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DETERMINATION OF PEA APHID THRESHOLDS IN VINING PEAS

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ABSTRACT

The results of seven experiments carried out in vining peas over two years, showed that significant yield increases were obtained following sprays to control pea aphid *(Acyrthosiphon pisum)*. The largest mean yield increases were obtained from a single application of pirimicarb at either the visible bud stage (GS 202) or at first flower (GS 203). The mean percentage of infested shoots at these stages were 52.3 and 50.1 respectively. A spray at the late vegetative stage gave a similar yield increase, but one made at first pod did not produce a significant yield increase compared with no treatment.

INTRODUCTION

Pea aphid *(Acyrthosiphon pisum)* is one of the most common pests of peas and can infest crops at any time during the growing season. Damage resulting in yield loss can be incurred by direct feeding, the production of honeydew which encourages the development of saprophytic moulds and virus transmission (Biddle, 1985). In combining peas, significant yield responses were obtained from single applications of pirimicarb applied at specific growth stages (Lane & Walters, 1991) and this and subsequent work defined treatment thresholds for both infestation and crop growth stage for economic response.

In vining peas, the growing season is much shorter and no specific threshold for treatment has been determined. Most of the crop in the UK is sprayed routinely and this is not based on either crop growth stage or aphid infestation level. The object of this work was to determine a treatment threshold in vining peas.

METHODS

Experiments were carried out in commercial vining pea crops at three sites in 1991 and four sites in 1992:-

1991	1992
1. Gedney Hill, Lincolnshire	1. Gorefield, Cambridgeshire
2. Crimplesham, Norfolk	2. Moulton-Seas-End, Lincolnshire
3. Carrington, Lincolnshire	3. Thorney, Cambridgeshire
	4. Crimplesham, Norfolk

Each treatment was replicated five times in a randomised block design. At all sites, single sprays of pirimicarb (Aphox) were applied by precision plot sprayers at a rate of 280 g product per hectare in 200 or 250 litres of water. The details and intended crop growth stages, as defined by Knott (1987), were as follows:-

1. late vegetative growth stage	(GS 107)
2. visible bud	(GS 202)
3. first flower	(GS 203)
4. first pod	(GS 204)

Assessments of aphid infestation were made at each of the growth stages immediately prior to spraying, by examining the growing shoots of 25 randomly selected plants on each plot and recording the number of plants infested with one or more aphids. A final aphid assessment was made 7 or 10 days after the last spray.

Harvesting was carried out at the appropriate crop stage, i.e. either freezing or canning stage. Plots 5 m x 2 m were cut by hand and the total haulm weight recorded. The haulm was then vined using a plot viner and the weight of vined peas recorded. The results were expressed as tonnes/ha. Where possible, pea maturity of each plot was measured by tenderometer (TR).

RESULTS

Aphid species present

Pea aphid *(Acyrthosiphon pisum)* was the main species present at all sites in 1991 and 1992. In 1991, however, black bean aphid *(Aphis fabae)* was recorded in addition to the pea aphid at Carrington and Crimplesham. Pea aphid populations built up rapidly during the latter part of the season at all sites in 1991.

In 1992, aphid infestation was relatively high quite early in the season and at most sites continued to rise. However, at Moulton-Seas-End, the population declined rapidly from the end of June and had fallen to zero by 23 July. At some sites, there was re-invasion of aphids following the early application of aphicide.

Weather conditions

In 1991, adverse weather conditions prevented spray applications at some of the intended growth stages at Carrington. In 1992, a heavy rain storm at Crimplesham (Table 8), in the middle of July, may have been responsible for the reduction in aphid infestation.

Aphid infestation

On average, 27% of the plants were infested with aphid at the late vegetative growth stage. Peak infestation generally occurred at first flower and reached 50% (Table 1).

TABLE 1. Aphid infestation: results from 3 sites in 1991 and 4 sites in 1992

Treatment growth stage		% aphid infested shoots Vegetative	Visible bud	1st flower	1st pod	1st pod +7-10 days
Late vegetative	(107)	27.2	19.8	21.7	28.0	18.8
Visible bud	(202)	-	52.3	16.7	11.9	14.7
First flower	(203)	-	-	50.1	10.5	14.7
First pod	(204)	-	-	-	45.5	13.1
Untreated	-	27.2	41.5	50.3	45.7	23.3

Yield responses to treatments

Significant yield responses following treatment were obtained at Crimplesham in 1991 and at three sites, Thorney, Crimplesham and Gorefield in 1992. The yield and maturity data for the individual sites are shown in Tables 2 - 8, together with the level of aphid infestation present at the growth stages at which the spray was applied.

TABLE 2. Gedney Hill, cv. Darfon - 1991

Treatment growth stage	% infestation	Yield t/ha	% of untreated	Maturity (TR)
GS 107	14.0	4.79	102	102
GS 202	25.0	4.71	100	100
GS 203	26.7	5.38	114	101
GS 204	55.0	4.99	106	99
Untreated	-	4.71	100	98
SED @ P = 0.05		NSD		NSD

TABLE 3. Crimplesham, cv. Puget - 1991

Treatment growth stage	% infestation	Yield t/ha	% of untreated
GS 105	30.0	5.16	124
GS 202	72.8	5.12	123
GS 203	96.0	4.68	113
GS 204	10.4	4.67	112
Untreated	-	4.16	100
SED @ P = 0.05		0.33	

TABLE 4. Carrington, cv. Small Sieve Freezer - 1991

Treatment growth stage	% infestation	Yield t/ha	% of untreated
GS 201	39.2	7.98	99
GS 203	49.6	8.22	102
GS 204	67.2	8.34	103
GS 205	56.0	7.92	98
Untreated	-	8.08	100
SED @ P = 0.05		NSD	

TABLE 5. Gorefield, cv. Waverex - 1992

Treatment growth stage	% infestation	Yield t/ha	% of untreated	Maturity (TR)
GS 107	50.0	3.26	106	121
GS 202	37.3	3.78	123	121
GS 203	36.0	3.71	121	121
GS 204	70.1	3.57	117	122
Untreated	-	3.06	100	118
SED @ P = 0.05		0.20		NSD

TABLE 6. Moulton-Seas-End, cv. Markana - 1992

Treatment growth stage	% infestation	Yield t/ha	% of untreated	Maturity (TR)
GS 107	10.0	5.95	98	109
GS 202	62.7	6.06	100	110
GS 203	2.7	5.95	98	109
GS 204	2.7	5.76	95	108
Untreated	-	6.07	100	110
SED @ P = 0.05		NSD	NSD	NSD

TABLE 7. Thorney, cv. Scout - 1992

Treatment growth stage	% infestation	Yield t/ha	% of untreated	Maturity (TR)
GS 107	15.0	4.46	120	99
GS 202	53.6	5.40	145	95
GS 203	79.2	5.23	141	96
GS 204	90.4	4.36	117	95
Untreated	-	3.72	100	97
SED @ P = 0.05		0.49		NSD

TABLE 8. Crimplesham, cv. Puget - 1992

Treatment growth stage	% infestation	Yield t/ha	% of untreated	Maturity (TR)
GS 107	32.0	8.27	113	148
GS 202	64.8	8.21	112	143
GS 203	43.2	8.32	114	147
GS 204	33.6	7.73	106	143
Untreated	-	7.32	100	140
SED @ P = 0.05		0.21		1.9

At most sites, aphid populations built-up throughout the season until just prior to harvest, but there was clear evidence of a sharp population decline which occurred earlier at Moulton-Seas-End in 1992. No single reason could be given for this and a similar decline has been noted in work carried out by ADAS in a MAFF-funded project on pea aphid in combining peas. In some cases there was significant aphid re-infestation which occurred after spraying at the earlier growth stages. This may have contributed to the lower yield increases obtained from these treatments. Re-infestation

may have occurred from adjacent plots - a problem which is inherent in small plot experiments. However, re-infestation on a field scale may not be significant unless there is a continuous migration of aphids from overwintering sites or other crops.

A multi-site analysis of the trials in both 1991 and 1992 (Table 9) showed that sprays applied at visible bud or first flower growth stages gave statistically significant yield increases compared with the untreated control. Yield increases averaged 12% across all sites with a maximum increase of 45% at Thorney in 1992 (Table 7) where plant infestation had exceeded 50% by the visible bud stage. Early sprays were not so effective in providing yield increases and sprays made at first pod were too late to give a significant yield increase.

