Integrated control of insect pests in the Netherlands

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pudoc

Wageningen
Centre for Agricultural Publishing and Documentation
1980

Cover drawing. *Adoxophyes orana* (F.v.R.) drawn by H. Beeke, Research Station for Fruit Growing, Wilhelminadorp (Zeeland).

ISBN 90 220 716 2

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Introduction

Thirty five years ago, the first synthetic insecticides astounded all those engaged in the control of pests with their unprecedented effectiveness. Not very long after their introduction in agriculture, however, scientists began to question the unqualified benefit that many expected from pesticides. In the Netherlands, Voûte (1946), Kuenen (1948) and Briejèr (1949) expressed their doubts about the course plant protection was taking. The need to resume research on non-chemical methods of pest control was increasingly recognized and, in 1956, Professor De Wilde of the Agricultural University's Department of Entomology established a committee to coordinate and stimulate research on such methods. On 20 September 1958, this committee received official status within the auspices of the TNO National Council for Agricultural Research as the Working Party on Integrated Control and obtained additional funds to initiate research on integrated control further to what cooperating research institutes were already doing. De Wilde and De Fluiter of the Research Institute for Plant Protection initiated a wide variety of activities. Until his untimely death in 1970, De Fluiter was also active in the international scene.

During its early years, the Working Party concentrated on basic studies in insect physiology, biochemistry and population dynamics as a background to integrated control. Later the emphasis gradually shifted to practical application. The acquisition by the Working Party of its own experimental fruit farm in 1965 contributed to this shift; the approach advocated could there freely put to the test.

The Working Party has now come of age and this book sums up what has been achieved for readers in other countries, describing some sixty projects on the theory and practice of integrated control. It brings together and updates information from scattered publications and provides key references for those interested in the details.

During the Working Party's existence, considerable progress has been achieved in background knowledge and practical application of integrated control of pests. Supervised control is now generally accepted in the policy of plant protection. We must take care and indeed have reason to fear that those concerned with pest control will look for the easy way out and compromise the methods of supervised control. Biological control is successfully applied in some situations; and a number of selective pesticides have reached the market. There are promising novel techniques awaiting implementation. Pesticides known to be injurious to the environment have been largely withdrawn.

Over the same period of time, however, the use of chemical pesticides has greatly increased. Fungicides and aphicides are used as a routine in cereal crops, the use of herbicides has expanded, and in protected cultivation as well as certain crops in the open, soil disinfectants are lavishly used. Agriculture has become increasingly dependent on the quick--and-easy push on the chemical button. It is easy for all engaged in plant protection to continue with the established means of chemical control. So we cannot take much satisfaction with the progress achieved. But it is perhaps unrealistic to think that a fundamental change in the approach to plant protection could have been achieved within twenty years. What has been done in this period is to show that integrated control is feasible and in some cases is economic. It is our task in the next decades to implement this method of plant protection to the full, no easy matter. The growing awareness of the consumer of products grown with a minimum use of pesticides will perhaps help.

Only a comprehensive plan of action, carefully designed and resolutely executed in an international framework, offers any chance of shifting the habits of decades. This task awaits concerted action by the International Organization of Biological Control and the European Communities. This book tries to summarize what we in the Netherlands have to offer.

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Acknowledgments

We gratefully acknowledge the cooperation with Pudoc in publishing this book. Several projects were supported by grants from the National Council of Agricultural Research TNO.

1 Fruit crops

Since the early days of the Working Party, a large part of the effort in putting the principles of integrated control to practice has concentrated on orchards. Much work has been devoted to analysis of the relationships of phytophagous arthropods in orchards with their parasites and predators (1.6). Fruit-tree red spider mite, *Panonychus ulmi*, and apple pygmy moth, *Stigmella malella*, could be controlled to a satisfactory degree by native beneficial arthropods (1.4, 1.1, 1.7). For other major pests, such as aphids and leaf-rollers, control by naturally occurring or mass-reared and released beneficials proved insufficient (1.5, 1.6). Leaf-rollers still pose a difficult problem, because the main species in Dutch orchards, *Adoxophyes orana*, resists selective control and because other tortricids rise to pest status under a selective programma (1.3). The latter holds for other minor pests as well (1.5, 1.1). Although the sterile-male technique gave satisfactory results against *Adoxophyes orana*, it proved uneconomical (1.8). Fortunately, other selective methods against leaf-rollers show promise (6.3, 8.4, 9.2). Since work began, the number of selective pesticides put on the market by chemical industry has gradually increased, with the result that a satisfactory program integrating biological and chemical control is now running on an experimental scale in commercial orchards (1.1, 1.2). Supervised chemical control has been adopted by about 20% of the fruit growers in the Netherlands (1.2). Work on the antagonistic relations between micro-organisms on apple trees and other approaches aimed at reducing the profuse application of fungicides now common in a moist climate started some years ago (1.9, 1.10).

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1.1 Development of an integrated control program for orchards

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INTRODUCTION

Orchards are subject to several fungal diseases and many arthropod pests. In the 1960s, routine spray programs of about 6-8 insecticide and 14 fungicide sprays were habitual, resulting in improved harvest but in the well-known complications, including resistance of *Panonychus ulmi* to pesticides (Section 1.4).

Initially modified spray programs, designed to permit natural control factors to operate, were tested in plots on private holdings. Since the commercial interests of the owners and the needs of research were difficult to reconcile, an orchard was set aside for the purpose in 1965. The work there aimed at developing an economically acceptable system of control with minimum use of chemical pesticides. It began in 1967.

METHODS

The area of this orchard, called De Schuilenburg, at Lienden near Wageningen is 12 ha. It consists predominantly of spindlebush apple-trees on dwarfing rootstocks. In long-term comparative trials on plots of about 1 ha, the effect of three experimental factors, spray program, nitrogen fertilizer and presence of flowering weeds, was tested by pest incidence, effectiveness of natural enemies of pests, and amount and quality of harvest. Initially the factor interfering most drastically with the beneficial fauna, spray program, was set at four levels: (1) no insecticide; (2) a conventional spray program; (3) a moderate program composed of certain commercially available pesticides chosen because of their presumed relative mildness towards non-target organisms; (4) pesticides considered strictly selective. Later, when supervised chemical control became accepted in practice, Spray Program 3 was stopped and variants of Selective Program 4 were added.

Besides these long-term trials, the orchard is being used for field tests on a small scale of new control techniques, such as selective chemicals including insect growth regulators (Section 8.4), pheromones (Section 6.3), viruses (Section 9.2), and nematodes (Section 9.5), and for studies on relationships between microorganisms inhabiting the surface of the apple plant (Section 1.10).

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Monitoring techniques were devised and preliminary economic thresholds laid down and tested (Van Frankenhuyzen & Gruys, 1971), as a basis for supervised chemical control, the procedure of which was subsequently developed in detail in a series of plots elsewhere (Section 1.2).

In the moderately and the selectively sprayed plots, some species became severe pests in the first years *(Panonychus ulmi, Stigmella malella)*, contrary to the situation in the plot receiving no insecticides. This difference was due to the absence of certain beneficials (typhlodromids; *Chrysocharis prodice*). They did not readily migrate into the moderate and selective plots and, particularly in the former, were suppressed by insufficiently selective pesticides. After they had been released in the selective plots, biological control of these pests became successful within 2-3 years (Fig. 1; Section 1.7).

High nitrogen, contrary to earlier findings did not preclude successful biological control of *P. ulmi*. A ground flora of sowed flowering weeds did not reduce pest numbers, as might be expected on the assumption that it stimulates entomophagous arthropods. On the contrary, it increased the numbers of the noxious bug, *Lygus pabulinus*.

Adoxophyes orana, a major pest in orchards in the Netherlands, declined first in the selectively treated plots but has increased again in recent years. Although mortality of the caterpillars by parasites is heavy in summer, it is not sufficient or not sufficient in every year. Work is now going on with the chalcid, *Colpoclypeus florus*, to increase its parasitic impact on *Adoxophyes*.

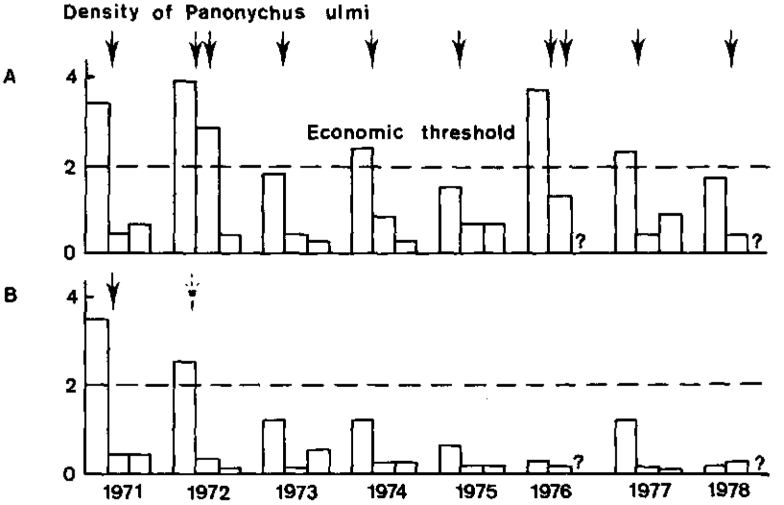

Fig. 1. Densities of *Panonychus ulmi* in winter, June, and August, 1971-1978.
A: a plot under conventional chemical control, in which predatory mites are lacking.
B: a plot under integrated control, in which predatory mites have been introduced and are present since.
Density of *P. ulmi* is in scale 0-6, where 6 corresponds to over 100 per leaf. Arrows indicate applications of specific acaricides; broken arrow indicates application to part of area.

Natural control of several other common harmful insects has been insufficient in the selectively sprayed plots as well as in the plot receiving no insecticides. These included *Operophtera brumata*, aphids and *Lygus pabulinus*.

In the selectively treated plots, certain other insects gradually developed into pests, because there were no broad-spectrum sprays restraining them or because the orchard became a more favourable habitat to them as it grew older, or both. These included several tortricids (such as *Pandemis heparana*, *Spilonota ocellana*, *Archips podanus* and *Laspeyresia pomonella)*, *Hoplocampa testudinea; Eriosoma lanigerum*, *Lepidosaphes ulmi*, *Anthonomus pomorum* and *Phyllobius oblongus*. Some other potential pests did not increase to injurious numbers in these trials, but did so in others elsewhere *(Orthosia* spp.; *Archips rosanus; Argyresthia conjugella; Dasineura mali;* (Section 1.2).

Some of these pests are probably susceptible to one form or another of ecological control. Although leads are available, we have not yet worked out which factors should be manipulated to achieve this. The best defence for the time being is selective pesticides. Comparative tests were started, as variants within the selective spray program, on the effectiveness of some of these compounds and their compatibility with biological control.

Diflubenzuron has been found indispensable to approach the proportion of blemish-free fruit required at present. Its use is compatible with biological control of *P. ulmi*, and it is effective against a broad range of pests including Lepidoptera, *H. testudinea* to a - probably sufficient - degree, *P. oblongus* and *A. pomorum*. Control of Coleoptera is achieved by reduced fertility of the adults. Diflubenzuron is, however, not effective against certain tortricids *(A. orana*, *P. heparana*, and *A. rosanus)*, although it is a potent insecticide against others members of the family *(L. pomonella*, *S. ocellana*, and *A. podanus)*. How can this gap be filled?

Bacillus thuringiensis has not proved satisfactory (Section 9.1). The experimental juvenile hormone mimic, epofenonane, gave good control of *A. orana* if applied in sufficiently isolated orchards or large areas; it proved helpful in suppressing other pests (such as other species of tortricids, aphids, including *Eriosoma lanigerum;* and *Lepidosaphes ulmi)* and compatible with biological control of *Panonychus ulmi*. It is disheartening that commercial conditions are restraining the development and marketing of this useful insecticide. The experimental pyrazoline insecticide PH60-41 (Mulder et al., 1975) also meets the requirements for *A. orana* control in an integrated program, but it seems subject to similar commercial delay. Viruses of *A. orana*, and confusion of males by pheromones, have shown promise in preliminary tests (Section 9.2 and 6.3).

The choice of fungicides is essential for the success of biological control of *P. ulmi*. Captan, one of the fungicides applied against *Venturia inaequalis*, proved harmless for predatory mites. Neither did it upset biological control of *P. ulmi* by physiological stimulation of the spider mites, a negative effect sometimes reported of captan. Dodine and thiophanate-methyl, used incidentally, also proved applicable. However, the fungicide mancozeb was found to kill typhlodromids and preclude biological spider mite control, though sometimes recommended in integrated programs elsewhere because of its suppressive effect on spider mites. The same was found for other manganese-containing bisdithiocarbamates (Section 7.5). The conventional fungicides used against *Podosphaera leucotricha*, such as sulphur, dinocap, and binapacryl, prove detrimental to the biological component of integrated control, but the newer fungicides against mildew, bupirimate, nitrothal-diisopropyl, and triadimefon can be used.

The control techniques used at present in our integrated program for orchards are listed in Table 1. This program (Fig. 2) gives a harvest similar to conventional chemical control, though using only 4 to 5 applications of selective insecticides per year. This number can be reduced to 1 to 3, or *Bacillus thuringiensis* can be substituted for certain chemical treatments, without any danger to the orchard, though yield may suffer and incidence of insect-blemished fruit increases (Table 2; Gruys, 1975; Gruys & Mandersloot, 1978).

Table 1. Control measures in the integrated program for orchards.

Acari	
Panonychus ulmi	typhlodromids release! white oil, benzoximate, fenbutatinoxide[3]
Lepidoptera	
Operophtera brumata *Orthosia* spp.	Dimilin (diflubenzuron)
Adoxophyes orana	parasites, incl. Colpoclypeus florus; epofenonane[1], Dipel[2] *(Bacillus thuringiensis)*
other tortricids	Dimilin (not effective against all species), epofenonane[1]
Laspeyresia pomonella *Pammene rhediella*	Dimilin
Stigmella malella	*Chrysocharis prodice* release! Dimilin[3]
Homoptera	
Eriosoma lanigerum	endosulfan, in early spring; pirimicarb, in growing season; *Aphelinus mali*
aphids	pirimicarb
Hemiptera	
Lygus pabulinus	suppress dicotyledonous weeds by frequent mowing; white oil at bud burst
Hymenoptera	
Hoplocampa testudinea	Dimilin (suppresses next generation); thiophanate-methyl
Coleoptera	
Anthonomus pomorum	Dimilin, in early spring
Phyllobius oblongus	Dimilin: strong suppressive effect on next generation
Diptera	
Dasineura mali	endosulfan: spray at low temperature
Fungi	
Venturia inaequalis	dodine, captan; do not use Mn-containing fungicides!
Podosphaera leucotricha	bupirimate, triadimefon[1], nitrothal-diisopropyl; do not use sulphur nor dinocap
Pezicula spp.	captan, thiophanate-methyl
Fruit thinning	naphthalene acetamide

1. Experimental; not on the market.
2. Effectiveness rather unsatisfactory.
3. Not necessary once the program functions satisfactorily.

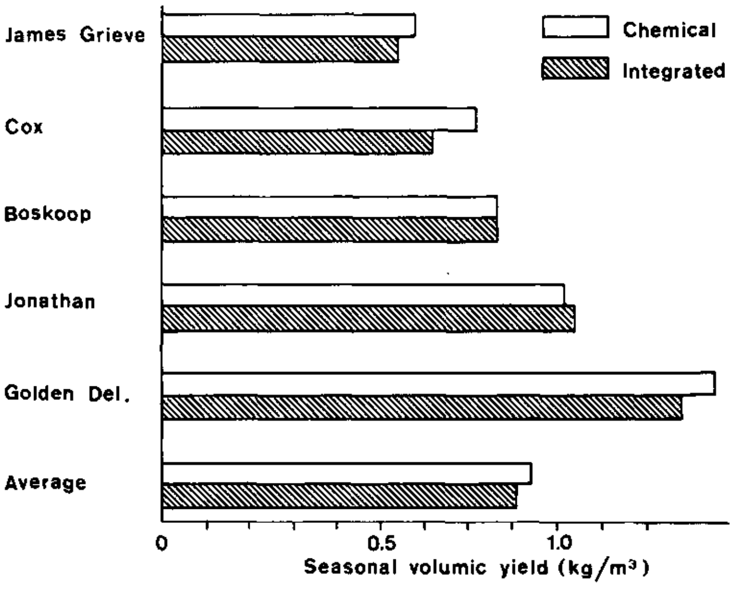

Fig. 2. Average seasonal yield divided by tree volume (kg/m^3) to make results independent of differences in tree size.

Table 2. Proportion of fruit low-classed because of insect blemishes under routine chemical control and different variants of the integrated program in 1977.

	Sprays against Lepidoptera		Sprays against other arthropods, number	Low-classed fruit (%)
	number	insecticides		
routine chemical	5	organophosphorus, carbaryl	3	1
integrated	0		1-3	17
integrated	4	Dipel 0.2%	1-3	11
integrated	1	Dimilin 0.1%	1-3	6
integrated	3	Dimilin 0.1%	1-3	3

CONCLUSIONS AND PROSPECTS

The availability of an orchard in which different approaches to pest control can be tried freely has been indispensable for the progress of integrated control in fruit growing.

Biological control, by native parasites and predators, contributes essentially to the integrated program. It can be combined successfully with chemical pesticides, provided these are sufficiently selective. Further research on non-chemical techniques for insect control will undoubtedly reduce the amount of selective chemicals used in the program.

Moreover, greater emphasis should be placed on developing and growing varieties (partly) resistant to fungal diseases, because it is their control that requires the greater part of the pesticides used in orchards in the Netherlands. For this purpose, a collection of old

Dutch varieties has recently been put together, and an orchard has been planted with certain of these and some scab-resistant apple varieties from the United States and elsewhere.

Satisfactory functioning of an integrated program under experimental conditions does not necessarily mean it will be appropriate in practice. Practical trials were needed and are described in Section 1.2.

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1.2 Implementation of integrated control in orchards

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INTRODUCTION

From 1950 to 1970, the number of sprayings in apple orchards increased in the Netherlands. Usually routine treatments were arranged at the phenologically correct time but irrespective of the presence of noxious species. Roosje (1967) mentions an average of 22 sprays against injurious fungi and arthropods in the 1960s.

Intensive as it has become, chemical control in orchards in the Netherlands has never evoked side-effects so grave as to force the growers to adopt a form of pest control different from routine schedules. The process of introducing integrated control into fruit-growing must therefore be gradual. Our approach is to test whether methods are applicable within the economic margins, and to demonstrate and explain to the growers that these methods will not impair financial returns and will probably be beneficial, in the long term, to society as a whole.

In this gradual process, we find it useful to distinguish clearly between supervised chemical and integrated control. Supervised chemical control consists of pest monitoring and applying conventional pesticides if and when the pests' numbers, relative to economic thresholds, indicate the need to do so. In integrated control in the strict sense, utilization of environmental factors restraining pests (such as natural enemies) is primary and the application of pesticides based on pest monitoring and economic thresholds is secondary though often indispensable. The functioning of effective biological controls requires drastic adaptation of the pesticides used. So an integrated program is a separate and more difficult phase to introduce.

TESTS OF SUPERVISED CONTROL

Preliminary guidelines for monitoring techniques and control thresholds of pests, based on the experience obtained in trials on integrated control (Section 1.1) were tested in 1971-1974 in orchards of 1 to 5 hectares. The test plots were situated in commercial holdings, and pests were monitored by experienced technicians. Insecticide and acaricide treatments were restricted to those indicated by the assessments and were done by the orchardist

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in the usual way. Proportion of fruit damaged by insects, the number of sprayings and the time needed for monitoring were used as criteria in assessment of the results. Any loss in yield could not be determined reliably because of the nature of the trials, but the control thresholds made such losses highly improbable. At first, the results for the test plots could be compared with those of the rest of the orchards, treated according to the judgment of the orchardists. In the course of the test, however, this standard lost its usefulness because the orchardists adapted their decisions to the treatment of the test plots. Hence, the results had to be judged against a general knowledge of conventional chemical control.

Through supervision, the number of sprayings against pests could be reduced to 3.2 per year on average, with an acceptable level of damage to the fruits (Table 1). Figure 1

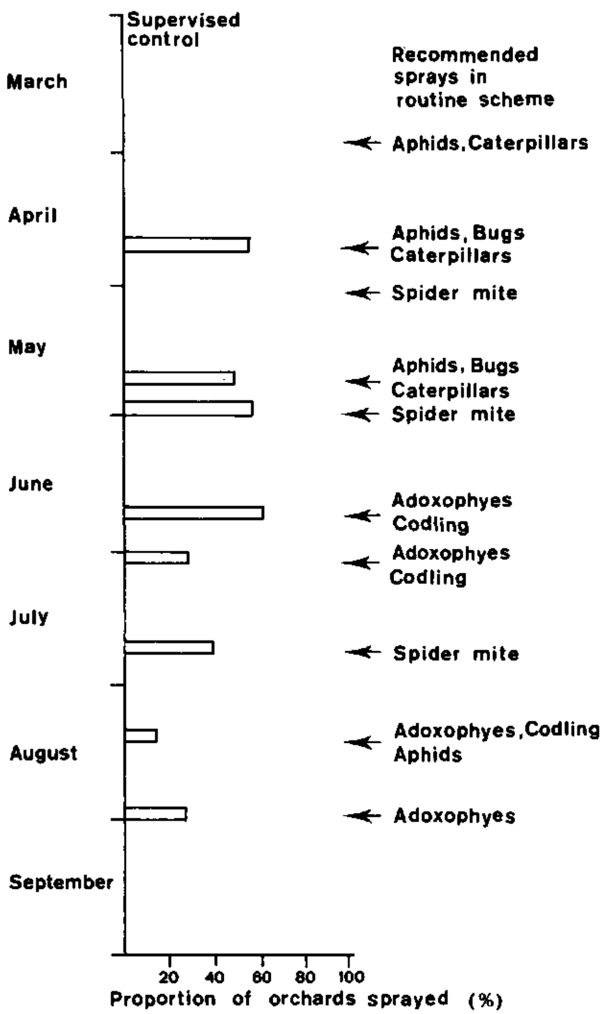

Fig. 1. Sprays needed under supervised control (left, average % test orchards sprayed from 1971-1974) in comparison to sprays recommended for a routine schedule (right).

Table 1. Trials on supervised control.

Year	Number of orchards	Average number of sprayings			Proportion of insect damage to fruits (%)
		insecticides	acaricides	total	
1971	11	2.1	0.8	2.9	2.3
1972	29	2.8	0.9	3.7	1.3
1973	24	1.7	1.1	2.8	2.7
1974	23	2.5	1.0	3.5	0.8

shows how far the sprayings of the routine program recommended by the advisory service at that time were really necessary. The average grower applied only some 6-8 of the 10 recommended treatments. With the 10 recommended sprayings, the damage would not exceed 1%, but haphazard omission, as the average grower did, gave no better results than those obtained by the supervised system.

The time needed for pest monitoring in a year for a given area was rather variable, depending on characteristics of the orchards such as size and number of varieties grown, without much difference between years (Fig. 2). Simple calculations showed that the savings on pesticides outweighed the costs of monitoring.

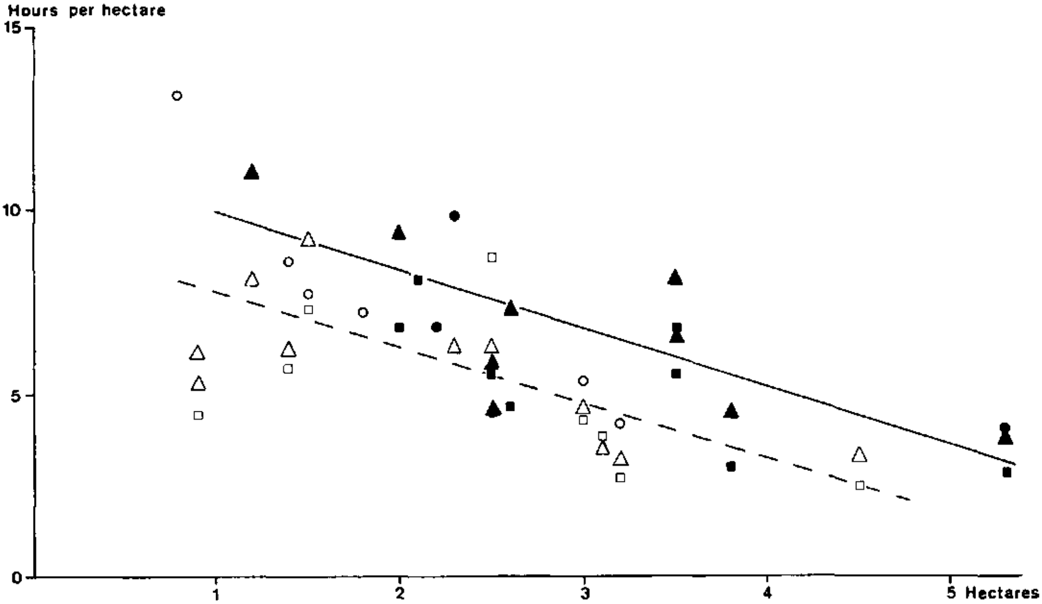

Fig. 2. Time needed per hectare per year for assessments of pests in test orchards.
---- simple orchards, i.e. 1-3 varieties, in 1971 (0), 1972 (Δ), and 1973 (□)
—— complicated orchards, i.e.>3 varieties, in 1971 (●), 1972 (▲), and 1973 (■).

INTRODUCTION OF SUPERVISED CONTROL IN PRACTICE

In 1973, one of the specialists on plant protection of the State Horticultural Advisory Service, in cooperation with 8 orchardists, took up the challenge to test supervised control against the routine schedule. After thorough instruction, each of the growers applied the supervised system to part of his holding and the routine program to the rest of it. The success of this trial prompted the advisory service to start introducing the supervised system all over the country. The principle has been that the orchardists should remain responsible for pest control, and perform the necessary assessments themselves; instruction, however, was, and is, provided by the advisory service.

Instruction to the orchardists began in winter, when backgrounds of pest control were explained in a number of meetings. Initially, much attention was paid to the techniques of pest assessment and economic thresholds. Since many growers are now familiar with these matters, the emphasis has shifted to side-effects and choice of pesticide. During the growing season, groups of 10-15 orchardists each meet four times, guided by advisory staff, to practise the assessments to be performed in the period concerned. Moreover, general information on the phenological development of pests and timing of assessments and sprays is provided by the regionally operated telephone warning systems.

The development of supervised control since 1973 is shown in Table 2. About 20% of the orchardists now use the system. The remaining 80% have also reduced their number of sprays, as a result of the attention paid to these matters during recent years. A sample taken in 1978 showed the average number of sprays against animal pests on conventionally operated holdings to be 6, which is somewhat less than before 1973. The initial economic thresholds, at first issued in a loose-leaf booklet (Van Frankenhuyzen & Gruys, 1971), were somewhat simplified and adapted in the course of practical application. Their present nature is summarized in Table 3. Any changes deemed necessary are mentioned in an advisory leaflet on pest control in fruit growing, issued annually (Rijksvoorlichtingsdienst, 1972).

Table 2. Supervised control in practice.

Year	Number of advisory groups	Number of growers	Average number of sprayings			Proportion of insect damage to fruits (%)
			insecticides	acaricides	total	
1973	1	8	0.6	1.3	1.9	1.9
1974	15	175	2.4	1.0	3.4	1.4
1975	37	400	2.2	1.4	3.6	1.0
1976	41	400	3.5	1.5	5.0	0.9
1977	45	600	3.4	1.4	4.8	1.8
1978	41	700	3.0	1.0	4.0	0.4

Table 3. Assessment of pests and economic thresholds.

Time	Pest	Sample/ orchard	Economic threshold (spray if over)	Time for treatment; remarks
Before blossom	*Panonychus ulmi*	40 branches	eggs more abundant than sporadic	petal fall
	Archips rosanus	5 x 10 spindles or 5 x 3 bushes	5 egg masses	petal fall
	Anthonomus pomorum	200 mixed buds at bud burst	30 with feeding damage	assess only if capped blossoms present in previous year
	Operophtera brumata	100 mixed buds	10 larvae	before blossom
	Rhopalosiphum insertum	100 mixed buds	50 with over 4 aphids	before blossom
	Dysaphis plantaginea	whole orchard	presence	before blossom
	Eriosoma lanigerum	whole orchard	presence	before blossom on early varieties
Before and during blossom	*Lygus pabulinus*	beat 100 branches	4 larvae	before blossom to petal fall
	Dysaphis plantaginea	beat 100 branches	presence	before blossom to petal fall
	Operophtera brumata *Orthosia* spp.	beat 100 branches	5 larvae	before blossom to petal fall
	Adoxophyes orana	beat 100 branches	1 larva	late June, when eggs hatch
	Adoxophyes orana	200 trusses	1 larva	late June, when eggs hatch
Petal fall	*Dysaphis plantaginea*	200 trees	10 trees infested	continue sampling to 15 June; petal fall to June
	Hoplocampa testudinea	100 trusses	10 egg insertions	asses only if attack present in previous June
Early June	*Orthosia* spp.	500 shoot tips	12 larvae	early June
	Psylla pyri	whole orchard	abundant young larvae	early June
	Eriosoma lanigerum	whole orchard	presence	early June
Late June	*Panonychus ulmi*	50 leaves of clusters	5 leaves with over 5 mites	late June
	Stigmella malella	200 leaves of clusters	20 mines	July
	Aphis pomi	500 shoot tips	25 severely infested	late June
	Laspeyresia pomonella	400 fruitlets	4 entries	late June; repeat sampling
Late July	*Adoxophyes orana*	200 shoot tips	10-30 infested	spray when larvae hatch in August twice if larvae hatch before 20 August, once if larvae hatch after 20 August
			30 infested	spray twice when larvae hatch in August
	Panonychus ulmi	50 leaves	5 leaves with over 5 mites	late July
	Laspeyresia pomonella	400 fruits	4 entries	late July; repeat sample in mid August
Late August	*Stigmella malella*	whole orchard	30% of leaves with mines	petal fall next year

TESTS OF INTEGRATED CONTROL

In 1972, when the integrated program began to function satisfactorily (Section 1.1), practical tests of integrated control were initiated on plots of 0.5 to 2 hectares in commercial holdings. The number of plots was extended to about 20 in 1975.

The program is as depicted in Table 1, Section 1.1, with *Bacillus thuringiensis* (Dipel) and Ryania instead of diflubenzuron (Dimilin) and epofenonane, which were not available at the time, cyhexatin as an acaricide, and a less restricted choice of fungicides.

Although the system functioned properly in some plots, it did not in several others. Circumstances imputable for the difficulties encountered included the following.

- *Adoxophyes orana* was a persistent pest in most plots, much more so than at De Schuilenburg, and *Bacillus thuringiensis* (Dipel) proved disappointing as a control agent. The cause of this difference is not clear. Small plots relative to a large supply of moths from the routinely treated surrounding orchards and the lack of an integrated history in the new plots, may have something to do with it.
- Small size of plots and large disturbances by drift of unselective sprays from neighbouring orchards, prevented success of biological control of spider mites in some cases.
- The use of certain pesticides, whose toxicity to predatory mites was not known at the time, interfered with biological control of spider mites. Cyhexatin, used to suppress *Panonychus ulmi* when the typhlodromids were not yet sufficiently effective, caused much harm. This acaricide acts insidiously, since it is hardly harmful to adults but very toxic to eggs and larvae, as tests in the laboratory have shown since (Section 7.5). Similarly, manganese-containing bisdithiocarbamates proved fatal to the populations of predatory mites if used repeatedly, as some orchardists prefer to do because of the benevolent effect of these fungicides to the quality of foliage.
- The trials in many different places confronted us with a number of "minor" pests against which selective control procedures still had to be devised.

Thanks to the many mishaps, the project has been very useful in identifying practical deficiencies of the integrated program. One considerable improvement has been the substitution of diflubenzuron (Dimilin) for *Bacillus thuringiensis* and Ryania. This insecticide, however, is ineffective against *A. orana*.

The new design of the trials in 1978 should achieve further improvements. Efforts are now concentrated on a smaller number of larger, rather isolated, orchards. These conditions allow use of the juvenile hormone mimic, epofenonane, against *A. orana* (Section 8.4). Laboratory and small-scale field trials on the selectivity of pesticides (Sections 7.5 and 1.1) have enabled us to adapt the applications of pesticides to the requirements for biological control of spider mite.

Male confusion by pheromones, another technique requiring tests on a large scale, will be included as a possible control of *A. orana* and codling moth in some of the test orchards in 1979.

In the latest edition of the instruction book (Van Frankenhuyzen & Gruys, 1978), guidelines on the integrated program are given for the north of the country where *A. orana* never develops into a pest.

CONCLUDING REMARKS

The success of these trials as a nucleus from which integrated control can extend gradually into practice fully depends on the availibility of adequate control measures against certain pests, notably *A. orana*. Some control measures require large-scale field trials to evaluate their efficacy, for instance juvenile hormone mimics. Trials like this then offer an excellent opportunity for government research and industry to cooperate in developing such compounds into marketable pesticides. Pilot projects are indispensable for making an experimental integrated program sufficiently foolproof to be generally applicable.

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1.3 Tortricids in integrated control in orchards

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INTRODUCTION

Orchard-dwelling tortricids include the fruit-piercing species, *Laspeyresia pomonella*, *Pammene rhediella* and *Pammene argyrana* and several leaf-rollers damaging the skin of fruits, as do *Adoxophyes orana*, *Archips podanus*, *Archips rosanus*, *Hedya nubiferana*, *Pandemis cerasana*, *Pandemis heparana* and *Spilonota ocellana*. All of these are general pests except *A. rosanus*, which is usually local.

Forty years ago, codling moth, *Laspeyresia pomonella*, was a major pest in apple and pear orchards in the Netherlands, and other tortricids were of some importance. The decrease in infestation with the codling moth since then coincided with a gradual substitution of shrubs and dwarf trees for standard trees. Since shrubs and dwarfs have far less bark-scales, which serve as hibernation sites for the codling larvae, they are a less favourable habitat for this insect than standard trees. Moreover, insect control is generally more successful on dwarf trees.

In 1939, the summer-fruit tortrix moth, *A. orana*, was observed as a new species in Dutch orchards. It spread rapidly through the country and soon became one of the most serious orchard pests, on which control was concentrated. The timing of sprays against *A. orana* and codling moth largely coincided, contributing to the decline of codling moth. Similarly, in routine spray programs, the other leaf-rollers are suppressed by treatments directed against other insects.

Today, *A. orana* is the main potential tortricid pest in orchards in most regions of the Netherlands. Damage is prevented by adequate supervised control programs with broad-spectrum insecticides. In such programs, the other tortricids including codling moth are also controlled. In integrated programs, *A. orana* cannot be controlled, because the insecticide epofenonane (see under control) is not approved nor available. In strict supervised programs and integrated programs, the other tortricids including codling moth can regain some of their former status and so also require attention.

Bionomics and ecology

A. orana usually has two generations per year, but a partial third generation may occur when the season is exceptionally early. The larvae of the first generation, present on the trees in July, inflict the greatest harm. Those of the second generation cause less damage, only small feeding scars, because they hibernate in the third instar, they are much more harmful, however, when they give rise to a third generation in the same year. After hibernation, the caterpillars are reactivated in April and feed in the flower clusters to pupate at the end of May or the beginning of June. They do not cause economic damage at that time. When growers shifted to small trees and stimulated shoot growth by increased pruning and fertilization, *A. orana* became important as a pest; in the first generation, many larvae inhabit the shoot tips and young leaves during the greater part of their life.

Initially, a large number of sprays in spring and summer seemed necessary to achieve adequate control, particularly if the insecticides used were not persistent. A detailed study of the bionomics of the species and habits of the larvae showed the hatching larvae of the first generation in June to be highly susceptible to contact insecticides, and allowed a more accurate timing of the sprays. Sprays directed against the hatching larvae in June appeared more effective than treatments of the hibernated larvae in April (De Jong & Gruys, 1975). A procedure was developed to calculate the hatching period of the larvae in June and the right time for spraying from catches of moths in light traps, and the relationship between temperature and embryonic development (De Jong et al., 1965). A successful warning system based on this procedure and a network of light traps has been operated by the State Horticultural Advisory Service since 1953. In the 1970s, the light traps were replaced by pheromone traps.

Since *A. orana* is a polyphagous insect, immigration from surrounding woodland and orchards may influence the population of an orchard. Immigration may be decisive for the success of certain control techniques and was therefore studied in detail. Release of mass--reared moths showed that males may cover distances of up to 200 m in a week. Females covered distances of only 20 m in the same period.

Several hymenopterous and dipterous parasites were reared from the larvae of *A. orana* (Section 1.6), but parasitism was never sufficient to suppress the population below the economic threshold.

Sampling

For decisions on control later in the season, *A. orana* populations are sampled in May. Since other tortricid larvae may be present, keys for identification of larvae and pupae were developed (Evenhuis et al., 1973; De Jong & Vlug, 1974). The sample size prescribed in the instructions on supervised control is 200 clusters and is time-consuming for the fruit growers. Therefore, a sequential sampling procedure was developed (De Jong & Van Dieren, 1974).

The second major period for sampling the population is in July, when the larvae of the first generation are present in the shoot tips. The numbers present at that time and the subsequent phenology determine whether control will be needed in August. In years with an early flight in August, the economic threshold should be reduced because many larvae will complete their development and threaten the fruit more seriously than when entering diapause at their third instar. In orchards, the fraction of the population giving rise to a third flight and the rate of damage to the fruits are closely related (De Jong & Beeke, 1976). Quantitative phenological information, based on the critical photoperiod for the induction of diapause in *A. orana* determined by Ankersmit, enables us to predict the danger from a third generation and to adjust the economic threshold. The occurrence of a third flight also has a positive effect, namely that many of the larvae issued from it will be unable to reach the instar required for successful hibernation. This reduces the population of the next spring.

A computer model has been developed including both the natural factors in the population dynamics of *A. orana* and the effect of insecticide treatments (De Jong & Van Dieren, 1974). It is used to study the suitability of newly developed insecticides in pest management programs. For public health and to improve predatory activities in summer time, some of these programs aim at applying less poisonous insecticides and at restricting use of insecticides to the period before mid-July. These programs are now implemented in long-term field trials and may contribute to safer pest management for man and environment.

Control

The use of mass-reared parasites was studied. Some strains of *Trichogramma embryophagum cacoeciae* (Hymenoptera, Trichogrammatidae), were mass-reared and released in experimental orchards to control *A. orana*. Satisfactory results were obtained in some trials, but bad weather often limited the activity of the parasites so the effects were unreliable. A lack of hosts in commercial orchards seriously limited survival in winter and repeated releases of this egg parasite were necessary. Moreover, the parasites proved extremely susceptible to pesticides. So this means of control has been abandoned.

So far, expensive preparations of *Bacillus thuringiensis* have proved inadequate for control of *A. orana*. A nuclear and cytoplasmic polyhedrosis virus discovered in larvae of this leaf-roller controlled young larvae satisfactorily in trials in a greenhouse. In orchards, however, the results were less promising initially. Bad weather hampered the effectiveness of the virus and the period over which the larvae hatched was too long to keep the virus preparation without deterioration. Recent trials with the virus show more promise (Section 9.2).

Trials on release of sterile males against the summer fruit tortrix showed that the population could be reduced below the economic threshold. Reinfestation from surrounding vegetation, however, would probably interfere with success on a practical scale. Moreover, the increase in population of other tortricids and winter moth (*Operophtera brumata*) in the absence of broad-spectrum sprays reduces the effectiveness of the technique. More details are given in Section 1.8.

The selective insecticide diflubenzuron hardly affects the population of *A. orana*. The juvenile hormone mimic, epofenonane, to which only fifth-instar larvae are susceptible, adequately controls *A. orana* in spring. Treatment in spring gives sufficient control for the rest of the year as long as reinfestation from surrounding orchards and other vegetation is negligible. So cooperation between adjoining holdings in an orchard area is necessary to achieve adequate control with this type of insecticide. Epofenonane is, however, not approved for orchard application and will not be produced for economic application. Further details are given in Section 8.4.

Reduction in treatment with broad-spectrum chemicals increases the incidence of codling moth and certain leaf-rollers mentioned in the introduction. Ryania is a selective insecticide able to control codling moth, *Laspeyresia pomonella*, to some degree, but it is not approved in the Netherlands. Since codling moth is effectively and selectively controlled by diflubenzuron, it poses no great problem in integrated control. Trials on the use of viruses and male disruption by pheromones against it are under way (Section 6.3 and 9.2). *Pammene* spp. causing early worminess in apple are occasionally a problem in integrated programs, but can be controlled with diflubenzuron.

Of the other tortricids mentioned, *Hedya nubiferana* is not a really harmful leaf-roller on apple and pear; *Archips podanus, Spilonota ocellana* and *Pandemis* spp., however may be harmful. Diflubenzuron is effective against these three species but not against *Pandemis*, which may also continue to cause trouble in integrated programs.

CONCLUSION

The initial problem posed by leaf-rollers in integrated control has broadened and has not yet been solved to our satisfaction. Some promising selective control techniques may bring the problem into practical control in the future.

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1.4 Population regulation of the fruit-tree red spider mite Panonychus ulmi by predators

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DESCRIPTION OF THE PROBLEM

The fruit-tree red spider mite, *Panonychus ulmi*, has been a problem in commercial orchards in the Netherlands since the mid-1930s, when pesticides - mainly tar-oil winter washes and anorganic fungicides - were introduced and when cultural practices like pruning, fertilization and fruit-thinning were intensified. Tar-oil winter washes are now known to be highly injurious to mite and insect predators of the fruit-tree red spider mite. Most of the synthetic insecticides, introduced in the 1940s and 1950s, such as the organophosphates and DDT, are extremely toxic to the natural enemies of *P. ulmi*; moreover, DDT has a stimulative effect on the oviposition of *P. ulmi*.

The biology and taxonomy of this mite was studied by Geijskes (1938); Kuenen (1947, 1949) studied the biology, host-plant relationships and the influence of some predator species on the population development of this mite. In the Netherlands, the fruit-tree red spider mite passes through 5-7 generations in a season. The successive generations may increase drastically; a small population in spring may give rise to damaging densities within two generations. Damage consists of puncturing the leaves by the mites, followed by bronzing and leaf-drop (Van de Vrie, 1956). Consequently photosynthesis and respiration are impaired and the number of flower buds in the following spring decreases. In late summer and autumn, diapausing winter eggs are deposited on the branches and twigs; they hatch the next spring.

Organophosphorus compounds were introduced in the early 1950s for control of many orchard pests, including the fruit-tree red spider mite. After an initially successful introduction, resistance to these acaricides was noticed in 1953 and became widespread in the following years. Resistance to chlorbenside (4-chlorobenzyl 4-chlorobenzyl sulphide), fenson (4-chlorophenyl benzenesulphonate), and chlorofenson (4-chlorophenyl p-benzenesulphonate) developed widely after their introduction in the mid-1950s.

Destruction of natural enemies and resistance to acaricides are the main reasons for the emergence of *P. ulmi* as a major pest in Dutch orchards. Once a species has been freed from natural and chemical control, a greater pest status is inevitable. Natural enemies are rare in commercial orchards, contrary to the situation in neglected orchards where predacious mites (Phytoseiidae) and predacious bugs (Myridae) hold the phytophagous mites in check. Neglected orchards differ in two main aspects from commercial orchards. The species composition of the arthropod fauna is more diverse and the chemical composition of the host plant is different, mainly in a lower nitrogen content of the leaves.

In environments where broad-spectrum pesticides are used, the enemies are often worse affected than the fruit-tree red spider mite and outbreaks of the latter may occur. Some pesticides even stimulate the reproductive capacity of *P. ulmi*.

AIM AND MOTIVATION OF THE RESEARCH.

The ability of *P. ulmi* to develop resistance to acaricides, the drastic reduction in predators that could reduce *P. ulmi* populations, and the danger of contamination of the orchard environment with biologically active pesticides were motives for experiments on management of these natural enemies. As general predators like *Anthocoris nemorum* or *Orius minutus* leave fruit trees in search for other prey before *P. ulmi* is reduced to acceptable low levels (Kuenen, 1947), most attention was on phytoseiid mites. Some species are specialized on *P. ulmi* and the fruit-tree habitat, as are *Amblyseius potentillae*, *A. finlandicus* and *Typhlodromus pyri*. Tests were conducted on relative rates of increase in predator and prey species, prey stage preferred, functional, numerical and total response, of the predator, and searching capacity. These tests were followed by experiments under different conditions of initial numbers of prey and predators.

RESULTS AND DISCUSSION

There are two main avenues to understanding mite outbreaks:

- improved nutritional conditions for the phytophagous mite;
- detrimental effects of pesticides on the natural enemies.

Increases in mite populations have commonly been associated with higher nitrogen content of the leaves. Kuenen (1947) was the first to show that *P. ulmi* multiplied much more on leaves of fruit trees under good management than in neglected orchards; in well-kept orchards, the leaves contained more nitrogen. The population dynamics of these findings were studied by Post (1962). She showed that the physiological condition of the host plant was crucial. In an orchard where *P. ulmi* occurred only sporadically, an increase in population density corresponded to an increase in nitrogen content. In a plot with a high initial population of *P. ulmi* situated in a well-kept orchard, the population dropped considerably after the nitrogen content of the leaves had been reduced by stopping nitrogen dressing, soil cultivation and pruning. Post concluded that predacious mites and insects were not sufficient to counteract the increased development of *P. ulmi* induced by cultural measures.

The influence of host-plant quality on mite development and the influence of mites on the physiology of the host plant was studied by Storms (1971); Section 4.4.

Van de Vrie & Kropczyńska (1965) studied the influence of predacious mites on the population development of *P. ulmi* on apple under semi-controlled laboratory conditions. They showed that *A. potentillae* under these conditions had a strong regulatory effect on populations of *P. ulmi* without eliminating the prey entirely. They attributed the effect to (1) distrubution of prey and predator, and their fluctuations during the season on individual apple leaves; (2) the preference of the predator for the juvenile stage of the prey; (3) the functional, numerical and total response of the predator to prey densities; (4) phenological similarities of predator and prey. They chose *A. potentillae* as a model predacious mite, because it could easily be identified on apple leaves, it could be easily cultured with *T. urticae* as food and is quite common in undisturbed habitats. Other species, which also frequently occur on apple are *Amblyseius finlandicus* and *Typhlodromus pyri*, but they have not been studied in the same detail as *A. potentillae* in the Netherlands.

Van de Vrie & Boersma (1970) conducted experiments with *P. ulmi* and *A. potentillae*, introducing the findings of Kuenen (1947), Post (1962), and Storms (1971) on the influence of host-plant condition on population development of *P. ulmi*. In their experiments, apples were grown under three levels of nitrogen fertilization and with *A. potentillae* absent or present. The total number of eggs produced on high-nitrogen trees was more than four times that on low-nitrogen trees, and the females lived one-third longer on the high-nitrogen trees, thus confirming the results of Kuenen, Post, and Storms (Fig. 1). Figure 2 shows

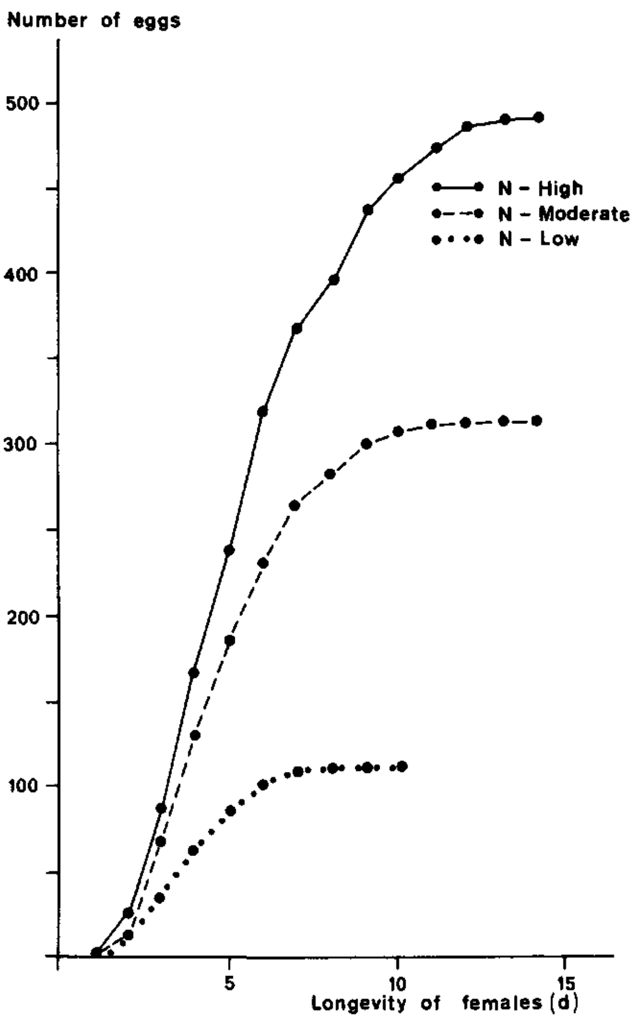

Fig. 1. Number of eggs produced by 20 females of *Panonychus ulmi* on leaves from apple trees with different levels of nitrogen fertilization. After Van de Vrie & Boersma, 1970.

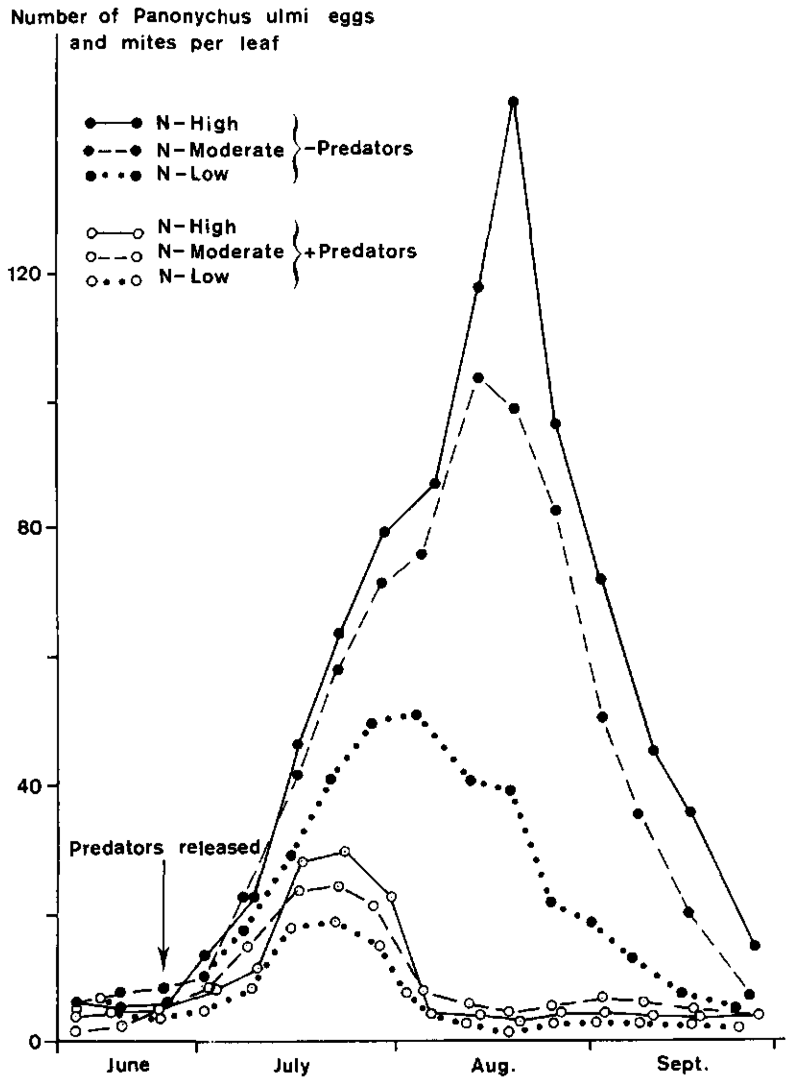

Fig. 2. Populations of *Panonychus ulmi* on apple grown with low, medium and high levels of nitrogen fertilizer, with and without the predacious mite *Amblyseius potentillae*.

the efficiency of *A. potentillae* in curtailing the *P. ulmi* population increase in spite of this inherently increased fecundity assumed to be near its maximum during June - August. In the absence of predators on the high- and intermediate levels of nitrogen damaging populations developed. It is very interesting to note that even on the high-nitrogen trees no damaging populations could develop, provided that predatory mites were present. This is due to the effect of the predatory mites that prevented *P. ulmi* from reaching high densities, and because of the shorter period during which these populations were present. It is also highly interesting to note that the predators never annihilated their prey.

Also McMurtry & Van de Vrie's studies (1973) explain the effects of *A. potentillae* on the population development of *P. ulmi* in terms of prey consumption, dispersion, and total response of the predator to its prey density.

These findings are contrary to Post's opinion on the capacity of predatory mites and insects. A critical consideration is whether Post in her field studies was dealing with the best predator species for reducing or preventing *P. ulmi* outbreaks. *Typhlodromus aberrans* was reported to be the most numerous phytoseiid species; very little is known about this species whereas *A. potentillae, A. finlandicus* and *T. pyri* have been reported to be efficient against *P. ulmi*. Probably, in Post's experiments the predators did not have sufficient time to develop, or the isolated position of the experimental plot was responsible for the observed phenomena. However, Post's transferring experiments with *P. ulmi* populations on potted apple trees from well-kept orchards to neglected ones, and vice versa, are indicative for the efficiency of the local predator complex.

Predatory mites are, in general, more susceptible to pesticides than their prey. Some insecticides and acaricides are, however, selective in their action. Pirimicarb and diflubenzuron may be applied without reducing the predator populations. Several fungicides, like bipurimate and captan, also can be applied without detrimental effects for predacious mites.

Resistance to acaricides or insecticides in phytoseiid mites has not yet been observed in orchards in the Netherlands. This phenomenon was manifested in Canada, the United States, and New Zealand in local populations of some species. This resistance has stimulated the use of predacious mites considerably.

SIGNIFICANCE FOR AGRICULTURAL PRACTICE

The material presented above indicates proper methods of managing phytoseiid predators for effective control of *P. ulmi*, consistent with profitable fruit production. This effect may be realized more easily considering the general trend to reduce the amount of nitrogen fertilization of fruit trees.

FUTURE OUTLOOK

Although the practicality of an integrated control approach for the fruit-tree red spider mite has been shown, more information is needed to make handling the system by the fruit grower more easily. Resistance in phytoseiid predators to generally used pesticides, as has been shown in other fruit growing areas, makes introduction and maintenance of these predators more feasible and reliable. Yet our methods of production, handling and conserving predacious mites need improvement. Considering the regulative capacity, the phenological similarity, and the general occurrence of these predators, long-term control of *P. ulmi* populations below injurious levels can be realized.

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1.5 Biology and control of secondary insect pests in fruit crops

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INTRODUCTION

Some species of harmful insect and mites in orchards can develop explosively under certain conditions, and acceptable measures for control were not always known. In order to prevent economic damage, a technique for observation and adequate control of such pests is being developed under the auspices of the Working Party on Integrated Control TNO.

Failure to prevent explosive increase in population can be due to several factors: Insufficient knowledge on the biology and ecology of the insect; inadequate familiarity with current pesticides and transition to new ones; technique of application, which should fit the ecology of the insect. Restricted spraying schemes can lead to an increase in non-target insects. Denser planting as in multi-row systems also hamper crop protection. Changes in culture patterns can hinder crop protection and create new problems, as for strawberry blossom weevil. The ban on persistent pesticides has also caused difficulties, which in part still have to be solved.

One must therefore be alert to unexpected problems. Research on bionomics and phenology of the susceptible stages of the pest is often necessary alongside tests on pesticides. Although the problems are frequently related to a single insect species, research should be directed to complete schemes of plant protection.

At Wilhelminadorp (Zealand), research is being done on insects in top fruit and on strawberry blossom weevil in soft fruit.

APPLE CLEARWING MOTH, AEGERIA (SYNANTHEDON) MYOPAEFORMIS

This moth has a 2-year cycle and oviposits near callus tissue. The larvae mine that tissue, causing canker. The species is known in older standard trees and bush orchards but seldom causes economic damage. When the moth emigrates to young apple orchards nearby, they especially attack the graft union and can destroy small trees. Field research has been done on the biology and ecology of this insect as support in the search for suitable protection measures, observation methods and damage thresholds. The 2-year cycle makes it possible to tackle the insect at different times. It was already known that a spring application with several pesticides controlled it to a certain degree, but it is not yet clear if this suffices to curb heavy infestations. Treatment in autumn is also possible, but control during flight and oviposition may be preferable because of the possibility of combined control of leaf-rollers and leaf-miners. As in France, a sampling method for supervised schemes can be used, based on the presence of empty pupal cases in the canker lesions. Control is necessary if one or more empty pupal skins per tree are found at an inspection of a sample of 50 trees per hectare.

LEAF-MINERS

Two species of leaf-miner, *Lithocolletis blancardella* and *Stigmella malella*, play a major role at the moment. A third species, *Stigmella pomella*, is only of local interest and *Leucoptera scitella* used to be very injurious locally. Leaf-miners are usually not very harmful because they are controlled together with more important pests in complete control schemes. Restricting the number of sprayings, however, can undesirably increase the population and make special measures necessary.

Unfortunately application and maintenance of the Hymenopterous parasite *Chrysocharis prodice*, which can keep *S. malella* under control, is not possible in simple supervised control schemes. Acceptable chemicals had to be found. Larvae of the leaf-miners enter the leaf directly from the bottom of the egg. The newly hatched larvae can be killed by penetrative phosphorus compounds, but the older larvae in the mines are hard to control. Neither the new pyrethroids nor diflubenzuron can penetrate the leaf, so their use should be adapted accordingly. Pyrethroids must be applied at the beginning of the flight period, and diflubenzuron has a satisfactory and selective ovicidal action and can also be used in integrated control schemes by application before egg hatch. Control with diflubenzuron can also frequently be combined with that of other insects.

PEAR PSYLLID, PSYLLA PYRI

In the last few years, psyllids have caused increasing trouble on pear, including supervised control schemes. In 1977, we found that the technique of spraying influenced control. The low-volume technique of mist spraying often resulted in unsatisfactory penetration of the insecticide into the foliage and penetration was essential in psyllid control. The older stages usually live in leaf axils and near the shoot base where the mist does not penetrate sufficiently. To limit the use of insecticides, control must be combined with that for other insects.

ARCHIPS ROSANUS

This leaf-roller usually occurs locally. The caterpillar population in spring must not be too high. Control with juvenile hormone analogues has proved feasible.

STRAWBERRY BLOSSOM WEEVIL, ANTHONOMUS RUBI

After DDT was prohibited, no suitable control agent was found for strawberry blossom weevil. The behaviour of the insect excluded the use of diflubenzuron, because the larva completes development within a closed flower bud. The new pyrethroids, however, offer some control, as the weevil is active when the weather is warm. Control is seriously hampered by the need to protect honey-bees and to avoid poisonous residues on the fruits. Sampling the insects to assess their number on soft-fruit crops is possible, but a complication is that this species occurs on various crops: strawberry, raspberry and blackberry. The insect is also known from some wild Rosaceae and therefore we are examining the significance of nearby sources of infection such as wild brambles. More knowledge of the dispersal of the weevil is essential in view of the mutual influence of the many soft-fruit plots in an area. The blackberry cultivars Thornless Evergreen and Thornfree seem to be unattractive for oviposition. In 1978, a pyrethroid during the period the weevil emerged in spring up to the flowering of the crop yielded satisfactory results in raspberry. In 1979, the results were less satisfactory because of the influence of rapid dispersal of weevils from nearby plantations.

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1.6 Relation between insect pests of apple, and their parasites and predators

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APHIDS

Though the woolly apple aphid, *Eriosoma lanigerum* (Hemiptera, Aphididae), was introduced accidentally from eastern North America as early as the end of the 18th Century, its monophagous parasite in its native area, *Aphelinus mali*, (Hymenoptera, Aphelinidae), was only introduced in France for biological control after World War I. From France, it was distributed to other European countries and in 1924 material was received by the Plant Protection Service in Wageningen. Yet several years passed before the parasite became established in the Netherlands.

It has been a matter of debate whether introduction into the Netherlands markedly influenced its host. As in other countries, the ecology of host and parasite were studied.

Reproduction, mainly expressed in terms of progeny number and rate of development, much favoured the aphids over its parasite. However, winter mortality was high for the aphid and much less for the parasite. So parasitism is high at the beginning of the season, about the end of May. It could even be 100% if the female parasites were to find all aphid hosts available. As this is not so, there remains an unparasitized aphid population, which increases faster than the population of the parasite, causing a gradual decrease in parasitism. Parasitism is lowest at the end of July or the beginning of August, but increases afterwards as a result of a decline in aphid population caused by a changing physiological condition of the food plant, apple, which does not affect *Aphelinus mali*. During September and October, parasitism decreases as the parasite enters diapause, whereas the aphid still reproduces. The increase in parasitism and the decline of the aphid population in July and August is often wrongly interpreted as cause and effect.

In the Netherlands, the parasite cannot adequately control the woolly aphid. No doubt, however, it must be effective to some degree, both in early spring and in summer when the host population is lowest and parasitism highest. Then the small aphid populations are still more strongly reduced and the rapid build up is impeded.

The other major apple aphids, namely the apple-grass aphid *(Rhopalosiphum insertum)*, the rosy apple aphid *(Dysaphis plantaginea)* and the green apple aphid *(Aphis pomi)* are parasitized by a number of Aphidiidae(Hymenoptera), each aphid having one main parasite, *Monoctonus cerasi*, *Ephedrus persicae* and *Trioxys angelicae*, respectively. *M. cerasi*, though mentioned in the literature from several aphid species on a number of food plants, seems a strictly specialized parasite of *R. insertum* on apple. It has two generations in spring, corresponding with the two spring generations of its aphid host on apple. When the aphid migrates to grasses, its summer host-plants, the full-grown larva of the parasite enters diapause. When the aphid returns in September and October to its winter host, apple and related trees and shrubs, some of the parasites come out of diapause and the female *M. cerasi* pararitizes the oviparae. This may be of real economic importance, as the number of oviparae parasitized is proportional to the reduction in number of winter eggs. When parasitism is, for instance, as high as 50%, as observed occasionally, the economic threshold for control of the apple-grass aphid may not be reached, so that chemical treatment is unnecessary.

E. persicae is a rather polyphagous parasite of aphids living within leaf curls on several trees and shrubs and *T. angelicae* is a parasite of several aphid species, mainly of the genus *Aphis*.

Hyperparasites may seriously interfere in the action of the primary aphidiid parasites, especially in *A. pomi*, which aphid may stay on young apple-trees with growing shoots for the greater part of the summer season. *T. angelicae* may be nearly completely eliminated by hyperparasites at the end of the summer.

The three host—parasite complexes, *R. insertum* —*M. cerasi*, *D. plantaginea* — *E. persicae* and *A. pomi*— *T. angelicae* have four hymenopterous hyperparasites in common, namely *Dendrocerus carpenteri* (Proctotrupoidea, Ceraphronidae), and *Asaphes vulgaris*, *Asaphes suspensus* and *Pachyneuron aphidis* (Chalcidoidea, Pteromalidae). Cynipidae Alloxystinae (= Charipinae) seem generally more specialized and are represented for the three complexes by *Phaenoglyphis villosa*, *Alloxysta arcuata* and *Alloxysta pleuralis*, respectively.

As to aphid predators, adult Coccinellidae, especially *Adalia bipunctata*, *Coccinella decempunctata* and *Propylaea quatordecimpunctata* may be important in early spring. They can then destroy a large proportion of fundatrices before these reproduce. The role of other predators like the larvae of Syrphidae and Neuroptera is less evident. The species frequenting apple aphids are polyphagous aphid predators, except for *Cnemodon vitripennis*. The larvae of this species are, at least in apple orchards, restricted to the woolly apple aphid as a prey.

LEAF-MINERS

The apple leaf-miner *Stigmella malella* (Lepidoptera, Nepticulidae) is the most important apple leaf-miner in the centre of the Netherlands. Its most effective parasite, *Chrysocharis prodice*, controls the larvae within the mines at a low population density (Section 1.7). Two other common larval parasites of the leaf-miner are *Cirrospilus vittatus* and *Achrysocharella chlorogaster*, whose numbers in apple orchards are in a proportion of about one to three or four. When the population density of the leaf-miner increased, the density of the parasite *C. vittatus* generally increased more than proportionally, indicating that the parasite can control the leaf-miner. As the balance is far above the economic threshold of the leaf-miner, the parasite seems of little use in biological control of *S. malella*.

Another drawback of *C. vittatus* is that the time of emergence of parasite and leaf-miner do not closely coincide. The same holds for the autumn, end of September and beginning

October, when hardly any adequate stages of the host are available. This suggests that *C. vittatus*, which is a pantophagous parasite of leaf-miners on trees and shrubs, must have one or more alternative hosts that are more suitable, perhaps in quite a different habitat.

LEAF-ROLLERS

As leaf-rollers (Lepidoptera, Tortricidae) are major pests in apple orchards, their parasite complex has been studied. More than 50 species of parasitic Hymenoptera and a few species of Tachinidae (Diptera) were reared from caterpillars and pupae on an artificial diet. The hosts belong to the eight more common leaf-rollers of apple, *Adoxophyes orana* being the most important. Almost all biological categories of parasites were represented: ectoparasites and endoparasites; solitary and gregarious parasities; monophagous, oligophagous and polyphagous parasites; egg, larval, pupal, egg-larval and larval-pupal parasites; primary and secondary parasites.

Host specificity was studied especially in two *Ascogaster* species, namely *A. rufidens* and *A. quadridentatus*. The former was found to be an egg-larval parasite of Tortricidae Tortricinae, the females of which lay their eggs in batches, the latter of Tortricidae Olethreutinae, which lay their eggs separately. The searching behaviour of the female parasite was supposed to be responsible for the difference in discrimination between types of host.

Identification of the parasitic Hymenoptera gave large problems, because most of these Hymenopterous groups need thorough biotaxonomic revisions. As long as these revisions do not exist, reliable identification is impossible.

One chalcid species, namely *Colpoclypeus florus*, seems of particular importance in control, especially of *A. orana*. This oligophagous gregarious ectoparasite of the older larvae of Tortricidae has many generations a year. In Dutch apple-orchards, however, it was practically never observed before the second half of July, after which it reproduced rapidly. Thus parasitism in September may be almost complete. The drawback of the parasite is that its last generation develops to adults at a time when there are only overwintering young larval stages of the host and these are not accepted by the female adult parasite. Thus these parasites die before winter without progeny and recolonization has to start next year from outside the orchard.

As *C. florus* is an oligophagous parasite of Tortricidae, large numbers of leaf-roller caterpillars were collected from as many plant species in as many habitats as possible. The caterpillars were fed separately on an artificial diet and resulted ultimately in many leaf-roller species, but also in many Hymenopterous parasites and some Dipterous parasites. However *C. florus* could not be recovered in the Netherlands. Thus how this parasite hibernates remains a mystery.

Many other parasitic species were reared, different from those obtained from apple leaf-rollers. Even the same species of leaf-rollers collected from apple and from other food plants, often yielded different parasites or the same parasites in quite different proportions. So the value of alternative leaf-roller hosts on the various food plants inside and outside apple orchards is doubtful.

Several strains of *Trichogramma embryophagum cacoeciae* were reared in mass and applied against the eggs of the apple leaf-rollers, especially of *A. orana*. Though this sometimes gave good results, the effect was generally unreliable. This was often ascribed to bad weather. Moreover, the parasite proved susceptible to a variety of pesticides used against other apple pests and against diseases (Section 1.3).

