SESSION 7D

ADVANCES IN HORTICULTURAL CROP PROTECTION

Session Organisers	Dr P R Ellis and Dr J G White *HRI, Wellesbourne*
Poster Papers	7D-1 to 7D-13

INFLUENCE OF SOIL-LESS CULTIVATION ON DEVELOPMENT AND CONTROL OF SPIDER MITES ON VEGETABLE CROPS IN GLASSHOUSES

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ABSTRACT

The influence of soil-less cultivation on two spider mite species, *Tetranychus cinnabarinus* and *T. urticae*, was investigated in glasshouse tomatoes and cucumbers in both spring and autumn crops. In spring crops the dynamics of a *T. cinnabarinus* population was not influenced by the kind of substrate (rockwool, polyurethane foam, peat), in which tomatoes and cucumbers were grown, but depended on the time of pest appearance. On autumn crops, the highest density of *T. cinnabarinus* was found on plants grown in a peat substrate, while *T. urticae* was always most abundant on crops in this substrate. Effective control of both spider mite species in different substrates was achieved by biological and chemical control.

INTRODUCTION

The two spider mite species *Tetranychus cinnabarinus* and *T. urticae*, are a constant threat to glasshouse tomatoes and cucumbers in Poland (Szwejda, 1993), and it is essential that these pests are controlled. In recent years, the area of vegetable crops grown in soil-less substrates has steadily increased. The present experiments were done to investigate the effects of various substrates used in glasshouse crops on the population density of these pests. Also, the effectiveness of biological and chemical methods used in the control of these two mite species was assessed.

MATERIAL AND METHODS

Four experiments were done on cucumber and five experiments on tomatoes in glasshouses at the Research Institue of Vegetable Crops in 1993 and 1994. The plants were grown in 4 types of substrates: two of rockwool: Grodan and Flormin; polyurethane foam: Dynamite; and peat substrate in bottom-less plastic cylinders (20 x 20cm), placed on a peat layer. Slabs containing 3 plants each were laid down in two rows at a spacing of 100 x 50cm. The analogical design was used for the peat cylinders. Equal rates of macro- and micronutrients applied in drip feeds were supplied to all treatments in the experiments. The area of a trial covered either $400m^2$ ($50m^2$ per combination) or $80m^2$ ($20m^2$ per combination). The air temperature was recorded between plants during the growing period and is shown in Table 1.

The plants in each combination were divided into four replicates. Each species of spider mites was introduced separately at a rate of 50 adults per 10 plants, when the tomatoes or

cucumbers had reached 70-90cm in height. Two weeks after treatment, pests were counted at weekly intervals until the end of season. Eight leaves of tomato (cv 'Fiorin') and four of cucumber (cv 'Rawa') were removed from each replicate using the Henderson-McBurnie,s appliance.

Phytoseiulus persimilis (0.5 specimens per $1m^2$) was used for the biological control of red spider mites. The following acaricides were used for spider mite control: Danirun 110 EC, Nissorun 05 EC, Talstar 100 EC and Torque 50 WP (tomato: 1500 l water/ha, cucumber: 2000 l water/ha). The timing of biological and chemical control is indicated in the Tables. Statistical analysis was based on the Newman - Keuls test, at $P = 0.05$.

RESULTS

Tetranychus cinnabarinus

There were no significant differences between the preferences of *T. cinnabarinus* in attacking tomatoes grown in rockwool, polyurethane foam and peat substrate, although on the spring crop there were significant differences found in its population density at certain times (Table 2a, b and c). However, on the autumn crop *T. cinnabarinus* was most abundant on tomatoes grown in the peat substrate (Table 2d). At harvest time, these plants were much taller (mean 147cm) when compared with other treatments (88-99 cm). In the spring crop these differences were not significant, since tomato plants grown in the peat substrate were not taller. The air temperature recorded between the plants differed, depending on the kind of substrate. During the entire growing season the temperature was always lowest in peat substrate treatments (Table 1).

On cucumbers, *T. cinnabarinus* was most abundant during the season on the crop grown in Grodan rockwool (Table 3a and b). In this treatment the plants were largest, reaching an average height of 290.5cm, as compared to those grown in Flormin rockwool - 248.8cm, Dynamite polyurethane foam - 262.6cm and in the peat substrate - 267.7cm. The highest air temperatures (minimum and maximum) between the plants were also recorded in the Grodan rockwool treatment (Table 1).

Biological control with *P. persimilis* resulted in a significant reduction of *T. cinnabarinus* on tomatoes and cucumbers, irrespective of the substrate used (Table 2c and Table 3a). The effectiveness of chemical control with the acaricides (Danirun 110 EC, Nissorun 05 EC, Talstar 100 EC) did not depend on the substrate (Tables 2a,d and Table 2c).

Table 1. Air temperature (°C) recorded between plants during the growing season. Glasshouse experiment, Skierniewice 1994

Crop	Grodan		Flormin		Dynamite		Peat	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Tomato	18.4	25.7	17.6	24.8	18.5	26.7	15.7	22.5
Cucumber	21.2	27.4	21.2	27.2	21.0	26.6	20.1	25.9

Table 2. Dynamics of a *Tetranychus cinnabarinus* population and its biological and chemical control on glasshouse tomatoes cultivated in different types of substrate.

a) Experiment 1, spring cultivation, 1993.

Substrate	Dates of observation							
	22/3	19/4	10/5	31/5	7/6*	14/6	21/6	5/7
Grodan	0	0a	0a	16a	29a	10a	23a	58
Flormin	4	6a	108b	191b	312c	65b	82b	85
Dynamite	0	0a	0a	8a	16a	12a	25a	54
Peat	0	29b	166b	148b	190b	47b	56ab	96

* chemical control: Nissorun 05EC (0.06%)

b) Experiment 2, spring cultivation 1993

Substrate	Dates of observation			
	19/5	25/5	4/6	19/6
Grodan	53	56	157b	183b
Dynamite	47	51	143b	177b
Peat	50	57	90a	125a

c) Experiment 3, spring cultivation 1993

Substrate	Dates of observation							
	25/4	10/5	17/5	30/5*	14/6	21/6	27/6	5/7
Grodan	0.4	1	10	69	119	165b	103b	41
Flormin	0	0	11	69	84	82a	47a	43
Peat	4	1	24	67	104	114a	62a	52

* biological control: *Phytoseiulus persimilis*

d) Experiment 4, autumn cultivation 1994

Substrate	Dates of observation							
	25/10*	2/11*	9/11	17/11	23/11	30/11	8/12	14/12
Grodan	21	16a	6a	10a	12b	18b	22b	25b
Flormin	23	16a	6a	10a	6a	9a	11a	13a
Dynamite	25	19b	6a	10a	11b	13ab	15ab	17ab
Peat	26	21b	9b	15b	25c	32c	51c	53c

*chemical control: 21.10 - Talstar 100 EC (0.04%), 2.11 - Danirun 110 EC (0.06%)

Table 3. Dynamics of a *T. cinnabarinus* population and its biological and chemical control on glasshouse cucumber in different types of substrate.

a) Experiment 1, spring cultivation 1994

Substrate	Dates of Observation							
	22/4	6/5	10/5	17/5*	31/5	10/6	16/6	30/6
Grodan	2	40b	138b	475c	337c	67b	107b	48a
Flormin	0	0a	1a	3a	12a	9a	21a	84b
Dynamite	0	5a	21a1	72b	121b	35ab	60a	29a
Peat	0	0a	15a	21a	39a	45b	55a	51a

* biological control: *Phytoseiulus persimilis*

b) Experiment 2, spring cultivation 1994

Substrate	Dates of observation							
	22/4	4/5	18/5	1/6	15/6	23/6*	27/6	1/7
Grodan	0	13	74b	123c	321b	378b	316b	208b
Flormin	0	6	17a	20a	118a	141a	84a	61a
Dynamite	0	0	0a	0a	16a	30a	18a	22a
Peat	0.2	9	20a	53b	340b	450b	278b	85a

* chemical control: Danirun 110 EC (0.06%)

Tetranychus urticae

On both tomato and cucumber crops *T. urticae* populations were most numerous on plants grown in soil-less substrates (Table 4), whereas there was no development of this pest on tomatoes grown in peat (Table 4a).

The effectiveness of biological (*P. persimilis*) and chemical (Nissorun 05 EC) control was not influenced by the substrate used for the crop (Tables 4a and b).

DISCUSSION

The present study demonstrated a relationship between the type of substrate and the dynamics of *T. cinnabarinus* and *T. urticae* populations. Both species preferred tomatoes and cucumbers grown in rockwool and polyurethane foam. It can be concluded that this preference resulted from abiotic factors existing between plants. When compared with a peat substrate, soil-less crops had a lower humidity and a higher air temperature as well as a more stable and uniform supply of nutrients (Wysocka-Owczarek, 1993). A reduced humidity and a higher temperature stimulate spider mite development (Northcraft & Watson, 1987; Brandenburg & Kennedy, 1987). These experiments have shown that an increase in weight of plant foliage favours the development of spider mites, when compared with smaller plants.

Table 4. Dynamics of a *T. urticae* population and its biological and chemical control on plants cultivated in different types of substrate.

a) Experiment 1: tomato 1993

Substrate	Dates of observation							
	31/3	26/4	17/5	31/5	7/6*	14/6	21/6	5/6
Grodan	0	0	0	6a	16a	3	5	18
Flormin	0	0	0	7a	15a	2	12	30
Dynamite	1	0	0	25b	30b	8	13	13
Peat	0	0	0	0a	0a	0	0	0

* chemical control: Nissorun 05 EC (0.1%)

b) Experiment 2: cucumber 1993

Substrate	Dates of observation							
	10/5*	18/5	25/5	2/6	8/6	15/6	22/6	30/6
Grodan	7a	10	8	19	40	87	145b	196b
Flormin	23b	36	27	24	53	67	188b	234b
Dynamite	44c	37	20	15	34	53	95a	148a
Peat	19ab	23	14	7	12	31	88a	90a

* biological control: *Phytoseiulus persimilis*

c) Experiment 3: cucumber 1993

Substrate	Dates of observation					
	2/6	9/6	17/6	25/6	2/7	8/7
Grodan	20b	18	26b	29b	31b	32
Dynamite	20b	18	22ab	27b	30b	32
Peat	10a	14	16a	17a	20a	22

REFERENCES

Brandenburg, R L; Kennedy G G (1987) Ecological and agricultural considerations in the management of two spotted spider mite *Tetranychus urticae* Koch. *Agric. Zoology* **2**, 186-236.

Northcraft, P D; Watson, T F (1987) Developmental biology of *Tertranychus cinnabarinus* (Boisduval) under three temperature regimes. *Southwestern Entomologist* **12**, 45-50.

Szwejda, J (1993) Injury symptoms and control of two spider mite species: Tetranychus urticae and *T. cinnabarinus* occurring on cucumbers and tomatoes. *Materialy 33 Sesji Nauk.* IOR, Poznañ,Cz.**1**, 128-135.

Wysocka-Owczarek, M (1993) Pomidory pod osionami. Hortpress, Warszawa, 169pp.

INSECTICIDAL CONTROL OF FOLIAR AND ROOT APHIDS ON OUTDOOR LETTUCE

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ABSTRACT

Pirimicarb, demeton-S-methyl, pymetrozine, triazamate and an experimental seed treatment were tested for efficacy against foliar aphids (*Nasonovia ribisnigri* and *Macrosiphum euphorbiae*) and lettuce root aphid (*Pemphigus bursarius*) on outdoor iceberg lettuce crops in the UK in 1994 and 1995. In three separate experiments, the best control was given by the experimental seed treatment. Of the foliar sprays, triazamate consistently gave the best level of control in terms of efficacy and persistence. In a separate experiment, the efficacy of different dose rates of the experimental seed treatment were tested against *P. bursarius* and foliar aphids. A low level of *P. bursarius* infestation was controlled at all dose-rates, though by harvest control at the lowest rate was declining. Foliar aphid control was moderate and not related to dose-rate.

INTRODUCTION

Foliar- and root-feeding aphids are the most serious pests of outdoor lettuce in the UK. They regularly cause severe crop loss, either through direct feeding damage or by contamination of harvested heads with live and dead aphids. Lettuce root aphid (*Pemphigus bursarius*) is potentially the most serious pest as severe infestations can result in total crop failures. Attacks are most likely in dry summers (Dunn, 1959), and it is therefore a particular problem in the drier areas of the UK such as eastern and south-eastern England. Since the withdrawal of diazinon in 1993, the UK lettuce industry has lacked Approved insecticides with label recommendations for the control of lettuce root aphid on transplanted outdoor lettuce. Recent years have also seen an apparent increase in problems with foliar aphid infestations on iceberg lettuce, particularly the currant-lettuce aphid (*Nasonovia ribisnigri*), the potato aphid (*Macrosiphum euphorbiae*) and to a lesser extent, the peach-potato aphid (*Myzus persicae*). Peaks of foliar aphid activity tend to occur in mid-summer (June/July) and in the autumn (Ellis *et al.*, 1995). Control of foliar aphids during peaks of infestation can only be achieved by making routine insecticide applications at no more than weekly (and often less) intervals throughout the life of the crop (e.g. Mackenzie *et al.*, 1988, Ester *et al.*, 1993). This intensive approach is necessary to prevent aphid colonies, particularly of *N. ribisnigri*, establishing in the wrapper leaves (Mackenzie & Vernon, 1988) where they become a difficult target for control with insecticides. However, this approach is probably unsustainable due to the over-reliance on a few insecticides, the risks of increased pesticide residues (Sances *et al.*, 1993) and pressure from consumers and retailers for a more rational 'managed' approach to pesticide use.

Research is currently under way in the UK aimed at developing an IPM programme for aphids on outdoor lettuce (Ellis *et* al., 1995). Part of this programme involves testing newer insecticides as candidates for inclusion in an IPM programme. These efficacy experiments, done in 1994 and 1995, were primarily directed at foliar aphid control. In 1995, additional industry-funded work on an experimental seed treatment for *P. bursarius* control was also done. The results of all these experiments are reported here.

MATERIALS AND METHODS

Experiment location and timing

All experiments were done during 1994 and 1995 in commercial iceberg lettuce crops (cv. 'Saladin' in all cases) in two major lettuce production areas. Locations were Experiment 1: Burscough, Lancashire (1994); Experiment 2: Barway, Cambridgeshire (1994); Experiment 3: Scarisbrick, Lancashire (1995); Experiment 4: Stretham, Cambridgeshire (1995). The timing of the experiments coincided either with the early autumn peak of foliar aphid activity (Experiments 1 and 2) or with the June/July foliar aphid peak (Experiments 3 and 4). This latter peak coincides with the peak risk period for *P. bursarius*.

Treatments

The treatments used in each of the experiments are given in Tables 1 and 2.

Table 1. Treatments used in Experiments 1,2 and 3.

Code	Treatment	Rate: g a.i. ha^{-1}
A	untreated	Nil
B	pirimicarb	125
C	demeton-S-methyl	244
D	pymetrozine	200
E	triazamate	56
F	seed treatment A	90 (g 100,000 $seeds^{-1}$)

All foliar spray treatments (treatments B, C, D and E, Table 2) were applied to previously untreated lettuce crops approximately 14 days after planting. Treatments were applied in 500 l water ha^{-1} through Lurmark F80-03 flat-fan nozzles using a CO_2-powered hand-held precision boom sprayer operating at 2.0 bar pressure.

In 1994, seed treatment A was applied to small lots of seed using an experimental film-coating seed treatment apparatus. In 1995, a commercial film-coating seed treatment process was used to apply seed treatment A to large (> 2 kg) seed lots.

Table 2. Rates of Seed Treatment A used in Experiment 4.

Code	Rate (g a.i. 100,000 seeds^{-1})
I	Nil (blank seed treatment)
II	180
III	90
IV	60
V	30

Experiment design

All experiments were randomised complete block designs. In Experiments 1, 2 and 3 each insecticide treatment was replicated five times. Untreated plots were replicated 10 times. In Experiment 4, all treatments (including untreated plots) were replicated four times. In all experiments, plot size was 10 m x 1 bed (bed width approx. 1.83 m, 4 rows of lettuces per bed). Raw data were transformed using $\log_e (n+1)$ or $\sqrt{(n + 0.5)}$, followed by analysis of variance. Where F-tests were significant, differences between means were tested using Dunnett's Test (Experiments 1, 2 and 3) or Duncan's Multiple Range Test (Experiment 4).

Assessments

In Experiments 1, 2 and 3, assessments of foliar aphid infestation level on all plots were done 2 and 10 days after treatment (2 DAT and 10 DAT respectively). Fifteen plant per plot were assessed on each occasion. Plants were removed from the field by cutting off the plant at ground level. Cut plants were bagged and returned to the laboratory where they were destructively searched for aphids. At the 10 DAT assessment only, the root balls of plants removed for foliar aphid assessments were assessed in the field for the level of *P. bursarius* infestation using an index system. This technique categorises the aphid infestation level on the roots of each plant according to a logarithmic index ranging from 0 (no infestation) to 7 (severe infestation).

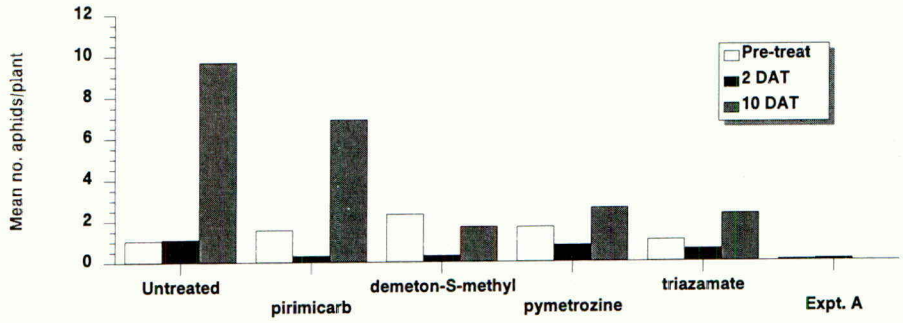
In Experiment 4, assessments of *P. bursarius* and foliar aphid infestation levels on all plots were made at hearting (i.e. when the heart leaves started to form an identifiably dense head) and at harvest. Assessments for *P. bursarius* infestation were made on the roots of 20 plants per plot using the index system described above. For foliar aphid assessment, 10 plants per plot were assessed. As in the other experiments, plants were removed from the field and assessed for aphids destructively in the laboratory.

RESULTS AND DISCUSSION

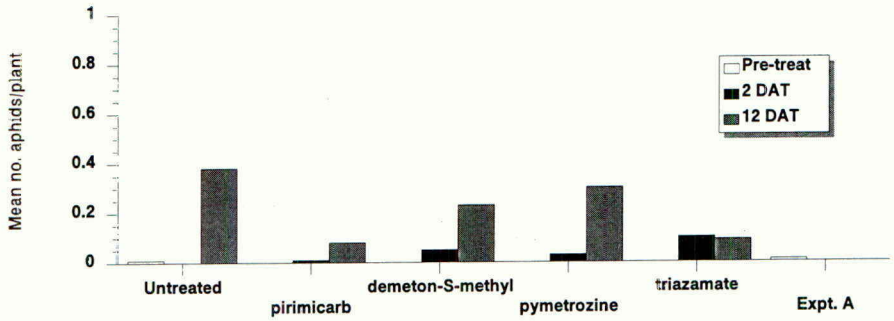
Moderate infestations of *N. ribisnigri* and *M. euphorbiae* developed on Experiments 1 and 3, with the former species predominating. Low numbers of *M. persicae* were also found. Only a low infestation developed on Experiment 2. Untreated aphid populations generally increased during the course of the experiments (Figure 1).

Figure 1. Effect of treatment on mean number of foliar aphids plant^{-1} 2 days after treatment (2 DAT) and 10 or 12 days after treatment (10 or 12 DAT). Pre-treat. = mean number of aphids plant^{-1} immediately prior to foliar treatment application. Expt. A = seed treatment A.

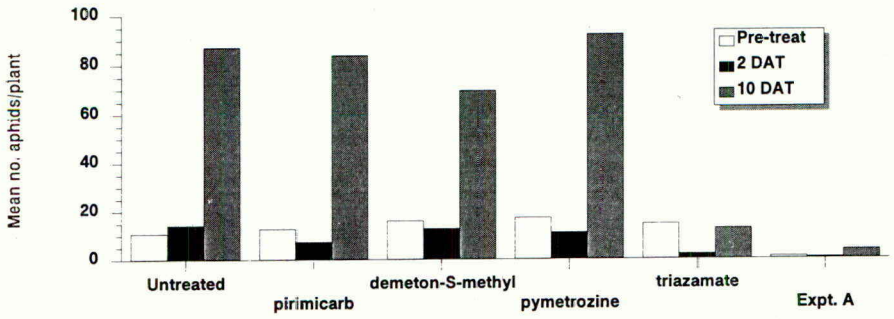
a) Experiment 1.



b) Experiment 2.



c) Experiment 3.



In Experiment 1 (Figure 1a), treatment differences were significant for all three assessments (Pre-treatment, F=5.08, 25 d.f., P=0.002; 2 DAT, F=3.53, 25 d.f., P=0.015; 10 DAT, F=8.43, 25 d.f., P<0.001). Significant differences (Dunnett's Test, P<0.05) were found between the Pre-treatment (i.e. before foliar sprays were applied) and 2 DAT means for untreated and Seed Treatment A plots. Results for all foliar spray treatments 2 DAT were not significantly different from the untreated mean. At 10 DAT, mean counts for demeton-S-methyl, triazamate and Seed Treatment A were significantly lower (P<0.05) than the untreated mean. Aphid counts from Experiment 2 were very low (Figure 1b) and there were no significant differences between treatments at any assessment. In Experiment 3 (Figure 1c), treatment differences were again significant for all three assessments (Pre-treatment, F=4.20, 24 d.f., P=0.007; 2 DAT, F=8.22, 25 d.f., P<0.001; 10 DAT, F=12.20, 25 d.f., P<0.001). Pre-treatment differences were again due to the significantly lower Seed Treatment A mean compared to the untreated mean. At 2 DAT and 10 DAT, means for triazamate and Seed Treatment A were significantly lower (Dunnett's Test, P<0.05) than untreated means. No other foliar spray treatment gave a significant level of control. *P. bursarius* infestations were light in all three experiments and no meaningful conclusions could be drawn from the data.

These experiments demonstrated that the current commercial standards for foliar aphid control on lettuce (pirimicarb and demeton-S-methyl) have poor efficacy and persistence, at least as single treatments, in comparison to some newer insecticides. Triazamate was particularly effective, even when aphid pressure was high (e.g. Experiment 3), and its suitability for use in an IPM programme is currently being investigated further. Seed Treatment A gave excellent foliar aphid control in Experiments 1 and 3, (though less so in Experiment 4; see below), and clearly has potential for substantially altering the pattern of insecticide use on outdoor lettuce in the UK. Pymetrozine was broadly comparable to pirimicarb; it is possible that this product might be useful as part of an insecticide resistance management strategy. However, there is no confirmed insecticide resistance in *N. ribisnigri* and *M. euphorbiae* in UK lettuce crops at present.

Table 4. Effect of different rates of seed treatment A (g/100,000 seeds) on mean lettuce root aphid infestation index and mean number of foliar aphids/plant at hearting (14 July) and harvest (27 July). Means are back-transformed from $\sqrt{n + 0.5}$, S.E.D. given is for transformed means. Means followed by the same letter are not significantly different at p = 0.05.

