SESSION 9C

CROP PROTECTION IN HORTICULTURAL CROPS

SESSION ORGANISER DR P. R. ELLIS

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POST-HARVEST ROTS OF AVOCADOS IN NEW ZEALAND AND THEIR CONTROL

W.F.T. HARTILL

DSIR Plant Protection, Private Bag, Auckland, New Zealand

ABSTRACT

The post-harvest rots of avocados in New Zealand are anthracnose, caused by *Colletotrichum* spp., and stem-end rots caused by *Botryosphaeria* spp. and *Phomopsis* spp.

The rots can be controlled by a combination of field sprays and post-harvest dips. The incidence of *Botryosphaeria* spp. was reduced by monthly applications of copper hydroxide, that of *Phomopsis* spp. by pre-harvest applications of benomyl, and that of *Colletotrichum* spp. by post-harvest dipping in prochloraz. The orchard sprays probably reduced the inoculum present in the tree canopy rather than protecting the fruits directly The long term problems that may arise from using this schedule, and its possible replacement by alternative treatments, are discussed.

INTRODUCTION

Avocados are a relatively new crop in New Zealand with most of the produce coming from orchards planted 8 - 16 years ago. The major area of production is the Bay of Plenty, and there are also significant plantings in the Gisborne region and in the far north of the country. The predominant cultivar is 'Hass'. The trees flower from October to December and the fruits are normally ready for picking in the following November. However, ripening does not occur until the fruits are picked, and mature fruit may be held on the tree until May. The major export market in Australia requires fruits to be picked from mid-December until February. Except for occasional cases of scab (*Sphaceloma persea* Jenkins) the fruits generally remain apparently disease-free until after they are harvested. However, rots frequently develop as the fruits ripen. A proportion of the immature fruits (c. 20 - 50%) do fall in their first autumn, but the relative importance of pathological and physiological factors causing this fall has not been established.

Orchards are usually divided into blocks 4 - 6 rows wide, each block being surrounded by shelter belts of fast growing tree species or of giant bamboo to provide protection from wind.

The present investigations into the cause and control of post-harvest rots of avocados started in 1982 and full-scale field trials three years later following claims that there had been a high incidence of the rots in fruits sold in Australian markets.

METHODS

The most common commercial cultivar, Hass, was used in all experiments.

Isolations

Fruit rot pathogens were isolated by taking small pieces from the advancing edge of diseased areas and plating onto potato dextrose agar containing 50 units/litre streptomycin (PDAS).

Field sprays

Sprays were applied to run-off (c. 40 litres/tree) using a hand lance at 2800 kPa. The treatments were copper hydroxide (Kocide 101, 150g product/100 litres) applied monthly throughout the year, benomyl (Benlate 50 DF, 50g product/100 litres) applied monthly for 3 months prior to harvest, or a combination of these treatments, i.e. copper applied monthly until 3 months before harvest, and subsequently benomyl.

Post-harvest dipping

Freshly-harvested fruits were dipped in prochloraz (Sportak 45 EC, 5.5ml product/10 litres) for 2 minutes and intermittently agitated while in the dip. They were then air dried before being packed into boxes.

Post-harvest rot assessment

Fruits were ripened for 7-20 days in boxes on laboratory benches at ambient temperature (c. 23°C). Every 1-2 days the fruits were examined for post-harvest rots and those with external rots were removed and isolations were made of any fungi present. When ripe, the remaining fruits were each cut in half and assessed for internal rots. The extent of rotting in each fruit was estimated as follows:

nil	=	no rots
trace	=	shallow (c. 1 mm deep) rot only
marginal	=	rots confined to a small segment of the fruit
rotten	=	rots spreading beyond initial infection site.

A rot severity index was derived for each treatment by multiplying the number of fruits in each category by a factor, related to the extent of rot found in that category, and then combining these values and converting the sum to a percentage of the maximum severity possible. Fruits with no more than a trace of rot were considered to be commercially acceptable for consumption.

Rot severity index (%) =

$$\frac{[(\text{number trace}) + (4 \times \text{number marginal}) + (10 \times \text{number rotten})] \times 100}{10 \times \text{total fruits in sample}}$$

RESULTS AND DISCUSSION

The pathogens

The identity of the fungi isolated from post-harvest rots and confirmed as pathogenic is given in Table 1. Their incidence and cultural characteristics were described by Hartill (1991). The list differs somewhat from other international lists (Snowden, 1990; Ohr *et al.*, 1991), because in New Zealand *Colletotrichum acutatum* is as important as *C. gloeosporiodes* as a cause of anthracnose, and because a revision of the stem rot pathogens usually included under *Dothiorella* has indicated they are more correctly placed in *Botryosphaeria* and its *Fusicoccum* anamorphs (Pennycook & Samuels, 1985). It may be significant that avocado orchards are frequently adjacent to kiwifruit orchards typical of those from which the latter authors collected their isolates. *B. parva* and *B. dothidia* frequently cause body rots, and the anthracnose pathogens also cause neck rots, particularly if fruits are harvested when wet. In this paper "neck rot" is used as a general term for all rots starting at the stem end of the fruits, and "body rot" for rots starting elsewhere.

TABLE 1. Pathogens isolated from post-harvest rots.

Anthracnose:	*Colletotrichum acutatum* Simmonds ex Simmonds
	C. gloeosporioides (Penzig) Penzig & Saccardo
"Stem-end" rots:	*Botryosphaeria parva* Pennycook & Samuels
	B. dothidea (Mougeot ex Fries) Cesati & de Notaris
	Fusicoccum luteum Pennycook & Samuels
	Phomopsis spp. (at least 2 species)
Others (rare):	*Fusarium* spp. (5 species)
	Botrytis cinerea Persoon
	Rhizopus stolonifera (Ehrenberg) Vuillemin
	Trichothecium roseum (Persoon) Link

Control measures

The development of control strategies based on good hygiene (removal of dead wood and mummified fruits from within the canopy), routine orchard sprays and post-harvest dips has been described by Hartill *et al.* (1986, 1991). Some of these treatments have been applied on a continuous basis for 3-4 seasons as it was thought that the full effects of the treatments might take time to become apparent. Trees, located in a single orchard block, which in two trials in previous years had received the effective treatments, based on the use of routine copper sprays and good hygiene in one trial and the use of pre-harvest benomyl sprays in the other, were combined into a single experiment in 1991-92 to determine an optimum strategy for growers' use. In two of the treatments (unsprayed and monthly copper sprays) dead wood removal was continued where it had been tested on a split-block basis in previous years this was continued. Half of the fruits harvested from each tree were dipped in prochloraz within 2 hours of picking.

Table 2. The incidence and severity of post-harvest rots isolated from fruits from trees with different disease management strategies (treatment means).

Fungicide	Treatment: dead wood removed	Treatment: dipped in Sportak	Post-harvest rots: acceptable fruits (%)	Post-harvest rots: rot severity (%)	Post-harvest rots: body rots (%)	Post-harvest rots: neck rots (%)
Orchard treatments:						
none	no	---	50	42.4	25	51
	yes	---	46	47.9	24	60
Kocide	no	---	66	26.3	6	42
	yes	---	69	26.8	11	41
Benlate	no	---	64	27.5	29	29
Kocide/ Benlate	no		78	16.5	10	27
		S.E.	± 6.9	± 6.3	± 4.4	± 7.3
L.S.D.	P =.05		20	18.1	13	21
Post-harvest dip treatment:						
---	---	no	50	41.5	25	52
---	---	yes	74	21.0	10	32
		S.E.	± 4.0	± 3.7	± 2.5	± 4.2
L.S.D.	P =.05		11	10.4	7	12

Note: --- = combined data

Post harvest dipping in prochloraz was effective in reducing both body rots and neck rots (Table 2). In contrast to the results obtained in previous years (Hartill *et al.*, 1991), orchard sprays had a lesser effect, and only the copper hydroxide followed by pre-harvest benomyl treatment reduced overall post-harvest rots to a significant extent. Copper hydroxide sprays reduced the proportion of fruit with body rots, and pre-harvest benomyl sprays reduced the proportion of fruits with neck rots.

The different treatments had differential effects in reducing the incidence of the various pathogens (Table 3). Fruits from trees sprayed with copper hydroxide had a reduced incidence of *Botryosphaeria* spp., and had some indication of a reduced incidence of *C. acutatum*. There was an increased incidence of *C. acutatum* in fruits from trees sprayed only with benomyl, but a decreased incidence of *Botryosphaeria* spp. and of *Phomopsis* spp.

Post-harvest dipping in prochloraz reduced the incidence of both *Colletotrichum* species and, to a lesser extent, of *Botryosphaeria* spp.

Table 3. The incidence of pathogens isolated from fruits from trees with different disease management strategies (treatment means).

Fungicide	Treatment: dead wood removed	Treatment: dipped in Sportak	*Colletotrichum*: *C. acutatum*	*Colletotrichum*: *C. gloeosporioides*	*Botryosphaeria* all species	*Phomopsis* all species
Orchard treatments:						
none	no	---	12	8	38	21
	yes	---	10	15	35	22
Kocide	no	---	5	16	12	15
	yes	---	7	22	7	14
Benlate	no	---	26	6	23	5
Kocide/ Benlate	no		12	14	8	6
		S.E.	± 3.1	± 5.2	± 4.0	± 2.8
L.S.D.	P = .05		9	-	12	9
Post-harvest dip treatment:						
---	---	no	16	21	24	16
---	---	yes	8	6	17	11
		S.E.	± 1.8	± 3.0	± 2.3	± 1.8
L.S.D.	P = .05		6	9	7	-

Note: --- = combined data
- = differences not significant

In contrast to previous results dead wood removal did not affect the severity of post-harvest rots nor the incidence of individual pathogens.

These results indicate that a range of treatments are required to obtain optimum control of post-harvest rots of avocados in New Zealand. There is considerable evidence that infections occur in newly formed fruits, remain latent until the fruits are harvested (Binyamini & Schiffmann-Nadel, 1972). However, in inoculation experiments I have found that fruits inoculated up to two months after fruit set tend to fall prematurely, and that most infections causing post-harvest rots appear to occur close to harvest (unpublished data). The pathogens, especially the *Colletotrichum* spp., are present in dead twigs within the canopy, in mummified fruits, and in the living branches, twigs and pedicels (Johnson *et al.*, 1991; Hartill, unpublished data). It seems probable that the need to maintain fungicide cover throughout the season is primarily because it is important to suppress the development of infection sources within the canopy rather than to provide direct protection for the fruits. Removal of dead wood may only be helpful when there are few other sources of infection.

The long term use of large volumes of copper hydroxide and benomyl may have adverse effects on soil microflora and fauna in the orchards. Alternative strategies now under investigation include using low volume sprays, and the possible replacement of all or part of the spray schedule with applications of phosphonate-based fungicides. Potassium phosphonate has been effective in reducing the incidence of *Botryosphaeria* spp. in a preliminary trial and may act by stimulating the natural diene defence compounds present in the fruits (Prusky *et al.*, 1983). There is also some indication from preliminary work that the post-harvest dipping treatment may be more effective if carried out at elevated temperatures (*c.* 50°C).

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CAULIFLOWER CULTIVAR SUSCEPTIBILITY AND THE EFFECT OF COPPER SPRAYS ON BACTERIAL LEAF SPOT

J M Ll DAVIES

ADAS Terrington, Terrington St Clement, King's Lynn, Norfolk PE34 4PW

D E STEAD

Central Science Laboratory, MAFF, Hatching Green, Harpenden, Herts AL5 2BD

ABSTRACT

Trials carried out in the winters of 1987/8 and 1988/9 evaluated the susceptibility of 21 cauliflower cultivars to various inoculated strains of bacterial leaf spot, *Pseudomonas syringae* pv *maculicola* (Psm). Moderate to high levels of disease developed in 1988/9 with Danish Perfection being the most susceptible cultivar. There was a trend towards greater susceptibility in early summer cultivars. The effects of foliar sprays of copper hydroxide or copper oxychloride on cv. Danish Perfection applied pre- and post-inoculation with Psm and in various combinations were investigated. Copper sprays had little effect on disease prevention or eradication and there was no significant reduction in spread. In a 1988 field trial, one, two and three-spray programmes of copper hydroxide and five half-rate sprays were compared with an untreated control. High disease levels developed and copper sprays had no effect on disease or marketable yields.

INTRODUCTION

Bacterial leaf spot, bacterial blight or pepper spot caused by *Pseudomonas syringae* pv *maculicola* (Psm) can affect most brassicas. It is particularly important on cauliflowers as it infects the curds and renders the crop unsaleable. It is not a very common disease in the United Kingdom. However, it was recorded annually on one farm in South Lincolnshire for many years and in the mid/late-1980s it was a serious disease of early summer cauliflowers on both seedlings and field crops in that county. Limited information exists on control measures but those based on copper sprays as Bordeaux mixture applied in the seed bed were ineffective in controlling this disease (Clayton, 1924). The objectives of the work described here were to assess the susceptibility of a range of cauliflower cultivars to Psm during propagation and to evaluate the effectiveness of foliar sprays of copper for disease control both in propagation and in the field.

MATERIALS AND METHODS

Cultivar susceptibility

Twenty three cauliflower cultivars, all October sown, were assessed for susceptibility to Psm in 1987/8. In 1988/89 a similar range of 21 cultivars were tested Table 1. Each SWC polystyrene module tray of 308

plants was cut and divided into four sections. Three sections were each inoculated with a different strain of Psm (10^7 colony forming units/ml) in peptone phosphate buffers. The trial design was 21 cultivars randomised within each treatment, i.e. 3 Psm strains and uninoculated controls. In both years as a control, plants were inoculated with the peptone phosphate buffer only. The strains used in 1987/88 were: 41/A ex Brussels sprouts ex Cambridge ADAS, 82/B ex cauliflower ex Cambridge ADAS and NCPPB 1766 ex cauliflower and in 1988/89 were: NCPPB 3572 ex cauliflower ex Kirton 1988, NCPPB 1766 ex cauliflower and 41/A ex Brussels sprouts ex Cambridge ADAS. Inoculation was made using an ultra low volume spinning disc sprayer (Micron Ulva 8). The plants were sprayed to run off from above and sides and also one row of plants in each tray was stab inoculated in the stems and leaves. Plants were inoculated on 25 February and re-inoculated on 25 March in 1988 and on 24 January in 1989 and incubated under a polythene sheet for 24 hours prior to being laid out. Disease was assessed as mean % lowest leaf area affected with Psm and plant death.

The effect of copper sprays on disease control in propagation

Plants of cv Danish Perfection were raised in Hassy 308 modules from an October sowing in both years. In 1987/88 the treatments were copper hydroxide (Kocide 101 77% w/w Chiltern) 1.1 g product/l @ 50ml/tray applied as a foliar spray one week prior to inoculation with Psm (27 February). Eight weekly foliar sprays of copper hydroxide 1.1g/product/l @ 50 ml/tray or copper oxychloride (Cuprokylt 50% w/w Unicrop) 3.75g product/l @ 50ml/tray plus 0.25ml/l PBI spreader were applied starting 48 hours after inoculation with Psm. Pre- and post-inoculation sprays i.e. combinations of the above treatments with and without inoculation with Psm were compared with an inoculated but unsprayed and an untreated control. Each treatment was applied to one half of the plant tray, the other half remaining untreated. The strain of Psm used was NCPPB 1766. In the 1988/89 trial the inoculation treatments were modified. The central 16 plants (4 x 4 cells) in each tray were replaced with plants which had been inoculated previously, as described above, with Psm NCPPB 3572. Four foliar sprays of copper, either of copper hydroxide at 1.1g product/l or of copper oxychloride 3.75g product/l were applied at 50ml/tray at weekly intervals pre-inoculation. The disease was introduced into the trays on 8/9 February. Five weekly post-inoculation foliar sprays applied either as copper hydroxide or copper oxychloride and in various combinations were compared with an inoculated and an untreated control. In both years the treatments were replicated four times, and the copper sprays were applied with a hand held sprayer (Polyspray 2-ASL). Disease was assessed as mean % leaf affected. The above trials were carried out in an unheated polythene tunnel at the Kirton Area Laboratory; the plants were fed and watered and sprayed for downy mildew as per commercial practice.

The effect of copper sprays on disease control in the field

In a naturally infected site at Saracen's Head, Lincolnshire in 1988, plots of cv. Danish Perfection, each of 4 rows of 14 plants, were treated with copper oxychloride at 1.1kg product/2001 water/ha. Single, double and triple applications on 1, 15 and 20 June were compared with 5 applications of copper oxychloride at 0.5kg product/2001 water/ha applied on 1, 6, 15, 20 and 24 June and with an untreated control. (Due to wet weather between 6 and 15 June, no sprays were applied and two 3-spray programmes were identical but only one has been shown in the results). Copper sprays were applied using an MDM Precision Sprayer. Assessments were made on 20 plants

of the middle 2 rows of each plot. Leaf disease assessment was made using the ADAS swede powdery mildew key (Anon., 1976) and giving percentage leaf area affected as indices ie, 5% index 1; 10% index 2 etc. A harvest assessment was made on marketable plants, plants with affected curds and blind plants, i.e. no curds produced. All data were subjected to analysis of variance. Treatment means followed by the same letter within any one column do not differ significantly P=0.05 (Duncan's Multiple Range Test).

RESULTS

Cultivar susceptibility

In 1988, leaf spot symptoms were first seen on 3 March but little further disease development occurred and no differences among cultivars were noted. On 25 March the plants were re-inoculated and initial symptoms were confirmed. Again no further disease developed and no differences were apparent among cultivars. In 1989 leaf spot symptoms were first seen on 6 February. Differences between cultivars were detected (Table 1). In this table cultivars are listed in order of maturity.

TABLE 1. Incidence of Psm leaf spot and plant survival in 1989 (mean of 3 strains).

Maturity season/ cultivar	Plants affected 10 March		Leaf area affected 10 March		Plant survival 5 April	
	%	Ang trans	%	Ang trans	%	Ang trans
Early Summer						
Danish Perfection	100.0	90.0b	68.7	57.2d	61.0	51.8a
Michalese Carillon	63.3	57.3a	25.2	26.9c	62.6	65.7bc
Alpha Jubro	60.0	51.1a	19.7	20.4bc	83.0	66.8bc
Alpha Begum	56.7	48.4a	4.4	10.9ab	87.0	69.1bcd
Alpha Selsto	40.0	38.2a	2.2	7.7a	84.0	66.7bc
Oberon	40.0	34.9a	2.1	6.8a	88.3	70.2bcd
Corvilia	40.0	28.9a	2.0	6.6a	83.7	66.4bc
Erfu	36.7	36.1a	2.6	6.5a	94.3	76.8cd
King	33.3	30.0a	1.7	4.4a	83.7	66.2bc
Fortuna	43.3	41.1a	3.0	7.9a	86.7	68.8bcd
Summer and Autumn						
White Summer	50.0	49.9a	3.1	9.5ab	95.4	80.0d
Andes	43.3	45.0a	4.7	9.1ab	93.7	75.7bcd
White Fox	23.3	24.1a	1.5	5.7a	81.0	64.4b
Linas	36.7	36.1a	1.6	7.2a	92.3	74.2bcd
Dok Elgon	40.0	38.9a	2.3	8.0a	87.7	69.9bcd
Cervinia	43.3	40.4a	1.9	7.3a	88.0	69.9bcd
Revito	36.7	36.1a	1.3	5.9a	96.3	78.9d
White Rock	40.0	38.9a	1.5	6.9a	89.0	71.3bcd
Gigo	50.0	45.0a	2.0	6.6a	90.0	71.9bcd
Elby	40.0	38.9a	1.5	5.4a	86.7	68.7bcd
Alice Springs	53.3	42.8a	4.5	9.8ab	86.7	68.6bcd
SED (40df)		14.4		5.3		4.7

The results showed that Danish Perfection was consistently the most significantly susceptible cultivar with a higher number of plants showing symptoms, more green leaf area affected and lowest survival. Also there was significantly more plant death recorded in Danish Perfection. Early summer cauliflowers were generally more susceptible than later maturing cultivars. Apart from Danish Perfection, Michalese Carillon, Alpha Jubro and Alpha Begum were the most susceptible of the early summer cauliflowers. King, Revito and White Fox appeared to be the least susceptible cultivars. Differences between the various strains were detected with NCPPB 3572, a local cauliflower strain, the most pathogenic, and strain 41/A from Brussels sprouts, the least pathogenic (mean 13.4% and 3.7% leaf area affected, respectively).

The effect of copper on disease control in propagation

In all inoculated treatments, infection and subsequent disease spread occurred. The results of the two disease assessments are given in Table 2. No disease was detected in trays which had not been inoculated. On the 17 March the most severe symptoms seen were in plants in the inoculated half of trays following copper oxychloride sprays. Disease levels in this treatment were significantly higher than in those which had received the similar treatment of copper hydroxide. However, disease was recorded in all inoculated trays treated with copper. None of the copper treatments prevented infection. The uninoculated tray halves were assessed on 25 March. No disease spread was recorded in the uninoculated treatments. Foliar sprays of copper failed to prevent subsequent disease spread. No significant differences between the two copper formulations were detected.

TABLE 2. Mean % leaf area affected with Psm following inoculation and copper fungicide treatments in 1988.

Treatment	Inoculated half tray 17 March 1988 (based on 32 plants/ treatment)	Uninoculated half tray 25 March 1988 (based on 15 plants/treatment)
Copper hydroxide spray pre-inoculation	5.85ab	3.3b
Inoculation followed by copper hydroxide sprays	7.78ab	2.6b
Copper hydroxide sprays pre and post-inoculation	5.16ab	1.3ab
Inoculation followed by copper oxychloride sprays	15.28c	0.8ab
Copper hydroxide sprays only no inoculation	0.0a	0.0a
Inoculation only	10.13bc	1.7ab
Control no inoculation or copper treatment	0.0a	0.0a
SED (18df)	2.46	1.12

In the 1989 trial, inoculated plants in the middle 4 x 4 cells showed severe bacterial leaf spot symptoms. On 13 March (planting out stage) significant spread was recorded in trays containing the inoculated plants albeit at a low level (Table 3).

TABLE 3. Mean % leaf area affected with Psm following inoculation and copper fungicide treatments in 1989.

Pre-inoculation copper sprays	Inoculated with Psm	Post-inoculation copper sprays	Mean % leaf area affected 13 March	5 April
No spray	-	No spray	0.0a	1.9a
No spray	+	No spray	2.4cd	2.3a
Copper hydroxide	-	No spray	0.43abc	0.8a
Copper hydroxide	+	Copper hydroxide	2.2bcd	4.0a
No spray	+	Copper hydroxide	2.9d	5.6a
Copper oxychloride	-	No spray	0.2ab	1.1a
Copper oxychloride	+	Copper oxychloride	1.5abcd	3.3a
No spray	+	Copper oxychloride	2.3bcd	3.3a
SED (14df)			0.89	1.35

There was no control of bacterial leaf spot with either copper formulation applied pre- and post-inoculation or applied post-inoculation. Limited cross infection occurred but there was no significant difference between the mean % leaf area affected in the two copper controls and the uninoculated control. The trial continued for another 3 weeks and by 5 April no significant differences were detected between treatments.