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1.7 Natural control of the leaf-miner Stigmella malella in apple orchards

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INTRODUCTION

In apple orchards in the Netherlands, several species of leaf-miner occur, including *Stigmella malella, Stigmella pomella, Lithocolletis blancardella, Lithocolletis corylifoliella, Leucoptera scitella, Lyonetia clerkella, Ornix guttea,* and *Phytomyza heringiana.* From a practical point of view, *S. malella* is the most important, as it may cause persistant outbreaks which, until recently, were not easy to control even with chemical insecticides. Heavy attack by *S. malella* causes premature leaf-drop, which may result in insufficiently developed fruits. Outbreaks of *S. pomella, L. blancardella,* and *L. scitella* are rare, and the other species are not of practical importance.

The trouble caused by *S. malella* during the first years of trials on integrated control at De Schuilenburg (Section 1.1) prompted an investigation into the causes of outbreaks, aimed at ecological or selective chemical control.

METHOD

Relative population densities of *S. malella*, in terms of number of mines per leaf, was assessed in different plots at De Schuilenburg. The assessments were concentrated on three situations: two neighbouring plots receiving no insecticides, aged 25-35 and 3-10 years, of 1 and 0.5 ha, respectively, and a selectively sprayed plot of 1 ha, 5-15 years old, part of a larger selectively sprayed area 200 m away from the unsprayed plots, and separated from these by a cherry orchard and a heavily sprayed apple orchard. Mortality was assessed and the causes of it identified as far as possible, by analysing samples of mines and breeding parasites from these. The reproductive potential of *S. malella*, potential predators, and some details of the biology of the most numerous parasites were studied in the laboratory.

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RESULTS

Biology and natural control factors

In the Netherlands, *S. malella* has two full and a small, practically negligible, partial third generation. The tiny delicate moths are sensitive to wind and low relative humidity. They fly during the day at wind speeds below about 3 m/s, temperatures over 15 ^{o} C, relative humidities over 60%, and an overcast sky. Under experimental conditions, the average number of eggs deposited per female is about 60. The flattened eggs are attached to the underside of the leaves, which the larvae enter directly without going into the open. The larvae produce an elongated gallery about 4 cm long, which they leave when full-grown to spin a cocoon and pupate in the litter and soil. The insect hibernates as a pupa in the soil.

Predators of *S. malella* larvae include the larvae of Chrysopidae, which pierce and suck them with their jaws, *Forficula auricularia*, which eat the larvae from the mines, and predatory bugs.

Parasites, all of them chalcids, probably cause more larval mortality than do predators. There are several larval ectoparasites, of which *Cirrospilus vittatus* rates first and *Achrysocharella chlorogaster* second in abundance. They kill the *Stigmella*-larvae at oviposition. The adult parasite emerges from the mine in the same year or hibernates in the mine, in the fallen leaf. *C. vittatus* also kills *Stigmella* larvae through host-feeding. It is rare and causes little mortality during the first generation of *S. malella* but can be abundant during the second.

The larval endoparasite, *Chrysocharis prodice*, is inconspicuous because it emerges from its host after the latter has fallen to the ground and spun its cocoon, but before it pupates. Contrary to *C. vittatus*, which attacks many species of leaf-miners, this parasite is specialised on *S. malella*. It is well synchronized with its host, emerging about three weeks after the moths of *S. malella*, at the time when the new generation of mines is present. *C. prodice* attacks all larval instars of *S. malella*. Hibernation occurs as a pupa in the soil, within the cocoon of *S. malella*. *C. prodice* can cause considerable mortality to both the first and the second generation of *S. malella*.

A third and minor category of mortality to the larvae of *S. malella* is through what appears to be a hypersensitivity reaction of the leaf, resulting in a necrotic patch in which the mine stops and the larva in it dies.

A large part of the larval mortality in the orchard is difficult to attribute but, judging from exclusion experiments, probably mainly of a biotic nature.

Nothing is known about the causes of mortality during the pupal stage, in the soil, but its rate appears to be high.

The fluctuations in population of *S. malella* are characterized by relatively low numbers in the first, and relatively high numbers in the second generation, resulting in a zig zag pattern when numbers are plotted against generation (Fig. 1.). Apparently, generation mortality is (far) below that required for the population to be stable in the first generation, and (far) above that limit in the second generation. In the 'unsprayed' plots, the numbers of the mines fluctuated at a low level (Fig. 1 A); in the selectively sprayed part of the orchard, they were so high in 1969-1971 that suppression with selective insecticides was

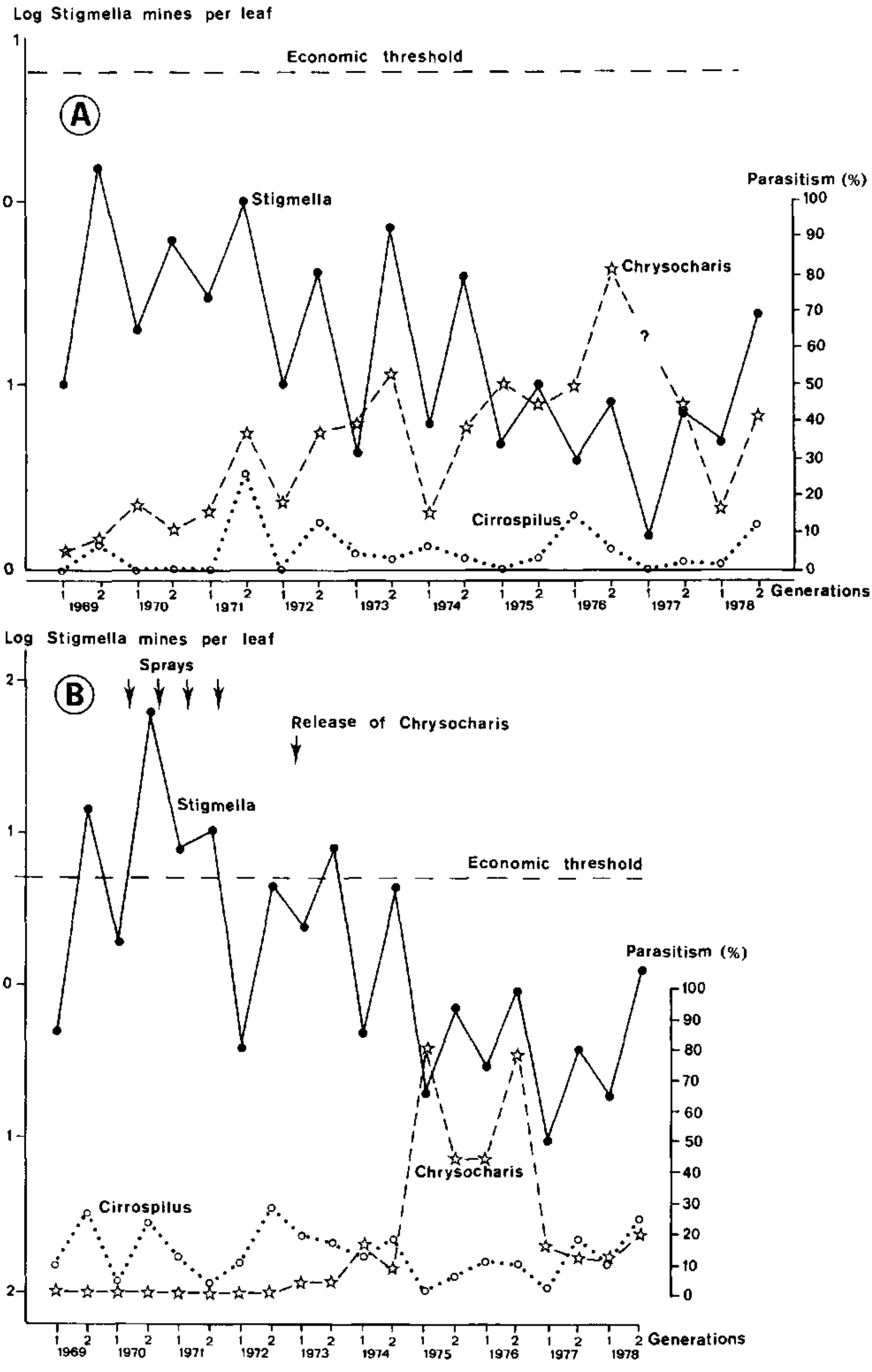

Fig. 1. Fluctuations in the number of *Stigmella malella* mines per leaf and in parasitism by two parasites. A. Orchard not sprayed with insecticides. B. Orchard sprayed with selective insecticides.

necessary (Fig. 1 B).

The mortality of *S. malella* in the two situations was found to differ in certain respects. The parasite *C. prodice*, occurring in the 'unsprayed' plot from the beginning and causing a high mortality in some years, was absent in the selectively sprayed plot.

Since the delayed density-dependence of mortality caused by this parasite suggested that it might be the agent regulating the population of *S. malella*, it was collected and released in the selectively sprayed part of the orchard (Gruys, 1975). Its increase was associated with a gradual decline in the population of its host (Fig. 1B). Presumably, it did not enter the selectively treated area earlier, on its own, because this was effectively screened from the 'unsprayed' area by the intensively sprayed orchard in between.

An attempt to obtain experimental proof of the effectiveness of *C. prodice* in biological control, by creating plots in the selectively treated area with and without the parasite, failed because, once introduced, it rapidly dispersed over the whole area.

The polyphagous parasite *C. vittatus* was present in the selectively sprayed area in the years of high population density, but did not control *S. malella*. So it is ineffective for biological control.

Besides the original difference in the occurrence of *C. prodice*, there are other differences in the pattern of mortality between the selectively sprayed and the untreated areas, particularly during the second generation. In the selectively sprayed area, mortality during the larval stage in the mines was highest, whereas population reduction in the period from pupation to the next generation (mortality in the soil?) was least. High mortality of the larvae, of unknown cause, was at least partially responsible for the sharp decline in population in the selectively sprayed plot in 1973-1974.

A more detailed analysis of mortality in natural control of *S. malella* is in progress.

Insecticides

There are few chemicals capable of controlling *S. malella* effectively. The organophosphates azinphos-methyl and particularly methidathion are effective, if application is carefully timed and repeated. However these insecticides are unsuitable for integrated control since they upset, for instance, the spider mite — typhlodromid predator relationship. The selective botanical insecticide, Ryania, gave sufficient control if carefully timed and applied repeatedly. However, from a practical point of view, it is not a very satisfactory control agent, being expensive and difficult to spray.

Recently, two new and very effective agents for the control of *S. malella* have come onto the market: diflubenzuron and the synthetic pyrethroids. Whereas the latter do not hold much promise for integrated control, diflubenzuron has been included successfully in the integrated program, to control several pests (Section 1.1). One of its side-effects is to reduce the population of *S. malella* and its specific parasite *C. prodice* dramatically. The effect of diflubenzuron on the parasite is indirect: it is due to reduction in the numbers of the host. In the few hosts still present after several years of a spray schedule including diflubenzuron, the parasite was still present, and able to find its scarce hosts. Apparently, it possesses a high searching power.

PROSPECTS

Although the evidence presented for the effectiveness of *C. prodice* in control of *S. malella* is circumstantial, the parasite is used in the integrated program. Practical introduction of this program will not bring further evidence on the indispensability of *C. prodice* as long as diflubenzuron is another essential part of it.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

1.8 The sterile-male technique in control of Adoxophyes orana (Lepidoptera: Tortricidae)

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The leaf-roller *Adoxophyes orana* is presently controlled in our apple orchards with broad-spectrum insecticides like azinphos-methyl, permethrin and carbaryl. These cannot be included in integrated control programs currently being developed. Alternative selective methods are therefore needed.

The sterile-male technique could be such a method. It requires a good knowledge of all major aspects of the biology and population dynamics of the insect, a good sterilization method, cheap mass-rearing, good mating competitiveness on the part of the released males, a low initial population density of the pest in the area of release, low immigration into the release area, and suitable methods of release and monitoring.

BIOLOGY

A. orana has been subject to many studies in the Netherlands (De Jong et al., 1971). This polyphagous insect thrives on a wide variety of perennials. It usually has two flight periods per year, one in June and one in August and September. The species hibernates as a third-instar larva on the tree. These larvae become active in early spring, feed on the buds and leaves, and are usually full-grown by the end of May. Their offspring, the summer generation, feed on leaves and fruits. They cause most of the damage. The offspring produced during the second flight are more numerous but cause only superficial damage to fruits.

STERILIZATION

The first problem in sterilization is a suitable method and a suitable moment in the life cycle. Chemical sterilization was not studied because of toxicity and environmental hazards. Irradiation with X-rays was therefore used.

The effects of this sterilization method were extensively studied by Snieder et al. (1973). The adults proved to be most suitable for irradiation. Doses of 250 Gy of X-rays to male moths reduced hatching by 90-95% in eggs laid by untreated females to which the males were mated. The few surviving offspring were mostly sterile. Because of this effect, this dose was expected to suffice in later release trials. The dose caused complete sterility in females. This allowed simultaneous release of male and female moths. Almost complete sterility could only be reached with a dose of 350 Gy. This dose would probably have a more deleterious effect on the quality of the released moths.

MASS-REARING

An artificial medium was developed (Ankersmit et al., 1977a) and the rearing technique was optimated by analysing the rearing system and modelling it with a computer program written in Fortran IV. It indicated a rearing cost per moth of ƒ 0.015-ƒ 0.030 in 1973, depending on wages and size of culture. Conditions of rearing were 20 °C, 70% relative humidity and light and dark phases of 16.5 and 7.5 h.

A higher temperature, though speeding up development, decreased production per rearing unit and therefore raised cost. At higher relative humidity, moulds developed on the medium. This may serious reduce the daily output of moths. In our culture, a daily production of 5 000-10 000 moths was obtained. This sufficed for a release trial in orchards of 1-2 ha. In the culture, a multiplication rate per generation of 15 times was found. This allowed rapid adaptation of culture size.

QUALITY CONTROL.

Competitiveness in mating and flight activity are the most important aspects. Quality can be affected by the use of a laboratory strain, the rearing medium or by irradiation effects. Competitiveness in mating was studied with moths labelled with ^{32}P. These moths produce labelled spermatophores which can be distinguished in the bursa copulatrix from unlabelled ones. Tests with labelled and unlabelled moths in field cages indicated that the dose of 250 Gy had little effect but with the laboratory strain mating was reduced by 40-50% as judged by spermatophore transfer. The selection of a laboratory strain, however, seems the less likely explanation or this selection must occur within one generation as mating competitiveness in newly collected strains was similar to that of the laboratory strain after one generation. An effect of diet on mating competitiveness seemed possible.

Flight activity of reared irradiated moths was reduced too. When equal numbers of irradiated and unirradiated moths were released, recapture in pheromone traps of irradiated males was reduced by 20%. All moths were marked with Rotor dyes (ICI, London). The effect of marking had to be studied in trials outside the natural flight period. Recapture of marked moths was reduced by about 25%.

MIGRATION

Calculations with the Berryman model for the sterile-male technique showed that immigration of native fertile moths easily upsets all release programs.

Dispersal of *A. orana* was studied by Barel (1973). Released males can be recaptured to a distance of a few hundred metres. Only 1-2% of the male population emigrated from an orchard. Immigration into the orchard depended much upon population density in the surrounding area. As the moths tended to avoid open spaces, meadows may provide a barrier for migrants.

During release trials, an effort was made to measure immigration. We assumed that the

Table 1. Calculation of the number of immigrated males *Adoxophyes orana*. From Ankersmit et al. (1977b).

Year	Flight	Corrected ratio of released moths	Expected ratio of native moths	Counted native males	Immigration (approx.)
1973	June	0.11	129/1170	490	700
	August	0.05	51/1020	800	200
1974	June	0.07	251/3586	160	3400
	August	0.28	203/725	90	600

ratio of released moths recaptured to total number released would be nearly the same as the number of captured native moths to the total numbers of native moths. As flight activity of reared irradiated marked moths was lower, the number recaptured was corrected by a factor 0.6. From the ratio and the number of captured native moths, we calculated the actual number of moths. As an absolute estimate was made by counting, the difference between calculated and counted was attributed to immigration. At the low population density, emigration was neglected. The results (Table 1) indicate an immigration of usually a few hundred male moths to an isolated orchard of 1.9 ha; in one estimate, even a few thousand. So immigration seriously interferes with release trials of *A. orana*. Immigration by females is not evaluated in this way. Besides active flight, *A. orana* may disperse as first-instar larvae floating on their threads in the air.

RELEASE TRIALS

In view of immigration, eradication seemed unlikely. It was decided to aim at keeping the population low. According to calculations, a population of 1000 moths in June could be reduced in two flight periods to 100 with a release ratio of 20:1, provided immigration were of minor importance.

Two trials were made, one in 1971 on 1.4 ha near Overberg (Utrecht) and a second in 1973 and 1974 on 1.9 ha near Lienden (Betuwe) (Ankersmit et al., 1977 b). Population in both was reduced by chemical treatment before the trial. Flight activity was monitored at Overberg with light traps and at Lienden with pheromone traps. According to these traps, the attempted 20:1 ratio was hardly ever achieved, even when considerable numbers were released (Table 2).

Moths were collected from the mass culture. The total yield per day was weighed, then sampled for individual weight and checked for sex ratio. This allowed estimation of total number. Subsequently they were marked and divided over a large number of small refrigerator boxes. Irradiation was at room temperature with 250 Gy of X-rays from a ^{60}Co source at the Pilot Plant for Food Irradiation in Wageningen. They were released by walking at constant low pace through the orchards. By this method, only active moths were released.

Counts were made when larvae were full-grown in May and July. Additional counts on young larvae in September gave some preliminary impression of the result. In most trials whole trees were examined in order to obtain an absolute estimate. Counts must be finished before

Table 2. Capture and release data of the two trials. All figures refer to number of male *Adoxophyes orana* in the whole orchard.

	Number caught		Ratio	Number
Flight period	native	released	native: released	released
Overberg June 1971	244	929	1:4	41 000
August 1971	423	5368	1:13	56 000
Lienden June 1973	129	1133	1:9	17 000
June 1974	251	2379	1:9	55 000
August 1974	203	4313	1:21	26 000

the moths emerge or the larvae enter diapause. This restricts the counting period and therefore sample size. Statistical analysis of the counts was impossible because of the few larvae found. In September, when larvae are still more numerous, we estimated the population with $S(\bar{x})/\bar{x} = 0.28$ in 1973, and 0.21 in 1974. The small number of larvae itself and the continuous decrease in number of *A. orana* can be regarded as strong evidence for effect to the method (Table 3). Winter mortality reduced the population from September 1971 to May 1972 to 16%; from September 1973 to May 1974 to 13% and from September 1974 to May 1975 to 6%. In the last period parasitism was high.

Spilonota ocellana increased rapidly from less than 100 per ha in 1973 to almost 5000 per hectare in 1975. *Operophtera brumata* became numerous too. Leaf-roller damage to fruits by other species than *A. orana* amounted to 4% in 1973 and 9% in 1974.

The most likely explanation of increase in other pests is omission of insecticidal applications, but as larvae of *A. orana* are agressive and attack any intruder in their territory, competition may also contribute.

The sterile-male technique can reduce the population to an extremely low level, but the ultimate goal is not reached as chemical control against other leaf-rollers remains necessary. The method requires continuous release of large numbers of moths per generation to deal with immigrants and seems therefore too costly.

Table 3. Numbers of *Adoxophyes orana* and *Spilonota ocellana* per ha in the orchard near Lienden.

Date	Numbers of *A. orana*	Numbers of *S. ocellana*
May 1973	500	65
July 1973	830	
May 1974	170	620
July 1974	100	
May 1975	40	4830

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

1.9 Threshold of economic injury for apple powdery mildew and scab

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INTRODUCTION

Podosphaera leucotricha, causing apple powdery mildew, overwinters in fruit and wood buds. The disease occurs as 'primary mildew' on flower trusses and shoots emerging in the spring. Mycelium of 'secondary mildew', which arises from conidial infections on leaves, can spread and invade axillary and terminal buds. Conidia can also infect buds directly.

Epidemics of apple scab, caused by *Venturia inaequalis*, originate from ascospores discharged from perithecia present on overwintered leaves on the ground. Under conditions favouring the fungus, liberation of the ascospores leads to visible infection of the leaves and the fruits, and subsequently to production of conidia during the rest of the season. In the Netherlands, ascospores are discharged in April, May and June, reaching a peak at the beginning of May.

Damage by powdery mildew comprises reduction of shoot growth and number of leaves on extension shoots, loss of fruit quality and especially of fruit yield in the next year. Scab causes mainly loss of fruit quality. Until now, fungicide applications have been necessary for control of the diseases on susceptible cultivars of apple. In practice, most of the spray applications after flowering are composed of a fungicide mixture to control both diseases at the same time.

Critical economic circumstances and an increasing concern for environmental pollution will cause a cut-back in use of fungicides. A supervised control program meets this need. To adopt supervised control of apple powdery mildew and scab in practice, economic thresholds of injury and the epidemiology of both diseases have to be known in detail. So from 1975, the spread of powdery mildew and scab, the effect of fungicidal sprays, and the effect of the diseases on fruit quality and yield was studied on four apple cultivars.

MATERIALS AND METHODS

The trial included full-grown apple trees of the cultivars Cox's Orange Pippin, Golden Delicious, James Grieve, and Jonathan. The trees were about 3 m in height, planted in five isolated fields, each subdivided into equal-sized plots, with 6-8 trees of a cultivar in a row depending on the rootstock type, which did not affect the development of the diseases and will therefore be disregarded.

Until 1976, the trial was managed as usual, with a mixture of captan and dinocap for control of scab and powdery mildew. In the spring of 1976, scab was not observed and primary mildew was observed only occasionally, except for two plots with trees of the cultivar Jonathan on which a minor attack was noticed. In 1976, 1977 and 1978, fungicide sprays were applied regularly if necessary to slow down the spread of scab and powdery mildew: captan 83% (w/w) wettable powder (1.0 kg of active ingredient in 180 litres of water per hectare) and bupirimate 50% (w/w) wettable powder (0.225 kg of active ingredient in 110-180 litres of water per hectare) or triadimefon 25% (w/w) wettable powder (0.04 kg of active ingredient in 180 litres of water per hectare) were used for this purpose.

In 1976, captan was not applied for scab control. In that year, bupirimate was applied weekly in 3 fields from mid-June to the end of July, to slow down the spread of secondary mildew on plots where more than 20% of the leaves was mildewed. In 1977, all fields were sprayed with captan and bupirimate from the beginning of June to the end of July, the one half of each field receiving applications once a week and the other half once a fortnight. In 1978, all fields were sprayed with captan and triadimefon once a week (before blossom once per 10 days) as soon as circumstances for infection became favourable. On one half of each field the applications were omitted after the beginning of June for control of scab or after the end of June for control of powdery mildew. On the other half of each parcel, spraying was continued until the beginning of August, triadimefon being replaced by bupirimate.

In August and September of 1976 and 1977, captan was applied 6 times for control of fruit rot. In August and in September 1978, thiofanate methyl 70% (w/w) wettable powder (0.84 kg of active ingredient in 180 litres of water per hectare) was applied once for that purpose.

RESULTS AND DISCUSSION

Some of the results have recently been published (Van der Scheer, 1978). With the same (small) amount of primary mildew in spring, secondary mildew spreads faster on Golden Delicious than on Cox's Orange Pippin and Jonathan, although the last two cultivars are known to be more susceptible. However the fungus overwintered better in the buds of Cox's Orange Pippin and Jonathan than in those of Golden Delicious and this parameter strongly determined how quickly the disease spread. A second factor contributing to the ultimately higher susceptibility of Cox's Orange Pippin and Jonathan to *P. leucotricha* is the higher tolerance of Golden Delicious to the pathogen. With the same proportion of attacked leaves, the general leaf condition was visually better on Golden Delicious than on Cox's Orange Pippin or on Jonathan.

As in supervised control of insects, it was hoped that control measures could await infection. For powdery mildew, that procedure was successful in 1976, because the plots were almost free of overwintering inoculum (Table 1). In 1977, however, primary mildew occurred, after the build-up in 1976. Spraying with bupirimate in 1977, after diagnosing the amount of primary mildew after flowering, limited the spread of the disease only to some extent in that year and russeting due to mildew was seen on Jonathan apples. In con-

Table 1. Effect of fungicide spraying on average incidence of powdery mildew on four apple cultivars.

Year	Times of spraying	Number of emerging buds with primary mildew in a 10-m row of trees	Proportion of leaves with secondary mildew (%)	
			June	July/August
1976	occasionally in July	5	10	7
1977	from June every 7 days	36	26	14
	from June every 14 days	14	32	36
1978	every 7 days	-	1	2
	every 7 days until end of June	-	1	3

trast to the situation in 1977, the fungus had no chance in 1978 to spread rapidly because of regular spraying in good time. Termination of spraying after 22 June 1978 on parts of the fields led to an average increase of 1% in diseased leaves in August.

In 1978, the yield was not affected by the amount of diseased leaves in the same year, but by that of diseased leaves in the preceding year. An average increase of 22% in diseased leaves in 1977 led to a decrease in yield in 1978, which proved to be correlated significantly at the 5% level only in the Jonathan cultivar (r = - 0.64). Regression analyses showed that an increase in diseased leaves in 1977 on Jonathan led to a decrease in yield in 1978 of 0.1 kg per tree. This agrees with the results obtained by Butt et al. (1977).

Incidence of scab on the leaves started in July 1976 and the disease spread explosively. However the proportion of scabbed apples in that year was limited, also by the spraying with captan against fruit rot, and exceeded 1% only in some plots. This epidemic resulted in over-

Table 2. Effect of early termination of spraying with fungicide on incidence of scab on four apple cultivars.

Cultivar	Termination of spray applications	Shoots with scabbed leaves (%)		Scabbed fruits (%)
		3 August	at fruit picking time	at picking time
Cox's Orange Pippin	7 June	2 a[2]	3 a	0.26 a
	10 August	1 a	1 a	0.02 a
Golden delicious	7 June	21 b	63 b	1.60 a
	10 August	1 a	1 a	0.40 a
Jonathan	7 June	13 b	30 b	0.20 a
	10 August	1 a	3 a	0.08 a
James Grieve[1]	7 June	29 a	35 b	5.22 b
	10 August	15 a	8 a	1.20 a

1. Premature leaf drop due to scab attack.
2. a, b: pairs of values with the same letter are not statistically significantly different.

Table 3. Correlation between the proportion of shoots with scabbed leaves at picking (y) and of scabbed fruits (x) in four apple cultivars.

Cultivar	Regression	Correlation coeff.
Cox's Orange Pippin	$x = 0.11\ y - 0.0004$	0.77
Golden Delicious	$x = 0.02\ y + 0.0019$	0.61
Jonathan	$x = 0.01\ y - 0.0004$	0.84
James Grieve	$x = 0.13\ y + 0.0035$	0.61

wintering inoculum and subsequently in attack of the rosette leaves from the blossom buds in 1977. Regular spraying with captan after diagnosing the amount of scab on the rosette leaves, was not sufficient to prevent fruit attack. So in 1978 spraying was started as soon as circumstances favoured infection with scab and was stopped at the beginning of June on half of each field. The proportion of scab that occured in 1978 on the four apple cultivars is summarized in Table 2, and the relation between the proportion of shoots with scabbed leaves and of scabbed fruits in Table 3. The cultivars differed widely in susceptibility to scab, James Grieve being the most susceptible, with a low ratio between proportion of shoots with scabbed leaves and of scabbed fruits.

FUTURE OUTLOOK

The first steps towards supervised control of fungal diseases have been taken. If scab or powdery mildew is seen in a previous year, control cannot await disease symptoms. This is in contrast to the management in supervised control of insects. A decrease in the number of sprayings for control of powdery mildew seems possible at the end of the routine spray program without economic injury. For control of scab, this manner of disease management is somewhat more risky than that for control of powdery mildew, especially on susceptible cultivars with a low ratio between proportion of shoots with scabbed leaves and that of scabbed fruits.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

1.10 Fungi from phyllosphere and carposphere in relation to integrated control in an apple orchard

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DESCRIPTION OF THE PROBLEM

In De Schuilenburg experimental orchard at Lienden, the last twelve years have been spent in trials on biological and integrated control of insects. However, fungal diseases were not included. So in 1975, a project was started to find ways for integrated control of fungal diseases, such as apple scab.

In laboratory experiments, the following fungi were used: *Venturia inaequalis*, the cause of apple scab; whose conidial stage is called *Spilocaea pomi*, *Nectria galligena*, the cause of apple canker; and *Nectria cinnabarina*, which causes dying of branches in deciduous trees. These three strains were obtained from the Central Bureau of Fungal Cultures at Baarn.

The following saprophytic strains were isolated from the phyllosphere of apple at the orchard in Lienden: *Alternaria alternata*, *Cladosporium cladosporioides*, *Phoma macrostoma* and several yeast strains.

AIM AND MOTIVATION OF THE RESEARCH

Because fungal diseases may cause great losses of yield in orchards, a program of fungicidal sprayings was established. A spray schedule with 12-16 treatments a year is not uncommon. The costs for such spraying programs are high and the fungicides used till now have a wide action spectrum.

Little is known about the interaction between various fungal species in the phyllosphere or leaf ecosystem, and the carposphere or fruit ecosystem. The aim of this study was to learn more about these mutual relations in order to develop an effective integrated control of plant diseases.

WORKING PROCEDURE

The research comprised three steps.

- Identifying the fungi present in the phyllosphere and carposphere of apple under natural conditions, by the plate dilution method.
- Isolation of fungi from leaves of apple and testing their mutual relations.

- Study of the influence of pollen, present while the tree is in flower, in relation to germination of spores of phyllospheric fungi.

RESULTS AND DISCUSSION

The fungal population

In the orchard the composition of the fungal populations in the phyllosphere and carposphere in different plots was determined after various pretreatments with pesticides:
- intensive chemical control with insecticides and fungicides (dodine, captan and dinocarp);
- integrated control of insects, combined with the fungicides dodine, captan, and bupirimate;
- untreated.

For the survey the saprophytic fungi were divided into eight groups of genera. The results gathered in 1977 on the phyllosphere of the apple cultivars 'Schone van Boskoop' and 'Golden Delicious' indicated the following:
- The counts of fungi, especially white and pink yeasts, in the phyllosphere of 'Schone van Boskoop' were higher than in the phyllosphere of 'Golden Delicious'.
- There is also a great difference in the number of the phyllosphere fungi between the untreated plots and the two treated plots. The *Aureobasidium* group and the *Phoma* group were even absent in the treated plots of the variety 'Schone van Boskoop'.
- White and pink yeasts were the dominant fungi found in the phyllosphere.
- In the untreated plot, most groups (white yeasts, pink yeasts, the *Aureobasidium, Phoma* and *Cladosporium* groups) showed two peaks during the season. The first coincided with the spread of apple pollen and the beginning of fruit setting. The second peak was at the end of July or August, and is as yet unexplained.
- There were sometimes differences in the density of the fungal population with integrated control and heavy spraying.

The survey of the carposphere of 'Schone van Boskoop' in the untreated plot from 23 May till 2 October 1976, indicated the following:
- Highest numbers were of the white yeasts, followed by the *Cladosporium* group and then by the pink yeasts.
- These three groups showed two peaks. The *Phoma* group also has two peaks but just after those of the three groups mentioned. The *Aureobasidium* group has only one peak at the end of August. The *Alternaria, Penicillium* and the unidentified group followed a sinusoidal course during the season, with an increase for two groups at the end of the season.

Interrelations of fungi isolated from the phyllosphere

The counted phyllosphere fungi and their interrelations are so numerous that a choice had to be made on cultivation methods and the combinations of interacting fungi including the study of the influence of some parasites on yeasts strains from apple leaves. The influence of metabolic products of hyphal fungi, *Alternaria atrata, Cladosporium cladosporioides, Phoma macrostoma* and three parasites *Venturia inaequalis, Nectria galligena* and *N. cinnabarina* were tested on a white and a pink yeast, the latter being stimulated in growth by metabolic products of the phyllosphere fungi and the three parasites.

The experiment with the three phyllosphere fungi was repeated with eight yeast strains isolated from apple leaf, 4 white and 4 pink. The metabolic products of the three hyphal phyllosphere fungi stimulated growth of the yeasts, especially those of *C. cladosporioides*.

The influence of the metabolic products of three hyphal phyllosphere fungi, two yeasts and the three parasites upon germination of the conidia of *C. cladosporioides* and *P. macrostoma* was as follows:

- Metabolic products of *A. atrata* strongly inhibited spore germination of both species.
- The conidia of *C. cladosporioides* germinated less in all metabolic products tested than in the control.
- The conidia of *P. macrostoma* resulted in a higher percentage germination in the metabolic products of the three parasites, *C. cladosporioides* and *P. macrostoma* itself.

The influence of apple pollen

Apple pollen stimulated germination of the spores of the phyllosphere fungi *C. cladosporioides* and *Botrytis cinerea*. This effect was smaller for conidia of *A. atrata*.

PROSPECTS

For integrated control of apple scab, it is necessary to know the micro-organisms in phyllosphere and carposphere and their mutual relations with each other and with *Venturia inaequalis*. The laboratory experiments have shown some aspects of these mutual relations. To reach an effective integrated control, it is most promising to use organisms from the phyllosphere and carposphere of apple trees, which show no or little damage by *V. inaequalis*. Such a situation approximates fairly well the endemic relation between the pathogen and the host. The economic loss caused by *V. inaequalis* is small and shows a sinuous course during the years. The idea is now to cut off the highest peaks of this sinusoid by manipulating the micro-organisms or other biotic factors such as pollen in the phyllosphere and carposphere. The metabolic products of *A. atrata* that inhibit the germination of the conidia of other fungi may allow this. Another approach would be to stimulate the saprophytic phyllosphere fungi by pollen in order to inhibit germination and infection by *V. inaequalis*.

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2 Field crops

In North-West Europe, aphids are the major insect pests on arable crops. As vectors of virus diseases, they can quickly spread viruses in potato and sugar-beet fields. A network of observation points to monitor aphid flights and to establish early lifting dates for seed potatoes has been operating for more than 25 years and can be considered as one of the oldest examples of integrated control (2.1). In addition, the mechanisms of the transmission of the potato leaf-roll virus has been described in detail in a fundamental study (2.2). In sugar-beet, the effect of chemical control of aphids has been investigated (2.3). Although the aphid species feeding on cereals are not virus vectors, they can cause much damage (2.4). Finally, a study has started on the significance of predators on the development of aphid populations (2.5).

In sugar-beet crops, changing cultural practices have increased the chance of infestations by pests such as soil arthropods (e.g. springtails) and mangold beetles. The problem has now been identified and new lines of research are suggested (2.6). A study on the occurrence of soil arthropods and other components of the soil mesofauna has been initiated (2.7).

In vegetable crops, the sterile-male technique for control on the onion fly was a major topic in research. This project started about 15 years ago. After many years of preparatory studies on rearing, sterilization and ecology of the insect, a release trial on a practical scale over 20 ha of onions was started in 1978, and extended to almost 40 ha of onions in 1979. The results are encouraging: chemical control was not longer needed. At present, a private company is preparing to take over further practical application (2.8). Another approach to control the onion fly may be genetic, by use of chromosome rearrangements (2.9).

A second project on integrated control in outdoor vegetable crops was started some years ago. Particular attention is being paid to pest insects in cabbage and carrots: the cabbage root fly, cabbage aphids and various lepidopterous species in cabbage, and the carrot rust fly. There is an urgent need for selective chemical control with selective insecticides or if not possible, by selectively acting formulations or dosages of the insecticides. Establishment of a warning system is in progress. Attempts are being made to improve conditions for predators and parasites by intercropping (2.10).

Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

2.1 Integrated control of aphid-borne viruses of potatoes

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INTRODUCTION

Over a hundred years before leafroll was recognized as a very noxious virus disease transmitted by the aphid *Myzus persicae* (Oortwijn Botjes, 1920), seed potato growers had already discovered methods to reduce infection: the tubers had to be planted as early as night frosts permitted, and the crop harvested as early as possible. Roguing, removing plants with virus symptoms, became usage after the nature of the disease was known. By and by more virus diseases of potatoes, transmitted by aphids, were discovered, but the entomological aspects received little attention.

POPULATION STUDIES

Elze (1927) studied the life-history of 4 of the 9 aphids living on potato. In 1936, the author was asked to look into the relation between aphid numbers and virus spread. Spread of leafroll could be correlated with the time and density of aphid colonization shortly after the plants came up. The results were considered classified information and were not published. Attempts to describe quantitatively abiotic and biotic factors influencing the development of populations of potato aphids were discontinued in 1940. Development of 5 syrphids, 3 coccinellids, 3 chrysopas and one braconid was studied at 5 temperatures and their impact examined by providing a surplus of prey, daily renewed, in micro Petri dishes. The parasitization of aphids and larval predators collected in potato fields was followed by rearing. The network of interactions between the various aphids and some 50 other insects present in randomly chosen potato fields was far too complicated for quantitative analysis. However a rapid and very simple sampling method, threshing standing plants over a 50 cm x 40 cm board divided into squares of 10 cm x 10 cm, which dislodged prey and predators alike, made it possible to obtain information on the population and its future development. Recognition of the aphid species was taught to a number of co-workers and threshing data could be used for determining more sensible dates for various parts of the country.

EFFECT OF A CONTACT INSECTICIDE

A trial in 1938 on aphid control, in which small plots with virus sources were doused twice a week from above and below with 0.2% nicotine sulphate had no measurable effect on the transmission of leafroll and Y virus, although throughout the trial the aphid population on the treated plants consisted of only some alatae with young progeny. A sticky trap consisting of a 50 cm x 50 cm copper screen mounted vertically on the rim of a bicycle wheel on a perpendicular axis, placed in the field gave some information about flights but was considered impractical for general use.

OVERWINTERING OF MYZUS PERSICAE

In 1945, the project 'Overwintering of *Myzus persicae*' was started under the auspices of the Netherlands Agricultural Research Council. In that same year, British aphid workers sought contact and an advisorship to that group within the British Agricultural Research Council lead to the solution of many problems.

Peach *(Prunus persica)* and dwarf almond *(P. tenella)* (probably the original winter host), were known as suitable winter hosts though many eggs were laid on other *Prunus* spp. (Hille Ris Lambers, 1946). In 1946, it was found that *P. serotina*, an American birdcherry, planted in millions by foresters, could be an excellent overwintering host for *M. persicae* and therefore a source of vectors in areas where peach trees were extremely rare (Hille Ris Lambers, 1951). Methods for estimating spring flights from these various trees were developed and introduced into practice. But *M. persicae* can also overwinter on herbaceous plants as colonies, and the diversity and distribution of these host plants make a reasonable direct estimate of the importance of that mode of overwintering impossible. However, by utilizing the morphological differences between winged specimens born on herbs and those born in the first 2-4 generations on peach and the other *Prunus* spp. as first found by Gillette & Taylor (1908), it was easy to determine what arrived from the *Prunus* spp. and from herbs early in summer.

CORRELATIONS BETWEEN APHID NUMBERS AND VIRUS SPREAD

Nobody in the Dutch—British group could find satisfactory correlation between the aphid populations in fields and virus spread in those same fields. But Broadbent (1950) found a strong correlation between the total annual catch of winged vectors on sticky traps and virus spread in fields. That made the author conclude that there must be a correlation between catches per day and virus transmission per day. For that study, very large catches were necessary.

THE MYZUS CERTUS COMPLICATION

However, before large-scale trapping of aphids could begin, another problem had to be solved. Since 1929, I had known that another *Myzus* species occurred in the Netherlands, colonies of which could easily be distinguished. It was *Myzus certus*. It lives without host alternation on some herbaceous plants but not potato, can transmit Y virus but not leafroll, and it was suspected to fly earlier than *Myzus persicae*. If the winged aphids could not be distinguished from those of *M. persicae* and flew earlier, considerable losses in yield would result if lifting dates were based on flights of *Myzus*. It took all of two years before dead winged *M. certus* could be reliably distinguished from *M. persicae* by a trained and very experienced specialist. Trapping could begin. The trap catches in following years showed the great need to distinguish between alatae of the two species (Hille Ris Lambers, 1959).

TRAPS AND TRAPPING

A battery-driven suction trap was developed but just before the order for mass-production had to be given, Moericke's (1950b) paper on his yellow waterpans came in. The Paint Institute TNO selected a dye with a maximum remission in the wavelength found optimum for probing in earlier research by Moericke (1950a) and 132 large Moericke traps were in operation within a few weeks. Initially 6 traps were placed in each of the fields, but later two were placed in each field. All traps were emptied daily and the catches were mailed to "Bennekom" for immediate examination. After 3 years, regional NAK[1] laboratories sorted the catches first.

Through a fluke, the system became an immediate success. In 1951 in Friesland no more than 10 aphids were found per plant but at the same time trap catches of alate vectors were as large as they were in the south-west where very large populations of aphids were present on the crop. Against vehement opposition, NAK based the lifting dates on the trap catches, not on the aphid counts on plants. Friesian growers who left their crop in for even a few days beyond the lifting date found it ruined by leafroll. An aphid flight from an area about 90 km away had carried leafroll virus into the Friesian fields. The number of traps had to be increased to 250, the maximum that could be handled. From then on, interest in aphid counts in the field dwindled and the wholesale use of the method was later abandoned.

A SYSTEMIC INSECTICIDE AS A RESEARCH TOOL

In 1951 too, Reestman, Schepers and the author began experiments with a systemic insecticide, Systox, against aphids in connexion with virus-spread. I found double rows of oats effective against the spread of virus from one plot to the next in research on beet yellows, and the same was found in work with potato viruses. The results were very interesting (Fig.1). (Hille Ris Lambers et al., 1953; Schepers et al., 1955).

Spread within the field from the virus sources to healthy plants was virtually stopped by the earliest three applications of Systox when leafroll was involved. Later applications had very little effect. The method of planting, lifting and replanting made it possible to distinguish between spread within the crop and infection from outside. If, however, Y virus, a non-persistent virus (see below), was investigated in the same way, there was no measurable difference in virus-spread between plots treated with Systox and those treated with water. Only when a large number of experiments on Y virus over a number of years and all over the country were pooled was a small statistically reliable difference found between Systox and water plots.

1. General Netherlands Inspection Service for Seeds and Plants.

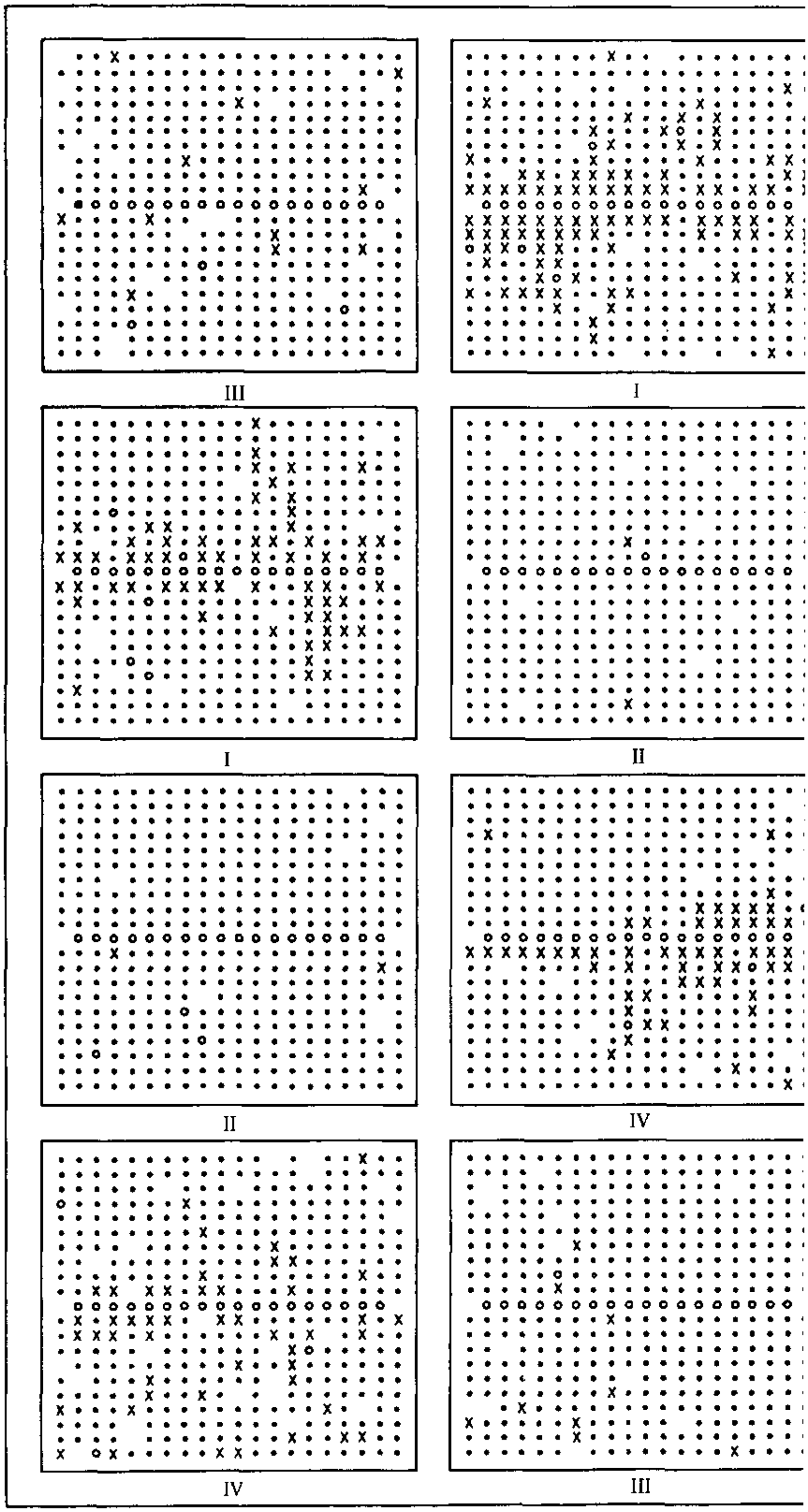
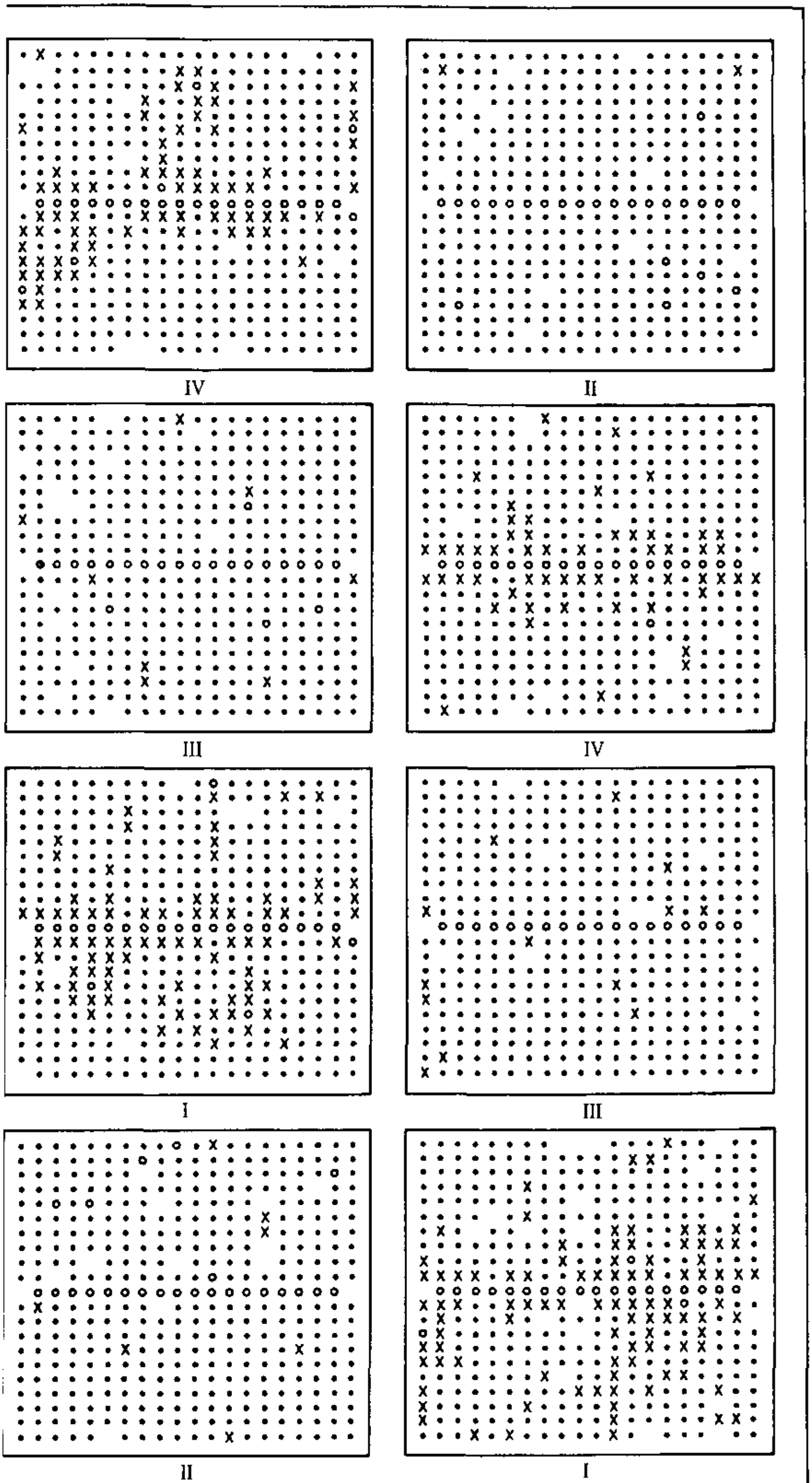


Fig. 1. Spread of leafroll (after Schepers et al.).



I= Sprayed with water (8x)

II= Sprayed with Systox (8x)

III= Sprayed with Systox (3x)

IV= Sprayed with DDT (5x)

o= implanted second season leafroll

x= spread of leafroll

•= no leafroll

CONCLUSIONS

Experiments over many years proved several things.

- A vector free of virus landing on a leafroll source poisoned with Systox ingested the insecticide with the virus and was disabled or dead before the circulation time of the virus in its body, 24-52 h, had passed. That accounted for the suppression of spread within the field.
- A vector with leafroll virus ready for use could not be prevented from infecting at least one potato plant on which it started feeding. So infection from outside the field could not be stopped.
- The effect of later treatments of a series was much less than that of the earlier treatments, despite later, much larger, aphid flights. Apparently leafroll infection occurred early in the growing season.
- Since treatments with nicotine had no effect on leafroll transmission although the aphid population was reduced to about the same level as with Systox, alate aphids coming from outside the field were responsible for both spread of virus within the field and spread from outside the field.

For Y virus, this was even more conspicuous because virus spread in a field with, say, 3000 vectors per plant, was very nearly equal to spread in a field with near zero aphids per plant, provided the aphids were not shaken from plants.

IMPORTANCE OF EARLY FLIGHTS

These results focused attention on early aphid flights. It had long been known that potato plants were much better sources for virus when they were young, and more susceptible to virus infection. Studies on peach trees showed that between the beginning of flight from peach and the first *Myzus* catch in a Moericke or suction trap, there was an interval of 3-6 weeks. In other words, traps were useless for registering early flights of low density. If indirect control of leafroll spread by insecticides should be used at all, it was necessary to poison the plants as early as possible, preferably before they came up.

Consequently the emigration from peach had to be studied more intensively, and the method was introduced of 1000-plant counts, (each plant being used as one trap), in which up to 3000 small potato plants per area were threshed over a small rimmed board. The catches were sent in, the species of winged aphids determined, and the *M. persicae* sorted according to provenance. Advice to spray with systemic insecticides, as well as advice not to spray, was given on the basis of studies on peach and on counts of what had landed on potatoes. With the combination of insecticide application and an early lifting method based on trap catches of *M. persicae*, leafroll ceased to be the most important virus problem in seed potatoes.

PROBLEMS OF NON-PERSISTENT VIRUSES

Before 1959, non-persistent viruses were not a serious problem. The old type Y virus caused such conspicuous symptoms on even very young plants that they could be rogued straight away. But in 1959, a new type of virus, known as new Y or Y^n penetrated from Germany, and was soon found everywhere in the country. The customary measure, early roguing, was often not very effective because the symptoms of the disease often appear late, and then roguing may result in dissemination of viruliferous aphids, with a negative effect. Chemical control of aphids had no significant effect: no poison could kill a winged aphid within the few minutes that it needs to transmit a non-persistent virus. Work in America on some non-persistent viruses had shown that very many aphid species incapable of living on the virus host were nevertheless efficient vectors of such virus. An experiment was made in 1963 with one of the most unlikely aphid species, *Rhopalosiphum padi*, the birdcherry-oat aphid, in which field conditions were imitated in a tripartite cage (3 compartments separated by double wide-mesh screen, vector culture in the first, virus source in the second, healthy plants in the third). The cage could rotate 180^o and the positively phototactic alatae produced in the first flew towards the light to the third. After some time, the cage was rotated resulting in flight in the opposite direction. The operation was repeated many times. Nineteen out of twenty potato plants became infected with Y^n virus. Work by Eastop in Africa and Moericke in Germany had shown that colour attraction differed between aphid species, and that our Moericke traps, though highly attractive for the vectors of leafroll, gave a distorted picture of the abundance of other potential vectors of Y^n virus. So in 1970, a 40-foot suction trap, developed and owned by Rothamsted Experimental Station, was erected near Wilhelminadorp (Province of Zeeland). It randomly sampled volumes of air for organisms ranging from aphids to birds. Catches were examined daily: of the many aphid species caught, 42 were separately counted as potential virus vectors.

SHORTCOMINGS OF TRAPS

Hopes that this trap might be able to register flights of *M. persicae* earlier than Moericke traps proved idle. The 1000-plant counts remained necessary. However data about the abundance of the various suspected aphid species became available, though information about their efficiency as vectors was lacking. Later work by Dr van Hoof supplied some information. Even if complete information on the efficiency of suspected vectors were available, this kind of trap will probably never provide sufficient information about what happens in the most dangerous period of potato development, the 3-6 weeks after coming up (Woodford 1975). Aphid studies hardly altered the Y^n virus problem which was further complicated by an immense increase in diseased groundkeepers. Recent work on Y^n virus control via mineral oils would seem to be more promising.

BY-PRODUCTS OF THE RESEARCH

Others had begun investigations into the feasibility of growing potatoes as a second crop. The great difficulty was that late in the season, potato blight could often not be controlled. When a variety (Spartaan) was bred that was resistant to all familiar physio's of blight, the project became feasible. Studies of the aphid population on plants and of vector flights, had made it possible to choose such a planting date that just when flights had ceased, the plants came up. A sudden drop to zero occurred only where the flights had been very large. And only there could a second crop of seed potatoes with very low virus infection be obtained. In areas where flight was small, it never reached zero or, if it did, far too late and second crops grown there became heavily infected with virus. After several years of success, a new physio of the blight fungus attacked Spartaan which put an end to this economically sound practice. In this case, the earliest possible date for planting had to be forecast long before flights reached zero.

Late roguing of spring crops, hardly avoidable for Y^n virus, could disseminate viruliferous aphids, with negative effects. Therefore spraying with an aphicide four days before late roguing was advised. But many of the now aphid-wise farmers added the ultra-cheap parathion to all their sprays against potato blight, an unnecessary threat to the environment. But though potatoes are sometimes seriously affected by toproll caused by a variety of the aphid *Macrosiphum euphorbiae*, the measure may have prevented the problem in seed potatoes, how ever badly ware potatoes were affected.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

2.2 Transmission of potato leafroll virus

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Potato leafroll is one of the most important virus diseases of the potato plant and is characterized by necrosis of the phloem. A vector of this virus is the green peach aphid, *Myzus persicae*. The aphid feeds mainly in the phloem, to which the virus is restricted; its stylets penetrate the plant tissue to reach the sieve tubes. The virus is ingested with the liquid food and circulates in the digestive tract, from which it passes into the haemolymph. Thence the virus is distributed to the various internal organs including the salivary glands, which are responsible for the transmission of the virus with the saliva into the phloem (Fig. 1).

The stylet bundle lies in a dorsal groove running along all three segments of the labium (proboscis). There are two pairs of chitinous stylets, one maxillary, one mandibular. The maxillary pair is always firmly interlocked and forms a single structure enclosing two minute canals formed by opposing grooves. The dorsal canal or food canal communicates with the pharyngeal duct, while the ventral one joins the salivary pump. The mandibular stylets are closely applied to the maxillary pair.

Each stylet originates from a retort-shaped organ situated in the posterior part of the head. These organs are responsible for the secretion of a new stylet at each larval moult. The four stylets are each conducted by a groove of the epipharynx on the ventral face of the clypeo-labrum and labrum. They are so arranged that the stylets fit snugly into the grooves, being held firmly in position, at the same time having perfect freedom of movement for protraction or retraction. After leaving the head, the stylets cross over into the longitudinal labial groove and extend along it to the extremity of the rostrum. The stylet bundle shows along its length a torsion of 180° and the labium telescopes to expose the stylets during feeding.

The pharynx can be divided in three parts : the pharyngeal duct, valve, and pump. The pharyngeal duct is a prolongation of the food canal and is formed by the epipharynx and the hypopharynx lip. This portion does not exert any sucking action, and is simply a duct, which conveys the plant juices into the pharyngeal pump. The epipharynx is marked by a thick sclerotized plate and reveals a median row of eight sensillum pores, which communicate with the pharyngeal duct. The cup-shaped part of the floor before the valve is provided with two sensillum pores.

The pharyngeal duct is separated from the pharyngeal pump by a valve, of which both the dorsal wall and ventral wall are marked by two cuticular dome-shaped protuberances, the

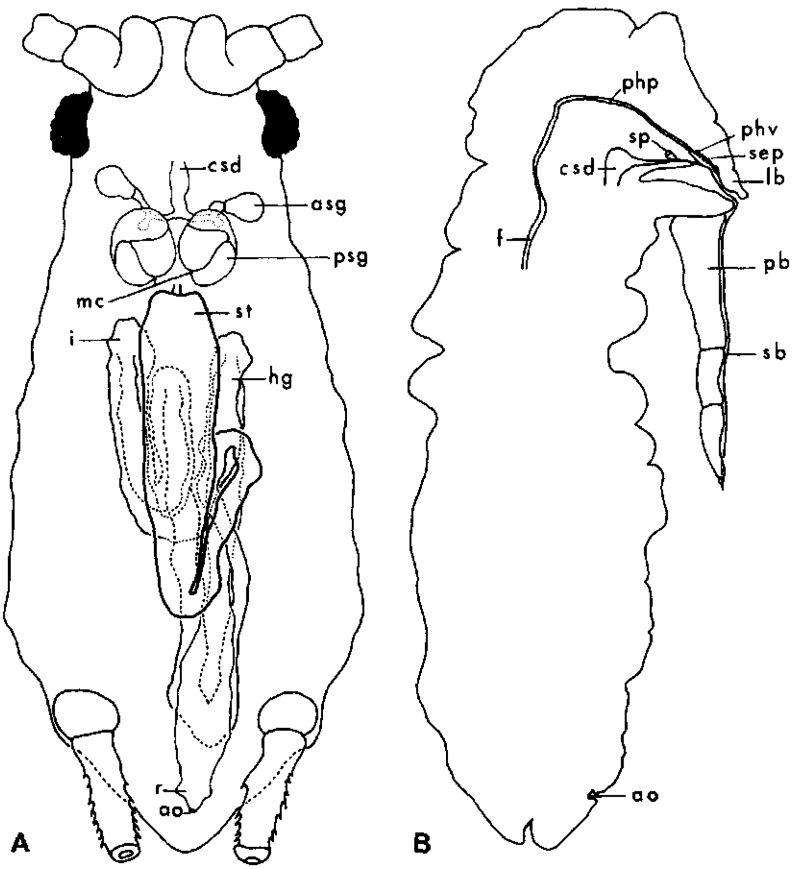

Fig. 1. Dorsal (A) and sagittal (B) view of a one-day-old *Myzus persicae* larva. ao: anal opening; asg: accessory salivary gland; csd: common salivary duct; f. foregut; hg: hindgut; i: intestine; lb: labrum; mc: myoepithelioid cell; pb: proboscis; php: pharyngeal pump; phv: pharyngeal valve; psg: principal salivary gland; r: rectum; sb: stylet bundle; sep: sensillum pores; sp: salivary pump; st: stomach.

pharynx protuberances. The dorsal wall of the valve is controlled by two pairs of muscles, and each lateral side by only one muscle. In a closed position, the opposite pharynx protuberances and the valve itself fit closely together.

The pharyngeal pump or sucking pump continues from the valve and traverses the middle region of the head to join the foregut in front of the tentorial bar. It acts as a pumping organ to bring the liquid food through the food canal and to force it into the foregut. The movement of the flexible dorsal wall of the sucking pump is controlled by 29 pairs of dorsal elevator muscles. Close to the tentorial bar the floor of the pump is attached by muscles, one pair originating from the tentorium, and one pair from the piston of the salivary pump.

The foregut (oesophagus) is a uniform thin tube which traverses the thorax to terminate at the oesophageal valve. It consists of a single layer of squamous epithelial cells which are lined with a chitinous intima, forming a stellate narrow lumen. The oesophageal valve is an invagination of the foregut into the stomach. The role of the valve is to prevent regurgitation of ingested food from the stomach to the foregut, although it does not have any muscle fibres.

The stomach is a dilation of the midgut and is lined with a single layer of cells. The anterior and posterior region of this organ consists of cuboidal epithelial cells, while the middle region is occupied by tall, finger-like columnar digestive cells. The latter secrete material by constricting apical cell parts. The cells of the posterior region secrete material by forming buds. Both processes continue during larval life without any degeneration or multiplication of cells. The intestine is the tubular portion of the midgut and after seven loops in the abdominal cavity it empties into the hindgut. In the first part of the intestine the squamous cells form a stellate narrow lumen, while in second division the strongly vacuolated cells are situated around a wide lumen.

The hindgut is a highly transparent, sac-like organ consisting of a single layer of flattened epithelial cells. The inner wall of the cells is strongly folded and coated by a delicate intima, which continues in that of the rectum. The latter is a tubular continuation of the hindgut and is composed of columnar cells. It opens into an invagination of the epidermis in which the anal opening is situated.

From the food canal in the maxillary stylets the plant juices with the virus passes into the pharynx, foregut, stomach, intestine, and finally the hindgut, the last organ of the digestive system. From the hindgut, they are excreted through the anal opening as honeydew. This viscous fluid which still contains the virus is deposited on plant leaves but the virus is not transmitted in this way.

The mechanism of virus transport from the gut lumen to the haemolymph is presumably restricted to the tubular part of the midgut. This part of the gut is lined with epithelial cells which have a large luminal surface and a well-developed microvillar system. Moreover, Forbes (1964) mentions the presence of membrane vesicles in these cells from which he concludes that transport of virus particles through the gut wall to the haemolymph takes place by pinocytosis. After multiplication in the fat cells, it is then transported to the salivary glands. The virus may subsequently be secreted again with the saliva into the sieve elements of the plant.

The salivary gland system is paired and each half of the system is composed of the principal and the accessory gland. The ducts from both principal glands join to a single one to form, together with the duct of the accessory gland, a common salivary duct. It runs forwards and passes into the afferent duct forming an S-shaped flexure to enter the pump chamber at the ventral side of the pump cylinder.

The accessory gland is composed of 3-4 cells of uniform size. The basal part of these cells shows laminated structures and in the cytoplasm many branching canaliculi occur which cross the cuticular lining of the salivary duct.

The principal gland has two lobes, each containing anteriorly six cover cells and posteriorly fifteen principal cells, which are situated around the internal salivary duct. Each cell is connected with the chitinous lumen of the salivary duct by an intercellular canaliculum. All these cells harbour a mass of secretory granules which are released by the cell into the canaliculi. The distal part of the two lobes of each principal gland is connected by a myoepithelioid cell. The bulk of the cytoplasm is occupied by myofibrils oriented in an interwoven pattern. This contractile mass has the opportunity to admit haemolymph into the lumen of both internal salivary ducts. The admission of haemolymph into the lumen of the salivary ducts presumably serves to dissolve the granular secretory products extruded by the acinar cells of the principal gland, and to remove waste material from circulation with the saliva. Via the duct, the haemolymph with saliva is transported through the salivary pump into the plant.

The salivary pump lies in the hypopharynx beneath the pharyngeal pump. It consists of a chitinous tulip-shaped cylinder and a pumpstem; in its open end, a U-shaped piston fits, connecting with their lips on the edge of the cylinder. The piston is provided with a tendon, to which muscles are attached originating from the tentorial bar and the pharyngeal pump. On both sides, the cylinder has a chitinous projection to which muscles are attached; these muscles originate from a chitinous ridge leading from the hypopharynx wall to the tentorial bar. The exit opening from the pumpchamber is situated on the distal end of the cylinder, and leads into the pumpstem as a narrow duct. At the foot of the pumpstem, the duct has two sensillum pores. The duct passes into the efferent salivary duct to terminate in the salivary canal enclosed by the maxillary stylets.

The salivary glands of *M. persicae* alternately produce a watery secretion and a viscous one (Bradley, 1959). According to Miles (1968), the nongelling watery secretion contains enzymes, which serve to dissolve the layers of the mid-lamellae during penetration of the stylets into plant tissues, while the viscous fluid forms the stylet sheath. The latter acts as a supporting structure for the stylets.

The extensive system of minute intracellular canaliculi in the accessory gland cells points to a rapid discharge of excretory products to the chitinous lumen of the salivary duct. These glands presumably excrete the watery suspension which, during its discharge to the salivary pump, is dehydrated by the duct cells to form the final viscous fluid. This fluid contains waxy droplets which render the stylet sheath waterproof.

On the other hand, the cells of the principal salivary glands contain a mass of secretory granules. They are released through the intercellular canaliculi into the lumen of the salivary duct to dissolve finally in the haemolymph. The haemolymph is pumped into the lumen of both ducts by pulsations of the myoepithelioid cell. Through the duct,, the watery saliva with its enzymes is transported by the salivary pump into the plant. The enzymes in the watery secretion serve to dissolve the layers of the mid-lamellae during penetration of the stylets into plant tissues, and in the sieve tubes to stimulate the sap stream, to prevent coagulation of the sap, and to assist in digestion (extra-intestinal digestion). The virus circulating in the haemolymph may be transported through the myoepithelioid cells and injected with the watery saliva into the lumen (Ponsen, 1972).

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

2.3 Aphids in sugar-beet

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DESCRIPTION OF THE PROBLEM

In sugar-beet fields in the Netherlands, aphids can be considered a major pest, being responsible for the transmission of virus diseases. Two viruses cause yellowing and one causes mosaic symtoms. As far as is known, these viruses are not transmitted through seed, so beet seedlings are virus-free.

The green peach aphid *(Myzus persicae)* and the bean aphid *(Aphis fabae)* are vectors of beet yellows. The green peach aphid overwinters as egg on peach and on *Prunus serotina*. The bean aphid overwinters in the egg stage, mainly on *Euonymus europaeus*. Numerical data on the overwintering stages are useful in forecasting later occurrence and spread of beet yellows.

However, more factors are involved. The beet yellows overwinter in seed beet, mangold clamps, beet volunteers and in some weeds. Neither the shifting of seed propagation from the south-west of the Netherlands to other regions, nor the early removal of the mangold clamps solved the virus problems. There were presumably other virus sources. In England, the green peach aphid was shown to hibernate in mangold clamps carrying the beet yellows viruses.