	Root aphid index		Mean no. aphids/plant	
Treatment	**Hearting**	**Harvest**	**Hearting**	**Harvest**
Untreated	0.56 a	0.66 a	18.7 a	9.3 a
180 g	0.40 b	0.43 c	7.5 b	1.3 b
90 g	0.40 b	0.42 c	12.4 ab	1.8 b
60 g	0.42 b	0.43 c	13.6 ab	2.8 b
30 g	0.43 b	0.55 b	8.7 b	2.1 b
F ratio	9.33 (p=0.001)	17.12 (p<0.001)	4.91 (p=0.019)	8.32 (p=0.002)
S.E.D.	0.066	0.075	2.83	2.04

Experiment 4

A low lettuce root aphid attack developed on this experiment. However, no visible signs *of P. bursarius* damage were observed in the crop at any time. A significant infestation of *M. euphorbiae* developed on the foliage. The results of the foliar and root aphid assessments are given in Table 4. All rates of the seed treatment significantly reduced *P. bursarius* infestation at hearting. At harvest, the overall level of control was maintained for the three highest seed treatment rates but was reduced for the 30 g rate. However, as the *P. bursarius* infestation pressure was low overall, it is likely that the higher rates would be required to suppress a serious attack. Foliar aphid infestations were significantly lower on some treatments at hearting (Table 4), but the level of control was moderate in comparison to the results achieved in Experiments 1 and 3, and was not related to insecticide dose. It is possible that there was a heavy influx of alate aphids at this time. By harvest, the level of aphid infestation had dropped by *c.* 50% on untreated plots, and the level of aphid control given by the seed treatments had improved to an average of 78% across all dose rates.

ACKNOWLEDGMENTS

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REFERENCES

Dunn, J A (1959) The biology of lettuce root aphid. *Annals of Applied Biology*. **47**, 475-491.

Ellis, P R; Tatchell, G M; Collier, R H; Chandler, D; Mead, A; Jukes, P L; Vice, W E; Parker, W E; Wadhams, L J (1995) Integrated pest management of aphids on outdoor lettuce. *BCPC Symposium Proceedings No. 63: Integrated Crop Production: Towards Sustainability?* pp 115-122.

Ester, A; Gut, J; van Oosten, A M; Pijnenburg, H C H (1993) Controlling aphids in iceberg lettuce by alarm pheromone in combination with an insecticide. *Journal of Applied Entomology* **115**, 432-440.

Mackenzie, J R; Vernon, R S (1988) Sampling for the distribution of the lettuce aphid, *Nasonovia ribisnigri* (Homoptera:Aphididae), in fields and within heads. *Journal of the Entomological Society of British Columbia* **85**, 10-14.

Mackenzie, J R; Vernon, R S; Szeto, S Y (1988) Efficacy and residues of foliar sprays against the lettuce aphid, *Nasonovia ribisnigri* (Homoptera:Aphididae), on crisphead lettuce. *Journal of the Entomological Society of British Columbia* **85**, 3-9.

Sances, F V; Gaston, L K; Campos, R; Dusch, M; Toscano, N C (1993) Multiple insecticide treatments affect harvest residues of lettuce. *Journal of Economic Entomology* **86**, 1781-1785.

DAMAGE TO STRAWBERRY FRUITS BY THE EUROPEAN TARNISHED PLANT BUG, *LYGUS RUGULIPENNIS*

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ABSTRACT

In field experiments on late-season strawberries, amounts of fruits showing symptoms of malformation were significantly higher on plots with large numbers of the capsid *Lygus rugulipennis* than on plots where numbers had been reduced with an insecticide. Caging of this species on individual flowers or on developing fruits prevented normal fruit development, with the symptoms dependent on the age of the flower or fruit when feeding occurred.

INTRODUCTION

In recent years the U.K. season of strawberry fruit production has been extended from the conventional June period to include the months July through to October. This has been achieved by growing the June-bearer cv 'Elsanta' as a '60-day plant', i.e. planting cold-stored runners in sequence in early summer so that they fruit *c.* 60 days later, or by growing day-neutral (everbearer) cvs that flower and fruit continuously through summer and into the autumn.

The fruit from these late-season crops is of high value, but many growers have suffered severe economic losses as a result of a high proportion of the fruit showing malformation. There are several possible causes of mis-shapen fruit, including poor pollination, but feeding by insects on the flowers or young fruit is another possible cause. Research at HRI East Malling has shown that large numbers of the European tarnished plant bug (*Lygus rugulipennis*) can occur in late-season strawberry fields (Easterbrook, 1996 and unpublished) and experiments were done to ascertain whether this species was causing damage to fruit.

MATERIALS AND METHODS

Glasshouse experiments

Strawberry runners cvs 'Tango', 'Calypso' and 'Elsanta' were potted into 2 litre pots. When flowers developed on these plants they were hand-pollinated using a sable hair brush. Nymphs or adults of *L. rugulipennis* were caged on individual flowers or developing fruits at various times after the flower opened. The insects were removed after 48 h. A cage consisted of a plastic pot of 4.5 cm diameter and 3 cm height, with a hole cut out of the base so that it could be slipped over the flower. The plastic lid of the pot also had a hole cut out and covered with nylon gauze. Clingfilm was placed around the bottom of the pot and attached to the flower stalk to prevent escape of the insects. The cage was taped to a cane to provide support. Control cages were set up in the same way, but no insects were

introduced. Wherever possible, different treatments were blocked on a plant, and there were 8-10 replicates of each treatment. In one experiment (No. 6), twelve whole plants were enclosed in nylon mesh cages after three or four flowers on each plant had been tagged and hand-pollinated and 3 adult *L. rugulipennis* per plant introduced onto half of them. Plants were kept in a glasshouse and the fruit inspected when they started to turn red. They were then graded for the degree of malformation.

Field experiments

In 1994, on a planting of cv 'Rapella', plots of 10 plants were covered with horticultural fleece supported by wire cloche hoops. There were three treatments:-

1. plants left untreated,

2. plants treated with 0.41 g a.i./litre heptenophos (Hostaquick) on 5 August and 0.028 g a.i./litre cypermethrin (Ambush C) on 12 August,

3. plants treated with 0.41 g a.i./litre heptenophos on 5 August and 2 *L. rugulipennis* 2nd/3rd instar nymphs per plant added on 9 August.

Insecticides were applied at high volume with a knapsack sprayer. On three occasions, 9, 15 and 23 August, 30 flowers per plot were hand-pollinated and tagged. Fruits developing from these flowers were graded into one of four categories of malformation, i.e. normal, slight, moderate or severe malformation, at maturity.

Two experiments were done on uncovered plants, where numbers of *L. rugulipennis* were reduced in parts of the field, while other areas were left untreated. In 1993 chlorpyrifos (Dursban) at 480 g a.i./ha, malathion (Malathion 60) at 456 g a.i./ha and cypermethrin (Ambush C) at 28 g a.i./ha were applied to parts of a field of '60-day' 'Elsanta' on 10 August, using a boom sprayer at 400 litres/ha. There were four replicate plots of each of these treatments and four untreated; each replicate plot consisted of a 2 row x 30 plant section of four beds. In 1995 parts of a field consisting of alternate rows of the everbearing cvs. 'Calypso' and 'Rapella' were treated with cypermethrin at 28 g a.i./ha on 4 August applied at 300 litres/ha using a Hardi-Mini air-assisted sprayer, while other parts were left untreated. There were four replicates of each treatment, each replicate plot consisting of a section of four raised beds, each with two rows of 38 plants.

In both experiments, *L. rugulipennis* were sampled at weekly intervals before and after spraying, using a modified petrol-engined garden sweeper/vac (MacLeod *et al*, 1994). Forty plants per plot were vacuumed in 1993, and 50/plot in 1995. Samples of 20 flowers/plot were also taken at weekly intervals to check for infestation by other insects. At the time of spraying, 30 flowers were tagged and observed to maturity, to relate to fruit harvests which were made weekly. Samples of 100 ripe fruit per plot were inspected and graded for malformation. The samples of insects and fruit were taken from the two central beds in each plot.

RESULTS

Glasshouse experiments

Table 1. Effect of *L. rugulipennis* nymphs or adults on strawberry flower/fruit development.

				Numbers of fruit in each category									
				- capsid					+ capsid				
Expt	cv[1]	Capsid stage	Age of flower	nd[2]	nor	sl	m	s	nd	nor	sl	m	s
1	T	nymph	1 day	0	8	0	0	0	5	0	0	0	3
2	C	nymph	1 day	0	7	3	0	0	9	0	0	0	1
3	C	adult	3 day	2	4	2	0	0	3	0	0	2	3
4	C	nymph	1 day	2	5	1	0	0	8	0	0	0	0
4	C	nymph	6 day	2	5	1	0	0	3	0	1	0	3
5	E	nymph	10 day	2	4	2	0	0	0	1	3	0	4
6	E	adult	3-10 day	0	20	0	0	0	0	8	2	1	10

[1] T = 'Tango', C = 'Calypso', E = 'Elsanta'
[2] n d = flower not developed, nor = normal shape, sl, m, s = slight, moderate or severe distortion

When capsids were caged on young flowers, the flower often failed to develop into a fruit at all, or very few achenes on the receptacle developed (Table 1). Caging the insects at a later stage, where the flower had already begun to develop into a fruit before capsid feeding, resulted in an increased incidence of severely mis-shapen fruit, often with a typical 'snub-nosed' appearance where the achenes and surrounding receptacle at the tip of the fruit failed to develop.

Field experiments

In the experiment with fleece cages the double-insecticide treatment reduced the amount of fruit damage significantly (Table 2). The proportion of fruit showing moderate or severe malformation in the treated plots was one third to one half that in the plots with *L. rugulipennis* present.

At the time the capsids were introduced in treatment 3, numbers on the untreated plots averaged 1 nymph per plant, though by a week later there were 2 nymphs per plant, i.e. similar to treatment 3. Few nymphs were found on the plants treated with insecticide.

Table 2. Comparison of fruit malformation on strawberries with low or high numbers of *L. rugulipennis*.

Harvest	Mean fruit damage score[1]			SED	df	*P*
	Untreated	Heptenophos + cypermethrin	Heptenophos + 2 nymphs/plant			
1	2.17	1.55	2.24	0.151	12	<0.01
2	1.86	1.63	2.23	0.192	10	<0.05
3	1.92	1.55	2.31	0.120	10	<0.001

[1] Scoring: normal = 1, slight malformation = 2, moderate = 3, severe = 4.

Table 3. Fruit malformation on '60-day' 'Elsanta' with contrasting populations of *L. rugulipennis*, 1993.

Treatment	Capsid nymphs/40 plants (log n+1)				% of fruit in normal + slight classes	
	2 DAT		15 DAT		7 Sept	15 Sept
untreated	9.3	(2.26)	7.5	(2.10)	91.3	86.8
chlorpyrifos	0.25	(0.17)	1.8	(0.72)	95.0	94.4
malathion	0.5	(0.35)	3.0	(1.24)	94.0	94.7
cypermethrin	0.25	(0.17)	2.0	(1.04)	95.3	91.9
SED		(0.248)		(0.376)	3.0	3.1
d f		9		9	9	9
P		<0.001		<0.05	n.s.	<0.05

Where numbers of *L. rugulipennis* were reduced by insecticide treatments in the 1993 experiment, the percentage of fruit showing malformation was significantly reduced in the pick on 15 September (Table 3). At that time, the fruits that had been at the flower stage when the insecticides were applied had reached maturity.

In the 1995 experiment the percentage of fruit classified as normal or with only slight distortion (i.e. Class 1 fruit) was significantly higher on the plots treated with an insecticide, except for the first harvest (Table 4). The flowers/young fruit present when the insecticide was applied did not mature until the second harvest, so any effect of reducing capsid numbers would not be evident until that and subsequent picks. On 7 August (3DAT) there were no *L. rugulipennis* adults and a mean of 0.5 nymphs per 50 plants on the insecticide treated plots, compared with 5.0 adults and 10.8 nymphs per 50 plants on untreated plots.

Table 4. Fruit malformation on strawberries with or without an insecticide treatment to reduce numbers of *L. rugulipennis*, 1995.

	% of fruit in normal + slight classes				
Harvest	Cypermethrin	Untreated	SED	df	*P*
'Rapella'					
1	97	92	2.3	3	ns
2	99	87	2.0	3	<0.01
3	100	86	1.7	3	<0.01
4	97	87	1.5	3	<0.01
'Calypso'					
1	94	90	1.4	3	ns
2	97	87	1.7	3	<0.05
3	96	83	2.7	3	<0.05
4	92	82	2.0	3	<0.05

DISCUSSION

Reduction of *L. rugulipennis* numbers in field plantings of strawberries led to fewer malformed fruits. Suction sampling and direct observation of flowers showed that numbers of other insects that might cause damage, e.g., other capsid species, thrips and pollen beetles, were very low. This suggests that *L. rugulipennis* is a major cause of fruit malformation in late-season strawberries, and is confirmed by evidence from the glasshouse cage experiments where similar symptoms were observed when this species was confined on developing fruits. Handley (1990) also found that different symptoms resulted according to the flower or fruit stage exposed to a related North American species, *L. lineolaris*. With both species, confinement on young flowers prevented fruit development completely, but it is not yet known whether this type of damage occurs in the field.

Even where numbers of *L. rugulipennis* were reduced to very low levels, there remained a 'background' of mis-shapen fruit, presumably from other causes. However, there is clearly great potential to reduce damage by controlling *L. rugulipennis*, and research is in progress to improve monitoring of this species and timing of control measures.

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REFERENCES

Easterbrook, M A (1996) Capsid control for shapely fruit. *Grower* **125(23)**, 28-29.

Handley, D T (1990) Strawberry fruit development and the effects of feeding by the tarnished plant bug (*Lygus lineolaris*). In: *The Strawberry into the 21st Century, Proceedings of the 3rd North American Strawberry Conference*. Dale, A & Luby, J J (Eds). Timber Press Inc., Portland, Oregon, 209-216.

MacLeod, A; Wratten, S D; Harwood, R W J (1994) The efficiency of a new lightweight suction sampler for sampling aphids and their predators in arable land. *Annals of Applied Biology* **124**, 11-17.

OPTIMISING THE EFFICACY OF SOIL-APPLIED INSECTICIDES

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ABSTRACT

A method has been developed for establishing rapidly the most persistent, and therefore potentially the most effective, soil-applied insecticide for the control of carrot fly in individual soils. The test can be completed within 2-3 weeks, and could be adapted for other soil-applied chemical and crop/pest combinations.

INTRODUCTION

Recent studies of microbial adaptation have revealed the extent to which this phenomenon can limit the persistence, and hence the duration of biological efficacy, of virtually all soil-applied insecticides available currently in the UK (Suett, 1991). Soil properties, especially pH, can influence the induction and stability of the phenomenon but the range, magnitude and duration of this influence varies with different insecticides. There is thus scope for development of a rapid, flexible and relatively inexpensive method for establishing the degradative characteristics of individual soils before selection of insecticides for specific crop-pest soil treatments.

This paper describes the development of such a system and summarises its use in assessing the potential activity of insecticides in soils from 16 carrot farms.

METHODS

The procedure is based on the simultaneous incorporation into each test soil of all the insecticides approved currently for control of carrot fly (*Psila rosae*) in the UK. Soils are then incubated at elevated temperature and moisture levels and residue loss is monitored using a single multiresidue analysis.

Duplicate soil samples were collected from commercial carrot growing areas of East Anglia and from Horticulture Research International, Wellesbourne. Field soils were sampled, sieved and stored at 5 °C until treated. The moisture holding capacity (mhc) was estimated by measuring the moisture content of saturated soil which had been allowed to drain overnight (16 hours). The pH of a suspension of air dried soil (10 g) in distilled water (25 ml) was also measured and total organic content was based on loss by combustion at 375 °C.

Soil incubation studies

The four soil-applied insecticides currently approved in the UK for carrot fly control were added simultaneously to all the soil samples. Granular formulations of phorate and disulfoton

(0.5% a.i.) were mixed with partially air-dried soil (1000 g dry soil equivalent) and spread out on polythene sheets. Then aqueous suspensions of chlorfenvinphos and carbofuran were added to give a final concentration of 10 mg kg^{-1} dry soil for each insecticide. Further water was added to adjust the final water content to 85% of the mhc. After equilibrating and mixing, duplicate portions were transferred to polythene jars which were partially sealed. The jars were placed in loosely covered water filled containers and incubated in the dark at 20 °C (Suett & Jukes, 1988). Samples were taken 0, 3, 7, 11 and 14 days after application and weekly thereafter.

Chromatographic analysis

Residues of carbofuran, chlorfenvinphos, phorate, phorate sulphoxide (PSO), phorate sulphone (PSO_2), disulfoton, disulfoton sulphoxide (DSO) and disulfoton sulphone (DSO_2) were extracted from soil samples (20 g) with acetone:hexane (9:1, 50 ml) after the addition of anhydrous sodium sulphate (20 g). Extracts were analysed by capillary gas chromatography using a Hewlett Packard HP5890 with a septum-purged packed inlet. A 12 m × 0.53 mm (id) BPX5 (film thickness 1 µm) column (SGE) was used and detection was by nitrogen-phosphorus detector. The oven temperature was programmed from 100 to 180 °C (7.5 °C min^{-1}) and the carrier gas was helium (3.5 ml min^{-1}).

Retention times (minutes) were 5.3 (phorate), 6.4 (carbofuran), 8.1 (disulfoton), 13.9 (PSO), 14.5 (PSO_2), 17.8 (chlorfenvinphos), 20.5 (DSO) and 21.0 (DSO_2). All components gave a linear detector response to standards at concentrations between 0.1 - 5.0 µg ml^{-1}. Analytical efficiencies were assessed by fortifying untreated soil with the insecticides and metabolites at levels of 1-10 mg kg^{-1}. Recoveries always exceeded 90% and results were not corrected for analytical losses.

RESULTS

Some physico-chemical properties of the soils tested are summarised in Table 1. The mhc ranged from 20 - 72%, pH from 5.8 - 8.0 and organic matter content from 3.5 - 62.7%.

The mean times for 90% breakdown (T_{90}) of all insecticides are displayed in Table 2. All compounds were generally less persistent in soils with higher pH (soils 11-15). Carbofuran was the least persistent of the four insecticides in most soils, with none of the insecticide remaining after 4 weeks in 9 of the 16 soils. In contrast, chlorfenvinphos and disulfoton were similarly more persistent with T_{90} >60 days in 10 of the 16 soils.

The parent compounds of both phorate and disulfoton degraded at a similar rate in all soils. However, there were marked differences between the rates of accumulation and loss of the sulphoxide and sulphone oxidation products. Thus, in soil 2, PSO_2 reached a maximum equivalent to 46% of the initial dose applied after 56 days. In contrast, in soil 11, it reached a maximum of only 2% after just 3 days. Similarly, DSO_2 reached a maximum of 94% after 63 days in soil 10 and only 10% after 5 days in soil 11. The maximum accumulations of PSO_2 and DSO_2 in each soil are displayed in Figure 1 and the contrast between the degradation patterns in soils 2 and 11 can be seen in Figure 2.

Table 1. Physico-chemical properties of soils (± se)

Soil No.	mhc	pH	% organic
1	51.5 ± 0.4	6.7 ± 0.1	26.4 ± 2.3
2	49.3 ± 0.5	6.0 ± 0.3	26.5 ± 2.0
3	53.8 ± 0.7	7.0 ± 0.1	22.4 ± 4.2
4	50.5 ± 3.5	6.6 ± 0.4	24.0 ± 1.0
5	65.5 ± 0.8	6.9 ± 0.5	45.3 ± 0.8
6	59.3 ± 8.5	6.5 ± 0.7	36.1 ± 12.4
7	48.5 ± 2.4	6.7 ± 0.1	23.9 ± 1.6
8	72.0 ± 1.4	6.1 ± 0.3	62.7 ± 2.4
9	71.1 ± 1.0	5.9 ± 0.3	60.3 ± 5.4
10	70.5 ± 1.0	5.8 ± 0.3	62.5 ± 1.8
11	24.0 ± 1.0	7.7 ± 0.2	2.3 ± 0.0
12	22.9 ± 2.0	7.6 ± 0.2	2.1 ± 0.0
13	22.2 ± 0.9	7.9 ± 0.1	1.7 ± 0.0
14	29.2 ± 2.7	7.8 ± 0.1	5.0 ± 0.1
15	21.1 ± 0.6	8.0 ± 0.0	1.8 ± 0.2
16	20.0	6.9	3.7

Table 2. Times (days) for 90% loss of freshly applied insecticides (T_{90}) and % insecticide remaining after 4 weeks (% rem) in 16 soils incubated at 20°C and 85% mhc. Data for phorate and disulfoton represent loss of total residues (parent + sulphoxide + sulphone).

Soil No.	carbofuran		chlorfenvinphos		phorate		disulfoton	
	T_{90}	% rem	T_{90}	% rem	T_{90}	% rem	T_{90}	%rem
1	31	18	>63	92	61	48	>63	73
2	>63	81	>63	84	>63	85	>63	90
3	31	15	>63	71	35	11	>63	75
4	16	2	>63	67	>63	60	>63	54
5	9	0	>63	81	>63	83	>63	95
6	29	6	>63	82	44	21	>63	63
7	11	0	17	0	27	11	54	64
8	52	51	>63	90	28	11	>63	90
9	20	0	>63	90	33	14	>63	99
10	31	12	62	38	32	13	>63	96
11	18	0	25	11	35	40	41	48
12	18	0	20	4	36	21	37	27
13	16	0	29	20	44	51	50	80
14	16	0	29	15	48	49	52	65
15	14	0	25	10	36	32	39	39
16	20	0	>63	78	>63	61	>63	83

Figure 1. The maximum percentage of (a) phorate sulphone and (b) disulfoton sulphone as a proportion of total phorate and total disulfoton residues respectively following application of fresh insecticide to 16 soils.

