# EULOPHUS PENNICORNIS: A POTENTIAL BIOCONTROL AGENT AGAINST THE TOMATO MOTH

# G.C. MARRIS, J.P. EDWARDS

Central Science Laboratory, (MAFF), London Road, Slough, Berks. SL3 7HJ

#### ABSTRACT

Introduction of the parasitoid *Eulophus pennicornis* lessens the extent of leaflet damage and significantly reduces the overall amount of crop-consumption measured in *Lacanobia*-infested tomato plants. Although percentage parasitism recorded at high wasp densities (20 wasps/larva) was significantly greater than parasitism achieved when few wasps were used (2 wasps/larva), these levels of wasp release are equally effective in suppressing feeding activity. This suggests that the observed reduction in *Lacanobia*-inflicted damage is not only due to parasitism, but also to the disruptive effect of searching *Eulophus* on larval behaviour.

#### INTRODUCTION

The Tomato moth, Lacanobia oleracea (Lepidoptera: Noctuidae), produces large, polyphagous larvae which are capable of causing economic damage to a variety of glasshouse crops (Lloyd, 1920; Speyer & Parr, 1948; Foster, 1981). Lacanobia cannot thrive on tomato foliage alone due to an intolerance to poisons present within leaf tissues and, as a result, larvae also attack the pith, stem or unripe fruit in order to fulfil their dietary requirements (Lloyd, 1920). Consequently, while extensive defoliation by caterpillars can lead to stunted plants with reduced yield, even a low incidence of scarred fruit can amount to a serious crop loss. Although control can be achieved chemically (Foster, 1980) increasing deployment of predatory or parasitic biocontrol agents against other horticultural pests has resulted in a general reduction in conventional insecticide usage. This requirement, coupled with changing cultivation methods (e.g. reduced steam-sterilisation of soil) which allow Lacanobia pupae to persist in relatively large numbers, has led to an increased pest status for this species (Foster, 1979) and by 1993 the Tomato moth was reported as inflicting significant damage to commercially grown tomatoes in the Channel Islands (N. Trainer, pers. commun.). There is therefore a need to develop alternative control measures for use against this pest which are compatible with modern growing techniques and with existing biocontrol practices.

An extensive series of laboratory trials into the biology of the gregarious ectoparasitoid *Eulophus pennicornis* (Hymenoptera: Eulophidae) has revealed that this wasp possesses many traits which could allow it to perform well as a biological control agent against *Lacanobia* larvae (Marris & Edwards, in prep.). Important characteristics identified include high wasp fecundity, a rapid rate of development, and a rapid and marked reduction in food consumption by parasitised hosts. Given the proven ability of this parasitoid species to suppress *Lacanobia* damage in the laboratory, the current study was designed to provide a preliminary assessment of the performance of *Eulophus pennicornis* against small-scale field

populations of moth larvae feeding on glasshouse tomato plants.

# MATERIALS AND METHODS

# Food plants

Tomato plants used in these trials were the true-breeding variety Moneymaker. Seeds were germinated in propagators and seedlings maintained in an environmental cabinet ( $15^{\circ}C$ , 65% r.h., 16h light/8h dark (L/D 16h/8h)) until they were approximately 25cm high. They were then transplanted, in groups of 20, into raised beds (soil depth c.20cm) contained within geodesic glasshouses (diam. 3.05m x height 2.10m). Plants were spaced at intervals of approximately 30cm, allowing the observer access to all leaves. The photoperiod in each house was L/D 16h/8h, and temperature was thermostatically controlled such that it never fell below 18°C. Ten houses were stocked with plants, 9 of which were subsequently infested with *Lacanobia* and used to compare the amount of crop damage inflicted in the presence or absence of *Eulophus*. The remaining house contained control plants maintained without *Lacanobia*.

#### Lacanobia larvae

Early fifth instar *Lacanobia* larvae were obtained from culture and maintained without access to their normal food medium for 24h (25°C, 70% r.h., L/D 16h/8h). Hosts of this developmental stage were selected because laboratory studies have shown them to be preferred oviposition sites for *Eulophus pennicornis* (Marris & Edwards, in prep.). In order to infest plants, larvae were placed in groups of 5, onto each plant, such that 9 houses each contained a total of 100 evenly-distributed feeding caterpillars.

#### Parasitoids

Newly-emerged adult *Eulophus pennicornis* were collected from laboratory culture and maintained without access to hosts for 48h. During this time they were supplied with food (50% volume by volume honey solution) and maintained at 25°C, 70% r.h., under a constant photoperiod (L/D 16h/8h). This delay prior to release allowed mating to occur, but prevented premature oviposition. Female wasps were then separated into the required densities to be used in trial releases. Two alternative densities were used, and each density was replicated 3 times:

Low density: 2 wasps/larva = 10 wasps/plant = 200 wasps/glasshouse

High density: 20 wasps/larva = 100 wasps/plant = 2000 wasps/glasshouse

Each wasp density was divided into 20 glass vials, such that each vial contained either 10 (low) or 100 (high) female wasps. At the time of release, individual vials were opened and placed at the base of each of the 20 *Lacanobia*-infested plants present in each glasshouse. To provide wasps with access to a fresh source of food after release, cotton wool pads soaked in 50% V/V honey solution (4 pads/house) were placed at regular intervals on the soil surface and replaced every 4d.

#### Monitoring crop damage

To reflect the fact that Tomato moth larvae cause different types of crop-damage, we chose to use 2 indices to measure the effects of parasitoid release on foraging caterpillars: Firstly, by recording the proportion of leaflets on infested plants bearing any marks of *Lacanobia* feeding, we sought to compare the pest's potential to scar plant tissues in the presence or absence of wasps; secondly, by ascertaining the overall weight lost by infested plants, we aimed to evaluate the wasp's ability to reduce total volume of crop consumed.

All plants were inspected at 48h intervals over an 8d period, and the number of leaflets which bore any marks caused by the feeding activity of *Lacanobia* was recorded. After 8d, the total number of leaflets/plant (i.e. damaged + undamaged) was counted, and all plants were harvested by cutting them off at their base as close as possible to the soil surface. Each plant was then placed into a hot oven (95°C) for 24h, before being crushed and weighed.

#### Monitoring parasitism

Throughout each trial *Lacanobia* larvae infesting the houses containing either density of *Eulophus* were inspected for the presence of wasp eggs or larvae, and the number of parasitised individuals was recorded.

#### RESULTS

The data collected was used to provide 2 different indices of crop damage (Table 1). The proportion of leaflets damaged in the presence or absence of wasps was calculated by expressing the number of leaflets/plant bearing marks of *Lacanobia* feeding as a percentage of the total number of leaflets/plant. In order to measure the total amount of crop consumed in each trial, the final dry weight of each infested plant was expressed as a percentage of the mean dry weight of the 20 control plants (i.e. those not exposed to caterpillars). Total weight loss was then calculated by subtracting these relative percentage weights from 100. Figure 1 compares the mean proportion of tomato leaflets attacked by fifth instar *Lacanobia* (0 wasps/larva), with the number of leaflets attacked in the presence of low or high densities of the parasitoid *Eulophus pennicornis*. Figure 2 shows the mean damage (weight loss) in infested plants in each type of trial. Figure 3 illustrates the mean percentage parasitism achieved at each wasp density.

Table 1.	Indices used	to measure dar	mage to tomato plants.	
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INDEX OF DAMAGE	CALCULATION
Proportion of leaflets damaged (%)	no. damaged leaflets/infested plant x 100 total no. leaflets/infested plant
Total weight loss (%)	100 - $\left( \frac{\text{dry weight infested plant x 100}}{\text{mean dry weight undamaged plant}} \right)$

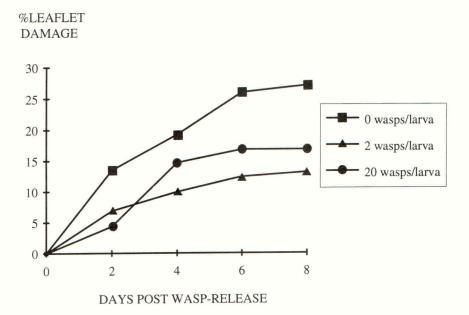
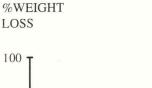
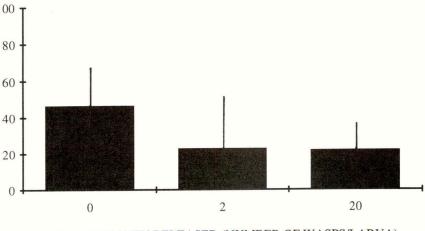


Figure 1. Percentage of leaflets damaged by *Lacanobia* larvae feeding in the absence or presence of low or high densities of *Eulophus*.

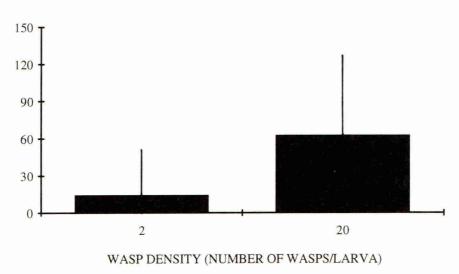
Figure 2. Plant damage (weight loss) caused by *Lacanobia* in the absence or presence of low or high wasp-densities (bars = 95% confidence limits).





WASP DENSITY RELEASED (NUMBER OF WASPS/LARVA)





#### %PARASITISM

#### DISCUSSION

The proportion of leaflets/plant damaged by *Lacanobia* in the absence of wasps was consistently higher than comparable proportions recorded in houses containing the parasitoid *Eulophus* (2 wasps/larva or 20 wasps/larva)(Figure 1). This decrease in feeding was apparent within 48h of wasp-release, but although the effect became more pronounced as trials progressed, the final number of leaflets attacked by foraging caterpillars after an 8d exposure to wasps was not significantly reduced: little variation existed between leaflet damage recorded at the 2 different parasitoid densities, and statistical analyses confirm that any slight effect *Eulophus* may have on the extent of *Lacanobia*'s feeding activity was not density-dependent ( $F_s$ =1.83, P>0.05, d.f.=2,6). While neither of the wasp-densities used in these trials significantly reduced the potential of *Lacanobia* to inflict leaf scars, weight loss in infested plants (0 wasps/larva) was almost twice as great as in similar plants grown in the presence of wasps (Figure 2). Statistical comparisons show that these differences between plant dry weights are significant ( $F_s$ =7.38, P<0.05, d.f.=2,6), and that high- and low-density releases were equally effective in reducing the amount of leaf tissue consumed ( $F_s$ =0.02, P>0.05, d.f.=1.6).

The mean level of parasitism which followed high-density releases (62.82%) was significantly greater than the number of successful attacks recorded in low-density houses (14.30%) ( $F_S$ =7.88, P<0.05, d.f.=1,4). Although far more hosts were parasitised at high wasp densities, both wasp levels resulted in an equal reduction in damage (weight of plant tissue consumed). This suggests that the observed reduction in damage to the plants cannot be attributed exclusively to successful parasitism. It has been documented that, as a defence

reaction to mechanical disturbance, Tomato moth larvae fall to the ground (Speyer & Parr, 1948) and they may not regain the same foodplant. It is possible that the activities of hostinspection which precede parasitism by *Eulophus* species (Shaw, 1981) induce a similar behavioural response in *Lacanobia* larvae, such that the mere presence of foraging wasps reduces the number of caterpillars actually on the crop.

Although a low density of *Eulophus* may bring about a short-term reduction in the amount of crop consumed, a correspondingly low rate of successful parasitism means there can be little expectation that the wasp population will become self-sustaining. By contrast, high densities of *Eulophus pennicornis* (eg. 20 wasps/larva) not only significantly reduced plant damage, but also resulted in a high proportion of parasitised hosts. This suggests that, providing sufficient numbers of female wasps are released in an initial dose, this species has the potential to be used as an inoculative agent to give an effective level of crop protection over successive pest generations.

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# WASP-INDUCED INFECTIONS IN A PARASITOID-HOST ASSOCIATION

# G. C. MARRIS, J. NAYLOR, N. PARKINSON, J. P. EDWARDS

Central Science Laboratory (MAFF), London Road, Slough, Berks. SL3 7HJ

### ABSTRACT

The parasitoid *Pimpla hypochondriaca* causes high Tomato moth mortality. Comparisons between microflora of healthy hosts, dead hosts and of adult wasp tissues, reveal that many deaths are not due to parasitism but may be the result of wasp-induced microbial infections. Density gradient centrifugation of wasp reproductive tissues show that female *Pimpla* harbour particles similar to polydnaviruses implicated in host immune disruptions in other parasitoid associations. It is therefore possible that observed death rates reflect altered susceptibility to microbial infection in wasp-attacked, immunocompromised *Lacanobia*.

# INTRODUCTION

The wide host-range exploited by the solitary endoparasitoid Pimpla hypochondriaca (Hymenoptera: Ichneumonidae) includes several pest Lepidoptera (Thompson, 1946). Although Pimpla lacks traits to allow use as a conventional biocontrol agent against the Tomato moth, Lacanobia oleracea (Lepidoptera: Noctuidae), experiments have shown that this wasp has a profound, adverse effect on Lacanobia survival (G.C. Marris, unpublished data). A comparison between the proportions of moth-emergence and mortality recorded in Lacanobia populations reared in either the presence or absence of foraging Pimpla reveals that while very few pupae are successfully parasitised (approximately 10%), less than half remaining wasp-exposed pupae actually produced moths. This level of host-survival is much lower than the number of moths produced in unexposed populations (70%). Pimpla is synovigenic, and some mortalities may arise when wasps feed on pupae to gain nutrients necessary for egg maturation. During host-feeding the wasp repeatedly penetrates a pupa with her ovipositor before enlarging the resulting wound with her mouthparts and imbibing host fluids. As a result pupae become mutilated and death rapidly ensues (Fitton et al., 1988). Each mutilated pupa can be readily identified by the presence of an obvious wound. However, initial observations of wasp-exposed Lacanobia reveal that this is not the only cause of death: Some pupae are dry and powdery; others contain a black, foul-smelling liquid. Since none of these dead pupae bear the marks of mutilation, their existence suggests that a significant number of deaths following exposure to Pimpla may be caused by microbial infection.

There are several potential mechanisms of infection for wasp-exposed pupae: Unparasitized hosts may act as reservoirs of pathogenic microbes which are transmitted between pupae via the ovipositors of foraging wasps; *Pimpla*'s mouthparts and/or reproductive tracts may harbour microorganisms which pass from wasp to pupa during parasitism; the inert granular substrate vermiculite, used to support *Lacanobia*-pupation in the laboratory, may contain opportunistic microorganisms which enter parasitised or mutilated pupae through wounds inflicted during wasp attack. Finally, many parasitic Hymenoptera possess mechanisms which protect their larvae from the immune defenses of their host (Vinson, 1990). Mechanisms vary according to species, but several Ichneumonidae produce specific polydnaviruses in their female reproductive tracts (Krell, 1991) and these have been widely implicated in the disruption of host immune defences (Vinson, 1990). It is possible that *Pimpla* introduces a similar host-regulatory factor into each pupa during parasitism, and that this substance alters the immune system of the host such that it is vulnerable to microbial invasions to which it would otherwise have a degree of resistance.

Given current interest in the use of microbial insecticides, if microorganisms are found to play a role in wasp-induced pest mortality then they may influence the design of successful biological control strategies. Moreover, any substance which reduces pest-ability to raise an immune response has the potential to greatly enhance the potency of an array of existing biocontrol measures (eg. baculoviruses, nematodes, parasitoids). The aims of this study were to carry out preliminary identification of the main types of microorganism in the *Lacanobia*/*Pimpla* association and to ascertain which of the above sources are important in their transmission to wasp-exposed pupae. Since initial electron microscope studies suggest that *Pimpla* contains particulate material in the reproductive tracts (G.C. Marris, unpublished results) adult female *Pimpla hydrochondriaca* were also screened for the presence of polydnaviruses using sucrose density gradient centrifugation techniques.

#### MATERIALS AND METHODS

# Preparation of Lacanobia, Pimpla and vermiculite samples for microbial investigation

Fifty *Lacanobia* pupae were obtained from a laboratory culture. Ten of these individuals were 48h post-pupation (without exposure to wasps) and presumed to be healthy; ten had been maintained for many weeks without wasp-exposure but had not yielded moths and were assumed to be dead; ten had been maintained with *Pimpla* and were mutilated; twenty individuals were shrivelled and showed signs of decomposition, but although they had been wasp-exposed they were unmutilated. In order to assess the quantity and variety of microorganisms on external surfaces, each pupa was moistened with 1ml of Buffered Peptone Water (BPW) and wiped over the surface of a series of 3 agar plates, each containing a different medium designed to favour the growth of distinct microbial types (Table 1). To reveal the presence of microbes within *Lacanobia* tissues without contaminating internal contents with surface-born organisms, pupae which had been used to prepare external plates were surface-sterilised for 2min in hypochlorite (chlorine 0.35%) prior to maceration in 5ml BPW. 0.1ml of each resulting solution was spread onto the 3 alternative agar plates.

Ten adult female *Pimpla* were anaesthetised by chilling and prolonged exposure to CO<sub>2</sub>. Individuals were then decapitated and their ovipositors cut off. Wasp abdomens were surface sterilised prior to removal of entire reproductive systems. Heads were moistened in BPW and used to prepare external plates using the method described above, before crushing to produce internal samples. Ovipositor sections were placed directly onto agar plates; reproductive tissues were macerated prior to inoculation.

In order to obtain fresh vermiculite a small volume (1.0g) which had not been exposed to any insects was heated to 100°C for 8 h. Similar volumes of substrate were obtained from both the *Lacanobia* culture (ie. exposed to pupae) and from the *Pimpla* culture (ie. exposed to pupae and wasps). Samples were divided into equal volumes and used to inoculate the 3 alternative media.

PLATE-TYPE	AGAR MEDIUM	INCUBATION CONDITIONS
FUNGAL	2% Malt Chloramphenicol	25°C 4-7 days (aerobic)
BACTERIAL	Starch Yeast Glucose Phosphate	30°C 2 days (anaerobic)
BACTERIAL	Brain Heart Infusion	25°C 2 days (aerobic)

TABLE 1. Alternative plate-types used for microbial growth.