Especially in mild winters, *M. persicae* hibernates also on weeds growing in sheltered conditions. The aphid is also continuously present in greenhouses, from which it can escape into the open. Under these conditions, there is no bisexual reproduction: the life cycle is anholocyclic. Under suitable conditions, for instance in greenhouses, the anholocyclic forms multiply rapidly and, when they later feed on virus-infected plants, become very early vectors of the virus.

Other aphid species, especially those hibernating anholocyclically, may be significant vectors of beet yellows. Little is known, however, of their role.

Alatae are possibly the main means of virus spread. If apterae were significant in spread killing of all aphids in the field should limit primary infections. Failure of insecticides to limit primary infection cannot be attributed to ineffective spraying. So the alatae are incriminated for transmission.

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SPECIFIC PROBLEMS AND AIMS OF RESEARCH

The control threshold is that level of infection or infestation at which the loss in yield of sugar equals the cost of control. The cost of control can easily be calculated, but the loss in yield is difficult to assess. For this assessment, healthy or nearly healthy fields are needed but in field trials such fields with a low percentage of infection are rare. Moreover, two forms of yellows occur simultaneously and are difficult to distinguish in the field. These difficulties must be faced to determine the control threshold as accurately as possible, in order to reduce the enormous amounts of pesticides used on beet, and to improve the profitability of sugar-beet growing.

WORKING METHODS

In experimental fields with plots of 100 m^2, all plants in a middle row were infected with *M. persicae* originating from beets with yellows. In such trials, there was no certainty which type of virus was used nor whether the aphids remain on the infested plants.

The day after the aphids were transferred, the infected plants were treated with an aphicide: TEPP, oxy-demeton-methyl, fenvalerate, pirimicarb and water as control were compared. Systemic long term aphicides can be effective against the spread of persistent viruses. From that moment, further spread of the disease was followed accurately. The strong type of yellows virus (BYV) is considered to be semi-persistent, and the weak type (BMYV) to be persistent. It was expected that the aphid would infect the plant with BYV before an applied systemic insecticide could stop it.

To obtain an impression of the influence of virus on production, the yield and sugar contents of the artificially infected rows were compared with outer rows in which less than 6% of the plants had been virus-diseased over several years. It was not practical to test each plant from the field since as many plants in an aphid-free greenhouse would be needed as there were in the field. By electrophoresis, we investigated the extent to which aphid strains resistant to certain pesticides occurred.

RESULTS AND DISCUSSION

Treatment with the aphicides had little effect on the number of infected plants. The artificially infested rows became completely diseased, but in the outer rows many plants remained healthy during the growing period. In 1973 and 1974, years with a generally high incidence of yellows, almost all plots became heavily infected. In those years, there were no clear differences in root yield and sugar content between artificially infected and outer rows. Surprisingly also in the 1976 and 1977 with very low general virus infection, there were no clear differences in yield of beets and sugar content between the treatments. The insecticides were no more effective in heavily infected areas than in less heavily infected areas. Indeed in 1978, the sugar content of diseased beets was 0.4% percentage unit less than that of healthy plants, but the mean root weight was 10.6% higher, which amply compensated the lower sugar contents (Table 1). Difference in the composition of the yellows virus complex could have played role. Insecticide resistance in aphids could be an explanation, but no evidence on this matter was found.

Table 1. Relative yields of sugar-beet and of sugar (%) from heavily and slightly infected rows in field trials in 1976, 1977 and 1978.

		More than 90% diseased					Less than 6% diseased					
		water	TEPP	fenva-lerate	oxy-demeton-methyl	pirim.	water (control)	TEPP	fenva-lerate	oxy-demeton-methyl	pirim.	absolute values for control (kg/ha)
1976	roots	93	81		69		100	91		85		60×10^3
	sugar	91	79		66		100	92		82		9.3×10^3
1977	roots	99	100		102		100	108		106		52.7×10^3
	sugar	97	94		97		100	108		105		8.89×10^3
1978	roots	106		110	104	110	100		90	94	96	46.74×10^3
	sugar	98		104	102	105	100		86	93	95	8.05×10^3

COST-EFFECTIVENESS OF CHEMICAL CONTROL

Beet growers use chemicals to control weeds, nematodes, various insects and fungi. Aldicarb (Temik G) does not only kill the beet cyst nematode but also various insects, including aphids. This dual effectiveness has much encouraged its use. In 1977, treatment with that chemical cost 270 guilder per hectare, in 1978 230 guilder per hectare.

Oxy-demeton-methyl (Metasystox R) soon after applications kills only insects sucking on beet plants. It is used against virus yellows and can reduce feeding damage by *Aphis fabae*. Costs are less then 100 guilder per hectare. Pirimicarb (Pirimor) is a carbamate with a strong aphicidal effect and is selective enough to spare natural enemies. Its use is increasing, mainly because resistance is growing up against organophosphorous compounds. Sometimes it is used in combination with oxy-demeton-methyl, which considerably reduces the selective effect of pirimicarb. A combined treatment, commonly pirimicarb, aldicarb and oxy-demeton-methyl, can easily cost 350 guilder per hectare.

In view of current sugar prices, an insecticide treatment would have to prevent a loss in root weight of 7% to be remunerative. In our field trials, this limit was not reached. Establishing losses from natural infection by the yellows is extremely difficult. In years with much yellows, a strange irregularity becomes apparent. In the Netherlands, very hot summers coincide with a high incidence of yellows. However, this type of weather also favours root production and sugar content of the beets, and can sometimes compensate the effect of the disease.

FUTURE OUTLOOK

As for oxy-demeton-methyl, the frequent use of pirimicarb can lead to resistant aphid clones. Recently in the 1978 season, an aphid was found that was almost completely resistant to pirimicarb. Against heavy infestations with *A. fabae*, pirimicarb can be useful for the time being.

In the future, advice on chemical control will still be hampered by unknown factors that appear in every season. Research should provide methods for rapid identification of virus both in plants and in aphids with immunological, electrophoretical and electron microscopical techniques.

Present knowledge of control thresholds compels us to view the use of insecticides critically. In the Netherlands, at least a quarter of the area is treated with aldicarb (300 000 kg per year) and about a third is treated twice with oxy-demeton-methyl (64 000 l per year). Any reduction in these amounts that can be achieved with well-founded advice is worth while, not only for the environment but also to avoid selecting for insecticide resistance.

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2.4 Aphids on wheat

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INTRODUCTION

The general concern of growers about the occasionally explosive increase in cereal aphids in past years resulted in world-wide research programs. Current research at the Research Institute for Plant Protection in Wageningen started in 1974. At a later stage, researchers and students of the Agricultural University cooperated. The research aimed to clarify the main aspects of production physiology and production ecology in the aphid-wheat relationship. It was considered that such fundamental research could yield knowledge necessary to develop schemes for supervised and integrated control. The program was organized to answer the following questions. What is the significance in aphid population development of temperature, parasites, predators and pathogens, host plant and density-dependent effects? What factors are involved in loss of yield and what are the quantitative aspects of yield loss? Ankersmit & Rabbinge (Section 10.2) studied aphid population dynamics in relation to abiotic factors as temperature, and natural enemies. They prepared a preliminary simulation model for population development, which was related to a damage model by Rabbinge & Vereijken (1979). We studied the influence of age of the plant, nitrogen dressing and feeding site on population development. We determined the influence of these factors and also of aphid species and honeydew on loss of yield. The effect of density on population development and loss in yield was studied with *Sitobion avenae*, which appeared to be the most injurious cereal aphid species.

RESULTS AND DISCUSSION

Influence of the host plant on the population development of Sitobion avenae, Metopolophium dirhodum and Rhopalosiphum padi

In trials in a climate room, the population development of 3 cereal aphid species on wheat was studied from the time the ear became visible. At the same time, the possible influence was established of flag leaf and ear as substrate, the age of the plant and nitro-

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gen dressing. Special attention was paid to efficiency of food utilization by *S. avenae*.

Before the milky-ripe stage of the grain, *M. dirhodum* multiplied on the flag leaf in numbers twice as quickly and in biomass 3 times as quickly as *S. avenae* and *R. padi*. On the ear, however, *S. avenae* multiplied twice in numbers and more than 3 times in biomass more quickly than on the flag leaf. On the ear, *R. padi* multiplied in biomass less than half as quickly as *S. avenae*. In the course of the milky-ripe stage, the multiplication rate on the flag leaf decreased strongly with *M. dirhodum* and hardly changed with *S. avenae*. On the ear, the multiplication rate decreased both with *R. padi* and with *S. avenae*, but with *R. padi* the rate was greater. In the second half of the milky-ripe stage, the multiplication rate of *S. avenae* on the ear had diminished by half.

In *M. dirhodum* and *R. padi*, alate production in reaction to crowding was less rapid than in *S. avenae*, which produced alatae sooner on the flag leaf than on the ear. Nymphal mortality in *M. dirhodum* and *R. padi* was twice as great as in *S. avenae*.

On the flag leaf, *S. avenae* produced 2 to 3 times as much honeydew as on the ear. Of the nitrogen, phosphorus and potassium present in the food, nitrogen was utilized best: 65% from the flag leaf sap and 83% from the rachis sap. In view of the energy budget of *S. avenae*, it was estimated that for the production of 1 mg of aphid, 5 mg of phloem sap from the ear and 11 mg of phloem sap from the flag leaf was needed, based on dry matter. Based on energy, 29% and 13% of the sap would be converted into aphid dry matter, respectively. *M. dirhodum* feeding on the flag leaf utilized its food about as efficiently as *S. avenae* at the same feeding site. Efficiency of food utilization by *S. avenae* on the ear increased and alate production and consequently migration decreased, when the plant was given an extra nitrogen dressing. Though there was also a rise in mortality, the increase in the number of apterous adults in the colonies led to a markedly higher population level in the next generation.

Population development of Sitobion avenae, especially in relation to crowding

In the river-clay area in the environment of Wageningen, field observations were made in the years 1974 to 1977. Migrants of *Sitobion avenae* colonized winter wheat especially during heading and flowering. Immigration reached its peak in the weeks after flowering. In view of the large number of apterous adults subsequently born in the crop, immigration in this phase could hardly contribute any more to population growth.

Field observations and laboratory trials suggested the following general conclusions. As soon as the ears are visible, the immigrants prefer to settle on them. Apterous adults developing on the leaves also move on the ear. On the leaves, a relatively small number of aphids remains. *S. avenae* is very susceptible to crowding: in colonies with an average of 5 or 15 individuals, 50% or 95% of the newborn nymphs develop wings. Consequently the second generation developing on the ear is already mostly winged. The majority of the young alatae emigrates so the number of adults increases less strongly. At a certain moment, more adults die than are added to the colony, which reaches its maximum density when the rapidly accumulating sum of emigration and mortality is balanced by the decreasing birth rate. After that, the population quickly decreases.

From laboratory research, it seems that crowding also adversely effects longevity, weight and thereby rate of reproduction. However the rate of reproduction is also directly reduced.

If the influence of natural enemies and the ripening of the ear are not taken into account, wing formation followed by emigration limits population growth and even results in a population decrease by more than half.

Injury and damage to wheat, caused by Sitobion avenae, Rhopalosiphum padi and Metopolophium dirhodum

Field and laboratory trials on injury and damage were performed with wheat from the stage of flowering onwards. Aphid infestation can lead to a decrease in the average weight of the grains. Normally the number of grains is not influenced. No difference could be detected in the effect on yield of *R. padi* and *S. avenae*, both feeding on the ear, or the effect of *M. dirhodum* and *S. avenae*, feeding on the flag leaf. Infestation of the ear leads to loss in yield at lower population densities than infestation of the flag leaf. *S. avenae* lives mainly on the ear, in contrast to the two other species, and is therefore comparatively the most injurious. There was no effect of time of infection and nitrogen dressing on the decrease in yield at comparable densities.

Honeydew on the plant stimulates the development of fungi, especially black moulds. In 3 field trials with *S. avenae*, this caused about as much damage as the uptake of sap. After correction for the mould effect, the established losses caused by *S. avenae* did not differ much from those calculated on the basis of its energy budget. Therefore stylet insertion and injection of saliva have no notable influence on the growth of the grains.

Quantitative aspects of attack by Sitobion avenae and its effect on yield; their relevance to supervised and integrated control

The aphid index of Rautapaa (1966) is less suitable for relating damage to the intensity of attack. It is better to use the aphid population maximum, which is correlated to damage to the same extent but does not have to be calculated.

In a system of supervised control, the criterion for treatment can best be expressed as the percentage of infected ears, which is closely correlated with the average number of aphids per ear and can be determined relatively simply and quickly (Fig.1).

Until 1978, we assumed that damage by *S. avenae* was reasonably predictable from biomass production and efficiency of food utilization. The fungus effect could be neglected because a fungicide treatment of the wheat at the beginning of flowering was a routine measure in the Netherlands. The results of a Dutch research group on the improvement of wheat yield (De Vos, pers. commun.) and the results of Dutch growers in 1978 (Rijsdijk, pers. commun.) changed our opinion. For the first time, we were confronted with crops that did not ripen prematurely under the influence of diseases, heat or lack of minerals and water and thereby yielded 2-3 t/ha (0.2-0.3 kg. m^{-2}) more than the average 5-6 t/ha. (0.5-0.6 kg. m^{-2}). Only in these long-living crops did honeydew have the time to realize its full yield-reducing capacity, considering the unexpectedly high losses caused by moderate infestations. This effect suggested that honeydew has also a direct effect on production. In our trials with the usual crops, the honeydew effect was apparently neutralized for the major part by the combined effect of various other yield-depressing factors.

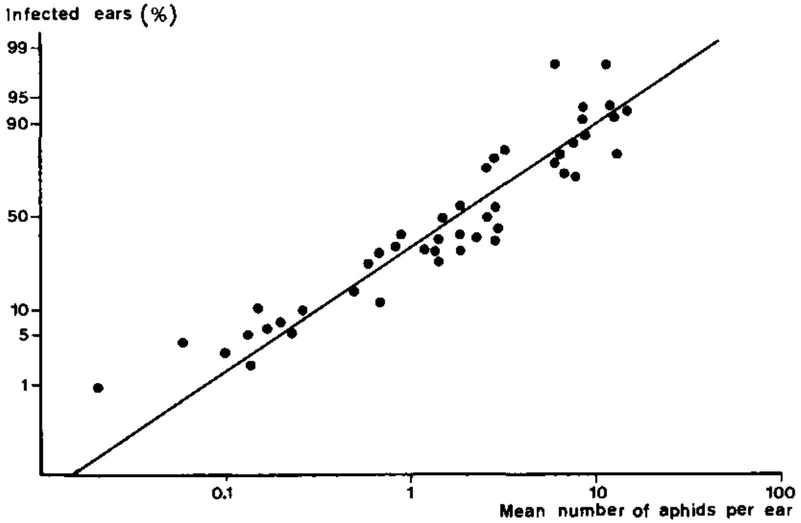

Fig. 1. The percentage of infected ears as a function of the average number of aphids/ear before the population maximum. Correlation coefficient R = 0.94. Regression formulas; probit y = 1.58 + 1.52 log 100 x, 100 log x = -0.69 + 0.59 probit y. Calculations were made on 49 samples of 100 ears, taken in the years 1973-1977.

So aphid damage is interrelated with a complex of other stress factors. Damage will only be predictable if both aphid population development and crop growth can be predicted from factors such as availability of water and minerals, weather, diseases and honeydew. The simulation model approach seems promising in forecasting but much of the quantitative data for it is still lacking.

Growing techniques are improving rapidly and so is yield. Consequently, an increasing significance of honeydew can be expected. Therefore, we suggest that the current criterion of control should be lowered from 15 aphids per ear to 5 and 10 aphids per ear equalling 80% and 90% of the ears infected for the respective periods before and during the milky-ripe stage. Until the above models are available, this advice may serve as a preliminary compromise with the demand of the growers, not to risk a serious loss in yield and the environmental requirement of minimal use of pesticides.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

2.5 Significance of predators on the development of insect pests in crops

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INTRODUCTION

The many reports that Carabidae and Staphylinidae were major predators of insects, injurious to crops, caused the Research Institute for Plant Protection (IPO) to start trials on the usefulness of predators in controlling insect pests in agrcultural and horticultural crops.

AIM

The aim of our investigations, from 1975 to 1978, was to establish the occurrence of carabids mainly on fields of winter wheat in the IJsselmeer Polders, the results of which have partially been published (Basedow et al., 1976). The feeding behaviour of some species have been studied and we tried to find out whether undersowing with spurry influenced the carabid population in vegetable crops. Field trials were started to establish whether changed populations of carabid beetles are inversely correlated with those of some vegetable--eating insects.

WORKING METHODS

Carabid populations in wheat fields were assessed by 10 simple pitfall traps made from 10 cm diameter plastic pots buried flush with the soil surface, about 50 m from the border and at intervals of at least 10 m. The traps had been filled with a 1% formalin solution and they were protected by a transparent plastic lid against rainfall. The catch was counted fortnightly.

The feeding behaviour was studied in the field and in the laboratory by using labelled prey animals and by the application of fluorescing red powder around plants with pest insects. The traces of the carabids could be observed later by exposing the plants to ultraviolet radiation in total darkness. Carabids were labelled with radioactive isotopes and examined for radio-activity by J.Ph.W. Noordink (IPO).

To establish whether changed populations of carabid beetles were inversely correlated with those of some vegetable-eating insects, plots about 20 m square, planted with cabbages and undersown with spurry, were enclosed with plastic barriers 20 cm high and to a depth of 5 cm. Five pitfall traps were placed in each plot. On some plots, the carabids were eliminated by trapping; on other plots, the population of carabid beetles was artificially increased by addition of beetles from elsewhere.

RESULTS AND DISCUSSION

Pitfall trapping on fields of winter wheat, from May to August showed that these fields supported an abundant population of carabid beetles and that their activity was affected by weather and by applications of herbicides and insecticides. The catches indicated that the following species were dominant: *Agonum dorsale, Anisodactylus binotatus, Bembidion ustulatum, Pterostichus cupreus, P. niger, P. strenuus* and *P. vulgaris*. Other common species were *Amara familiaris, Bembidion rupestre, Harpalus pubescens, Nebria brevicollis, Pterostichus vernalis, Stenolophus mixtus, Trechus discus* and *T. quadristriatus*. *Pterostichus vulgaris, Harpalus rufipes* and *Nebria brevicollis* are known to eat a wide range of food, but *Agonum dorsale* feed primarily on aphids. Basedow (1973) stated that in Germany predatory arthropods caused losses of 43-58% to larval populations of the yellow wheat midge (*Contarinia tritici* Kirby) and of 0-43% to the orange wheat midge (*Sitodiplosis mosellana* Géhin). According to Basedow, the abundance of individuals in cereal fields, as determined by means of grid counting, was 6 000-12 000 per hectare for the carabids *Agonum dorsale* and 6 000-16 000 for *Pterostichus vulgaris*.

In 1975, the insecticide lindane (pellets) and the herbicide 2,4-D/MCPA/MCPP reduced carabids to a low level by about 85% on a field of winter wheat in South Flevoland. However, investigations, based upon the pitfall trap method in 1976, showed no further effect of this chemicals on the beetle population.

Laboratory tests showed that the carabids *Amara convexiuscula* and *Pterostichus vulgaris* ate aphids (*Myzus persicae*) on potato plants. *Bembidion ustulatum, Pterostichus cupreus* and *P. vulgaris* ate the aphids *Metapolophium dirhodum* and *Sitobion avenae* on wheat plants. *Agonum dorsale, Clivina fossor, Harpaleus aeneus, H. pubescens* and *Notiophilus biouttatus* ate dead and living aphids from the soil surface. Larvae of *Mamestra brassicae* and *Evergestis forficalis* and also the aphid *Brevicoryne brassicae* were eaten by *Amara convexiuscula, A. spreta, Broscus cephalotes, Calathus erratus, Harpalus aeneus, H. pubescens, Nebria brevicollis, Pterostichus cupreus* and *P. vulgaris*.

Field tests in cabbage fields with labelled prey animals demonstrated that *Bembidion lampros, Broscus cephalotes* and *Calathus erratus* fed on *Brevicoryne brassicae* and *Mamestra brassicae*. Labelled 3rd-instar larvae of *Evergestis forficalis* indicated that they were eaten by *Amara spreta, Broscus cephalotes, Calathus erratus, C. melanocephalus, Nebria brevicollis* and *Pterostichus vulgaris*.

Investigations with pitfall traps near Lienden (Schuilenburg) showed distinct differences between experimental vegetable fields with and without undersowing of spurry (Spergula arvense). In the period 18 May-18 October 377 carabids (of 26 species) were caught on plots with and 169 carabids (of 20 species) without spurry. Den Ouden & Theunissen (Section 2.10) observed a significant effect of spurry on the occurrence of cabbage insects. Carabids, staphylinids, spiders and other predators might have played some role.

In 1978, trials were set out to determine the importance of carabids, staphylinids and spiders as predators of insect pests on Brussels sprouts and cauliflower. Though we succeeded in increasing only the carabid population on one plot by about 100%, no distinct influence on the development of cabbage aphid, cabbage diamond back moth, cabbage moth, cabbage rootfly and cabbage white butterfly was observed. However, compared with 1977, populations of predators and cabbage insects were small in that area.

CONCLUSION

Much work still needs to be done in the Netherlands to determine whether and in how far predators can control insect pests. Thorough studies of their occurrence in cultivated crops, their feeding behaviour, the influence of cropping measures such as crop rotation and undersowing), of chemicals and of weather on their population density are urgently needed. If we obtain a positive impression of their activities, it would be worth while taking them into account when establishing integrated programs in the growing of agricultural and horticultural crops.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

2.6 Approaches to integrated control of soil arthropods in sugar-beet

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DESCRIPTION OF THE PROBLEM

In the past twenty years, cultural practices in sugar-beet growing have changed drastically. The drilling distance in the rows has increased and developed into planting to a stand on a large part of the sugar-beet area. Weed control has improved by increased application of soil herbicides at drilling. These changes have had the following consequences for infestations by soil arthropods:
- equal pest populations cause more damage than in the past;
- the tolerance to the pest has decreased, since less plants can be missed;
- weeds that served as an alternative food supply for polyphagous arthropods are no longer present, so that concentration around sugar beet seedlings increases.

The use of insecticides as a seed dressing, seed furrow treatment or overall soil treatment for control of infestations by pygmy mangold beetles (*Atomaria linearis*) and springtails (*Onychiurus* spp.) has increased drastically. Overall treatments can have serious implications, because predators are killed too and soil and crops are more contaminated with pesticides. So an integrated approach was investigated. *Onychiurus* species can under certain conditions cause damage, but they are also beneficial by preying on noxious nematodes.

INTEGRATED CONTROL OF ONYCHIURUS ARMATUS

We aimed at a combination of local chemical control or seed treatment and biological methods like creating alternative food supply between the rows of sugar-beet or modification of seed-bed preparation. The influence of insecticides, methods of weed control and cropping practices, like drilling distance and seed-bed preparation, on the population dynamics of *Onychiurus spp.* (mainly *O. armatus*) and the resulting damage were studied in field trials and by simulation in greenhouse and laboratory. The populations were assessed by taking soil samples, from which the Collembola and *A. linearis* were extracted by different techniques.

Soil samples from some arable fields showed that *O. armatus* hibernated in deeper layers. When soil temperatures rise above 5 ^{o}C in early spring, they might already become active and can migrate to the seedbed. *O. armatus* is highly sensitive to desiccation and therefore its presence in the seed-bed is largely dependent on moisture content. If the water content of the seed-bed increases, many *O. armatus* migrate to the surface near the seedlings. So damage is considerable in a spring with periods of cool and rainy weather. It also migrates a short distance horizontally if weeds offer an alternative food supply between the sugar-beet rows. In plots where soil herbicides had not been applied and weeds were controlled mechanically after singling, a part of the damage was prevented and concentration of *O. armatus* in the beet rows was considerably less.

The physical structure of the seed-bed also influences the extent of damage, as springtails have difficulty in penetrating a compacted soil. The layout of a shallow seed-bed and compaction before drilling had a beneficial effect. *O. armatus* can feed on soil fungi and has a preference for some species that are common in arable soil. If the space between rows was treated with molasses, a byproduct of sugar industry that stimulates fungal growth, fewer *O. armatus* migrate towards the sugar-beet rows, but this method cannot completely prevent damage and has to be combined with supplementary seed treatment.

Multiplication of *O. armatus* in the soil is stimulated by the growing of clovers and grasses as green manure and by leys or grass for seed production.

ROLE OF SOIL ORGANISMS IN SEEDLING ESTABLISHMENT

This project is coordinated by the International Organisation for Biological Control (IOBC) and the International Institute for Sugar-Beet Research (IIRB). The influence of different insecticide, herbicide and crop treatments on the composition of the soil fauna and infestations of seedling pests has been studied for several years. Special attention has been paid to the predators of springtails and the predatory activity of *Onychiurus* spp. on nematodes.

A permanent trial on the effects of crop rotation, application of aldicarb and lindane and soil herbicides on the composition of the soil fauna and the interrelations between predators and preys was laid on an experimental farm. All soil arthropods were extracted from the different soil samples by Tullgren funnels and the surface-dwelling arthropods were caught in pitfall traps. Cyst-forming and free-living nematodes were extracted from separate soil samples with appropriate elutriators. Soil fungi were identified from the rhizosphere of seedlings. Damage was assessed by seedling counts and different weed species were counted after emergence. At harvest, sugar-beet samples were collected from every plot to determine root weight, sucrose content and juice quality. Though the weather was dry in the first year of the trial (1976) there was considerable damage by *O. armatus*. Adequate plant protection was obtained by overall soil treatment with lindane and to a lesser extent seed furrow treatment with aldicarb. Modified weed control (no soil herbicides, chemical or mechanical control at singling) also proved effective. The same results were obtained in 1977, when we found an increased occurrence of weeds in the lindane-treated plots since part of the springtail population feeding on weed roots had been killed. Only slight infestations could be observed in 1978.

The number of *Collembola* and *Acari* particularly *Cryptostigmata* in the short term were affected by lindane and to a lesser extent by aldicarb. The number of *Tarsonemini* increased in the aldicarb-treated plots. Some decrease in the diversity of species could be detected after application of either lindane or aldicarb. All these effects were temporary. In the plots with continuous sugar-beet fewer *Pergamasus* spp., *Isotoma notabilis* and *Cryptostigmata* were found than in those with wheat as an alternative crop.

The numbers of beet cyst nematodes *(Heterodera schachtii)* remained low also in the plots with three years of continuous sugar-beet. The same was true for free-living pathogenic nematodes. In the plots with sugar-beet for one year and winter wheat for two years *Pratylenchus* spp. increased in numbers. Infestations of seedlings by pathogenic soil fungi were very low. The number of fungi in the rhizosphere and the diversity were small.

Only in plots treated with aldicarb and lindane a significant increase in root yield over untreated could be demonstrated.

BIOLOGICAL CONTROL OF SEEDLING PESTS BY NEOAPLECTANA CARPOCAPSAE

In two trial fields with a high initial population density of *O. armatus* and *A. linearis*, respectively, suspensions of *Neoaplectana carpocapsae* were sprayed in the open seed furrow at drilling. Populations of seedling pests and plant establishment were assessed as above.

Seed-furrow application at a dose of 250 000 per m of row had no effect on the occurrence of and damage caused by *O. armatus*, which was confirmed by laboratory tests. This was also found for *A. linearis* at rates of 80 000 and 1000 per m of row in a field with serious damage.

SIGNIFICANCE FOR AGRICULTURAL PRACTICE AND PROSPECTS

Part of the damage caused by *Onychiurus* can be prevented by a firm seed-bed, in which the seed is drilled shallow, avoidance of clover and grass crops in the rotation, and weed control after singling. The effect of the last method, however, depends on the population density of the weeds. Application of molasses between rows might solve the problem, but further research is needed before this can be done on a practical scale.

The investigations into the role of different soil organisms in seedling establishment have taught us much about the predator-prey interrelationship in the soil and provides a base for control of polyphagous arthropods in such a way that their beneficial effect on other pathogens is not affected. Trials are planned in which the mesofauna and particularly nematode predators are stimulated by application of organic material. In this way we hope to control *Heterodera schachtii*, which is the main pest of sugar-beet in the Netherlands.

Integrated control of pests in the Netherlands. Pudoc, Wageningen, 1980.

2.7 Mesofauna of arable soil

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INTRODUCTION

After Protozoa and Nematoda, mites (Acarina) and springtails (Collembola) are the most abundant animals in soil. These small animals respond quickly to changes in their environment. As a consequence of their small size, mites and springtails have a restricted home-range, which is mostly several cubic centimetres or decimetres. The faunal composition of microarthropods extracted from soil cores can be taken as a response to the environment and to events that had happened in and near the soil core. For this reason and because their ubiquity, abundance and range of species, springtails and mites are useful indicators of disturbances of the environment, especially of pollution, for instance by pesticides in the soil.

Unfavourable conditions for the soil fauna are bare soil, extremely high and low temperatures, drying out of the soil, compactness of the soil, shortage of organic matter, mechanical disturbance of the soil, large dressings of mineral fertilizers and application of pesticides. Most of these unfavourable conditions occur regularly in arable soil and become more frequent with intensive farming, which may decrease the diversity of the soil fauna.

The impoverished soil fauna becomes more unstable and certain pests and diseases on the crops become more likely. A well known example is the springtail *Onychiurus armatus*, which may become an important pest on germinating seeds and young plants of sugar-beet.

For a better understanding of the relations between the different species of the soil fauna and with the microflora, the weed flora and with crop plants, a thorough knowledge is needed of the fauna of arable soil. Such data can improve the success of investigations on integrated control and an indication can be obtained of side-effects of pesticides on the soil fauna.

AIM OF THE INVESTIGATIONS

The aim of the investigations was to establish the occurrence of springtails and mites in the soil of arable fields. Little was known of the mesofauna in Dutch arable soils under normal farming practice. So the investigations concentrated on fields of sugar-beet and of winter wheat where sugar-beet had been grown the previous year. Side-effects of aldicarb, lindane and certain commonly applied herbicides on the soil fauna were studied, in particular by Heijbroek et al. (Section 2.6).

WORKING METHODS

From each of 10 sites all over the Netherlands, 24 soil samples of 125 ml were taken during the winters of 1975, 1976 and 1977.

During spring of 1976 and 1977, 24 packets of filter paper weighing 3 g were buried at the same sites 5 cm under the surface of the soil. After six weeks, they were recovered and extracted in a cylinder-extractor.

Each soil sample represented a core of 0-20 cm depth. They were taken with an auger of diameter 5,7 cm. Each soil core was divided into a part 0-10 cm and a part 10-20 cm.

The soil from each part of the core was gently broken, crumbled and mixed. Afterwards the mixed soil of each of these two layers was put into cylinders separately and was extracted by a modified Tullgren extraction apparatus, a cylinder-extractor (Van de Bund, 1965). All the extracted specimens of mites and springtails were transferred into cavity slides filled with lactic acid, counted and identified to the species or, when specific identification was not possible, to the smallest taxonomic level possible.

A disadvantage of all dry extraction methods is that a direct comparison of the fauna extracted from different types of soil is often not easily possible. A considerable number of microarthropods will be trapped and remain in the soil samples during extraction because of shrinkage of the soil in samples that have a high content of silt, for instance clay or loess soils. Also the number of extracted microarthropods from samples of arable soil is often very low.

For these reasons, a trapping method was also used. Certain amounts of organic matter (filter paper) in bags of nylon gauze of mesh 1,5 mm were buried 5 cm under the surface of the soil for six weeks. Natural products like straw or tree leaves may be less suitable, because dead plant material may vary considerably in structure and chemical composition, and may be contaminated with unknown amounts of applied pesticides or may be subjected to polluted air. So natural products may cause unknown and undesired effects on the number and species of the extracted soil fauna. By contrast, filter paper is homogeneous industrial product consisting mainly of pure cellulose. The breakdown of cellulose is one of the main biological activities in the soil.

The technique does not measure population density but indicates the activity of soil animals that migrate and become established in the filter paper from the surrounding soil in association with micro-organisms that attack and break down the cellulose.

RESULTS AND DISCUSSION

The number of springtails and mites was generally low, but there was observed a considerable variation in number in the investigated sites (Tables 1 and 2). There were more individuals in sandy soils than in clay soils. The number of microarthropods were larger in the loess soil than in the clay. There was not much difference in the number of species from clay and sandy soil, but more species were extracted from loess soil.

After 15 years of investigations on the soil fauna, I have recorded 100 species of mite and 41 of springtail in Dutch arable soils. From the 10 sites in the trial described, 88 species of Collembola and Acarina were extracted from the soil samples and from filter paper.

Table 1. Number of individuals and species of microarthropods in 3 litres of arable soil. Mean between sites; 10 samples of 125 ml soil (0-20 cm depth) at each site. The figures in parenthesis are standard deviations.

	Number of individuals		Number of species	
	Collembola	Acarina	Collembola	Acarina
Clay soil (4 sites)	68 (± 45)	88 (± 57)	6,8 (± 3,5)	16 (± 3,5)
Loess soil (1 site)	154	178	11	27
Sandy soil (5 sites)	282 (± 183)	380 (± 282)	6,4 (± 1,8)	15,6 (± 4,9)

Table 2. Number of individuals and species of microarthropods in 3 g of filter paper buried in arable soil. Mean between sites; 24 replicates at each site. Figures in parenthesis are standard deviations.

	Number of individuals		Number of species	
	Collembola	Acarina	Collembola	Acarina
Clay soil (4 sites)	262 (± 160)	305 (± 196)	7,3 (± 3,1)	11,8 (± 6,9)
Loess soil (1 site)	108	1332	7	22
Sandy soil (5 sites)	308 (± 196)	1415 (± 183)	5,8 (± 2,4)	14,4 (± 3,8)

Few species were ubiquitous. Only one species, the mite *Pygmephorus blumentritti*, was recovered at all sites. Only 12 species of mite and 6 of springtail were represented in more than half the sites (Table 3). Also the frequency of occurrence in the soil samples and filter papers was low and did not exceed 50% for nearly all species. Only 9 species of springtail

Table 3. Number of sites (of 10 arable fields) in which microanthropod species extracted from soil and buried filter paper. Species occurring on half or less of the sites are not listed.

	Soil samples	Filter paper
Springtails		
Hypogastrura denticulata (Bagnall)		7
Onychiurus armatus (Tullb.)		6
Tullbergia krausbaueri (Börner)	9	8
Isotoma notabilis (Schäffer)	8	6
Folsomia candida Willem	9	7
Folsomia fimetaria (L.)	7	7
Mites		
Alliphis siculus (Oudemans)	9	9
Arctoseius cetratus (Sellnick)	7	7
Hypoaspis aculeifer (Canestrini)		6
Rhodacarellus silesiacus (Willman)	8	
Histiostoma feronarium (Dufour)	9	9
Tyrophagus dimidiatus (Hermann)	8	6
Rhizoglyphus robini (Claparède)	6	6
Oppia nova (Oudemans)	6	6
Pygmephorus blumentritti Krczal.	9	10
Pygmephorus sellnicki Krczal.		7
Pygmephorus sp. mainly juv.	9	7
Variatipes quadrangularis (Paoli)	6	

Table 4. Frequency of occurrence of springtail and mite species respectively extracted from 240 soil samples of 125 ml and 240 filter paper packets of 3 buried into the soil at 10 sites on Dutch arable land. Represented are only the species with a frequency of > 10% of the extracted soil samples or the filter paper packets. 0 = relative abundancy of > 10% in at least on site; + = frequency of occurrence of > 50% in the samples in at least one site.

	Number of soil samples	Number of filter paper packets
Springtails		
Hypogastrura denticulata (Bagnall)		59, 0, +
Hypogastrura manubrialis (Tullb.)		39, 0, +
Onychiurus armatus (Tullb.)	42, 0, +	
Tullbergia krausbaueri (Börner)	153, 0, +	102, 0, +
Isotoma notabilis Schäffer		76, 0, +
Folsomia fimetaria (L.)	51, 0, +	27, 0, +
Folsomia candida Willem	45, 0, +	45, 0, +
Isotomina sp.	0	24, 0, +
Pseudosinella alba (Packard)		37, 0, +
Mites		
Mesostigmata		
Arctoseius cetratus (Sellnick)	33, 0, +	99, 0, +
Arctoseius semiscissus (Berlese)		32, 0, +
Alliphis siculus (Oudemans)	50, 0, +	82, 0, +
Hypoaspis aculeifer (Canestrini)	0	25
Dendrolaelaps rectus Karg	25	
Dendrolaelaps lasiophilus Hirschmann		40
Rhodacarellus silesiacus Willm.	49, 0, +	
Astigmata		
Histiostoma feronarium (Dufour)	0, +	85
Tyrophagus dimidiatus (Hermann)	0, +	111
Rhizoglyphus robini Claparède	0, +	104
Histiostigmata		
Oppia nova (Oudemans)	50, 0, +	60, +
Prostigmata		
Pygmephorus sellnicki Krczal	43, 0, +	110, 0, +
Pygmephorus blumentritti Krczal	0, +	55, 0, +
Pygmephorus sp. most juv.	24, +	40, 0, +
Variatipes quadrangularis (Paoli)	24	
Tarsonemus sp.		25, +

and 15 of mite occurred in at least 10% of the extracted filter papers or soil samples. (Table 4). They may be considered as the common species in arable soil. It was striking that the springtail *Onychiurus armatus* was mainly common in clay soils. It was usually absent or so scarce in sandy soil that we did not succeed in extracting it from soil samples at several sandy sites. Other common species but less frequent are *Isotomodes productus* in some sandy arable lands, *Proisotoma minuta* in soil with large amounts of decaying organic matter, *Isotoma olivacea*, *Isotoma viridis* and *Isotomorus palustris*, whose adults occur mainly on the surface. In soil samples and in filter papers, juvenile forms were more usual. That was also so for *Lepidocyrtus cyaneus* but adults were sometimes observed in the soil. A very common species, *Bourletiella hortensis*, occurred regularly on the surface and in the herbaceous layer but was rarely represented in the soil samples.

The predacious mites *Pergamasus runcatellus*, *Pergamasus mirabilis*, *Pergamasus quisqui-*

liarum, Pergamasus longicornis, Pergamasus norvegicus, Veigaia nemorensis and *Veigaia planicola* occurred regularly but in a low frequency and small numbers in the soil samples. They can be considered as major predators of Collembola and may be of importance as predators of *Onychiurus armatus*. *Pergamasus* and *Veigaia* species occurred especially in soil with a loose structure, near and in clods of organic matter and near the roots of plants. Most of the observed predacious mites were predators on nematodes. These predators were the most common species and were well represented, often in considerable numbers in the soil samples and filter papers.

The interrelations between nematodes and microarthropods can be considered as important (Bunt, 1975; Van de Bund, 1972 a and b). We observed by ourselves preying on nematodes by *Onychiurus armatus, Onychiurus bicampatus, Folsomia fimetaria, Folsomia candida, Alliphis siculus, Hypoaspis aculeifer, Lasioseius penicilliger, Lasioseius fimetorum, Rhodacarellus silesiacus, Rhodacarus calcarulatus, Rhodacarus roseus* and *Rhodacarus ancorae*. I observed *Onychiurus armatus* in vitro feeding on cysts of *Heterodera trifolii* and on free-living nematodes. Gilmore (1970) also observed feeding on free-living nematodes by some Isotomidae and Entomobryidae including *Isotoma viridis* and *Isotomurus palustris*. These species are common as surface dwellers on arable land in the Netherlands. Feeding on nematodes by Collembola has been reported also by Doncaster (1962) and Murphy & Doncaster (1957). They observed *Onychiurus armatus, Achorutues* spp., *Folsomia* spp. and *Isotoma* spp. feeding on *Heterodera* cysts.

Also the feeding on nematodes by the Cryptostigmatic mite *Pergalumna* spp. (Rockett & Woodring, 1966) and the feeding on plantparasitic nematodes by the bulbmite *Rhizoglyphus echinopus* Fum. et Rob. (Sturhan & Hampel, 1977) indicate that the habit of feeding on nematodes be common among many species that are considered saprophagous or microphytophagous.

CONCLUSION

Although the number of individuals of mites and springtails is smaller in arable soil than in forest soils and grassland soils, these arthropods can occur in large numbers near and on the root systems of crop plants where they can have a large influence on plant growth. This may be indicated by the occurrence of many springtails and mites on diseased parts of roots and rotting spots on the roots where they can interfere with the populations of nematodes and micro-organisms. Predation on nematodes is a widespread habit amoung Acarina and Collembola species that are common in arable soil.

There is little information on how these interrelations affect and are affected by the growing conditions of the crop. That interaction can be further studied in trials on integrated control of spoilpests.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

2.8 Sterile-insect technique for control of the onion maggot, Delia antiqua

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INTRODUCTION

In 1962 onion growers were alarmed by the failure of the chlorinated hydrocarbon insecticides to control the onion maggot. By 1965, resistance had spread all over the Netherlands and, although organophosphates became available, concern about future control called for research on non-chemical measures. Development of resistance to the insecticide dichlofenthion in 1968 confirmed the fears. Gradually over the years, the call for an integrated approach to pest problems as a means of reducing the pesticide load in the environment provided an additional justification for research.

Onion growing is concentrated in the centre and in the south-west of the country, and the sterile-insect technique seemed a promising avenue, having shown its merits against the screw-worm fly in the United States. Releases of sterilized onion flies in these two areas would provide protection to some 90% of the area under onions.

As the sterile-insect technique is a complex biological method, a considerable number of aspects had to be studied concurrently, among them rearing evolving into mass rearing, sterilization including quality of the reared and sterilized insects, ecology of the fly with emphasis on dispersal.

REARING

Initially a rearing method was available (Ticheler & Noordink, 1968), using onions in trays as oviposition site and as medium for the larvae. When full-grown, the larvae pupated in the soil in which the onions were planted. The adults fed on yeast as a slurry, honey and water.

It soon became clear that the well-being of the person in charge of rearing would depend on the development of a semi-artificial diet for the larvae. Some beautiful pupae were obtained when larvae fed on a diet of carrot powder and yeast, like that developed in Hawaii for Mediterranean fruitfly. The yield, however, expressed as number of pupae per hundred young larvae or eggs remained extremely low. Tedious playing around with nutritional value, vitamins, lipoids, different types of yeast, different bases of diet like carrot, wheat germ, wheat bran and lucerne, and with antibiotics did not produce tangible results. However when ground filter-paper was added to the original diet, the yield rose to 55-60% and has remained around that level ever since. The adult flies are now fed on a dry mixture of sugar, milk powder, brewer's yeast and soya bean hydrolysate.

For egg-laying, Noorlander (Ticheler, 1971) developed an ingenious device. It consists of the bottom half of a clay flower pot, under which an onion slice is placed. The pot is put upside down in a Petri dish and the females lay their eggs between roughened rim and dish. Eggs can be stored in a refrigerator for six days without loss of fertility. Mass rearing is greatly facilitated by the keeping of two-day-old pupae in a coldroom at 3 ^{o}C for periods up to a year. Histologically this stage is comparable to diapause, with the added advantage that development can be resumed at any chosen moment. Temperature in the rearing chambers, 23 ^{o}C, was chosen to provide for a weekly rhythm of operations, leaving weekends free except for egg collection. The scale of mass-rearing increased over the years from the production of a few pupae to a maximum of 1.2 million in a week.

Quality of insects

The reared insects have to compete, when released, with their wild counterparts, at least in mating behaviour and dispersal. So their quality is of utter importance. The aim has been to rear insects comparable in size, as measured by pupal weight. The main factor is adding as little antimicrobial agents as one dares, balancing on the brim of deterioration of the medium. Another factor is to exert as little laboratory selection as possible. Two ways are followed to achieve this, namely by adaptation of the rearing system to the insect rather than the reverse and the success is proven by the later generations doing equally well in the laboratory as the first generations. And also by regular collection of fresh wild stock, which made it possible to release insects in sufficient numbers after laboratory rearing for 3-6 generations only.

STERILIZATION

In several experiments (Ticheler & Noordink, 1968; Noordink, 1971), irradiation proved a suitable tool for the induction of sterility in the onion fly. X-rays of 7.7 kC/kg (3 kR) emitted by either X-ray equipment or electron generator, or gamma-rays emitted by a ^{137}Cs or ^{60}Co source, applied to pupae the day before hatching started, sterilized almost 100% of the sperm cells of the males and prevented development of the ovaries of the females. Irradiation earlier in the pupal stage harmed the insect.

Quality of the sterilized insects

Cage experiments (Noordink, 1971) with mixed populations of irradiated and unirradiated flies in various ratios showed that competitiveness was not impaired by the radiation treatment. Field-release trials support this conclusion.

Histology and radiopathology One aspect of quality control was considered of prime importance, namely the influence of the radiation treatment on the insect. Quality in this respect includes both effective sterilization by induction of dominant lethals in the sperm of the males and the absence of side-effects. The problem was approached by histological tests. Methods used included phase-contrast optics for the study of living cells. By light microscopy, fixed cells were investigated for normal and pathological histology of the onion fly, including chromosome aberrations. Electron microscopy served to elucidate some aspects of the normal histology of the testis.

It soon became apparent that detectable histopathological effects of irradiation in the dose range of about 8 kC/kg could be found only in the reproductive organs : ovaries and testes. No signs of such effects were found in other tissues and organs, which coincides with the absence of cell division. So the emphasis of the study was laid on the normal histology and on the radiopathology of these reproductive organs. Identification of the various germinal and somatic cell types in untreated ovaries and testes was the basis to establish radiation effects (Theunissen, 1976). Characteristic patterns of reactions to radiation have been found in the different cell types of males and females treated with various doses of hard X-rays (Theunissen, 1977).

ECOLOGICAL RESEARCH AND RELEASE TRIALS WITH STERILIZED INSECTS

This part of the research was started in 1970, aiming at assessment of the field situation for population density, reproduction and dispersal and at development of release and evaluation methods.

A series of four release trials started in 1971 (Theunissen et al., 1975), and was concluded by a 1-ha trial in an onion-growing area in 1974 (Loosjes, 1976).

Methods

In the field research, several methods had to be developed or adjusted to the onion fly. Hence, a considerable part of the time was spent on this. Population dynamics and dispersal, with an emphasis on the latter, were analysed by release-recapture techniques with simultaneous releases of different G marked insects from different sites. Flies were marked at emergence with daylight-fluorescent dye-powders in up to thirty different colours and mixtures of two or three colours. For recaptures, a flight-interception trap was developed, but recently we shifted to a simple type of shelter trap. Marking was checked by squeezing out the ptilinum under a microscope with ultraviolet light. Combination of tests on population dynamics and dispersal with large-scale releases for control provided data on tens of thousands of recaptured flies. Also, dispersal was analysed by computer simulation with a model that accounted for heterogeneity both in space and time.

Release methods were developed, reaching the level of 1.6 million flies released per flight from a small aeroplane, at up to forty sites. The main problem in releases was predation. This could only be prevented by releasing flies instead of pupae, and by an immediate extensive spread of the flies, requiring aircraft. The quality of the flies was shown not to be impaired by the sudden flow of air at 160 km/h (45 m/s) that was used to blow the flies out of the containers.

The effect of sterile-fly releases was at first evaluated by the ratio of sterile to fertile amoung the flies trapped, the steriles being identified by their dye-mark or, in females, by inspection of the ovaries. Later we tried to establish the ratio among the eggs, but the development of an efficient egg-trap has so far failed.

Damage was observed on randomly placed control plots, preferably of 1 m of onion row, generally with some stratification of the onion field. On a larger scale, rough estimates were obtained from pseudo-random plots, chosen by stick-throwing shortly before harvest. The relation of damage to number of pupae was established for different control methods by soil sampling and extraction of pupae by simple flotation.

Results

Some aspects of the onion fly's life-cycle were analysed, e.g. prediction of emergence and incidence of diapause. Also, the tests yielded some data on the fly's niche, e.g. on *Entomophthora* infection.

Analysis of the spatial distributions of damage and pupae showed that these did not fit the negative binomial. Due to low population and high aggregation, no reliable confidence limits could be obtained. Densities of diapausing pupae in chemically treated fields are generally some 1.5 per square metre, ranging from less than 0.01 to over 5 per square metre.

Oviposition frequencies were estimated from recaptures and analysis of the ovaries. From them and the life-span data, fecundities were estimated at about 40 and 15 for the two subsequent flights, with corresponding reproduction factors of 7 and 3. These were estimated from populations of pupae, incidence of diapause and ratio of sterile to fertile flies.

Dispersal was found to be dependent on temperature, but in general independent of wind direction. It could be described by a diffusion process, with diffusion coefficients of about 2000 m^2/day in onion fields and 14 000 m^2/day outside these, values found by optimization of parameters in the computer model. The difference between the two coefficients accounts for the accumulation of the flies in onion fields. Under the prevailing circumstances, this means dispersal distances of rarely more than 2 km. When released in an onion field, nearly half the males leave before mating. Migration is reduced during periods of mating and oviposition. The impact of immigration into an area treated with sterile insects is limited by the limited migration of females between mating and subsequent oviposition. In the field trials too, isolation proved no problem.

Preliminary field trials with sterile insects were set up near Wageningen, far from onion--growing areas as dispersal was then unknown. The results were promising (Theunissen et al., 1975). In 1974 on Flakkee, one of the major onion-growing areas, successful control by sterile males was obtained on an unisolated 1-ha field. The population was reduced to a quarter, whereas on a control plot the reproduction factor on a year base was 13. Damage to the crop was negligible, 2.4%.

SEMI-COMMERCIAL TRIALS

By the end of 1974, the research phase was concluded and, after ample consideration of the economic and other prospects, it was decided to undertake at least part of the development phase. Now the main aim was to show the applicability of the sterile-insect technique on a semi-commercial scale, some 20 ha of commercial onions, in 1978 and 1979.

As from mid 1976, methods were developed for mass production of the millions of insects required and a number of facilities were built. Rearing began by the end of that year with the parental generations. A field laboratory was installed in a farmhouse and a choice was made for release from aircraft.

The first year of the semi-commercial program was successful. On the eight onion fields, the population was reduced to a tenth on average, and the damage was 0.5 to 2.5%. Only on a silverskin onion field did the population escape the desired level of control for several reasons, although no noticeable damage occurred.

Outlook

Control of the onion fly with the sterile-insect technique is within practical reach. However at the moment, it competes with control with a biocide that also reduces eelworm damage. So there are problems in application of the steriles on heavier soils, which form a suitable habitat for stem eelworm. For efficient control, isolation does not seem to be a relevant factor. The main problem is the predominance of expenditures in the first year or two of application. The steriles should be applied for at least five years to make them economically attractive.

The present development phase has been limited in order to convince the farmers that the method works under practical circumstances. To achieve efficient control, there is still need to develop simple methods of estimating population and to learn more about the reproduction factors in rate. In mass-rearing, labour-saving methods await development.

Although the farmers are in general positive and a private company is preparing to introduce sterile onion flies, the government bodies are still undecided. On that decision, depends the future of this control method.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

2.9 Genetic control of the onion fly, Delia antiqua, with chromosomal rearrangements

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The onion fly *Delia antiqua* is the key pest of the onion crop in the Netherlands. Repeated occurrence of resistance to insecticides prompted the development of alternative techniques for control and preliminary research was begun in 1965 to develop the sterile-male technique. Later in 1970, it was decided to try to develop other ways of genetic control by chromosomal translocations. These could have the advantage that after isolation and subsequent release into the field, they could conceivably reduce costs. The work on the induction and isolation of translocations has been carried out at the Institute for the Application of Atomic Sciences in Agriculture (ITAL) and at the Department of Genetics of the Agricultural University in Wageningen.

WORKING METHODS

Genetic control techniques have been developed at ITAL using mainly, but not exclusively, chromosomal translocations; these are changes in the position of the genetic material between two chromosomes. Such changes do occur naturally but their frequency can be greatly increased by radiation. The expertise in radiation dosimetry and the different types of radiation available at ITAL, facilitate induction of such chromosome changes.

As related to genetic control, translocation heterozygotes have two important properties.
- Such an individual when mated with a normal individual causes a reduction in fertility by about half.
- The surviving progeny of such a mating consist for about half of normal individuals and for half of translocation heterozygotes, so that the translocation is inherited over many subsequent generations.

A radiation-induced translocation can be selected by 2 methods. Firstly by observing the phenomenon of inherited semi-sterility and secondly by cytological examination of the chromosomes. A translocation can be observed quickly when the exchanged pieces of chromosome are different in length. After isolation, the translocation line can be increased in numbers by crossing with normal individuals.

In a population containing translocation heterozygotes and normal individuals, matings can occur between translocation heterozygotes and a proportion of the progeny will be translocation homozygotes. Such individuals are fully fertile and breed true, just as the normal individuals and, when they are mated with normal individuals, all the progeny will be translocation heterozygotes (semi-sterile). The production of different homozygous translocation lines following such inbreeding constituted the backbone of the project. The lack of genetic markers in the onion fly greatly increased the need for screening, because parents of a particular cross could only be identified after cytological examination of their progeny.

Translocations can also be used an as aid for sterile-insect release in the following way. During releases of sterile insects it is often advantageous if only males are released for the following reasons:

- Females, although sterile, can still cause damage to the crop.
- If only males had to be reared in the mass-rearing system, there would be a considerable financial saving.

It is therefore advantageous to produce genetically altered lines which produce only males and as indicated earlier, translocations in which the male-determining chromosome is involved can be used te regulate the sex ratio of laboratory populations before release.

What is required is simply the linkage of a particular gene to the chromosome that determines whether an individual is a male, so that every male carries that gene and females do not. For example, such a gene could be a mutation conferring resistance against a certain insecticide. There are many examples of such conditional lethel genes. If males carrying such a gene are irradiated, a translocation between the male-determining chromosome and another chromosome carrying the resistance gene can occur, and the resistance gene and maleness become linked. The result of this is that a stock can be built up in which all males are insecticide resistant and all females are susceptible. Subsequently in a mass-rearing system a treatment of the population with a low discriminative dose of a certain insecticide would eliminate all females but the males would remain alive and could be used for release in the field.

RESULTS AND DISCUSSION

Seventy-one chromosomal translocations have been induced with radiation and isolated; both X-rays and fast neutrons have been used. Scheme 1 shows the classification of these translocations. Sixty were reciprocal translocations with an average fertility of 63.13% and of them 17 were symmetrical exchanges (i.e. the exchanged pieces were about equal in size); these were not studied further. The asymmetrical translocations were easier recognized cytologically. The group of translocations exhibiting more complexity were relatively more sterile and the stocks of many translocations were lost because of high sterility. For instance cyclic translocations (Scheme 1) contain 3 breakpoints and so are more sterile. In 47 of the reciprocal translocations, it was possible to establish which chromosome arms were involved and, on the assumption that each chromosome has a chance relative to its length of being included in a translocation, chromosomes 2 and 6 were preferentially included in the translocations.

Fourteen of the asymmetric translocations were inbred to produce homozygotes and two lines have been produced (Fig.1). This compares favourably with data from other insects, but many more homozygous stocks are needed to make crosses to produce double heterozygotes for release. On many of the tested lines many homozygous individuals were identified, but

Scheme 1. Chromosomal rearrangements isolated in the onion fly after irradiation (numbers and fertilities)

Translocations			
60 reciprocal (63.13%	42 asymmetric (64.07%)	7 with "duplication larvae"	(71.10%)
		35 without "duplication larvae"	(62.15%)
	17 symmetric (60.17%)		
	1 unidentified -		
9 triple (51.54%)	6 cyclic (48.10%)		
	3 a-cyclic (57.27%)		
1 quadruple (31.75%)			
1 two translocations (44.6%) (from one event)			
Inversions			
2 pericentric (77.0%)			
Translocation and inversion			
3			

low fitness prevented the lines from being established. After release of a translocation homozygote into a field cage, it became apparent that the sensitivity to diapause of the laboratory strains had greatly increased. This significant result lead to an expansion of the work on the genetics of diapause induction and, after 5 generations of selection, a non-diapausing strain was isolated.

For development of a genetic sexing system for the onion fly, dieldrin-resistant and dieldrin-susceptible lines have been obtained from Canada and, with a male-linked translo-

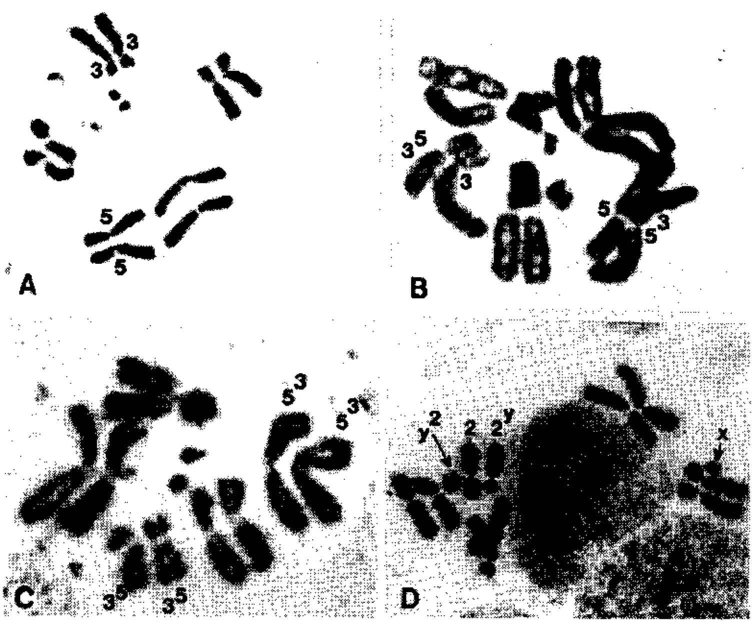

Fig. 1. A: Normal pair of chromosomes of the onion fly; B: Translocation-heterozygote; C: Translocation-homozygote of the same translocation; D: Sex linked translocation between the chromosomes 2 and Y.

cation (Fig. 1D), a genetic sexing system is being developed. First the resistance gene was introduced into the genome of the male-linked translocation by an appropriate crossing scheme. This has been accomplished and the line has been irradiated to link the gene to the male-linked translocation.

SIGNIFICANCE FOR AGRICULTURAL PRACTICE

The demonstration in 1978 that the fertility of a field cage population of onion flies could be reduced by 50% illustrates the potential of this method for insect control in agriculture. However, to succeed, much more work is needed first in the laboratory and then in the field to test different system.

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2.10 Integrated control of pests in outdoor vegetable crops

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AIM AND MOTIVATION

Insect pests in outdoor vegetable crops in the Netherlands include those of the cabbage pest complex: cabbage root fly, *(Delia brassicae)* cabbage aphids *(Brevicoryne brassicae)* and various Lepidoptera. Other root fly problems are caused by the carrot rust fly, *Psila rosae*, and onion fly, *Delia antiqua*. Several crops are attacked by cutworms, especially *Agrostis segetum*. Most pests are controlled with insecticides. The central problem, however, is the lack of knowledge on pest biology and pest management to enable growers to use insecticides in a rational way.

This can be achieved by selecting suitable insecticides, formulations and doses and by improving the timing of applications. A proper timing requires a functional national or regional warning system and simple and cheap monitoring systems for target species to establish their presence locally. Studies on the relation of population size and loss of yield can be used to establish control thresholds.

A long-term goal is to develop methods that can serve as elements in a total integrated control program to minimize or even exclude pesticides.

WORKING METHODS

Two items are described here to illustrate the approach and its results in some more detail. One subject, the development of slow-release formulations of biologically active compounds had its origin in consideration of the tarred ring around the stembase of cabbage plants, introduced in the last decades of the Nineteenth Century (Slingerland, 1894). It was considered to be an acceptable technique for control of the cabbage root fly at that time. From 1918 till about 1950, the repellent naphthalene was applied for that purpose (Rostrup, 1918). It is a component of tar and had a similar effect but repeated application was necessary because of its volatility.

In our trials, a more modern encapsulation of naphthalene than the old tarred ring was used. We applied Curasol, a non-phytotoxic polymer dispersion sold for prevention of soil erosion, which after evaporation of the solvent forms a water-permeable sheet and almost saturates the soil surface. Another matrix was Aqualith (Heslinga, 1976), a mixture of poly (vinyl acetate) and a styrene-maleic anhydride copolymer that is subject to pH adjust-able degradation by chemical and physical factors. Granules and sheets loaded with naphthalene were spread or sprayed as a disc around the stem-base of cauliflower. Stand scores, counts of pupae and estimation of root damage indices served as criteria of success.

The other item is investigation on intercropping in integrated systems of pest control. For two years, intercropping has been studied in field trials. In plots of Brussels sprouts, *Spergula arvensis* had been undersown before planting the crop. The density of the *S. arvensis* ranged from zero (= bare-soil under the Brussels sprouts) through a variable number of strips of *S. arvensis* in the crop to complete (100%) cover of the soil. At regular intervals, the presence and abundance of caterpillars of various species and incidence of cabbage aphid were systemacically recorded by taking samples, counting the caterpillars and evaluating the aphid incidence. At harvest the degree of infestation of the sprout buttons by the 3rd-generation cabbage root fly was assessed.

RESULTS AND DISCUSSION

The application of the polymer discs resulted in a considerably improved development of the crop over the very poor stand of untreated plants. Addition of naphthalene to Curasol gave a better development of the crop than application without the repellent. This combination almost equalled the effect of the insecticide trichloronate.

Aqualith granules with 30% naphthalene did not perform well, in contrast to naphthalene--loaded discs of that material. None of the treatments reduced root damage to zero, as obtained by trichloronate. Consequently Pupae were not very low under treated plants. In untreated root systems very low numbers developed because of overcrowding of the maggots. So 100% mortality was not obtained by application of the discs. Mechanical barriers with or without the repellent naphthalene partly or temporarily protect the plant and a considerable level of tolerance can be build up in the root system in the first month after planting (Den Ouden & Theunissen, 1980).

Intercropping Brussels sprouts with *S. arvensis* seemed to reduce populations of many pests. *Mamestra brassicae* and *Evergestis forficalis* gradually decreased, whereas *Pieris rapae* did not respond to intercropping. Populations of *Brevicorine brassicae* were significantly lower only in plots with maximum intercropping. Infestation of sprout buttons by *Delia brassicae* larvae also showed a gradual decrease in plots with increasing densities of *S. arvensis*.

The reasons for the phenomena observed are probably complex. Factors could be enhanced survival and activity of predators, interference with oviposition and attraction, and influence on microclimate in plots with intercropping (Theunissen & Den Ouden, 1980).

Current pest control practices in outdoor vegetable growing in the Netherlands differ much with crop and pest, but they have in common that chemical control methods are applied only. In crops like white cabbage, one to three sprays are sufficient to prevent any damage of foliage by insects, whereas in Brussels sprouts weekly sprays with parathion for many weeks do not prevent damage by cabbage gall midge. Some pests are readily controlled, e.g. caterpillars. Others again are virtually uncontrollable at this moment, e.g. cutworms or cabbage root fly attack on sprout buttons. Chemical control of root flies is routine but probably often unnecessary. Most vegetable pests are controlled effectively by chemical treatments, but the agricultural and economic efficiency of these treatments is doubtful. This is where research on a more rational use of insecticides can cause considerably savings by the use of better dosages and formulations and proper timing of the application by means of monitoring and warning methods. Replacement of chemical methods by other control methods is a long-term goal. Combined methods like using trap-crops or intercropping and use of repellents or attractants should eventually result in an efficient protection of the crop. To achieve both immediate and long-term goals, more information has still to be collected on the biology of pest species.

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3 Greenhouse crops

Vegetables in greenhouses are another crop on which the Working Party has concentrated attention, because heavy use of insecticides induced resistance of spider mites at an early date.

Breakthroughs in the development of biological control under glass were Dosse's discovery of the predatory potentials of *Phytoseiulus persimilis*, and mass production of this mite by a commercial firm by the end of the 1960s. Its success prompted research into other beneficial arthropods. Conditions for the successful use of *Encarsia formosa* against glasshouse whitefly were established, as a result of fruitful cooperation of investigators primarily interested in fundamental aspects of parasite-host relations and workers engaged in the practice of biological control (3.1). These beneficial arthropods are now produced on a large scale and being applied by a commercial firm over 30-40% of the area under these crops. Under glass as well as in orchards, selective control programs must deal with minor pests. Hence, apart from the need for new biocontrol agents and selective pesticides, resistance of useful predators (and parasites) to pesticides may prove indispensable (3.2).

The shortcoming of *Phytoseiulus persimilis* to survive periods of prey scarcity evoked the screening of other predator species, like *Amblyseius bibens*, which species might be useful in greenhouses if regulation over a longer period is considered (3.3).

In flower crops under glass, spider mites are the most serious problems to the growers, particularly to the rose growers. Intensive application of a wide variety of synthetic pesticides in the period between 1950 and 1965 resulted in multiple resistances (3.4). Surprisingly, mite populations inside the greenhouses were different from their outdoors relatives, not only in resistance to insecticides, but also in ecological and reproductive barriers (3.6). In fact, the spider mites from a greenhouse can be considered as "residents", with adaptations well preserved in a genetic continuum. Some investigations have aimed at breaking this continuum, for instance by genetic control (3.7) and by exploiting factors governing the induction and termination of diapause (3.5).

3.1 Integrated control of vegetable pests in greenhouses

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DESCRIPTION OF THE PROBLEM

In the Netherlands, 4600 ha of greenhouses, including 1000 ha of unheated houses, are used for the production of vegetables (data for 1978).

Our research on integrated control and guidance to extension officers is limited to tomato, cucumber, sweet pepper and gherkin. These crops constitute 80% of the economic value of food crops under glass. Integrated control in lettuce (the third most important crop) is not yet feasible.

The two-spotted spider mite *(Tetranychus urticae)* was a major pest in cucumbers during the 1950s and 1960s. Because of increasing resistance to some pesticides, Bravenboer developed a good method of biological control with the predatory mite *Phytoseiulus persimilis*, but this method was not used in practice until the end of the 1960s (Hussey & Bravenboer, 1971).

Because powdery mildew and spider mite were the two main pathological problems in cucumber growing, some pesticides active both as fungicide and as acaricide became popular.

In 1969, the introduction of the systemic mildew fungicide dimethyrimol (Milcurb), which does not kill the spider mites but can be integrated with biological control of spider mite, encouraged a commercial firm, Koppert Company, to produce and distribute the predatory mite *P. persimilis*. These two long-working and labour-saving agents were used by 30 growers in 1969 and 200 growers in 1970.

But in 1971 the greenhouse whitefly *(Trialeurodes vaporariorum)* became such an epidemic that frequent application of insecticides against this pest largely prevented the use of *P. persimilis*. At the same time, powdery mildew became resistant to Milcurb. So growers again started to use the double-action pesticides. Very soon, however, several new more specific fungicides against powdery mildew became available. For biological control of spider mites, a specific method of control against whitefly was also necessary. Research on this complex matter started in the early 1970s.

For the history of research and use of biological control under glass in Europe until 1973, see Greathead (1976, p. 52-64).

AIM AND MOTIVATION

In developing biological control methods, we are concentrating on developing complete schemes for a particular crop, because if control of one pest continues to depend on the use of pesticides, the future of biological control of any pest on that crop remains uncertain.

At the start of the 1970s, integrated control in greenhouses was applied on a small scale. It was the result of private enterprise and its basis was not firmly founded. Because of the successes, however, research was started, both to establish a stable basis for introduction of biological control and to search for biological control methods against several new pests.

The starting position for research was rather good because biological data about two main pests were present, since an English research group had already developed a scheme for integrated control of pests in cucumbers. This scheme did not, however, suit the rather high standards of quality and low population density of the pest required by Dutch growers.

The positive attitude of the growers towards biological control is an important factor. Although biological control requires more knowledge about pest and control organism, many growers prefer it for the following reasons.