TABLE 9. Yield responses from aphid control - mean of 7 sites in 1991 and 1992

Treatment growth stage	% infestation	Yield t/ha	% of untreated
late vegetative (GS 105-107)	27.2	5.73	108
visible bud (GS 202)	54.3	5.94	112
first flower (GS 203)	50.1	5.93	112
first pod (GS 204)	45.5	5.59	105
untreated	-	5.31	100
SED @ P = 0.05		0.15	

CONCLUSIONS

The growth stages at which spraying for pea aphid give an economic yield response have been identified. However, the level of infestation, either as number of infested shoots or numbers of aphid per shoot, at which such yield responses are achieved, is not known. Further work is required to evaluate the aphid threshold level at each susceptible growth stage in order to provide firm recommendations for aphid control in vining peas.

ACKNOWLEDGEMENTS

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CONTROL OF CABBAGE APHID: PROLONGED EFFICACY AND REDUCED OPERATOR EXPOSURE WITH DEEP SIDE-PLACEMENTS OF DISULFOTON

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ABSTRACT

The control of cabbage aphid (*Brevicoryne brassicae*) on Brussels sprouts by deep side-placement of disulfoton at various depths and intervals after planting was compared with a sub-surface band application at planting time. It was shown that deep side-placement offered prolonged efficacy without increasing harvest-time residues and with the added advantage of eliminating planter exposure to insecticide vapours.

INTRODUCTION

Aphid infestations are a continual problem affecting a wide range of vegetable crops. Inadequate control results in reduced crop quality especially in brassicas where marketable yield can be reduced by plant malformations, aphid-transmitted plant viruses and the presence of aphids in produce. In the UK, the systemic aphicide disulfoton is applied as a granular formulation at planting to control cabbage aphid (*Brevicoryne brassicae*) on Brussels sprouts. This treatment has been shown to give prolonged control of the pest, but the period of control is often insufficient to keep sprouts aphid-free over a whole season and additional treatments are often needed, especially in very dry seasons when insecticide availability can be reduced considerably (Suett & Padbury, 1976). A study was therefore done to establish if the period of efficacy could be extended by deep side-placement (DSP) of the insecticide at different depths and intervals after planting. Such an application would present the additional benefit of eliminating the exposure of planters to insecticide vapours, an aspect which is currently of much concern to growers.

METHODS

Equipment for deep side-placement of granules

Existing machinery was modified at Horticulture Research International, Wellesbourne (HRI,W) to allow the DSP of insecticide granules. Mild steel tubing, 16 mm i.d. was attached to the leading edge of an angled coulter blade. Two blades were angled, at 38° from the vertical, in opposite directions to allow DSP with the minimum of plant disturbance. The coulters were attached to the horizontal framework of a Stanhay drill unit so that accurate side-placement relative to the plant rows and depth adjustment could be achieved.

Experimental design

Brussels sprouts cv Golfer were sown into 2.5 cm peat blocks containing

chlorfenvinphos (78 mg AI/l peat) to protect against cabbage root fly (*Delia radicum*) and were raised in an unheated plant raising house. The field experiment was established at HRI,W on a light sandy-loam on 14 May 1992. The land received a base fertiliser application (240 kg P,K/ha) before power-harrowing and 190 kg N/ha shortly before planting. The experimental area comprised 4 blocks of 9 randomised, treated plots, each plot comprising 2 rows of 20 plants hand-planted at 60 cm between plants and 75 cm between rows. Table 1 shows the times and placements of the nine treatments with disulfoton (Disyston FE10, 10% AI, Bayer plc). All applications were made at a dose-rate of 10.5 g AI/ 100 m row which, at a spacing of 75 cm, was equivalent to the maximum recommended rate of 1.4 kg AI/ha. No untreated controls were included as the object of the experiment was to compare the efficacy of DSP with a sub-surface band at planting. The sub-surface bands (Treatment 1) were applied via Leeds coulters immediately before planting. DSP treatments were applied along one side of each row, the angled coulters "lifting" the plants slightly. During the 7-week application (Treatments 8 and 9), many of the larger plants were also struck by the applicator framework.

TABLE 1. Dates in 1992 and positions of disulfoton treatments.

Treatment number	Date	Weeks after planting	Distance from row (cm)	Depth (cm)
1	14/5	0	0	sub-surface
2	14/5	0	10	10
3	14/5	0	15	15
4	3/6	3	10	10
5	3/6	3	15	15
6	18/6	5	10	10
7	18/6	5	15	15
8	2/7	7	10	15
9	2/7	7	15	20

Infestation assessments

Cabbage aphid infestations were assessed by counting the numbers of aphids on every fourth plant at intervals from 23 June to 29 September. The numbers of aphids in sprout buttons were assessed at intervals from 27 August to 3 November by taking one mature button from every plant, removing the outside leaves and counting numbers of aphids.

Insecticide residue analysis

Insecticide uptake was monitored by taking the youngest unfurled leaf from alternate plants at fortnightly intervals from 28 June until 2 September. Residues in the buttons were assessed in the buttons taken for aphid assessment. Duplicate samples were obtained by combining leaves or buttons from diagonally opposed blocks. The samples were macerated and a sub-sample was stored at -15^{0}C until analysed.

The plant samples (50 g) were macerated with dichloromethane:methanol (9:1, 100 ml). The extract was filtered, evaporated to dryness, re-dissolved in cyclohexane:ethyl acetate (1:1, 3 ml) and cleaned up on a carbon (0.7 g)/cellulose (2.2 g) column. The column was

eluted with ethyl acetate (100 ml) which was then evaporated to dryness. The residue was re-dissolved in acetone (5 ml) and oxidised with 0.1 M potassium permanganate (20 ml, 30 min) and extracted with dichloromethane (3 x 25 ml) which was evaporated to dryness and the residue re-dissolved in acetone. The disulfoton metabolites present (disulfoton, disulfoton sulphoxide, disulfoton sulphone and the oxygen analogues of these three) were oxidised to the sulphone (DSO_2) and its oxygen analogue ($DOASO_2$). Residue concentrations of the two compounds were determined by gas chromatography using a Hewlett Packard 5890 fitted with a 12 m BP1 (SGE) widebore capillary column and nitrogen-phosphorus detector. Nitrogen (13 ml/min) was used as carrier gas and the injection, oven and detector temperatures were 195, 193 and 225°C respectively. The two components were separated with retention times of 3.9 ($DOASO_2$) and 5.5 (DSO_2) minutes. Recovery efficiencies, assessed by analysing fortified (0.01 and 0.1 mg/kg) untreated samples, exceeded 90%. The detection limit was 0.001 mg/kg and all results are expressed as disulfoton equivalents.

RESULTS

Control of cabbage aphid

The mean number of aphids on the leaves of sprout plants from 23 June (6 weeks after planting) are shown in Figure 1. Initially, planting-time treatments (1-3) were more effective than the post-planting treatments. By mid-July all treatments were performing similarly except the 7 week treatments (8 and 9) which had only just begun to reduce aphid numbers. From late July to mid-September all treatments kept plants virtually aphid free until, in late September, aphid infestations began to increase again. Infestations remained lowest in the treatments (6 and 7) applied 5 weeks after planting.

Changes in the numbers of aphids in sprout buttons from 27 August are presented in Figure 2. Mean numbers of aphids per button never exceeded 2 and were generally below 1 in all treatments. The greatest reduction in infestations continued to be achieved with treatments 6 and 7, applied 5 weeks after planting, with the treatment applied 10 cm deep (treatment 6) performing marginally better than that at 15 cm deep (treatment 7). By the end of September the shallower treatment was keeping buttons free from aphids and they were re-infested only slowly but by early November there were no significant differences ($P = 0.05$) between the 5 week applications (treatments 6 and 7) and the band application (treatment 1). Side placements at planting (treatments 2 and 3) were less effective than the sub-surface band (treatment 1) and again the shallower application (treatment 2) was generally more effective than the deeper application (treatment 3). Performances of applications made after 3 weeks (treatments 4 and 5) and 7 weeks (treatments 8 and 9) did not differ significantly ($P = 0.05$) from that of the sub-surface band (treatment 1).

Insecticide residues

The mean residues of total insecticide present (DSO_2 + $DOASO_2$) in leaf samples are depicted in Figure 3. There were no consistent differences in the residue concentrations between either treatment date or depth. However maximum concentrations in the 5 and 7 week treatments occurred 3 to 6 weeks after application and coincided with reductions in aphid numbers in the early assessments. Residue concentrations in sprout buttons sampled in September are shown in Table 2. There was no difference between treatments and residues in these and subsequent samples were always below 0.01 mg/kg.