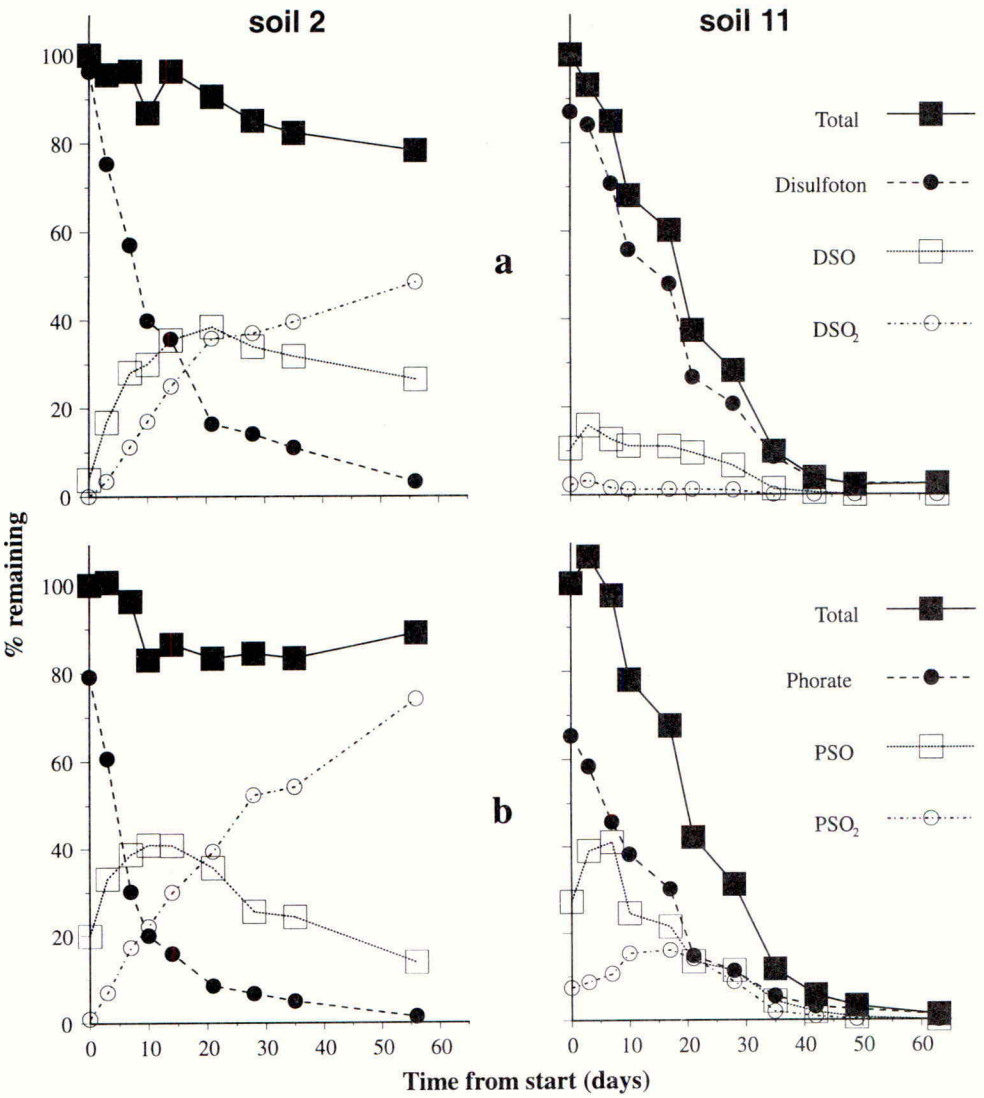

Figure 2. Degradation of (a) phorate and (b) disulfoton in soils 2 and 11

DISCUSSION

The wide range of stabilities encountered with these insecticides in the sixteen soils emphasises the extent to which the duration of biological availability of a specific soil treatment can differ from site to site. Only one of these soils (soil 2) maintained stability of all four insecticides for long enough to suggest that they would all offer optimum protection against carrot fly. In contrast, it seems likely that none of the insecticides would have provided even limited protection in soils 7 and 11-15.

Soil properties such as organic matter content and pH can have a marked effect on insecticide stability (Goring *et al.*, 1975). These characteristics commonly differ from site to site, so that between-site differences in degradation rates are fundamental and inevitable. It is now evident that such differences can be augmented by increased microbial activity against individual insecticides in soils which have been treated previously with the same, or similar, insecticide. Knowledge of previous treatments may sometimes indicate which insecticides should not be selected. However, interactions between all of the above components are potentially so complex that reliable prediction of the stability of future treatments in individual soils is virtually impossible.

The between-site differences between the sixteen carrot soils were so marked that it is difficult to justify continued selection of any individual treatment without preliminary determination of potential interactions between a target soil and the full range of insecticides approved for the control of a specific pest. The present study emphasises the merits of using such a mixture, with clear evidence of the insecticides which will be most persistent in each soil. The procedure is simple and reliable and, by using an elevated moisture content (85% mhc), enables results to be achieved within 2-3 weeks. It is also highly flexible and could be adapted readily to provide relevant information for other crop-pest problems.

Much progress has been made recently towards the development of models which use weather data to predict the impact of microbial adaptation on insecticide degradation and availability in the field (Jukes *et al.*, 1996). Clearly, the potential of these models will be achieved only by ensuring that the most appropriate insecticides are selected for individual crop-pest situations. It must be recognised that, without this essential input to decision making, many soil insecticide treatments will remain less than fully effective and significant reductions in insecticide use will not be achieved.

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REFERENCES

Goring, C A I; Laskowski, D A; Hamaker, J W; Meikle, R W (1975) Principles of pesticide degradation in soil. In: *Environmental Dynamics of Pesticides*, R Haque & V Freed (eds), New York: Plenum Press, pp. 135-172.

Jukes A A; Suett D L; Lynn J R (1996) Effects of soil moisture and temperature on the stability of carbofuran in previously-treated and -untreated soils. *Proceedings of COST 66 Workshop - Pesticides in Soil and the Environment, Stratford-upon Avon.* 123-124.

Suett D L (1991) Enhanced insecticide degradation in soil - implications for effective pest control. *Pesticide Outlook* **2(3)**, 7-12.

Suett, D L; Jukes, A A (1988) Accelerated degradation of aldicarb and its oxidation products in previously-treated soils. *Crop Protection* **7**, 147-152.

THE USE OF THE SELECTIVE INSECTICIDE PIRIMICARB FOR INTEGRATED PEST MANAGEMENT OF PLUM APHIDS IN UK ORCHARDS

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ABSTRACT

Current control measures for plum aphids involve tar oil winter washes and broad-spectrum insecticide sprays, both of which are damaging to natural enemy populations. In the absence of natural enemies, populations of pesticide-resistant damson-hop aphid (*Phorodon humuli*) increase unchecked. The effects of pirimicarb, a selective insecticide, on aphids and their natural enemies were assessed in plum orchard trials. The results confirmed the effectiveness of pirimicarb against spring populations of leaf-curling plum aphid (*Brachycaudus helichrysi*) and showed no damaging effects on polyphagous predators. With these predator populations intact, the mid- to late-season populations of *P. humuli* were heavily predated and remained below economic thresholds. The selectivity of pirimicarb has made it a valuable component of an integrated pest management programme for plum aphids.

INTRODUCTION

Aphids are frequently the most important pests of plum (*Prunus domestica*)(Ward, 1969; Gratwick, 1992). In the UK, three potentially damaging species overwinter on a range of both wild and cultivated *Prunus* species; the leaf-curling plum aphid (*Brachycaudus helichrysi*), the mealy plum aphid (*Hyalopterus pruni*), and the damson-hop aphid (*Phorodon humuli*). In addition to causing direct feeding damage and contaminating fruit as a result of sooty mould (*Cladosporium* spp.) development on honeydew, all three aphid species are vectors of plum pox virus ('Sharka')(Conti, 1986). Of the three species, *B. helichrysi* is probably the most common, and certainly causes the most severe damage (Alford, 1984).

Current chemical control of plum aphids is problematic. *Phorodon humuli* is resistant to all the pesticide groups currently registered for use against plum aphids (Muir, 1979; see also numerous papers in Campbell & Hrdý, 1988). The obvious concern over *P. humuli* resistance has prompted a move back towards the traditional use of dormant sprays which act against the overwintering egg stage and any eggs that have hatched. While tar oil winter washes are accredited with toxicity against resistant aphids (Gratwick, 1992), recent field trials suggest that they are relatively ineffective in reducing spring populations of *B. helichrysi* (R. Umpelby, ADAS Worcester, pers. comm.). In addition, tar oil winter washes are highly toxic to all natural enemies overwintering within plum orchards. In spring, adequate control of *B. helichrysi* and *H. pruni* can still be achieved through the use of available pesticides. However, the prophylactic use of these non-selective pesticides can destroy natural enemies, allowing populations of the pesticide-resistant *P. humuli* to increase uninhibited. The absence of an effective pesticide for *P. humuli* makes it the greatest concern for growers.

In order to relieve the selection pressure for resistance, and minimise detrimental side-effects of pesticides on natural enemies, an integrated approach to aphid management in plum orchards must be developed. Indigenous natural enemies are probably the major form of biological control available for IPM in orchards (Luck *et al.*, 1988). In unsprayed orchards, aphids are often of no economic importance because they are limited by predators (Niemczyk, 1966). In experimental orchards where broad-spectrum pesticides are not used, most foliage-feeding pests are controlled by naturally-occurring enemies (Lawson *et al.*, 1994). In order for the full potential of natural enemies to be exploited, orchard management practices, primarily concerning pesticide use, must be modified to encourage biological control.

This paper describes the development of a pest management strategy for plum aphids which integrates the conservation of indigenous natural enemy populations with the rationalised use of the selective insecticide, pirimicarb.

MATERIALS AND METHODS

Pesticide application

Field experiments were carried out during 1995 in a plum orchard (cv. 'Victoria') at East Malling. No tar oil washes had been applied in the previous winter. The orchard was divided into 12 equally-sized plots, each containing 16 trees. An additional row of guard trees separated each individual plot from all adjacent plots. On six randomly selected plots a single application of pirimicarb (Aphox 50% wt/wt, water soluble granules, Zeneca) at 0.14 g a.i./litre (equivalent to 280 g a.i./ha) was applied (to run off) on 31 March using a hand-lance attached to a Berthoud 600 sprayer. The remaining six plots were left as untreated controls.

Monitoring plum aphids

Weekly leaf samples were examined to monitor the aphid population development within the plum orchard from late April to early November. Separate samples of 100 leaves were selected without bias from both the untreated and pirimicarb-sprayed orchard plots. The species and numbers of all aphids present were recorded.

Predator exclusion experiments

The impact of predators on spring populations of *P. humuli* was determined using exclusion cage techniques.The cages were white polyester net bags (60 cm wide by 100 cm long, with mesh holes *c.* 0.1 mm^2), slipped over a branch and supported internally by two wire hoops (diameter *c.* 50 cm) which had been cross-braced onto the branch *c.* 50 cm apart. The experimental design consisted of six blocks (where a single tree constitutes a "block"), each containing the following three treatments: (1) uncaged, predators allowed access, (2) closed-caged, net bag tied close and predators removed, and (3) open-caged, net bag pegged open, predators allowed access. The open-caged treatment was used to address microclimate modification, a recognized disadvantage of net exclusion cages (Luck *et al.*, 1988). The experimental trees were located within the six pirimicarb-sprayed orchard plots. The three treatments were allocated at random to three similar-size branches, each bearing *c.* 100

dormant buds within the treatment area. Each treatment branch was inoculated with five fourth-instar *P. humuli* nymphs, reared from field-collected fundatrices, and left for 14 days to allow the introduced aphids to disperse and settle. After 14 days, and at weekly intervals thereafter, *P. humuli* numbers were monitored non-destructively; 10 leaves were selected without bias from within each treatment, and the numbers of aphids on these leaves were counted.

Monitoring aphid predators

A beating tray covered with white muslin cloth (*c.* 110 cm x 86 cm) was used to sample the aphid natural enemies within the plum orchard. The tray was held beneath a branch, and the branch was sharply struck four times with a 0.5 m long club, padded on its striking end to avoid injury to the tree. Branches below 2 m, and of roughly equal size, were sampled. Each set of four strikes constituted one "beat", 25 random beat samples being taken at weekly intervals from both the untreated and pirimicarb-sprayed orchard plots. Beneficial insect species that fell on the tray were identified, sexed where possible, and recorded.

Statistical treatment of data

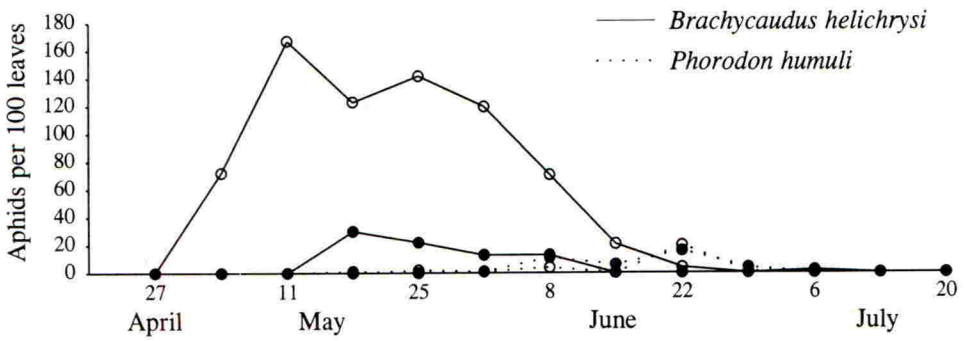
Data were transformed [$y = \text{Log}_{10}(x+1)$, where x= untransformed counts] to stabilize variances, and analyzed using an analysis of variance (ANOVA). The LSD test was used for means separation at $p<0.05$, when the F-statistic for the treatment effect was significant at $p<0.05$.

RESULTS

Aphid phenology

No plum aphids were observed in the initial leaf sample on 27 April. In the following two weeks *B. helichrysi* populations on untreated plots built-up rapidly, peaked over the period 11-25 May, and steadily declined over the following seven weeks (Figure 1). The number of *B. helichrysi* found on the untreated plots was significantly greater than that found on the pirimicarb-sprayed plots ($p<0.001$).

Figure 1. Plum aphid phenology in untreated (○) and pirimicarb-sprayed (●) orchard plots.



The delayed build-up and early decline of *B. helichrysi* populations within pirimicarb-sprayed plots reduced the period of infestation to 5 weeks, compared to that of 11 weeks in untreated plots (Figure 1).

Populations densities of *P. humuli* did not differ significantly between untreated and pirimicarb-sprayed plots. In all plots, populations built-up slowly from mid-May and peaked on 22 June (Figure 1). In the plum orchard the period of *P. humuli* infestation was six weeks in both the pirimicarb-sprayed plots and the untreated plots. The peak populations of *P. humuli* in all plots were smaller than the peak populations of *B. helichrysi* in pirimicarb-sprayed plots (Figure 1). No spring populations of *H. pruni* were found in leaf-samples during 1995.

Aphid predators

The impact of natural enemies on *P. humuli* populations is shown in Figure 2. From late May onwards aphid numbers were significantly higher for the closed-caged treatment than for any other ($p<0.05$)(Figure 2). Aphid numbers for the open-caged treatment were not significantly different from aphid numbers on the uncaged control branches (Table 1), showing that any

Figure 2. Average number of *P. humuli* in closed-caged, open-caged and uncaged populations.

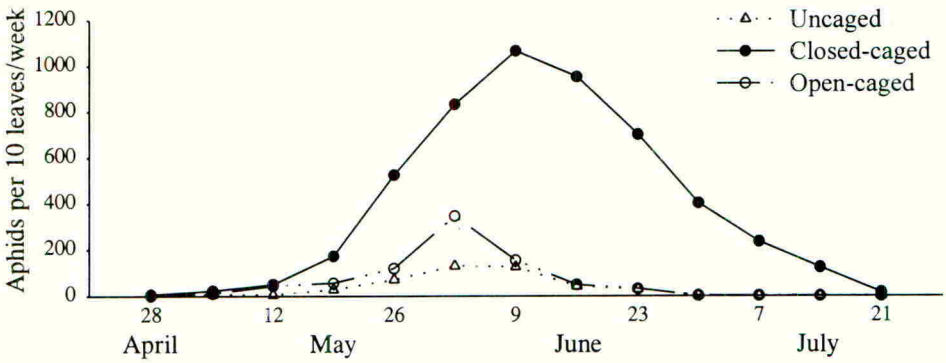


Table 1. Effect of various levels of predator exclusion on the number of *P. humuli* on plum[1].

Treatment	Log (n+1) mean *P. humuli* counts[2]
Uncaged	2.01[b]
Closed-caged	4.95[a]
Open-caged	2.26[b]
SED (0.05, 30 d.f.)	0.162

[1] Summary of 13 weekly records.
[2] Means subtended by the same letter are not significantly different ($p<0.05$), by an LSD test.

alterations to microclimate caused by the net bags were not sufficient to affect *P. humuli* development.

Aphid predators from eight arthropod families were identified from beating-tray samples (Table 2). The only predators that were abundant during the period of *P. humuli* infestation were polyphagous Araneae and *Forficula auricularia* (Dermaptera). The numbers of these polyphagous predators sampled within the untreated plots and the pirimicarb-sprayed plots were not significantly different. Differences in abundance of predator groups between the untreated and pirimicarb-sprayed plots were evident for aphid-specific species, most notably the Coccinellidae. The catches of Coccinellidae were dominated by larvae (64%) which were

Table 2. Total predatory arthropods caught during the period of *P. humuli* infestation (11 May - 13 July), using beating-tray sampling within untreated and pirimicarb-sprayed plum orchard plots .

Arthropod taxa	Untreated plots	Pirimicarb- sprayed plots
Araneae	359	245
Forficula auricularia	333	255
Coccinellidae	97	6
Cantharidae	4	4
Anthocoridae	50	13
Miridae	64	55
Hemerobiidae[1]	4	0
Chrysopidae[1]	16	0
TOTAL	927	528

[1] Larval stages only.

found exclusively within the untreated plots. Predatory ladybirds usually require abundant supplies of prey as ovipositional stimuli. Hence, the total absence of coccinellid larvae from pirimicarb-sprayed plots reflects the low numbers of aphid prey available, i.e. the absence of any ovipositional stimuli for adults. In contrast, the flourishing populations of *B. helichrysi* within untreated plots provided abundant prey and oviposition stimuli for early-season aphidophages. Once adult, these predators become more mobile, and consequently were equally abundant in both untreated and sprayed orchard plots.

DISCUSSION

These studies have shown the value of the selective aphicide, pirimicarb, for the integrated control of plum aphids. Control of severely damaging *B. helichrysi* populations can be

achieved with a single, accurately-timed application of pirimicarb. The rationalised use of this selective aphicide has no detectable effects on the most abundant indigenous natural enemies present in the orchard. These predator populations remain intact to prevent the build-up of pesticide-resistant *P. humuli* populations later in the season.

Pirimicarb is currently used for the IPM of plum aphids elsewhere in Europe, and for the control of aphid pests on other *Prunus* species in the UK, such as cherries. In addition to reducing the environmental impact, the use of pirimicarb within such IPM strategies can reduce the number of treatments and overall pesticide costs by 40%, compared to orchards where conventional pest management is practised (Malavolta *et al.*, 1995). Future availability of pirimicarb, possibly through a specific off-label approval, would considerably enhance the prospects for integrated control of plum aphids.

ACKNOWLEDGEMENTS

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REFERENCES

Alford, D V (1984) *A Colour Atlas of Fruit Pests; their Recognition, Biology and Control.* London: Wolfe Publishing Ltd., pp. 36-47.

Campbell, C A M; Hrdý I (1988)(eds.) Integrated pest and disease control in hops *International Organization for Biological Control/West Palaearctic Regional Section Bulletin* **XI** (5), 99 pp.

Conti, M (1986) Epidemiology and vectors of plum pox virus of stone fruits. *Informatore Fitopatologico* **36**, 23-26.

Gratwick, M (1992)(ed.) *Crop Pests in the UK: Collected edition of MAFF leaflets.* London: Chapman & Hall, pp. 56-59.

Lawson, D S; Ressig W H; Nyrop J P; Brown S K (1994) Management of arthropods on columnar apple trees using exclusionary cages. *Crop Protection* **13**, 346-356.

Luck, R F; Shepard B M; Kenmore P E (1988) Experimental methods for evaluating arthropod natural enemies for integrated pest management. *Annual Review of Entomology* **33**, 367-391.

Malavolta, C; Ponti I; Pollini A; Galassi T; Cravedi P; Molinari F; Brunelli A; Pasini F; Missere D; Scudellari D; Pizzi M (1995) The application of integrated production on stone fruits in Emilia-Romagna (Italy). *International Organization for Biological Control/West Palaearctic Regional Section Bulletin* **XVIII** (2), 55-69.

Muir, R C (1979) Insecticide resistance in damson-hop aphid, *Phorodon humuli* in commercial hop gardens in Kent. *Annals of Applied Biology* **92**, 1-9.

Niemczyk, E (1966) Predators of aphids associated with apple orchards. *Ecology of Aphidophagous Insects, Proceedings of Symposium 27 September-1 October 1965, Prague-Liblice*. Prague: Academia, pp. 275-276.

Ward, L K (1969) A survey of the arthropod fauna of plum trees at East Malling in 1966. *Bulletin of Entomological Research* **58**, 581-599.

A NEW BIOLOGICAL PRODUCT FOR CONTROL OF MAJOR GREENHOUSE PESTS

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ABSTRACT

The patented *Beauveria bassiana* JW-1 strain of fungus is active as a mechanical (non-chemical) mode of action against major greenhouse pests including whiteflies, thrips and mites. Studies indicate that beneficials such as *Encarsia* spp., *Eretmocerus* sp., *Chrysoperia* spp., and others are not impacted by the *B. bassiana* JW-1 strain. The target pests must come into contact with the fungal spores after direct application, movement on treated surfaces, or bodily contact with other target pests already exposed (horizontal transfer). Application of the flowable formulation can be by electrostatic, air-assist, pulse foggers, injection/water systems, ultra-low volume (ulv) or universal hand equipment. The *B. bassiana* JW-1 strain can be tank mixed with most pesticides and fertilizers but not with most fungicides. Some identified fungicides are compatible; however, a time interval of 48 h before or after application of fungicides is suggested. Extensive evaluation has yielded no observed phytotoxicity. Toxicological studies indicate that the *B. bassiana* JW-1 strain does not have an adverse impact on humans, livestock, birds, fish, beneficial insects, crops, waterways, or groundwater resources.

INTRODUCTION

Biological or natural alternatives for pest control have been sought for many years with few successes. The development of fungi as an alternative has been considered for many years, mainly by academics. Problems with activity, stability, formulations, production, economics, limited markets, registrations, commercial protection, etc., were associated with the lack of commercialization of fungi in the pest control arena. Considerable effort by Troy Biosciences Inc in collaboration with the United States Department of Agriculture (USDA) initiated a successful fungal research program which eventually established that the JW-1 strain of *B. bassiana* was commercially viable.

NATURALIS® is a commercial formulation containing *B. bassiana* JW-1 that has demonstrated excellent efficacy against major pests of cotton, vegetables, ornamentals, and turf. In this paper we will present experiments designed to control the major greenhouse pests, aphids, whiteflies, thrips and mites with examples from the United States, Spain, New Zealand and Egypt.

MATERIALS AND METHODS

Whiteflies

The *B. bassiana* JW-1 strain is a flowable formulation that can be applied with conventional application equipment, including backpack sprayers, aerial, conventional ground, air-assisted, electrostatic equipment, etc. In Spain, replicated trials were carried out against the greenhouse whitefly (*T. vaporariorum*) on tomatoes and peppers using rates of 750 ml/hl and 1000 ml/hl of formulated *B. bassiana* JW-1. Comparative activity was made with buprofezin (25%) at 70 g/hl and untreated controls. A total volume of 666 l/ha was used. Control for the whitefly was determined by counts on 10 plants (3 replicates) at 3, 7, and 14 days after treatment on tomatoes. For peppers, comparative activity was evaluated at 3 and 7 days and then a second application was applied and evaluations were made at 3 and 7 days after this second application. The Henderson-Tilton calculation was used to reflect the control activity.

In New Zealand, efficacy of *B. bassiana* JW-1 was evaluated against the adult greenhouse whitefly at the rate of 75 ml/100 l on a mixture of potted broad bean (*Vicia faba*) and dwarf bean (*Phaseolus vulgaris*) plants. Comparisons were made with taufluvalinate (240g D-valinate/l) at 20 ml/100 l; pyrethrum (14 g/l); and piperonyl butoxide (56.5 g/l) at 250 ml/100 l. Mortality was observed at 24 and 28 h after treatment and for *B. bassiana* JW-1 mortality was taken daily up to 7 days after treatment. The whitefly's mortality response to the treatments was analyzed using Analysis of Variance (ANOVA). The corrected values (in response to natural mortality) were calculated using Abbott's formula.