- Chemical control of whitefly and spider mite is difficult, and more time is required to apply chemicals than to distribute and check natural enemies.
- During the first weeks of a crop, several manipulations have to be carried out with every single plant, so there is plenty of time to make the necessary checks for early symptoms of pest attack and to introduce the natural enemies. Thereafter the system is reliable for several months with only occasional checks. As soon as harvesting starts, the grower has little time for crop protection. By contrast, chemical control requires continuous attention and action.
- Young plants are susceptible to the toxic effects of pesticides, especially in winter, when the plants are weak. Pesticides can also cause abortion of flowers and young fruits.
- In cucumbers, gherkins, sweet peppers and tomatoes, fruits have to be harvested twice or thrice weekly. After application of chemicals, at least three days must pass before picking is allowed again. This safety period is not required when biological control is used.

In contrast to outdoor cropping, indoor cropping has the following specific advantages for development and introduction of biological control methods.

- Because of the annual removal of plant debris and soil disinfection, nearly all pests are killed before the new cropping season, so pest populations always start low.
- The number of pest species in one crop is very limited.
- Testing of control capabilities of natural enemies in greenhouses is rather easy, since we have identical houses with controlled climate.
- Insect populations, especially in winter, can be considered as island populations. So biological control can be applied by individual growers without interference from neighbouring crops.

WORKING METHODS

The usual sequence in this kind of research is collecting natural enemies, study of relevant biological properties for selection of candidates, assessment of use for pest control in field tests, and development of a method of introduction and mass rearing. Existing knowledge about the natural enemies we studied was very varied. Some were already used in control and other species were new to taxonomy.

RESULTS AND DISCUSSION

The two-spotted spider mite (Tetranychus urticae)

A method of mass rearing and introduction for a natural enemy of the two-spotted spider mite were already available when our research was started. In cucumbers, crop protection is directed to powdery mildew and spider mite. Against both organisms, effective pesticides are available and can be used in mixed preparations, so that chemical control is both easy and cheap in labour. Despite this, nearly half the growers use *Phytoseiulus* against spider mite in the main crop (Table 1). This is possible because of the availability of several powdery-mildew fungicides that do not harm *Phytoseiulus* seriously. We expect that use of *Phytoseiulus* will increase even more as soon as the powdery-mildew resistant cucumber is introduced on a larger scale.

In gherkin, we also find an increasing use of *Phytoseiulus* against the spider mite: in 1978 it was applied on 16 of the 220 ha of heated greenhouses. Disease control raises some problems because BCM fungicides (e.g. benomyl) against *Phomopsis sclerotoides* interfere with *Phytoseiulus*.

Biological control of spider mite in sweet pepper is effective, but integration with chemical control of other pests is difficult.

Biological control of the mites is not advised for tomato crops as results are not often

Table 1. Area (ha) of the three most important greenhouse food crops in the Netherlands in which biological control is applied (except hothouses). Total area in the main cropping period, the area and proportion (%) of successful biological control of greenhouse whitefly by *Encarsia formosa* in tomato and of two-spotted spider mite by *Phytoseiulus persimilis* in cucumber and sweet pepper.

	Tomato			Cucumber			Sweet pepper		
	total	*Enc.*	%	total	*Phyt.*	%	total	*Phyt.*	%
1969	2200	-	-	860	25	2	-	-	-
1970	2380	-	-	870	200	25	-	-	-
1971	2430	4	-	750	75	10	70	-	-
1972	2290	20	1	840	100	12	75	-	-
1973	2040	120	5	790	150	20	150	10	7
1974	2090	400	20	785	150	20	160	12	10
1975	2060	500	25	780	200	25	160	15	10
1976	2040	600	30	720	300	40	160	20	12
1977	2090	550	25	770	350	45	175	30	15
1978	2000	530	25	750	400	50	180	40	20

good. Probably there are two reasons for poor control by *Phytoseiulus*: low temperatures and too slow pest population development in tomato.

The greenhouse whitefly (Trialeurodes vaporariorum)

The first years of *Encarsia* application against the whitefly (1971 and 1972) gave both good and bad control results in cucumber and tomato, successes in tomato were inexplicably more frequent. Therefore we started to develop biological control for tomatoes, and gave the advice not to apply *Encarsia* in cucumber and eggplant.

First the reliability was tested of the introduction schedule developed by Woets (1978) introductions of 1 parasite per plant (at two-week intervals). Data of an intensive sampling program in a normal greenhouse (0.75 ha) showed that (1) the number of wasps introduced was sufficient to control the whitefly population, (2) the introduction of wasps in a regular pattern, instead of putting most parasites at infested patches, is sufficient, and (3) multiple introductions are required to stabilize fluctuations in population. One introduction is certainly insufficient (Van Lenteren et al., 1976). The introduction schedule has been successfully applied for 6 years now.

In the autumn cropping period, an increasing number of growers do not spray against whitefly: they wait till *E. formosa* 'spontaneously' enters the greenhouse, as is not unusual in areas with many tomato growers and extensive *Encarsia* applications during the main cropping period.

Research in the long term was aimed at understanding the relationship between the parasite *E. formosa*, its host *T. vaporariorum* and several host-plant species, in order to improve biological control for those crops where control had failed up till then.

Flight experiments and observation of the behaviour of whiteflies after landing on four plant species (eggplant, cucumber, tomato and sweet pepper) showed us that it was only after landing that the whitefly could detect whether it had landed on a suitable host plant.

The behaviour of the whiteflies on plants of the four species differs widely. After landing, the whitefly probes a plant with its stylets. On some host plants (eggplant and cucumber), they remain quiet after the first probe, hardly changing their position, and they do not often leave the plant. On other plants (tomato and especially sweet pepper), they often change position and often leave the plant. In cage experiments with the four different host plants together where whiteflies were released, a fast and consistant change was observed in all experiments after the first landings: the number of flies on eggplant and cucumber always increased and on tomato and sweet pepper the number decreased. Most flies were present on eggplant and in decreasing numbers in the order cucumber, tomato and sweet pepper.

We studied the possible mechanical barriers that might prevent a whitefly adult or larvae from feeding on a plant (Van Lenteren et al., 1977). Neither the distance between the leaf cuticle and vascular tissue of the host plants was too large to prevent the insect from reaching its food source, nor could we find any mechanical barriers to prevent penetration of the insect's mouthparts. The phloem contents of the four species of host plant has not yet been analysed chemically.

The rate of development of whitefly populations on the four plant species was estimated to find out whether the preference order reflects a host-plant quality order. We therefore measured life-span, oviposition frequency, developmental period and mortality of developmental stages on the four species of plant. The following correlations were found: the higher the number of whiteflies on a plant after some time (in decreasing order eggplant, cucumber, tomato and sweet pepper), the larger the number of eggs laid per female, the higher the oviposition frequency, the longer the life-span of the females, the shorter the complete developmental period, and the lower the mortality of all stages. So for those four host--plant species, host-plant selection apparently has a biological significance: the more a plant is chosen, the better is its quality for the whitefly.

Recently part of the experiments was conducted for three other crops: melon, gherkin and gerbera. Within the Cucurbitacaeae and Solanaceae, the general picture remains the same: a high oviposition frequency correlates with a large number of eggs, a long life-span, a high rate of development and a low mortality of developmental stages. The only plant on which this correlation was not found was gerbera: certain aspects measured indicate a good quality (life-span, total number of eggs), whereas other suggest a bad quality (oviposition frequency, rate of development and mortality of developmental stages). Therefore preference alone cannot simply be used to determine host-plant quality. More reliable information about host-plant quality might be obtained with a combined test of oviposition frequency and mortality of developmental stages.

To estimate the success of whitefly control by *E. formosa*, the data of oviposition frequency and life-span were transformed into simple lines from which the potential rate of development and generation time could be calculated (Fig. 1). The plot indicates that biological control of the fly would be easiest on sweet pepper and hardest on eggplant. Some of the field data do support this conclusion: control is easy on sweet pepper, tomato and melon, difficult on eggplant, cucumber and gherkin. However we found some exceptions. Control on eggplant, for example, proved to be easier than on cucumber where almost every trial on biological control ended in a failure, although whitefly populations developed faster on eggplant (for both species a much higher number of parasites was introduced per plant than for tomato). There must be at least one other factor that influences the control: apparently either the host-plant or the host influences the parasite differently.

Encarsia formosa females do not show a preference for a certain host-plant species. They do, however, prefer plants infested with whitefly larvae to uninfested plants, and they can trace infested spots from a long distance by an odour produced by the host insects.

After a wasp arrives on the underside of a leaf with hosts, searching becomes random. All hosts that the parasite encounters are examined thoroughly. Larvae of the third and fourth stages are selected for oviposition and they produce the highest percentage of offspring. Host feeding occurs predominantly on young larvae and 'pupae'. Parasites can distinguish between parasitized and unparasitized hosts and they reject parasitized hosts for oviposition. If most or all hosts encountered in a certain patch are parasitized, the wasp will leave the site. This phenomenon is important for the parasitization efficiency of this wasp.

During the observations on finding and accepting hosts, we saw that the leaf structure of the plant strongly influenced the walking speed and pattern of *E. formosa*. We therefore measured these on the leaves of various plant species. The hairy cucumber and gherkin both

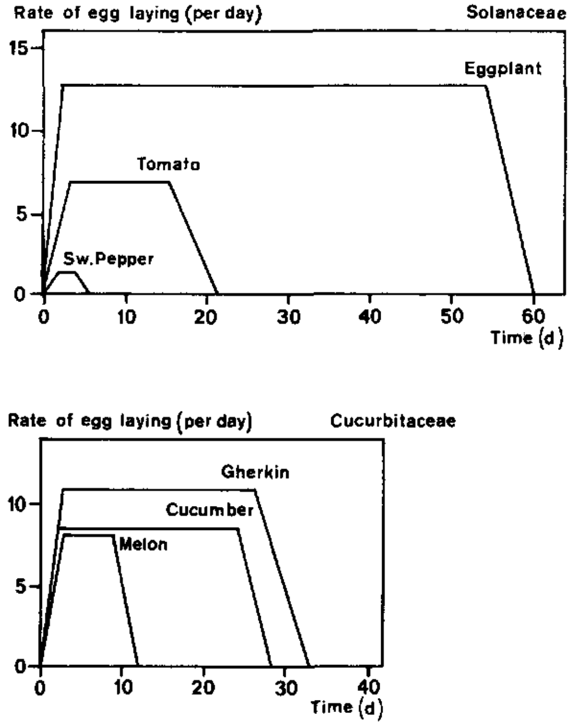

Fig. 1. Number of ovipositions per *Trialeurodes vaporariorum* female per day on different vegetable crops (simplified graphs).

have large stiff hairs on the leaves, while tomato and gerbera have much smaller hairs. Eggplant has relatively few, but large and stellate hairs. A hairless cucumber mutant and sweet pepper have no hairs at all. Highest walking speeds are reached on the smooth leaves and walking speed is inversely proportional to hairiness.

These results support the hypothesis that the structure of the leaf surface influence the walking speed and therefore the parasitization efficiency. The data may explain why control on cucumber is worse than on eggplant, though whitefly develops faster on eggplant. In comparison with eggplant, the wasps are much more hindered by the stiff hairs on cucumber leaves, which reduce their walking speed more and thus diminish their parasitization efficiency more drastically. This effect is strengthened by the larger amount of honeydew that is retained on leaves with large hairs than on smooth leaves. On a leaf with a lot of honeydew, the wasps spend much time preening their bodies, which become covered with honeydew. Therefore the parasitization efficiency will be more reduced than estimated from walking speed only. The practical implications of these results will be clear. It is very probable that biological control can be improved by using varieties of plant with few hairs of none. Our measurements on the hairless cucumber mutant that De Ponti (Section 5.8) recently discovered show that *E. formosa* walks 3.5 times as fast on this mutant as on a hairy variety. A greenhouse experiment showed that the parasitization efficiency was about 20% higher on that mutant.

Some more greenhouse tests have to be done to show that 'smooth' leaves alone increase the parasitization efficiency on cucumber sufficiently for reliable control. If not, it will be necessary to check whether there are cucumber varieties that are partially resistant to whitefly. Integration of both strategies will make biological control of whitefly possible in the second largest greenhouse crop.

A future problem will be the continuation of biological control of whitefly in tomatoes. Because of high energy consumption in greenhouses, much research is under way to develop varieties that produce well at lower temperatures. *Encarsia* cannot then keep the pests at low population levels. To cope with this problem, we are collecting and comparing data on parasitization efficiency of new whitefly parasites and strains of *E. formosa*.

The tomato leaf-miner (Liriomyza bryoniae)

Chemical control of leaf-miners makes biological control of the whitefly impossible. At present, leaf-miner pests are more frequent than before, both in chemically and integrated controlled tomato houses, and the area of biological control of whitefly on tomato is starting to decrease (Table 1). Leaf-miner can become a pest by pupae that survive in the soil from one growing season to another, if soil is not properly disinfected each year.

Three parasites are quite common and appear spontaneously, in Dutch greenhouses: *Opius pallipes, Dacnusa sibirica* and *Diglyphus isaea,* and if leaf-miners occur late in the season (from May onwards) they are apparently kept at low levels by this natural biological control.

Laboratory experiments showed that the parasites *Opius* and *Dacnusa* are promising candidates for biological control: their development from egg to adult is quicker than that of the host, adult life-span and egg production are about the same as that of the host. Also, mass production is not difficult, but probably expensive. As yet, we have not tested *Diglyphus,* an ectoparasite, because mass production will be more difficult. This parasite, however, has proved to be a good natural enemy of the chrysanthemum leaf-miner *Phytomyza syngenesiae.*

The onion thrips (Thrips tabaci)

Pesticides used in greenhouses are mainly broad-spectrum but short-action organophosphorus compounds, and more specific chemicals against fungi, mites and aphids. The onion thrips is killed by organophosphorus compounds, but because of the presence of unvulnerable stages (eggs in the leaves, pupae in the soil), repeated applications are necessary to keep numbers low. Incidental use of organophosphorus compounds is no problem for the biological control of spider mites (see this book, p. 227), but frequent use may disturb the balance between pest and predator. In sweet pepper, thrips causes cosmetic damage to the fruits, so the tolerance to the insect is low in this crop.

Integration of chemical control of onion thrips and biological control of whitefly is completely impossible.

Entomophthora epizootics may destroy thrips populations in summer and autumn. This process was observed both out of doors and in greenhouses, and details about the biology of two *Entomophthora* spp. were studied in vitro.

Research on the practical value of these pathogens is hampered because methods of reactivating resting spores and of cultivating the fungi on artificial diets are unknown.

In autumn, greenhouse crops are frequently inhabited by predatory mites of the genus *Amblyseius*, preying mainly on young thrips larvae (Ramakers, 1978). Both prey and predator are destroyed by the annual soil disinfection, but new thrips populations develop more quickly by immigration of adults flying in from adjacent greenhouses. After 'spontaneous' occurrence and after artificial introduction of these predators, low population densities of thrips could be achieved and maintained. Dispersal of the predators over the greenhouse after introduction at one spot is quite fast. Methods for mass rearing are being studied. Probably an alternative host will be necessary, because mass rearing of the onion thrips is not easy.

Aphids (Myzus persicae, Aphis gossypii)

In integrated control in greenhouses, there is no immediate need for biological control of aphids, because they can be destroyed by the selective chemical pirimicarb. However, increasing resistance of aphids to this chemical is being reported. In the literature, several natural enemies are mentioned as candidates for aphid control in greenhouses. Two of them were tested for their long-term effect on populations of *Myzus persicae* on sweet pepper.

The braconid *Aphidius matricariae* is frequently observed as a parasite of *Myzus persicae* in hothouses throughout the year. Mass rearing and cold storage are possible. The parasite completes its larval development faster than other known parasites of *M. persicae*, and has a shorter average generation time than this aphid. Adult parasites oviposit very rapidly; they prevent wastage of eggs by discriminating between parasitized and unparasitized aphids ('t Hart et al., 1978). Besides killing aphids by parasitization, the parasite causes loss of aphids, probably by disturbance. When the proportion of parasitized hosts reaches about 5% of the aphid population, this population stops increasing and within a few weeks the majority of the population is destroyed. The parasite controls the aphid until the end of spring, when the balance may be upset by hyperparasites. The possible use of this parasite might therefore be restricted to winter and spring.

The cecidomyiid *Aphidoletes aphidimyza* preys on greenhouse aphids from June (immigrating adults from outdoor populations) until October (start of diapause, induced by short photoperiod). After artificial introduction, this predator can be used from March onwards, and with prolonged photoperiod during the entire year. Methods of mass rearing have been developed and applied in Russia and Finland. Unlike several aphid predators observed in greenhouses, it establishes a permanent population, so one or only few introductions per season may be necessary (Markkula & Tiittanen, 1977). A single predator larva may complete development by feeding on less than 10 aphids, but kills many more aphids when density is high. (Markkula, pers. comm.). In hothouses, we obtained rapid destruction of populations of *Myzus persicae* and *Aphis gossypii* by introducing this predator. At the moment, we are studying the quality of control at low aphid density.

SIGNIFICANCE

Two factors influence the use of integrated control in greenhouses. First, the availability of effective natural enemies, together with a method of mass rearing and introduction, being *P. persimilis* against spider mite, and *E. formosa* against whitefly. Secondly, the availability of approved specific pesticides (insecticides and fungicides). *T. urticae* and *T. vaporariorum* can be controlled by natural enemies, but not in all crops (Table 1).

The Koppert company produces both natural enemies. From this producer or his dealers, growers buy the agents, and specialized guidance, which is essential for successful biological control, is included in the price. State extension officers can give more general information. Guidance on biological control deals with recognition of early symptoms of pest attack, introduction of the control agent, estimation of the quality of control and integration with chemical control of other pests and diseases.

Each year, a governmental advisory committee for disease and pest control in vegetables issues a plant protection guide, which includes integrated control schemes for the four crops mentioned above. The guide also contains a table providing data about susceptibility to pesticides of natural enemies and honeybees (this book, p. 227).

Besides general advantageous aspects of biological control (labour saving; safety for applicant, consumer and environment; absence of resistance problems), there are some specific positive aspects in its use for greenhouses (see "Aim and motivation"). This branch of horticulture has high investment and can achieve high profits. Because of this, there are high demands for quality in the plants as production units and in the fruit as economically valuable parts. In spite of these demands, which are usually seen as limitations for biological control, the area with integrated control has increased markedly in the last seven years (Table 1).

FUTURE OUTLOOK

Future projects for research have been mentioned. The prospects for application of new methods of biological control are good: growers ask for, rely on and use biological control.

Governmental organizations do all research on biological control and extension work; other governmental organizations provide legislation for pesticides used and check correct application by the growers. This strong influence on all these aspects allows the government to develop an active policy on biological control, which, to say the least, is lacking now, by (1) organizing and stimulating research on biological control and extension, (2) a better check on the illegal use of pesticides, and (3) changing the policy on pesticide approval, that is asking the pesticide industry to provide data about side-effects of products on natural enemies and allowing no new chemicals that interfere too strongly with biological control.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

3.2 A strain of Phytoseiulus persimilis (Acari: Phytoseiidae) resistant to organophosphorus compounds

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INTRODUCTION

The predatory mite *Phytoseiulus persimilis* is frequently used for the control of spider mites in greenhouses. The use of this predator in integrated control programs is limited because of its susceptibility to pesticides. To enlarge the scope of integrating chemical control with biological control with *P. persimilis*, a selection program for resistance to organophosphorus compounds was started.

WORKING METHODS

The base population originated from a small culture that had been maintained in the laboratory for Experimental Entomology (Amsterdam) for about 4 years. This strain was derived from the original *P. persimilis* strain in Naaldwijk (the Netherlands) about the time the original strain had become available commercially.

The susceptible strain was obtained from the Glasshouse Crops Research Institute, Littlehampton, Sussex, G.B.

Selection for resistance was done by regular spraying of bean plants infested with a resistant strain of *T. urticae* and with *P. persimilis* with increasing concentrations of parathion (a.i.) in water, starting with 50 mg/litre, aimed to obtain a 50-70% mortality at each spraying.

Susceptibility to the pesticide was tested by the slide dip method. Twenty egg-laying females about 1 week old were stuck on a slide and dipped for 1 min in the insecticidal solution. Mortality was determined after 24 h. During this period the slides were kept over water in a closed dessicator at 25 ^{o}C. Each test was repeated four times.

For crossing procedures, *P. persimilis* strains were maintained on detached-leaf cultures with *T. urticae* as food at 25 ^{o}C at relative humidity 70-80%. For crossing experiments, single eggs or larvae were placed on detached-leaf cultures and some days later males of the opposite strain were added. As soon as egg production started the females were pooled and their progeny was tested.

To determine the effect of pesticide residues on egg deposition and juvenile development in the resistant strain, detached bean leaves were sprayed with 2 ml of insecticide solution with a spray (Potter) tower. The insecticides tested were commercially available emulsifiable concentrates, which were diluted to concentrations used in practice. Small cells were made of 1-cm-thick Plexiglass with a circular hole 3 cm in diameter. The top of the cell was closed by a small glass plate and the bottom by a sprayed bean leaf which was kept fresh with moist filter paper. The leaf was infested with resistant spider mites and a day later with a fertilized female of *P. persimilis*. After 4 days, the mortality was assessed and the number of deposited eggs and juveniles was determined. Each experiment was replicated 15 times.

RESULTS AND DISCUSSION

After 8 selections, a strain was obtained tolerant to spraying with parathion (a.i.) in water at 1000 mg/litre. The susceptible strain succumbed to 25 mg/litre.

Using the slide dip method, dosage—mortality lines were determined in the susceptible and resistant strain for parathion and demeton-S-methyl. A resistance factor of over 100 was found for parathion and of about 30 for demeton-S-methyl. The F_1 of the cross: susceptible x resistant strain and its reciprocal had a similar susceptibility to parathion and demeton-S-methyl as the resistant strain. Testing of the backcross (susceptible x resistant) x susceptible clearly showed the presence of two categories of susceptible and resistant mites in an almost 1 : 1 ratio over the concentration range of 250-2000 and 125-200 mg/litre for parathion and demeton-S-methyl, respectively. So both resistances are caused by a dominant major factor for which the symbol OP has been proposed.

The commercially available strain proved later to have an even higher resistance than the selected strain. This makes it likely that other factors may contribute to the ultimate resistance as well. With the slide dip method, the cross-resistance pattern to a number of insecticides was determined. The resistance factor to organophosphorus compounds was rather variable, e.g. parathion > 143; diazinon 292; demeton-S-methyl 33; mevinphos 23 and phosphamidon 2. No cross-resistance could be established to the carbamate pirimicarb but by another test method (on residues) resistance was detected to propoxur. No cross-resistance was found to endosulfan, lindane and pyrethrins + piperonyl butoxide.

By breeding the resistant strain on residues as found under practical conditions of the mentioned organophosphorus and carbamate insecticides, it could be demonstrated that the resultant strain produced about the same number of offspring as on untreated leaves.

An organophosphorus resistant strain of *P. persimilis* can thus be used in conjunction with other pesticides in integrated control.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

3.3 Evaluation of the predacious mite Amblyseius bibens

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INTRODUCTION

The study of the predacious mite *Amblyseius bibens* Blommers (Acarina: Phytoseiidae) followed on investigations in Madagascar on the feasibility of using local phytoseiid mites for control of spider mite in crops. The activities in Madagascar revealed the presence of several new phytoseiid species. A preliminary evaluation of these species drew special attention to the species *A. bibens*, which is commonly found in various areas of the island. The field studies in Madagascar indicated that *A. bibens* is an important mortality agent of *Tetranychus* species on truck crops and cotton in more humid and irrigated areas (Blommers, 1976a).

The environment of greenhouses in temperate zones seems to meet the abiotic requirements for *A. bibens* to some extent. Investigations were initiated in Amsterdam to evaluate the use of this species for control of spider mite in greenhouses. We examined reproduction and predation of *A. bibens* under experimental conditions. Special attention was paid to the study of the survival of *A. bibens* at low densities of prey. In greenhouses, mite predators have restricted numbers of prey. The great reproductive capacities of phytoseiid mites and their specificity towards spider mites, could easily cause extinction through starvation. The ability of a predator to survive periods of extreme scarcity of prey is an essential condition for prolonged regulation of spider mites in a greenhouse.

EXPERIMENTS AND RESULTS

Experiments on the mite's life history were conducted in cabinets at constant temperature and humidity, using leaf discs of haricot beans for substrate. As prey, various stages of spider mite were offered in ample supply.

The reproductive qualities of *A. bibens* met the numerical requirements for successful control of all *Tetranychus* species, particularly at higher temperatures and humidities (Fig. 1 and Table 1). Because the natural mortality was almost zero in well fed juveniles and young females, the speed of development (egg to egg) and egg production, plus the sex ratio, sufficed for the estimation of the intrinsic rate of natural increase (r_{max}).

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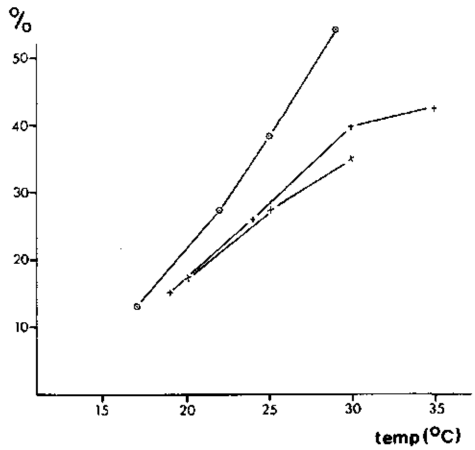

Fig. 1. Maximum population growth (percentage per day = 100 (e^{r_m} −1)) in *A. bibens* and 2 of the most prolific spider mites in relation to temperature. ⊗ = *A. bibens* at relative humidity 70%; + = *Tetranychus cinnabarinus* at relative humidity 38% (after Hazan et al., 1973); x = *T. urticae* at relative humidity 50% (after Gutierrez, 1974). Humidities are near optimum.

The r_{max} to 17 °C (Fig. 1) was estimated in this way. Because the developmental speed and the daily egg production of young females increased almost linearly with the temperature in the range 17 to 31 °C, the temperature-time sum can be used to estimate a reliable r_{max} in conditions of changing temperatures.

A. bibens attacks all stages of the spider mite, but tends to feed predominantly on *Tetranychus* eggs. The rate of consumption of prey eggs was related linearly to ambient temperatures between 17 and 31 °C. This relation can be described: number of prey eggs consumed per day is 1.3 times the value of temperature in degrees Celsius minus 19.3.

The rates of increase in experiments on potted plants corresponded to the r_{max} calculated from results obtained with individual predators on leaf discs. The maximum rate of increase as determined experimentally, represents the real rate of increase of *A. bibens* in the presence of sufficient prey. Ordinary control (= exclusion) methods together with the results mentioned so far will most probably permit us to forecast quantitatively the ability of this phytoseiid mite to check outbreaks of *Tetranychus* sp. in operational situations.

Whether *A. bibens* is also able to maintain low densities of prey more permanently is

Table 1. Average fecundity, maximum length of oviposition period and intrinsic rate of increase of *A. bibens* at 3 temperatures. Relative humidity 70 ± 10%.

	Temp. (°C)		
	22	25	29
Fecundity (eggs/♀)	65.0	64.1	62.4
Oviposition period (days)	43	27	23
r_m (intrinsic rate of increase)	0.242	0.326	0.434

Table 2. Average fecundity and duration of oviposition in relation to the number of fresh eggs of spider mite offered daily to individual female predators. Temp. 25 °C and relative humidity 70%. *N* = number of observations.

Prey offered (eggs/day)	Fecundity (eggs/♀)	Oviposition period (days)	*N*
20	57.1	20.1	8
10	58.7	23.1	9
5	56.5	47	2

still open to research. Whereas this species has a pronounced appetite for tetranychids, it accepts pollen of various plants when the preferred prey is absent. Although propagation in pollen-fed *A. bibens* is slow, a mass-rearing exclusively with pollen of *Vicia faba* on detached leaves could be continued for over a year. An experiment on potted plants indicated that the presence of pollen enables the predator population to survive and multiply in the absence of prey.

Life-table studies showed that *A. bibens* has an extraordinarily long life-span. The proportion of females alive at the end of the oviposition period under ideal conditions is 0.8 or more. The post-ovipositional periods are as long as 1 month, compared with a duration of the oviposition of hardly 3 weeks at 29 °C. Whereas such an extended life-span is clearly of little use under ideal conditions, it seems of major importance under conditions of prey scarcity. It is related obviously to the faculty of numerical response, because it permits the maintenance of fecundity over a wide range of suboptimal prey densities (Table 2). The long post-reproductive life, as presents itself under ample prey conditions, reflects in fact the reproductive opportunism of *A. bibens*. This phenomenon provides — in our opinion — circumstantial evidence on the adaptedness of this predator to conditions of low prey density.

A delay in reproduction can also be achieved by limitation in sperm supply. In *A. bibens*, no form of parthenogenesis occurs, eggs being produced only after fertilization. If mating is held up till mid-life, the total egg production is achieved in the second half of the lifetime.

DISCUSSION

In discussing the applicability of *A. bibens* for control of spider mite under greenhouse conditions, it is appropriate to compare *Phytoseiulus persimilis* Athias-Henriot. These phytoseiids have much in common. They are both specialized predators of tetranychid mites with great reproductive and predatory capacities. Both can exterminate colonies of spider mite in the greenhouse in a short time. *P. persimilis* is the most prolific species; its maximum rate of increase exceeding that of *A. bibens*, especially at high temperatures. In situations where the aim is only instantaneous control, *P. persimilis* is to be preferred. The result is, however, ephemeral, since this phytoseiid will become extinct because of its complete dependence on tetranychid prey. Re-infestation by spider mites, therefore, has to be followed by re-introduction of *P. persimilis*.

Although also a specialized predator, *A. bibens* has a definite chance of surviving periods of low prey density. In the absence of tetranychid mites, *A. bibens* will accept alternative food, as demonstrated. So in schemes for integrated control in greenhouses, this species is a candidate for regulation of spider mites.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

3.4 Genetics of resistance in spider mites from rose houses

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DESCRIPTION

Soon after parathion had been introduced in 1950 for pest control in the glasshouse area of Aalsmeer, Holland, spider mites developed resistance. Within two years, parathion resistance was fully established in that area. Thereafter the spider mite populations became resistant in succession to more effective organophosphorus compounds such as demeton and azinphos-methyl, to specific acaricides such as tetradifon, chlorobenzilate, chloropropylate and dicofol, and finally to binapacryl, within a period of 15 years. None of these compounds was effective for more than three years.

In 1966 a new specific acaricide, dienochlor, was put on the market for use on ornamentals, and this compound has been effective ever since its introduction. No cases of resistance to dienochlor have been reported so far.

The succession in development of various types of resistance aroused interest in the genetics of resistance. More knowledge of the genetics of resistance in spider mites might shed light upon problems in chemical control in greenhouses.

WORKING METHODS

Resistance was analysed genetically by means of Mendelian crosses. A favourable point in genetic studies of spider mites is that reproduction is based on arrhenotoky. Unmated females produce haploid (male) offspring only, whereas mated females produce haploid and diploid (male and female) offspring. This peculiarity allows special crossing schemes for analysis. By mass crossing of susceptible to resistant spider mites and by testing the hybrids and the haploid progeny of the hybrids successively with a series of dosages of the pesticide, a direct analysis of the genetic basis should be obtained.

By repeated backcrossing with susceptible males as recurrent parent under insecticide selection pressure, the gene(s) for resistance are introduced into the genome of the susceptible strain, at least if the type of resistance is inherited dominantly. By producing a strain thereafter that is homozygous for the resistance gene(s), the proper characteristics of the gene(s) for resistance can be studied. Information can then be obtained about the degree of protection that the gene or genes provide, the cross-resistance pattern, and the fitness of the bearer of the gene(s).

Where the action of a pesticide against adults was studied, toxicological tests were performed by exposing adult females to the residue on the leaves of pesticide-treated bean plants, the females being kept in Plexiglass cages on the leaves (Helle, 1962), or by the slide-dip technique (Schulten, 1968), in which adult females were glued dorsally to double-sided sticky tape that was fixed on microscope slides and dipped in suspensions of the pesticide. The residue method was also used for selection at discriminating dosages, also to select males if required.

The action of ovolarvicides was measured by spraying eggs that had been laid on detached bean leaves placed on wet cotton wool. Leaves with eggs, either purely haploid eggs or mixtures of haploid and diploid eggs were treated. Total mortality was measured, i.e. mortality of eggs and juvenile stages (Overmeer, 1967).

RESULTS AND DISCUSSION

Resistance to parathion proved due to the operation of a single nearly dominant gene (Helle, 1962). Cross-resistance was found to include also other organophosphorus compounds. Populations that were homozygous for this gene withstood a concentration of parathion some 250 times as high as for susceptible, as measured by the residue method. The resistance factor for diazinon, was of a similar magnitude, about 200. Schulten (1968) confirmed these results by the slide-dip technique. He found that the gene for resistance, which he called OP, was also responsible for a decreased sensitivity towards other organophosphates, such as demeton and azinphos-methyl. However, the resistance factors were much lower, 12 for demeton and 11 for azinphos-methyl. Schulten demonstrated that a high level of resistance to the latter compounds was due to the action of modifiers. The gene OP, however, is basic for resistance to all organophosphates.

The underlying mechanism for OP resistance is an altered cholinesterase that is less sensitive to organophosphates. This type of resistance is common in Aalsmeer, as elsewhere in the world. Another type of OP resistance, based on an increased rate of hydrolysis, which was found in the USA, has not been found in the Netherlands.

Schulten (1968) demonstrated allelic relationships between the resistance factors in different strains. He found that two different alleles are to be distinguished, which he called OP^B and OP^L, respectively. Competition experiments revealed that OP^L gave a slight disadvantage to its bearer compared with the + allele, whereas OP^B did not. For that investigation, homozygous backcross strains were produced in which OP^B and OP^L, respectively, were introduced into the genome of S.

Resistance is generally assumed to be preadaptive, resistance genes arising as a consequence of random mutation. If prolonged selection is applied, for instance use of insecticides for control purposes, resistant populations can arise. In an attempt to measure the mutation frequency for such a resistance gene, an ambitious program was conducted. Starting from a 100% susceptible colony, we bred a large number of individuals and tested them daily over a period of approximately one year. Some 3.4 million individuals were tested for OP resistance at a diagnostic dosage of parathion, but no positive results were obtained; all descendants of the colony appeared susceptible to organophosphates.

Resistance to tetradifon took longer in appearing in the Aalsmeer area than to parathion, three years. Also here resistance was found to be based on a single nearly dominant gene. The resistance factor was more than 200 (Overmeer, 1967).

Tests for allelism indicated that resistance to tetradifon such as this in various populations was due to a mutation at the same locus. Cross-resistance was found to include CPAS and animert, but not organophosphates, dicofol nor chlorbenside. The gene for tetradifon resistance did not seem disadvantageous to its bearer.

Also for resistance to tetradifon, an attempt was made to measure the mutation frequency starting from a 100% susceptible colony. Over 1 million eggs have been tested in successive generations with a low discriminating dose, but no resistance has been observed.

By contrast, in a search for visible mutations at our laboratory, there was a mutation rate of 10^{-4} for albinism and white eye in an initially 100% genotypically wild type colony.

Resistance to dicofol was due to a single nearly recessive gene. The gene provided its carrier with a resistance factor of about 15. Cross-resistance was found to chlorobenzilate and chloropropylate, but not to other acaricides or organophosphates. Dr. J.E. Cranham found resistance to binapacryl to be under monogenic control (Helle & Overmeer, 1973).

So far no resistance has been found to dienochlor. Occasionally samples of populations were sent to our laboratory suspected of showing decreased susceptibility, but in toxicological tests there was no sign of resistance.

FUTURE

At present, chemical control of spider mites on ornamentals in glasshouses is with dienochlor only. Spider mites are subjected to organophosphates as well, when the grower uses such compounds against other pests. But specific acaricides other than dienochlor have been discontinued in rose houses. If resistance to dienochlor eventually arises, further investigations into pesticide resistance in spider mites may be necessary. However, we can only speculate whether resistance to dienochlor will ever appear.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

3.5 Induction and termination of diapause in the spider mite Tetranychus urticae

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INTRODUCTION

A large body of literature has accumulated on the ecology and control of the spider mite *Tetranychus urticae*. Diapause has been studied in some detail. Knowledge of the factors inducing and terminating diapause is of importance for methods of control of many pest species in temperate zones. A more profound study of the physiological mechanisms of initiation and termination of diapause in *T. urticae* was initiated after preliminary experiments, which demonstrated a great sensitivity to photoperiod. Work on diapause was also stimulated by the presence in our Laboratory of a series of unique pigment mutants, which were expected to be useful for the analysis of the first steps of the induction process. Notwithstanding its fundamental nature, the research on spider mite diapause was included in the projects of the Integrated Control Working Party, since it was felt that it might lead to a new means of control, especially in greenhouses.

The main aims of this study were to analyse how photoperiod and temperature exert their combined influence during induction and termination of diapause, to identify the pigments involved in photoperiodic induction, and to analyse the mechanism of reception of photoperiod in spider mites. This paper summarizes results so far.

RESULTS AND DISCUSSION

In *T. urticae,* only adult females may undergo a facultative diapause, induced by short--day photoperiods during the larval and nymphal stages. The most conspicuous characteristic of the diapause is the deep orange colour of the diapausing females, as compared with the greenish-yellow of the 'summer' females. This change in colour is based on a more than two-fold increase in the ketocarotenoid content of diapausing females (Veerman, 1974). Further differences from the summer form are morphological, physiological and behavioral, the most important being arrest of feeding and oviposition.

The photoperiodic response of *T. urticae* is of the long-day type, showing a sharply defined critical daylength of 14 h in the strain tested. The response curve for induction of diapause in this strain shows a remarkable trait, which these mites have in common with only a few species of insects. In constant darkness, diapause is not induced, but with a photoperiod with light and dark phases of 1 and 23 h, diapause incidence is already complete

(Veerman, 1977a). This abrupt change of the response between 0 and 1 h light per day indicates that photoperiodic timing still proceeds at unnaturally short daylengths, but is not operative under aperiodic conditions like continuous darkness or continuous light.

The influence of temperature on diapause induction was investigated in a series of experiments, in which a thermoperiod was applied in combination with a photoperiod or in continuous darkness. With short days, diapause induction was abolished completely by constant temperature of 25 ^{o}C and above, while at 15 ^{o}C diapause incidence was complete. Combination of light and dark phases of 12 h each with temperature phases of 15 and 25 ^{o}C, which alternated regularly at the light/dark and dark/light transitions, strongly induced diapause when the low temperature coincided with the scotophase, but diapause incidence was negligible under the reversed conditions. So only the dark phase seems temperature-responsive for diapause induction in *T. urticae*. When the mites were exposed to the same thermoperiod in constant darkness, no diapause occurred, which demonstrates the absence of a thermoperiodic response. Although temperature influences diapause induction in these mites to a considerable extent, low or periodically fluctuating temperatures alone do not induce any diapause. When the combined influence of temperature and photoperiod is considered, temperature appears to have only a modifying effect, which emphasizes the predominating importance of photoperiod for the induction of diapause in this species of spider mites.

Evidence for the functional involvement of carotenoids in the photoperiodic reaction of spider mites was obtained in genetic experiments with some albino mutants of *T. urticae*. Diapause induction is hampered in albino mites, the diapause responses of four albino mutants ranging over 0 - 80% in different tests under experimental conditions where all other pigment mutants as well as the wild type strains show a 100% diapause. The ability to diapause could not be improved in albino by selection for diapause over six generations, nor by a series of back-crosses to wild type, sothat the albino locus must exert a pleiotropic effect, influencing both pigmentation and diapause induction. In crosses between wild type and albino, full diapause in albino was achieved only if the albino daughters came from hybrid phenotypically wild type mothers. Apparently a maternal effect is responsible for the complete induction of diapause in albino females (Veerman & Helle, 1978). So maternally derived carotenoids are probably utilized by the embryo in the formation of a receptor pigment concerned in the induction of diapause.

An important question in research on photoperiodism is still how organisms measure the length of day or night, often with such precision. A current hypothesis attibutes a central role in the timing mechanism of insects to circadian rhythms, but in some cases so-called 'hourglass' mechanisms have been found as well. The main difference between both theoretical mechanisms of time measurement is that the 'hourglass' mechanism requires a new starting pulse every time it completes a time measurement (the 'hourglass' has to be 'turned over'), whereas the circadian oscillations need to be started only once, after which they may run for several or many successive cycles. To analyse the photoperiodic time measurement in spider mites a mathematical model has been developed, consisting of two interacting 'clocks', both operating according to the 'hourglass' principle. The model shows a good fit for the photoperiodic response curve and for a number of other experiments based on a period length of 24 h. These consist of night interruption experiments with 1 h 'light breaks', which systematically 'scan' through the dark phase, and experiments in which photoperiods are applied containing two light pulses of equal duration, one of which 'moves' through the dark in relation to the other. The results of night interruption experiments in *T. urticae* show a remarkable similarity to those obtained with some species of insects. Two discrete points of sensitivity to the light breaks were revealed in the long night of a photoperiod with light and dark phases of 10 and 14 h. One point of sensitivity occurs early in the night and a second late in the night. In the middle of the night sensitivity to light interruptions seems considerably less. The great sensitivity to light interruptions and the sharp responses make the strain of mites used in the experiments eminently suitable for analysis of the mechanism of photoperiodic time measurement in spider mites. The validity of the methematical model will be tested in further experiments with 'ahemeral' photoperiods, in which the cycle length is either longer or shorter than 24 h.

Diapause in *T. urticae* is terminated in nature by a period of chilling. However, diapause development has been shown to be under photoperiodic control as well (Veerman, 1977b). In the first two months of diapause, mites can be reactivated by exposing them to long daylengths and temperatures of 17-23 ^{o}C, short daylenghts being far less effective. For example at 17 ^{o}C, half the mites terminated diapause in long-day conditions after a cold rest at 4 ^{o}C on only 8 days, but in short-day conditions diapause in half the test population was terminated only after a cold rest of 54 days. These experiments showed that adult females of *T. urticae* are still sensitive to photoperiod, at least during the first few months of diapause. About 2.5 month after the beginning of diapause, the photoperiodic sensitivity of diapausing females had apparently disappeared; the complete population was reactivated from that moment in short or long days. Since the mites have long lost this photosensitivity in the spring when diapause terminates in nature, the ecological significance of this sensitivity of diapausing mites to photoperiod is not to be sought in the termination of diapause. Rather it seems to be functional in retaining the diapause state in the autumn, when occasional warm days might break diapause in part of the population, while during the winter, diapause would be sustained and prolonged by prevailing low temperatures. This view is supported by the fact that the photoperiodic mechanism works only at higher temperatures, but is absent during the cold rest at 4 ^{o}C. For the termination of diapause, a response curve could be determined by exposing diapausing mites that had spent 20 days in cold storage to different photoperiods at 19 ^{o}C. In the range of ecologically significant daylengths, this response curve appeared almost to mirror the response curve for induction. The critical daylength for diapause termination corresponded almost exactly with that for diapause induction. These results seem to indicate that the same photoperiodic mechanism is at work both at the induction of diapause in larvae and nymphs, and during diapause in overwintering females.

Although practical application of the results in not yet feasible, further research on diapause in spider mites is certainly promising.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

3.6 Isolation mechanisms in populations of spider mite in rose houses

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STABILITY OF ACARICIDE RESISTANCE IN GREENHOUSE POPULATIONS

The introduction of the acaricide dienochlor about 1966 put a sudden end to the problem of controlling spider mite in rose houses in the Aalsmeer district. At that time, resistance to several acaricides e.g. several organophosphorus compounds, tetradifon, chlorobenzilate, dicofol and binapacryl, had become widespread and interfered with adequate control of the two-spotted spider mite, *Tetranychus urticae* particularly in rose houses. Dienochlor proved to be highly effective against the multiply resistant mites, and after 1966 only dienochlor has been used for spider mite control in rose houses. Specific acaricides such as tetradifon, chlorobenzilate and dicofol were discarded. So selection for resistance to the acaricides in greenhouse populations ceased in 1966. The question arose whether absence of selection in these populations would result in reversion to susceptibility. To obtain information about this process, samples from ten populations of *T. urticae* were collected from rose houses in the Aalsmeer district in 1973, and have been reared since then under laboratory conditions as separate strains. A similar procedure was followed for mite populations collected from outdoor plants, such as elder, nettle, ground-ivy and rose, at different distances from rose houses. With a series of dosages of pesticides (parathion, teradifon and dicofol), dose-mortality curves for each of the strains were worked out (Overmeer, et al., 1975; Van Zon & Overmeer, 1975).

The results of the survey can be summarized as follows.

Populations sampled from greenhouses showed partial resistance to parathion, tetradifon and dicofol. A high frequency of resistant phenotypes occurred for parathion, as expected since cholinesterase-inhibitors are still used in most greenhouses for control of other pests. But also for tetradifon, high frequencies of resistant phenotypes occurred in most samples. For dicofol, resistant phenotypes were found in 9 of the 10 samples examined, in varying frequencies.

Samples of populations taken from outdoor plants exhibited full susceptibility to parathion, tetradifon and dicofol, as long as the samples had been taken some distance from a greenhouse.

The most salient conclusion is that eradication of spider mites cannot be achieved by regular chemical control over a period of more than six years. Genes for resistance to tetradifon and dicofol can be considered as genetic markers for rose-house populations, and specifically typical for populations originating in 1966 and before. The presence of these genes in 1973, roughly 100 generations later, unequivocally demonstrates that resident populations maintain themselves in spite of intensive chemical control measures.

A second conclusion is that immigration from outside seems insignificant, if one considers the relatively high frequencies, for instance, of tetradifon-resistant phenotypes.

These findings were reason to investigate the factors that hamper introgression from outside. Three points come to the fore. The first is whether mites from outside can readily integrate genetically into a greenhouse population and mix with it. The second point is whether the ecological conditions of a greenhouse can be withstood by the immigrants. The third is whether the occasional use of organophosphorus compounds may retard reversion to susceptibility in general. These points will be considered below.

HYBRID STERILITY

When crossing different strains of *T. urticae* , the hybrid females may exhibit some degree of sterility: part of the eggs (F_2) produced by these hybrids do not hatch. An estimate of this kind of hybrid sterility can be given by the percentage of unviable haploid eggs produced by unmated hybrid females. In crosses within a strain, the percentage unviable eggs is usually less than 5%, but in crosses between strains, the percentage is considerably higher and may be up to 100%.

Overmeer & Van Zon (1976) investigated nine different populations sampled from rose houses in an area of nearly 25 km^2 in the Aalsmeer district. Reciprocal crosses between these populations were made, implying 72 combinations, and the degrees of sterility were estimated.

The results are striking. Each population possessed its own genetic identity since partial hybrid sterility was found to occur in all 72 combinations. In 38 of the combinations, lethality of the haploid eggs exceeded 50%, and some combinations had completely unviable eggs (100% mortality). Similar hybrid sterilities exist between indoor and outdoor populations in the Aalsmeer area (Van Zon & Overmeer, 1975).

The results raised the question how far hybrid sterility was a normal feature for adjacent populations of *T. urticae*. So we investigated the genetic compatibility between *T. urticae* populations from wild plants within an area of 25 km^2 in the dunes in Holland.

The study revealed that crosses between *T. urticae* from different hollows in the dunes do not result in hybrid sterility: practically all gave a fully viable haploid F_2. So hybrid sterility in *T. urticae* may be typical for populations on crops, for instance in greenhouses but the origin of reproductive barriers is still obscure. *T. urticae* is regularly transported over large distances, and the populations in greenhouses may be of different geographic origin. However they may be endemic, reproductive barriers having built up by spatial and ecological isolation.

We first suspected chromosomal rearrangements as reason for the genetic incompatibility between populations (Helle & Overmeer, 1973), but recent experiments have shown that the basis of hybrid sterility is probably genic and not chromosomal (Overmeer & Van Zon, 1976).

ABILITY TO DIAPAUSE

The greenhouse environment holds, an ecological paradox for *T. urticae*: short days in the autumn induce diapause, but the required period of chilling necessary for reactivation of the diapausing females is absent in heated rose houses. A survey (Van Zon & Overmeer, 1975) showed that *T. urticae* populations from rose houses do not respond or not fully to short-day treatment. The populations from outdoors respond normally by entering diapause. In roses subjected to a resting period in the winter, the mite population belonging to it appeared mixed in ability to diapause. In rose houses heated throughout the winter, diapause was induced only in a low percentage of the population. Doubtless, immigrant mites from outside are trapped by the peculiar environment, which acts as an ecological barrier between the greenhouse population and outdoor populations.

INSECTICIDE SELECTION

T. urticae from rose houses is resistant to organophosphorus compounds whereas local mites from wild plants are not. In fact, the latter are very sensitive to most of the cholinesterase-inhibiting pesticides used in rose houses. There is no cross-resistance to tetradifon and dicofol, and the resident populations of the mid-sixties cannot be considered homozygously resistant to the three pesticides mentioned. However the use of organophosphorus pesticides undoubtedly increased the frequency of the genes for resistance to tetradifon and to dicofol, as indicated by comparison of the fate of multiply resistant individuals and susceptible immigrants. Any reversion to susceptibility will definitively be retarded by the use of organophosphorus compounds.

DISCUSSION

Comparative studies on populations of *T. urticae* in greenhouses and outdoors revealed that free introgression of local *T. urticae* material from outdoor plants into resident rose house populations are hampered if not prevented for various reasons. *T. urticae* from rose houses is resistant to organophosphorus compounds whereas local mites from wild plants are not, in fact the latter are very sensitive to most of the cholinesterase-inhibiting pesticides used in rose houses. An isolating mechanism is the different responses towards short--day conditions of resident and outdoor material. A considerable reproductive barrier protects the rose-house population against introgression from outdoor *T. urticae*.

The degree of isolation of resident greenhouse populations and the ability of resident populations to survive intensive chemical control over long periods are aspects that deserve special attention. Studies on how to eradicate resistent greenhouse populations from time to time are felt most appropriate. A possible extermination of multiply resistant mites by any alternative method would be far more useful than regulation of pest numbers by chemicals.

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3.7 Genetic control of the greenhouse spider mite Tetranychus urticae

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Since 1965, different genetic methods of eradicating spider mites *Tetranychus urticae* in greenhouses have been seriously considered in order to counter the establishment of successive types of resistance to pesticides in resident populations (Section 3.6). Helle & Pieterse (1965) found partial incompatibility between adjacent greenhouse populations of *T. urticae*. The idea arose that incompatibility resulting from a cross between a translocation hemizygous male and a wild-type female could reduce the population fertility so much that this could be used in control. The effectiveness of this method could be increased significantly if it were possible to release translocation hemizygous males which were also resistent to an acaricide to which the pest population was susceptible. A fundamental condition was that a 100% incompatibility barrier be created between the released material and the wild-type material in order to prevent escape of the resistence genes into the wild population. High incompatibility barriers did not occur in nature, so artificial induction of chromosomal translocations was attempted. The induction by X-rays of structural chromosomal aberrations and breeding them for homozygosity was very successful. The aim of finding a structural chromosome mutation with a 100% reduction in fertility in heterozygous condition was not achieved. However fundamental knowledge of the radiobiological properties of *T. urticae* were seriously lacking and other topics of genetic control needed to be evaluated. This research field was assigned to me in 1971.

RADIOBIOLOGICAL STUDIES

The radiobiological studies demonstrated that significantly different results were obtained after irradiation of either sperm or oocytes with X-rays or neutrons. The dose required to obtain sterile males was 240 Gy and 160 Gy for X-rays and neutrons, respectively. Females were sterilized by 80 Gy for neutrons but for X-rays a higher dose was needed. Many heritable effects (structural chromosome mutations and recessive lethals) were induced in sperm but not in oocytes. To induce heritable effects in sperm, X-rays were more effective than neutrons. Irradiation with X-rays of mature sperm at 40 Gy resulted in fully sterile F_1 females, whereas the same dose of neutrons to sperm left 8% of the females still fertile. The dose of 40 Gy of X-rays is suitable for obtaining semisterile males, which can be used in a genetic control program. However there was a significant increase in male fertility within 7 days of irradiation treatment. A significant increase in F_1 female fertility was observed in the successive broods from the irradiated males. Studies on competition in male mating showed that irradiation of males with 40 Gy of X-rays impaired mating and accelerated ageing. So further studies on the feasibility of the sterile insect techniques had to await results from studies on other topics of genetic control before a proper assessment could be made of which method should be studied in more detail.

The next observations on the induction of structural mutations of chromosomes were relevant to the use of the translocation technique for pest control. After a doze of 15 Gy of X-rays to sperm, there was a maximum percentage of structural chromosome mutations associated with recessive lethals. The frequency of association of recessive lethals with a structural mutation was very low after a dose of 10 Gy of X-rays to sperm, whereas the percentage of structural mutations without recessive lethals was maximum at that dose. This is an important observation because it is difficult or impossible to obtain a chromosomal rearrangement homozygous if recessived lethals are associated with such a rearrangement. Neutrons of energy 1.5 MeV were less effective than 250 kVp X-rays of 250 keV peak value for induction of structural chromosome mutations after irradiation of mature sperm.

TRANSLOCATION TECHNIQUE

Experiments were started to obtain 17 lines, homozygous for different chromosomal rearrangements. The lines were then crossed with each other and with wild type. Some of the lines showed very high fitness. High levels of incompatibility were found. No cross was 100% incompatible, as necessary for a successful application of this method. So it was then decided to re-irradiate a line, homozygous for a structural mutation in order to isolate lines with higher incompatibility, due to a more intricate chromosomal rearrangement. Four lines were obtained homozygous for a structural chromosomal rearrangement. Their levels of incompatibility were higher than before. The decrease in fitness of the new lines cast doubt on whether re-irradiation of structural chromosome mutation homozygous lines was the best way of creating lines. It remains debatable whether lines with 100% incompatibility can be obtained by irradiation of sperm. A much larger test would be needed to answer that question. A positive result of the work is that good and stable lines, homozygous for a structural chromosome mutation, can be obtained by irradiation of mature sperm with X-ray or fast neutrons.

POPULATION DISPLACEMENT

A preliminary assessment of the ability of a strain homozygous for a structural chromosome mutation to displace a wild-type population was made by estimating life-table data and competitiveness of males in mating.

The life cycle was studied to compare the wild type with a particular strain homozygous for a structural mutation that showed very good rearing characteristics. Both strains had the same rate of development and about the same productivity under standardized experimental conditions. The intrinsic rate of increase (r_m) for the wild type and the newly synthesized strain were 0.264 and 0.278, respectively.

Behavioral differences between strains escape quantitative comparisons, though they are next to the latter of paramount importance for the success of population displacement. The studies of male mating competitiveness of the same promising line that was used for the life table studies showed that this line had a reduction of only 12%. The age-dependent reduction in mating competitiveness was no different from the wild type.

Studies on fitness traits showed that population displacement of the wild karyotype by the newly synthesized karyotype was possible if the reduction in mating competitiveness of the new strain was artificially compensated.

The experiments were performed by releasing inseminated females of both the standard strain and of a strain homozygous for a structural chromosome mutation on 3 whole bean plants (*Phaseolus* spp.) at three different initial ratios, 10:10, 13:7 and 16:4 for the females with chromosome mutations and standard females, respectively.

Of 14 lines tested so far, 3 showed an unstable equilibrium around 65%. Fixation of the rearranged karyotypes in the experimental population was observed at an initial release ratio of 80% of the rearranged karyotype.

Equilibrium or either slow fixation or extermination of the rearranged karyotypes of these 3 lines was observed at an initial ratio of 65%. Rapid extermination of the rearranged karyotypes from the experimental population was observed for all 14 lines tested at an initial ratio of 50%.

Sensitivity analysis on some population parameters in computer simulation of karyotype displacement revealed that the probability of a female mating with a male of corresponding genotype is the pivot for either fixation or extermination of a genotype. The probability of displacement of the standard karyotype by a rearranged karyotype was significantly increased if males of the line with chromosome mutation were released when the first-generation teneral females appear in the population. The population experiments showed that the release of males with chromosome mutation increased significantly the probability of population displacement of the strain with the corresponding chromosome mutation if the initial release ratio of the chromosome mutation females was close to the point of unstable equilibrium (0.65).

OUTLOOK

At the moment, experiments are going on, designed to isolate conditional lethals, more specifically temperature-sensitive lethals. Conditional lethals, such as temperature-sensitive lethals, can be induced in a large number of genes. By treating a line, homozygous for a structural mutation with a mutagen (ionizing radiation or ethylmethanesulphonate) a variety of positions on the chromosome can contain a temperature-sensitive lethal. It is very likely that some of these lethals are so closely associated with the breakage point of the rearrangement, that recombination with the wild allele is absent. After breeding these temperature-sensitive lethals true, there is a prospect of practical application. Because of the short generation time and the large number of generations per year, population displacement of a resident greenhouse population of *T. urticae* should be possible within a fraction of a year with temperature-sensitive population. The use of temperature--sensitive lethals as a control technique is promising for application in greenhouses, because of isolation properties and of the artificially temperature-controlled greenhouse environment. Cost-benefit analysis as an indication of economical feasibility for different greenhouse crops must await greenhouse trials.

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4 Insect-host plant relations

Research on the causes of plant infestation by insects has several aspects. There is the problem of how individual insects recognize certain plants as acceptable hosts, analysis on a population level of nutritional relationships between an insect species and its host, and population-dynamic consequences of such interactions. These various levels have been investigated by several entomologists in close cooperation in the laboratory, under standardized conditions in the field and under natural conditions. These studies include analysis of the sensory physiology of feeding behaviour (4.1, 4.2), effects of variations of the nutritional status of crop plants on population development of insects (4.3) and mites (4.4) and the effect of food quality as a consequence of host plant locality and host-plant density on insect fecundity and pest outbreaks (4.5).

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4.1 Host-plant selection in the Colorado potato beetle

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More than a century ago, the Colorado potato beetle *Leptinotarsa decemlineata* was an innocuous feeder on buffalo bur *Solanum rostratum*, on the foothills of the Rocky Mountains in North America. The cultivated potato *Solanum tuberosum* was then introduced into its habitat. This new food source turned out to be quite acceptable, initiating an extension of the beetle's territory eastwards, even across the Atlantic Ocean, to Europe where the Colorado potato beetle was reported as early as the 1920s in the Bordeaux region of France. As the dispersal of this new insect pest over Europe brought about substantial damage to potato production, the beetle's specialized feeding habits soon attracted attention.

Initially a basic question was what makes potato foliage acceptable and why are non--solanaceous and several solanaceous species rejected for feeding and oviposition. Nowadays, in observing the complex interactions of insects and plants, it is realized that any attempt to give an unambiguous answer to this question, would oversimplify the process of host selection. Through a long period of association of the genus *Leptinotarsa* with a number of solanaceous species, and under the constant action of natural selection, both the nutritional requirements and feeding habits of the Colorado potato beetle have adapted to the chemical characters of its food plants. The potato proved to fulfil the beetle's physiological requirements - being suitable and acceptable-, and at present potato crops are the main food resource for maintaining this insect pest in most of its territory.

Nevertheless, from time to time the beetles lack food. For instance because of crop rotation, post-diapause beetles emerge in non-host plant crops and have to search for suitable plants. Henceforth these beetles have to rely on their own abilities to find their host plants, in directing their feeding and oviposition act towards particular plant species. Besides behavioral components of exploration in space time is an essential aspect in host selection, since in the course of time the proportion and constitution of successive generations are affected by the various degrees to which plants meet the physiological requirements of both larvae and adults.

The present paper focuses attention on the restrictive elements in space and time, as deduced from a series of investigations in the Netherlands over thirty years. The sequence of events in host selection follows rather the historical than the functional line, in order to explain the progress in our understanding of this insect-host relationship. In this way, the paper emphasizes that every single event is a decisive element in the establisment of the Colorado potato beetle on a particular plant. The paper draws out some of the principles in the associations of phytophagous insects with food crops.

LARVAL FEEDING AND GROWTH

The larvae of the Colorado potato beetle exhibit remarkable discriminative powers, apparent from the extreme preference for a few species of solanaceous plants. The larvae starve to death in the absence of such palatable food plants. For a proper understanding of the word palatable, observations on the behavioral patterns are briefly summarized (Chin, 1950; De Wilde, 1958).

The odour of the resistant plant *Solanum demissum* is as attractive to the larvae as the odour of potato foliage. After coming into contact with the leaves, the larvae bite this new substratum, their biting response being initiated by the odour. However, contrary to the feeding action performed on potato foliage, the larvae do not feed continuously and spend most of their time resting and wandering. For feeding, *S. demissum* is rejected by the larvae. Besides *S. demissum*, a number of other solanaceous species do not possess one of the essential qualities of the normal foodplants, an acceptable taste. The selective feeding habits of the larvae are caused by avoidance of deterrent factors in a range of solanaceous species. These factors have been attributed to a typical class of secondary plant substances distributed in Solanaceae, i.e. alkaloids and their parent alkaloid-glycosides.

Petunia hybrida is an example of the solanaceous species that cause adverse effects in another way. Larvae are attracted by the odour of *Petunia* leaves and on arrival they eat a relatively large amount of the foliage. However *Petunia* is poisonous and feeding kills the larvae. Unlike the avoidance of feeding on *S. demissum*, feeding action is not prevented. Apparently the main advantage from rejection of unpalatable species is avoidance of toxic principles. The toxic nature of secondary plant substances seems to be the principal reason for the selective feeding habits of the larvae.

The nutritive ingredients of plants are essential for feeding and growth of phytophagous insects. The nutritional requirements of the Colorado potato beetle instars have been studied with artificial diets (Wardojo, 1969). These requirements do not differ essentially from the requirements of many other phytophagous insects, feeding on a diversity of plant species. However the suitability of the diet to support growth closely depends on the concentration ratios of the individual nutrients. Besides this metabolic aspect, the act of feeding has a behavioral aspect. The amount consumed is affected by the proportions of several nutrients (Ritter, 1967).

Through perception of both secondary plant substances and nutrients, poisoning and malnutrition of the larvae are prevented. In general, young larvae hatch from the egg pod, deposited by the female Colorado beetle on the proper food plant. Even so, there is no question about the advantage of discriminative powers, if one examines the probable casualties, when larvae fall to the ground during heavy rains or run out of food. Larvae removed from their food plant search in a random fashion until they are guided by olfactory and optic stimuli. They bite the substrate and so are informed of its chemical and physical properties.

ADULT FEEDING, OVIPOSITION AND EXPLORATION

In the acceptance of suitable feeding and oviposition sites the adults are even more selective than the larvae. The principles underlying the acceptance of their solanaceous hosts seem to require discrimination of the same chemical characteristics as required in the selective feeding habits of instars. The be-all and end-all of adult life is reproduction. The number of eggs deposited on plants suitable for larval feeding and growth reflects the degree to which individual beetles overcome the restrictive elements in space and time.

Lack of alignment of adult feeding and oviposition has been observed in the oviposition preference for *Solanum luteum* (Bongers, 1970). When female Colorado potato beetles, previously fed on potato plants, are given the choice between the foliage of potato and leaves of *S. luteum*, they show a marked preference for ovipositing on the latter plant. However, in the course of time, the beetle's fecundity is suppressed since feeding on *S. luteum* is avoided. Though *S. luteum* is preferred to potato as an oviposition substratum, reproduction on this species - succession in time - is impeded.

Exploration in space is thought to be essential for finding of host plants. The odour of solanaceous species releases an upwing locomotory response in Colorado potato beetles, which brings the beetles in the vicinity of these plants (Visser & Avé, 1978). In other words, through olfactory discrimination over a long range, exploration is to some extent confined to a relevant part of the vegetation. Chemical analysis of potato plant odour revealed the complex of 'general green leaf volatiles', like cis-3-hexen-1-ol, cis-3-hexenyl acetate, trans-2-hexenal and trans-2-hexen-1-ol. The concentration ratios of these volatile components are decisive for the release of a positive anemotactic response in the Colorado potato beetle. When the particular ratio in this complex is changed artificially, the release of the upwind locomotory response is prevented. Complexes of the 'general green leaf volatiles' form a predominant aspect of all leaf odours, discernable in the different and particular concentration ratios. In mixed vegetation, interaction of the different leaf odours might prevent the release of long-range olfactory orientation, in this way hiding plants from insects. This conception might give a new dimension to the onset of host-plant selection, as the beetle's ability to explore in space is not affected by the host plant's spatial distribution, but also by the heterogeneity of plant odour in the vegetation.

CONCLUSIONS

The sequential order in the process of host selection is illustrated in Figure 1. The gaps between the lines symbolize the instant a plant character fulfils one of the physiological requirements of a particular insect. As demonstrated in the larval feeding act on *S. demissum* and *P. hybrida*, and in the oviposition preference for *S. luteum*, non-host species occasionally meet some of the physiological requirements of the Colorado potato beetle but these species are devoid of other crucial qualities (Fig. 1). The scheme indicates that both nutrients and secondary plant substances - antibiotics - are the principle barriers for feeding, growth and reproduction of insects on the majority of plant species. In the Colorado potato beetle, successive behavioral patters have been evolved to evade non-host species, whose lack of fitness becomes apparent in the course of time. Through this, the

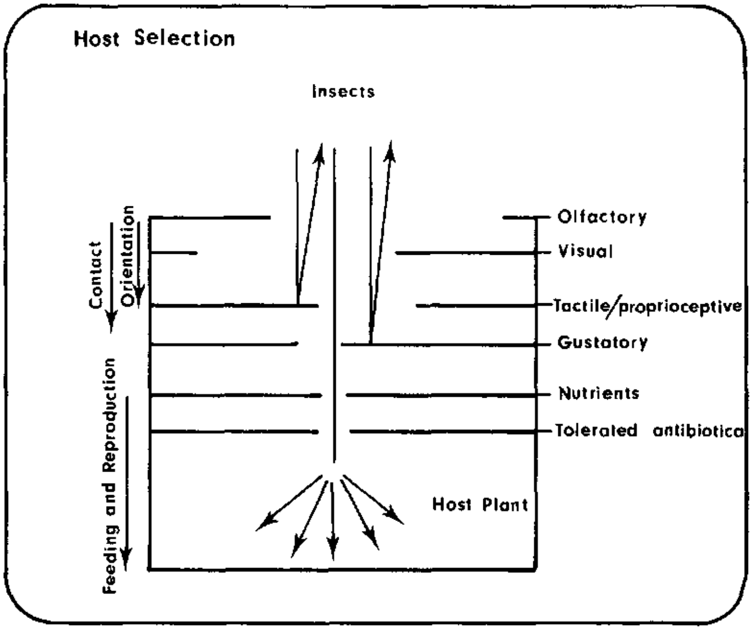

Fig. 1. General scheme of host selection in phytophagous insects. The width of a gap between lines symbolizes the specificity of a particular plant character that meets one of the physiological requirements of an insect. Host-plant selection in the Colorado beetle is illustrated in the centre arrow, leading to reproduction on *Solanum tuberosum*; whereas the unfitness of non-host plants is shown by the other two situations, e.g. in the light arrow the 'unpalatability' of *S. demissum*.

selection process involves a second major aspect, exploration in space. The alignment of events in space and time, of successive behavioral patterns and plant characters favourable for reproduction, determines the process of host selection.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

4.2 Role of chemoreceptors in host-plant recognition by insects

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NO INSECT ATTACKS ALL PLANTS

One of the crucial features about infestations of plants by insect pests is the specificity of the relations between insects and their food plants. Even 'polyphagous' species do not feed on all plants and show clear preferences for certain plant species within their host spectrum. These generalists, however, are fairly rare in the insect world.

