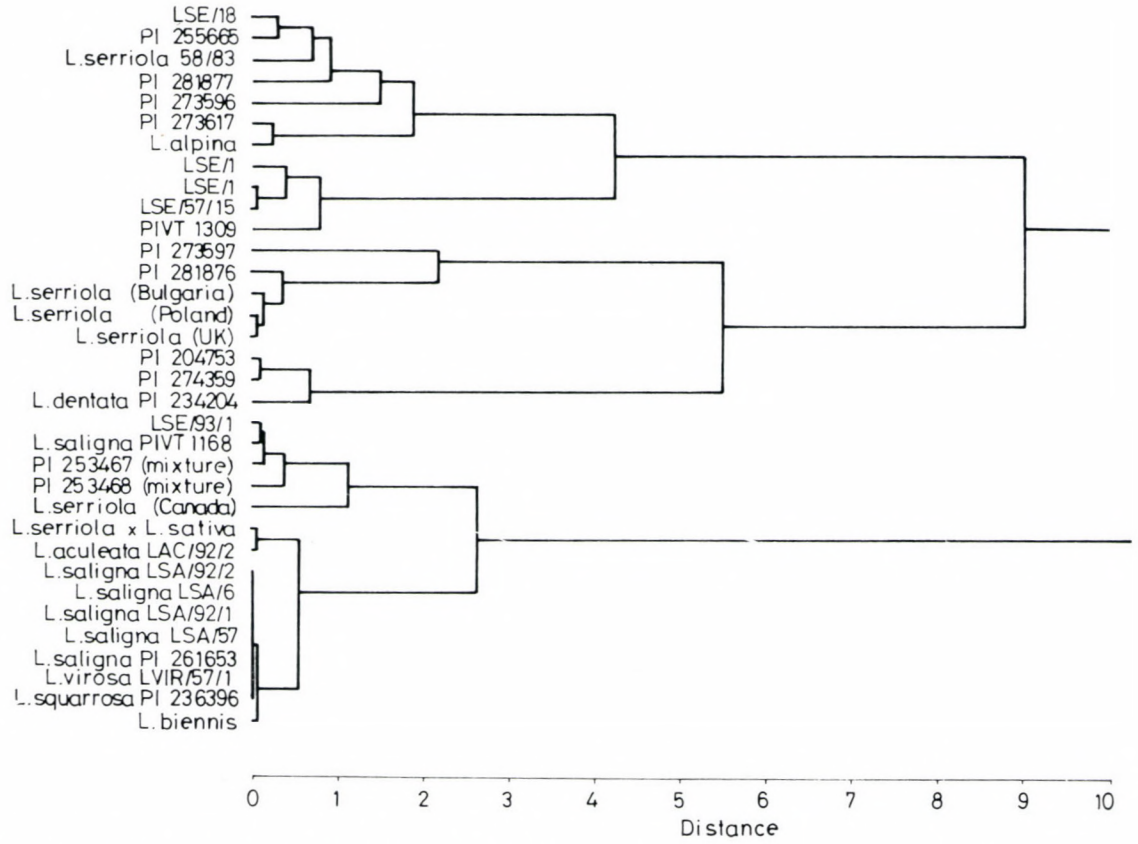

Fig. 2. A dendrogram (Ward-Wishart method) showing similarity and successive clustering of 34 *Lactuca* species and accessions (adult plants)

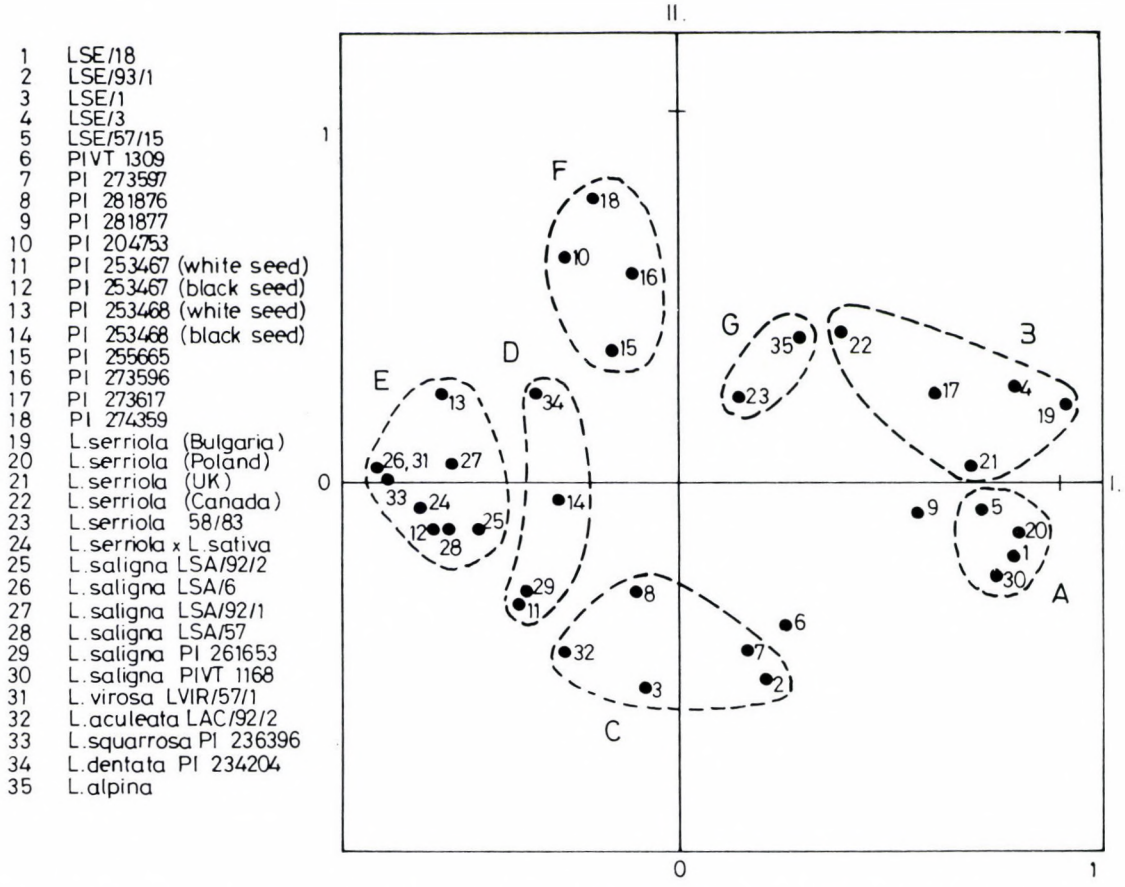

Fig. 3. Clusters of 35 *Lactuca* species and accessions (seedling stage) as projected onto the plane of two principal components

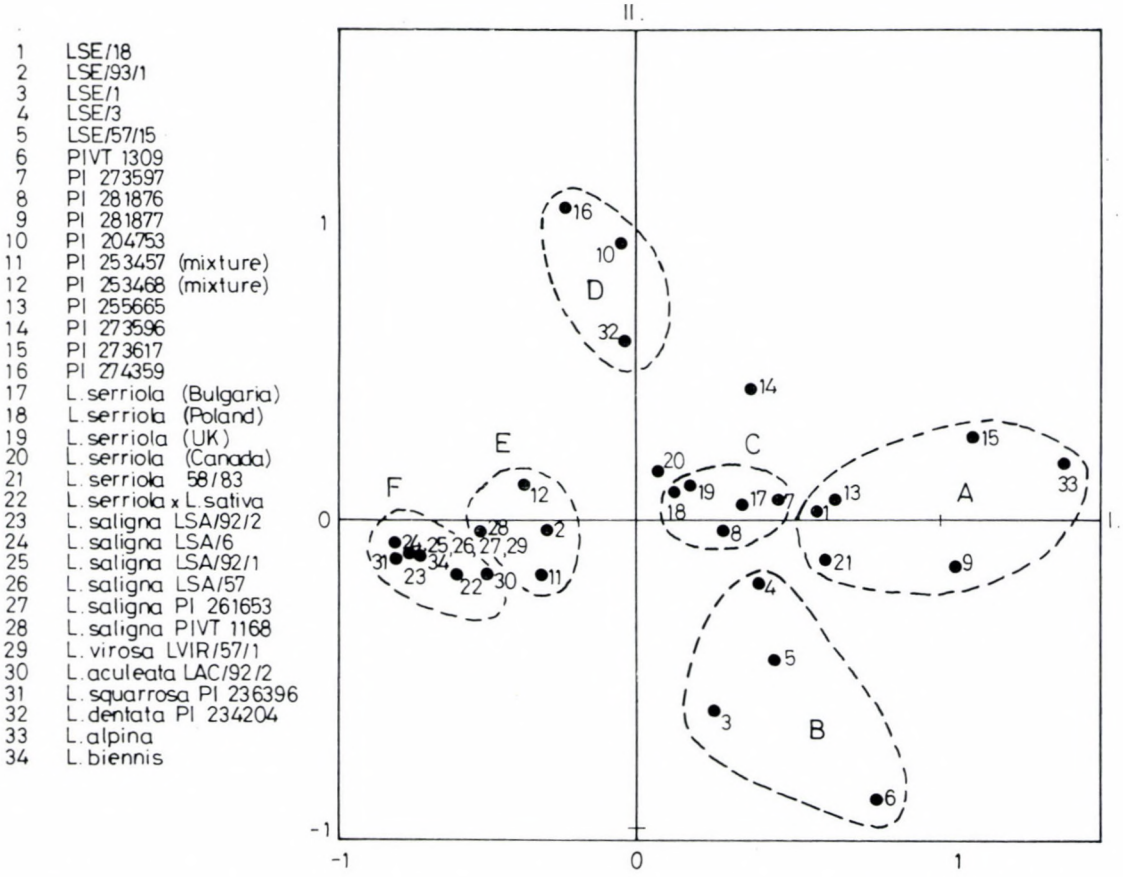

Fig. 4. Clusters of 34 *Lactuca* species and accessions (adult plants) as projected onto the plane of two principal components

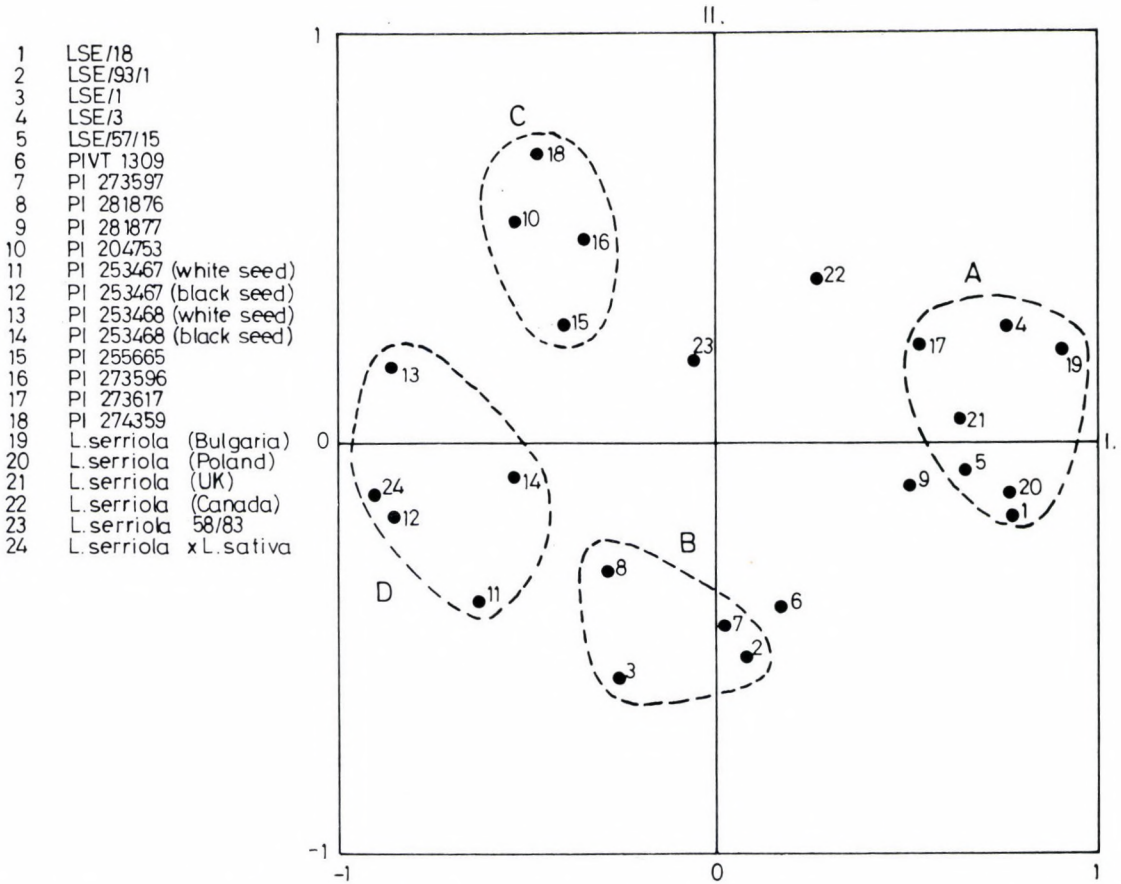

Fig. 5. Clusters of 23 *Lactuca serriola* accessions and one *L. serriola*× *L. sativa* hybrid as projected onto the plane of two principal components (seedling stage)

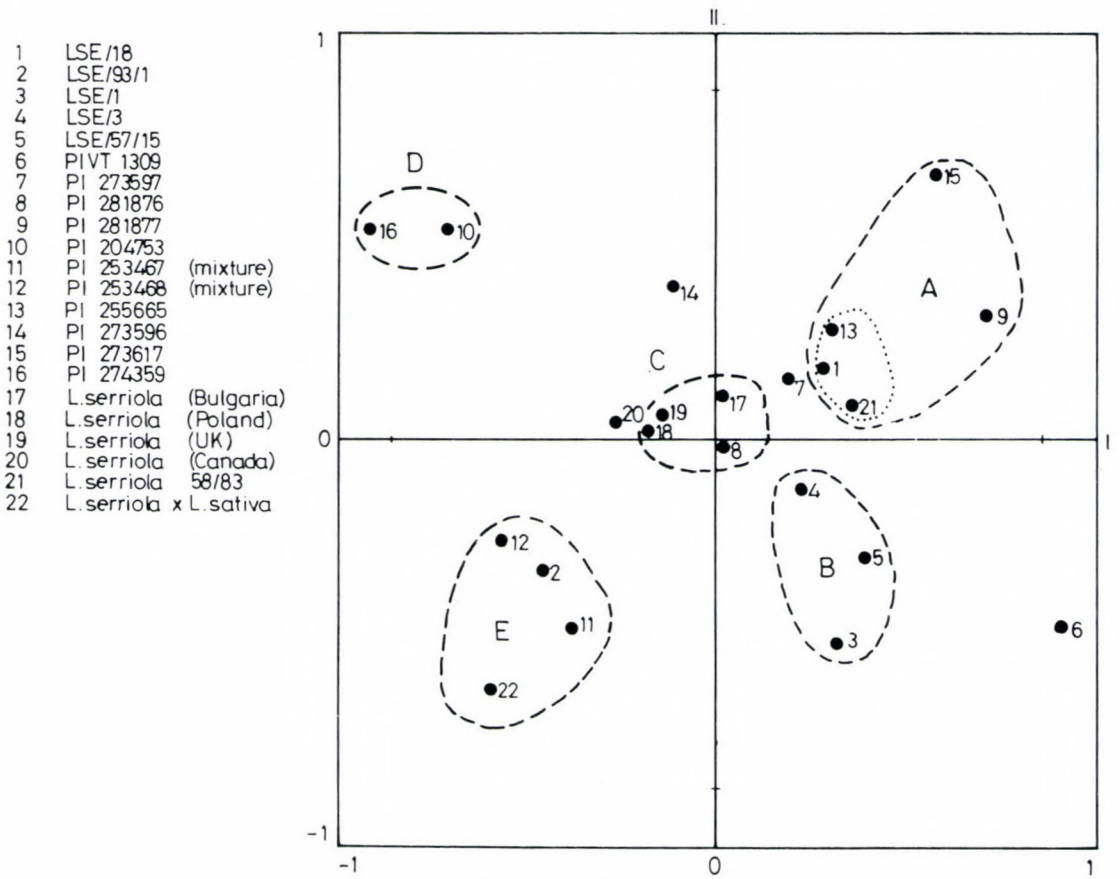

Fig. 6. Clusters of 21 *Lactuca serriola* accessions and one *L. serriola*× *L. sativa* hybrid as projected onto the plane of two principal components (adult plants)

Some differences were observed in adult plants (Fig. 4). The clusters showed one substantial difference from the seedling analysis. In the central part of Fig. 4 there was a cluster (C) representing accessions with different levels of general susceptibility. It is possible that this represents quantitative race non-specific resistance which Lebeda (1986) has drawn attention to in PI 281876. Accessions in cluster E probably exhibit a high level of race non-specific resistance. The compactness and independence of cluster F suggests the expression of non-host resistance in *L. saligna*.

Determination of similarity among Lactuca serriola accessions

Studies in the interaction between *Lactuca serriola* and *Bremia lactucae* isolates from this species were of particular interest, so analysis of the *L. serriola* accessions only was conducted. Relationships were examined with principal components analysis. The first principal component was susceptibility to isolates 12/81, 26/81, 27/81 and 2/82. It is clear from Fig. 5 (seedlings) that individual accessions are widely distributed indicating a diversity of response. Cluster A includes accessions with a high level of susceptibility and without any marked race-specific response. Accessions of cluster D probably exhibit a high level of race non-specific resistance. The second principal component is response to isolates 1/82 and 4/82. Cluster B is represented by accessions exhibiting a race-specific reaction characterized by susceptibility to most isolates, but resistance to one or both isolates 1/82 and 4/82. Cluster C exhibits a clear-cut differential response.

When the data for adult plants of *L. serriola* was analysed some regrouping occurred (Fig. 6) compared to seedlings. Both principal components were the same as for adult plants. Compact clusters were apparent with more clear-cut responses. Individual clusters were similar to those from analysis of seedling data. The central cluster (C) was characteristic of the adult plant stage and results from a splitting of cluster A (Fig. 5). This cluster is characterized by general susceptibility, but of a lower level in comparison with cluster A accessions.

Determination of similarity among Bremia lactucae isolates for pathogenicity on Lactuca spp.

Similarity relationships among isolates were examined by Euclidean metrics. A matrix of distances was analyzed using Ward-Wishart's method. From a dendrogram (Fig. 7) based on analysis of seedling data it can be seen that the set of seven isolates breaks into two large clusters. Isolates are clustered differently on the basis of differences in pathogenicity which more or less correlated with their origins. There is a great similarity between isolates 12/81 and 26/81 from adjacent localities. A similar conclusion can be drawn for isolates 1/82 and 4/82; 2/82 and 3/82 (Lebeda, 1984b). It is interesting that isolate 27/81 is relatively distant from isolates 12/81 or 26/81, since 27/81 came from the same locality

as 26/81 (Lebeda, 1984b). This indicates that in this wild pathosystem there are differences in pathogenicity among isolates coming from the same locality. Similar pathogenicity characteristics (related to localities) presumably result from a degree of genetic homogeneity in pathogen populations, possibly related to host homogeneity. Similarity in isolate pathogenicity was also seen when

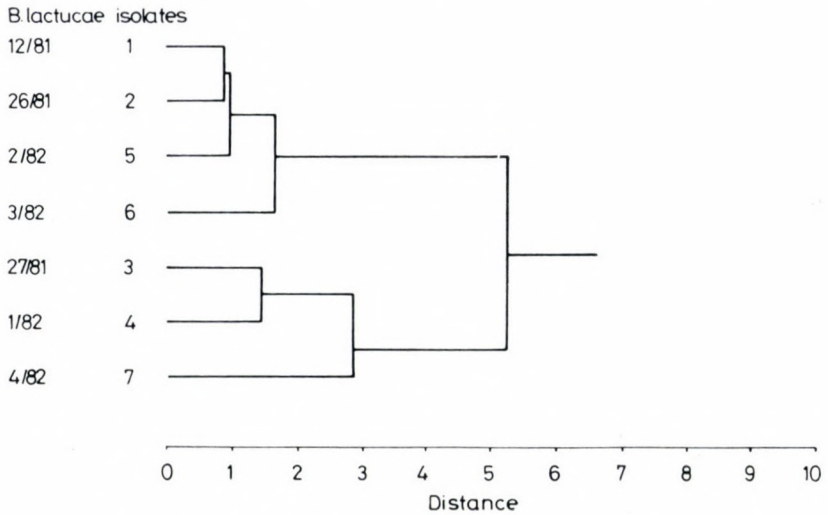

Fig. 7. A dendrogram (Ward-Wishart method) showing similarity and successive clustering of 7 *Bremia lactucae* isolates (data from *Lactuca* spp. seedlings)

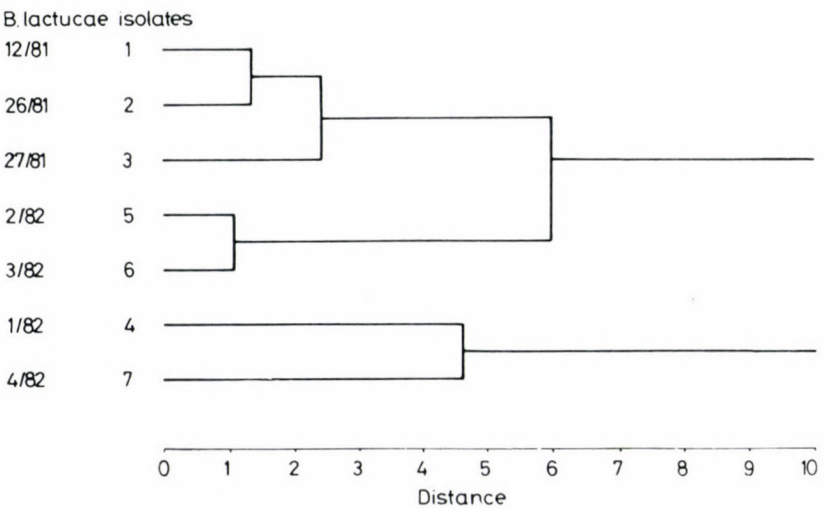

Fig. 8. A dendrogram (Ward-Wishart method) showing similarity and successive clustering of 7 *Bremia lactucae* isolates (data from *Lactuca* spp. adult plants)

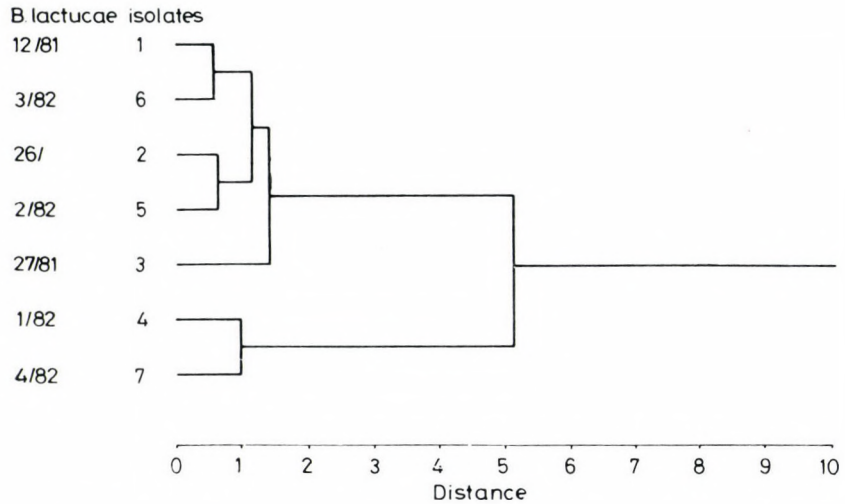

Fig. 9. A dendrogram (Ward-Wishart method) showing similarity and successive clustering of 7 *Bremia lactucae* isolates (data from *Lactuca serriola* seedlings)

data from adult plant tests were examined. Cluster formation (Fig. 8) was comparable to that for the seedlings data. The only exception was isolate 27/81 which formed a joint cluster with isolates 12/81 and 26/81. The three clusters correspond entirely with the origins of the isolates. It seems evident that the pathogen exists as local populations or subpopulations with relatively clear-cut pathogenicity characteristics in relation to *Lactuca* species. The use of other clustering methods confirmed the above informations.

Determination of similarity among Bremia lactucae isolates for pathogenicity on Lactuca serriola

In comparison with the whole data set there were some changes in clustering when only responses of *L. serriola* seedlings were considered. Two large clusters were apparent in this case (Fig. 9) but there was not such a clear dependence between isolate origin and their pathogenicity. The isolates 1/82 and 4/82 were exceptions and were differentiated by their pathogenicity on accessions of *L. saligna.*

Discussion

The cluster analysis and principal components analysis complement each other. The method of principle components, co-ordinates and factor analysis are typical representatives of multivariate analysis applied for reduction of data dimension. The main goal of the methods under consideration is to reveal cryptic

independent values called principal components and/or factors explaining variation and dependence of the original measurable variables. These new values cannot be directly measured and evaluated, however, they may have certain objective interpretation. Consequently, the method of principal components makes it possible to analyze the structure of relationships in a set of dependent variables.

Interpretation of the principle components (for adult *Lactuca* spp. plants) can be demonstrated as follows (Table 1). The first principal component explains approximately 61% total data variation, having positive significant factor loads with all variables (i.e. parasite isolates), and thus it represents general susceptibility. The second principal component explains 17% total variation. There are positive factor loads with variables 1/82 and 4/82, and negative factor loads with the reamaining isolates. It represents a factor of specific resistance to the respective solates. The third component (approx. 10% total variation) is characterized by positive factor loads to the first 4 isolates and negative factor loads to the others. It represents another race-specific resistance factor to the respective isolates. Generally, on the basis of localization either in dendrograms or graphs of principal components it was possible not only to sort out the set into groups, bupto reach some judgement on the forms of resistance expressed by each group. Such a race-specific resistance of *Lactuca sativa* was also demonstrated in our previous paper (Lebeda and Jendrulek, 1987a).

The results presented in this paper correspond with information on *Lactuca* spp. derived from studies of isozyme markers. Data presented by Kesseli and Michelmore (1986) showed that *L. saligna* and *L. virosa* formed separate clusters (being expressed by genetic distances between groups) considerably isolated from *L. serriola*. *L. serriola* was broken up into several clusters on the basis of isozyme patterns (Kesseli and Michelmore, 1986). Interestingly in the present

Table 1

Component proportion in total variation of studied set of *Lactuca* spp. (adult plants)

Variable (isolates of *B. lactucae*)	Component/Loadings		
	1.	2.	3.
12/81	0.39	−0.24	0.20
26/81	0.33	−0.24	0.29
27/81	0.40	−0.10	0.46
1/82	0.35	0.55	0.36
2/82	0.45	−0.17	−0.46
3/82	0.42	−0.22	−0.47
4/82	0.26	0.71	−0.31
Cumulative proportion of variation	60.50%	77.80%	87.30%
Variation explained	60.50%	17.30%	10.00%

study *L. serriola* accessions such as LSE/18, LSE/57/15 and PI 281877 were relatively similar (high levels of susceptibility; see Fig. 1). These three accessions also formed a relatively compact cluster on the basis of isozyme similarity.

The aim of this study was to demonstrate the application of methods of analysis based on quantitative data clustering to data resulting from laboratory tests. Nevertheless, these methods can also be applied in epidemiological investigations (Lebeda and Jendrulek, 1987b, 1988) for comparison of different resistance genotypes. Further development of multivariate analysis in plant pathology esp. research of host-parasite specificity may involve the use of factor analysis which is the objective of continuing work (Lebeda and Jendrulek, 1986).

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Results on the Application of the Screening Method for Resistance to Viruses in Potato Breeding*

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Paper presents some methods and results regarding research on virus resistance in potato in the German Democratic Republic.

Relating to the actual virus situation, some check methode established in the GDR are mentioned.

At present, 70% of all GDR varieties are classified as "highly resistant to the complex of economically relevant viruses" (potato virus, PLRV; potato virus Y, PVY; potato virus M, PVM; potato virus X, PVX). Nevertheless, assessment of specific resistance to one single virus is becoming more and more important.

Paper summarizes some results regarding the laboratory determination of quantitative resistance to PLRV, PVX and PVM using virus concentration in primary infected plants.

Virus situation in the GDR

Progress is evident in the level of virus resistance in the GDR potato assortment (Table 1). At present, 70% of all GDR varieties are classified as "highly resistant to the complex of economically relevant viruses" (potato leafroll virus, PLRV; potato virus Y, PVY; potato virus M, PVM; potato virus X, PVX). Nevertheless, assessment of specific resistance to one single virus is becoming more and more important. Analysis of the virus spectrum – i.e., of the overall and specific appearance of economically important viruses and their isolates or strains – is an essential prerequisite for the development of suitable methods.

The high relevance of such observations to the organization of virus control illustrates the relationship between PLRV and PVY and the shares of these viruses in total virus infection in seed potatoes (Fig. 1).

After years of PLRV dominance, an increasing share of PVY has been stated since 1974, against the background of declining total percentage of virus-infected seed potatoes (Podelleck et al., 1987; Schenk et al., 1989). The tendency of increasing PVY infection has been observed in several countries of Central Europe (Weidemann, 1986; Dziewonska, pers. communication). PVM and PVX have not become economically important in the GDR. In connection with the occurrence of GDR varieties showing PVS symptoms, the situation of PVS

* Text of a lecture, held at the "Meeting on screening methods of detection of virus resistance of plants" in September 1988 in Hódmezővásárhely, Hungary.

Table 1

Development of virus resistance in the potato assortment of the GDR

Year	Number of varieties	Percentage of varieties in different groups of complex virus resistance		
		low resistance (4)	moderate resistance (3)	high resistance (2)
1950	23	61	30	9
1955	23	57	26	17
1960	20	40	25	35
1965	22	18	46	36
1970	25	20	52	28
1975	20	5	55	40
1980	18	—	22	78
1985	21	—	29	71
1986	22	—	27	73
1987	23	—	30	70

infection has to be reconsidered. Sporadic occurrence of PVA was the reason why we included the diagnose of that virus in the seed potato certification system.

Two conclusions regarding the strategy of breeding for virus resistance are derived from the alternating occurrence of particular viruses and their strains:

1. The whole complex of economically important viruses should be considered;
2. Polygenicly controlled pathotyp-independent quantitative (relative) virus resistance should be given special attention.

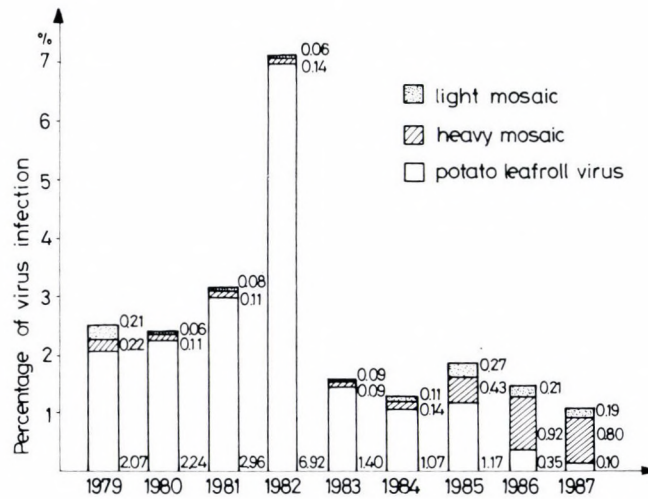

Fig. 1. Total virus infection of GDR seed potatoes (Schenk et al., 1989)

Established methods for evaluation of potato resistance to viruses

The evaluation of virus resistance in the GDR is based on a system of field observations over a period of 10 years, beginning in the third breeding year; it includes laboratory tests (Fig. 2).

The following laboratory tests are included in the program of the Institute of Potato Research Gross Lüsewitz:

1. Preselection in greenhouse of about 100,000 seedlings per annum for extreme resistance to PVY, using the spray gun method;
2. Testing of about 320 breeding lines per annum for extreme resistance to PVX and PVY, using spray gun, aphid and graft inoculations;
3. Testing of about 350 breeding lines per annum for quantitative (relative) resistance to PLRV, using aphid inoculation;

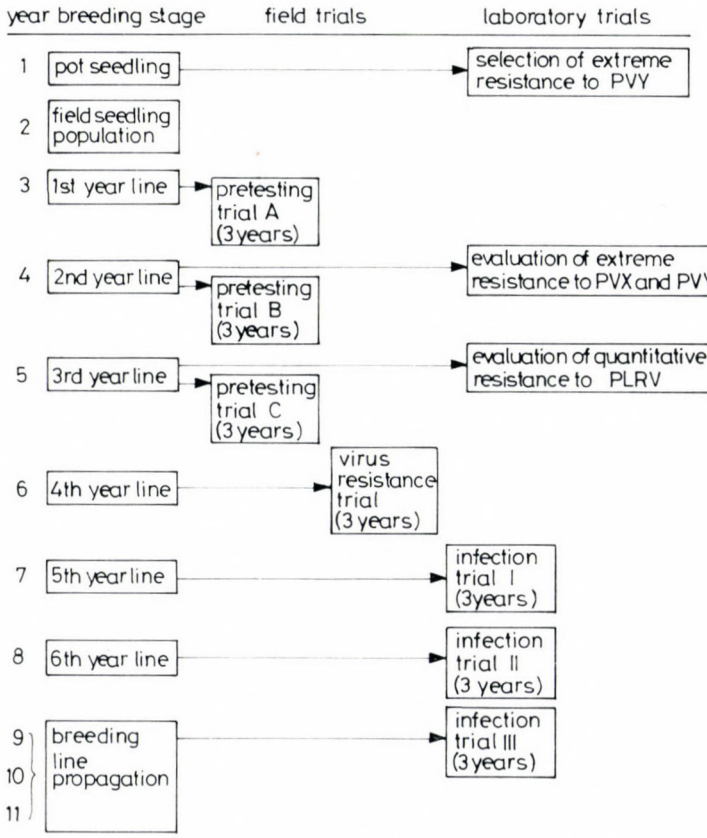

Fig. 2. Evaluation of virus resistance in GDR potato breeding (Vogel unpublished)

4. Testing of about 40 breeding lines per annum for quantitative (relative) resistance to both PVX and PVM, using mechanical (hand) inoculation.

Some examples of established laboratory testing of the qualitative and quantitative types of resistance are:

- Laboratory test for extreme resistance to PVX and PVY acc. to Neitzel et al. (GDR standard TGL 31994/01);
- Laboratory test for quantitative (relative) resistance to PLRV acc. to Hamann 1956; Hamann et al., 1968.

Development of new virus resistance tests

Complex quantitative resistance, including the careful introduction of extreme resistance, is the long-term target of breeding for virus resistance in GDR,

Therefore investigations are concentrated on the traits of quantitative virus resistance and factors influencing this type of resistance and on the development of practical methods for resistance testing.

On the one hand, quantitative resistance to viruses may be connected with traits protecting against inoculation and penetration; this may become manifest in a reduced number of plants infected or showing symptoms (resistance to infection) and/or in an extended incubation period.

On the other hand traits may exist that affect postinfectional processes, i.e. mechanisms of virus multiplication and translocation; this would lead to low virus concentration and/or absence of any systemic spread of viruses in plants. Recently, the degree of virus accumulation has become important as one of the most interesting traits of quantitative virus resistance. Methods of quantitative ELISA have been the essential prerequisites for evaluation of this trait. These methodes are suitable for evaluation of absolute and relative virus concentrations. For evaluation of absolute virus concentration results are compared by standard curves of known virus concentration. Data are obtained in absolute weight units (ng/ml). Evaluation of relative virus concentration is mostly sufficient; absorbance values are compared with dilution of freeze-dried laboratory samples of unknown but stable concentration. These standard samples are applied to each microtitre plate in the trial. The samples are compared with standards. Even comparison of evaluated absorbance values will be sufficient in some cases.

At the Institute of Potato Research Gross Luesewitz, all ELISA tests are realized with photometre unit "SUMAL-PE 1" (VEB Kombinat Carl Zeiss Jena, GDR) and ELISA sets received from the Institute of Phytopathology Aschersleben of the Academy of Agricultural Sciences of the GDR. Special computer software (Schenk et al., 1988) is used for recording and interpretating of results.

Results

Potato virus X (PVX)

Two potato varieties of high (Karella N, Schwalbe) and two of low quantitative resistance to PVX (Ogonjok, Turbella N) were artificially inoculated under in vitro, greenhouse and field conditions (Kürzinger and Schenk, 1988). At several dates, beginning 7 dpi. virus was detected by ELISA (Fig. 3).

The resistant genotypes Schwalbe and Karella N included significantly higher percentages of infected plants with inhibited virus concentration (i.e. plants with absorbance values lower than or equal to a defined threshold of 1.0). Even with simplified quantitative evaluation, the percentage of plants with inhibited virus multiplication reflects the known differences of resistance in four varieties not only under field and greenhouse conditions but also in vitro.

After extensive investigation of 24 genotypes a method was developed for evaluation of quantitative resistance to PVX in greenhouse-grown plants

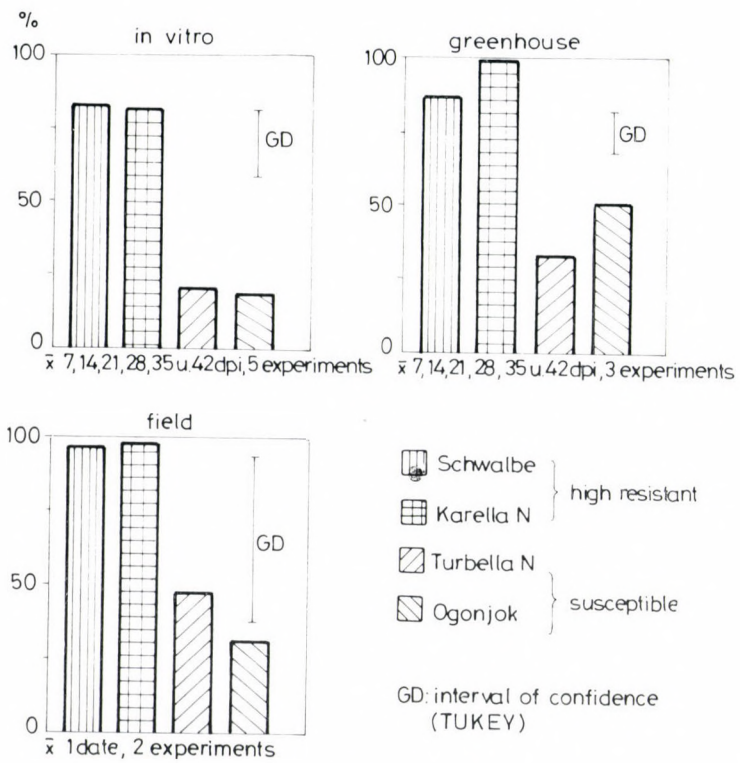

Fig. 3. Percentage of PVX infected plants with restricted virus multiplication ($A_{405} = 1.0$) under in vitro, greenhouse (primary infection) and field conditions (secondary infection) (Kürzinger and Schenk, 1988)

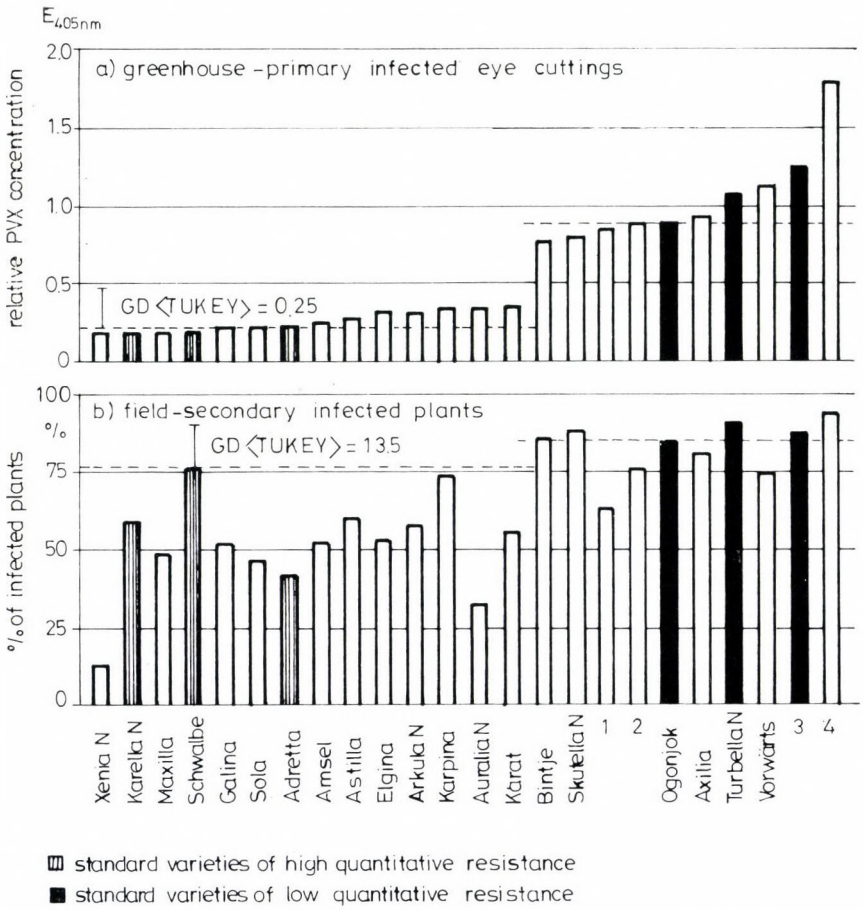

Fig. 4. PVX concentration in primary infected eye cuttings (greenhouse, 4 years) in comparison to the percentage of field infected plants (3 years) (Kürzinger and Schenk, 1989)

from eye cuttings (Kürzinger and Schenk, 1989). Comparison with standard varieties of high quantitative PVX resistance (Schwalbe, Karella N, Adretta) and low quantitative PVX resistance (Ogonjok, Turbella N, genotype 3), allows to assign the tested varieties to two groups (Fig. 4a):

- High quantitative PVX resistance:
  Xenia N, Karella N, Maxilla, Schwalbe, Galina, Sola, Adretta, Amsel, Astilla, Elgina, Arkula, Karpina, Auralia N, Karat;
- Low quantitative resistance:
  Bintje, Skutella N, genotype 1, genotype 2, Ogonjok, Axilia, Turbella N, Vorwärts, genotype 3, genotype 4.