In Egypt, *B. bassiana* JW-1 was assessed against the whitefly (*B. tabaci*). Infested plants were treated at the 75 ml/100 l rate and compared with etofenprox, actellic and imidacloprid at normal rates. Counts were made at 1, 3, and 5 days after application.

In the United States, the product was evaluated in commercial poinsettia production. The rate used was 1000 ml\1000 l, applied with standard high volume sprayers. Comparisons were made with the growers' standard insecticide program which consisted of recommended rates of endosulfan, pyrethroids, and insect growth regulators. Treatments were made at a 3-day interval as infestation of the whitefly was severe and evaluations made at 3-day intervals.

Thrips

In the United States, staked tomatoes with infestations of the western flower thrips (*F. occidentalis*) were treated at the rate of 750 ml/1000 l. Evaluations were made as to the number of thrips in 10 flowers per treatment (40 flowers total per 4 replicates). Comparisons of activity were made with tebufenozide 70W at 142 g/h, imidacloprid 1.6F at 54 g/h and cyfluthrin 2EC at 39 g/h. All data were analyzed using ANOVA and means were separated by using LSD (P=0.05). On chrysanthemums with infestations of the western flower thrips (*F. occidentalis*), the *B. bassiana* JW-1 product was evaluated against immatures and adults in separate replicated tests. Rates used were 750 ml/1000 l and counts were made at 5, 7, and 20 days after treatment. Data were analyzed using ANOVA and means were separated by using LSD (P=0.05).

Mites

On roses infested with two-spotted spider mites (*T. utricae*), evaluations against the adult and eggs were made separately in replicated tests. Rates used were 750 ml/1000 l and compared with the program of recommended rates of a mixture of avid and orthene as well as an untreated check.

Aphids

Broccoli infested with aphids (*M. persicae*) was treated with *B. bassiana* JW-1 at the rate of 750 ml/1000 l. Counts were made at 1-day intervals thereafter for 10 days after treatment (three replicates). On Siberian elm seedlings in 3.7 l pots infested with aphids, the product was applied at the rate of 750 ml/1000 l until drip using a 1 l hand-held sprayer. Evaluations were made at 2, 6, and 7 days after treatment. Aphids that failed to move after repeated proddings or that were shriveled and discolored were considered dead.

Pumpkins infested with aphids (*A. gossypii*) were treated with the product at the rate of 750 ml/1000 l. Each treatment was replicated 4 times in a randomized complete block design. Applications were begun 3 days after plants emerged from 3 different planting dates and applied weekly. Comparison of activity was made with permethrin 3.2 EC (448 ml/h) as well as an untreated control. Standard toxicological evaluations were performed as well as assessments against nontarget species.

RESULTS

Whiteflies

For whitefly (*T. vaporarium*) control on tomatoes in Spain, there was no significant difference between the *B. bassiana* JW-1 rates of 750 and 1000 ml/hl and buprofezin (25%) at 70 g/hl, which is the standard used for evaluation of materials for whitefly control (Table 1).

Table 1. Control of whiteflies (*Trialeurodes vaporariorum*) on tomatoes in Spain.

Treatment	Dose	% control at 3 DAT	% control at DAT *
B. bassiana JW-1	750 ml/hw	71.0 a	67.5 a
B. bassiana JW-1	1000 ml/hw	74.5 a	70.8 a
buprofezin	70 gr/hl	76.2 a	74.5 a

* Means with the same letter are not significantly different (P=0.05)

For control of whitefly (*T. vaporarium*) on peppers in Spain, the *B. bassiana* JW-1 treatment of 1000 ml/hl was significantly better than the standard of buprofezin. The rate of *B. bassiana* JW-1 at 750 ml/hl was the same as the buprofezin. This significant activity was demonstrated

consistently at 3 and 7 days after the first treatment and also at 3 and 6 days after the second application. These evaluations were performed in what was described as heavy infestations of the whitefly (Table 2).

Table 2. Control of whiteflies (*Trialeurodes vaporariorum*) on peppers in Spain.*

Treatment	Rate	DAT Application 1		DAT Application 2	
		3	7	3	7
B. bassiana JW-1	750 ml/hl	65.5 b	30.8 b	72.3 b	50.5 b
B. bassiana JW-1	1000 ml/hl	85.3 a	62.8 a	84.3 a	74.3 a
B. bassiana JW-1	70 g/hl	72.0 b	44.5 b	78.5 b	59.2 b

* Means with the same letter are not significantly different (P = 0.05)

The *B. bassiana* JW-1 was compared to taufluvalinate and a pyrethrum/piperonyl butoxide mixture against *T. vaporariorum* on broad beans and dwarf beans in New Zealand. The *B. bassiana* JW-1 product was significantly better than either the taufluvalinate or the pyrethrum/piperonyl mixture at 24 and 48 h after treatment (Table 3). The evaluation continued for the *B. bassiana* JW-1 product up to 7 days after treatment and mortality increased significantly at each daily interval and reached more than 95% after 7 days, at which time evaluation was ceased (Table 4).

Table 3. Efficacy of *B. bassiana* JW-1 against the whitefly (*Trialeurodes vaporariorum*) on broad bean and dwarf beans in New Zealand. *

Treatment	Rate	Mortality DAT	
		1	2
B. bassiana JW-1	75 ml/100 l	22.7 b	48.4 a
taufluvalinate	20 ml/100 l	14.4 b	22.6 b
pyrethrum/piperonyl butoxide	250 ml/100 l	13.6 bc	17.2 b

* Means with the same letter are not significantly different (P = 0.05)

Table 4. Efficacy of *B. bassiana* JW-1 on the mortality of the whitefly (*Trialeurodes vaporariorum*) over seven days on broad bean and dwarf beans in New Zealand.

Treatment	Rate	% of mortality (corrected) DAT						
		1	2	3	4	5	6	7
B. bassiana JW-1	75 ml/100 l	22.7	48.4	60.1	78.9	82.0	94.1	95.4

In Egypt, the *B. bassiana* JW-1 was evaluated against the whitefly, *B. tabaci*, on tomatoes. Comparisons against etofenprox, actellic, and imidacloprid indicated that no significant differences

in mortality occurred among the treatments. *B. bassiana* JW-1 was as effective as the other products in the control of the *B. tabaci* on tomatoes (Table 5). However, *B. bassiana* JW-1 had a cumulative mortality effect against the whitefly as data over a 7-day period yielded more than 92% mortality (Table 6).

Table 5. Control of the whitefly (*Bemisia tabaci*) on tomatoes in Egypt.

Treatment	Rate	% mortality (corrected)
B. bassiana JW-1	750 ml/hl	79.4 a
entofenprox		85.0 a
actellic		74.9 a
imidacloprid		70.4

Table 6. Cumulative effect of *B. bassiana* JW-1 on *Bemisia tabaci* on tomatoes in Egypt.

Treatment	% mortality (corrected)			
	DAT			
	1	3	5	7
B. bassiana JW-1	79.4	87.9	90.2	92.1

In the United States, evaluations for the control of whitefly (*B. argentifolii*) on poinsettia indicated that the *B. bassiana* JW-1 product was significantly more effective for the control of adults and immatures (Table 7) than the commercial program of endosulfan, pyrethroids, and insect growth regulators.

Table 7. Control of whitefly (*B. argentifolii*) on poinsettias in the United States. *

Treatment	Rate	Stage	DAT			
			3	6	10	17
B. bassiana JW-1	750 ml/1000 l	adults	1.8 a	0.8 a	0.0 a	0.0 a
endosulfan/pyrethroids/IGR	program	adults	4.4 b	5.4 b	6.0 b	0.4 a
B. bassiana JW-1	750 ml/1000 l	immatures	25.5 a	8.6 a	9.6 a	12.6 a
endosulfan/pyrethroids/IGR	program	immatures	39.8 a	18.4 b	55.9 b	38.4.b

* Means with the same letter are not significantly different (P = 0.05)

Thrips

Infestations of the western flower thrips (*F. occidentalis*) and the tobacco thrips (*Thrips tabaci*) on tomatoes in the United States were treated with the *B. bassiana* JW-1 and compared with tebufenozide, imidacloprid, and cyfluthrin. Populations of the thrips were reduced significantly by all treatments; however, there were no significant differences among the treatments. The *B. bassiana* JW-1 product was as effective as the commonly used insecticides (Table 8).

Table 8. Reduction of western flower thrips (*Frankliniella occidentalis*) and tobacco thrips (*T. tabaci*) on tomatoes in the United States. *

Treatment		Rate	No. of thrips/10 flowers
B. bassiana JW-1		750 ml/ha	0.3 a
tebufenozide	70 W	142 g/ha	0.3 a
imidacloprid	1.6 F	54 g/ha	0.8 a
cyfluthrin	2 EC	39 g/ha	0.8 a

* Means with the same letter are not significantly different (P = 0.05)

On chrysanthemums infested with the western flower thrips (*F. occidentalis*), *B. bassiana* JW-1 was evaluated against immatures and adults. The results in Table 9 indicate a cumulative effect for mortality against the immatures and the adults of *F. occidentalis* on tomatoes. After 14 days, more than 91% mortality occurred in the life stages of the thrips. The cumulative effect of *B. bassiana* JW-1 reflects the mechanism of contact activity by *B. bassiana* JW-1 in that thrips are well protected by the flowers and when the thrips are active, contact is made with the *B. bassiana* JW-1 and subsequent horizontal transfer may occur which increases the mortality.

Table 9. Results of B. bassiana JW-1 against immatures and adults of the western flower thrips (*Frankliniella occidentalis*) on chrysanthemums in the United States.

Treatment	Rate	Stage	% control DAT		
			4	7	14
B. bassiana JW-1	750 ml/ha	immatures	50.4	73.0	91.4
		adults	46.4	81.7	91.7

Mites

Roses infested with the two-spotted spider mite (*T. utricae*) were treated with *B. bassiana* JW-1 and compared to a greenhouse grower program of abamectin and acephate. The results in Table 10 show that the *B. bassiana* JW-1 was more effective in controlling *T. utricae* than

the program of the combination of abamectin and acephate as a cumulative effect was again noted in that mortality increased significantly with time. The eggs of the mites were also affected by both treatments.

Table 10. Control of two-spotted spider mites (*Tetranychus urticae*) on greenhouse roses in the United States.

Treatment	Rate	Stage	% control DAT		
			4	11	16
B. bassiana JW-1	750 ml/100 l	adults	30.0	60.0	94.2
abamectin & acephate	program	adults	15.0	0	0
B. bassiana JW-1	750 ml/1000 l	eggs	0	75.0	54.0
abamectin & acephate	program	eggs	0	80.0	28.0

Aphids

Broccoli infested with aphids (*M. persicae*) was treated with the *B. bassiana* JW-1 and evaluations were made at 2, 4, 7 and 10 days after treatment. The results in Table 11 show significant control by the *B. bassiana* JW-1 product with an increase in mortality from day 2 of 78.4% to 91.3% at 10 days after treatment. In Table 12, aphids on Siberian elm seedlings were controlled with a single application of *B. bassiana* JW-1, and at 2 and 4 days after treatment, 100% mortality had occurred.

Table 11. Control of aphids (*Myzus persicae*) on broccoli in the United States.

Treatment	Rate	% control DAT			
		2	4	7	10
B. bassiana JW-1	750 ml/1000 l	78.4	80.0	82.5	91.3

Table 12. Control of aphids on Siberian elm seedlings in the United States.

Treatment	Rate	% control DAT	
		2	4
B. bassiana JW-1	750 ml/1000 l	100	100

In Table 13, the efficacy of *B. bassiana* JW-1 on aphids (*A. gossypii*) on pumpkins in the United States was compared to that of permethrin. Control by the two products was equal

until at the latter part of the evaluations at 30 days after treatment (Table 13). The efficacy of permethrin declined significantly as compared to that of the *B. bassiana* JW-1.

Table 13. Efficacy of *Beauveria bassiana* JW-1 against aphids (*Aphis gossypii*) on pumpkins in the United States.

Treatment	Rate	% control of DAT		
		10	20	30
B. bassiana JW-1	750 ml/1000 l	65.4	84.7	74.7
permethrin	3.2 EC	73.3	79.6	7.3

DISCUSSION

These trials have confirmed that the activity of the *B. bassiana* JW-1 product is as effective as the standard insecticides in use today for control of important greenhouse pests, such as whiteflies, thrips, aphids, and mites. Additionally, the *B. bassiana* JW-1 product achieves significant cumulative mortality when compared to standard insecticides. This activity is probably an expression of the mechanism of activity of the *B. bassiana* JW-1 and may reflect additional horizontal transfer to other unexposed pests.

An analysis of the toxicological and environmental data for *B. bassiana* JW-1 may be summarized as having no immediate or potential negative effect against tested organisms. Numerous studies in different greenhouse environments have determined that the product does not affect key predators and parasites of pest species, such as *Encarsia* spp., *Eretmocerus* spp., *Chrysoperla* spp., *Geocoris* spp. and various arachnids. Results of a 30-day dietary and contact study with the honeybee, *Apis mellifera*, indicate that the *B. bassiana* JW-1 strain does not significantly affect this important pollinator. Fresh water toxicological studies indicate no effect on fish embryos, larvae, or adults and also no effects were indicated on water fleas. Dermal, oral, and inhalation toxicity studies with Sprague Dawley rats indicate a level of non-toxicity and no pathogenicity. Additional toxicological studies with other test organisms yielded the same conclusions: the *B. bassiana* JW-1 product is non-toxic and non-pathogenic.

Pesticide resistance is a major issue in insecticide efficacy; and in these trials with *B. bassiana* JW-1 with other insecticides, this was not considered, although the authors have reason to believe that many of the populations of insects that were tested had levels of resistance according to local authorities. The *B. bassiana* JW-1 provides effective control of susceptible and resistant strains of greenhouse pests. The mechanism of action is not biochemical but is mechanical as the targeted hosts act as substrates.

APHID-RESISTANT HOPS - THE KEY TO INTEGRATED PEST MANAGEMENT IN HOPS

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ABSTRACT

Despite much research effort, integrated pest management in hops has had only very limited success. This is because the key element of host plant resistance to the main pest, damson-hop aphid (*Phorodon humuli*), has not been present in any commercial variety. A screening of accessions held in the hop germplasm collection at HRI-Wye revealed a male genotype from Japan with strong resistance to this pest. Subsequent studies have shown that the characteristic is highly heritable, being controlled by two dominant genes with undiluted transmission between generations. Feeding experiments have shown the biology of the aphid on plants with such resistance to be affected: aphid progeny are smaller, less numerous and with longer intervals between generations. Thus, the aphid population size is reduced. Farm trials have demonstrated that this feature, in conjunction with predator activity and use of compatible sprays, can provide, for the first time, commercially acceptable levels of control of this pest in hops without the need for sole reliance on pesticides.

INTRODUCTION

The damson-hop aphid (*Phorodon humuli*) is a hop pest of worldwide importance. In Britain, winged aphids migrate from primary hosts (certain *Prunus* spp.) from late May through July, giving birth to viviparously-reproducing wingless offspring on hop (*Humulus lupulus*). Heavy infestations reduce hop plant vigour and may induce defoliation. Even light infestations of the harvested hop cones markedly reduce their economic value. The commercial threat posed by this aphid encouraged the prophylactic use of pesticides and, consequently, provided intense selection pressure for individuals resistant to insecticides. Resistance to organophosphate, carbamate and synthetic pyrethroid insecticides is now widespread (Hrdy & Hrdlickova, 1981; Campbell & Hrdy, 1988). The problems posed by insecticide-resistant aphids stimulated research into a more sustainable programme of integrated pest management that combined an early soil-applied aphicide with control exerted by predators later on in the season (Neve, 1977). However, this strategy has proved unreliable on current commercial hop varieties (Neve, 1977; Cranham, 1982). The build-up of aphids in June can be too rapid for naturally occurring predators to contain, a problem sometimes exacerbated by limited aphicide uptake from dry soils. Campbell (1983) argued that an aphid-resistant hop variety could provide the key to improved integrated pest management on hop, but noted that opportunities for

exploiting the relatively low levels of aphid-resistance then present in European hop varieties were limited. This paper presents evidence that such hop varieties, with much enhanced levels of aphid resistance, are now in prospect.

IDENTIFICATION OF HOST-PLANT RESISTANCE

The hop germplasm collection at HRI-Wye contains more than 500 accessions comprising wild hops, breeding lines and cultivars. Over the period 1981-3, all accessions were left untreated with insecticides until mid-July, about five weeks after the start of the migration of aphids to hops from the winter host. Assessments were made of the degree of natural infestation and damage before insecticides were applied to prevent excess damage to the collection.

Forty five accessions appeared less damaged than expected and these were propagated and planted in a replicated field trial which included reference varieties. As in the preliminary screen, the trial was untreated with insecticides during the migration period. Assessments were made in 1985 and 1986.

Table 1. Varietal differences in infestation by *P.humuli*

Variety	Mean no.aphid progeny per lateral	Tibia-3 length (μm)
Reference varieties:		
'Northern Brewer'	3089	850
'Wye Northdown'	4364	821
'Tolhurst'	3160	829
Accessions:		
33/75/9	1478	823
29/80/8	1258	768
27/76/8	630	754
19/65/29	524	802
11/68/15	256	766
INT 101	60	-*

* No adult aphids were present in the samples

In 1985, six accessions were found to have significantly fewer wingless aphids on the foliage than reference varieties (Table 1), a result which was confirmed in 1986. As a measure of the

impact of these varieties on aphid size, the average lengths of the tibial segment of the hind leg was determined for adult aphids from the foliar samples. Aphids on resistant accessions were generally smaller than on standard varieties.

Several of these were breeding lines derived from European varieties including cvs. 'Hallertau Mittelfrüh' and 'Record'. However, outstanding resistance to colonisation was shown by INT 101, a wild male accession collected from the mountainous region of central Japan: an area where damson-hop aphids are not found.

Feeding experiments

Apterous clonal *P.humuli* were reared in groups of four in clip-on-leaf cages, each enclosing 1.27 cm^2, on plants of the 9 genotypes (Table 1) in a controlled environment room at 18°C and 18L/6D photoperiod. Two cages were used per plant, and the experiment was replicated five-fold in a randomised complete blocks design. Cages were examined daily and carefully transferred to newly expanded leaves twice weekly. The daily age schedules of births and deaths were used to calculate the population doubling time which reflects the hosts impact on nymphal development, reproduction and survival rates (Campbell, 1983).

Table 2. Population increase rate for *P.humuli*

Variety	Doubling time (days)
Reference varieties:	
'Northern Brewer'	3.19
'Wye Northdown'	3.09
'Tolhurst'	3.15
Accessions:	
33/75/9	3.56
29/80/8	3.63
27/76/8	3.39
19/65/29	3.35
11/68/15	3.98
INT 101	4.02

Results from the feeding experiments agreed broadly with the field studies, with the shortest population doubling times on the standard varieties, whereas those on the resistant genotypes, particularly 11/68/15 and INT 101, were extended (Table 2).

INHERITANCE OF HOST PLANT RESISTANCE

The six genotypes, identified as resistant to damson-hop aphid from the germplasm screen and from caged feeding experiments, were used as parents to examine the mode of inheritance of this trait.

With the exception of INT 101, all selections were breeding lines, presenting no barriers to crosses being made. Small numbers of plants of each F1 family were raised and exposed to natural infestation in field plots during 1988 and 1989. Several of the progeny supported fewer aphids than reference varieties. However, of 160 progeny assessed, only one individual (a seedling of 19/65/29) showed resistance similar to that of the original parent. This suggested a low heritability for the trait.

INT 101 was poorly adapted to UK conditions and flowering was too late to coincide with other breeding material. However, technical difficulties were overcome and it was used as a parent in 1987. A large number of plants of a progeny family (55/87) were established in 1988 and assessed for field resistance to colonisation by damson-hop aphid in subsequent years.

Table 3. Segregation for resistance to *P. humuli.*

Family	No. resistant plants*	No. susceptible plants
Original family:		
55/87	184	70
Families derived from 55/87:		
4/89	25	21
10/89	75	21
11/89	60	20
12/89	90	33
13/89	22	14
Families derived from 4/89-13/89:		
10/92-35/92	763	496

* resistance defined as <3000 aphids per lateral.

Results showed a good agreement with a 3:1 ratio of resistant to susceptible plants, indicating the action of two dominant major genes. This hypothesis has been confirmed in subsequent generations (Table 3) where clear segregation and undiluted expression of the characteristic

have been observed. Individual families showed either a 1:1 or 3:1 segregation ratio depending on whether one or two genes, respectively, were present in the resistant parent.

The phenotypes observed in the progeny of 55/87 were found to be stable between seasons (Darby, 1994). Unfortunately, 55/87 plants and their own progeny inherited not only resistance to aphids but many 'wild' characteristics from INT 101 including poor cone structure, low yield, poor aroma and late maturity. Breeding work between 1992 and 1996 aimed to rectify these deficiencies and incorporate the many features necessary in a commercial variety (Darby, 1995). It is hoped that suitable parental material has been developed and crosses made in 1996 are the first aimed at developing a commercial variety resistant to aphids.

FARM TRIAL

It was considered important to assess the robustness of the resistance in a commercial environment before embarking on a breeding programme. Thus, in 1993, a selection (coded 11/89/39, F2 generation from INT 101) was established as a stand of 80 plants within a field of cv. 'Wye Target' at a hop farm in Kent where organic methods of production were used, conforming to the guidelines of the Soil Association. Only soft soap applications were used to control aphid populations.

By harvest 1993, all plants of cv. 'Wye Target' were severely infested with aphids; cones were small and discoloured, and all foliage was covered in sooty moulds. The crop was ruined and unsaleable (Lovelidge, 1994). Cones of 11/89/39, in contrast, were free of aphids and showed no discoloration or malformation. Aphid colonisation of the test plants was observed during 1994 and 1995. Aphids were able to colonise the plants but exponential population growth did not occur, confirming the expectations of Campbell *et al* (1993). Samples of aphids found on a few cones at harvest were tested in cage experiments on resistant plants under controlled conditions and results were the same as those described previously. These aphids did not represent selection for a sub-population able to overcome the host plant resistance. During the 1995 season, all test plants were infested during aphid migration but significant populations failed to establish. Predators were abundant and, by mid-July, no living aphids were found on any of the leaves sampled. In contrast, surrounding plants of cv. Wye Target had residual populations and spots of sooty moulds, despite exceptionally high air temperatures.

Although not a commercial variety, the cones from 11/89/39 were harvested and used with satisfactory results in a pilot-scale brew by a regional brewing company.

The farm trial confirmed that host plant resistance of this level, in combination with compatible sprays and natural predator activity, could consistently achieve a standard of control equal to that obtained in commercial practice at other farms by the use of pesticide applications.

CONCLUSIONS

Rapid progress is being made towards producing a commercially acceptable aphid-resistant hop variety. Such a variety is likely to become the cornerstone for integrated pest

management on hop in the next century as it provides the best opportunity for eliminating the need for aphicides. Although growth and reproduction of damson hop aphid is impaired on aphid-resistant genotypes, an additional component, such as the natural build-up of predators, is needed to curtail aphid population increase. The high level of aphid-resistance first identified in the germplasm collection has been shown to be heritable and undiluted in successive crosses.