The effect of copper sprays on disease control in the field

When the site was first inspected on 21 May approximately 5% of the plants were affected with bacterial leaf spot. On 1 June approximately a third of the plants were affected and 3 weeks later nearly all of the plants were affected. No significant differences between the number of plants affected were detected in any treatments. Similarly, on 1 June disease severity was low (0.46 disease index in the untreated) and by 13 June it had dramatically increased (approximately tripled to 1.16) but with only a slight further increase a week later (1.40). Copper treatments had no significant effect on disease severity. Serious losses of marketable crop were attributed to Psm. At harvest, no significant differences between treatments were detected in marketable plants, diseased curds nor on plant blindness with a mean of 31%, 14% and 7.5% plants respectively in the untreated. More blind plants were affected with Psm than non-diseased ones 10.6% and 4.6% plants respectively in the untreated. Only one head in the whole trial was considered unmarketable due to blue spotting from the copper sprays.

DISCUSSION

Insufficient disease developed in the 1987/88 trial to show differences between cultivars. The plants were grown very "hard" and it was felt that softer grown ones may have been more susceptible. Plants for the copper trials but derived from a different source were treated with the same bacterial inoculum, from which successful infection was obtained. The cultivar trial was re-inoculated on 25 March but by this time it was felt that the weather was too warm for disease development to occur. Although Psm was confirmed from initial symptoms little further disease developed following both inoculations. It is possible that because the plants were raised and grown 'hard' that the leaves may have acted as a physical barrier to infection following spray inoculation. However, this does not explain the lack of symptoms following stab inoculation. At a similar time of the first inoculation one of the strains used NCPPB 1766 produced good symptoms in the copper disease control trial in softer grown plants. The reason why 'hard' grown plants were not very susceptible cannot be explained. In the 1988/89 trial early summer cultivars were the most susceptible. However, Erfu and King were the least affected of this group. Young plants of cv. Michalese Carillon in this trial were moderately susceptible. However, observations of field outbreaks of Psm in Lincolnshire made in 1987 revealed that where grown together in the same field, cv. Michalese Carillon was only slightly affected compared with severely affected crops of cv. Danish Perfection. Copper sprays had little effect on disease eradication or on reducing disease spread. This agrees with previously published work (Clayton, 1924). Psm can be seed-borne but tests on stocks of seed which were implicated with disease outbreaks failed to detect the pathogen (J D Taylor, personal communication). During late spring of 1989 outbreaks of Psm were confirmed on crops of cv. Danish Perfection covered with polythene; symptoms were seen when the covers were removed. These were the last recorded outbreaks of this disease and may reflect the diligence of both seedsmen and propagators to supply disease-free seed and to achieve a high standard of hygiene respectively.

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EVALUATION OF FUNGICIDES FOR CONTROL OF RINGSPOT AND LIGHT LEAF SPOT IN BRUSSELS SPROUTS

P. GLADDERS

ADAS Boxworth, Boxworth, Cambridge CB3 8NN

O.W. JONES

ADAS Starcross, Staplake Mount, Starcross, Exeter EX6 8PE

D.D. SLAWSON

Ministry of Agriculture, Fisheries and Food, Pesticides Safety Division, Rothamsted, Harpenden, Herts AL5 2SS

ABSTRACT

The fungicides benomyl, chlorothalonil and triadimenol were applied alone or in two-way tank mixtures as 3-spray programmes to Brussels sprouts in Devon (1989), Cornwall and Kent (1988). Severe ringspot and light leaf spot developed at two sites and rendered 88-99% buttons unmarketable. Good control of ringspot and light leaf spot was obtained with all treatments except triadimenol and benomyl + wetter on cv. Dolmic harvested on 18 October 1989 but light leaf spot was poorly controlled by all treatments on the same site on cv. Gabion harvested on 21 November 1989. In 1988, chlorothalonil did not control severe light leaf spot on buttons in Cornwall but gave good control of low levels of the disease in Kent. The addition of wetter improved the performance of triadimenol at 2 sites and benomyl gave better results with 1% or 2% mineral oil than with wetter. Resistance to benomyl was confirmed in ringspot from Devon, the first record in England.

INTRODUCTION

Vegetable growers currently face the apparently conflicting demands of producing high quality produce whilst minimising pesticide usage. To help meet these demands, growers need up to date information on product efficacy. In addition, there is concern that reliance on benzimidazole fungicides for control of two major diseases of brassicas, ringspot (Mycosphaerella brassicicola) and light leaf spot (Pyrenopeziza brassicae) (Wafford et al., 1986) could lead to selection of fungicide resistant strains of these pathogens.

This paper presents recent experiments to evaluate fungicides for the control of light leaf spot and ringspot in Brussels sprouts and discusses the opportunities to diversify fungicide usage.

MATERIALS AND METHODS

Experiments evaluating 3-spray programme of various fungicides or fungicide mixtures were carried out in commercial crops of Brussels sprouts at Mitchell, Cornwall and Dunkirk, Kent in 1988 and at Clovelly, Devon in 1989. Each plot consisted of 4 rows of 14-16 plants (plant spacing averaged 0.50 x 0.75 m) and treatments were replicated 3 (in 1989) or 4 (in 1988) times in a randomised block design. At Mitchell, cv. Richard was planted on 13 June on clay loam soil and treatments were applied on 23 August, 16 September and 10 October. At Dunkirk, cv. Rampart planted on 24 May on silty loam soil received spray treatments on 17 August, 7 September and 30 September. Adjacent blocks of cvs. Dolmic and Gabion were sprayed on 3 August, 23 August and 12 September after planting out on 18 May on sandy, clay-loam soil at Clovelly. All fertiliser and pesticide treatments (other than fungicides) followed local practice. Sprays were applied by an Oxford Precision sprayer in 600 l water/ha through F110.03 nozzles operated at 300 kPa pressure.

The fungicides used were benomyl 'Benlate Fungicide' 0.55 kg AI/ha, chlorothalonil 'Bravo 500' 1.50 kg AI/ha, triadimenol 'Bayfidan' 0.125 kg AI/ha and chlorothalonil + metalaxyl 'Folio 575 FW' 1.00 + 0.15 kg AI/ha. Various combinations of fungicides were also evaluated as indicated in Tables 1-5. A non-ionic wetter alkyl phenol ethylene oxide 'Agral' 0.162 kg AI/ha or mineral oil 'Actipron' at 5.82 or 11.64 l AI/ha was used with some fungicides (see Tables 1-5).

Assessments were made on 10 plants in the two central rows of each plot at intervals during the growing season. At harvest, a sample of 100 buttons/plot was collected and assessed for diseases and blemishes. Buttons with >5% surface blemish were considered unmarketable. Ringspot was isolated on to potato dextrose agar (PDA) and its sensitivity to benomyl noted by recording presence or absence of growth on PDA containing 2 mg/l benomyl. Isolates growing at 2 mg/l were classified as resistant to benomyl.

RESULTS

In 1988, ringspot levels were low in August at the site in Cornwall, but defoliation was apparent in early October (Table 1) and infection continued to develop rapidly during October. All treatments gave control of foliar symptoms (Table 1) but good control of ringspot on buttons was only achieved by treatments with benomyl (Table 2). Light leaf spot control was poor but significant increases in marketable buttons were recorded with all treatments except chlorothalonil in Cornwall (Table 2). In Kent, disease severity was low (0.6% surface area affected in untreated) and all treatments controlled light leaf spot on buttons from the middle of the stem (Table 3).

In 1989, severe ringspot and light leaf spot developed on two cultivars in Cornwall. All treatments gave control of foliar symptoms on both cultivars (data for cv. Dolmic not presented) (Table 4) and button infection on cv. Dolmic. Control of button diseases on cv. Gabion was poor particularly with triadimenol and benomyl + wetter. Oedema was also detected on leaves and its incidence was significantly reduced from 15% leaf area affected in untreated to 0.7-5.0% by treatments with

chlorothalonil. Fungicide resistance tests on ringspot isolates (2 from untreated and 6 from benomyl treated plots) showed that half the isolates from both the untreated and the benomyl sprayed plots were resistant to benomyl.

TABLE 1. Effect of fungicide treatment on retention of leaves and severity of ringspot on leaves, Cornwall 1988.

Treatment	Mean Index* of leaf retention (0-5 scale) 10 October	Mean % leaf area affected	
		10 October	31 October
Untreated control	3.8 d	3.7 b	18.5 c
Benomyl + wetter	2.3 bc	1.0 a	1.4 a
Benomyl + 1% mineral oil	1.5 ab	0.1 a	0.0 a
Benomyl + 2% mineral oil	1.0 a	0.1 a	0.0 a
Chlorothalonil + wetter	2.3 bc	0.7 a	1.9 a
Triadimenol	2.3 bc	0.7 a	6.3 b
Triadimenol + wetter	2.8 c	0.7 a	1.6 a
Benomyl + chlorothalonil	1.8 ab	0.2 a	0.6 a
Benomyl + triadimenol	2.0 bc	0.3 a	0.9 a
Chlorothalonil + triadimenol	2.0 bc	0.4 a	1.1 a
SED (27 df)	0.42	0.49	1.95

* Index 1 - good >80% retention 5 - poor <20% retention

Treatment means followed by the same letter do not differ significantly ($P = 0.05$)

TABLE 2. Effect of fungicides on button diseases and marketability at harvest on 16 November, Cornwall 1988.

Treatment	% Buttons with		% Marketable buttons
	Ringspot	Light leaf spot	
Untreated control	52.3 c	83.5 d	1 a
Benomyl + wetter	2.3 a	60.3 abc	34 bcd
Benomyl + 1% mineral oil	0.0 a	40.0 a	53 e
Benomyl + 2% mineral oil	1.5 a	47.5 ab	48 de
Chlorothalonil + wetter	21.0 b	70.0 cd	17 ab
Triadimenol	15.5 ab	56.3 abc	26 bc
Triadimenol + wetter	10.0 ab	52.8 abc	36 cde
Benomyl + chlorothalonil	0.0 a	43.8 ab	54 e
Benomyl + triadimenol	1.3 a	57.8 abc	38 cde
Chlorothalonil + triadimenol	10.0 ab	63.3 bc	25 bc
SED (27 df)	7.21	8.87	8.6

Treatment means followed by the same letter do not differ significantly ($P = 0.05$)

TABLE 3. Incidence of light leaf spot on buttons on 20 December, Kent 1988.

Treatment	% Buttons affected	
	Middle of stem	Top of stem
Untreated control	14.5 b	5.5 ab
Benomyl + wetter	3.0 a	3.3 ab
Benomyl + 1% mineral oil	0.3 a	0.8 a
Benomyl + 2% mineral oil	0.8 a	1.3 ab
Chlorothalonil + wetter	0.0 a	0.3 a
Triadimenol	3.0 a	6.8 b
Triadimenol + wetter	1.8 a	0.5 a
Benomyl + chlorothalonil	0.3 a	0.3 a
Benomyl + triadimenol	0.0 a	0.3 a
Chlorothalonil + triadimenol	0.0 a	0.0 a
SED (27 df)	2.62	2.51

Treatment means followed by the same letter do not differ significantly ($P = 0.05$)

TABLE 4. Effect of fungicides on the severity of ringspot and light leaf spot infection on leaves cv. Gabion, Cornwall 1989.

Treatment	Mean % (Arcsin) leaf area affected			
	Ringspot		Light leaf spot	
	Lower leaves 2 Oct	Middle leaves 21 Nov	Middle leaves 2 Oct	Upper leaves 2 Oct
Untreated control	22.6 c	28.7 g	14.8 c	11.3 c
Benomyl + wetter	5.7 ab	19.5 de	1.9 ab	5.7 b
Benomyl + 1% mineral oil	3.8 ab	14.6 bc	0.0 a	1.9 a
Benomyl + 2% mineral oil	1.9 a	14.6 bc	0.0 a	0.0 a
Chlorothalonil + wetter	8.1 b	11.2 ab	3.8 ab	6.5 b
Triadimenol	6.5 b	24.4 f	3.8 ab	6.5 b
Triadimenol + wetter	5.7 ab	23.3 ef	3.8 ab	6.5 b
Benomyl + chlorothalonil	5.7 ab	11.2 ab	1.9 ab	5.7 b
Benomyl + chlorothalonil + metalaxyl	6.5 b	10.5 ab	0.0 a	1.9 a
Benomyl + triadimenol	5.7 ab	18.5 cd	3.8 ab	5.7 b
Chlorothalonil + triadimenol	5.7 ab	7.9 a	4.6 b	6.5 b
SED (20 df)	1.91	1.88	1.64	1.57

Treatment means followed by the same letter do not differ significantly ($P = 0.05$)

TABLE 5. Effect of fungicides on the incidence and severity of button diseases at harvest on cvs. Dolmic (18 October) and Gabion (21 November) in Devon 1989.

Treatment	% Buttons affected					
	cv. Dolmic			cv. Gabion		
	Ringspot	Light leaf spot	>5% area diseased	Ringspot	Light leaf spot	>5% area diseased
Untreated control	85 c	80 d	93 c	81 d	88 c	88 c
Benomyl + wetter	29 b	39 bc	45 b	50 c	86 c	86 c
Benomyl + 1% mineral oil	2 a	16 abc	16 ab	18 ab	81 bc	81 bc
Benomyl + 2% mineral oil	1 a	18 abc	18 ab	22 ab	56 a	56 a
Chlorothalonil + wetter	9 a	18 abc	19 ab	28 b	89 c	89 c
Triadimenol	29 b	42 c	44 b	56 c	78 abc	78 abc
Triadimenol + wetter	19 ab	18 abc	26 ab	59 c	81 bc	81 bc
Benomyl + chlorothalonil	0 a	7 a	7 a	8 a	61 ab	61 ab
Benomyl + chlorothalonil + metalaxyl	3 a	8 a	9 a	11 ab	77 abc	77 abc
Benomyl + triadimenol	11 ab	14 abc	22 ab	44 c	75 abc	75 abc
Chlorothalonil + triadimenol	7 a	5 a	11 a	11 ab	69 abc	69 abc
SED (20 df)	8.4	13.7	13.3	7.9	9.4	9.4

Treatment means followed by the same letter do not differ significantly ($\underline{P}$ = 0.05)

DISCUSSION

The fungicides benomyl, chlorothalonil and triadimenol all gave significant control of ringspot but control of light leaf spot was more variable particularly with chlorothalonil. Additional wetter improved the performance of triadimenol against ringspot (Table 1) and light leaf spot (Table 3) at some sites. Poor control of light leaf spot on cv. Gabion (Table 5) in comparison with cv. Dolmic suggested that the persistence of treatments was more limited for light leaf spot than for ringspot. Alternatively, a longer period of protection may be required to prevent light leaf spot developing on the buttons. Benomyl gave better disease control with mineral oil than with wetter under high disease pressure (Tables 4 and 5). There appeared little advantage in applying fungicides in mixtures except on cv. Gabion in 1989 but further work is needed to evaluate reduced rates of fungicides in mixtures.

Resistance to benomyl in ringspot was recorded for the first time in England at Clovelly, Devon in November 1989. However, the incidence of benomyl resistant strains may well have been low early in the season as benomyl treatments gave good disease control. Reports of resistance to benzimidazole fungicides in ringspot from Eire (Ryan, 1987) and Australia (Wicks et al., 1987) also indicated that chlorothalonil and a range of systemic fungicides including triadimefon (closely related to triadimenol used in these experiments) controlled resistant strains when applied on a 10-14 day schedule. Growers should use fungicides with different modes of action when applying spray programmes for control of ringspot and light leaf spot. Both chlorothalonil and triadimenol can give comparable results to benomyl, and will enable growers to diversify their spray programmes.

ACKNOWLEDGEMENTS

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CITRUS TRUNK APPLICATION OF FENAMIPHOS TO CONTROL TYLENCHULUS SEMIPENETRANS

B. J. MONKE, W. M. ZECK

Miles Inc., Agriculture Division, P.O. Box 1508, Vero Beach, FL 32961

K. A. NOEGEL

Miles Inc., Agriculture Division, P.O. Box 4913, Kansas City, MO 64120-0013

ABSTRACT

The citrus nematode (Tylenchulus semipenetrans) is successfully controlled with soil applications of fenamiphos. However, newer application methods may improve application efficiency and reduce environmental exposure. Utilizing absorption and basipetally systemic characteristics of fenamiphos, successful results have been obtained with applications to the trunks of citrus trees. Semi-annual applications of fenamiphos were made to trunks of grapefruit and orange trees at 5.25 to 30.0 g AI/tree. The annual broadcast soil application of 45.0 g AI/tree was the standard. Average nematode control with trunk applications for three years was similar to the soil treatment. Yields were also comparable to the standard with a minimum average increase over the untreated of 19% and 27% for oranges and grapefruit, respectively. The application of fenamiphos directly to the trunks of citrus is a viable option for controlling citrus nematodes and reduces environmental exposure and potential for leaching in soil.

INTRODUCTION

The use of nematicides has been acknowledged to increase citrus yields by 15-30% through control of the citrus nematode (Tylenchulus semipenetrans) (Duncan & Cohn, 1990). NEMACUR (fenamiphos) has successfully been used to control the citrus nematode in Florida and other citrus producing states of the U.S. as well as other countries. The conventional treatment is made with application of either liquid or granular formulations to the soil in a 50% band under the trees, followed by incorporation.

However, conventional application methods allow exposure to the environment and potential for leaching.

We have investigated a new method of applying fenamiphos to the trunks of citrus trees from where it is translocated directly into the root system. This is possible because of the exceptional basipetal systemic nature of fenamiphos (Zeck, 1971 and Flint, 1977). During the past three years we have demonstrated the efficacy of trunk treatments through hand application to individual trees. Tractor mounted equipment has been developed that allows for this application on a commercial scale.

MATERIALS AND METHODS

Two studies at Vero Beach, FL, utilizing trunk application of fenamiphos for nematode control in grapefruit and Valencia oranges are reported here. The undiluted liquid formulation comprised of 35% AI fenamiphos was applied directly to the bark of the trunk at the base of the trees. The trunk treatments were made semi-annually with a hand held Spraying Systems Meterjet™ Spray Gun to coincide with the spring and fall flushes of root growth. The applicator delivered a consistent pre-calibrated volume of spray solution at 20 psi with a TeeJet 8003VS nozzle. The conventional soil application was made once a year in the spring. The applications were made from 0.25-0.75 m above the base of the tree in a 360^{0} band around the trunk or in two spots, each one-quarter the circumference, on opposite sides of the trunk (2 X 90^{0}). Soil and root samples were taken at 3-5 months after each application for nematode analyses.

Grapefruit

Fenamiphos was applied semi-annually to the trunk at 5.25, 7.0, 10.5 and 14.0 g AI/tree in a 360^{0} band and one treatment of 2 X 90^{0} spots at 10.5 g/tree was included. Granular fenamiphos (15%) in a 50% band was broadcast under the tree at 45 g AI/tree, the maximum labelled rate for 247 trees/ha, as the standard. Each treatment was replicated 4 times and each replication was a 2 X 3 block of 6 contiguous trees. Trunk applications were made 24 April 1989, 3 November 1989, 15 May 1990, 8 November 1990, 7 March 1991, and 20 September 1991 with soil and root samples for nematode analysis taken 88, 91, 94, 113, 166 and 157 days after the aforementioned respective treatment dates. Only the two middle trees in each replicate were used for nematode and yield samples.

Valencia Oranges

Fenamiphos at 7.5, 15.0 and 30.0 g AI/tree was applied semi-annually to the trunk in either 360^{0} or 2 X 90^{0} spots for each rate. Soil applications of granular and liquid fenamiphos at 45 g AI/tree in 50% broadcast bands were used as standards. Treatments were replicated 7 times with a single tree per replication. Trunk applications were made 12 October 1988, 12 April 1989, 17 October 1989, 3 May 1990, 15 November 1990, 7 March 1991 and 21 October 1991 with samples for nematode analyses taken 89, 91, 97, 92, 109, 141 and 91 days after the aforementioned respective treatment dates.

RESULTS AND DISCUSSION

Grapefruit

The average nematode control in soil and roots is presented in Tables 1 and 2, respectively. It is evident that nematode control by the trunk application treatments approximated that from the conventional soil broadcast but there was no rate response.

TABLE 1. Average numbers of T. semipenetrans in soil of grapefruit trees treated semi-annually with fenamiphos by application to the tree trunks.

Appl. Method[1/]	g AI/ Tree	Nematodes per 100 g Soil					
		7/89	2/90	6/90	3/91	8/91	2/92
Untreated	-	267	462	1132	1331	727	1515
360^{o}	5.25	94	110	581	260	456	950
360^{o}	7.0	63	130	249	706	466	964
360^{o}	10.5	55	92	277	534	400	984
360^{o}	14.0	57	390	640	227	1510	524
2 X 90^{o}	10.5[2/]	96	116	618	249	406	414
Soil bcst	45.0[2/]	98	118	516	680	618	584
LSD (P=0.05)		227	892	801	539	788	528

[1/] 360^{o} = 360^{o} entirely around the trunk; 2 X 90^{o} = two spots on opposite sides of trunk.
[2/] Applied once a year as fenamiphos 15G.

TABLE 2. Average numbers of T. semipenetrans in roots of grapefruit trees treated semi-annually with fenamiphos by applications to the tree trunks.

Appl. Method[1/]	g AI/ Tree	Nematodes per gram of root					
		7/89	2/90	6/90	3/91	8/91	2/92
Untreated	-	3	2	12	20	85	140
360^{o}	5.25	1	1	3	13	24	39
360^{o}	7.0	7	2	4	10	62	80
360^{o}	10.5	22	1	3	10	70	44
360^{o}	14.0	13	20	3	13	63	70
2 X 90^{o}	10.5[2/]	3	1	3	8	61	51
Soil bcst	45.0[2/]	11	2	3	7	34	66
LSD (P=0.05)		26	7	3	5	57	48

[1/] 360^{o} = 360^{o} entirely around the trunk; 2 X 90^{o} = two spots on opposite sides of trunk.
[2/] Applied once a year as fenamiphos 15G.

Yield results presented in Table 3 show the trunk application at least equal to the conventional treatment, depending on rate. Differences from the untreated were substantial, though generally not statistically significant. The yield improvement compared to the untreated was the least in 1992 when ideal growing conditions removed most stresses on the trees except nematode pressure in the untreated. In 1990, the grove was subjected to drought and a severe freeze.

TABLE 3. Average yields of grapefruit trees treated semi-annually with fenamiphos by application to the tree trunks.