Following appropriate incubation (Table 1) all plates were inspected under the Light Microscope for the presence and abundance of different types microbial growth. To allow identification of any bacteria, samples from single colonies were used to prepare gram stains.

#### Preparation of wasp samples for viral screening

Sixty newly-emerged adult female *Pimpla hypochondriaca* were divided into 3 groups of 20 individuals each, anaesthetised and dissected to remove their entire reproductive systems. In order to prevent digestive enzymes which may be released into the abdomen during dissection from breaking down cell contents, isolated organs were immediately placed into 0.5ml of chilled insect saline. When all dissections were complete, each of the 3 final samples was spun at 13,000g for 10s to remove excess tissue debris and their supernatants were then layered onto sucrose density gradients (25% to 50% sucrose) held in a solution of Phosphate Buffered Saline (PBS). Samples (+ gradients) were spun for 1h at 20,000rpm. Following high speed centrifugation each gradient was placed below a very bright spot light in a photographic darkroom to reveal the presence of any bands of particulate material.

#### RESULTS

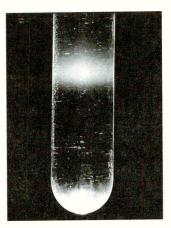
A variety of distinct microbial colonies were observed on the 3 growth media inoculated. Types of growth identified included at least 3 species of aerobic bacteria (grampositive cocci, gram-positive bacilli and gram-negative bacilli), anaerobic bacteria, yeasts and 3 fungii (*Rhizopus* sp., *Penicillium* sp. and *Mucor* sp.). Table 2 summarises the main types of microorganisms observed and indicates their relative abundance in each of the specimen types prepared for microbial investigation.

Each of the samples dissected from adult *Pimpla hypochondriaca* revealed a clear band of material following density gradient centrifugation (Figure 1). Single bands were relatively narrow (c. 1cm wide) and possessed a distinctive blue colouration characteristic of a tindalisation effect caused by scattering of light by microscopic particles. In each case the

TABLE 2. Occurrence of microorganisms in the *Pimpla/Lacanobia* association. Microbial abundance: +++ 100% of plates; ++ >50% of plates; + <50 % of plates; - absent. Microbial types: 1 gram +ve aerobic cocci; 2 gram -ve aerobic bacilli; 3 gram +ve aerobic bacilli; 4 anaerobic bacteria; 5 yeasts; 6 *Rhizopus*; 7 *Mucor*; 8 *Penicillium*.

Sample Type	1	2	3	4	5	6	7	8
Exterior healthy pupae	-	-	-	+++	+++	-	-	+++
Interior healthy pupae	+	-	-	++		-	Ξ.	-
Exterior dead pupae								
(not exposed to Pimpla)	+	-	-	+++	+	-	++	+++
Interior dead pupae								
(not exposed to Pimpla)	+	•	-	+	+	-	-	++
Exterior dead pupae (exposed	8 5 0 10	140				1.1.1		+++
to Pimpla)	+++	+	++	+++	+++	+++	-	TTT
Interior dead pupae (exposed					++	1		++
to Pimpla)	++	+	-	+++		+	-	TT 1.1
Exterior mutilated pupae	+++	+	++	+++	++	+++		++
Interior mutilated pupae	+++	+	-	+++	++	++	-	++
Pimpla ovipositor	-	-	×	-	+	-	-	8
Pimpla reproductive tissues	+	-	-	++	+++	+	-	-
Exterior Pimpla mouthparts	++	-	-	+	+	-	-	-
Interior Pimpla mouthparts	+	-	-	+	+	++	-	-
Clean vermiculite	-	-	-	-	-	-	-	-
Vermiculite exposed to								
Lacanobia	+	+	-	+++	+++	-	+++	+++
Vermiculite exposed to								
Pimpla/Lacanobia	+	-	-	+++	+++	+++	-	+++
L								

FIGURE 1. Results of sucrose density gradient centrifugation of reproductive tissues from 20 female *Pimpla hypochondriaca*.



depth on the gradient was similar (approximately 5cm) and the compact nature of each band suggests the presence of a large number of microscopic particles of similar size and density.

#### DISCUSSION

A wide array of microorganisms occurred in the various samples analysed including several types within the tissues of dead Lacanobia. However, if any individual microbial species is to be directly linked with wasp-induced mortality then it would be expected to be present in wasp-exposed pupae but absent from pupae which have not been encountered by Pimpla. Only three of the microbial types identified fulfil both these criteria: Gram-negative aerobic bacilli, gram-positive aerobic bacilli and the fungus Rhizopus. Differences in the patterns of occurrence of these microbes suggest that they differ in their routes of transmission in the Pimpla/Lacanobia association. Gram negative bacilli were found on the external surfaces and inside the bodies of wasp-exposed pupae. These bacteria were invariably absent from wasp mouthparts, ovipositors and reproductive organs, but did occur in a proportion of the vermiculite samples analysed. While it therefore seems unlikely that Pimpla plays a direct role in transmission, it is possible that these microbes are opportunists, entering hosts from their surrounding substrate through wounds inflicted during wasp attack. The fact that gram positive bacilli were present only on external surfaces of wasp-exposed pupae and on outer mouthparts of some wasps suggests that transmission from wasp to pupa may occur incidentally, during host-feeding or inspection. Rhizopus was found to occur on external surfaces of every wasp-exposed pupa tested, and was almost equally prevalent inside the same individuals. As several wasps were found to carry Rhizopus in their mouthparts and in their reproductive organs, it is therefore possible that Pimpla is the source of this fungus which is then transmitted from parasitoid to host during mutilation or oviposition.

Although the presence of these microbial types in the different insect samples tested show that their incidence in dead *Lacanobia* is correlated with the foraging activity of female *P. hypochondriaca*, whether any or all of them are actually responsible for observed levels of mortality is not clear. The pathogenicities of the bacterial isolates have yet to be assessed, and while *Rhizopus* species have been recorded in the corpses of other insects (Batra *et al.*, 1973), these fungi are commonly saprophytic. However, even if the bacteria and fungi recorded in this study are not normally lethal to *Lacanobia*, if *Pimpla* is equipped with some mechanism which alters the immune defence reactions of its hosts following oviposition, it is possible that vulnerable immunocompromised hosts may succumb to infective agents to which they would otherwise be resistant.

Density gradient centrifugation of *Pimpla*'s female reproductive tissues revealed large amounts of particulate material which is directly comparable in its site of origin and centrifugation properties to wasp-specific polydnaviruses isolated in other parasitoids (Krell & Stoltz, 1980). Such viruses have been widely implicated in disruptions to the immune responses of parasitized hosts (Vinson, 1990), their modes of action varying according to species. In some cases wasp eggs are enveloped in a particulate coat which mimics proteins found in host tissues such that progeny are not recognised as foreign. Eggs protected in this way fail to illicit an immune reaction, while the parasitised host retains its sensitivity to invading antigens. Other viruses are more destructive in action, blocking or destroying components of cellular immunity. Wasp eggs equipped with a viral complement of this type

may encounter a greatly reduced immune response, but the host is also rendered susceptible to other infective agents. If the particles possessed by *Pimpla* do regulate host immunity, then the observed high incidence of infection in wasp-exposed pupae suggests that they do so by inactivating the host immune system rather than through a mechanism which masks the presence of wasp eggs.

The alternative modes of operation of polydnaviruses have implications for the extent of parasitoid host-ranges. Viruses which serve as molecular mimics of particular host compounds are implicitly host-specific in their activity, and it follows that the parasitoids which harbour them will only benefit from their protective qualities in a relatively limited number of host species. Viruses which are more destructive may be less host-specific in their activity, enabling parasitoid offspring to develop in a potentially wider array of insect types. The fact that P. hypochondriaca successfully parasitises Lepidoptera, Coleoptera and Diptera (Thompson, 1946) may support the idea that viruses produced by this species could have a non-specific, destructive effect on host immunity. In terms of parasitoid survival strategies, although Pimpla sacrifices offspring in parasitising hosts later overcome by microbial infections, each female need expend less time or energy searching for a particular host species, being free to exploit many of the different insect types she encounters. Under these circumstances the high incidence of wasp-induced mortality in the Pimpla/Lacanobia association, coupled with Pimpla's extensive host range, should be viewed together as reflections of the relative costs and benefits incurred through the operation of a putative factor with powerful immunoregulatory properties.

#### ACKNOWLEDGEMENTS

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THE EFFECTS OF POLYPHAGOUS PREDATORS ON SPIDERS AND MITES IN CEREAL FIELDS

# K.D. SUNDERLAND, S.J. ELLIS

Horticulture Research International, Littlehampton, BN17 6LP

A. WEISS

Odenwaldstrasse 26, 69412 Eberbach, Germany

#### C.J. TOPPING

Scottish Agricultural College, Department of Land Resources, 581 King Street, Aberdeen, Scotland, AB2 9SX

#### S.J. LONG

Department of Pure & Applied Biology, University of Leeds, LS2 9JT

#### ABSTRACT

Some species of Carabidae, Staphylinidae and Nabidae were found to be voracious predators of spiders in the laboratory. Carabid density was manipulated in a field of winter wheat and the densities of Collembola, immature Araneae and predatory Acari were found to be greatest where carabid density was lowest. This may have resulted from predation by carabids, or from a numerical or aggregative response by the arachnids to their Collembola prey.

#### INTRODUCTION

There is now a general consensus for the need to develop biological pest control methods as a means of reducing chemical inputs in agriculture. Spiders, as generalist predators, have, for some time, been acknowledged as potentially valuable pest control agents in world agriculture (Riechert & Lockley, 1984). Money spiders (Linyphiidae) are extremely numerous in European crops (Sunderland, 1987) and can make a significant impact on cereal aphid populations in winter wheat (Sunderland *et al.*, 1986). Investigations were made, in the laboratory, of predation on spiders by other generalist predators, and manipulative experiments were done to investigate the impact of such predators on spider populations in the field. The results presented here are of relevance to programmes of predator augmentation. They must be regarded as preliminary because the laboratory observations were made under ecologically simplified conditions, atypical of field conditions, and the field trials were unreplicated.

#### MATERIALS AND METHODS

#### Laboratory observations of predation on spiders

Spiders and some of their potential predators were collected, by pooter, from a field of winter wheat in August and September 1993. They were stored individually on moist filter paper in 9 cm diameter plastic Petri dishes at room temperature in the laboratory. Before use in feeding observations (also at room temperature), predators were starved for a period sufficient to induce hunger but without causing mortality; this period varied considerably according to taxonomic group (Sunderland et al., 1987). A single predator was confined with a single spider and any immediate predation was recorded. Dishes were then re-examined at regular intervals (usually (i) <1 h after introduction of the predator, (ii) several hours later, (iii) the next morning, (iv) several times per day for up to two weeks) to check whether the spider had been eaten by the predator. Records of feeding at <1 h (Table 1) represent instances of observed predation, but for other records there is an increasing probability, with increase in elapsed time since introduction of the predator, that the spider may have died naturally and its body been eaten by the predator. In many cases the predator was also deliberately presented with a dead spider (and also other items of carrion, and mince) to check whether scavenging was part of its behavioural repertoire. Predators collected in autumn may have been in physiological preparation for overwintering, so a check was made to see if they would kill alternative foods, such as fruit flies, so that any individuals in an inappropriate condition for predation trials could be identified. The majority of predator species tested were offered a wide range of species and sizes of spider.

#### Predator density manipulation experiment in the field

This experiment was carried out in 1993 in a 3 ha field of winter wheat (cv "Haven") Three plots were on a silty brick earth soil at HRI Littlehampton, West Sussex. established on 17 March, 45 m from the field edge; the plots measured 22 m x 7.3 m (160.6 m<sup>2</sup>) and were separated from each other by 12 m. There was an unmanipulated field plot (F), a predator-reduced plot (R) and a predator-increased plot (I). Resources did not permit replication. The R plot was surrounded by a 45 cm deep trench, with a vertical inner wall and a gently sloping outer wall. Predators running on the ground surface and falling into the trench were more likely to leave the trench by the slope than the vertical wall, which would reduce their numbers in the plot. To increase the efficiency of this design, plastic-covered wooden boards (each 60 cm x 240 cm by 1 cm deep) were arranged horizontally above the vertical wall to give a 15 cm overhang. The I plot was identical to the R plot except that the vertical wall and overhanging wooden boards were on the field side of the trench, thus encouraging an increase of predators within the plot. On 6 April 231 pitfalls (7 x 9 cm plastic drinking cups containing a 4:1 mixture of water and ethylene glycol plus a little detergent) were established in a grid pattern within the R plot to further reduce numbers of surface-active predators. On 4 June a predator density sample was taken from all plots. This consisted of 10 x 0.1 m<sup>2</sup> sample units per plot. The method, similar to that of Topping & Sunderland (1994), involved taking a vacuum insect net sample (0.1 m<sup>2</sup>) followed by handsearching of the area of ground just sampled with the vacuum insect net. On 11 June pitfalls were removed from R and the wooden boards were moved into a

vertical position in R, I and F, to prevent further movement between plots and field by surface-active predators. A predator density sample  $(15 \times 0.1 \text{ m}^2)$  was taken on 20 July. Differences in the density of arthropods between plots were tested by Analysis of Variance.

#### RESULTS

#### Laboratory observations of predation on spiders

Results of the feeding trials are presented in Table 1. Amongst the Carabidae, P. melanarius and A. dorsale readily killed and ate spiders of a wide range of species and sizes and they also ate dead food (eg Araneae, Nabidae, Diptera, Staphylinidae, Carabidae) without hesitation. The other carabids were less voracious. T. quadristriatus, D. atricapillus, N. biguttatus and Bembidion spp. appeared to kill mainly hatchling spiders and the response to carrion varied between individuals. The staphylinid beetle Q. tristis was observed to kill and eat all species and sizes of spider offered, except for a large lycosid. In contrast, T. hypnorum killed no spiders but many refused to eat alternative foods, so they may have been preparing for overwintering. Earwigs (F. auricularia) were able to kill adult linyphiids and they also ate dead spiders. The heteropteran bug N. ferus was an efficient spider predator that successfully attacked any spider smaller than an adult Pachygnatha spp. The feeding trials showed that spiders of one species will sometimes kill spiders of another species (eg large immature lycosids killed adult linyphilds) and cannibalism is also possible. Cannibalism in Lepthyphantes tenuis has been observed amongst equal-sized hatchlings emerging from an eggsac, but most of the observations in Table 1 relate to cannibalism of hatchlings by conspecifics one instar larger.

#### Predator density manipulation experiment

On 4 June there were more than ten times as many adult Carabidae in I as in R (P < 0.001) (Table 2). Seven species of Carabidae were involved, the dominants being *N. biguttatus* and *T. quadristriatus*. Other predators (including Staphylinidae, beetle larvae, adult and immature Araneae, predatory Acari, Chilopoda and Dermaptera), belonging to at least 34 taxa, were not affected significantly. By 20 July at least 59 taxa of predators were present in the density sample and there were no significant differences between plots in the density of adult Carabidae. There were, however, 1.8 times more immature Araneae (P = 0.02) and 6.4 times more predatory Acari (P < 0.001) R as in I (Table 2). Densities of all other predators, including adult Araneae, did not differ significantly between plots.

*L. tenuis* was the only species of Araneae for which immatures could be identified to species. There were 2.4 times more (P = 0.02) immature *L. tenuis* in R as in I, but numbers of adult *L. tenuis* did not differ significantly between plots. Potential food categories (Aphididae, other Hemiptera, Diptera, Thysanoptera, Collembola) of polyphagous predators were recorded in the vacuum net sample of 20 July. The dominant category was Collembola . There were 1.9 times more (P = 0.01) Collembola in R as in I (both arthropleone and symphypleone Collembola were affected similarly), but densities of other food categories did not differ significantly between plots.

# TABLE 1. Laboratory observations of predation on spiders

Nu		Number of	Number of		% of observations					
Predator spe		individuals feeding						Failed to	Spider species killed	
		observed	observations	<1h	1h-1day	2-3days	>3days	<ul> <li>kill by</li> <li>&gt;3days</li> </ul>		
Carabidae	Pme	20	30	50	38	0	6	6	Lii,Lyi,Ci,Or,Bg,Pd,Pc	
	Ad	8	40	31	8	0	8	53	E,Lt,Oa,Bg,Pc	
	Nb	20	31	19	34	8	8	31	Lii,Or,Oa,Bg	
	Pma	19	31	11	24	18	29	18	Lii, Lyi, Pdi, Or, Dc, Bg, Of, Pd	
	Tq	13	38	3	37	9	18	33	Or,Of,Bg	
	Be	9	14	0	31	8	23	38	Or,Bg,Dc	
	Lp	3	7	0 °	20	0	20	60	Oa,Ci	
	Hr	8	24	0	15	0	8	77	Dc,Pd	
	Da	6	14	0	0	50	13	37	Or,Oa,Lt	
Staphylinidae	Qt	3	41	77	17	0	3	3	Lii, Lyi, Lt, E, Or, Oa, Bg, Dc, Pd, I	
	X	10	29	14	11	32	7	36	Lii,E,Or,Bg	
	Th	14	39	0	0	0	0	100		
Dermaptera	Fa	9	21	17	25	8	17	33	Lt,E,Dc,Oa,Mr	
Heteroptera	Nf	11	45	74	23	0	0	3	Lii,Ci,E,Bg,Dc,Lt,Oa	
Araneae	Lyi	3	15	50	14	7	7	22	Lii, Lt, Bg, Dc	
	Lt	8	9	33	33	0	11	23	Lt	
	Dc	3	8	20	20	20	20	20	Lii,Lt,Bg	
	Pd	3	6	0	34	33	0	33	Lii,Lt,Dc	
<b>KEY TO PRED</b> Pma = Pterostic Pme = Pterostic Be = Bembidion and Bembidion of	chus madidus chus melanariu i lampros	$\begin{array}{ll} x &= y \\ Xanthe \end{array}$	Agonum dorsal Cantholinus line Olinus longivent Nabis ferus	aris ar	nd Tq = r Th =	= Harpalu. = Trechus = Tachypo = Loricera	quadristri rus hypno	rum	Da = Demetrias atricapillus Nb = Notiophilus biguttatus Qt = Quedius tristis Fa = Forficula auricularia	
<b>KEY TO SPIDERS</b> Lii = Linyphiidae immature Pc = Pachygnatha clercki Or = Oedothorax retusus Bg = Bathyphantes gracilis		Ly Pd	= Erigone  spp. i = Lycosidae = Pachygnath a = Oedothorax	immatu a degeo	eri	Mr = Ci =	Meioneta Clubionid	<i>a concolor</i> <i>rurestris</i> ae immature <i>i</i> immature	Of = Oedothorax fuscu Lt = Lepthyphantes tenuis	

		I	F	R
4 June	Carabidae	5.0	1.9	0.4
20 July	Araneae imm.	9.5	11.5	16.9
	Predatory Acari	0.5	2.8	3.2
	L. tenuis imm.	2.8	4.5	6.7
	Collembola	26.4	43.6	51.2

TABLE 2. Mean number of arthropods,  $0.1 \text{ m}^{-2}$ , in a predator-reduced plot (R), a predator-increased plot (I) and an unmanipulated field plot (F).