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REFERENCES

Campbell, C A M (1983) Antibiosis in hop (*Humulus lupulus*) to the damson-hop aphid, *Phorodon humuli*. *Entomologia Experimentalis et Applicata* **33**, 57-62.

Campbell, C A M; Darby, P; Morgan, D (1993) Dynamics of damson-hop aphid on aphid-resistant hop plants. In: *Proceedings of the Scientific Commission 1993 International Hop Growers Convention*, R E Gunn & C P Green (eds), Wye College, Ashford, pp. 61-68.

Campbell, C A M; Hrdy, I. (1988) Integrated pest and disease control on hops. *International Organisation for Biological Control/West Palaearctic Regional Section Bulletin* **11**, 99 pp.

Cranham, J E (1982) Integrated control of damson-hop aphid, *Phorodon humuli*, on English hops: a review of recent work. *Agriculture and Environment* **7**, 63-71.

Darby, P (1994) Dwarfness and resistance to aphids: two novel traits in hop breeding. *European Brewery Convention Symposium on Hops*. Monograph XXII, Zoeterwoude, pp. 24-35.

Darby, P (1995) Current objectives in UK hop breeding. In: *Proceedings of the Scientific Commission 1995 International Hop Growers Convention*, E Seigner (ed), Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Freising, pp. 57-63.

Hrdy, I; Hrdlickova, H (1981) Integrated pest and disease control in hops. *International Organisation for Biological Control/West Palaearctic Regional Section Bulletin* **4**, 179 pp.

Lovelidge, B (1994) Sit back and leave it to the predators. *Grower* **44** Nov 3, pp. 38-39.

Neve, R A (1977) Integrated control of hop pests. *ARC Research Review* **3**, pp. 66-68.

INTEGRATING INSECTICIDE USE WITH BIOLOGICAL CONTROL OF TWO SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE*) BY *PHYTOSEIULUS PERSIMILIS* ON STRAWBERRY IN THE UK

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ABSTRACT

Four replicated field experiments at HRI Kirton and HRI Efford between 1992 and 1995 examined the effects of foliar sprays of insecticides, applied at varying intervals before or after introduction of the predatory mite *Phytoseiulus persimilis*, on biological control of two-spotted spider mite (*Tetranychus urticae*) on strawberry. Application of a foliar spray of chlorpyrifos, malathion, endosulfan or cypermethrin one week before or one week after introduction of the predator, or of chlorpyrifos or malathion at longer intervals pre-introduction, did not eliminate the predator nor prevent successful biological control.

A spray cover test at HRI East Malling in 1994 showed that high volume (2000 l ha^{-1}) hand lance and low volume (300 l ha^{-1}) air assisted spraying gave a high degree of spray cover (>80%) of the upper leaf surface. However, cover on the lower surface was poorer and more variable, particularly with the air assisted spraying method, providing many niches for survival of predatory mites from deposits of harmful insecticides. Directing a hand lance spray from above further reduced cover on lower leaf surfaces where predator and prey mites live, so providing opportunity for reducing possible harmful effects.

INTRODUCTION

Most strawberry cultivars currently grown in the UK are moderately or highly susceptible to two-spotted spider mite (*Tetranychus urticae*). In the absence of effective control methods, it is likely that *T. urticae* would cause significant economic damage in most crops in most seasons. Until the late 1980s, growers relied on acaricides for control of *T. urticae*. However, *T. urticae* rapidly develops multi-resistant strains to acaricides though the organo-tin acaricide cyhexatin, introduced in the 1970s, proved highly effective and durable and growers relied on it for many years. The withdrawal of the registration of cyhexatin in the UK in the late 1980s led subsequently to widespread commercial use of the predatory mite *Phytoseiulus persimilis* for biological control of *T. urticae* on field grown strawberry crops

in the UK. This predatory mite, though long known to be effective on protected strawberry crops (Cross, 1984), had not hitherto been considered sufficiently reliable in the field for commercial purposes. Though it was known that *P. persimilis* had developed some limited resistance to organophosphorus insecticides (Schulten *et al.*, 1976), integration with broad-spectrum insecticides to control other pests on strawberries was believed to be difficult because these pesticides had been classified as harmful based on standard laboratory tests (Ledieu, 1985; Oomen *et al.*, 1991). An 8 week pre-introduction safety interval was believed by growers to be necessary with chlorpyrifos.

The following paper reports the results of four replicated field experiments to determine the safe interval between application of four insecticides widely used on strawberry and introduction of *P. persimilis*. A further experiment examined the degree of spray cover achieved with different methods of spray application. Strawberry spraying practice varies widely in the UK but low volume (down to 150 l ha^{-1}), air-assisted spray application is becoming increasingly prevalent (Cross & Berrie, 1995).

MATERIALS AND METHODS

The field experiments were done in 1992 and 1993 at HRI Kirton, Boston, Lincolnshire, and in 1994 and 1995 at HRI Efford, Lymington, Hants. The same experimental plots at each site were used for the experiments in the two successive years. A further experiment at HRI East Malling, Kent, in 1994 examined the effects of different methods of application of sprays of chlorpyrifos or cypermethrin, but it failed because high populations of naturally occurring insecticide-resistant phytoseiid mites (*Amblyseius* sp.) overran the plots, eliminating *T. urticae* and preventing successful establishment of *P. persimilis*. However, tests were conducted to determine the leaf-cover achieved with the different methods of spraying, and these are described below.

At HRI Kirton, the experimental site was planted in bare, flat-surfaced soil with cold-stored strawberry runners, cv 'Hapil', on 17 March 1992. It consisted of a 9 x 4 rectangular array of 36 plots, each plot consisting of a double staggered row of 64 plants with 0.3 m spacing between and within rows, and with the double rows (and hence the plots) spaced 1.8 m apart. There were 2 m gaps of bare soil between plots in the row. Each plant was artificially infested with *T. urticae* on 16 June 1992 and on 4 June, 6 and 29 July 1993 by placing an infested strawberry leaflet, collected from a heavily infested crop, amongst the foliage of each plant. In 1992, treatments consisted of a foliar spray of chlorpyrifos (Dursban 4, 480 g l^{-1} EC) at 720 g ai in 1000 l water per ha 1, 2, 4 or 8 weeks before *P. persimilis* introduction at a rate of 1 per plant on 8 July 1992, or of malathion (Malathion 60, 600 g l^{-1} EC) at 1140 g ai in 1000 l water per hectare 2 weeks after or 1, 2 or 4 weeks before predator introduction. *P. persimilis* were formulated in admixture with vermiculite as is normal commercial practice. In 1993, the same treatments were used except that intervals of application for both insecticides were 4, 2 or 1 week before or 1 week after predator introduction at a rate of 1 per plant on 17 August 1993. Sprays were applied with a hand lance using an Oxford Precision sprayer. The foliage of the plants was ruffled immediately in advance of the lance to allow penetration of the spray. A randomised complete block design with 4 replicates of 9 treatments, including an untreated control, was used. In 1992,

a sample of 25 expanded, trifoliate leaves was taken per plot on 22 July, 5 August and 19 August, 2, 4 and 6 weeks after predator introduction. In 1993, samples were taken on 18 and 31 August, 14 and 28 September and 18 October, 0, 2, 4, 6 and 9 weeks after predator introduction. The number of eggs and motiles of *T. urticae* and *P. persimilis* were counted using the leaf-brushing technique (Morgan *et al.*, 1955). Analysis of variance was done on the data, with and without $\log_{10}$ (x + 1) transformation.

At HRI Efford, the experimental site was planted in bare, flat-surfaced soil with cold-stored strawberry runners, cv 'Hapil', on 15 April 1994. It consisted of a 9 x 4 rectangular array of 36 plots, each plot consisting of a double staggered row of 30 plants with a 0.3 m spacing between and within rows. However, 4 m of bare soil separated adjacent plots on all sides. Artificial infestation with *T. urticae* was not necessary as the plants were already infested. Treatments in both years consisted of a foliar spray of chlorpyrifos (Dursban 4, 480 g l^{-1} EC) at 720 g ai ha^{-1}, of malathion (Malathion 60, 600 g l^{-1} EC) at 2280 g ai ha^{-1}, of endosulfan (Thiodan 20, 200 g l^{-1} EC) at 1000 g ai ha^{-1} or of cypermethrin (Ambush C, 100 g l^{-1} EC) at 28 g ai ha^{-1} each applied one week before or one week after predator introduction, and an untreated control. Sprays were applied with a hand lance at 2000 l ha^{-1} targeted to cover both leaf surfaces. *P. persimilis* was introduced at a rate of 5 per plant on 25-26 July 1994 and 18 July 1995. In 1994, a sample of 25 trifoliate leaves was taken on 2 and 22 August and 5 and 26 September, 1, 4, 6 and 9 weeks after predator introduction respectively. In 1995, a sample of 50 leaflets was taken from each plot on 10 and 25 July and 9 and 22 August, 1 week before and 1, 3 and 5 weeks after predator introduction. Numbers of mites were estimated and statistical analysis was done in the same way as in the previous experiments.

A further experiment at HRI East Malling in 1994 compared the spray cover achieved with three different methods of spray application *viz*, 1) low volume (lv) air-assisted spraying at 300 l ha^{-1} using the Hardi Mini Variant sprayer most commonly used in commercial practice, 2) high volume (hv) hand lance spraying at 2000 l ha^{-1} moving the spray nozzle amongst and round the strawberry canopy to maximise cover on the undersurfaces of leaves and 3) downward-directed hand lance spraying at 600 l ha^{-1} with plants sprayed from above only with minimal nozzle movement to cover the upper leaf surface only. A UV fluorescent, water soluble tracer dye, Tinopal CBS-X, was used at 1.0% concentration. Sprays were applied to single plots of established strawberry plants (cv 'Elsanta') grown on raised, polythene-mulched beds on 12 July 1994. As soon as the spray deposits were dry, a sample of 200 fully expanded leaves was taken from each plot. Using an Optomax V image analyser, the percentage of the leaf surface covered with deposit was determined by direct image analysis of the fluorescent deposit on a randomly selected 2 cm x 2 cm square area of each leaf surface illuminated with UV light in a dark chamber. The mean and coefficient of variation of the percentage leaf area covered with spray deposit was determined on each leaf surface for each method of spraying.

RESULTS

Log_{10} (x+1) transformation was necessary for valid analysis of variance of the data. However, for reasons of space, only mean mite counts, and not $\log_{10}$ (x+1) transformed values, are given in this paper. Standard errors of differences of untransformed data are

given to indicate the degree of variability of the data only, and should not be used for comparisons between treatment means.

In the first experiment at HRI Kirton in 1992 (Table 1), none of the spray treatments with chlorpyrifos or malathion, even those one week before or, in the case of malathion, 2 weeks after *P. persimilis* introduction, had any significant effect on successful biological control of *T. urticae*. Predators had established well by 2 weeks after introduction. Predator : prey ratios reached approximately 1:10 by 4 weeks after predator introduction and *T. urticae* was completely eliminated by 6 weeks after introduction.

Table 1. Mean numbers of *T. urticae* and *P. persimilis* per leaf 2, 4 and 6 weeks after introduction of *P. persimilis* at a rate of 1 per plant on 8 July 1992 to plots that had received a spray of chlorpyrifos or malathion or no spray at varying intervals before (negative values) or after (positive value) predator introduction at HRI Kirton in 1992.

Insecticide treatment	interval (weeks)	*T. urticae*/leaf			*P. persimilis*/leaf		
		2 wk	4 wk	6 wk	2 wk	4 wk	6 wk
untreated control		2185	761	0	2.7	46	2
chlorpyrifos	-1	2707	501	0	0.7	47	1
	-2	3553	354	-	3.7	35	-
	-4	2646	-	-	2.7	-	-
	-8	1446	-	-	6.0	-	-
malathion	+2	2384	1005	0	3.3	66	1
	-1	3157	217	-	1.7	28	-
	-2	2167	-	-	6.0	-	-
	-4	2663	-	-	3.0	-	-
s.e.d. (≥ 12df)†		698	233	-	3.1	19.5	-

†analysis of variance of $\log_{10}$ (x+1) transformed data revealed no statistically significant differences between treatments. S.e.d. values from analysis of variance of untransformed data are given to indicate degree of variability in data only, not for comparisons between means.

Note: - = not sampled.

At HRI Kirton in 1993 (Table 2), the biological control interaction was much slower and less convincing than in 1992. None of the spray treatments with chlorpyrifos or malathion, even those one week before or one week after predator introduction, eliminated *P. persimilis* from the plots. Numbers of *T. urticae* declined steadily through the 9 week period of monitoring, but it was difficult to determine whether this was due to biological control by *P. persimilis* or other factors such as unfavourable weather.

Table 2. Mean numbers of *T. urticae* and *P. persimilis* per 25 leaves 2, 4, 6 and 9 weeks after introduction of *P. persimilis* at a rate of 1 per plant on 17 August 1993 to plots that had received a spray of chlorpyrifos or malathion at varying intervals before (negative values) or after (positive values) predator introduction at HRI Kirton in 1993.

Insecticide treatment	Interval (weeks)	*T. urticae*/25 leaves				*P. persimilis*/25 leaves			
		2 wk	4 wk	6 wk	9 wk	2 wk	4 wk	6 wk	9 wk
none		4026	1183	23	2	1.0	2.3	0	0.5
chlorpyrifos	+1	1642	1323	400	12	1.0	1.8	0.8	2.5
	-1	2492	2361	712	68	0.8	1.0	2.0	1.5
	-2	5440	3016	444	23	0.3	2.0	1.8	0.5
	-4	3228	2441	500	26	1.3	1.5	2.8	0.5
malathion	+1	4198	1914	763	52	1.0	0.5	3	3.5
	-1	4176	2420	274	32	2.3	3.8	1.5	1.0
	-2	3939	1917	172	4	1.3	1.0	1.0	0
	-4	4250	2185	238	12	0	1.3	0.3	0.5
s.e.d. (24 df)†		1658	905	361	30	1.1	1.0	1.2	1.2

† Analysis of variance of $\log_{10}$ (x+1) transformed values revealed no statistically significant ($P \leq 0.05$) treatment differences. S.e.d. values from analysis of variance of untransformed data are given to indicate variability in data only.

At HRI Efford in 1994 and 1995 (Tables 3 overleaf and 4 below respectively), sprays of chlorpyrifos, malathion, endosulfan or cypermethrin, either one week before or one week after predator introduction, did not eliminate *P. persimilis* nor significantly affect the biological interaction between predator and prey. In 1994, there were no statistically significant treatment differences except in the counts of *T. urticae* 9 weeks after predator introduction, where significantly ($P<0.05$) greater numbers of mites occurred with the chlorpyrifos and cypermethrin sprays applied one week after predator introduction. The variability in the data together with the lack of statistically significant differences in numbers of *P. persimilis*, mean that evidence of a slight delaying effect in biological control caused by these treatments is, at best, weak.

Table 4. Mean numbers of *T. urticae* and *P. persimilis* per leaf 1, 3 or 5 weeks after *P. persimilis* introduction at a rate of 5 mites per plant on 18 July 1995 to plots that had received a spray of chlorpyrifos, malathion, endosulfan or cypermethrin or no spray one week before (negative values) or one week after (positive values) predator introduction at HRI Efford in 1995.

Insecticide treatment	Interval (weeks)	*T. urticae*/25 leaflets			*P. persimilis*/25 leaflets		
		1 wk	3 wk	5 wk	1 wk	3 wk	5 wk
chlorpyrifos	-1	3728	582	40	20.0	4.3	0.3
	+1	2423	249	13	6.0	3.5	0.3
malathion	-1	2711	572	44	3.0	3.3	0.3
	+1	2956	1508	32	9.3	4.0	0.5
endosulfan	-1	4247	499	43	2.7	11.3	0.8
	+1	1405	493	25	2.7	1.3	0
cypermethrin	-1	1646	731	87	17.3	0.0	0.3
	+1	275	149	38	6.7	0.8	0.5
untreated control		2003	471	36	1.0	3.5	0.5
s.e.d.(24 df)†		1352	494	32	10.5	2.4	0.5

† Analysis of variance of $\log_{10}(x+1)$ transformed values revealed no significant difference between insecticide treatments and the control using a 2-sided Dunnetts test. S.e.d. values from analysis of variance of untransformed data are given here to indicate degree of variability in data only.

The spray cover measurements at HRI East Malling in 1994 (Table 5) showed that all three methods of spraying tested gave a high percentage cover of spray deposit on the upper leaf surface. The hv hand lance treatments gave a higher and less variable percentage cover on the upper leaf surface than the lv air-assisted sprayer. Spray cover was less, and more variable, on the lower than the upper leaf surface. Levels of spray cover were least, and most variable, from the downward-directed hand lance sprayer and greatest and least variable with the hv overall hand lance treatment. The lv air assisted sprayer gave intermediate values.

Table 3. Mean numbers of *T. urticae* and *P. persimilis* per 50 leaflets 1, 4, 6 and 9 weeks after introduction of *P. persimilis* at a rate of 5 per plant on 25-26 July 1994 to plots that had received a spray of chlorpyrifos, malathion, endosulfan or cypermethrin or no spray one week before (negative values) or one week after (positive values) predator introduction at HRI Efford in 1994.

Treatment (insecticide) and interval (weeks)		*T. urticae*/25 leaves				*P. persimilis*/25 leaves			
		1 wk	4 wk	6 wk	9 wk	1 wk	4 wk	6 wk	9 wk
chlorpyrifos	-1	933	52	13	3	1.5	3.3	4.0	2.8
	+1	232	74	59	17*	1.0	1.5	6.3	8.5
malathion	-1	395	54	25	6	0.3	0.3	3.3	0.3
	+1	445	57	36	5	0.8	3.3	6.3	2.0
endosulfan	-1	670	52	31	2	1.5	0.3	2.3	0.5
	+1	652	36	17	2	0.5	0.3	1.5	0
cypermethrin	-1	1276	48	41	7	1.5	0.3	1.8	2.3
	+1	423	159	57	27*	2.0	0.8	4.5	5.5
untreated		326	25	14	2	0.8	1.8	0.3	0.5
s.e.d. (24 df)†		279	44	26	5.1	0.94	1.66	1.97	1.90

* significantly ($P<0.05$) greater than mean untreated control using 2 sided Dunnetts test on $\log_{10}(x + 1)$ transformed values
† s.e.d. values from analysis of variance of untransformed data are given to indicate variability of data only.

Table 5. Mean percentage of the leaf surface covered with spray deposits of the fluorescent tracer dye Tinopal CBS-X and, in parenthesis, the coefficient of variation (cv%) resulting from three different methods of spray application tested at HRI East Malling in 1994.

Spraying method	Leaf surfaces targeted	spray volume ($l\ ha^{-1}$)	% cover (cv%) upper leaf surface	lower leaf surface
lv air assisted sprayer	both	300	79 (34)	40 (78)
lance	both	2000	91 (12)	77 (37)
downward lance	upper	600	88 (17)	21 (119)

DISCUSSION

Although laboratory tests show that the insecticides evaluated are harmful to *P. persimilis*, (Ledieu, 1985; Oomen *et al.*, 1991), in these experiments field applications had little or no perceptible harmful effect. The spray cover measurements indicate that, even with high volume hand lance spraying, spray cover is likely to be far from complete and that there are likely to be many niches on the undersides of strawberry leaves where predators can escape the immediate harmful effects of pesticides. Directing spray from above reduces deposits on the undersides of leaves where predator and prey mites live and breed so reducing possible harmful effects.

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REFERENCES

Cross, J V (1984) Biological control of two-spotted spider mite (*Tetranchus urticae*) by *Phytoseiulus persimilis* on strawberries grown in 'walk-in' plastic tunnels, and a simplified method of spider mite population assessment. *Plant Pathology* **33**, 417-423.

Cross, J V; Berrie, A M (1995) Strawberry. A review of published literature and available information on spray application for pests and disease control on commercial crops. *Report to HDC No. SF42* issued December 1995, 36pp.

Ledieu, M (1985) Evaluation of side-effects of pesticides by the Glasshouse Crops Research Institute. In: *Biological Pest Control, The Glasshouse Experience Eds. N W Hussey and N Scopes, Blandford Press, UK,* 153-165.

Morgan, C V G; Chant, D A; Anderson, N H; Ayre, G L (1955) Methods for estimating orchard mite populations, especially with the mite brushing machine. *Canadian Entomologist* **87**, 189-200.

Oomen, G; Romeijn, G; Wiegers, G L (1991) Side effects of 100 pesticides on the predatory mite *Phytoseiulus persimilis*, collected and evaluated according to the *EPPO Guideline*. *Bulletin OEPP/EPPO Bulletin* **21**, 701-712.

Schulten G G M; van de Klashorst, G; Russel, V M (1976) Resistance of *Phytoseiulus persimilis* to some insecticides. *Zeitschrift für Angewandte Entomologie* **80**, 337-341.

PRODUCTION AND MEASUREMENT OF A BIOSURFACTANT FROM *BACILLUS BREVIS* FOR BIOCONTROL OF *BOTRYTIS CINEREA*

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ABSTRACT

Cultures of the biocontrol agent *Bacillus brevis* Nagano mutant-strain E-1 (gramicidin S negative) were tested for the presence of biosurfactant *in vitro,* using techniques of foam persistence and surface run-off. Quantitative measurement was difficult but was achieved using a 5-minute foam persistence test and comparison with commercial surfactants. Serial dilution was used to quantify the activity in the sample. Qualitative analysis was carried out by centrifugation, pH, and temperature treatments, and by preliminary purification with ammonium sulphate and ethanol.

INTRODUCTION

The plant pathogen *Botrytis cinerea* infects fruits, vegetables, cereals, legumes, flowers, and ornamentals (Verhoeff *et al.*, 1992). As the pathogen has developed a wide-ranging resistance to fungicides including benzimidazoles and dicarboximides, chemicals can no longer be considered as the sole means for the control of this pathogen (Edwards *et al.*, 1994). *B. cinerea* is therefore an obvious target for biocontrol studies.

Field testing of 14-day cultures of *B. brevis* against *B. cinerea* on Chinese cabbage showed disease control on a par with iprodione fungicide treatment (Edwards & Seddon, 1992) at about 70%. Treated leaves dried four times faster after irrigation in glasshouse trials than control leaves, indicating that the supernatant contained a biosurfactant which reduced leaf wettability (Edwards *et al.*, 1994). This was the first reported case of biological control by reduction of leaf wetness duration.

Biosurfactants enable microbial growth on water-insoluble substrates by reducing the surface tension at the phase boundary and enabling uptake and metabolism. Molecules may aggregate in micelles which accumulate at interfaces and "mediate between phases of different polarity...acting as wetting agents on solid surfaces" (Fletcher, 1992). Measurable properties include foaming, lowered surface tension, wettability, and emulsifying power (Rosen & Goldsmith, 1972).

This work set out to develop methods to measure biosurfactant activity of *B. brevis* cultures in order to monitor production levels and characterise the biosurfactant.

EXPERIMENTAL WORK

Growth conditions of *B. brevis* for biosurfactant production

B. brevis Nagano E-1 (Iwaki *et al.*, 1972) was grown in 500 ml Tryptone Soya Broth (TSB) (Oxoid) in a 2 L flask at 37°C on an orbital shaker at 150 rpm (Edwards, 1993).