Appl. Method[1/]	g AI/ tree	kg/Tree 1990	1991	1992
Untreated	-	72	220	260
360^0	5.25	105	273	312
360^0	7.0	109	256	296
360^0	10.5	126	296	347
360^0	14.0	121	293	313
2 X 90^0	10.5	115	257	274
Soil bsct	45.0[2/]	107	295	305
LSD (P=0.05)		34	86	94

[1/] 360^0 = 360^0 entirely aroung the trunk; 2 X 90^0 = two 90^0 spots on opposite sides of trunk.
[2/] Applied one a year as fenamiphos 15G.

Oranges

The nematode control in the Valencia oranges presented in Tables 4 and 5 show the trunk application treatments were effective in reducing nematode numbers but not as good as the conventional application. Only after early 1990 were treatments significantly different from the untreated. There was no rate response or differences between application placement on the trunk.

TABLE 4. Average numbers of T. semipenetrans in soil of Valencia orange trees treated semi-annually with fenamiphos by application to the tree trunks.

Appl. Method[1/]	g AI/ Tree	Nematodes per 100 g soil 2/89	7/89	1/90	8/90	3/91	7/91	1/92
Untreated	-	2306	2990	80	285	190	447	676
360^0	7.5	1800	2321	355	315	126	33	209
2 X 90^0	7.5	1226	1844	77	21	117	13	157
360^0	15.0	920	1486	65	33	48	102	324
2 X 90^0	15.0	5143	2191	416	121	87	163	397
360^0	30.0	1077	2240	442	217	98	65	479
2 X 90^0	30.0	1497	805	16	16	61	8	72
Soil bcst[2/]	45.0	737	176	94	8	63	116	184
Soil bcst[3/]	45.0	951	525	46	18	112	53	193
LSD (P=0.05)		3429	2432	500	347	135	311	503

[1/] 360^0 = 360^0 entirely around the trunk; 2 X 90^0 = two 90^0 spots on opposite sides of trunk.
[2/] Applied once a year.
[3/] Applied once a year as fenamiphos 15G.

TABLE 5. Average numbers of T. semipenetrans in roots of Valencia orange trees treated semi-annually with fenamiphos by application to the tree trunks.

Appl. Method[1/]	g AI/ Tree	Nematodes per gram of root						
		2/89	7/89	1/90	8/90	3/91	7/91	1/92
Untreated	-	23	35	22	101	501	102	92
360°	7.5	54	29	45	13	212	47	49
2 X 90°	7.5	54	35	152	5	124	38	29
360°	15.0	67	53	30	5	133	62	30
2 X 90°	15.0	41	25	27	4	283	81	28
360°	30.0	91	63	103	7	167	34	48
2 X 90°	30.0	57	40	10	11	66	9	17
Soil bcst[2/]	45.0	162	28	9	2	96	43	24
Soil bcst[3/]	45.0	43	26	9	4	83	33	20
LSD (P=0.05)		103	44	151	29	359	74	61

[1/] 360° = 360° entirely around the trunk; 2 X 90° = two 90° spots on opposite sides of trunk.
[2/] Applied once a year.
[3/] Applied once a year as fenamiphos 15G.

Yields for trunk applications (Table 6) were also less than the conventional application, but still approximately 20% or more greater than the untreated, though generally not significantly different.

TABLE 6. Average yields of Valencia orange trees treated semi-annually with fenamiphos by application to the tree trunks.

Appl. Method[1/]	g AI/ Tree	kg/Tree			
		1989	1990	1991	1992
Untreated	-	77	52	113	114
360°	7.5	93	70	147	134
2 X 90°	7.5	87	66	154	140
360°	15.0	94	67	151	140
2 X 90°	15.0	86	66	134	122
360°	30.0	95	68	116	122
2 X 90°	30.0	127	68	193	164
Soil bcst[2/]	45.0	117	111	176	185
Soil bcst[3/]	45.0	142	91	195	200
LSD (P=0.05)		47	31	74	63

[1/] 360° = 360° entirely around the trunk; 2 X 90° = two 90° spots on opposite sides of the trunk.
[2/] Applied once a year.
[3/] Applied once a year as fenamiphos 15G.

The age (over 40 years) and relatively poor conditions of these orange trees may have resulted in less efficiency in translocating the fenamiphos into the roots, but these factors would not affect nematode control from soil application. Thus, the nematode control and subsequent yield responses for the conventional application was generally greater than the trunk treatments in this trial.

CONCLUSIONS

These trials demonstrate that the application of fenamiphos to the trunks of citrus trees is an effective way of treating for nematode control, resulting in at least an average 20% increase in yield over the untreated. Tests are also now underway to demonstrate this concept with systemic insecticides as well as fenamiphos on other tree and vine crops. Trunk application may or may not be as effective as the soil application. However, variety of citrus fruit, age of trees, conditions and maintenance of the groves and environmental factors can impact degree of response.

Because of the potential, a tractor mounted applicator has been developed which is capable of treating an entire grove. This prototype is being used to demonstrate the feasibility of this concept on a commercial basis.

The trunk application of fenamiphos to citrus may be a viable alternative to the conventional soil application. It could be an important technique of reducing environmental exposure and reducing potential for leaching.

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FENAZAQUIN FOR THE CONTROL OF TWO-SPOTTED SPIDER MITES ON ORNAMENTALS

R.T. POLLAK, P. BLACKBURN,

DowElanco, Latchmore Court, Brand Street, Hitchin, Herts, SG5 1HZ

D.W.F. BUTLER,

Department of Agriculture, The University, Earley Gate, Reading. RG6 2AT.

ABSTRACT

Fenazaquin 4-[2-{4-(1,1-dimethylethyl)phenyl} ethoxy] quinazoline, is a new contact acaricide. The compound, formulated as a 200 g/l SC, (EF1127) has been assessed in forty-two replicated trials on potted ornamentals in the UK. It has been shown to control motile stages of the two-spotted spider mite, *Tetranychus urticae.* at rates of 0.1 and 0.2 l/hl, applied in water volumes of 200 or 1000 l/ha. Where good spray coverage was achieved, excellent knockdown and persistent control of two-spotted spider mite was accomplished with the lower of the two rates tested, for up to three weeks. Variation in the speed and extent of control was attributable to dose rate, spray volume and structural diversity between the target plant species.

Dose related selectivity against adult *Phytoseiulus persimilis,* was recorded. The lowest mean mortality in contact and persistency tests was 76% and 26% one and two days after application respectively with 0.05 l/hl EF1127 in a water volume of 200 l/ha.

Application of EF1127 at a rate of 0.2 l/hl, in water volumes of 200 l/ha or 750 l/ha did not cause damage on any of the twenty nine species of potted ornamental plants tested.

INTRODUCTION

The resurgence of two-spotted spider mite, *(Tetranychus urticae)* due in part to widespread insensitivity to synthetic pyrethroids, has fuelled the search for new products to control phytophagous mites on many crops, including potted, glasshouse-grown and outdoor ornamentals.

Fenazaquin, 4-[2-{4-(1,1-dimethylethyl)phenyl} ethoxy] quinazoline, was discovered by DowElanco. It is a new chemical from the quinazoline group. Fenazaquin has excellent contact activity but is not systemic and has no translaminar or vapour activity. (Longhurst *et al*. 1992). All trials reported in this paper were undertaken with EF1127, a 200 g/l SC formulation of fenazaquin.

MATERIALS AND METHODS

Selectivity

In 1992, EF1127 was applied to twenty-nine species of hardy ornamental and potted house plants and onto one species of potted strawberry runner 'Elsanta'. The following hardy ornamental species were tested; *Camellia* sp., *Caryopteris x clandonensis* 'Heavenly Blue', *Cordyline* cultivars 'Torbay Dazzler' and 'Sundance', *Choisya ternata* 'Sundance', *Elaeagnus pungens* 'Maculata', *Fuchsia* cultivars 'Genii' and 'Howlett's Hardy', *Hedera* sp., *Lavatera olbia* 'Rosea', *Lonicera periclymenum* 'Serotina', *Pieris japonica* 'Variegata', *Picea glauca* var. *albertina* 'Conica', *Skimmia japonica* 'Rubella', *Stranvaesia davidiana*, *Viburnum tinus* 'Variegatum', *Pyracantha coccinea*. The following species of ornamental house plants were tested; *Codiaeum variegatum*, *Cordyline terminalis*, *Tolmiea menziesii*, *Hedera helix*, *Pilea cadierei*, *Asparagus setaceus*, *Strobilanthes* sp., *Saintpaulia ionantha*, *Gynura sarmentosa*, *Chlorophytum comosum* 'Variegatum', *Cyclamen persicum* 'Pastel Compacta', *Solanum capsicastrum* 'Thurino'.

Three or six replicate pots of each species were sprayed in May,June or July, with the product EF1127, applied at a concentration of 0.2 l/ hl in a water volume equivalent to 200 or 750 l/ha. Treatments were made to "run off," using a CP5 knapsack sprayer fitted with a flat fan nozzle or were applied at the lower water volume, at 210 KPa pressure, using a hand-held Azo sprayer fitted with hollow cone nozzles. Temperatures inside glasshouses at the time of treatment were between 15°C and 20°C.

Plants were assessed for phytotoxic symptoms and differences relative to untreated control plants 2-3 weeks after treatment, on a scale of 0-100 where 0 was equal to no damage.

Efficacy

Motile two-spotted spider mites

Six replicated trials were undertaken, two on each of three plant species, *Solanum capsicastrum* (Thurino), *Cyclamen persicum* 'Pastel Compacta' and strawberry 'Elsanta'. Plants were artificially infested three days prior to treatment with motile two-spotted spider mites reared on pinto beans. Four replicate plants per plot were sprayed with EF1127 in June at product rates of 0.1 or 0.2 l/hl, at two water volumes, 200 or 1000 l/ha. Treatments were applied at 210 KPa at a temperature of 18 °C, using a hand-held Azo sprayer fitted with hollow cone nozzles. Bifenthrin (10% EC) applied at 0.04 l/hl in a water volume equivalent to 1000 l/ha, was included in each trial as a control. After treatment, plants were placed on rectangles of capillary matting isolated in water troughs. This prevented mites from escaping and eliminated the use of overhead watering. Plants were retained in a polythene tunnel for the duration of the tests. Counts of motile mites were undertaken 3, 14 and 21 days after treatment. An assessment of mite feeding damage was made 7 and 28 days after treatment on a percentage scale where 0 was equivalent to no damage.

Two-spotted spider mite eggs

A replicated trial was undertaken on strawberry runners with 3-5 expanded true leaves, transplanted into 100 cm^2 pots and artificially infested with two-spotted spider mites. Six days later, plants were sprayed with EF1127 at a rate of either 0.1 or 0.2 l/hl at 210 KPa, using a hand held Azo sprayer fitted with a hollow cone nozzle, at a water volume of 1000 l/ha. Temperature at the time of treatment (July), was 23°C. Immediately after treatment, one replicate leaflet per plant was cleared of sessile and motile two-spotted spider mites and placed, lower surface uppermost on a bed of moist cotton wool in a Petri dish. The perimeter of each leaf was then covered with a 1cm. wide strip of damp cotton wool. Petri dishes were maintained in ambient laboratory conditions with their lids removed. An assessment of hatched eggs was made 3, 7 and 10 days after treatment.

Phytoseiulus persimilis adults

Five replicated trials were undertaken on *Cyclamen persicum* 'Pastel Compacta' to assess the level of contact or persistent activity of EF1127 against the predatory mite *P.persimilis*. In the contact trial, plants were artificially infested with a mixed population of motile two-spotted spider mites and *P. persimilis*, six days prior to treatment. The mean number of *P. persimilis* adults pre-treatment was 7.2/leaf. Five replicate plots were sprayed at 210 KPa using a hand held Azo sprayer fitted with a hollow cone nozzle. EF1127 was applied at product rates of 0.05 or 0.1 l/hl, in a water volume of 200 or 1000 l/ha. Assessments of predator mortality were made one day after treatment.

In persistency trials, five replicate plots were artificially infested with two-spotted spider mite, prior to treatment with EF1127 applied at 0.05 or 0.1 l/hl, in a water volume of 200 or 1000 l/ha. Treatments were made with similar equipment and at equivalent rates to those in the "sister" trial described above. One, three, seven and fourteen days after treatment adult *P. persimilis* were introduced at a target rate of 100 adult mites/plant and predator mortality was assessed the following day.

RESULTS

Selectivity

No phytotoxicity or reduction in vigour was recorded where EF1127 was applied at 0.2 l/hl and at two water volumes, onto any of 29 plant species tested. Potted strawberry runners were also unaffected when treated with EF1127 at this dose rate.

Efficacy

Motile two-spotted spider mites

Against a background of consistently rising numbers of motile two-spotted spider mites, rapid and persistent control was achieved for up to three weeks after treatment with high volume sprays, applied to plants with penetrable leaf canopies.

This result was similar to that achieved with the reference product bifenthrin (10%EC). Percent mortalities of adult and larval two-spotted spider mites, 3 and 14 days after treatment, are given in Tables 1 and 2 below.

TABLE 1. Mean percent control of two-spotted spider mite adults following application of fenazaquin 200 g/l SC, 3 and 14 days after treatment.

Treatment	Rate Product ml/hl	Winter Cherry		Cyclamen		Strawberry		Water Volume l/ha
Days		3	14	3	14	3	14	
Untreated*		0a	0a	0a	0a	0a	0a	
		(4.7)	(6.1)	(9.7)	(10.3)	(12.3)	(18)	
EF1127	100	79b	93b	53b	74b	82b	52b	200
EF1127	200	91c	99b	68b	90bc	88bc	83c	200
EF1127	100	99d	100b	73bc	95c	95c	96c	1000
EF1127	200	99d	100b	85c	97c	94bc	95c	1000
Bifenthrin	40	100d	100b	93c	90bc	87c	87c	1000

* values in parentheses are mean mite numbers in untreated. Values in columns followed by the same letter are not significantly different; P= 0.05 (Duncan)

Table 2 Mean percent control of two-spotted spider mite larvae following application of fenazaquin 200 g/l SC, 3 and 14 days after treatment.

Treatment	Rate Product ml/hl	Winter Cherry		Cyclamen		Strawberry		Water Volume l/ha
Days		3	14	3	14	3	14	
Untreated*		0a	0a	0a	0a	0a	0a	
		(1.2)	(1.4)	(4.1)	(4.3)	(1.6)	(8.1)	
EF1127	100	86b	95b	88b	83b	71b	46b	200
EF1127	200	86b	100b	96b	98b	96b	84c	200
EF1127	100	100b	100b	89b	98b	100b	97c	1000
EF1127	200	100b	100b	96b	100b	98b	97c	1000
Bifenthrin	40	100b	100b	98b	99b	100b	98c	1000

* values in parentheses are mean mite numbers in untreated. Values in columns followed by the same letter are not significantly different; P= 0.05 (Duncan).

The excellent control of motile two-spotted spider mites was reflected in a commensurate reduction of feeding damage on leaves. The extent of the damage appeared limited on *Cyclamen persicum* 'Pastel Compacta', due to the fleshy nature of the leaves. Damage ratings on leaves of the remaining two species, *Solanum capsicastrum* and strawberry, are given below in Table 3.

TABLE 3. *Mean two spotted-spider mite feeding damage on leaves following treatment with fenazaquin 200 g/l SC, 7 and 28 days after treatment.

Treatment	Rate Product ml/hl	Winter Cherry		Strawberry		Water Volume l/ha
Days		7	28	7	28	
Untreated	100	4.2	19.1	20.0	54.1	
EF1127	200	0.8	3.3	12.5	35.8	200
EF1127	100	0	0	7.5	18.6	200
EF1127	200	0	0	3.3	3.6	1000
EF1127	40	0	0	3.7	4.5	1000
Bifenthrin		0	0	3.3	7.3	1000

* Assessed on 0-100 scale where 0 = no damage and 100 = necrotic

Two-spotted spider mite eggs

Ovicidal activity was recorded from both EF1127 treatments with no significant difference between either ($P = 0.05$). Seven days after application, against a background level of 11% eggs unhatched in untreated plots, 34% to 36% control of egg hatch was recorded. This trend continued ten days after treatment. Results are recorded in Table 4.

TABLE 4. Mean % hatch of two-spotted spider mite eggs following application of fenazaquin 200 g/l SC, 3,7 and 10 days after treatment.

Treatment	Rate Product ml/hl	Strawberry runners			Water Volume l/ha
Days		3	7	10	
Untreated*		8a(105)	88a(13)	95a(6)	
EF1127	100	3b(128)	61b(51)	72b(34)	1000
EF1127	200	4b(169)	71b(34)	80b(23)	1000

* values in parentheses are mean unhatched egg numbers in respective plots.

Phytoseiulus persimilis adults

Dose related selectivity against *P. persimilis* was achieved in both the contact and residual tests. Spray treatment of established *P. persimilis* populations caused greater mortality than the introduction of the predator to previously treated plants. Reduced mortality of *P. persimilis* adults was recorded in plots receiving the lowest rates of fenazaquin sprayed in the smallest water volume tested. No residual effect was recorded fourteen days after treatment.

TABLE 5 Mean percent mortality of *P. persimilis* 1, 2, 4 and 8 and 15 days after treatment with fenazaquin.

Treatment	Rate	Contact Trial	Persistency Trials				Water Volume
	ml/hl	1#DAT	2#DAT	4#DAT	8#DAT	15#DAT	l/ha
Untreated*		(7.2)a	(12.8)a	(10.4)a	(11.0)a	(4.7)a	
EF1127	50	76b	26.5b	38.0b	36.0b	8.0a	200
EF1127	100	90bc	75.0c	76.0c	37.0b	0.0a	200
EF1127	50	94c	85.1c	82.0c	53.0bc	10.0a	1000
EF1127	100	99c	96.0c	93.0c	62.0c	0.0a	1000

* values in parentheses are mite numbers/leaf. Values in columns followed by the same letter are not significantly different; P= 0.05 (Duncan). #DAT = days after treatment

DISCUSSION

Blemish-free ornamental crops are of the utmost importance. When tested against twenty-nine species of pot-grown, hardy ornamental and house plants, EF1127 was found to be safe to all tested species at a rate of 0.2 l/hl, a dose in excess of that required for excellent control of motile two-spotted spider mite.

Variation in the speed and extent of two-spotted spider mite control was attributable to the differences in shape and size of the target ornamental species, the dose rate applied and the water volume used. Good coverage of all foliage was shown to be essential for swift and persistent control. A low level of ovicidal activity against two-spotted spider mite was recorded on up to six day old eggs.

Contact treatment of established *P. persimilis*. populations was found to be more harmful than the introduction of mites to previously treated plants. Lower water volumes and commensurately reduced application rates were less damaging to *P. persimilis* populations in both contact and persistency trials. EF1127 demonstrated excellent control of two-spotted spider mite motiles, yet incomplete ovicidal activity coupled with short bioavailability and useful selectivity against *P. persimilis*. These characteristics may prove beneficial in long term, integrated pest management systems in ornamental crops.

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CGA 215'944 - OPPORTUNITIES FOR USE IN VEGETABLES

C. R. FLÜCKIGER, R. SENN AND H. BUHOLZER

Plant Protection Division, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland

ABSTRACT

CGA 215'944 is a new insecticide with a unique mode of action, representing a new class of insect control agents. It was discovered by Ciba-Geigy and is now being developed worldwide. The compound can be used against susceptible and resistant aphids and whiteflies in many different crops. Its is especially useful in vegetables where the compound has been tested since 1987 in W. Europe, the United States, Japan, South East Asia, Africa and Brazil. At rates of 10-20 g AI / 100 l it provides excellent control of aphids such as *Myzus persicae* and *Aphis gossypii*, and against the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum*. CGA 215'944 is harmless to natural enemies and is therefore especially useful in IPM programmes, also in combination with biological control agents. The major use of the compound will be as a foliar spray, but application of granules and seed treatment are also possible.

INTRODUCTION

Aphids and whiteflies have become important pest problems in vegetables worldwide. The severity has increased because many species of both pest groups have become resistant to existing insecticides (Voss, 1988). This has resulted in intensive spray programmes with mainly broad spectrum insecticides, and beneficial organisms were adversely affected. Today, modern pest control programmes emphasise the advantages of natural enemies. CGA 215'944 is safe to beneficials and therefore especially useful in IPM programmes (Flückiger *et al.*, 1992). In some areas, particularly in protected crops, the release of natural enemies has become a feasible addition to chemical control methods. In these cases selective insecticides are essential and CGA 215'944 is a new answer to this demand. It is of unprecedented chemical structure and possesses a new mode of action.

MATERIALS AND TEST TARGETS

CGA 215'944 was tested in three formulations : WP 25, GR 5, WS 50

Aphids : The aphid species that were tested in the field represent the major aphid problems in vegetables worldwide. CGA 215'944 was tested in different vegetable crops (Table 1)

TABLE 1. Aphid pests tested

Pest	Crops	Countries*	No. of trials
Myzus persicae (Green peach aphid)	Tomato, pepper, eggplant cabbage, potato	CH, E, F, I, J, USA	43
Aphis gossypii (Melon aphid)	Okra, eggplant, melon, cucumber potato	E, ET, F, I, J	18
Aphis fabae (Bean aphid)	Broad bean	F, I, CH	30
Brevicoryne brassicae (Cabbage aphid)	Cabbage, cauliflower, oil seed rape, brussels sprouts	BR, CH, ET, F, ZA	23
Macrosiphum euphorbiae (Potato aphid)	Potato	CH, I	17
Acyrthosiphon pisum (Pea aphid)	Pea, alfalfa	CH, USA	4

* countries: BR, Brazil; CH, Switzerland; E, Spain; ET, Egypt; F, France; I, Italy; J, Japan; ZA, South Africa.

Whiteflies : The two most important species of whiteflies in vegetables are the sweet potato whitefly (*Bemisia tabaci*) and the greenhouse whitefly (*Trialeurodes vaporariorum*). They were tested on tomatoes, peppers, eggplants and beans in Switzerland, Spain, Egypt, Italy, Brazil and Japan and on ornamentals in the USA.

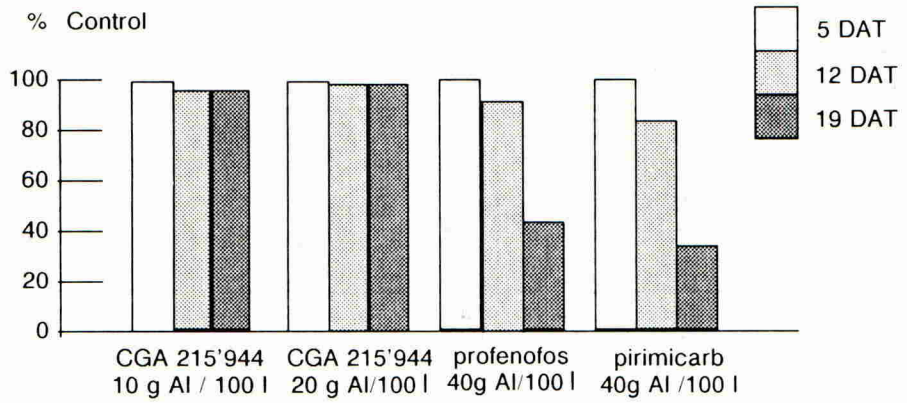
FIELD TRIALS AGAINST APHIDS

Myzus persicae

Resistance of this pest to standard insecticides has become an increasingly important problem in vegetables (Voss, 1987). Although distributed worldwide, resistance reports are concentrated in Europe. The general trend has been for initial resistance to organophosphates, to extend to carbamates and then to pyrethroids. The efficacy of the standard products used in our trials varied greatly from trial to trial depending on the resistance status of *M. persicae* in that particular location.