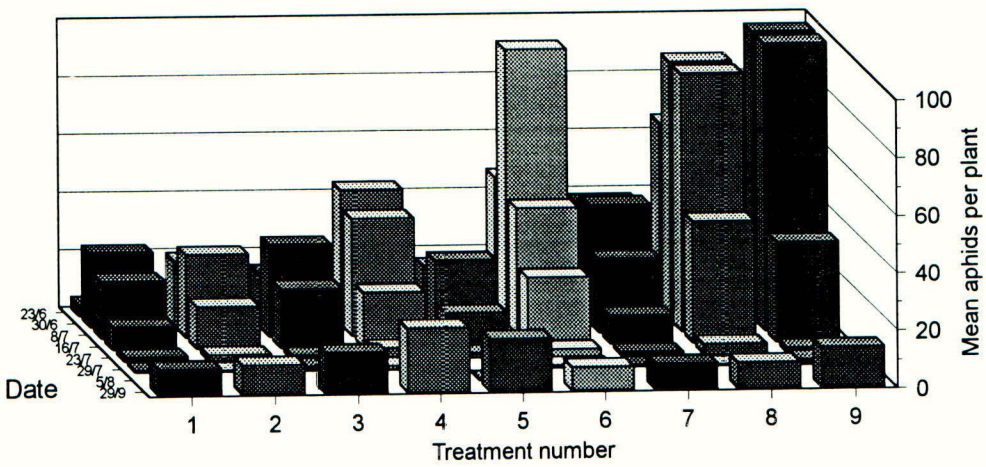

FIGURE 1. The mean number of aphids on leaves of disulfoton treated Brussels sprout plants in 1992. Treatment 1 - sub surface band, 2 - DSP (10 cm) and 3 - DSP (15 cm) at planting, 4 - DSP (10 cm) and 5 - DSP (15 cm) after 3 weeks, 6 - DSP (10 cm) and 7 - DSP (15 cm) after 5 weeks, 8 - DSP (15 cm) and 9 - DSP (20 cm) after 7 weeks.

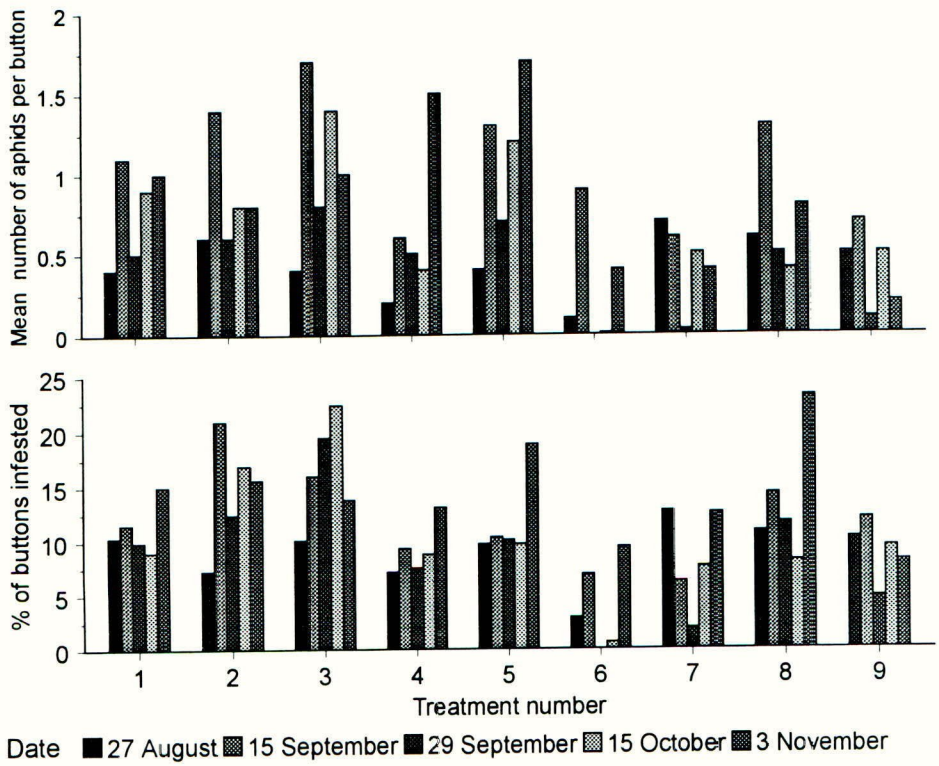

FIGURE 2. The mean numbers of aphids and % of infested buttons on Brussels sprout plants treated with disulfoton in 1992. Treatments 1 - 9 as in Figure 1 above.

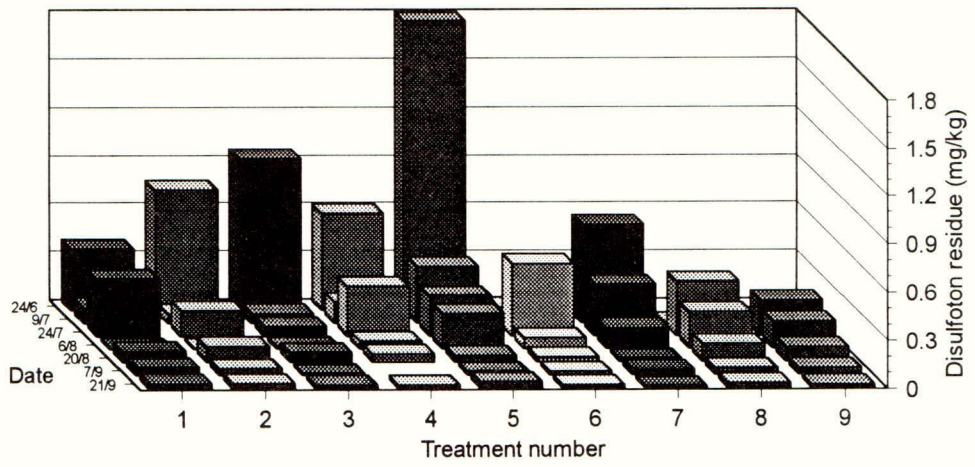

FIGURE 3. Disulfoton residues in sprout leaves in 1992. Treatment 1 - sub surface band, 2 - DSP (10 cm) and 3 - DSP (15 cm) at planting, 4 - DSP (10 cm) and 5 - DSP (15 cm) after 3 weeks, 6 - DSP (10 cm) and 7 - DSP (15 cm) after 5 weeks, 8 - DSP (15 cm) and 9 - DSP (20 cm) after 7 weeks.

TABLE 2. Disulfoton residues in sprout buttons sampled 15/9/92

Treatment number	1	2	3	4	5	6	7	8	9
Disulfoton residue (ug/kg)	4.5	3.0	1.0	1.5	2.5	2.5	2.5	2.0	2.0

DISCUSSION

The study showed that deep side-placement of disulfoton granules alongside established sprout plants could be an effective alternative to conventional planting-time treatments, giving extended efficacy without increased residues in the mature crop. Applications made 5 weeks after planting proved to be the most efficient but this may have been a seasonal effect as, in the summer of 1992, rainfall was greater than average (Figure 4) and July was particularly wet. Insecticide availability, and therefore residue uptake into the plant, would thus have been near-optimum during this time and the consequences of root disturbance are likely to have been minimal. However, this prolonged rainfall would also have maximised leaching of the water soluble DSO_2, the predominant disulfoton oxidation product in soil (Suett, 1975; 1977). In drier years, residue availability from the sub-surface band would be more limited than from the deeper treatments. The optimum application depth is therefore likely to be a compromise to suit a range of soil conditions, being sufficiently deep to maintain availability during drier seasons but not so deep that leaching during wet seasons removes residues from the zone of most efficient uptake by the root system. Similarly, the optimum application distance from rows should provide maximum insecticide availability without excessive plant disturbance. Applications closer than 10 cm are likely to cause too much plant disturbance

and around 25 cm has been reported to be the limit for an effective treatment (Suett & Padbury, 1977).

Despite the wet conditions, disulfoton residues declined steadily after early August, probably due largely to the dilution effects of the growing plant. Residues were therefore barely detectable in the buttons and it seems most unlikely that residue levels at harvest would exceed the maximum residue limit (0.5 mg/kg) irrespective of seasonal or positional differences. Deep side-placement clearly has the potential to prolong the duration of insecticide efficacy as well as to eliminate planter exposure to insecticide vapours. It therefore merits further evaluation and development in order to optimise and realise this potential.