#### DISCUSSION

The laboratory observations provide a first indication of the extent to which various species of predator are physically and behaviourally capable of killing and eating spiders; much more information would be required to determine which species actually do so, to any significant extent, in the field. Both N. ferus and Q. tristis were able to track spider movement very efficiently and they used bristles on the forelegs to secure their prey. The willingness to attack immobile spider prey (tested here by using carrion) is also of significance; spiders are extremely vulnerable to predation during moulting, and when paralysed by sub-lethal doses of insecticide (Everts et al., 1991). The horizontal barrier technique, as reported earlier (Sunderland et al. 1980), is an effective method for manipulating carabid density in the field. Recovery of adult carabids by 20 July in R was due to large numbers of T. quadristriatus which probably flew into the area. This migration is a well known phenomenon (Sunderland, 1992). When T. quadristriatus were excluded from the data, carabid densities per 0.1 m<sup>2</sup> were 1.3 in I, 0.9 in R and 0.8 in F. Densities of large carabids, such as P. melanarius (which was a voracious spider predator in the laboratory) were underestimated by the methods used here (Sunderland et al., 1994), and so the differences between plots, in carabid densities, may well have persisted to 20 July and beyond. Although treatments were unreplicated, it seems likely that the differences between plots in the density of Collembola, immature Araneae and predatory Acari were caused by the initial differences in the density of adult Carabidae resulting from the experimental manipulations. Carabids have previously been recorded to prey on Collembola (eg Ernsting, 1977), but there are few previous records of their impact on Collembola populations. The effect of carabids on small predators could result from (a) direct predation by carabids, (b) a numerical response to Collembola density, (c) redistribution of small predators in relation to prey density. The laboratory observations established that immature Araneae could be killed by most of the carabid species tested, and spider remains have been found in the guts of carabids collected from fields (eg Sunderland, 1975), but no quantitative data are available for carabid predation on spiders and mites under field conditions. The numerical response hypothesis is probably feasible for the period 4 June to 20 July because some linyphilds have a generation time as short as 40 days under optimal conditions (DeKeer & Maelfait, 1988). There is some evidence that adult linyphilds can aggregate in prey-rich areas, by showing a greater tendency to balloon when hungry (Weyman et al., 1994). However, an aggregative response is probably not the cause of the differences between treatments in this study because adult linyphiids were not affected; limited evidence, to date, suggests that adult linyphilds are more aeronautic than immatures (Sunderland, 1993). The observed results are most likely to be due to predation or a numerical response or a combination of these. Discovering the mechanism requires further work, but this study does suggest that one

group of predators can have a marked effect on the density of others; this has significance for programmes of predator augmentation and could have a knock-on effect to pest abundance (Rosenheim *et al.*, 1993).

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# THE APHID SEX PHEROMONE : A NOVEL HOST LOCATION CUE FOR THE PARASITOID *PRAON VOLUCRE*

# R. LILLEY, J. HARDIE

Department of Biology, Imperial College at Silwood Park, Ascot, Berks SL5 7PY

# W. POWELL, L.J. WADHAMS

Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ

# ABSTRACT

The sex pheromone of the blackberry-cereal aphid Sitobion fragariae, comprises a single monoterpenoid - (+)-(4aS, 7S, 7aR)-nepetalactone, which is released by sexual females (oviparae) to attract males. The aphid parasitoid *Praon volucre* (Hymenoptera : Braconidae) uses this sex pheromone as a host location cue. In the laboratory, female *P. volucre* were attracted to synthetic nepetalactone and to sexual aphids (oviparae). Male parasitoids did not respond to either cue and neither sex of parasitoid was responsive to asexual summer aphids (virginoparae) or autumn migrant aphids (gynoparae). Oviparae are only present in the field during autumn, however, female *Praon* are attracted to water traps releasing nepetalactone from mid-June through to the end of November. Simultaneous suction trap catches indicate the presence of both male and female *Praon*, suggesting that only female parasitoids, possibly searching for aphid hosts in which to oviposit, are responding to nepetalactone. Aphid parasitoids of the genus *Aphidius* were also collected in water traps, but did not show a preference for traps releasing nepetalactone.

# INTRODUCTION

The monoterpenoid (+)-(4aS,7S,7aR)-nepetalactone has been identified as a component of the sex pheromone of a number of aphid species (Dawson *et al.*, 1987; Pickett *et al.*, 1992) and is the only constituent in the sex pheromone of the blackberry-cereal aphid, *Sitobion fragariae* (Hardie *et al.*, 1992). Aphid parasitoids of the genus *Praon* (Hymenoptera : Braconidae) have been shown to respond to nepetalactone, both in the field (Hardie *et al.*, 1991; 1994) and in the laboratory (Lilley *et al.*, 1994). In the present laboratory studies with a Pettersson olfactometer, the behaviour of male and female *P. volucre* was observed when exposed to air passing over three different adult morphs of *S. fragariae*; oviparae, virginoparae and gynoparae. In a field study, clear water traps were used to investigate the responses of aphid parasitoids to nepetalactone released from glass vials.

# METHODS AND MATERIALS

# Insect rearing

A clone of S. fragariae was established from a single individual collected

from blackberry, *Rubus fruticosus*, at Dornoch, Scotland in the spring of 1992. Virginoparae were reared on barley, *Hordeum vulgarae* var. *Igri*, at 20±2°C in long days (LD 16:8). Gynoparae and oviparae were produced by transferring aphids to short days (LD 11:13) and collecting the first and second generation of aphids respectively.

Adult aphids were used in all experiments. Parasitoids were maintained on a clone of the grain aphid, *Sitobion avenae*, on barley and individual mummies were collected and isolated until emergence. Virgin wasps, 2-3 days old, were used in the laboratory bioassay.

#### Laboratory bioassay

A Pettersson olfactometer was used to assess parasitoid responses to the three different aphid morphs. Five aphids of each morph were transferred to a single excised leaf of either barley (for virginoparae) or blackberry (for gynoparae and oviparae), which was inserted into moist sorbarod (Ilacon U.K. Ltd). Aphids were allowed to settle and begin feeding before each leaf was placed in a 100 ml glass bottle, from which air was drawn into the olfactometer. A single aphid treatment was run against three excised leaf controls. In all experiments, wasps were released individually into the centre of the olfactometer and their activity observed for 12 minutes at  $20\pm1^{\circ}$ C. The number and duration of visits to each odour field (see Vet *et al.*, 1983) was recorded. The olfactometer was thoroughly cleaned after each replicate and rotated through 45° to remove any directional bias.

#### Field trial

Glass vials (08-CPV, Chromacol) with a 1 mm hole drilled in the plastic lid were prepared with either 10 mg nepetalactone in 50  $\mu$ l diethyl ether or without nepetalactone. Two nepetalactone and two control water traps were set up at four sites in Silwood Park in April 1993. Traps were checked three times a week and aphid parasitoids collected. Vials were changed every two weeks from April to November 1993. At two of the trap sites, 1.1 m high suction traps were used to sample air (326 m<sup>3</sup> per hour) and were emptied three times per week.

#### RESULTS

#### Laboratory bioassay

Female *P. volucre* showed a significant preference for air passing over oviparae and made more entries for longer periods into this odour field compared to controls (Table 1).

TABLE 1. Response of <i>P. volucre</i> to five adult sexual females (oviparae) of <i>S.</i>	
fragariae on an excised blackberry leaf (Treatment) with blackberry leaf controls	
(Control) in a Pettersson olfactometer.	

When a set and work there are a to the second of			
Activity	Sex	Treatment	Control
Number of visits	F	6.1±1.0**	2.7±1.2
Time spent (min)	F	8.4±0.4**	$1.2 \pm 1.5$
Number of visits	М	$9.0\pm2.0$	9.2±1.7
Time spent (min)	М	$3.1\pm0.6$	3.0±0.5

In contrast, male parasitoids showed no preference for this treatment. Neither sex responded to odours from virginoparae (Table 2) or gynoparae (Table 3). Results are presented as the mean number of visits or mean time spent in each odour field for eight individual insects per experiment.

TABLE 2. Response of *P. volucre* to five adult summer forms (virginoparae) of *S. fragariae* on an excised barley leaf (Treatment) with barley leaf controls (Control) in a Pettersson olfactometer.

Activity	Sex	Treatment	Control
Number of visits	F	6.8±2.0	5.7±1.9
Time spent (min)	F	$2.4 \pm 1.0$	$2.2\pm0.9$
Number of visits	Μ	7.8±3.2	$5.1 \pm 1.8$
Time spent (min)	Μ	$2.8\pm0.8$	2.1±0.6

TABLE 3. Response of *P. volucre* to five adult autumn migrants (gynoparae) of *S. fragariae* on an excised blackberry leaf (Treatment) with blackberry leaf controls (Control) in a Pettersson olfactometer.

Activity	Sex	Treatment	Control
Number of visits	F	7.3±2.3	5.8±1.7
Time spent (min)	F	3.6±1.3	$2.8\pm0.7$
Number of visits	Μ	9.9±2.9	9.7±2.2
Time spent (min)	Μ	$2.9 \pm 0.5$	3.0±0.6

\*\* P< 0.01, Chi-square test . \*\* P<0.01 T-test

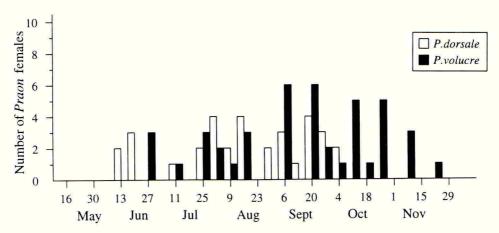


Figure 1. *Praon* spp. females caught in water traps associated with (+)-(4aS, 7S, 7aR)-nepetalactone from July to November 1993.

Aphid parasitoids of the genera *Aphidius* and *Praon* were caught in water traps during the field trial. Two *Praon* species, *P. dorsale* and *P. volucre*, were caught in water traps from the middle of June to the end of November (Figure 1). Only female *Praon* were caught and all were found in traps releasing nepetalactone (*P. dorsale* = 34, *P. volucre* = 43). In comparison, catches of male and female *Aphidius* spp. were restricted to an eight week period from the middle of May until the middle of July. Only seven male and five female *Aphidius* spp. were collected, of which four and two individuals respectively were found in traps releasing nepetalactone.

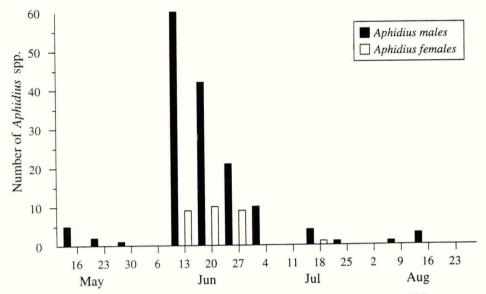


Figure 2. Aphidius spp. caught in suction traps from July to November 1993.

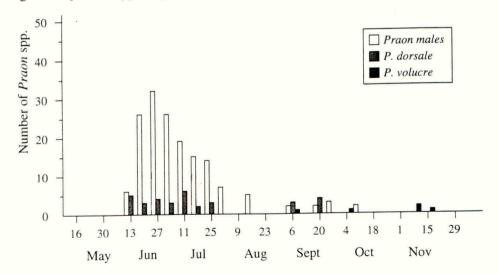


Figure 3. Praon spp. caught in suction traps from July to November 1993.

Suction traps caught both *Aphidius* and *Praon* spp. parasitoids together with small numbers of male and female *Trioxys* spp. Male parasitoids predominated in suction trap catches, with males comprising 85 % of the total *Aphidius* catch (N = 167) (Figure 2) and 81 % of the total *Praon* catch (N = 159) (Figure 3). More female *P. dorsale* (N = 24) than *P.volucre* (N = 4) were found in suction trap collections. Most parasitoids were caught in suction traps in June and July with male *Aphidius* recorded as early as the middle of May and with *P. volucre* females present until the middle of November.

#### DISCUSSION

In the laboratory bioassay, female *P. volucre* were attracted to odours from oviparae of *S. fragariae*, but did not show this response to either virginoparae or gynoparae. Previous studies have shown that *S. fragariae* oviparae release the sex pheromone (+)-(4a*S*, 7*S*, 7a*R*)-nepetalactone to attract males (Hardie *et al.*, 1992) and that this compound is also attractive to aphid parasitoids of the genus *Praon* in the field (Hardie *et al.*, 1991; 1994). The responses of female *P. volucre* indicate that the oviparae are releasing a pheromone which is acting as an attractant for the parasitoid. Male *P. volucre*, which are not attracted to nepetalactone in the field, are unresponsive to oviparae in the olfactometer. A number of studies have indicated that aphid parasitoids can detect odours from the host / plant complex (e.g. Wickremasinghe and van Emden, 1992), however, this response is usually developed following previous encounters (e.g. Turlings *et al.*, 1992). In contrast, responses of inexperienced *Praon* females to nepetalactone (Lilley *et al.*, 1993; 1994) and oviparae of *S. fragariae* which release nepetalactone, indicate that they have an innate attraction this compound.

In the field study, females of two *Praon* species, *P. dorsale* and *P. volucre*, were identified in both water and suction trap catches. Female *Praon* were only found in water traps releasing nepetalactone, confirming earlier observations of the attraction of this species to this compound (Hardie *et al.*, 1991; 1994). *Praon* males were not found in water traps, however, more male than female *Praon* were collected in suction traps. This indicates that males were active close to water trap sites, but were unresponsive to nepetalactone. Few *Aphidius* spp. were found in water traps (N=12), despite their presence in relatively large numbers in suction trap catches. This contrasts with a recent laboratory study in which male and female *Aphidius matricariae* and male *Aphidius ervi* were shown to be attracted to nepetalactone (Hardie *et al.*, 1993). Responses of *Aphidius* spp. in the field may be more subtle than those of *Praon* spp. and this is currently being investigated further.

The observed responses to aphid sex pheromone offer opportunities to manipulate *Praon* populations in the field with a view to improving aphid control. In a preliminary study, Lilley *et al.* (1994) placed aphid colonies in the field close to nepetalactone release sites to monitor the resulting levels of parasitism. This work indicated that parasitism by *Praon* spp. is increased in colonies associated with nepetalactone, when compared to control colonies. An aphid control strategy involving the use of nepetalactone to attract *Praon* females into areas close to crops, which have been seeded with non-pest host aphids, is being developed. These parasitoids would form a reservoir population which would move into the crop and limit the rate of aphid population development.

This would be of particular importance in the early part of the season when synchrony between colonising aphids and parasitoids is essential for subsequent control. Additionally, nepetalactone may prove useful in monitoring the presence of *Praon* females in the field, both to assess the impact of these parasitoids on aphid populations and to provide information for developing I.P.M. strategies.

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# A STUDY OF THE EFFECTIVENESS OF CROP COVERING WITHIN IPM, USING AN AMOCO NON-WOVEN FLEECE AS A BARRIER TO APHIDS, WHITEFLIES AND THEIR ASSOCIATED PLANT VIRUSES

# I.D.BEDFORD, P.G.MARKHAM

Department of Virus Research, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH

# P.A.STRAUSS

Growing Enterprises U.K., Strella House, 385 Alfreton Road, Nottingham, NG7 5NW

#### ABSTRACT

The recent rise in insecticide resistant strains of insects has resulted in unprecedented infestations and damage to certain crops worldwide. Their control is now involving the use of Integrated Pest Management systems. Results from testing the effectiveness of a non-woven fleece, as a physical barrier to *Myzus persicae* and *Bemisia tabaci* has demonstrated its use as an integral part of IPM. This crop-covering is shown to exclude both *M. persicae* and *B. tabaci* and also prevent them feeding through the covering to transmit cauliflower mosaic virus and bean calico mosaic geminivirus respectively. Further foreseeable uses for this type of crop-covering as a contained environment for both field and glasshouse pest management are discussed.

# INTRODUCTION

In areas where insecticides have often been over-used, pests have developed resistance and in an environment often free from predators and parasites, which had been killed by the insecticides, these resistant strains have increased to cause serious infestations. The most damaging insect pests to world agriculture include aphids, leafhoppers, thrips and whiteflies, mainly because members of these groups can acquire and transmit plant viruses. Concern is presently being shown worldwide over the appearance of a highly resistant strain of the tobacco whitefly Bemisia tabaci called the "B" biotype. (Bedford et al., 1993). B. tabaci has always been a pest of about 300 different plant species worldwide (Mound & Halsey, 1978). each distinct regional population however, has only been associated with a few plant species. These regional populations still have the potential to transmit most whitefly-transmitted geminiviruses (Bedford et al., 1994a). The "B" type, however, has a vastly increased host range (Wool & Greenberg, 1990) of around 600 plant species. It is also more fecund (Bethke et al., 1991) and can cause phytotoxic damage (Bedford et al., 1992). Its spread is associated with the trade in ornamental plants such as poinsettias and cut flowers, and it is now found in the Americas, the Caribbean basin, the Middle East, South Africa, Japan, southern Europe and the glasshouses of northern Europe (Bedford et al., 1993). Its appearance in southern USA in 1991 caused around \$500m damage to the winter harvest (Perring et al., 1993). Crops in areas of Europe where the "B" type has appeared are now at risk from B. tabaci infestation and in some cases, phytotoxic damage and virus infection (Bedford et al., 1994c).