More specialized insects, like the larvae of the large white butterfly *(Pieris brassicae)* and Colorado potato beetles, would rather die of starvation than feed on non-host plants. Apparently their host plants contain something that stimulates feeding and is absent from non-host plants. When some chemicals typically occurring in certain plants (like sinigrin in cabbage and other cruciferous plants) became available, about 60 years ago, E. Verschaffelt showed that various insect species recognized their host plants by these compounds. Meticulous behavioral observations by V.G. Dethier, G.S. Fraenkel and others have extended knowledge about typical secondary plant substances, not only as attractants to some insects living on the plants containing them, but also as deterrents to insects not adapted to these plants. That was the stage of knowledge when, about 15 years ago, we started an analysis of the chemical senses in host-plant recognition by electrophysiological methods.

WHAT IS THE SENSORY BASIS OF FEEDING BEHAVIOUR?

Once it had been established that certain key chemicals play a role in food recognition, the question arose what is the sensory capacity of insects in terms of sensitivity and specificity of their chemoreceptors. Do insects only perceive the key chemicals that typify their host plants or do they obtain a more complete chemical image of their food? Do insect species differ in their chemical senses? And do they therefore react differently to different plant chemicals? How consistent is the relation between sensory input and behavioral output?

The selectivity shown by most insects to their food is undoubtedly innate, but the possibility remained that some adaptation occured within inborn limits. If so, where and to what extent is such flexibility present and what is its physiological basis?

Is oviposition based upon the same sensory information as food selection? If not, what are the differences?

We aimed to solve many questions of this sort in the hope of extricating the subtle discriminatory behaviour of phytophagous insects. An understanding of the mechanisms of plant recognition might then help us to disrupt some undesired insect-plant relationships.

The role of the olfactory sense in recognizing food plants will be discussed by Visser & De Wilde (Section 4.1). The present paper will focus on the gustatory sense.

HOW ARE SENSORY RESPONSES TO PLANTS MEASURED?

Electrophysiological methods enable us to record the nerve impulses elicited in the receptor cells after stimulation by chemicals. These trains of impulses contain a code, on which the brain bases a behavioral reaction. The chemical senses are incorporated in specialized hairs or papillae on the external mouthparts, in the buccal cavity, on the antennae and on the feet. Often each sensillum is equipped with 3-4 receptor cells. Some insects, such as locusts and flies, have thousands of such sensilla, whereas others possess only a limited number. Lepidopterous larvae have in total only about 30 of such sensilla containing about 120 chemosensory cells of which only about 40 serve the gustatory sense. This remarkably small number is one reason that most of our research was on these insects. If we ever hope to obtain a complete picture of the sensory message sent to the brain, it will be in this simple system.

FEEDING BEHAVIOUR GUIDED BY SENSORY INPUT

Sensory reactions to general and to typical plant chemicals

On the basis of known behavioral reactions to chemicals typical of certain plant taxa (e.g. sinigrin), the presence of specific receptors for such compounds might be expected. Larvae of the large white butterfly do have 4 cells on their maxillary taste hairs that react to sinigrin and some closely related substances. In addition, however, they have cells stimulated by sugars, salts or amino acids. Four other cells are specialized to sense the presence of feeding-inhibitory substances, including several alkaloids and terpenoids (Ma, 1972). Thus the gustatory sense not only reacts to compounds that typically occur in certain plants only (sinigrin, alkaloids), but also to substances with a general distribution. Apparently, quantitative information on such chemicals is also relevant to the insect. It probably helps them to find the nutritionally optimum place on a plant, as does the polyphagous larva of the gypsy moth, which carefully selects leaves of alder picked from the south side of the tree and hardly touches leaves (of the same tree) collected at its north side, if conditions are ideal.

Though there are certainly not enough receptor types to record all chemicals present in a certain plant, insects obtain a sufficiently detailed indication of its chemical composition to recognize the plant species, and to sense more subtle characteristics about plant variety, physiological condition and age. All such details are encoded in the subtle and complex pattern of sensory signals travelling to the brain (Schoonhoven, 1977). In an elegant and detailed study, the sensory input to the brain was compared to the behavioral output in quantitative terms. Relatively simple relationships between input and output were found, indicating that central processing involves a fairly uncomplicated system (Blom, 1978).

Sensory coding: species related differences

Probably no two insect species exist with an identical set of chemoreceptors. Although some compounds, like sucrose and salts, are perceived by most insects, there is a remarkable specialization. Sometimes these specializations have an obvious adaptive value, as do the sorbitol receptors in several insect species restricted to rosaceous plants or the sinigrin receptors in cruciferae-feeding insects. Other chemoreceptors, like the fairly generally occurring inositol-receptor or the chlorogenic acid receptor in Colorado beetle larvae have less obvious significance (Schoonhoven, 1972).

The finding that even in some very closely related species the receptors vary considerably in physiological characteristics may give us clues about the evolution of the sensory system. For instance, some small ermine moths (*Yponomeuta* spp.), which are taxonomically very close, feed on different plants. Their gustatory receptors differ markedly, and seem adapted to perceive the chemicals typical of each of their respective host plants. This indicates that the receptors, though morphologically conservative, possess a high degree of physiological flexibility in an evolutionary sense.

WHERE DOES LEARNING OCCUR?

The tobacco hornworm (*Manduca sexta*) feeds exclusively on solanaceous plants. If it is reared on tomato leaves, the last-instar larvae show a preference for tomato over potato leaves. If reared on potato, its preference is reversed. Still more curious is that larvae reared on an artificial diet, lacking any chemicals specific to a host-plant, also accept some plants outside their normal host plant range, e.g. cabbage, dandelion or dogwood. During larval development on a normal host, some learning apparently takes place, altering the food preferences. Undoubtedly a major component of such learning processes is situated in the central nervous system. But, interestingly, the gustatory receptors also altered in sensitivity when the insect was reared on different diets. Consequently, part of the "learning" process is due to changes in the periphery, leading to alterations in the sensory input. This type of adaptation of chemoreceptors is hitherto unknown for animals (Schoonhoven, 1977).

OVIPOSITION

The ovipositing female, of course, largely determines what plant her offspring are exposed to. She discriminates, also on the basis of chemical information, between acceptable and unacceptable plants. The large white butterfly (*Pieris brassicae*) has tarsal receptors that respond to sinigrin, as electrophysiological information has revealed. Before egg deposition, she "taps" with her front legs, making good contact between those receptors and leaf chemicals.

Although she needs a cabbage plant for oviposition, the butterfly searching for a place to oviposit avoids cabbage leaves that already bear eggs. Together with eggs, females produce some unknown pheromone, that deters other butterflies (Rothschild & Schoonhoven, 1977). This water-soluble pheromone is sensed by antennal (olfactory) receptors, as electrophysiological recordings have shown, as well as by contact chemoreceptors on the tarsae.

Apparently, oviposition behaviour is also governed by a complex and subtle stream of sensory messages.

APPLICABILITY

The combined approach of electrophysiological studies and behavioral analysis has considerably enlarged our understanding of the factors governing food selection by insects (Beck & Schoonhoven, 1980). These studies mainly lead to a theoretical understanding, although by working with pest insects one might encounter useful information. The isolation of an oviposition-deterring pheromone of the cabbage large white butterfly, for example, may have some promise as a cabbage-protecting natural substance. Likewise, feeding inhibitors, which are relatively easily assayable by the methods mentioned, could play some role in crop protection.

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4.3 Physiological condition of the potato plant and population development of Myzus persicae

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DESCRIPTION OF THE PROBLEM

Aphids are a permanent pest in our potato culture. They transmit different harmful viruses and are a continuous menace to crops of seed potatoes. Even 160 years ago, Friesian seed-potato growers lifted crops early to prevent 'the curl' (potato leafroll). Insecticides are only partly effective to prevent spread of the persistent leafroll virus, and are almost ineffective against spread of non-persistent viruses, like potato virus Y. The aphids may die, but too late, as the virus has already been transmitted before the insecticide does its job.

The infection of the tubers has to be prevented by killing the leaves or lifting shortly after an intolerable high immigration of a number of vector aphids.

The precise date of lifting is of great importance. Even one day's difference in early lifting runs to a difference in yield of millions of dollars over the total area under seed potatoes.

AIM AND MOTIVATION OF THE RESEARCH

As insecticides provided only partial protection against virus transmission, Dr Hille Ris Lambers proposed a project to investigate ways of influencing development and flight of aphids in such a way, that the crop could be lifted later. The research started with *Myzus persicae*, because it is the principle vector of leafroll and it is also an important vector of non-persistent viruses. Also quite a lot is known about its life cycle, anatomy and physiology, and behaviour.

The winged emigrants colonizing the summer hosts produce individuals, which will multiply parthenogenetically for several generations. Interaction between individuals can result in the production of the winged morph. Therefore it seemed logical to reduce population density by influencing the physiology of the host plant in order to delay or reduce the production of alatae.

WORKING METHODS

The available literature on field trials on the relationship between host-plant quality and population development of aphids revealed a number of contradictions. It was deemed necessary to investigate whether we could influence development and reproduction of *M. persicae* by the mineral nutrition of the potato plant.

In 1966, Johnson reliably demonstrated the direct influence of the host plant on alary dimorphism in *Aphis craccivora*. As *M. persicae* mainly feeds on phloem sap, which is difficult to analyse, it seemed wise to use artificial diets to study the significance of dietary factors in wing dimorphism. Consequently, three main lines were followed in our research.

- The use of soilless culture to establish the effect of mineral nutrition of the host plant on population development of *M. persicae*.
- The relationship between the composition of chemically defined diets and wing production.
- Field trials to put the new knowledge into practice.

Special gravel culture systems were constructed to study the development of *M. persicae* on the potato plant in relation to N, S, K, Mg and Ca levels of the nutrient solutions. The technique was also used to investigate whether wing production and reproduction rate of *M. persicae* were similarly dependent on the nutritional status of the potato plant.

Aphids were reared on artificial diets by a technique developed by Mittler & Dadd (1962). Diets were modified to obtain optimum growth and reproduction, and a continuous-flow artificial-feeding device was developed to allow standardization of conditions for disturbance. A new method was developed to measure diet uptake and acceptance, by isotope techniques. The specific effect of a number of nutrients on wing dimorphism could be studied with a special micro-injection device, which eliminated gustatory effects on developmental processes.

In field trials different sampling methods were compared to improve statistical treatments. Assessment of the quality of the host plant included chemical analysis and reflection spectrophotometry.

RESULTS AND DISCUSSION

The nutritional value of the potato plant for *M. persicae* depended on the supply of the elements N, P, K and Mg in particular, of which N, P and Mg stimulated growth and reproduction, and K above a certain level reduced them. Besides nutrition, age of the plant was determinative for the nutritional value of the phloem sap. Although only a few nutrients were essential in a biochemical way, about 20 amino acids and amides influenced growth and development of *M. persicae*. Growth was highly stimulated by amides, particularly asparagine. The amino acid methionine also acted as a phagostimulant and had to be present in the phloem sap, together with sucrose.

Changes in the host plant resulting in reduced reproduction were also responsible for a smaller proportion of alatae. This result contradicted the generally accepted statement that more alatae were produced on host plants with a suboptimal nutritional value. Experiments with artificial diets demonstrated that these results were just as valid off the plant. Apterae were produced when the amino acid ratio in the food was unfavourable to normal development of *M. persicae*. They were also produced, however, on very young plant parts, especially on seedlings, which permitted normal growth and development. Yet on a suboptimal diet, alatae were produced, if the restlessness of the aphids was increased.

Host-plant substances influencing morph can be divided into several classes, with different modes of action. Some have a twofold effect, as they affect population development (and contact between individuals) and the system governing wing dimorphism. This is especially true for methionine, isoleucine and histidine, but also for the ratio of asparagine to glutamine and to unessential amino acids. For a fuller report of these results see Harrewijn (1977).

Some compounds (trace elements, phytohormones and growth substances) have an extrasensory effect on wing dimorphism, as well as affecting the activity of neurosecretory cells that regulate polymorphism. Micro-injection experiments demonstrated that biogenic amines derived from the aromatic amino acids tryptophan and tyrosine had a mediating function in the regulation of wing dimorphism in *M. persicae*. A few trace elements, like lithium, interfered with the activity of these biogenic amines, thus shifting the regulatory mechanism of wing dimorphism towards the apterous course of development.

Field trials on regulation of population development of *M. persicae* by the mineral nutrition of the potato plant confirmed the results obtained in the laboratory. Both population development and production of alatae of this aphid were influenced by nitrogen dressing and by the amount of potassium available to the crop. The magnitude of the effects obtained in soilless culture and in the field differed considerably, although the trend of the effect was the same as long as artificial fertilizers are used. This cannot be said of organic manure, which reduced population development in the field. However, when potato plants were raised on nutrient solutions with and without extracts of stable manure, but with an equal ion concentration and ratio of the major nutrients, the performance of *M. persicae* was optimum in all trials (80-100 larvae per female).

Low rates of N fertilizer reduced the total amino acid content of the leaves. K reduced glutamine, histidine, methionine and tyrosine, and increased proline.

With results of field trials from 1972 to 1978, a fertilizer scheme has been developed (combined treatment with N, P and K) that can reduce populations of *M. persicae* and *Macrosiphum euphorbiae* to 20% of normal, without loss in yield. Wing production is reduced from 70%-30%. Trials with trace elements to delay wing development in the field are now under way.

SIGNIFICANCE FOR AGRICULTURAL PRACTICE

Mackauer & Way (1976), in their discussion on alternative methods to control aphids, refer to the unique sensitivity of *M. persicae* to the physiological condition of its host plants. They state that manipulation of the physiology of the plant could be invaluable in a program of integrated control for this aphid. This project has provided satisfactory proof that manipulation of the potato plant can make a major contribution towards a scheme of integrated control.

In the Netherlands, the proportion of winged morphs of *M. persicae* (although exceptionally) can reach 30% in the field by the end of June. Our basic scheme will reduce this to about 8% and it is expected that proper timing of lithium treatment will further reduce this figure to less than 1%, or proportionally lower if less winged aphids are normally produced. This may be sufficient to omit 'cleaning' of the crop with insecticides before selection for the presence of non-persistent viruses, and for control of leafroll.

When applied to plots surrounded by other crops, the anti-aphid treatments will be most effective against persistent viruses and will only reduce infection by a non-persistent virus in so far as it is spread within the plot by a developing aphid population. That aspect differs from year to year, as it depends on flight activity of non-residents, also able to transmit non-persistent viruses. It can be calculated that in some years the treatments should reduce infection with non-persistent viruses by less than 50%, but in those years insecticides would give no better protection, would be more expensive and harm natural enemies.

FUTURE OUTLOOK

Van Hoof (in prep.) recently found that ware potatoes may be a serious source of non-persistent viruses (like PVY^N), that can spread into a neighbouring seed potato field. These results provide an interesting basis to extend our treatments to ware potatoes. A further improvement in control of aphid flight may be obtained by application of trace elements like lithium at the proper time. In seed potatoes, a delay in wing production by one generation will be sufficient; for ware potatoes, time schedules have to be worked out.

Spraying mineral oils helps to reduce infection with non-persistent viruses. Once started, one has to continue spraying at regular intervals, so a yield reduction is inevitable. If our anti-aphid treatments could be combined with this technique, it would be possible to stop oil spraying after the threat for early infection with virus transmitted by non-host aphids is over. This type of integration will be studied in the near future.

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4.4 Nutritional relationship between host plants and phytophagous mites

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DESCRIPTION OF THE PROBLEM

At the beginning of the nineteen sixties, the prospects of introducing a scheme of integrated control in orchards were not very encouraging. Phytophagous mites became a serious problem, because of the alarming development of resistance to the usual insecticides and acaricides. More or less selective acaricides that would keep natural enemies of the pests alive were not available and the study to introduce specific natural predators was in its infancy. Moreover, there was a tendency to enhance nitrogen dressings in apple orchards. This led Kuenen (1962) to question whether predators could cope with a possibly accelerated development of spider mites under these circumstances.

There was, however, some evidence that changes in the chemical composition of apple leaf brought about by differences in manuring could be a major factor in controlling the population development of spider mites. In particular, Post (1962) found that the rate of egg production of *Panonychus ulmi* was significantly lower on apple trees in neglected orchards than that in well kept orchards. The level of leaf nitrogen in neglected orchards was lower than in the well kept ones.

In contrast, population development of *P. ulmi* in neglected peach orchards in Canada did not seem to be retarded. This made it difficult to develop fertilizer schemes for apple orchards that would fit into a scheme of integrated control.

AIM AND MOTIVATION OF THE RESEARCH

The project was started to elucidate the role of mineral nutrition of the apple tree in population development of spider mites and to investigate the influence of a mite population on the physiology of the host in different periods of the year. Main attention should be given to application of fertilizers within the range expected in practice.

WORKING METHODS

The research developed along two main lines:
- the reaction of a mite population to alteration in the chemical composition of the host plant;

- the reaction of a host to the feeding activities of a developing mite population.

The problem was first approached in laboratory experiments. One-year old apple rootstocks were grown in gravel culture on different nutrient solutions. It was thought that knowledge of the nutritional demands of spider mites would help in evaluating chemical composition of the host plants. So the development of artificial diets was started. Preliminary results showed that *P. ulmi* was not a suitable mite for the chosen experimental techniques. In its place, *Tetranychus urticae* was used with apple and bean as host plants.

Phytophagous mites damage the crop by decreasing the assimilatory capacity. I observed that they may also interfere with some chemical processes in the plants. With autoradiography, the fate of saliva injected into the plant, and auxin relationships were investigated. Little was known about the magnitude of the damage caused by a developing mite population. More information on this topic was thought to be obtainable by studying transport of minerals in a closed system. This type of experiment was set up as balance studies; that is to say, the whole of nutrient solution together with plants and mites was arranged as a closed system for centain nutrient elements. In this way, the effect of mite attack on the distribution of a certain element could be evaluated.

Field trials were set up to induce a different N content in the leaves of apple trees. Both artificial and organic fertilizers were used.

RESULTS AND DISCUSSION

An increase in total nitrogen content of leaves of apple rootstocks grown in gravel culture increased longevity and egg production of *P. ulmi* and *T. urticae*. Difference in the K^{+}/Mg^{2+} ratio in the nutrient solutions were not correlated with mite reproduction. Phosphorus promoted mite development. It was difficult to find a close relationship of soluble N and P in the leaves with mite development. Dabrowski & Bielak (1978) arrived at similar results for N and P content of leaves of apple cultivars and the fecundity of *P. ulmi*.

Balance studies on *T. urticae* on bean plants showed that mites cause a marked change in the distribution of P in the plant. Infestation increased transport of P to the growing parts of the plant and also to the parts occupied by the mites. Population densities between 1 and 4 females per square centimetre resulted in a growth stimulation. If the density exceeded 10 cm^{-2} growth inhibition occurred.

Mites can transform the amino acid tryptophan into an indole derivative. As they inject saliva into the plant, the equilibrium of growth substances may thus be influenced by a mite infestation. There was some evidence that an auxin-like substance and a gibberellin play a role in growth regulation of the plant, after infestation by mites. The first thing was that growth was stimulated and, when the mite population grew, it was reduced. So a mite infestation in an orchard may even be beneficial, as long as it develops slowly. In this way, predators can stay alive and 'stand by' for action. It was thought that in most summers the nutritional quality of the apple trees must be influenced in some way to allow slow population growth of mites. Although population development of mites in the greenhouse was found to be positively influenced by nitrogen fertilization, field trials mainly revealed an increase in longevity of spider mites (up to 28% after nitrogen dressing at more than 300 kg/ha) and only minor differences in reproduction capacity. Van de Vrie & Boersma (1970) found that the development of *Panonychus ulmi* on apple trees reacted positively to an increased content of N in the host plant, but only so in the absence of predators, especially *Typhlodromus potentillae*. This means that *T. potentillae* is able to reduce the population density of the prey *P. ulmi* under various conditions of the host plant. It could also explain why Storms found that N at more than 300 kg/ha in orchards increased longevity of individuals of *P. ulmi*, but had little effect on population development. A more detailed knowledge of the chemical relationships between fertilizer and mite development was hard to obtain, as rearing of mites on artificial diets was not very successful. Although *T. urticae* could be kept alive for some weeks, no diet could be formulated that enabled it to complete its life cycle. The best results were achieved when energy-rich phosphates, stearic and palmitic acid were added to a diet containing amino acids at a mass fraction of no more than 90 mg/kg. This concentration is lower by a factor 7-8 than expected in leaf juice of bean (0.5-0.6 g/kg).

Glumatic acid was not quite omitted from the diet, although its presence seemed to have a negative effect on the performance of *T. urticae* (Storms, 1971). Dabrowski & Bielak (1978) ascribed the low sensitivity of the rose cultivar Baccara to *T. urticae* to its high content of glumatic acid.

SIGNIFICANCE FOR HORTICULTURE AND FUTURE OUTLOOK

Studies of the nutritional relationships between host plants and phytophagous mites have revealed many facts, but only a narrow basis for an integrated control. In the Netherlands, N dressings have been substantially reduced in the last couple of years. This has resulted in a solution of the mite problem for most cultivars. Rabbinge (1976) constructed a number of simulation models for the relationship between *P. ulmi* and the predator *Amblyseius potentillae* on a basis of field trials. As long as leaf nitrogen was between 2.0 and 3.0% of dry weight, food quality could be neglected in simulation models of field situations.

In young orchards on new sites, where predator activity is low, it will be wise to use fertilizers carefully. The same holds for apple orchards where insecticides are used that harm the predators. For these situations beneficial effects may be obtained by special combination of N, Mg and K fertilizers. Another possibility is the development of cultivars with, for instance, a high content of glumatic acid in the leaves. This project, however, was stopped in 1971. Since then, only a little research has been done on the development of artificial diets for mites. It is to be hoped that somewhere in the world financial support and patience is found to resume this useful and interesting work.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

4.5 Elements in the population dynamics of Euproctis chrysorrhoea on roadside trees

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INTRODUCTION

The Netherlands has only little forest but many trees along roadsides and canals. One of the pests attacking these trees is the browntail moth, *Euproctis chrysorrhoea*, notorious not only for defoliating oaks and other trees but also for causing nuisance by its hairs, which irritate the skin of susceptible people.

E. chrysorrhoea reaches the northern limit of its range in the Netherlands. In the interior, it is restricted to the South part of the country, crossing the River Waal, which is the southern most and largest branch of the Rhine, flowing almost east-west through the middle of the country, only occasionally, during severe outbreaks. In the sand dunes on the coast of the North Sea, where the species dwells on sea buckthorn *(Hippophaë rhamnoides)*, it extends right up to the northernmost end of the Netherlands, the Friesian Isles. The caterpillars sometimes invade camping sites after defoliating their host plants and are dreaded by tourists at the seashore.

The work reported in this paper aimed at understanding the causes of the outbreaks of *E. chrysorrhoea* in the interior of the country, as a basis for ecological control of the species.

LIFE CYCLE AND OUTBREAKS

E. chrysorrhoea has one generation per year. The adults fly in July, depositing their egg masses on the leaves near the tip of shoots. After hatching in August, the larvae reduce the leaves to skeleton and spin a nest at the end of a shoot. The nests begin as rather loose webs but are gradually strengthened to become tough structures in which the caterpillars hibernate. All the larvae hatched from one egg mass, or those from two or more masses, co-operate in building the nest. Early in spring, when the temperature rises, the caterpillars leave their nests and start feeding when the buds burst. If the caterpillars are so abundant as to defoliate the trees, they descend in search of new food plants. The larvae are full-grown in June and pupate on the trees to give rise to adults in July. Although several species of tree and shrub can serve as hosts, the main food plants inland are oak, hawthorn, and fruit trees.

Outbreaks of *E. chrysorrhoea* in the Netherlands have been recorded since 1902. The ups

Fig. 1. Outbreaks of *E. chrysorrhoea* in the south of the Netherlands; --- local —— severe.

and downs in abundance are rather irregular, and the duration of outbreaks shows an increase from 3 years initially in 1917 to 10 years at the present time (Fig.1).

From 1962 to 1977, a survey of the dispersion and abundance of *E. chrysorrhoea* was made yearly in winter, when the nests are conspicuous, by inspecting roadside trees along a fixed route in the southern part of the country from a car. In periods of low numbers, the population of *E. chrysorrhoea* maintains a footing in certain places in the region where it is indigenous, and extends its area from these centres when conditions for the species become favourable.

OUTBREAKS AND FOOD QUALITY

The idea that the quality of the food might be a key factor in outbreaks was prompted by the observation of a fixed order in which different tree species were attacked by *E. chrysorrhoea* in the course of outbreaks (Voûte & Van der Linde, 1963). Rearing experiments confirmed the supposed differences in suitability of different tree species as food for the larvae: hawthorn, apple, and oak in that order proved decreasingly suitable. Moreover, larvae reared on the same tree species in different places of the country differed strikingly in success of completing their development. Those reared north of the Rhine were less successful than those reared in the south (Van der Linde & Voûte, 1967).

More detailed rearing experiments in an insectarium on cut-off branches, showed that foliage from different parts of a tree differed widely in suitability as food for the larvae in spring, i.e. from hibernation to pupation. Females from caterpillars reared on foliage from the top or the south side of oak trees produced several times as many eggs as females from larvae reared on branches from the north side of the same trees. Again, hawthorn proved more suitable as food than oak (Van der Linde, 1968).

In the period from hatching to hibernation, the origin of the food also influenced larval development and hence the eventual fecundity of the population. This was concluded from experiments in which larvae were reared in situ on different parts of oak trees. The total number of eggs produced by the groups of larvae with which the experiment was started was greater on the old foliage (i.e. spring shoots) than on the young (i.e. summer shoots) and, within old foliage, greater on the foliage from the south side than from the north side of the trees. The most favourable type of food showed the highest content of protein and sugar (Van der Linde, in preparation).

The nature of the food has a great impact on the development of the larvae of *E. chrysorrhoea* and, presumably, on the fluctuations of the population. The quality of the foliage, determined by tree species and site, and influenced by weather conditions, must be a key factor in the abundance of *E. chrysorrhoea*.

It has not been possible to put this assumption to a complete test, which would consist of ascertaining the development of *E. chrysorrhoea* nests and populations, and analysing the foliage of the trees, on different sites during a number of consecutive years. Some preliminary work, although it did indicate the merits of this approach, was not sufficient for conclusive evidence.

NEST BUILDING AS A CRITICAL FACTOR FOR SURVIVAL

An analysis of nests on different tree species in certain localities suggested that nest building is a critical factor for the success of hibernation of the larvae inhabiting the nest, and may be decisive for survival of local populations. Larvae of small groups, when building their hibernacula, were found to contribute a greater amount of nest material per larva than caterpillars from large groups. The former weighed less after hibernation, with suggested lower vitality, since the percentage survival per nest was positively correlated with the weight of the larvae after hibernation. Apparently, nest building is heavy labour and, if it has to be performed with too few fellow caterpillars, it detracts from survival during winter. The minimum number of larvae per nest successfully surviving was 34. Food quality and amount of foliage available on a branch as material to construct the nest are factors determining the success of nest building.

E. CHRYSORRHOEA IN AND CLOSE TO FORESTS

Single trees and roadside trees are more prone to heavy attack by *E. chrysorrhoea* than trees in or near forests, a difference clear at medium densities but becoming less pronounced in years of severe outbreaks. The nests in the forest localities were smaller, on average, and contained fewer living caterpillars in spring than those in open country. Bird damage, mainly by titmice, was greater in or near forests; but parasitization, predominantly by the chalcid, *Eupteromalus peregrinus*, was lower than in open areas.

CONCLUDING REMARKS

The work on *E. chrysorrhoea* has focused attention on a factor too often neglected in the population dynamics of phytophagous insects: the quality of their food. Although it has not yet traced a full and coherent picture of the relationships determining the pattern of rise and decline so characteristic for this species, it suggests an approach that might improve control.

At present, extensive chemical control campaigns by aircraft or powerful spraying machinery are initiated when outbreaks develop. It would be better to find the footholds of the population during periods of population depression, carefully monitor them, and treat then at the first sign of increase.

Recently started work in the Netherlands has concentrated on late-leafing oak types as a possible means of controlling the browntail moth. This approach might be particularly effective if this type of oak were planted at the sites now permitting *E. chrysorrhoea* to survive during inclement periods.

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5 Host-plant resistance

The importance of host-plant resistance as a basic component of integrated control has been insufficiently recognized for a long time. In the Netherlands, relations of insect and host-plant have been extensively investigated since the end of the forties (Chapter 4) but host-plant resistance only received close attention in the early seventies. Despite a relatively short period of activities, results are encouraging.

Much effort was devoted to development of reliable and efficient tests (5.1, 5.4, 5.5, 5.6, 5.7). Simultaneously sources of resistance were traced in either commercial varieties (5.2, 5.3), varieties from world collections (5.1, 5.4, 5.7) or related species (5.3, 5.5, 5.6). Resistance was improved by recurrent selection (5.2, 5.3) and intercrossing of partially resistant varieties (5.7). A start was made with transferring resistance from related species to commercial varieties (5.5, 5.6).

Breeding varieties better suited to parasites and predators (3.1, 5.8) is another, rather peculiar, contribution of plant breeding to integrated pest control.

Some of the newly bred material has already been released to commercial breeding firms (5.7, 5.8), which will further develop it into commercial varieties.

Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

5.1 Resistance to aphids in barley and wheat

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DESCRIPTION OF THE PROBLEM

Cereal aphids used to play a minor role as pests in the Netherlands. There is some evidence that in the last decades the average level of infestation is growing higher in many European countries. Three aphid species are involved, the bird-cherry oat aphid, *Rhopalosiphum padi,* which occupies the culms, the rose grain aphid, *Metopolophium dirhodum*, which can be found mainly on the lower leaves, and the English grain aphid, *Sitobion avenae,* which inhabits the ears and upper leaves. Since *S. avenae* is the most numerous aphid in the ripening stages of cereals and is generally assumed to be the most harmful, investigations were centred on this species.

MOTIVATION AND AIM OF THE PROJECT

Though the data on direct harm caused by cereal aphids are rather limited, there is a consensus that high population densities reduce grain yield. The economic injury level is about 20-30 aphids per tiller, or at least 15 aphids per ear. Although the aphid population only seldom exceeds the economic injury level and only in restricted regions of the country, a tendency can be observed to spray preventively. Large-scale application of insecticides would cause a severe environmental load, taking into account that the total area of grain cereals is at least 20 million hectares in Western Europe. Therefore it is of importance to develop methods of biological control to lower the general level of infestation. Genetic resistance to cereal aphids seems to be the most appropriate way to attain this goal. Consequently the main objective of the present project is to trace sources of host-plant resistance to cereal aphids. This can only be achieved after the second objective, the development of simple, efficient and reliable screening methods. In the first phase of the project, much attention is given to that aspect.

MATERIALS, METHODS AND CONSIDERATIONS

Barley was chosen as the major test crop. Between 1973 and 1977, some 800 accessions were screened, including commercial varieties, semi-wild populations and botanical species. In 1977, hundred wheat accessions entered the program too.

Screening methods were developed at three levels: natural infestation; artifical inoculation in field cages or greenhouse compartments; single plants under controlled conditions. A concise outline of the pros and cons of the various test methods can be found in Van Marrewijk & De Ponti (1975).

Natural infestation is the most simple way of testing. This method is used to screen large numbers of accessions for major differences in aphid resistance. Accessions are planted in small plots, rows or tussocks in a randomized block design and assessed for aphid density at fixed intervals. Due to the hazardous build-up of epidemics and the irregular distribution of aphids a relative large number of replicates is necessary to detect significant differences.

Plants were infested artificially in the field with modified Reitzel cages as described in Van Marrewijk & Dieleman (1977) at about phase 7-8 of the Feekes scale with a random sample of laboratory-reared *S. avenae*. The initial density was about 1 aphid per tiller. High costs of the trial made this kind of test only feasible for an unreplicated check on natural infestation trials or to test small numbers of selected accessions again. Tests under abnormal growing conditions, including greenhouse tests, are only meaningful alongside field trials.

In tests on single plants, small numbers (1-10) of fourth-instar larvae or young adults were placed on individually caged pot plants. As each plant can be considered a separate treatment, these tests can be done in many replicates. The tests on single plants were used for renewed tests on accessions selected in the field, cage or greenhouse for a genetic background of resistance, to find the mechanism of resistance, and to find optimum conditions for expression of resistance. The criteria used were directly or indirectly related to factors determining the rate of increase of an aphid population.

RESULTS AND CONCLUSIONS

Only a general outline of observations and results is presented.

Natural infestation trials did not yield any variety completely resistant to *S. avenae* nor *M. dirhodum*. No conclusions could be drawn about *Rh. padi* because of the very low density in all years of observation.

Natural infestation trials were characterized by a considerable amount of variation, within and between blocks, caused by irregular distribution of aphids, local activity of predacious insects, and other external factors. This seriously handicapped tracing clear--cut and statistically significant differences in resistance. Nevertheless there was considerable evidence that heritable differences in aphid resistance occurred between the barley accessions tested.

Both in field and greenhouse tests, there was a retarded population growth of *S. avenae* on late or non-bolting barley accessions. In one experiment, a significantly higher aphid density was measured on the types that had come into ear.

A high correlation was found between aphid density per tiller and the number of infested culms, when the general level of infestation was not too low. This is in accordance with observations made by Basedow (1975) and may lead to a simplified method of assessment.

There was a conspiceous lack of conformity in population development between accessions in field cages and in naturally infested plots. No explanation could be given so far.

Two-row barley accessions contained less aphids than four-row or six-row types. It is doubtful whether this reflects group differences in aphid resistance. A more obvious explanation is the greater accessibility of two-row barley to predaceous insects, especially coccinellids.

Artificial inoculation in the open (in order to accelerate population development) proved to be a failure. Within two weeks, aphid density fell to the level of naturally infested plots.

Differences in resistance observed in the field were generally not confirmed by tests in the greenhouse or on single plants. An exception was barley accession CI-16145, which showed a low aphid density at natural infestation and was less susceptible than other accessions in a single plant test. When inoculated at Feekes stages 5 or 6-7, the reduced susceptibility was expressed by a low reproduction, a high larval and adult mortality and a relatively long generation time.

SIGNIFICANCE FOR FARMING

Introduction of cereal varieties with an increased level of resistance to aphid attack will be a valuable contribution to crop farming. In the Netherlands, however, commercial varieties of cultivated crops are bred exclusively by private companies. These will only be willing to include aphid resistance in their breeding programs if appropriate sources of resistance, preferably as partly bred material and if well established screening methods are available. Therefore the present project is prerequisite for commercial breeding of resistance to aphids.

FUTURE OUTLOOK

The project started in 1973 as a joint activity of the Departments of Entomology and of Plant Breeding of the Agricultural University, Wageningen. Despite the promising results so far, it will not be continued in its present form after 1978 because of its laborious character and because of restrictions in staffing.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

5.2 Breeding of carrot (Daucus carota) for resistance to carrot fly (Psila rosae)

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INTRODUCTION

Since the large-scale introduction of synthetic pesticides, insecticides have been used routinely in the Netherlands against the carrot fly *(Psila rosae)* in carrots *(Daucus carota)*, although it is doubtful whether this is always necessary.

The use of these insecticides also became common practice in selection fields, so that susceptible plants were no longer eradicated or recognized. Based on other agronomic characters some of these plants may have been selected and participated in the pollination and fertilization processes of this cross-fertilized crop. This procedure may have caused a gradual shift to a lower resistance in recent carrot varieties and selections.

Because of the rapid reduction in germination of carrot seed, it is not possible to compare the resistance of old varieties and selections with that of recent ones. For the same reason, old varieties and selections cannot be used as possible reservoirs of resistance.

This project aims at tracing variation for resistance between and within varieties and selections. By careful selection, it is intended to breed a carrot population with a higher resistance, which can be used as germplasm in commercial breeding.

WORKING METHODS

Over six years, about 190 varieties and selections of mainly West European origin have been screened for resistance in field trials in an area where the carrot fly is generally sufficiently active. Insecticides were not used and herbicides only before the plants emerged.

In May, the varieties were sown in 3-8 replicate rows 1.5 m long, according to the amount of seed available. The first-generation attack of carrot fly, which only rarely killed plants, was generally less important than the second-generation attack, which in some years was heavy. In October, the carrots were harvested, washed and classified as attacked or unattacked. From those varieties that were repeatedly least attacked, about 20 unattacked carrots were selected from the most heavily attacked replicates, where the selection pressure had been the strongest. The selection criterion was altered from year to year, depending on the average level of attack. By selfing, seed was grown for each selected plant in the next year and a year later the resulting inbred (I_1) lines were compared with the parental material. From the best I_1 lines, individual plants were again selected and selfed and the I_2 lines were screened for resistance two years later.

In 1978, the first series of I_2 lines originating from 7 varieties were tested. Tests included 35 varieties, 107 I_1 lines and 62 I_2 lines.

RESULTS AND DISCUSSION

The behaviour of the carrot fly results in a very uneven distribution over the field. Consequently the differences in attack between replicates are always large. The year 1978 was suitable for selection, because incidence on susceptible varieties was up to 90%.

Figure 1 shows the results of the 1978 trial. The lines were generally tested in fewer replicates than the varieties because only limited amount of seed were harvested from

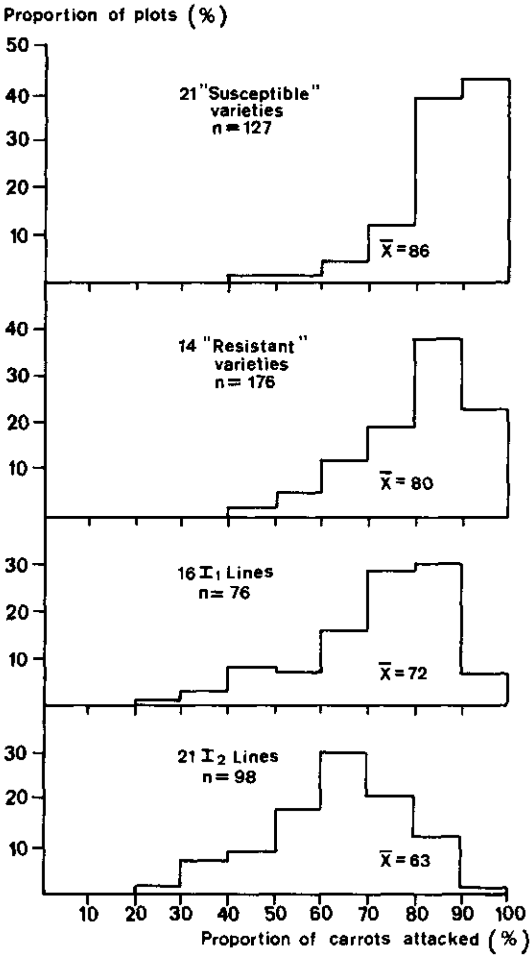

Fig. 1. Proportional frequency distributions of replicated plots of 'susceptible' and 'resistant' varieties and of selected I_1 and I_2 lines from the latter by incidence of carrot fly. n = number of plots; $\bar{x}$ = mean proportion (%) of carrots attacked.

single plants. The varieties were divided into a more "susceptible" and a more "resistant" group. The first two histograms of Fig. 1 demonstrate that the differences in resistance between varieties are small. This agrees with previous experience (Nieuwhof, 1977).

Line selection during the first two generations have resulted in an increase of about 20% in resistance of the selected lines over the parental generation. Of the I_1 lines, only a few (16 of 107) met the selection criterion. This indicates that many carrots selected in the parental material were not more resistant, but merely escaped attack. Of the I_2 lines, a larger proportion (16 of 62) met the selection criterion. This shows that the selected I_1 lines possessed a higher resistance than the parental material. The effectiveness of line selection for culling of wrongly selected material is thus demonstrated.

Figure 2 shows that within some varieties resistance varies. Improved I_2 lines were selected from the varieties 'Nantes', 'Pioneer', 'Signal' and 'Vertou'. Other varieties are still under investigation.

Although prolonged line selection might result in a further increase in resistance, this selection procedure will not be continued uninterruptedly because of the decline in fitness, quality, yield, seed yield and seed quality, with repeated inbreeding. Resistance might increase by bringing together resistance genes from different varieties. So the selected I_2 lines will be intercrossed and the seed harvested for each plant or for each line. The families thus obtained will again be subjected to line selection for one or two generations. This procedure can be repeated as long as progress is made. During each intercross generation, I_2 lines of newly discovered resistant varieties can be crossed with the existing resistant population. All resistances found will eventually be brought together in one population with a maximum resistance.

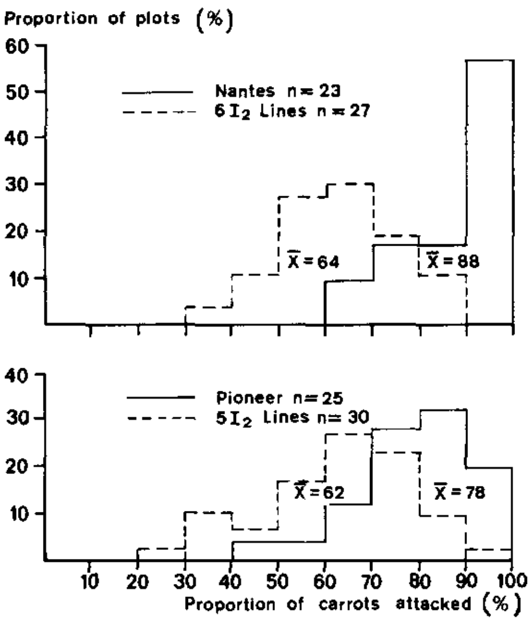

Fig. 2. Proportional frequency distributions of replicated plots of the varieties 'Nantes' and 'Pioneer' and of selected I_2 lines from them by incidence of carrot fly. *n* = number of plots; $\bar{x}$ = mean proportion (%) of carrots attacked.

FUTURE WORK

When seeds are harvested in intercross generations for each line, larger amounts of seed will become available, allowing essential background studies. The limited amounts of seed from single plants necessitated experimental designs with one-row plots. We must investigate how far the differences in resistance hold in larger plots, which better represent the situation in crops. On the resistant carrot families, the population development of the carrot fly can be studied in terms of oviposition, egg viability and larval development. Also chemical and biophysical characteristics of the leaves and roots of resistant families should be compared with these of susceptible ones to discover any essential factors responsible for host selection and utilization by the carrot fly.

SIGNIFICANCE FOR AGRICULTURE

The results indicate that resistance can be improved step by step and that the ultimate level of resistance cannot be predicted. Possibly additional control measures will remain necessary, but total dependence on chemical control will be over. Partial resistance as one of the components of integrated control of carrot fly might improve the effectiveness of other factors, such as open windy fields, which are ecologically less suitable for the carrot fly, and slow-release insecticides.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

5.3 Breeding onion (Allium cepa) for resistance to onion fly (Delia antiqua)

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INTRODUCTION

The aim, motivation and philosophy of this research project were similar to those of the project on carrot fly (Section 5.2).

WORKING METHODS

Over 6 years, about 60 varieties and selections of *Allium cepa* mainly of European origin, have been screened for resistance to the onion fly *(Delia antiqua)*. The material also contained 20 breeding populations of the Polish variety 'Rawska', which, according to field observations in Poland, were less prone to onion fly. From botanic gardens, 60 accessions of *Allium fistulosum* were received, a species which seemed more resistant than *A. cepa*.

This material was screened for resistance in field trials at Wageningen, where the onion fly was generally sufficiently active. Because the trials were always set out in the same area, the population of onion fly increased from year to year, so that in some years the selection pressure tended to be too heavy. In 1978, however, the weather resulted in a very small population of onion fly and consequently the selection of valuable inbred (I_1 and I_2) lines was hindered. This illustrates the shortcomings of testing in the field. This method is, however, necessary for collection of reliable data on the resistance (Van Marrewijk & De Ponti, 1975).

The experimental design and selection procedures were identical to those used in screening for carrot fly resistance, with one exception. The first generation of the onion fly mostly caused serious damage. It destroyed the young seedlings, which were not detectable at harvest. Therefore all plots are counted three times: after emergence of the seedlings (May), at the end of the first generation of onion fly (July) and at harvest (September). At harvest, the bulbs are classified as attacked and unattacked. Thus selection can be based (possibly stepwise) on first-generation attack or on all-season attack.

RESULTS AND DISCUSSION

In 1976, the susceptible varieties were attacked up to 90%. This level of attack proved suitable for selection. Varieties of *Allium cepa* were found with some degree of resistance

Table 1. Proportion of plants attacked of some onion varieties and of some accessions of *A. fistulosum* as recorded in a field test in 1976. Values are averages for all plots for the whole growth season.

Material	Origin	Proportion of plants attacked (%)
A. cepa 'Jumbo'	Netherlands	90
A. cepa 'Hiberna'	Czechoslovakia	54
A. cepa 'Wolska'	Poland	58
A. cepa 'Yellow Makoi'	Hungary	64
A. cepa 'Rawska sel. 1'	Poland	64
A. cepa 'Kastika'	Hungary	65
A. fistulosum	Hungary	32
A. fistulosum	Czechoslovakia	34
A. fistulosum	West Germany	34
A. fistulosum	Sweden	35
A. fistulosum	West Germany	71
A. fistulosum	West Germany	73

to the onion fly (Table 1). Within the related species *A. fistulosum*, markedly higher resistance was present, although not all accessions of the species were highly resistant.

From the most resistant *A. cepa* varieties and *A. fistulosum* accessions, unattacked plants were selected and selfed. The I_1 lines of the varieties were tested in 1978, when selection was hindered by a low frequency of attack (susceptible control only 35%). Nevertheless after selection in one generation, incidence in the best I_1 lines was about 10 percentage units less than that of the parental varieties. The I_1 lines of *A. fistulosum* will be tested in 1979.

Resistance was found mainly in eastern European varieties, perhaps because insecticides were introduced there later or to a lesser extent, at least in selection fields.

FUTURE WORK

Selection for resistance will be continued by the same procedures as for carrot fly. At first, the *A. cepa* and the *A. fistulosum* material will be bred separately and even in alternate years, because the resistance of *A. fistulosum* seemed to be underestimated, when that species grew mixed with *A. cepa*. It seems that the onion flies attracted by *A. cepa* will readily attack *A. fistulosum*. After one or two cycles of selection, the two kinds of population will be hybridized and further treated as one kind of breeding population. When larger seed samples become available by family selection, background studies similar to those described for the carrot fly can be done.

SIGNIFICANCE FOR AGRICULTURAL PRACTICE

It is difficult to predict which level of resistance to the onion fly will ultimately be reached, but it is almost certain that additional control measures remain necessary. Partial resistance as one of the components of an integrated control system of the onion fly might promote other components like the release of sterile males. For it is expected that on slowly growing insect populations the frequency and size of the releases can be diminished, thus increasing the profitability of this strategy.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

5.4 Resistance in lettuce (Lactuca sativa) to the green peach aphid (Myzus persicae)

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INTRODUCTION

The green peach aphid, *Myzus persicae*, causes problems in greenhouse lettuce and has to be controlled with insecticides. Other methods of insect control are needed because of the expense of insecticides, adaptation of aphids to some insecticides and the risk of residues, especially in a vegetable with a large area of consumable leaf. Plant resistance could offer an inexpensive and safe solution.

MACROTEST FOR RESISTANCE

For a rapid screening for resistance of a large collection of plant genotypes of the IVT *Lactuca* gene bank, a macrotest was developed (Eenink & Dieleman, 1977a). Plant genotypes to be tested are grown in a greenhouse (15-25 °C) in four replicates with five plants per genotype in each replicate. Individual plants (5-8 leaves) are inoculated with 10-15 aphids reared on Chinese cabbage. After 3-4 weeks, the number of aphids per plant is estimated on a scale from 0 (absolute resistant, no aphids at all) to 90 (very susceptible, many aphids). By this method, mostly relative differences between plants can be observed. Nevertheless there is a good relationship between estimates and counts of the number of aphids (Eenink & Dieleman, 1977a).

By the macrotest, large differences were found between about 800 lettuce genotypes (Fig.1). About 15 selected partially resistant genotypes were used for more detailed investigations on the aphid-lettuce relationship, for studies on the inheritance of resistance and for the improvement of the level of resistance by breeding.

In general, test procedures are determined by characters of the plant, the aphid and the plant-aphid relationship. So rather detailed information is needed about factors such as plant age, type of leaf, larval growth (weight of the larvae), fecundity and mortality of larvae and adults. The interdependence of some of these criteria for resistance, the genetic and environmental influences on the expression and stability of resistance should also be investigated.

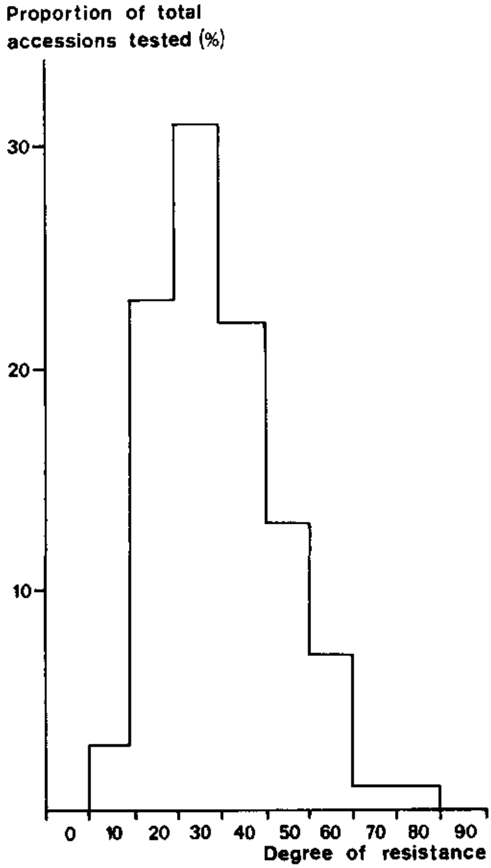

Fig. 1. Frequency distribution of 266 IVT Lactuca accessions over a scale for resistance. 0 = absolute resistant; 90 = susceptible. Values per genotype are means of 20 plants.

INFLUENCE OF PLANT AND LEAF AGE

Colonizing aphids often change their position on a leaf and over different leaves of the same plant. The ultimate result of plant development and ageing of the plant, and population growth of the insect is a distribution dependent on the age of the leaf. For instance, on a fully grown lettuce plant, most aphids are on the lower, senescent leaves.

The characteristic distribution of aphids over a plant may influence the outcome of resistance tests. Table 1 lists some results of the influence of plant age of a partially resistant and a susceptible genotype on aphid behaviour and development. There were large differences for larval mortality, insect weight and fecundity between the tested genotypes. In both genotypes, insect weight and production of larvae were influenced by plant age. The older plants seemed to be somewhat more susceptible than the younger ones. The difference in resistance between the two genotypes measured by weight of insect was somewhat bigger with young plants. The difference between resistant and susceptible plants for larvae production did not change with plant age.

Experiments with a partially resistant and susceptible genotype also showed the influence

Table 1. Influence of plant age on larval mortality, insect weight and number of larvae produced per aphid (in four days) for a partially resistant and a susceptible genotype. Plant age 22 and 15 days at the time of infestation.

Quantity	PIVT 313 susceptible		PIVT 339 partially resistant	
	22 days	15 days	22 days	15 days
larval mortality (%)	23	23	50	58
insect weight (μg)	566	502	398	304
number of larvae produced per aphid in 4 days	18.6	16.4	9.9	7.8

of leaf age and leaf parts on weight of larvae and reproduction (Eenink & Dieleman, 1977a). For these experiments, leaf cages were used (2 cm diameter). Weight of insect and reproduction were higher on old leaves, both of the partially resistant and the susceptible genotype. Significant differences between leaf parts, measured by insect weight and larvae production, were only observed on the partially resistant genotype. Near the tip of the leaves, the highest values for these characters were measured. The differences between resistant and susceptible plants for weight and production of larvae hardly changed with leaf age or part of the leaf.

INFLUENCE OF APHID DENSITY

Some evidence is available that the rate of increase is density-dependent. This implies that the criteria for resistance in our tests are also density-dependent. The influence of aphid density on factors like larval mortality, production of alatae, insect weight and larvae production was investigated with leaf cage tests. Some results for susceptible and partially resistant genotypes are given in Table 2. For all criteria, large differences existed between the two genotypes. The influence of aphid density on mortality seemed to be absent or very weak. On both tested genotypes, aphid density positively influenced the frequency of winged aphids and negatively influenced weight of larvae and net production.

Table 2. Influence of number of aphids on larval mortality, proportion of winged aphids (%), insect weight and number of larvae produced with a resistant and a susceptible genotype.

Quantity	PIVT 180 partially resistant			PIVT 197 susceptible		
	larvae per cage			larvae per cage		
	1	5	10	1	5	10
larval mortality (%)	50	53	63	23	23	24
winged aphids (%)	13	19	32	4	9	14
weight per aphid (μg)	225	173	163	437	384	294
number of larvae produced per aphid in 7 days	9.4	7.8	7.3	31.5	24.0	20.0

Table 3. Comparison of behaviour of two biotypes of Myzus persicae, (WM-1 and WM-2), on two lettuce genotypes.

Plant genotype	Insect weight (μg) after 7 days		Larval mortality (%)		Proportions of adults after 7 days (%)	
	WM-1	WM-2	WM-1	WM-2	WM-1	WM-2
PIVT 313	637	268	4	48	84	24
PIVT 339	326	110	22	96	53	0

The difference between the susceptible and resistant genotype for weight and production of larvae seemed to decrease with increasing aphid density.

APHID BIOTYPES

The initial population of *M. persicae* was collected from commercial crops of lettuce. In the laboratory, the aphids were reared on Chinese cabbage. From this population, two biotypes were selected, WM-1 and WM-2. In several experiments, the behaviour was compared of these biotypes on resistant and susceptible plant genotypes. Some of the results are given in Table 3. For both biotypes, large differences were found between the tested genotypes for all criteria. Larvae of WM-2 developed slower on both plant genotypes and had a lower weight after 7 days and a higher mortality. Both the genotypes susceptible and resistant to WM-1 were classed as bad foodplants for the WM-2 biotype. Consequently all our tests for resistance were with the WM-1 biotype.

ADAPTATION

In aphid populations, physiological (short-term) adaptation (conditioning) can interfere with the results of resistance tests. After transfer of *M. persicae* from Chinese cabbage to lettuce, the strongest conditioning was found in alatae, less strong in apterous aphids and negligeable in young larvae. So first instar larvae were always used for microtests (Eenink & Dieleman, 1977a).

Genetic variation within the aphid population was already demonstrated by the occurrence of biotypes. This variation might result in a genetic adaptation of the aphid population to resistant plant genotypes. Therefore several experiments were done with susceptible and partially resistant plant genotypes to investigate such an adaptation, resulting in a more or less gradual breakdown of resistance. Preliminary results suggest that no adaptation occurred within 3 generations. Similar results were found for other plant genotypes and aphid populations over 12 insect generations. Further investigations have to be carried out with aphid populations originating from different parts of the country.

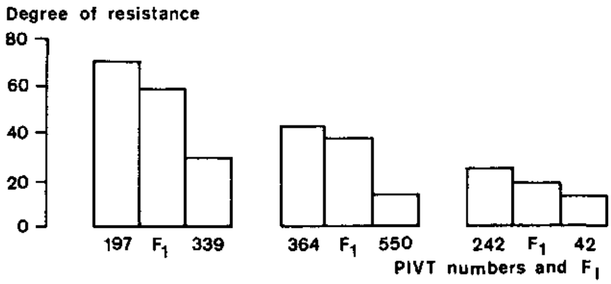

Fig. 2. Degree of resistance of IVT accessions and of F_1 from crosses. 0 = resistant; 90 = susceptible.

INHERITANCE OF RESISTANCE

Diallele crosses were made between susceptible and resistant genotypes. The parents, and F_1, F_2 and F_3 populations were evaluated for resistance. At least for some genotypes, resistance was inherited in additive fashion (Eenink & Dieleman, 1977b). So the level of resistance of the F_1 was intermediate between that of the parents (Figure 2).

The genetic variation in F_2 populations from crosses between susceptible and resistant parents was larger than the variation in the F_2 populations from crosses between two resistant parents. So probably at least some genes for resistance in the two parents were the same. As yet, no transgression of resistance could be obtained by crossing resistant parents.

CONCLUDING REMARKS

Partially resistant lettuce genotypes obtained so far could contribute to aphid control in lettuce. The retarded population development of the aphids, for instance by high mortality and low fecundity, allow less frequent application of insecticides. The resistance level may be improved in the near future with transgression by intercrossing resistant genotypes.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

5.5 Breeding lettuce (Lactuca sativa) for resistance to the aphid Nasonovia ribisnigri

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INTRODUCTION

Nasonovia ribisnigri is the most common aphid colonizing outdoor lettuce. Effective control of this aphid requires frequent application of insecticides because of repeated secondary infestation by winged aphids. The aphids are mainly present on the younger leaves, inside the heads of lettuce. This behaviour makes pesticides, especially contact ones, less effective. So use of insecticides confronts consumers both with insecticidal residues and dead aphids. Resistant lettuce varieties could offer a solution to these problems.

APHID BEHAVIOUR AND TEST PROCEDURE

Tests for resistance have to be adapted to aphid behaviour and characteristics of the host plant. The specific feeding behaviour and rapid disturbance of settled aphids makes artificial infestation, manipulation and aphid counting more difficult then for *Myzus persicae*, another lettuce-infesting aphid. *N. ribisnigri* is also sensitive to crowding, and winged aphids are already produced at low population densities. This phenomenon limits the duration of tests.

In certain tests, young lettuce plants(20 days old, 2 replicates and 5 plants per genotype per replicate) were inoculated with about 10 aphids of mixed ages and development. After two weeks, the population increase per plant differed widely between plant genotypes. As a result of a large scatter, the significance of these differences for partial resistance was not always clear. However among 300 genotypes tested, some accessions of the wild species *L. virosa* could be clearly distinguished from all other genotypes because of the very high resistance to *N. ribisnigri* (Dieleman & Eenink, 1977).

Resistance was expressed quantitatively by the following more detailed test procedure. Ten first-instar larvae were put in the centre of lettuce seedlings (5 plants per genotype). These larvae were born by young alatae to avoid the production of winged aphids on the plants to be tested. After 7-10 days, larval mortality was assessed and 5-7 days later the total number of aphids per plant was counted, during the first generation of aphids born on the tested plants. Table 1 illustrates the difference in resistance between a susceptible genotype and the resistant *L. virosa*. Under natural and artificial conditions, settling of *N. ribisnigri* on resistant *L. virosa* was observed, but colonization always failed.

Table 1. Differences between a resistant and a susceptible genotype of *Lactuca* after artificial infestation with 10 first-instar larvae per plant (5 plants per genotype). Surviving larvae and larvae produced were counted 10 and 15 days after infestation, respectively.

L. serriola (susceptible)		*L. virosa* (resistant)	
Number of larvae surviving	Number of larvae produced	Number of larvae surviving	Number of larvae produced
5	70	0	0
7	78	0	0
8	70	0	0
9	70	0	0
10	140	0	0

TRANSFER OF RESISTANCE

Because resistance was present in accessions of a wild *Lactuca* species, which was rather distant from the cultivated species *L. sativa*, great barriers for transfer of the resistance to butterhead lettuce had to be taken. Another susceptible wild species, *L. serriola*, was used as a "bridge parent" between *L. virosa* (resistant) and *L. sativa* (susceptible). The crossing scheme is outlined in Table 2. Plants of F_1 seemed to have the same resistance level as the male parent, so that resistance may be governed by one or more dominant chromosomal genes. Only a few of the F_1 plants showed some female fertility, and all plants were completely male-sterile. Many hundreds of backcrosses to *L. serriola* and *L. sativa* were made, resulting in a few B_1 seed. After further backcrossing with *L. serriola* and *L. sativa*, and cultivation of seed on nutrient medium in test tubes, finally some plants were obtained with a rather good male and female fertility. After testing, some of these plants seemed to have the same resistance level as the resistant parental genotype (Table 3).

Table 2. Crossing scheme for the transfer of resistance from *L. virosa* to *L. sativa*.

L. serriola (susceptible)	x	*L. virosa* (resistant)
F_1 (resistant)	x	*L. serriola* *L. sativa*
B_1	x	*L. serriola* *L. sativa*
B_2	x	*L. serriola* *L. sativa*
B_3 (susceptible or resistant)	x	*L. sativa*

Table 3. A sample of resistant and susceptible selections (B_3 generation) obtained by backcrossing and compared with the original susceptible and resistent parents. Each plant was inoculated with 10 first-instar larvae. There were 6 replicate plants for *Lactuca serriola* and 5 for *L. virosa*.

Genotype	Number of larvae surviving (after 10 days)	Number of larvae produced (after 15 days)
L. serriola (susceptible)	8	85
L. virosa (resistant)	0	0
Selections from backcrossing		
resistant	1	3
	1	0
	0	0
	1	2
susceptible	8	100
	9	170
	9	120

PROSPECTS

It is feasible to include the resistance from our selections in lettuce varieties. Because of high resistance, *N. ribisnigri* cannot colonize the resistant varieties. So the use of insecticides to control this aphid will be superfluous. However further investigations are needed on the inheritance and stability of resistance and on the chemical basis of resistance.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

5.6 Breeding tomato (Lycopersicon esculentum) for resistance to the greenhouse whitefly (Trialeurodes vaporariorum)

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INTRODUCTION

In the Netherlands, the greenhouse whitefly *(Trialeurodes vaporariorum)* in tomato is successfully controlled with the parasitic wasp *Encarsia formosa* on a large scale (Van Lenteren et al., 1977). Environmental changes such as occasional hot summer periods or intentional lowering of temperatures for new varieties can, however, disturb the equilibrium between pest and parasite. The reliability and consequently the applicability of the system of biological control can be promoted by use of varieties less susceptible to the greenhouse whitefly, so retarding development of the whitefly. Unlike other components of integrated control, resistance is generally not influenced by environmental changes, thus benefiting the entire system.

The present project was aimed at finding sources of resistance in cultivated and related species of tomato. Simultaneously reliable and efficient test methods had to be developed.

SOURCES OF RESISTANCE

The search for sources of resistance was started by screening 83 accessions belonging to 8 species of the genus *Lycopersicon* and 2 accessions of *Solanum pennellii*. To prevent choice by the whitefly, the accessions were tested separately in a greenhouse in large cages made of cheesecloth. In each cage, three plants were infested with 75 whitefly emerged from pupae the day before. After six weeks, reproduction of the first generation developing in cages was assessed by estimating the number of adults, the number of empty pupae and the number of new eggs. Within the cultivated species *L. esculentum*, no usuable levels of resistance were traced. Only the related species *S. pennellii* and *L. hirsutum* were selected as partially resistant (De Ponti et al., 1975).

TEST METHODS

To transfer the resistance from the wild to the cultivated species, *S. pennellii* and *L. hirsutum* var. *glabratum* were crossed with susceptible commercial varieties. For the screening of the segregating F_2 and following generations, plants were grown in pots. At the stage with 2-3 true leaves, each plant was covered with a transparant plastic cylinder and infested with 15 female whitefly emerged the day before. After 5 days, living whiteflies were counted and then killed with sulfotep fumigant. Three weeks later, pupae were counted. The resistance of selected lines of F_4 and further generations was checked in practical tests under normal growing conditions. For that purpose, 18 plants of each line and of the susceptible and the resistant parent were separately planted in small isolated compartments of a greenhouse. Each plant was infested with 30 female whiteflies and the development of the populations was followed by estimating the number of adult whiteflies in the second and third generation after infestation. In these tests, many lines from plants selected in pot tests were as susceptible as the susceptible control, so the reliability of the pot test was doubtful.

In 1978, 30 lines were extensively tested by both means. In addition to the visual estimation of the numbers of adult whiteflies in the consecutive generations, another method, developed by a guest-worker, Dr M.J. Berlinger (Regional Experiment Station, Gilat, Israel) was introduced. In each compartment were placed 5 yellow Petri traps (yellow-painted plastic Petri dishes, greased with Tanglefoot). After 48 h the number of trapped whiteflies was electronically counted with a Biotran II Colony Counter. In general these samples of the populations agreed with the visual estimate. As the correlation between the pot test and the practical test was, indeed, very poor, the pot test, in its original form, was judged inadequate.

In the meantime, Berlinger started a detailed study of the relationship between the whitefly and its hosts *L. esculentum, L. hirsutum* var. *glabratum* and *S. pennellii*. He used material whose susceptibility or resistance had been determined with certainty in practical tests. Under controlled conditions, the following characteristics were assessed: adult survival, oviposition rate, egg and larval mortality, sex ratio and duration of development. These data were compared with those on population development in a greenhouse for three generations. The preliminary results indicated that the differences in survival and oviposition rate on tomato and its wild relatives were small during the first days of the whitefly's adult life, whereas later the differences increased. This agrees with the experiences of Woets & Van Lenteren (1976), who studied the suitability of different vegetable crops as hosts for the whitefly. The study of population development showed also that the resistance of *S. pennellii* depended on its growth stage: the first leaves were rather susceptible whereas the later-developed leaves were completely resistant. Both findings might explain the failure of the pot test. An improved test is being developed.

RESULTS AND DISCUSSION

The shortcomings of the pot test have retarded the development of resistant breeding lines. After several practical tests, many lines, unjustly selected in pot tests, had to be discarded. From *L. esculentum* x *L. hirsutum* var. *glabratum* crosses, only one resistant F_7 line and some F_3 lines were left. Table 1 shows the resistance of this F_7 line and the resistance of *S. pennellii*. In segregating F_3 lines from crosses between *L. esculentum* and *S. pennellii*, resistant plants were selected. In Table 1, the resistance of *S. pennellii* is underestimated, because development of the whitefly population on *L. esculentum* was retarded by heavy contamination with parasitic wasps. Contamination with *E. formosa* is a major

Table 1. Numbers of greenhouse whiteflies collected in 5 Petri dish traps over 48 h in the third generation after inoculation in two different practical tests on the tomato cultivar 'Heinz 1370', two related species and a breeding line.

Material	Number of whiteflies trapped	
	Test 1	Test 2
L. esculentum cv. Heinz 1370	3215	605
L. hirsutum var. *glabratum*	44	-
F_7 (Heinz 1370 x *L. hirsutum* var. *glabratum*)	166	-
S. pennellii	-	112

problem in the practical tests, because that wasp cannot be selectively controlled.

To investigate whether resistant plants can be recognized when they are grown between susceptible plants, *L. esculentum*, *L. hirsutum* var. *glabratum*, the resistant F_7 line and one susceptible F_7 line were planted randomly in a greenhouse. After inoculating each plant with 30 female whiteflies, the plants were judged as in the practical test. In that situation too, the differences between resistant and susceptible plants were clear. So that method too seems suitable for selecting segregating populations.

SIGNIFICANCE FOR HORTICULTURE PRACTICE AND FOR FUTURE WORK

In view of the levels of resistance found, it may be possible to breed varieties that do not need any additional control measures.

The sticky exudate of the glandular hairs of *S. pennellii* is the most striking mechanism of resistance of that species. However from crosses between this species and the tomato, some F_3 plants were selected with the same level of resistance as *S. pennellii*, but with markedly less sticky exudate. So other factors, such as components of the leaf sap, must also contribute to the resistance. In breeding resistant lines, we will avoid incorporation of the characteristic sticky exudate, in which the parasitic wasps are also trapped. If not, resistance would become incompatible with the use of *E. formosa*.