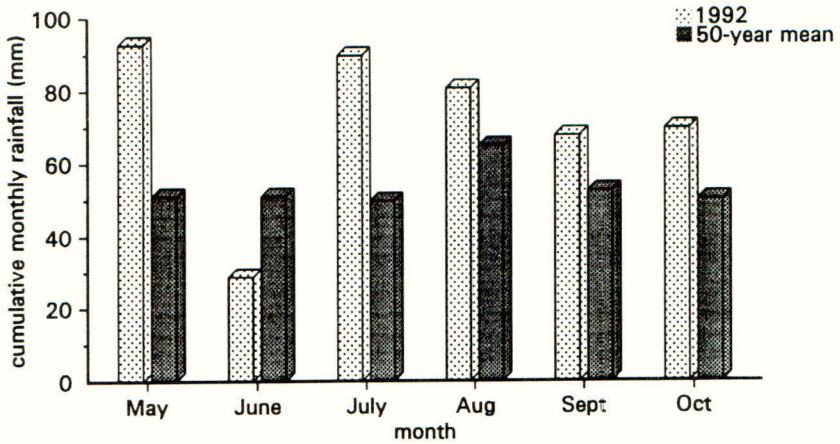

FIGURE 4. Rainfall in 1992 and the 50-year average as measured at HRI Wellesbourne.

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PROGRESS TOWARDS INTEGRATED PLANT PROTECTION IN STRAWBERRY PRODUCTION IN THE UK

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ABSTRACT

Integrated plant protection methods for the major pests, diseases and weeds of strawberries in the U.K. are discussed and an example of an integrated plant protection programme is given. The aim is to minimise pesticide use, including reliance on soil sterilisation, so minimising environmental impact whilst maintaining commercially acceptable control. For many diseases, resistant cultivars are the only sure means of achieving this objective. For pests, many alternative approaches are possible and the most promising are identified. For weed control, difficulties depend on the system of production (polythene mulching is effective), but use of multiple applications of selective short-persistence, contact-acting herbicides may be preferred to reliance on persistent residual compounds, several of which are prone to leaching.

INTRODUCTION

Strawberries are subject to attack by many damaging pests and diseases, several of which are intractable. Control of weeds is also difficult with the restricted range of herbicides available. Fortunately, few weed species are from the same plant family as strawberry (Rosaceae). Profitable production currently relies on intensive use of a wide range of pesticides, often including pre-planting soil sterilisation. Traditional 'main crop' cultivars bear fruit in June (June-bearers). Much effort has been directed to extending the season and improving yield and quality by use of cultural techniques especially crop covers. Cold storage of runners or tip propagation have enabled great variation in planting date, and manipulation of time of fruiting. Plant breeding has made a significant impact by the introduction of everbearer cultivars which fruit continuously from July to October. However, they are subject to attack by several additional 'new' pests, and diseases which are in many cases difficult to control.

In the following paper we briefly review progress made in the development of integrated plant protection methods for strawberries. The use of pest and disease assessment methods and economic thresholds and of cultural, biological and genetic control methods are included. Each of the main crop protection problems is covered. An example of an Integrated Plant Protection Programme, which minimises pesticide use whilst ensuring high standards of control acceptable in commercial practice, is given (see table 1).

STRAWBERRY DISEASES

Verticillium wilt

Almost all commercially acceptable strawberry cultivars are susceptible to wilt *(Verticillium dahliae)*

which has the potential to cause extensive plant losses. Approximately 80% of UK production relies on the cultivar Elsanta which best fulfils supermarket fruit quality standards, but this cultivar is particularly susceptible. Intensive production of susceptible cultivars relies on the routine use of pre-planting soil sterilisation, usually with methyl-bromide. There are no effective fungicide treatments. The development of resistant cultivars which meet the quality requirements of supermarkets is the only sure means of overcoming dependence on sterilants. The current alternative approach is to avoid planting in infested soil and avoid other host crops of the disease (e.g. potatoes, linseed) in the rotation. A test to quantify levels of *Verticillium dahliae* sclerotia in soil (Harris *et al.*, 1991) can be used to assess wilt risk to determine the need for sterilisation or identify wilt-free fields. Since its introduction in 1991 the test has been widely used by growers.

Phytophthora diseases

Most commercial cultivars, and in particular Elsanta, are susceptible to red core (*Phytophthora fragariae*) and crown rot (*P. cactorum*) which can devastate the crop. Both can be introduced into clean land by planting infected runners, and once present remain indefinitely. Soil sterilisation is not a reliable means of control. Current control of red core relies on an integrated approach using a combination of cultural and chemical methods including healthy planting material, the use of raised beds to improve drainage and routine annual treatment with fosetyl-aluminium or metalaxyl + copper oxychloride. For crown rot, disease-free planting material is important but where crops are planted in infected soil, fungicide treatment is of some benefit. Whilst new cultivars have been bred with resistance to red core the existence of many races of the disease means that they cannot be relied on. There is little prospect of improvement in the current strategy for controlling these diseases though for crown rot, the health status of planting material could be improved and there is a good prospect of crown rot resistant cultivars.

Grey mould

Most fungicide treatments are directed against fruit rot caused by grey mould *(Botrytis cinerea)* which consistently causes economic losses that are considerable when conditions favour the disease. In June-bearer crops a programme of at least four fungicide sprays (dichlofluanid or iprodione) is applied from early flowering. In ever-bearer crops, many more sprays are needed. All cultivars are susceptible and prospects for breeding resistant cultivars are remote. In addition, the ability of *Botrytis* to readily develop resistance to certain fungicide groups further complicates the current control strategy.

Much research has been directed to alternative approaches including the use of biological control (Janisiewicz, 1988). Two commercially available formulations of the antagonistic fungus *Trichoderma* have been evaluated in the field and under protection but results have been disappointing. Removal of infected plant material, including dead leaves and rotting fruit, is already widely practised. In reality, the use of fungicides is likely to remain the principle means of control for the foreseeable future. There is considerable scope for improving control and reducing fungicide use by better timing of spray application guided by a disease risk assessment system.

Powdery mildew

All aerial parts of the plant may be attacked by powdery mildew *(Sphaerotheca macularis).* Infection of flowers and fruits reduce yield directly. On June-bearers, losses during fruiting are generally small, the main epidemic occurring post-harvest. Previous work (Freeman & Pepin, 1969) indicated that post-harvest epidemics had no effect on yield the following season but preliminary results with Elsanta show that yields can be reduced. In contrast, on susceptible everbearers losses during fruiting can be serious. Because of difficulties of quantifying infection and the ability of the disease to increase rapidly in favourable conditions, control relies on routine application of fungicide sprays starting at early flower and continuing until the end of the growing season. Cultivars vary markedly in their susceptibility, but Elsanta and most everbearers are highly susceptible. The recently introduced everbearer cultivar Evita exhibits a high degree of resistance and is of good commercial quality. The development of resistant cultivars is the only promising strategy for minimising fungicide use.

STRAWBERRY PESTS

Aphids

The strawberry aphid (*Chaetosiphon fragaefolii*) and the shallot aphid (*Myzus ascalonicus*) are the most important species in the UK. Direct damage to the plant, especially by the shallot aphid, can be considerable and all species can contaminate and downgrade fruits. Strawberry aphid is a vector of the persistent virus diseases crinkle and yellow-edge and of the semi-persistent virus disease mottle. The shallot aphid is a vector of the latter two diseases. Plant symptoms are slight when these viruses occur singly, but they frequently occur in mixtures which severely debilitate the plant. Vein banding virus, which occurs widely in Europe though not in the UK, is aphid transmitted and poses a significant threat.

All the aphid species are readily controlled with aphicides. Pirimicarb is favoured for integrated plant protection, because of its selectivity. To avoid routine treatments, crops should be inspected at fortnightly intervals throughout the growing season and an aphicide applied if significant populations are found. Close examination of the young emerging leaves is necessary. Economic thresholds have not been determined, but a nominal threshold of 10% of plants infested is acceptable in practice. Populations must not be allowed to increase to an extent that plant to plant movement of aphids occurs. There has been little progress in developing biological control agents for aphids on strawberry. It is likely that the response of insect predators and parasites would be too slow to prevent aphids spreading virus infection.