The correlation between the ranks from clxssification by virus concentration in eye cuttings (Fig. 4a) and classification by percentage of infected field plants (Fig. 4b) was significant ($r = 0.75$, $n = 24$, $\alpha < = 0.05$).

The test for quantitative PVX resistance in primary infected eye cuttings is the first practical method based on reduced virus multiplication in GDR potato breeding. One test year with two dates will be sufficient to verify the degree of resistance for $\bar{x} = 92\%$ (67 . . . 100%) of resistant varieties and $\bar{x} = 83\%$ (56 . . . 100%) of susceptible ones.

Potato leafroll virus (PLRV)

In seven genotypes of known resistance grown in the greenhouse virus concentration was determined 15, 22 and 29 dpi (GASE et al., 1988). For inoculation a PLRV isolate was used which had been identified as moderately virulent.

Inoculation was realized by *Myzus persicae* Sulz. Classification in three following groups was significant at each of the test dates (Table 2):

Susceptible:	Sieglinde, Astilla
Moderately resistant:	Adretta, Galina
Highly resistant:	3 breeding lines

Table 2

PLRV concentration and percentage of infected plants of potato genotypes with different degree of quantitative PLRV resistance (GASE et al. 1988)

Genotype	Degree of PLRV resistance	PLRV concentration (A_{405nm}, significance at $\alpha < = 0.05$)	Percentage of infected plants (%)
Sieglinde	low	2.40a	100
Astilla	low	2.12b	97
Adretta	moderate	1.48c	68
Galina	moderate . . . high	1.42c	56
I–71.17/6 N + B	high	0.61d	21
I–78.2063/11	high	0.59d	25
I–77.198/135 N	high	0.54d	19

Absorbance values correlate with the estimated percentage of infected plants ($r = 0.988$, $B = 0.976$).

Virus concentration in the highly resistant GDR breeding lines is about 10% of that in susceptibility standard Sieglinde.

Potato virus M (PVM)

In primary PVM-infected potato varieties differing in their degree of quantitative resistance were also observed differences in virus concentration (Fig. 5).

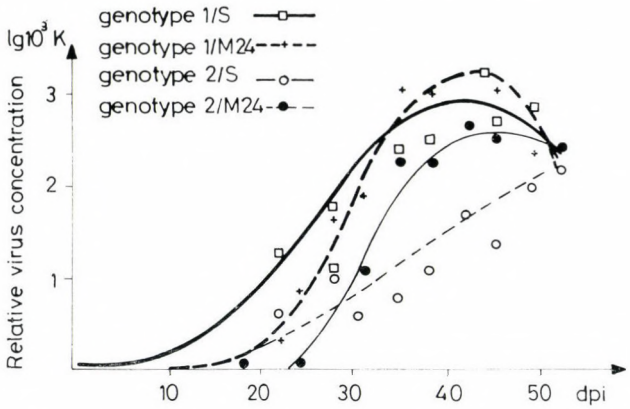

Fig. 5. Dynamics of PVM concentration in primary infected potato plants of two genotypes with different degree of quantitative resistance to PVM (isolates PVM-Saco, PVM-M24)

A moderately resistant genotype shows significantly lower relative virus concentration than the highly resistant one (PVM-M 24: 41 . . . 82%, PVM-Saco: 26 . . . 71%, $\alpha <= 0.05$, n = 10 . . . 18) over a relative long period (PVM-M 24: 28 . . . 45 dpi., PVM-Saco: 28 . . . 49 dpi.). 22 and 52 dpi. the two genotypes could not be differentiated.

Investigations will be continued to develop methods for determining quantitative resistance to PVY, PLRV, PVM and PVS, and to facilitate complex and specific evaluation of virus resistance in potato breeding material.

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New Artificial Cruciferous Hosts to *Erysimun* Latent Virus (Tymovirus Group) in the Genera *Crambe* and *Erysimum*[1]

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The three *Crambe* species (*C. abyssinica* Hochst. et R. E. Frees, *C. filiformis* Jacq., *C. hispanica* L.) and five *Erysimum* species [*E. aureum* Rieb., *E. cheiranthoides* L., *E. crepidifolium* Rchb., *E. decumbens* (Schleich.) Dennst., *E. rupestre* DC.] inoculated with the original strain of *Erysimum* latent virus (ELV) proved equally susceptible. The new host plants of ELV — of which *Crambe abyssinica* is a fodder crop of growing importance, and the various *Erysimum* species are indigenous annual, biennial and perennial weed and medicinal plants — may play an important role in the epidemiology of the virus.

Erysimum latent virus (ELV) was first isolated from symptomless *Erysimum helveticum* (Jacq.) DC. plants and described by Shukla and Schmelzer (1972). The host range, *in vitro* properties, particle morphology, sedimentation behaviour, transmissibility by beetles (*Phyllotreta* species) and serological relationships of the virus were studied by Shukla and Schmelzer (1973a, b, c), Shukla et al. (1973, 1975), Shukla and Gough (1980). Further characterization of the ELV (e.g. molecular weight of the capsid protein, base composition of the RNA, electrophoretic- and further serological properties, cytopathological effects) is given in another paper by Shukla et al. (1980).

According to our present knowledge *Crambe* species have not so far been known to be susceptible to the ELV, although their susceptibility to the turnip yellow mosaic virus (TYMV) which belongs to the same tymovirus group, as well as to other typical cruciferous viruses [radish mosaic virus (RMV), comovirus group; turnip mosaic virus (TuMV), potyvirus group; cauliflower mosaic virus (CaMV), caulimovirus group] is a long since established fact (Thornberry and Phillippe, 1965; Horváth, 1972; Horváth et al., 1973, 1981; Horváth and Besada, 1980).

At present five *Erysimum* species are known to be naturally and two of them to be artificially susceptible to ELV (Table 1). It is remarkable that mixed virus infections frequently occur in various *Erysimum* species. In the case of *Erysimum crepidifolium* Rchb. and *E. perovskianum* Fisch et Mey. three viruses (ELV + RMV + TuMV, and ELV + broad bean wilt virus (BBWV) + cucum-

[1] Dedicated to J. Szirmai D.Sc. on the occasion of his 80th birthday.

Table 1

Natural and artificial susceptibility of *Erysimum* species to *Erysimum* latent virus

Erysimum species	Susceptibility		Literature
	Natural	Artificial	
E. allionii hort.	No	Yes	Shukla and Schmelzer (1972)
E. crepidifolium Rchb.	Yes	No	Shukla et al. (1975)
E. helveticum (Jacq.) DC.	Yes	Yes	Shukla and Schmelzer (1972)
E. perovskianum Fisch. et Mey	Yes	No	Shukla et al. (1975)
E. pulchellum (Willd.) Boiss.	Yes	No	Shukla et al. (1975)
E. silvestre (Cr.) Scop.	Yes	No	Shukla et al. (1975)

ber mosaic virus (CMV), respectively), in *E. odoratum* two viruses (CMV + TuMV and CMV + RMV, respectively) and in *E. silvestre* ELV and RMV induced mixed infection (Shukla and Schmelzer, 1975). There are two *Erysimum* species (*E. aurantiacum* Leyb. and *E. linariifolium* Tausch.) in which the virological examinations have not detected virus infection so far.

Considering that on the ELV susceptibility of the *Crambe* species and on the artificial virus susceptibility of the *Erysimum* species – except for two of them (see Table 1) – no data exist in the world literature, we carried out investigations with some *Crambe* and *Erysimum* species to find out what role they may play in an occasional natural ELV infection.

Materials and Methods

In our virological laboratory young *Crambe* and *Erysimum* plants [*Crambe abyssinica* Hochst. et R. E. Frees, *C. filiformis* Jacq., *C. hispanica* L., *Erysimum aureum* Rieb., *E. cheiranthoides* L., *E. crepidifolium* Rchb., *E. decumbens* (Schlech.) Dennst., *E. rupestre* DC.] were inoculated by carborundum spatula technique with the original strain of ELV maintained in *Brassica chinensis* L. propagation host. With the exception of the *Crambe* species, *Erysimum cheiranthoides* and *E. rupestre* the mentioned plants are new in the plant virology literature. Of the experimental plants listed above the three *Crambe* species are not members of the Hungarian flora; the annual *Erysimum cheiranthoides* and the annual or biennial *E. crepidifolium*, on the other hand, are characteristic Hungarian weed and medicinal species of the genus *Erysimum* (Priszter, 1986).

From leaves of *Brassica chinensis* plants previously infected by ELV tissue sap was made in a mortar, which then was filtered through sterile gauze, diluted with distilled water at a ratio of 1 : 1, and rubbed onto leaves of *Crambe* and *Erysimum* plants previously dusted with carborundum (400 mesh). The inoculated plants were determined for virus infection on the basis of the symptoms appearing on the infected plants, and by the back-inoculation method. Back-inoculation was carried out both from the inoculated leaves (marked with a sterile plastic

sucking stick pierced through them in the course of inoculation) and from the non-inoculated ones to the ELV susceptible *Brassica napus* var. *napobrassica* (L.) Tchb. local-lesion host too.

Results and Conclusions

1. Crambe species

Of the three *Crambe* species examined *C. abyssinica* and *C. hispanica* responded to the inoculation with local chlorotic lesions and later with brown necrotic lesions (Fig. 1). After the appearance of the local symptoms characteristic systemic mosaic developed in the non-inoculated leaves, followed by mosaic and necrosis even extending to the axillary shoots. As regards the symptoms *Crambe abyssinica* proved highly susceptible (Fig. 1A and B). *Crambe filiformis* did not show symptoms following the inoculation. In the course of the back-

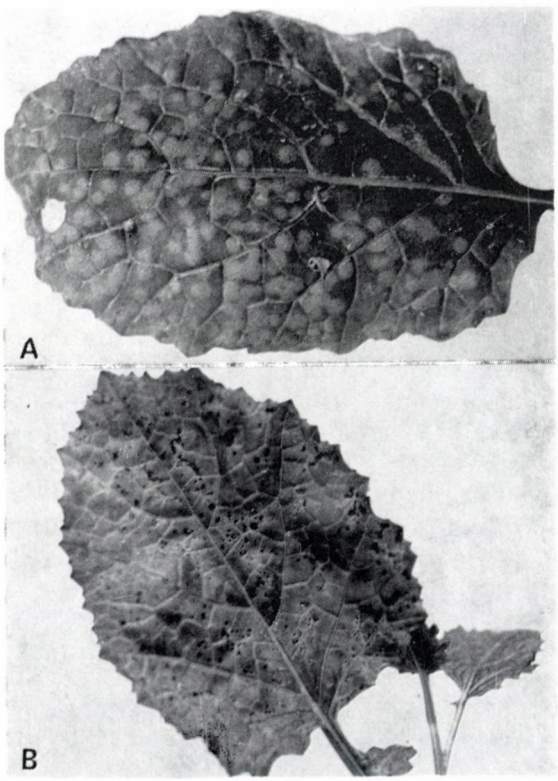

Fig. 1. Symptoms on *Crambe abyssinica* to artificial inoculation with *Erysimum* latent virus. A: local chlorotic lesions, B: systemic necrotic lesions

inoculation test (*Brassica napus* var. *napobrassica* as assay plant) *Crambe filiformis* unambiguously proved to be a local and latent systemic host for ELV. Considering that the *Crambe* species as fodder crops play an ever growing role in the United States of America and Europe, the danger of their infection by ELV must not be underrated even if the occurrence of the virus has only been pointed out in the German Democratic Republic so far. According to our knowledge the ELV is not transmissible by *Brassica chinensis* seeds, but we do not know whether or not it can be transmitted by seeds of other plants, e.g. of *Crambe* species. It is known that the ELV is transmissible by four species of flea-beetles of the genus *Phyllotreta*. It would be interesting to known whether or not the beetle pests of *Crambe abyssinica* seeds (*Ceuthorrhynchus quadridens* Panz. and *C. assimilis* Payk.) (cf. Eisentraut 1967) are potential ELV carries. If so, the *Crambe* plants and the supposed beetle vectors would play an important role in the epidemiology and ecology of the virus.

2. Erysimum species

Of the five *Erysimum* species examined *E. aureum* and *E. decumbens* are new experimental plants in virology. Both of them showed chlorotic lesions and systemic mosaic in response to inoculation with ELV.

Erysimum cheiranthoides reacted to the ELV with large local chlorotic-necrotic rings, severe systemic chlorotic rings and mosaic (Fig. 2). This plant showed the most severe symptoms among the *Erysimum* species examined. According to the results of experiments carried out so far in connection with its virus susceptibility this plant is susceptible to CMV, TuMV, turnip crinkle virus (TuCV) and turnip rosette virus (TuRV) (Schmelzer and Wolf, 1971). Since *Erysimum cheiranthoides* occurs in Hungary, it can be regarded as a potential host for ELV in our country. In the course of investigations with the species *Erysimum crepidifolium* latter was found to be locally (chlorotic spots) and systemically (mosaic) susceptible to ELV. According to the relevant literature the species is a natural host for ELV (see Table 1) and RMV (Shukla and Schmelzer, 1973d). Shukla et al. (1975) isolated ELV from symptomless *Erysimum crepidifolium* plants collected in the Botanical Garden of Eberswalde (German Democratic Republic) and from plants collected in the Dresden Botanical Garden and showing flecks and mosaic symptoms, alike.

Erysimum rupestre – which according to the results of earlier investigations (Schmelzer and Wolf, 1971) was only susceptible to TuMV – showed in our experiments local (chlorotic-necrotic lesions) and systemic (mosaic) susceptibility to ELV.

On the basis of literary data it can be established that the natural and artificial host-virus relations between *Erysimum* species and ELV, as well as the natural relations between *Erysimum silvestre* and ELV are latent (Shukla and Schmelzer, 1972, 1973; Shukla et al., 1975). In our own experiments – carried out at high light intensity, in long-day summer months – latency was not pointed

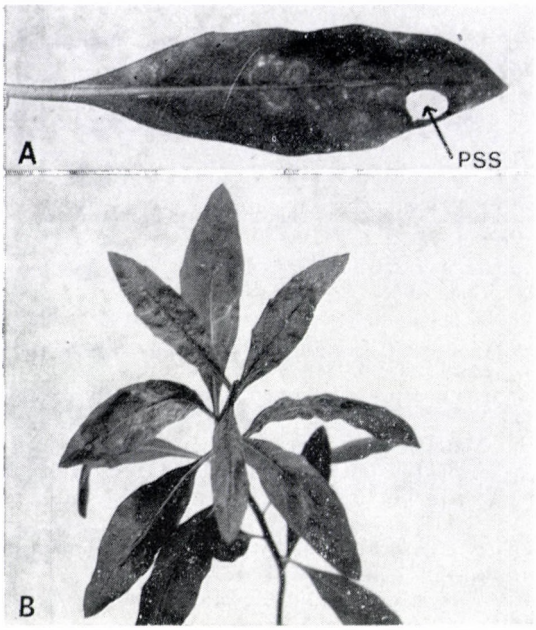

Fig. 2. Symptoms on *Erysimum cheiranthoides* to artificial inoculation with *Erysimum* latent virus. A: chlorotic-necrotic local rings, B: systemic mosaic. PSS: Place of the inoculated leaf pierced through by a sucker stick

out in any host-virus relation. It is a well-known fact that light intensity and its duration do influence the virus concentration and the development of symptoms in the various host-virus relations. In general, high light intensities and long days favour virus multiplication in the host plants (e.g. Pound and Garces-Orejuela, 1959; Opel, 1970). Considering that the virus strain used in our experiments is identical with the original ELV, the development of symptoms could not be influenced by differences in virus strain. The manifestation of ELV in symptoms appearing in the new *Erysimum* species is supposed to be connected with a new type of reaction by the so far unknown *Erysimum* species, or even more so with the higher intensity and longer duration of light compared to the experimental conditions of the German Democratic Republic.

On the basis of the data published by Shukla and Schmelzer (1972, 1973a, b, c, d), Shukla et al. (1973, 1975) and of the present results of our experiments the appearance of ELV in other cruciferous *(Brassicaceae)* plants and in countries outside the German Democratic Republic can be reckoned with in the future. This foreboding is supported by a recent result of our experiment carried out with a new vegetable plant in Hungary (Pak-Choy, *Brassica campestris* L. var. *chinensis* cv. *Japro RS 2701*) to settle the question of its susceptibility to ELV (Horváth et al., 1989). The eight native annual, biennial and perennial *Erysimum* species of Hungary (cf. Priszter, 1986) are potential virus hosts.

Acknowledgements

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Reaction of Some New Bolivian Tuber-Bearing *Solanum* Species to Different Potato Pathogenic Viruses

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Host-virus and resistance relations between 9 wild *Solanum* species (3 of them are botanically new) collected by the Bolivian German-Netherlands Potato Expedition in 1980 and 9 isolates of 6 viruses were studied.

Of the 74 new host-virus relations become known during the investigations resistance was pointed out in the case of 3 wild *Solanum* species and 5 viruses: *Solanum neocardenasii* (potato virus Y, PVY), *S. paucissectum* (tobacco rattle virus, TRV), *S. violaceimarmoratum* (alfalfa mosaic virus, AMV; cucumber mosaic virus, CMV; potato virus X, PVX).

Hypersensitive resistance was found between 8 wild *Solanum* species and 5 isolates of 4 viruses in 14 host-virus relations: *Solanum alandiae* (PVX, PVY, TRV), *S. astleyi* (PVX), *S. circaeifolium* ssp. *quimense* (PVX), *S. mochicense* (AMV, PVX), *S. neocardenasii* (AMV), *S. neorossii* (AMV, PVX), *S. paucissectum* (AMV, PVX), *S. violaceimarmoratum* (PVX).

Further, between the wild *Solanum* species and viruses examined one locally latent relationship (*Solanum alandiae* — CMV) and 48 local and systemic, – as well as in the case of 5 wild *Solanum* species and 4 viruses 6 systemic host-virus relations were pointed out.

As it is known, after Peru Bolivia is the country richest in tuber-bearing wild *Solanum* species and primitive forms (Van Soest, 1980; Van Soest et al., 1980; Hondelmann and Van Soest, 1980). In spite of this relatively little is known of the resistance characteristics of the Bolivian wild *Solanum* species and primitive forms. One of the reasons for this is the minor importance attached for a long time to the Bolivian collection much of which has even been destroyed; besides, systematic research work concerning the *Solanum* species has but recently begun. The excellent work by Ross and Rowe (1965) is an exception; it contains data on the resistance of the Bolivian *Solanum acaule*, *S. ajanhuiri*, *S. megistacrolobum*, *S. sucrense* and of the *S. tuberosum* to potato virus A (PVA), potato virus X (PVX), potato virus Y (PVY) and potato leaf roll virus (PLRV). Subsequently our own papers supply data on the compatibility of several Bolivian wild species with PVY and tobacco mosaic virus (TMV), and on their incompatibility (hypersensitive reaction) to PVY (Horváth, 1968a, b). Since beginning of the eighties an increasing number of research results concerning wild *Solanum* species of Bolivian origin have been published. In one of these publications Van Soest and

Seidewitz (1981) supplied valuable information on 23 tuber-bearing wild *Solanum* species of Bolivian origin. Accordingly, two accessions of *Solanum tarijense* (BGRC 008230, BGRC 008231) are susceptible to potato virus M (PVM). To PVX 9 wild *Solanum* species showed immunity, and 13 accessions of 9 wild *Solanum* species susceptibility. According to data related to PVY 4 accessions of 10 wild *Solanum* species proved immune and 17 accessions of them susceptible.

In connection with the resistance of wild *Solanum* species of Bolivian origin in recent years Van Soest (1983), Van Soest and Hondelmann (1983) published data concerning 240 accessions of 34 wild *Solanum* species. The resistance examination related with various viruses covered responses by 45 accessions of 16 wild *Solanum* species to PVX and by 47 accessions of 20 species to PVY.

As to the latest results of research interesting are the works by Hanneman and Bamberg (1986), and Hoekstra and Seidewitz (1987). In the course of examining 22 wild *Solanum* species of Bolivian origin they established numerous compatible and resistant relations with four viruses. According to the data published the number of susceptible relations found in the case of the wild *Solanum* species examined were 15 for PVA, 45 for PVX, 46 for PVY and 144 for PLRV. The resistant relations were: 10 with PVA, 18 with PVX, 6 with PVY and 18 with PLRV (Hanneman and Bamberg, 1986). The most detailed data concerning some 52 wild *Solanum* species of Bolivian origin are found in the work by Hoekstra and Seidewitz (1987). To PVM further *Solanum* species (*S. leptophytes*, BGRC 008222) has proved susceptible. Thirty accessions of 10 wild *Solanum* species were found to be immune of, and 99 accessions of 25 wild *Solanum* species susceptible to PVX. In the case of PVY 7 accessions of 3 wild *Solanum* species proved immune, while 111 accessions of 30 wild *Solanum* species susceptible. The most recent data are found in the work of Huaman (1987), among others on the responses of wild *Solanum* species of Bolivian origin to PVX, PVY and PLRV. In his inventory he numerates a total of 46 compatible relations in 7 wild *Solanum* species: 17 with PVX, 21 with PVY and 8 with PLRV. The author describes 1 resistant relation between *Solanum andigenum* and PVX and 1 between *S. juzepczuki* and PVX, and 2 resistant relations between *S. andigenum* and PLRV. It cannot be left unmentioned either that one accession of the *Solanum tuberosum* spp. of Bolivian origin and several other wild *Solanum* species are resistant to important pathotypes of *Globodera rostochiensis* and *G. pallida* important (Ro_1, Pa_2, Pa_3), to *Phytopthora infestans*, *Synchytrium endobioticum* and to *Erwinia carotovora* var. *atroseptica* (Van Soest, 1980; Van Soest and Hondelmann, 1983).

The German-Netherlands Potato Expedition to Bolivia in 1980 collected many wild species and primitive cultivars. Among them five new, botanically not yet described species belonging to 3 series were found: *Solanum soestii*, *S. circaeifolium* ssp. *quimense* (Series VII *Circaeifolia*), *S. astleyi* (Series XV *Megistacroloba*), *S. neorossii*, *S. okadae* (Series XVIII *Tuberosa*). Considering that the virus resistance characteristics of these new species as well as of other species are not known as yet, our experiments were aimed at studying the resistance

of some species of Bolivian origin. Here we note that in the latest work by Hoekstra and Seidewitz (1987) resistance examination data are found for two species (*Solanum astleyi, S. okadae*). One of 3 accessions of *Solanum astleyi* (BGRC 027381) and 3 of 9 accessions of *Solanum okadae* (BGRC 02737, 027039, 027162) are regarded as susceptible to PVY.

Materials and Methods

In the experiments the following 9 Solanum species – of which 3 were botanically new – were included: *Solanum alandiae* (BGRC 27326), *S. astleyi* (BGRC 27381), *S. circaeifolium* ssp. *quimense* (BGRC 27163), *S. mochicense* (BGRC 18578), *S. neocardenasii* (BGRC 28001), *S. neorossii* (BGRC 18587), *S. okadae* (BGRC 027037), *S. paucissectum* (BGRC 8162), *S. violaceimarmoratum* (BGRC 28037). Young plants grown from seeds of *Solanum* species free from potato spindle tuber viroid (PSTV) were inoculated with 9 isolates of 6 viruses (Table 1). The inoculation was carried out by carborundum-spatula method using water diluted

Table 1

List of viruses

Viruses	Strains or isolates	Propagation hosts	Assay plants	Literature or virus gene bank[1]
Alfalfa mosaic virus (AMV-)	L/K_2	*Nicotiana tabacum* cv. *Xanthi-nc*	*Chenopodium amaranticolor*	Peczner (1972)
Cucumber mosaic virus (CMV-)	U/246	*N. glutinosa*	*C. amaranticolor*	Schmidt and Horváth (1982)
Henbane mosaic virus (HeMV-)	W/H	*N. tabacum* cv. *Xanthi-nc*	*Datura stramonium*	Horváth et al. (1987)
Potato virus X (PVX-)	HB,N	*N. tabacum* cv. *Xanthi nc*	*Gomphrena globosa*	IPC, Lima, Peru (L. F. Salazar); IP, Aschersleben, GDR (K. Schmelzer)
Potato virus Y (PVY-)	^{C}-2C, ^{N}-201, ^{O}-RP	*N. tabacum* cv. *Xanthi-nc*	*G. amaranticolor*, *Solanum demissum A6*-hybrid	EMP, Vitoria, Spain (F.J. Legorburn); IPC, Lima, Peru (L. F. Salazar)
Tobacco rattle virus (TRV-) (syn.: Potato stem mottle virus)	H	*N. tabacum* cv. *Xanthi-nc*	*N. glutinosa*	Horváth (1976, 1977)

[1] IPC, International Potato Center, Lima Peru; EMP, Estacion de Mejora la Patata, Vitoria, Spain; IP; Institut für Phytopathologie, Aschersleben, GDR.

(1 : 1, v/v) tissue sap of previously infected virus-containing propagation hosts (see Table 1). Since we had only 50 seeds of each *Solanum* accession and obtained 45–50 plants from them, we only could inoculate 5 plants per virus and isolate. After the inoculation the plants were sprayed with water. The responses of the *Solanum* species to viruses were symptomatologically determined. Then from inoculated and non-inoculated leaves of the *Solanum* species back-inoculation to virus specific assay plants was carried out (see Table 1) in order to find out whether possible latent infections can be pointed out too. Those *Solanum* plants were regarded as resistant from which the reisolated virus could not be transmitted to assay plants. Considering that in the case of the resistant plants grafting could not be carried out for technical reasons, their immunity could not be determined

Results and Discussion

The responses of the 9 *Solanum* species examined to 6 viruses and their 9 isolates were highly varied both symptomatologically and as regards resistance.

The host-virus relations detected in the course of the experiments can practically placed in two large groups: (A) incompatible host-virus relations and (B) compatible host-virus relations.

The incompatible host-virus relations were of resistant (1), and hypersensitive (2) nature. In the resistant host-virus relations the *Solanum* species could not be infected with viruses, and from the inoculated plants the viruses could not even be reisolated to assay plants; on the basis of the terminology of Cooper and Jones (1983) we called these plants nonhosts. The hypersensitive host-virus relations symptomatologically could be placed in three sub-groups: (a) necrotic local lesions with leaf drop, (b) dark-green islands on the leaves, yellowing, and later leaf drop, (c) dark-green islands on the inoculated leaves and necrotic lesions.

The compatible host-virus relations can be placed in three groups. To the first (1) group those host-virus relation belong in which the inoculated leaves are latent susceptible. In the latent local relations the inoculated plants did not show disease symptoms but the virus could be reisolated from the inoculated leaves. To the second (2) group those host-virus relations belong in which both local and systemic relations were established between the wild *Solanum* species and the viruses. The local and systemic relations can be symptomatologically placed in 7 sub-groups on the basis of partial differences between the symptoms: (a) necrotic local lesions, leaf drop, and systemic veinal necrosis, (b) necrotic local lesions, leaf drop, and systemic mosaic, (c) necrotic local lesions, leaf drop, systemic veinal necrosis, and death of the whole plant, (d) necrotic local lesions without leaf drop and mosaic, (e) necrotic local lesions, leaf drop, and systemic veinal necrosis as well as death of the whole plant, (f) no symptoms on the inoculated leaves, but latent infection, (g) no symptoms on the inoculated leaves and non-inoculated leaves, but latent in infection in both of them. To the third

(3) group of the compatible host-virus relations those relations belong in which only the systemic could be proved to be present. In this relation symptoms did not appear on the inoculated leaves, and even in the back-inoculation test the plants proved free from viruses. On the non-inoculated leaves mosaic appeared; from these leaves the virus could be re-isolated.

The relationships between accessions – as populations – and viruses did not show essential symptomatological differences, probably due to the small number of plants. In a single case, in the relationship between *Solanum alandiae* (BGRC 27326) and PVY^N–201 we found that 4 of the 5 inoculated plants showed local and systemic symptoms, and 1 plant was symptomless (Fig. 1A). In a back-inoculation test, however the latter proved to have latent virus infection.

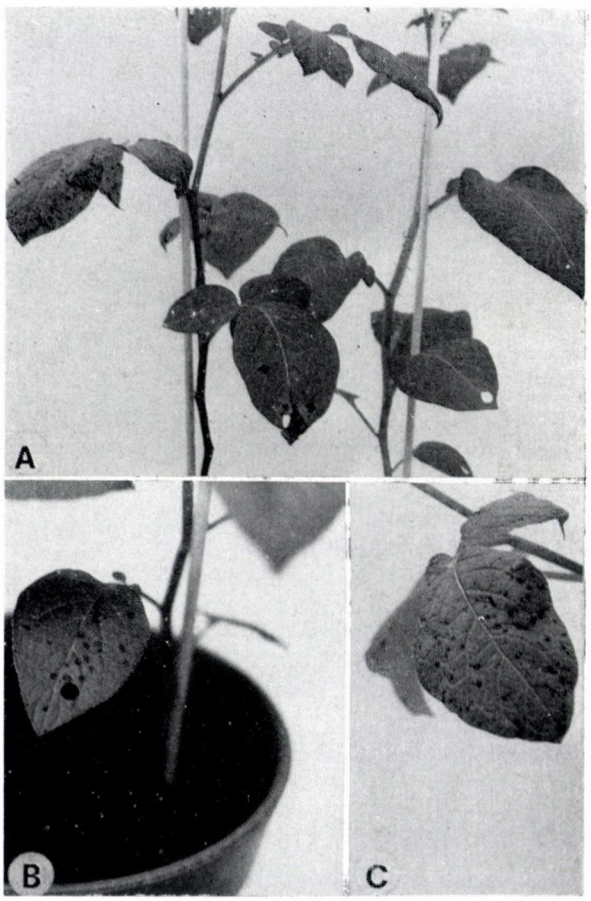

Fig. 1. A: Local and systemic symptoms on *Solanum alandiae* (BGRC 27326) inoculated with PVY^N–201 (left) and resistant plant (right). B: Local lesions and C: systemic symptoms on *Solanum alandiae* (BGRC 27326) inoculated with PVY^N–201

On the basis of the above between the *Solanum* species and viruses examined the following relations were found:

A. *Incompatible host-virus relations*

1. *Resistant relationship*

Solanum neocardenasii: PVY^{O}–RP; *S. paucissectum:* tobacco rattle virus (TRV); *S. violaceimarmoratum:* alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), potato virus X (PVX–N).

2. *Hypersensitive host-virus relations*

a. Solanum alandiae: PVX–HB, PVY^{O}–2C; *S. astleyi:* PVX–HB; *S. mochicense:* AMV, PVX–HB; *S. neocardenasii:* AMV: *S. neorossii:* AMV, PVX–HB; *S. paucissectum:* AMV, PVX–HB, PVX–N; *S. violaceimarmoratum:* PVX–HB.

b. Solanum alandiae: TRV.

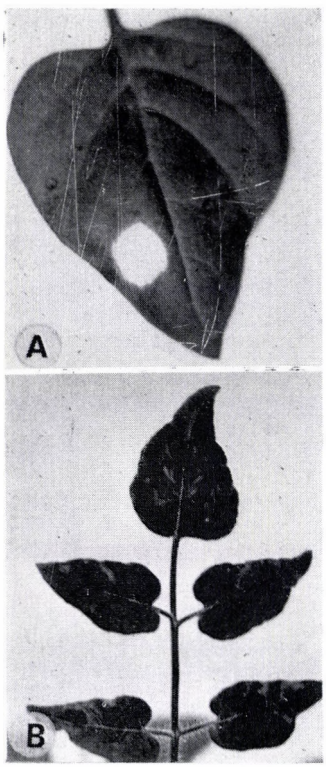

Fig. 2. Local (A) and systemic symptoms (B) on *Solanum neocardenasii* (BGRC 28001) inoculated with PVX–HB

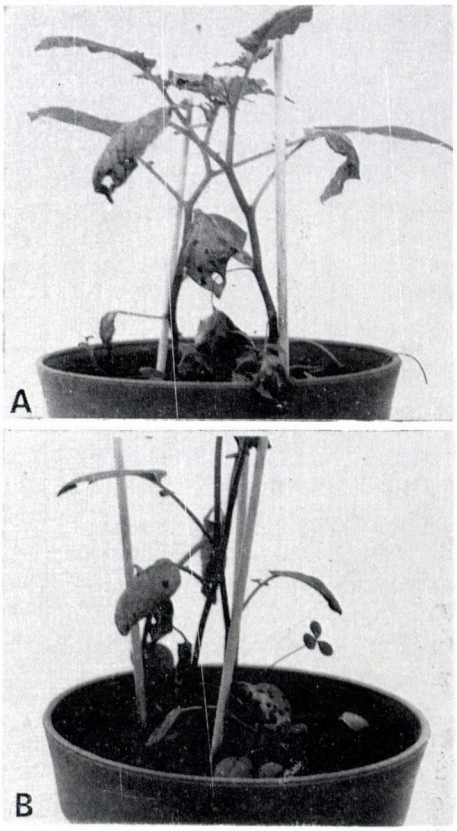

Fig. 3. A: Local and systemic symptoms on *Solanum mochicense* (BGRC 18578) and B: *Solanum neocardenasii* (BGRC 28001) inoculated with HeMV

c. Solanum circaeifolium ssp. *quimense:* PVX–HB,

B. *Compatible host-virus relations*

1. *Locally latent relationship*
Solanum alandiae: CMV.

2. *Local and systemic susceptibility*

a. Solanum alandiae: henbane mosaic virus (HeMV), PVY^{N}–201. (Fig. 1B and C); *S. circaeifolium* ssp. *quimense:* PVY^{C}–2C; *S. mochicense:* CMV; *S. neocardenasii:* PVX–N; *S. neorossii:* PVY^{C}–2C, PVY^{N}–201; *S. okadae:* TRV.

b. Solanum alandiae: PVX–N, PVY^{0}–RP; *S. astleyi:* PVY^{N}–201; *S. circaeifolium* ssp. *quimense:* AMV, CMV, PVX–N, PVY^{0}–RP, TRV;

S. mochicense: PVY^C–2C, PVY^N–201, TRV; *S. neocardenassii:* PVX–HB (Fig. 2A and B); *S. naorossii:* CMV, PVX–N, TRV; *S. okadae:* AMV, CMV, PVX–HB, PVX–N, PVY^C–2C, PVY^N–201, PVY^0–RP; *S. violaceimarmoratum:* PVY^0–RP.

c. Solanum astleyi: HeMV, PVY^0–RP; *S. mochicense:* HeMV (Fig. 3A); *S. neocardenasii:* HeMV; *S. neorossii:* HeMV (Fig. 3B); *S. okadae:* HeMV; *S. paucissectum:* HeMV; *S. violaceimarmoratum:* HeMV.

d. Solanum astleyi: CMV, PVX–N; *S. mochicense:* PVX–N.

e. Solanum circaeifolium ssp. *quimense:* HeMV.

f. Solanum circaeifolium ssp. *quimense:* PVY^N–201; *S. neocardenasii:* CMV, TRV; *S. neorossii:* PVY^0–RP.

g. Solanum neocardenasii: PVY^0–201.

3. *Systemic susceptibility*

Solanum alandiae: AMV; *S. astleyi:* AMV, TRV; *S. mochicense:* PVY^0–RP; *S. paucissectum:* PVY^N–201; *S. violaceimarmoratum:* TRV. In the course of the experiments we threw light upon some 74 new host-virus relations between the 9 *Solanum* species and 9 isolates of 6 viruses. In the case of four *Solanum* species and 6 viruses (*Solanum astleyi* – PVY^C–201; *S. neocardenasii* – PVY^C–2C; *S. paucissectum* – CMV, PVY^C–2C, PVY^0–RP; *S. violaceimarmoratum* – PVY^C–2C) the examinations could not be performed for technical reasons.

On the basis of the results it can be established that resistance could be pointed out for 3 wild *Solanum* species and 5 viruses. Hypersensitive resistance was found in the case of 8 *Solanum* species with 5 isolates of 4 viruses in 14 host-virus relations. Among the compatible relations one wild species *(Solanum alandiae)* reacted without symptoms (latent susceptible), 9 wild *Solanum* species gave local and systemic responses to 9 isolates of 6 viruses in 48 host-virus relations. Systemic susceptibility alone was observed in 5 wild *Solanum* species to 5 isolates of 4 viruses in 6 relations.

According to the literary data available earlier virological data can be found for 6 wild *Solanum* species out of those included in our experiment (Table 2).

The experiment results related with *S. circaeifolium* ssp. *quimense*, *S. neocardenasii* and *S. violaceimarmoratum* can be regarded as new in respect of the species too, while in the case of the other species they mean the disclosure of new host-virus relations. In this place attention is called to the PSTV susceptibility of *Solanum circaeifolium* ssp. *quimense* and *S. mochicense* (Singh and Slack, 1984), which originate from the collection of the Interregional Potato Center, Sturgeon Bay, Wisconsin, USA. Considering that in the course of examinations including 555 plant introductions of 81 tuber-bearing *Solanum* species the author did not find plants immune of PSTV, a continuous screening of *Solanum* germ plasms to PSTV is highly necessary in the case of wild *Solanum* species of Bolivian origin too.

Table 2

Reaction of some wild *Solanum* species to potato pathogenic viruses and potato spindle tuber viroid

Solanum species	P. I. or BGRC number	Origin	Viruses[1]		Literature
			Susceptible	Resistant	
Solanum alandiae	498087	Bolivia	PLRV		Hanneman and Bamberg (1986)
	498089				
	498090				
S. astleyi	027381	Bolivia	PVY		Hoekstra and Seidewitz (1987)
S. mochicense	238616	Peru	PVX, PSTV		Singh and Slack (1984)
	283114		PVX		Hanneman and Bamberg (1986)
	338616		PVY		
S. neorossii	473429	Argentina	PLRV		Hanneman and Bamberg (1986)
	473429		PLRV		
S. okadae	320327	Argentina	PVY	PVX	Hanneman and Bamberg (1986)
	320328	Argentina		PVX	
	027037	Bolivia	PVX		Hoekstra and Seidewitz (1987)
			PVY		
	027039	Bolivia	PVY		
	027162	Bolivia	PVY		
S. paucissectum	473489	Peru		PLRV	Hanneman and Bamberg (1986)

[1] Viruses or viroids were: PLRV, potato leaf roll virus; PVX, potato virus X; PVY potato virus Y; PSTV, potato spindle tuber viroid.

Out of the 3 botanically new species *Solanum astleyi* is particularly remarkable for its hypersensitive resistance to PVX–HB, and systemic susceptibility to PVY^N–201, HeMV, PVY^C–RP, CMV, PVX–N, AMV, TRV. Among the new species *Solanum neorossii* – which so far had only been regarded as susceptible to PLRV (cf. Hanneman and Bamberg, 1986) – proved susceptible to the following viruses too: PVY^C–2C, PVY^N–201, CMV, PVX–N, TRV, HeMV, PVY^O–RP. The third botanically new species, *Solanum okadae* had been known to be susceptible to PVY and resistant to PVX (see Table 2). In our own experiments the latter plant proved susceptible to the following viruses: TRV, AMV, CMV, PVX–HB, PVX–N, PVY (all of its three isolates) and HeMV.