CGA 215'944 successfully controlled *M. persicae* at a rate of 10 g AI / 100 l (Figure 1). The degree of resistance of *M. persicae* towards OP and carbamate insecticides was not investigated. In other trials successful control was obtained with the granule formulation at the rate of 0.05 g AI per cabbage plant.

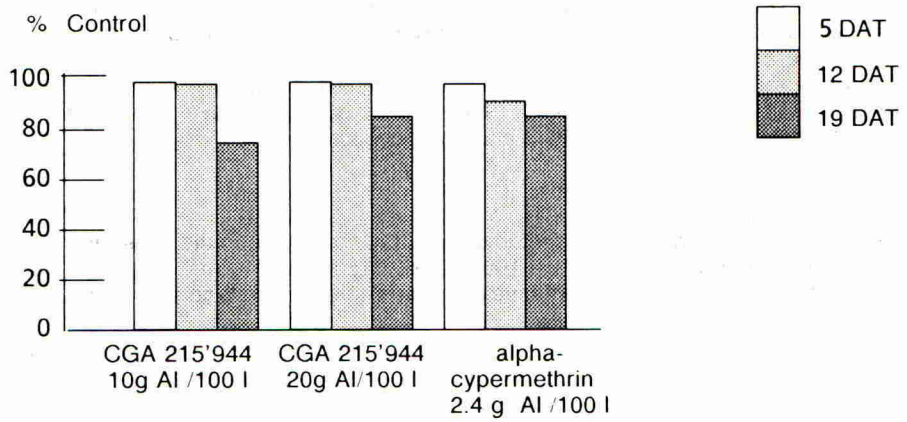
Figure 1: Control of *Myzus persicae* on tomatoes (Spain, 1990)



Aphis gossypii

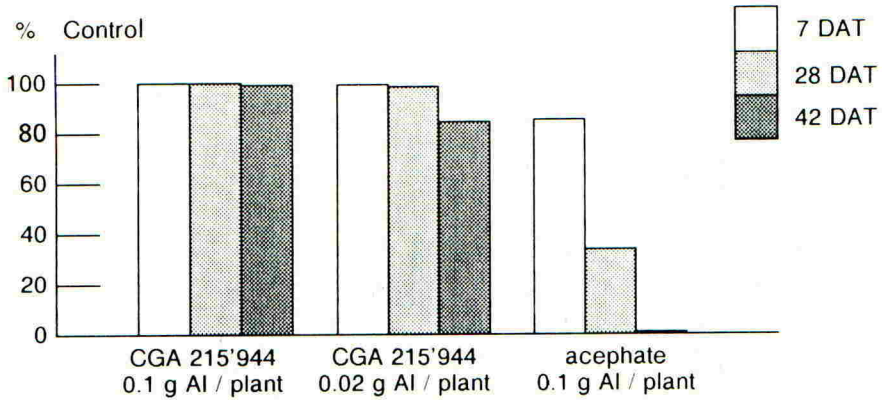
This pest has become important very recently in many vegetable areas due to resistance problems with standard products. CGA 215'944 successfully controlled *A. gossypii* at 10 g AI / 100 l (Figure 2).

Figure 2: Control of *Aphis gossypii* on cucumbers (Italy, 1990)



Granules of CGA 215'944, applied at planting time, controlled *A. gossypii* for 42 days in 3 trials (Figure 3).

Figure 3: Control of *Aphis gossypii* on cucumbers with granules (Japan, 1991)



Other aphids

CGA 215'944 also performed well against *Aphis fabae*, *Brevicoryne brassicae*, *Macrosiphum euphorbiae* and *Acyrthosiphon pisum* at rates of 10-20 g AI / 100 l.

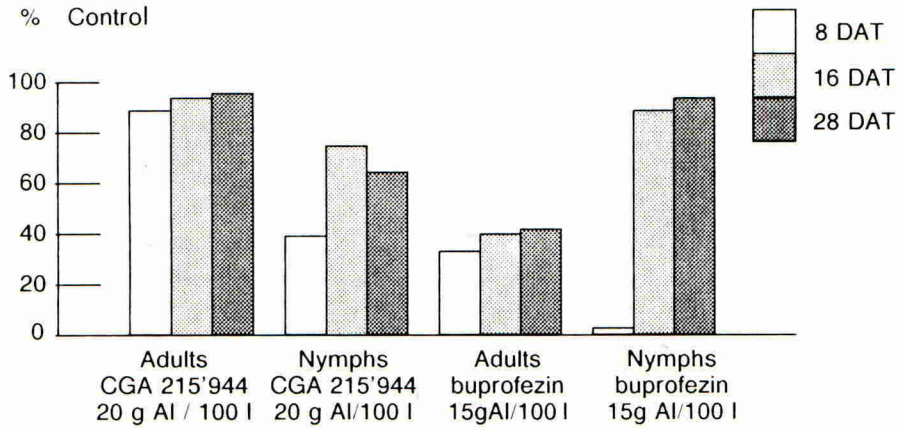
FIELD TRIALS AGAINST WHITEFLIES

Whiteflies have become a major pest in vegetables as a consequence of intensified crop production. A wide spectrum of insecticides frequently used to maintain high crop yield and quality exerted a strong selection pressure on whitefly populations. Resistance of whiteflies against standard insecticides is common today in many areas. With the growing trend of farmers to apply IPM principles in plant protection, more specific insecticides are needed. Thus the chemical control of whiteflies with broad spectrum insecticides is not acceptable when the two-spotted mite *(Tetranychus urticae)* is controlled by the predatory mite (*Phytoseiulus persimilis)* (Koppert, 1978). When whiteflies are prevailing, CGA 215'944 offers control possibilities without harming beneficials.

Trialeurodes vaporariorum

The most important whitefly in vegetables has traditionally been the greenhouse whitefly (*T. vaporariorum)*. At 20 g AI / 100 l CGA 215'944 performs very well against populations of this pest (Figure 4) that have become resistant to standard insecticides in many areas (Voss, 1987).

Figure 4: Control of *Trialeurodes vaporariorum* on tomatoes (Italy, 1990)



Bemisia tabaci

Recently the sweet potato whitefly *(B. tabaci)* has become a key pest in many areas worldwide and has caused considerable yield losses in vegetables. CGA 215'944 performed very well against this pest at the rate of 20 g AI / 100 l (Figure 5).

Figure 5: Control of *Bemisia tabaci* on eggplants (Egypt, 1990)

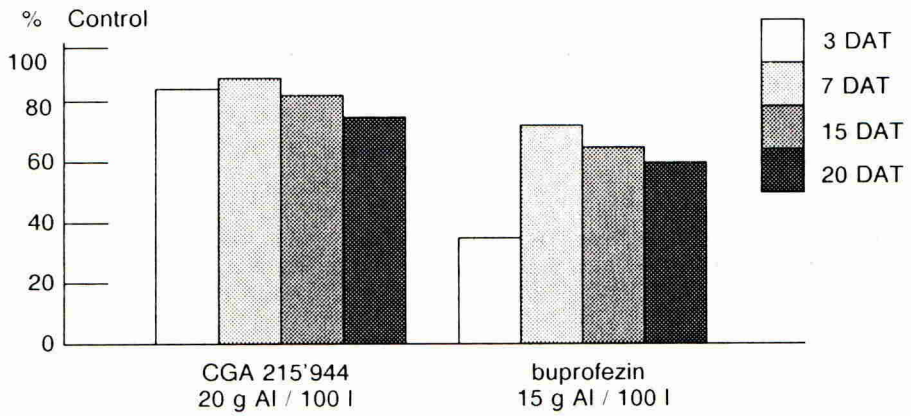
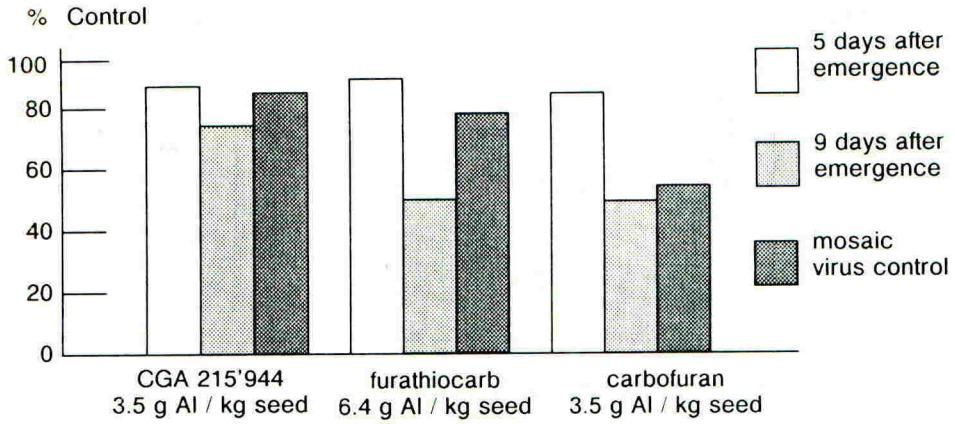


Figure 6 shows the results of a trial where CGA 215'944 was applied as a seed treatment. It effectively reduced the pest and the mosaic virus that is transmitted by *B. tabaci* at the rate of 3.5 g AI / kg seed (Figure 6).

Figure 6: Control of *Bemisia tabaci* on french beans and reduction of mosaic virus symptoms by seed treatment (Brazil, 1991)



CONCLUSION

Extensive field evaluations have demonstrated that CGA 215'944 is very well suited as a control agent against aphids and whiteflies in vegetables. Having a new mode of action, the compound shows excellent activity also against resistant aphids and whiteflies. Being safe to natural enemies CGA 215'944 is also especially useful in IPM programmes.

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MATING DISRUPTION UTILIZING LEPIDOPTEROUS SEX PHEROMONES: THREE YEARS OF TESTING IN APPLE ORCHARDS IN THE NETHERLANDS

P. VAN DEVENTER, A.K. MINKS, L.H.M. BLOMMERS

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

U. NEUMANN, K. JILDERDA

BASF, Agricultural Research Station, Limburgerhof, Germany, resp. Arnhem, the Netherlands

ABSTRACT

A "twin-dispenser" formulation developed by BASF, consisting of pheromone components of four lepidopterous orchard pests, was tested in the Netherlands between 1989 and 1991. One of the "twin dispensers" released the one-component pheromone of the codling moth, *Cydia pomonella*, while the other released a single pheromone component common to many leafroller species, such as *Adoxophyes orana*, *Archips podana* and *Pandemis heparana*.
Mating disruption, as measured by, a) reduced numbers of males captured in pheromone-baited traps, b) decreased numbers of larvae and pupae collected in field samples, and c) improved quality of fruit at harvest, occurred for codling moth as well as for the above-mentioned three leafroller species, and satisfactory control was obtained in all test orchards.

INTRODUCTION

In integrated pest management (IPM)-treated apple orchards in Europe selective control of codling moth and leafrollers is an essential requirement. As only a few selective insecticides are available, other control methods such as mating disruption by means of permeating the air with sex pheromones would be a welcome addition to the range of control possibilities. Good control by mating disruption was recently reported with the "twin-dispenser" formulation developed by BASF Aktiengesellschaft (Germany), for *Cydia pomonella* (Charmillot, 1990; Neumann *et al.*, 1990) and for *Adoxophyes orana* (Charmillot, 1989; Neumann *et al.*, 1990).

We present here the results of a 3-year test of the effectiveness of BASF twin-dispensers in apple orchards in the Netherlands. Mating disruption was tested on the codling moth, *C. pomonella* by using 'codlemone', its major sex pheromone component, and on the leafroller moths, *A. orana, Archips podana* and *Pandemis heparana*, by using a single compound common to the pheromone blend of all three test species.

MATERIALS AND METHODS

Experimental orchards

There were three orchards used for our tests at: 1) Eck en Wiel, 2) Maurik and 3) Kesteren. All orchards were located 20-30 km southwest of Wageningen. Orchard 1 consisted of 2.3 ha 7-year old spindle bush apple trees (cvs Cox's Orange Pippin, Elstar, and Gloster) and had been under IPM treatment since 1986. A block of 1 ha of apple trees, similar to those treated with pheromone and situated at the north side with a buffer zone of 25 m, served as the control plot. As the latter area was felled in 1990 an adjacent block of pears served as the control plot in 1991, although it was treated with fenoxycarb against leafrollers. Orchard 2 was 1.6 ha and was planted with 10-year old spindle bush apple trees (cvs Cox's O.P., Jonagold, James Grieve and Suntan), and IPM treated since 1988. This orchard was surrounded by grassland along three sides, while on the fourth side a 9-year old apple orchard of 0.7 ha situated at a distance of 44 m served as the

control plot in 1989 and 1990. In 1991 this area had to be treated with diflubenzuron against codling moth and with fenoxycarb against leafrollers, thus serving as a chemically-treated control plot. Orchard 3 comprised 1.5 ha of spindle bush apple trees, 7-12 years old (cvs Cox's O.P. and Elstar). It had been under an IPM regime since 1986. In this orchard a block of 1 ha of apple trees similar to those in the treated plot served as the control plot. A buffer zone of 25 m was situated between the treated and control plot.

Mating disruption treatment

The "twin-dispenser" formulation produced by BASF-Germany was used in our tests. It is a red coloured plastic matrix with two containers. One container was filled with codlemone (*E,E*-8,10-dodecadien-1-ol), the major component of the sex pheromone of the codling moth, *C. pomonella*, (in 1989 with 220 mg, in 1990 with 370 mg and in 1991 with 310 mg). Twenty percent of tetradecan-1-ol acetate were added to keep the codlemone liquid. The second container was supplied with *Z*-11-tetradecen-ol acetate, a compound present in the pheromone blend of many economically important leafroller moths such as *A. orana, P. heparana* and *A. podana*, (in 1989 with 270 mg, in 1990 with 400 mg and in 1991 with 300 mg). According to the recommendations of BASF, 500 dispensers were used per ha (approx. 5 m spacing) and installed at a height of 1.7 m in the trees. Along the borders of the treated plots dispensers were suspended at double density (2.5 m spacing). Additional dispensers were installed in adjacent orchards in a zone of 25 m around the treated plots.

Insecticidal/acaricidal treatments

Although the owners applied IPM in the test orchards and agreed to a minimum use of pesticides, it was necessary to occasionally use control measures against other insects or mites. Sprays mainly took place against green capsid bug (propoxur), mussel scale (propoxur or phosalone), aphids (pirimicarb) in all plots. Fenoxycarb was applied against leafrollers in the control plot and buffer zone in orchard 1 on 5 May 1989 and in orchard 2 on 24 May 1989.

Assessment of mating disruption effect.

1) Pheromone trap captures (codling moth and leafrollers). Sex pheromone traps were used to monitor trap catch reduction. In all three orchards, two Delta-type traps (with replaceable sticky bottom) were suspended in the trees at a height of 1.75 m, and evenly distributed in each treated as well as each control plot. Traps, baited with 1 mg sex pheromone, were used for each of the four following tortricid species: *C. pomonella, A. orana, P. heparana, A. podana* and also, for the eye-spotted budmoth, *Spilonota ocellana*. Traps and dispensers came from the Research Institute for Plant Protection, Wageningen, the Netherlands. All traps were installed well in advance of the start of the flight of the moths to be monitored. Trap catches were checked weekly. The dispensers were refreshed around mid-July.
2) Mid-season check of fruit (codling moth). Two mid-season inspections of fruit were made by checking randomly chosen samples of 1000 apples from all plots for entries of *C. pomonella* larvae.
3) Damage assessment of fruit at harvest (codling moth). Randomly chosen samples of 2000 apples were taken from all plots to inspect them for damage by larvae of *C. pomonella*. Premature fruit drop was also recorded.
4) Check of hibernating larvae (codling moth). The presence of hibernating *C. pomonella* larvae was checked in just the treated plots by placing standard treebands, made of corrugated cardboard, jute and waxy paper, around 60 trees per site. The treebands were placed in early summer, they were collected and checked for larvae in the following winter.

5) Spring inspection of flower clusters (leafrollers). The spring population of leafrollers in all plots was estimated by checking 300 randomly chosen flower clusters per plot for larvae. This check was done twice or three times per season: in April-May.
6) Mid-season check of growing shoots (leafrollers). The numbers of the first-generation larvae of *A. orana* in all plots were estimated by inspecting 800 randomly chosen growing shoots per plot. This was done once or twice per season around mid-July.

7) Damage assessment of fruit at harvest (leafrollers). Randomly chosen samples of 2000 harvested apples from both treated and control plots were checked for leafroller damage (samples including premature fruit drop).

RESULTS

Pheromone trap captures of codling moth

Table 1 shows the captures of male codling moths in the sex pheromone traps. A substantial reduction in captures can be seen in the treated plots, varying from 80.5% (orchard 2 in 1990) to 96.8% (orchard 1 in 1989). It should also be noted that the captures in orchard 3 were much higher than in the other orchards due to a high codling moth infestation migrating from a neighbouring orchard.

Table 1: Total number of male codling moths caught in pheromone treated and untreated control plots: numbers in 2 sex pheromone traps per plot in 3 successive seasons (1989-1991).

	1989		1990		1991	
orchard/plot	phero.	contr.	phero.	contr.	phero.	contr.
1.	2	61*)	5	26	5	95
2.	1	27	17	87	4	84*)
3.	6	105	21	121	36	232

*) = fenoxycarb treated control plot.

Mid-season check of fruit for codling moth

Samples of 1000 young apples per plot were checked for infestation by codling moth larvae twice in the middle of the growing season. Table 2 presents the results, from which it can be seen that the infestation level was low, and that the number of entries in the treated plots tended to be lower. In orchard 3 on 15 August 1991, an increase in the number of infested apples was noted, in particular in the control plot. In orchard 1 no observations were made on 13 August 1990, nor in 1991 on 25 July and 15 August in the control plots.

Table 2: Numbers of codling moth entries counted in mid-season samples of 1000 apples per plot in pheromone treated (ph) and untreated (co) plots in 1989-1991.

	1989				1990				1991			
	11 July		25 July		26 July		13 Aug.		25 July		15 Aug.	
orchard/plot	ph	co	ph	co	ph	co	ph	co	ph	co	ph	co
1.	0	0*	0	0*	0	0	-	-	0	-	1	-
2.	1	2	2	6	3	6	4	13	0	1	1	0*
3.	0	2	1	2	0	4	2	10	0	5	12	58

* = fenoxycarb treated control plot
- = no observations

Codling moth damage on harvested fruit

Table 3 presents the percentage of fruit damaged by codling moth larvae in samples of 2000 harvested apples taken from each plot in the three experimental orchards. The damage levels were generally low, except in 1991 in the control plot of orchard 3. In 1989 damage in the pheromone treated plots was higher in orchards 2 and 3, but this reversed in 1990 and 1991. Unfortunately in 1990 no observations could be made in orchard 1 due to severe hail damage in the last week of August and untimely felling of trees in the control plot. In 1991 harvest of apples in the control plot of orchards 1 and 2 took place before they could be checked for damage.

Table 3: Percentages of codling moth entries in ± 2000 harvested apples in pheromone treated and untreated control plots in 3 successive seasons (1989-1991).

	1989		1990		1991	
orchard/plot	phero.	contr.	phero.	contr.	phero.	contr.
1.	0.0	0.0*)	-)	-)	0.3	-)
2.	0.9	0.5	0.2	2.3	0.3	-)
3.	1.7	0.6	0.6	1.4	1.4	6.6

*) = fenoxycarb treated control plot
-) = no observations

Table 4: Total captures of 4 leafroller species in pheromone treated and untreated control plots supplied with 2 sex pheromone traps per plot in 1989-1991.

	1989		1990		1991	
species/ orchard	phero.	contr.	phero.	contr.	phero.	contr.
A.orana						
1.	0	14*)	0	16	0	5*)
2.	2	97	1	25	0	48*)
3.	2	95	0	22	0	25
P.heparana						
1.	1	5*)	1	22	4	37*)
2.	1	21	0	12	0	20*)
3.	3	51	0	13	8	61
A.podana						
1.	0	8*)	0	39	0	115*)
2.	0	139	0	185	0	147*)
3.	0	78	0	94	0	146
S.ocellana						
1.	68	47*)	64	41	149	111*)
2.	70	173	30	89	47	177*)
3.	325	160	164	75	259	225

*) = fenoxycarb treated control plot

Pheromone trap captures of leafrollers

In Table 4 captures of four species of leafrollers are presented. The reduction in trap catches of all three pheromone treated species was evident, ranging from 86.9% (1991 in orchard 3 for *P. heparana*) to 100% in all cases for *A. podana* and in several other cases for *A. orana* and *P. heparana*. No reduction in catches could be recorded for *S. ocellana*, which is not surprising because this moth species was not included in the treatment.

Check of growing shoots for leafrollers

During inspection of first generation *A. orana* larvae by checking a sample of 800 growing shoots per plot in the three orchards in July from 1989-1991 hardly any larvae could be found, neither in the treated nor in the control plots.

Leafroller damage on harvested fruit

The total leafroller damage found in samples of 2000 harvested apples from each plot in the three experimental orchards is shown in table 5. Damage levels in the treated plots were in all cases lower than in the controls in all 3 years. Unfortunately no observations could be made in the control plot of orchard 1 in 1990 and 1991, nor in the control plot of orchard 2 in 1991 for the same reasons as stated for the codling moth.

Table 5: Percentages of total leafroller damage found on ± 2000 harvested apples in pheromone treated and untreated control plots in 3 successive years (1989-1991).

	1989		1990		1991	
orchard/plot	phero.	contr.	phero.	contr.	phero.	contr.
1.	0.05	0.27*)	0.13	-)	1.37	-)
2.	0.90	1.37	0.26	1.31	0.12	-)
3.	1.03	1.45	0.12	1.60	2.50	4.73

*) = fenoxycarb treated control area
-) = no observations

DISCUSSION

We can conclude that the mating disruption treatment with the twin-dispenser formulation resulted in satisfactory control of codling moth and leafrollers in all 3 test orchards throughout the 3 years. The results obtained for the leafrollers were particularly encouraging, because they clearly showed that it is possible to disrupt more than one species by using one single pheromone component common to all three species instead of the three different complete pheromones of each of the three species. With regard to the codling moth, damage of harvested fruit in the treated plots was kept at an economically-acceptable level throughout the test (Table 3). In 1989 there were more codling moth entries in the treated plots of orchards 2 and 3 than in the controls, probably because of premature termination of pheromone evaporation from the dispensers in August. No replenishment was available for the rest of the season. Tables 1-3 also show that in orchard 3 the codling moth population increased continuously, in particular in the control plot, due to adjacent highly infested orchards. Despite this unfavourable situation, control in the treated plot remained remarkably good, whereas damage in the control plot increased far beyond acceptable limits. Acceptable damage levels in harvested apples (Table 5) were also obtained for the
leafrollers, although it did not fully meet our expectations when taking into account the favourable data collected earlier in the season, as shown in Table 4 and in the text on p.5. The problem here is that leafroller damage cannot be distinguished for the different species. It may well be that most leafroller damage must be attributed to *S. ocellana*. This moth species, not utilizing *Z*-11-tetradecen-1-ol acetate as a pheromone component and thus escaping mating disruption, occurred in large numbers throughout the test period

(see Table 4). Inclusion of *S. ocellana* in future mating disruption formulations is highly recommended.