Biosurfactant assay

Foam persistence (until complete surface cover was no longer observed) of 2 ml culture samples in 10 ml plastic tubes, was measured after shaking for 5 minutes on a reciprocal shaker (Griffin & George). Samples foamed for in excess of 300 minutes, whereas fresh medium alone foamed for less than 2 minutes. Samples were centrifuged at 3500g for 20 minutes (MSE Chilspin) to remove cells and then autoclaved at 121°C for 15 minutes (to prevent regrowth), with no loss in biosurfactant activity. The technique was improved by using a faster 5 second vortex shake of 2 ml samples, in 20 ml glass tubes which yielded an easily observed and more persistent foam. The measurement was improved by recording the positive foam persistence (that is, complete surface cover of foam) of twenty tubes after 5 minutes. Measurements were made at room temperature (20 - 25°C) unless otherwise stated.

Assessment of biosurfactant activity

For test purposes, dilutions were made with sterile distilled water to yield solution concentrations as a factor of the original autoclaved culture supernatant (ACS). Foam persistence was measured over a range of dilutions until activity was lost. Minimum measurable activity was found to be between 5 and 10% of the original 12 day ACS. Comparison was made with solutions of the agricultural surfactant Cittowet and detergent Triton X100. The 10% ACS solution exhibited comparable activity to 2% solutions of these reagents with 25-60% of the tubes showing positive activity.

Effect of temperature

The foam persistence activity of the biosurfactant was highest in the temperature range 5 - 50°C and fell off markedly but reversibly above 70°C to zero at 85°C under the conditions of assay used. Freezing (-18°C) and thawing lead to little or no loss of activity and harvested culture samples were therefore batch frozen at -18°C, thawed at room temperature and subsequently stored at +4°C prior to testing.

Precipitation

Precipitation of biosurfactant was achieved (as measured by subsequent foam persistence tests) by sequentially increased saturation of ACS with ammonium sulphate to70% (Dawson *et al.*,1969). The majority of biosurfactant was removed at 45% saturation by sequential 10 minute mixes of the supernatant, followed each time by centrifugation at 3500g for 15 minute periods in clean plastic universals; the brown pellet re-dissolved in sterile distilled water within 2 hours in the original volume. The

sample was further processed by the addition of ethanol to a final concentration of 80%, resulting in a milky white precipitate after boiling for 2 minutes (Dawson *et al.*,1969). After cooling and centrifugation, the cream-coloured pellet was resuspended in water at pH 9.3 with recovery of at least 80% of activity.

Effect of pH adjustment

The measured pH of the TSB culture increased from 7.3 to 9.3 when inoculated with *B. brevis* Nagano E-1 and incubated over a 12 day period. Post-harvest supernatant-adjusted pH was measured in the range pH 1-13, at 10% concentration. A major peak of foaming activity was observed between pH 8-10, with zero activity in the range pH 2.5-5.5 and a minor contribution below pH 2 (see Figure 1).

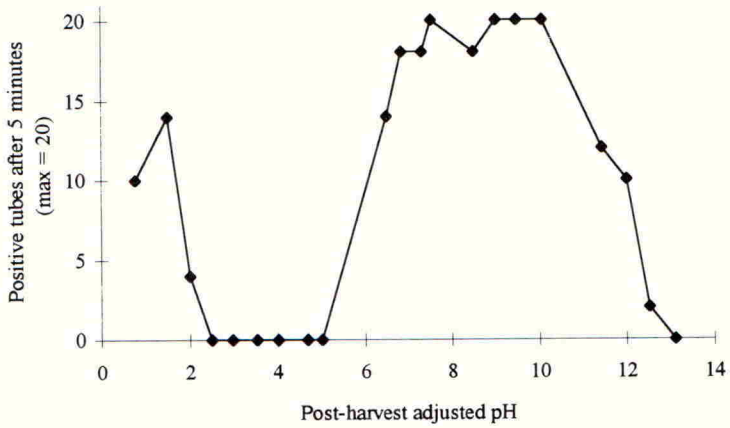

Figure 1. Foam persistence at various pH. Twenty tubes of sample were vortexed and the number of tubes with complete foam cover after five minutes were scored positive, up to a maximum of twenty.

Precipitation of calcium and magnesium ions

Soaps are known to precipitate calcium and magnesium ions, forming an insoluble 'scum' or precipitate. Addition of 1.0 M sulphate salts to ACS yielded less than 15% difference between foaming activity of treated and filtered samples and untreated samples. The low difference in both cases suggests that the surfactant is not a soap.

Evaporation and run-off of supernatant

The major finding of Edwards (1993) in respect of the biosurfactant is that treated and irrigated leaves dried some 4 x faster than untreated leaves. Faster drying times may result from increased spread over treated surfaces as a function of altered surface tension. The droplet sizes required to achieve total run-off of 10% supernatant and of water from an artificial surface were measured. Ten droplets of specified size and sample, were placed equidistant on horizontal glass and polystyrene Nunc Bioassay

plates (Life Technologies) and on polystyrene coated with paraffin wax. The number of droplets which moved at least 0.5 cm when the plate was held vertical for 60 seconds was recorded. Droplet size (volume) was increased until total run-off was achieved (Figure 2). ACS did not wet polystyrene, and achieved run-off at smaller droplet sizes than was observed with water, whereas greater spread and wetting ability was observed on waxed polystyrene.

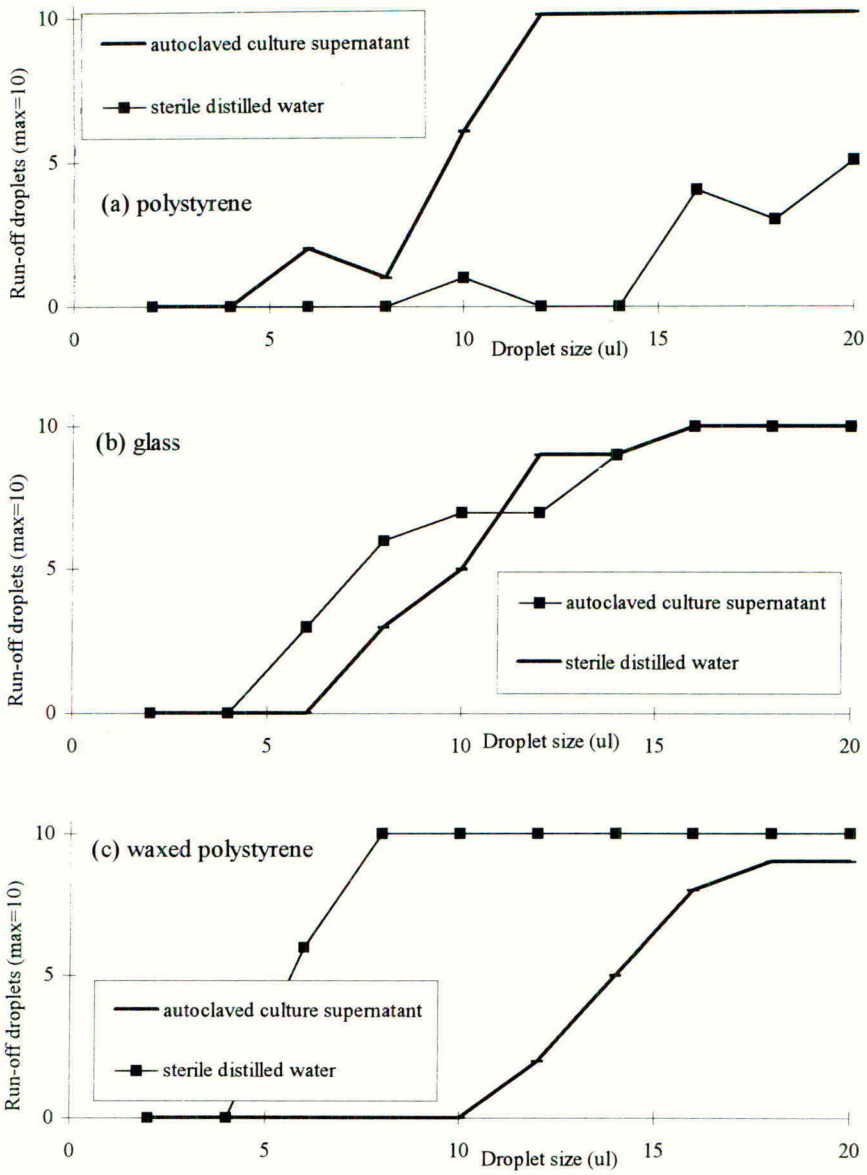

Figure 2. Run-off of sterile distilled water and autoclaved culture supernatant droplets from (a) polystyrene, (b) glass and (c) waxed polystyrene surfaces.

Production rate

Production of biosurfactant by *B.brevis* Nagano E-1 cultures measured over a 6 day period, was apparent at between 1-2 days of media inoculation with production levelling off at the upper limit of measurement by day 4 (Figure 3).

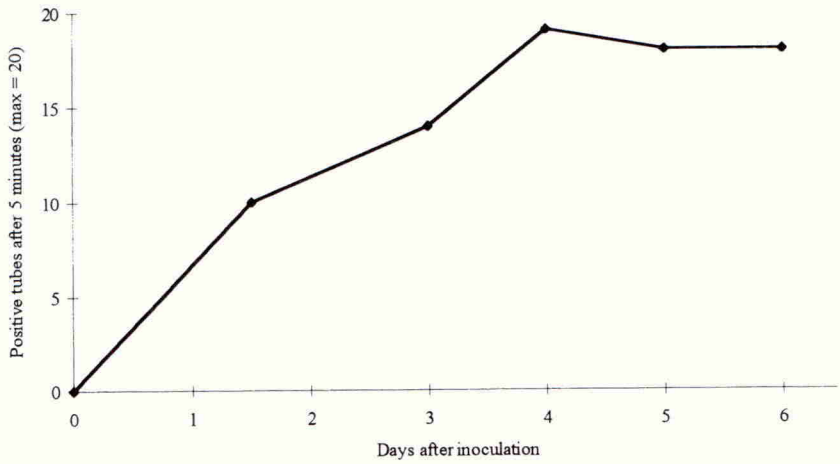

Figure 3. Production of biosurfactant activity by *B.brevis* Nagano E-1 culture over time. ACS was treated with 45% ammonium sulphate as described in the text; the precipitate was collected by centrifugation and redissolved in sterile distilled water to equal the original volume. The resultant sample was pH-adjusted to 9.3 and diluted to 10% of the original concentration for measurement of foam persistence.

DISCUSSION AND FUTURE WORK

A 10% solution of the autoclaved *B. brevis* Nagano E-1 culture supernatant exhibited shorter foam persistence than 2% standards of Cittowet and Triton X100, whereas a 20% solution was greater. If the supernatant contained less than 10% pure biosurfactant (and this is very likely), the foaming activity of the biosurfactant would be greater than the strong surfactant standards used.

The ability of the biosurfactant to adhere to, and wet a waxed surface, may indicate a lipophilic moiety as part of the molecule. This suggests that the use of polystyrene is not a suitable surface for these studies and that a waxed surface is more appropriate given that wettability of plant surfaces is reduced by cuticle wax. Further work must be carried out on leaves, although difficulties of standardisation will occur as most leaves are not flat. Some study of the physiological requirement by *B. brevis* for a biosurfactant is anticipated. This will be investigated by varying the composition of Tryptone Soya Broth in order to increase the production of biosurfactant so that purification and identification may be facilitated.

ACKNOWLEDGEMENTS

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REFERENCES

Dawson, R M C; Elliott, D; Elliott, W H; Jones, K M; (1969) Data for Biochemical Research. 2nd edition. Clarendon Press, Oxford. p.616.

Edwards, S G; Seddon, B; (1992) *Bacillus brevis* as a biocontrol agent against *Botrytis cinerea* on protected Chinese cabbage. In: *Recent Advances in Botrytis Research.* Pudoc Scientific Publishers, Wageningen. pp.267 - 271. Verhoeff, K; Malathrakis, N E; Williamson B; (eds)

Edwards, S G; (1993) Biological control of *Botrytis cinerea* by *Bacillus brevis* on protected Chinese cabbage. PhD thesis, University of Aberdeen.

Edwards, S G; McKay, T; Seddon, B; (1994) Interaction of *Bacillus* species with phytopathogenic fungi - Methods of analysis and manipulation for biocontrol purposes. In: *Ecology of Plant Pathogens.* Blakeman, J P; Williamson, B; (eds) CAB International, Wallingford. pp.101-118.

Fletcher, A; (1992) Biosurfactants: moving towards industrial application. *Trends in Biotechnology* **10**: 208-217.

Iwaki, M; Shimura, K; Kanda, M; Kaji, E; Saito, Y; (1972) Some mutants of *Bacillus brevis* deficient in gramicidin S formation. *Biochemical and Biophysical Research Communications* **48**: 113-118.

Rosen, M J; Goldsmith, H A; (eds) (1972) Systematic analysis of surface-active agents. 2nd edition. *Chemical Analysis Volume 12.* Elwing P J; Kalthoff I M; (eds) Wiley-Interscience, New York.

Verhoeff, K; Malathrakis, N E; Williamson B; (1992) *Recent Advances in Botrytis Research.* Pudoc Scientific Publishers, Wageningen.

DETECTION OF APPLE CHAT FRUIT DISEASE

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ABSTRACT

Chat fruit disease cannot be detected visually in commercial apple cultivars although it can reduce cropping by over 30%. Control is severely hampered by graft assays using fruiting indicators, cv. Lord Lambourne, in which symptom production is erratic and which take several years to complete. Two similar experiments were conducted in consecutive years to examine the development of symptoms in detail and to attempt to produce them reliably in potted trees of cv. Lord Lambourne grown under glass in controlled conditions. In both years there was a significant reduction in the initial fruit set in trees inoculated with chat fruit, compared to control mock-inoculated trees, although the flowers were normal in appearance. Shoot extension growth and trunk girth increment were greater in the inoculated trees. These results accord with earlier field observations and show that part of the disease syndrome can be reproduced in the glasshouse. However, early fruit drop in all treatments in both years precluded observations of fruit symptoms and is an obstacle to development of an improved assay.

INTRODUCTION

Chat fruit is an economically damaging disease of apple (*Malus sylvestris*) that can reduce yields by over 30%, even in cultivars that show no obvious symptoms (Luckwill, 1963; Campbell & Sparks, 1986; Posnette, 1986). The disease spreads slowly in orchards (Campbell & Hughes, 1974) but a vector has not been identified; the only variety to show clear symptoms is Lord Lambourne, the only means of experimental transmission is by grafting and the causal organism is not known.

Chat fruit is detected by a graft-inoculation procedure. Chips of test material are budded into fruiting trees of the biological indicator cv. Lord Lambourne and the fruits monitored for symptoms over a period of three years (Posnette & Cropley, 1965). Symptoms in the indicator are expressed erratically; the fruit on infected trees are small, poorly coloured and occasionally develop dark green spots with red pigment at the circumference. Affected fruits are unevenly distributed on an infected tree and the appearance of symptoms is inconsistent, with warm summers being detrimental to symptom expression (Posnette *et al.*, 1976). These characteristics hamper research into the disease and slow down the testing procedures for viruses and virus-like diseases that are necessary before planting material is provided to the propagation and fruit growing industries.

This is a preliminary report of experiments aimed at improving the speed and reliability of testing for the presence of chat fruit by growing young inoculated trees in controlled conditions.

MATERIALS AND METHODS

Two similar experiments were conducted, each lasting for 3 years, in which pot-grown trees of the indicator cv. Lord Lambourne were inoculated with chat fruit in year one, kept in a field plot for a year to allow the disease to become systemic and for fruit buds to develop, and grown in a temperature-controlled glasshouse in the third year for observations on symptom development (Figure 1).

Inoculum

The virus content of budwood was verified by grafting to standard woody indicators in the field (OEPP/EPPO, 1992). Four sources of inoculum were used, all cv. Lord Lambourne growing at HRI East Malling: M1 - a tree infected with chat fruit plus apple chlorotic leaf spot virus and spy decline disease originating from apple cv. Daniels; M2 - a tree infected with chat fruit alone, originating from an unknown apple variety; M3 - a tree infected with chat fruit alone originating from apple cv. Fiesta; C - uninfected control.

Year 1 (inoculation)

EMLA2 MM106 rootstocks were grafted at Blackmoor Nurseries, Hampshire, with cv. Lord Lambourne provided from the control source tree at East Malling. The trees were grown in pots at the nursery and supplied to East Malling in August when each tree was grafted with four buds from the appropriate source; the first bud was placed 15 cm above the scion/rootstock union and the remaining three at 5 cm intervals above, at 90 degrees to the previous inoculation point. During the winter the trees were re-potted into 10 litre containers in a standard peat-based compost and sunk into the ground, inclined at an angle of 45 degrees to reduce vigour and encourage flower bud formation. They were pruned in the second winter to promote the development of fruiting spurs for year 3.

Year 2 (incubation)

Any developing fruits were removed in the spring. During the growing season each tree was watered via a drip irrigation system delivering 1 litre of water twice a day. This was gradually reduced to 0.5 litres per day later in the season to induce slight water stress to encourage flower bud formation. Foliar feed was applied weekly.

Year 3 (symptom development)

The trees were lifted while dormant, repotted and pruned to remove excess vegetative growth. They were placed in one of two glasshouse compartments, each containing 14 blocks of four trees plus six cv. Virginia Crab pollinator trees. Each tree received 0.25 litres of water twice a day via a drip irrigation system. Nutrients were supplied as a slow release fertiliser incorporated into the potting mixture. At flowering time a hive of communal bumble bees (Biobest Biological Systems, Westerlo, Belgium) was placed in each chamber to aid pollination. In addition, flowers were pollinated by hand with pollen from cv. Golden Delicious stored from the previous year at 4°C. At weekly intervals, for 10 weeks following full bloom, all laterals were pruned to restrict growth to 10 cm to reduce premature fruit loss. After this, pruning was stopped to maximise leaf area.

Glasshouse temperature was controlled within +/- 2°C. One compartment received a standard regime, the other was 2°C warmer. The standard regime was based on meteorological data from an East Malling site in 1993 where, that year, clear chat fruit symptoms were seen. Compartment temperatures were reset each week in line with the mean day and mean night temperatures for the corresponding week in 1993.

Experiment design and analysis

The trees were planted in the field in year 1 in randomised blocks, each containing a single tree of each treatment. Spare blocks were included to substitute for blocks containing any trees which died or were accidentally damaged. The blocks were transferred to the glasshouse compartments in year 3. Observations were made in year 3 on the appearance of the flowers and fruit, and records were taken of: flower buds per tree, flowers per flower bud, initial fruit set, date of opening of each 'king' flower and (1995 experiment only) trunk girth increment and shoot extension growth. The data were subjected to analysis of variance.

RESULTS

In 1995 and 1996 flowering occurred in the warmer chamber one day earlier than in the standard one. Apart from this the results followed similar trends and data from the standard chamber are summarised in Table 1. The numbers of flower buds per tree and flowers per flower bud were largely unaffected by the treatments but the initial fruit set was consistently lower in the trees with chat fruit disease; this effect was not greater in trees that were also infected with spy decline and apple chlorotic leaf spot virus (treatment M1). Isolate M3 had the most severe effect and in 1995 the fruit set in the standard house represented an average of 24% of the flowers per tree setting fruit compared with an average of 67% for the uninfected controls.

Shoot extension growth and trunk girth increments were measured at the conclusion of the 1995 experiment and both variables were greater in the infected trees, indicating greater vigour, although most of the fruit fell from the control trees as well as from the infected ones within a month of fruit set. The lack of developed fruit prevented meaningful observations in 1995 or 1996 on fruit size, colour and seed content and no ring spots typical of chat fruit were seen on the few fruit to develop.

DISCUSSION

The results clearly demonstrate that chat fruit disease reduces fruit set, a key factor in determining final crop yield. This is in agreement with earlier experiments on cv. Cox's Orange Pippin (Campbell & Sparks, 1986) which suggested that fruit set was reduced not by any effect on the pollen but by early abortion of the ovules. The increased vigour of infected trees and the lack of symptom enhancement in the presence of other viruses (treatment M1) also supports previous observations (Luckwill, 1963; Posnette & Cropley, 1965); the vigour did not seem to be associated with the lack of fruit as all treatments, including the controls, lost most of their fruit at an early stage of development. We could

see no visible signs of infection in the flowers in contrast to the misshapen flowers seen by van der Meer (1980) in cv. Lord Lambourne affected by chat fruit.

Although the experiments have successfully reproduced part of the chat fruit syndrome, insufficient fruits were retained on the trees in the glasshouses to allow useful observations of visible fruit symptoms. Young trees, such as those used in these experiments, often shed most of their fruitlets at 'June drop' when grown in pots under glasshouse conditions, irrespective of the efficiency of pollination/fertilisation. It is not therefore known if the glasshouse temperatures would have been suitable for fruit symptoms to develop. A different rootstock, pruning or fertigation regime may be required to provide the appropriate conditions for fruit retention if this approach to improving the assay for chat fruit is to be successful.

Table 1. Floral data (treatment averages) and growth measurements of trees cv. Lord Lambourne infected with chat fruit isolates M1, M2 or M3 or uninfected (C) and grown in a 'cool summer' temperature regime under glass.

Parameter	Year	Treatment				Treatment LSD ($P < 0.05$)
		C	M1	M2	M3	
Flower buds per tree	1995	5.4	5.7	6.1	6.2	2.2
Flowers per bud		6.6	6.2	6.6	6.2	0.4
Initial fruit set per tree		21.7	16.4	13.6	8.7	6.4
% fruit set		66.9	50.9	44.2	24.4	17.3
Shoot extension (cm)		428	423	500	448	89
Trunk girth increment (cm)		0.46	0.49	0.61	0.62	0.16
Flower buds per tree	1996	7.9	6.1	7.0	6.7	3.4
Flowers per bud		6.1	6.1	6.3	6.2	0.3
Initial fruit set per tree		27.2	16.2	16.3	13.1	11.3
% fruit set		58.2	36.9	32.5	31.9	15.8

1995 residual df 39
1996 residual df 38

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REFERENCES

Campbell, A I; Hughes L (1974) Chat fruit of apple. *Report of Long Ashton Research Station for 1976* 37-38.

Campbell, A I; Sparks T R (1986) The effect of chat fruit on Cox's Orange Pippin apple. *Acta Horticulturae* **193**, 319-322.

Luckwill, L C (1963) Apple chat fruit. *Technical Communication No. 30 Commonwealth Bureau of Horticulture and Plantation Crops* 27-28.

Meer, F A van der (1980) Kleinvruchtigheid bij appels. *De Fruiteelt* **18**, 608-611.

OEPP/EPPO (1992) Certification scheme for virus-free or virus-tested fruit trees and rootstocks Part 3: testing methods for viruses of fruit trees present in the EPPO region. *Bulletin OEPP/EPPO* **22**, 265-275.

Posnette, A F (1986) Chat fruit disease in Cox's Orange apple. *Acta Horticulturae* **193**, 335.

Posnette, A F; Cropley R (1965) Field experiments with chat-fruit virus disease of apple. *Annals of Applied Biology* **55**, 439-445.

Posnette, A F; Campbell A I; Cropley R (1976) The re-indexing of EMLA scionwood trees and rootstocks on special stock nurseries. *Acta Horticulturae* **67**, 275-278.