In the inventory of Ross and Rower (1965) there are two species (*Solanum mochicense*, Peru, P. I. 283114; *S. violaceimarmoratum*, Bolivia, P. I. 258856) which were included in our experiments reported in this paper. Our results concerning these species also can be regarded as new, since the work of Ross and Rowe (1965) does not contain data on their virological resistance. In our experiments *Solanum mochicense* showed hypersensitive resistance to AMV and PVX–HB, local and systemic susceptibility to CMV, PVY^C–2C, PVY^N–201,

TRV, HeMV, PVX–N and systemic susceptibility to PVY^{O}–RP. *Solanum violaceimarmoratum* proved resistant of AMV, CMV, PVX–N and showed hypersensitive resistance to PVX–HB. Local and systemic relation was pointed out for the latter plant with PVY^{O}–RP and HeMV, and systemic relation with TRV.

Of the viruses studied in our experiments PVY, PVX and TRV are widespread and have a high economic importance (MacLeod, 1962; Horváth, 1967; Spaar and Hamann, 1977; Hooker, 1981; Rich, 1983; De Bokx and Van der Want, 1987). Therefore the new results of examinations concerning the resistance of these viruses are important from both virological and breeding points of view.

AMV and CMV do not belong to the dangerous virus pathogens of potato, yet, their importance must not be underestimated (Anonymous, 1985; Somerville et al., 1987). Each of the two polyphagous viruses possesses a wide range of hosts (Hull, 1969; Beczner, 1973; Horváth, 1979, 1980, 1981b), and has been known as a pathogen of potato for more than fifty years (Porter, 1935; Folsom and Bonde, 1936; Black and Price, 1940; Horváth, 1963, 1981a, b; reviewed by MacLeod, 1962; Beczner and Horváth, 1973; Schmelzer and Spaar, 1975). For this very reason we think that the results of our present investigations, namely, that the species *Solanum violaceimarmoratum* is resistant of both viruses mentioned above are remarkable. The hypersensitive resistance of *Solanum mochicense*, *S. neocardenasii*, *S. neorossii* and *S. paucissectum* to AMV is also important. And the new compatible host-virus relation pointed out for 7 wild *Solanum* species with the two viruses (AMV: *Solanum alandiae*, *S. astleyi*, *S. circaeifolium* ssp. *quimense*, *S. okadae;* CMV: *S. alandiae*, *S. astleyi*, *S. circaeifolium* ssp. *quimense*, *S. mochicense*, *S. neocardenasii*, *S. neorossii*, *S. okadae*) is important from the point of view of virus prognosis.

HeMV was included in the experiments because – while its spontaneous occurrence in potato has not been proved so far – recently it has been found to be transmissible to potato and wild *Solanum* species under experimental conditions (Horváth et al., 1987, 1988). According to our present experiment results all *Solanum* species examined are susceptible to the virus. This fact made it reasonable to examine new wild *Solanum* species for HeMV.

From our experiments – which threw light upon new host virus relations and upon the virus resistance of botanically new wild *Solanum* species – we have drawn the conclusion that between accessions of the wild *Solanum* species collected in Bolivia and various viruses not only compatible, dangerous host-virus relations may exist which must be reckoned with under natural conditions, but these plants may be sources of virus resistance possible important for potato breeding. It is known that the genetic base of potato breeding needs permanent broadening (Ross, 1979, 1986; Horváth, 1984, 1988) which involves seeking out new botanically unknown wild *Solanum* species and new indispensable resistance sources.

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Asclepias syriaca L. (Common Milkweed), a New Natural Host of Cucumber Mosaic Virus in Hungary and Yugoslavia*

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Cucumber mosaic virus (CMV) was isolated in Hungary and Yugoslavia from wild-growing *Asclepias syriaca* L. (common milkweed) plants showing mosaic, vein-clearing, line-pattern or mosaic symptoms. 5–35 per cent of the individuals were found to be affected in different places.

Three isolates of CMV (Asc/B76 and Asc/H from Hungary as well as Asc/Y from Yugoslavia) were identified on the basis of reactions of test plants and by agar-gel double diffusion serological tests using antisera to CMV. The isolate Asc/B76 was also characterized by host range, serological comparison with the To and D serotypes of CMV as well as by particle morphology, aphid transmission, cross protection tests and physical properties in plant extract. Based on these results, this isolate was classified within the To serotype (B pathotype) of CMV.

Preliminary studies revealed that the Asc/H and Asc/Y isolates of CMV differ from the CMV/B76 by infecting some leguminous species (e.g. *Vigna sinensis)* systemically.

Asclepias syriaca as a perennial plant plays an important role in the maintenance and infection chain of CMV in the Carpatic Basin.

Viruses or virus-like pathogens have been reported infecting several plant species that belong to the family *Asclepiadaceae* (Thornberry, 1966; Schmelzer and Wolf, 1977). The majority of information refer to the species *Asclepias syriaca* (syn.: *A. cornuti*, common milkweed), which has been found to be naturally infected by cucumber mosaic virus (CMV) in North-America and Europe as well as by alfalfa mosaic virus (AMV) in North-America, Europe. As far as the other members of the milkweed family are concerned, relatively few data are known about their spontaneous viral infections (Table 1). However, the discovery of a new potyvirus, *Araujia* mosaic (AjMV) in *Araujia* and *Morrenia* species in South-America and the attempts to use it for the biocontrol of the weed *Morrenia odorata* (cf. Charudattan et al., 1976, 1978, 1980) call the attention to this, virologically little known family of plants.

Since 1975, specimens of *Asclepias syriaca* have been found affected by mosaic, vein-clearing and line-pattern symptoms in Hungary and Yugoslavia,

*In memoriam Dr. L. Beczner, Ph.D. († 10. November 1988).

Table 1

Natural occurrence of viruses pathogenic to the family *Asclepiadaceae*

Plant species	Viruses[1]	Countries	Literature
Araujia angustifolia	AjMV	Argentina, Uruguay	Charudattan et al. (1976, 1978, 1980)
A. species (?)	AjMV	Argentina, Uruguay	
Asclepias curassavica	CMV	Brazil	Silberschmidt (1955)
A. curassavica	CMV	Europe	Schmelzer and Wolf (1977)
	RLP	Ecuador	Dollet and Gargani (1989)
A. syriaca	CMV	USA	Doolittle (1916), Doolittle and Walker (1926) Rist and Lorbeer (1989)
A. syriaca	UV	USA	Newhall (1923)
A. syriaca	CMV	Canada	Koch (1942)
A. syriaca	CMV	Poland	Kochman and Stachyra (1960)
A. syriaca	CMV	Bulgaria	Kovachewsky (1965)
A. syriaca	CMV	Hungary	Salamon (1978, 1986), Horváth et al. (1983)
A. syriaca	CMV	Yugoslavia	Horváth et al. (1983), Mamula et al. (1986)
A. syriaca	AMV	GDR	Schmelzer et al. (1973)
A. tuberosa	CMV	the Netherlands	Oudshorn (1963)
Cynanchum acutum	AMV	Italy	Savino and Gallitelli (1976)
Morrenia odorata	AjMV	Argentina	Charudattan et al. (1980)
	CMV	USA	
M. species (?)	AjMV	Argentina	

[1] Meaning of abbreviations: AjMV — *Araujia* mosaic virus, AMV — alfalfa mosaic, CMV — cucumber mosaic virus, RLP — rhabdovirus-like particles are found in symptomatic leaves of Asclepias. UV — identified virus.

suggesting that some viruses were spread in the common milkweed populations in both countries. As far as we know, there were approximately 30 thousand hectars covered with infected common milkweed in Hungary in 1985 (Varga, 1986; Hunyadi and Varga, 1988). Severe infestation occurs on sandy soil in the middle of Hungary on both sides of the Danube river.

Materials and Methods

In the years of 1975–1988 leaf samples of diseased *Asclepias syriaca* plants were collected from several localities in Hungary and in the sorroundings of Zagreb, Yugoslavia. For the isolation of virus(es), leaf samples were washed with 2 per cent NaOH and rinsed with cold tap water, then ground in sterile mortars with a pestle by adding ca. one part (w/v) of 67 mM phosphate buffer (pH, 7.0)

to each one. Using the inocula prepared as above *Chenopodium* spp., *Cucumis sativus* and *Nicotiana* spp. known to be susceptible to a wide range of viruses were rubbed manually or with glass spatula. Carborundum (400 mesh) was used as an abrasive.

Two virus isolates, designated Asc/B76 and Asc/H from Hungary as well as the third one marked Asc/Y from Yugoslavia were selected for host range and serological investigations. *Nicotiana tabacum* cv. *Xanthi-nc* and/or *N. megalosiphon* served as propagative hosts of each isolate throughout this work.

Serological double diffusion tests were performed in 0.75 or 1 per cent agar (Difco Noble) or agarose (Reanal) gels dissolved in various buffers. The gels contained 0.02 per cent NaN_3. In routine serological tests virus antigens were prepared from original *Asclepias* leaves and from systemically infected leaves of the propagative hosts by grinding them in 0.5 M Na_3-citrate (pH, 6.5) buffer. The extracts were low-speed centrifuged before use. Control antigens were taken from healthy tobacco plants. In the case of serological investigations of the Asc/H and Asc/Y isolates, an antiserum to a carnation isolate of CMV, kindly supplied by Dr. D. E. Luisoni, Turin, Italy was used. This antiserum had a homologous titre of 1 : 128 (Štefanać et al., 1981). For the serological comparative studies the Asc/B76 isolate was partially purified according to the method of Lot et al. (1972). Purified antigens of the To and D serotypes of CMV as well as antisera to them marked by CMV–D606 and CMV–W609 were kindly supplied by Dr. J. C. Devergne, Antibes, France. In the spur tests some other isolates of CMV, e.g. CMV–SnTz2 (Salamon, 1989) were also used as antigens.

The isolate Asc/B76 was further characterized by particle morphology, aphid transmission and cross protection tests as well as by physical properties.

A drop of the partially purified preparate of this isolate was loaded on formvar-carbon coated grid, and stained with 2 per cent phosphotungstic acid (pH 6.5). The negatively stained virions were examined in an OPTON EM 9 S2 electron microscope.

Aphid transmission experiments were carried out using *Myzus persicae* as vector. The aphids reared on virus-free turnip *(Brassica rapa* var. *rapa)* plants, were starved for overnight, and allowed to feed for 10 minutes on tobacco *(Nicotiana tabacum* cv. *Xanthi-nc)* leaves infected by Asc/H and Asc/B76. The aphids were transferred to healthy tobacco plants (ten aphids pro plant), kept there for 10 minutes and then killed by Pirimor insecticide.

In cross protection tests a total of 12 young tobacco *(Nicotiana tabacum* cv. *Xanthi-nc)* plants were inoculated by each of the isolates Asc/H and Asc/B76. Six days later 10 plants out of them were challenge inoculated with the white strain of CMV (CMV–W) (Skiebe and Schmelzer, 1967). Healthy tobacco plants inoculated by CMV–W alone served as control.

The physical properties (thermal inactivation point, dilution end-point and longevity *in vitro*(of the three isolates were determined as proposed by Bos et al. (1960). Infected *Nicotiana tabacum* cv. *Xanthi-nc* plants were used as virus donor and *Chenopodium amaranticolor* as the assay species.

Results and Conclusion

Viral symptoms on *Asclepias syriaca*

Disease symptoms, characteristics of viral infections, e.g. conspicuous mosaic spotting, yellow line pattern and rarely vein clearing on the leaves of *Asclepias syriaca* have been found in distant localities in Hungary and near Zagreb, Yugoslavia (Fig. 1). In the wild-growing milkweed populations surveyed symptomatologically in 1975–1988, the percentage of symptom bearing plants was usually 5–15 per cent, but in some places it reached 30–35 per cent.

Isolation and identification of cucumber mosaic virus (CMV)

Each virus isolate caused necrotic local lesions without systemic symptoms on *Chenopodium amaranticolor*, *C. quinoa*, while *Cucumis sativus* and *Nicotiana*

Fig. 1. Symptoms on the leaf of *Asclepias syriaca* naturally infected with cucumber mosaic virus (isolate *Asc/H*)

tabacum cv. *Xanthi-nc* plants became infected both locally and systemically. The only local susceptibility of *Chenopodium amaranticolor* and. *C. quinoa* and the type of symptoms which appeared on the systemically susceptible indicator plants strongly suggested that CMV alone was isolated from *Asclepias syriaca.* For further characterization the isolate marked by Asc/B76 and Asc/H from Hungary and Asc/Y from Yugoslavia were selected as representatives.

Host range

Inoculation of the isolate Asc/B76 to different plant species (a total of 28 species belonging to 10 families) indicated, that this isolate has a wide host range characteristic of CMV (Horváth, 1976, 1980; Kaper and Waterworth, 1981).

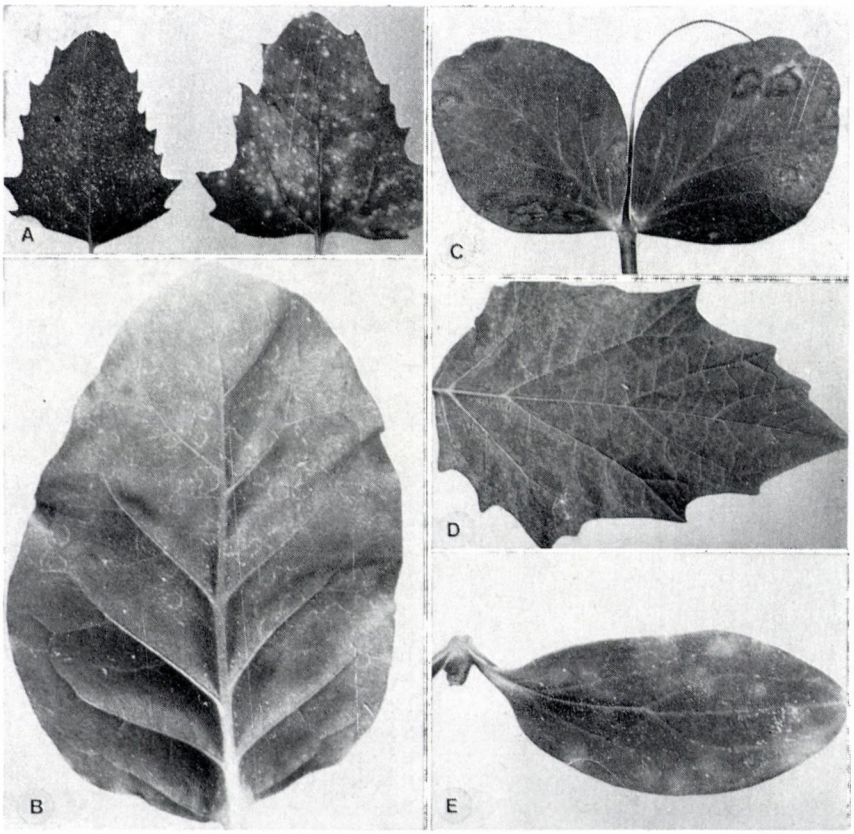

Fig. 2. Symptoms on different test plants infected with cucumber mosaic virus (isolate *Asc/B76*). A: local lesions on the leaf of *Chenopodium amaranticolor* (left) and *C. quinoa* (right). B: necrotic local etched rings on *Nicotiana tabacum* cv. *Xanthi-nc*. C: local necrotic lesions on the leaf of *Pisum sativum*. D: systemic mosaic symptoms on the leaf of *Datura stramonium*. E: chlorotic local lesions on the primordial leaves of *Cucumis sativus*

Table 2

Host range and symptomatology of CMV-Asc/B76 isolated from naturally infected *Asclepias syriaca*

Families and species[1]	Symptoms
AMARANTHACEAE	
Gomphrena globosa	L: chlorotic spots S: chlorotic spots, mosaic
ASCLEPIADACEAE	
Asclepias curassavica	L: necrotic spots and rings S: necrotic spots, mosaic
A. incarnata	L: necrotic spots and rings S: mild mosaic
A. rubra	L: necrotic spots and rings S: mosaic
A. syriaca	L: chlorotic spots S: yellow spots, mosaic
CHENOPODIACEAE	
*Chenopodium amaranticolor**	L: pin-point necrotic lesions (Fig. 2A) S: not infected
C. murale	L: brown necrotic lesions S: not infected
*C. quinoa**	L: reddish necrotic lesions (Fig. 2A) S: not infected
COMPOSITAE	
Ageratum houstinianum	L: chlorotic lesions S: deformations, stem necrosis
CRUCIFERAE	
Brassica rapa var. *rapa*	L: not infected S: not infected
Crambe abyssinica	L: not infected S: not infected
CUCURBITACEAE	
Cucumis sativus cv. *Delicatesse*	L: chlorotic lesions (Fig. 2E) S: mosaic, deformations
LABIATAE	
Ocimum basilicum	L: chlorotic spots S: mosaic
LEGUMINOSAE	
Phaseolus vulgaris cv. *Pinto*	L: not infected S: not infected
Pisum sativum cv. *Rajnai törpe*	L: necrotic lesions (Fig. 2C) S: not infected
Vicia faba cv. *Lippói*	L: necrotic lesions S: not infected
Vigna sinensis cv. *Black Eye**	L: necrotic lesions S: not infected
SOLANACEAE	
Capsicum annuum cv. *Almaalakú*	L: chlorotic spots S: leaf narrowing, mosaic, partial recovery
*Datura stramonium**	L: latent infection S: mosaic, yellow spots (Fig. 2D)

Families and species[1]	Symptoms
Hyoscyamus niger	L: chlorotic spots S: mosaic, ring spots
Lycopersicon esculentum cv. *Primset*	L: latent infection S: leaf narrowing, deformations
Nicandra physaloides	L: latent infection S: mosaic, necrotic spots
Nicotiana clevelandii	L: chlorotic spots S: mild mosaic, deformations
N. debneyi	L: chlorotic spots and rings S: mild mosaic, deformations
*N. megalosiphon**	L: necrotic rings S: necrotic rings, and lines, recovery
N. tabacum cv. *Xanthi-nc*	L: etched necrotic rings (Fig. 2B) S: etched rings, line pattern, recovery
Vestia lycioides	L: not infected S: not infected
UMBELLIFERAE *Ammi majus*	L: symptomless infection S: symptomless infection

[1] Plants marked with asterisk were only studied for susceptibility and reactions to Asc/H and Asc/Y, respectively.

The majority of the inoculated species, listed in Table 2, became infected both locally and systemically and showed symptoms typical of CMV. The local and systemic reaction of *Nicotiana tabacum* cv. *Xanthi-nc* was similar to those described characteristic of the strain B of CMV (Marrou et al., 1975). In our inoculation experiments four *Asclepias* species could be easily infected by the isolate Asc/B76 even when the inocula were prepared from tobacco plants. In contrast to this, Silberschmidt (1955) reported that he was able to infect *Asclepias curassavica* with difficulties if tobacco was used as the donor plant. The symptoms induced by the isolate Asc/B76 on *Asclepias syriaca* under experimental conditions were similar to those observed on spontaneously infected plants supporting the causal relationship between the isolated virus and the investigated disease.

Concerning the pathological properties of the isolates Asc/H and Asc/Y, the species marked by asterisks in Table 2 were so far inoculated with them. Most of the inoculated plants reacted to these isolates as to Asc/B76 except *Vigna sinensis* which became infected systemically. This reaction of *Vigna sinensis* shows that strains of CMV differ from the isolate Asc/B76 occur in *Asclepias syriaca*.

Serology

In double gel-diffusion test partially purified preparation of the isolate Asc/B76 reacted strongly with the CMV antisera W609 and D606. When the antiserum to CMV–To was placed in the central well and antigens of To sero-

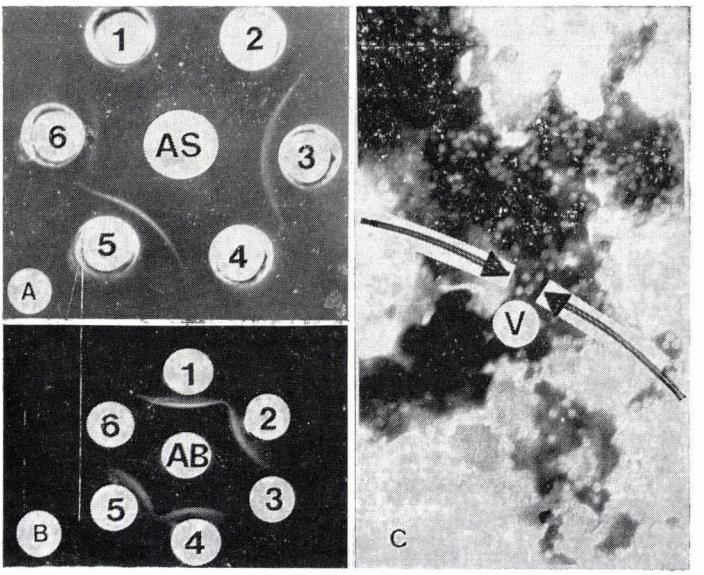

Fig. 3. Results of the serological (A and B) and electron microscopical (C) investigations. A: Gel-diffusion test using partially purified isolates of cucumber mosaic virus. AS — cucumber mosaic virus *D606* antiserum, 1–cucumber mosaic virus SnTz2 antigen, 2 — healthy plant sap from *Nicotiana tabacum* cv. *Xanthi-nc*, 3 — cucumber mosaic virus *To* standard antigen, 4 — cucumber mosaic virus *W* antigen, 5 — cucumber mosaic virus *Asc/B76* antigen, 6 — cucumber mosaic virus *D* standard antigen. B: Gel-diffusion test with isolates of *Asc/H* and *Asc/Y* of cucumber mosaic virus. AS — antiserum to carnation isolate of cucumber mosaic virus, 1 and 4 — sap of *Nicotiana tabacum*cv. *Xanthi-nc* infected with *Asc/H*, 2 and 5 — sap of *Nicotiana tabacum* cv. *Xanthi-nc* infected with Asc/Y, 3 and 6 — sap of healthy *Nicotiana tabacum* cv. *Xanthi-nc*. C: negatively stained virions in partially purified preparation of the isolate *Asc/B76* of cucumber mosaic virus. → V–virions

type of CMV and of Asc/B76 in the peripherial wells the precipitation lines against the antigens coalesced without spur formation. However, spur was formed between the precipitin lines of the D serotype of CMV and the Asc/B76 isolate (Fig. 3A). These results have showed that our Asc/B76 isolate belongs to the To serotype of CMV. In other set of serological experiments Asc/H and Asc/Y isolates reacted with the antisera prepared against the carnation isolate of CMV (Fig. 3B). The titre of this antiserum to the Asc/H and Asc/Y isolates was 1 : 64, closely to the homologous titre.

Electron microscopy

Spherical particles of c. 28 nm diameter were seen in partially purified preparations of the isolate Asc/B76 (Fig. 3C). Other type of virions have not been found.

Aphid transmission

Two isolates (Asc/H and Asc/B76) from *Asclepias syriaca* could be easily transmitted by *Myzus persicae* aphids from tobacco to tobacco plants in a non-persistent manner.

Cross protection

The isolates of Asc/H and Asc/B76 protected tobacco plants against the infection of the CMV–W.

Physical properties

Thermal inactivation point of isolates was found to be at 62–65 °C, when assayed on *Chenopodium quinoa* and *Nicotiana megalosiphon* plants. The dilution end-point 10^{-3}–10^{-4}. The virus isolates remained infective in plant extracts after storage 4 to 6 days at room temperature.

Discussion

Asclepias syriaca, the perennial species was introduced to Europe from North-America where it is endemic and known to be noxious weed (Bhowmick and Bandeen, 1976). During the past decade *Asclepias syriaca* has been found to spread in the ruderal flora and in some agricultural crops both in Hungary and Yugoslavia. Our investigations have shown that *Asclepias syriaca* is not only a weed, but a very important host for some viruses, e.g. CMV. The role of the *Asclepias syriaca* as virus host in Hungary and Yugoslavia is the same as described by some authors in USA (Doolittle, 1921; Doolittle and Walker, 1926). Considering that *Asclepias syriaca* is known to be susceptible to a number of economically important plant viruses other than CMV (Kable and Parker, 1968; Thornberry, 1966; Schmelzer and Wolf, 1977), further investigations are needed to demonstrate whether this species is or is not affected by these viruses in our countries.

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Physiology of Rice Tungro Virus Disease: Changes in Leaf Pigments due to Infection

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Alterations in the concentrations of various leaf pigments caused by tungro, a virus complex in a sensitive (Taichung Native 1), and intermediate (IR 20) cultivars and in a tolerant line (CR 44–955) was examined *vis-à-vis* in senescing leaf blade segments of the tungro-sensitive rice cultivar. The rate of disease-induced reductions in total chlorophyll contents increased with the disease development and was maximum in a sensitive cultivar. Changes in chlorophyll contents of a tolerant line were less significant when compared with the other two cultivars. Diseased leaf blades of sensitive and intermediate cultivars contained higher amounts of carotenoids than the healthy leaf blades even during the early stages of disease development. This explains the characteristic leaf discolouration caused by the disease. The concentrations of anthocyanins and flavonols decreased due to tungro infection The disease-induced reduction in these pigments was greater in the sensitive cultivar than in intermediate and tolerant line. The concentrations of total chlorophylls, anthocyanins and flavonols decreased in senescing healthy leaf blade segments, while that of carotenoids increased during senescence. Kinetin retarded and abscisic acid accelerated the loss of chlorophyll, and the rate of accumulation of carotenoids in senescing leaf blade segments. Although kinetin retarded the loss of anthocyanins and flavonols, abscisic acid did not have any marked effect on these pigments. The possible role of hormones like cytokinins and abscisic acid in the disease-induced changes of leaf pigments is discussed.

Tungro is a virus complex caused by rice tungro spherical virus and rice tungro bacilliform virus transmitted in a semipersistent manner by green leafhoppers (Hibino et al., 1978; Saito et al., 1981). The disease is characterized by stunting and discolouration of leaves which ranges from various shades of yellow-orange to orange-red besides other associated symptoms. Localized or general yellowing is a common symptom of virus infection in leaves (Esau, 1968; Smith, 1972; Matthews, 1981). Although it has been recognised that many of the physiological and biochemical changes occurring in virus-infected plants are not specific to virus replication, but may be caused by ageing and by certain environmental conditions (Farkas and Solymosy, 1965; Matthews, 1981), the cause of alterations in pigments in virus-infected plants is little understood. In this paper,

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we explore the similarity of changes in leaf pigments between tungro-affected and senescing rice leaf blades and discuss the possible causes that induce these changes.

Materials and Methods

Plant materials and inoculation. Rice (*Oryza sativa* L.) cultivars Taichung Native 1 (sensitive) and IR 20 (intermediate) and the line CR 44–955 (tolerant) differing in their reaction to tungro by producing severe, intermediate and mild symptoms when infected with tungro were used. The conditions of plant culture are the same as described in an earlier paper (Mohanty and Sridhar, 1982). Twenty-five-day-old plants at 3 to 4 leaf stage were caged individually in galvanized iron-wire-net cages and were inoculated by confining two viruliferous green leafhoppers [*Nephotettix virescens* (Distant)] in each cage for 8 hr (Sridhar, et al., 1976). Control plants were similarly treated with nonviruliferous insects.

Sampling. The symptoms of the disease appeared in the form of interveinal chlorosis on the fifth leaf blades of the plants, which had emerged fully six days after inoculation. The fifth leaf blades were collected separately from 10 healthy and 10 virus-infected plants at 3 day intervals from 6 to 21 days after inoculation (zero to 15 days of leaf emergence).

Senescence studies. Pigment changes in senescing healthy leaf blade segments were studied following the floatation method (Tetley and Thimann, 1974). Five-cm apical segments of fully expanded second leaf blades from 20-day-old healthy seedlings of tungro sensitive cultivar (Taichung Native 1) were floated with their adaxial side up in groups of five on 20 ml of either distilled water of 2×10^{-5} M kinetin (Loba Chemie, Austria) or abscisic acid (Sigma Chemical Co., U.S.A.) in 10-cm Petri dishes. The Petri dishes were incubated in the dark at 28 ± 2 °C for 3 days. The leaf segments remained floating throughout the experiment. The senescing leaf blade segments were collected at day zero and at 24 hr intervals for three days, and were used for the determination of leaf pigments. The loss of chlorophyll pigment is used as a measure of senescence.

Extraction and estimation of pigments. Leaf blade samples blotted to remove the water from the surface, and cut into small pieces of 1 to 2 mm, were extracted twice with 10 volumes of boiling 80 per cent (v/v) ethanol on a hot water bath (Mahadevan and Sridhar, 1986). The concentrations of total chlorophyll (Arnon, 1949) and anthocyanins and flavonols (Swain and Hillis, 1959) present in the alcohol extract were determined spectrophotometrically. Carotenoids were extracted from a different set of leaf blade samples in acetone and were partitioned with peroxide-free ether before and after saponifying the extract to remove chlorophyll and interfering lipids. The concentration of the pigment finally dissolved in ethanol was spectrophotometrically measured (Harborne, 1973). All the extractions were carried out under white fluorescent light.

Number of determinations. Data represent the mean of four determinations performed in duplicate with each of two identical extracts. The experiments were repeated once, and the data presented are from a typical experiment.

Results and Discussion

Total chlorophyll. In accordance with the earlier reports (Raychaudhuri et al., 1969; Chowdhury and Mukhopadhyay, 1974; Sridhar et al., 1976, 1978; John and Rao, 1979), this study has also shown that tungro infection caused marked reductions in chlorophyll contents particularly in sensitive and intermediate cultivars (Table 1). Tungro infection significantly reduced the total chlorophyll contents in the leaf blades of sensitive cultivar even from the initial sampling on six days after inoculation. The rate of disease-induced reduction in the concentration of pigments in leaf blades of both sensitive and intermediate cultivars increased with the development of the disease. Sensitive cultivar recorded greater amount of reduction in the content of the pigments than the intermediate cultivar. The infection induced significant reduction in total chlorophyll content in tolerant line only 15 days after inoculation.

The low chlorophyll content of the diseased plants may be either due to a stimulation of normal cell enzymes that affect chlorophyll or utilization of plastid protein for the synthesis of virus protein (Bawden, 1956). Even though the decrease in chlorophyll content in tungro-diseased leaves of sensitive and intermediate cultivars correlated with the increase in infectivity titer of the plants (Sridhar

Table 1

Total chlorophyll content in leaf blades of healthy and tungro-infected plants differing in their reaction to the disease. (Rice cultivars/line, sensitive (Taichung Native 1), intermediate (IR 20) and tolerant (CR 44-955) to tungro were inoculated at 3–4 leaf stage, and the fifth leaf blades were analysed. Data represent the mean of four determinations, and are in mg chlorophyll per g fresh wt of tissue)

Cultivar/line	Condition	Days after leaf emergence					
		0	3	6	9	12	15
		Days after inoculation					
		6	9	12	15	18	21
Sensitive	Healthy	1.15a	1.28b	1.41a	1.46a	1.39a	1.25a
	Inoculated	1.07bc	0.97c	0.91b	0.84c	0.74d	0.61c
		(− 7)	(− 24)	(− 35)	(− 42)	(− 47)	(− 51)
Intermediate	Healthy	1.08bc	1.31ab	1.41a	1.46a	1.35a	1.21ab
	Inoculated	1.05c	1.02c	0.93b	0.88c	0.82c	0.81d
		(− 3)	(− 22)	(− 34)	(− 40)	(− 39)	(− 34)
Tolerant	Healthy	1.13ab	1.33ab	1.38a	1.44a	1.24b	1.16bc
	Inoculated	1.16a	1.36a	1.28a	1.26b	1.18b	1.14c
		(+ 3)	(+ 2)	(− 7)	(− 12)	(− 5)	(− 2)

Values within a column followed by a common letter do not differ significantly at P = 0.05, according to Fischer's least significant difference test.

Figures in parentheses represent per cent difference over healthy (+, increase; −, decrease).

et al., 1978) until 18 and 15 days after inoculation, respectively, such a correlation was lacking in still aged plants. That the loss of chlorophyll in virus-infected plants is not necessarily related to virus titer has been reported (Pratt, 1967).

In response to tungro infection, severe chlorotic symptoms developed only in young leaves which had emerged after inoculation and not in old leaves. No appreciable leaf discolouration occurred in old leaves which were present at the time of inoculation. Concentration of total chlorophyll in the fifth leaf blades of healthy plants increased with leaf maturation until nine days after their emergence and declined subsequently in all the three cultivars. The diseased leaf blades of sensitive and intermediate cultivars contained lower amounts of chlorophyll, and, in addition, its concentration gradually declined with the age of the tissues. Similar observations have been made by Jenson (1968) in barley infected with barley yellow dwarf virus. Apparently, reduction in the chlorophyll pigments in virus infected leaves is related to inhibition of pigment synthesis (Crosbie and Matthews, 1974).

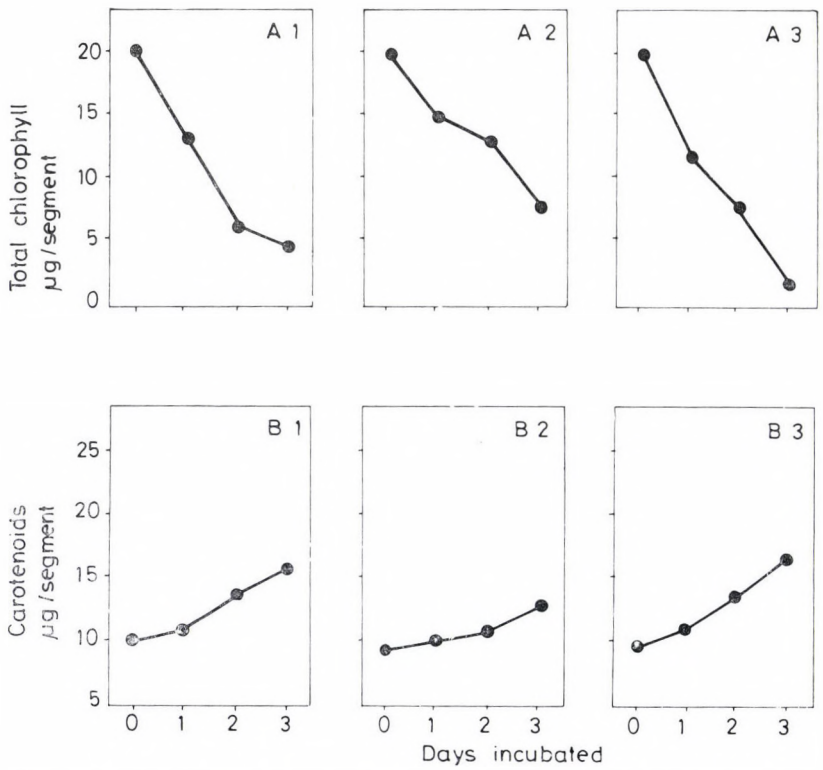

Fig. 1. Time course of total chlorophyll (A) and carotenoids (B) contents of detached leaf blade segments during senescence. (Leaf blade segments of rice cultivar, Taichung Native 1 were floated on water (1) and on 2×10^{-5} M kinetin (2) and on abscisic acid (3) in the dark)

Disappearance of chlorophyll from the virus-infected leaves due to accelerated senescence should also be considered while studying the pigment changes in diseased tissues, for similar to diseased leaves, total chlorophyll content decreased markedly in senescing detached leaf tissues (Fig. 1).

The loss of chlorophyll from the detached and dark-incubated (senescing) leaf blade segments of tungro-infected less-susceptible cultivar is delayed when compared to that of healthy leaves (Sridhar et al., 1978), and, in contrast, the loss of pigment during accelerated senescence from the leaf blade segments of tungro-infected leaves of a susceptible cultivar is more rapid than that from the healthy leyves (Mohanty et al., 1979). The delay in the loss of chlorophyll, and an enhanced rate of pigment disappearance have been related with disease-induced changes in cytokinin- (Sridhar et al., 1978) and abscisis acid- (Mohanty et al., 1979) like substances in the diseased plants. The data presented herein (Fig. 1) show that kinetin retarded and abscisic acid accelerated the loss of chlorophyll in the senescing leaf blade segments (Thimann, 1980; Thomas and Stoddart, 1980). Cytokinins are known to delay leaf senescence (Letham, 1967; Thomas and Stoddart, 1980). In light of these observations, our assumption that the loss of chlorophyll is induced by premature or accelerated leaf senescence of diseased leaves, presumably controlled by the altered hormonal status of the plants cannot be excluded.

Tungro infection has been shown to affect the root development (Ling, 1972). The impairment of root system is more severe in sensitive cultivar than in intermediate cultivar and in tolerant line (Mohanty et al., unpublished). Cytokinins are produced in the roots and transported in the transpiration stream to leaves and other aerial parts (Kende and Sitton, 1967; Letham, 1967; Wareing et al., 1977). Presumably, tungro-infected sensitive cultivar lacks the ability to synthesize and transport cytokinins to the foliage. On the other hand, no significant effect on chlorophyll concentration of the tolerant line was noticed. This is perhaps due to the normal cytokinin supply from their root system which is not adversely affected by the viruses.

The low level of total chlorophyll content in infected intermediate cultivar whose root system also was not affected by the virus could possibly by explained by the disease-induced inhibition of pigment synthesis and to the probable increase in abscisic acid level which would have affected the cytokinin-abscisic acid balance. Evidently, the decreased concentration of chlorophyll in tungro-infected rice leaves especially during the later stages of disease development is under the control of delicate balance between different endogenous hormones.

Reports on involvement of chlorophyllase in chlorophyll degradation are contradictory. Despite the role of chlorophyllase in chlorophyll synthesis has been established (Holden, 1961; Shimizu and Tamaki, 1963), it is not clear whether this enzyme is responsible for chlorophyll biosynthesis or for its degradation (Rebeiz and Castelfranco, 1973; Jackson, 1976). Therefore, it appears reasonable to conclude that the reduced chlorophyll contents of tungro-diseased leaves especially in those of sensitive and intermediate cultivars is due to both

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Table 2

Carotenoid content in leaf blades of healthy and tungro-infected plants differing in their reaction to the disease. (Rice cultivars/line, sensitive (Taichung Native 1), intermediate (IR 20) and tolarent (CR 44–955) to tungro were inoculated at 3–4 leaf stage, and the fifth leaf blades were analysed. Data represent the mean of four determinations, and are in μg carotenoid (β-carotene equivalent) per g fresh wt of tissue)

Cultivar/line	Condition	Days after leaf emergence					
		0	3	6	9	12	15
		Days after inoculation					
		6	9	12	15	18	21
Sensitive	Healthy	348.26b	354.85c	373.56c	384.17c	386.67e	388.57e
	Inoculated	351.18b	368.33b	392.51ab	416.67b	431.55bc	455.00b
		(+1)	(+4)	(+5)	(+9)	(+12)	(+17)
Intermediate	Healthy	351.22b	364.61bc	388.19abc	409.99b	416.23cd	421.44d
	Inoculated	369.33a	382.71a	401.67a	457.30b	466.26a	469.34a
		(+5)	(+5)	(+4)	(+12)	(+12)	(+11)
Tolerant	Healthy	356.08b	370.06b	376.67bc	394.23c	406.54d	435.74c
	Inoculated	351.19b	359.64bc	389.70abc	409.99b	443.29b	450.81b
		(−1)	(−3)	(+4)	(+4)	(+9)	(+4)

Values within a column followed by a common letter do not differ significantly at P = 0.05, according to Fisher's least significant difference test.

Figures in parentheses represent per cent difference over healthy (+, increase; −, decrease).

inhibition of pigment/synthesis, particularly during early stages of leaf growth and development, and to degradation of the pigment caused by the premature and accelerated leaf senescence during later stages of infection.

Carotenoids. The carotenoids concentration of the leaf blades from both the healthy and inoculated plants increased with their growth and development (Table 2). The rate of increase in the level of carotenoids with ageing of the leaf blades was more in those from tungro-infected plants.

The literature records wide variations over the changes of carotenoids due to virus infections. It has been observed earlier (Rao et al., 1979) that carotenoids increase in leaf blades of sensitive rice cultivar following infection with tungro. Tungro infection generally increased the amounts of carotenoids. The disease-induced accumulation of carotenoid pigments was greater in sensitive and intermediate cultivars than in the tolerant line. These changes were more pronounced during 18 and 21 days after inoculation. This observation together with that of Rao et al. (1979) serve to establish that tungro infection enhances the concentration of carotenoids in rice leaf blades, specially in sensitive and intermediate cultivars.