As soon as the pot test has been improved, the breeding of resistant lines can be enhanced. Large amounts of F_2 seeds of crosses between the resistant wild species and the cultivated tomato are still available. The search for new sources of resistance within the related species of tomato will also be repeated.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

5.7 Breeding cucumber (Cucumis sativus) for resistance to the two-spotted spider mite (Tetranychus urticae)

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INTRODUCTION

In the Netherlands, about 1000 ha of cucumbers are grown in greenhouses. The control of the two-spotted spider mite *(Tetranychus urticae)* requires permanent attention and effort by the grower to prevent economic loss. After an enthusiastic start in the early seventies, biological control with the predatory mite, *Phytoseiulus persimilis* has ceased to expand (Section 3.1). Although this control system generally functions well, it is sensitive to environmental change, incurs costs and requires much care by the grower.

The two-spotted spider mite thrives less on resistant varieties, which might promote biological control and diminish the frequency and dose of acaricide sprays. So breeding and use of resistant varieties promote integrated control (De Ponti, 1977a). The present project investigated the breeding of cucumber varieties highly resistant to the two-spotted spider mite.

WORKING METHODS

Kooistra (1971) selected 12 partially resistant varieties after three greenhouse trials in which 400 varieties of the IVT cucumber collection were screened for resistance by a simple damage rating. With the selected varieties, the breeding of resistant lines of greenhouse cucumber was started, but the screening techniques soon proved inadequate for the purpose. So a thorough study of the host-parasite relationship was started, with cucumber varieties different in resistance. The intention was to trace those aspects of population development of the two-spotted spider mite that were influenced by resistance. According to its level, resistance reduced adult lifetime, oviposition, egg viability, and larval and nymphal survival (De Ponti, 1977b).

Based on this study, two resistance tests were developed.

- In the laboratory test (De Ponti, 1977b), the degree of acceptance and reproduction was scored on potted plants and leaf discs, respectively. After greasing the petiole of the first true leaf with 'Tanglefoot', 20 female deutonymphs were placed on that leaf. After 10 days, the mites left were counted as a measure of acceptance. From these mites (adults about eight days old), five were placed on separate leaf discs of the second leaf and after three days, oviposition was observed as a measure of reproduction. These tests are carried out under controlled conditions in climate rooms.

- In the practical test (De Ponti, 1978a) under normal growing conditions in a greenhouse, 10 adult female mites were placed on the third leaf of each plant to be tested in the third—leaf stage. Over about eight weeks, the development of the spider mite population was rated on a damage scale from 0 to 5.

After an additional screening of 400 varieties, altogether 800 varieties were tested. The 50 least damaged ones were extensively retested in laboratory and practical tests. Thereafter the research was continued by investigating the genuineness of resistance and ways of increasing it. During all phases of the project, the role was considered of the bitter principle cucurbitacin-c in this resistance. Van Keulen (1980) developed an efficient method of estimating this compound.

RESULTS AND DISCUSSION

Of the 50 selected varieties, only 9 (out of 800!) were significantly different from the susceptible control for acceptance, reproduction and damage index (De Ponti, 1978b). Table 1 shows the differences in acceptance and reproduction between some of these varieties and the susceptible control. The implications of these partial resistances for development and control of the two-spotted spider mite are best shown in the practical test. The slow increase in damage of the partially resistant varieties is demonstrated in Figure 1. The horizontal line at a damage index of 1.9 represents the economic injury level, which for the most resistant varieties was passed about 3 weeks later than for the susceptible control.

The reduction in reproduction after moving the mites from a susceptible variety, on which they are normally reared, to other (resistant) varieties might only be tempory. If so, the resistance of the selected varieties would not be genuine. To investigate this, both resistance tests were repeated after the mites had been reared on the selected varieties for 10-20 generations. The degree of acceptance and reproduction and the damage index decreased rather than increased, providing evidence for the genuineness of the resistance (De Ponti, 1978b).

Attempting to increase the level of resistance found in the partially resistant varieties, I intercrossed these varieties and subjected the successive generations to selection in laboratory and practical tests. Table 2 and Figure 2 clearly demonstrate that this breeding pro-

Table 1. Degree of acceptance and fecundity of the two-spotted spider mite on 5 partially resistant cucumber varieties and the susceptible control.

Variety	Geographic origin	Acceptance (%)	Number of eggs per female in 3 days
PI 220860	Korea	24	11.6
Hybrid LGP	United States	36	18.5
Taipei no 1	Taiwan	39	21.1
PI 178885	Turkey	35	11.5
Ohio MR 200	United States	37	18.3
Susceptible control	Netherlands	76	26.5

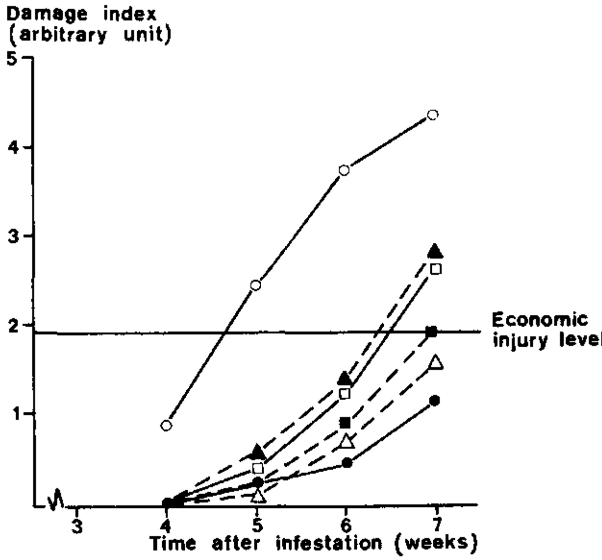

Fig. 1. Increase of damage on the susceptible control (o) and on 5 partially resistant cucumber varieties after infestation with 10 female two-spotted spider mites per plant. The economic injury level is at damage index 1.9.

cedure was successful. Some F_5 lines were selected with a markedly lower reproduction than the parental varieties. The 15 most resistant lines were released in 1978 to private breeding firms in the Netherlands, which will try to combine this resistance with other useful agronomic characteristics to develop varieties resistant to two-spotted spider mite.

All resistant varieties are bitter, as would be expected since the world assortment of cucumber contains 99% bitter varieties. Although resistant and bitter varieties generally did not contain more cucurbitacin-c than susceptible and bitter ones, there was an unintended increase in the amount of cucurbitacin-c in the selected F_5 lines over the parental varieties. However many bitter-free and resistant plants were found in F_2 and backcross generations after crossing resistant and bitter with susceptible and bitter-free lines.

Table 2. Degree of acceptance and fecundity of the two-spotted spider mite on a susceptible control, on 2 partially resistant cucumber varieties and on 2 lines derived from crosses between these varieties.

Variety or line	Acceptance (%)	Number of eggs per female in 3 days
Hybrid LGP	36	14.1
Robin 50	45	15.1
F_5 (HLGP x Robin 50)	45	8.4
F_5 (HLGP x Robin 50)	34	9.4
Susceptible control	72	21.0

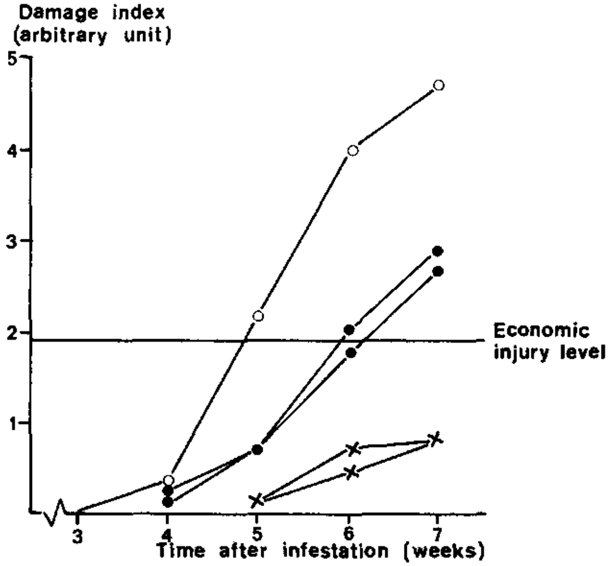

Fig. 2. Increase of damage on the susceptible control (o), on two partially resistant varieties (•) and on two F_5 lines derived from crosses between the varieties (x).

SIGNIFICANCE FOR HORTICULTURE AND FOR FUTURE WORK

Control of the two-spotted spider mite can be advanced by the introduction of resistant varieties. Figure 2 shows that a combination of resistance with acaricidal control will cause a reduction in frequency of spraying to half or a third. Biological control will also be favoured by retarded development of the mites, although the exact quantitative effect has not yet been investigated. According to the extent of spontaneous infections, resistance might often be enough for us to abandon any additional control.

The inheritance of the resistance is still under study, but appears to be polygenic. So the bitter principle cucurbitacin-c, whose presence is governed by one dominant gene, cannot be entirely responsible for resistance. Further analysis of the genetics of the resistance in relation to that of cucurbitacin-c should clarify the role of that substance. The influence of resistance on the efficiency of chemical and biological control will also be investigated and attemps will be made to trace biochemical or biophysical factors responsible for the resistance. Since the release of resistant lines to breeding firms, these firms have been and will be supported in their efforts to breed cucumber varieties resistant to two-spotted spider mite.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

5.8 Breeding glabrous cucumber (Cucumis sativus) varieties to improve the biological control of the greenhouse whitefly (Trialeurodes vaporariorum)

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INTRODUCTION

In the early 1970s, the greenhouse whitefly *(Trialeurodes vaporariorum)* became a major pest of greenhouse cucumbers *(Cucumis sativus)* in the Netherlands. In 1976, 200 varieties of the IVT cucumber collection were screened for resistance to the whitefly. Techniques developed to screen tomato for resistance to the same insect (De Ponti et al., 1975) were used. Significant differences between varieties were not found. This may be due to a lack of differences in resistance or, more likely, to inadequacies in the screening techniques. Also in the tomato project, these techniques raised doubts (Section 5.6). Until improved techniques become available, screening of the cucumber collection has been postponed.

Contrary to the situation with tomato, sufficient control of the greenhouse whitefly by the parasitic wasp *Encarsia formosa* is rarely achieved in greenhouse cucumbers, despite repeated large introductions of the wasp. Van Lenteren et al. (1977) ascribed this failure of control to the many large hairs on the cucumber leaf and the honeydew on the hairs. These factors would reduce the mobility and consequently the parasitism by the wasp. Therefore varieties were sought with a leaf morphology that would hinder the wasp to a lesser extent.

WORKING METHODS

With a stereomicroscope, the 200 varieties mentioned above were judged for the length and density of the hairs on the leaves and the thickness and density of the veins. Differences were traced by comparison with a standard variety. On aberrant varieties the number of hairs were counted and divided by leaf area.

RESULTS AND DISCUSSION

Of the 200 varieties, only 7 showed clearly deviating leaf characteristics (Table 1). To what extent these characteristics might favour the mobility of the parasitic wasp was not studied, because we then came across a publication of Strelnikova & Mashtakova (1973) about two glabrous cucumber mutants that sounded promising for the purpose. After receiving seed of these mutants from the Vavilov All-Union Institute of Plant Industry in Lenin-

Table 1. Description of leaf characteristics in which some cucumber varieties deviate from the standard.

Variety	Origin	Description
PI 167223	Turkey	40% fewer hairs and shorter hairs
PI 171609	"	50% fewer hairs
PI 173674	"	40% fewer hairs
PI 179676	India	40% fewer hairs
PI 176950	Turkey	shorter hairs
PI 137846	Iran	lower vein density
Zia Stetga Si	Vietnam	thinner veins

grad, I multiplied it and gave it to Van Lenteren and Woets, who found that on the glabrous leaves the wasp was no longer hampered by the hairs and travelled at a speed 3.5 times as great as on normal hairy leaves (Hulspas-Jordaan & Van Lenteren, 1978). The expected positive influence of this enhanced mobility on parasitism will be investigated in greenhouse and laboratory tests.

Anticipating the results of these tests, I have started breeding glabrous cucumber varieties by crossing the mutants with greenhouse cucumber breeding lines of IVT. Recently 8 advanced breeding lines with the glabrous characteristic have been released to breeding firms in the Netherlands. Because of the simple inheritance (1 recessive gene) and selectability of the character, release of glabrous varieties may soon be expected.

FUTURE WORK

Although parasitism by the wasp might be enhanced by glabrousness, the reproduction rate of the greenhouse whitefly is still rather high and about the same as on normal hairy varieties. Biological control of this insect can be further improved by breeding varieties resistant to the greenhouse whitefly, so that the reproduction rate is reduced. As soon as efficient screening techniques are developed, investigations into resistance to the whitefly will be resumed.

SIGNIFICANCE FOR HORTICULTURE

With glabrous cucumber varieties, biological control of the greenhouse whitefly with the parasitic wasp *Encarsia formosa* will be possible. The consequent decline in use of insecticides will also promote biological control of the two-spotted spider mite *(Tetranychus urticae)* with the predatory mite *Phytoseiulus persimilis*. A forthcoming release of varieties resistent to powdery mildew *Sphaerotheca fuliginea* will also facilitate the biological control of both pests.

So far, no unfavourable characteristics of glabrousness have been found. On the contrary, it is expected that growers will welcome these varieties because they cause less skin irritation.

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6 Pheromones and attractants

Since 1967, Dutch research has played a leading role in the field of insect pheromones. Inspired by the successes of Dr W.L. Roelofs in the United States, it was decided to concentrate all efforts first on orchard pests, in particular on the tortricid (or leaf-roller) moths. The Institute for Pesticide Research at Wageningen, and the Division of Technology for Society TNO at Delft cooperated closely. The latter institute did the chemical analysis, namely isolation and identification (6.1). The former institute tested the pheromones in the field and, after positive results, worked out methods for practical use of the pheromones either for monitoring or for control by disruption of mating (6.3).

Most of the pheromone compounds were synthesized and purified in Wageningen and a large collection of such compounds was built up. This collection has made it possible to find a number of sex attractant mixtures for several moth species by empirical screening (6.2).

At present, the research program is no longer exclusively devoted to pests of fruit orchards. Much attention is now also being paid to pests in vegetable and flower crops.

Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

6.1 Isolation and identification of pheromones

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DESCRIPTION OF THE PROBLEM (INCLUDING HISTORY)

In 1959, A. Butenandt et al. isolated and identified the first insect pheromone: bombykol, the sex pheromone of the silkworm moth. Soon after this pioneering work, attempts were made, mainly in the United States, to identify pheromones of pest insects to find ways of selective control. With the techniques then available, that was a tricky job and several chemical structures published in that period had to be withdrawn later.

Concurrently with the chemical research, field work was done with 'sex traps' containing life virgin females to find out whether sex pheromones could, in principle, be used in pest control. In the Netherlands, Minks did that in 1967 for the summerfruit tortrix moth *Adoxophyes orana* and, in later years, he demonstrated the potential use of sex pheromones for monitoring purposes in supervised or integrated control of that insect in orchards. But as the traps were not sufficiently effective to replace light traps, it was decided to try to isolate and identify the sex pheromone, in the hope that the synthetic pheromone would yield better results.

This joint project was started in 1970 by the Laboratory for Insecticide Research (now Institute for Pesticide Research), which did the biological work, and the Central Laboratory TNO (now Division of Technology for Society), which did the isolation and identification. The latter had already gained some experience in this field with work on cockroaches and termites, and had identified (Z)-3, (Z)-6, (E)-8-dodecatrienol as a trail-following compound for the French termite *Reticulitermes santonensis* in 1969, a year after the compound had been identified in the United States in American *Reticulitermes* spp. After identification of the sex pheromone of *A. orana* in 1971, the project was extended to the identification of the sex pheromones of other lepidoptera and of the American cockroach, the alarm pheromone of grain aphids and the trail pheromone of the Pharaoh's ant.

AIM AND MOTIVATION

The aim of the project is the identification of pheromones for synthesis and use in selective pest control.

Its motivation is different for different insects. The tortricids *Adoxophyes orana*, *Archips podanus* and *Pandemis heparana*, for example, are three major leaf-roller pests in

Dutch orchards, as also in other European countries. The cabbage leaf-roller *Clepsis spectrana* is a serious pest in outdoor and greenhouse roses in the Netherlands.

The potato tuberworm moth *Phthorimaea operculella* is not a common pest in the Netherlands, but its sex pheromone was identified as a part of a program to develop a method of detecting imported infestations. The insect is, however, a major pest of potatoes in Mediterranean countries in Europe and also in tropical and subtropical regions throughout the world.

Aphids are major pests all over the world. In the Netherlands they are a significant pest of young cereal plants and they are serious vectors of virus diseases of potatoes and sugar-beet.

The reasons for the isolation and identification of pheromones of non-agricultural pest insects are different. Termites, for example, are economically among the most serious pests in the world, particularly those of the genus *Reticulitermes*. In the Netherlands, they seldom pose a problem, but their pheromone could be used to detect infestations in imported timber. Cockroaches, in particular *Blattella germanica*, but to a lesser degree also *Periplaneta americana*, and the pharaoh's ant *Monomorium pharaonis*, are serious urban pests in many countries, including the Netherlands.

WORKING METHODS

Usually whole insects, abdomens, glands or other tissues are extracted from a few hundred up to about 30 000 insects. The isolation and identification steps are monitored by a behavioral assay and often also by electro-antennography. The main techniques of separation and purification are high-pressure liquid chromatography and gas chromatography. The chemical structures of the pheromones are determined by several chemical and physical microtechniques such as hydrogenation, ozonolysis, reaction chromatography, mass spectrometry, infrared spectroscopy and nuclear magnetic resonnance. The structure must ultimately be confirmed by synthesis and by testing the activity of the synthetic pheromone.

RESULTS AND DISCUSSION

The sex pheromone of *A. orana* was soon isolated and its structure was reported in 1971. It was the first time a sex pheromone was found, in which the presence of two isomers, (Z)-9 and (Z)-11 tetradecenyl acetate, was prerequisite for biological activity. The ratio of the two compounds was crucial: in sex traps the largest number of males were trapped with a ratio of 9:1. Other ratios gave much smaller catches. Now these facts are hardly surprising for pheromone scientists but they were very much so in 1971.

The two components mentioned are *isomers*, differing only in the position of their double bond. Together with Roelofs (Cornell University), we found the same two isomers in another tortricid moth, *Clepsis spectrana*, which is sympatric with *A. orana* and has its flights in the same periods. The ratio of the two sex pheromone components in *C. spectrana* was the reverse of that of *A. orana*. The difference seems to ensure their reproductive isolation.
The joint efforts of the teams at Wageningen and Delft yielded another surprise when the structure of the sex pheromone of a third tortricid moth, *Archips podanus*, was elucidated

and reported in 1973: again a binary pheromone. The components were cis-trans isomers: (Z)-11 and (E)-11 tetradecenyl acetate, in a ratio of about 1:1. This was quite unexpected at that time as it was generally believed among pheromone scientists that cis-trans isomers of sex pheromones were inhibitors rather than synergists. It is now known from experiments in many countries that such a synergism between cis-trans isomers is a very common phenomenon, not only in mono-unsaturated pheromones but also in those containing two double bonds. An example of the latter is gossyplure, a 1:1 mixture of (Z,Z) and (Z,E) 7,11-hexadecadienyl acetates. This gossyplure is the first successful commercial disruptive of an agricultural pest (the pink bollworm); it was registered by the United States Environmental Protection Agency in 1978.

Even the first sex pheromone ever identified (Butenandt's bombykol) recently proved to be a component of a more complex secretion, which also contains one of its cis-trans isomers. The biological function of the latter is not yet known.

An example of synergism between sex pheromones only differing in the number of double bonds was found in the potato tuberworm moth *Phthorimaea operculella.* Its structure was again elucidated by a joint effort of the teams at Delft and Wageningen. The components, (E)-4, (Z)-7-tridecadienyl acetate and (E)-4, (Z)-7, (Z)-10-tridecatrienyl acetate, are rare examples of sex pheromones with an uneven number of carbon atoms. The three double bonds in the second component were also unprecedented among sex pheromones.

In the United States, the alarm pheromone of the rose, pea, greenbug and cotton aphids was reported to be (E)-β-farnesene in 1972, and its presence in *Myzus persicae* was reported in 1973, being confirmed in the same year by Wientjes et al. in our laboratory. In collaboration with Hille Ris Lambers, the grain aphids *Macrosiphum (Sitobion) avenae, Rhopalosiphum padi* and *Metopolophium dirhodum* were mass-cultured and (E)-β-farnesene was found to be the alarm pheromone of these aphids too.

Although not relevant to agriculture, the results of research on termites, cockroaches and ants were also regularly discussed and reported to the Working Party on Integrated Pest Control. Persoons and Ritter were awarded the Royal Dutch/Shell Prize 1978 for their work on the American cockroach and on pharaoh's ant. Two sex pheromones of *Periplaneta americana* have been isolated: periplanone A ($C_{15}H_{20}O_2$) and periplanone B ($C_{15}H_{20}O_3$). The structure of the latter proved to be (1Z, 5E)-1,10(14)-diepoxy-4(15), 5-germacradiene-9-one, which was confirmed by synthesis by Still in the United States. The odour trail of the pharaoh's ant was found to contain two biologically active heterocyclic compounds, monomorine I and III, and, in addition, a very potent trail pheromone, called faranal. An interesting feature of its structure, (6E,10Z)-3,4,7,11-tetramethyl-6,10-tridecadienal-1, is its resemblance to juvenile hormone II.

SIGNIFICANCE FOR AGRICULTURE

The sex pheromones of *Adoxophyes orana* and *Archips podanus* are now used routinely by the Horticultural Advisory Service in the Netherlands as a monitoring agent in sex traps. Promising field results for direct control of *A. orana* by the confusion technique are about to be put into practice. The other pheromones described are still being evaluated for their practical use in monitoring devices or direct control, for example by disrupting mating through permeation of the air with sex pheromones.

FUTURE OUTLOOK

In the past, attempts to use pheromones in pest control have often started prematurely: later the structures often appeared to be incorrect or incomplete. In particular, the significance of isomers of the main pheromone and of other minor components was neglected. We now know many examples of binary and multicomponent pheromones. Some of the secondary or minor components are essential for biological activity under field conditions; others enhance the efficiency considerably or have a vital function in the total system of attraction, excitation and mating. For several species, we have evidence that, although the main components of the total pheromone blend are known, some minor but highly important components are still to be identified. Further analytical work is then necessary. Sex traps containing pheromones will probably find many more uses in the near future. But also the use of pheromones in direct control seems to be within reach. The results obtained in the United States with the direct control of the pink bollworm, are particularly encouraging. A crucial matter for the success of these approaches is the development of suitable controlled-release formulations and techniques.

The practical use of pheromones is lagging far behind scientific knowledge, largely because of economic factors. The specificity of these agents is a great advantage for the environment but makes them only attractive for industry when they can be used on pests of great economic importance. The expense incurred in obtaining government approval for registration of a product is a main reason for the reluctance of the pesticide industry to enter this field. For further progress, close collaboration between government, industry and universities or other non-profit organizations is therefore necessary.

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6.2 Synthesis and investigation of potential sex pheromones of insects

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INTRODUCTION

For environmental and economic reasons, research and extension on pest control is aimed at reducing amounts of insecticides, for instance by applying them only when necessary and at the right time. An exact knowledge of the presence or absence of the noxious insect species is indispensable. Sex pheromones and attractants of insects can be used in collecting this information and estimating flight activities. Sticky traps, baited with 1 mg or less of the pheromone, usually attract only one insect species. So the start of the flight period of an insect species can be easily established. If no insects are trapped, no control measures are necessary. If so, trapping data can be helpful to set up an efficient and economic program of control.

Also chemical communication between males and females of an insect species can be disrupted by evaporating large amounts of their sex pheromone into the air. This method could become a very selective way of controlling insects and is being studied in several parts of the world.

RESULTS AND DISCUSSION

Our attention was initially concentrated on leaf-rollers (Lepidoptera: Tortricidae) in apple and pear orchards; later other insect species were studied too. Many unsaturated straight-chain acetates were synthesized to support current identification programs and to screen these compounds in orchards, singly or mixed in several combinations on their attractivity. Figure 1 shows a typical reaction scheme to obtain (Z)-9-tetradecen-1-ol acetate (Z-9-TDA). With slight modifications, most positional and geometrical isomers can be prepared (Voerman, 1979a).

A 9:1 mixture of Z-9-TDA and Z-11-TDA is the sex pheromone of *Adoxophyes orana* (Lepidoptera: Tortricidae). Later, we found that a 1:1 mixture of E-11-TDA and Z-11-TDA is the sex pheromone of the moth *Archips podanus*. The E compound can be obtained by reduction of 2-(11-tetradecynyloxy) tetrahydropyran with sodium in a mixture of liquid ammonia and tetrahydrofuran.

Now about 100 mono-unsaturated acetates are available with varying chain length (10-18 carbon atoms) and position of the double bond in the chain. They are used in field screening

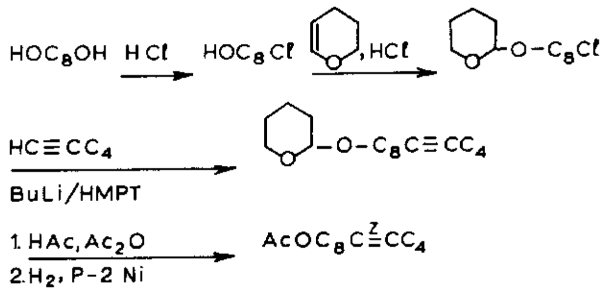

Fig. 1. Reaction scheme for obtaining (Z)-9-tetradecen-1-ol acetate.

and as model compounds in identification programs. During field screening Minks et al. (1976) discovered that a 1:1 mixture of (E)-9 and (Z)-9 dodecen-1-ol acetate was attractive for male *Enarmonia formosana* moths (Lepidoptera: Olethreutinae).

High isomeric purity is essential. Small amounts of E-9 or E-11 TDA much decrease the attractivity of the sex pheromone of *Adoxophyes orana*. Because it is almost impossible to synthesize geometrically pure compounds, synthetic compounds must ultimately be purified to remove small amounts of the unwanted isomer. This can be achieved by argentation chromatography: a liquid-chromatographic method in which weak interactions are used between silver ions and ethylenic bonds. The E compounds form a weaker complex with silver ions than the Z compounds and therefore pass quicker through a column containing silver ions than the Z compounds with a suitable mobile phase. This principle can also be used to estimate the isomeric purity of a sample. Gas—liquid chromatography is also indispensable (Voerman, 1979a).

More sophisticated methods are needed to produce poly-unsaturated straight-chain acetates. All 4 geometrical isomers of 3,13-octadecadien-1-ol acetate can be made by chemical conversion of 9-tetradecen-1-ol acetates as is shown in Figure 2 (Voerman, 1979b). The Z, Z isomer is an attractant for the male clearwing moth *Synanthedon myopaeformis* (Lepidoptera: Sesiidae). Addition of a small amount of one of the stereoisomers probably results in a mixture that is more attractive than the single compound.

The potato tuberworm moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) is a serious pest in tropical and subtropical areas. Its sex pheromone was identified and consists

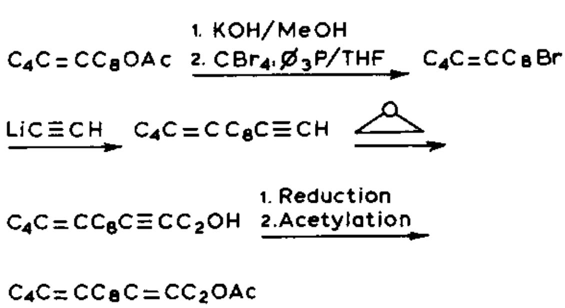

Fig. 2. Chemical conversion of 9-tetradecen-1-ol acetates to 3, 13-octadecadien-1-ol acetates. The symbol ϕ stands for phenyl group.

Table 1. Composition of sex pheromone or sex attractant of insect species for which dispensers are prepared regularly.

Insect species	Composition of sex pheromones or sex attractant[1]
Adoxophyes orana	0.9 mg Z-9-TDA + 0.1 mg Z-11-TDA
Archips podanus	0.5 mg E-11-TDA + 0.5 mg Z-11-TDA
Archips rosanus	0.9 mg Z-11-TDA + 0.1 mg Z-11-TDOH
Clepsis spectrana	0.1 mg Z-9-TDA + 0.9 mg Z-11-TDA
Laspeyresia nigricana	1 mg E-10-DDA
Enarmonia formosana	0.5 mg E-9-DDA + 0.5 mg Z-9-DDA
Grapholitha funebrana	0.04 mg E-8-DDA + 0.96 mg Z-8-DDA + 1 mg $C_{12}OAc$
Grapholitha molesta	0.07 mg E-8-DDA + 0.93 mg Z-8-DDA + 1 mg $C_{12}OH$ + 0.003 mg Z-8-DDOH
Laspeyresia pomonella	1.5 mg E-8,E-10-DDOH
Lithocolletis blancardella	1 mg E-10-DDA
Lithocolletis corylifoliella	1 mg PTM 1
Pandemis heparana	0.2 mg Z-9-TDA + 0.4 mg Z-11-TDA + 0.2 mg Z-9-TDOH + 0.2 mg Z-11-TDOH
Phthorimaea operculella	0.4 mg PTM 1 + 0.6 mg PTM 2
Ptycholoma lecheana	0.25 mg Z-11-TDA + 0.75 mg Z-11-TDOH
Sitotroga cerealella	1 mg Z-7,E-11-HDDA
Spilonota ocellana	1 mg Z-8-TDA
Synanthedon myopaeformis	1 mg Z-3,Z-13-ODDA (incl. about 5% ZE,EZ, and EE)
TDA = tetradecen-1-ol acetate	ODDA = octadecadien-1-ol acetate
TDOH = tetradecen-1-ol	PTM 1 = E-4,Z-7-tridecadien-1-ol acetate
DDA = dodecen-1-ol acetate	PTM 2 = E-4,Z-7,Z-10-tridecatrien-1-ol acetate
DDOH = dodecen-1-ol	$C_{12}OAc$ = dodecan-1-ol acetate
HDDA = hexadecadien-1-ol acetate	$C_{12}OH$ = 1-dodecanol

1. Z and E stands for cis and trans

of two compounds: (E)-4, (Z)-7-tridecadien-1-ol acetate (PTM 1) and (E)-4, (Z)-7, (Z)-10-tridecatrien-1-ol acetate (PTM 2). Mixtures of PTM 1 and PTM 2 are very attractive. The ratio of the two components does not seem critical (Voerman & Rothschild, 1978).

CONCLUDING REMARKS

So far, our work had led to the commercial availability of several sex pheromones and attractants. Some of them are used in pest control or in entomological research. Table 1 gives a survey of insects species for which attractive caps are produced on a fairly regular basis. Some formulations can certainly be improved. Identification programs are under way for some important insects. A list has been compiled of insect species for which the isolation, identification, and synthesis of the sex pheromones is regarded as useful. Much work remains to be done on field screening of several potential attractants.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

6.3 Use of sex pheromones for the control of leaf-rollers in orchards

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INTRODUCTION

In the Netherlands, several leaf-roller moth species (Tortricidae) are important pests, particularly in fruit crops, because they can seriously affect the quality of fruit.

The moths are predominantly nocturnal. Sexually mature female moths release an odour signal by means of sex pheromones, that draw males to females in the dark for mating, even when the population of moths is very low. This odour relationship works efficiently. Extremely low concentrations of sex pheromone can already stimulate the male moths and guide them over long distances to their destination, the 'calling' female.

THE SEARCH FOR PHEROMONES AND ATTRACTANTS

The strong long-distance attraction of lepidopterous pheromones has evoked much research on possible applications (Sections 6.1 and 6.2). In the Netherlands, investigations on sex pheromones of noxious insects were started in 1967. As main experimental insect was chosen: the summer fruit tortrix moth, *Adoxophyes orana*, the major lepidopterous pest in Dutch apple and pear orchards since several decades.

Soon the production of an effective pheromone by the females of this species could be established. This pheromone was isolated and purified from the extract of the abdominal tips of mass-reared female moths. In 1971, it was identified in cooperation with the Central Laboratory TNO at Delft. This was the first lepidopterous sex pheromone that turned out to consist of more than one component, the positional isomers cis-9 and cis-11 tetradecenyl acetate. They were attractive only in each other's presence and also only in proportions close to 9:1.

Since that time effective sex attractants have been found for a number of other leaf-roller species, in most cases also of importance in fruit orchards. They were found either by identification of the natural sex pheromones or by screening of appropriate compounds or blends in the field. Such investigations for other species are still in full progress.

From the beginning of the investigations attention was concentrated on how the pheromone of *A. orana* could be used for control of this important pest (Minks 1975; Minks 1977).

The larvae of *A. orana* mostly hide very well in their host plants, either in leaves or in leaves and fruits spun together. Control is not easy and spraying is only effective against newly hatched larvae while they are searching for fresh leaves. As soon as fresh leaves are found, the larvae start spinning, and then become difficult to reach with sprays. Therefore correct timing of the sprays is essential to be effective.

The newly hatched larvae, however, are so tiny and inconspicuous that a sufficiently accurate determination of the first moment of egg hatching on the basis of visual observations in the orchard is impossible.

A method was developed to forecast how long after the beginning of a moth flight the first eggs of the new generation would hatch, based on the relation between temperature and time of development of the eggs: heat summation. For practical application of this method, only moth flights and temperature need be recorded.

At first, light traps were used to determine the start and trend of the flight of *A. orana*. Since 1968, sticky traps have been tried too, baited at first with living virgin female moths or crude abdominal extracts, but after elucidation of the chemical structure of the pheromone, the synthetic mixture was used (Section 6.2). These sex-pheromone traps proved easy to handle and to register the flight periods reliably. In combination with the forecasting method, they made it simple to determine the right spraying times (Minks & De Jong, 1975). For several years, the system has been used by the Horticultural Advisory Service (Plant Protection) all over the country with excellent results, thus increasing the effectiveness of insecticidal sprays and decreasing the number of them.

PHEROMONE ATTRACTION AND INHIBITION

Strong attraction in the field with synthetic pheromones can only be achieved if the proportions of the pheromone components be as close as possible to the natural pheromone blend and if chemical purity be extremely high. A contamination of the pheromone preparation with a few per cent of related chemicals as positional or geometrical isomers may destroy the attraction. For *A. orana*, addition of 2-6% of the opposite geometrical isomers trans-9 and trans-11-C_{14}OAC in 9:1 mixtures or alone reduced catches by more than half (Minks, 1975).

DIRECT CONTROL WITH SEX PHEROMONES

The synthesis of pheromones and availability in relatively large amounts have opened new prospects for direct control of insect pests. The sex pheromone communication system of a tortricid species is essential for successful reproduction and therefore constitutes a promising point of attack, by preventing fertilization of the female by the male moths.

For this purpose, sex pheromones can be applied in two ways. The first way is mass trapping: using the strong long-distance attraction to lure as many males as possible into sticky traps, thus eliminating them from the reproduction process. The second way is confusion or mating-disruption: interrupting the pheromone communication system of the pest insect by permeation of the air, which might affect the mate-finding ability of the males and thus the reproductive capacity of the population.

Three different effects of air permeation with pheromone are possible:

- Disorientation: males can no longer find the 'calling' female moths.
- Adaptation: the relatively weak signals of the female moths can no longer be smelled, because of an increase in the sensitivity threshold of the male's receptor cells, which perceive the pheromone.
- Habituation: natural pheromone signals no longer elicit the adequate behavioral reactions, because of an increase in the threshold of the cerebral neural system.

How far should mate-finding ability of the leaf-rollers be suppressed before satisfactory control of the insects can be achieved? Experience from experiments with the sterile-male technique indicate that if only 10% of the females can mate, effective control of the population is possible. So for mass-trapping, at least 90% of the males should be trapped in the first nights of the flight, preferably before the first mating. For mating-disruption experiments an even higher efficiency of catching is needed, because all male moths of the populations remain present continuously during the flight period and are potentially able to mate during each night of the flight period.

EXPERIENCE WITH MATING-DISRUPTION EXPERIMENTS

Minks et al. (1976) obtained promising results in a first mating-disruption experiment on *A. orana* by spraying of micro-encapsulated antipheromones (trans-9 and trans-11 tetradecenyl acetate, ratio 9:1). However, these results were not confirmed in later years, when results were variable and sometimes even contradictory. Only one thing could be concluded from this first series of experiments: namely that for *A. orana* the best prospects of effective disruption of mating are not by use of antipheromones but by use of the pheromone itself in a blend that should mimic the natural pheromone as closely as possible.

In 1978, strong evidence was found that mating-disruption for *A. orana* was technically feasible. In our experimental orchards, pheromone evaporated from widely separated dispensers, evenly distributed at a density of about 400 per hectare and 5 metres apart. Less than 1 g of pheromone per hectare per flight period suppressed catches of the moths by 95-98%. Somewhat higher doses gave a suppression of 99-100%. A dose of not more than 10 g pheromone per hectare per season seems sufficient to achieve satisfactory control of the mating activities of the *A. orana* populations.

MORE FUNDAMENTAL RESEARCH IS NEEDED

More fundamental understanding of the behaviour, the communication system and population dynamics of the insects is needed before we can manipulate pheromones. So since 1976, more attention has been paid to the study of behavioral responses of the insects after the application of pheromones.

PLANS FOR THE FUTURE

For the coming years, a large-scale pheromone experiment has been planned in a large part of the Experimental Orchard 'De Schuilenburg' at Lienden. An attempt will be made to control *Adoxophyes orana* and also the codling moth *Laspeyresia pomonella*, exclusively with synthetic sex pheromone. Data about mating success of female moths and long-term effects in the development of the population of the leaf-roller moths will be collected.

If the results of these experiments are favourable, there will be good prospects for future applications of pheromones as control agents, not only in fruit growing, but also in other areas of horticulture, particularly in situations with one or two major pests. Greenhouses seem ideal for mating-disruption experiments. However the chemical structure of the pheromones of some lepidopterous pests in glasshouses is not yet known.

Side-effects on the environment are hardly conceivable as a consequence of specific action, the low toxicity and the extremely low dosages. In contrast to most insecticides, pheromones might allow recovery or might not even affect natural control agents of pests. This sort of agents can play an important role in the transition from intensive chemical treatments to integrated or biological control.

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7 Selective insecticides

There are two kinds of selectivity of pesticides, that between large classes of organisms, like arthropods, fungi, higher plants, vertebrates, and that between much more closely related species.

Many pesticides show a considerable degree of the former type, but generally little attention is paid to the selectivity between related species, which is of particular importance in integrated control.

In the studies presented here, several aspects of selectivity of both kinds have been investigated. These studies relate to such varied aspects as the general basis of selectivity (7.1), the mode of action of the selective insecticide diflubenzuron (7.3) and possible side-effect of this compound on nestling birds (7.4), the mode of action of some selective fungicides (7.2) and studies on the effect of various pesticides on beneficial mite species (7.5).

Such studies are essential in promoting development and use of selective pesticides and consequently of integrated control, which is strongly dependent on the availability of compounds with as little disturbing influence as possible in the biological systems in which they have to be employed.

Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

7.1 Processes in selective toxicity of insecticides to insects

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GENERAL PRINCIPLES

One can distinguish two kinds of selectivity, that between broad classes of animals, such as arthropods and vertebrates, and that between much more closely related species. The latter is of particular importance in integrated control. If the use of insecticides is to be integrated with other important means of restraining insects, notably predators and parasites, insecticides should be available that enable us to kill the pest without eradicating its natural enemies.

When looking for such selective compounds, an alternative to large-scale screening could be fundamental research on those processes that determine the susceptibility of insect species to insecticides, and application of its results to the development of new compounds or the use of existing ones.

One way to set about such research is to study what mechanisms are involved in one of the most obvious cases of selective toxicity: that of differences in susceptibility between strains of one and the same species. In such a case, only a single or very few factors usually cause the difference and their effect can be relatively easily assessed by comparison of appropriate strains. Another way is to investigate the major processes contributing in one way or another to the toxic effect of an insecticide and to describe quantitatively how they interact and how the complex dynamic system they represent functions as a whole.

For the first approach, strains are used that have developed resistance in the field to insecticides. This seems in particular justifiable since it has been shown in some cases that differences between strains resemble those responsible for differences between species (Dyte, 1969).

The study of resistance mechanisms of course provides information on other aspects of insect toxicology than just selectivity. We hope that it should contribute to the prevention or overcoming of resistance to insecticides.

EXAMPLES

A few examples of this kind of approach, the nature of the results, and its possible usefulness in practical problems will be given here.

Combined genetic and biochemical research has shown that single genes are often responsible for differences in susceptibility by a profound influence on one of the toxicological parameters, such as detoxication and site of action. Simple genetic techniques allow or facilitate separation, combination and characterization of the various factors. As an example, resistance to DDT in houseflies can be due to three different causes, each dependent on a single gene: two kinds of detoxication and one kind of change in sensitivity at the site of action. These three kinds of resistance have diverse characteristics: the two dependent on detoxication can be synergized by different synergists (overcome by means of non-toxic detoxication-blockers), the one caused by changed sensitivity of the site of action also causes resistance to pyrethroids, an example of cross-resistance.

Interaction of factors is sometimes quite marked. An acetylcholinesterase with a 20-fold decreased sensitivity to organophosphorus inhibitors causes little resistance when introduced into a strain in which detoxication to the inhibition (tetrachlorvinphos in this case) is virtually absent. To another insecticide, paraoxon, there is much more resistance, because this compound is detoxicated by oxidation. If oxidation is blocked, resistance is greatly reduced (Table 1).

An interesting question is whether these resistance studies indicate what factors are important for toxicity. Detoxication and site of action often cause resistance, whereas penetration and binding do not figure as important resistance factors. Are the latter unimportant also in differences between species, or are they absent because they cannot readily be changed with the available genetic variation?

The effect of penetration on toxicity follows from the simple observation that resistance factors are usually much smaller after injection than after topical application of an insecticide. In topical treatment, there is also a well known effect of the solvent , which shows the practical importance of the rate of entry in the use of pesticides. Rate of entry, however, is only one of many processes that contribute to toxicity. Internal transport, reversible binding, distribution, detoxication and reaction with the target are also relevant. They can, moreover, be highly interactive (Table 1).

Such a system can be represented in simplified form as a deterministic compartmental model. A number of processes that seem toxicologically relevant are selected and represent-

Table 1. Interaction of detoxication and reduced sensitivity of site of action.

Strain	Synergist	Tetrachlorvinphos	Paraoxon
S	-	0.025	0.036
	+	0.013 (2 x)	0.004 (9 x)
R	-	0.098	1.890
	+	0.081 (1 x)	0.010 (189 x)

The figures represent the median lethal dose (LD_{50}) in µg per fly of topically applied insecticide of two strains of housefly, S and R, that differ only in the sensitivity of their acetylcholinesterases. The rate of inhibition of this enzyme is reduced 18 and 23 fold for tetrachlorvinphos and paraoxon, respectively. The insecticides are applied in the presence or absence of the synergist sesamex, which blocks oxidative detoxication. Synergistic factor's are given in parenthesis.

Tetrachlorvinphos is hardly degraded by oxidation, so neither synergist nor reduced sensitivity of acetylcholinesterase have much effect. Paraoxon is actively degraded oxidatively, which has a large effect when the acetylcholinesterase with reduced sensitivity is present.

ed by differential equations. By solving them numerically, the fate and action of toxic compounds can be simulated. Many experimental measurements are required to obtain the parameters needed in the equations. The expertise and insight obtained earlier in resistance studies is of great help for such a complete description but the development of models also shows where essential knowledge is still lacking.

This approach to insecticidal action is rather new and it has still to prove its usefulness. Preliminary results suggest that the rate of cuticular penetration and the extent of adsorption of lipophilic compounds by tissue constituents are factors of similar weight to detoxication.

PRACTICAL RESULTS

Fundamental studies of this nature do not generally lead to direct practical results. A few examples can nevertheless be presented.

In many strains of organophosphorus-resistant houseflies, organophosphates can be hydrolysed. This only applies to methyl and ethyl compounds, *n*-propyl compounds blocking the hydrolytic enzyme. Such compounds can therefore act as synergists. In the housefly, this is unfortunately of little help, since detoxication by oxidation also developed, and did not have the restricted scope of the hydrolytic defence mechanism. Our finding led however to the development by a Japanese firm of the new insecticide propaphos, used for controlling various rice insects (Sakai, 1978). Curiously enough, it proved useful against resistant insects with a completely different mechanism, depending on an altered acetylcholinesterase. The compound was more toxic to green rice leafhoppers resistant to methyl-carbamate than to the susceptible one!

During a study of oxidative detoxication of organophosphates, we found that minute amounts of toxic or non-toxic thionophosphates blocked detoxication. Such compounds were extremely powerful synergists for the strains of houseflies involved.

We feel that the scarce examples of direct practical applicability do not constitute the main output of our approach. The main output should be the contribution to the general knowledge of insect toxicology, which in many subtle ways should allow us to develop and refine the control of insects.

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7.2 Fungitoxic mechanism of organophosphorus compounds

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INTRODUCTION

In crop protection, organophosphorus compounds are used mainly as insecticides and generally have a low fungal toxicity. However since 1960, organophosphorus compounds have also been developed as fungicides, the first one being triamiphos. The chemical is systemic and selectively active against powdery mildew fungi on several ornamental plants. Since that time, many other organophosphorus fungicides have been introduced such as Kitazin and edinfenphos, selectively active against *Pyricularia oryzae* on rice, and pyrazophos active against several powdery mildew fungi. Some of these chemicals had a useful side-effect against aphids and spider mites. The mechanism of fungicidal action of organophosphorus fungicides was unknown. We supposed that knowledge on the mechanism of insecticidal activity might provide clues for the elucidation of their fungicidal action, and so the subject was investigated from 1968 up to 1974 at the Laboratory of Phytopathology in Wageningen, in co--operation with the Working Party on Integrated Control of Pests in the Netherlands.

RESULTS AND DISCUSSION

Attention was concentrated on pyrazophos (*O,O*-diethyl *O*-(5-methyl-6-ethoxycarbonylpyrazolo(1,5-a)pyrimidin-2-yl phosphorothioate). This chemical displayed both a protective and curative action against the powdery mildew fungus *Sphaerotheca fuliginea* on cucumber; toxicity to *P. oryzae* on barley was demonstrated too.

Both pyrazophos and its phosphate analogue (PO-pyrazophos) inhibited carboxylesterases of *S. fuliginea*. But since no correlation could be established of inhibition of the activity of these enzymes in vivo by pyrazophos and PO-pyrazophos with fungitoxicity, this effect probably does not account for the action of pyrazophos. In further experiments with *P. oryzae*, Kitazin (*O,O*-diethyl *S*-benzyl phosphorothioate) and edinfenphos (*O*-ethyl *S,S*-diphenyl phosphorodithioate) influenced the permeability of cell membranes. At fungitoxic concentrations, these two substances caused a leakage of (^{32}P) orthophosphate from mycelium into the culture medium. Effects on chitin synthesis were supposed to be secondary, since fungi that do not synthesize chitin like *Pythium debaryanum* are also sensitive to these chemicals. Pyrazophos had no effect on cell membrane permeability nor chitin synthesis. Pyrazophos also hardly affected nucleic acid and protein synthesis of *P. oryzae*, while oxygen uptake was only slightly inhibited. So pyrazophos did not have a pronounced short-term effect on any of the metabolic processes tested.

In short term experiments with an incubation time of 2 h, pyrazophos was less toxic to fungal growth by a factor 100 to 1000 as in experiments in which growth was assayed after incubation for one week. These results can be at least partly explained by the metabolic conversion of pyrazophos in the longer term into two fungitoxic breakdown products, PO-pyrazophos and 2-hydroxy-5-methyl-6-ethoxycarbonylpyrazolo(1,5-1)pyrimidine (PP). In short term experiments, PP even proved to be considerably more toxic for mycelial growth in suspensions buffered at pH 4.0 than pyrazophos and PO-pyrazophos and so might be the actual fungitoxic principle of pyrazophos. This view is supported by the finding that PP, in contrast to pyrazophos, also inhibited synthesis of nucleic acids and proteins and uptake of oxygen by the fungus. The weak effects of pyrazophos on these processes and on mycelial growth in short term experiments were probably due to an insufficient conversion of pyrazophos into PP under these conditions. *Pythium debaryanum* and *Saccharomyces cerevisiae* are insensitive to pyrazophos. Upon incubation of these fungi with the fungicide, no breakdown products could be detected. So sensitivity of fungi to pyrazophos seems to result from selective uptake of pyrazophos or from its conversion into PO-pyrazophos and PP, as has been demonstrated for *P. oryzae*.

In *P. oryzae*, PP could act in one of two ways. It could specifically inhibit oxygen uptake and so indirectly inhibit cellular synthetic processes like nucleic acid and protein synthesis or it could react aspecifically with cellular components and so directly affect both oxygen uptake and biosynthetic processes.

The diethylthionophosphate group of pyrazophos is of little importance in the intrinsic fungitoxic action, in contrast to organophosphorus insecticides. In pyrazophos, the diethylthionophosphate substituent may act as an inbuilt formulation factor, which facilitates the uptake of pyrazophos. The fungicide has to be hydrolysed intracellularly before it can exert its toxic action.

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7.3 Mode of action of diflubenzuron

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DESCRIPTION OF THE PROBLEM

Much was already known about the action of diflubenzuron on insects when we started our study. Insects treated with diflubenzuron have a weak cuticle that disrupts easily, causing death during moulting. The amount of chitin in affected cuticles is greatly reduced indicating that chitin synthesis is impaired. Diflubenzuron inhibits chitin synthesis soon after application, suggesting that it interferes directly. The details of the mode of action, however, had still to be resolved. Although it was generally assumed that chitin synthetase (the polymerization enzyme) was the receptor of diflubenzuron, evidence was not convincing. If one of the reactions is blocked in a step-by-step process like chitin synthesis the precursor of that reaction should accumulate. So when chitin synthetase is blocked, its substrate uridinediphosphate *N*-acetylglucosamine (UDPGlcNAc) should accumulate. Indeed, Marks & Sowa (1976) found a build-up of UDPGlcNAc in insect tissue treated with diflubenzuron, though Deul et al. (1978) did not find an increase in UDPGlcNAc in treated larvae of *Pieris brassicae*.

AIM AND MOTIVATION OF THE RESEARCH

Most insecticides have identical sites of action in vertebrates and insects. Then selectivity of an insecticide may be ascribed to differences in penetration, distribution, metabolism and rate of reaction at the site of action of the compound in insects and vertebrates. This selectivity depends on quantitative differences between one or more of these toxicological parameters.

Selectivity of diflubenzuron seems to be one of the few examples of qualitative selectivity, as diflubenzuron interferes with synthesis of chitin, a process that is mainly found in arthropods and fungi but is absent from other organisms.

A study of the mode of action of diflubenzuron could tell us more about the complex processes in chitin synthesis and cuticle formation in insects. Moreover if we could find out where it acts, we could establish whether an enzyme were involved that is unique to insects and fungi, as is chitin synthetase. That would indicate the nature of the selectivity of the compound and possible toxicological hazards to other organisms.

Table 1. Accumulation of uridinediphosphate *N*-acetylglucosamine (UDPGlcNAc) in body walls of housefly larvae treated with inhibitors of chitin synthesis.

	Control	Diflubenzuron	Polyoxin D
UDGPlcNAc	0.72	1.28	1.27
Chitin	0.63	0.03	0.03

Data represent amount of labelled precursor converted into UDPGlcNAc and chitin (pmol) in a test system with body walls from quadruplicate samples of 4 maggots.

METHODS

Since it has been impossible so far to study chitin synthesis of insects in a cell-free system, and studies on whole insects have several disadvantages, an attempt was made to study chitin synthesis and its inhibition in isolated organs. This compromise avoids many of the complexities of studies on whole organisms, but may not provide complete answers on the molecular nature of the toxic action.

So a system was developed consisting of body walls of actively growing larvae of the housefly *Musca domestica*. During incubation of the body walls with a radioactively labelled precursor, radioactivity was readily incorporated into chitin and the effects of diflubenzuron on chitin synthesis could be studied.

Polyoxin D has been shown to be a competitive inhibitor of chitin synthetase in fungi. When properly administered, it also inhibits chitin synthesis in insects. Since its mode of action is most likely the same in insects as in fungi, the compound was included in our studies for comparison.

RESULTS AND DISCUSSION

The effects of diflubenzuron and polyoxin D on synthesis of chitin in body walls of housefly larvae are summarized in Table 1. With either compound, exactly the same effect was obtained: chitin synthesis was inhibited almost completely, and UDPGlcNAc increased. The increase corresponded to the decrease in incorporation in chitin. The accumulation of UDPGlcNAc indicates that chitin synthetase is the receptor not only for polyoxin D but also for diflubenzuron, though final proof of this still has to await studies of the insect enzyme in a cell-free system. Since fungal chitin synthetase is not inhibited by diflubenzuron, a consequence of this conclusion would be that there is a difference in susceptibility of the chitin synthetases to diflubenzuron between fungi and insects.

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7.4 Impact of the insecticide diflubenzuron on insectivorous birds

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INTRODUCTION

Diflubenzuron, a recently developed insecticide, is a stomach poison which interferes with the deposition of chitin in the insect's skin, thus causing death during the moulting process.

Insects sensitive to diflubenzuron are not immediately killed by this compound, but only after a longer or shorter time in the following moult (Van Daalen et al., 1972).

Diflubenzuron may well be included in integrated control programs, because it saves certain categories of beneficial arthropods.

However, as a consequence of its delayed action, insectivorous birds could possibly take in and feed their nestlings with so much diflubenzuron, that poisoning might ensue. This was considered possible for the cuckoo *(Cuculus canorus)*, the oriole *(Oriolus oriolus)* and the red-backed shrike *(Lanius collurio)*, which consume the caterpillars of the browntail moth *(Euproctis chrysorrhoea)*. In some regions of the Netherlands, these caterpillars are a severe pest in shade trees, which can be controlled by diflubenzuron.

METHOD

Cuckoos, orioles and red-backed shrikes, however, cannot be subjected to experiment. Therefore, birds in a comparable situation have been selected, namely great tit *(Parus major)* in orchards and coppice. Tits using nestboxes can easily be studied and their food consists largely of caterpillars. Tree sparrows *(Passer montanus)* were also included in the research because of their great number in the study orchards.

RESULTS AND CONCLUSION

No significant effect of diflubenzuron on breeding and on growth of nestlings of the examined birds was found in comparison with birds in nearby unsprayed experimental fields.

On the basis of measurements of the composition of the food of the nestlings and the contents of diflubenzuron in the leaf-eating insects in the food, the actual and maximum possible daily intake of diflubenzuron by nestlings were estimated (Table 1).

The maximum possible daily intake by nestlings of the great tit was about a hundredth

Table 1. Actual daily intake of diflubenzuron in terms of bodyweight (mg/kg) by nestlings estimated in the experimental fields and maximum possible daily intake (mg/kg) calculated for nestlings of the great tit, tree sparrow and the red-backed shrike.

Age of nestlings (days)	Actual intake			Maximum possible intake		
	orchard		ash coppice	orchard		shade trees
	great tit	tree sparrow	great tit	great tit	tree sparrow	red backed-shrike
1-2	4	0.5	4	15	5	3
3-7	3	0.9	2	13	10	4
8-12	3	0.9	0.6	11	10	3
13-..	2	0.6	0.3	10	7	2

of the highest daily intake by bobwhite quail and mallard duck in eight-day dietary toxicity tests (Philips Duphar, 1975) which revealed no observable signs of toxicity. Poisoning of insectivorous birds with diflubenzuron in orchards and shade trees is therefore unlikely.

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7.5 Effect of pesticides on predacious mites

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INTRODUCTION

In integrated systems for control of pests and diseases, chemical pesticides must be carefully selected. The pesticides should be selective with little or no adverse effect on beneficial arthropods, such as parasitoids, predators and pollinators. Broad-spectrum pesticides should be avoided in any case.

Among the beneficial arthropods, phytoseid mites play a important role as predators of spider mites. A well known example is *Phytoseiulus persimilis*, already used for several years in control of spider mites in greenhouses. Its success, however, entirely depends on proper selection of pesticides simultaneously used with the predator, e.g. fungicides against mildew and other fungus diseases. In orchards, phytoseid mites of the genera *Amblyseius* and *Typhlodromus* are probably of great importance in the regulation of spider mite populations. These mites can only act as control agents, if they are not seriously affected by pesticides.

The present study was set up in order to gather information about adverse effects of pesticides on predacious mites, as an aid in a better selection of pesticides on crops where phytoseid mites help to regulate phytophagous mite populations.

Part of the work is in collaboration with the Working Group 'Pesticides and Beneficial Arthropods' of the International Organization of Biological Control. This working group is attempting to standardize procedures for the assay of pesticides against a large number of different species of beneficial arthropods.

WORKING METHODS

Of the predacious mites, *Phytoseiulus persimilis* was chosen, because it is already commercially used for control of spider mites. *Amblyseius bibens*, a mite from Madagascar (Blommers, 1973), was used as a representative of the genus *Amblyseius* and the closely related genus *Typhlodromus*, both of importance in orchards. It was chosen rather than one of the west European *Amblyseius* or *Typhlodromus* species, because it was available in our laboratory and because of its behavioral characteristics, which make it more suitable as test object than the other species.

The susceptibility of the mites towards the chemicals are only tested in the laboratory.

Besides pesticides, other compounds were tested: phytoauxins, foliar fertilizers and cosmetics, used for improving the appearance of fruits. Test procedures are as follows.

Predator mites are placed on cultures of detached *Phaseolus vulgaris* leaf on moist cotton wool. The leaves are heavily infested with the spider mite *Tetranychus urticae*, which serves as prey for the predator. The predator mite is allowed to adapt itself to the leaf for one day, and the leaf is then treated with the pesticide by means of a Potter precision spray tower. For *P. persimilis*, the chemicals are tested in 3 concentrations: the concentration recommended by the manufacturer and double and half that concentration. In tests with *A. bibens*, only the recommended concentration is tested. Mortality, egg production and hatching are observed over 8 days. For some pesticides, regression lines of log dose against mortality were determined by eye and median lethal concentrations were estimated (5 concentrations with 30 adults per concentration) (Table 1).

When adults are tested, 5 individuals are placed on each leaf culture, 10 juveniles or 20 eggs for each leaf are taken for assay of other stages. Conditions during the tests were set at 25 °C, 70% relative humidity and a light phase of 18 h in a period of 24 h.

Pesticides mentioned in the text and table contain the following active ingredients:
Pallinal M (BASF): a mixture of 44% metiram, 15% maneb, and 12½% nitrothaldiisopropyl;
Nimrod (ICI): 25% buprimate;
Plondrel 50W (Dow Chemical): 20% ditalimphos;
Funginex (Asepta): 20% triforine;
Morestan (Bayer): 25% quinomethionate;
Fungaflor (Philips Duphar): 20% imazalil;
Karathane (Aagrunol): 22½% dinocap;
SM 55 (Ligtermoet): 45% sulphur and 20% captan;
Goldion (Philips Duphar): 60% sulphur and 20% mancozeb;
Malonoben (Philips Duphar): 50% 3,5 di-tert-butyl-4-hydroxybenzylidenemalonitrile;
Benlate (Philips Duphar): 50% benomyl;
Curamil (Hoechst): 30% pyrazophos;
Pirimor (ICI): 50% pirimicarb;

Table 1. Recommended concentrations for application of a number of pesticides and their median lethal concentration (LC_{50}) towards adults of *Amblyseius bibens* at different times after treatment. The concentrations are expressed traditionally in '%', meaning here grams of active ingredient per litre of diluted preparation.

Pesticide preparation	Recommended concentration ('%' = 0.1 g/l)	LC_{50} (%) after			
		1 day	4 days	7 days	10 days
Pirimor	0.05	0.1	0.1	0.1	
Plictran	0.1		0.2	0.15	0.1
Benilate	0.05	>1.0	>1.0	0.1	
Karathane	0.06-0.1[1]	0.15	0.1	0.1	
Torque	0.05	>1.0	1.0		0.5
Thiodan	0.1	0.15	0.15	0.15	
Luxan Maneb 80	0.2	0.4	0.3	0.2	
Luxan Zineb (70%)	0.05	>0.8	>0.8	0.5	

1. Concentration dependent on intervals between applications

Plictran (Philips Duphar): 25% cyhexatine;
Karathane (Aagrunol): 22½% dinocap;
Torque (Shell): 50% phenbutaninoxide;
Thiodan (Hoechst): 50% endosulfan;
Luxan Maneb 80: 80% maneb.

RESULTS AND DISCUSSION

Eleven fungicides have so far been tested for their action on *P. persimilis* (Van Zon & Wysoki, 1978). The fungicides Pallinal M, Nimrod, Plondrel, Funginex, Morestan and Fungaflor caused no damage to any of the stages of the predator. Karathane affected juvenile stages and eggs to some degree. SM 55, Golden Ssp and Malonoben had little effect on adults, but killed most eggs and juveniles.

About 40-50 chemicals have as yet been tested for their action on *A. bibens*, including most of the pesticides used in orchards in the Netherlands. Most of the pesticides were fungicides. Only adulticide and ovicide action were determined. Chemicals known to have a high toxicity for a broad spectrum of insects (e.g. parathion) were not included in the study.

Fungicides containing manganese in general show an unacceptable effect on *A. bibens*, especially if one takes into account the frequent application of fungicides in orchards. Only a few acaricides were tested, as it was felt that these pesticides are not needed in integrated control, where spider mites are controlled by predacious mites. Two insecticides were tested: endosulfan and pirimor. Both compounds showed a moderate effect at the recommended dose, but a slight excess may cause considerable damage.

None of the foliar fertilizers, cosmetics and phytoauxins showed any adverse effect on the predators.

The data on median lethal concentrations are still tentative, as the tests have not been replicated. The concentration recommended for application is also given. Some chemicals, such as Benomyl and Zineb can be safely used. For others, however, caution is needed.

The test method does not closely follow the specifications of test conditions prepared by the IOBC Working Group during a meeting in Colmar, France in 1974. We had to deviate for several reasons.

The Working Group specified that test animals be placed on surfaces already containing a residue of the chemical to be tested. In our method, the chemical is applied to leaves with the predator mite already on it. This is done because an adaptation period is required for the mite: the mites will otherwise leave the leaf and become trapped in the cotton wool. Also contrary to the specification is that eggs are tested and the hatchability of eggs laid by treated females is calculated. For some pesticides, specific action was found on eggs or on offspring. If the 'Colmar' specifications were followed, such effects would not be observed. The Working Group also preferred the use of inert substrates, such as glass or plastics instead of, for instance, leaves. The use of inert substrates gave considerable difficulties: mites tend to leave the treated surface. Efficient isolation barriers were needed, as we had to work with open cells in order to avoud vapour action of the chemicals. It was possible to use talcum powder as isolation barrier for adults of *P. persimilis*, but this system failed for the juvenile stages of this same species and for the smaller *Amblyseius* species.

SIGNIFICANCE FOR AGRICULTURE

Data on the action of pesticides on beneficial arthropods are still few but are essential in integrated control. If pesticides are needed in such schemes, they should be selective and harmless to parasitoids and predators.

Knowledge on the effect of agricultural chemicals on a large number of beneficial arthropods of different taxonomic groups is very welcome. The IOBC working group was formed for this purpose. Information on the effect of pesticides on beneficial arthropods should be added to the label of any pesticide preparation.

FUTURE OUTLOOK

Most of the results were obtained by using the tropical predator mite species *A. bibens*. Research will be needed to determine whether this particular mite species reacts in the same way as the predator mites that occur naturally in the orchard. The susceptibility of *A. bibens* may be different from that of west European species. Comparative studies are planned.

Many pesticides and other agricultural chemicals still need to be tested on predator mites.

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8 Insect hormones

In different institutions for fundamental and applied entomology, it was realized that insect hormones could be exploited as possible substitutes for conventional insecticides. This chapter describes the multidisciplinary study of insect hormones. The first section (8.1) deals with the delineation of endocrine processes in the Colorado potato beetle. Environmental signals influence the behaviour of adults of this model insect through the intervention of the hormonal system and in particular the juvenile hormone. Several of the principles found were of use for the planning and interpretation of the other experiments with hormones. Juvenile hormone is also the key hormone in caste differentiation and adult behaviour of the honey bee. Yet, due to the selective action of certain analogues, bee colonies will probably tolerate the presence in the orchards of the powerful analogue epofenonane (8.2). Laboratory studies were conducted to determine how insects react and under what conditions analogues can be used to control the summer fruit tortrix moth, a serious pest in apple orchards (8.3). The last section (8.4) reports on the use of epofenonane as a pesticide to control this moth and a variety of other species under field conditions. The compatibility with other methods of biological control is discussed.

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8.1 Endocrine regulation of seasonal states in the Colorado potato beetle, Leptinotarsa decemlineata

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INTRODUCTION

In the Section 'Insect Hormones and Pheromones' of the Working Party on Integrated Control, one of the topics regularly discussed was endocrine regulation of adult diapause in the Colorado potato beetle studied by the Department of Entomology in Wageningen. This work was gradually extended to include the endocrine regulation of reproduction, phase formation in locusts, caste formation in the honey-bee, and endocrine aspects of host-parasite synchronization. The present chapter gives an account of the results of these fundamental studies. When the Working Party on Integrated Control was started, research on the identification of juvenile hormone and ecdysone had been undertaken by several groups, because of the potential use of these substances as selective insecticides. Little was then known of the involvement of the two insect hormones in ecological relations, and our investigations have provided an understanding of the complexity of their effects. They have also prompted suggestions for hormonal insect control by revealing specific sites of action. Examples are given in the following paragraphs.

SEASONAL ADAPTATION IN THE COLORADO POTATO BEETLE - ROLE OF JUVENILE HORMONE

Insects are regularly faced with conditions unsuitable for breeding. To survive such unfavourable conditions they have developed behavioural and physiological mechanisms for adaptation, including migration and diapause. These mechanisms can be considered as integral parts of the life history of the insect, and enable the insects to survive, either by escape in space (migration) or in time (diapause). Both mechanisms are usually facultative, which means that they can either occur or be omitted from the life history. They enable the insect to synchronize its developmental and breeding activity to the most favourable seasonal state of the environment.

As both reproduction and adult diapause can be influenced by the corpora allata, which are controlled by the brain, the neuroendocrine system was considered as the centre governing these processes. This linked ecophysiological and endocrinological work on the life-history of insects in our laboratory.

In 1946, De Wilde found that in the Netherlands the adult Colorado potato beetle may enter a reproductive diapause at the end of July. He subsequently started a series of observations and experiments to find the environmental factors involved and the internal neuro-endocrine processes responding to these signals. Photoperiod proved to be the main factor governing diapause. Under long-day conditions (e.g. 18 h light and 6 h dark), more than 70% of the beetles feed and reproduce, but short days invariably induce diapause, the critical photoperiod being about 15 h. Old potato leaves induced diapause under long-day conditions. Some Russian research showed that temperatures above 30 ^{o}C antagonized the short-day effect. The work progressed in two directions: ecophysiology and endocrinology.

Ecophysiological work

This work showed which external factors influenced the phenology of the beetle and it showed that geographic dispersal modified the photoperiodic response throughout the European area. Our laboratory population showed considerable adaptability. Relatively quick changes were observed in photoperiodic response, fecundity, and the duration of some latent periods. This work has grown out into an international network of observations on seasonal development of populations in some critical areas in Europe and Near Asia, to study the adaptivity of the beetles strategy of phenological development in relation to survival and pest outbreaks. We also need more information on whether migration is an integral part of the life-history of the beetle and on factors promoting this behaviour. Alongside the field observations, work is done at the laboratory in Wageningen on photoperiodic response in a large number of geographic strains.