As yet, only one *B. tabaci*-transmitted virus, tomato yellow leaf curl, is found in Europe, however, many European crops are already under attack from virus infection and feeding damage caused by other insect pests such as aphids. Crops susceptible to insect-transmitted viruses usually require the feeding of only a few viruliferous insect vectors to become infected. Total eradication of these vectors is virtually impossible once they have become established within a crop and so the virus infection continues to spread.

Viruses transmitted by whiteflies are presently causing total losses to melon crops in the Yemen (Bedford *et al.*, 1994b), and large losses to tomato crops throughout the Middle East, the Americas, Asia and the Mediterranean (Mansour & Al Musa, 1992). Subsistence crops in Africa such as cassava are affected (Fargette *et al.*, 1993), and in Pakistan over 1 million hectares of cotton is affected by a whitefly-transmitted virus (Mansoor *et al.*, 1993).

Aphids cause massive losses to crops throughout the world through feeding damage and virus transmission. They too exhibit a strong predisposition to develop resistance to insecticides. One of global agricultures most important aphids is the cosmopolitan peachpotato aphid (*Myzus persicae*). It is capable of transmitting in excess of one hundred and eighty different viruses (Kennedy *et al.*, 1962). In order to reduce the risk of evolving resistant insect strains and placing harmful toxins within the food chains integrated control strategies should be used whenever possible. This is most necessary where several insect problems are common such as in the hotter climates of the Middle East, Africa and Asia when early crop protection is essential to prevent high or total yield loss. This study was undertaken to establish whether  $MyPex_R$  crop cover (Amoco Fabrics), a non-woven  $17g/m^2$  polypropylene fleece, can offer essential protection to certain crops as part of integrated pest management. It aimed to show whether this fleece could act as an effective barrier against pests such as the whitefly *B. tabaci* and the aphid *M. persicae*. It also investigated the possibilities of these insects feeding through the fleece and transmitting two associated plant viruses, bean calico mosaic geminivirus and cauliflower mosaic caulimovirus, respectively.

#### METHODS AND MATERIALS

#### Origins and maintenance of insects

The tobacco whitefly *B. tabaci* ("B" biotype), was collected in California USA in 1991 and kept under quarantine conditions within an insectary. They were maintained in culture as colonies on cotton, *Gossypium hirsutum* var. Delta Pine at  $25^{\circ}$ C and 16h daylength.

Colonies of the peach-potato aphid *M. persicae* were maintained in culture on turnip plants, *Brassica campestris* var. Just Right, at 22°C and 16h daylength.

# Origin and maintenance of viruses

Viruses used in these tests were:- (i) bean calico mosaic virus (Brown et al., 1990), a whitefly-transmitted geminivirus infecting *Phaseolus* beans in the Americas which was maintained in french bean plants, *P. vulgaris* var. Top Crop by insect transmission and (ii) cauliflower mosaic virus (Day & Venables, 1961), an aphid transmitted caulimovirus found worldwide in *Brassica spp.*, maintained in turnip plants, *B. campestris* var. Just Right, by mechanical inoculation and by insect transmission.

# Comparison of fleece inter-fibre sizes with insect sizes

To compare the inter-fibre sizes of the fleece in relation to the insects under test, a Cam Scan series 4 scanning electron microscope was used. Juvenile and adult aphids were observed and photographed on the fleece using the microscope cold stage at  $-180^{\circ}$ C.

Whiteflies could not be viewed successfully by scanning electron microscopy since the high vacuum within the microscope distorted their wings. Macro-photography using a Sinar revolving horseman back camera, Leitz lenses and a fibre optic light source was used instead.

# The fleece as a physical barrier to insect movement

Ten 9 cm plastic petri dish bases were sealed with a layer of the fleece taped around the rim. Through a hole in the base 200 whiteflies were put into 5 of the dishes and 200 aphids into the remaining 5 dishes. The holes were then sealed with cotton wool. The dishes were placed under cotton plants in a perspex cage (90cm x 45cm x 45cm) and left at  $25^{\circ}$ C with 16h daylength and observed every 2 days for insects that had passed through the fleece.

The fleece as a physical barrier to the insect transmission of plant viruses.

# (a) "Choice" test.

Trays of 15 (7x7cm) plastic pots were sown with turnip, *B. campestris* var. Just Right, one per pot. When germinated, one tray was covered with a sleeve of fleece and placed inside a perspex cage next to an uncovered tray. About 2,000 *M. persicae*, fed for 3hrs on a cauliflower mosaic virus infected turnip plant, were released into the perspex cage. The experiment was left under 16h florescent illumination at 22°C.

This test was repeated with french beans, *P. vulgaris* var. Top Crop using about 2,000 whitefly, *B. tabaci*. Whitefly were kept as a stock colony on french beans infected with bean calico mosaic virus. This was left at  $25^{\circ}$ C with 16h florescent illumination. After 15 days the fleece was removed from all covered trays and plants checked for insects. All plants from the covered and open trays were then fumigated and moved to a glasshouse where infected plants were recorded. Tests were replicated three times.

# (b) "No choice" test.

To see if aphids and whiteflies could feed through the fleece and transmit viruses, a "no choice" experiment was designed. Two petri dish cages, as used in the "physical barrier" experiment, were clamped either side of a turnip leaf on a young plant (fleece sides touching the leaf). Twenty *M. persicae*, fed on cauliflower mosaic virus infected turnip plants for 3hrs, were put into each of the cages and left under 16h florescent illumination at 22°C until they died. Plants were then moved to a glasshouse where they were observed for infection.

This test was repeated using french bean plants and 50 whitefly, *B. tabaci* "B" biotype, per cage, previously fed for 24 hours on beans infected with bean calico mosaic virus.

Control tests, allowing viruliferous insects to feed on an unprotected leaf on plants of the same age as those tested with the fleece were also made. Tests were repeated five times.

#### RESULTS

# Comparison of fleece inter-fibre sizes with insect sizes

Scanning electron microscopy revealed the irregular structure of the fleece and showed that the inter-fibre distances were small enough to exclude adults and juveniles of M. *persicae*. Macro-photography showed that it should also exclude the whitefly B. *tabaci*. Occasional less dense areas were found in the fleece, but the inter-fibre spaces were still small enough to exclude M. *persicae* and B. *tabaci*. The smallest aphid seen in these tests, was 0.744mm in length x 0.423mm in width and the smallest whitefly 1.150mm in length x 0.405mm in width. The larger gaps between the fleece fibres measured 0.60mm x 0.40mm.

# The fleece as a physical barrier to insect movement

All insects contained within the petri dish cages failed to pass through the fleece and subsequently died in the cages within 2 days.

The fleece as a physical barrier to the insect transmission of plant viruses.

#### (a) "Choice" test.

After 15 days, all turnip plants within protected trays were free from infestation by *M. persicae*. One covered plant however, became infected with cauliflower mosaic virus. All non-protected control plants were heavily infested and all were infected with the virus which resulted in a high level of stunting and mortality of the plants.

Results from testing covered french beans against B. tabaci viruliferous with bean calico mosaic virus showed all to be free from infestation and infection. Uncovered plants however, were all infested with eggs, larvae and adult B. tabaci and all were infected with the virus.

Leaves on these covered plants were noticeably crumpled, but in general, plant growth was not significantly stunted during this test period.

#### (b) "No choice" test.

*B. tabaci* and *M. persicae*, viruliferous to bean calico mosaic virus and cauliflower mosaic virus respectively, failed in all cases to transmit these viruses through the fleece to the test plants. All control plants exposed to insects without the fleece became infected.

#### DISCUSSION

These results have clearly shown that this non-woven fleece can be used as an effective barrier against *M. persicae*, and *B. tabaci*. Within these tests, these insects were unable to pass through the fleece. We have also shown that the fleece is an effective barrier against the feeding of these insects on a covered crop and the subsequent transmission of an associated plant virus. Scanning electron microscopy however, identified occasional areas within the fleece where the fibre was noticeably less dense. Although the inter-fibre distances appeared small enough to prevent *M. persicae* and *B. tabaci* passing through, the possibility of them feeding through these areas and transmitting viruses could not be dismissed and would require further investigation. This could explain the appearance of one infected turnip plant

in the covered trays in the "choice test" study. Amoco Fabrics have subsequently advised that technical modifications to the production equipment will be made to overcome this. The no-choice tests where the fleece contained no weak areas, showed that under these conditions insects were deterred from feeding and transmitting viruses into the covered leaves.

Under field conditions the effectiveness of this fleece will depend on other important factors. Primarily, the standard to which it is applied to the crop will govern whether pests could ingress and secondly the condition of the soil and plants at the time of covering will reflect the future status of the protected crop. If this crop covering is properly applied when seeds or seedlings are first sown or planted into the field, it will repel a future infestation from aphids and whiteflies throughout its recommended lifetime. It should however be suggested, that crops and soil are initially sprayed with insecticide before covering to remove pests that may already be infesting the area. Further treatments against smaller pests such as thrips and mites, that could possibly pass through the fleece, should also be considered.

Results from field trials with a similar fleece on courgettes in Morocco, showed that in its absence, all the harvested courgettes showed signs of infection from aphid transmitted viruses and only 2.3% were saleable (25g per plant). By using a fleece, yield was significantly increased (1,228g per plant), although the best results were obtained when the fleece was removed as plants reached the flowering stage (1,693g per plant) (Reyd *et al.*, 1993). A study to compare different types of coverings for protecting Californian honeydew melons, has shown that different products vary in their level of effectiveness. However, all coverings resulted in increased fruit yield (Natwick & Laemmlen, 1993).

Possibilities now exist for extending the uses of this covering within modern crop protection. This fleece, applied to crops that are already infested and where chemical application is undesirable, could provide a contained and protected environment suitable for Integrated Pest Management. Beneath the fleece, specific predators and parasites could be introduced along with chemicals designed to harm pests and not the beneficials. The fleece could also enable fungal pathogens such as *Verticillium lecanii* to be used in pest control (Helyer, 1993). Irrigation spray pipes laid under the covering could deliver the fungal pathogens to the underside of crop leaves and increase the humidity around the crop to the levels required for these pathogens to be most effective (Helyer *et al.*, 1992). It has already been shown in field trials that by using a non-woven fleece on crops, a humid microclimate can be retained much better than in uncovered crops (Reyd *et al.*, 1993). However, it is apparent that certain crops, mainly those that grow nearer ground level, are more suited to complete covering than others. Taller crops may present a greater problem with continuous cover, but could benefit from an early covering or selected later coverings in order to provide contained environments for establishing an IPM system.

The use of a non-woven fleece on field crops as a barrier to indigenous insect pests is already essential throughout the Middle East. The possibilities now exist to extend the role of this material as a major component within Integrated Pest Management of field and glasshouse crops throughout the world.

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# THE ROLE OF CROP HUSBANDRY PRACTICES IN WHEAT PRODUCTION FOR DISEASE CONTROL

# L. BASTIAANS

DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands Netherlands Grain Centre (NGC), Costerweg 5, 6702 AA Wageningen

# R.A. DAAMEN

DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands

# ABSTRACT

An inventory of cropping practices at integrated and conventional farms was made to characterize wheat production in both farming systems. Integrated cropping of winter wheat was characterized by a broad crop rotation, late sowing, no application of growth regulators, mechanical weed control and a reduced input of pesticides. Only small differences were found in choice of cultivar and nitrogen application rate. The comparison also included surveys, in which the prevelance and incidence of the major diseases and pests of winter wheat were determined. The reduced number of pesticide applications at integrated farms did not result in a higher disease pressure. For *Septoria tritici* integrated wheat production resulted in a clearly reduced disease intensity, whereas slight reductions of stem-base diseases were observed.

# INTRODUCTION

Integrated farming tries to combine ecological and economic objectives, by reducing chemical inputs while maintaining net financial return. This objective should be achieved by substituting expensive and potentially polluting inputs, like fertilizers and pesticides, with both agricultural and ecological knowledge, labour and non-chemical husbandry techniques (Vereijken, 1989). Wijnands and Darwinkel (1990) formulated a strategy for wheat production within an integrated farming system. Prevention of diseases, pests and weeds should result in a reduced dependence on pesticides. A minimum use of pesticides and a reduced supply of fertilizers should result in a reduction of costs and an enhanced environmental safety. Finally, the production of wheat with a good baking quality should be stimulated to improve both market position and financial return. Based on these objectives, an integrated crop protection strategy for cereal diseases in winter wheat was formulated (Wijnands & Darwinkel, 1990). This strategy consists of two elements. Prevention of diseases and pests is realised through various crop husbandry practices, such as a broad crop rotation, resistant cultivars, late sowing, no application of growth regulators and a moderate N-fertilization. In situations where the preventive measures are inadequate to keep disease intensity at a low level,

pesticides are used as last resort.

Between 1974 and 1986, systematic annual surveys of diseases and pests in winter wheat were conducted in the Netherlands (Daamen, 1990). In 1993 a new project was started as a follow-up to the previous disease surveys. The objectives of this project are to determine whether a large scale introduction of integrated wheat production will result in a shift in the importance of the major diseases and pests in winter wheat, and to evaluate the effectiveness of various crop husbandry practices for disease control at the farm level. Disease surveys were conducted in wheat fields on conventional and integrated farms. The surveys were combined with an inventory of cropping practices. In this paper the results of the first year of the project are presented. Differences between conventional and integrated wheat production in the Netherlands are summarized and the intensity of the major diseases and pests of winter wheat in conventional and integrated farming systems are compared.

#### MATERIALS AND METHODS

In the Netherlands, the development of an integrated arable farming system was started in 1979 at the national experimental farm for development and comparison of alternative farming systems at Nagele (Vereijken, 1986). Between 1989 and 1993 a project was conducted in which the concept of integrated arable farming was validated at 38 pilot farms, located all over the country. This project was jointly conducted by the Research Station for Arable Farming and Field Production of Vegetables (PAGV), Research Institutes (CABO-DLO, LEI-DLO) and the Extension Service (IKC, DLV). The associated study described in this paper was conducted in 1993. Single wheat fields on 28 of the pilot farms, mentioned above, were used as experimental units. The combined results from all fields were used to represent the integrated farming practice. Similarly, an identical number of wheat fields at farms in the neighbourhood of the pilot farms were selected and used as representatives of the conventional farming practice. The paired location of fields was used to minimize differences in soil type and weather conditions between fields used to represent both farming systems. Accordingly, differences in disease intensity between farming systems can be attributed to differences in cropping practices.

Crop husbandry practices in the selected wheat fields were collected through questionnaires. The questionnaire requested information on soil type, land preparation, crop rotation, seed quality, choice of cultivar, fertilization, weed control and application of growth regulators and pesticides. Disease surveys were carried out four times a year (during stem elongation [Decimal Code (DC) 30-31 (Zadoks et al., 1974)], around booting [DC 38 - 57], around anthesis [DC 61 - 73] and during milk development [DC 75-77]). In all fields an area of 250 x 50 m was selected for sampling and for each survey 50 tillers were collected along a diagonal transect across the selected area. Tillers were screened for symptoms of stem-base, leaf and ear diseases, and for symptoms of injury by insect-pests. Disease intensities for the two farming systems were expressed as the fraction of fields with disease symptoms (incidence). The latter measure was expressed on a basis of infected

fields only. For stem-base diseases and cereal aphids a tiller was used as basic plant unit. For leaf diseases and insect-pests a green leaf was used as basic plant unit. For ear diseases an ear was divided in about forty segments, depending on the size of the ear. In this case a basic plant unit consisted of glumes of all kernels in a segment.

#### RESULTS

#### Crop husbandry practices

Preventive measures that were employed at integrated farms were a broad crop rotation, late sowing and no application of growth regulators (Table 1). Differences in choice of cultivar were small and induced by cultivar differences in baking quality, rather than by differences in disease resistance. Differences in N-fertilizer application rate were remarkably small.

A clear reduction in the number of pesticide applications was observed in the integrated farming system. The difference between farming systems resulted from a reduced number of fungicide applications against foliar diseases (mainly powdery mildew) before flowering, and a reduced number of insecticide applications against aphids during grain filling. In total, the number of pesticide applications was reduced by 35%. An even larger reduction was observed for the amount of active ingredient (60%). This extra reduction was realized by using pesticides with a lower amount of active ingredient (fungicides and insecticides) and by applying pesticides in a reduced dosage (insecticides).

At 40% of the integrated farms mechanical weed control was employed, with on average 2-3 treatments per crop. This represents a clear difference from the conventional farms, where mechanical weed control was employed at only 10% of the farms and all with only a single treatment. Consequently, the number of herbicide applications was reduced on integrated farms. The difference between the two farming systems was fully explained by the mechanical weed control employed at integrated farms, since no obvious differences were found between conventional farms, and integrated farms without mechanical weed control.

#### **Disease** intensity

The results of the fourth disease survey, conducted during milk development, are presented in Table 2. The results are a good representation of the differences observed during the first three disease surveys. Disease intensity of stem-base diseases on wheat grown in the conventional farming system was generally higher than on wheat produced in the integrated system. For brown foot rot (*Fusarium* spp.) and eyespot (*Pseudocercosporella herpotrichoides*) the differences were only marginal. However, prevalence of sharp eyespot (*Rhizoctonia cerealis*) was considerably higher in the conventional farming system.

For foliar diseases an obvious difference in incidence of speckled leaf blotch (*Septoria tritici*) was observed, with incidence in the conventional farming system about twice as high as in the integrated sytem. This difference was observed from

Table 1. Crop husbandry practices related to wheat production in conventional and integrated farming systems. The characteristics of each farming system is based on an inventory of cropping practices at 28 farms, conducted during 1993.