Similar to tungro-diseased leaves, the contents of carotenoids also increased in senescing leaf blade segments (Fig. 1). Kinetin retarded and abscisic acid

Table 3

Anthocyanin content in leaf blades of healthy and tungro-infected plants differing in their reaction to the disease. (Rice cultivars/line, sensitive (Taichíng Native 1), intermediate (IR 20) and tolerant (CR 44-955) to tungro were inoculated at 3–4 leaf stage, and the fifth leaf blades were analysed. Data represent the mean of four determinations, and are in A_{525} per 100 mg fresh wt of tissue)

Cultivar/line	Condition	Days after leaf emergence					
		0	3	6	9	12	15
		Days after inoculation					
		6	9	12	15	18	21
Sensitive	Healthy	1.21b	1.12b	0.90b	0.79b	0.76b	0.71b
	Inoculated	0.78d	0.52f	0.35f	0.30e	0.23e	0.15e
		(35)	(54)	(61)	(62)	(70)	(79)
Intermediate	Healthy	1.47a	1.40a	1.27a	1.07a	1.05a	0.96a
	Inoculated	1.00c	0.81d	0.52e	0.40d	0.38d	0.35d
		(32)	(42)	(59)	(62)	(64)	(63)
Tolerant	Healthy	1.18b	0.99c	0.85c	0.83b	0.62c	0.50c
	Inoculated	1.08c	0.72e	0.64d	0.55c	0.43d	0.35d
		(8)	(27)	(25)	(34)	(31)	(30)

Values within a column followed by a common letter do not differ significantly at $P = 0.05$, according to Fisher's least significant difference test.

Figures in parentheses represent per cent decrease over healthy.

augmented that rate of pigment accumulation in detached senescing leaf blade segments.

The biochemical significance of increases in the levels of carotenoids in rice leaves due to virus infection and as a consequence of leaf senescence is yet to be evaluated.

Chlorophylls and carotenoids are synthesized via two separate biosynthetic pathways, and, therefore, the disappearance of one and increase in the concentration of the other may not be related. As chlorophyll disappears, the yellow colour of carotenoids is unmasked in tungro-diseased leaves. This might explain the characteristic discolouration of rice leaves infected with tungro viruses to different shades of yellow-orange.

A previous study (Mohanty et al., 1979) from this laboratory indicated that tungro-infected rice plants contain more abscisic acid-like substances than the healthy. Abscisic acid is biosynthesized from mevalonate (Milborrow, 1974; Walton, 1980) by either direct pathway from farnesyl pyrophosphate or from a C_{40} carotenoid-type precursor (Milborrow, 1978). Since knowledge on the biosynthetic sequences in abscisic acid biosynthesis is limited, it is difficult to distinguish between the two possibilities (Walton, 1980). The structural similarity of abscisic acid to the end parts of many carotenoids and certain circumstantial evidences (Taylor and Smith, 1967) led to the hypothesis that carotenoids might

Table 4

Flavonol content in leaf blades of healthy and tungro-infected plants differing in their reaction to the disease. (Rice cultivars/line, sensitive (Taichung Native 1), intermediate (IR 20) and tolerant (CR 44-955) to tungro were inoculated at 3–4 leaf stage, and the fifth leaf blades were analysed. Data represent the mean of four determinations, and are A_{500} per 100 mg fresh wt of tissue)

Cultivar/line	Condition	Days after leaf emergence					
		0	3	6	9	12	15
		Days after inoculation					
		6	9	12	15	18	21
Sensitive	Healthy	0.36a	0.31b	0.25a	0.19a	0.12b	0.08b
	Inoculated	0.23a	0.23c	0.20a	0.14a	0.06c	0.01f
		(36)	(26)	(20)	(26)	(50)	(87)
Intermediate	Healthy	0.65a	0.59a	0.34a	0.27a	0.19a	0.11a
	Inoculated	0.57a	0.31b	0.28a	0.26a	0.13b	0.06cd
		(12)	(47)	(18)	(4)	(32)	(40)
Tolerant	Healthy	0.67a	0.57a	0.26a	0.19a	0.08c	0.05d
	Inoculated	0.63a	0.30b	0.21a	0.15a	0.06c	0.03e
		(6)	(47)	(19)	(21)	(25)	(40)

Values within a column followed by a common letter do not differ significantly at $P = 0.05$, according to Fisher's least significant difference test.

Figures in parentheses represent per cent decrease over healthy.

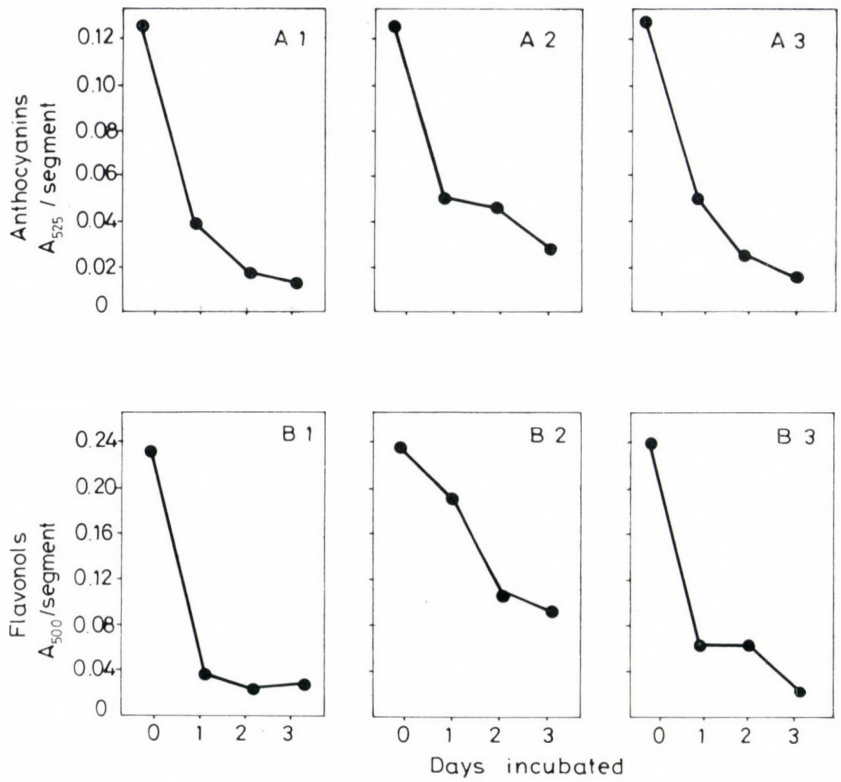

Fig. 2. Time course of anthocyanins (A) and flavonols (B) contents of detached leaf blade segments during senescence. (Leaf blade segments of rice cultivar, Taichung Native 1 were floated on water (1) and on 2×10^{-5} M kinetin (2) and on abscisic acid (3) in the dark)

be the natural precursors of abscisic acid (Milborrow, 1974). The present results, though do not provide any evidence for carotenoid destruction in tungro-diseased plants, the increased presence of carotenoids in diseased plants might be favourable for abscisic acid synthesis.

Anthocyanins and flavonols. Anthocyanins (Table 3) and flavonoids (Table 4) decreased with ageing in leaf blades of both the healthy and tungro-diseased plants. Inoculation with tungro generally reduced the contents of both anthocyanins and flavonols. The rate of disease-induced reduction was markedly greater in the sensitive cultivar than in the intermediate cultivar and tolerant line. Augmented levels of anthocyanins due to tungro infection in a purple pigmented rice line has been reported by Rao et al. (1979). However, this could not be confirmed in the present study with the normal rice plants.

Senescence of leaf blade segments induced the loss of anthocyanins and flavonols (Fig. 2). Kinetin retarded the pigment loss in senescing rice leaves.

However, abscisic acid did not have any marked effect on the loss of these pigments.

Tungro infection has earlier been shown (Sridhar et al., 1976; John and Rao, 1979) to decrease the concentration of total phenolic compounds, especially in a sensitive cultivar. This study clearly showed that the concentrations of both anthocyanins and flavonols declined in tungro-infected leaf blades, and also in senescing leaf blade segments. Obviously, it is evident that these classes of phenolic compounds (anthocyanins and flavonols) may not participate in the leaf discolouration due to tungro infection in rice leaves and carotenoids might be the most important source of yellow colour of the diseased foliage.

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Nonpersistent Transmission of Plant Viruses by Aphids

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Two main classification systems have been used to categorize virus transmission by aphids. One system is based on the length of time that vectors retain a virus, the other on where and how a virus is carried by the vector. The former system, the older of the two, was proposed by Watson and Roberts (1939). According to this system the viruses transmitted are classified as (i) *nonpersistent*, (ii) *semipersistent* (Sylvester, 1956), or (iii) *persistent* depending on whether virus is readily retained (i) for minutes, (ii) for several hours to days, or (iii) from weeks to a life time (through a moult). This method of classification has the advantage of utilizing easily determined property, but its value is lessened somewhat by the fact virus retention can vary with factors such as ambient temperature and vector probing activity before of after virus acquisition.

The second system was proposed by Kennedy et al. (1962). They classified viruses as *stylet-borne* or *circulative*. The stylet-borne viruses include all those familiar as nonpersistent, together with a few semipersistent and even persistent ones; the circulative viruses include the bulk of the persistent viruses. The term circulative refers to a process in which the virus is imbibed in infected sap, absorbed through the gut wall, transferred to the salivary gland and eventually inoculated into plants in virus-laden saliva. Circulative viruses which also multiply in their vectors were described as *circulative-propagative* (Smith, 1965). This system of classification was widely adopted, but it suffers from a lack of evidence that the so-called stylet-borne viruses are actually transferred via the styles. There is good evidence for some persistently-transmitted viruses that the virus circulates through the vector and more recently it has been proposed that the term *circulative* should be retained but that *stylet-borne* should be replaced by *noncirculative* (including nonpersistent and semipersistent subcategories) (Harris, 1976, 1977a, 1977b, 1979, 1981). However, the assignment of most viruses to one or the other category is still bgsed on persistence in the vector and in this review, the terms non-, semi- and persistently-transmitted viruses have been retained.

General reviews on nonpersistent virus transmission are (Sylvester, 1962; Bradley, 1964; Smith, 1965; Pirone, 1969; Watson and Plumb, 1972; Garrett, 1973; Pirone and Harris, 1977; Harris, 1983).

Characteristics of nonpersistent transmission

Effect of preacquisition fasting

The transmission rates of nonpersistent viruses are increased by fasting the aphids prior to acquisition (Watson, 1938) even for periods as brief as 15 min. Only slight increases occur with fasting periods longer than 1 h and the effect is cancelled out if fasted aphids are allowed to probe a leaf for more than a few minutes (Watson, 1938, 1972; Watson and Roberts, 1939, 1940).

Different hypotheses have been postulated in order to explain this phenomenon. Watson (1938) and Watson and Roberts (1939) postulated that transmission by nonfasted aphids is hampered by a virus-inactivating substance (s) which the aphids secrete during feeding presumably in the saliva. Keeping aphids off plants for some time arrests or slows down the production of this inactivator, and the virus transmission by fasted aphids remains relatively unaffected until probing and feeding activity again stimulate the production of inactivator(s). However, aphids fasted in cellophane-covered petri dishes salivate considerably while probing against the side of the container and through the cellophane top but subsequent virus acquisition is still enhanced (Hashiba and Misawa, 1969).

Fasting also affects aphid feeding behaviour. Thus, when fasted aphids are put on a leaf they almost always make one or more brief probes during which they may be sampling sap to test its host-status whereas unfasted aphids often make long probes to initiate feeding. Accordingly, the few unfasted aphids that made brief probes within two minutes of being placed on a virus source plant transmitted virus almost as well as fasted aphids (Bradley, 1961). During these brief, presumed sap-sampling, probes aphids may be alternately inbibing and egesting sap (Harris, 1977a) and this behaviour may ensure good acquisition of virus. Van Der Want (1954) and Bradley (1952, 1964) have also suggested that virus acquisition may be good during these brief probes because aphids do not secrete salivary sheaths, but this now seems unlikely (Mclean and Kinsey, 1964, 1965; Hodges and McLean, 1969; Hashiba and Misawa, 1970; Harris and Bath, 1973). Whatever the cause of enhanced virus transmission by fasted aphids, epidemiologically it may be important as it makes aphids migrating into a crop likely to be more efficient vectors than those bred on the crop (Broadbent and Martini, 1959).

Duration of acquisition probes

Aphids can acquire a virus during probes as brief as 5 sec. (Swenson, 1968; Harris, 1977a). However, uninterrupted probes of 15–60 S are generally optimal,

and longer access to source leaves results in poor acquisition. Thus, only 5 *Myzus persicae* (Sulz.) of 45 tested transmitted PVY after 4 h of acquisition access whereas 25 transmitted after 2 min access (Watson and Roberts, 1939). Hodges and McLean (1969), using electrical conductivity to accurately measure the duration of probes, demonstrated that the pea aphid *Acyrthosiphon pisum* (Harris), acquired bean yellow mosaic virus (BYMV) during probes of 16 ± 4 s. Acquisition and inoculation thresholds were 4.50 S and 4.25 S respectively. As duration of probes increases beyond 1 min, there is a decrease in acquisition (Watson, 1940, Hashiba and Misawa, 1969).

Latent period

An aphid is infectious immediately after it has probed an infected plant. Thus, unlike persistently-transmitted viruses which require time for the virus to circulate through the aphid, nonpersistently-transmitted viruses have no latent period and this is part of the evidence that viruses are carried in the region of the mouthparts or foregut. In practice, this is important because it allows nearby plants to be infected (Doncaster and Gregory, 1948; Dungan et al., 1956).

Duration of inoculation probes

The minimum time required for inoculation is slightly shorter than that for acquisition (Sylvester, 1950; Hamlyn, 1953; Sylvester, 1955; Hodges and McLean, 1969) but unlike acquisition long probes can give high rates of inoculation (Bradley, 1964). However, viruliferous aphids generally become non-viruliferous following prolonged probes on healthy plants (Hashiba and Misawa, 1969).

Retention period

Following acquisition, the chance of an aphid transmitting a nonpersistently transmitted virus diminishes gradually with time. At about 20 °C, an aphid usually remains viruliferous for no more than a few hours following access to a source leaf, although this varies with particular virus-vector combination. For example *M. persicae* retains maize dwarf mosaic virus (MDMV) for up to 30 min (Thongmeearkom et al., 1976), but peanut mottle virus (PeMotV) for at least 12 h (Paguio and Kuhn, 1976). Aphids remain infective somewhat longer off than on a healthy leaf; for example, when aphids carrying potato virus Y (PVY) probed into healthy tobacco plants, they usually ceased to be viruliferous within 1 h whereas some aphids kept in a glass tube remained viruliferous up to 4 h (Bradley, 1959). The rate at which they become noninfective is similar on leaves of plants that are, and are not, susceptible to the virus (Bradley, 1959). At low temperatures aphids may remain infective for longer (Kassanis, 1941; Bradley, 1954; Sylvester, 1954; Cockbain et al., 1963). Nonpersistent viruses are not retained through ecdysis and this characteristic provides a clear-cut means of distinguishing non- and persistently-transmitted viruses.

A common reason for attempting to determine how long aphids retain the ability to infect relates to the spread of virus in the field. In most experiments, conditions have not been particularly close to those that might exist under field conditions but Cockbain et al. (1963) attempted to simulate field conditions by allowing tethered aphids to fly for various times in an air current. The infectivity of alatae of *M. persicae* and *Aphids fabae* Scop. carrying either pea mosaic virus (PMV) or beet mosaic virus (BMV) diminished at about the same rate regardless of whether they were flying or kept fasting in a glass container. Few aphids transmitted these viruses if they were held at temperatures above 30 °C for 30 min.

Virus groups transmitted nonpersistently

Potyviruses

Most viruses that are transmitted in the nonpersistent manner are potyviruses. The virions are flexuous rods with modal length from about 680 to 900 nm. Those so far characterized have helical symmetry and contain about 5% single-stranded RNA (Shepherd, 1977). Common members of this group are PVY, BYMV, BMY, PeMotV, plum pox virus (PPV), tobacco etch virus (TEV), potato virus A (PVA), turnip mosaic virus (TuMV), lettuce mosaic virus (LMV), soybean mosaic virus (SoyMV), and potato virus V (PVV) (Fribourg and Nakashima, 1984; Jones and Fribourg, 1986).

Cucumoviruses

This group includes cucumber mosaic virus (CMV) and tomato aspermy virus (TAV). The virions of members of this group are isometric particles of about 30 min. Those characterized contain about 18% single-stranded RNA in four different-sized particles (Shepherd, 1977).

Alfalfa mosaic virus group

This is a monotypic group for which no formal name has been proposed. At least four types of virions occur, of which three are bacilliform, 58, 48, and 36 min in length and 18 nm in diameter, and one is spheroidal, about 18 nm in diameter; all contain about 16% single-stranded RNA. A number of strains of alfalfa mosaic virus (AMV) have been described (Pirone and Harris, 1977).

Carlaviruses

Common members of this group are potato virus S (PVS), potato virus M (PVM), carnation latent virus (CarLV), red clover vein mosaic virus (RCVMV) and pea streak virus (PeaSV). Viruses are slightly flexuous rods with modal lengths

of 620–690 nm. Those characterized have helical symmetry and contain about 6% single-stranded RNA. Not all members of this group have shown to be aphid transmissible (Shepherd, 1977).

Potexviruses

Typical members of this group are transmitted only by sap, although aphids may be able to transmit them in artificial conditions (Pirone and Kassanis, 1975). However, potato aucuba mosaic virus (PAMV) although a potexvirus (Matthews, 1982), is naturally transmitted by aphids but only when it is in association with certain potyviruses (Kassanis and Covier, 1971a).

Physical properties of nonpersistent viruses

Nonpersistent viruses are relatively stable and are generally easily sap transmissible. They are inactivated by drying, but not completely by freeze drying, by heating for 10 min at 60 °C, and by treatment with acid or alcohol. Longevities *in vitro* (minutes to days) and dillution end-points very considerably depending on the virus (Harris 1977a).

The role of helper component

In 1936 Clinch et al. reported that they were unable to transmit tuber blotch virus (PAMV) by aphids from infected potatoes unless the potatoes were also infected with PVA. The problem was reinvestigated by Kassanis (1961). None of twelve strains of PAMV was transmitted by *M. persicae* from plants infected with PAMV alone but they were transmitted from plants which were also infected with either PVA or PVY. Kassanis suggested several explanations for the phenomenon: phenotypic mixing leading to the protein of PAMV containing material from PVY or PVA which conferred aphid transmissibility, increase in PAMV concentration in certain tissues a change in the position of PAMV in the infected cells so that it becomes available to the aphids and a mechanism by which the helper virus may cause the particles of PAMV to aggregate with each other or with those of the helper viruses and thus form larger virus units which can attach to aphid mouthparts. Exchange of genetical determinants has also been postulated to explain the transmission of potato virus C (PVC) a virus not normally aphid-transmissible, from plants also infected with PVY (Watson, 1960). However, Kassanis and Govier (1971a) showed that PAMV and PVC are transmitted by *M. persicae* not only from plants also infected with PVY, but from plants infected with PAMV and PVC alone, provided the aphids fed first on plants infected with PVY. However, when the order was reversed they were not transmitted.

Pirone and Megahed (1966) transmitted both CMV and AMV by aphids probing into purified preparations. However, neither they nor Watson et al. (1967) could obtain transmission of purified potyviruses such as Henbane mosaic virus (HMV), PVY, TuMV. However, like PAMV, purified PVY could be transmitted by aphids which had first fed on a PVY-infected leaf, the leaf having been irradiated with ultraviolet light to inactivate the virus in the leaf (Kassanis and Govier, 1971b). It was further demonstrated that a virus-free extract from PVY-infected leaves promoted the transmission of purified PVY and PAMV (Govier and Kassanis, 1974a, 1974b). The active principle in these extracts was termed helper component. Characterization studies indicated that helper component is a protein of between 100.000 and 200.000 daltons, which is serologically distinct from PVY coat protein or inclusion protein (Govier et al., 1977).

The evidence available suggests that aphids in order to transmit possibly all potyviruses must acquire the helper component normally induced in plants as a result of infection by these viruses. Purification separates the virus from its helper component so aphids cannot transmit the virus. Nontransmissible isolates of potyviruses, as have been reported for PVY, certain strains of PeMotV, TuMV and TEV probably induce either none or a defective helper component. They can be transmitted if aphids acquire helper component induced in the same plant by another potyvirus or from another infected plant (Kassanis and Govier, 1971a; Paguio and Kuhn, 1976; Simons, 1976; Sako, 1980; Mossop, 1982).

In most research, *M. persicae* was used as a vector; however *Aphis gossypii* Glov. also requires helper component to transmit (Pirone, 1981) a virus. A requirement for helper component has been demonstrated in many potyviruses including PVY, TEV, tabacco vein mottling virus (TVMV), watermelon mosaic virus (WMV), BYMV, and TuMV, so helper component-dependency may occur for all aphid-polyviruses-combinations. Serologically-distinct helper component proteins are produced in response to specific potyvirus infection suggesting that helper component is a virus coded polypeptide (Thornbury and Pirone, 1983; Hellmann et al., 1983; Hiebert et al., 1984) and that induced by one virus may not be *(as)* ffective for transmitting another (Pirone, 1981; Sako and Ogata, 1981). Thereealso indirect evidence that the particular helper component induced by a virus is volved in determining whether or not an aphid species is a vector (Sako, 1981; Sako et al., 1984). However, transmissibility of at least TEV is a function of the virus particles as well as of the helper component (Pirone and Thornbury, 1983). So far, the existence of helper component regulating transmission of nonpersistent viruses has been documented only for potyviruses; CMV (Pirone and McGahed, 1966), AMV (Pirone, 1964; Pirone and Megahed, 1966), and two carlaviruses (Weber and Hampton, 1980) retain transmissibility after purification.

The mode of action of helper component has yet to be determined. Recent hypotheses are that it may act either by enabling a virus to bind to receptor sites in the aphid from which it subsequently can be released or by affecting the ability to ingest virus, preventing the breakdown of aggregation of particles of

preventing virus from being bound to parts of the alimentary tract (Govier and Kassanis, 1974b; Pirone, 1977; Pirone and Harris, 1977; Lopez-Abella et al., 1981; Raccah and Pirone, 1984).

More recently it has been shown that helper component has no apparent effect on virus uptake (Berger and Pirone, 1986). However, their data certainly supports the hypothesis that helper component may function via a binding mechanism, although they do not rule out alternate or additional functions for helper component.

Sites of virus acquisition and inoculation in plant

Uninterrupted probes of 15–60 sec are, in general, optimal for acquisition of nonpersistent viruses. During these probes the stylets penetrate only a few (< ten) micrometers into the epidermis and this distance is less than the depth of an epidermal cell (Bradley, 1964; Misawa and Hashiba, 1967). As acquisition probes last beyond one minute transmission rates decline (Watson, 1940, 1946; Hashiba and Misawa, 1969) and this is about the time it takes an aphid's stylet to penetrate to sub-epidermal tissues (Roberts, 1940; Misawa and Hashiba, 1967). This suggests that virus is not acquired from sub-epidermal cells. To explain this Bawden et al., 1954; Bradley (1954) and Watson (1958) have postulated that the virus in sub-epidermal cells is less abundant or in a less aphid-transmissible form than virus in epidermis, but BYMV is actually less abundant in the epidermis than in the underlying tissue, aphids allowed acquisition probes into exposed sub-epidermal tissue transmitting the virus at three times the rate of aphids probing into stripped epidermis (Hashiba, 1970). Other studies with leaves that have been stripped off epidermis have also shown that virus in mesophyll cells is at least as available for acquisition as virus in the epidermis (Van Hoof, 1958; Naba, 1962; Normand and Pirone, 1968).

Whether brief probes are made into or between epidermal cells remains unclear. Thus stylet insertion has been reported to be intercellular (Yosmii, 1966), intracellular (Swenson, 1962) or both (Roberts, 1940; Bradley, 1952; Hashiba, 1969). These differences may result from the use of different aphid-plant systems. However, 90% of the initial probes of fasted *M. persicae* on *Vicia fabae* were intracellular (Hashiba, 1969) and it was these probes that acquired BYMV (Hashiba and Misawa, 1970). Lopez-Abella and Bradley (1969) found that 50% of the probes that were initiated intercellularly by *M. persicae* eventually became intracellular as the stylet tips penetrated an epidermal cell and they also considered that acquisition of CMV by *M. persicae* was intracellular (Lopez-Abella and Bradley, 1969, 1970). Intracellular acquisition would also seem to agree more with the high rates of transmission observed for many nonpersistant viruses since, if acquisition occurs between cells, seemingly inefficient mechanisms, such as acquisition from broken plasmodesmata (Yoshii, 1966), must be postulated.

Fewer studies have been made on the sites of stylet insertion during inoculation probes, but the sites involved may include the sub-epidermal tissues as well as epidermal as both long and brief probes are effective at virus inoculation.

Sites of transmitted virus in/on the aphid

Currently, there are two main contenders for the site of transmissible virus in/on the aphid.

The stylet-borne hypothesis

There are several indications that nonpersistant viruses may be transmitted on aphid stylets. Thus, transmission occurs during brief probes, infectivity is lost when aphids shed their stylets along with the exoskeleton, fore and hindgut during ecdysis and otherwise persists for only a few hours or less if aphids feed. Although Gamez and Watson (1964) failed to get transmission by artificially inserting aphid stylets first into HMV-infected, then into healthy leaves, Barnett and Pirone (1966) obtained transmission of CMV, although at a low rate, after dipping the stylets of anaesthetized aphids into capillary tubes containing purified virus. Furthermore, treating stylets with formalin or exposing them to ultraviolet (UV) irradiation rendered aphids carrying PVY nonviruliferous (Bradley and Ganong, 1955a, 1955b). However, UV irradiation of the aphid stylets before access to infected leaves also prevented aphids transmitting PVY suggesting that it is the aphid that is affected rather than the virus (Bradley, 1964). Also, exposing the stylets of viruliferous aphids to other antiviral agents such as 8-azaguanine, ribonuclease, milk and saliva did not diminish virus transmission (Bradley, 1959; Simons and Moss, 1963; Nishi, 1969; Hashiba and Misawa, 1970).

Aphid stylets posses ridges and grooves on the mandibles that might function to hold virus particles (Van Hoof, 1958). However, examinations of the stylets of ten aphid species, revealed no substantial differences in stylet structure (Schmidt et al., 1974), although they differ in vectoring ability (Kennedy et al., 1962).

Direct evidence that viruses might by stylet-borne has been sought by electron microscopy. Taylor and Robertson (1974) found a few virus-like particles lining the distal 20 μm of the maxillary food canal in transverse sections of the stylets of *M. persicae* which had probed leaves infected with TEV. Lim and Hagedorn (1977) detected pey seedborne mosaic virus (PSbMV) or its protein on the inner surfaces of the mandibles of *Macrosiphum euphorbiae* (Thos.), using scanning electron microscopy and labelled antibody. However, detection of stylet-associated virus is not proof that this virus would be transmitted.

The ingestion-egestion hypothesis

The concept that nonpersistant viruses were acquired by ingestion carried into the anterior portion of the alimentary canal where they might adhere and

were inoculated by regurgitation has been favoured by Watson and co-workers (Watson and Roberts, 1940; Gamez and Watson, 1964; Watson and Plumb, 1972). Aphids feeding on artificial diets can be observed to ingest and egest carbon black particles (Harris and Bath, 1973) and when they probed for less than 10 min on plants labelled with P^{32}, they contained considerably less P^{32} after 6–8 min probes than after probes that lasted 3–5 min, suggesting that the tracer has been regurgitated (Garrett, 1973). There was a correlation between the number of *M. persicae* which ejected 570 μm^3 or more of P^{32} labelled sap and the number that transmitted CMV. This sap volume exceeds by ten times the volume of the food canal of the stylets suggesting that egested sap was from the fore-gut where CMV is concentrated (Gera et al., 1979).

At first sight, the stylet-borne hypothesis accounts well for the short retention and the brief acquisition and inoculation thresholds which typify nonpersistent transmission. However, virus vector specificity might be better explained by the ingestion-egestion hypothesis via some form of selective attachment to the membranes lining the alimentary canal which are biologically active tissues rather than to the stylets. The ingestion-egestion hypothesis is also attractive because the explanation of the preacquisition fasting effect based on increased frequency of sap-sampling probes by fasting aphids fits well and because it provides for the uptake of relatively large volumes of virus containing sap into protected areas and its subsequent inoculation into a plant. However, at present there is no clear experimental evidence discriminating between whether nonpersistent viruses are transmitted via the stylets or ingested into the pharynx or foregut.

Vector specificity

Nonpersistent viruses are usually transmitted by more than one aphid species, for example, at least sixteen aphid species transmit PVY (Kennedy et al., 1962), but vectors of one virus are not necessarily vectors of another. Furthermore, some species, such as *M. persicae*, are vectors of many viruses whereas others transmit few if any (Kennedy et al., 1962). Even within an aphid species, there may be large differences in vectoring ability between aphid seasonal forms (Paine and Legg, 1953; Orlob, 1962), growth stages, morphs (Sylvester, 1955; Broadbent, 1960; Cockbain et al., 1963; Thottappilly et al., 1972; El Kady et al., 1973), clones and biotypes (Simons, 1959, 1966; Sohi and Swenson, 1964; Kvicala, 1968; Upreti and Nagaich, 1971; Jurik et al., 1980; Singh et al., 1983).

Different hypothesis have been proposed to explain virus-vector specificity. Thus, Van Der Want (1954) proposed a mechanical surface adherence hypothesis in which specificity was an expression of differences in the structure and surface adherence properties both of the stylets of different aphids and of particles of different viruses. However, there does not appear to be a relationship between stylet-tip morphology and vector specificity (Proeseler et al., 1972; Forbes, 1977). For example, Schmidt et al. (1974) found no substantial differences in stylet

morphology on ten aphid species, which included *M. persicae*, a good vector of PVY and *Rhopalosiphum padi* (L.), a poor vector of PVY (Van Hoof, 1980). However there is evidence for differences in the surface adherence of the stylets. Thus, large amounts of labelled virus were detected on the inner surface of mandibles of a biotype of *M. euphorbiae* that transmitted PSbMV efficiently whereas only small amounts were on those of a biotype that transmitted PSbMV inefficiently (Lim et al., 1977). Some virus isolates have become non-transmissible by some aphid species whilst still being transmitted by others (Badami, 1958) and others are not transmissible by the normal range of vectors (Lucas and Hill, 1980). Helper component has also been suggested as a factor which may play a role especially for potyviruses, in determining factor specificity (Sako, 1981). In CMV, which does not require a helper component, the coat protein of the virus particle may determine rates of transmission (Gera et al., 1979).

Virus inactivating properties of saliva have also been suggested as an explanation of vector specificity (Bradley, 1952; Day and Irzykiewicz, 1954; Sylvester, 1954; Van Der Want, 1954; Hashiba and Misawa, 1969; Nishi, 1969). Saliva may well play a role in restricting virus transmission; for example, by limiting persistence in the aphid (Loebenstein and Raccah, 1980), as this can diminish virus infectivity (Nishi, 1969; Pirone, 1970) but attempts to demonstrate selectivity of saliva on viruses have failed (Pirone, 1970).

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A Comparative Immunochemical Study of Potato Virus X, Potato Aucuba Mosaic Virus, Hydrangea Ringspot Virus, and White Clover Mosaic Virus by the Sandwich and Blocking Methods, Elisa, Double Diffusion Test, Virobacterial Agglutination, Rocket Immunoelectrophoresis and Immune Electron Microscopy

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Evidence has been obtained on the antigen interaction of potato virus X, potato aucuba mosaic virus, white clover mosaic virus and hydrangea ringspot virus by means of double diffusion test, virobacterial agglutination, rocket immunoelectrophoresis, the sandwich and blocking methods ELISA, and immune electron microscopy.

All the viruses studied were shown to have affined antigen determinants. The most closely related viruses are potato virus X and white clover mosaic virus (over 90% affinity) and the less related one is hydrangea ringspot virus (up to 40% affinity), potato aucuba mosaic virus occupies an intermediate position (up to 60% affinity), and this allows with certainty to assign in to definitive potexvirus.

The purpose of this work was to study the antigen affinities of potato virus X (PVX), potato aucuba mosaic virus (PAMV), hydrangea ringspot virus (HRSV), and white clover mosaic virus (WCMV), to reveal their common and individual determinants. We are still unaware of works devoted to comparative characterization of the given viruses and to the study by immunochemical methods of the group affinity of PAMV to PVX, albeit comparative serological and electron microscopic studies of WCMV, HRSV and PVX were performed by Bercks and Brandes (1961). However, there is still no direct evidence of serological affinity of PAMV to other potexgroup representatives (Koenig and Lesemann, 1978; Matthews, 1982).

Materials and Methods

To accumulate PVX and PAMV, we used *Datura stramonium L.*, *Phaseolus vulgaris L.* and *Hydrangea macrophylla L.* to accumulate WCMV and HRSV, respectively. Maximum PVX concentration in the test-plants was noted on the

7th–10th days following inoculation; maximum concentration for PAMV and WCMV was observed on the 12th–14th and 30th days, respectively. HRSV concentration usually retained a high level during autumn and winter.

The PVX-infected material was homogenised in 0.05 M phosphate buffer, pH 7.5, adding 0.3% Trilon B; PAMV-infected material was homogenised in 0.05 M phosphate buffer with 0.5 M NaCl, pH 7.5, adding 0.1% 2-mercaptoethanol. To resuspend the PAMV-containing residues, we used an 0.2 M borate buffer, pH 7.6, adding 0.03% NaN_3. WCMV-infected leaves were homogenised in an 0.05 M phosphate buffer, pH 7.8–8.0, adding 0.2% EDTA and 0.1% Na_2SO_3. The virus-containing residues were suspended in an 0.13 M borate buffer, pH 8.4–8.6. The HRSV-containing material was ground in an 0.5 M borate buffer, pH 7.8, adding 0.1% Na_2SO_3 and 0.1% ascorbic acid. Extracts with PVX, HRSV and PAMV were clarified with a mixture: chloroform (v/v = 1\7) and butanol (8%). The WCMV-containing homogenate was centrifuged for 30 min at 6.000–7.000 rpm, and the supernatant acidified with 20% acetic acid to pH 5.0.

PVX, PAMV, HRSV and WCMV were precipitated by adding 4–5% PEG 6.000 and 0.3 M NaCl to the clarified extracts. Further purification was achieved by 2–3 cycles of differential centrifugation (36.000–40.000 rpm 90 min, 5.000 rpm 10 min).

Antisera were obtained by immunizing rabbits intracutaneously and subcutaneously in the lymphonodes (along the spine, over the lateral lines, in the popliteal holes, and abdominal regions). The immunization scheme included 3–4 injections at one-week interval. The antigen (0.4–1.0 mg) was mixed with an equal amount of Freund's complete adjuvant ("Difco", USA). The titres of the resultant sera were determined in a double diffusion reaction in agar.

The antigen correlations between the given viruses were studied by double diffusion test (Ouchterlony, 1953), virobacterial agglutination (Gnutova and Sibiryakova, 1983), rocket immunoelectrophoresis (Laurell, 1966), the sandwich method ELISA (Lister and Rochow, 1979) and blocking method ELISA (Hermann, 1981), and immune electron microscopy (Milne and Luisoni, 1975).

Since the potexviruses hardly pass via the agar pores due to their large size, the viral particles were broken down into fragments by ultrasound (MSE 150 Watt Ultrasonic Disintegrator MK-2). Different modes were tested, i.e. 5, 10, 15 and 20 min at 5, 10, 15, 18 and 20 KHz. Besides, the virus antigens were treated with sodium dodecyl sulphate (SDS) in various concentrations, i.e. 0.05, 0.1, 0.2, 0.5 and 0.1% Tween 20.

Immunoglobulins (IgG) were isolated by two-fold reprecipitation with ammonium sulphate at 25% saturation with subsequent purification on DEAE-Sephadex A-50. Specific antibodies were marked by covalently binding the molecules by the periodate technique of Nakane and Kawaio (1974), using peroxidase from horseradish (Rz = 2.4–2.7). To determine peroxidase activity the 1% aminoantipirin was used as a substrate in 0.1 M NaH_2PO_4 with 0.01% H_2O_2. The degree of digestion of the substrate by the enzyme was determined on a spectrophotometer ("Specol-21") at 405 nm, and visually by staining intensity.

In sandwich method ELISA for each of the potexviruses studied, we priorly selected optimal IgG concentrations, working dilutions of conjugates, and incubation temperature and time. For IgG tested concentrations from 1 to 100 mg ml, for conjugates – dilutions from 1 : 50 to 1 : 500 (0.025–0.25 mg of peroxidase from horseradish-Ph in 1 ml). The plates with coated IgG were maintained for 1, 2, 3 h at 37 °C and for 18 h at 4 °C with antigens and conjugates – 1, 1.5, 2 and 3 h at 37 °C. The substrate reaction was recorded in 30, 40 and 60 min at 37 °C. The optimal IgG amounts were those conjugate dilutions and incubation temperature and time were such that their use was characterised by an extinction coefficient of up to 0.8–1.0 as a result of the reaction, since at E higher than 1.5 the error of the recording instrument would increase. The suggested version of sandwich method was performed three times. The preparation of heterologous tobacco mosaic virus was used as a control in testing of viruses in the purified preparations. Leaves of healthy plants served as control in the test of plant material. In addition the non-specific conjugate binding (holes in which an antigen is substituted for buffer with bovine serum albumin-BSA). A concentration of the test object with the registered value of optical density of a product of peroxidase reaction being exceeded the optical density in control assay by 2.0 unit was taken as sensitivity of the method.

The competitive ELISA version with our modifications was as follows:

1. An antigen (200 μl) was introduced into plate holes, in 0.1 M Na_2CO_3 with 0.15 M NaCl, pH 9.5. The mixture was incubated for 18 h at 4 °C, unbound reagents were washed with 0.01 M NaH_2PO_4, 0.15 M NaCl, pH 7.5, containing 0.05% Tween 20.
2. Solution of the antigen tested (50 μl) and serume which was homologous to an antigen sorbed on the plate were successively introduced in 0.01 M NaH_2PO_4 with 0.1% BSA. Holes were washed after incubation for 4–5 h at 37 °C.
3. Solution of the antispecific conjugate was added into the holes (IgG of donkey, against IgG of rabbit labelled by Ph) in 0.01 M of NaH_2PO_4 with BSA. Contents of the holes were incubated for 1–1.5 h at 37 °C and washed.
4. A substrate was added and enzymatic activity determined.

Since an assay proceeds in the equilibrium mode and time of its performance in basically limited by the time needed to attain equilibrium in the antigen-antibody reaction, minimal amounts of the sorbed antigen and antibody concentrations ensuring the binding of 50% of the sorbed antigen in the absence of the blocker were used to achieve maximum sensitivity. An essential condition was to determine the minimal antigen concentrations, the optimal dilutions of antisera for each of the viruses studied and conditions of the competition stage (temperature, incubation time and minimal concentrations of antigen).

Minimal concentrations of antigen were determined by the immunoenzymatic indirect method. The antigen solution (200 μl) was introduced into the plate holes within the range of concentrations from 0.25 to 12 ng/ml in a buffer

for sorption. Plates were washed after incubation for 18 h at 4 °C. Then antiserum (200 μl) was introduced in a buffer with BSA at dilutions 1 : 125, 1 : 250 and 1 : 500.

Three antiserum dilutions were taken for more accurate selection of minimal concentration of the antigen sorption. The hole content was incubated at 37 °C for 1 h and then washed. An antispecific conjugate (200 μl) in working dilution 1 : 250 (0.05 mg/ml Ph). Following incubation at 37 °C for 1–1.5 h and washing, the substrate was poured.

Antisera working dilutions were selected by the above-described scheme of indirect ELISA. Various antigen concentrations (0.1, 1.0, 10 ng/ml) were used to determine accurately the antisera optimal dilutions and minimal concentration of antigen sorption. The homologous serum was introduced into the holes also in various dilutions, i.e. 1 : 125, 1 : 250, 1 : 500, 1 : 1000, 1 : 2000.

The optimal values of temperature and time for competition stage were determined in the following manner. The antigen solution was introduced into each hole (200 μl, 1 ng/ml), in a buffer for sorption and incubated for 18 h at 4 °C, then washed. The competitive antigen (100 μl, 1 ng) and homologous serum in working solution were added. The hole content was incubated for 1, 2, 3, 4 and 5 h at 37 °C or for 18 h at 4 °C and washed. An antispecific conjugate in working dilution was introduced, incubated for 1–1.5 h at 37 °C and washed. The substrate was introduced into holes.

Electron microscopic 0.8–1% carbon-formvar coated grids were placed onto a drop of viral preparation and maintained for 1–5 min at room temperature in a humid chamber. The excess preparation was removed and the grids placed for 15 min onto a drop of antiserum which dilution was selected by experimental. Antibodies unbound with homologous antigen were removed phosphate buffer and distilled water. Preparation were contrasted with 2% uranylacetate, pH 5.0 and examined in an electron microscope EMB-100 AK (USSR) × 31000–45000.

Results and Discussion

All the potexviruses proved good immunogens. However, the PVX, WCMV and HRSV were characterized the strongest immunogenic properties. The following antisera with the following titres were obtained: PVX-1 : 256–1 : 512, WCMV-1 : 128–1 : 256, HRSV-1 : 128–1 : 512 and PAMV-1 : 16–1 : 32.