The laboratory strain kept for 20 years in the Department of Entomology in Wageningen has doubled its rate of reproduction and has shortened its critical photoperiod by about 2 h. Also, the incidence of pre-diapause oviposition has increased.
In several natural European populations, especially in those of southern origin, the effect of short-day in inducing diapause is considerably less than in the original French population. In some Turkish populations, long days (16 h) no longer prevent diapause. Most marked, however, is the considerable individual variation in adult responses to photoperiod in each population, as expressed for instance in the final effect (diapause or reproduction), and in the duration of transient phases such as pre-diapause and pre-oviposition. This variability assures survival even under unfavourable summer conditions. A reasonably quick adaptation occurs under changed patterns of selection pressure, both in the field and in the laboratory (De Wilde et al., 1978).

Endocrine basis of diapause and reproduction

The corpus allatum, which produces JH-III (C_{16} juvenile hormone), has emerged as a master gland controlling diapause and oogenesis. About 1960, we found that allatectomy and short-day had similar effects, and in subsequent work we clearly established that corpus allatum activity was under photoperiodic control. Biological assay of the concentrations of juvenile hormone with the Galleria wax-test showed that under long-day conditions high concentrations of JH were present in the haemolymph, while under short-day conditions only minute amounts of JH were detected soon after adult emergence. Thereafter the titre rapidly decreases and during diapause the titre of hormone is below the detection limit of the bioassay (De Wilde et al., 1968). Chemical analysis confirmed that high concentrations of JH-III were circulating in the haemolymph under long-day conditions, while only minute concentrations were present in short-day beetles. (Fig. 1.).

NEURO-ENDOCRINE INTEGRATION: JUVENILE HORMONE TITRE

Our investigations on the role of the corpus allatum in the adult beetle deal principally with two aspects: how the environmental stimuli are translated into a neuro-endocrine response; how the corpus allatum (CA) affects the target tissues.

The neurosecretory cells of the brain represent the final common pathway of neuro-endocrine integration. The photoperiodic information, after being received by the brain, is passed on to the neuro-endocrine system, together with information from a variety of other sources. After integration of these signals, the cerebral neurosecretory cells influence the activity of the CA, which needs constant stimulation to produce juvenile hormone. This conclusion is based on detailed studies on factors involved in regulation of the hormone titre in haemolymph.

The steady state concentration of hormone is maintained by equilibrium between synthesis by the CA and degradation in haemolymph and tissues. Degradation of the hormone seems very rapid. After injecting (^{3}H) JH-I, we found a half-life of only 25 min. Juvenile hormone

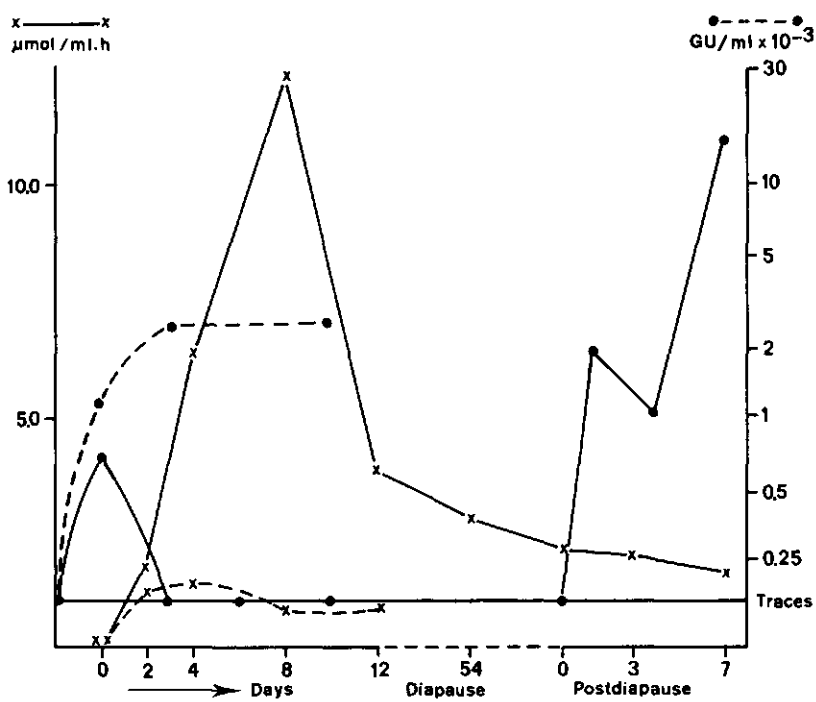

Fig. 1. Juvenile hormone titre and hormone carboxyl esterase activity in haemolymph of Colorado beetles reared under different light regimes. Broken line, long day; solid line. short day; short day; • titre data; x activity of esterases. GU, Galleria assay units. After De Kort et al. (1978).

can be degraded by esterase attack at the methyl ester function or by hydration of the epoxide group by the enzyme epoxide hydratase. Studies on metabolism of the hormone showed that degradation by esterases was the major pathway in the Colorado potato beetle and that the hormone breaks down most rapidly in the haemolymph, which contains an active JH specific carboxyl esterase. The physiological significance is indicated by the observation that the esterase activity shows drastic changes closely correlated with hormone titre (Fig. 1). Apparently JH esterase activity is under strict control. High titres of the hormone coin-

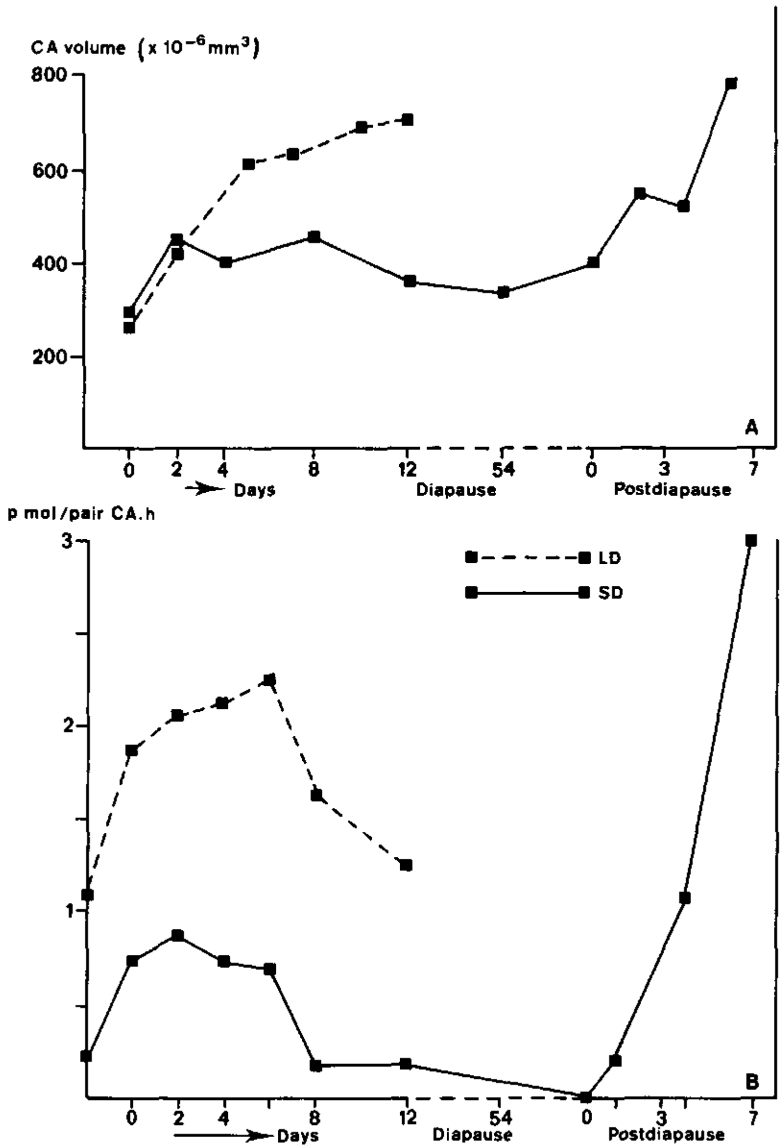

Fig. 2. Volumes of corpora allata (CA) (A) and rates of synthesis in vitro of juvenile hormone per pair of corpora allata (B) from beetles reared under different light cycles. Broken line, long day; solid line, short day. After Schooneveld et al. (1977) and Kramer (1978).

cide with relatively low JH esterase activity and low titres of hormone with high esterase activity, e.g. in the last larval instar and under short-day conditions. However, even under long-day conditions, the JH esterase activity is higher than in the haemolymph of other insect species studied (De Kort et al., 1978). The problem remains of how hormone titres can rise in the presence of such high esterase activity. We investigated whether a carrier protein that protected the hormone against breakdown was present in haemolymph. Indeed, a high-molecular lipoprotein has been shown to bind the hormone with a low dissociation constant ($K_d \simeq 10^5$ mol/l), but it protects the hormone only partly against the esterases (De Kort et al., 1978).

Under conditions of rapid degradation and little protection, the occurrence of high titres of juvenile hormone can be explained only if synthesis by the CA is rapid. Indeed, by a short-term radiochemical assay in vitro, Kramer (1978) showed rapid synthesis by CA. The rate of synthesis in vitro changed drastically in beetles of different age and different photoperiodic treatment (Fig. 2). The CA activity was much higher in long-day beetles than in short-day beetles. In diapause, the rate of synthesis was very low, as would be expected. Rates of synthesis found by this assay in vitro correlated nicely with titres of hormone in the haemolymph. So the photoperiodic effect on the hormone titre in haemolymph is primarily caused by differences in the rate of synthesis by the CA (Kramer, 1978). The changes in the activity of the esterase in haemolymph are a consequence of low titres of hormone rather than the cause. The high esterase activity found during pre-diapause is probably required to eliminate the last traces of the hormone, thus enabling the beetles to enter diapause (De Kort et al., 1978).

CONTROL OF CORPUS ALLATUM ACTIVITY

If a low activity of corpora allata (CA) is the main cause of diapause, the question arises how that activity is regulated by the brain. Are corpora allata controlled by neural pathways or by humoral stimuli? Severing of the nerves to the CA does not change gland activity compared to controls in either long-day or short-day beetles, when the glands are examined with the assay system in vitro 24 h after cutting nerves (Schooneveld et al., 1979). So probably denervated corpora allata remain active under long days, and do not become activated by removing them from the brain under short days. Thus no neural inhibition from the brain is evident in the Colorado potato beetle. Indeed, De Wilde showed that severing of the nervi corpori allati never prevented the onset of diapause if carried out in short-day animals. So humoral stimuli, most probably from the brain, seem primarily involved in regulation of the corpora allata.

We are just beginning to understand what the function of neurohormones may be in the Colorado potato beetle. It is likely that at least one of the neurosecretory factors plays a role in the control of the CA. Indeed, diathermic cauterization of the pars intercerebralis of the protocerebrum inactivates the glands. Moreover, De Wilde showed that diapause after allatectomy can easily be prevented by implantation of one pair of corpora allata from active reproducing females. This does not happen in beetles in short-day diapause. It takes many more pairs of corpora allata to prevent onset of diapause. This can easily be explained if we assume that corpora allata implanted into a short-day beetle rapidly lose their activity, while in long-day allatectomized beetles the implanted glands retain their activity. Thus, the brain produces a substance stimulating CA (allatotropin) under long-day conditions, but not with short days. Alternatively with short days, an inhibiting substance is produced (allatohibin), which prevents the activation of corpora allata under short-day conditions. The search for either type of humoral factors from the brain will be continued.

If the titre of hormone in haemolymph is homoeostatically regulated by changes in the activity of the corpora allata governed by the brain, there should also be a feedback mechanism to keep the titre at a desired level. To find out whether such a feedback mechanism operates, the following experiments were designed. Unilateral allatectomy was performed in long-day beetles and the titre of hormone measured seven days after the operation, and the activity of the remaining gland was tested in the assay system in vitro. The single corpus allatum had increased its activity significantly and the hormone concentration remained the same as in the controls. So the neuroendocrine system accurately compensates for the loss of one CA by increasing the activity of the other. On the other hand, when extra corpora allata are implanted in long-day animals the volume of the CA of the recipient beetles remained low. Moreover, topical application of juvenile hormone to long-day beetles depressed the volume of the CA, and the assay in vitro of CA activity clearly showed a decrease in synthesis.

JUVENILE HORMONE AND FLIGHT MUSCLE DEVELOPMENT

Diapause is characterized by a dramatic reduction in metabolic rate. The main cause proved to be the degeneration of the flight muscles. The question arises whether juvenile hormone directly affects flight muscles. At emergence, the flight muscles have small myofibrils and only few mitochondria. Rapid development takes place during the first 8 days under long--day conditions. During pre-diapause, development is incomplete and the muscles degenerate after the onset of diapause. Termination of diapause leads to rapid regeneration of the flight muscles. These developmental changes in the flight muscles correlated nicely with the titre of hormone in haemolymph. High titres result in complete flight muscle development; low titres in incomplete development; extremely low titres in complete flight muscle atrophy. The interpretation that JH regulates flight muscle development directly is unsatisfactory. Flight muscle degeneration also occurs in other insect species but mostly during reproduction when the corpora allata are presumed to be active. Indeed, allatectomy inhibits degeneration in those species, while treatment with hormone stimulates breakdown of flight muscle. These examples demonstrate that flight muscle development and degeneration are correlated with the activity of the corpora allata, but the response of these muscles to hormone can be different in different insects. This presents a serious difficulty in correlating flight muscle development and degeneration with hormone titre. Perhaps there is rather a correlation with locomotory activity.

In migratory insects, flight occurs before reproduction; flight muscles degenerate when migration has ended. In the Colorado potato beetle, degeneration occurs with diapause, a state of almost complete immobility in the soil at a depth of 40-60 cm. The effect of the hormone can well be mediated by the nervous system. Indeed, diapause is accompanied by a drastic decrease in acetylcholinesterase activity in the brain. Moreover, severance of the nerve to the flight muscle leads to a failure of normal flight muscle development. Apparently flight muscle development and degeneration has been so correlated with diapause that it fits into the phenological strategy of the Colorado potato beetle.

ENDOCRINE REGULATION OF REPRODUCTION

Reproduction in the Colorado potato beetle occurs under long-day conditions. Two days after emergence the adult beetle starts producing yolk. Mating starts on Day 2 or 3 and from Day 5 onwards oviposition occurs daily in batches of more than 60 eggs per female. The majority of the yolk proteins consists of 2 proteins, vitellins, which are immunologically identical with 2 proteins in the haemolymph (vitellogenins). The vitellogenins are synthesized at a high rate in the fatbody of long-day females after Day 2, transported to the ovaries, and specifically taken up by pinocytosis.

Under short-day conditions, vitellogenin synthesis by the fat body is very slow and yolk is no longer produced. However, the fat body then synthesizes a large number of other proteins. Among these, there are three proteins stored in large amounts after Day 6 in 'protein granules'. Large differences exist in the fat body between long-day and short-day beetles, both functionally and structurally. These differences in function are under the control of hormones, especially juvenile hormone. Allatectomy of long-day beetles leads to a decrease in synthesis of vitellogenins. Treatment of short-day beetles with juvenile hormone leads to increased synthesis of vitellogenins by the fat body. However the hormone alone is not the only factor responsible for protein metabolism in the fat body. Increase in protein synthesis also occurs in allatectomized diapausing animals if they are transferred to long-day.

Moreover, when 1-day-diapausing beetles were treated topically with a large dose of juvenile hormone, they entered the soil again immediately. Several days later, some beetles emerged again but all returned to the soil after a few days to several weeks. Very few females oviposited during that period, even when JH doses were increased to lethal amounts. Mimetics that were known to be more potent than natural juvenile hormone in other tests also demonstrated high activity in their potency to break diapause. But all compounds only induced temporary activation and limited reproductive activity.

The other factor required to break diapause permanently proved to be a long-day regime. Long days, in combination with juvenile hormone in a dose normally activating 75% of the diapausing beetles terminated diapause and gave persisting reproductive activity in 100% of the beetles. The beetle's corpora allata hardly play a role under these conditions and may be removed without any noticeable effect over 2 months. Since exogenous hormone is rapidly degraded once it arrives in the blood, reproduction under these conditions must continue in spite of very low titres of hormone. This finding requires clarification.

OUTLOOK

In 1967, the chemical structures of both the juvenile hormone and ecdysone were elucidated, and world-wide investigations on the practical use of the hormones and their analogues were under way. About 6 years later, the responsible authorities were confronted with requests for admission of the first hormonal insecticides. Work done by our Working Party on Integrated Control has contributed to these developments.

Our investigations have helped to provide background information to judge the consequences for insect life in general of spraying or dusting with insect hormones. Break of diapause, interference with oecomorph development are matters in point. Our finding that queen-like characteristics develop when honey bee larvae are treated with small doses of juvenile hormone came just in time for the plant protection authorities to be alerted. We were first in elucidating that titres of juvenile hormone are responsible for seasonal states and oecomorphs (De Wilde, 1978).

Now that the endocrine basis has been elucidated, the whole conception of seasonal strategies in insects and the impact of token stimuli is on much firmer footing.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

8.2 Effects of juvenile hormone and some analogues on caste differentiation and adult behaviour in the honey bee, Apis mellifera

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INTRODUCTION

The development of a female larva into a queen or worker bee depends completely on the conditions prevailing in the cells in which they are reared. From experiments on rearing of larvae in vitro, the following hypotheses have been put forward in the literature. The process of queen differentiation is regulated by

- the presence of a determining principle in the food of queen larvae;
- the higher moisture content of the food of young queen larvae, resulting in a higher food intake than by worker larvae;
- the higher content of sugars in the food of young queen larvae, inducing a higher food intake than by worker larvae;
- a difference in hormone balance in the early stages of larval development between queen and worker larvae.

The aim of our research in the laboratory of Entomology was to attain a better understanding of the process of caste differentiation by testing some of these hypotheses in vivo. The laboratory interest directed research to the effects of hormonal insecticides on colony development.

ROLE OF JUVENILE HORMONE IN CASTE DIFFERENTIATION

In a comparative anatomical study, Wirtz (1973) demonstrated that the corpora allata in queen larvae were larger than in worker larvae. An increase in size and activity of the corpora allata occurred after transferring 2-day-old worker larvae into queen cells. The Galleria wax-test showed that the titre of the juvenile hormone in the haemolymph of 3-day-old larvae was about 10 times as high as in worker larvae of the same age.

To prove that the hormone plays a role in caste differentiation, it was applied topically to worker larvae (Wirtz, 1973). Application of 1 µg of JH-I to 3.5-day-old larvae resulted in the development of worker-sized individuals with obvious queen characteristics, even though treated larvae received normal amounts of worker jelly from the nurse bees and grew in narrow cells. In this project, J. Naisse demonstrated that the degeneration of ovarioles in young worker larvae was inhibited by artificially increasing the titre of the hormone in haemolymph.

Queen differentiation was also obtained after topical application of several juvenile hormone analogues (JHA). Copijn et al. (1979) found that application of 1 µg of JH-I or ZR-512 to worker larvae 3.25 - 3.50 days old resulted in the highest degree of expression of queenlike characteristics.

After it became known that JH-III was the only natural juvenile hormone in the adult, the effect was studied of application of this substance on caste differentiation in the larvae. Rather than affecting all measured characteristics by JH-I application, one dose of 3-14 µg of JH-III caused only some features within an individual to become queenlike. However thrice repeated applications of 4 µg of JH-III improved the differentiation into a queenlike individual. The different effects of JH-I and JH-III on caste differentiation and the extremely low mortality after application of JH-III suggest that JH-III plays a role both in the adult bee and in larval development.

De Kort et al. (1977) demonstrated that JH-I was not degraded by specific esterases in the haemolymph of larvae of the last three instars; a very low activity of JH-III esterase was found only in the 5th instar. As it is very unlikely that enzyme activities differ towards JH-I and JH-III, we assume that the different results obtained after application of these two hormones may be due to a different rate of release from the cuticle to the haemolymph. After application of JH-I, the titre remained high two days after application (Copijn et al., 1979). An effective JH-III titre was probably maintained only briefly. The induction of queen characteristics should then depend on the coincidence of a high titre in the haemolymph and a sensitive period of separate imaginal discs.

Final evidence of the significance of the hormone in the process of queen-worker differentiation was obtained by administration of the corpus allatum inhibitor Precocene-II, a compound extracted from the herb *Ageratum houstonianum*. Goewie et al. (1978) found that the corpora allata partially or completely disappeared after topical application of Precocene-II to queen larvae. Although all larvae had consumed large amounts of royal jelly, which should enhance queen differentiation, the resulting adults developed worker-like characteristics.

FUNCTION OF THE BRAIN

It is still unknown how the perception of food quality or differences in food intake regulate the production of juvenile hormone in the corpora allata. Recent studies by Goewie (1978) revealed that the medial neurosecretory cells in the brain are not involved. These cells are hardly differentiated in larvae up to 60 h. However, at the end of the 4th and 5th larval instar, differentiation of these cells is completed and a considerable number of neurosecretory granules are transported in the axons running from the neurosecretory cells to the corpora cardiaca and to the corpora allata. The appearance of neurosecretory material in the corpora allata coincides with a decrease in activity of these glands. So during these stages of larval development, the median neurosecretory cells must inhibit production of juvenile hormone.

PERCEPTION OF FOOD QUALITY

With scanning electron-microscopy, Goewie (1976) described the development of the sense organs on the larval mouth parts. After preliminary experiments by Wirtz (1973), Goewie (1978) studied the histology and function of these sense organs. All chemoreceptors proved sensitive to sugars and salts. However no other tested component of larval food, including Rembold's determining principle stimulated the sense organs. This result was confirmed when comparing receptor responses generated by diluted royal jelly and worker jelly. A water receptor could not be found. So sugars may function as phagostimulants and no determining principle or other food component is involved in caste differentiation at the stage of perception.

FURTHER RESEARCH ON CASTE DIFFERENTIATION

The main problem to be solved is the different behaviour of the nurse bees when feeding larvae in queen or worker cells.

OTHER EFFECTS OF JUVENILE HORMONE AND ITS ANALOGUES

Copijn et al. (1979) demonstrated that several analogues topically administered in various doses to young worker larvae caused a high mortality, both in the larval and pupal stages. The adult bees interfered with the development of the treated brood after the cells had been sealed by them. Though sealed cells containing treated and untreated worker larvae appear identical to the human eye, the bees discriminated between the two situations. Soon, all hormone treated larvae and pupae were removed from their cells by the bees (Wirtz, 1973). Thickness of the cocoon, ultimate orientation of the pupating larva or contact pheromones may inform the bees of the presence of the treated brood. Colonies repeatedly used to rear treated larvae neglected the brood and some times the queen disappeared (Wirtz, 1973). Because of these observations, our group assumed that the behaviour of the nurse bees had been affected as well.

EFFECT OF JUVENILE HORMONE ANALOGUES ON COLONY DEVELOPMENT

By feeding small colonies on analogues or by spraying analogues onto a crop visited by bees, Beetsma & Ten Houten (1975) found a considerable mortality in the brood nests. Not only the brood was affected but also the adult bees. Food glands degenerated completely and wax glands were small.

Treatment of colonies with some analogues resulted in the death or loss of the queen. Since queens are normally fed by the bees, the observed effect is presumably caused by degeneration of food glands of the workers. Queen-rearing behaviour was affected as well. Dequeening a colony normally results in transformation of some worker cells into queen cells by the workers. However, no queen cells were built when the queen disappeared from analogue treated colonies.

CONCLUSION

If juvenile hormone analogues are to be used in pest control, the effects on the development of useful insects must be tested first. The analogue epofenonane, which hardly affected parasitic hymenoptera, seems also inactive against the honey bee. Since no increase in mortality of adult honey bees could be demonstrated after contact with analogues (Beetsma & Ten Houten, 1975), the current method of screening pesticides for toxicity to honey bees should be used with caution. Effects on brood development and adult behaviour should certainly be studied too.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

8.3 Use of insect growth regulators with juvenile hormone activity to control Adoxophyes orana: laboratory studies

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The endocrine system in insects controls several processes associated with embryogenesis, growth and development, reproduction, diapause, and various aspects of behaviour. Experimental changes in hormone balance, brought about at specific stages of the life cycle are likely to induce permanent disturbance of these processes, which may reduce the chances of normal development of the individual or its fitness for survival.

Studies on hormonal insect control are focused on the finding of weak spots during development, in which hormonal balance may be upset. This paper considers the use of synthetic compounds with juvenile hormone activity, often called insect growth regulators (IGR), in control of summer fruit tortrix moth *Adoxophyes orana*, which is a pest in apple orchards in the Netherlands and which is hard to control with selective insecticides (Sections 1.3, 1.8 and 8.4). Introductory laboratory research was conducted to determine the biological activity of different compounds. This paper stresses the evaluation of factors of special importance to IGR action in the field.

MATERIALS AND METHODS

Adoxophyes orana larvae were mass-reared on artificial diet at 20 °C, unless otherwise specified. Freshly moulted 5th-instar larvae were collected from the culture boxes, kept isolated, and treated topically with one of the following compounds: methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (IGR-1); 6,7-epoxy-3,7-dimethyl-1-3,4-(methylenedioxy)phenoxy-2-nonene (IGR-2); 6,7-epoxy-3-ethyl-1-(*p*-ethylphenoxy)-7-methylnonane (IGR-3). All compounds were used as mixtures of isomers dissolved in acetone, and were supplied by Dr R. Maag A.G. (Dielsdorf, Switzerland). Pupae and adults were inspected for effects by criteria outlined in the text.

RESULTS AND DISCUSSION

Dose-response relationship

One needs methods to determine and compare the biological activity of different compounds in order to select the most suitable one.

For *A. orana*, the compounds were applied topically within one day of the moult to the 5th (last) larval instar. The effect was assessed after pupation. Small doses yielded pupae with minor effects, such as retention of larval prolegs or palpi (score 1); larger doses resulted in the retention of more larval characteristics including green coloration. The strongest effect was the formation of a supernumerary perfect 6th-instar larva (score 7) (Abdallah, 1972). When sufficiently large numbers of larvae were treated, the average effect of a given dose was predictable. Plotting the log of the dose against the average numerical response resulted in a sigmoid curve, which made it easy to read the dose that gave a score of, say, 3. The rather potent compounds selected for further evaluation, IGR 2 and 3, were a hundred times more active than IGR-1 and the critical dose was about 0.1 μg per larva.

The larvae show an optimum sensitivity (critical period) about the middle of the 5th intermoult period. Earlier application results in partial breakdown of the compound before the sensitive period; later application is ineffectual since the sensitive period has largely elapsed. Spraying in the field must be precisely synchronized to the development of the population; alternatively a deposit must be applied on the substrate, such that every individual in a mixed population receives sufficient of the compound at its sensitive stage.

Effect of temperature

Temperatures in the field vary considerably. As it was unknown whether the action of compounds was modified by low or high temperature, treated larvae were kept at temperatures in the range from 13 to 32 °C and pupal scores were compared (Schooneveld & Wiebenga, 1974). Low temperature gave clearly less pronounced effect but greatly extended the sensitive period. High temperatures gave dramatically decreased effects and reduced the sensitive stage to only 2 days, even if high temperatures lasted only a few hours each day in a regular day/night temperature regime, as obtained in the field. The results are as yet unexplained but one would expect that hot periods in the field would be a disadvantage for such control.

Effect on adult emergence

To determine the significance of juvenile hormone analogues on pupation, the further life history of treated animals was followed. There was a clear relation between the effects of compounds on pupation and the condition of the adults emerging from such pupae. Pupae with scores of 1 and 2 usually gave rise to externally normal adults. Adults from pupae with scores of 3 had crumpled wings and moved much less. Those from pupae with a score of 4 never emerged. 'Pupae' scored 5 or higher sometimes moulted once or twice more but never reached adulthood.

Adult emergence was reduced dramatically when treatment was postponed until the second half of the fifth instar, i.e. when no visible effects on pupation were obtained. Treatment 1 to 2 days before pupation prevented almost all moths from emergence (Fig. 1). The 'critical period' was thus extended considerably.

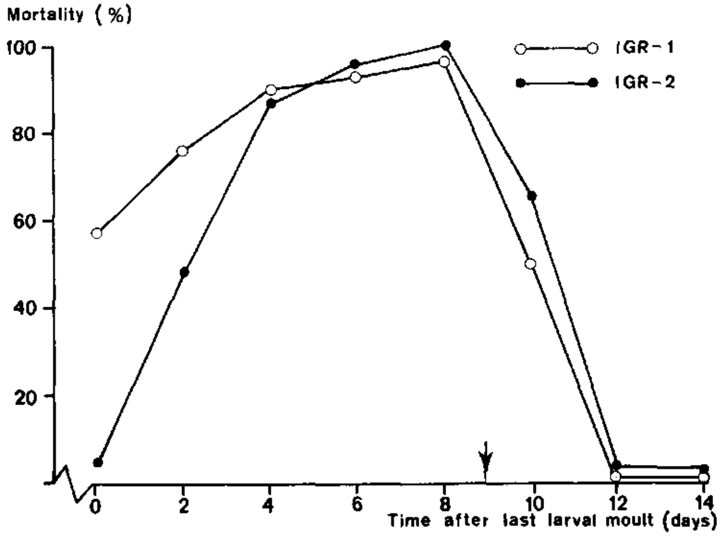

Fig. 1. Mortality before or during adult ecdysis after treating last-instar larvae or pupae at one of the indicated intervals with 5 µg of IGR-1 or 0.05 µg of IGR-2. Arrow indicates pupation.

Effect on behaviour

Preliminary experiments indicated that treatment influenced two aspects of behaviour. Fifth-instar larvae feeding on bean plants sprayed with a high concentration of IGR-3 fell from the plant more often than larvae on plants sprayed with lower doses. When these fallen larvae were collected and fed on untreated artificial diet, a positive correlation was found between the morphogenetic effect at pupation and the incidence of falling. So locomotion in larva might be impeded by the compounds.

Spontaneous movement of moths was visibly less when the pupae from which they emerged showed only minor juvenile characteristics. Normally male moths reacted to synthetic sex pheromone with a display of wing movement. Pheromone at a concentration that would normally activate nearly all males activated fewer males the more of a compound had been applied to the larvae. Only a few males from pupae of scores 1 or 2 reacted, whereas the duration of the activity was less.

Each of these effects on behaviour may reduce chances of survival and reproduction.

Effect on reproduction

Moths from treated pupae were less fecund; several induced abnormalities accentuated that effect (Schooneveld & Abdallah, 1975). In some individuals, the ovaries looked abnormal at ecdysis.

In some females, nurse cells and oocytes developed irregularly and oocytes degenerated early or later during oogenesis. In others, egg maturation was slowed down and oviposition was retarded but mating with normal males remained possible, although the frequency was reduced. Treatment of the adults did not disturb reproduction or fertility of the eggs. None

Table 1. Relationship between degree of pupal juvenilization (scores) and number of offsprings from the resulting moths.

Pupal scores		Relative number of offspring	
♀	♂	IGR-1	IGR-2
untreated	untreated	100[1]	100[1]
0	0	.	99
1	1	4	16
2	2	0	0
untreated	0	51	103
untreated	1	26	14
untreated	2	0	8
0	untreated	90	138
1	untreated	21	56
2	untreated	7	20

1. Reference value 100% for untreated sex partners kept as isolated pairs.

of the compounds tested had a high ovicidal action when applied directly to batches of eggs.

Factors contributing to the number of offspring are mating, fertilization of eggs, and number of eggs matured and deposited (Table 1). Each of these factors was affected after larvae were treated with the compounds. Male sexual behaviour was more inhibited than female responsiveness. Analyses were made by studying separate couples in which none, one or both partners were treated. The number of offspring was reduced to 16% of normal if both partners or only the male originated from pupae scored 1, i.e. showed barely visible aberration. Adults from pupae scored 2 or more did not reproduce at all. In the field, they would not contribute to population increases.

These findings indicate that visible signs of juvenilization reflect developmental anomalies of much greater impact than had been realized.

Conclusions and prospects

The developmental stage during which *A. orana* in the field may be reached and affected by juvenile hormone analogues is restricted to the 5th-instar larva. Younger larvae are not affected; pupae are well hidden and difficult to reach with sprays; adults are insensitive and possibly too mobile; eggs are hardly affected. Hormonal disturbances cause visible aberrations in the pupal or imaginal instar, depending on the timing of the application. Larvae in the field are not strictly synchronized, so for control we need a compound that is sufficiently persistent on the tree to disrupt development of both fast and slowly developing individuals, as does IGR-3. Small outdoor trials with IGR-3 have demonstrated its potential for control of *A. orana* (Schooneveld et al., 1976).

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8.4 Use of the insect growth regulator, epofenonane, in integrated control in orchards

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INTRODUCTION

In orchards in the Netherlands, *Adoxophyes orana* is feared as a pest because the extent of outbreaks is unpredictable. The larvae, hiding against leaves attached by a web to fruits, may damage several tenths of an apple crop in July and, to a lesser degree, again during the second generation of the tortricid in September. With routine chemical control, outbreaks of *A. orana* can be severe. Although beneficial insects seem to suppress these outbreaks to some extent, directed chemical control seems indispensable (Sections 1.3 and 1.8).

The insecticides that have proven highly successful against *A. orana*, such as azinfos--methyl, methidathion, and recently certain synthetic pyrethroids, are unselective and not suited to integrated control. Of the approved selective caterpillar insecticides, diflubenzuron is not effective against *A. orana*, and *Bacillus thuringiensis* not sufficiently reliable (Sections 1.1 and 9.1). Since laboratory and small field trials showed the effectiveness of certain insect growth regulators, juvenile hormone analogues, in control of *A. orana* (Section 8.3), the usefulness for integrated control of the most promising of these, epofenonane, was tested on a large scale.

Three questions were raised:

- Given the fact that only fifth-instar larvae are susceptible to this insecticide, can sufficient control be achieved?
- Will other noxious insects be controlled at the same time?
- Is the use of epofenonane compatible with biological control of other pests, in particular *Panonychus ulmi*?

Though it is the fifth instar that is susceptible to epofenonane, i.e. a period in the life cycle after the caterpillar has damaged the plant, this insecticide may succeed in controlling caterpillars. However the rationale of control is different from that for conventional insecticides.

After hibernation in their second or third instar, the larvae of *A. orana* feed on the young foliage in April and May without causing economic damage at the densities occurring at this time. If the population could be reduced to such a degree before the next harmful

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generation that it could not rise to a harmful level within the same year, and if immigration of moths were unimportant, acceptable control would be achieved. This was the approach in our trials. Conventional contact insecticides are not particularly successful against *A. orana* when applied in spring; they are, however, effective when used at hatching of the eggs of the first and second generation of the year in June and August.

METHOD

We compared the development of *A. orana* and other insects in entire apple orchards treated with ACR 2019 E and untreated orchards. Swiss colleagues organized parallel trials.

ACR 2019 E contains a mixture of 6,7-epoxy-3-ethyl-1-(*p*-ethyl-phenoxy)-7-methylnonane and 6,7-epoxy-1-(*p*-ethylphenoxy)-3,4,7-trimethylnonane(epofenonane). If directed against *A. orana*, it was applied twice between early and full bloom, when about 30 and 90% of the larvae were in their 5th instar. If aimed against other tortricids, aphids and *Lepidosaphes ulmi*, spraying dates were adapted to the phenology of these insects. Dosages ranged from 0.6 to 1.5 kg a.i. per hectare.

The effect of the sprays was assessed with individual *A. orana* as well as by development of the population. Larvae of *A. orana* were either collected after spraying and reared in the laboratory on untreated artificial diet or treated foliage or kept in gauze bags in their natural position on the trees and examined about two weeks after spraying. The morphogenetic effects were scored as in laboratory tests (Section 8.3).

To ascertain the influence of the sprays on the population of *A. orana*, relative densities of the larvae were determined, in samples of blossom in spring and of shoots in summer. Absolute densities, expressed as larvae per tree, were sometimes assessed in order to estimate the multiplication factor from the treated generation to the next one.

In one of the orchards in which biological control of *Panonychus ulmi* was functioning, the effect of the epofenonane sprays on the spider mite-predator relationship was assessed.

EFFECT ON ADOXOPHYES ORANA

The delayed action of epofenonane made it difficult to assess its effect in the field, on the individual insects and on the population.

When larvae collected in the field 1-3 weeks after treatment were reared on untreated artificial diet, the effect was underestimated; but when they were kept on treated foliage, it was sometimes overestimated. Larvae enclosed in gauze bags on the trees after treatment presumably give the most reliable results.

Two sprayings caused morphogenetic effects, as found in the laboratory in all or almost all enclosed specimens. They died either as larvae or pupae or, if they did develop into adults, they deposited few viable eggs, if any. The final effect of preventing a next generation was estimated as almost complete; and the greater part of the effect seemed to be due to the first spraying. The second spraying may be superfluous under certain weather conditions or when the density of the hibernated larvae is low.

Traps baited with synthetic female pheromone showed considerably smaller catches in sufficiently isolated test orchards than in untreated areas. Catches in traps on the borders of test plots neighbouring untreated controls, however, suggest that re-immigration may rapidly neutralize the effect if a source of *A. orana* is nearby.

In sufficiently isolated orchards, the effect on the populations of treatments in spring lasted throughout the summer and a satisfactory level of control was achieved; damage by *A. orana* to the fruits remained below the acceptable limit.

The trials are being continued in some orchards separated by up to 800 m from the next orchard, to estimate the degree of isolation required for this control technique to function properly. The first results suggest that about 100 m may be sufficient.

EFFECT ON OTHER TORTRICIDS

Ptycholoma lecheana, Hedya nubiferana, Archips podanus, Archips rosanus, Spilonota ocellana and *Pandemis heparana* may increase in numbers with an integrated programme instead of conventional chemical control. The fifth larval instar of the former two species, which are hardly harmful, coincides with that of *A. orana*, and they are about as susceptible. Hence, they are controlled with the sprayings directed against *A. orana*. The fifth instars of the other tortricids mentioned, which are injurious, are more dispersed over May and June. Epofenonane sprays tend to induce a normally functioning supernumerary larval instar in these species, thus protracting the noxious stage. Therefore, the usefulness of epofenonane to control other species of orchard tortricids than *A. orana* is as yet questionable.

EFFECT ON OTHER INSECTS

Aphids such as *Rhopalosiphum insertum, Dysaphis plantaginea* and *Eriosoma lanigerum*, and the coccid *Lepidosaphis ulmi* are controlled to a large and possibly satisfactory degree if sprays are properly timed: early spring for the aphids and May, partially coincident with the sprays against *A. orana*, for *L. ulmi*. The later spraying may also act against the geometrids *Operophtera brumata* and *Chloroclystis rectangulata*.

COMPATIBILITY WITH BIOLOGICAL CONTROL

Sprays of epofenonane applied for three consecutive years to an orchard in which *Panonychus ulmi* was kept at a low level by predatory mites, mainly *Typhlodromus pyri*, have not upset the relationship of spider mite and predator. This corroborates the results of laboratory tests in which epofenonane was not toxic to predatory mites.

CONCLUSION AND PROSPECTS

The evidence so far suggests that control of *A. orana* by sufficiently persistent juvenile hormone analogues is practical, if the area treated is sufficiently isolated or large. Although several insects from different orders are susceptible to epofenonane, separate sprayings would probably be needed to achieve satisfactory control because timing is so critical with this insecticide.

Since control of *A. orana* and certain other insects could be increased or maintained after several years at a high level with less ingredient, the trials with epofenonane are being continued as part of large-scale tests on integrated control.

Regrettably, epofenonane will not be marketed as an insecticide because the economic prospects are not favourable for this expensive chemical. Nevertheless we consider it useful to continue these tests because economically more promising chemicals with an identical mode of action are being developed, and similar complications will be associated with other control agents acting with delayed effects, as have pheromones used for male disruption.

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9 Micro-organisms, viruses and nematodes

Nematodes, fungi, viruses and the spore-forming bacterium *Bacillus thuringiensis* were studied as potential organism in the biological and integrated control of some insect pests. Preparations of *B. thuringiensis* gave insufficient and variable results against the summer generation of *Adoxophyes orana*, whereas promising results were obtained in the laboratory (9.1). In field trials, an attractive reduction in fruit damage was observed after application of the granulosis and nuclear polyhedrosis virus of *Laspeyresia pomonella* and *A. orana*. The nuclear polyhedrosis viruses of *A. orana* and *Mamestra brassicae* were not cross--infective so that *M. brassicae* could not be used to produce *A. orana* nucleopolyhedrosis virus (9.2) as earlier work had suggested. To demonstrate the differences between these viruses a new tool in the form of an analysis of the viral DNA after digestion by restriction enzymes was used. This method will be reliable as a technique to identify viruses and virus isolates (9.3).

Studies on the use of the nematode *Neoaplectana carpocapsae* are in progress with emphasis on the control of *Synanthedon myopaeformis* and *Sciapteron tabaniformes* (9.5). The significance of some new isolates of rhabditids will be studied. A feasibility study has started on use of *Entomophthora* spp. in the control of aphids (9.4).

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9.1 Trials with Bacillus thuringiensis against lepidopterous pests

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DESCRIPTION AND AIM OF THE RESEARCH

One of the most effective insect pathogens used for control of insect pests is undoubtedly the spore-forming bacterium *Bacillus thuringiensis*. It acts mainly by means of the δ'-endotoxin, contained in a cellular inclusion (crystal or parasporal body) formed simultaneously with the spore. The δ'-endotoxin is highly selective and is active against larvae of lepidopterous insects.

Research on this pathogen started in our laboratory in the late 1950s and was at that time partly in collaboration with the Park Department of the Municipality of Amsterdam. This work solved the regular problem of the tent caterpillar *Malacosoma neustria* in elm trees in parks and along the streets of Amsterdam: commercial *B. thuringiensis* formulations were an effective means of control and have been used in the city since the early 1960s (Van der Laan & Wassink, 1962). These formulations replace other insecticides, in particular Derris powder, which often gave difficulties, for instance by killing fish in private ponds.

Later research on *B. thuringiensis* in our laboratory has been concerned with integrated control in orchards or with methods of biological assay for standardization of *B. thuringiensis* preparations and determination of their effectiveness.

The work on *B. thuringiensis* with orchard pests was concentrated on the leaf-roller *Adoxophyes orana*, one of the key pests in orchards. In field tests, trees 3-4 years old were treated with the following commercial preparations of *B. thuringiensis:* Dipel, Thuricide HP and Biotrol 183. The first two products contain the potent HD-1 strain (Dulmage, 1970), Biotrol 183 is an older product and much less effective. Some treatments with *B. thuringiensis* were combined with low doses of insecticides (azinphos methyl and tetrachlorvinphos) to detect any possible synergistic action. The treatments were in spring against the overwintering generation and against the first summer generation of the year.

In the laboratory, several factors were studied, such as temperature and ultraviolet light. In addition, the effect of chitinase was studied, as it was reported that this enzyme enhances the action of *B. thuringiensis* (Smirnoff & Valero, 1972). The reaction of

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A. orana was also studied after ingestion of the spore-crystal complex by feeding larvae on suspensions of pure spores, crystals or combinations, as there was some evidence that this species could only be killed by the combined action of spores and crystals, a relatively rare phenomenon called type III reaction (Heimpel & Angus, 1959). Spores and crystals were obtained from the commercial preparation Dipel. They were separated by a flotation method, by density-gradient centrifugation or by killing the spores with radiation or lysozyme treatment.

Another aspect of the research on this pathogen was the development of methods of biological assay to test the effectiveness of different preparations of *B. thuringiensis*. These tests were mainly on *Pieris brassicae* (Van der Geest & Wassink, 1972). By a modification of that method, many isolates of *B. thuringiensis* of several serotypes were tested as a contribution to an international program on the spectrum of activity of different *B. thuringiensis* serotypes organized by the United States Department of Agriculture. Several other laboratories in North America, Japan and Europe participated. The program allowed study of the activity of many isolates towards many insect species, including lepidopterous and dipterous larvae. Mosquito larvae were studied, since recent investigations showed activity of certain strains of *B. thuringiensis*. It is not yet known, whether that activity was caused by the action of the δ'-endotoxin or by other actions. Other Diptera were included in the program to detect possible presence of other toxins in the preparations, such as β'-exotoxin.

RESULTS AND DISCUSSION

In the first year of field trials, acceptable control was obtained against the summer generation of *A. orana* in several tests with formulations based on the HD-1 strain of *B. thuringiensis*. It was not possible to obtain satisfactory control in the spring of overwintering larvae. No synergistic effect was detected of *B. thuringiensis* and low doses of azinphos methyl or tetrachlorvinphos.

Later results against the first summer generation were quite variable and in certain years insufficient, even when treatments are repeated every 7-10 days. The larvae were quite susceptible to *B. thuringiensis* in laboratory experiments. The disappointing results in the field are attributed to the behaviour of larvae, which are for most of their life hidden underneath webs or between folded leaves. They can only be reached by insecticides, when they move to other shelter. Insecticides with a contact action are more effective against these migrating larvae than insecticides with a stomach action, such as *B. thuringiensis*. Control of larvae may be improved by increasing persistence of the insecticides, for instance by adding stickers or ultraviolet protectants, such as benzyl cinnamate, which proved effective in the laboratory at a concentration of 10 g/l. The action of *B. thuringiensis* could not be improved by adding the enzyme chitinase.

Another factor in the action of *B. thuringiensis* is temperature. At temperatures of 15 °C or less, control was much poorer, unless six times as much was applied as at 22 °C. However in spring the insecticide have been diluted by the rapid growth of foliage notwithstanding the low temperatures prevailing in the Netherlands in that time.

In laboratory tests, *A. orana* proved to belong to the group of insects with the type II reaction (Heimpel & Angus, 1959) after ingestion of the spore-crystal complex. The crystals caused paralysis of the alimentary tract soon after uptake. Death ensued after about 4-5 days. No spores were needed to kill the insect. The median lethal dose of crystals for 2nd-instar larvae was about 250.

For the International Cooperative Program on the spectrum of activity studies of *B. thuringiensis* serotypes, more than 300 different isolates were tested. Results from all participants will be published as a Technical Bulletin of the United States Department of Agriculture. Subgroups within serotypes differed in reaction towards different species of insects, probably by differences in the δ'-endotoxin composition. Against *P. brassicae*, the highest proportion of active isolates were found in the variety *kurstaki* (serotype 3a,b). A moderate proportion of active isolates was found also in the varieties *thuringiensis* (serotype 1) and *alesti* (serotype 3a). Most of the other varieties comprised isolates with low toxicity, e.g. *galleriae* (serotype 5a,b), *kenyae* (serotype 4a,c) and *aizawai* (serotype 7). Some of the isolates tested in the program were found to be more effective than the HD-1 strain presently included in most of the present commercial formulations and will undoubtedly be exploited in the future.

Poor performance of *B. thuringiensis*, especially in the spring, can sometimes be attributed to the low temperatures. It is not known whether any of the isolates tested in the international program performed better at low temperatures than the HD-1 strain.

Bacillus thuringiensis is still little used in the Netherlands. It has been successfully used against *Malacosoma neustria*, *Euproctis chrysorrhoea* and *Stilpnotia salicis*. In experimental fields, results have been variable against *A. orana* and *Operophthera brumata*. Preparations based on this pathogen have a potential use against cabbage pests, such as the highly susceptible *P. brassicae* and the less susceptible *Mamestra brassicae*. Under certain circumstances, they can also be used for the control of insects such as *Ephestia* spp. in stored products.

SIGNIFICANCE FOR AGRICULTURAL PRACTICE

The selectivity of insecticides with *B. thuringiensis* as agent makes this type of product attractive against insect pests, especially in integrated control and where use of chemical toxins should be avoided, as in recreational areas. It is disappointing that good control is not possible for the main lepidopterous pests of horticulture and agriculture by means of *B. thuringiensis*. As a result of the international program, more active formulations of this bacterium may be marketed in the future. New formulations active at lower temperatures are needed for some pests in the Netherlands.

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9.2 Baculoviruses as agents in biological control

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Viruses, especially the baculoviruses, which infect often a small range of species, may offer a means of selective pest control in agriculture and forestry.

ACTIVATION OF LATENT INFECTIONS IN MAMESTRA BRASSICAE AND ADOXOPHYES ORANA

To use baculoviruses in control of insect pests, large amounts of virus has to be produced on a commercial scale and at costs comparable with those of insecticides. Much research is directed to developing techniques to grow insects on a large scale on cheap artificial diets. The use of large insects to produce these viruses is one approach. Ponsen & De Jong (1964) tried to use larvae of the cabbage moth *Mamestra brassicae* to produce the nuclear polyhedrosis virus of the summer fruit tortrix moth *Adoxophyes orana*. Larvae inoculated with the virus went down with nuclear polyhedrosis. Nuclear polyhedrosis viruses contain one (single-embedded) or more (multiple-embedded) nucleocapsids per envelope. The virus of *A. orana* is a single-embedded virus. However no single-embedded viruses were found in the cross-inoculated *M. brassicae* but only a multiple-embedded one like those usually encountered in naturally polyhedrosis-diseased larvae of *M. brassicae*. In back-inoculations of this virus of *M. brassicae* to *A. orana* larvae, single-embedded viruses were detected in the diseased larvae. These observations were reason for a more thorough study on the relations of the nuclear polyhedrosis viruses occurring in *M. brassicae* and *A. orana*. The results of Ponsen & De Jong could be interpreted either as activation of latent infections occurring in both species or as one virus differently enveloped by the two hosts.

Further studies (Jurkovičová, 1979) confirmed that the two viruses were singly and multiply embedded viruses. The polyhedra of these viruses differed in size and shape. Most of the polyhedra in *A. orana* were globular and ranged in diameter from 1 to 2 µm. Those in *M. brassicae* have a hexagonal or pentagonal outline and ranged in diameter from 1.5 to 4 µm. On comparison of the polyhedral and viral proteins by polyacrylamide gel electrophoresis, no characteristic differences were detected. These comparative studies did not reveal any distinction other than the way the nucleocapsids were enveloped. To learn more about the viruses used and produced in the cross-inoculation experiments the attention was now directed towards the nucleic acids of these viruses.

The molecular weight of the circular double-stranded nucleic acids (DNA) of the viruses from *A. orana* and *M. brassicae* was estimated to be 67×10^6 and 89×10^6 from length measurements on electron micrographs and from reassociation kinetics. By the latter technique, no homology was found between the DNA of these viruses when assayed by competition hybridization. These DNA molecules were also differentiated by analysis of the fragments obtained by digestion of these molecules with restriction endonuclease Eco RI. This enzyme gives a fragmentation pattern specific for each DNA. No fragments with the same size were detected in the DNA digests of the two viruses. The DNA of the two viruses differed also in their buoyant density in CsCl solution, indicative of differences in base composition. The content of guanine plus cytosine calculated from buoyant density of the DNA of the two viruses was consistent with that derived from thermal denaturation. All these results indicate that the two viruses are different entities that can easily be distinguished by the properties of their genomes.

In particular, analysis of the DNA fragments from digestion with restriction enzymes gives irrefutable answers. This technique was used to analyse the virus from cross-inoculation experiments. Larvae of *A. orana* and *M. brassicae* were each inoculated with virus from the other species. The number of larvae containing polyhedra was significantly higher for the cross-inoculated group than for the uninoculated group. DNA was isolated from the polyhedra of cross-inoculated larvae and compared with DNA from virus used as inoculum. The DNA fragmentation pattern of the virus isolated from cross-inoculated larvae was identical with that of the virus naturally occurring in that host. So the virus of *A. orana* and *M. brassicae* are not cross-infective.

Latent infections were demonstrated by the presence of viral DNA in seemingly healthy larvae. DNA was extracted from larvae grown from surface-sterilized eggs and was analysed by reassociation with viral DNA. About 2.5 viral DNA copies were detected per diploid host genome of *M. brassicae*. The amount of viral DNA in *A. orana* was much less. Every copy of host genome contained 0.04 copy of viral DNA.

The mortality rate of larvae was much lower in these experiments than found by Ponsen & De Jong, who noticed that 80 to 90% of the population died after cross-inoculation. This difference in mortality would be due to the more sterile conditions we used in our laboratory for the rearing of the insects.

To explain latent infections, the virus has to be transmitted to the progeny. This transmission from generation to generation may take place in two ways. The virus may be transmitted at the surface of the egg and within the egg itself. Polyhedra were detected in larvae grown from surface-sterilized eggs as well as from untreated eggs. More larvae with polyhedra were found in the second group, indicating that both modes of transmission occur.

As shown, the viruses of *A. orana* and *M. brassicae* are different types that cannot infect the other host. Symptoms observed after cross-inoculation are probably due to activation of latent infections, which presumably occur more in field than in populations maintained for a long time in the laboratory. Uncontrolled outbreaks of polyhedrosis in field and laboratory populations can be explained by the spontaneous activation of latent infections.

BIOLOGICAL CONTROL WITH VIRUSES

Recently a small program was initiated to study the effects of two baculoviruses in the control of two moths in Dutch orchards. Experiments were with the granulosis virus of the codling moth, *Laspeyresia pomonella* in 1977 and 1978, and with the nuclear polyhedrosis virus of the summer fruit tortrix moth *Adoxophyes orana* in 1978. In the summer of 1977, 2 and 3 sprays of *L. pomonella* virus were applied in different plots at doses of about 5.10^{10} granules per tree. Both schedules of spraying reduced damage by the codling moth by about 75%, with most of the apples having a stopped entry. Insecticide treatments gave a reduction by 95%. In the control plots, 19% of the apples were damaged either by superficial or deep bores. Better results were obtained in the summer of 1978. The number of moths was low, probably because of the cool season. Only 2.5-3.8% of the apples in the control plots were affected by the codling moth and 7% by the fruit tortrix.

Attemps were made in 1978 to estimate the population of the codling moth and the fruit tortrix by sampling diapausing larvae. The few specimens found and their distribution did not allow any conclusion. The activity of the two viruses was checked by biological assays during both seasons. In 1977 the virus from *L. pomonella* was completely inactivated in about two weeks. In 1978, the inactivation was much slower, both viruses showing a drop in activity by half over three weeks. This difference in rate of inactivation was probably due to the difference in duration of sunshine in 1977 and 1978.

The results encourage continuance of the studies. The effects of the mixture of viruses on the codling moth will receive attention.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

9.3 Restriction endonucleases: a new tool in baculovirus identification

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INTRODUCTION

Nuclear polyhedrosis viruses (NPV) and granulosis viruses form a group of viruses (baculoviruses) that are pathogenic for invertebrates, mainly insects. These viruses are natural enemies of insects and they can reduce the population density of insects by epizootics. Among insects, host ranges of baculoviruses are often limited in orders, families or even species. The high specificity and natural occurrence make these viruses suitable for control of insect pests without creating heavy burden on the environment. The baculoviruses have been promoted by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) as good alternatives to chemical pesticides in biological pest control (WHO/FAO, 1973).

AIM OF INVESTIGATIONS

In using these baculoviruses in nature, it is a prerequisite to establish unequivocally the identity of the virus before and after field use. For example, in studies to determine host ranges, one must show whether the virus used had caused virosis in a target host or had provoked a hitherto latent infection giving rise to disease. Several techniques are available for virus identification, but they all are in themselves unsatisfactory for unequivocal identification. It is difficult to identify the virus on the basis of its host range, since different viruses can have similar host ranges. Moreover, the biological assays are particularly laborious and time-consuming. Serological techniques give only indications as to the nature of the virus, because many viruses react positively with heterologous antisera. However serology is now one of the most rapid and sensitive techniques for virus identification. Analysis of viral proteins on polyacrylamide gels is emerging as a tool for identification but, because of the presence of proteolytic activity in viral preparations, is less attractive. Therefore, an urgent need remains for a reliable and sensitive method to identify baculoviruses by stable characteristics. We hope to meet these requirements by using restriction endonuclease analysis of viral nucleic acids.

TOOLS

Baculoviruses are rod-shaped and consist of enveloped nucleocapsids containing deoxyribonucleic acid (DNA) as genetic element. Many virus particles, singly or in bundles, are surrounded by a protein matrix giving a final structure of 0.3 to 15 μm diameter that is polyhedral or granular in shape. Baculoviruses contain a circular, double-stranded DNA molecule in the form of a superhelix. Recently, bacterial enzymes have been found that recognize and cleave double-stranded DNA at specific nucleotide sequences (Roberts, 1976). Each of these 'restriction endonucleases' will, therefore, generate a set of DNA fragments that is unique for a given virus. Restriction endonucleases Eco RI (from *Escherichia coli* BS5), Bam HI (from *Bacillus amyloliquefaciens* H) and Sma I (from *Serratia marcescens* S_b) have been used in this study on the identification of the nuclear polyhedrosis viruses of the alfalfa looper *Autographa californica* (AcNPV), the cabbage moth *Mamestra (Barathra) brassicae* (MbNPV) and the fall armyworm *Spodoptera frugiperda* (SfNPV). Fragments of DNA, generated after cleavage of viral DNA, were analysed by electrophoresis on agarose gels and were numbered alphabetically starting with A for the largest fragment. The molecular weight of the fragments is expressed in megadaltons (10^6 daltons).

BACULOVIRUS IDENTIFICATION

The fragmentation pattern of SfNPV DNA digested with Eco RI was unique for SfNPV, and was completely different from the Eco RI cleavage pattern of MbNPV DNA and AcNPV DNA (Fig. 1). Fragments with common electrophoretic mobilities were rarely found, suggesting that there is only limited homology between these baculoviruses. Similar results were obtained when these viral DNAs were treated with other restriction endonucleases such as Sma I and Bam HI. The DNA fragments generated by the enzymatic degradation irrefutably identify each of the three viruses. The apparent different make-up of nucleotide sequences is also reflected by the large number of biological differences between these viruses, for instance in morphology, host range and biological activity.

From the cabbage moth *M. brassicae*, two NPV isolates were obtained and compared. One isolate was found near Rhenen in the Netherlands ($MbNPV^{Nl}$), the other near Darmstadt in Germany ($MbNPV^{D}$). Their biological and biochemical properties appeared to be similar, if not identical. After analysis of their DNA with restriction endonuclease Bam HI, Eco RI and Sma I the two virus isolates were differentiated by the presence of unique DNA fragments (Fig. 2). However the two types of DNA also share many fragments with the same electrophoretic mobility in gels suggesting a large degree of sequence homology. The similarity in biological properties of the NPV is a reflexion of this homology and these two viruses should be considered as strains rather than different types of virus. Since MbNPV appears to be a factor limiting the population of *M. brassicae* in the field, the Department of Virology plans to relate the regional distribution of other isolates of MbNPV with the divergence of DNA sequence and biological properties (Vlak & Gröner, 1980).

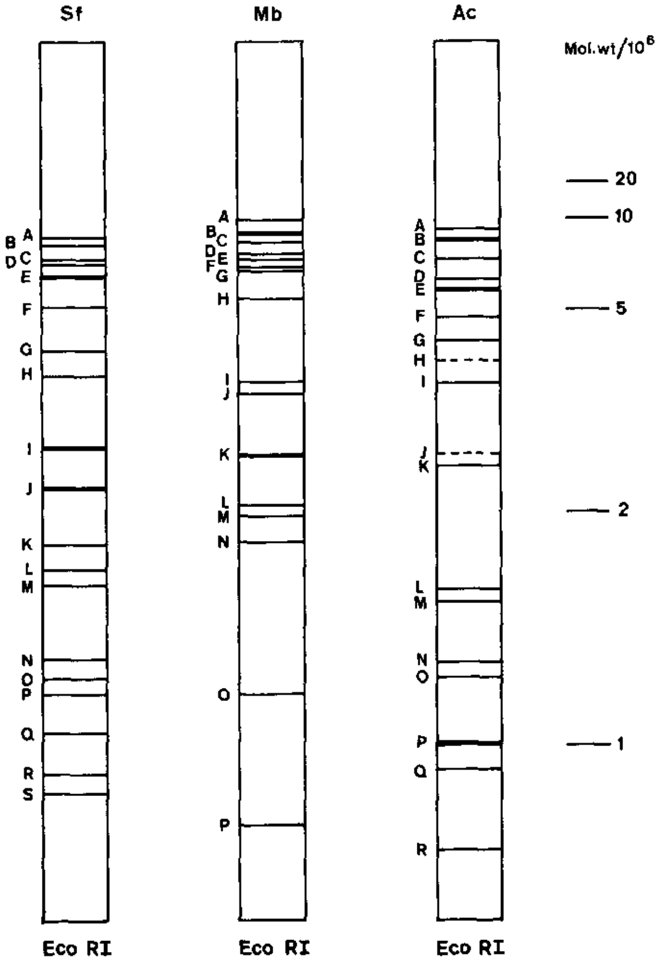

Fig. 1. Scheme of deoxyribonucleic acid (DNA) from *Spodoptera frugiperda* (Sf) nuclear polyhedrosisvirus (NPV), *Mamestra brassicae* (Mb) NPV DNA and *Autographa californica* (Ac) NPV DNA digested with endonuclease Eco RI. The DNA fragments were separated by size electrophoretically in agarose gel of concentration in water 10 g/l and were stained with ethidium bromide.

FURTHER CHARACTERIZATION OF BACULOVIRUS DNA

DNA of the NPV from the alfalfa looper *A. californica*, a lepidopteran endogenous in America, was studied in more detail. This AcNPV can be multiplied in established insect cell lines of several species, and its biology is the best studied of all baculoviruses. The DNA was isolated from extracellular virions as covalently closed, double-stranded molecules. The substance (mole) fraction of guanine plus cytosine in AcNPV DNA was estimated from its buoyant density in CsCl and its melting point to be about 0.427. From velocity sedimentation in alkaline and neutral sucrose gradients, a molecular weight of about 80 x 10^6 was calculated for AcNPV DNA (Vlak & Odink, 1979).

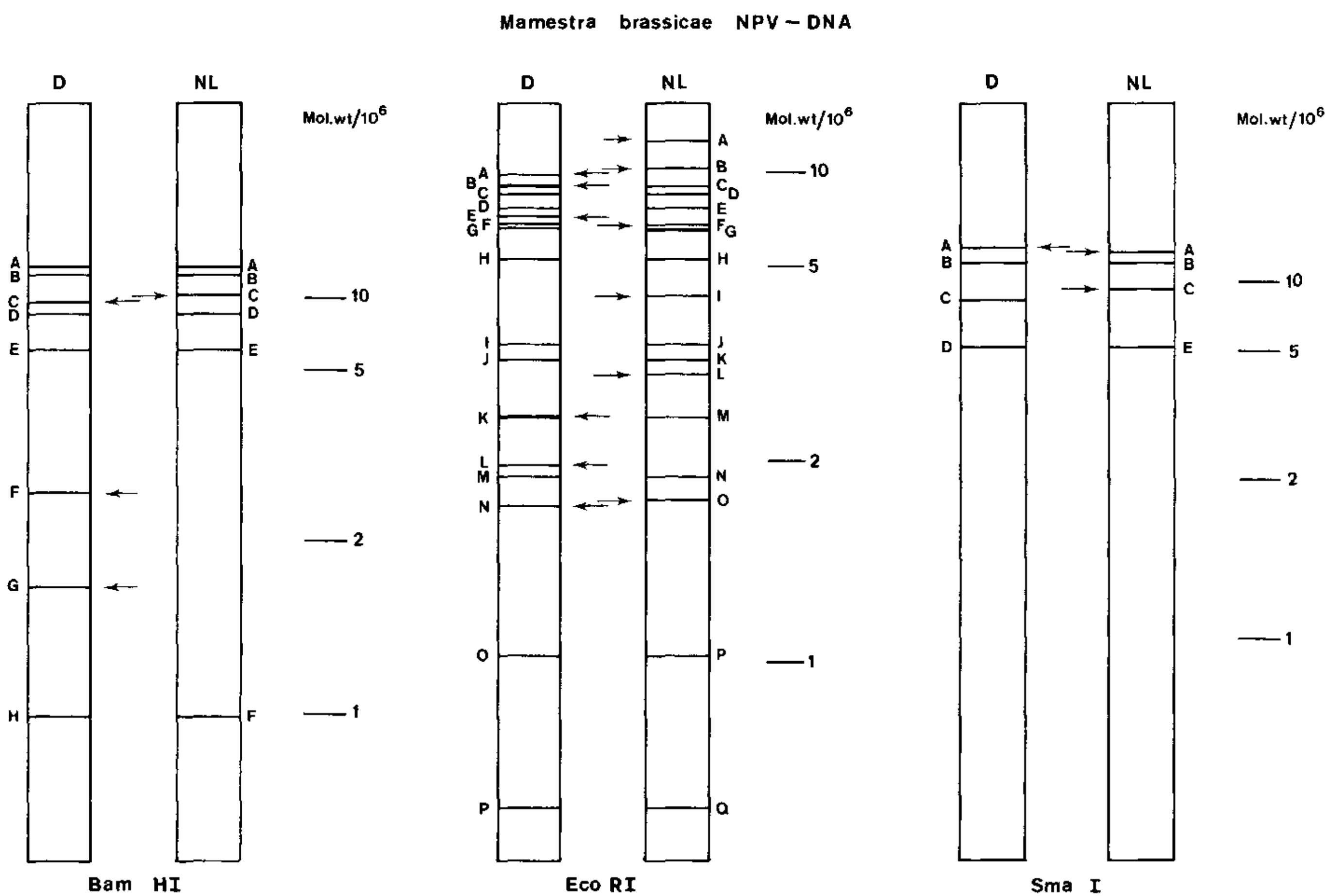

Fig. 2. Scheme of deoxyribonucleic acid (DNA) from *Mamestra brassicae* nuclear polyhedrosis virus (NPV) of the Dutch (NL) and German (D) strain, digested with endonucleases Bam HI, Eco RI and Sma I. The DNA fragments were separated by size electrophoretically in agarose gel of concentration 8 g/l and were stained with ethidium bromide. The arrows indicate the position of DNA fragments unique for each MbNPV.

After digestion of AcNPV DNA with Bam HI, seven fragments were generated and separated by electrophoresis (Fig. 3). The molecular weight of the fragments ranged from 55 x 10^6 for Bam HI-A to 0.69 x 10^6 daltons for Bam HI-G. AcNPV DNA was cleaved by Sma I into four fragments ranging in size from 47 x 10^6 for fragment Sma I-A to 9 x 10^6 for Sma I-D. Twenty-one fragments were generated by digestion of AcNPV-DNA with Eco RI, with molecular weights ranging from 9.3 x 10^6 for Eco RI-A to 0.65 x 10^6 for Eco RI-R (Fig. 3). Most fragments were present in equimolar amounts. In some cases, however, two (Eco RI-B, Eco RI-K and Eco RI-P) or three (Eco RI-E) fragments of similar size, but with different nucleotide sequences were detected (indicated by thicker lines). By summation of the molecular weights of the fragments, generated by each of these restriction endonucleases, a molecular weight of about 82 x 10^6 was deduced for AcNPV DNA. At present, I am constructing a map with the physical order of the Bam HI, Eco RI and Sma I fragments.