In our tests initial pheromone quantities in the BASF-formulation were rather high, but for *C. pomonella* 220 mg/dispenser was not sufficient for the whole season in 1989. Analysis of the dispensers showed that in 1990 on average only 140 mg out of the initial 370 mg, and in 1991 154 mg out of 300 mg of the *C. pomonella* pheromone evaporated (0.96 mg/day, resp. 0.7 mg/day). For the leafroller component the evaporation rate was 154 mg out of the initial 400 mg in 1990, and 204 mg out of 300 mg in 1991 (1.05 mg/day, resp. 1.30 mg/day). As economic considerations are of crucial importance for further development of this technique, we recommend testing this formulation at a lower density (e.g. 300 dispensers/ha). This will save on the number of dispensers, which are rather expensive.
One of the major conditions for successful mating disruption is a low intitial population of the insect species to be controlled. For this purpose we used treebands to get an idea of the number of overwintering codling moth larvae and a spring inspection of flower clusters to check the number of leafrollers. The results indicate rather low numbers throughout the test period, so that our tests could proceed without the need of applying corrective measures.

Finally, it is worth mentioning that the pesticide treatments occasionally applied to other insect and mite pests did not have any detrimental effect on the outcome of our tests.

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FIELD SELECTION OF THE PREDATORY MITE *TYPHLODROMUS PYRI* FOR RESISTANCE TO PYRETHROIDS

J.D. FITZGERALD, M.G. SOLOMON

Horticulture Research International, East Malling, West Malling, Kent ME19 6BJ.

ABSTRACT

Over a period of 6 years, populations of *Typhlodromus pyri* have been subjected to applications of various rates of deltamethrin in the field at HRI East Malling. *T. pyri* was virtually eliminated from plots receiving 6.3 g AI/ha deltamethrin, but persisted throughout the trial at low levels in plots receiving 3.1 and 1.6 g AI/ha. Numbers of *T. pyri* in plots treated with 0.8 g ai/ha deltamethrin were similar to numbers in untreated control plots.

In bioassays of mites collected from plots that had been treated with the three lowest rates of deltamethrin and the untreated plots, the 24 hr LC_{50} for the strain that had received 3.1 g AI/ha deltamethrin in the field was 3.75 times higher than that obtained for the strain from the untreated plot. The LC_{50}s of the other two strains were intermediate in value.

INTRODUCTION

The fruit tree red spider mite *Panonychus ulmi* is a potentially serious pest in apple; resistance has developed in this species to most of the acaricides used to control it (Cranham & Helle, 1985). Another important mite pest on apple is *Aculus schlechtendali*, the apple rust mite. Feeding by large numbers of this mite on developing fruitlets early in the season has been shown to cause russeting of the fruit (Easterbrook & Fuller, 1986).

In the UK, integrated pest management in apple is based on the regulation of phytophagous mites by the predatory mite *Typhlodromus pyri*. The strains of *T. pyri* found in many commercial orchards in SE England are resistant to most organophosphate insecticides (OPs) and carbaryl (Kapetanakis & Cranham, 1983; Solomon & Fitzgerald, 1984) so these chemicals can be used to control other pests when necessary, without affecting biological control of phytophagous mites (Solomon, 1987; Solomon *et al.*, 1993). Synthetic pyrethroids (SPs) have a lower mammalian toxicity than OPs and carbamates, so are safer to use. However, these chemicals are more toxic to phytoseiids than to tetranychid mites and have a stimulatory effect on tetranychid fecundity (Gerson & Cohen, 1989), so their use in apple has often resulted in mite management problems. The aim of the current trial was to attempt to induce SP resistance in OP-resistant strains of *T. pyri*, and thus to increase the range of available insecticides compatible with IPM.

MATERIALS AND METHODS

Deltamethrin (Decis 2.5% e.c. Hoechst) was applied at 6.3, 3.1, 1.6 and 0.8 g AI/ha by tractor-drawn air-assisted power sprayer. Each rate was applied to 2 replicate plots of 18 trees; two further plots were left unsprayed as a control. Treatments were applied twice in 1986 and three times per year in 1987-1991. A sample of 25 leaves was taken from each plot before, and about a week after, each deltamethrin application. Leaves were taken to the laboratory and mites removed onto sticky discs using a mite brushing machine (Leedom Engineering, California, USA). Mites were counted under a stereomicroscope. *T. pyri* was collected from plots in which reasonable numbers had survived in September of each year (except 1989) and kept on artificial culture plates until numbers were sufficient to enable bioassays to be undertaken. Mites from plots treated with different rates of deltamethrin in the field were cultured separately.

In 1987 *T. pyri* was bioassayed by attaching individuals, dorsal side down, on double-sided sticky tape on glass slides. Twenty adult females were used for each concentration of deltamethrin. In subsequent years the bioassay technique was modified to allow the use of survivors for setting up new cultures. In 1988 mites were treated on leaf discs, with thirty mites used per treatment. In 1990 forty mites per treatment were sprayed on plastic arenas confined by an annulus of wet filter paper, and in 1991 the same procedure was used except that twenty mites were sprayed per treatment. Deltamethrin was diluted to the concentrations shown in Table 1 using 0.01% aqueous Agral solution (ICI Agrochemicals), and 0.01% Agral was used as the non-toxic control. Mites were sprayed with 3 ml of pesticide or Agral under a Potter tower. This quantity of liquid gives a spray deposit equivalent to high volume spray deposits in the field. In all bioassays the spray deposit was allowed to dry for *c.* 1 h. Where leaf or arena was used as the substrate for the bioassay, the mites were then transferred to clean, sub-divided culture plates and provided with pollen as a food source. Mites were maintained in a CT room at 20°C with 18L/6D photoperiod and mortality assessed after 24 h. The data obtained in 1990 were analysed using the Maximum Likelihood Programme for probit analysis (Lawes Agricultural Trust) and an LC_{50} derived for each strain of *T. pyri*.

RESULTS

At the beginning of the trial in July 1986, *T. pyri* was present in all plots at about 1/leaf. Very few *T. pyri* were found in the plots treated with 6.3 g AI/ha deltamethrin throughout the trial. On the plots treated with 3.1 and 1.6 g AI/ha numbers of *T. pyri* declined initially but persisted at low levels during 1987 and gradually increased, with maximum numbers of 1/leaf in the 3.1 g AI/ha treatment (Figure 2) and 0.8/leaf in the 1.6 g AI/ha treatment (Figure 1) in 1991. *T. pyri* survived in plots treated with 0.8 g AI/ha deltamethrin; numbers were similar to those in the untreated plots, with maximum numbers exceeding 1/leaf.

TABLE 1. 24 h % mortality of *T. pyri* strains collected from plots treated with different rates of deltamethrin and assayed with deltamethrin

Year	Field rate (g AI/ha)	Conc. deltamethrin (ppm AI) in bioassay					
		0	0.39	0.78	1.6	3.1	6.3
1987	untreated	10	--	90	100	--	--
	0.8	10	--	25	60	--	--
1988	untreated	3	--	35	67	--	--
	0.8	--	--	31	46	67	--
	1.6	--	--	8	37	59	--
1990	untreated	5	11	58	82	94	--
	0.8	0	6	43	53	76	--
	1.6	0	10	39	42	58	--
	3.1	0	3	18	21	47	--
1991	untreated	0	--	--	90	100	100
	3.1	5	--	--	12	25	68

Figure 1. Numbers of *T. pyri* per 50 leaf sample collected from plots treated with 1.6 g AI/ha deltamethrin during 1986-1991. Arrows indicate dates of deltamethrin applications.

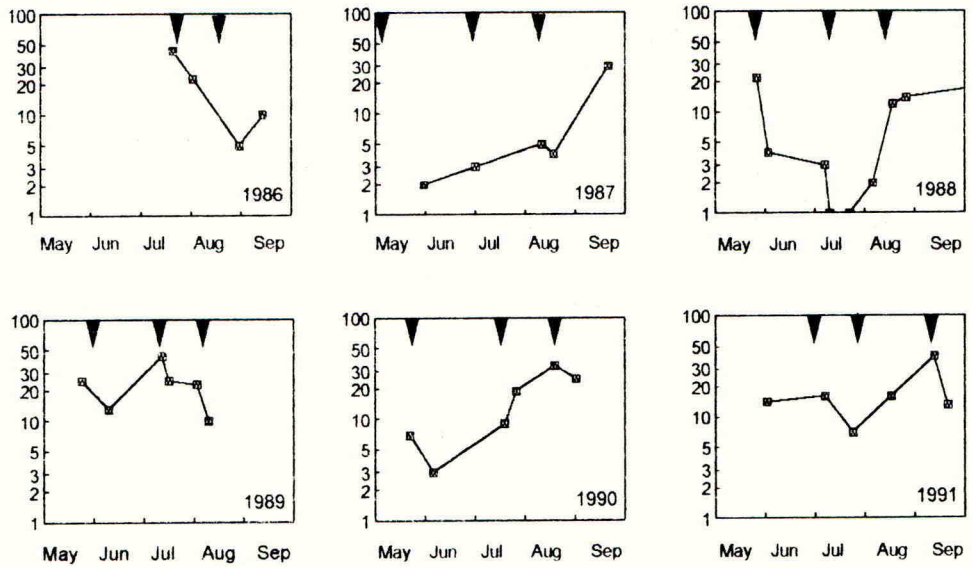
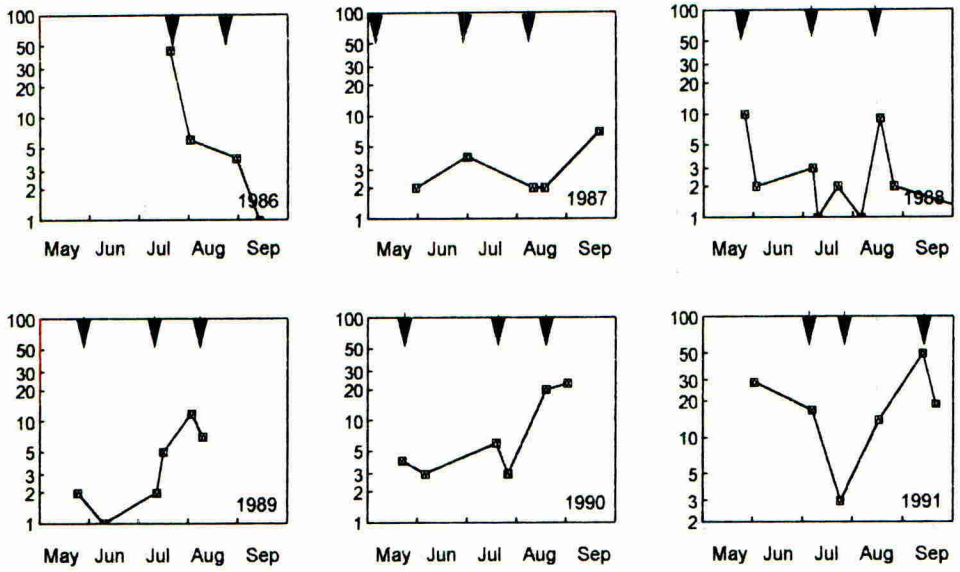


Figure 2. Numbers of *T. pyri* per 50 leaf sample collected from plots treated with 3.1 g AI/ha deltamethrin during 1986-1991. Arrows indicate dates of deltamethrin applications.



In 1990 and 1991 *P. ulmi* numbers in the lowest rate deltamethrin plots and the untreated plots were very low. However, *A. schlechtendali* numbers were generally over 20/leaf in mid-August on all plots throughout the trial, so prey numbers were not limiting *T. pyri* populations.

The results of bioassays of *T. pyri* with deltamethrin are shown in Table 1. In each year there was a progressive decrease in mortality at any one deltamethrin concentration from the mites collected from the control plots to mites collected from plots treated with higher concentrations of deltamethrin in the field. In 1987 mites from the untreated and the lowest rate deltamethrin plots were bioassayed (Table 1); survival of mites on all other plots was very low. By 1991, mites were numerous enough in the plots treated with 3.1 g AI/ha for collection and culturing.

Probit analysis of bioassays undertaken in 1990 gave an LC_{50} of 0.40 ppm AI for the strain collected from the untreated plots and 1.50 ppm ai for the strain collected from the plots treated with 3.1 g AI/ha. The LC_{50}s of the other two strains were intermediate in value.

DISCUSSION

There appeared to be a gradual increase in resistance within a strain of *T. pyri* with time (Table 1 and Figures 1 & 2). In 1987 there were few survivors in the plots treated with 3.1 g AI/ha; after the final deltamethrin application they were present at only 0.02/leaf, but after the final spray in 1991 they were present at 0.4/leaf (Figure 2). In a pilot selection programme two hundred and twenty adult females from these plots were treated with 3.1 ppm AI deltamethrin. There was 39% mortality, which was in line with the results of bioassays of this strain in 1990 and 1991 (Table 1).

The bioassay results in 1990 indicated that the LC_{50} for deltamethrin for the different strains of *T. pyri* was related to the field exposure concentration of each strain. Markwick (1986) selected New Zealand strains of *T. pyri* with cypermethrin, increasing the selection pressure when *c.* 25% of mites survived any one rate of insecticide. She obtained a 10-fold increase in resistance over a susceptible strain with 6 selections at increasing rates. The four-fold increase in resistance to deltamethrin indicated by the 1990 bioassay results was obtained with 11 selections, but without any increase in insecticide concentrations. The LC_{50} obtained for the strain of *T. pyri* treated with 3.1 g AI/ha in the field was 1.5 ppm AI. The probit line calculated for this strain suggested a mortality of *c.* 80% is to be expected when mites are exposed to a concentration of 4.4 ppm AI deltamethrin, which is equivalent to the high volume field concentration.

Future work will include both laboratory and field selections of strains of *T. pyri* with increased rates of deltamethrin with the objective of producing a strain that can survive full field rates of deltamethrin.

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THE ROLE OF THE OLFACTORY SYSTEM OF THREE CROP PESTS: APHID, WHITEFLY AND THRIPS IN THE DETECTION OF SEMIOCHEMICALS.

M. ANDERSON, P. EDMUNDS, H.E. MELLOR, M.H. WALBANK

School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT

ABSTRACT

An investigation of the gross morphology and fine structure of the antennal sensilla of the lettuce aphid, three whitefly species and the western flower thrips has revealed some unique chemosensory structures. Within the brain of the aphid clear regions have been located where the information from the antennal sense organs is likely to be processed.

BACKGROUND and INTRODUCTION

Aphids, whiteflies and thrips rank among the most destructive insect pests attacking a wide variety of glasshouse and field crops with the total value of the crop threatened representing many millions of pounds. All three insect groups feed by sucking plant fluids and this can cause damage in itself when the insects are present in large numbers. Indirect damage is caused by the production of the excretory product honeydew (aphids and whitefly) which acts as a suitable growth medium for moulds and other plant fungi. In addition, the pest status of these 3 insect groups is greatly enhanced by their ability to transmit serious disease causing agents such as viruses and bacteria.

Currently aphids, thrips and whiteflies are controlled primarily by conventional insecticides. There are a number of reasons why new control measures are being sought. First of all public opinion is now such that any chemically treated food-product is considered less desirable for consumption whereas products which have been produced in a "chemical-free" way are deemed more acceptable. This, linked with overuse of chemicals which has contributed to resistance development, population increase due to the removal of the natural enemies and environmental damage has led to a desire to reduce the use of chemical insecticides. However, it has to be appreciated that in order to maintain high agricultural and horticultural output there has to be some alternative control measures developed to use against these insect pests.

Biological agents - insect parasitoids, mites, fungi, etc., - have been used to control aphids, thrips and whiteflies (e.g. van Emden, 1989). The successes of using these natural enemies are, however, limited since their efficiency depends upon a large number of parameters such as climatic conditions, (e.g. Russel, 1962), host specificity, (e.g. Hart *et al.*, 1978), correct timing of natural enemy releases and nature of continuity of the crop.

Integrated programmes, in which two or more control methods are combined, have been developed for reducing populations of whiteflies, thrips and aphids. Examples of these are the early attempts at integrated control of whitefly undertaken by McClanahan (1970), who investigated the suitability of oxythioquinox, several organophosphates and endosulfan for a combined use with release of chalcid parasitoids. Likewise integrated control of *T.vaporariorum* is practised in the former USSR by integrating the use of *Encarsia* sp and insecticides such as pirimiphos methyl (Popov & Zabudskaya, 1984). Being insensitive to many insecticides, fungi can be used with or shortly after chemical treatments. Hall (1985) suggested the use of insecticides on high whitefly populations prior to the use of fungi.

Host location by insects.

To locate their host plants and conspecifics, insects respond to a number of cues which are detected by chemosensory structures (generally located on their antennae) or by visual structures. It has been thought for a number of years that manipulation of this behaviour could be used to develop new control methods. The visual response of insects has been exploited in the production of a variety of traps and lures, but it has been recognised that such traps are generally only useful as monitoring devices. The development of a trap for crop pests combining olfactory with visual stimuli has yet to be proven successful.

At the University of Birmingham studies of the antennae of aphids have identified sense organs which detect a large number of plant volatile chemicals (Bromley & Anderson, 1982; Anderson & Bromley, 1987). This work has been continued in collaboration with Rothamsted Experimental Station and has confirmed the important role of olfactory sensilla in determining aphid behaviour (see Pickett *et al.*, 1992)

This paper reviews morphological studies of the antennae of whiteflies and thrips to ascertain the structure and function of the antennal sensilla. In addition, we report on studies of the aphid olfactory system, in particular on the connections of the olfactory processes within the central nervous system. All these studies form part of a comprehensive investigation of the host-finding behaviour of aphids, whiteflies and thrips and how this behaviour can be manipulated in the development of suitable control methods.

RESULTS AND DISCUSSION

The antennal morphology of whiteflies

Three species of whitefly have been studied:- the glasshouse whitefly, *Trialeurodes vaporariorum*, the cabbage whitefly, *Aleyrodes proletella*, and the tobacco whitefly, *Bemisia tabaci*. The antenna of the adult whitefly consists of a basal scape, a bulbous pedicel and a long flagellum composed of five segments. The final segment is tapered and terminates in a long hair-like spike.

Six types of sensillum were found on the antennae of the three species studied:-trichoid sensilla; campaniform sensilla; chaetae; sensory cones; primary sensilla; "subsidiary" sensilla. Sensory cones, primary sensilla and the subsidiary sensilla are unique to the antennal flagellum of the whitefly and because of their relative importance are discussed here.

The sensory cones with their distinctive shape are extensively pitted externally. The primary sensilla are composed of a central vertically, grooved peg surrounded by inwardly directed microtrichia (rhinaria type). Both the sensory cones and the primary sensilla are found on the ventral surface of the flagellum of the antennae. There are minor differences in the number and distribution of the sensory cones and primary sensilla within the three species, a factor which may be useful for classification of adults.

The "subsidiary" sensilla occur adjacent to the sensory cone present on antennal segment six (flagellar segment 4) of *T. vaporariorum* and *A. proletella*, and is referred to as "subsidiary" since it is always found in association with this sensory cone. In each species it appears to have a slightly different form and precise position.

None of the three species studied exhibits a significant sexual dimorphism with respect to antennal structure or number and distribution of antennal sensilla. In each species the female is the larger of the sexes but the antennae of both sexes are very similar in size. There is

however, a significant size difference in the body and antennae of the three species i.e. *Aleyrodes* > *Trialeurodes* > *Bemisia.*

Transmission electron micrographs of sections taken of trichoid sensilla of *T. vaporariorum* revealed that these sensilla are not associated with neurones. They appeared to be non-cellular cuticular processes (acanthae). Longitudinal sections, of the central grooved peg of primary sensilla revealed possible pores in the cuticle of the peg. Cross sections of sensory cones of *T. vaporariorum*, revealed that branched dendrites almost fill the lumen of the cone and pores are present in the single wall of the cuticle. The ultrastructure of the sensory cones is typical of an olfactory sensillum. Whole cross sections of the antennae of *T. vaporariorum* revealed pores in the cuticle. Some of the micrographs showed the pores to be associated with what appear to be "tubes" running along the length of the antennae. The function of these structures is, as yet, unknown.

The antennal morphology of thrips

The species of thrips studied was the western flower thrips *Frankliniella occidentalis.* Scanning electron microscopy revealed the antennae of *F. occidentalis* to comprise a scape, a pedicel and a flagellum, but in thrips there are six flagellar segments which become progressively smaller towards the tip, the most distal two being so small as to be hard to distinguish from each other.

Four types of structurally different hairs were found on the antennae, these being:-

i. long, thin hairs ending in a sharp tip, on segments 1-3,
ii. small, sharp-tipped hairs found on segments 1-4 in large numbers,
iii. large forked hairs with pointed tips; only two of these are present one on segment 1 and one on segment 2.
iv. 4 hairs of intermediate length with blunt tips on segment 6.

These findings are in close agreement those of Slifer & Sekhon (1974) who worked on the flower thrips, *Frankliniella tritici.*

Transmission electron microscopy and the work of others (e.g. Zarcharuk, 1980) has helped to assign functions to these hairs. The large pointed hairs, both the forked and single-tipped, have thin walls and are packed with many dendritic branches. The walls also appear to contain many pores. This is the typical morphology of an olfactory chemoreceptor.

The blunt-ended hairs have a large, apparently empty lumen and thick walls which is characteristically associated with gustatory chemoreceptors. The small, sharp, pointed hairs have no lumen and would therefore appear to have a mechano-sensory function, if any. It can therefore be concluded from the above results that thrips have the capability to detect both olfactory and gustatory stimuli with their antennae.

Another interesting finding revealed by transmission electron microscopy is the presence of a continuous ring of canals, tubules or pores which appear to run the entire length of the antennae similar to those found in the whitefly antennae. It was at first thought that these canals were artefacts of the chemical fixation processes, but recent use of cryofixation methods, which involve no chemicals, have also revealed the presence of these structures. Their function is as yet unknown, however, Hunter & Ullman (1992) found similar structures in the mouthparts of *F. occidentalis* and found they contained a single dendrite which innervated a single sensillum. This close association of the tubules with a single sensillum is not apparent on the antennae.