Double diffusion test in agar gel. The precipitation lines formed both at the interaction of the antisera with homologous antigens and with all the potexvirus under study (Fig. 1). Addition to the antigen of SDS in various concentrations, Tween 20, and also the sound disintegration showed that the last technique to be the most effective for potexviruses, namely ultrasonic treatment of viral antigens for 15 min at 15–20 kHz. The use of 0.2% and 0.5% SDS led to arisal of non-specific precipitates formed from the interaction with serum proteins. When using SDS (concentration 0.05–0.1), non-specific reactions were not observed.

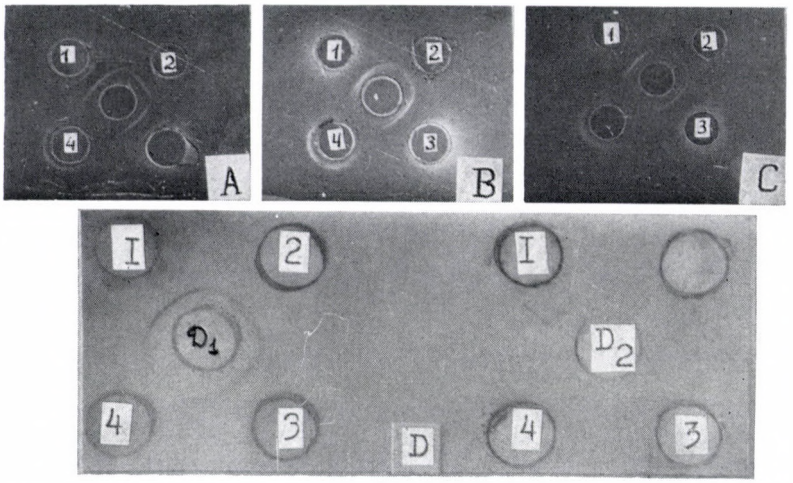

Fig. 1. Revealing potexvirus antigen affinity in double diffusion test. In central holes: antisera to PVX (A), PAMV (B), HRSV (C), WCMV (D_1) and normal serum (D_2). In peripheral holes: antigens — 1: PVX, 2: PAMV (A, B, C), 3 (A, B, C) — HRSV, 3 (D) — WCMV, 4 (A, B, C) — fresh sap healthy plant, 4 (D) — HRSV

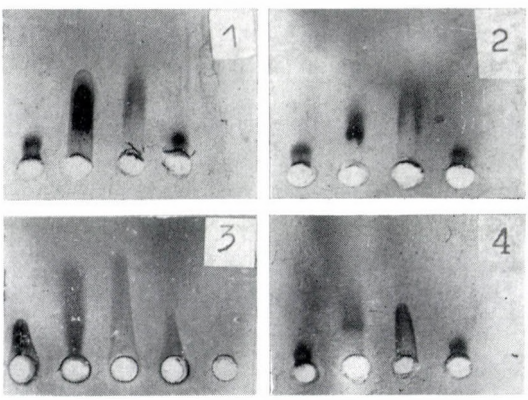

Fig. 2. Identification of antigen determinants in potexviruses using RIEP. 1 — in gel, antiserum to PVX; in holes from left to right: antigenes of PVX, PAMV, HRSV, and WCMV. 2 — in gel: antiserum to WCMV; in holes: antigenes of WCMV, PAMV, HRSV, and PVX. 3 — in gel: antiserum to PAMV; in holes: antigenes of PAMV, WCMV, HRSV, and PVX. 4 — in gel: antiserum to HRSV; in holes: antigens of HRSV, PVX, PAMV, and WCMV

However, in this case too, strict controls are essential: 0.05% and 0.1% SDS in physiological solution. To break down the potexvirus particles, one may apply 0.1% Tween 20; however, in this case, the precipitation lines would be of diffusive nature.

Rocket immunoelectrophoresis (RIEP). In all systems precipitation peaks obtained in the form of rockets were indicative of the antigen relationship of

Table 1

Cross titration of virus antigens in homo- and heterosystems using virobacterial agglutination

Antigen	Antiserum			
	PVX	PAMV	WCMV	HRSV
PVX	1 : 256	1 : 64	1 : 128	1 : 64
PAMV	1 : 32	1 : 64	1 : 16	1 : 16
WCMV	1 : 128	1 : 32	1 : 256	1 : 32
HRSV	1 : 64	1 : 32	1 : 64	1 : 128
sap of healthy plant	—	—	—	—

given potexviruses (Fig. 2). To form a distinct rocket, we selected equivalent doses of the antigen (0.5–1.0 mg/ml) and optimal antisera dilutions (1 : 50 for PVX and WCMV, 1 : 30 for PAMV and 1 : 40–1 : 50 for HRSV).

Virobacterial agglutination was performed with antisera whose working dilution was 1 : 100–1 : 200 for PVX and HRSV, 1 : 200–1 : 300 for WCMW, and 1 : 100–1 : 400 for PAMV. The virus antigen concentration did not exceed 0.5–1.0 mg/ml.

Besides, we titred the viruses present in the sap of infected plants. Homologous and heterologous sera were taken in a 1 : 100 dilution. The titration results for virus antigens in homo- and heterosystems are shown in Table 1.

The obtained evidence is indicative of the presence of affined antigen determinant groups in all the four viruses studied.

Enzyme-linked immunosorbent assay (ELISA). In applying the sandwich method, we revealed that optimal IgG sorption concentration corresponded to

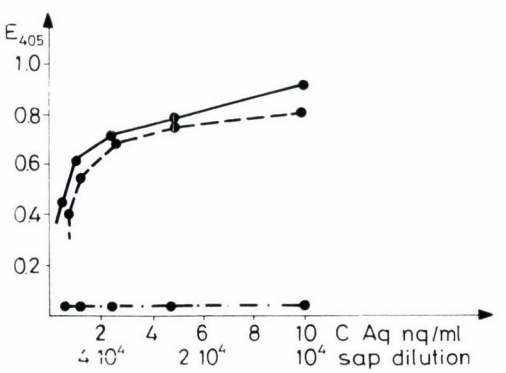

Fig. 3. Determination of virus concentration in sap infected plants by meyns of calibration curves obtained from using sandwich method ELISA. ●——● — preparation; ○----○ — sap of infected plant; ▲——▲ — sap of healthy plant. System PVX (conjugate dilution 1 : 75)

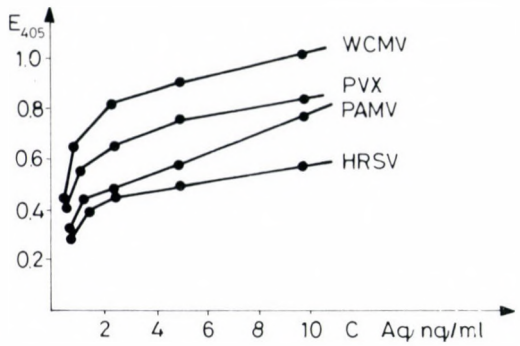

Fig. 4. Antigen correlation of potexviruses revealed with sandwich method ELISA. System WCMV (conjugate dilution 1 : 200)

10 μg/ml for HRSV, 5 μg/ml for PVX and PAMV, 1 μg/ml for WCMV. The conjugate working dilutions corresponded to 1 : 50–1 : 75 (0.175–0.25 mg/ml for peroxidase) for PVX, 1 : 100–1 : 150 (0.0875–0.125 mg/ml for PAMV and HRSV, and 1 : 100–1 : 200 (0.065–0.125 mg/ml) for WCMV. The obtained data showed complete IgG sorption to occur at 4 °C for 18 h, 1–1.5 h long incubation at 37 °C is sufficient for the antigens and conjugates to sorb. Substrate reaction showed most distinctly in 30–40 min at 37 °C.

Calibration curves (Fig. 3) were obtained for titration of the potexvirus preparations studied by the sandwich method ELISA. The selected conditions and the high sensitivity of the method allowed to reveal 10^{-6} mg of the virus. In carrying out the sandwich method with infected plant sap titration curves were obtained (Fig. 3) and determined the potexvirus concentration in the sap. The results were as follows: 0.23 mg/ml for WCMV, 0.44 mg/ml for HRSV, 0.8 mg/ml for PVX, and 0.18 mg/ml for PAMV.

By conducting cross reaction in each of the systems of potexviruses studied, we obtained titration curves for homologous and heterologous preparations (Fig. 4). The presence of cross reactions and the similar character of curves in all the systems were indicative of antigen affinity between the viruses studied. By analysing the arrangement of the curves, one may note that PVX and WCMV are closest in antigen properties, HRSV closer, and PAMV occupies an intermediate position. Unfortunately, this method does not allow to afford distinct quantitative assessment of the antigen affinity. Toward this end we have modified competiting method ELISA, which was previously used only for animal viruses (Hermann, 1982). This method is based on the competition between the antigen sorbed on the solid phase and competitive (affined) antigen for centers of binding in antibodies which are homologous to the sorbed antigen. As a result of selection of conditions for carrying out the competitive version of ELISA it was established that minimal antigen concentration for sorption in all the studied potexviruses amounted to 1 ng/ml and working antisera dilutions – 1 : 250 for

PVX and HRSV and 1 : 250–1 : 500 for WCMV and PAMV. 4–5 h incubation of ingradients at 37 °C is sufficient for the stage of competition and attaining equilibrium in the system. Our experiments showed that optimal conditions for competition arose if 50 μl of the competitive antigen and 150 μl of serum homologous to the sorbed antigen used and also if a reaction was carried out with constant shaking. Preselected conditions for running the ELISA version allowed to examine comparatively the potexviruses in homo- and heterosystems. The antigens for competition were induced in concentrations 0.05–10 g/ml.

The blocking curves (Fig. 5) were obtained which were plotted along the coordinate axis $y = \frac{A}{A_0} \times 100$ and $x = \lg C$, where A means extinctions obtained for holes in the presence of the competitive antigen; A_0 means extinctions for holes in the absence of competitor (as an alternative 50 μl of buffer with BSA was introduced); lgC-logarithm for concentration of the competitive antigen. A degree of affinity (in %) between the studied viruses was estimated due to the antigen concentrations with 50% blocking of a reaction of the sorbed antigen with homologous antiserum and calculated by a formula $\frac{\lg C_1}{\lg C_2} \times 100$, where C_1

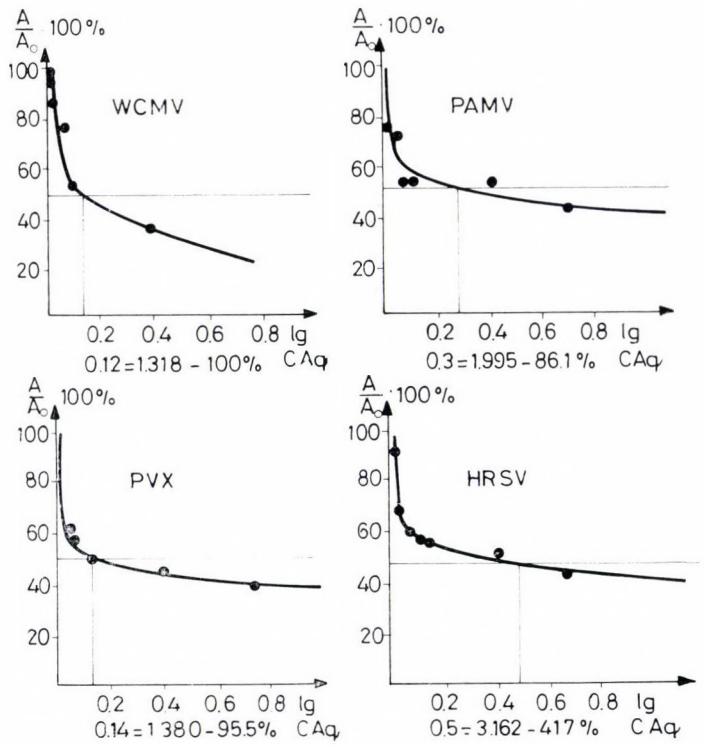

Fig. 5. Blocking curves. System WCMV

is a concentration of the homologous competitive antigen with 50% blocking; C_2 is a concentration of the heterologous competitive antigen with 50% blocking. Each system is characterized by its own percentage of affinity. This may be associated with different affinities of the antibodies used.

Data on the degree of affinity of the given viruses are given in Table 2. In analysing the obtained data, one may state that most closely affined are PVX

Table 2

Degree of the antigenic affinity (in %) of potexviruses identified in the Far East

System	Antigens			
	PVX	WCMV	PAMV	HRSV
PVX	100.00	95.50	39.80	26.30
WCMV	95.50	100.00	66.10	41.70
PAMV	63.07	28.80	100.00	24.50
HRSV	20.90	47.85	24.00	100.00

and WCMV (over 90% affinity); HRSV is less affined (up to 40% affiinity); and PAMV occupied an intermediate position (up to 60% affinity).

The thermodynamic characteristic of the immunochemical reaction has decisive significance in estimating the efficacy of the binding techniques; the corresponding indicators of that characteristic are the association constants (AC) of the antibodies with antigens. By analogy with radioimmunological analysis, we calculated the AC of antibodies with homologous and heterologous antigens in all the four systems studied. The calculation was performed proceding from the blocking curves showing the form of Sketchard graphs (Chard, 1981).

The ratios of the enzymatic activity of bound and plate-sorbed free antigens, $\frac{A}{A_0-A}$, we plotted along the ordinate axis. The total plate-sorbed antigen concentration and the competitive antigen concentration were plotted along the abscissae axes. The antigen concentration was given in mol/l (M/l) basing on the molecular masses of structural proteins of the studied potexviruses which were determined by polyacrylamid gel electrophoresis technique according to Hedrick and Smith (1968) in our laboratory (Artyukova, 1985). 25000 for PVX, 28000 for PAMV, 24120 for HRSV and 22880 for WCMV.

The concentrations of plate-sorbed antigens were determined graphically, basing on the curves showing the minimal concentration of antigen sorption. The perpendicular to the curve from the intersection point in the continuation of the curve branches afforded a plates-sorbed antigen concentration equal to 0.94 ng for PAMV, 0.72 ng for HRSV, 0.36 ng for PVX and 0.54 ng for WCMV.

The Sketchard graphs had the form of straight lines (Fig. 6) whose inclination was numerically equal to the AC, while its intersections with the horizontal

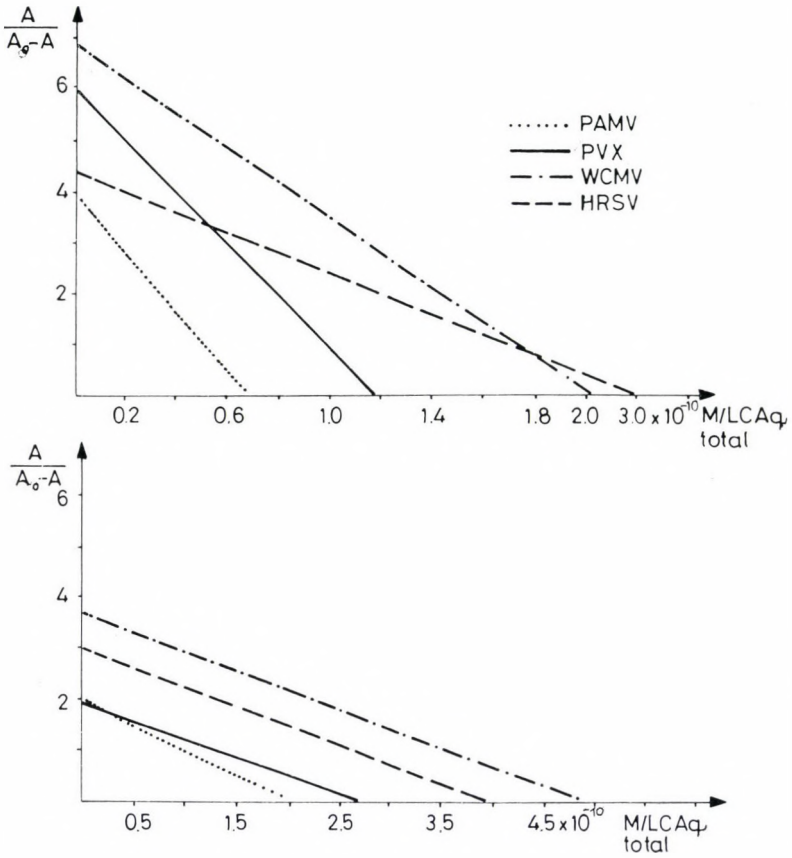

Fig. 6. Sketchard's graphs for determining association constants of antibodies with homologous and heterologous antigens. System PAMV

axis gave the total antibody binding concentration (n). The AC was calculated by a formula based on the equation describing the law of mass action:

$AC = \frac{(AbAg)}{(Ag)(Ab)}$, where the ratio $\frac{(AbAg)}{(Ag)}$ is equal to $\frac{A}{A_0-A}$, and $(Ab) = n$. The calculated AC of the studied potexviruses with homologous and heterologous antibodies are given in Table 3.

Two Sketchard graph branches and two AC's in all the systems are indicative of the presence in the antisera used in experiment of antibodies of high affinity (AC_1) and low affinity (AC_2). The obtained constants additionally evidenced the presence and degree of antigen affinity between the given potex-group viruses.

Immune electron microscopy (IEM) clearly showed the sorption of specific antibodies not only homologous particles of potexviruses, but heterologous viral

Table 3

Association constants of the studied potexviruses with homologous and heterologous antibodies ($1M^{-1}$)

Antiserum	Antigens			
	PVX	WCMV	PAMV	HRSV
AC_1				
PVX	1.37×10^{10}	1.24×10^{10}	1.05×10^{10}	0.84×10^{10}
WCMV	2.09×10^{12}	2.10×10^{12}	1.29×10^{12}	0.59×10^{12}
PAMV	3.58×10^{10}	3.11×10^{10}	5.55×10^{10}	1.41×10^{10}
HRSV	7.4×10^{10}	10.4×10^{10}	8.3×10^{10}	15.1×10^{10}
AC_2				
PVX	0.08×10^{10}	0.078×10^{10}	0.73×10^{10}	0.056×10^{10}
WCMV	1.11×10^{10}	2.165×10^{10}	0.609×10^{10}	0.44×10^{10}
PAMV	0.77×10^{10}	0.77×10^{10}	1.05×10^{10}	0.74×10^{10}
HRSV	0.7×10^{10}	0.87×10^{10}	0.85×10^{10}	1.26×10^{10}

Table 4

Interaction of potexviruses in homo- and heterosystems (results of IEM)

Antigen	Antiserum				
	PVX	PAMV	WCMV	HRSV	Normal
PVX	100/++++	12/+	90/+	0/−	−
PAMV	0/−	100/++++	36/±	83/++	−
WCMV	50/±	85/±	100/++++	77/+	−
HRSV	79/+	0/−	0/−	100/++++	−

particles, albeit in different degrees. Taking the intensity to which the particles are covered with antibodies in a homologous system for 4 "+", we plotted diagrams by which it was possible to judge of the antigen relationships between the viruses of the given potexgroup (Table 4).

Note. Figures show the number of particles that interact with antibodies (calculated per 100 particles). The dominator shows the intensity with which viruses were covered with antibodies.

Thus, the obtained results allow to consider PAMV like PVX, WCMV and HRSV to be a typical representative of potexviruses.

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Immunologic Characteristics of Brome Mosaic Virus and two Rhabdoviruses of Cereals

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The active and strictly specific antisera against two isolates of brome mosaic virus (from wheat grass and *Spodiopogon sibiricus* Trin.) were obtained.

A high sensitivity of indirect method ELISA allowed to find out about 10 g of the virus in purified preparations and to identify the virus with dilution up to 10^5 in a sap of infected plants. The virus concentration was estimated in barley leaf sap cv. Viner as 1.2 mg/ml and cv. Primorsky 89 as 0.6–0.8 mg/ml.

Viruses of cereal mosaic and pupation showed a weak immunogenic activity. A titre of antisera in double diffusion reaction was 1 : 4–1 : 32. Conditions for intensification of immune response were chosen (a dose of immunogene, the number of injections, adjuvant and immunization scheme).

Serological affinity among three rhabdoviruses, namely cereal mosaic virus, cereal pupation virus and cereal north mosaic virus was demonstrated by the rocket immunoelectrophoresis.

Studies of viruses affecting cereals are caused by the injurious effect of these viruses. Diagnostics is hindered owing to symptom resemblance. Due to this fact the immunologic methods are essential for identification and studies of cereal viruses.

We have aimed at studying the antigenic and immunogenic characteristics of brome mosaic virus, cereal mosaic and cereal pupation viruses and at the search for ways of increase, in the immunogeneity to obtain active specific antivirus sera.

Brome mosaic virus was identified in our region by E. E. Sokolova et al. (1977) whereas cereal mosaic and cereal pupation viruses by Fedotina (1974) and Krylov et al. (1981).

Materials and Methods

Antiserum against brome mosaic virus (BMV) was obtained for virus isolate identified on *Spodiopogon sibiricus* Trin. The virus was reproduced in barley plants cul. Viner and cul. Primorsky 89. The technique proposed by Hull (1972) assumed as a basis with our modifications was used to obtain a purified virus preparation.

In the 12–15th day after inoculation leaves were homogenized in 0.2 M acetate buffer, pH 5.0 adding 0.1% of ascorbic acid and 0.1% 2-mercaptoethanol. 1/5–1/7 part of chloroform was added to homogenate for clarification. A pap was isolated by centrifuging at 6000 g for 20 minutes. Virus from the clarified homogenate was sedimented with 7% PEG (6000) adding 0.35 M of sodium chloride. After an hour a precipitate was isolated by centrifugation at 6000 g for 20 minutes. The virus was resuspended in 0.5 M of acetate buffer, pH 5.0. Further purification was conducted using a double differential centrifugation (70000–90000 g for 100 min and 15000–18000 g for 20 min).

The first attempt to obtain antiserum against BMV was initiated by us in the middle seventies relative to the isolate identified from wheat grass (Sokolova et al., 1977). The virus was accumulated in barley plants cul. Viner. 1.4 mg of purified virus was injected within a cycle of immunization including five intravenous injections.

The immunization scheme allowing to obtain an antiserum with a high titre of specific antibodies was developed for the BMV identified on *Spodiopogon sibiricus* Trin.:

the first day – 500 g immunogene were injected intracutaneously in 10–15 dots along the spine, and over the lateral lines of animal body. Supplementarily, the nonspecific stimulation of immune response in addition to Freund's adjuvant, which was mixed with immunogene, is provoked with intramuscular introduction of 0.25 ml AWDA (adsorbed whooping-cough-diptheria-tetranuseous anatoxin);

the eighth day – 1 mg of immunogene intracutaneously in 10–15 dots along the spine, over lateral lines, in corners of shoulder-blades and popliteal holes in subcutaneously;

the twenty third day – 1 mg of virus – intravenously.

130 days after the last injection the animals were reimmunized by means of combined method (500 g of immunogene intravenously and 500 g intradermally).

Purified preparations of cereal mosaic (CMV) and cereal pupation (CPV) viruses were obtained at our laboratory by L. A. Minskaya. Immunogenes were injected into animals intramuscularly and by a combination of methods, namely intramuscularly and intravenously. A common dose of virus ranges from 2 to 4.5 mg. Immunization scheme included from 7–8 to 3–4 injections with a week interval. To stimulate immunogenic response the following adjuvants were used: sodium alginate, Freund adjuvant, oil adjuvant "Al-Spain-Oil" (Czech) and pertussoid-diphtheritic-tetanic anatoxin.

A titre of specific antibodies was determined in the reaction of double diffusion (DDR). To carry out the reaction of double diffusion 0.8–1.0% of "Bacto"-agar (Ferak, F. R. G.) and "Difco" (USA) were used in a buffer for isolation of virus or in 0.85% solution of sodium chloride adding into agar in both cases to show more distinctly the precipitation lines of 1.5% PEG (6000). Degradation of rhabdovirus virions before DDR was performed by the ultrasonic

treatment (5, 10 and 15 min at 15 kHz) or by adding of 0.1% Tween-20 0.05–0.1% sodium dodecylsulphate.

Radial immunodiffusion reaction (RID) was used to determine the BMV concentration in a sap of infected plants used for obtaining of virus preparations. Melted agar was mixed with an experimentally chosen aliquot of antiserum (5%) at 50 °C. 4.0–4.5 ml of the mixture obtained was coated on a slide. 10 g of virus antigen or sap of infected plants were introduced into holes at certain dilutions. Virus concentration in the preparation under study was determined according to diameters of rings formed in RID. For this purpose the calibration lines of control antigen with a known concentration were used.

The indirect method ELISA (Sibiryakova et al., 1987; Koenig, 1981) was used for BMV identification in a sap of infected plants and for preparing the immunodiagnosticum. The preparations of homologous and heterologous (TMV) antigens were titrated within the range of concentration from 5 hg to 200 mkg/ml. Dilution of infected sap and a sap of healthy plants was 1 : 50. The working dilution of antiserum and antispecific conjugate (IgG of donkey against IgG of rabbit) amounted to 1 : 100–1 : 200.

Antigenic relationships of the rhabdoviruses under study were tested in reactions of the rocket immunoelectrophoresis (RIEP). 1% solution of agarose ("Serva", F. R. G.) was used in 0.075 M veronal-acetate buffer, pH 8.6. Experimental dilutions of antisera amounted to 1 : 10 and 1 : 15 for CMV and CPV respectively. 5 l of preparations with the concentration 0.5 mg/ml or a sap of infected plants were put into holes. Reactions were carried out for 2 h at 35 mA, 70 v or for an hour at 45 mA, 90 v.

Results and Discussion

Protein electrophoresis of the purified virus preparation demonstrated presence of the only protein zone. The virus was sedimented with one peak at the analytic centrifugation with a constant of sedimentation equal to 86–88 S in acetate buffer, pH 5.0. Molecular weight of capsid protein was 2000 ± 500 daltons thus conforming to the literature data. Examination of virus transmission by aphid *Myzodes persicae* Sultz. shoed an absence of transfer.

The antiserum against the isolate BMV identified from wheat grass was strictly specific and responded to homologous virus both in drop agglutination and double diffusion reaction in agar. Titre of antiserum to virus isolate on *Spodiopogen sibiricus* Trin. in DDR was corresponded 2048. As a result of reimmunization on the 130th day after the last injection we succeeded again in increasing in the titre from 32 up to the previous level (Fig. 1).

BMV concentration in a sap of barley leaves cul. Viner equal to 1.2 mg/ml (Fig. 2) was determined by means of RID. This allowed to trace the process of virus accumulation in plant material and got rid of the necessity of virus purification in most cases thus simplifying our work.

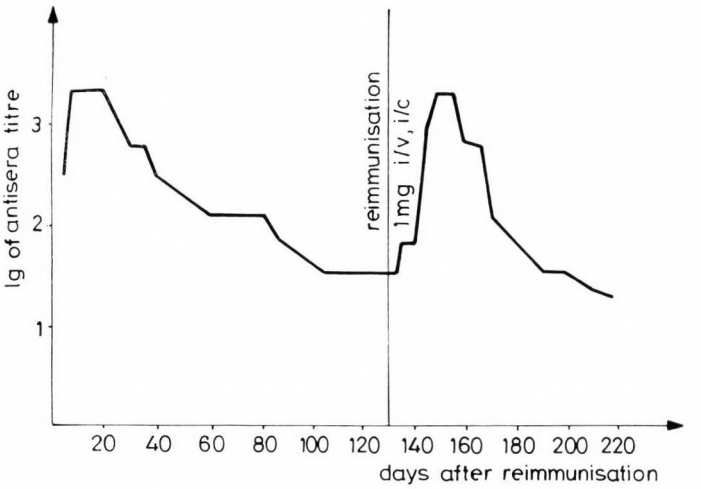

Fig. 1. Accumulation of BMV antibodies in reimmunized animals

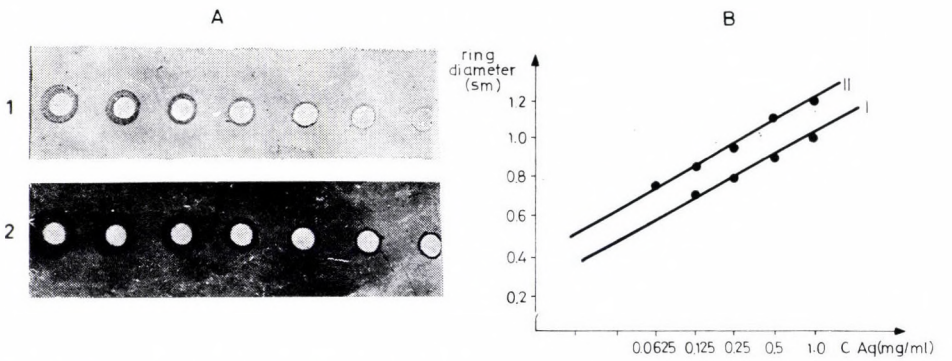

Fig. 2. Determination of BMV concentration according to RID results in sap of infected plants. A) In holes: 1 — preparation dilution C = 1, 0.5 . . . 0.15 mg/ml, 2 — sap dilution of barley leaves cv. Viner infected by BMV — undiluted, 1 : 2, 1 : 4 . . . 1 : 64. In agar 5% (0.35 ml) of antiserum to against. BMV. B) Calibration straight lines. I — virus preparation, II — sap of leaves infected by BMV

A high sensitivity of indirect method of ELISA permitted to reveal about 10 g of virus in purified preparations and in plant sap diluted to 10^5. The BMV concentration was estimated in leaf sap of barley cv. Primorsky 89 as 0.6–0.8 mg/ml (Fig. 3).

Weak immunogeneity and extreme lability of phytorhabdoviruses (Minskaya et al., 1987; Gnutova, 1985; Herold, 1972; Jackson, Christie, 1977) complicated preparing of the active specific antisera against CMV and CPV. Due to instability of those viruses we had to inject sometimes only a partially purified preparation.

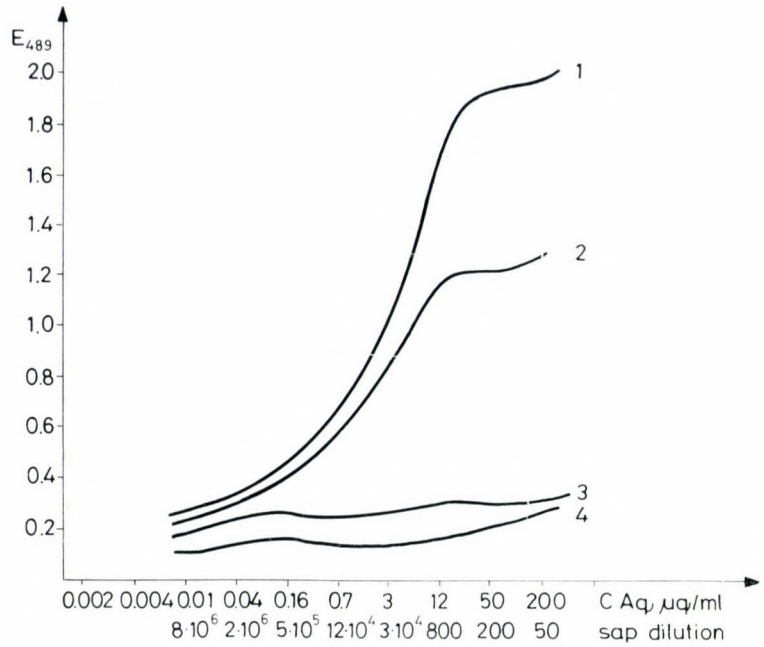

Fig. 3. Determination of BMV concentration by ELISA indirect method. 1 — virus preparation, 2 — sap of barley leaf of the cv. Primorsky 89, infected by BMV, 3— TMV preparation, 4 — sap of healthy plant leaves. Dilution of antiserum and antispecific conjugate — 1 : 200

Rhabdoviruses under study are characterized by a low concentration in a sap of diseased plant.

Specific sera with titres from 1 : 4 to 1 : 32 were obtained against virion preparations of CMV and CPV, thus indicating the weak immunogenic activity of rabdoviruses studied. It was shown that increase in the number of immunizations to 7–8 did not result in better effect in comparison with 3–4 time injection of immunogene.

The problem of interaction between CPV and CMV was discussed (Minskaya et al., 1987) because the only transmitter *Myzodes persicae* Sulz. for those viruses has been found. Therefore we verified their immunologic relationships. The rocket immunoelectrophoresis demonstrated the presence of common and individual antigenic determinants in phytorhabdoviruses under study (Fig. 4). Precipitation peaks with the antiserum against the cereal north mosaic virus (CNMV), kindly offered by Dr. Yu. Shirako from Japan, were obtained as well (Fig. 5). Thus, the serological affinity was shown among CMV, CPV and CNMV.

So the active and strictly specific antisera against two isolates of BMV were obtained. Preparing of the antisera against rhabdoviruses is more complicated task due to their lability and low concentration. It is necessary to choose carefully

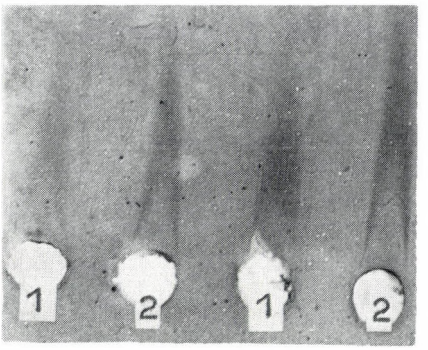

Fig. 4. Identification of antigenic determinants of CMV and CPV by RIEP. In gel: antiserum to CMV dilution 1 : 10; in holes: 1 — CMV preparation (C = 0.5 mg/ml), 2 — CPV preparation

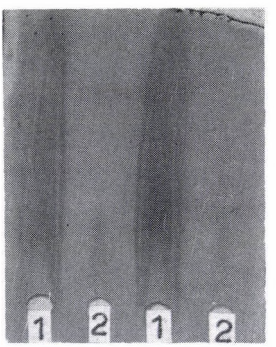

Fig. 5. Identification of antigenic determinants of CMV, CPV and CNMV by RIEP. In gel: antiserum to CNMV dilution 1 : 25; in holes: 1 — CMV preparation (C = 0.5 mg/ml), 2 CPV preparation

one of the factors affecting immunogenesis of animal, a dose of immunogene injected, way of injection and the adjuvant used, choise of immunization scheme and reimmunization. Maximum intensification of the process for producing specific antibodies against CMV and CPV is feasible only with complex interaction of all factors. Immunologic relationship between three phytorhabdoviruses, namely CPV, CMV and CNMV was demonstrated.

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In vitro Activity of Some Essential Oils toward *Erwinia amylovora* (Burril) Winslow et al.

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The *in vitro* activity of 15 essential oils against some isolates of *Erwinia amylovora* was tested. The oils of origanum, garlic, savory, camomile and white thyme had a minimum inhibitory concentration of 112.5 mg/l up to 900 mg/l.

Origanum oil was the only one with a bactericidal action (at 900 mg/l) against 2 isolates. Four groups of oils were distinguished on the basis of their antibacterial activity: a) none, b) slight, c) fair and d) good. Their antiseptic activity was compared to that of streptomycin sulphate and gentamycin sulphate.

The antiseptic activity of essential oils has long been known (Chamberland, 1887) and there have been extensive studies of these properties *in vitro* in relation to human bacteriology (Bello, 1942; Gorg and Kasera, 1983; Maruzzella and Lichtenstein, 1956; Melegari et al., 1985; Morris et al., 1979; Piacentini, 1948; Prasad et al., 1986), as well as some practical applications (Belaiche, 1983). In bacterial phytopathology study of the bacteriostatic and bactericidal activity of these natural compounds from the vegetable kingdom has been rather neglected, even if Turkmenoglu et al. (1974) found good results on the control of *Agrobacterium tumefaciens* (Smith and Townsend) Conn on grapevine by oil of *Juniperus oxycedrus* L.

The study reported in this paper was designed to discover if the essential oils tested possesses antibacterial activity against certain isolated of *E. amylovora.* Methods recently developed to more correctly identify the minimum bacteriostatic and bactericidal inhibitory concentration of these compounds were adopted for the purpose (Allegrini and Simeon de Bouchberg, 1972; Al-Meshal et al., 1982; Melegari et al., 1985; Sherris, 1986).

Materials and Methods

Essential oils (E. O.) of commercial origin* were used. These had been obtained by distillation in a current of steam and de-terpenated. Oils from 15 species were studied: *Pinus silvestris* L. (Scots pine); *Cupressus sempervivens* L. (Italian cypress); *Juniperus communis* L. (common juniper); *Rosa canina* L. (dog rose); *Citrus bergamia* R. and P. (bergamot); *Tilia tormentosa* Moench

* Pharcos company, Cisterna di Latina (LT)-Italy.

(silver linden); *Menta×piperita* (peppermint) ; *Rosmarinus officinalis* L. (rosemary); *Salvia sclarea* L. (sclarea sage); *Ocimum basilicum* L. (basil); *Thymus vulgaris* L. (white thyme); *Origanum vulgare* L. (red origanum); *Satureja hortensis* L. (savory); *Ma icaria chamomilla* L. (camomile); *Allium sativa* L. (garlic).

A small quantity of E. O. (0.5 ml), filter sterilized (millepore 0.22 μm), was mixed with a basal solution of 10^{-2} M dimethylsulphoxide (DMSO) and diluted successively with DMSO to obtain the following concentrations: 900 mg/l; 450 mg/l; 225 mg/l; 112.5 mg/l; 90 mg/l; 45 mg/l; 22.5 mg/l; 9 mg/l.

Antibacterial activity was tested on 4 isolated of *E. amylovora:* WC-31; RIF NY; WV-55 JOINEE (these received from T. Van der Zwet, Appalachian Fruit Research Station, West Virginia); and NCPPB 595. For the tests the isolates were grown in nutrient broth for 48 hours at 25 °C, centrifuged and washed in sterile physiological solution (0.85% NaCl) and resuspended in the same medium to obtain a concentration of 10^5 colony forming units (CFU)/ml.

Tubes were prepared containing 4.5 ml of liquid culture medium 523 (Kado and Heskett, 1970: saccharose 10 g/l, acid hydrolysed casein 8 g/l; yeast extract 4 g/l; K_2HPO_4 2 g/l, $MgSO_4 \cdot 7H_2O$ 0.3 g/l), to which were added 1 ml of bacterial suspension and 1 ml of E. O. at each of the concentrations. Two tubes were prepared for each treatment. After Vortex shaking, the optical density (O. D.) of the suspension was measured at 620 nm using an ELISA micro-plate and a photometer (Titertek-Multiscan MCC). The tubes were incubated in a water bath at 25 °C.

The MIC (minimum inhibitory concentration: the lowest concentration with an action inhibiting microbe growth for at least 24 hours) was determined on the following day; MIC was considered present when the O. D. was the same as on the previous day.

After 3 days the O. D. was measured a second time and, if present, the MBC (minimum bactericidal concentration: the lowest concentration that yields fewer than 0.1% survivors) was recorded.

Moreover, in order to detect partial bacteriostatic activity of E. O., during the trial the bacterial concentration corresponding to each O. D. was determined by collecting 0.1 ml from controls and inoculated tubes and counting CFU in Petri dishes containing nutrient agar (NA). Partial antibacterial activity of the

Table 1

MIC and MBC (mg/l) of E.O. towards *E. amylovora* isolates

Isolates E. O.	WC 31		RIF NY		WV 55 JOINEE		NCPPB 595	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Origanum	450	—	450	900	450	900	900	—
Garlic	900	—	225	—	112.5	—	900	—
Savory	900	—	112.5	—	—	—	900	—
Camomile	—	—	—	—	900	—	450	—
White thyme	900	—	—	—	—	—	—	—

E. O. was pointed out when there was a decrease in growth, compared to the controls, of at least 10^2 CFU/ml after 1 day and at least 10^3 CFU/ml after 3 days. In the case of the oils demonstrating bactericidal activity, an aliquot was taken daily for a week from the test-tube and smeared on the Petri dishes with NA to check the absence of growth. For each E. O. tested were set up in parallel a) sterility control (uninoculated test-tubes with the substrate, containing the E. O. under examination); b) fertility controls (inoculated test-tubes without E. O. with the addition of 1 ml of DMS solvet); c) vitality controls (test-tubes with only inoculated substrate) and d) volatilization controls (test-tubes with E. O. inoculated after 3 days of storing at 20 °C).

The bacteriostatic of the E. O. was compared with that of streptomycin sulphate (SIGMA) (200 mg/l) and gentamycin sulphate (SIGMA) (20 mg/l) added after filter sterilization, to the inoculated test-tubes.