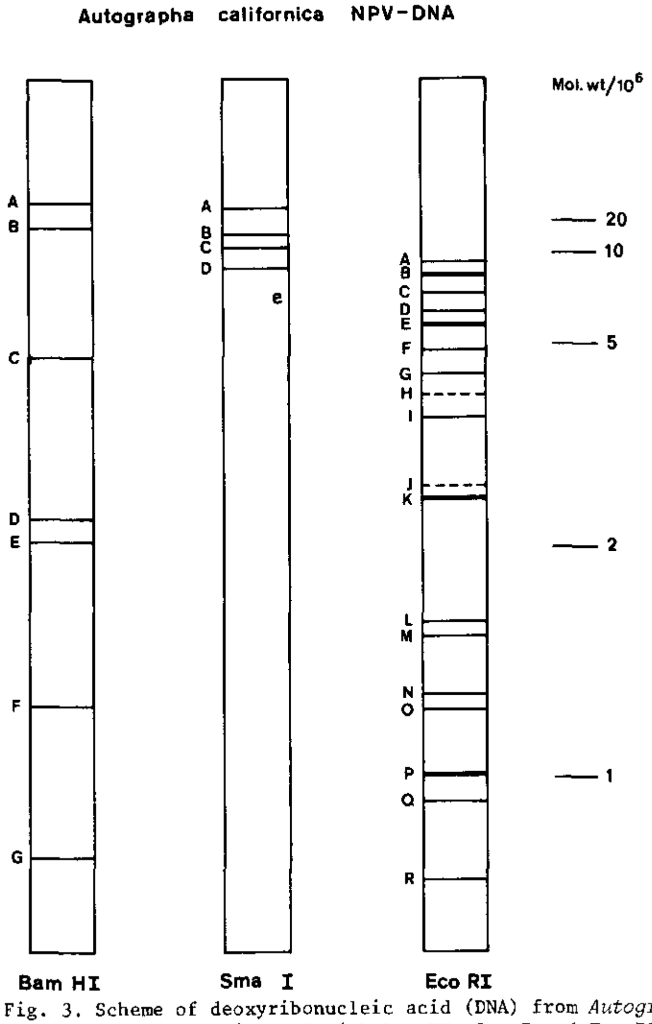

Fig. 3. Scheme of deoxyribonucleic acid (DNA) from *Autographa californica* nuclear polyhedrosis virus (NPV) digested with Bam HI, Sma I and Eco RI. The DNA fragments were separated by size electrophoretically in agarose gel of concentration 10 g/l and were stained with ethidium bromide.

Analysis of AcNPV DNA with Eco RI (Fig. 3) also revealed the presence of two minor fragments (Eco RI-H and Eco RI-J, dotted lines). Viral DNA of clones of AcNPV that were selected by plaque assays on insect cells, lacked these two minor fragments. This result may indicate that the original isolate of AcNPV could be considered as a population of different but related viruses. This heterogeneity has not yet been correlated with biological properties. If heterogeneity is a more general phenomenon, it may play a role in the maintenance of these baculoviruses in nature and their adaptation to non-target insects. This should be a matter of concern when uncloned virus is applied to control insect pests.

CONCLUDING REMARKS

Restriction endonuclease analysis of DNA is a useful procedure to discriminate between nuclear polyhedrosis viruses and probably also granulosis viruses occurring in nature, and adds a new technique for identifying baculoviruses. The sensitivity of the method based on recognition of nucleotide sequences is high and meets the requirements for an unequivocal identification of baculoviruses. Even subtle differences between strains can be detected. Genetic changes in the virus when exposed to nature can now be accurately monitored with this enzymic analysis of DNA. Other restriction endonucleases than those mentioned may be useful too in baculovirus identification. Studies on the molecular biology of baculoviruses as presented here may contribute to the safer use of these viruses in control of insect pests.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

9.4 Control of aphids with insect pathogens

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DESCRIPTION OF THE PROBLEM

Aphids are serious pests in a variety of agricultural crops, either by their mere presence in vast numbers or by their ability to transmit plant viruses. There is considerable economic loss in crops such as potato and sugar-beet, where *Myzus persicae* acts as the vector to several virus diseases. Even a very low population density of *M. persicae* early in the year may cause great economic loss to a sugar-beet crop by virus transmission.

Aphids have been controlled for many years with chemical insecticides. Recently the use of chemicals against aphids has increased tremendously, as control of aphids in cereals has been found to increase yields. This increased use of chemicals is undesirable for the environment, especially since cereals are grown over wide areas. Resistance to chemical insecticides have been reported from several places. Control methods other than chemical should therefore be studied more closely.

AIM AND MOTIVATION

Field studies have shown that aphid populations are sometimes infected by entomogenous fungi, especially fungi of the genus *Entomophthora*, mainly the species *E. aphidis*, *E. fresenii* and *E. thaxteriana*. Under favourable conditions, aphid populations may be wiped out over large areas. In Brittany, *Aphis fabae* populations are controlled every year naturally by *Entomophthora* species (Missonier et al., 1970).

It was always felt that the occurrence of epidemics of *Entomophthora* in aphid populations depended only upon the proper climatic conditions and that the amount of infective material present was never a limiting factor. However, it is possible that insufficient infective material is available to cause epidemics in aphid populations. Introduction of infective material, in the form of spores or as infected dead or living aphids under favourable climatic conditions may thus control the aphids.

The present research was set up in order to study whether *Entomophthora* spp. could control aphids in annual crops. Complete control of aphids is not always required: a delay in the building up of aphid populations in potatoes and sugar-beet would already be valuable.

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WORKING METHODS

Several *Entomophthora* species received from other laboratories (Institut Pasteur, Paris, France and Rothamsted Experiment Station, Harpenden, Herts, England) were available: *E. aphidis*, *E. fresenii*, *E. phalloides*, *E. thaxteriana* and *E. virulenta*. All these species except *E. fresenii* grow on artificial media.

Four phases are planned for the research.

1. Laboratory studies, to obtain information about climatic conditions favourable for *Entomophthora* infection in aphids. Experiments were started to study germination of spores at different temperatures and relative humidities. Preliminary experiments were also conducted to follow the spread of the fungi in *Aphis fabae* populations in small cages under different relative humidities at a temperature of 21 ^{o}C. Infective material was introduced in the form of dead infected aphids.
2. Mass cultivation of promising *Entomophthora* species.
3. Listing of *Entomophthora* species on insects in the Netherlands, in particular on aphids.
4. Small experiments on greenhouse and outdoor crops, on which these fungi will be used as control agents for aphids.

Phases 1 and 2 have started.

RESULTS AND DISCUSSION

Spore germination was studied in more detail for *E. virulenta*. At 21 ^{o}C and 100% relative humidity, germination started after 3-4 h but a reasonable level (40-50%) of germination was only reached after 20 h. Germination at lower relative humidities, even at 98%, was considerably less. For the other species of fungi, no detailed data are yet available.

For the cage experiments, dead aphids infected with *E. aphidis* were used as infective material. Even at high humidities, only a few aphids became infected in this way. Perhaps the spores were not sufficiently spread throughout the cage in this manner. Spraying of the plants in the cages with a suspension of spores may give a much higher infection of the aphids. Such tests are planned.

In 1978, only a few infected aphids were observed in the Netherlands. Most of these aphids collected appeared to have been killed by *E. aphidis*.

So a high relative humidity is needed for satisfactory germination of spores. We should, however, interpret these data carefully: to obtain an epidemic in an aphid population, a high proportion of germinated spores may not be needed. Only a few germinated spores is needed to infect and kill an aphid. By treating a crop with a high dosage of spores, sufficient germinating spores may be obtained even under conditions that are suboptimal for the fungus.

FUTURE OUTLOOK

When enough data about climatic conditions favourable for *Entomophthora* infections are obtained, attention will be paid to the mass rearing of the fungi in order to start field application. Most of the fungi under study can be cultured on artificial media: the growth, however, is slow for most species; only *E. virulenta* grows easily. This characteristic makes this species seem more suitable as a biological insecticide. Which fungus will be chosen for the field work is, of course, dependent also on other characteristics of the species.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

9.5 Control of insects with nematodes

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Friesland College of Agriculture, Leeuwarden, the Netherlands.

INTRODUCTION

Nematodes associated with insects have been known since the Eighteenth Century and at present over three thousand relationships have been described (Poinar, 1975). Of these, a fair number can be fatal to the insect host; others may cause sterility; often no apparent harm is done to the insect. About the natural regulatory role of parasitic nematodes in insect populations, knowledge is still limited. Parasitism has been shown to range from less than 1% to 100% for specific groups like mermithids. Often the occurrence of a high level of parasitism has led to speculation whether the nematode concerned may play a major role. In their review of this subject, Gordon & Webster (1974) say "Biological control of insect pests and disease vectors by nematodes is one of the more promising alternatives to over-reliance upon persistant chemical insecticides".

Successful insect control has been achieved with *Romanomermis culicivorax* against various mosquito larvae and with *Deladenus siricidicola* against the wood-wasp, *Sirex noctilio* in Australia. *Neoaplectana* species have been used in many experiments with varying success. In the Netherlands, studies on the use of entomophagous nematodes started in 1975 on a limited scale.

METHODS

Staring from insect problems, two approaches can be considered.

1. Searching for parasitic nematodes in insect populations in either outbreak areas or in natural habitats. When found, investigating their usefulness as control agents. This means studying their biology, testing their usefulness for practical control, including ecological studies, and establishing a procedure for mass culture.
2. Trying to use known aggressive (or less specific) parasitic nematodes, for instance members of the Steinernematidae (Rhabditida), that have proved promising elsewhere.

An ideal working method would include both, but limited resources have directed our choice to Approach 2 in the first instance and *Neoaplectana carpocapsae* is being tested. This nematode may be regarded as a vector of its own deadly symbiotic bacterium.

Recently the search for indigenous parasites has been included in the program on a limited scale.

Neoaplectana carpocapsae

At temperatures below 15 °C, the pathogenicity of *N. carpocapsae* decreases rapidly. Since Dutch soil conditions can vary considerably the nematode's suitability for use under field conditions had to be established. For that purpose, soil was sprayed with nematodes against the susceptible beetle larvae of rape, *Psylliodes chrysocephala*, in late summer and autumn.

As a rule, laboratory tests – treating larvae with nematodes in a Petri dish – preceded field or pot trials.

All experiments were conducted in moist habitats in soil and in insect tunnels in trees, and preferably on arthropods whose chemical control had proved difficult or faced objections.

Susceptible insects with promising rates of mortality in field or pot trials will be further tested for the effect of soil, nematode, insect and application factors (Simons, 1978), in order to determine whether the nematode can suppress the insect population to an economically acceptable level.

In all initial trials in soil, dosages of nematodes were standardized at 100 per square centimetre and were applied by spraying the soil surface with an aqueous nematode suspension.

Indigenous rhabditids and pest-specific nematodes

Soil samples of one litre were taken from uncultivated light soils with a natural vegetation. In the laboratory, five or six wax moth larvae, *Galleria mellonella*, were put into each soil sample (Bedding's trap) and, after one week, dead larvae were examined and put onto a White trap for further culture and studies of the rhabditid parasites found. Before undertaking morphological studies, a mutual crossbreeding schedule was carried out to determine relationships of the different nematode populations. Along with morphological and taxonomic studies, the nematodes' pathogenicity to various insect larvae will be investigated.

The search for pest-specific parasitic nematodes by collecting live and dead insects in cropping areas suffering from the insect has received little attention so far. One fortuitos discovery will be mentioned.

RESULTS AND DISCUSSION

Neoaplectana carpocapsae (as insect-control agent)

Infective stages of *N. carpocapsae* can be stored at high densities (100 million per litre) in formalin at a concentration 1 ml/l at 5 to 8 °C with continuous aeration. The number of live nematodes decreases slowly. After three months, most nematodes are still alive and in good condition. Aeration at room temperature for a few days before storage increases survival. After two years, storage losses are considerable and also virulence decreases when applied to soil.

Survival in soil at detectable densities seems to be a matter of weeks (8 to 15), depending on temperature and soil type, i.e. biological activity in the soil. Detailed studies will be necessary to determine 'residual' effects on populations of pest and other insects. *Psylliodas chrysocephala* (Coleoptera: Chrysomelidae). Numbers of young larvae in rape plants were reduced by up to 60% by September sprayings with nematodes. Spraying in November gave poor control. Similar field trials the following year yielded no positive results, because major larval invasion began in November, when soil temperatures were too low for the nematode. Further trials with this insect were considered useless, as it had become clear that *N. carpocapsea* might be useful only at soil temperatures prevailing in Dutch summers.

Agrotis segetum (Lepidoptera: Noctuidae), a universal polyphagous pest insect. Pot experiments gave up to 80% control of 3rd - 5 th instar larvae, when the nematodes were sprayed on the soil or applied in a moist bran mixture. The reduction in damage to young lettuce plants was statistically significant but economically insufficient. Further work is going on to improve control.

Otiorrhynchus sulcatus (Coleoptera: Curculionidae), a pest in crops such as strawberries and ornamentals. Resistance to chemical insecticides has become a real problem and in strawberries aldrin is the only alternative for the moment, until, its use is prohibited completely. Successful laboratory tests with *N. carpocapsae* were followed by field trials in two successive years, but the results were unpromising (See also under 'Indigenous rhabditids and pest-specific nematodes').

Hoplocampa testudinea (Hymenoptera: Tenthredinidae). Last-instar larvae of this insect drop out of young apples onto the soil to form a cocoon and overwinter at a depth of 10 to 20 cm. If nematodes are present when the larvae arrive at the soil surface, they might be able to penetrate. Pot and field trials have proved this to be possible but soil conditions may strongly influence control. This study is being continued.

Sciapteron tabaniformis (Lepidoptera: Sesiidae). Injection of nematodes into tunnels in young poplars killed all caterpillars in the trees examined (tests by L. Wouters, Lelystad). This offers good control for small-scale application, as in field trials or in tree nurseries, but is otherwise far too laborious. Simply spraying the surface of the affected areas on a tree has no effect, since the nematodes cannot penetrate the mass of bore-meal in the tunnels.

Synanthedon myopaeformis (Lepidoptera: Sesiidae). Sprays on apple trunks allowed nematodes to penetrate the rather superficial tunnels and kill the larvae. Tests are being continued.

Scolytus multistriatus (Coleoptera: Ipidae), a vector of Dutch elm disease. Even under favourable laboratory conditions, the nematodes could not penetrate the bore-meal in the tunnels underneath the bark.

Delia brassicae (Diptera: Muscidae) maggots, *Atomaria linearis* (Coleoptera: Cryptophagidae) imagos, *Onychiurus armatus* (Collembola: Onychiuridae) nymphs, *Scutigerella immaculata* (Symphila: Scutigerellidae) nymphs. In all cases, both laboratory and field or pot tests have shown the lack of susceptibility of these organisms to *N. carpocapsae*. Close examination has shown that penetration of the nematodes through the small natural openings of *S. immaculata* is impossible. In the case of *O. armatus* it has been observed that the nematodes scare the springtails as soon as they come in contact.

In a pathogenicity test, young Chinese grass-carp *(Ctenopharyngodon idella)* were kept in water at about 22 °C with a maximum of 10 000 *N. carpocapsae* per litre for 18 days. No injurious effect could be detected.

Indigenous rhabditids and pest-specific nematodes

Fourteen nematode populations have been isolated from soil samples and cultures on *Galleria mellonella*. All populations consist of *Neoaplectana* species. A cross-breeding schedule is being carried out and the first results have indicated some relationships. Taxonomic studies and pathogenicity tests will follow.

Recently a few red dead larvae of *Otiorrhynchus sulcatus*, containing a *Heterorhabditis* species, were found on strawberry plants in pots. This nematode also carries a symbiotic bacterium in its intestinum. The utility for biological control of *O. sulcatus* and other insects will be investigated. A preliminary test in the laboratory and with pots have indicated a high degree of susceptibility of *O. sulcatus* larvae.

FUTURE OUTLOOK

Knowledge of the usefulness of nematodes in insect control is still scanty, but even the hitherto acquired insight will allow development of good methods. To compete with chemical insecticides, the method must be reliable. If reliability cannot be achieved with one nematode species alone, combined use with other pathogens or low dosages of non-persistent selective insecticides should be considered.

Practical control of insects cannot be achieved by one means alone. Neither will nematodes bring the ultimate solution. These long-neglected organisms can be expected to take on a useful role in the control of insects in the Netherlands.

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10 Supporting techniques

Many techniques have been used in research on integrated control, and three merited description in some detail.

Systems analysis and computer simulation, techniques holding much promise as tools in basic research and possibly in the practice of integrated pest management as well, have been successfully used as an aid for analysing prey-predator systems of mites and the epidemiology of cereal aphids (10.1, 10.2).

Detailed meteorological assessments in support of studies were indispensable (10.3).

Finally, experiences with radiobiological techniques are summarized. They were found indispensable in an array of problems encountered in physiological and ecological studies by members of the Working Party (10.4).

Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

10.1 Systems analysis and simulation as an aid to the understanding of acarine predator-prey systems

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INTRODUCTION

During the last decade systems analysis and simulation have gained increased interest in crop protection. In the Working Party on Integrated Control of Pests, these techniques were so far used mainly to analyse and understand biological control with mites.

The experience with introduction of endemic predatory mites and modified spraying schemes and products indicate that these predatory mites can reduce and maintain populations of fruit-tree red spider mite below the economic threshold. At present, predatory mites are coming into wide use in the control of spider mites in apple orchards, and a large proportion of the growers of vegetables in greenhouses are using predatory mites for control of spider mites in cucumber and sweet peppers. For sweet peppers the predatory mites can be used as an acaricide. A permanent control system is desirable for all crops but it is questionable whether it can be achieved. This study investigates the possibilities for such control in relation to prey and predatory mite species, their spatial distribution, webbing activity and behaviour. In the two crops, the changes with introduction of predatory mites have been measured, but the mode of control is still poorly understood. This understanding should be based on integration of the knowledge of the underlying ecological processes. Our study with simulation models aimed at such an integration and at closer insight in the acarine systems and their sensitivity to disturbance. It may thus pave the way for simple prescriptions on how to manage the prey-predator system in the course of time in relation to crop status and climate. Simplified models can thus lead to advice in sprayings and interventions with predatory mites or alternate prey; they may also help in planning observations during the monitoring phase.

MODELLING TECHNIQUE

The models used in this study are of the state-variable type, and contain state variables, that characterize quantitative properties of the system, such as the number of eggs and females, the amount of biomass or the content of the predator's gut, rate variables that express the rate of change of the state variables, such as development rate, oviposition rate or the predator's digestion rate, and driving variables that characterize the influence from outside, such as macrometeorological variables.

Often a hierarchical approach is used. It is based on the idea that only two levels of causal depth should be distinguished in a model. For example the rate of assimilation may be explained from knowledge of the stomatal behaviour, the physiology of cells may be explained from the underlying biochemical processes. However, a problem in using this principle is that in complicated systems the causal relations are so manifold that a relational diagram looks like a spider's web rather than a pyramid. Usually the causal connections are more numerous in some places than in others, so that it is sometimes possible with some skill and effort in modelling, to distinguish regions with relatively many relations inside and only a few outside. These regions are called submodels.

Often simplified and short mathematical relations based on calculations with these submodels are used in the higher-ordered models of the whole system. Models on walking behaviour of a predatory mite may, for example, constitute one of the models on subprocesses of the predation process, which in its turn is one of the elements of a model on the population dynamics of predacious and prey mites in a crop system.

POPULATION MODELS

For both prey and predator, population models are based on knowledge of the effect of humidity, temperature, daylength and food quality. Relations between rates of development, mortality, oviposition, ageing, diapausing on one hand and temperature or other physical factors on the other hand are introduced based on data from the literature, on estimates and on laboratory experiments. To account for the different morphological stages, age classes are distinguished. The residence time in each age-class depends on the length of that development stage of the animals. Dispersion in time during development and ageing is also taken into account. For example eggs of one batch laid at the same moment hatch at different times. To do so, a special 'boxcar' routine is developed with which development through different stages is simulated. This subroutine mimics the dispersion in time during development and ageing and adapts the mimicked dispersion to external conditions. Basically this is done by distinguishing artificial age-classes within the morphological age-classes, which are passed at different rates.

PREY-PREDATOR INTERACTIONS

The core of the population models of prey and predatory mites consists of the interactions between the two populations. These interactions include functional responses of the predator to prey density and quantitative responses of the predator to increasing or decreasing prey densities. To grasp the complicated prey-predator relation, Fransz (1974) unravelled behavioral components of the predation process in detail. He found that the predator's behaviour was governed by the satiation level of the gut and that this induces changes in prey preference and prey utilization. Fransz's studies were done with the two-spotted spider mite and the predatory mite *Metaseiulus occidentalis*. The adult female predator (the most voracious stage) shows a strong preference for the younger stages of the prey, but this preference decreases when the predators are "hungry". To measure prey preference and prey utilization in relation to satiation level of the predator, the predation process was analysed in detail.

A time series of the predation process with all its elements was made. A searching predator encounters a prey and this may result in a killing. What happens depends on the satiation level of the predator. In hungry predators, the success ratio (number of successful encounters divided by the total number of encounters) is high and in satiated predators low. The handling or killing of the predator takes some time (handling time), after which the predator may start feeding. The length of the feeding period depends again on the satiation level of the predator since hungry predators may stay long with their prey, even after the prey's body content is completely consumed, whereas satiated predators may leave their prey before they are completely consumed.

SATIATION LEVEL OF THE PREDATOR

Apparently the satiation level (gut content) is one of the most important state variables in the system. Several ways to measure the satiation level are possible. For fruit-tree red spider mite and the predatory mite *Amblyseius potentillae*, gut content can be scored visually because well-fed predators are dark and reddish, while hungry predators are whitish and transparent. A colour scale was therefore developed that related the amount of leaf and animal pigments in the predator, which together constitute the colour, to the relative predation rate and prey utilization. These relations were introduced into the population models. Oviposition rate and development rate of the predator (quantitative response) also depended on the satiation level and temperature and were similarly introduced into the population model. If a visual characterization of the satiation level is impossible, the weight of the predators may be used as a state variable that governs predatory behaviour.

STOCHASTIC CHARACTER OF THE PREDATION PROCESS

Two events in the predation process show a clear stochastic character. These are the encountering and abandoning of the prey. Fransz (1974) showed that because of the curvilinear relationships of the encountering rate and abandoning rate with the predator's satiation level, stochastic models have to be used. With linear relationships, the differences between deterministic and stochastic models were absent. Since the relations mentioned were non-linear, a stochastic model was necessary. This tedious affair required much computer time and so the development of other techniques was necessary. Especially for population models of prey-predator interactions, simplified models are urgently needed.

Fransz (1974) therefore introduced a new simulation method called compound simulation. This was basically a deterministic simulation model for classes of individuals with a certain satiation level, which are iteratively reclassed in each time interval. When the number of classes is properly chosen, the results of this model scarcely deviate from the stochastical model and the computer time is considerably less.

Another way to account for the stochastic character of some elements of the predation process is to apply queuing theory, as proposed by Taylor (1976). In this approach, the predator is considered to be a service facility, for example a dentist, and the prey to be a client. This client may enter the waiting room (gut) at a certain rate in expectance of the service (digestion). To evaluate the mentioned predation models, i.e. deterministic, stochastic, compound and queuing, their outcomes were compared with the results of experiments on the predation rate of *M. occidentalis* on eggs of two-spotted spider mite. The deterministic model gives erroneous results and the outcomes of the three other models fall within the confidence intervals of the measurements.

The model calculations further showed that if the number of two-spotted spider mites was kept constant with one standardized[1] *M. occidentalis* predator on a leaf disc, an equilibrium was reached within a few hours. In that situation, the degree of satiation of the predatory mite oscillates with a small amplitude at a level that depends mainly on predator and prey density and on the temperature of the system. In this steady state, unique relations exist between the predation rate and the density of prey and predator, so that simplified relations may be introduced in the population models. In the situation of the fruit-tree red spider mite the relative predation rate (predation rate divided by prey density in the steady state), and prey utilization expressing the degree of consumption of a prey are introduced as functions of the satiation level. Temperature affects both these relations and evidently also the rate of digestion and thus the decrease in satiation level.

MICROWEATHER

Since the mites operate in the small laminar layer around the leaf, they are exposed to the microweather there. To evaluate the effect of microweather on the biological parameters, a microweather simulator was coupled to the population models. For the fruit-tree red spider mite — predatory mite system, the result of this combined model calculation does not differ from the population model calculations in which air temperature was used as a driving force. Thus the wide variety of leaf temperatures is such that the few places with temperatures much higher than the air temperature must be cancelled out by a high number of leaves with temperatures slightly lower than the air temperature.

VALIDATION OF THE POPULATION MODELS

Evaluation procedures should be performed by comparing model output with the results of independent experiments on the population level. The simplest evaluation of the population models of prey and predator mites is by measurement of the population growth of prey and predator in the course of time in small ecosystems under controlled conditions in situations with and without predators. The results for the fruit-tree red spider mite are shown in Figure 1 and are reached with a model in which the difference in size of the system is introduced by simple extrapolation of the surface on which the predation process experiments are done (5.6 cm^2) to the surface of the ecosystem. Although the results of this evaluation were reasonable, it was questionable whether such a simple extrapolation is permissible, since in many cases errors due to the heterogenous distribution of the prey and the specific walking pattern of the predator may counterbalance but also cause considerable deviations from the simulated pattern. For the two-spotted spider mite, this question was examined. In

1. The predatory mites used in the predation experiments were reared under constant conditions and are of a well defined age, gut content and food history.

that system, prey are distributed in clusters and interfere with the mobility of the predator by the production of a dense web. Within these webbed colonies, very high densities of prey, 20-100 cm^{-2}, are reached. Outside and inside the colony, behaviour is quite different. Outside the colonies, the predators walk in fixed patterns, guided by leave fringes and veins, inside the colonies their walking pattern is more random. Not all morphological stages of prey and predator participate in the dispersal process. In the two-spotted spider mite, dispersal is mainly by the adult females before oviposition whereas in the predatory mites both adult females and males may be active in dispersal during their whole imaginal life, depending on food conditions. Therefore to evaluate the population models in these situations, a provision was introduced to account for the dispersal. The dispersal submodels of the predator and prey were developed from detailed studies of walking behaviour of both. Dispersal by wind was not considered for these situations, since the wind speed is too low to induce take off of the predatory (Johnson & Croft, 1977) and prey mites under greenhouse conditions. Basically these models were so constructed that population models were applied to different spatial unities coupled by different dispersal rates. These simulation models included the clear preference of the predatory mites *M. occidentalis* and *P. persimilis* for the webbed areas. Their effectiveness in searching behaviour outside the colonies guided by leave fringes and leave veins make their residence time outside the colonies negligible. This does not hold for other predatory mite species such as *Amblyseius potentillae* that cannot move around in the webbed areas and shows a clear preference for the thickest parts of the ribs, as a resting place.

Model calculations with population models in which these aspects of dispersion were considered showed that, in systems of limited size, regulation of the population of prey was possible, as in the experiments shown in Figure 2.

EVALUATION FOR THE FIELD SITUATION

To evaluate the simulation models under field conditions, the simulated fluctuations in population of fruit-tree red spider mite and the predatory mites was compared with the averaged results of some apple orchards. Figure 3 gives the observed average densities and the simulated densities for adult females of the prey and predator. The simulated functions are based on an initial number per 100 leaves of 4 prey females that have just matured, 1 predator female on 1 June, the observed surface of the leaves and the weather data of 1974 in the orchards. There was a good overall agreement between the simulated and measured results, especially for the maximum numbers of prey and predator, the time lag between these maxima and the density of prey and predator with respect to each other. Also the observed and simulated colour values (the indicator of satiation level) of the adult predator were in good agreement.

From the simulations, the number of generations can easily be deduced and, by changing parameters and structural elements in the model, their contribution to the behaviour of the system may be determined. None of the changes had a major effect on the behaviour of the acarine system in orchards. A wide range of prey-predator ratios may be tolerated in spring without leading to too high prey densities in the summer. The predation activity of the younger stages and the adult males of the predatory mite was relatively insignificant and

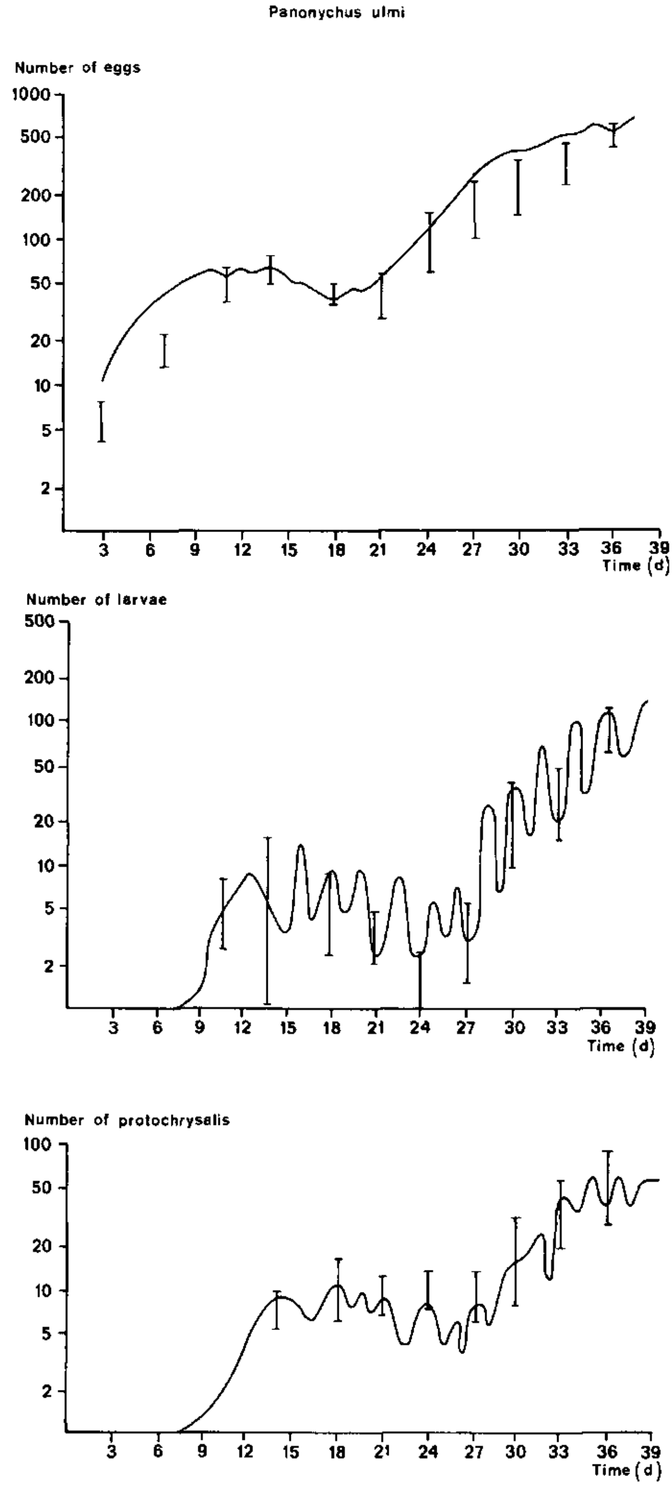

Fig. 1. Simulation of population dynamics of fruit-tree red spider mite and the predatory mite *Amblyseius potentillae* in a small apple ecosystem in comparison with the experimental outcomes (Rabbinge, 1976). Simulated numbers of the spider mite are given as drawn lines and measured figures as confidence intervals (α=0.05) (p. 286). Simulated numbers of the spider mite are given as dotted lines and measured figures as confidence intervals (α=0.05) (p. 287).

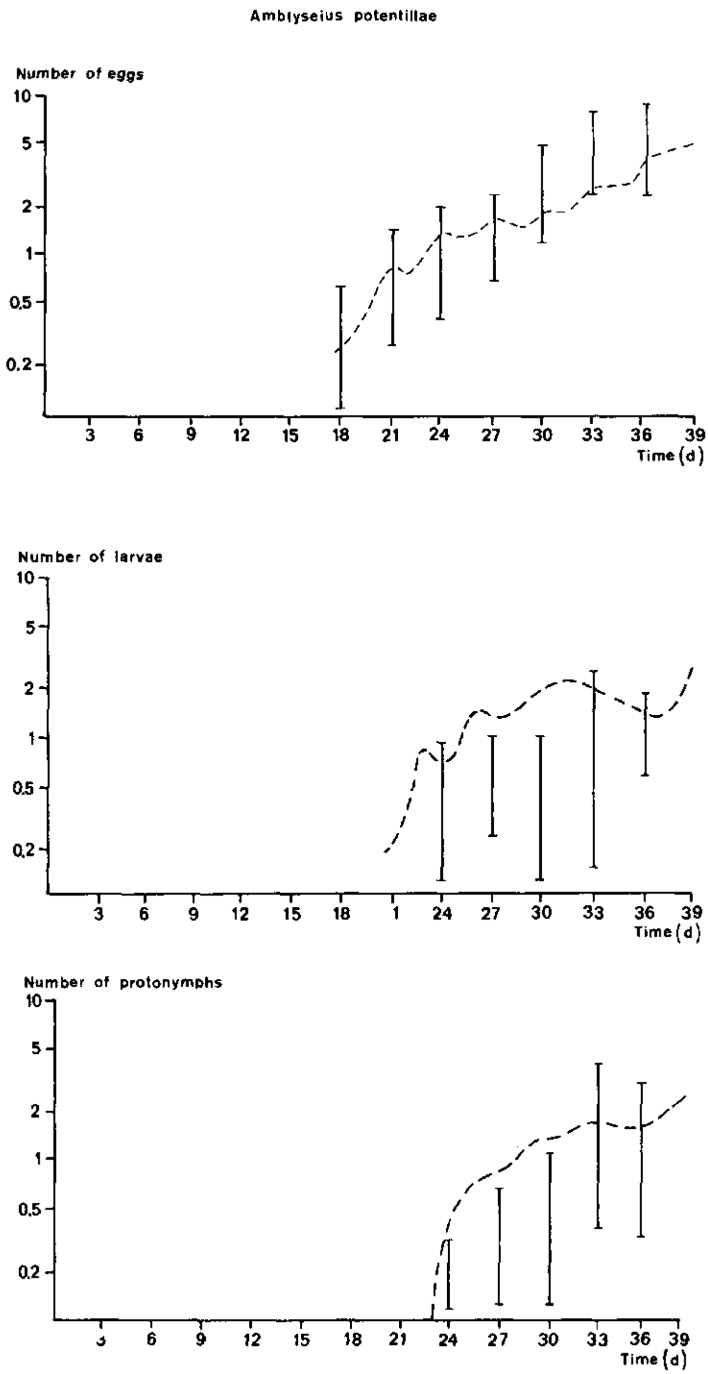

Fig. 1 continued.

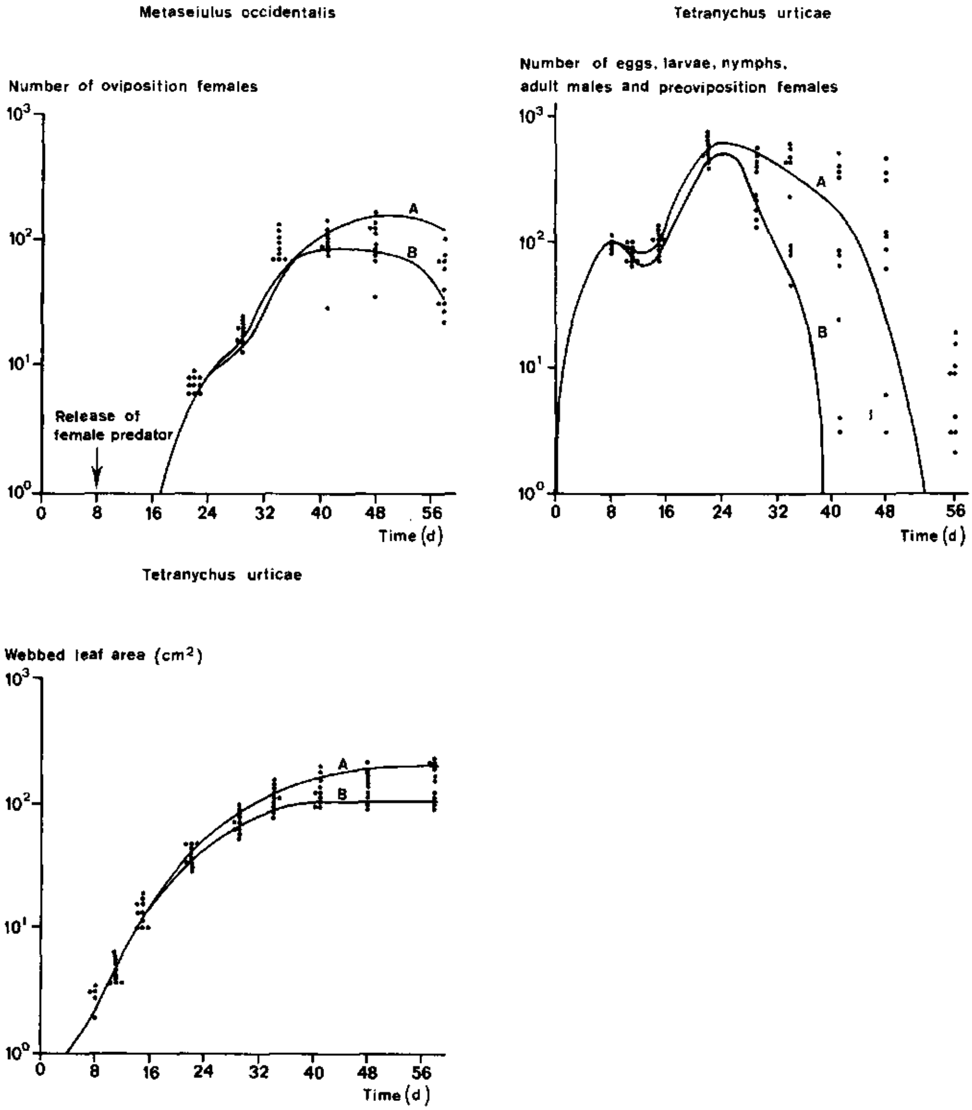

Fig. 2. Simulation of the population dynamics of two-spotted spider mite *Metaseiulus occidentalis* in roses in 8 leaf ecosystems (Sabelis, 1980).
a A twig with 8 spider mite colonies with effects of mutual interference on predation.
b A twig considered as one colony, without mutual interference effects and neglecting dispersal.

the adult female predatory mite was the major regulator because of its high predation capacity, its long life and its numerical response to increasing prey densities. The system was rather sensitive to length of the juvenile period of the prey and the delay in development of the predator if it was not sufficiently fed. The latter effects may be prevented if alternate prey like gall midges (Eryiophidae) or mildew spores and honeydew are present.

For the acarine system in roses, dispersal of the two-spotted spider mites and the predatory mites in the crop was of major importance. The simulations showed that dispersal of

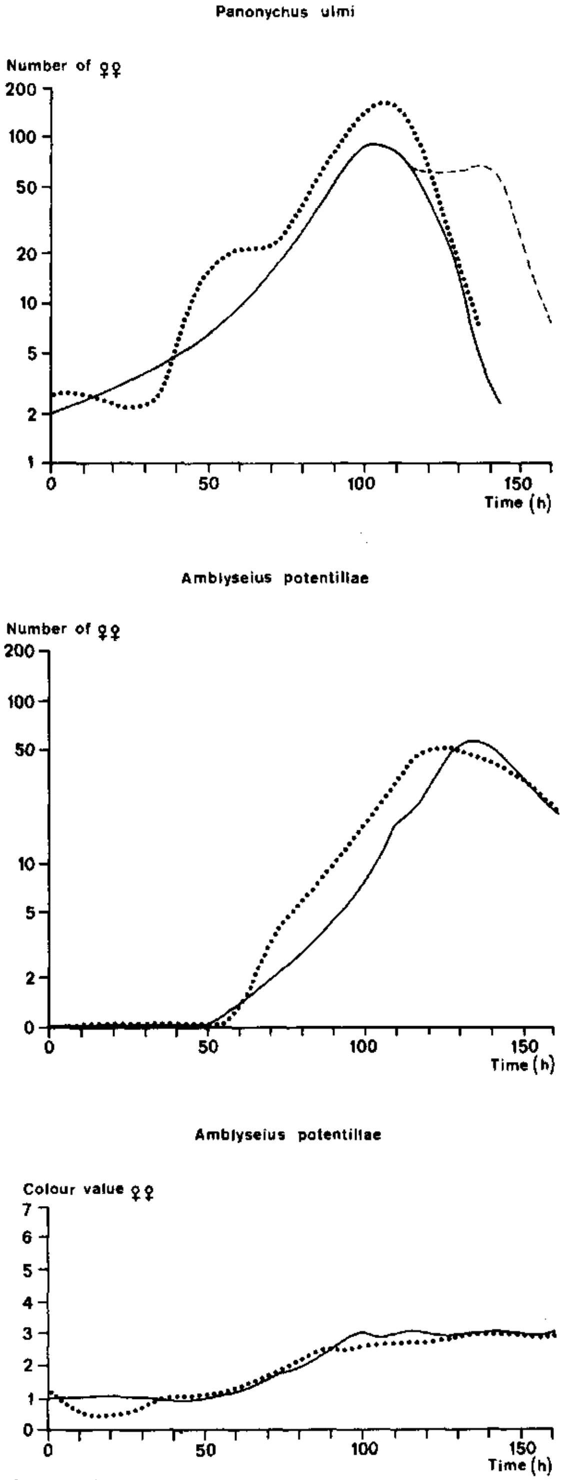

Fig.3. Simulated (dotted lines) and experimental results (drawn lines) of the population dynamics of fruit-tree red spider mite and the predatory mite *A. potentillae* in apple orchards (Rabbinge, 1976). Data are number of mites per 100 leaves.

the predators caused a considerable delay in the predator-prey interactions. This delay was partly due to the predatory mite frequently entering and leaving the colonies at low prey densities and also perhaps to the relatively high predator densities. As a consequence of this behaviour the variability in prey numbers increases considerably (Fig. 2).

MANAGEMENT AND PROSPECTS

For management, the models are too complicated and too time-consuming. Therefore other simplified relations are needed. This has been met for the fruit-tree red spider mite system by sensitivity analysis. Thus prescriptions were developed which show acceptable prey-predator ratios. The acceptable ratio of prey females to predator female was 10 on 25 May, and decreased to 3 on 15 August. Prescriptions of this type may be used by the extension services and the monitoring growers.

The model calculations with the two-spotted spider mite system showed that dispersal behaviour of the prey and predatory mites had a great effect on the behaviour of the system. So far only the dispersal of walking prey and predatory mites has been considered and it seems necessary to investigate the dispersal of prey and predatory mites by wind. Some knowledge of these effects is available (Johnson & Croft, 1977), but this information is still insufficient to gain more understanding of the dispersal of mites. In roses in greenhouses, dispersal by wind seems negligible since wind velocities are never high enough for departure. Other aspects that need more research are the possibilities for other food resources for the predatory mites to survive periods of food scarcity. The models showed that these alternate food sources were necessary in the orchard system since prey densities may reach very low levels. This is even more needed for clustered prey, for instance two-spotted spider mites in roses. The models were a help in formulating the conditions that had to be fulfilled to reach a reliable control system.

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Integrated control of insect pests in the Netherlands. Pudoc, Wageningen, 1980.

10.2 Epidemiology of the cereal aphid, Sitobion avenae

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In the past decennium, cereal aphids became a serious problem to wheat growing in the Netherlands. Three species are often numerous, one of them, *Sitobion avenae*, was considered the most serious pest and was studied in more detail. This species can rapidly increase in the few weeks between the onset of flowering and the milky-ripe stage. It feeds mainly on the ear. It damages not only by sucking nutrients from the plant but it also produces large amounts of honey dew that disturb the physiology of the plant and stimulate the growth of saprophytic and pathogenic fungi.

The explosive character of population growth makes forecasting difficult. Therefore growers tend to spray their fields preventively with pirimicarb or organopohsphorus compounds early in the flowering period. In view of the vast area under wheat, this means a considerable increase in the use of environmentally hazardous toxicants.

The aim of this study was to develop simulation models that can help to explain population development and that can be used for forecasting over periods of 3-5 weeks (Rabbinge et al., 1979). Possibly these models can pave the way for development of simpler rules for prognosis and of a reliable crop protection system. Moreover they may help to explain population development and guide research to the more significant relationships between aphids, their host plants and the natural enemies. The study is based on analysis of physiological and ecological phenomena at the individual level. An existing model describing the growth of wheat is simplified and coupled to the aphid population growth model to calculate the effect of the aphids on the plant making use of detailed studies on the aphid - host plant relations (Vereijken, 1979).

MODEL OUTPUT

Figure 1 shows output from the population model in comparison with field observations in 1976. The correspondence seems reasonable. The period of rapid growth as well as the peak are well described as confirmed by simulations of the epidemics of 1975 and 1977. Somewhat less correspondence exists for the collapse of the population. At that phase, mortality by predation and *Entomophthora* spp. seems important as well as emigration of alates. The action of *Entomophthora* spp. is not incorporated in the model because of lack of experimental data.

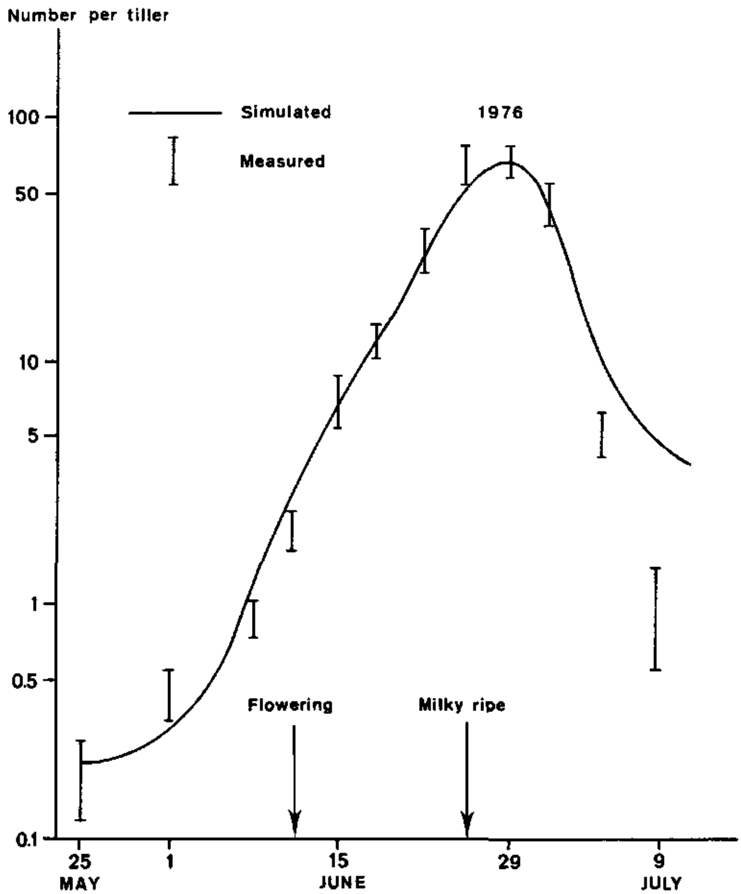

Fig. 1. Simulated and counted total number of *S. avenae* in 1976. The control numbers are given in terms of 95% confidence intervals.

AGE-DISTRIBUTION

From field observations, we may deduce that alates immigrate until around flowering of wheat. Afterwards, the number of immigrated alates on the plants no longer increases. As the offspring of these alates consists of apterae, these constitute the main driving force of population growth. Their number largely explains future growth. The offspring of apterae are mainly alates that are assumed to emigrate. Simulation with the above assumptions on immigration and emigration incorporated in the model gives a reasonable correspondence between simulated and observed age distribution as expressed by the L_4 instar (Fig. 2). The model includes parameters for development and reproduction from literature data and laboratory studies. The correspondence indicates that our laboratory data on development and reproduction suffice to understand the population growth in the field.

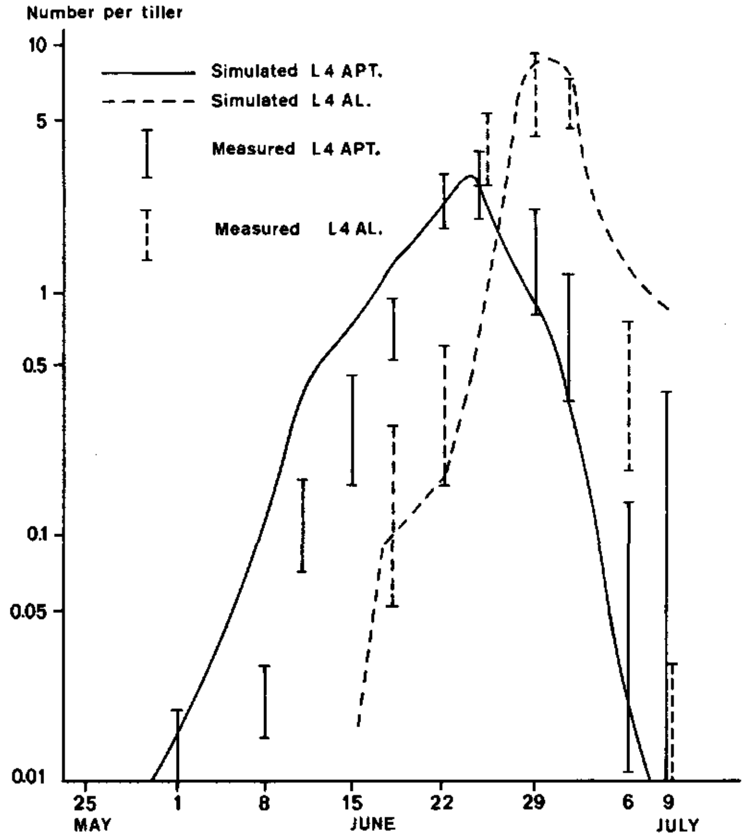

Fig. 2. Simulated and counted number of alate and apterous L_4. The counted numbers are given in terms of 95% confidence intervals (Figure from Neth. J. Pl. Path.).

SENSITIVITY ANALYSIS

The modelling effort may help to pin-point black spots in our knowledge and to evaluate the relative significance for the population dynamics of different phenomena. To determine the contribution of different processes, sensitivity was analysed. The computer model was run with some of the relations changed or with subprocesses omitted and replaced by simple algorithms. This sensitivity analysis gives the following results.

Immigration

Ignoring immigration in the simulation after the end of May had little effect on ultimate growth in 1976 but a distinct one in 1975 and 1977. In 1976 immigration before the end of May determined later population level.

Emigration

The offspring of alates are generally apterous like those of non-crowded apterae but the latter situation rarely occurs in the epidemic. Crowding L_1 causes immediate wing development in some specimens. Crowding of L_4 and apterous adults results in many alate offspring. The fate of these alates is vital for understanding the epidemic. Do they emigrate or do they stay in the field and reproduce? Field observations indicate that most of them emigrate but some may reproduce. No correlation was found between the number of alates swarming above the field after the middle of June and the number in the field on the plants. No increase in the number of L_1 was found in that period. Moreover the number of apterae decreased in contradiction to an important role of alates remaining in the field and reproducing, as these alates should have mainly apterous offspring.

In the model, we assumed that in this phase no immigration occurred and that alates were lost from the system. As the curves of simulation and field observations reasonably coincide, this supports our hypothesis on alate behaviour.

Predation

With emigration alone, the population collapse was not completely predicted. The calculations showed the necessity of an important mortality factor. Besides *Entomophthora* spp., predators like Syrphids were important. In the final phase of the epidemic, such factors that eliminate apterous adults are vital to the collapse. Predation on larvae is less important then, as most of these develop into emigrating alates. The role of parasites too has to be considered with this aspect in mind. When they prefer to attack only younger stages, they are unlikely to contribute much to population collapse.

The model calculations revealed that the place of the breaking point in the functional response curve for predator's predation rate on prey density was essential in describing its effect.

ABIOTIC FACTORS

In the simulation model, a decrease of temperature delayed population growth but the population finally reached a higher level, perhaps because of differences in response to temperature of growth rates of plant an aphid.

Not incorporated in the model were the parasites and diseases. The aphidiid parasites *Aphidius uzbekistanicus* resembling *A. ervi*, *A. picipes* and *Praon volucre* were found regularly.

Each year, another species predominated. Only in 1977, a clear effect of parasites was found when they were probably responsible for a 60% decrease in the number of apterae during the growth phase of the epidemic. In the final phase most aphidiids are attacked by hyperparasites. In 1977, a very high percentage of aphids was attacked by *Entomophthora* spp. It was not incorporated in the model as little was known of the biology.

POPULATION COUNTS

In field work, all larval stages, apterae, alates, mummified aphids, predators and aphids attacked by *Entomophthora* spp. were counted. We tried to obtain an accuracy level of $S(\bar{x})/\bar{x} < 0.1$ for the total number of living aphids. So sample size was constantly changing. As a rule, the number of samples decreases when the number of aphids increases. With a doubling time of about three days, the population grows by some 25% per day. So in order to keep the variance low, all counts have to be completed within a day.

Weather strongly affects variance. When the weather is bad, aphids become more evenly distributed over the plants. It is yet unclear how this factor will affect the future growth rate of the population. In the notably bad summer of 1978, this more even distribution was clearly observed.

PROSPECTS

Knowledge about the processes in the system is lacking. As more experimental data become available for use in the model, our confidence can increase. Of much importance is the help of the model in integration of fragmentary knowledge and guidance in experiments.

The development of a forecasting model forms just one part of the warning system. More knowledge is needed of the effect of the aphids on their host plant and of host on aphid to reach a reliable management system. The results of a combined population model and plant model are encouraging (Rabbinge & Vereijken, 1979) but still much more research is needed to make these models applicable in practice.

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10.3 Meteorology and integrated pest control

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INTRODUCTION

Populations of plants and animals meet with the influences of their biotic, chemical and physical environment. The quantitative assessment of these influences on the development of populations of a plant or an insect is a task for biologists. Assistance from other disciplines, however, can be helpful. So it was decided, when the experimental orchard of the Working Party on Integrated Control of Pests was set up at Lienden, to provide supporting meteorological observations.

METEOROLOGICAL OBSERVATIONS IN THE EXPERIMENTAL ORCHARD

Since 1969, temperature, humidity, wind and radiation have been recorded in the orchard with classical meteorological instruments such as thermometers, thermographs, hygrographs, a Moll-Gorczynski meter for diffuse radiation and a Lambrecht cup anemometer for wind speed and direction. The data have been used in biological investigations. Notable is the investigation of Rabbinge (1976; Section 10.1) on the feasibility of biologocal control of the fruit-tree red spider mite (*Panonychus ulmi*). For that project, the meteorological measurements at Lienden were intensified in selected situations during the years 1973-1975. Especially attention was paid to the temperature regime in the orchard.

METEOROLOGICAL ASPECTS OF RESEARCH ON THE BIOLOGICAL CONTROL OF THE FRUIT-TREE RED SPIDER MITE

From laboratory research, Rabbinge (1976) found that the influence of temperature on development and fluctuations of a population of *Panonychus ulmi* is of such significance that in a mathematical model, which simulates development of a population of the mite, the temperature should be introduced as a driving variable.

For outdoor research and the application of the model in practice, it was recognized that the individual mites will be subject to the temperature of the tree leaves, which may be different from the air temperature as measured in a standard meteorological cabinet. These differences originate because the temperature of the leaf is not only determined by the temperature of surrounding air but also by processes like radiation and evaporation.

A good starting point for the assessment of the circumstances under which and to what extent the temperature of a leaf can deviate from the temperature of the air is the notion that the storage capacity for energy of a leaf is negligible. So the total incident energy fluxes equal the energy rate of loss. Now energy is gained by the leaf in various forms: as heat when the leaf is colder than the surrounding air, as radiation from sun and sky and as radiation emitted or reflected from the ground and from objects in the environment. Energy is lost by the leaf in the form of heat when the leaf is warmer than the air, as thermal radiation emitted by the leaf and as latent heat due to evaporation of water.

So when there is considerable incident radiation, the leaf's temperature rises sufficiently that the heat flux and the flux of water vapour to the environment keep the energy fluxes balanced.

In bright sunshine, such deviations of the leaf temperature from the air temperature may amount to over 10 °C, as is demonstrated in Figure 1 where observations are recorded of a typical summer day in the orchard at Lienden.

Leaf temperatures can nowadays be easily measured with radiation thermometers, but can also be calculated from the mathematical expression of the energy balance described above. A drawback both for measurements and for calculations is the variability of leaf temperatures. Firstly there is the variability in time, corresponding to variations in the energy fluxes. But at a certain moment, the temperature of an individual leaf of a tree depends strongly on the exposure of that leaf to sunshine or shadow.

The detail in which meteorological quantities, such as leaf temperatures, have to be

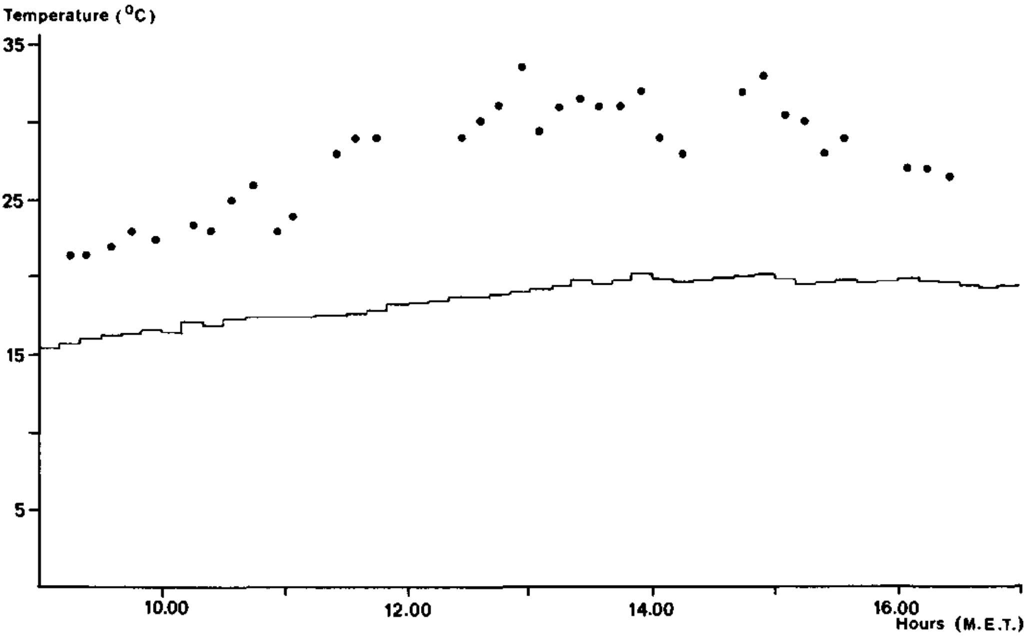

Fig. 1. Temperatures of leaves and air in the experimental orchard on a bright summer day (28 August 1973). ● Temperature of leaves exposed to bright sunshine. —Temperature of the air at 1.50 m (thermograph).

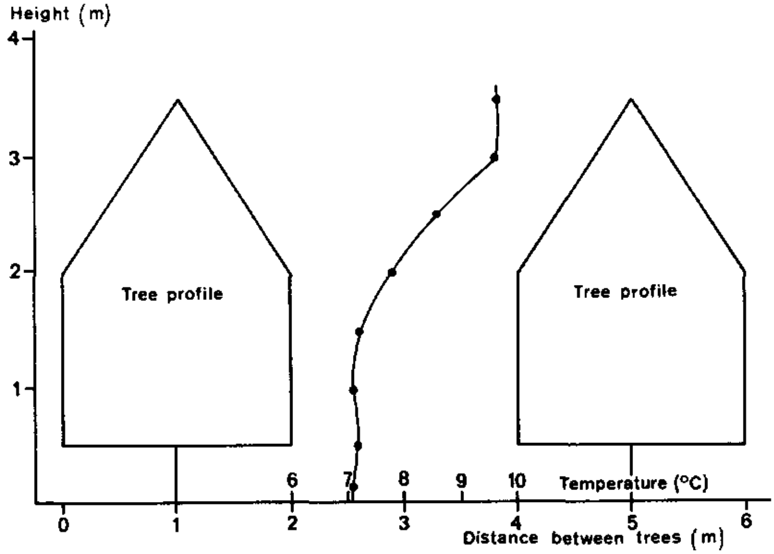

Fig. 2. Temperature profile in the orchard on 20 June 1974 at 00:35 h and the geometry of the orchard.

known depends on the nature of the biological problem under study. Rabbinge therefore tested the sensitivity of his model calculations with populations of *Panonychus ulmi* to variations in the detail of the meteorological sub-model. He found that calculations with specified leaf temperatures and calculations where average air temperatures were used gave only slight differences in the results of the simulation of the population of *P.ulmi*, so for that purpose, the air temperatures measured in a cabinet at height 1.50 m could be used to represent the temperature of the population in the simulation model.

Besides the difference between air and leaf temperature, variations of temperature with height in the orchard could also be neglected, since vertical temperature gradients in orchards are small (Landsberg et al. 1973). The development of steep temperature gradients such as occur over open terrain during calm clear nights is strongly impeded in orchards by the vegetation. The cooling of branches and leaves due to their energy loss as infrared radiation causes a cooling throughout the depth of the orchard and the cooling of the ground is hampered by the infrared radiation from the vegetation. A typical example of the nocturnal cooling in a clear night, as observed in the orchard at Lienden is shown in Figure 2.

CONCLUDING REMARK

The requirements for meteorological support to biological or agricultural research range from the supplying of meteorological data that can easily be gathered to the study of micrometeorological problems that go beyond existing knowledge or available research capacity. It is therefore advisable that in the planning stage of biological research it be recognized whether the physical environment will play an important role and that the feasibility be investigated of adequate support from meteorological or physical disciplines.

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10.4 Radionuclides and ionizing radiation in research on integrated control

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During the past twenty years radionuclides have been used extensively in entomological research. Some aspects are described to give an impression of the role isotopes played in research on integrated control.

DISPERSAL

One day starved predatory mites, *Amblyseius potentillae,* were labelled with ^{32}P. mixed with honey and released in an apple tree. After 2 weeks it was possible to find some of them with a small portable Geiger counter. Their dispersal was found to be about 2 m, but most of the released specimens were still at the point of release.

In integrated control, predatory wasps like *Trichogramma* spp. and *Colpoclypeus* spp. can play a major role. To study dispersal of these insects, isotopes were used. For *Trichogramma*. eggs of *Sitotroga cerealella* were sprinkled over sticky pieces of cardboard distributed in an orchard. *Trichogramma* labelled with ^{32}P were released and after a week the pieces of cardboard were collected. When autoradiographed, the blackening of the film over some *Sitotroga* eggs proved parasitization by the radioactive wasps, thus establishing their dispersal.

Colpoclypeus florus was labelled with various isotopes such as ^{14}C, ^{32}P and ^{65}Zn in studies on dispersal and effectiveness. It was found that ^{14}C-leucine was a satisfactory label that was transferred from the labelled parent to its progeny where it could be detected by autoradiography.

Some trials were done with ^{32}P-labelled *Adoxophyes orana* to study its flight range in the field and the effect of its pheromone. Tagged male specimens were released in an orchard. Specially designed sex traps were distributed in the orchard and the insects caught were assayed for radioactivity. Minimum night temperatures below 12 °C lowered the flight activity and also the sex trap catches. Data collected by using a release centre showed that the wind is a determining factor for the upwind orientation of the male moths to the attractive females (Minks & Noordink, 1971).

In preliminary trials with the strawberry blossom weevil, *Anthonomus rubi,* several isotopes were used to label the adults (^{22}Na, ^{32}P, ^{60}Co and ^{65}Zn). The first results indicated that ^{32}P was an excellent tracer for dispersal studies with the overwintered generation. Even after 2 months, the initial mark could be detected autoradiographically, not only in the parent generation but also in its progeny. The other isotopes will be used to label the overwintering generation because longer half lives and more penetrative radiation are needed.

PREY-PREDATOR RELATIONSHIP

In trials on the prey-predator relationship between the predatory mite *Amblyseius potentillae* and its prey *Tetranychus urticae*, the prey was labelled with ^{32}P and offered to the predator for a measured time. Then the radioactivity in predator and prey was determined separately in a scintillation counter by the Cerenkov technique. From the ratio of the radioactivity between predator and prey, the relevant data could be calculated.

The labelled *Colpoclypeus florus* was used to study the effectiveness of laboratory-reared specimens on the parasitization of leaf rollers in the field. Also the ratio could be estimated between number of insects released in the orchard and immigrating insects.

DIETARY PREFERENCE

The influence of some chemical constituents of a synthetic diet on diet uptake and egg production of young *Tetranychus urticae* females was investigated with $Na_2H^{32}PO_4$. The highest dietary uptake and egg production occurred at pH 10 with the standard diet. Addition of adenosine triphosphate increased the rate of ingestion.

In another trial, the turn-over of phosphorus was determined by putting specimens of the two-spotted spider mite on plants made radioactive with ^{32}P. Every 2 h, the eggs produced during that time were collected and mounted on discarded X-ray film. After 24 h, the assembled eggs were autoradiographed and exposed for 2 weeks. By 5 h after their transfer to the radioactive plant, the mites produced radioactive eggs, so that turn-over of phosphorus was rapid. When the mites that had produced the labelled eggs were put on normal plants, it took them 5 days to deposit normal, not radioactive eggs. The females themselves, however, were still very radioactive. This implies that the mites use only recently acquired phosphorus for egg production.

Radiophosphorus was used to study the taste perception of *Myzus persicae*. With parafilm sachets aphids were offered a choice between different solutions and, by making one of them radioactive the aphids proved easily and quickly to distinguish between a basic diet and a sugar solution. Autoradiographs were made of aphids which had an hour's choice between two solutions of different nutritive value, one of which was radioactive (Harrewijn & Noordink, 1971).

MOVEMENT OF APHID SALIVA IN SEEDLINGS

Translocation of aphid saliva in an apple seedling was proved by containing ^{32}P-labelled *Dysaphis devecta* immediately below the cotyledons. Non-radioactive aphids were placed at the apex of the seedling where they fed for a week. The leaves, roots, cotyledons and the aphids that had fed at the shoot tip were autoradiographed for 3 weeks. The blackening of the plant parts and the receptor aphids showed that the radioactive saliva of the initially labelled aphids was transported throughout the plant and was subsequently taken up by the aphids feeding at the plant apex (Forrest & Noordink, 1971).

STERILE MALE TECHNIQUE

In the mid-sixties, it became clear that the onion fly, *Delia antiqua*, had developed resistance to a number of organochlorine insecticides. To ensure the future control of the fly, it was decided to study the feasibility of the sterile male technique. Apart from scientific and technical aspects such as dispersal and rearing, the appropriate radiation dose had to be established. After a number of trials it was decided to use 30 Gy (3 krad) of hard gamma rays, emitted by ^{60}Co, to sterilise the flies as pupae. Receiving that dose the males were sterile for more than 98% whereas the females were infecund after a dose of 20 Gy (2 krad). With several isotopes, the polygamy of the females, the dispersal of irradiated flies, the overwintering mortality and the length of spermatogenesis were established. Now, about 15 years later, there is evidence that this technique is suitable for implementation in commercial onion growing (Ticheler & Noordink, 1968).

NEMATODES

With ^{14}C-labelled oxamyl, a nematicide/insecticide, several trials were done to establish its systemic behaviour. Leaves of *Vicia faba* plants were treated with a solution of concentration 4 g/l and after a certain time dried, mounted on filter paper and autoradiographed. All plant parts became radioactive in both the symplastic and apoplastic direction from the point of treatment. Extremely large plants whose middle leaf was treated also showed a general distribution of radioactivity in other leaves and in the roots. By thin-layer chromatography and subsequent autoradiography, root exudates were examined. Besides some breakdown products, a small amount of ^{14}C-oxamyl applied to the leaves was present in the root exudates (Bunt & Noordink, 1977).

MISCELLANEOUS

Biogenic amines labelled with ^{14}C were injected in very small amounts into *Myzus persicae*. When the next generation were to be apterous, certain cells of the protocerebrum and of the abdominal ganglion and the brain of the unborn embryo became labelled within 15 min of the injection. No such reaction took place when the embryo would produce an alate. In apterous larvae, the serotonergic activity in the wing buds was not enhanced. So substances affecting this process have a direct impact on the brain and can influence wing dimorphism very quickly.

The efficiency to detect radioactivity of X-ray films was compared with that of a thin-window Geiger counter. Unter the circumstances of our trial, the film was about seventy times as sensitive to ^{32}P radiation as the electronic counter (Noordink & Minks, 1970).

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