Aphid olfactory structures.

When viewing the aphid antenna it is possible again to see that it is made up of three main parts, a scape, pedicel and flagellum. There are few sense organs on the scape or the pedicel . The flagellum of the aphid antenna is made up of five or six segments and is the structure which carries the main olfactory organs. There are mechanoreceptive hairs distributed over the entire flagellum. At the tip there are four to six hairs which are different from the structures found on the rest of the flagellum, these apical hairs have a chemoreceptive function as well as a mechanosensory role.

The other main sensory structures on the flagellum are the rhinaria, primary and secondary, and these are the principal olfactory sense organs of the aphid. The primary rhinaria are located on the two distal flagellar segments and have basically the same structure and composition in all stages. The proximal rhinarium is a single placoid sensillum with a typical olfactory structure i.e. small surface pores and a large number of neurones and dendritic branches. The distal rhinarium is made up of two small olfactory placoid sensilla and three coeloconic pegs which are probably thermo/hygro receptors. The secondary rhinaria are more numerous than the primary and are found on alate, apterous, male and female aphids (see Anderson & Bromley, 1987).

As a continuation of the structural studies of the peripheral sensilla recent work on the lettuce aphid, *Nasonovia ribis-nigri*, has identified in aphids clear olfactory pathways within the brain (Edmunds & Anderson, in preparation.)

The sense organs on the antennae all feed their axons containing the sensory information into an antennal nerve. This nerve enters the brain in a region known as the deutocerebrum. Using various cobalt dye-filling techniques the antennal nerve in aphids has been filled with a dye and by sectioning the brain it has been possible to follow the path taken by the antennal nerve in the brain.

Immediately after entering the deutocerebrum the antennal nerve branches. Each of the branches terminates in a clearly defined region. The filling techniques have identified three main areas in the deutocerebrum where branches end:-

1. **The glomeruli**: these are areas of synapses (junctions) between the incoming antennal nerve and the internal deutocerebral neurones. Glomeruli have been found in several insect species (Rospars, 1988) and are closely associated with olfactory stimulation.

2. **The macro-glomerular complex** (MGC): this consists of many glomeruli closely packed together with a clearly defined border, it also represents an area of junctions between incoming and internal neurones. In other insect species this type of MGC has only been found in male individuals where it has been linked to the processing of information relating to the female sex pheromone .(Boeckh *et al.*, 1984).

3. **The antennal mechano-motor centre** (AMMC): this is again an area of the deutocerebrum where junctions between external and internal neurones occur. This region deals with the mechanosensory information (Rospars, 1988).

The presence of these structures in the aphid brain indicates that the central olfactory processing is sophisticated. The presence of an MGC in both male and female individuals is particularly interesting. It could point to a wider functional role for this structure other than female sex pheromone recognition. In female aphids it may be used to process other olfactory cues (e.g. alarm pheromones, host plant volatiles, etc.).

CONCLUSIONS

Previous light microscopy studies have revealed the general structure of the antennae of whiteflies, thrips and aphids as a scape, a pedicel and a segmented flagellum. The present scanning and transmission electron microscopy studies have confirmed the general structure and have revealed a complex distribution of the primary sense organs on the antennae. The location of clear central processing regions in the aphid brain has confirmed the dependence of the aphid on olfactory information.

Behavioural and electrophysiological studies being carried out on the whiteflies, thrips and aphids indicate that the process of host location is a complex interplay of visual and olfactory information. These studies emphasise that there is potential to influence host-finding behaviour by changing the olfactory and visual environment in the area of potential host crops.

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DAMSON-HOP APHID CONTROL IN UK TRIALS WITH IMIDACLOPRID, A NITROGUANIDINE INSECTICIDE

T.J. MARTIN, P.A. BIRCH, D.J. BLUETT

Bayer plc, Crop Protection Business Group, Eastern Way, Bury St Edmunds, Suffolk IP32 7AH

ABSTRACT

Imidacloprid as a 5% granule and a 70% water dispersible granule was tested in UK hop plantations in 1991 and 1992. Good control of damson-hop aphids *(Phorodon humuli)* was obtained from a surface granule application around the plant crown in April. A bine-base spray in May, as recommended for mephosfolan, the standard, was also very effective. Results with experimental rates from 0.0085 g AI to 0.25 g AI per plant suggest that 0.017 g AI to 0.034 g AI is necessary for performance to the level, or above, that of the standard. More trials will be done to examine the value of earlier applied bine-base sprays and to test further the reliability of a potential recommendation.

INTRODUCTION

Imidacloprid is a new nitroguanidine insecticide (Elbert *et al.*, 1990). In the UK and elsewhere good control of aphids and soil pests of sugar beet was obtained when it was included in the seed pelleting process. The consequent reduction in sugar beet yellowing virus infection has also been reported (Dewar & Read, 1990; Schmeer *et al.*, 1990). Treatment of winter barley seed with imidacloprid resulted in effective control of barley yellow dwarf virus and its aphid vectors in UK field experiments (Schmeer *et al.*, 1990). Its activity as a systemic aphicide highlights its potential in many other crops.

Damson-hop aphid, *Phorodon humuli*, is a very damaging pest of hops. If uncontrolled it is capable of crop destruction in the worst situations, but more usually reduces yield and quality. It is also important as a vector of Hop Mosaic Virus. Many insecticides have been used to control it including soft soap, quassia and nicotine. During the more modern era of crop protection some of the most widely used chemicals included organo-posphorus (OP) compounds, carbamates, and synthetic pyrethroids. Unfortunately the pest has become resistant to virtually all available materials except mephosfolan applied as a bine-base/soil spray (Neve, 1991).

Imidacloprid is of importance to UK hop growers and the future control of *P. humuli* because it acts on the postsynaptic nicotinergic acetylcholine receptors (Abbink, 1991). This is different from the action of the other aphicides still available in the hop growers' armoury.

In 1991 the first field experiments with imidacloprid were conducted in UK hops and further trials were initiated in 1992.

MATERIALS AND METHODS

In 1991 five trials of a 4 replicate randomised design were conducted in hop plantations in the two hop growing areas, the South East and the West Midlands. Each plot consisted of five hills or stocks (hop plants).

A 5% granular formulation of imidacloprid (5 GR) was applied by pepper-pot to an area of *ca.* 0.25 m^2 around the base of the bines. The 70% water dispersible granule formulation (70 WG) was applied as a diluted spray using a Lurmark hollow cone nozzle (disc 5, core 23) attached to the hand lance of a carbon dioxide pressurised knapsack sprayer working at a pressure of 300 kPa and delivering 100 ml to the base of the hop bines (20-30 cm) and to *ca.* 0.1 m^2 of soil immediately surrounding. In the South East the granules were left on the surface of the soil whilst in West Midland trials an attempt was made to lightly incorporate with a hand rake. The standard was an emulsifiable concentrate of mephosfolan (250 EC) applied in a similar manner to that described above for imidacloprid (70 WG) using 114 ml of diluted spray per plant.

Granule treatments were applied in mid-late April whilst the bine-base sprayed treatments were made a month later in mid-late May.

Aphid infestation was assessed by counting the number of aphids (apterae) on 10 randomly-selected young leaves in each plot at intervals after treatment. In some trials a final assessment was made by recording presence or absence of aphids in 50 randomly-selected cones from each plot.

Six small replicated plot trials were similarly arranged in 1992. The site details for both years are given in Tables 1 and 2.

RESULTS

Results of aphid counts have been summarised in Tables 3 and 4. The overall means for actual numbers of aphids on 10 young leaves for each assessment occasion have been provided because, for those involved in hop-damson aphid research work, these are of practical importance. The per cent reduction of aphid numbers by treatments in each trial on each assessment occasion were meaned to produce the figures shown in the tables and therefore the weighting of each trial is the same and this procedure corrects for those with very high aphid populations.

Results on each assessment occasion for each trial were statistically examined by analysis of variance using aphid numbers per 10 leaves transformed to sq rt (x + 0.5). The values for per cent infected cones were transformed using the angular transformation (arc-sine sq rt). Space does not allow all of these data to be tabulated but some important findings may be highlighted. On all assessment occasions in 1991 trials, treated hops had a significantly lower aphid infestation than those left untreated except in WR/21 on 7 June when the 0.034 g rate was not significantly different from untreated (P = 0.05). On most occasions treatments were not significantly different from one another though mephosfolan was significantly poorer than imidacloprid treatments in SR/19 on 22 July and 30 August (at harvest); in WR/22 on 7 June (except in the

TABLE 1 Site details for 1991 trials

Trial number	SR/18	SR/19	WR/21	WR/22	WR/24
Site	Tonbridge	Faversham	Bishops Frome	Bromyard	Munderfield
Cultivar	Target	Northdown	Fuggle	Fuggle	Fuggle
Timing (GR)	22 April	22 April	25 April	18 April	18 April
Crop stage	30 cm	50 cm	20-40 cm	20-30 cm	20-40 cm
Timing (WG)	30 May	30 May	15 May	15 May	15 May
Crop stage	1.75 m	2.0 m	0.5-1.0 m	0.5-0.75 m	0.75-1.0 m
Assess. date *	12 June	25 June	7 June	7 June	7 June
Crop stage	2.3 m	4.5 m	1.5-1.7 m	1.0-1.2 m	1.5 m
Assess. date *	23 July	22 July	11 July	11 July	11 July
Crop stage	pin	pin	pin	pin	pin
Assess. date *	-	-	21 July	31 July	31 July
Crop stage			burr	burr	burr
Assess. date **	-	30 August	12 Sept.	4 Sept.	6 Sept.
Crop stage		harvest	harvest	harvest	harvest

* aphids per 10 leaves
** % infested cones

TABLE 2 Site details for 1992 trials

Trial number	SR/12	SR/13	SR/14	WR/16	WR/18	WR/36
Site	Tonbridge	Faversham	Tonbridge	Mun'field	Bromyard	Eggleton
Cultivar	Target	Northdown	Target	Fuggle	Fuggle	Challenger
Timing (GR)	13 April	14 April	15 April	17 April	17 April	15 May
Crop stage	40-45 cm	60 cm	60 cm	30-40 cm	30-50 cm	50 cm
Timing (WG)	22 May	21 May	22 May	13 May	13 May	2 June
Crop stage	3.0 m	3.0 m	3.0 m	1.5-2.0 m	1.0-1.5 m	1.0-1.2 m
Assess. date *	18 June	17 June	18 June	18 June	18 June	18 June
Crop stage	pin	pin	pin	pin	pin	pin
Assess. date *	21 July	21 July	20 July	13 July	15 July	13 July
Crop stage	early cone	early cone	early cone	early burr	burr	pin

* aphids per 10 leaves

TABLE 3 Mean numbers of apterae on 10 leaves, mean percent infested cones and percent reduction by treatments in 1991 trials

Number of trials		5		5		3		4	
Days after GR applic.		43 - 64		77 - 92		97 - 104		130 - 141	
Days after WG applic.		13 - 26		53 - 57		77		92 - 120	
Treatment	g AI/plant	A	B	A	B	A	B	A**	B
Imid. 5 GR	0.034	143.6	73.9	10.0	98.7	5.3	99.6	0.4	99.1
Imid. 5 GR	0.081	128.0	69.9	5.2	99.6	8.5	99.8	0	100.0
Imid. 70 WG	0.034	250.3	72.3	29.2	95.3	10.6	99.6	2.1	95.1
Imid. 70 WG	0.081	182.9	73.0	8.4	99.1	1.9	99.9	0	100.0
Imid. 70 WG	0.25	97.4	89.4	2.3	99.9	0.3	100.0	0	100.0
Mephos. 250 EC	1.00	237.1	74.2	72.6	96.3	22.5	99.4	9.5	90.0
Untreated		868.3	-	1097.4	-	3146.5	-	56.8	-

A - apterae per 10 leaves
B - % reduction (mean calculated from % reduction figures for each trial)
** - % infested cones

TABLE 4 Mean numbers of apterae on 10 leaves and percent reduction by treatments in six 1992 trials

Days after GR applic.		34 - 66		58 - 99	
Days after WG applic.		16 - 36		41 - 63	
Treatment	g AI/plant	A	B	A	B
Imidacloprid 5 GR	0.034	1.1	99.8	276.1	99.1
Imidacloprid 70 WG	0.0085	38.8	92.5	9449.4	67.8
Imidacloprid 70 WG	0.017	3.7	99.3	2657.6	93.3
Imidacloprid 70 WG	0.034	0.7	99.9	1223.2	97.0
Imidacloprid 70 WG	0.047	0.5	99.9	12.1	99.9
Imidacloprid 70 WG	0.081	0.8	99.9	6.1	100.0
Mephosfolan 250 EC	1.00	11.0	98.8	4224.1	89.5
Untreated		505.6	-	21929.6	-

A - apterae per 10 leaves
B - % reduction (mean calculated from % reduction figures for each trial)

case of imidacloprid 5 GR at 0.081 g AI) and in WR/24 at harvest. Of the imidacloprid treatments in SR/19 on 25 June the bine-base spray (0.034 g AI) was significantly poorer than the other imidacloprid treatments though this difference had disappeared by 22 July, whilst in WR/24 on 7 June the bine-base spray (0.25 g AI) was significantly better than all other treatments. Once again differences were not maintained.

In the 1992 trials on only three occasions were any of the treated hops not significantly less aphid infested than those left untreated. In SR/13 on 21 July aphid numbers on hops treated with imidacloprid bine-base spray (0.0085 g AI) or mephosfolan were statistically indistinguishable from untreated populations; in SR/14 on 20 July the same two treatments were, once again, not significantly different from the untreated and in WR/18 on 15 July only the imidacloprid bine-base spray at the lowest rate (0.0085 g AI) was poorer than all other treatments and not significantly different from the untreated value.

These trials will continue until harvest as in 1991; at the time of writing only two assessments have been made at each site.

DISCUSSION

The control of damson-hop aphid by imidacloprid used as a surface granule or bine-base spray treatment was effective. The lowest rate of 0.0085 g AI/plant as a bine-base spray in 1992 trials was insufficient and it is likely that 0.017 g to 0.034 g AI/plant may be necessary for consistency of performance. There was some variability in effectiveness between sites for both imidacloprid and mephosfolan using these application techniques. A number of factors are thought to affect the performance of mephosfolan including dry weather at and following application, the cleanliness or otherwise of the hop bine-bases in terms of weeds or other unwanted hop vegetation and possibly the emergence of bines well clear of the treated main crown (Caldicott, 1973). More work will be required to evaluate the influence of these factors on the performance of imidacloprid.

Bines affected by mechanical damage or disease which causes severe girdling appear not to transport sufficient active ingredient and this phenomenon was observed in one trial (WR/16) on a plant treated with imidacloprid. One bine on a treated plant was noticeably infested with aphids and examination showed that it was almost severed at soil level.

The imidacloprid results reported here should be regarded as preliminary. There is still a need for further work in hops to allow a clearer decision to be made on rate of use. There is also an interest in the possibility of applying imidacloprid much earlier onto the crown and soil when conditions are damper. The 1992 results in which the early granule application was better in July than the equivalent rate of active ingredient applied a month later supports this suggestion.

Campbell (1990) reported good control of damson-hop aphid by releasing the larvae of the predatory lacewing, *Chrysoperla carnea*, in experiments on dwarf hops. Mephosfolan can be used in integrated control programmes because it is not sprayed over the whole plant in a manner

which would damage introduced predators. Imidacloprid as a basal treatment is also likely to be useful for integrated pest management systems though the appropriate experimentation has still to be done.

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ACCELERATED DEGRADATION OF PHORATE: IMPLICATIONS FOR PEST CONTROL IN THE UNITED KINGDOM

D.L. SUETT, A.A. JUKES

Horticulture Research International, Wellesbourne, Warwick, CV35 9EF

ABSTRACT

In laboratory incubations with mineral and organic soils, total phorate residues declined more quickly in previously-treated soils than in similar untreated soils. Accelerated degradation was induced by a single application of the recommended dose of phorate and was still being expressed more than 2.5 years later. Reduced stability was associated with increased loss of phorate sulphoxide and sulphone. Gamma-irradiation and autoclaving of previously-treated soil greatly reduced the degradation of freshly-applied phorate.

INTRODUCTION

In the UK, only 13 carbamate or organophosphorus compounds are available currently to control a range of insects and nematodes in soil and it is unlikely that this limited range will increase significantly - if at all - in the foreseeable future. With evidence that the behaviour of most of these compounds is being influenced by the accelerated degradation of their residues in previously-treated soils (Suett, 1991), the efficacies of many widely-used crop protection measures are threatened. The potential threat of this phenomenon to UK horticultural crops is therefore being studied at Horticulture Research International-Wellesbourne (HRI-W).

The UK carrot crop, with a current farm-gate value of £80-90 million, would be vulnerable to further limitations in insecticide treatments. Most of this crop is susceptible to damage by carrot fly (*Psila rosae*) and is protected by applying granular formulations of insecticides at sowing time. Phorate (Campbell's Phorate, MTM Agrochemicals) is particularly effective against this pest in organic soils and, for more than 20 years, has remained the most widely-used soil insecticide on this crop. Following evidence of changes in its behaviour in previously-treated soils (Suett & Jukes, 1988), the stability of the phenomenon in mineral and organic soils was studied at HRI-W. This paper summarises the results of these studies.

EXPERIMENTAL

Soils

Soils 1, 2, 11 & 12 (Table 1) were organic fen soils from farms in Cambridgeshire, soils 3-6 were organic soils from a Lancashire farm and soils 7-10 were sandy-loams (mineral) from HRI-W. The farm soils had been treated in order to protect commercial carrot crops and the HRI-W soil was from an area which had been established to study long-term effects of soil-applied insecticides. Treatment histories and organic matter contents of the soils are shown in Table 1. All previous treatments had been applied at the commercially-recommended rate of either 3.4 kg AI/ha (organic soil)

or 1.7 kg AI/ha (mineral soil). Soils were sampled and prepared for incubation studies as described previously (Suett & Jukes, 1988).

TABLE 1. Sources, treatment histories and properties of soils

Soil No.	Site	Treatment history	Date sampled	Organic matter (%)
1	Cambs	Treated 1987	21/10/87	42.2
2		Treated 1986		41.0
3	Lancs	Treated 1989	23/11/89	61.1
4		Treated 1988		63.0
5		Treated 1987		62.9
6		Previously untreated		72.9
7	HRI-W	Treated 1987, '88 & '89	12/2/90	4.6
8		Treated 1987 & '88		4.6
9		Treated 1987		4.6
10		Previously untreated		4.6
11	Cambs	Treated 1986 & '87	15/2/88	50.6
12		Treated 1986 & '87		75.1

Soil sterilisation

The extent to which soil microorganisms were contributing to changes in the degradative capacities of phorate-treated soils was examined by exposing portions of soils 11 and 12 to gamma-irradiation and to autoclaving. Gamma-irradiation, at a dose of 50 kGy, was done by Isotron plc (Swindon, UK) and further samples were autoclaved for 30 min at 121°C.

Laboratory incubations

Each soil was dried to *c.* 50% of its moisture-holding capacity and treated with phorate, at a dose equivalent to 10 mg/kg dry soil, by adding portions of "blank" Fullers earth granules which had been fortified with an acetone solution containing technical phorate (85% AI). After adjusting the water content to the appropriate value at 33 kPa, each soil was mixed by sieving, sampled and duplicate portions were incubated in the dark at 15°C. Moisture contents were maintained and samples were taken for analysis 1, 2, 3, 4, 6, 8 and 12 weeks after treatment.

Analytical methods

Phorate residues were extracted by mixing soils with anhydrous sodium sulphate powder and tumbling for 30 min with hexane:acetone (4:1). Extracts were washed acetone-free and analysed by glc (Packard 438) using a thermionic detector. A 60 cm x 2 mm glass column containing 3% OV-101/Chromosorb W-HP was used for parent phorate (P) and 2% DEGS/Chromosorb W-HP was used to separate phorate sulphoxide (PSO) and phorate sulphone (PSO_2). Recoveries of the three residue components at 1-10 mg/kg were >90% and results were not corrected for analytical losses.

RESULTS

Figures 1-3 show differences in insecticide behaviour within and between the soils from the four sites. Total phorate residues were most persistent in the previously-untreated soils 6 and 10, with 70% and 80% respectively of the initial dose remaining after 12 weeks. In contrast, almost 90% of the initial dose was lost from soil 1 after 2 weeks and initial half-lives of 2 weeks or less occurred also in soils 2, 7 and 12. In these four soils, as well as in soils 3 and 11, <5% of the initial dose remained after 12 weeks. The differences in total residue levels resulted from marked differences in the formation and stabilities of the different residue components. Thus, in the previously-untreated soils, PSO_2 became the major residue component within 2-3 weeks of treatment and, after 12 weeks, comprised >90% of the total residue remaining. In the previously-treated soils, the behaviour of PSO_2 was more variable. In soil 1, PSO_2 was scarcely detectable and the residue comprised predominantly parent P (Figure 1). Relatively small proportions of PSO_2 (<20% of initial dose)

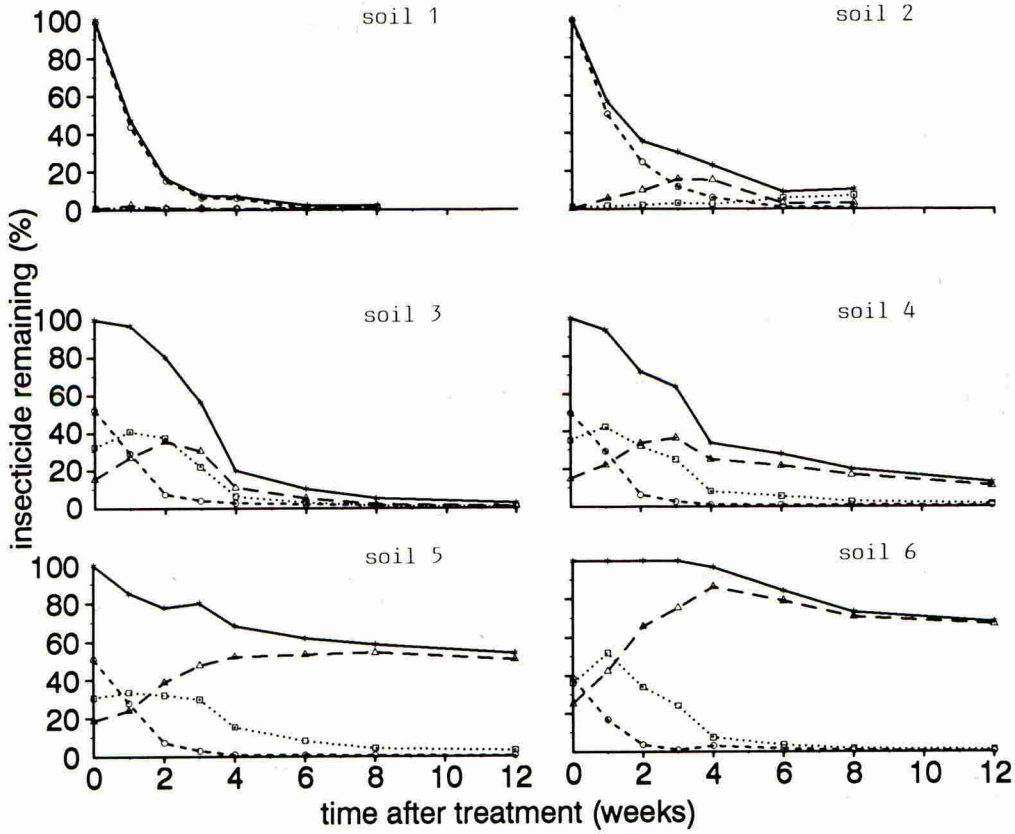

FIGURE 1. Behaviour of freshly-applied phorate in soils 1-6.
+_____+ total residues; o-----o parent phorate; □.....□ phorate sulphoxide; △— — —△ phorate sulphone.

occurred also in soils 2, 7, 11 and 12. Within each group of soils from each site, the accumulation and stability of PSO and PSO_2, as well as total residue persistence, could be correlated with treatment history. Thus degradation in soil 2, which had been treated 18 months previously, was slower than in soil 1, which was treated only 6 months earlier. A similar trend was evident in soils 3-5 and soils 7-9, with most rapid degradation in the most recently- or most frequently-treated soils (soils 3 and 7 respectively). In both groups, degradation in soils treated once with phorate 2.5 years earlier (soils 5 and 9) was greater than in the corresponding untreated soils 6 and 10.