Results

MIC and MBC

E. O. from Origanum, Garlic, Savory, Camomile and white Thyme showed MIC ranging from 112.5 mg/l to 900 mg/l (Table 1).

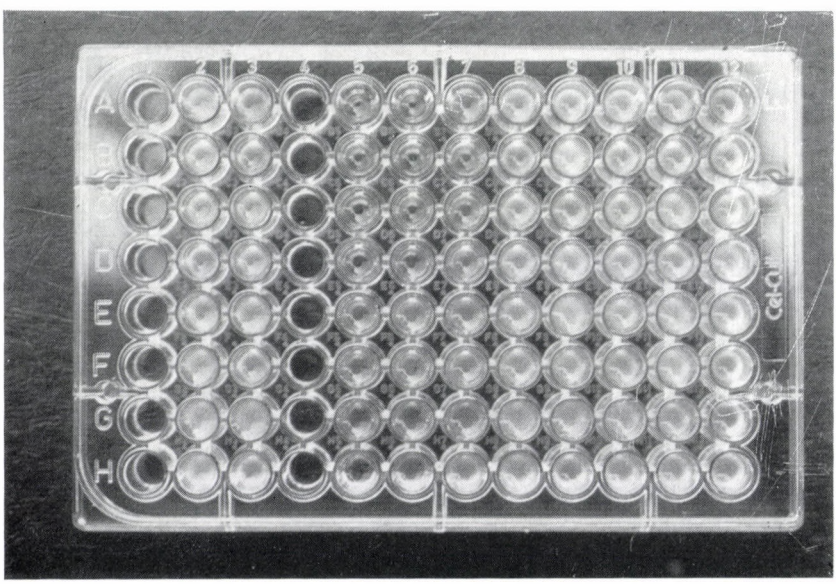

Fig. 1. Minimum inhibitory concentration of origanum essential oil. Line A, B: RIF NY; line C, D: WV 55 Joinee; line E, F: NCPPB 595; line G, H: WC 31. Column 2: vitality control; column 3: fertility control; columns 5–12: essential oil of origanum from 900 mg/l to 9 mg/l. It is possible to observe no bacterial growth on lines A, B, C, D, G, H-columns 5 and 6 and on lines E, F-column 5 while bacterial growth is present in columns 2 and 3

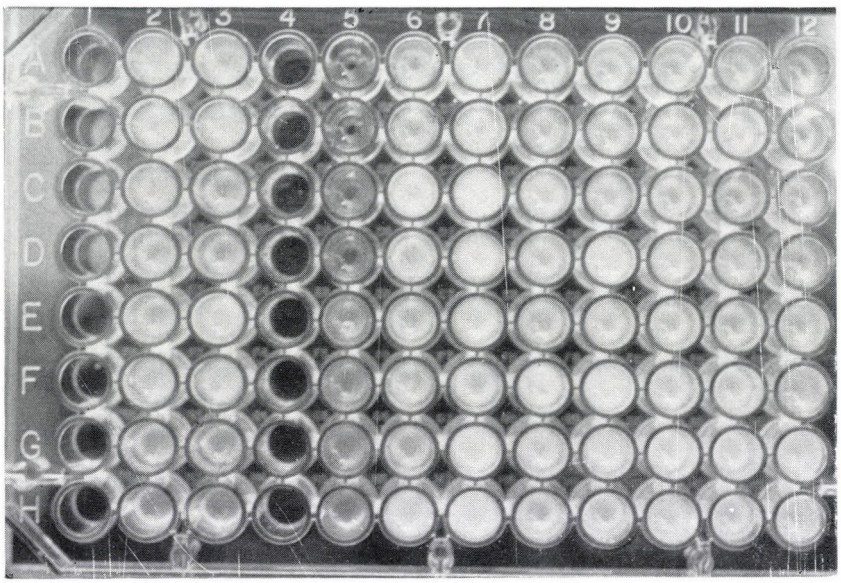

Fig. 2. Minimum bactericidal concentration and partial antibacterial activity after 3 days of origanum essential oil. Line A, B: RIF NY; line C, D: WV 55 Joinee; line E, F: NCPPB 595; line G, H: WC 31. Column 2: vitality control; column 3: fertility control; columns 5–12: essential oil of origanum from 900 mg/l to 9 mg/l. It is possible to observe no bacterial growth only on lines A, B, C, D-column 5 (900 mg/l)

Figure 1 shows the effect after 1 day of the different concentrations of origanum E. O. compared to vitality and fertility controls. MIC is between 450 and 900 mg/l.

The only E. O. to demonstrate bactericidal activity was that of origanum (at 900 mg/l) against RIF NY and WV 55 JOINEE (Table I and Figure 2). Even at higher concentrations, the other 10 E. O. failed to demonstrate any growth inhibitory action on the isolates examined, not even for a single day. Streptomycin sulphate inhibited the growth of all the isolates in the 24 hours following inoculation, however it did not demonstrate subsequent bactericidal activity. Gentamycin sulphate exerted a bacteriostatic action against all the isolates and a bactericidal action against NCPPB 595 and RIF NY (Figs. 3, 4, 5).

Partial antibacterical activity

The E. O. can be classified into four groups on the basis of their partial antibacterical activity.

A) E. O. with nil antibacterical activity (no bacteriostatic effect on any isolate): Italian cypress and common juniper.

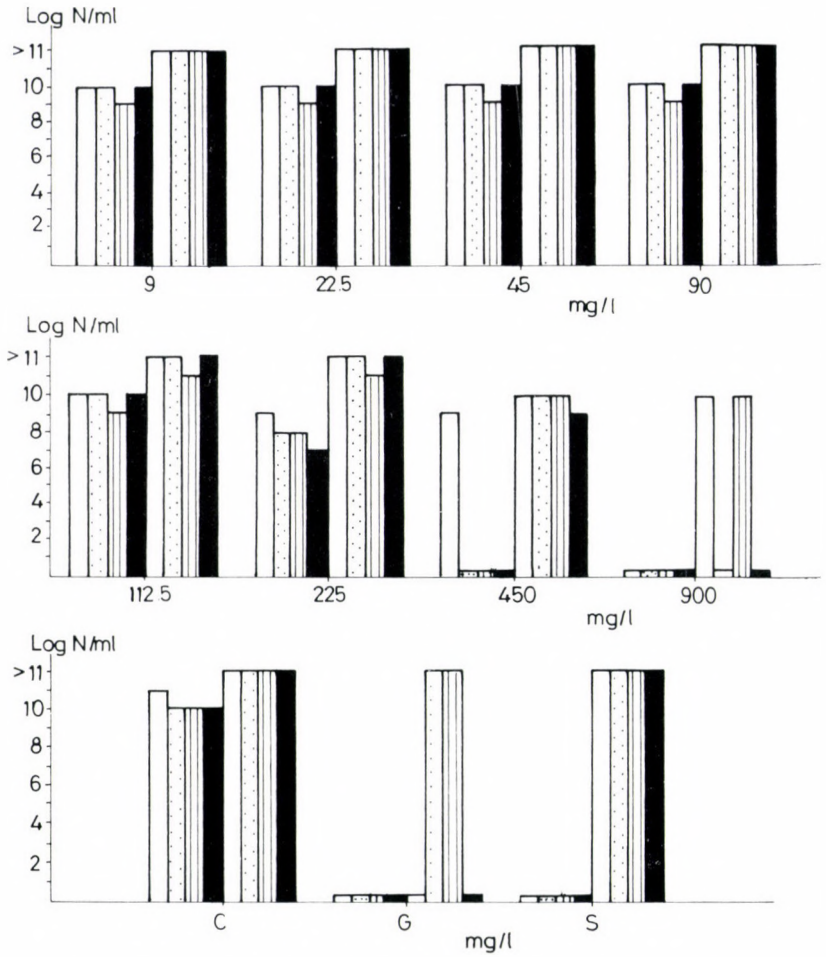

Fig. 3. Activity of origanum E. O. on bacterial growth (Log N bacterial cells/ml) compared to gentamycin sulphate (20 mg/l), streptomycin sulphate (200 mg/l) and vitality control (C). First four columns represent growth after 1 day from inocula. Second four columns represent growth after 3 days from inocula. □: NCPPB 595, ⊞: 55 Joinee; ⊞⊞: WC 31; ■: RIF NY

B) E. O. with slight antibacterial activity (bacteriostatic effect, after 1 or 3 days, only on some isolates at the highest concentrations): Scots pine, dog rose, rosemary, sclarea sage, basil.

C) E. O. with moderate antibacterial activity (bacteriostatic effect, after 1 or 3 days, on all isolates, or effective one some isolates at the lower concentration): white thyme, peppermint, bergamot, silver linden, camomile.

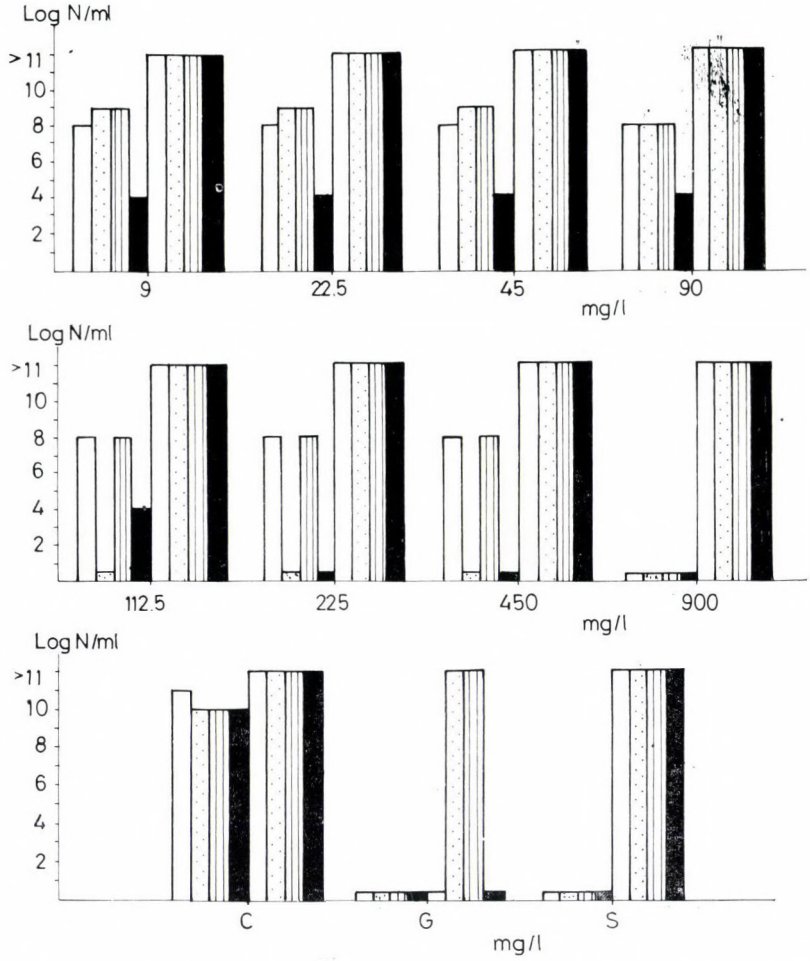

Fig. 4. Activity of garlic E. O. on bacterial growth (Log N bacterial cells/ml) compared to gentamycin sulphate (20 mg/l), streptomycin sulphate (200 mg/l) and vitality control (C). First four columns represent growth after 1 day from inocula. Second for columns represnt growth after 3 days from inocula. □: NCPPB 595; ⊞: WV 55 Joinee; ⊞⊞: WC 31; ■: RIF NY

D) E. O. with good antibacterial activity (bactericidal effect and/or bacteriostatic effect on all isolates after both 1 day and 3 days): origanum, savory, garlic.

The E. O. of origanum not only possessed bactericidal activity but was also the one which exerts an antibacterial action on the isolates, after 1 day, at relatively low concentrations (225 mg/l) (Fig. 3). After 3 days, partial antibacterial activity towards all isolates was detected from 450 mg/l (Fig. 3). E. O. of

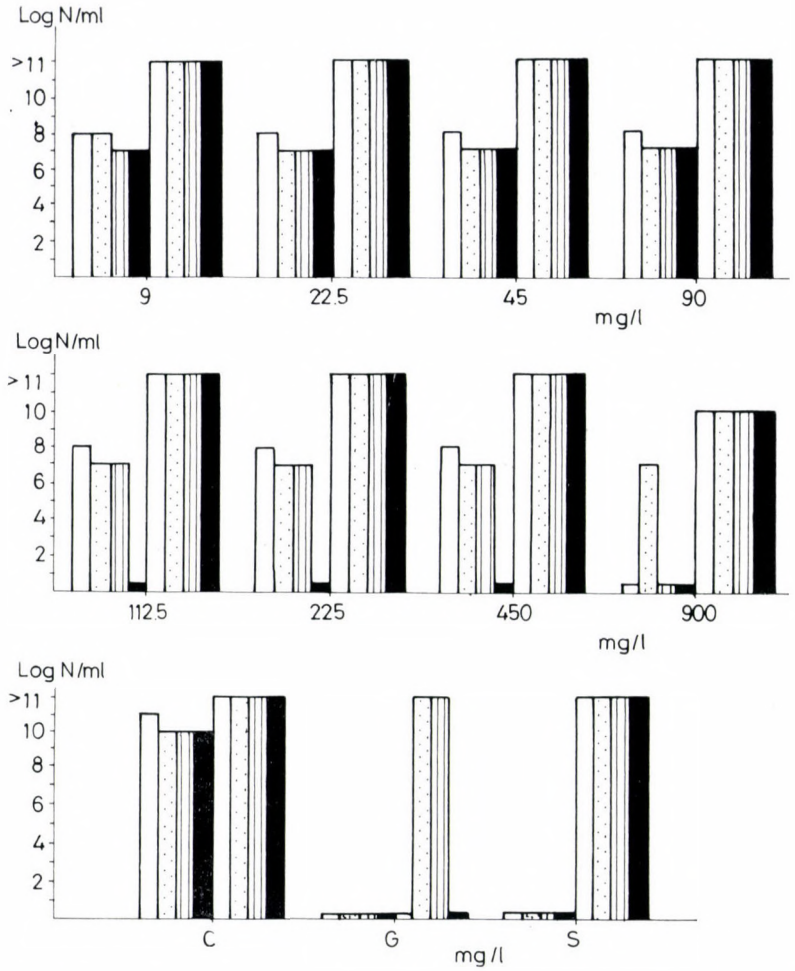

Fig. 5. Activity of savoty E. O. on bacterial growth (Log N bacterial cells/ml) compared to gentamycin sulphate (20 mg/l), streptomycin sulphate (200 mg/l) and vitality control (C). Firts four columns represent growth after 1 day from inocula. Second four columns represent growth after 3 days from inocula. □: NCPPB 595; ⊞: WV 55 Joinee; ⊞⊞: WC 31; ■: RIF NY

garlic, after 1 day, demonstrated partial bacteriostatic activity at very low concentrations (9 and 22.5 mg/l against RIF NY and NCPPB 595),whereas at 3 days activity was encountered only at the highest concentrations towards WC 31 (Fig. 4).

E. O. of Savory exerted a partial antibacterial activity towards all isolates after 1 day also at the lowest concentration; whereas after 3 days this action was encountered only at the highest one (Fig. 5).

It was noticed that after 3 days the bacteriostatic activity of the E. O. had decreased greatly or disappeared. It was probably due to the partial volatilization of E. O. after 3 days. Infact tubes with E. O. of group D, inoculated after 3 days, showed, the day after inoculation, a higher overage growth (10^2–10^3) compared to that observed with "fresh E. O." of the same group. It should be noted that the conifer oils either had no effect (Italian cypress, common juniper) or else the effect was slight (Scots pine).

It was also noticed a different reaction towards E. O. of the isolates tested: NCPPB 595 proves most susceptible to them, with 13 E. O. demonstrating an inhibitory effect against it, including minimum effects; RIF NY showed no sensitivity to 6 E. O. although it was one of the most susceptible to origanum oil.

Streptomycin sulphate demonstrated an activity comparable to that of the E. O. in group D. Gentamycin sulphate behaved in the same way towards WV 31, whereas it showed a higher antibacterial activity (10^4 CFU/ml) against WV 55 JOINEE.

The solvent used (DSMO) did not influence bacterial growth, the values for O. D. in the fertility controls being similar to those for the vitality controls, while the tubes containing only culture medium with the E. O. remained sterile (no growth of any contaminant) during the trial.

Discussion

The results of this study confirm effectiveness of some deterpenated E. O. in inhibiting the growth of phytopathogenic, as well as other, bacteria. It also confirm the good bacteriostatic action exerted by the E. O. of origanum towards the *Enterobacteriaceae* (Morris et al., 1979) and the interesting action of savory and garlic oil. The values of MIC and MBC encountered can be considered good (Morris et al.), even though comparable data on *E. amylovora* are lacking.

Concerning antibacterial activity of E. O. further studies are required, however, to point out I) the most active compounds, II) the best time to harvest plants, III) the spectrum of action at different temperatures, on different bacterial isolates and concentrations and with different time of inoculation, IV) the action of the same E. O. undeterpenated (in general their antiseptic power is greater).

Moreover, studies of the phytotoxicity of the E. O. needed prior to any practical application in the control of "fire blight", as well as *in vivo* studies on their effectiveness in controlling *E. amylovora*.

These compounds could, however, also be tested in mixtures with the copper products traditionally used, or as additions to antibiotics. The synergistic increased effectiveness shown by the addition of E. O. to antibiotics (Halbeisen, 1956; Stickl, 1955) might make it possible to use the latter at lower concentrations.

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Influence of Culture Age on the Plasmid of *Pseudomonas solanacearum*

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Pseudomonas solanacearum grown on catechin showed differences in plasmid DNA during its growth cycle. A correlation existed between UV intensity and the quantity of plasmid. Plasmid DNA was maximum in stationary phase of 36 h old culture and least in the cells from lytic phase (48 h old).

Currier and Morgan (1981) claimed the presence of a plasmid in *Pseudomonas solanacearum*. Subsequently, Rosenberg et al. (1982) isolated a megaplasmid with a molecular weight of 675 kb from the cultures. Morales and Sequeira (1985) screened 32 strains of *P. solanacearum* collected from different regions for plasmids and reported plasmids of size ranging from 7.5 to 750 kb in 22 strains. Only one strain harboured 2 plasmids. Boominathan and Mahadevan (1987) demonstrated the involvement of a plasmid pAMB in *P. solanacearum* associated with the dissimilation of catechin. In this note, results on the level of plasmid DNA in the cells collected from different growth phases are reported.

P. solanacearum originally isolated from infected stems was grown in BG broth (Boucher et al., 1977). At prefixed interval, cells were suspended in TES buffer (Sucrose 0.3 M; Tris buffer 25 mM; EDTA 25 mM; pH 8.0), diluted to 1.40 O. D., having a viable count of 2.47×10^9 cells/ml and used for plasmid isolation. Alkaline lysis of the cells was made (Boominathan and Mahadevan, 1985). The amount of lysate loaded on gels was kept constant for each analysis. The plasmid DNA was electroeluted from the unstained gel using TE buffer (Tris 10 mM; EDTA 1 mM; pH 8.0) and estimated at 260 nm. DNA (CSIR Centre for Biochemicals, Delhi) suspended in TE buffer was used as standard.

The experiment was conducted in duplicate and repeated. Matching results are reported.

The intensity of plasmid DNA band in 8 h culture was faint but in 20 h and 36 h cultures, it was high and in 48 h culture, it declined. Subsequently, the density of the plasmid DNA from different growth phases of the culture was estimated (Fig. 1). A correlation existed between UV intensity and the quantity of plasmid DNA. According to Rownd et al. (1966), the amount of plasmid DNA was maximum in stationary culture of *Proteus mirabilus*. In *Escherichia coli*, plasmid DNA declined in exponentially growing cells (Collins and Pritchard,

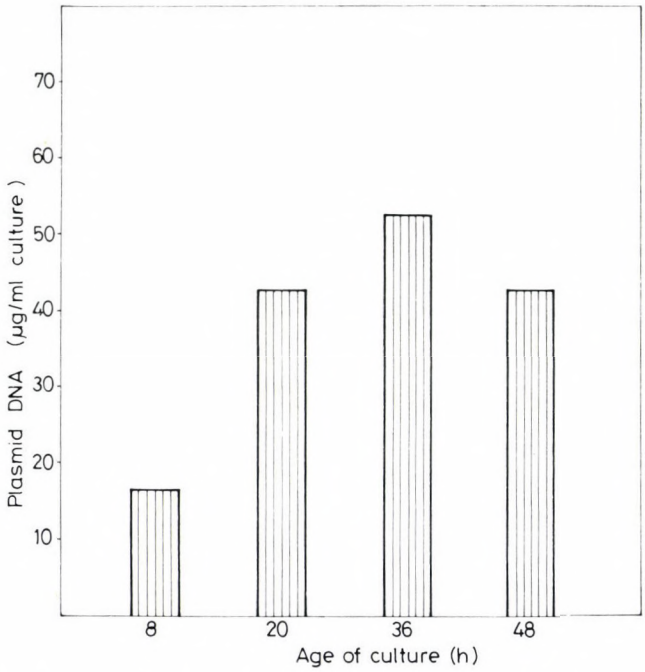

Fig. 1. Plasmid DNA in *P. solanacearum* at different growth stages

1973). Broda (1978) concluded that age of the culture significantly influenced the plasmid copy number in bacteria. Previous investigators (Rosenberg et al., 1982; Morales and Sequeira, 1985) have used only exponentially growing cells and have assumed that the copy number was high at this stage. Our results indicate that the copy number of plasmid in *P. solanacearum* was high only in 36 h (stationary phase) old culture. We therefore suggest that the level of plasmid DNA varies in different growth stages of the bacterium.

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Some Properties of the Fauna of Carabid Beetles in the Southern Urals

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The object of this investigation was to study the properties of carabid fauna of the Southern Urals and Urals foothills on the material collected in Bashkiria. The historical survey of the study of carabids of the given region adduced. During this investigation generally accepted methods of field study of invertebrate animals have been used. The goal of this work is to discuss inhabitants, areas and some ecological data of carabids of the forest zone (with subzone of southern taiga and broad-leaved forest) as well as forest-steppe and teppe zones.

The attempt of zoogenographical analysis of zonal groups of carabids permits us to draw a number of conclusions:

– Among carabids of the Southern taiga species with broad holarctic and transpalaearctic areas predominate.

– The subzone of broad-leaved forest is richer in the quantity of species, in variety of areas. The central place in its fauna is occupied by westpalaearctic species.

– Among the species of forest-steppe zone the predominance of any area group has not been found.

– The steppe zone is characterized by the tendency of the predominance of European-Kasakhstan, Kasakhstan-Mongolian and Euroasian species.

According to the quantity (abudance of species as well as according to their role in natural biogeocenosis and agrocenosis *Carabide* family is one of the most important among *Coleoptera.* Overwhelming majority of the carabid fauna in the USSR is predatory. When carabid beetles are abundant in numbers they assist to regulate the reproduction of a number of dangerous pests in agriculture and forestry. At the same time a lot of carabid species injure agriculture crops. Stenotopyty of a lot of carabid species used to indicate successfully edaphic phytologic conditions and the capability of them to respond to a change of the environment make them a convenient object for biological monitoring.

The inventory of carabid beetles of the USSR demands detailed data through the whole territory. If there are rather detailed data about the carabid fauna in a number of regions of the country, the carabid fauna of the Urals remains not yet enough investigated.

The Urals do not make sharp zoogeographical boarder between the faunas of Eastern Europe and Western Siberia. It is rather a place where the representatives of both these faunas cohabit (Krizhanovsky, 1983). Moreover, the carabid

fauna of the Southern Urals and the Urals foothills is still more heterogeneous on account of penetrating Kasakhstan elements from the South and boreal elements along the ridges. The study of carabid fauna of the Souther Urals is of great interest because this region belongs to two landscape countries and the biographical borders of all landscape zones which are focused here keep moving to the South.

The carabid fauna of the Southern Urals and the Urals foothills has been investigated rather poorly up to now. The first data about carabids in Bashkiria appeared in the XVIII century. P. S. Pallas (1773–1788) found two species of *Carabus* and four species of *Cicindela L.* The next discoveries were made only in the XX century. In the handbook by G. G. Jacobson "The beetles of Russia, West Europe and contiguous countries" (1905–1916) 16 species of carabids have already been found in Umfimskaya Gubernia. Some attention is paid to carabids of Bashkiria in the book "The animal world of Bashkiria". K. S. Nikiforuk (1949) gave a list of carabic species with not many species involved. But he again drew attention to the genra *Carabus*, *Cicindela* and *Calosoma Web.* The next two works (Yafaeva, Chanislamov, 1977; Yafaeva, Girfanova, 1977) repeated in the main that list of the species adding to it six species of *Carabus.* At the beginning of the 80's the investigations of carabids of this region went more actively. The data about carabids of Southern Urals reservation were published (Kashevarov, 1983). The work by A. B. Matveyev (1983) is devoted to carabid fauna of hardwood forests of Bashkirian Urals foothills. Then the data about carabid dynamics on the pastures of the Southern Urals and about the natural zoogeographical barries of the distribution of some species of *Carabus* (Kashevarov, 1985a ; 1985b). A. B. Matveyev in his abstract (1985) and U. I. Korobieinikov (1986) paid attention to the dispersed carabids inhabiting the mountaneous tundra of the Southern Urals. Finally the work about the genus *Carabus* of the region was published (Matveyev, 1986). The author has also investigated carabids of the town of Ufa.

The material for the present investigation served the author's collections and observations which were conducted in more than 200 places in Bashkiria during 1977–86. Since the largest part of the Southern Urals and Urals foothills are in Bashkiria one may consider the data obtained exact enough for the whole region. Besides the author's finding the collections of the Bashkir State United Museum, Zoomuseum of Moscow State University as well as the private collections were used. The material was collected both in expeditions and at stations as well. Generally accepted methods of field study of invertebrate animals were used (Inayeva, 1965; Paliy, 1966; Phasulaty, 1971; Gilayarov, 1975). As soil traps metal cans with the hole diameter of 75 mm filled on 1/3 with 4 per cent formol were applied. In order to avoid filling with rainwater the cans were covered with bark. To our mind the latter is more convenient than plastic or metal covers because it imitates carabid's natural shelter. Carabids were also collected by hand from the soil surface, from their shelters and other methods such as moving grass and flotation as well as catching carabids by means of incandescent and mercury lamps were used too.

Landscape regions of Bashkiria are well determined in a number of works (Kadilnikov, 1966, 1974; Makunina, 1968 and others), therefore we shall not dwell on them. On the territory of the given region we may pick out three landscape zones: forest, forest-steppe and steppe zones. The forest zone is divided into the subzone of southern taiga and broadleaved forests.

The fauna of the sothern taiga is presented rather richly, possibly, it is in relation with its border situation. One of the characteristic features of this fauna is a great number of species with broad areas. So we can meet here holarctic *Notiophilus aquaticus L.*, *Nebria gyllenhali Schoenh*, *Loricera pilicornis F.*, *Dyschirius politus Dej.*, *Tachyta nana Gyll.*, *Bembidion obscurellum Motsch.*, *B. petrosum Gebl.*, *Agonum mannerheimi Dej.*, *A. quadripunctatum Dey.*, *Amara lunicollis Schidt.;* transpalaearctic *Cicindela sylvatica L.*, *Carabus clathratus L.*, *Dyschirius globosus Hbst.*, *Elaphrus riparius L.*, *Bembidion argen-mteolum Ahr.*, *B. littorale Ol.*, *B. tetracolum Say.*, *Agonum impressum Pz.*, *Calathus icropterus Duft.*, *Badister bipustulatus F.*, *Cymindis vaporariorum L.*, *Dromius quadraticolis Mor.* and European-Siberian group which has almost the same distribution as transpalaearctic is comaratively abundant too. Within it there are a number of species distributed from West Europe to the Baikal. *Cicindela campestris L.* and *Carabus cancellatus Ill.* are met at the border where southern taiga passes into mixed forest. Usual European-Westsiberian *Carabus glabratus Pk.* and easteuropean-siberian *C. henningi F. W.* inhabit here. In pine larch forests European-westsiberian *Notiophilus palustris Duft.* is distributed as well as *Trechus rubens F. Bembidion* genus *B. bruxelle nse Wesm.*, *B., fellmani Mnnh.*, *B. schueppeli Dej.* are dispersed here too. Usual habitat of *Pterostichus mannerheimi Dey.* is in the soutern taiga but it is often represented in broad-leaved forests too. West-palaearctic group is also represented by littoral euryzonal *B. femoratum Sturm.*, eurytopic polyzonal *B. quadrimaculatum L.*, *Poecils cupreus L.*, *Pterostichus oblongopunctatus F.* and *P. strenuus Pz.* which are also met in more southern broad-leaved forests. Represented in this region polyzonal *Amara apricaria Pk.*, *A. familiaris Duft.*, *A. nitida Sturm.* have also westpalaearctic area. The territory of Bashkiria is the extreme limit of the distribution of some of European and Siberian species, which do not cross the Urals but some of them do so but move not far. So European *Carabus hortensis L.*, *C. nitens L.* and Siberian *C. regalis F. W.* inhabit here. The very eastern findings of *Bembidion pygmaeum F.*, *Amara montivaga Sturm.* were discovered in Bashkiria. We met Siberian *Pterostichus magus Esch.*, European *Patrobus assimilis Chd.*, Easteuropean *Pterostichus uralensis Motsch.* The latter, as it has recently been discovered, has a very great ecological plasticity – it inhabits almost all forest types, it is also met in altitudinal belt as well as in town parks but more seldom near river banks or lake shores.

Finishing the review of *Carabidae* zone of southern taige we consider necessary to pay attention to the specific fauna of carabids of the altitudinal belt. We'll speak about the highest central part of the Southern Urals the mountains Yaman-Tau, Iremel, Zigalga chain. In spite of being not very high (1400–1650 meters above sea level elevation) these mountains have strongly pronounced

altitudinal zonality. The investigation of the altitudinal belt gave 19 carabid species. The most interesting of them is *Carabus karpinskii Kryzh. et Matv. (in litt.)*. It is a new species though it is related to *C. Odoratus Motsch.* It is likely to be eudemic for the Southern Urals. *Pterostichus urengaicus Juirec.* is also endemic for the region but it is more eurytopic; *P. kaninensis Popp.* is Ural boreomontane subendemic and at last Ural endemic *Nebria uralensis Glas.* Found here *P. kokieli Mill.* has a specific discontinuous area. Before it was only met in the North and Polar Urals and in the mountains of Middle Europe. *Tachycellus curtulus Motsch.* is a new species for Europe. Before it was only known in Eastern Siberia.

As it is very difficult to delimit the faunas of the mixed forest and southern taiga not only on the territory considered but on the territory of the whole European part of the Soviet Union (Krizhanovsky, 1983). Therefore our goal is not to consider detailed investigation of *Carabidae* of mixed forests. We must only mark that though the fauna of mixed forests includes a number of boreal elements in the whole, it is more comparable with the fauna of broad-leaved forests.

Passing to the discussion of the fauna of broad-leaved forests we must mark that the main distinction from the fauna of mixed forests is the falling out of a number of boreal species of the taiga complex. Among the carabid species of this subzone only few of them have broad areas. Few species with holarctic area or transpalaearctic area confined to intrazonal stations – the banks of rivers and they scarcely can be characteristic (*Loricera pilicornis F., Clivina fossor, Dischirius aeneus Dej., D. politus Dej., Bembidion articulatum Pz.*, and not many others). More abundant is the group of European-Siberian species, such as *Cicindela campestris L. and C. hybrida L.* Among *Carabus* we often met *C. cancellatus Ill.* and easteuropean-Siberian *C. schoenherri F.-W.*, which is mainly in lime forests. Easteuropean-Siberian subspecies *C. violaceus aurolimbatus Dej.* is very rare. On the banks of ponds *Elaphrus cupreus Duft.* and along river banks and creeks *Dyschirius tristis Steph., Asaphidion flavipes* (which often moves into the depth of woods), *Bembidion andreae polonicum Müll., B. dentellum Thunb., B. ruthenum Tschitt., B. striatum F., B. unicolor Chaud., Pterostichus anthracinus Ill., P. diligens Sturm* are inhabit. The same areas have *Agonum ericeti Pz., A. fuliginosum Pz., A. gracile Gyll., A. obscurum Hbst., A. piceum L., A. sexpunctatum L., Amara fulva Deg., A. majuscula Chaud., A. municipalis Duft., Curtonotus aulicus Pz.*

Westpalaearctic *Cicindela germanica L., C. maritima sahlbergi F.-W.* (the area of the latter is not yet cleared up) appear here. In oak forests is also distributed phytophilous *Calosoma sycophanta L.*, which dispersed in western Palaearctic and it is biologically related with its prey Gupsy Moth. Widely spread in broad-leaved forests is *Carabus convecsus F.* which has specific westpalaearctic area. Further to this group belong comparatively rare *Elaphrus uliginosus F.*, a number of species living near water, such as *D. thoracicus Rossi., Perileptus areolatus Creutz., Tachys bistriatis Duft., T. micros F.-W., Bembidion azurescens D.-Torre., B. minimum F., B. properans Steph., B. punctulatum Drap.*, euryzonal

B. quadrimaculatum L. From *Pterostichini* and *Agonini-Poecilus cupreus L., P. subcoeruleus striatopunctatus Duft., Pterostichus gracilis Dej., P. strenuus Pz.*, the inhabit of wood edges *P. lepidus Leske.*, eurytopic *P. oblongopunctatus F., P. vernalis Pz., Agonum assimile Pk., A. muelleri Hbst.* We shall also mark *Amara consularis Duft., A. eurynota Pz., A. familiaris Duft., A. ingenua Duft., A. similata Pz., Badister uni pustulatus Bon., Chlaenius nitidulus Schrank., Oodes helopioides F., Panagaeus cruxmajor L., Odacantha melanura L., Lebia chlorocephala Hoffm., L. cyanocephala L., Paradromius linearis Ol., Microlestes minutulus Gz., Brachinus crepitans L.* Such a great specific weight of westpalaearctic species in the fauna of broad-leaved forests confirms the point of view by L. V. Arnoldy (1953) that this fauna is connected with those regions that were not covered with glacier and which preserved partly preglacier pliocenal fauna. After the glacier retreated this fauna moved eastward and reached the Urals the Southern Urals (A. M.) and even the Enisei River.

It is significant to note the appearancy of European-Mediterranean elements in the fauna of broad-leaved wood *Carabidae*. Comparatively common here is *Calosoma inquisitor L.* On the banks of rivers and lakes one can meet *Bembidion biguttatum F., B. guggula F., B. octomaculatum Gz., B. quadripustulatum Serv.*, in flood plain forests *Pogonus luridipennis Germ., Agonum lugens Duft., Acupalpus exiguus Dej., Chlaenius vestitus Pk.* are distributed.

There are not very many species with typically European area in this subzone: *Carabus hortensis L., C. nemoralis Müll., Cychrus caraboides L., Leistus ferrugineus L.*, and Siberian species are completely absent here. May be it is partly connected with a number of great differences in the composition of the forests of the Urals foothills and Western Siberia, for example the absence of oak trees as one of the main forest making species.

To our regret the shortage of the material do not allow us to dwell on the carabid fauna of forest-steppe zones with such details as on the carabid fauna of the forest zone. Therefore we mark only the most interesting or characteristic species.

Forest-steppe zone occupies a considerable territory of the Southern Urals. One can consider the carabid fauna of forest-steppe as an independent fauna complex, because there are a number of species that are characteristic only of this zone (Krizhanovsky, 1983). Though, without doubt, forest, steppe and eurozonal species predominate here numerically. Of characteristic forest-steppe species we'll note Easteuropean-Westsiberian *Carabus estreicheri F.-W. and C. stscgeglovi Munh.* A number of literary sources (Nikiforus, 1949; Yafaeva, Chanislamov, 1977; Uafaeva, Girfanova, 1977) give indication for *C. haeres F.-W.* But we think that the data are erroneous. Instead of *C. haeres F.-W.* that is spread more westward they mistook *C. sibiricus F.-W.* For this zone the following species are characteristic: European *Cicindela solute Dej., Broscus cephalotes L.* and Siberian *Curtonotus fodinae Munh* (having the west border of its area on the territory of Bashkiria). The findings of the Mediterranean *Bembidion laticolle Duft.* and Pontic *B. menetriesi Kol.* are of interest. For the latter Meleuz region of Bashkiria is the north-eastern point of disperse.

The steppe zone of Bashkiria is being subjected to a great anthropogenic influence. Intensive agriculture has effected the largest part of the steppe area of the Southern Urals. That has changed the carabid fauna, these being manifested in the increase of the specific weight of euryzonal forms, such as *Poecilus cupreus L.*

The steppe regions of the Southern Urals and the Urals foothills in zoogeographical relation are the part of Sarmat (Kasakhstan-Siberian) province of the steppe zone. In relation to it the main indicators of carabids here are Kasakhstan-Siberian *Carabus cribellatus Ad.*, Kasakhstan-Mongolian *Harpalus brevis Motsch.*, *H. ellipticus Ball.* European-Kasakhstan *Amara timida Motsch.*, *H. serripes Quens.*, European-Caucasus *Ophonus cordatus Duft.*, *O. punctatulus Duft.*, Eurasian *H. froelichi Sturm.*, *H. fuscipalpis Sturm.*, *H. zabroides Dej.* are represented. Typical for this zone are westpalaearctic *Calosoma auropunctatum Hbst.* and European-Kasakhstan *C. denticolle Gebl.* Easteuropean-Siberian *Carabus sibiricus F.-W.* and European-Siberian *Poecilus sericeus F.-W.* are often met here. *P. crenuliger Chd. and Pterostichus macer Marh.* are considerably rarely met here.

So the attempt of zoogeographic analysis of carabids zonal groups of Bashkiria allows to observe a number of properties. So among the carabid beetles of Southern Urals taiga the species with broad holarctic and transpolaearctic areas predominate. The subzone of broad-leaved forests is richer in the quantity of species, in variety of areas and westpalaearctic species occupy the main place in its fauna. We could not find any area group predominant among species of forest-steppe zone. In steppe regions we mark a tendency of the predomination of European-Kasakhstan, Kasakhstan-Mongolian and Eurasian species. In conclusion we'd like to say that in spite of the considerable quantity of the list of carabid fauna of Bashkiria (233 species from 49 genera) the study of the regional carabid fauna is far from completion. More than 40–50 carabid species are likely to be found here and mainly on the account of the investigation of the Southern regions of Bashkiria.

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Investigations on the Acquisition Period of Plum Pox Virus by *Myzus persicae* Sulzer

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The author has established by reason of the former data and the results of his subsequent transmission experiments that the virginoparous apterae of *Myzus persicae* are able to acquire plum pox virus (PPV) during the first brief probe (28 seconds) from the infected leaves and transmit that to other plants even in that case when it is not its host plant. The obtained data indicate that there is no possibility of preventing the natural spread of PPV by vector-resistant varieties of apricot, peach and plum.

The acquisition periods — or rather the lengths of time during which the aphids were kept on the infected plants — in the different experiments carried out with plum pox virus (PPV) were changed between 2 and 5 (Kassanis and Šutić, 1965; Leclant, 1973), 5 and 10 (Kunze and Krczal, 1968, 1971; Krczal and Kunze, 1976), 5 and 30 (Maison, 1975), 30 and 120 minutes (Marénaud and Massonié, 1977; Massonié and Maison, 1976). In several experiments the PPV was transmitted from peach to peach by aphids in spite of the fact that peach is not their host plant. In our former experiments the specimens of *Myzus persicae* Sulzer have acquired PPV from the infected apricot (*Armeniaca vulgaris* Lam.) whereas again the latter is not its regular host plant. According to the results of the former experiments carried out with a non-persistent virus (Sylvester, 1949, 1950), these data refer to the fact that the aphids are able to acquire PPV from the infected leaves in a shorter time than earlier assumed. Therefore we have studied the possibility of the acquisition of PPV by aphids under the time of the first brief probe.

Materials and Methods

The transmission experiments were performed with virginoparous apterae of *Myzus persicae* which were mass-reared on *Raphanus sativus* L., kept in an insectproof cage, under 16 hours light. As a virus source we used an isolate obtained from peach (*Persica vulgaris* Mill.) that had been propagated on peach seedlings (*P. vulgaris* cv. GF 305) as well as on an infected apricot (*Armeniaca vulgaris* Lam.) tree in the orchard of Érd Experimental Station of Research Institute for Fruitgrowing and Ornamentals at Budapest. Both isolates have

developed characteristic symptoms of the necrotic strain of PPV on the *P. vulgaris* cv. GF 305. As indicator plants we used seedlings of *P. vulgaris* cv. Elberta and cv. GF 305, with 6–10 leaves. The aphids were first starved for 2 hours then they were placed on the infected leaves. The leaves infected by PPV were kept in a Petri-dish padded with wet filter-paper. The behaviour of the aphids on the infected leaves was observed by stereo microscope and the duration of first brief probe was time measured. In the average 28 ± 17.3 seconds were needed for the probe period of 200 specimens of virginoparous *M. persicae*. Immediately following the first brief probe the aphids were transferred for 24 hours to healthy indicator plants, 25 specimens to each plant. For each test series an identical number, or at least 20 healthy seedlings were used as check plants. All experiments were carried out in an insectproof greenhouse. The development of symptoms followed for a whole year after inoculation. The seedlings were investigated visually and some of them by DAS-ELISA serological test.