Two-spotted spider mite

Strawberry cultivars vary considerably in their susceptibility to two-spotted spider mite (*Tetranychus urticae*) which is often introduced on planting material. The withdrawal of the acaricide cyhexatin in 1987 led to the widespread commercial use of the predatory mite *Phytoseiulus persimilis* as a biological control agent. This method, though long known, had not hitherto been considered sufficiently reliable in the field for commercial purposes. Recently, commercial use of the mite has become standard practice. Spider mite populations should be assessed at fortnightly intervals (Cross, 1984) and the predator introduced at a rate dependent on the population density (Cross, 1992) during settled weather in spring. Naturally occurring Phytoseiids, including organophosphate (OP) resistant *Typhlodromus pyri* and an *Amblyseius* sp. which appears resistant to OP and pyrethroid insecticides (Easterbrook, pers. comm.), may also assist in regulating spider mite populations.

Though *P. persimilis* has developed some limited resistance to OPs, integration with pesticides used to control other pests, especially blossom weevil and flower pests (see below), is difficult. Pyrethroid insecticides should not be used as they are particularly harmful to *P. persimilis* and naturally occurring Phytoseiids. Recent work has shown that the OP insecticides malathion or chlorpyrifos can be successfully integrated with predators. It is probable that toxicity to the predator can be reduced by downward directed spraying of the upper leaf surfaces only, and this approach is being investigated.

Strawberry mite

Strawberry mite (*Tarsonemus pallidus fragariae*) is especially troublesome on everbearer crops which cannot be sprayed with endosulfan because of the long safe-to-harvest interval. Use of the predatory mite *Phytoseiulus persimilis* for control of two spotted spider mite depresses populations, but does not provide adequate control. Recently we have had favourable results by introducing *Amblyseius cucumeris* and *A. barkeri* in spring. These predatory mites are available at very low cost from biological control suppliers.

Vine weevil

Since the withdrawal of the persistent organochlorine soil insecticides, growers have struggled to control this pest *(Otiorhynchus sulcatus)* which is often locally devastating. High volume drenching with chlorpyrifos which is currently the usual means of control is unpopular because it is expensive and labour

intensive. Other factors which have led to an increased prevalence of this pest in recent years are the widespread use of soil sterilants and of methiocarb slug pellets which are harmful to predatory insects, and the increased use of polythene mulches which favour the pest by providing a protected environment. Limited success in controlling vine weevil using entomopathogenic nematodes (*Steinonema carpocapse*) applied through irrigation systems in late summer or spring has recently been reported (Kakouli *et al.*, 1994). However, the nematodes are of only short persistence in soil and their reliability in commercial practice has yet to be proven. They are effective if applied to warm ($>15^{o}C$) moist soil against young larvae. We have obtained success with incorporation of controlled release chlorpyrifos granules in the substrate for module raised plants, a method that greatly reduces insecticide use. However, crops established from module raised plants comprise only about 15% of current production.

Strawberry blossom weevil

Strawberry blossom weevil (*Anthonomus rubi*) is prevalent on most intensive strawberry farms. Adult weevils partially sever individual flower stalks after laying an egg in the unopened flower bud. A spray of chlorpyrifos is applied against adults before flowering with subsequent sprays as necessary. Control on everbearer cultivars is complicated because insecticides harmful to bees should not be used during flowering. Alternative approaches to control of this pest have not been adequately explored. Work by us is in progress to determine economic thresholds. The degree of damage compensation by the crop is likely to vary considerably according to method of production and time of season. There is scope for investigation of alternative selective or biological control methods.

Caterpillars

Several leaf rolling tortricid species are minor pests attacking leaves and flowers and certain noctuiids (e.g. *Phlogophora meticulosa*) defoliate plants. Crops should be inspected fortnightly throughout the growing season and a spray applied if significant infestation is detected. *Bacillus thuringiensis* is effective, especially against the noctuiids, providing temperatures are sufficiently high for active feeding by caterpillars. Chlorpyrifos or malathion, applied to control blossom weevil or flower pests are also effective. Registration of an insect growth regulator for use on strawberry would be useful for control of several pests.

Slugs and strawberry seed beetle

Ripening fruits are attacked. Damage by slugs (e.g. *Deroceras reticulatum)* is widespread and frequently economic, despite routine use of methiocarb pellets which are applied to the soil surface after flowering and shortly before strawing. Methiocarb is toxic to carabid beetles (adults and larvae) which may be useful predators of vine weevil. Metaldehyde may be a safer alternative but may be less effective against strawberry seed beetle, *Harpalus rufipes*, itself a carabid. Evaluation of this approach in the field is required.

Flower pests

With the advent of everbearer cultivars which flower continuously during the summer and autumn, several species of thrips and capsids (especially *Lygus rugulipennis*) have become significant pests (Easterbrook & Cross, 1993). Crops must be monitored regularly, at least at fortnightly intervals, and a spray of a short persistence OP such as malathion or heptenophos applied if significant populations develop. Downward directed spraying should minimise adverse effects on *Phytoseiulus persimilis*. Nominal thresholds of a mean of 1 thrip/flower (Cross, 1992) or 2 capsids/50 plants are proposed (Easterbrook, 1994).

WEED CONTROL

Achieving adequate weed control is a challenge to every strawberry grower. Current commercial methods vary according to the system of production. In matted row crops, where soil sterilisation is not widely practised, control relies on the use of herbicides pre- and post-planting. In polythene mulched

crops (possible only with single spaced plants), soil is often sterilised before planting, providing very effective (non-residual) control of the majority of weed species. The mulch itself provides an effective means of control, though weed growth close to the crown of the plant can be a problem.

Soil sterilisation is not favoured in integrated plant protection programmes though it is currently indispensable for control of *Verticillium* wilt on susceptible cultivars on intensive farms. Integrated weed control in strawberry production and the avoidance of soil sterilisation are worthy of consideration.

Standard growing practices such as proper rotations and ensuring freedom from perennial weeds by using glyphosate prior to planting is essential. Biological control offers few prospects, though there are often naturally occurring diseases and insects that decimate populations of a particular weed species. Mechanical weed control is also possible in theory, though detailed hand weeding would be needed in the crop rows as it is impossible in strawberry to set an acceptable weed threshold. There is scope for improvement in the strategy of use of herbicides, bearing in mind persistence (including adverse effects on the following crop), toxicity and other factors that affect environmental impact. Long-persistence herbicides (trifluralin, napropamide, isoxaben, propyzamide, chlorthal-dimethyl) especially and moderately persistent herbicides (diphenamid, lenacil, pendimethalin, simazine, chlorpropham) should be avoided if possible, especially where highly soluble in water and poorly adsorbed onto organic matter so posing a risk of leaching (e.g. simazine, diphenamid, lenacil). It is likely that a commercially-acceptable standard of weed control could be achieved using short persistence herbicides, especially selective contact-acting materials such as phenmediphan and clopyralid. Phenmediphan is active only against newly-emerged weeds up to the 2-leaf stage and it can be phytotoxic to some cultivars. Repeated applications in response to flushes of newly emerged weeds would be required. Addmixture with clopyralid (both products of reduced doses) for one application just before flowering and one immediately post harvest (the maximum permitted) would be beneficial where more mature weeds have established. Work at Weed Research Organisation on Cambridge Favourite (Davidson & Bailey, 1980) showed that this strategy could be effective with minimal crop damage. Use of the short persistence residual herbicide propachlor should also be considered pre-emergence. However, it is difficult to quantify the benefits of such a strategy in crop protection or environmental terms in comparison with current practice.

ACKNOWLEDGEMENTS

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TABLE 1. An integrated plant protection programme for the June-bearer disease susceptible cultivar Elsanta grown on raised polythene-mulched beds and planted in early August. This programme is suitable only for farms where suitable wilt free land is available.