There were marked differences in the behaviour of freshly-applied phorate in the sterilised and non-sterilised soils. Initial half-lives of 2-3 weeks in the non-sterilised soils 11 and 12 were extended to 8-9 weeks by gamma-irradiation (soils 11-S and 12-S) and to >12 weeks by autoclaving (soils 11A and 12A). Both of the sterilisation procedures prolonged the persistence of the individual residue components but differences were most pronounced following autoclaving. Thus PSO_2 accumulated to comprise 30-40% of the initial dose in soils 11A and 12A, compared with little more than 10% in soils 11S and 12S.

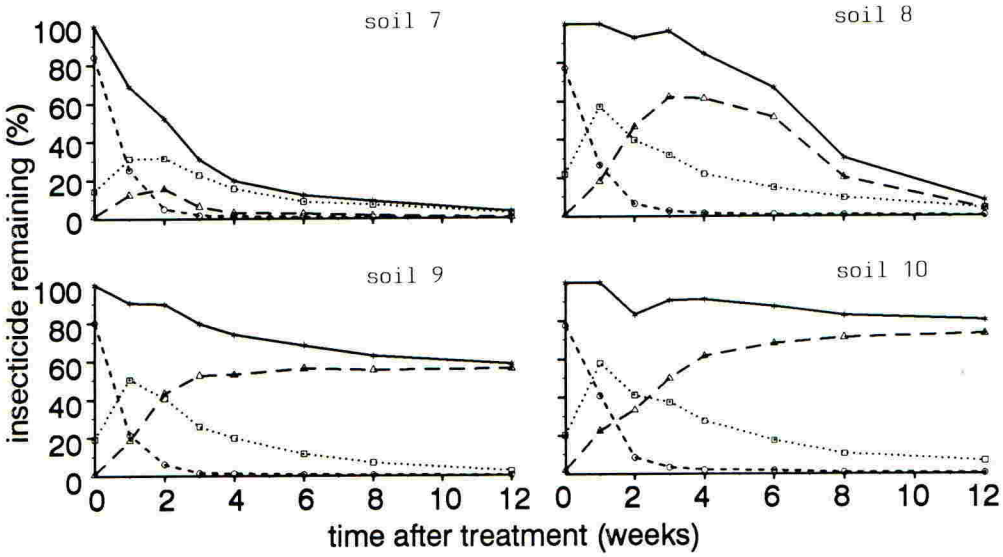

FIGURE 2. Behaviour of freshly-applied phorate in soils 7-10. +_____+ total residues; o-----o parent phorate; □.....□ phorate sulphoxide; Δ__ __ __Δ phorate sulphone.

DISCUSSION

The study confirmed the report (Suett & Jukes, 1988) that freshly-applied phorate behaves differently in previously-treated and previously-untreated soils. It confirmed also that a single application of a granular formulation of phorate at the recommended dose was sufficient to initiate a change in the degradative properties of mineral and organic soils. Other

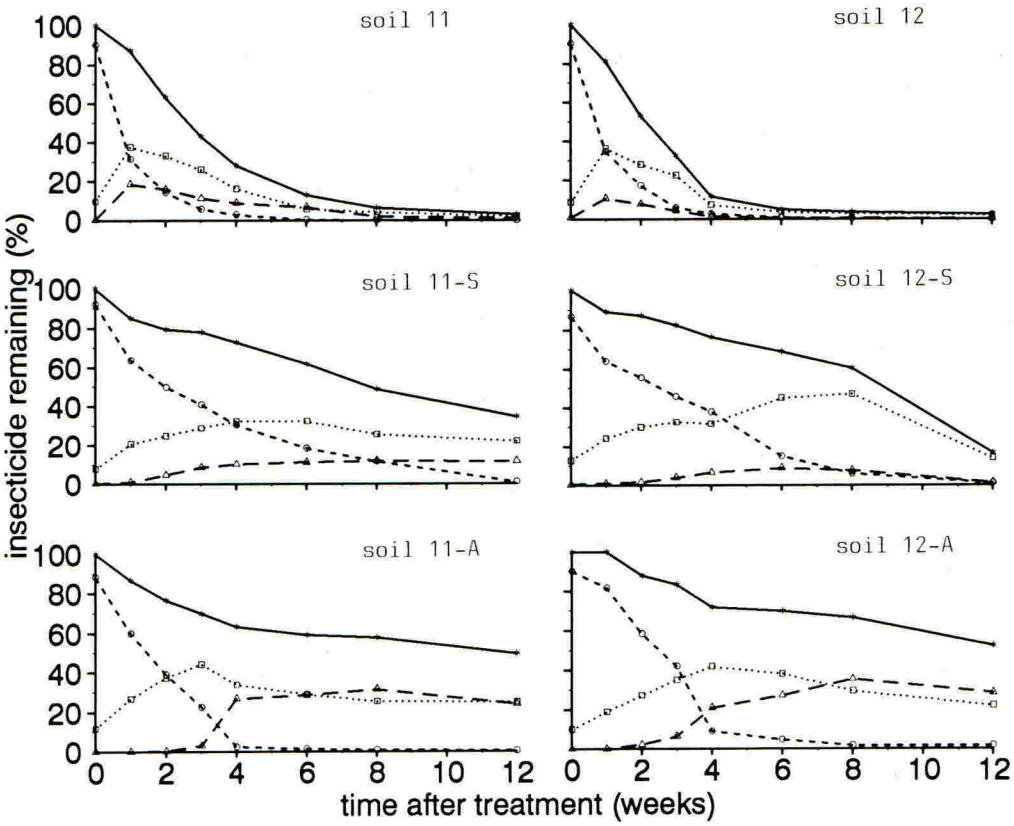

FIGURE 3. Behaviour of freshly-applied phorate in soils 11 and 12 before and after sterilisation by either gamma-irradiation (S) or autoclaving (A). +_____+ total residue; o-----o parent phorate; □.....□ phorate sulphoxide; △ — — — △ phorate sulphone.

studies have shown that accelerated degradation of phorate residues can be induced by a single application of the insecticide. Racke & Coats (1988) induced the phenomenon in the laboratory by adding a dilute solution of radio-labelled insecticide to previously-untreated soils. In the field, Harris *et al.* (1988) showed that a band application of a granular formulation of phorate at the recommended linear dose rate had induced accelerated degradation of the insecticide within 6 weeks. The present study of soils used commercially to grow carrots emphasises that the phenomenon can be induced readily in different soils by a single treatment with phorate and that, contrary to much expressed opinion, it does not result solely from repeated or excessive dosing.

The differences in phorate residue composition observed in these soils were similar to those reported previously in the UK (Suett & Jukes, 1988) and North America (Harris *et al.*, 1988). In each group of soils, parent P degraded at similar rates in previously-treated and -untreated soils. However, most of the prolonged bioactivity of phorate against a pest such as carrot fly results from the persistence of PSO and, especially, PSO_2 (Suett, 1971). In the present study, the formation and prolonged

persistence of these metabolites in the previously-untreated soils was in marked contrast to their transience in some of the previously-treated soils, notably soils 1, 2, 3, 7 and 11. The practical implications of such rapid loss have not, to date, been correlated unequivocably with reduced performance in the field. However, it seems significant that control of late second-generation carrot fly in organic soils, achieved for many years with a single sowing-time application of phorate, can now be maintained only with the addition of mid-season supplementary treatments. Clearly, it would seem unrealistic to expect prolonged efficacy from a phorate application to most of the above soils. The present study suggests that the effects of a single application of phorate are likely to reduce the persistence, and hence the performance, of a second application for at least 2 years.

The changes in residue behaviour induced by the sterilisation treatments are further evidence of the central role of soil microorganisms in the development of accelerated degradation. The greater inhibitory effect of autoclaving was not unexpected. Gamma-irradiation at the level selected would have destroyed all microorganisms but is likely to have had only limited effect on intra- and extra-cellular enzymes, which would have been deactivated by autoclaving. Together with evidence of increased enzyme activity in soils treated previously with organophosphorus insecticides (Sikora *et al.*, 1990), it would seem that prospects for controlling fully the effects of accelerated degradation in the field by using biocides are limited. This emphasises the importance of recognising and defining the potential impact of this phenomenon on crop protection.

ACKNOWLEDGEMENTS

The technical assistance of Mrs S Sime is acknowledged gratefully. The study was supported by the Ministry of Agriculture, Fisheries and Food.

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BEHAVIOUR AND EFFICACY OF CARBOFURAN AND CARBOSULFAN APPLIED AS SEED TREATMENTS IN PREVIOUSLY-TREATED AND PREVIOUSLY-UNTREATED SOILS

A.A. JUKES, D.L. SUETT

Horticulture Research International, Wellesbourne, Warwick, CV35 9EF.

P. CHAMRASKUL

Agricultural Toxic Substances Division, Department of Agriculture, Paholyotin Road, Bangkhen, Bangkok, 10900, Thailand

ABSTRACT

The field performances of carbofuran and carbosulfan, applied as film-coat treatments to radish seed, were assessed against cabbage root fly (*Delia radicum*) in soil treated previously with the recommended dose of carbofuran and in previously-untreated soil. Both insecticides performed significantly better in the previously-untreated soil. Laboratory incubation studies showed that carbofuran, either freshly applied or produced by hydrolysis of carbosulfan, degraded more rapidly in the previously-treated soil than in the previously-untreated soil. Parent carbosulfan declined at a similar rate in both soils but the carbofuran produced by hydrolysis of carbosulfan accumulated in only the previously-untreated soil.

INTRODUCTION

Microbial degradation of pesticides in soil has generally been considered advantageous for the breakdown of potentially hazardous residues. However, adaption of soil microorganisms to use pesticides as an energy source can lead to the accelerated degradation of subsequent applications of the same chemical. This well-documented phenomenon (Suett & Walker, 1988) can result from a single application of a recommended dose (Suett & Jukes, 1991). It can persist for several years even when no further chemical is added and may affect the behaviour of other chemically related-pesticides.

Despite increasing evidence that accelerated degradation of many pesticides occurs widely, there are relatively few reports of field performance being reduced. It is known that conditions used in laboratory studies can differ significantly from those encountered in the field and may show up differences which will not necessarily cause problems in practice (Suett & Jukes, 1991). It has also been suggested (Suett & Walker, 1988) that this continued efficacy is due to the recommended doses generally being in excess of those needed to control most pests. However, increasing pressure from environmental lobbies and the public in general to reduce pesticide usage is likely to lead to the adoption of procedures which use reduced dose rates. The impact of accelerated degradation may therefore become evident much more frequently as doses decline and the pressure on the chemical is increased.

Amongst several reduced dose systems which have been shown to be effective against insect pests, seed treatment is particularly flexible and

convenient. The recent development of film-coating allows the accurate and uniform application of insecticides, some of which have been shown to be highly effective with as little as 10% of conventional dose rates (Salter & Smith, 1988). The impact of accelerated degradation on the efficacy of seed treatments was therefore studied in field and laboratory experiments at Horticulture Research International, Wellesbourne (HRI-W) in 1991. The insecticides used, carbofuran (Yaltox, Bayer UK Ltd.) and the pro-carbofuran insecticide carbosulfan (Marshall, Rhone Poulenc Crop Protection), are both currently approved for the control of cabbage root fly (*Delia radicum*) on UK brassicas.

MATERIALS AND METHODS

Soil sampling and incubation

Sites in two fields at HRI-W were selected. One (soil T) had been used 1 year previously to grow a brassica crop which had been treated with carbofuran at the recommended rate (a spot treatment of 20 mg AI/plant). The other (soil U) had never been treated with carbofuran. Soil, a sandy loam, from the two sites was sampled 4 weeks before sowing and prepared for incubation studies as described previously (Suett & Jukes, 1988). The pH and moisture holding capacities respectively were 6.9 and 12.3% for soil T and 6.6 and 13.5% for soil U.

For the incubation studies, 1 kg samples of air-dried soil were treated with carbofuran or carbosulfan at doses equivalent to 25 mg/kg dry soil, using aliquots of an aqueous solution of the liquid formulations described below. Water was then added to adjust the moisture content to the appropriate 33 kPa value. After an equilibration period, samples were mixed thoroughly and duplicate portions were incubated in the dark at 15°C. Moisture contents were maintained and samples were taken for analysis 0, 1, 2, 3, 4, 6 and 8 weeks after treatment.

Seed treatments

Radish seeds, cv. French Breakfast, were film-coated at HRI-W in a fluidised-bed unit (Maude & Suett, 1986). Carbofuran (35 g AI/l liquid seed treatment) was applied at target doses of 7.5 and 15 g AI/kg seed and carbosulfan (30 g AI/l liquid seed treatment) at 15 and 30 g AI/kg seed. A polymer sticker (polyvinylacetate, Vinamul formulation R18160) was used at a concentration of 25 g/kg seed for all applications and a polymer-only treatment was included. The treatments were applied over 15 min at a column temperature of 30°C.

Field performance against cabbage root fly

Both field plots were power-harrowed to prepare 8 beds, 1.52 m wide and 5 m long. On 1 July the treated seeds, together with untreated controls, were sown at a mean rate of 64 seeds/m row and 3 rows per bed using a tractor-mounted Stanhay seed drill. This gave 4 replicate rows of each treatment randomised within a 24-row plot. The crop was harvested on 7 August, 5 weeks after sowing. All roots were taken from the central 3m of each row, washed and assessed for the presence or absence of damage caused by cabbage root fly larvae. For each treatment an estimate of the % reduction of numbers of larvae present was made by comparison with data from the untreated rows (Wheatley & Freeman, 1982).

Residue analysis

Seeds

Seed loadings were determined by extracting 3 weighed samples of 100 seeds per treatment with methanol (100 ml). Seed-to-seed variability was assessed by extracting 25 single seeds per treatment with methanol (1 ml).

Solution concentrations were determined by hplc using a Spectra-Physics model 8810 pump with a 10 cm Spherisorb C8 cartridge column. For carbofuran the mobile phase was water + acetonitrile (65 + 35, 1.6 ml/min) and for carbosulfan the mobile phase was water + acetonitrile (20 + 80, 1.3 ml/min). Retention times were 3.2 min and 2.5 min respectively. Detection was by Cecil 2112 variable wavelength uv detector set at 220 nm. All samples were injected automatically using a Spectra-Physics 8775 autosampler. Peak areas were measured by a Spectra-Physics 4270 computing integrator and were quantified by comparison with external standards.

Soils

Residues of carbofuran and carbosulfan in soil were extracted by tumbling the soils (25 g) for 1 h with methanol (50 ml) and filtering through Whatman no. 1 qualitative filter paper. Extracts were analysed by hplc as described above. Analytical efficiencies, assessed by fortifying untreated soils at 1.0-10 mg/kg, exceeded 95%; the results were therefore not corrected for analytical losses. The detection limit was <0.1 mg/kg.

RESULTS

Accuracy and uniformity of film-coated seed treatments

Table 1 shows the doses achieved with the four seed treatments, together with estimates of variation in seed-to-seed dose. All treatments were within ± 15% of target doses and the variability in dose between seeds ranged from 7.7 to 15.7%.

Table 1. Accuracy and uniformity of film-coated seed treatments (All doses expressed as g AI/kg seed)

	Bulk seed samples		Single seed samples	
Target dose	mean loading	% of target	mean loading	% CV
carbofuran 7.5	8.57	114.3	8.0	15.7
carbofuran 15	15.1	100.7	15.1	11.2
carbosulfan 15	13.7	91.1	14.2	11.0
carbosulfan 30	29.5	98.3	28.0	7.7

Laboratory incubation studies

The degradation of freshly applied carbosulfan and carbofuran in the 2 soils is shown in Figures 1 and 2 respectively. Results are expressed, for each sampling occasion, as a percentage of total residues in the

initial sample. Carbosulfan values are based on carbofuran equivalents. Each result represents the mean of single analyses of duplicate samples. The mean coefficient of variation (% CV) between duplicate samples was 10.0% and 85% of results were obtained within a CV range of 0.1 - 12.4%.

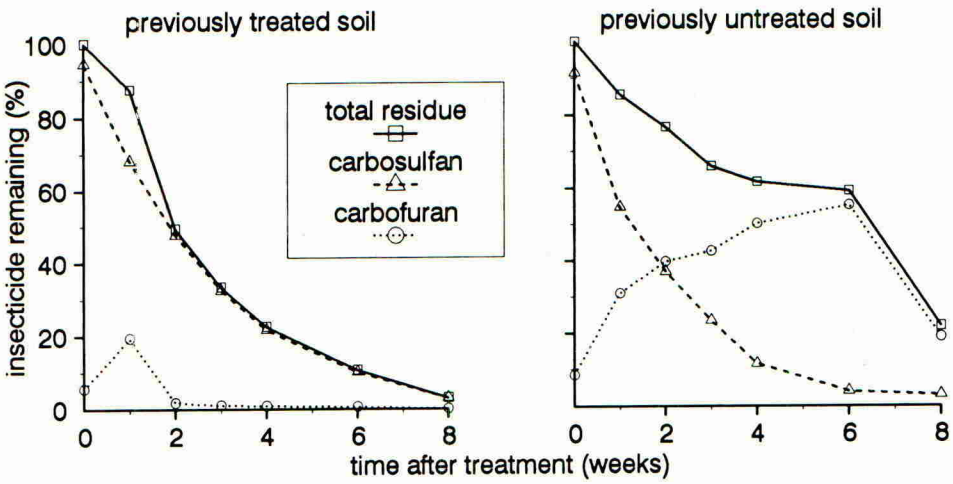

FIGURE 1. Degradation of carbosulfan in previously-treated and -untreated soils.

Carbosulfan residues (Figure 1) declined at a similar rate in both soils. Initial half lives were 12 days in soil U and 14 days in soil T. The concentration of the principal hydrolysis product, carbofuran, increased steadily in soil U until, after 6 weeks, it reached a maximum equivalent to 55% of the total initial residue. In contrast, in soil T it reached a maximum of only 20% of the total initial residue after 1 week and after 2 weeks it was virtually undetectable. Residues of freshly applied carbofuran (Figure 2) declined far more rapidly in soil T than in soil U. Initial half lives were 10 and 45 days respectively.

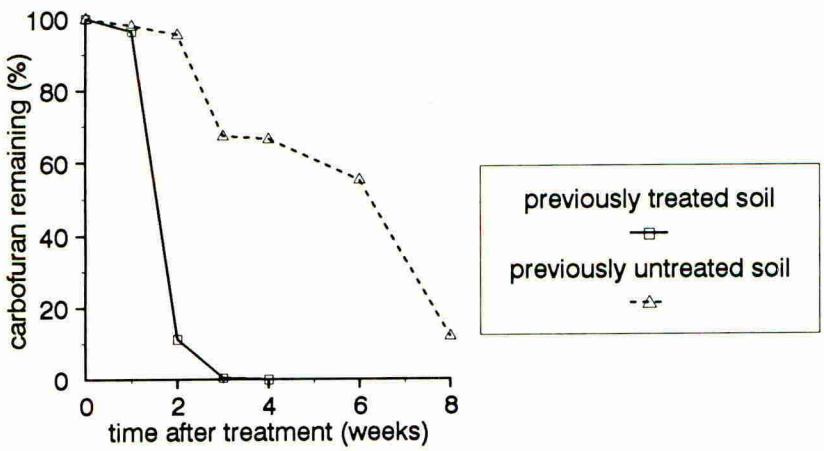

FIGURE 2. Degradation of carbofuran in previously-treated and -untreated soils.

Field performance against cabbage root fly

A heavy infestation of cabbage root fly at both sites damaged 83% (78.9 - 89.4%) and 95% (93.9 - 97.0%) of the untreated roots in soils T and U respectively. The relative performances of the seed treatments are shown in Table 2. All 4 insecticide treatments were significantly less effective in soil T than in soil U. In the latter, damage was reduced by 54-93%, compared with only 19-32% in soil T. Carbosulfan at 30 g AI/kg seed (which is equivalent to 17.4 g carbofuran/kg seed upon hydrolysis) was less effective than carbofuran at 7.5 g AI/kg seed in soil U. In both soils the performance of the polymer-only treated seeds was slightly better than that of the untreated seeds.

Table 2. Comparison of insecticide performance in soil U (previously untreated soil) and soil T (previously treated soil): percentage of radish damaged by cabbage root fly and estimated percentage reduction in numbers of larvae.

	Mean % attacked		Estimated % reduction in numbers of larvae (± sd)	
Treatment	Soil U	Soil T	Soil U	Soil T
carbofuran 7.5	39.8	73.0	83.2 (1.2)	24.7 (11.4)
carbofuran 15	18.3	72.6	93.3 (1.2)	25.7 (7.1)
carbosulfan 15	73.7	76.4	54.5 (9.4)	19.2 (10.3)
carbosulfan 30	52.6	69.4	75.1 (4.2)	32.1 (11.0)
polymer only	88.4	77.3	28.0 (7.4)	15.1 (3.2)
polymer only	95.0	82.5	-	-

DISCUSSION

This study showed the extent to which accelerated degradation can impair the performance of a potentially effective reduced-dose control measure. At a sowing rate of 64 seeds/m row, the carbofuran seed treatment at 7.5 g AI/kg seed would have yielded a dose rate of 5 mg AI/m row, equivalent to <10% of the recommended linear dose of 62.5 mg AI/m row. In the previously-untreated soil this greatly-reduced dose achieved high levels of control, reducing numbers of cabbage root fly larvae by 83%. This level of control would have been more than adequate for most crop production systems (Wheatley, 1973). In contrast, in the soil which had received a single application of the recommended dose of carbofuran the previous year, this treatment achieved only 25% reduction of damage.