	Conventional farming system	Integrated farming system
Crop rotation		
- proportion wheat in crop rotation	0.36	0.27
- years since previous wheat crop	3.0 years	4.3 years
Choice of cultivar		
- proportion fields with Ritmo or Vivant	0.80	0.50
- proportion fields with other cultivars	0.20	0.32
- proportion fields with cultivar mixtures	0.00	0.18
Sowing date - average sowing date Growth regulator	November 3	November 20
- proportion fields with $\geq 1$ application	0.71	0.14
N-fertilization		
- average N-application rate	182 kg ha <sup>-1</sup>	172 kg ha <sup>-1</sup>
- number of applications	2.7	2.9
Fungicides and Insecticides - number of sprayings used to control		
- eyespot	0.07	0.00
- leaf diseases	0.64	0.25
- leaf and ear diseases	0.93	0.89
- aphids	<u>0.68</u> 2.32	<u>0.39</u> 1.53
- total amount of active ingredient	1.39 kg ha <sup>-1</sup>	$0.55 \text{ kg ha}^{-1}$
Weed control - mechanical - proportion fields with mechanical weed		
- proportion needs with mechanical weed	0.07	0.40
- number of treatments per treated field	1.0 x	2.5 x
- herbicides	1.0 A	2.0 /
- number of sprayings to control weeds	1.50	1.14
- total amount of active ingredient	1.90 kg ha <sup>-1</sup>	1.11 kg ha <sup>-1</sup>

the first disease survey onwards. For the other foliar diseases, ear diseases and insect-pests no obvious differences between the two farming systems were found.

Table 2. Proportion of fields with disease symptoms (prevalence), and proportion of basic plant units with disease symptoms in infected wheat fields (incidence) of crops grown in conventional and integrated farming systems, from the 4th disease survey (DC 75-77). The definition of a basic plant unit differs per disease and is specified in the text.

	Prevalence		Incidence	
Disease/pest	conventional	integrated	conventional	integrated
Stem-base diseases				
- brown foot rot	1.00	1.00	0.26	0.20
- eyespot	0.71	0.57	0.16	0.16
- sharp eyespot	0.57	0.25	0.09	0.08
Foliar diseases				
- yellow rust	0.04	0.00	0.02	0.00
- brown rust	0.36	0.36	0.20	0.25
<ul> <li>powdery mildew</li> </ul>	0.96	0.96	0.23	0.27
- speckled leaf blotch	0.93	0.86	0.25	0.13
- leaf blotch	0.61	0.64	0.07	0.08
- snow mould	0.46	0.50	0.04	0.07
Ear diseases				
- glume spot	0.07	0.00	0.00	0.00
- head blight	0.79	0.71	0.02	0.02
<ul> <li>powdery mildew</li> </ul>	0.43	0.29	0.05	0.15
Insect-pests				
- cereal leaf beetle	1.00	1.00	0.16	0.18
- leaf miner	0.96	1.00	0.10	0.11
- aphids	0.96	1.00	0.47	0.53

#### DISCUSSION

During 1993 cropping practices and disease intensities of wheat grown in conventional and integrated farming systems were determined and compared. Results of a single growing season are inadequate to serve as a basis for general conclusions. Nevertheless, the inventory of cropping practices gives a good indication of the most important differences between conventional and integrated wheat production in 1993. The differences in cropping practices between both farming systems did not result in a clear change in the importance of the major diseases and pests of winter wheat. Furthermore, a reduced use of pesticides did not result in increased disease pressure. Differences in disease intensity were limited to a reduced intensity of speckled leaf blotch and slight reductions in intensity of

stem-base diseases at integrated farms. Yield estimates by the farmers demonstrated that in 1993 the average grain yield at integrated farms was about 0.9 t ha<sup>-1</sup> lower than on conventional farms (8.4 and 9.3 t ha<sup>-1</sup>, respectively). Assuming an equal price per tonne, the lower yields are insufficiently compensated for by cost reduction based on the lower inputs of pesticides and fertilizers. However, the existance of (local) organizations willing to pay a higher price per tonne, provided the wheat is grown according to specified integrated (or ecological) production practices, makes it impossible to draw a general conclusion on the economics of conventional and integrated wheat production.

Although average differences in disease intensity between conventional and integrated farms were small, clear differences were observed between individual farms. The same was found for differences in cropping practices. The variation in disease intensity and cropping practices among individual farms will be used to evaluate the effectiveness of various crop husbandry practices for disease control at the farm level. A preliminary analysis of differences in incidence of speckled leaf blotch illustrates this approach. The analysis demonstrated that large differences in incidence of speckled leaf blotch between neighbouring farms were mainly present between farms with a large difference in sowing date. Late sowing resulted in a reduced incidence of speckled leaf blotch. A major objective of this project is to define such relationships, which ultimately should result in the development of an integrated control strategy for cereal diseases and pests in winter wheat. It is only then that the replacement of pesticides by non-chemical husbandry techniques will gain wide acceptance.

#### ACKNOWLEDGEMENTS

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# UNDERSOWING BRASSICAS WITH CLOVER TO INCREASE THE ACTIVITY OF CARABID BEETLES

# G. ARMSTRONG

SAC Aberdeen, 581 King Street, Aberdeen, AB9 1UD

## R.G. McKINLAY

SAC Edinburgh, West Mains Road, Edinburgh, EH9 3JG

# ABSTRACT

Carabid beetles, which are beneficial predators, were collected in significantly higher numbers by pitfall trapping, from plots of swedes undersown with clover, whether these were treated with insecticide or not, than from swedes grown on bare, herbicide-treated soil. However, in organic cabbages, most of the species of carabid present were collected in greater numbers from plots with a natural weed cover than from plots undersown with clover. In both the swedes and cabbages, cabbage root fly damage was not reduced by undersowing, probably because the plant cover had not developed before the first generation flies appeared. The yield of cabbages was less from the undersown plots, and so in this case, undersowing gave no advantage over the natural weed cover in the nonundersown plots.

# INTRODUCTION

Carabid beetles (Coleoptera: Carabidae) are major predators of the eggs and larvae of the cabbage root fly (Delia radicum) (Coaker, 1965), which is the most important invertebrate pest of root and leaf brassicas in Scotland. The pest usually has two generations a year of which the first can kill seedlings and the second can severely scar the developing root brassicas. It is normally controlled by insecticidal granules and sprays, but increasingly, the market is demanding minimal use of pesticides. A possible alternative to insecticide for controlling cabbage root fly and other pests in brassicas is intercropping. Cabbages and Brussels sprouts have been grown with a diverse range of other plant species including clover (O'Donnell and Coaker, 1975; Theunissen et al., 1992), grass and beans (Tukahirwa and Coaker, 1982), and spurry (Theunissen and den Ouden, 1980), and in general, fewer pests have been found in the mixed crops. One reason for this may be that the mixed crops provide a more favourable environment for predatory insects, including carabid beetles (eg Dempster and Coaker, 1972). An alternative explanation is that flying pests are less efficient at locating and reacting to their host plants in mixed crops (Finch, 1993; and see Altieri and Letourneau, 1982, for other hypotheses), but even so, carabids are known to prey on pests, therefore any method of increasing their abundance or activity is potentially beneficial. Enhancing the effect of naturally occurring predators would be particularly useful in organic crops, but these tend to be more weedy than conventionally grown ones, and may, therefore, already provide a varied environment for carabids, which cannot be improved by mixed cropping.

The research described in this paper was carried out over two years to determine the effect of undersowing with clover on the activity of carabids and the incidence of cabbage root fly damage in brassica crops. In the first year (1992) undersowing was contrasted with more conventional methods of swede turnip production in eastern Scotland. The following year, the role of intercropping in organic crops was investigated in northern Scotland, using cabbages either undersown with white clover or not undersown, but with a natural weed cover.

## MATERIALS AND METHODS

In 1992, swede turnips were sown on ridges in 20x20m plots at Old Cambus East Mains, near Cockburnspath in East Lothian. There were 36 plots, of which 12 were undersown with white clover, 12 were undersown with red clover and 12 were not undersown. The clover was confined to the furrows between the ridges, and herbicide was used for weed control in those plots which were not undersown. Six plots of each of the 3 treatments were also treated with insecticide in the form of carbofuran granules at sowing and chlorfenvinphos sprays on 24th July and 7th August. Carabid beetles were collected using 4 pitfall traps in each plot. These were emptied every two weeks from 9th June until 10th November, when the turnips were harvested. A mean, cumulative catch of each species was calculated for each treatment. Cabbage root fly damage was assessed on 1.5m of row in the centre of each plot.

In 1993, cabbage seedlings were transplanted into 40 plots, each 10x5m, at Aldroughty farm, near Elgin in Moray. Half of the plots were sown with white clover a few days before the cabbages were planted. No weed control was used on the plots which were not undersown, except between 17th and 23rd August, when the vegetation was thinned out using a rotary cultivator between the cabbage rows, and the uprooted weeds were removed from the site. Carabids were collected using 3 pitfall traps in each plot between 18th and 25th May, 20th and 27th July, and 24th and 31st August. The first two weeks coincided with the appearance of the first and second generations of cabbage root fly, as determined using white water traps to collect the adults, and the third week followed the cultivation of the non-undersown plots. The mean number of each carabid species collected per plot from each treatment was calculated for each week's pitfall trapping. Plant cover over the whole trial area was estimated in the field on 25th May, and from a photograph of the ground in each plot on 27th July and 31st August. The percentage of marketable plants in each plot and mean weight of the marketable plants were also recorded.

### RESULTS

### 1992

The clover cover in the undersown plots developed slowly, and reached its fullest extent at the end of July, which was two months later than the appearance of the first generation of cabbage root fly, but corresponded to the appearance of the second generation. There was a complete cover of clover between the ridges, to a height of 10cm, but this did not extend to the swede plants on the ridges. Table 1 shows that, in general, more carabids were collected from the treatments with clover than without, and from the treatments which did not receive insecticide than from those which did. Carabids were also collected in greater numbers from the insecticide treatments which were undersown than from that which was not; the difference was significant for the treatment undersown with white clover.

TABLE 1. Carabid beetles collected by pitfall trapping in
1992 from swede turnips in 20x20m plots treated as shown.
Numbers are totals from all traps per plot (4) and all
recording dates (11), averaged over all replicates (6).

Treatment	Number
No insecticide	144.0
Insecticide	114.7
No insecticide + white clover	186.7
No insecticide + red clover	174.5
Insecticide + white clover	172.3
Insecticide + red clover	143.8
LSD (p<0.05)	39.2

There was significantly less root damage in all of the insecticide treatments than in the treatments without insecticide (Table 2), and the combination of insecticide and white clover gave the least damage. Both of the unsprayed, undersown treatments had more damage (significantly for red clover) than the unsprayed treatment which was not undersown.

TABLE 2. Cabbage root fly damage to swedes, 1992, assessed on a 1.5m length of row in the centre of each plot.

Treatment	Root damage index score
No insecticide	0.124
Insecticide	0.053
No insecticide + white clover	0.160
No insecticide + red clover	0.193
Insecticide + white clover	0.031
insecticide + red clover	0.050
LSD (p<0.05)	0.029

### 1993

The amount of plant cover in each treatment at the end of each week of pitfall trapping is summarized in Table 3. The ground cover on 25th May was uniform over the whole trial and consisted of cabbage, weed and clover seedlings, but the area was mostly bare soil. On 27th July and 31st August, the cover in the undersown plots consisted almost entirely of dense clover from the soil surface upwards. The apparent reduction in the area covered by the cabbage plants between the two dates is a result of the clover covering some of the smaller plants. By contrast, the ground cover in the plots which were not undersown consisted mostly of weeds, which gave a more open vegetation structure.

TABLE 3. Mean total plant cover and crop cover (%) in cabbages either undersown with white clover or not undersown, estimated when traps retrieved at the end of each of 3 weeks' pitfall trapping.

	Not u	ndersown	Undersown		
	Total	Cabbage	Total	Cabbage 5	
25th May	20	5	20		
27th July	67	12	100	10	
31st August	64	27	100	7	

There was no difference in the total number of carabids collected from each treatment in May, but in July and August, more carabids were collected from the plots which were not undersown, and this difference was significant in August (Table 4). The differences in the total numbers of carabids collected in July and August were reflected in the catches of most of the common species, whereas in May, most species were collected in similar numbers from both treatments (Table 5).

TABLE 4. Mean number of carabids per plot collected during 3 weeks of pitfall trapping in 1993 from organic cabbages undersown with white clover (u/s) or not undersown but with natural weed cover (n-u/s). Three traps were used in each 5m x 10m plot and there were 20 plots of each treatment.

	n-	·u/s	u		
	Mean	SEM	Mean	SEM	
18 - 25 May	15.6	1.3	16.1	1.2	
20 - 27 July	17.7	1.7	14.0	1.2	p<0.07
24 - 31 August	13.9	1.1	9.4	1.0	p<0.002

TABLE 5. The number of species of carabid which were more abundant ( treatments differed by >10% of the total catch from both ) in pitfall catches from organic cabbages either undersown with white clover (u/s) or not undersown (n-u/s). Only species of which more than 10 individuals were collected are included.

	n-u/s	u/s	no difference
18 - 25 May	0	2	6
20 - 27 July	8	4	1
24 - 31 August	5	1	2

The mean plant stands were not significantly different between treatments, but the percentage of marketable cabbages, and their mean weight were significantly less from the undersown treatments (Table 6).

TABLE 6. Plant stand on 7th July (as % of original number of transplanted seedlings), marketable percentage at harvest, and mean weight at harvest of organic cabbages either undersown with white clover (u/s) or not undersown (n-u/s).

	n-u/s	u/s		
Plant stand (%)	59.8	56.1		
Marketable %	67.3	45.5		
Mean weight (kg)	13.8	5.8		

#### DISCUSSION

Undersowing with clover was found to be an effective way of increasing carabid activity in the swedes, and this is in keeping with the findings of other workers (Theunissen et al., 1992; O'Donnell and Coaker, 1975). In the undersown plots which were treated with insecticide, the carabids were probably protected from the sprays by the plant cover, (Dixon and McKinlay, 1992) and, being active at or just below the soil surface, they would not have come into contact with the insecticide from the granules at seed depth. In the cabbages, more carabids were collected from the plots with natural weed growth, even after these had been mechanically weeded. The reason for this may lie in the difference in the density of the vegetation between the two treatments, particularly at ground level. Pitfall trapping provides a combined measure of activity and population density, and the activity of carabids is restricted by dense vegetation (Greenslade, 1964), therefore the higher numbers of most species collected from the non-undersown plots may reflect a higher level of activity. However, carabids must be active both to play a part in controlling pests and to be collected in pitfall traps. Therefore in this study, pitfall catches were indicators of the general level of carabid activity, and an open vegetation structure supported more of this than a dense cover of clover, although in the swedes, the clover was better than bare soil.

Undersowing the swedes with clover was obviously not as effective as insecticide at preventing root scarring by second generation cabbage root fly. This was to be expected, but it was more surprising that, of the unsprayed treatments, damage was worse in the undersown plots than the non-undersown. This may be because second generation flies, emerging within the crop, were confined in the vicinity of the swede plants by the clover, which had grown too late to give any protection from the first generation. In any case, the greater level of carabid activity in the undersown plots was not enough to prevent this damage. No benefit of undersowing was seen in the cabbages either, but as in the swede trial, the clover cover had not developed when the first generation flies appeared, and it is the first generation which affects plant stand. The yield from the undersown cabbages was probably reduced as a result of competition with the very vigorous clover sward, but this competition might be reduced in future by maintaining a cleared strip of soil along each cabbage row, or by sowing alternate rows of cabbage and clover (eg. O'Donnell and Coaker, 1975).

This work has confirmed that the activity of carabids in general can be increased by undersowing with clover, but has also shown that this activity might be increased further by management of the ground cover to give a more open vegetation structure. However, the benefits of undersowing as a means of crop protection were not seen, largely because of the late development of the ground cover. The need for an early development of ground cover is clearly a major obstacle to the use of this technique in Scotland, but one which might be overcome by sowing the previous autumn.

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## THE INFLUENCE OF FOOD PLANTS ON FOOD UTILISATION AND MIDGUT MICROSOMAL OXIDASES IN LARVAE OF THE COTTON LEAFWORM SPODOPTERA LITTORALIS (LEPIDOPTERA : NOCTUIDAE)

# P. FISCIAN, R.M. WILKINS

Department of Agricultural and Environmental Science, Newcastle University, Newcastle upon Tyne, NE1 7RU

# ABSTRACT

The effects of food plant species on feeding and the responses of cytochrome P-450 in *Spodoptera littoralis* was investigated in the laboratory. Larvae were maintained on a semi-artificial diet up to their last instar. On moulting, they were fed on various food plants. Larval food intake was severely reduced on maize, millet and sorghum. The levels of midgut cytochrome P-450 were influenced significantly by the food plants. Topical treatment of the larvae with  $\beta$ -naphthoflavone increased the larval food consumption of maize, millet and sorghum. The levels of midgut cytochrome provide resulted in a reduction of maize intake. The results indicated that P-450 levels may influence larval food intake.

# INTRODUCTION

Spodoptera littoralis is a polyphagous insect which attacks a wide range of economically important crop plants (Sanino *et al.*, 1987). Various control measures, are being used to reduce crop losses due to *S. littoralis* but the main method of control is the use of chemical insecticides. One of the problems with chemical control is that, after only a few seasons of use, the larvae develop resistance to the insecticides (Campion and Hosny, 1986).

One of the factors that could influence the susceptibility of the larvae of *S. littoralis* to insecticides is the presence of microsomal mixed-function oxidases (MFO). These enzymes have been found in several insect species, degrade xenobiotics and may play a role in the food preference of phytophagous insects (Krieger *et al.*, 1971). Since food plants affect the susceptibility of larvae to insecticides, the role of the MFO's in the preference of food plants requires further study.

The present study is part of a research programme investigating the interaction between food plants and cytochrome P-450, and the implications for integrating plant resistance and insecticides in the management of S. *littoralis*.