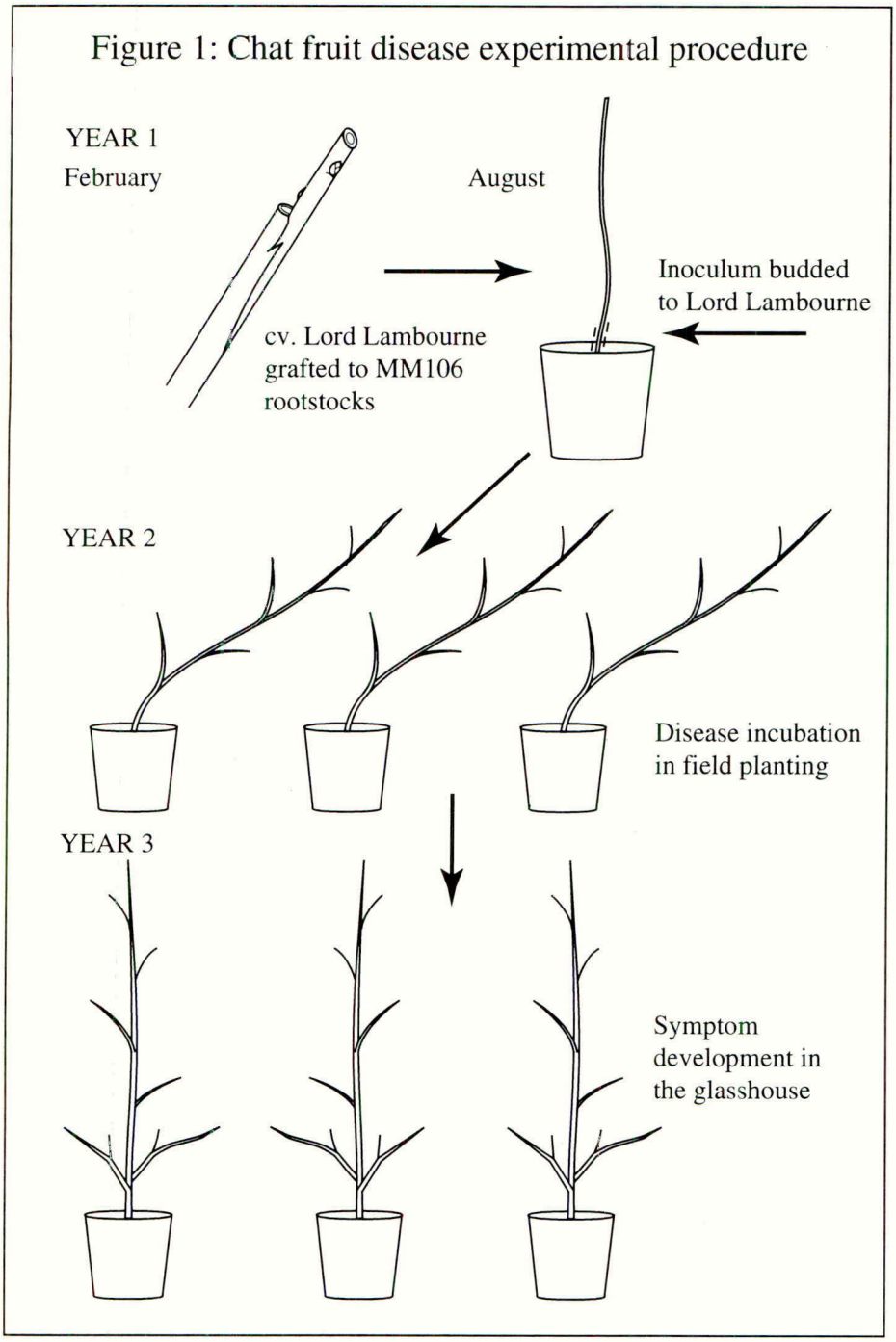
Figure 1: Chat fruit disease experimental procedure
YEAR 1
February
August
Inoculum budded
to Lord Lambourne
cv. Lord Lambourne
grafted to MM106
rootstocks
YEAR 2
Disease incubation
in field planting
YEAR 3
Symptom
development in
the glasshouse

ALTERNATIVE STRATEGIES FOR CONTROLLING STORAGE ROTS IN COX'S ORANGE PIPPIN APPLES WITHOUT THE USE OF POST-HARVEST FUNGICIDES

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ABSTRACT

The suitability of pre- and post-harvest treatments to control storage rots in Cox's Orange Pippin apples were investigated. Late season sprays of captan reduced the incidence of *Nectria*, *Phytophthora* and *Monilinia* rots. Selective harvesting reduced the incidence of *Monilinia* rots in fruit from one orchard. The biological control agent *Candida oleophila* applied post-harvest reduced *Botrytis* rots, either on its own or in conjunction with a low dose of carbendazim (0.005% wt/v a.i.). Drenching with chlorine was not a viable alternative, and in some cases increased the amount of rotting compared to undrenched controls.

INTRODUCTION

The uncertain future of post-harvest fungicide treatments for the control of storage rots in apples and pears has created a need to research alternative methods of control.

The losses caused by fungal rotting depend on variety, orchard site and season. The amount and type of inoculum present in an orchard is affected by cultural practices and weather conditions during the year. These factors also affect conditions for infection and the susceptibility of fruit to infection (Edney, 1983). Cox's Orange Pippin, the main UK dessert variety, is host to a variety of fungal pathogens including *Monilinia fructigena*, *Gloeosporium spp*, *Nectria galligena*, *Phytophthora syringae* and *Botrytis cinerea*. Control of these diseases is currently dependent on post-harvest application of fungicides. However, even when post-harvest fungicides are used, fungal rotting can still cause substantial economic losses if the pathogen has become insensitive to the fungicide (*Botrytis* strains resistant to benomyl) or available fungicides are ineffective (none of the currently available fungicides for use on top fruit control *Mucor*).

This paper describes work carried out to develop a package of measures to circumvent the use of post-harvest fungicides. The alternative strategies under review include post-harvest treatment with chlorine (calcium hypochlorite), and a biological control agent, *Candida oleophila*, late season orchard sprays of captan and selective harvesting.

Chlorine has been used as a means of reducing inoculum levels on fruit both prior to storage, and ex-store prior to grading and packing (Kupferman and Waelti, 1992).

Recent work on biological control of post-harvest diseases has resulted in commercial products becoming available in the USA, and includes a formulated preparation of *Candida oleophila* (Hofstein *et al.*, 1994).

In some EU states late-season fungicide sprays have been used as an alternative to post-harvest application of fungicides. Ensuring that only sound fruits are harvested for storage and excluding fruits close to the soil was also beneficial in reducing storage rots (Johnson, 1993).

MATERIALS AND METHODS

Drenching with chlorine

In 1993, Cox apples from two orchards with different rotting potentials were harvested into bulk bins and treated with 100 ppm chlorine (calcium hypochlorite) as a single treatment, or drenched in chlorine prior to treatment with a proprietary fungicide containing carbendazim/metalaxyl (0.05%/0.01% wt/v a.i.); other treatments included fungicide only and an undrenched control. The following year the chlorine, fungicide and control treatments from the 1993 experiment were repeated, and an additional treatment was given of chlorine mixed with a non-ionic surfactant containing alkyl alcohol ethoxylate (0.3% v/v). In both years fruits were stored in an experimental controlled atmosphere (CA) store, where carbon dioxide concentration was maintained below 1% and oxygen concentration was controlled at 1.2% by automatic ventilation. In each year the store was opened in February for the fruit to be graded, and rots were removed, weighed and identified. Rotting was expressed as a percentage of the total weight of fruit in the bin. The number of fruit affected by individual pathogen types was recorded and the percentage by weight of rotting caused by the different fungal pathogens was estimated by assuming that all types of rot contributed equally to the total weight of rotten fruit.

Biological control

Cox apples were harvested to commercial standards into wooden bulk bins on 12 September 1996. Five bins of fruit were drenched in chlorine as described previously. After drenching, the bins were drenched again in water to remove any chlorine residues from the fruit surface and were subsequently treated with a wettable granule preparation of the yeast *Candida oleophila* (0.3% wt/v). Five bins which received no chlorine treatment were drenched in *C. oleophila* while five more were treated with a mixture of *C. oleophila* and a 1/10 dose rate of carbendazim (0.005% wt/v ai). A fungicide treatment of carbendazim (0.05% wt/v ai) and metalaxyl (0.01% wt/v ai) was also included, as was an undrenched control. All bins of fruit were stored under conditions described previously for the chlorine drenching experiment. In February 1996 the bins were removed from the store and fruits were graded and rots removed as in the previous trial.

Late-season captan sprays, selective harvesting and drenching with chlorine

In 1995 a factorial trial was carried out in two orchards (CW109 and TL109) at HRI, East Malling, which had provided fruit for the chlorine drenching trials in 1993 and 1994. Both orchards received a standard pesticide spray programme during the growing season. In addition, within each orchard, selected rows were sprayed on 25 July, 14 August and 5 September with captan at a rate of 2.7 kg ha^{-1} a.i., in a volume of 200 litre ha^{-1}. Rows of trees which were treated either with captan or received no captan were divided into groups. Groups of trees were either picked to normal commercial standards, or selectively harvested, whereby

fruit closer than 0.5m to the ground were omitted from the storage bins, along with cracked, diseased and insect-damaged fruit. After harvest, fruit was either drenched in chlorine (100 ppm) or left undrenched. Fruit was stored under conditions described previously until February, 1996, when fruit was graded, rots removed, weighed and identified.

RESULTS

Drenching with chlorine

The two years of chlorine drenching trials produced variable results. In the first year, in the orchard where *Botrytis* was the predominant pathogen (CW109), there was a significant increase in the number of storage rots following treatment with chlorine (Table I). However, in the other orchard (TL109) where, in addition to *Botrytis*, the incidence of *Nectria galligena* was also high, a significant reduction in the overall incidence of storage rots was achieved with chlorine treatment. Carbendazim/metalaxyl proved the most effective treatment, reducing rotting in fruit from both orchards to 4% .

Table I. The overall effect of post-harvest drenches on percentage rotting in bins of Cox's Orange Pippin apples stored in 1.2% O_2, <1% CO_2 at 3.5°C in 1993/94.

Orchard	Control	Chlorine	Chlorine + Carbendazim/ metalaxyl	Carbendazim/ metalaxyl	SED 24 df
CW109	7.0	10.7	4.6	4.0	1.70
TL109	8.7	6.6	3.9	4.0	0.87

In the following year chlorine failed to reduce significantly the incidence of storage rots in fruit from either orchard, and the addition of a non-ionic wetter failed to enhance its efficacy. Carbendazim/metalaxyl again proved the most effective treatment, reducing the amount of rotting in fruit from both orchards (Table II).

Table II. The overall effect of post-harvest drenches on percentage rotting in bins of Cox's Orange Pippin apples stored in 1.2% O_2, <1% CO_2 at 3.5°C in 1994/95.

Orchard	Control	Chlorine	Chlorine + wetter	Carbendazim/ metalaxyl	SED 24 df
CW109	3.8	4.5	4.0	1.4	0.48
TL109	10.3	8.7	10.5	7.0	1.53

Biological control

The incidence of rotting in untreated bins was low and hence the opportunity for treatment effects was diminished. Despite this, *C. oleophila*, alone or in combination with a 10% dose of carbendazim, significantly reduced the amount of *Botrytis* rots compared to untreated controls (Table III). However, the biocontrol agent did not reduce rotting caused by other pathogens.

Table III. The effect of 'biocontrol' and other post-harvest treatments on the incidence (% by weight) of fungal rotting in stored Cox's Orange Pippin apples.

Pathogen	Control	*Candida oleophila*	*Candida oleophila* + Carbendazim	Chlorine + *Candida oleophila*	Carbendazim + metalaxyl	SED 23 df
Overall rotting	1.60	1.31	1.66	1.49	1.01	0.284
Botrytis	0.37	0.13	0.18	0.23	0.32	0.073
Monilinia	0.75	0.62	0.83	0.64	0.44	0.201
Phytophthora	0.09	0.11	0.16	0.08	0.01	0.042
Nectria	0.12	0.12	0.13	0.14	0.07	0.056
Penicillium	0.07	0.10	0.05	0.19	0.02	0.058
Mucor	0.04	0.05	0.09	0.09	0.07	0.039

Late season captan sprays, selective harvesting and chlorine drenching

Selective harvesting reduced the overall incidence of rotting in fruit from orchard CW109 (Table IV, $P \simeq 0.05$) and significantly reduced the incidence of *Monilinia* rots in orchard TL109, although the difference in overall rotting was not significant (Table IV).

Late season spraying of captan was the most effective component of the package, and significantly reduced storage rots in fruit from both orchards (Table IV); in particular losses from *Phytophthora*, *Nectria* and *Monilinia* were reduced significantly (Table IV). Drenching with chlorine was not effective in reducing rotting.

Table IV. Overall effect of harvesting method, fungicide application, and chlorine drenching on the amount of rotting (% by weight) caused by different pathogens in bins of Cox's Orange Pippin apples stored in 1.25%O_2, <1%CO_2 at 3.5°C

Orchard	Pathogen	Harvesting		Spraying		Drenching		SED
		Normal	Selective	Yes	No	Yes	No	7 df
	Overall rotting	1.45	0.98	0.59	1.84	1.19	1.24	0.211
CW109	*Botrytis*	0.10	0.06	0.04	0.13	0.10	0.06	0.045
	Monilinia	0.33	0.24	0.15	0.43	0.35	0.22	0.097
	Penicillium	0.10	0.03	0.10	0.03	0.04	0.09	0.066
	Phytophthora	0.29	0.17	0.06	0.40	0.27	0.18	0.153
	Nectria	0.56	0.37	0.26	0.67	0.43	0.50	0.116
	Mucor	0.02	0.04	0.02	0.04	0.01	0.05	0.024
	Overall rotting	7.10	6.25	4.61	8.74	6.20	7.15	1.687
TL109	*Botrytis*	0.34	0.38	0.34	0.38	0.36	0.36	0.128
	Monilinia	1.29	0.77	0.84	1.22	0.82	1.24	0.206
	Penicillium	0.50	0.45	0.39	0.56	0.46	0.49	0.208
	Phytophthora	1.08	1.18	0.63	1.63	1.31	0.95	0.369
	Nectria	3.25	2.82	1.86	4.21	2.48	3.60	1.017
	Mucor	0.13	0.17	0.10	0.20	0.17	0.13	0.073

DISCUSSION

Chlorine drenching of Cox apples gave variable results and does not provide a viable alternative to current post-harvest fungicides. Although chlorine prevents the build-up of fungal inoculum in the drench tank, it is unable to penetrate well into wounds (Spotts & Peters, 1982) and even with the addition of a non-ionic surfactant, it failed to reduce rots caused by pathogens that infect fruit in the orchard, which are either well established or latent at harvest time.

Late season sprays of captan have been used to reduce the incidence of *Nectria* fruit rot in orchards with high levels of cankers (Berrie, 1992), and have some effect against *Phytophthora* (Edney, 1978). Consistent with these reported effects, late season sprays of captan tended to reduce the occurrence of both these types of rot and also significantly reduced *Monilinia* rots in fruit from one of the orchards.

The knowledge that in orchards with bare soil management there is an increased potential for infection by *Phytophthora syringae* through soil splash (Harris, 1981) has lead to the concept of selective harvesting, whereby low hanging fruit (within 0.5m of the ground) in the zone of soil splash are harvested separately and not put into storage bins destined for long-term storage. In this trial there was a generally low incidence of *Phytophthora* rots, despite wet conditions at harvest, and there was little indication of a benefit from selective

harvesting. However, brown rots were significantly reduced in fruit from orchard TL109, where large numbers of fruit infected with *Monilinia fructigena* littered the ground at harvest.

In 1995/96 the incidence of *Botrytis* was considerably lower than in previous years, and consequently the effectiveness of a captan and selective harvesting package could not be evaluated fully. Captan had little effect on *Botrytis* storage rots, which agrees with results obtained by Johnson (1993).

The biological control agent *Candida oleophila* was effective in reducing the incidence of *Botrytis* rots. This yeast functions as an antagonist, competing for nutrients and preventing germination of *Botrytis* spores (Hofstein *et al.*, 1994). It is unlikely that *Candida oleophila* will stop the development of *Botrytis* rots that are already established before harvest (so-called 'Dry eye rots') but it may prevent secondary spread of primary rots to adjacent sound fruits.

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REFERENCES

Berrie, A M (1992) Comparison of fungicide sprays for the control of canker *(Nectria galligena* Bres.) in apple cvs. Cox's Orange Pippin and Spartan. *Acta Phytopathologica et Entomologica Hungarica* **27** (1-4), 103-109.

Edney, K L (1978) The infection of apples by *Phytophthora syringae*. *Annals of Applied Biology* **88**, 31-36.

Edney, K L (1983) Top Fruit. In: *Post harvest pathology of fruits and vegetables*, C Dennis (ed.), Academic Press, pp. 43-71.

Harris, D C (1981) Herbicide management in apple orchards and the fruit rot caused by *Phytophthora syringae*. In : Pests pathogens and vegetation. J M Thresh (ed.). Pitman Publishing Inc, pp. 429-436.

Hofstein, R; Fridlender, B; Chalutz, E; Droby S (1994) Large scale production and pilo testing of biological control agents for postharvest diseases. In: *Biological control of postharvest diseases theory and practice*. C L Wilson & M E Wisniewski (eds), CRC Press, pp. 89-100.

Kupferman, E; Waelti, H (1992) Management practices to minimise post-harvest decay of apples and pears.*Tree Fruit Post-Harvest Journal* **3**, 5-9.

Johnson, D S (1993) Use of late season fungicides sprays and selective harvesting as an alternative to postharvest fungicides for the control of storage rots in Cox apples. *Proceedings from the Sixth International Controlled Atmosphere Research Conference Cornell University Ithaca, New York*, pp. 373-385

Spotts, R A; Peters B B (1982) The use of surfactants with chlorine to improve pear decay control. *Plant Disease* **66**, 725-727

INTEGRATED CONTROL OF *PHYTOPHTHORA* FRUIT ROT (*PHYTOPHTHORA SYRINGAE*) IN STORED COX APPLES

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ABSTRACT

An integrated minimum-pesticide approach to control of *Phytophthora syringae* fruit rot was developed and tested. A system of rot risk assessment prior to harvest was developed based on certain factors. These factors were orchard rot history, fruit quality, % bare ground, % of the crop < ½ metre above the ground and rainfall. Using this system, pre-harvest predictions of rot due to *P. syringae* in fruit from 13 orchards agreed closely with the incidence of rot recorded in March. It is concluded that pre-harvest assessment of *Phytophthora* rot risk can be used to determine the need for fungicide treatment. Orchard experiments have shown that pre-harvest sprays of captan, metalaxyl + mancozeb, selective picking or the use of a straw mulch to cover the soil surface to prevent soil splash were all effective in reducing *Phytophthora* fruit rot. An integrated scheme for disease management based on rot risk assessment and available control measures is proposed.

INTRODUCTION

Phytophthora syringae can cause serious losses in stored apples, particularly cv. Cox, following wet weather at harvest (Edney, 1978; Upstone, 1978; Berrie, 1989). The fungus is soil-borne and orchards most at risk are those with a high proportion of low hanging fruit prone to soil splash. *Phytophthora* rot may develop in the orchard, but it is the fruits infected at or near harvest which are picked before symptoms develop that initiate rotting post-harvest. The rot first caused substantial losses in store in the 1970's following a change in orchard management from overall grass to bare soil or herbicide strips combined with the use of dwarfing rootstocks (Upstone, 1978; Harris, 1981). The introduction in the late 1970s of metalaxyl + carbendazim (Ridomil mbc 60 wp) as a post-harvest treatment (Edney and Chambers, 1981a) gave effective economic control and this, until recently, was used routinely when wet weather prevailed during harvest, regardless of need.

The use of post-harvest fungicides is not liked by consumers, mainly because of the residues left following such treatments. In addition, there is a risk of reduced efficacy due to the development of metalaxyl-resistant strains of *P. syringae*. Isolates of the pathogen with reduced sensitivity to metalaxyl have been detected (Berrie and Koomen, 1994) but as yet there is no evidence of control failure. For these reasons alternative strategies for control of *Phytophthora* rot are being explored.

Fungicide treatment is only necessary if fruit is at risk from infection. If a method of assessing risk could be developed, unnecessary usage of fungicide could be avoided. The results of recent surveys of storage rots in untreated Cox apples (Berrie, 1994) showed that rot incidence is related to site. Thus, even under conditions favourable to *Phytophthora* rot the disease does not necessarily develop. Factors likely to influence rot incidence, which will be included in rot risk assessment are - fungal inoculum, fruit susceptibility to rotting and weather conditions.

Alternative control measures to post-harvest treatments for control of *P. syringae* must also be investigated. Cultural practices such as mulching the soil surface to prevent fruit coming into contact with soil, and harvesting low hanging fruit separately, should reduce rotting. Also the use of protective fungicides (e.g. captan, metalaxyl) applied to low hanging fruit and/or the soil-surface should reduce rot incidence (Edney and Chambers, 1981b).

This paper describes work to develop a scheme for management of *Phytophthora* rot based on rot risk assessment and available control methods.

MATERIALS AND METHODS

Criteria for rot risk assessment

Over the period 1991-93 studies were conducted on fruit from 20-25 Cox orchards. The same orchards were used each year. Pre-harvest assessments on a range of factors likely to affect rot risk were carried out. The factors assessed were fruit mineral composition, orchard type (overall grass, overall bare ground, herbicide strip assessed as % bare ground), the presence of weed cover or straw mulch on the herbicide strip, fruit quality (russet, cracking), % crop (by number) liable to soil splash (taken as that $< \frac{1}{2}$ metre above the soil), fungal inoculum (Nectria canker, incidence of brown rot) and weather (rainfall from May - harvest). Additional information on the likely fungal inoculum in the orchard was taken from packhouse records of rot incidence. At harvest a random sample of 500 fruits was picked from each orchard. These were stored in controlled atmosphere ($< 1\%$ CO_2, 1-1.25% O_2 at 3.5°C) until March when losses were assessed and the rots identified. Statistical analysis was carried out to evaluate the relevance of the assessment criteria as determinants of rotting.

Testing rot risk assessment for *Phytophthora* rot

A prediction of *Phytophthora* rot was made for 1993-95 crops for each orchard, based on the criteria identified in the preliminary studies described above and a knowledge of the biology of *P. syringae*. The fruit mineral composition was used to determine storage potential (Luton, 1987) (Table 2). For orchards with a history of moderate/high incidence of *Phytophthora* rot, a high % bare ground (40% or more), $> 10\%$ of the crop liable to soil splash, *Phytophthora* rot risk was assessed as high enough to justify control. Wet weather (10 mm or more rainfall) in the seven days up to and including harvest would confirm the high *Phytophthora* risk. Orchards were deemed to be of lower risk if the % bare ground was very low (e.g. overall grass or straw mulched or with weed cover), or most of the crop was $> \frac{1}{2}$ metre from the soil and less likely to be subject to soil splash. At harvest a random sample of 500 fruits was picked from each orchard and stored as before. In March losses

were assessed and the incidence of *Phytophthora* fruit rot recorded and compared to that predicted.

Alternatives to post harvest chemicals

A field experiment with large plots (4 rows of 90 trees) was set up in a Cox orchard on M9 (dwarfing) rootstock with overall herbicide soil management and a known history of *Phytophthora* rot. There were three main fungicide treatments each of which was split to include three sub-treatments of different cultural management regimes (Table 1). Each fungicide treatment was replicated three times in a randomised block design. Fungicide treatments were applied at 500 l/ha using an axial fan orchard air blast sprayer.