Results and Discussion

The data presented in Table 1 unanimously prove that the specimens of *M. persicae* are able to acquire PPV during the first brief probe (in an average 28 ± 17.3 seconds) from the infected leaves. According to the data of Leclant (1973) the specimens of *Aphis craccivora* Koch and *A. spiraecola* Patch were able to acquire PPV from the leaves of peach although the latter is not their host plant. In our experiments we have established that the acquisition period of PPV by aphids agrees with the acquisition period of the non-persistent viruses published by Sylvester (1949, 1950).

After Krczal and Kunze (1972) the specimens of *Brachycaudus cardui* L., *M. persicae* and *Phorodon humuli* Schrk. were able to transmit PPV from plum

Table 1

Acquisiton period of PPV by *Myzus persicae* Sulzer

Virus source	Indicator plant	Acquisition period	Symptoms	
			infected plants*	control plants*
Peach	cv. Elberta	27.6± 13.2	2/5	0/5
Peach	cv. Elberta	32.5± 22.5	1/5	0/5
Peach	cv. Elberta	28.6± 17.3	2/5	0/5
Peach	cv. Elberta	24.9± 16.7	1/3	0/3
Peach	cv. Elberta	26.2± 20.6	3/6	0/6
Peach	cv. Elberta	28.0± 17.3	10/28	0/20**
Apricot	cv. GF 304	28.0± 17.3	20/43	0/20

* Infected/inoculated plants
** Plants investigated by ELISA test

and myrobalan to apricot, consequently, to such a plant which is not a host plant of these aphid species. These data agree with those of Sylvester (1949, 1950), in other words the period of brief probe is also sufficient to transmit PPV.

In conclusion the results of this study indicate that there is no possibility of preventing the natural spread of PPV by vector-resistant varieties of apricot, peach and plum.

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Preliminary Report Biology of Rose Leafhopper *(Edwardsiana rosae* Linné, *Homoptera: Auchenorrhyncha)* in Hungary

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Ayn.: *Cicada rosae* L.; *Typhlocyba rosae lactifera* Rey.; *T. rosae subcarnea* Rey.; *T. rosae manca* Rib.

Food plant

The leafhopper feeds mainly on roses, but is not infrequently found on other shrubs and trees belonging to the family *Rosaceae*. From them it flies over fruit-trees. Apple and quince are its favourites. In the case of mass reproduction it attacks cherry-, sour-cherry- and plum-trees too. Further feed plants for the pest are: *Ribes nigrum L.*, *Ribes spicatum Robs.*, *Rubus caesius L.*, *Crataegus* spp. Now and then it can be collected from peach and pear as well (Sáringer, 1979). From apricot- and walnut-trees it has not even been collected in the case of mass reproduction. According to Müller (1956) it is a species occurring on *Ulmus* spp., *Quercus* spp. and *Fagus* spp., on the basis of penis examinations it did not prove to be *E. rosae L.*

Lehmann (1973a, b) found it to be dominant among 26 leafhopper species living on apple- and cherry-trees in the GDR.

Symptoms of damage

On the adaxial surfaces of the infested leaves tiny yellowish-white spots appear in increasing numbers. When the plant that shows such symptoms is shaken a multitude of leafhoppers spring down and fly off.

The yellowish-white spots arising in consequence of sucking by the rose leafhopper always appear scattered over the leaves. The change of colour thus occurring resembles the damage of pear lace bug (*Stephanitis pyri* Fabr.). The traces of sucking caused by the latter are, however, confined to certain parts of the leaf-blade. A further difference between the two syndromes is the absence of black drops of excrement on the abaxial surface of leaf damaged by the rose leafhopper.

The damage of rose leafhopper often can be seen on apple leaves too; the pest sometimes attacks them in masses. In such cases it is particularly important to make distinction between the damages of the two pests.

Both the adult and the larva keep sucking on the abaxial surface of the leaf. The saliva dissolves the palisade cells of the leaf with the consequence that in the place of bite the leaf becomes white. When 3 or more individuals keep sucking a leaf they destroy a large part of the palisade tissue and the leaf becomes white-mottled all-over (Vereshchagina, 1962). Frost and Craighead (1924) in the USA, then Lathrop (1927) in Canada observed that the leaf curled following the sucking, and the larvae and/or nymphs left sticky saliva on it. According to Rupajsz (1961) in consequence of rose leafhopper sucking the leaves of roses (*Rosa* spp.) the amount of chlorophyll may even be reduced by 50 per cent in them.

Till the American and Canadian authors could observe the shiny secretion on the fruit too, European researchers gave account of damages and contamination of fruits (Müller, 1956; Vereshchagina, 1962).

Biology

The rose leafhopper has two, seldom three generations a year (Müller, 1956). According to Balás (1966) two generations of the pest develop every year. During our investigations carried out between 1968 and 1975 at Keszthely (South-Western Transdanubia) it had three generations a year, the first of which lived on rose or wild rose, the second and third on apple and quince (Sáringer, 1979). Plum-, almond-, pear-, cherry-, sour-cherry- and sometimes peach-trees are only visited by them with the purpose of feeding; they do not lay eggs on them.

Overwintering takes place in the form of egg exclusively in the epidermis of rose- and wild rose shoots. At the end of summer or in autumn (September-October) the adults fly from the fruit-trees to roses where they feed till late in the autumn and lay eggs. In the mild winter of 1975 we even collected adults in January in a state of quiescence (Balatongyörök). Before laying eggs the female makes a crescent-shaped cut with its spiculum in the epidermis of the rose shoot and places 2–8 eggs one above the other in it. At the site of egg laying the shoot shows a slight protrusion and becomes discoloured. Eggs are found first of all in the shoots, mostly near the apices, and only seldom in two- or three-year parts of plant.

Hatching starts at the end of April and lasts a month. When spring comes early, as e.g. in 1961, the larvae begin to emerge in the first week of April already. Having emerged from the egg the larva casts its film-like skin at once. The skins cast off are shiny white and usually hang out of the cut. The small larvae with their sluggish movement are hardly perceptible. The larvae cast their skins every 5–10 days depending on the temperature. Their development takes 25–35 days. The first generation adults developed by the end of May – beginning of June suck the young rose leaves for 5–6 days then fly over to apple- or quince-trees. Transmigration takes about 10–15 days. They cannot cover great distances; according to Vereshchagina (1962) they only can get as far as 200–300 metres flying step by step.

The second generation lays the eggs into the leaf veins of apple and quince. The larvae begin to emerge in the second half of June. This generation takes a month to develop. The third generation similarly develops on apple- and quince-trees. At the end of August the adults begin to fly back onto roses where they may feed for some more time then lay their overwintering eggs. In 1974 we found overwintering eggs as early as on 16 September (Balatongyörök) (Balás and Sáringer, 1984).

The reasons for the change of feed-plants by the second generation have not been cleared until now. That much has become known for us that the younger the apple orchard and the more intensive the growth of the shoots and leaves, the later the autumn transmigration of the adults to roses will start. That is why adults sometimes occur on apple or quince as late as at the beginning of November.

Apart from apple and quince on the food plants listed only adults can be observed in the course of summer, and very seldom more developed nymphs which must have got there through migration.

Vereshchagina (1962) is the only author who has reported on the occurrence of overwintering eggs outside the rose, namely in 1959 she found some in the shoots of a Champagnei renet apple-tree.

The distribution of leafhoppers within the crown of a tree is not uniform. According to our observations they mostly live on leaves in the inside of the lower level of crown. In the case of a large individual number they occur on outside leaver of the crown too. According to Vereshchagina (1962) the uneven distribution can be traced back to the different morphological and biochemical properties of leaves within the crown. In her opinion the abaxial surfaces of younger leaves in the upper and lateral parts of the crown are more tomentose than those of older leaves in the inside of the crown. Besides, older leaves contain less water and more dry matter, further, the concentration and osmotic pressure of their cell-sap are higher. Beyond all this the differences in light conditions are also likely to play some role in the dispersity of the individual number.

Economic importance

According to our observations made at Keszthely in a mixed orchard the leaves of Téli aranyparmen- and Kanadai ranett trees were always damaged more severaly in years of gradation than the Jonathan leaves. Plants in sunny hot places (e.g. rambler roses beside walls) suffer most from the attacks of leafhoppers. Several-year observations testify that mass reproduction occurs in dry summers following mild winters.

Natural enemies

The most important parasites of the rose leafhopper – *Anagrus epos* Gir., *A. bartheli* Tullgr. and *A. armatus* Ashm. var. *nigriventris* Gir. (Mymaridae) – infest the eggs according to Müller (1956) and Vereshshagina (1962). The latter

author also made biological observations of *Anagrus* spp. and found that they equally infested summer- and winter eggs. The parasites swarm at the same time when the leafhopper larvae emerge or somewhat later, and unlike the larvae they do not come out through the cut of the bark but with their huge mandibles chew tiny round holes in the bark of the shoot. On the basis of the number of holes the percentage of egg parasitization can even be established. According to the results of investigations carried out by Vereshchagina between 1959 and 1961 in Moldavia eggs were parasitized in summer to 17.4 per cent in a regularly controlled apple orchard and to 74.7 per cent in a quince stand left without plant protection.

On the bodies of the adults *Aphelopus melaleucus* Dalm. *(Drynidae)* larvae may occur as ectoparasites (Müller, 1956). Vereshchagina (1962) observed *Trombidiidae* spp. individuals on leafhopper larvae at various development stages. On a single larva 1–3 mites may even occur. Neither the mites nor the members of the family *Dryinidae* limit essentially the individual number.

Müller (1956) lists further natural enemies: *Chrysopa californica* Co., *Hemerobius pacificus* Banks, *Thrips* spp., *Scatophaga merdaria* F. (larvae); predatory bugs: *Plagiognathus politus* Uhl., *Hyaloides vitripennis* Say, *Diaphnidia pellucida* Uhl., *Nabis alternatus* Parsh.

Directives of control

Since wild rose and rose are the reservoir plants of winter eggs it is reasonable to keep them off the orchard. The distance should be a least 300 m.

With the shoots of rose cut off and burnt early in spring many overwintering eggs can be destroyed.

Young larvae on roses in spring before budding, and on apple- and quince-trees in summer can be efficiently controlled with insecticides containing phosphoric acid ester as active agent. In the course of control operations carried out in summer the soil under the plants and the wall or fence – if there is one – beside them had better be sprayed too in order to destroy the leafhoppers possibly jumping there.

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Synthesis of Novel 2,2-Dimethylchromene Derivatives and their Toxic Activity on Larvae of *Pieris brassicae (Lep., Pieridae)* and *Leptinotarsa decemlineata (Col., Chrysomelidae)*

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Precocene 1, 2 and 3 as well as 36 derivatives of 2,2-dimethylchromene were tested for direct toxicity on newly moulted 3rd and 4th instar larvae of *Pieris brassicae* and 4th instar larvae of *Leptinotarsa decemlineata*. The chemicals were applied to the insects topically or by treated food assay.

In topical tests the highest activity was observed in both species when 6,7-dialkoxy analogues, especially those having a 7-(2-propynyloxy) group, had been used. In most cases a further increase of toxicity was found if two chlorine atoms were introduced to the C3 and C4 position. In *P. brassicae* larvae the higher toxicity of some 6,7-dialkoxy derivatives was in accordance with the greater size of the alkoxy group at C7. In the same test series, among disubstituted 7-alkoxy analogues the positive influence of 5-methyl or 8-methoxy substituents was also detected. Stricter structural requirements of chromene toxicity were demonstrated in *L. decemlineata* than in the other test insect.

In the treated food assay less informative data were obtained than in the topical ones, especially in *L. decemlineata*. However, two compounds bearing cyclopentyloxy substituents at C7 exhibited fairly good toxic action on *P. brassicae* larvae in the treated food assay. A few compounds also showed some antifeedant and growth retardant activity.

Ageratochromenes, isolated from *Ageratinae (Eupatorinae)* species (Alertsen, 1961; Kasturi and Manithomas, 1967; Bohlmann et al., 1978) have been found to possess allatocidal activity in some Exopterigota species and have been termed "precocenes" (P) due to their capacity to induce precocious metamorphosis (Bowers, 1976; Bowers et al., 1976; Bowers, 1977; Bowers and Martinez-Pardo, 1977).

In sensitive Exopterigota species precocenes as "suicide substrates" (pro-allatocidins) are biotransformed into highly reactive 3,4-epoxy derivatives by microsomal cytochrome P-450-dependent monooxygenase, which bind to cellular macromolecules causing rapid, irreversible degeneration in glandular parenchyma cells of the *corpora allata* (CA) (Pratt et al., 1980; Pratt, 1983). Regarding other allatotoxins, a conversion into reactive quinone-methids has been suggested (Bowers et al., 1982). Also some QSAR studies and quantum chemical considerations do not support the theory on epoxide formation (Dinya et al., 1985).

Acute toxicity of precocenes is a known effect in Exopterigota species (Pener et al., 1981; Farag and Varjas, 1981; Fridman-Cohen et al., 1984; Brooks et al., 1985a; Darvas B. et al., 1985; Bélai et al., 1987) and in "insensitive" Endopterygota species (Cupp et al., 1977; Kelly and Fuchs, 1978; Rembold et al., 1979; De Loof et al., 1979; Unnithan, 1983; Fluri, 1983, Mathai and Nair, 1983; Wisdom et al., 1983). There is no information on the mechanism of acute toxicity of the precocenes, although the allatocidal effect may be sublethal manifestations of a more general toxic effect which is seen at higher doses (Brooks et al., 1985a). However, in Endopterygota species, the effective dosage for precocious metamorphosis may be very close to a toxic concentration (Kiguchi, 1982; Bowers, 1985).

Materials and Methods

Synthesis. The original procedure (Fig. 1) for the synthesis of the new alkoxy-chromenes (**C**) was based on reduction of the appropriate alkoxy-4-chromanone (**A**) to the corresponding alcohol (**B**), the dehydration of which was expected to yield the alkoxy-chromenes (**A**) (Ohta and Bowers, 1977).

For the preparation of the novel alkoxy substituted 3,4-dichloro-chromenes (**D**) we used the method reported earlier (Camps, 1979) to obtain the **7** and **8** and other analogues (see in Fig. 2).

Insects. Caterpillars of *Pieris brassicae* L. (large white cabbage butterfly) were reared on cabbage (savoy) plants at 25 °C (16 h photoperiod). Larvae of *Leptinotarsa decemlineata* Say. (Colorado potato beetle) were reared on potato plants at 25 °C (16 h photoperiod).

Bioassays

Topical application. Duplicated groups of 20 newly moulted 4th instar larva of *P. brassicae* or 3rd instar larva of *L. decemlineata* were treated with test

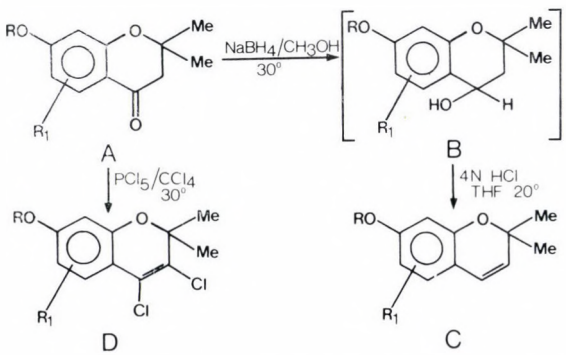
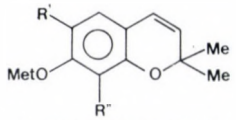
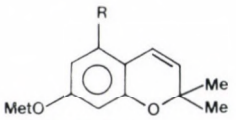


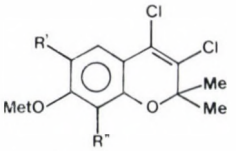
Fig. 1. General scheme for preparation of chromenes (**C**) and 3,4-dichlorochromenes (**D**)



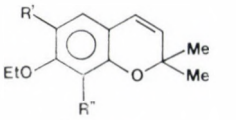
(1; PRECOCENE 1; R'=R"=H; Bowers, 1976)
(2; PRECOCENE 2; R'= MetO–, R"=H; Bowers, 1976)
(3; R'=H, R"= MetO–; Bowers, 1977)
(5; R'=H, R"= Me–)



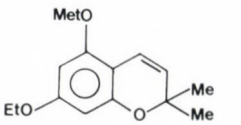
(4; R = MetO–; Bowers, 1977)
(6, R = Me–; Ottridge, 1981)



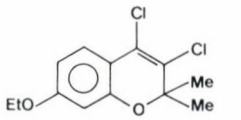
(7; R'=R"=H)
(8; R'=MetO–, R"=H)
(9; R'=H, R"=MetO–)
(10; R'=H, R"=Me–)



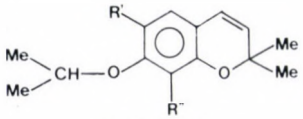
(11; R'=R"=H; Bowers, 1977)
(12; PRECOCENE 3; R'= MetO–, R"=H; Bowers, 1977)
(14; R'=H, R"=Me–)



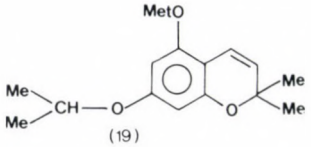
(13)



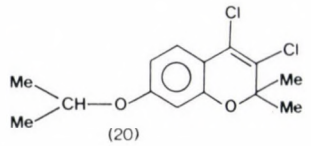
(15)



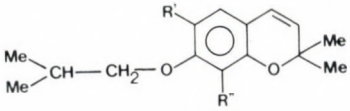
(16; R'=R"=H)
(17; R'=MetO–, R"=H; Bowers, 1977)
(18; R'=H, R"=MetO–;)



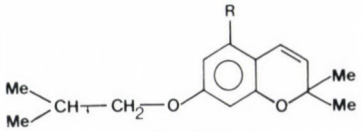
(19)



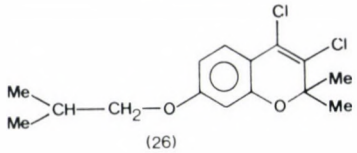
(20)



(21; R'=R"=H)
(22; R'=MetO–, R"=H)
(23, R'=H, R"=MetO–)



(24; R=MetO–)
(25; R=Me–)



(26)

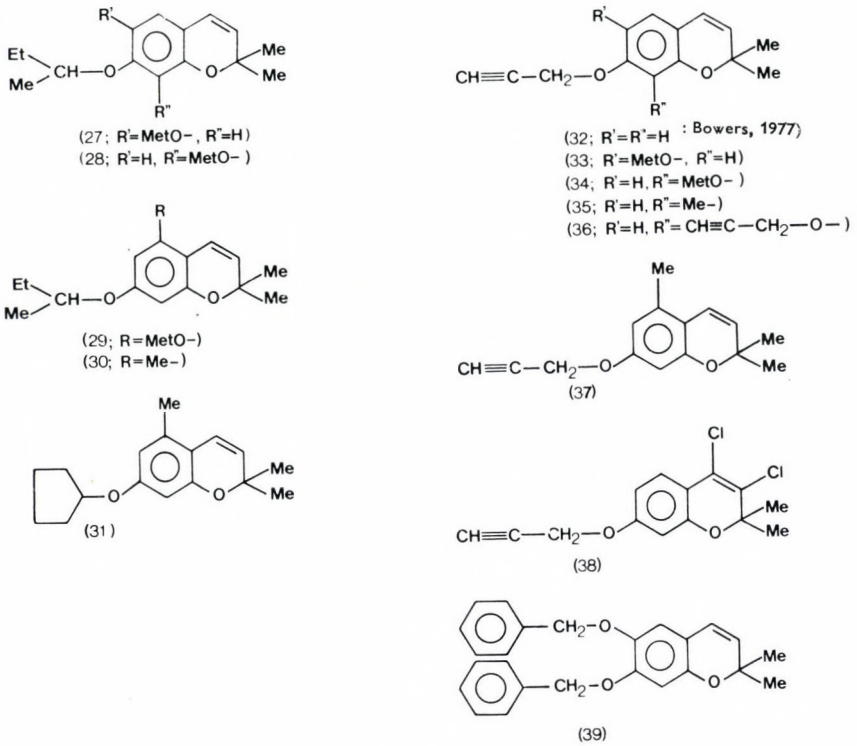


Fig. 2. Compounds

chemicals (1–200 μg in 2μl acetone) by topical application to the dorsal thoracic surface.

Treated food assay. The foliage of the food plants was sprayed with a "Rofra" sprayer until "droplet run off" was obtained with serial dilutions (500–2000 ppm) of aqueous emulsions or suspensions of the test chemical. Duplicated groups of 20 newly moulted 3rd instar larva of both insects were placed onto the treated leaves in plastic boxes. Four days later the numbers of dead larvae were counted. LD_{90} and LC_{90} values were calculated by linear regression of log dose or concentration against probit mortality. Data were expressed in nmol or mM per insect, respectively.

QSAR studies. Attempts were made to find quantitative structure-activity relationships (QSAR) between the chemical descriptors of the 2,2-dimethylchromene derivatives and their toxic activity (LD_{90}). The following 11 chemical descriptors were considered: Hammett's electronic constant (Hansch and Leo, 1979), Hansch-Fujita's constant (pi) characterizing hydrophobicity (Hansch and Leo, 1979); hydrophobicity parameter (log P) calculated using the PrologP 1.2 expert system (Darvas F. et al., 1987); Swain and Lupton's electronic param-

eters (F an R) (Hansch and Leo, 1979); STERIMOL steric parameters (B1, B4, L) (Verloop et al., 1976), molar refractivity (MR) (Hansch and Leo, 1979); IA and ID indicator variables 1 for electron donor and electron acceptor capacity of the substituent, respectively. Stepwise regression analysis gave no significant equation (F-test) between any of the parameters or their combination and LD_{90} because the activity contribution of the individual substituents of the test compounds was strongly influenced by the neighboring substituents.

Results and Discussion

Toxicity 2,2-dimethylchromenes to caterpillar P. brassicae

Topical application. On *P. brassicae* larvae (Table 1), it was observed that among monosubstituted 2,2-dimethylchromenes (structural type **C**) the size of the 7-alkoxy substituent (**1, 11, 16, 21**) did not play a significant role in the determination of direct toxicity. The only exception was the 7-(2-propynyloxy) group (**32**) that conferred a highly toxic property to the molecule thereby masking any possible activity-modifying effect of other structural elements (**33—38**).

A second substituent on the aromatic ring practically always altered biological effectiveness. In the present study where the significance of methyl or methoxy substituents were examined, the C6 and C5 positions were found to be the most critical sites. The presence of an additional methoxy group at C6 always increased the toxicity of the molecule (except **32** and **33**). The activity of the disubstituted compounds, expressed by LD_{90} values, was about 1.5 to 4.5-fold (**2** versus **1**, **12** versus **11**, **17** versus **16**) or even 16-fold (**22** versus **21**) in comparison with the corresponding 7-monosubstituted compounds. Also these data suggest that alongside a more spacious alkoxy group at C7 the positive influence of a 6-methoxy substituent was still more striking.

The introduction of a methyl substituent at C5 significantly enhanced the toxicity of 2,2-dimethylchromene bearing also a 7-alkoxy group. By comparing the activities of these compounds, 1.5-fold (**25** versus **21**) or 9-fold (**6** versus **1**) differences in LD_{90} values could be demonstrated. In the same position, however, a methoxy group exerted a reverse effect by strongly diminishing the toxicity of the molecule (**4, 13, 19, 24, 29**).

A moderate increase of toxicity was found when a methoxy substituent was introduced to C8 (**3** versus **1**, **18** versus **16**, **23** versus **21**, **34** versus **32**), but our data were not sufficient to estimate the role of a methyl group in the same position (**5, 14, 35**).

The monosubstituted 7-alkoxy-3,4-dichloro analogues (structural type **D**) were always slightly more effective than their non-chlorinated counterparts (**7** versus **1**, **15** versus **11**, **20** versus **16**, **26** versus **21**), a property that could be detected even among the highly toxic 7-(2-propynyloxy) analogues (**38** versus **32**). In combination with other substituents, chlorine-containing 2,2-dimethylchro-

Table 1

Toxicity of 2,2-dimethylchromene analogues on *Pieris brassicae* and *Leptinotarsa decemlineata* larvae

Compound	*Pieris brassicae*		*Leptinotarsa decemlineata*	
	Topic. appl.[a] LD_{90} (nmol/larva)	Treated food[b] LC_{90} (mM)	Topic. appl. LD_{90} (nmol/larva)	Treated food LC_{90} (mM)
1 (P1)	389	I	525	I
2 (P2)	234	I	148	I
3	331	I	257	I
4	I	I	I	I
5	380	I	468	I
6	44	I	490	I
7	141	I	36	I
8	692	I	36	I
9	166	I	331	I
10	74	I	200	I
11	398	I	550	I
12 (P3)	269	3.9	83	I
13	427	6.8	98	I
14	282	8.5	204	I
15	132	I	151	I
16	437	I	794	I
17	98	2.5	178	I
18	275	6.5	224	I
19	807	6.5	589	I
20	229	I	257	I
21	398	I	282	I
22	25	5.8	191	I
23	135	6.2	457	I
24	I	6.3	562	I
25	263	5.3	295	I
26	246	6.6	316	I
27	324	4.6	151	I
28	195	7.6	263	I
29	I	5.8	617	I
30	32	4.8	407	I
31	170	1.6	479	I
32	49	3.3	195	2.0
33	43	4.5	51	I
34	54	NT	170	NT
35	37	3.1	398	4.7
36	62	3.4	62	4.4
37	22	6.0	98	I
38	21	4.4	15	3.3
39	I	2.3	538	I

a Tested on newly moulted 4th instar larva;
b Tested on newly moulted 3rd instar larva;
c Tested on newly moulted 3rd instar larva;
I = inactive: mortality below 50% at the highest dose (200 μg/larva) or concentration (2000 ppm); NT = not tested

menes were examined only in the group of 7-methoxy derivatives (**7** to **10**). In comparison with the "parent" molecule (**7**) a considerable reduction in activity was observed for the 6-methoxy (**8**) but not the 8-methoxy (**9**) substitution. On the contrary, a surprisingly high increase of toxicity to *P. brassicae* larvae could be demonstrated when a 8-methyl group (**10**) was the second substituent on the aromatic ring.

We also obtained some information on the potency of two compounds having cyclopentyloxy and benzyloxy substituents. In topical tests the 7-cyclopentyloxy-5-methyl analogue (**31**) showed only moderate activity, whilst the 6,7-dibenzyloxychromene (**39**) proved to be inactive.

Treated food assay. The most active compounds had as high as 1.5 to 2.5 mM or even higher LC_{90} values. This fact hindered good discrimination between toxicity of individual chemicals.

Under these circumstances all monosubstituted 7-alkoxy analogues (**1, 11, 16, 21**) except the 7-(2-propynyloxy) derivative (**32**), proved to be inactive. The same was found for the disubstituted 7-methoxy analogues (**2** to **6**). The corresponding 3,4-dichloro compounds having the same structural elements as the chemicals mentioned above (**7, 15, 20, 26** and **8, 9, 10**) were also inactive.

Disubstituted 2,2-dimethylchromenes exhibited only moderate activity. In some instances, however, the positive influence of a 6-methoxy (**12, 17**) or 5-methyl group (**25**) could be well demonstrated.

Seven-cyclopentyloxy-5-methyl-2,2-dimethylchromene (**31**) and 6,7-dibenzyloxy-2,2-dimethylchromene (**39**) showed a satisfactory activity.

In the highest concentration used, some compounds (**2, 13, 14, 17, 39**) considerably impeded the feeding of surviving insects and retarded larval growth. The majority of these larvae moulted only after a considerable time.

Toxicity of 2,2-dimethylchromenes to larvae of L. decemlineata

Topical application. On *L. decemlineata* larvae, monosubstituted 7-alkoxy-2,2-dimethylchromenes showed only moderate toxic action (Table 1). However, the introduction of some functional groups, did increase the toxicity of the molecule, although the number of analogues found to be effective against beetle larvae were smaller, than against the *Pieris* caterpillars. A particular combination of substituent groups seemed to be more essential in determining the toxicity to *L. decemlineata*. Only a few general trends regarding the role of some substituents could be established.

Generally the toxicity was consistently increased when 6-methoxy group was introduced as a second substituent (**2** versus **1, 12** versus **11, 17** versus **16, 22** versus **21, 33** versus **32**). On comparison with the activity of the corresponding analogue, the difference in LD_{90} values was 7-fold in the case of P3 (**12**) which was one of the most efficient compound used in this test series. Introducing two chlorine atoms to the C3 and C4 position increased toxicity (**7** versus **1, 15** versus **11, 20** versus **16, 38** versus **32**) except in the case of a 7-(2-methylpropoxy) de-

rivative (**26** versus **21**). This effect was particularly conspicuous in the chlorine-containing analogue of P1 (**7** versus **1**) demonstrated by a 15-fold difference in LD_{90} values.

The 7-(2-propynyloxy) group by itself positively influenced the activity of these chromenes and at the C7 position it was more efficient than any other alkoxy group. The presence of 7-(2-propynyloxy) group seemed to potentiate the influence of some other structural changes, for example the chlorine introduction to the C3 and C4 position (**38**), and the incidence of 6-methoxy (**33**) and 5-methyl (**36**) substitutions. The 5-methyl group remained relatively "silent" in other combination tested in *L. decemlineata* larvae (see compounds **6, 25**).

Treated food assay. Several 7-(2-propynyloxy) analogues (**32, 35, 36, 38**) caused moderate toxicity.

Comparative aspects of chromene toxicity in insects

Concerning the direct insecticidal action of certain chromenes some questions of both theoretical and practical significance arise: 1) What is the physiological and biochemical background of this lethal action?; 2) Is the mode of action the same as in their specific effect on CA?; 3) How does penetration, peripheral detoxification and other essential pharmacodynamic factors influence chromene toxicity?; 4) What kind of biotransformation, if any, occurs preceding the toxic effect of these chemicals?

At present we are not aware of any data on the sensitivity of individual larval tissues to precocenes the specific injury of which leads to the death of the insect. There is a general effect as well as special effect on the CA in larval tissues.

As to the mode of action in CA and other organs, some results obtained in precocene-sensitive species point to a kind of parallelism between the allatocidal and toxic effect when some simple chromenes had been used. The changes of precocene susceptibility in bugs and to some extent also in locusts regarding the morphogenetic and toxic action show the same trend in consecutive larval stages or within an instar (Pener et al., 1981; Fridman-Cohen et al., 1984; Farag and Varjas, 1981). The presence of a small alkoxy group at C6 in 7-alkoxy-2,2-dimethylchromenes markedly increased morphogenetic anti juvenile hormone activity (Bowers, 1977). The same C6 substitution in our compounds could also result in higher effectiveness against larvae of *P. brassicae* and *L. decemlineata.* The size and character of the C7 substituent, in general, strongly influenced, especially in monosubstituted analogues, the prothetelic action of the compounds but the toxic activity did not show similar variations (Brooks et al., 1985a). P3 was found to be morphogenetically more active in bugs but otherwise less toxic than P2 (Bellés and Baldellou, 1983). In our topical tests P3 exhibited the same or even higher toxicity to Endopterygota larvae than its more simple analogues P1 or P2.

As examinations with radiolabelled P2 revealed, the penetration of this compound through the cuticle was relatively rapid (Haunerland and Bowers,

1985). The same property of other lipophilic derivatives, like most of our compounds can also be assumed when they are used topically. In foliar application, however, when the chemicals may reach the hemolymph via another route, the role of some other transport barriers and strong attack by detoxifying enzymes (mixed function oxidases) located in the midgut should also be taken into consideration. A relatively rapid excretion of precocenes without any metabolic transformation was also observed (Haunerland and Bowers, 1985). One must be careful in comparing data obtained by different application modes and the factors mentioned above may have contributed to the smaller numbers of compounds showing activity in the treated food assay, especially in *L. decemlineata* larvae. The reason for the relatively good efficacy of the compound bearing a cyclopentyloxy substituent at C7 subsequent to spraying the food-plant of *P. brassicae*, is unknown as yet.

Metabolic inactivation and the excretion of polar metabolites are considered as the major transformation pathways determining the fate of precocenes in the insect body (Bergot et al., 1980). By not taking the sensitivity of the CA into account, the ratio of ED_{50} (morphogenetic 50% response dose) and D_{50} (toxic 50% response dose) values measured in locusts and also the age-dependent changes of precocene sensitivity in the same species were suggested to be in closely connected the degree of peripheral detoxification (Fridman-Cohen et al., 1984). Thus, the significant influence of metabolic inactivation to the rate of lethal action should not be neglected when investigating any type of chromene compounds. In Endopterygota the same enzyme reactions were found to convert precocene moieties to easily excretable molecules as in susceptible species (Bergot et al., 1980). It seems, however, that some changes in the chromene structure (introduction certain second substituents, and chlorine to the C3 and C4 position) may interfere even with some metabolic reactions. Some recent findings in the field of precocene pharmacodynamics (sequestration in fat body, carrier proteins in the hemolymph) make this picture still more complex (Bowers, 1985).

Most authors suggest that 3,4-epoxidation of precocenes by allatal monooxygenases must be the essential conversion step preceding the cytotoxic action of these compounds (Pratt et al., 1980; Pratt, 1983). It is also assumed that the presence or absence of epoxy hydrases in CA or in other tissues explain the selective allatotoxic activity of these chemicals (Pratt, 1983). As possible alternative reactions, quinone-methide transformation (Bowers et al., 1982) (originally described for isopentenyl-phenol) or O-demethylation (Bergot et al., 1980) (reported as the first step of metabolic deactivation) were published. Epoxide formation may not be the only one reaction of all precocene type chromenes in terms of anti-allatin effect (allato-toxic or MF-epoxidase inhibitor). Indeed the effects on other susceptible organs and tissues should also be taken into account. QSAR studies with 31 precocene analogues concerning their allatocidal activity in *Locusta migratoria* CA demonstrated that the biological effect was in good correlation with some steric properties on the aromatic rings (Dinya et al., 1985). The activity was influenced not only by the C7 and C6 but also by the C5 and

C8 substitutions. In this respect it should be remembered that in our topical application tests, especially in *P. brassicae* larvae, similar steric features determined direct toxicity. In the case of 6,7-dialkoxy-2,2-dimethylchromenes, the longer the chain length of the 7-alkoxy group the higher the toxicity to *P. brassicae* larvae. However, in *L. decemlineata* larvae, using the same series of compounds, only P3 was more active than P2. The toxicity of the compounds also increased, especially against *P. brassicae* larvae, when a 5-methyl or 8-methoxy group was introduced. Quantum chemical results outlined in the paper mentioned previously (Dinya et al., 1985) showed that the charge density of the 3,4 double bond of the precocenes was calculated to be practically constant and the position of the minimum of the electrostatic potential contour maps of the compounds govern the direction of the oxidative dealkylation reactions rather than epoxidation of the 3,4 double bond.

Two main structural changes of our 2,2-dimethylchromene derivatives deserve more detailed considerations: 7-(2-propynyloxy) substitution and introduction of chlorine atoms to the C3 and C4 position. Both of these substituents had noticeable and in most cases marked effect on toxicity. All chemicals having a 2-propynyloxy substituent at C7 proved highly toxic when applied topically to *P. brassicae* larvae, but only some combinations of other group (6-methoxy, 5-methyl, 3,4-dichloro, 2,8-dipropynyloxy) proved satisfactory effective against *L. decemlineata* larvae. It was reported that precocenes with a 7-(2-propynyloxy) group exhibited no or any moderate morphogenetic activity in *Oncopeltus fasciatus* larvae, but they were consistently toxic (Brooks et al., 1985a). The 2-propynyloxy functional group of several compounds is a well known inhibitor of cytochrome P-450 exerting its action by alkylating the heme-containing prosthetic group (Wilkinson, 1976; Ortiz de Montellano and Kunze, 1980; Ortiz de Montellano and Correia, 1983; Wilkinson and Murray, 1984). A number of 2-propynyl ethers were found to inhibit juvenile hormone III biosynthesis in excised CA of *Periplaneta americana* (Pratt and Finney, 1977; Brooks et al., 1984a; Brooks et al., 1984b; Brooks et al., 1985b; Feyereisen et al., 1985). It was recently demonstrated that 7-(2-propynyloxy)-3,4-dichloro-2,2-dimethylchromene, used also in the present investigations (**38** = FI-121) possessed marked inhibitory action on JH biosynthesis in CA when tested in the same *in vitro* system (Darvas B. et al., 1986).

Some chromene compounds that we tested for toxicity (structural type **D**), have 3,4-dichloro substitutions. At present the exact mode of biological activity of these analogues seems to be difficult to explain. Since chlorine atoms could have influenced first of all the steric and electric properties of the molecule at the 3,4-double bond, their activity-modifying effect is not surprising. It is interesting to note that the chlorine-containing analogues of P1 and P2, compounds **7** and **8,** respectively, proved to be more toxic only in *L. decemlineata* larvae, but on *P. brassicae* larvae this structural change did not caused as strongly decreased activity as it observed in the case of the other experimental species. The reason of this species-specific difference is still unknown. In an *in vitro* culture of *L. decem-*

lineata CA P2 did not show any activity (Khan et al., 1982). According to some biotests on *O. fasciatus* and *L. migratoria*, methyl substituents at C3 or C4 completely abolished morphogenetic anti-JH activity of the chromene molecule, but this structural change did not alter the direct toxicity of the same compounds (Brooks et al., 1985a).

Multiple stepwise regression analysis involving the above descriptors and biological activity data (Table 1) as well as the non-parametric Free–Wilson analysis failed to give a significant result. The lack of correlation between the chemical parameters and biological activity is probably due to the following factors: the compounds act indirectly after different metabolic activation steps at multiple sites; the compounds act by different activity mechanisms; the activity contribution of the individual substituents are not additive (they influence each other) thus the multiple linear regression model does not apply. The compounds in this study were not designed for QSAR analysis and the descriptor values of the set do not span the whole parameter space leaving open the possibility of finding activity maxima at some other regions in the future.

Our data are not sufficient to explain the antifeedant and growth retardant effect of some 2,2-dimethylchromenes tested on *P. brassicae* by spraying their food-plants. P2 was demonstrated to possess similar biological effects in *Spodoptera maurita* and *Heliothis zeae* larvae (Mathai and Nair, 1983; Wisdom et al., 1983).

In this area studies on *Mamestra brassicae*, *Hyphantria cunea*, *Myzus persicae*, *Acyrthosiphon pisum*, *Aleyrodes brassicae*, and *Trialeurodes vaporariorum* have already been reported (Darvas B. et al., 1987).

Experimental

All melting points (mp) are uncorrected. Infrared (IR) spectra were determined on a Perkin-Elmer 283 B spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker WP-200 SY spectrometer. Chemical shifts are reported as values in ppm relative to tetramethylsilane as an internal standard. Low resolution electron inpact mass spectra (MS) were obtained on a VG 7035 spectrometer. Thin layer chromatography (TLC) was made using precoated silica gel plates (60 F_{254}) supplied by E. Merck Column chromatography (CC) was done with Reanal Kieselgel 60. The result of elemental analysis was in agreement with calculated values (within 0.3% error).

Synthesis of compounds **1–6, 11–14, 16, 21, 25, 30–32** and **35–37** were described elsewhere (Timár et al., 1985; Timár et al., 1988a; Timár et al., 1988b; Timár et al., 1988c; Timár and Jászberényi, 1988).