# MATERIALS AND METHODS

The larvae of S. littoralis used in the study were from eggs obtained from The

Natural Resources Institute. The insect culture was maintained on a kidney bean based semi artificial diet (Dimetry, 1976). The diet was dispensed into rearing jars. On pupation the pupae were placed in jars containing Dupre Vermiculite. On emerging the adults were paired and placed in 1 litre Kilner jars and fed 20% honeyed water. The Insect culture was maintained in an incubator at 25 °C and 16:8 L:D photoperiod.

## Larval food consumption

Freshly moulted sixth instar larvae maintained on the semi artificial diet, were randomly selected and divided into groups. Each group consisted of 18 larvae per replicate and 4 replications. One group continued to feed on the semi artificial diet. Each of the other groups was placed on excised leaves of food plants (Table 1.). The plants were grown in trays in a green house and the Gramineae were used at the 4 leaf stage, while the Leguminosae and the Cruciferae were used at the 6 leaf stage. The larvae were provided with weighed amounts of excised leaves of the crop plants. To maintain the freshness of the leaves, the petri dishes used for the experiments were lined with moistened filter paper. After 48h uneaten leaves and the faecal pellets were separated, oven dried at 60°C for 48h, and weighed. The amount of foliage consumed by the larvae was then determined using the method of Waldbauer, (1964).

Family	Food plant	Source	Variety
Leguminosae	Broad bean	U.K	Imperial Windsor White
0	Soy bean	Mexico	Stan Rosa
	Cowpea	U.K	California BlackEye
Gramineae	Maize	Ghana	Dobidi
	Millet	U.K	HSM/025
	Sorghum	Ghana	Naga Red/Manga Nara
Cruciferae	Chinese		<b>-</b> 1 571
Crucinoria	cabbage	U.K	Wang Bok

Table 1. Food plants used.

## Extraction of enzyme

The midguts of groups of 20 larvae were dissected out and their contents removed. The guts (approximately 450mg), were then washed in 1.15% KCl and homogenised, for 30s, in ice-cold sodium phosphate buffer (25ml), pH 7.5, in a hand operated glass tissue grinder with a teflon pestle. The homogenate was then filtered through cheese cloth and centrifuged at 10,000gmax for 15mins. The pellets were discarded and the supernatant was then filtered through glasswool and recentrifuged at 100,000gmax for 1h. The microsomal pellets were resuspended in sodium phosphate buffer containing 30% glycerol. All operations were carried out at 3°C. Cytochrome P-450 concentration was measured using the methods of Gibson and Skett (1994). The protein content was determined by the methods of Bradford (1976). In a second experiment a known inducer of cytochrome P-450,  $\beta$ -naphthoflavone

# 8D-23

 $(19.5\mu g/g)$  and an inhibitor, piperonyl butoxide  $(21.2\mu g/g)$  were applied topically, in acetone, to 6th instar larvae (mean weight 532  $\pm$  35mg) prior to feeding on maize, millet and sorghum three crops that severely reduced larval food intake. Amounts of food ingested were determined after 48h.

#### RESULTS

In the present study, the total dry weight of food ingested by the larvae was significantly lower on maize, millet and sorghum than on the other food plants (Fig. 1). The highest intake (127mg) was on the cabbage and the lowest intake (13.4mg) was on the millet.

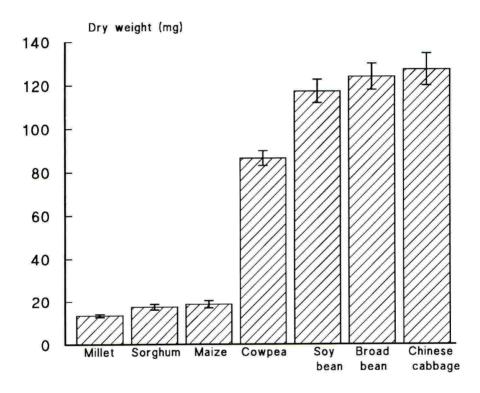


Fig. 1. Weight of foliage ingested by larvae

The specific content of cytochrome P-450 in the larvae of *S. littoralis* was influenced by the food plant on which it had fed (Table 2). It was lowest (0.14 nM/mg soluble protein) on millet and highest (0.41 nM/mg soluble protein) on the soy bean. There was also a 2 to 3 fold increase in the cytochrome P-450 content in the non-cereal food plants indicating an induction of the enzyme on these food plants. These results reflect those obtained by Yu (1986) who showed differences in levels of induction in the fall armyworm on different food plants. There was also a positive correlation (r=0.86) between the larval food intake

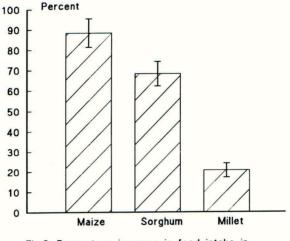


Fig.2. Percentage increase in food intake in B-Naphthoflavone trated larvae

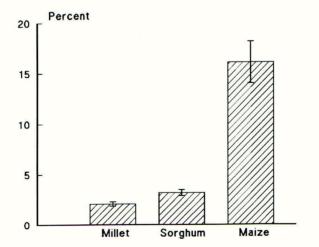


Fig.3. Percentage reduction in food intake in piperonyl butoxide treated larvae

and the specific content of cytochrome P-450 of the larvae which could be due to high food consumption rate resulting in a higher level of induction (Yu, 1983).

Table 2. The specific content of cytochrome p-450 of larval midguts of *Spodoptera littoralis* on the food plants.

Food plants	P-450 content (mean $\pm$ SE) (nM/mg soluble protein) in midgut	
Artificial diet	$0.10 \pm (0.02)a$	
Millet	$0.11 \pm (0.03)a$	
Maize	$0.13 \pm (0.04)a$	
Sorghum	$0.14 \pm (0.04)a$	
Cowpea	$0.23 \pm (0.06)b$	
Broad bean	$0.28 \pm (0.06)b$	
Cabbage	$0.35 \pm (0.08)c$	
Soy bean	$0.41 \pm (0.09)c$	

Means followed by the same letter are not significantly different at the 5% level according to the SNK test.

Topical Application of  $\beta$ -napthoflavone to the insect increased the weight of food consumed by the insect (Fig. 2) on the three graminous crops. The highest percentage increase (89%) was on maize and the least (24%) was on millet. Application of piperonyl butoxide resulted in a reduction of (17%)in the food intake of maize and a (2%) reduction in the intake of millet.

## DISCUSSION

The study indicates that even though *S. littoralis* is a polyphagous insect, larval feeding on cereal crop plants is severely reduced. In a previous study, it had rejected rye grass, wheat and rice seedlings. The severe reduction in the food intake could be due to chemical factors Stadler (1984). Bernays *et al.*, (1974) obtained an increase in food consumption by acridids when leaf surface chemicals were first removed from grasses.

The role of cytochrome P-450 in the metabolism of xenobiotics and in insecticide resistance is well documented. In spite of the importance of *S. littoralis*, the possible role of these enzymes in larval feeding has largely been ignored.

Even though the result of the present study is limited, it indicates that cytochrome P-450 may be a factor in the food plant preference of insects . Cytochrome P-450 content was higher in the food plants that were ingested in larger amounts. Little is known about the influence of  $\beta$ -Naphthoflavone and piperonyl butoxide on larval food intake. This study indicates that topical applications of a known inducer and an inhibitor of cytochrome P-450 influences the consumption of foliage of maize, millet and sorghum. Since the

larval fresh weight gain on the crop plants were also influenced, it could be inferred that the enzyme may play a role in the utilisation of food plants by the larvae of *S. littoralis*.

## ACKNOWLEDGEMENTS

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# USE OF PYMETROZINE IN IPM VEGETABLE PROGRAMS

# R. SENN, B. SECHSER, C.R. FLÜCKIGER

Crop Protection Division, Ciba-Geigy AG, CH-4002 Basel, Switzerland

# ABSTRACT

Pymetrozine is an insecticide with a new mode of action, representing a novel class of insect control agents (pyridine azomethines). It is highly active against susceptible and resistant aphids and whiteflies in vegetables and other crops. The compound has a low acute toxicity to mammals as well as terrestrial and aquatic organisms, and favourable ecochemical properties. The compound was tested in season-long spray programs based on economic thresholds and these confirmed its performance in both the field and the glasshouse. At 10 and 20 g AI/hl, pymetrozine required fewer sprays than the standard products for *Aphis gossypii* and *Myzus persicae* control. In glasshouses, pymetrozine gave control of aphids (*Macrosiphum euphorbiae, Myzus spp.*) and whiteflies (*Trialeurodes vaporariorum*) on tomatoes for four months. No negative influence on released beneficial arthropods (*Encarsia formosa, Aphelinus abdominalis* and *Bombus terrestris*) was observed. Because of its selectivity towards beneficial arthropods and its novel chemistry, pymetrozine is especially useful in IPM programs.

# INTRODUCTION

Pymetrozine (CGA 215'944, CHESS <sup>TM</sup>, PLENUM <sup>TM</sup>) is a new insecticide, representing a novel type of chemistry (pyridine azomethine), with a unique mode of action (Fluckiger *et al.*, 1992a). It is active against aphids and whiteflies in vegetables, field crops and fruits and against hoppers in rice. The favourable toxicity and ecotoxicity profile and its selectivity towards beneficial arthropods are characteristics of the compound which make it an important tool in integrated pest management programs. Although treated aphids may still live for up to four days, they stop sucking on the plants immediately and, therefore, also transmitting persistent viruses (Harrewijn and Piron, 1994). It can be assumed that the mode of action is different from that of all other currently known insecticides and this guarantees its activity against highly resistant strains of pests (Bolsinger *et al.*, 1993).

The biological control of glasshouse pests has become common practice in Central and Northern Europe to meet the requirements of residue reduction and resistance management (van Lenteren and Woets, 1988). Unique in this strategy is the use of the parasitoids *Encarsia formosa* and *Aphelinus abdominalis* for the control of whiteflies (*Trialeurodes vaporariorum*, *Bemisia tabaci*) and a complex of aphids (*Macrosiphum euphorbiae*, *Myzus* spp.), respectively. Failures of biological control measures can be corrected by chemical pesticides. These should not interfere with the host-prey interaction and belong to different chemical classes to avoid or at least delay the development of resistance (van Lenteren and Woets, 1988). New classes of pesticides are needed for the control of whiteflies and aphids to broaden the spectrum of available products. Heptenophos is the only remaining insecticide in Europe giving an acceptable control of sucking pests on vegetables in glasshouses. Pymetrozine offers a new alternative integrated control solution (Flückiger *et al.*, 1992b). This study tested the efficacy of pymetrozine against whiteflies, aphids and their parasitoids and its performance with regard to residual activity and selectivity by comparing it with the standard control practice in Swiss tomato glasshouses and in open field vegetables in Spain and Egypt.

## MATERIALS AND METHODS

### Field trials

Pymetrozine at 10 and 20 g AI/hl was tested against *Aphis gossypii* on okra in Kaha near Cairo in Egypt. It was compared to pirimiphos-methyl, carbosulfan and pirimicarb applied at standard label rates. All products were applied at weekly intervals, but only when the threshold level of 10 aphids per leaf was reached. The first application was made at the beginning of the infestation on July 27. In Almayate (Malaga) in Spain, pymetrozine applied at 10 and 20g AI/hl was tested against *Myzus persicae* on sweet pepper in 1993. It was compared to cypermethrin and pirimicarb. All products were applied only when the threshold level of one aphid per shoot was reached. The first application of all products was at the beginning of the infestation on May 17. These field trials were replicated 2-3 times. The plot size was 36-38 m<sup>2</sup>.

#### Glasshouse trials

The trial was carried out on tomatoes in four glasshouses in Croiz de Rozon near Geneva, Switzerland. The chemical and biological treatments are summarized in Table 1. The plants were treated with a spray gun to run-off. Whitefly adults and aphids were counted at approximately two-weekly intervals over the whole observation period of four months (May 18 until Sept. 16, 1993) on 10 plants in a diagonal transect in each glasshouse. The size of the glasshouses varied between 2000 and 4800 m<sup>2</sup>. Pymetrozine was the key insecticide in the glasshouse VUI 1, and heptenophos was planned to play the same role in the glasshouse BRE 2 received an early treatment with pirimicarb at 0.025% AI to keep the aphid populations low. Immature stages of aphids were counted on the green compound leaves of the plants, the number of which varied between 19 and 25 per plant. The average infestation on 10 leaves per plant was calculated. The number of whitefly adults was estimated for each of the 10 rated plants in each glasshouse.

#### **RESULTS AND DISCUSSION**

#### Field trials

For the season-long control of aphids on okra, pymetrozine applied according to the pest threshold levels needed fewer applications than the standard products (Table 2). At the lower dosage rate 4 and at the higher dosage rate only two applications of pymetrozine became necessary whereas the standard compounds had to be applied six times.

Glasshouse no	Product	Application rate % AI	Application date	Parasitoids released	Number of releases
VUI 1	Pymetrozine	0.02	May 7	Encarsia formosa	10 x
BRE 2	Pirimicarb	0.025	April 20	Encarsia formosa	8 x
	Heptenophos	0.05	May 18	Aphelinus	2 x
	Methomyl +	0.0375 +	Aug.12	abdominalis	
	Buprofezin	0.0125			
	Methomyl	0.0375	Sept. 4		

TABLE 1. Treatment scheme of tomatoes in glasshouses, Geneva, Switzerland, 1993

TABLE 2. Season-long control of Aphis gossypii on okra in Egypt, 1993

Product	Dosage (g AI /hl)	Numbe	er of apl	Application dates					
Sample date:		26.7.	1.8.	7.8.	10.8.	16.8.	22.8.	28.8.	-
Pymetrozine	20	9	1	6	10	14	59	1	27.7., 20.8.
Pymetrozine	10	5	2	14	17	13	124	2	27.7., 8.8., 20.8., 26.8.
Pirimiphos- methyl	125	4	10	20	35	183	317	18	27.7., 2.8., 8.8., 14.8., 20.8., 26.8.
Carbosulfan	36	7	12	16	12	58	261	4	27.7., 2.8., 8.8., 14.8., 20.8., 26.8.
Pirimicarb	30	6	36	103	225	597	2049	5	27.7., 2.8., 8.8., 14.8., 20.8., 26.8.
Untreated control		5	31	101	234	2004	1941	33	

TABLE 3. Season-long control of Myzus persicae on sweet pepper in Spain, 1993

Product	Dosage (g AI /hl)	Numbo	Number of aphids per 25 shoots						
Sample date:		27.5.	8.6.	23.6.	30.6.	8.7.	16.7.	27.7.	-
Pymetrozine	20	0	0	0	2	5	6	2	17.5.
Pymetrozine	10	0	0	6	13	23	0	0	17.5., 8.7.
Cypermethrin	10	5	27	65	10	38	2	1	17.5., 23.6., 8.7.
Pirimicarb	30	2	7	22	28	3	3	1	17.5., 30.6.
Untreated control		36	59	141	186	105	71	37	

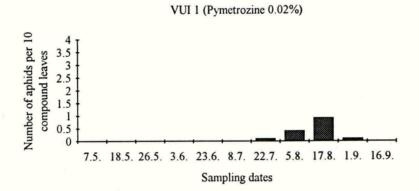
In sweet pepper, pymetrozine needed fewer applications than the standard products for season long control of aphids when applied according to pest threshold levels. At the lower dosage rate only two and at the higher dosage rate one application of pymetrozine had to be applied whereas the standard compounds had to be applied two or three times (Table 3).

#### Glasshouse

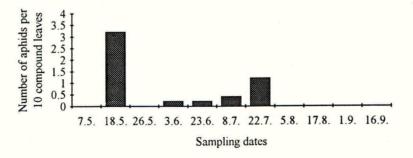
## Aphids

Aphids never became a problem after one treatment with pymetrozine (VUI 1) or the applications of pirimicarb followed by heptenophos, methomyl, buprofezin and methomyl alone (BRE 2) (Figure 1).

FIGURE 1. Population development of aphids on tomatoes in the two glasshouse treatment schemes, 1993 (average number of aphids per 10 compound leaves)



BRE 2 (Pirimicarb, heptenophos, methomyl, buprofezin)



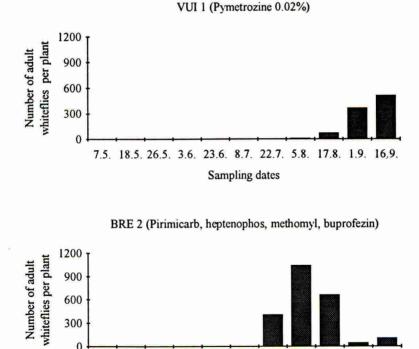
#### Whiteflies

Pymetrozine (0.02 % a. i.) suppressed the whiteflies for four months as shown by the counts of the whitefly adults (Fig. 2). In spite of higher numbers of whitefly adults in September there was no unacceptable infestation of nymphs for the grower. The fruits remained clean over the whole harvesting period.

In the glasshouses treated with heptenophos (BRE 2), there was a high incidence of whitefly adults (Fig. 2), and problems of severe spot attacks by nymphs of T. vaporariorum occurred. Such attacks are the main concern for the glasshouse grower. E. formosa parasitoids were released eight times. In spite of that, two additional sprays became necessary against whitefly nymphs with methomyl plus buprofezin and methomyl alone (Table 1).

The method adopted for counts of whitefly adults only gave an indication of the incidence of potentially unacceptable attacks and ignore local hot spots of infestation. Such strong local attacks can be missed by evaluating only 10 plants per glasshouse, but adding more plants was prevented by the resources available. The glasshouse grower however, is looking for such sources for mass outbreaks of pests, and decisions for spraying were taken in glasshouse BRE 2 in spite of low average whitefly attacks.

FIGURE 2. Population development of whitefly adults on tomatoes in the two glasshouse treatment schemes 1993 (average number of adults per plant)



Pymetrozine gave full control of whiteflies and aphids at the rate of 0.02 % AI over a period of four months and was compatible with the biological control of both pests through *E*.

7.5. 18.5. 26.5. 3.6. 23.6. 8.7. 22.7. 5.8. 17.8. 1.9. 16.9. Sampling dates formosa and A. abdominalis. From other reports the good selectivity of pymetrozine versus predators and parasitoids is well known (Flückiger *et al.*, 1992a). Heptenophos showed signs of increasing failures of the control of whitefly nymphs and required two additional insecticidal treatments.