Table 1. Treatments for control of *Phytophthora* rot

Treatment	Fungicide product	Active ingredient	Rate product /ha	Timing	Number of sprays
Main treatment					
A	PP Captan 83	captan	3.3 kg	1st week August + 2 weeks later	)) 2
B	Fubol 58 wp	metalaxyl + mancozeb	1.5 kg	one month pre-harvest	1
C	-	-			0

Sub-treatment
(i) Straw mulch applied to herbicide strip.
(ii) Selective picking of fruit > ½ metre from ground.
(iii) No extra treatment

At harvest, four 14 kg samples of apples were taken from each plot by picking an entire quarter section of each of eight trees from the plot to ensure a random fruit sample. The fruit was stored in a CA store (< 1% CO_2, 1-1.25% 0_2 at 3.5^{0}C) until March. The incidence of *Phytophthora* and other rots was then assessed. The trial was conducted over two seasons between 1993 and 94.

RESULTS

Criteria for rot risk assessment of *Phytophthora* rot

In 1991 and 1992 rainfall in August and at harvest was low and consequently the incidence of *Phytophthora* rot in store was also low. Nevertheless, preliminary statistical regression analysis of the assessed factors indicated a significant relationship between fruit mineral

composition, rot history, % bare ground, rainfall in August and the incidence of *Phytophthora* rot that developed subsequently in store.

Testing rot risk assessment of *Phytophthora* rot

In 1993 the weather in the seven days prior to harvest (5 September) was relatively dry (3.7 mm rain). The incidence of *Phytophthora* rot was therefore expected to be low, even in orchards potentially of high risk from the other characters assessed. *Phytophthora* rot occurred in fruit from two out of four orchards assessed as high risk (Table 2). Orchard 26 was harvested two weeks later than the other orchards (19 September) during which a further 58 mm of rain fell increasing the risk of rotting. Orchard 25 was harvested before significant rain fell but received regular overhead irrigation. *Phytophthora* rot also occurred in two out of six orchards predicted to be a low risk. In 1994 and 1995 there was 10 mm or more rainfall in the seven days prior to harvest. *Phytophthora* rot developed in most samples of fruit from orchards predicted to be high risk and in only one (orchard 19) out of seven samples of fruit from orchards deemed to be low risk. No *Phytophthora* rot developed in samples from orchard 18 in any of the three years, despite the high risk predicted.

Alternatives to post-harvest chemicals

In 1993 fruit was harvested in late September in the rain ensuring a high risk of *Phytophthora* rot which was the main rot that developed in store. Use of a straw mulch, selective picking and pre-harvest fungicides significantly reduced rotting due to *P. syringae* (Table 3). However control was not improved by combining fungicide treatment with selective picking or soil mulch. In 1994 although the fruit was again harvested in rain, the incidence of rotting was lower and differences between treatments were not significant, although trends were similar to those in 1993.

DISCUSSION

In the rot risk study in each of the years up to 1994 most of the fruit samples were harvested before significant rain had fallen. Hence the incidence of *Phytophthora* rot was low and factors such as the proportion of the crop liable to soil splash were not correlated significantly with the incidence of *Phytophthora* rot. Nevertheless the orchards predicted to be at high risk of rotting due to *P. syringae* usually experienced rotting. The discrepancies that arose can be explained to some extent. In orchard 6 (Table 2) *Phytophthora* infection may have arisen from soil contamination of bins during transit from orchard to packhouse and in orchard 19 assessment of orchard characteristics was conducted in early August. By harvest the proportion of the fruit liable to soil splash had increased due to the weight of fruit causing branches to drop. In 1995 this was taken into account at harvest. In future predictions, orchard 19 will be reclassified as high *Phytophthora* risk. Orchard 28 would merit a high *Phytophthora* classification, but the presence of a thick straw mulch reduced the risk. There are still a number of factors that need to be investigated to improve the system. While the importance of rain at harvest in relation to the incidence of *Phytophthora* rot is clear form previous work (Upstone and Gunn, 1978), there is no indication of the amount of rain or the period of fruit surface wetness required for infection. The present rainfall criteria are based on practical experience.

Table 2. Rot risk assessment - incidence of *Phytophthora* rots ex-store in Cox apples 1993-95 in relation to predicted risk at harvest.

Orchard code	Rot[1] history	Pre-harvest assessment (August)												Actual rotting % *Phytophthora* rot recorded ex-store in March		
		Storage[2] potential			% bare[3] ground			% crop < ½ m above soil			weather[4] at or near harvest					
		93	94	95	93	94	95	93	94	95	93	94	95	93	94	95
Predicted high *Phytophthora* risk																
11	R	M	L	M	40	44	39	30	35	30	d	w	w	0	1.1	0.4
18	r	S	S	M	40	42	37	5	30	40	d	w	w	0	0	0
25	R	M	M	S	64	63	56	50	50	45	d	w	w	0.4	0.8	0.2
26	R	S	S	S	47	66	40	40	15	10	w	w	w	3.2	0.9	0
30	R	-	L	M	-	66	68	-	30	30	-	w	w	-	1.1	0.2
31	R	-	M	M	-	66	63	-	20	30	-	w	w	-	4.0	0.7
32	R	-	L	S	-	76	76	-	35	40	-	w	w	-	4.5	0.2
Predicted low *Phytophthora* risk																
		93	94	95	93	94	95	93	94	95	93	94	95	93	94	95
2	o	L	L	L	46	45	50	10	10	5	d	w	w	0	0	0
5	r	S	S	S	24	49	50	10	15	10	d	w	w	0	0	0
6	r	S	S	S	35	56	6	5	5	10	d	w	w	0.8	0	0
19	o	M	L	L	56	36	24	10	10	45	d	w	w	0.4	0.7	0
23	o	S	M	-		0	-	10	10	-	d	w	-	0	0	-
28	R	L	L	M	47	0	0	20	30	15	d	w	w	0	0	0

1. Rot history 0 = not recorded, r = low incidence, R = moderate/high incidence.
2. Storage potential based on mineral analysis S = short term - Christmas, M = medium - February, L = Long - March/April
3. % bare ground estimated from width of herbicide strip and weed cover and mulch.
4. Based on rainfall in 7 days up to and including harvest w = wet = > 10 mm rain
   d = dry = 0 or < 10 mm rain

All the alternatives to post-harvest chemical control were effective in reducing rotting due to *Phytophthora* in the 1993 trial. The two fungicides used - captan and metalaxyl + mancozeb - were effective as protectants against *P. syringae*. However, captan would be the preferred choice in practice because it is also effective in protecting fruit against several rot fungi e.g. *Nectria galligena* (Berrie, 1992). Metalaxyl + mancozeb is specific to *Phytophthora* and, also whilst currently effective, its routine use in the orchard may increase the risk of metalaxyl-resistant isolates developing, not only with *P. syringae*, but also with *P. cactorum*, the cause of crown and collar rot in apple trees. While application of fungicides pre-harvest is an alternative to post-harvest treatment, it may increase the use of fungicides, since treatments against *Phytophthora* will need to be applied before the risk of the rot occurring has been determined. Where a post-harvest fungicide is planned, the decision on treatment can be delayed until harvest. It is likely that, in many seasons for many orchards, no treatment will be necessary.

Table 3. Effect of fungicide and cultural management on % *Phytophthora* rot in stored Cox apples.

Orchard fungicide treatment	Cultural management			Overall effect of fungicide
	Mulch	Selective picking	Nil	
captan	1.1 (5.8)	0.8 (5.1)	2.4 (8.7)	1.4 (6.5)
metalaxyl + mancozeb	1.7 (6.9)	1.9 (7.8)	2.0 (7.4)	1.9 (7.4)
untreated	2.1 (7.9)	1.4 (6.5)	8.8 (17.0)	4.1 (10.5)
overall effect of cultural management	1.6 (6.9)	1.4 (6.5)	4.4 (11.0)	

SED (4 df) Fungicide - (1.3)
SED (12 df) Cultural management - (1.1)
SED (12 df) Fungicide x cultural management (2.0)
Figures in parentheses are angular transformed values. These values should be used with SEDs for statistical comparisons.

The cultural management treatments were also effective in the 1993 trial. Selective picking may prove more costly and present practical difficulties, but some farms have adopted this practice already. The use of straw as a mulch is a cheap option compared to other possible mulches such as bark. Other possibilities might be the manipulation of a weed cover mulch, provided the weeds are low growing, and the use of contact herbicides such as glufosinate-ammonium. Overall herbicide treatments increase the risk of *Phytophthora* rot because of the high risk of soil being introduced into bins at harvest which can lead to rotting in store (Edney, 1978; Upstone and Gunn, 1978). In such orchards it may be better to introduce a grass strip which can be burnt off using contact-acting herbicides in the summer when competition for water is high, and allowed to regrow during winter.

An integrated scheme for management of *Phytophthora* rot is proposed in Table 4. The system assumes that the orchard to be assessed has fruit suitable for storage beyond December. Short-term stored fruit should not require treatment. Decisions on risk of *Phytophthora* can be used with either pre or post- harvest treatment. Where packhouse records of previous rotting for an orchard are absent or uncertain then an assessment procedure should be followed.

Table 4. Proposed rot riskssessment for *Phytophthora*.

Orchard rot history after wet seasons	Orchard type*	% crop in splash zone (0.5 m from ground) Aug	Rainfall	Treatment/ manaegment consequences
None recorded or trace				No treatment
Moderate, high incidence	overall grass herbicide strip/ mulched/weed cover			No treatment
	bare herbicide strip	<10%		Apply captan sprays pre-harvest
		>10%	monitor rain in Aug and at harvest: no rain	No treatment
			monitor rain in Aug and at harvest: wet harvest	Treat post-harvest or modify storage potential to early marketing
	overall herbicide			Apply captan sprays pre-harvest

1. Assessment of orchard factors (orchard type, % crop in splash zone) and rainfall is carried out in mid\late August.
2. Rot history must be based on reliable records. If the records are absent or unreliable then the rot incidence should be treated as 'moderate/high'.
3. The risk of *Phytophthora* rot is increased in overall herbicide orchards because of the high risk of soil being introduced into bins at harvest. For other orchard types it is assumed that the risk of soil introduction to bins is minimal.

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REFERENCES

Berrie, A M (1989) Storage rots of apple and pear in south-east England 1980-88 incidence and fungicide resistance. *Integrated control of pome fruit diseases, Vol 11, IOBC Bulletin XII/6,* 229-239.

Berrie, A M (1992) Comparison of fungicide sprays for the control of canker (*Nectria galligena* Bres.) in Apples cvs Cox's Orange Pippin and Spartan. *Acta Phytopathologica et Entomologica Hungarica* **27**, 103-109.

Berrie, A M (1994) Apple and Pear - A survey of rotting in stored apples cv Cox and pears cv Conference treated and untreated with a fungicide post-harvest. *East Malling Research Association Members' Day Report May 1994* pp. 4-6.

Berrie, A.M; Koomen I (1994) Benzimidazole and dicarboximide resistance in pathogens of stored apples and pears. *BCPC Monograph No 60: Fungicide resistance* pp. 207-217.

Edney, K L (1978) The infection of apples by *Phytophthora syringae*. *Annals of Applied Biology* **88**, 31-36.

Edney, K L; Chambers D A (1981a) Post-harvest treatments for the control of *Phytophthora syringae* storage rot of apple. *Annals of Applied Biology* **97**, 237-241.

Edney, K L; Chambers D A (1981b) The use of metalaxyl to control *Phytophthora syringae* rot of apple fruits. *Plant Pathology* **30**, 167-170.

Harris, D C (1981) Herbicide management in apple orchards and the fruit rot caused by *Phytophthora syringae*. In *Pests, Pathogens and Vegetation Edited by J M Thresh* pp. 429-436.

Luton, M T (1987) Preparing for storage of apples. *ADAS Leaflet P3103*.

Upstone, M E (1978) *Phytophthora syringae* fruit rot of apples. *Plant Pathology* **27**, 24-30.

Upstone, M E; Gunn E (1978) Rainfall and the occurrence of *Phytophthora syringae* fruit rot of apples in Kent 1973-76. *Plant Pathology* **27**, 30-35.

BIOLOGY OF CYMOXANIL ACTION AGAINST *PHYTOPHTHORA INFESTANS* INFECTION OF TOMATO AND POTATO

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ABSTRACT

We have conducted a qualitative and quantitative cytological analysis on the effects of cymoxanil on the host-pathogen interaction between the Late Blight pathogen (*Phytophthora infestans*) and leaf tissue of tomato. Qualitative observations were also made in potato. Both preventative and curative treatments of cymoxanil were employed. Preventative treatments of tomato affected development of the pathogen prior to penetration of the host surface, as evidenced by a reduced size of appressoria. After penetration, haustorium development was especially limited in the presence of cymoxanil relative to the numbers observed in untreated tissues. Both preventative and post-infection treatments with cymoxanil abated spread of the pathogen beyond the initial sites of infection, apparently by inducing a set of hypersensitive-type host responses. These host cell responses included a rapid onset of granulation, plasmolysis and yellowing of cytoplasm in penetrated epidermal cells, formation of host cell wall appositions (papillae), browning of mesophyll cells and walls of epidermal cells, and collapse of invaded host cells. As a consequence of cymoxanil action, sporulation by the pathogen was also prevented.

INTRODUCTION

Cymoxanil is an active ingredient in several crop protection products used worldwide on more than 15 crops grown over 10 million hectares (see Douchet et al., 1977; Bradshaw, 1992). First introduced over 15 years ago (Klöpping & Delp, 1980; Serres & Garraro, 1977), cymoxanil has been used effectively against major plant diseases such as Late Blight of potato and tomato, and Downy Mildew of grape, caused by organisms (formerly classified as fungi) belonging to the Peronosporales, including some resistant to other products (e. g. Samoucha & Cohen, 1988). It is used effectively in both preventative and post-infection (curative) applications and is known to inhibit sporulation by the pathogen (Klöpping & Delp, 1980; Serres & Garraro, 1977). To better understand the biology of these activities we conducted a cytological analysis of the Late Blight pathogen *Phytophthora infestans* infecting tomato and potato leaf tissue to establish when and where cymoxanil affects these host-pathogen interactions.

MATERIALS AND METHODS

Green house grown, 2-wk old tomato (cv. Pixie) and 3-wk old potato (cv. Benji) plants were used for these studies. Both pre- and post-infection applications of cymoxanil, 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide (CAS registry no. 57966-95-7), in the form of Curzate® 50WP (DuPont Company, Wilmington, DE USA), were applied at 935 l/ha in water with an automated sprayer either 1 h before (80ppm) or 18 h after (120 ppm) inoculation, equivalent to 80 and 120 g ai/ha, respectively. Inoculum consisted of 200,000

sporangia/ml in water and was applied to adaxial leaf surfaces using a hand sprayer. Following inoculation, plants were incubated at 20 C and 100% RH for 24h (preventative and control plants) or 18 h (curative and a second set of control plants), and were then moved to 20 C growth chambers held at 70% RH with an 18h photoperiod.

Microscopic Analyses

Observations of pathogen development and host cell responses were made with both light and cryo scanning electron microscopy. The latter methods, used only for observations of the pathogen on host surfaces, have been described previously (Braun & Howard, 1994). For light microscopy, plant tissue samples were taken from treated and untreated check plants at various intervals after inoculation for subsequent analysis. The youngest two leaves were harvested from plants, processed and mounted on microscope slides as described elsewhere (Heath, 1984). Differential interference contrast light microscopy was used for collecting most of the data.

For each leaf sample, detailed observations were made and recorded at 20 different host-pathogen encounter sites. At each site, information concerning the extent and type of pathogen development and host responses were tabulated. Categories included infection structures formed, penetration of host surface, number of haustoria, number of host cells invaded, extent of pathogen spread within the host tissue, conidiophore development, granulation of host cell cytoplasm, presence of papillae, and discoloration of host cell walls and/or cytoplasm. Quantitative data was obtained only from tomato plant tissues. Subsequent qualitative analyses of potato tissue samples were used for comparison with observations from tomato. Results are noted for potato only where they differed from those obtained with tomato, as interactions between *P. infestans* and these hosts normally exhibit very similar infection processes (Pristou & Gallegly, 1954).

RESULTS

Invasion of untreated host tissue

Infection of tomato was initiated between 6 and 12 h after inoculation by either of two paths. One path involved production of zoospores which (upon release and attachment to the host surface) encysted, germinated and penetrated directly, either with or without formation of an appressorium. A second path was by means of sporangial germination and production of a germ tube, again with or without an appressorium. By 12 h after inoculation, 80% of host-pathogen encounters had progressed to the point where the pathogen had penetrated into a host epidermal cell, or beyond: 15% had penetrated additional cells. Within the penetrated host cell a bulbous infection vesicle and several hyphal extensions of determinate growth (i.e. haustoria) usually formed. By 24 h, 90% of encounter sites were successful and 65% included spread of the pathogen into mesophyll parenchyma. After 48 h incubation, the pathogen had invaded the entire width of the leaf blade. The first response by host cells was not evident until 72 h, when yellowing and collapse of invaded cells commenced. The initial stages of sporulation did not appear until 96 h after inoculation.

Infection of potato was very similar to that described for tomato. The only differences included the time after inoculation when host responses first appeared (48 h), the presence of intracellular hyphae in mesophyll cells, and the time when sporulation was first evident (81 h).

Invasion of tissues treated before inoculation

Prior to penetration, an early effect on the pathogen was evidenced by a reduced size of appressoria in the presence of cymoxanil. This effect could not be confirmed in potato due to the slightly diffent timing of pathogen development relative to the harvest schedule of tissue samples. By 12 h after inoculation, 85% of host-pathogen encounter sites included penetration by the pathogen into an epidermal cell, or beyond (15% had exited the epidermal cell), but a number of apparent cymoxanil effects were detected at this stage: 65% of encountered epidermal cells exhibited granular cytoplasm, and 30% had formed a papilla in cells where penetration appeared blocked. At all sites no further spread of the pathogen occurred during subsequent incubation -- and most important, the number of haustoria did not increase. Instead, additional host responses began to appear 48 h after inoculation, including discoloration of penetrated epidermal cells and/or cell walls, and browning and sometimes collapse of adjacent mesophyll cells. Cells of the pathogen also appeared to discolor and collapse at this stage of the interaction. Together, these effects acted to confine the pathogen to the initial infection site. No signs of pathogen sporulation were evident 96 h after inoculation.

Invasion of tissues treated after inoculation

A host cell response was induced by cymoxanil at pathogen encounter sites just 2 h after application, in plants already infected for 20 h. Such host cell responses were evident in 65% of encounter sites and were characterized by granulation, plasmolysis and/or discoloration of epidermal cell cytoplasm (55%) and/or discoloration, and sometimes collapse, of epidermal cells (10%). Significantly, collapse of some haustoria was also detected at this very early stage of cymoxanil treatment. Very little spread of the pathogen was observed in treated tissues during subsequent incubation. Extensive collapse of apparently uninfected mesophyll cells (adjacent to infected mesophyll cells) was detected. In addition, cymoxanil treatment caused the apparent death of pathogen cells which were observed to turn brown and collapse within the host tissue by 48 h after treatment (66 h after inoculation). No signs of sporulation were evident.

DISCUSSION

We have examined the effects of cymoxanil on host-pathogen interactions during *Plasmopara viticola* infection of grape (Howard et al., 1996), and *Phytophthora infestans* infection of tomato or potato, finding very similar activities in all three disease situations. The apparent induction by cymoxanil of host cell responses, in the presence of the respective pathogen, was the common theme. These host cell responses, including papillae formation, granulation of cytoplasm, plasmolysis, discoloration of cytoplasm and/or cell walls, and apparent cell death, can be likened to the hypersensitive-type reactions that are believed to represent (or at least accompany) natural host defense mechanisms that are triggered by the presence of an incompatible strain of a particular pathogen. That is to say, a strain which is incapable of causing disease in that particular cultivar of the host.

In the present study we found that host penetration by *P. infestans* as well as spread to other cells following initial ingress were affected significantly by a preventative application of cymoxanil. These inhibitory effects were accompanied by the above described hypersensitive host cell responses and showed similarities to the cytological consequences of induced

systemic resistance described previously (Kováts et al., 1991). Post-infection treatments, where application occurred well after these initial penetration stages of the disease process, brought about an almost immediate onset of hypersensitivity by invaded plant cells. In both types of applications, subsequent invasion by the pathogen was severely limited or absent, an obvious cause for the observed lack of sporangium production in preventative- or curative-treated tissues.

Mycelial growth of *P. infestans* has been tested in vitro for sensitivity to cymoxanil and it was inhibited by 50% at concentrations in the 1µg/ml range. However, the primary mode of action of cymoxanil remains unknown (Ziogas & Davidse, 1987). Our studies on the biology of cymoxanil action suggest that the pathogens were inhibited while growing in treated plants as well. The early collapse of haustoria after post-infection application of cymoxanil supports this interpretation. We infer that cymoxanil treatment alters pathogen physiology such that the host cells then recognize the presence of the invading cells, where they would not otherwise. Cymoxanil action thus triggers the hypersensitive-type reactions which ultimately seem to prevent pathogen spread and disease development.

REFERENCES

Bradshaw N J (1992) The use of fungicides for control of potato late blight (*Phytophthora infestans*). *Aspects of Applied Biology* **33**, 101-106.

Braun, E J; Howard R J (1994) Adhesion of *Cochliobolus heterostrophus* conidia and germlings to leaves and artificial surfaces. *Experimental Mycology* **18**, 211-220.

Douchet, J P; Absi M; Hay S J B; Muntan L; Villani A (1977) European results with DPX 3217 a new fungicide for the control of grape downy mildew and potato late blight. *Proceedings 1977 British Crop Protection Conference*, Vol. 2: 535-540.

Heath, M C (1984) Relationship between heat-induced fungal death and plant necrosis in compatible and incompatible interactions involving the bean and cowpea rust fungi. *Phytopathology* **74**, 1370-1376.

Howard, R J; Ferrari, M A; Bourett, T M; Stidham, M D; Shillingford, C; Power, R J; Hamlen, R A (1996) Biology of Curzate® Action Against Plasmopara viticola Infection of Vitis. *Inoculum, Newsletter of the Mycological Society of America* **47**, p. 12 (abstr).

Klöpping, H L; Delp C P (1980) 2-(Cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)-acetamide, a new fungicide. *Journal of Agricultural and Food Chemistry* **28**, 467-468.

Kováts, K; Binder, A; Hohl, H R (1991) Cytology of induced systemic resistance of tomato to *Phytophthora infestans*. *Planta* **183**, 491-496.

Pristou, R; Gallegly M E (1954) Leaf penetration by *Phytophthora infestans*. *Phytopathology* **44**, 81-86.

Samoucha Y; Cohen Y (1988) Synergistic interactions of cymoxanil mixtures in the control of metalaxyl-resistant *Phytophthora infestans* of potato. *Phytopathology* **78**, 636-640.

Serres M; Garraro G A (1976) DPX-3217, a new fungicide for the control of grape downy mildew, potato late blight and other Peronosporales. *Mededelingen van de Faculteit, Landbouwwetenschappen, Rijksuniversiteit, Gent.* **41**, 645-650.

Ziogas B N; Davidse L C (1987) Studies on the mechanism of action of cymoxanil in *Phytophthora infestans*. *Pesticide Biochemistry and Physiology* **29**, 89-96.