General procedure for the synthesis of compounds **17–19, 22–24, 27–29, 33–34, 36** and **39**:

Alkoxy-4-chromanones (**A**) were dissolved in methanol (100 ml) and stirred at 30 °C for 2 hours. During this time $NaBH_4$ (5 g, 130 mmol) was added in

Table 2

Parameters of the *C* type compounds

Comp.	Yield (%)	Molecular formula	NMR ($CDCl_3$) δ	MS, m/z (rel. int. %)
17	82	$C_{15}H_{20}O_3$ (248.31)	1.36 (6H, d, J = 7Hz), 1.44 (6H, s), 3.78 (3H, s), 4.50 (1H, m,), 5.47 (1H, d, J = 10Hz), 6.25 (1H, d, J = 10Hz), 6.42 (1H, s), 6.55 (1H, s)	248 (M^+, 20), 233 (16) 191 (100) 176 (10)
18	80	$C_{15}H_{20}O_3$ (248.31)	1.35 (6H, d, J = 7Hz), 1.45 (6H, s) 3.85 (3H, s), 4.50 (1H, m), 5.50 (1H, d, J = 10Hz), 6.25 (1H, d, J = 10Hz), 6.40 (1H, d, J = 8Hz), 6.62 (1H, d, J = 8Hz)	248 (M^+, 22), 233 (21) 191 (100) 176 (17)
19	87	$C_{15}H_{20}O_3$ (248.31)	1.30 (6H, d, J = 7Hz), 1.40 (6H, s), 3.75 (3H, s), 4.47 (1H, m), 5.40 (1H, d, J = 10Hz), 5.96 (1H, d, J = 2Hz), 6.04 (1H, d, J = 2Hz), 6.57 (1H, d J = 10Hz)	248 (M^+, 20), 233 (21) 205 (10) 191 (100)
22	78	$C_{16}H_{22}O_3$ (262.34)	1.02 (6H, d, J = 7Hz), 1.42 (6H, s), 2.15 (1H, m), 3.74 (2H, d, J = 7Hz), 3.81 (3H, s), 5.46 (1H, d, J = 10Hz), 6.25 (1H, d, J = 10Hz), 6.41 (1H, s) 6.55 (1H, s)	262 (M^+, 18), 247 (40) 191 (199) 176 (8)
23	81	$C_{16}H_{22}O_3$ (262.34)	1.05 (6H, d, J = 7Hz), 1.47 (6H, s), 2.16 (1H, m), 3.75 (2H, d, J = 7Hz), 3.85 (3H, s), 5.50 (1H, d, J = 10Hz), 6.26 (1H, d, J = 10Hz), 6.40 (1H, d, J = 8Hz), 6.65 (1H, d, J = 8Hz)	262 (M^+, 30), 247 (75) 191 (100) 176 (25)
24	79	$C_{16}H_{22}O_3$ (262.34)	1.00 (6H, d, J = 7Hz), 140 (6H, s), 2.10 (1H, m), 3.68 (2H, d, J = 7Hz), 3.80 (3H, s), 5.44 (1H, d, J = 10Hz), 6.02 (2H, m, 6.57 (1H, d, J = 10Hz)	262 (M^+, 22), 247 (90) 191 (100) 176 (18)
37	85	$C_{16}H_{22}O_3$ (262.34)	0.97 (3H, t, J = 7Hz), 1.32 (3H, d, J = 7Hz), 1.42 (6H, s), 1.72 (2H, m), 3.80 (3H, s), 4.25 (1H, m), 5.48 (1H, d, J = 10Hz), 6.22 (1H, d, J = 10Hz), 6.41 (1H, s), 6.55 (1H, s)	262 (M^+, 15), 247 (10) 191 (100) 176 (10)
28	70	$C_{16}H_{22}O_3$ (262.34)	1.00 (3H, t, J = 7Hz), 1.33 (3H, d, J = 7Hz), 1.44 (6H, s), 1.73 (2H, m), 3.79 (3H, s), 5.50 (1H, d, J = 10Hz), 6.25 (1H, d, J = 10Hz), 6.25 (1H, d, J = 10Hz), 6.40 (1H, d, J = 8Hz), 6.62 (1H, d, J = 8Hz)	262 (M^+, 43), 247 (24) 191 (100) 176 (35)
29	80	$C_{16}H_{22}O_3$ (262.34)	0.96 (3H, t, J = 7Hz), 1.30 (3H, d, J = 7Hz), 1.44 (6H, s), 1.73 (2H, m), 3.78 (3H, s), 4.22 (1H, m), 5.44 (1H, d, J = 10Hz), 6.00 (2H, m), 6.56 (1H, d, J = 10Hz)	262 (M^+, 25), 247 (50) 191 (100) 176 (18)

Table 2 (continued)

Comp.	Yield (%)	Molecular formula	NMR ($CDCl_3$) δ	MS, m/z (rel. int. %)
31	88	$C_{15}H_{16}O_3$ (244.38)	1.42 (6H, s), 2.52 (1H, t, J = 2Hz), 3.80 (3H, s), 4.70 (2H, d, J = 2Hz), 5.50 (1H, d, J = 10Hz), 6.25 (1H, d, J = 10Hz), 6.55 (2H, s)	244 (M^+, 41), 229 (100) 189 (19) 161 (20)
32	77	$C_{15}H_{16}O_3$ (244.38)	1.50 (6H, s), 2.52 (1H, t, J = 2Hz), 3.90 (3H, s), 4.75 (2H, d, J = 2Hz), 5.55 (1H, d, J = 10Hz), 6.27 (1H, d, J = 10Hz), 6.57 (1H, d, J = 8Hz), 6.70 (1H, d, J = 8Hz)	244 (M^+, 32), 229 (100) 190 (41) 144 (15)
37	86	$C_{15}H_{16}O_3$ (268.30)	1.45 (6H, s), 2.42 (1H, t, J = 2Hz), 2.50 (1H,d, J = 2Hz), 4.72 (2H, d, J = 2Hz), 4.75 (2H, d, J = 2Hz), 5.25 (1H, d, J = 10Hz), 6.27 (1H, d, J = 10Hz), 6.55 (1H, d, J = 8Hz), 6.68 (1H, d, J = 8Hz)	268 (M^+, 31), 253 (100) 220 (15) 205 (46)
39	88	$C_{17}H_{24}O_3$ (372.44)	1.41 (6H, s), 5.05 (2H, s), 5.10 (2H,s), 5.45 (1H, d, J = 10Hz), 6.20 (1H, d, J = 10Hz), 6.49 (1H, s), 6.63 (1H, s), 7.25–7.55 (10H, m)	372 (M^+, 61), 281 (10) 144 (32) 91 (100)

portions to the reaction mixture. The solvent was removed and water (100 ml) was added to the residue. Thix mixture was extracted with CH_2Cl_2 (3 × 50 ml), dried with Na_2SO_4 and the solvent then evaporated. The residue was dissolved in THF (75 ml) and treated with 4M HCl (100 ml) at 20 °C and stirred for 2 hours. The reaction mixture was subsequently extracted with ether (3 × 50 ml), and the combined ether layers were washed with 2% NaOH solution (2 × 50 ml), water (3 × 50 ml) and brine (2 × 50 ml) and dried with Na_2SO_4. The solvent was evaporated under vacuum. The yields obtained and other data are given in Tables 2 and 4. Analytical samples were obtained by column chromatography using hexane : ether (9 : 1) as eluent.

General procedure for the synthesis of compounds **7–10, 15, 20, 26** and **38**:

Alkoxy-4-chromanone (**A**) (40 mmol) was dissolved in dry CCl_4 (200 ml) and stirred with PCl_5 (17 g, 80 mmol) at 30 °C for 10 hours. When the reaction was completed (followed by TLC), water (200 ml) was added and the mixture stirred for 1 hour. The organic layer was separated and washed with 1% NaOH solution (2 × 100 ml, water 2 × 100 ml) and brine (2 × 50 ml) and dried with $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography using hexane : ether (4 : 1) as eluent. The yields obtained and other data are summarized in Tables 3 and 4.

Table 3

Parameters of the **D** type compounds

Comp.	Yield (%)	mp (°C)	IR (KBr) γ (cm^{-1})	NMR ($CDCl_3$) δ	MS, m/z (rel. int. %)
7	75	oil lit. bp. 2	2920, 1030, 1610, 1500, 1150	1.55 (6H, s), 3.76 (3H, s), 6.40 (1H, d, J = 2Hz), 6.50 (1H, dd, J = 2Hz, J = 8Hz), 7.31 (1H, d, J = 8Hz)	258 (M^+, 30), 243 (100) 223 (38) 188 (10)
8	72	68–69 lit. mp. 2 69.5–70.5	2960, 1201, 1605, 1000, 1505, 1285	1.50 (6H, s), 3.70 (3H, s), 3.74 (3H, s), 6.33 (1H, s), 6.83 (1H, s)	288 (M^+, 28), 273 (100) 253 (54)
9	76	52–53	2920, 1108, 1605, 1010, 1497, 1290	1.60 (6H, s), 3.82 (6H, s), 6.50 (1H, d, J = 8Hz), 7.10 (1H, d, J = 8Hz)	288 (M^+, 67), 273 (100) 253 (75) 218 (20)
10	82	53–55	2940, 990, 1609, 1485, 1120	1.55 (6H, s), 2.07 (3H, s), 3.82 (3H, s), 6.48 (1H, d, J = 8Hz), 7.22 (1H, d, J = 8Hz)	272 (M^+, 28), 257 (100) 237 (25) 202 (4)
15	78	37–38	2980, 1150, 2920, 1015, 1610, 1495, 1380	1.41 (3H, t, J = 7Hz), 1.56 (6H, s), 4.00 (2H, d, J = 7Hz) 6.40 (1H, d, J = 2Hz), 6.50 (1H, dd, J = 2Hz, J = 8Hz), 7.32 (1H, d, J = 8Hz)	272 (M^+, 29), 257 (100) 237 (30) 229 (58) 209 (20)
20	80	54–55	2990, 1010, 1610, 1490, 1387, 1270	1.35 (6H, d, J = 7Hz), 1.57 (6H, s), 4.52 (1H, m), 6.40 (1H, d, J = 2Hz), 6.50 (1H, dd, J = 2Hz, 7.31 (1H, d, J = 8Hz)	286 (M^+, 18), 271 (23) 251 (5) 229 (100) 209 (28)
26	83	41–43	2980, 1170, 1610, 1030, 1495, 1285	1.00 (6H, d, J = 7Hz), 1.56 (6H, s), 2.09 (1H, m), 3.69 (2H, d, J = 7Hz), 6.39 (1H, d, J = 2Hz), 6.50 (1H, dd, J = 2Hz, J = 8Hz), 7.32 (1H, d, J = 8Hz)	300 (M^+, 20), 285 (55) 229 (100) 209 (38)
38	77	47–49	3385, 1615, 1490, 1170, 1030	1.57 (6H, s), 2.55 (1H, t, J = 2Hz), 4.67 (1H, d, J = 2Hz), 6.47 (1H, d, J = 2Hz), 6.60 (1H, dd, J = 2Hz, J = 8Hz), 7.35 (1H, d, J = 8Hz)	282 (M^+, 30), 267 (100) 247 (26) 228 (18) 212 (8)

Table 4

Elemental analytical results

Comp.	Molecular formula	Anal./Calcd.			Anal./Found		
		C (%)	H (%)	Cl (%)	C (%)	H (%)	Cl (%)
7	$C_{12}H_{12}Cl_2O_2$	55.61	4.66	27.30	55.42	4.70	27.51
8	$C_{13}H_{14}Cl_2O_3$	53.99	4.87	24.52	53.90	4.60	24.65
9	$C_{13}H_{14}Cl_2O_3$	53.99	4.87	24.52	53.90	4.60	24.41
10	$C_{13}H_{14}Cl_2O_2$	57.15	5.16	25.96	57.31	5.42	26.03
15	$C_{13}H_{14}Cl_2O_2$	57.15	5.16	25.96	57.01	5.45	26.04
17	$C_{15}H_{20}O_3$	72.55	8.12	—	72.30	8.00	—
18	$C_{15}H_{20}O_3$	72.55	8.12	—	72.60	8.41	—
19	$C_{15}H_{20}O_3$	72.55	8.12	—	72.71	8.13	—
20	$C_{14}H_{16}Cl_2O_2$	58.54	5.61	24.69	58.50	5.83	24.51
22	$C_{16}H_{22}O_3$	73.25	8.45	—	73.00	8.40	—
23	$C_{16}H_{22}O_3$	73.25	8.45	—	73.11	8.31	—
24	$C_{16}H_{22}O_3$	73.25	8.45	—	73.42	8.13	—
26	$C_{15}H_{18}Cl_2O_2$	59.81	6.02	23.54	59.60	6.24	23.29
27	$C_{16}H_{22}O_3$	73.25	8.45	—	73.20	8.40	—
28	$C_{16}H_{22}O_3$	73.25	8.45	—	73.51	8.18	—
29	$C_{16}H_{22}O_3$	73.25	8.45	—	73.03	8.52	—
33	$C_{15}H_{16}O_3$	73.72	6.60	—	73.91	6.71	—
34	$C_{16}H_{22}O_3$	73.72	6.60	—	73.86	6.53	—
36	$C_{17}H_{16}O_3$	76.10	6.01	—	76.23	5.86	—
38	$C_{14}H_{12}Cl_2O_2$	59.38	4.27	25.04	59.18	4.20	24.91
39	$C_{25}H_{24}O_3$	80.62	6.49	—	80.71	6.29	—

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Effects of Juvenoids on Prediapause and Postdiapause Females of *Epidiaspis leperii* Sign. (Hom., Diaspididae)

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Treating prediapause females of *Epidiaspis leperii* with the juvenoids kinoprene, methoprene, hydroprene, and fenoxycarb did not affect the hormonal regulation of adult diapause. This may be due to a high activity of JH-esterase in the prediapause phase.

Only when prediapause and postdiapause females of *E. leperii* were treated with juvenoids at a high concentration (0.5%) was vitellogenesis reduced.

Egg hatch was inhibited when the juvenoids methoprene and kinoprene, at a concentration of 0.1%, were applied to postdiapause females of *E. leperii*.

In vivo activated compounds that modify reproduction, development or growth of insects without affecting cholinesterase are likely become more widely used for controlling insects (Hedin, 1983). The synthetic analogues of juvenile hormones (JH) (Williams, 1967), known as juvenoids, mimic the biological effects of JHs. The most frequently used juvenoids are kinoprene, methoprene and hydroprene, which were discovered by Henrick et al. (1973). A relatively new juvenoid, fenoxycarb, has been described by Dorn et al. (1981).

It was Bagley and Bauernfeind (1972) who first drew attention to the possibility of controlling scale insects with JH analogues. Boboye and Charman (1975), treated first instar larvae of *Aonidiella aurantii* (Dispididae) with juvenoids and found that hydroprene was more active than methoprene. Methoprene was more effective on males of this species than kinoprene (Moreno et al., 1976). Peleg and Gothilf (1981) used methoprene at a concentration of 0.1% on 2nd instar larvae of *A. aurantii* and *Chrysomphalus aonidum*. They demonstrated that this juvenoid is highly effective against males, but not females. Second instar male larvae are more sensitive than female larvae of *Quadraspidiotus perniciosus* to kinoprene, methoprene and hydroprene, and hydroprene is the most active (Kozár and Varjas, 1976). Molnár and Sántha (1983) used kinoprene, methoprene and hydroprene to control *Q. perniciosus* in the field. They showed, that the following developmental stages were most vulnerable to sprays; 1) scale differentiation, 2) populations made up of 15 to 25% pronymps, and 3) 70 to 80% of pronymphs. Darvas et al. (1985) studied the persistence of foliar residues of hydroprene using 1st instar larvae of *Q. perniciosus*. Twenty-four hours after

spraying with an 0.1% emulsion of hydroprene, only half the initial activity could be demonstrated. This suggest a relatively rapid breakdown of this juvenoid.

Jászai-Virág and Darvas (1983) applied kinoprene and hydroprene to *Aspidiotus nerii* at a concentration of 0.05% with great success providing it is applied before overlaping scale masses form. Hydroprene has also been shown to control 1st instar larvae of *Carulaspis juniperi* and *Unaspis euonymi* (Vinis, 1983).

Darvas and Zsellér (1984; 1985) tested kinoprene and hydroprene against 1st instar larvae of *Pseudaulacaspis pentagona*. Higher rates of mortality were observed after the use of kinoprene but in each case the number of eggs deposited by surviving insects was reduced.

Dorn et al. (1981) showed that a concentration as low as 0.03% of fenoxycarb applied to first instar larvae of *A. aurantii*, prevented further development. However, females of *A. aurantii* proved to be significantly less sensitive than males (Peleg, 1982).

First instar larvae of *P. pentagona* treated with a 0.1% solution of fenoxycarb mainly died during the first moult and those that survived produced few eggs (Darvas and Zsellér, 1984; 1985).

Most authors find the 1st instar larvae of Diaspididae to be the most sensitive larval stage, and male more sensitive than female 2nd instar larvae to treatment with juvenoids (Darvas and Varjas, 1990).

The aim of this work was to evaluate the effects of kinoprene, methoprene, hydroprene, and fenoxycarb on prediapause and postdiapause females.

Materials and Methods

Chemicals

The effects of the following juvenoids, Enstar 5 E (65.3% kinoprene = = (2E,4E)-2-propynil-3,7,11-trimethyl-2,4-dodecadienoate; produced by Zoecon Co., USA), Viodat 50 EC (50% methoprene = (2E,4E)-1-methylethyl-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate; produced by EGIS, Hungary), EGYT 2669 20 EC (20% hydroprene = (2E,4E)-ethyl-3,7,11-trimethyl-2,4-dodecadienoate; produced by EGIS, Hungary) and Insegar 50 WP (50% fenoxycarb = = ethyl-2-(4-phenoxyphenoxy-ethyl)-carbamate; produced by Dr. R. Maag Ltd., Switzerland), were studied.

Field experiment

In a pear orchard (Nagykovácsi, Hungary), juvenoid preparations were sprayed using a hand pump pressure sprayer at concentrations of 0.5, 0.1, 0.01% on 23rd August, 1984, when freshly moulted *Epidiaspis leperii* females were abundant. In each treatment, 0.025% Nonit, was used as a wetting agent.

The number of live females were counted on 5×50 cm^2 pieces of bark/treatment collected on 1st October, and 15th December 1984, and 12th February,

and 14th March, 1985, and percentage of mortality was calculated using the Henderson–Tilton formula. Data were analyzed using a one way ANOVA and regression analysis.

Twentythree to thirtyeight females/treatment were collected on 19th June, 1985, to evaluate egg-production. Data were analysed using a one way ANOVA.

Laboratory experiment

Five 20 cm length of twigs, heavily infested with overwintered *E. leperii* females, were collected on 29th March, 1985 and sprayed with juvenoids as in the field. The treated twigs were kept at either 15 °C or 25 °C under either short-night or long-night conditions. The numbers of eggs laid by each females were counted. Data were analyzed using a one way ANOVA.

The number of larvae and eggs on five 10 cm length of twigs kept each of 15 °C and 25 °C were counted in the 4th and 8th week. After determining the % egg hatch, the data was analyzed using a one way ANOVA.

Results

Field experiment on prediapause females

The results are summarized in Table 1 and Fig. 1.

Table 1

Chemosterilizing activity of some juvenoids on prediapause females of *E. leperii* under field conditions

Juvenoids	Conc. a.i. %	No. of observed females	% of reproductive females	Mean No. of eggs/female ± SE
EGYT 2669 20 EC	0.5	26	84.6	15.4± 6.4b
(hydroprene)	0.1	24	91.7	18.1± 7.4a
	0.01	30	100.0	19.6± 6.6a
VIODAT 50 EC	0.5	25	92.0	15.4± 6.4b
(methoprene)	0.1	23	100.0	18.9± 7.4a
	0.01	29	100.0	21.3± 7.7a
ENSTAR 5 E (kinoprene)	0.5	31	93.5	14.2± 7.5b
	0.1	28	96.4	17.5± 5.6a
	0.01	25	96.0	19.8± 6.0a
INSEGAR 50 WP (fenoxycarb)	0.5	29	89.7	16.8± 6.3ab
	0.1	25	92.0	19.6± 5.9a
	0.01	35	94.3	21.2± 7.5a
Untreated control		38	100.0	21.1± 8.0a
SD 1%				4.8

Values labelled with the same latter in a column are not significantly different at the 1% level, respectively (one way ANOVA).

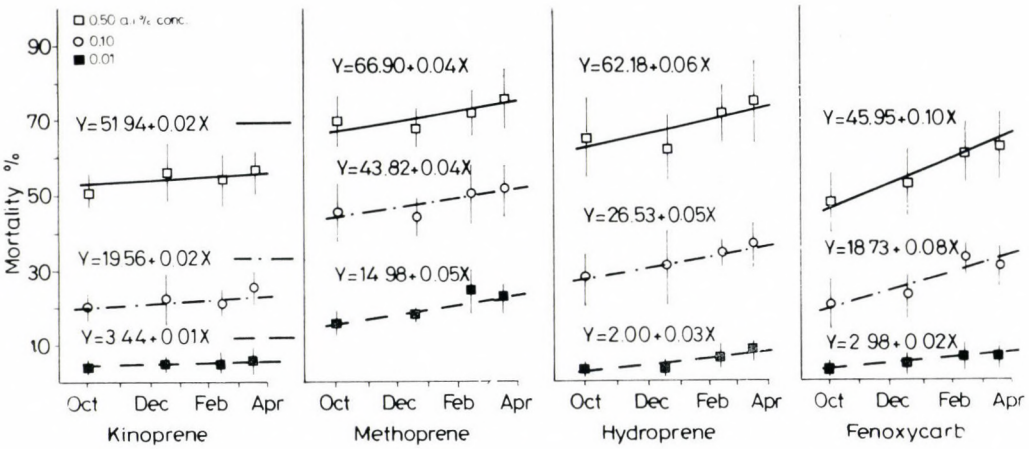

Fig. 1. Percentage mortality of prediapause females of *E. leperii* under field conditions after treatment with different concentrations of juvenoids, kinoprene, methoprene, hydroprene and fenoxycarb over the period October to April. SD 0.1%: 1st Oct. = 15.49%, 15th Dec. = 15.83%, 12th Feb. = 14.76%, 14th March = 16.47%

Table 2

Chemosterilizing activity of some juvenoids on postdiapause females of *E. leperii* under long or short night conditions at 15 °C

Juvenoid	Conc. a.i. %	12, h. scotophase			8, h. scotophase		
		No. of observed females	% of reproductive females	Mean No. of eggs/female ± SE	No. of observed females	% of reproductive females	Mean No. of eggs/female ± SE
EGYT 2669	0.5	40	92.5	16.9± 7.3a	59	100.0	17.5± 5.9a
20 EC	0.1	38	100.0	16.9± 6.1a	53	100.0	18.6± 9.2a
(hydroprene)	0.01	41	100.0	18.3± 7.7a	47	100.0	19.1± 5.9a
VIODAT	0.5	50	70.0	9.4± 4.0c	40	77.5	10.9± 4.4cd
50 EC	0.1	40	95.0	14.6± 5.5b	38	100.0	16.0± 3.6b
(methoprene)	0.01	48	100.0	17.4± 7.4a	40	100.0	18.5± 7.9a
ENSTAR	0.5	50	82.0	9.9± 8.0c	47	89.4	10.9± 5.1cd
5 E	0.1	36	91.7	11.2± 5.8bc	43	100.0	14.5± 5.6b
(kinoprene)	0.01	35	100.0	13.8± 7.3b	44	100.0	15.3± 4.0b
INSEGAR	0.5	40	97.5	13.0± 4.8b	52	92.3	14.5± 6.3b
50 WP	0.1	39	100.0	14.3± 4.5b	44	100.0	16.2± 4.9a
(fenoxycarb)	0.01	36	100.0	16.6± 8.1a	47	100.0	18.6± 3.3a
Untreated control		42	100.0	18.3± 3.7a	43	100.0	19.2± 3.8a
SD 1%				3.8			3.2

Values labelled with the latter in a column are not significantly different at the 1% level, respectively (one way ANAVA).

Table 3

Chemosterilizing activity and effect on egg hatch of some juvenoids applied to postdiapause females of *E. leperii* under long or short night conditions at 25 °C

Juvenoid	Conc. a.i. %	12 h scotophsae				8 h scotophase			
		No of observed females	% of reproductive females	Mean No. of eggs/female ± SE	Hatchability % + SE	No. of observed females	% of reproductive females	Mean No. of eggs/female ± SE	Hatchability % ± SE
EGYT 2669	0.5	65	100	19.1± 4.6ab	0.0± 0.0b	50	100.0	19.0± 5.7b	0.0± 0.0d
20 EC	0.1	66	100	19.7± 6.5a	4.7± 2.2b	50	100.0	20.3± 4.3a	11.3± 3.7c
(hydroprene)	0.01	59	100	20.7± 5.3a	20.4± 7.8a	69	100.0	21.8± 5.4a	21.3± 4.9b
VIODAT	0.5	55	100	14.4± 3.4cd	0.0± 0.0b	59	89.8	16.3± 3.8c	0.0± 0.0d
50 EC	0.1	61	100	19.1± 6.6ab	0.0± 0.0b	67	100.0	18.9± 5.0b	0.0± 0.0d
(methroprene)	0.01	47	100	20.9± 4.6a	4.4± 1.9b	58	100.0	20.4± 5.5a	6.3± 3.1c
ENSTAR	0.5	58	100	13.6± 3.9cd	0.0± 0.0b	76	97.4	11.4± 5.1d	0.0± 0.0d
5 E	0.1	66	100	18.6± 6.4b	0.0± 0.0b	57	100.0	18.6± 7.1b	0.0± 0.0d
(kinoprene)	0.01	49	100	20.1± 6.8a	6.5± 3.8b	71	100.0	19.3± 4.9b	12.0± 4.8c
INSEGAR	0.5	58	100	16.4± 4.4c	0.0± 0.0b	59	100.0	17.9± 5.9b	0.0± 0.0d
50 WP	0.1	57	100	20.0± 7.4a	0.0± 0.0b	55	100.0	20.3± 8.5a	5.7± 3.3c
(fenoxycarb)	0.01	60	100	20.4± 6.7a	7.6± 2.9b	63	100.0	20.7± 5.8a	16.8± 6.b
Untreated control		58	100	21.9± 4.2a	25.0± 8.4a	70	100.0	22.3± 5.5a	29.9± 8.0a
SD 1%				2.7				2.7	
0.1%					7.8				8.4

Values labelled with the same letter in a column are not significantly different at the 1% or 0.1% level, respectively (one way ANOVA)

The juvenoids kinoprene, methoprene, hydroprene, and fenoxycarb, at a concentration of 0.01%, did not affect survival or egg production. Methoprene, at a concentration of 0.1%, caused only a slight decrease in survival, but did not affect egg-production. Methoprene and hydroprene, at the high concentration (0.5%) caused a relatively high mortality and low egg-production.

Laboratory experiment on postdiapause females

The results are summarized in the Tables 2 and 3.

Oviposition rate was twice as fast at 25 °C (4 weeks), as at 15 °C (8 weeks).

At 15 °C, hydroprene had no affect on egg-production in *E. leperii*, but fenoxycarb had a slight affect. Kinoprene and methoprene, at concentrations of 0.1 and 0.5%, significantly decreased egg-production and gave good practical control (50% reduction) at a conc. of 0.5%. Rearing females under short- or long-night conditions did not significantly affect their egg-production.

At 25 °C, only kinoprene and methoprene, at a concentration of 0.5%, significantly reduced egg-production.

The greatest effect was on the hatchability of the eggs. After treatment of overwintered females of *E. leperii* with juvenoids at a concentration of 0.5% none of their eggs hatched. The results were the same for kinoprene, methoprene and fenoxycarb used at a concentration of 0.1%.

Discussion

JH-control of diapause in diaspidids

The two most important processes in insects, which are partly regulated by JH are diapause and vitellogenesis. Adult diapause is partly a developmental diapause, but mainly involves changes in behaviour, reproduction, and metabolism. All of these changes are induced by an endocrine deficiency, are synchronized and give the insect greater resistance to environmental stress (de Wilde, 1983). The most important changes in JH activity during adult diapause in *Leptinotarsa decemlineata* (Col., Chrysomelidae) may be summarized as follows: 1) *Corpora allata* of prediapause adults has a limited JH synthetic activity, which after day 6 is further reduced (de Kort, 1981); 2) There is a very high JH-esterase activity in prediapause adults. During diapause JH-esterase, synthetized in the fat body, decreases the level of JH to nearly zero (de Kort, 1981).

The level JH activity during larval and pupal diapause is different from that during adult diapause. The JH-level in diapausing larvae remains moderate to high (Chippendale, 1983). The third form of diapause is egg (embryonic) diapause, which is induced by a neurohormone, the diapause hormone (Yamashita, 1983).

In diaspidids every type of diapause is known, for example *Lepidosaphes ulmi* shows egg diapause, *Q. perniciosus*, *Q. ostraeformis*, *Q. pyri* larval diapause,

and *Q. marani* and *E. leperii* adult diapause. However, some like *A. nerii* do not diapause.

The hormonal regulation of diapause in scale insects has not been studied and there is only one study on the environmental control of diapause. Long-night conditions induce larval diapause in *Parthenolecanium corni* and temperature plays a role in the postdiapause phase (Saakyan-Baranova et al., 1971). There is an apparent contradiction between the results for aphids and coccids as long-night conditions induce alate forms in aphids, which have a low level of JH in their hemolymph (Hardie, 1984; Hardie et al., 1985), but diapausie scale insects (Saakyan-Baranova et al., 1971) have high level of JH in their hemolymph. However, treatment of the 1st instar larvae of *Q. perniciosus* with precocene 2 (pro-allatocidin) did not increase the proportion of diapause larvae (Darvas et al., 1985). Thus the increase in level of JH necessary for larval diapause in diaspidids may differ from that for other insects. It is likely that JH is not the only chemical involved the control of the survival strategies of Homoptera.

The survival of overwintering females of *Ceroplastes pseudoceriferus* (Hom., Coccidae), treated with methoprene, is not significantly different from the controls (Kamei and Asano, 1976). The hormonal control in the two species, *C. pseudoceriferus* and *E. leperii*, which belong to different families of scale insects, may be similar as they react similarly to juvenoids. It is possible that juvenoids do not interfere with the hormonal regulation of adult diapause in scale insects, because of the high level of activity of JH-esterase in the the prediapause phase.

JH-control of vitellogenesis in diaspidids

In most species, vitellogenin, the predominant yolk protein precursor, is produced by the fat body and then transported via the hemolymph to the growing oocytes (Wyatt and Pan, 1978). The JH-control of the synthesis of vitellogenin is thought to be the key to vitellogenesis (Engelmann, 1983). Engelmann (1971) found that following a single topical application of JH the maximum rate of vitellogenin synthesis occurred three days later.

Treatment of first instar larvae of the scale insect *Planococcus citri* (Hom., Pseudococcidae), which have telotropic meroistic ovarioles, with juvenoids induced the development of small adults which start to secrete an ovisac, but do not lay any eggs (Darvas, 1986). Histological observations on the ovaries of these insects revealed few, degererate trophocytes. Thus, vitellogenesis was completely inhibited. Treatment of 2nd instar larvae of *Q. perniciosus* (Kozár and Varjas, 1976) and *A. aurantii* (Peleg and Gothilf, 1981), and 3rd instar larvae of *Saissetia coffeae* (Hom., Coccidae) (Jászai-Virág and Darvas, 1983) with juvenoids all resulted in decreased egg-production.

Bielenin (1974) found that the medial neurosecretory cells exhibit maximal activity in young *Q. ostreaeformis* females during the development of oocytes, while the *corpora cardiaca-corpora allata* complex reaches its greatest size in the 2nd instar larvae and in young females prior to vitellogenesis.

Kamei and Asano (1976) found that after a topical application of methoprene, overwintered females of *C. pseudoceriferus* started to lay egg earlier than the controls. They suggested that high JH activity is necessary for oviposition.

In the case of freshly moulted females and overwintered females of *E. leperii*, only juvenoids at a high concentration (0.5%) affect vitellogenesis. Similar results have been obtained with freshly moulted females of the soft scales *P. corni* (Darvas etal., 1988) and *Physokermes inopinatus* (Darvas, Kulcsár and Tóth-Vilmos, unpubl. data). Increasing the JH-level in the hemolymph of the first 3 stages of scale insects, appears to disturb the normal development of the ovarioles, possibly by affecting germ cell cluster formation, and also slightly reduces the production of vitellogenin in the fat body and nurse cells of prediapause and postdiapause females. This contradicts Raabe (1984) who claims that only the ecdysteroids are responsible for germ cell cluster formation (meiosis, ovariole differentiation, and the first stage of oogenesis).

Kamei and Asano (1976) found that on increase in temperature affects the postdiapause phase. Similarly in *E. leperii* the rate of egg laying at 25 °C was double that at 15 °C.

JH-control of egg-hatch in diaspidids

Analogues of the juvenile hormone (dichlorofarnesoate) inhibit egg hatch in the nematode, *Haemonchus concortus* without obviously affecting the development of the embryos (Rogers, 1973). It is possible that JH interacts with a component of the membranes of the eggs (Rogers, 1980). It was suggested by Wigglesworth as early as 1969, that JH controls morphogenesis in insects by affecting membrane permeability. After treating prediapause females of *Eurygaster integriceps* (Hem., Scutelleridae) (Burov and Shekhtman, 1976) and *E. maura* (Németh and Varjas, 1976) with juvenoids, nearly all the first laid eggs failed to hatch and the embryos died within the eggs.

When treated with methoprene freshy moulted females of *C. pseudoceriferus* subsequently laid eggs that do not hatch possible because metoprene inhibits egg development (Kamei and Asano, 1976). Our results agree with their findings, but the exact mode of action of the juvenoids is still obscure.

The effects of treating prediapause and postdiapause females of *E. leperii* with juvenoids can be summarized as follows: 1) When applied during prediapause, juvenoids do not interfere with the hormonal control of adult diapause, possibly because of the high activity of JH-esterase. 2) When applied to prediapause and postdiapause individuals high doses of juvenoids markedly interfere with vitellogenesis. 3) Juvenoids applied to postdiapause females inhibit egg hatch.

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Book Review

Hale, M. G. and Orcutt, D. M.: *The Physiology of Plants Under Stress.* John Wiley and Son,s New York–Chichester–Brisbane–Toronto–Singapore. 1987. 206 pp. Paperbound

In this textbook — mainly for plant science students — the authors skilfully present an integrated view on plant stress physiology with useful appraisals of recent developments in this field and concise surveys of breeding for stress resistance. Knowledge on the mechanism of stress physiology is also essential to an understanding of resistance and survival mechanisms. The questions at the end of chapters stimulate thought and basic understanding. This book is not intended for specialists, rather, it will meet the needs of students including plant pathologists and plant breeders who want to acquire a sound reading knowledge of plant stress.

The main chapters are dealing with drought, temperature, nutrient, salt, irradiation and allelochemical stresses, as well as with the effects of stress on plant membranes, the role of phytohormones in stressed plants and the biotechnological aspects of increasing stress tolerance. In addition to Discussion Questions, at the ends of chapters general and specific References help the reader in understanding the subject.

In several places the effects of stresses on membranes are emphasized. Apparently, as a consequence of stress, there is some molecular reordering in the membranes and changes in membrane fluidity occur. It is also important that the role of stress proteins and other stress compounds such as proline, betaine etc. is not clarified as yet. These stress compounds may have role in the hardening process, too.

One paragraph deals with the phytohormone response to pathogens and insects. Different disease symptoms are evaluated in relation to increased hormonal activities.

The use of genetic engineering for incorporating stress resistance characteristics into plants is limited to traits controlled by single gene manipulations. As the authors emphasize, "unfortunately" multiple gene interactions are common in plant stress responses which will limiting future hopes. Although it seems ironic that a disease-producing organism *(Agrobacterium tumefaciens)* as a vector of genes is being used to understand stress inducing mechanisms and stress resistance, the primary problem is to characterize and understand the genes involved in different stress phenomena.

This book is highly recommended to students and young researchers in the field of plant biochemistry, physiology, biotechnology, agronomy, breeding and plant pathology.

Z. Király

D. Spaar, H. Kleinhempel and R. Fritzsche: *Diagnose von Krankheiten und Beschädigungen an Kulturpflanzen — Gemüse*.VEB Deutscher Landwirtschaftsverlag, Berlin 1986. With 151 color drawings and 67 black-and-white pictures on 406 pages. Price DM 140

The publication of the book "Gemüse", the first volume of the book series "Diagnose von Krankheiten und Beschädigungen an Kulturpflanzen" by D. Spaar, H. Kleinhempel and R. Fritzsche is welcomed by the readers. Among the German technical books which appear less and less frequently on the international book market it is one of the most original works which when later published in English will certainly become available for a wider reading public. That the book has been an "article in short supply" is proved by the fact that the German publication was quickly sold in the GDR. The chapters of the book were written by the most illustrous members of various research institutes and universities in the GDR: H. Bochow (Berlin) is coordinator and deals with fungi; R. Fritzsche (Aschersleben) is coordinator and responsible for insect pests and for keys; K. Neumann (Aschersleben) deals with diseases of bacterial pathogens; H. E. Schmidt (Aschersleben) with virus diseases; H. Decker (Rostock) with nematodes; W. Wrazidlo (Jena) with mineral deficiencies and toxicities. The special lines of H. Hartleb and K. Skadow are not indicated in the book. The authors' names guarantee the content, and H. Thiele undertook a great task when making the book illustrative with the 151 color drawings and 67 black-and-white pictures. The key occupies 79 (pp. 7–88) of the 406 pages of the book. It deals with identification of diseases, pests and injuries of cabbage (radish, horse radish), pea, bean, tomato, pepper, eggplant, cucumber, melon, squash, spinach, red beet, and Swiss chard, lettuce, endive and cichory, asparagus, black salsify, onion, garlic, leek, carrot, celery, parsles, rhubarb and mushroom.

On pages 90 to 391 (a total of 299 pages) the reader finds the descriptions of diseases and injuries of the above plants (on the left side of the book), and opposed to it their coloured and black-and-white pictures (on the right side) in the following order: injuries, viruses, mycoplasms, bacteria, fungi, insects, abiotic damages. This section of the book is followed by a list of the literary works used and recommended, 45 in number. It might be worth giving a more detailed list of literature, and refer thereby to works as e.g. G. R. Dixon: Vegetable crop diseases. MacMillan (1981), J. T. Fletcher: Diseases of greenhouse plants. Longman, London and New York (1984), M. J. Bassett: Breeding vegetable crops. Publ. Comp. Inc., Westport, Connecticult (1986), etc. The usefulness of the bibliography could have been increased and the orientation of the young professionals made easier if the authors had given the suggested fundamental works for the individual plants discussed. For the viruses besides the German names the authors give their English names too, which have become widely used by now. I think that the transcription of the Russian names of viruses in Latin letters in unnecessary, since for Russian speaking people they do not say anything in this form, while for those speaking other world languages the German and English virus names are sufficient. If the authors insisted on giving names of viruses in Russian languages too, they ought to have done so with Cyrillic letters.

The book is completed with a list of references to the origin of the pictures on one page, and a scientific and German register on 12 pages.

This first volume of the series "Diagnose von Krankheiten und Beschädigungen an Kulturpflanzen — Gemüse" is such a literary work which besides building up a fine reputation for the authors widens the knowledge of those doing professional work either in scientific life, or at universities or even in practice. The book "Gemüse" is warmly recommended to university students as well.

J. Horváth

Gy. Matolcsy, M. Nádasy and V. Andriska: *Pesticide Chemistry*. Akadémiai Kiadó, Budapest, 1988. 808 p.

This volume contains six individually written chapters, subdivided when necessary, each supported with a comprehensive list of references. Three chapters ("Anti-insect agents", "Acaricides", and "Nematocides") were written by Gy. Matolcsy, two ("Rodenticides" and "Fungicides") by M. Nádasy, and the chapter "Herbicides" was written by V. Andriska.

In a brief introduction the authors define their goal as to discuss mainly those active substances in pesticides that are of practical significance, may be of possible future importance, or represent research results and interesting trends, rather than to write an encyclopedic work on pesticides. The authors have ably met their goal and produced a book that brings together in one volume the complex subject of the chemistry of a number of groups of pesticides currently in use or under development, and gives a broad and balanced coverage of diverse aspects. These include details of the organic chemistry (structures, synthetic routes) as well as the broad spectrum of the biochemical/toxicological aspects (from the points of view of environmental safety to modes of action). In addition, the main trends of development in the field of pesticide research are also dealt with. Therefore, it is a special merit of this book that the usual fault of many volumes of this type, e.g. the lack of consistency between chapters, is not apparent.

As can be seen, the coverage is extensive. The detailed chemical pathways for the examples discussed are extremely well presented. In addition, the range of chemical compounds covered in the book seems to be most complete. Although the publishing date is 1988 most literature references are not later than 1984 (this explains why the section on herbicides, the area most familiar to this reviewer, lacks mentioning of a compound like tridiphane). Therefore, in rapidly moving fields such as the biochemical mode of action of pesticides more recent papers should also be consulted.

As the title indicates, the book is heavily weighed toward the chemistry of pesticides, and is an excellent source of structural formulae and generalized synthetic routes. Toxicological data are given for many chemicals, and are not limited, for the most part, to rat LD50's, but results on poultry, fish, and other organisms are frequently given. Metabolic routes of some active ingredients are discussed in detail as are some mechanisms of action, but this is not always applied consistently to different classes of compounds.

This volume is easy to read and it should prove to be a valuable addition to the libraries of pesticide scientists working in the fields of synthesis, biochemistry, and application, and it will also serve as an important general reference for undergraduate and graduate students studying and conducting research into the above areas.

T. Kőmíves

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