Time/growth stage	Target	Action
Pre-planting	Verticillium wilt,	Have soil tested for wilt risk and nematodes, avoid infested sites.
	Nematodes	Examine soil in lab, avoid sites infested with pest species, especially virus vectors.
	Weeds	Encourage germination by cultivation and control with glyphosate.
Planting	Virus, mites, nematodes root pathogens	Use clean, healthy planting material. Apply fosetyl-aluminium or metalaxyl + copper oxychloride as soon as new growth starts to control *Phytophthora* sp.
Establishment (autumn)	Aphids, caterpillars	Inspect fortnightly, apply pirimicarb for aphids or *Bacillus thuringiensis* for caterpillars if necessary.
	Spider mite	Introduce *Phytoseiulus persimilis* shortly after planting as soon as infestation reaches suitable level.
	Vine weevil	Examine foliage fortnightly for signs of adult feeding. Apply drench of entomopathogenic nematodes in late August/early September if infestation is detected.
	Weed control	Inspect soil round crown of plant for emerging weeds. Spray phenmediphan at 2 leaf stage as necessary.
	Mildew	Apply programme of sprays of bupirimate alternating with a DMI fungicide. Adjust interval according to disease intensity.
Pre-flowering (spring)	Aphids, caterpillars	As per autumn unless spray for blossom weevil to be applied (see below).
	Spider mite	As per autumn.
	Weed control	As per autumn. Add clopyralid to phenmediphan for one spray if established weeds present.
	Blossom weevil	Inspect crop carefully for adults when the stem of the first flower truss is extending. Spray chlorpyrifos if adults or damage significant.
Flowering	Caterpillars, spider mite	As per autumn.
	Botrytis	Apply programme of sprays of dichlofluanid starting at first flowering.
	Tarsonemid mite	Introduce *Amblysieus* if infestation detected.
	Mildew	Apply programme of sprays of a DMI fungicide starting at first flowering.
Fruit development	Botrytis	Apply sprays of iprodione alternating with chlorothalonil as necessary, depending on level of Botrytis and weather conditions.
	Mildew	Apply sprays of bupirimate if required, depending on weather and level of foliar mildew.
	Slugs, seed beetle	Apply metaldehyde pellets pre-strawing.
Fruiting	General	Avoid application of plant protection products if possible.
Post harvest	General	Mow off foliage and remove debris.
	Weeds	Spray phenmediphan + clopyralid if weeds present.

CHEMICAL CONTROL OF THE SOUTH AMERICAN LEAF MINER, *LIRIOMYZA HUIDOBRENSIS*

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ABSTRACT

The South American leaf miner (*Liriomyza huidobrensis*) continues to be introduced to the UK on imported plant material and is subject to statutory action. Laboratory trials at CSL Harpenden and glasshouse trials at ADAS Trawsgoed showed that the insecticides cyromazine and abamectin were very effective against larvae of *L. huidobrensis*, while triazophos and trichlorfon were also effective, but on occasions were phytotoxic to ornamental plants. Triazophos was effective against adult leaf miners but other organophosphorous materials, such as malathion and dimethoate were not sufficiently active except at rates greater than those used in the field.

INTRODUCTION

The South American leaf miner, *Liriomyza huidobrensis,* caused serious damage to a range of crops in Holland in 1989 (Bartlett *et al.*, 1989) and soon afterwards the first outbreaks were recorded in the UK. Primary introductions were largely associated with chrysanthemums but other crops were also soon infested. *L. huidobrensis* is highly polyphagous and infestations have been recorded on a wide range of plants from many families, with both protected ornamental and vegetable crops being at risk. In addition to chrysanthemums, early outbreaks occurred on celery, coriander, cucumber, *Dianthus*, lettuce, pak-choi, pansy, *Primula*, radish, spinach and *Verbena,* with over 100 outbreaks being recorded by the end of 1990 (Bartlett, 1993; Cheek *et al.*, 1993).

L. huidobrensis is a listed pest in the UK legislation (Anon., 1987) so all outbreaks are closely monitored and a number of controls are required, usually including intensive use of pesticides. However, *L. huidobrensis* exhibits a high level of tolerance to many pesticides, which suggests the presence of pesticide resistance (Macdonald, 1991), and chemical control can therefore be difficult. If control schedules are to be effective, it is important to know which pesticides are most effective against various stages of the pest. Avoiding the use of ineffective pesticides will also reduce the number of unnecessary applications with consequent economic and environmental benefits. Investigations were therefore carried out on a laboratory scale by the Central Science Laboratory and by ADAS in glasshouses, to screen a range of materials under controlled conditions. Because of the statutory status of the pest all tests were carried out in licensed quarantine facilities.

METHODS

Samples of *L. huidobrensis* used in these trials were originally collected from outbreaks on growers' holdings in 1990. Cultures of leaf miners were maintained in cages at 26 ± 3 °C, adults were given access to 10% sugar solution. For the glasshouse trials either tomatoes or chrysanthemums were presented to adults for oviposition. Mines were allowed to develop until pupae emerged and dropped into sand at the bottom of each cage. For glasshouse tests on larvae, four insects were introduced, as pupae, into cages, for each experimental plant. When mines were observed on the majority of leaves (normally 4-5 days after oviposition), pesticide treatments were applied to run off using an Oxford precision sprayer. For the trials on chrysanthemums the treatment was repeated one week later. Each test utilised 6-10 plants per treatment arranged in a randomised block design on the glasshouse bench.

For laboratory tests, insects were cultured on a range of plants. Tests on larvae used plants infested with approximately 100 larvae. Up to three plants were then individually sprayed to run off. The efficacy of treatments against larvae in leaves was determined by counting the number of pupae that successfully emerged. Tests on adult flies were carried out in the laboratory using adults anaesthetised with CO_2, placed on glass fibre filters in a petri dish and sprayed using a Potter tower. For each pesticide a logarithmic series of doses plus a control was tested. Controls were sprayed with distilled water. Mortality was assessed 18 hours after treatment and dose response curves calculated.

RESULTS

Laboratory tests

The LD_{99} for adult *L. huidobrensis* are compared with the maximum permitted field rates for the pesticides tested in Table 1. Those pesticides with LD_{99}s that are less than the maximum field rate are likely to be effective in the field. The results show that adult leafminers have a high level of tolerance to pyrethroids and to some organophosphorous insecticides. Trichlorfon and Heptenophos were both very effective at rates below those recommended for field use and triazophos was effective at rates approved for use on ornamental crops. Pyrethroids may have a repellent action on adult *L. huidobrensis,* which might help to protect crops but this would not be detected in these laboratory tests.

The initial laboratory screening work against larvae is presented in Table 2. These screens showed that abamectin, triazophos, trichlorfon and the new material imidacloprid could all kill larvae inside the leaf; thus preventing pupal emergence. Deltamethrin had some effect, reducing larval emergence by 40%, but this level of kill is unlikely to provide effective control of populations. Heptenophos, which had proved to be very effective against adult flies had no effect against larvae.

TABLE 1. The response of adult *Liriomyza huidobrensis* to insecticides.

Active Ingredient	LD_{99} (fiducial limits)	Max Field Rate
Dimethoate	10.6 (5.25-109)	0.636
Malathion	10.8 (4.04-287)	0.803
Nicotine	11.0 (7.97-27.7)	4.2
Fenitrothion	11.7 (7.24-27.6)	0.89
Triazophos	1.01 (0.629-2.46)	0.331 on vegetables 1.53 on ornamentals
λ-cyhalothrin	7.94 (2.87-309)	0.12
Deltamethrin	2.80 (1.86-8.57)	0.445
Trichlorfon	0.209 (0.171-17.3)	1.11
Heptenophos	0.391 (0.300-42.4)	2.39

All units are µl of formulated pesticide deposited onto a 90mm diameter circle.

TABLE 2. Effect of insecticides on *Liriomyza huidoibrensis* larval emergence +.

Active Ingredient	Spray rate (ml/100 litres)	% reduction in larval emergence from control
Abamectin	25	92-98
Deltamethrin	45	40
Dimethoate	21	0
Heptenophos	75	0
Teflubenzuron	50	0
Imidacloprid	25	100
Triazophos	50	89-98
Trichlorfon	150	97

+one spray of each insecticide was applied to plants 4-5 days after oviposition.

Glasshouse trials

Further trials were then carried out at ADAS Trawsgoed to verify some of these results in small plot trials in the glasshouse (Tables 3 and 4). In the trials on chrysanthemums all four pesticides tested produced significant levels of kill, with abamectin and cyromazine completely preventing pupal emergence. For the tests on tomato plants, teflubenzuron was ineffective giving no significant difference compared to the control in either the number of mines or the number of pupae per plant. Cyromazine had no significant effect on the number of mines developing, however the mines were much shorter than those in the control plants and pupal emergence was completely prevented. Trichlorfon was effective, especially at the high rate but caused some phytotoxic damage (necrosis).

TABLE 3. Numbers of *Liriomyza huidobrensis* pupae emerging after treatment: host plant chrysanthemums.

Treatment	Spray rate (ml/100 litres)	Mean number of pupae per plant‡
Control	-	10.0[a]
Triazophos	50	1.6[b]
Abamectin	50	0.0[c]
Cyromazine	100	0.0[c]
Deltamethrin	45	1.5[b]
SE ±	-	2.3

‡ Means followed by the same letter are not significantly different. (Duncan's multiple range test).