Hives with bumble bees (*Bombus terrestris*) from Biopest Biological Systems, Westerlo, Belgium, were placed in each glasshouse for pollination and renewed every six weeks throughout the whole growing season. The fruit setting was found to be satisfactory by the growers in all four glasshouses and none of the insecticides caused any negative impact on either bees or fruit set.

## CONCLUSION

The important features of pymetrozine in addition to its selectivity, are its novel chemistry and the different mode of action. In open field vegetables, pymetrozine needs fewer applications than the standard products for the season-long control of aphids when applied according to the threshold levels. In the glasshouse, pymetrozine gave full control of whiteflies and aphids at the rate of 0.02 % AI over a period of four months and was compatible with the biological control of both pests using both *E. formosa* and *A. abdominalis*. No negative influence on the pollination activity of bumble bees was observed. All these features make pymetrozine a highly suitable product for the integrated control of vegetable pests in glasshouses and in the open field.

#### ACKNOWLEDGEMENTS

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# SELECTIVITY OF DIOFENOLAN (CGA 59 205) AND ITS POTENTIAL FOR INTEGRATED SCALE CONTROL

# B. SECHSER, B. REBER, H. WESIAK

Crop Protection Division, Ciba-Geigy AG, CH-4002 Basel, Switzerland

# ABSTRACT

Diofenolan (CGA 59 205) is a new insect growth regulator with good activity against lepidopterous pests and scales in pome fruit and citrus. It has demonstrated excellent selectivity at rates of between 50 and 200 ppm against *Orius majusculus* and *Aphytis melinus* under laboratory conditions. In citrus orchards in Spain, populations of *Cales noacki* and other hymenopterous parasitoids, *Chrysoperla carnea, Conwentzia* spp., *Scymnus* spp. and spiders survived in the diofenolan plots at the same levels as in untreated plots. On citrus trees in Egypt, diofenolan caused only a low reduction of *Aphytis* spp. larvae on the citrus purple scale. Diofenolan showed no significant effect on the predatory mite *Typhlodromus pyri* in an Italian apple orchard in a control program against the codling moth. Preliminary laboratory tests showed that larvae of *Coccinella septempunctata* and *C. carnea* did not moult to the pupal stage due to the particular mode of action of the compound.

# INTRODUCTION

Diofenolan (CGA 59 205) is a new type of insect growth regulator with good activity against scales and lepidopterous pests in deciduous fruit, citrus, grapes and olives (Streibert et al. 1994). New insecticides with a different mode of action are required to control these pests because of potential resistance development. Pome fruit and citrus also have a long history of integrated pest management (IPM), in which beneficial arthropods often play a key role. This paper presents the results of a study of the selectivity of diofenolan for these beneficial arthropods.

# MATERIALS AND METHODS

# Laboratory studies

Diofenolan was tested in the laboratory, at 150 ppm, against three predator species: (i) *Orius majusculus* (L<sub>2</sub>), (ii) *Coccinella septempunctata* (L<sub>3</sub>) and (iii) *Chrysoperla carnea* (L<sub>2</sub>). Three replicates of all tests were undertaken. Cotton pads in petridishes were sprayed with diofenolan at the recommended rate, allowed to dry and 2nd instar stages of the 3 species were exposed for 10 days. Surviving larvae of C. septempunctata and C. carnea were observed up to 1 or 2 months, respectively.

## Citrus, Spain 1991

Diofenolan was tested with two applications in Spanish mandarines in the region of Valencia, where it was compared with one application of methidathion (ULTRACIDE<sup>®</sup>) and 2 applications of buprofezin (APPLAUD<sup>®</sup>). Each high volume treatment was applied to two plots each measuring1500 m<sup>2</sup>. Each chemical treatment plot had a small adjacent plot of 12 untreated citrus trees. The population development of the natural enemies was monitored at intervals by beating samples taken from 100 branches in each plot. Sample sizes for the untreated control were smaller but adjusted for comparison purposes.

### Citrus, Spain 1992

A single spray of diofenolan was tested at the appropriate time for the control of *Parlatoria pergandei* (July 30). The chemical treatment and an untreated control were replicated three times. Beating samples of a total of 100 branches were taken in each block pre-spray and 4 and 13 days after application to monitor the survival of the parasitoid and predator populations.

## Citrus, Egypt 1992

Mixed stages of the purple scale *Lepidosaphes beckii* parasitized by *Aphytis* spp. were evaluated on citrus trees in Northern Egypt. The plot size was a single tree. The sample size was 4 samples each consisting of five leaves, per tree, and 10 visits were made to each tree before and after treatment application. On each leaf, live larvae of *Aphytis* spp. were counted on 4 cm<sup>2</sup> of surface, i. e. a total of 80 cm<sup>2</sup> for each treatment. Four chemicals were used as treatment, diofenolan, methidathion, buprofezin and pirimiphos-methyl (ACTELLIC ().

### Apple orchard, Italy 1992

Diofenolan was applied three times to apple trees at 15 and 20 g/hl a. i. high volume. The standard treatments were diflubenzuron, fenoxycarb and teflubenzuron. The survival of the predatory mite *Typhlodromus pyri* was assessed at the end of the season by counting the number of mobile stages on 20 leaves in each of the three replicates per treatment.

#### RESULTS

## Laboratory studies

Due to the growth regulating activity of diofenolan, the observation period was lengthened beyond the normal time. The findings (Fig. 1) suggest a high degree of short term selectivity of diofenolan against all three species. Surviving nymphs of *O. majusculus* finished their development into normal adults. Larvae of *C. septempunctata* reached the 5th instar and remained alive for one month, but finally died. Last instar larvae of *C. carnea* attained a bigger than average size and finally died after two months. Further studies are required to elucidate the residual activity of spray deposits against these two predators.

# Citrus, Spain 1991

Diofenolan and buprofezin were found to be safe to hymenopterous parasitoids even in the samples taken only two days after spray application (Fig. 2). Methidathion had a strong knockdown effect, but in the samples taken 30 days after the single spray the parasitoid population density had recovered to the levels in untreated controls.

Larvae of *C. carnea* were scarce at the time of the first spray application but started to increase one month later (Fig. 3). The numbers in the diofenolan and buprofezin plots were comparable to the untreated control over the whole observation period, while the numbers in the methidathion treatment did not recover fully until six weeks after application.

*Conwentzia* spp. belong to the small neuropterous family of Coniopterygidae; they are general predators like *C. carnea*; but are much less common than the chrysopids. The data for the adults suggest that neither diofenolan nor buprofezin had a negative impact on them (Fig. 4). In the methidathion plots full recovery of populations occurred only after one month.

## Citrus, Spain 1992

The population densities of the beneficials were low at the time of spraying (Figure 5). The aphelinid *Cales noacki*, a parasitoid of the whitefly *Aleurothrixus floccosus*, had increased by 13 days after the spray to numbers comparable to those of the untreated controls. The same is true for the whole complex of spiders. Adults of *Scymnus* spp. were

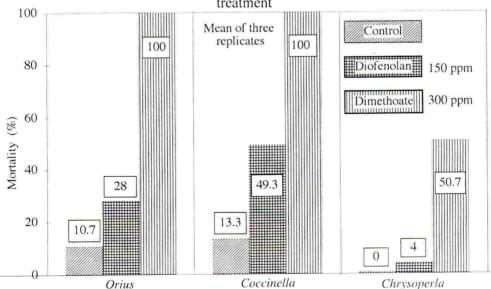
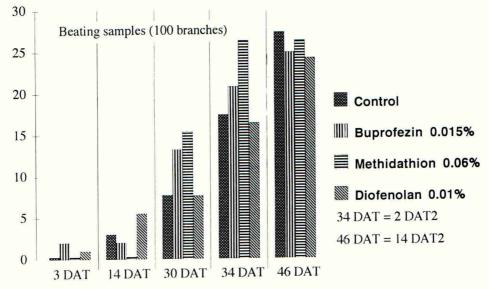
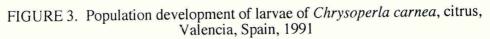


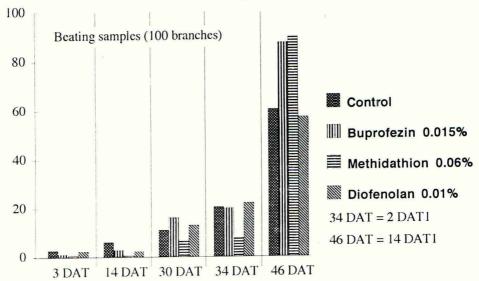
FIGURE 1. Population reduction (%) of three predator species 10 days after treatment

FIGURE 2. Population development of hymenopterous parasitoids, citrus, Valencia, Spain, 1991



Treatment dates: May 3 and June 4 (Buprofezin, Diofenolan), May 3 (Methidathion)



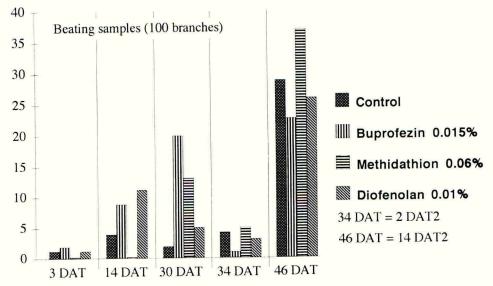


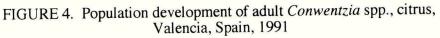
Treatment dates: May 3 and June 4 (Buprofezin, Diofenolan), May 3 (Methidathion)

still more scarce, but numbers went up in both the control and diofenolan block after two weeks. Thus, in summary, diofenolan had no negative impact on these three beneficial groups.

## Citrus, Egypt 1992

Of all chemical treatments diofenolan and buprofezin caused the lowest reduction of *Aphytis* spp. larvae during the evaluation period of 128 days. The suppression was quite severe with pirimiphos-methyl and methidathion.



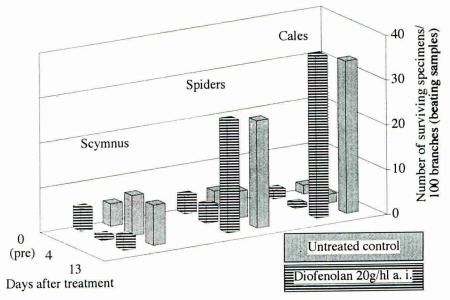


Treatment dates: May 3 and June 4 (Buprofezin, Diofenolan), May 3 (Methidathion)

### Apple orchard, Italy 1992

The numbers of *T. pyri* in all treatments were comparable to the figures in the control plot with 16 predatory mites per 20 leaves. The plots with diofenolan had an infestation of 22 (15 g) and 18 (20 g) *T. pyri* per 20 leaves. Therefore diofenolan can be rated as harmless to *T. pyri*.

Diofenolan has demonstrated excellent activity against various scale species in citrus and was harmles to most of the beneficial groups investigated. Immature stages of *C. septempunctata* and *C. carnea* did not moult to pupal stages, but it remains to be determined whether these effects occur in the field. The overall favourable selectivity and its novel chemistry make diofenolan suitable for an integrated program in citrus, where scales are the dominant pest problem.



# FIGURE 5. Surviving populations of citrus beneficial arthropods, Valencia, Spain, 1992

### ACKNOWLEDGEMENTS

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# DEVELOPMENT OF INSECTICIDES FOR INTEGRATED PEST MANAGEMENT OF KIWIFRUIT IN NEW ZEALAND

# A.R. TOMKINS

Horticultural & Food Research Institute of New Zealand Ltd, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand

# ABSTRACT

New Zealand's kiwifruit industry is strongly export oriented. The crop's key pests (armoured scale insects, leafrollers) used to be controlled by regular application of broadspectrum insecticides. However, an increasing proportion of the industry is now adopting a first stage Integrated Pest Management (IPM) system. This system relies on a narrow range of insecticides (petroleum oils, *Bacillus thuringiensis*) to manage pests. There is a need for further classes of insecticides. Insect growth regulators have very good potential for kiwifruit pest management, but adoption is hampered by their persistence. The effects of Maximum Residue Limits on the development of IPM systems for export fruit crops is discussed.

# INTRODUCTION

Kiwifruit (Actinidia deliciosa cv. 'Hayward') is a significant crop for New Zealand with annual sales of around \$NZ 550 million. However, kiwifruit is a minor crop worldwide with 22,900 ha of commercial plantings in 1983 producing an estimated 528,900 tonnes of fruit in 1990 (Warrington, 1990). In comparison worldwide production of apples in 1991 was 36,463,000 tonnes (FAO, 1992).

New Zealand's kiwifruit crop is grown entirely for export. Very high quality standards set by the industry plus the stringent quarantine requirements of some importing countries cause fruit to be rejected for a variety of defects, eg, 65-93% of unsprayed crops due to pest, disease and physical damage (Ferguson, 1980). Some reject fruit is consumed locally or processed, but lower financial returns to the orchardist plus the small size of the local market limit the potential of these outlets. A very high level of pest control is therefore required which until recently has been achieved by using calendar spraying.

However, the New Zealand kiwifruit industry has now begun to adopt a first stage Integrated Pest Management (IPM) system. Further progress to IPM will involve greater use of biological control. Nevertheless, in order to maintain the standards required for export, it is probable that insecticides will continue to play a key role within future IPM systems. Insecticides compatible with biological control are therefore required.

This paper discusses the development and registration of insecticides for use on New Zealand kiwifruit, with an emphasis on how this process is affecting progress towards IPM.

# HISTORY OF INSECTICIDE USE BEFORE THE DEVELOPMENT OF IPM

The slow rate of registration of insecticides to enable the production of export fruit hampered the pioneering commercial development of kiwifruit in New Zealand. In the 1960s and 1970s only three insecticides were registered (Table 1). Registration of further insecticides was complicated by the lack of Maximum Residue Limits (MRLs) for kiwifruit in many export markets. This situation was aggravated by the uniqueness of kiwifruit. It has improved as kiwifruit have become more widely grown, but initially required a considerable New Zealand input to collect residue decay data and negotiate with export markets and Codex to establish MRLs (Watts, 1988).

Insecticide	Use	Year of registration	Toxicity to pest natural enemies <sup>2</sup>
Organophosphates			
azinphos-methyl	pre-flowering	1966	3.96p
chlorpyrifos	full season	1974	-
diazinon	full season	1967	
etrimfos	pre-flowering	1985 (withdrawn)	-
methidathion	dormant	1990	-
phosmet	full season	1981	-
pirmiphos-methyl	full season	1981	4.60
Pyrethroids			
cypermethrin	non-bearing	1985	3.63p
deltamethrin	pre-flowering	1982	4.40
fluvalinate	pre-flowering	1986	2.60
permethrin	full season	1981	4.38p
Insect growth reg	ulators		
buprofezin	pre-flowering	1991	-
Others			
Bacillus	full season	1984	2.05
thuringiensis			
petroleum oil	full season	Experimental Use Permit - Limi	ited Sales

TABLE 1: Insecticides registered<sup>1</sup> for use on New Zealand kiwifruit.

<sup>1</sup> Agricultural Compound Unit, Ministry of Agriculture & Fisheries.

Theiling and Croft (1988). Rated toxicity to natural enemies (1=0%; 2=<10%; 3=10-30%; 4=30-90%; 5=90-100% mortality), p- parasitoids only; - no data presented.

No insecticides have obtained a registration for full season use on kiwifruit since chlorpyrifos in 1988, although a petroleum oil currently has an experimental use permit. During the registration of chlorpyrifos there were some bureaucratic delays obtaining MRLs (Watts, 1988) which was indicative of the difficulty meeting the stringent data requirements for setting of MRLs internationally.

The New Zealand kiwifruit industry now has a range of conventional insecticides. However, some are subject to re-registration procedures in the United States (diazinon, methidathion, permethrin, petroleum oil, phosmet; Stimmann and Ferguson, 1990). In some instances, this may lead to product withdrawal. Any reduction in the range of insecticide types available may increase the potential for resistance development which emphasises the need to develop IPM systems.

## INSECTICIDE USE IN A FIRST STAGE IPM SYSTEM

In 1990 a small proportion of the New Zealand kiwifruit industry began using a first stage IPM system. This system is based on monitoring for key pests with associated thresholds used to determine when to spray, coupled with the use of "soft" insecticides. The "soft" insecticides have low mammalian toxicity and greater compatibility with pest natural enemies. They include petroleum oil and products based on the bacterium *Bacillus thuringiensis* (Bt). This system also enables the production of fruit without residues at harvest. Diazinon may be used up to 4 weeks post-flowering due to its short residual life.

The development of Bt and oil for use on kiwifruit has been a significant step forward in the development of IPM. However, since the introduction of oil some limitations and threats to its use have occurred. Although oils are used to control pests including armoured scale insects on kiwifruit in Italy (Monaco, 1981), Italian authorities have recently specified that there be "no detectable residues of mineral oils in food" (Lunn pers. comm.). To meet this requirement in the kiwifruit IPM system, oils are not applied within 28 days of harvest. Of greater concern is a moratorium on the use of hydrocarbons on food by the United Kingdom (Anon., 1990), primarily to prevent postharvest use of waxes. These measures serve to illustrate that the longterm acceptability of any insecticide is not guaranteed.

#### DEVELOPMENT OF ADDITIONAL INSECTICIDES

The development of insecticides compatible with existing biological control agents (Table 2) will assist the viability of IPM. Most insecticides currently registered for use on kiwifruit have high toxicity to natural enemies (Table 1). Calendar spraying with these insecticides has therefore led to the absence of some biological control agents (Table 3).

A wide range of alternative insecticides including insect growth regulators (IGRs), soaps and other microbial insecticides are being screened for their suitability in kiwifruit IPM. Table 3 shows data from a field trial evaluating a range of IGRs against kiwifruit pests and their side effects on biological control agents. This trial was conducted on mature kiwifruit vines which had no previous spray history. A randomised block design was used with 6 single-vine plots. Seven applications (10/11, 7 and 22/12/88, 11/1, 9/2, 10/3 and

4/4/89) per treatment were applied with a handgun at 2250 litres/ha. One hundred fruit per vine sampled on 11/5/89 were microscopically examined and infestation by armoured scale insects and their parasitism as shown by emergence holes in scale caps recorded.