TABLE 4. Numbers of *Liriomyza huidobrensis* mines and larvae after treatment: host plant tomatoes

Treatment	Spray rate ml/100 litres	Mean number of mines per plant	* Mean number of pupae per plant
Untreated	-	3.75[a]	2.50[a]
Trichlorfon	100	1.0[b]	0.38[b]
Trichlorfon	150	0.0[b]	0.0[b]
Teflubenzuron	50	4.37[a]	2.50[a]
Cyromazine	100	3.87[a]	0[b]
SE ±	-	1.02	0.61

*Means followed by the same letter are not significantly different (Duncan's multiple range test).

DISCUSSION

These trials have confirmed that some insecticides have little effect on either adult or larval stages of *L. huidobrensis*. In the larval tests there was good agreement between the results of the initial laboratory screens and the larger glasshouse trials, carried out under more natural conditions. All the pesticides which showed promise as control agents in the laboratory tests proved to have at least some efficacy in the glasshouse trials. One pesticide, deltamethrin, showed a higher level of efficacy in the field trial (85% control) than had been expected from the laboratory screen (40% control). However this may have been a result of the double application of pesticides in the glasshouse, which was compared to only a single application in the laboratory. The initial, laboratory, screen also failed to pick up the phytotoxic effect of trichlorfon. Many of the pesticides that did not show any efficacy in the laboratory trials were not carried forward for further testing, so the possibility that some of these may be more effective than was indicated in these tests cannot be ruled out. However,

it would appear that small scale laboratory tests can give a good indication of the efficacy of a pesticide under field conditions, though further trails under more realistic conditions should always be carried out.

Abamectin, imidacloprid, trichlorfon and cyromazine all gave good control of larvae, trichlorfon was also effective against adult flies. Triazophos was also effective but only at the higher doses approved for use on ornamentals. Heptenophos gave good control of adults but had no effect on larvae.

Triazophos is known to cause damage to some ornamental crops and these trials show that trichlorfon may also cause some damage to crops. Abamectin is a translaminar pesticide so the toxicant forms a reservoir inside treated leaves (Lasota & Dybas, 1991) making it ideal for the control of leafminers. It has now been registered in the UK as Dynamec and has already proved valuable in controlling outbreaks of *L. huidobrensis* on ornamental crops. Cyromazine is an insect growth regulator and so will not kill larvae until one or more moults have occurred (Schlapfer *et al.*, 1986). This explains the development of mines that were later aborted in plants treated with this chemical. Although cyromazine shows a high level of efficacy against larvae as a foliar spray there are crops where the presence of mines may be unacceptable, this may limit the use of this chemical. There is currently no registration in the UK for cyromazine. The only registration for imidacloprid does not allow its use against leafminers on ornamentals.

These tests show that there is a very limited range of effective chemicals for use against *L. huidobrensis*. Pesticide resistance may also be a problem, and this may increase in the future. The existing effective chemicals should therefore be used with care, to avoid the further development of resistance. Different chemicals, from different chemical groups should be used in sequence and large outbreaks that are difficult to control should be monitored for the development of resistance.

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PYMETROZINE (CGA 215'944): A NOVEL COMPOUND FOR APHID AND WHITEFLY CONTROL. AN OVERVIEW OF ITS MODE OF ACTION

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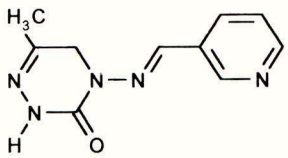
ABSTRACT

Pymetrozine is a new insecticide discovered and developed worldwide by Ciba. It is a pyridine azomethine and thus represents a novel chemical class of insecticides. Pymetrozine exerts selective activity against homopteran insects (aphids and whiteflies). Although it has no knockdown effect, pymetrozine rapidly affects the feeding behaviour of aphids. This was demonstrated in lab studies using the electrical penetration graph (EPG) technique. When pymetrozine was applied to the aphids topically, by injection or *via* the diet, insertion of the stylets into the plant was almost immediately blocked. The aphids died, probably by starvation, a few days later. The blockage of stylet insertion took place with a delay of several hours when pymetrozine was sprayed onto the plants or applied systemically *via* the roots. The block of aphid feeding activity was irreversible. No repellent action was observed. In the locust, studied as a lab model insect, pymetrozine acted on the nervous system and on the foregut by stimulating their spontaneous electrical activity and peristalsis, respectively. Whether these effects are related to pymetrozine's action at the cellular level is still open. In any case, no known insecticidal mode of action is involved. Taken together, pymetrozine is a fast acting compound for the control of plant sucking insects at the level of their feeding behaviour.

INTRODUCTION

Pymetrozine, CGA 215'944 is a new insecticide from a novel type of chemistry; its basic structure is a pyridine azomethine:



Pymetrozine is active against aphids and whiteflies on different crops and against hoppers on rice (Flückiger *et al.*, 1992a, b). Initial studies on its mode of action revealed that it affects feeding behaviour of the aphids by preventing feeding, and is not due to a deterrent action. Mortality is slow, probably due to starvation (Schwinger *et al.*, 1994). To get more information on the mode of action of pymetrozine the electrical penetration graph technique (EPG) was used to record distinct phases of feeding activity in individual aphids. Furthermore, we searched for the molecular mechanism(s) underlying the feeding inhibition by testing pymetrozine in a number of *in vitro* and *in vivo* tests on insecticidal targets.

MATERIAL AND METHODS

Aphid experiments

Aphids

Colonies of *Aphis fabae* were reared on bean plants, *Macrosiphum euphorbiae* on potato plants, *Myzus persicae* on chinese cabbage and lettuce plants and *Aphis gossypii* on cucumber plants. All plants were kept in a glasshouse at 19 ± 2°C.

Treatments

Topical application. For direct aphid treatment, stem solutions of pymetrozine in dimethyl-sulfoxide (DMSO) were appropriately diluted with acetone. Pymetrozine was applied in a volume of 25 nl with a pressure-operated micro needle. Controls received the solvent only. The treated aphids were immediately prepared for the EPG technique which took about 5 min.

Injection. For micro-injections, self-made calibrated glass needles were used. Stem solutions were made up in DMSO and diluted with aphid saline (mM: Na^+, 391; K^+ ,19; Ca^{2+}, 11; Cl^-, 302). Usually, the injected volume was 2 nl containing not more than 8% DMSO (by vol.). Controls were injected with the same amount of solvent.

Diet feeding. Feeding through a parafilm membrane was performed with diet nr. 144 (Harrewijn, 1983). The device contained two feeding chambers which could be switched within seconds.

Plant spraying. Plants were sprayed with pymetrozine solutions while standing on a turntable. Controls were sprayed with water only. Aphids settled after 24 h.

Systemic application. For systemic root uptake, plants were placed in solutions of pymetrozine. Care was taken that all of the solution was taken up which was then replaced by fresh distilled and aerated water. Tests were started 24 h later.

Electrical recording of penetration behaviour

The electrical penetration graph (EPG) technique was used basically as described by Tjallingii (1988) with modifications to be described elsewhere. All studies were done under climatically fully controlled and electrically isolated conditions. The aphids were connected to a gold wire of 20 μm diameter with a small droplet of silver paint. The signals, created when the aphid inserted the stylet into the plant, were amplified, displayed and recorded for later use.

Locust experiments

Locusts

Locusts (*Locusta migratoria)* were bred inhouse under a light:dark cycle of 12h:12h at constant temperature (28°C); they were reared on wheat seedlings. The test insects were not sexed. For the ganglion test, they were normally fed, but starved for one day for the foregut test.

Preparation and recording

Ganglia: The suboesophageal ganglion was used for a primary test of pymetrozine on neuronal activity. Parts of the cuticle were removed to get access to the suboesophageal ganglion. Nervous activity was recorded by drawing the cut salivary nerve into a sucking electrode which was connected to a differential preamplifier. The preparation was continuously superfused (2 ml/min) with saline (mM: NaCl, 180; KCl, 10; $CaCl_2 \cdot 6H_2O$, 10; $MgCl_2 \cdot 6H_2O$, 15; Hepes (4-(2-Hydroxyethyl)-piperazine-1-ethane-sulfonic acid), 5; pH 6.8.) Once activity was stable, pymetrozine, dissolved in locust saline, was added at the appropriate

concentrations. The metathoracic ganglion was similarly made accessible; the leg nerve was used to study its activity.