Table 3 shows that the parasitoids of armoured scale insects can survive repeated exposure to IGRs. Unfortunately, a number of promising insecticides have either not been registered or have been registered for pre-flowering use only. This group includes abamectin and some IGRs. For example, buprofezin which has a high level of activity against armoured scale insects on kiwifruit, has been registered for pre-flowering use only.

TABLE 2: Kiwifruit pests and their known biological control agents (Source: Steven 1990; J. Charles pers. comm.).

Pest	Biological control agents		
Key pests			
armoured scale insects			
	(Hemiptera: Diaspididae)		
	Encarsia citrina (Hymenoptera: Aphelinidae)		
	Hemisarcoptes coccophagus (Acari: Hemisarcoptidae)		
	Signiphora flavella (Hymenoptera: Signiphoridae)		
	S. merceti		
leafrollers (Ctenopseus	tis obliquana, C. herana, Cnephasia jactatana, Epiphyas		
	postvittana) (Lepidoptera: Tortricidae)		
	Dolichogenidea tasmanica (Hymenoptera: Braconidae)		
	D. "sicaria"		
	Glyptapanteles demeter (Hymenoptera: Braconidae)		
	Glabridorsum stokesii (Hymenoptera: Ichneumonidae)		
	Pales funesta (Diptera: Tachinidae)		
	Trigonospila brevifacies (Diptera: Tachinidae)		
	Xanthopimpla rhopaloceros (Hymenoptera: Ichneumonidae		
Secondary pests			
Scolypopa australis (H	Jemintera: Ricaniidae)		
Scotypopu dustralis (1	Centrodora scolypopae (Hymenoptera: Aphelinidae)		
	Comodora scolpopus (Lijinin p		
	(ari: Tetranychidae)		
Tetranychus urticae (A	ican. Terranyemeae)		

# INTRODUCTION OF NEW INSECTICIDES

Registration of insecticides for use on export fruit crops may be prevented by some countries not accepting residues of a product on imported produce which is not registered for use in their own country. The major reason why new insecticides have not been registered or only allowed for pre-flowering use on kiwifruit is the cost and delays of up to 8 years to obtain maximum residue limits (MRLs) in all major export markets.

Many IGRs have protracted residual lives, which may benefit insect control, but also means that residues persist on fruit which may affect the likelihood of their registration.

The development of IPM coincides with a general decline in insecticide use by the New Zealand kiwifruit industry. While this trend is desirable from the point of view of improving the ability to use biological control, it also means that the size of the insecticide market is decreasing. When a new insecticide must help to recoup worldwide costs of development and undergo 2-3 years of local efficacy and residue trials, any reduction in the market size may significantly effect the willingness of companies to develop new products.

Treatment (	Rate	% fruit	Scale parasitism	
	(g ai/100 litres)	infested	Total scale	% parasitised
unsprayed	-	37.4(38.9)	250	8.9
phosmet	112.5	-(0.5)	1	0.0
(Imidan 75WP)				
fenoxycarb	37.5	19.7(14.2)	73	9.6
(Insegar 25WP)				
XRD-473 (5EC	) 10.0	32.6(31.5)	234	9.0
buprofezin	12.5	5.8(2.4)	1	0.0
(Applaud 25WP	?)			
SED		6.44	·•	-

TABLE 3: Percent infestation of kiwifruit by armoured scale insects and parasitism of the third instar greedy scale on the fruit.

Infestation data angular transformed before ANOVA with raw means in brackets.

The ramifications of residues on marketing is now the dominant factor determining insecticide use on export crops, eg, the New Zealand Kiwifruit Marketing Board has limited use of phosmet to the pre-flowering period although it has a registration for use full season. This was because season-long use creates relatively high residue levels, although within MRLs. New Zealand kiwifruit is exported to 56 countries. To ensure access to all of these markets, MRLs are required from Codex, U.S. Environmental Protection Agency and the European Union. Until recently this problem was worsened by the fact that any consignment of the crop might be sent to any country, and that MRLs may vary between countries (Watts, 1988). As a result the lowest MRL in any market will determine the use pattern for the entire crop. In New Zealand spray programmes compiled by the producer boards determine how an export fruit industry uses insecticides to comply with the lowest MRL. The New Zealand Kiwifruit Marketing Board also has a rigorous monitoring programme to ensure that spray programme recommendations are adhered to.

## FUTURE USE OF INSECTICIDES

Research to find commercially available insecticides for use in kiwifruit IPM is continuing and there are some promising candidates. However, in future insecticides registered for full season use may only be products exempt residue tolerances. Two potential sources are microbial pesticides and those derived from natural products. Multinational firms are actively researching these options, but may not develop them for minor crops such as kiwifruit. This uncertainty has led to research in New Zealand to identify endemic sources of pesticides.

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# IPM FOR CACAO PRODUCTION

# R. N. WILLIAMS

Ohio State University, Wooster OH 44691, U.S.A.

# R. H. FULTON

33 Lake Villa Dr., Roswell, GA 30076, U.S.A.

# ABSTRACT

Integrated pest management (IPM) of cacao (*Theobroma cacao*) began seriously about fifteen years ago. It was a result of an overuse of organic agricultural pesticides which were first produced with the promise of complete eradication of crop pests. Pesticides were perceived as the panacea for protecting crops/commodities from insect, weed and disease plagues. Unfortunately, these organic chemicals met unforeseen difficulties - perceived, real or imagined, by the public and government entities. The IPM strategies described here will assist in better management of cacao problems in the field and storage.

## INTRODUCTION

Integrated pest management in cacao is complex, with many biological questions unanswered. For many years pesticides have been used as the panacea for protecting cacao production in the field as well as on the exportable beans in warehouse storage. This no longer is the case as public concern over pesticide residues in/on agricultural products has reached the crisis point. The public fear over food safety, has forced government policy makers to cancel many pesticide registrations, while enhancing analytical techniques that can detect residues as low as 0.001 ppm. So today, it is absolutely necessary that all realize that chocolate products can be as much at pesticide residue risk as cereals, flour or other daily diet staples.

# HEALTHY TREES: THE PEST PREVENTATIVE STRATEGY

The major impetus of IPM is to maintain pest populations below their economic thresholds; the key to this task is "preventative maintenance". This can be done by simply establishing and implementing agronomic practices to assure "healthy non-stressed" trees.

Site selection should be based on drainage feasibility, plans and implementation for surface drainage, and soil sampling coordinated with mapping of the area. Establishment of new plantings or rehabilitation requires use of successive machete cleaning. Glyphosate, paraquat and oxyflurofen herbicides are registered on cacao and quite effective based on their biological efficacy. They are soft on the environment, biodegradable, and tightly bound to soil particles therefore causing no threat to groundwater. Tank combinations of oxyflurofen with ither glyphosate or paraquat are synergistic, thus can be used at lower dosages. This in turn reduces worker exposure time to pesticides.

Cover cropping is an IPM strategy for fence rows, roadsides, waterways and poor soil types. Planting stock as the Perennial Peanut (*Arachis glabrata*) or *Desmodium* spp. are excellent covers. Cover crop practices negate the need for machete or herbicide weeding, and further serve as a home site nursery for beneficial and predatory insect species. Interesting wild flower mixes, as in the Umbelliferae or Asteraceae families, planted in harmony with these cover crops along fence rows serve as unique pollen/nectar food sources for the survival of natural enemies such as parasites and predators. This helps maintain natural enemies during periods of reduced populations of their primary hosts, the pest insects.

## SHORTEN TREE HEIGHT

In the Americas, 99% of all trees are too tall (20-50 ft.) for efficient harvesting. Under these conditions there is little or no chance for successful sanitation, and spraying, if deemed necessary, would be ineffective. Two of the three major pod diseases can be managed by sanitation. To date, this simple agronomic practice has failed to alleviate the problem; infective sources remain in the trees because they are too high or infected tissue (pods/brooms) can not be observed. Bean prices and convention are the excuses given for not pruning. Yet this practice is cheap, requires little supervision after initial training and meets the required goals.

Future research should be centered around grafting "high yielders" onto slow growing rootstocks to produce "dwarfed" trees which are common to the fruit industry. This in turn will trigger high density population efforts. Dwarfed high density plantings are a critical key, as good farm land in the tropics for plantation crops is becoming a rarity.

## SHADE TREE MANAGEMENT OPTIONS

For decades, "Madre de Cacao" (*Gliricidia sepium*) and Tropical Almond (*Terminalia catappa*) were considered shade trees of choice. Unfortunately, these serve as alternate hosts for cacao thrips, aphids and mealybugs.

By using alternative shade crops such as banana, plantain or papaya, they can also serve as a source of frequent cash or family food. They are not hosts for cacao pests, and banana or plantain trunks can be diced and left on the planting floor to serve as nesting sites for the midges which pollinate cacao (Young 1983). Banana and plantain debris are also high in potassium. Other diversified shade crops like acerola, citrus or coconut can also be used. Fertilizer formulas for these shade crops are in tune with those for cacao.

# THE BOUNDARY LAYER AND CACAO SEMIOCHEMICAL PRODUCTION AS REFERENCES FOR IPM OPTIONS

The Boundary Layer consists of a thin layer of static air completely surrounding all aerial

The laver represents a transition zone of air, motion, and temperature plant surfaces. differences between plant surfaces and open-air space. Within this Boundary Layer are varied amounts of water vapor, oxygen, carbon dioxide, and minute flows of exchanging air. The thickness is influenced by shape, size of the plant part, speeds of the wind and solar radiation. The layer depth may vary from ca. 10 to 1,000 microns. Vapors given-off by impacted pesticide droplets, such as chlorpyrifos, are entrapped within the Boundary Layer and its' fumes are deadly to any pest living or immersed within this zone. Attractant and/or repellent vapors called semiochemicals are given-off by the cacao tree. Fibrous rootlets release a Phytophthora zoospore attractant, pruning wounds give off vapor that attracts stem borers, while trees "under stress" release amino acid vapors through leaf/pod stoma, which in turn attract thrips (Selenothrips rubrocinctus) invasion. The deep locule creases on the pod surfaces serve to accumulate high concentrations of the semiochemical thrips attractant. This is evidenced by typical thrips damage occurring first in these creases then later spreading over the pod surfaces. Our IPM options for these phenomena are to encapsulate such semiochemicals, chelate them or modify the environment so as to increase tree health to stall or impede the plant's production of pest attractant semiochemicals.

### BLACK POD

Since the 1920 s, Black Pod (*Phytophthora palmivora*) has been cacao's primary fungal disease affecting production worldwide. Reviews of world literature disclosed existence of soils suppressive to *Phytophthora* on avocado in Australia. Common to such soils were high o.m., high exchangeable calcium and ammonium nitrate nitrogen, a pH of 6.0 to 6.5 and excellent drainage. Such soil modification has suppressed *Phytophthora* causing diseases on papaya and citrus. It has been common place to spray papayas 12-15 times per season to prevent rot. Now, with suppressive soils fungicidal applications, sprays have dropped to a few or none. Interestingly, major cacao research centers have not accepted this thesis of soil habitat modification as an IPM option. When the price of cocoa beans goes up everyone sprays copper.

Copper does not degrade in the soil, but instead it builds-up. High concentration of copper cause necrosis of fibrous rootlets and further enhance extensive zoospore infections of these rootlets. To reduce copper concentrations/solubility in the soil, lime is added. This works not only to tie-up copper, but also to increase the soil pH. By adding lime, the soil pH increases causing the reduction of *Phytophthora* oospore production, a sexual body that carries this soil living fungus through the dry season.

Seventy years of fungicide testing have yielded mediocre disease control and complete sanitation trials were unsuccessful. In the 1950 s research verified that the soil was the main reservoir of black pod inoculum. The soil environment can be modified by using lime. The companion element linked to lime is boron. Boron is known to increase pod set and reduce cherelle wilt. An annual foliar spray is considered the best method of introducing boron quickly into the cacao tree; an IPM option.

# MONILIA & WITCHES BROOM: SANITATION THE BASIC STRATEGY

Monilia pod rot (*Moniliophthora roreri*) and Witches Broom (*Crinipellis perniciosa*) have rudimentary life cycles that are completed on the tree. Pruning of affected plant parts is an IPM managerial strategy. However, if trees are too tall, survey/pruning teams cannot see all infections, nor can they reach them for effective cutting-out. Sanitation success depends on a 100% pruning success. Fungicides have failed to control these diseases and the inability of sprays to effectively cover tall trees, pesticide residues and high costs have intensified the problem.

This can be managed by pruning trees down to a height of 3.5-4.0 meters. Monilia completes its' entire life cycle on pods in the tree. Pod infection is identified by conspicuous bumpy swellings on the pod surfaces. This is not a difficult scouting task since Monilia is easily observed under proper pruning management. Within 12 days after noting the pod swellings, sporulation begins over the pod surfaces causing a tan discoloration.

In order to monitor for Monilia "sanitation sweeps" are made through the planting after pod set and continue on a 7-10 day schedule. This is well within the expected pod surface sporulation, which would disperse spores through trees during rain. Pods are at high risk from Monilia infection for the first 90 days after fruit set. Field monitoring for IPM sanitation is critical during this period, thus survey crews should be kept on schedule.

After a few months into the rainy season the "Witches Brooms" left on trees begin to sporulate, especially those in the upper canopy. This results in a flow of infective spores over the new foliage, flower and cushions. These brooms continue to sporulate as the season progresses resulting in newly infected Brooms. The new brooms sporulate the following season, while affected flower cushions and young distorted pods produce no spores, and are a complete loss for normal pod production. Young infected brooms are easily recognized and sanitation crews can easily prune them off "short" trees. Because of labor cost, pruned brooms are not removed from the ground area around trees. However, these brooms will sporulate in the next year's rainy season for further spore dispersal. Petroleum oil, like that used for Sigatoka on banana, must be applied as a soil spray onto the old pruned brooms. The oil should be applied before the rainy season starts. Petroleum oil is not a fungicide, but it stops the absorption of rain water into necrotic broom surfaces. It is moisture within the infected brooms that triggers the production of spores. Petroleum oil is not considered a pesticide, its action being physical. Furthermore, oil is not applied to new maturing cocoa pods, but only to the understory ground areas.

# MANAGING THE LEAF CUTTING ANT

The Leaf Cutter Ants (*Atta* spp.) have caused the most physical damage to cacao in the Americas. These ants defoliate mature cacao and tropical fruit shade trees within a few days. The cut-out leaf pieces are carried back to the subterranean nest to provide the growing substrate for the fungus (*Rozites gonglyophorathe*), the food, energy source for the ant colony. Continued scouting of fields to locate and flag nests is the basic strategy for assured success of this IPM program.

*Hymenaea courbarilis* is considered a shade tree of choice and works as a biological barrier used around cacao plantings. This tree produces "terpenoids" that not only repel the leaf cutters, but is also toxic to their fungal food source.

In order to reduce leaf cutter ant populations, chlorpyrifos powder is applied directly into the nest entrance. Chlorpyrifos produces a vapor phase that permeates the underground nest killing ants and the *Rozites* fungus as well.

To protect cash-crop cacao shade such as papaya, citrus or acerola employ Tanglefoot<sup>®</sup>, a non-toxic environmentally safe sticky pine resin that is applied as a "band barrier" to tree trunks.

## COCOA THRIPS

Thrips (*S. rubrocinctus*) attack leaves and pods, especially cacao trees under stress. The main problems are leaf drop causing die-back and pod surface discoloration making it impossible to distinguish between immature and ripe pods. Tree stress can be a multiple complex issue of soil compaction, end-row soil mounding, poor drainage, and loss of shade. The use of shade trees such as "Madre de Cacao" increases the potential of thrips infestation.

Thrips can be managed by visually monitoring on a weekly basis and by employing the yellow sticky card method in which counts of three thrips per card is the trigger threshold. High thrips count areas should be modified; addressing the causes of tree stress. Annual reviews of field monitoring notes would further verify the historical occurrence of thrips outbreaks at the same field sites. Again indicating that agronomic practices should be employed as a solution rather than pesticide application as a resolution.

#### HELOPELTIS AND COCOA POD BORER: MAJOR GLOBAL INSECT PROBLEMS

The annual deployment of broad spectrum insecticides to control, cocoa pod borer (*Acrocercops cramerella*, CPB); and *Helopeltis* (Caspidae) ended with pest tolerance, excessive expense for low cash return, and potential for detectable residues along with jeopardy of human safety.

A wide range of approaches need to be explored sustainable methods of controlling these pests, such as antifeedants, disruptants to mating, egg laying or growth regulators; altering pheromone communications; modifying the cocoa pod semiochemical attractants and suicidal attractant systems.

Localized concentrations of insects are usually two to three weeks in advance of populations in the rest of the planting. Herein, varied innovative tests are needed to determine economic thresholds for profitable results. Tree height has a major influence in these programs for ease of monitoring, placement of traps, pod surface modifications and sanitation.

Long chain fatty acids as oleic, palmitic or linoleic are ideal insect attractants and could

be employed on "false" branches with adhesives in the formulation to determine CPB attraction. The same fatty acids can act to disrupt egg laying. Another problem encountered is trap placement; CPB populations opt for higher levels on the underside of "hydrophobic" surfaces of the branches. Many spray droplets bounce off these targets. Thus, new options are required.

Field proven biorational systems are assured of early EPA registration. It is now in the hands of cocoa researchers to reduce uses of pesticides in fields. Changing of the target surfaces may be one answer, use of oils plus surfactants as leaf litter cocoon killers, another. Nonetheless, more information will be the key to insect IPM success.

### CONCLUSIONS

IPM is gradually making advances into cacao production. However, biorational approaches are lagging far behind expectations. Agencies are attempting to curb the use of broad spectrum pesticides which might lead to residues or permanent environmental damage. Many cultural, biological and chemical techniques are referred to in this paper which would enhance sustainability, if practiced. IPM is a dynamic, ever changing system. It requires constant observation and innovation.

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