Foregut: The isolated foregut was ligated at the oesophageal and proventricular regions. For isotonic measurements of the spontaneous rhythmic contractions (peristalsis) the preparation was mounted in a cylindrical glass chamber that was continuously superfused (5 ml/min) with aerated locust saline. The top of the glass chamber was open, allowing attachement of the thread from the anterior end of the foregut to a force-displacement transducer. The resulting contractions were displayed on a polygraph recorder.

RESULTS

Effect of pymetrozine on aphid feeding behaviour

Application to the aphids

Topical application of pymetrozine in a number of aphid species showed that this new insecticide had a rapid action, as the initial penetration behaviour was immediately disrupted. Though the treated aphids started walking on the leaf they did not try to insert their stylets. The representative data of two of the species studied (Table 1) demonstrated that it is the initiation of feeding which was effectively blocked. If the phloem was ever reached, it took much longer than normal, and the aphid ingested sap for a very short time only.

TABLE 1. Effects of pymetrozine after topical application to aphids.

	Myzus persicae Dosage (ng/aphid)			*Aphis gossypii* Dosage (ng/aphid)		
Effect on	0	50	100	0	50	100
Time to first penetration (min)	1.5	280	~	1.8	220	~
Longest feeding pattern E2 (min)	> 600	36	0	> 600	15	0
Mortality in first 24 h (%)	2.0	12	90	3	14	85
Offspring per aphid in 24 h	2.0	0.8	0	2.3	1.0	0

~ not reached

When pymetrozine was **injected** into *M. persicae*, the lowest active dose was 1.2 ng per aphid, which is about 3 mg per kg of body weight (c. 0.4 mg). Doses above this level resulted in immediate inhibition of the probing behaviour; the aphids did not recover but usually died within 24 h. Further dose increases immobilised the insect.

In the diet **feeding** studies, a concentration of 300 μg pymetrozine per ml of diet was sufficient to disrupt food uptake after only 5 to 10 min of feeding. Calculations on the resulting haemolymph concentration corresponded very well to those necessary by injection. As in the diet experiments, the aphids inserted their stylets into the diet and started feeding, which was interrupted only later, it is concluded that the feeding block was not due to a deterrent action by pymetrozine. This is in agreement with other observations.

Application to plants

When the plants (without aphids) were **sprayed** with pymetrozine, all aphid species started stylet penetration as normal. After a few hours, however, the E2 feeding pattern was not continued and, after the stylets were withdrawn, penetration was not resumed. If recovery occurred feeding was continued for a short period leading to uptake of more pymetrozine. Representative data on two species are given in Table 2.

TABLE 2. Effects of pymetrozine after spraying of the aphid food plants.

	Aphis fabae Spray conc. (mg/l)			*M. euphorbiae* Spray conc. (mg/l)		
Effect on	0	80	200	0	80	200
Time to first penetration (min)	4.5	5.0	4.2	2.5	3.2	2.8
Longest feeding pattern, E2 (min)	>600	<120	<60	>480	<120	<60
Mortality in first 24 h (%)	2.0	36	82	1.5	12	32
Offspring per aphid in 24 h	1.9	0.5	0	2.3	1.2	0

The results obtained after **systemic application** of pymetrozine *via* the roots were very similar to those of the spraying experiments. Pymetrozine concentrations of 80 and 200 mg/l were tested for effects on aphid feeding behaviour. As pymetrozine was in the plant only, as in the spray experiments, all aphids started normal penetration and sap uptake. Feeding was interrupted or completely stopped some time later which depended on the dose applied.

Irreversibility of pymetrozine action

When aphids which had ceased feeding due to the action of pymetrozine were placed on untreated plants they did not resume feeding. Detailed studies on *Aphis craccivora* (details not shown here) demonstrated that 3 h of exposure to pymetrozine were sufficient to let the aphids die which may take place after 1 or 2 days. Hence, the action of pymetrozine looks irreversible provided a certain amount has been taken up.

First choice studies

When food plants were systemically treated with pymetrozine (40/100 mg/l) and offered to *M. persicae* and *A. fabae* in a choice situation with untreated plants, there was no preference at all for any of the plants. These and other studies (to be reported elsewhere) convincingly demonstrated that the feeding inhibition effect of pymetrozine is not based on a repellent action.

Effects of pymetrozine on the nervous system and on the foregut of *Locusta migratoria*

As pymetrozine rapidly affected the behaviour of aphids it was speculated that this new compound might act on the nervous system. Though pymetrozine is selective for plant sucking insects, model studies had to be done with conveniently sized non-target species such as the locust, a routine lab insect. These experiments, designed to provide some insight into the cellular mode of action of pymetrozine, were performed at the organ or tissue level.

Effects on the nervous system

The effect of pymetrozine on neuronal activity was studied on two different ganglia *in situ*. Pymetrozine induced an increase of the spontaneous electrical activity of the metathoracic ganglion and the suboesophageal ganglion a few seconds after bath application (Figure 1). This activation decreased after 10 to 30 minutes. The threshhold level for the increase of the spontaneous activity of the suboesophageal ganglion was at 0.1 μM.

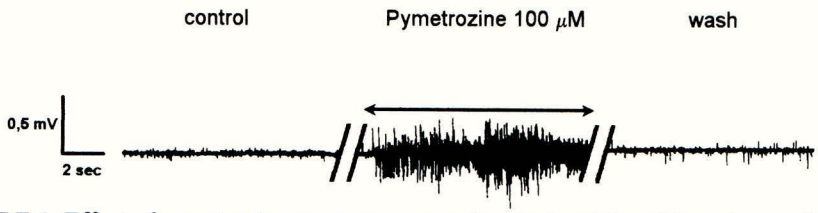


FIGURE 1. Effect of pymetrozine on spontaneous electrical activity of the suboesophageal ganglion in *Locusta migratoria*.

Effect on the foregut

Bath application of pymetrozine to the isolated locust foregut resulted in activation of its spontaneous contractions (peristalsis). Both, the frequency and the amplitude of the contractions were increased (Figure 2).

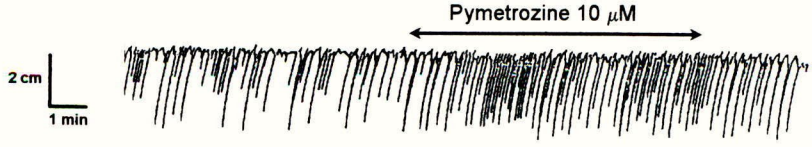


FIGURE 2. Effect of pymetrozine on spontaneous contractions of the locust foregut.

Taken together, physiological responses to the application of pymetrozine can be recorded at the nervous system and at the foregut in the locust as a lab model insect. It remains to be seen whether these effects reflect or are related to the mechanism of action of pymetrozine as observed in aphids.

Tests on the target mechanism of pymetrozine

A large number of diverse biochemical and electrophysiological assays, both *in vitro* and *in vivo*, were performed on all established modes of action of commercial insecticides in order to identify the target mechanism of pymetrozine action. All of these specific tests were clearly negative (manuscript in prep.). Hence, pymetrozine does not use any of the classical targets such as the sodium channel, acetylcholine esterase, the nicotinic acetylcholine receptor, the GABA receptor and chitin biosynthesis. Also mitochondrial respiration is not affected. The mode of action of pymetrozine is thus obviously a novel one affecting the nervous system without being of acute toxicity even to the target insects.

SUMMARY

Pymetrozine (CGA 215'944) represents a novel type of insecticides with a high selectivity for plant sucking insects. It can be applied in various ways and affects the feeding behaviour in a similar way in all aphid species studied. Its action is fast as it leads to an immediate feeding stop, respectively to a blockage in stylet penetration (feeding initiation). There is no knock-down effect, in contrast the aphids stay alive, walk around but do not feed again. This blockage of feeding behaviour is irreversible. Death, which may be due to starvation, occurs one or a few days after pymetrozine application depending on the species and the instar.

A consequence of the fast inhibition by pymetrozine of the probing behaviour is that the transmission of plant viruses by aphids is effectively inhibited. A detailed study proved that pymetrozine strongly reduced transmission of the persistent potato leafroll virus and partially reduced transmission of the non-persistent potato virus Y^N (Harrewijn & Piron, 1994).

The molecular mode of action of pymetrozine has not yet been identified. It it obvious, however, that none of the established modes of action of commercial insecticides is affected. Hence, the target mechanism of pymetrozine can be expected to be a novel one.

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