A previous study (Suett, 1987) showed that, dose-for-dose, band applications of granular formulations of carbosulfan and carbofuran were similarly effective against cabbage root fly on radish in previously-untreated soil. However, with the much smaller doses applied in the present experiment, the performance of carbofuran in the previously-untreated soil was always significantly better than that of carbosulfan. This suggests that, if cabbage root fly control by carbosulfan results

largely from the greater toxicity of its hydrolysis product, carbofuran, then during the period of most intensive larval infestation the carbofuran level was not sufficient to achieve adequate control. In the previously-treated soil both insecticides were similarly ineffective.

Continued evidence of the widespread occurrence of accelerated degradation emphasises the importance of establishing the treatment history and if possible the degradative characteristics of a soil before it receives any insecticide treatment. The present study suggests that such assessments are particularly vital when the efficacies of reduced-dose systems such as seed treatment are being examined. The implications for other pro-carbofuran insecticides, some of which are used in the UK within typical horticultural/agricultural crop rotations, should also be considered. Careful insecticide rotation must be employed to limit unduly intensive use of carbofuran or any of its pre-cursors.

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CONTROL OF VINE WEEVIL WITH CONTROLLED RELEASE CHLORPYRIFOS GRANULES IN CONTAINERISED NURSERY STOCK

J. H. BUXTON
ADAS, Woodthorne, Wolverhampton WV6 8TQ

J. V. CROSS
ADAS, Olantigh Road, Wye, Ashford, Kent TN25 5EL

B. J. EMMETT
ADAS, Lawnswood, Leeds, Yorkshire LS16 5PY

M. SAYNOR
ADAS, Coley Park, Reading, Berks RG1 6DT

ABSTRACT

ADAS Entomologists at 4 sites (Leeds, Reading, Wolverhampton and Wye) have investigated alternative insecticides to the previous standard, aldrin in a series of co-ordinated, replicated trials between 1989 and 1992.

Tests of persistence showed that chlorpyrifos controlled release granules (suSCon green) remained active in the compost and gave good control of vine weevil for periods of up to 3 years. The fonofos slow-release product (Cudgel) gave good control in the first year, but was less effective than the chlorpyrifos product in subsequent years.

Because of its low mammalian toxicity, ease of handling and mixing, and excellent control of vine weevil for extended periods of time, the chlorpyrifos controlled release granular product, is likely to be a satisfactory replacement for aldrin in the nursery stock market.

INTRODUCTION

Vine weevil (Otiorhynchus sulcatus) is probably the most serious pest of both field grown and containerised nursery stock in the UK. If not controlled, vine weevil can cause considerable losses of hardy ornamental plants. The main damage is caused by larvae feeding on the root systems and the bark of the stem at and below ground level. Severely damaged plants wilt and die, while lightly damaged plants may also be stunted. Infested liners are subject to rejection by the receiving nursery. Adult weevils also feed on the leaves, reducing quality and marketability of affected plants, especially high value subjects such as rhododendrons. The biology and life history of vine weevil has been well documented by Smith (1932).

Until recently, excellent control of vine weevil was achieved by routine incorporation of aldrin into the compost at 20 g. a.i. m^{-3} before use. This treatment was so effective that vine weevil was virtually eliminated from commercial nurseries, at very low cost. However, aldrin was withdrawn from use in the UK in 1989, because of its excessive persistence in the environment. Therefore there is an urgent need for alternative insecticides to be evaluated for vine weevil control.

The micro-encapsulated formulation of fonofos has now been Approved for use in the UK as a compost-incorporated treatment and also as a preventative or curative drench treatment. However, fonofos has a toxic hazard classification (it was previously specified as a Part II substance in the Poisonous Substances in Agriculture Regulations) and gloves are required when handling treated compost. This has proved to be a major obstacle to its commercial use.

The controlled release formulation of chlorpyrifos granules was designed to release the active ingredient over a period of two years or more, depending on the environmental conditions. Because chlorpyrifos itself is not a scheduled poison, it is possible that gloves may not be needed when handling treated compost. The work reported here was done to compare the efficacy of fonofos, and controlled release chlorpyrifos, and to determine the optimum rates of chlorpyrifos needed for vine weevil control.

METHODS

Experiments were carried out at four ADAS sites: Leeds, Reading, Wolverhampton and Wye. Bare root host plants were used, the species of plant varying from year to year. The bare root plants were normally potted direct into one litre rigid plastic pots containing the compost or insecticide mix being investigated. The type of compost used was normally a peat/grit mix in the proportion 90:10, with added slow release fertiliser (Osmocote 12-14 months) at 4 kg. m^{-3}.

Vine weevil cultures were maintained at each of the four ADAS sites; eggs were used to artificially inoculate each pot in the experiments. A shallow depression was scraped in the compost surface around each plant, the eggs were then carefully introduced, and covered to prevent desiccation. After inoculation with vine weevil eggs, plants were brought into the greenhouse, except at Leeds where they remained outside in a protected area. There were normally 10-15 pots (individual replicates) of each treatment, and 30 eggs per pot were used as the inoculum. In previous work, Blackshaw (1987) introduced test plants to an area containing vine weevils and allowed natural oviposition to occur.

Assessments for surviving vine weevil larvae were carried out the winter following inoculation, when the larvae were large enough to be easily observed.

RESULTS

Trials 1989 - 92

This series of trials compared various rates of controlled release chlorpyrifos granules, with aldrin and slow release fonofos, using vine weevil eggs to inoculate treated plants 4-6 months, 18 months, and 30 months after treatment. Plants were initially potted into one litre pots, but in the second year of the trial, remaining plants were potted on into three litre pots.

The results presented in table 1 show that controlled release chlorpyrifos gave excellent control of vine weevil at rates of 75-300 g. a.i. m^{-3} for up to 3 seasons at the Leeds site, while fonofos also gave good control. At Wolverhampton and Wye, chlorpyrifos worked well but fonofos did not. At Reading, results for the first year were good for all treatments, but in year 2 only slow release chlorpyrifos continued to exert control. However, aldrin pots had been repotted in untreated compost so this explains the lack of control. In year 3 at Reading, fonofos gave no control, and chlorpyrifos granules were less effective than the previous year.

Table 1 Mean number of vine weevil larvae recorded per pot three to four months after artificial infestation with eggs in August/September in 1989, 1990 or 1991

Site		Leeds			Reading			Wolverhampton			Wye		
Plant Species		Cotoneaster bullatus			Thuja plicata			Thuja plicata			Thuja plicata		
No of eggs applied		30			80			30			30		
Repotting Compost*		freshly treated 11.5.90			'stale' treated 1.5.90+			'stale' treated 7.3.90			untreated compost 25.4.91		
Interval (wks)**		20	68	176	16	72	123	21	66	118	14	72	124
Year	Rate g. a.i. m^{-3}	1989	1990	1991	1989	1990	1991	1989	1990	1991	1989	1990	1991
aldrin	20	0	0.1	0.1	0.1	2.6	23.3	0	0	0	0	0.1	0.1
chlorpyrifos	75	0.1	0.1	0	0	0.6	15.2	-	-	-	-	-	-
chlorpyrifos	150	0	0	0	0	0.1	6.7	0	0	0.1	0	0.2	2.7
chlorpyrifos	300	0	0	0	0.1	0.3	9.7	-	-	-	-	-	-
fonofos	43.3	0.1	0.1	0.4	0	2.2	26.8	0.3	0	3.3	0	5.3	1.7
untreated	-	12.5	13.9	16.4	0	2.5	26.1	5.7	4.0	13.5	3.9	4.3	1.3
SED (168 df)		-	-	-	-	-	5.1	-	-	-	-	-	-

* 'stale' treated compost indicates compost used for repotting was treated in April or May 1989 at the start of the experiment and stored for approximately one year.

** Numbers indicate the time in weeks between original treatment and artificial infestation with eggs.

\+ Except for the aldrin treatment where untreated compost was used at repotting on 1 May 1990.

Further trials 1990 onwards

A separate series of trials was set up in 1990 at three ADAS sites, to evaluate lower rates of chlorpyrifos controlled release granules and to check the effect of compost type on vine weevil survival. It had been observed that vine weevil infestations tended to be more frequent when bark was present in the compost, so peat/grit composts were compared with compost containing 25% bark by volume. The type of compost used can also affect insecticide efficacy (Nielsen and Roth, 1985). Bare-root plants were used at Leeds and Reading, but at Wolverhampton, untreated liners were potted into 1 litre pots. The results of these trials are summarised in Tables 2 and 3.

Table 2 Mean number of larvae recorded per pot after artificial infestation with vine weevil eggs

Treatment		Leeds		Wolverhampton		Reading	
plant species		Cotoneaster bullatus		Euonymus alatus		Thuja plicata	
		peat/grit	peat/bark grit	peat/grit	peat/bark/ grit	peat/grit	peat bark/ grit
Interval (weeks)+		10		5		14	
No. of eggs inoculated per pot		30		30		20 + 20	
	Rate (g ai m^{-3})						
Chlorpyrifos	37.5	0.3	0.5	2.7	2.9	0.5	0.9
Chlorpyrifos	50	1.2	0	0.2	2.4	0.2	0.6
Chlorpyrifos	75	0	0	0.5	2.6	0	0.3
Chlorpyrifos	100	0	0	0.4	1.6	0	0
Chlorpyrifos	150	0	0	0.6	0.6	0	0.1
Fonofos	43.3	0	0.1	0.9	1.7	3.0	1.2
Untreated	-	8.9	11.1	2.1	9.7	4.4	5.4

+ Time in weeks between treatment and egg inoculation

Survival of vine weevil larvae in untreated pots was higher in the peat/bark compost than in the straight peat/grit compost, at all three sites. At Leeds and Reading control of vine weevil was almost total at rates of chlorpyrifos at or above 75 g. a.i. m^{-3}. Fonofos worked well at Leeds but was more variable at the Reading site. At Wolverhampton the relationship between the rate of chlorpyrifos and control of vine weevil was not evident. This is because the liner compost was untreated and allowed vine weevil larvae to survive even though the surrounding compost was treated with insecticide. This result reinforces the need for vine weevil control to begin at the propagation stage if control is to be effective.

Table 3 Mean number of vine weevil larvae recorded per pot after artificial infestation with vine weevil eggs*

Treatment	Leeds		Wolverhampton		Reading	
	Cotoneaster bullatus		Chamaecyparis		Thuja plicata	
	peat/grit	peat/bark	peat/grit	peat/bark	peat/grit	peat bark
No of eggs inoculated per pot	30	30	30	30	80	80
Interval (weeks) +	61		11-13		66-72	
Rate g. a.i. m^{-3}						
suSCon green 37.5	0	0	0	0	6.1	2.6
suSCon green 50	0.1	0	0	0	3.4	1.8
suSCon green 75	0	0	0	0	0.4	1.0
suSCon green 100	0	0	0	0	2.1	0.6
suSCon green 150	0	0	0	0	0.0	0.4
Fonofos 43.3	0	0	0	0.1	16.8	15.3
Untreated -	2.8	9.5	9.7	10.3	21.6	16.3

* All plants repotted into freshly treated compost, except at Wolverhampton, where original 1990 plants were scrapped and the trial restarted using bare-root plants

\+ Time in weeks between original treatment and egg inoculation.

Approximately one year later (Table 3), all rates of chlorpyrifos and the fonofos treatment were still giving total control of vine weevil at the Leeds site. At Reading, rates of chlorpyrifos at or above 75 g. a.i. m^{-3} were very effective, but fonofos gave little or no control.

DISCUSSION

The earliest work with chlorpyrifos was carried out by Blackshaw (1984) who used standard chlorpyrifos granules (not controlled release) and obtained control of vine weevil for up to 26 weeks. The results of the trials to date have shown that controlled release chlorpyrifos granules gave good control of vine weevil for up to 3 seasons, providing that the rate was 75 g. a.i. m^{-3} or more. These granules were easy to mix into the compost and because they were bright green in colour, it was easy to see whether incorporation had been even or not. Fonofos slow release was usually effective for at least one year when incorporated into the compost, but after this the control was unreliable and sometimes poor. Because this product is a flowable liquid, it is not as easy to apply to the compost as a granule, which growers found very convenient to apply.

Even though chlorpyrifos granules are effective against vine weevil, the compost used at potting up must also contain the incorporated insecticide. This is because roots can grow out into new, untreated compost and vine weevil will then survive in this area.

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BEMISIA TABACI - BIOTYPE CHARACTERISATION AND THE THREAT OF THIS WHITEFLY SPECIES TO AGRICULTURE

I.D. BEDFORD, R.W. BRIDDON, P.G. MARKHAM

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

J.K. BROWN, R.C. ROSELL

Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721, U.S.A.

ABSTRACT

Bemisia tabaci were compared to determine biological and genetic characteristics associated with populations derived from different host plants and geographic locations worldwide. A scanning electron microscopy study of several representative populations showed nearly identical morphologies. Several populations could be distinguished by non-specific esterase patterns. The populations were assayed for their ability to transmit 15 different geminiviruses and for their ability to induce phytotoxic symptoms such as "silverleaf" and honeysuckle vein yellowing. Some populations had a broad host range, induced phytotoxic symptoms, have high reproductive capabilities and were efficient vectors of many viruses tested. These populations are referred to as the "B" biotype. The recent spread of this "B type" has been investigated. Originating in the Middle East, it is now found throughout the Mediterranean, in South Africa, the Caribbean and Central and North America.

INTRODUCTION

Some *Bemisia tabaci* populations have the potential to colonise a wide range of plants and are estimated to encompass a host range of at least 300 plant species in 63 families (Mound, 1963). This insect is present on every continent and is indigenous to most of the tropical and sub-tropical regions of the world (Cock, 1986). Recently it has become established in southern and southwestern USA, while in southern Europe it has been identified in Italy, Southern France, Spain, Portugal and Greece. Infestations of glasshouse crops have occurred even as far north as Scandinavia and occasionally in the United Kingdom and are linked with the importation and movement of certain ornamental plants such as *Euphorbia pulcherrima* (Poinsettia).

The largest and economically most significant group of plant viruses transmitted by *B. tabaci* are the geminiviruses. They include African cassava mosaic virus (ACMV) which devastates subsistence cassava crops throughout Africa, and the golden mosaic viruses of tomatoes and beans in South America. Whitefly-transmitted geminiviruses causing leaf curling and yellow leaf curl of tomatoes and peppers are some of the most widespread, occurring in Asia, the Middle East, North and Central America. The recent appearance of *B. tabaci* in Italy has been associated with the occurrence of tomato yellow leaf curl virus (TYLCV) in glasshouse-grown tomato crops. Previously this virus was found in the eastern Mediterranean region causing severe losses to commercial tomato crops. Similarly, problems with a number of geminivirus diseases of vegetable and fibre crops in the United States and Mexico have been linked to increased infestation levels and distribution of *B. tabaci* (Brown & Bird, 1992). *B. tabaci* caused an estimated $200 million loss in yield to the 1991 Californian winter vegetable crop (Culotta, 1991). With its ability to cause severe feeding damage to plants, the existence of populations resistant to pesticides and as the known vector of more than 60 geminiviruses, *B. tabaci* poses a major economic threat to world agriculture. Predicted increases in seasonal average temperatures due to "global warming" suggest that *B. tabaci* and possibly its associated viral diseases could become established in agricultural crops in more temperate regions.

The investigations described here were designed to investigate the biological and genetic variability of *B. tabaci* and the ability of geographically isolated populations of *B. tabaci* to transmit a range of geminiviruses

from around the world, including some that occur in ornamental plants in Britain.

MATERIALS AND METHODS

Origins and maintenance of insects

B. tabaci from field locations are listed in Table 1. The colonies were cultured in perspex cages (90cm x 45cm x 45cm) in growth-rooms at 25°C with a 16h daylength. Observations on the behaviour of the *B. tabaci* populations in culture revealed differences in both the feeding positions on the plant and the tendency for the insects to swarm to the top of the culture cages at certain times. These characters are subjectively given as "Movement" (Table 3). There was also a contrast between colonies in their survival when moved to different plant host species during transmission tests. This data has been summarised as "Host Range".

Scanning electron microscopy (SEM)

Morphological studies were made of individual adults and nymphs from the *B. tabaci* colonies, particularly the 4th instar/ puparium stage, and a detailed study of the vasiform orifice region was made using a Cam Scan series 4 scanning electron microscope.

TABLE 1. *Bemisia tabaci* colonies cultured at the John Innes Institute giving origins, culture hosts and colony code.

Country	Culture Host Plant	Code
Florida	Nightshade	FN
California	Cotton	CC
Arizona	Poinsettia	ArP
Arizona	Pumpkin	ArPu
Guatamala	Cotton	GC
Nicaragua	Cotton	MNC
Antigua	Watermelon	AnW
Benin	Asystasia	ABA
Nigeria	Ipomea	NI
Sudan	Cotton	SC
South Africa	Potato	SAP
Israel	Cotton	IsC
Cyprus	Cotton	CyC
Turkey	Cotton	TC
Yemen	Cotton	YC
Yemen	Watermelon	YW
Pakistan	Cotton	PC
India	Watermelon	IW

Viruses and Insect transmission

Plants infected with whitefly-transmitted geminiviruses were either collected from the location where the virus is endemic, or obtained from other centres (Table 2). Groups of approximately 500 non-viruliferous *B. tabaci* were placed on a virus infected plant in a perspex cage. The insects were allowed to feed on the virus infected source plant for a 24 hour acquisition access period (AAP) and then allowed a transmission access period (TAP) on 3-5 healthy test seedlings. After a minimal 48 hour TAP the test plants were removed from the insect cage after insects had been removed from the leaves. The plants were transferred to an insect proof glasshouse where they were fumigated with a carbamate-based insecticide (Propoxur, Octavius Hunt Ltd.) and every two days thereafter. Plants were checked daily for symptoms, and where symptoms were not conclusive,

TABLE 2. List of geminiviruses, their origins and maintenance method

Virus	Code	Country	Received	Source	Maintained*
Abutilon mosaic	AbMV	U.K.	-	JII	(v)
African cassava mosaic	ACMV-K	Kenya	1988	R.Markham	(v)
(Ogorocco)	ACMV-N	Nigeria	1990	S.Shoyinka	(v)
Asystasia golden mosaic	AGMV	Benin	1989	R.Markham	(i)(v)
Bean calico mosaic	BCMoV	Arizona	1992	J.K.Brown	(i)
Benin legume	BLV	Benin	1991	R.Markham	(i)(v)
Cotton leaf crumple	CLCV	Arizona	1990	J.K.Brown	(v)
Honeysuckle yellow vein mosaic	HYVMV	U.K.	-	P.Markham	(v)
Indian cassava mosaic	ICMV	India	1991	R.Venkitesh	(v)
Pseuderantheum yellow vein	PYVV	Yemen	1989	P.Jones	(v)(g)
Sida golden mosaic	SiGMV-CR	Costa Rica	1990	R.Markham	(i)(v)
	SiGMV-H	Honduras	1990	R.Markham	(i)(v)
Sida yellow vein	SYVV	Nigeria	1991	R.Markham	(i)(v)
Squash leaf curl	SLCV	Arizona	1990	J.K.Brown	(i)
Tomato yellow leaf curl	TYLCV	Yemen	1989	P.Jones	(i)(v)(g)
Tobacco leaf curl	TLCV	Yemen	1989	P.Jones	(i)(v)(g)
Watermelon chlorotic stunt	WCSV	Yemen	1989	P.Jones	(i)(g)

*(i) = Insect transmitted, (v) = Vegetative propagation, (g) = Grafting.

infection was confirmed by spot hybridisation (Maule *et al.*, 1983), using [^{32}P] labelled, full-length viral DNA clones. All experiments were repeated three times except where a very high insect mortality occurred when tests were repeated a number of times. Mortality levels varied greatly depending on the insect population and the species of virus host plants used.

When possible, the test plants included seedlings of the host species from which the virus culture originated. Other species including *Nicotiana benthamiana, N. tabacum* cv. Samson, *Lycopersicon esculentum* (cv. Kondine Red, Money Maker), *Datura stramonium, Gossypium hirsutum, Phaseolus vulgaris* (cv. Top Crop), *Citrullus vulgaris* (cv. Charleson Grey), and *Cucurbita pepo* (cv. Fordhook) were tested to identify common hosts and those more amenable for glasshouse culture and maintenance.

Bioassay for phytotoxic disorder

The ability of all colonies to induce a phytotoxic response in squash plants (*Cucurbita pepo* cv. Fordhook) (Yokomi *et al.*, 1990; Costa & Brown, 1991) was tested by placing single seedlings of squash in the insect stock cages. The potential for silvering of the upper leaf surfaces was noted approximately 10 days following initial exposure of whiteflies to the bioassay plants. Other hosts were observed for phytotoxin induced symptoms.

Non-specific esterase marker analysis

The method used was modified from Costa & Brown (1991). Individual adult whiteflies, previously frozen at -70^{0}C, were homogenised in 12 microlitres 0.1M Tris-Borate-EDTA buffer, pH 7.0, containing 10% sucrose. Samples were analysed by polyacrylamide gel electrophoresis (PAGE) on 7.5% vertical native gels (0.75mm thick) with a 3% stacking gel and using a Tris-glycine (pH 8.3) running buffer (at 15-milliamps at room temperature for 2.5-3hr). Gels were stained for the presence of esterases in 0.1M phosphate buffer pH 6.5 using alpha- and beta-naphthyl acetate as substrates and fast blue RR stain.

RESULTS

Adults from the *B. tabaci* populations varied considerably in length (mean ± SE), the smallest being ABA (males 0.64±0.013mm; females 0.70±0.016mm) and the largest being FN (males 0.85±0.023mm; females 1.11±0.014mm). The morphological study of *B. tabaci* fourth instar nymphs and pupa, in particular the vasiform orifice, showed all colonies to have typical *B. tabaci* characteristics (Martin, 1987). Differences were detected in the morphology of nymphs from the same populations depending on leaf surface morphology. Nymphs cultured on plants with glabrous leaves developed less setae and spines than those cultured on plants with hirsute leaves; as did nymphs from the same culture that had developed on the smooth upper leaf epidermis compared to the hairy lower epidermis of certain plants (e.g. Poinsettia) (Markham *et al.*, 1992). Esterase patterns were useful for distinguishing some colonies; and over 10 distinct esterase types were identified (Table 3). Half the populations tested so far have been assigned to the "B-Type".

B-type colonies contained highly active individuals and showed a tendency for the insects to swarm to the top of the culture cage at certain times. Colony TC never "swarmed" in 8 years of culturing under the same conditions. Both ABA and NI whiteflies were less active and did not swarm in culture. All B-type individuals survived and bred on a wide host range in contrast to colony ABA which bred on *Asystasia* and survived poorly on most other hosts tested. Colony NI survived well on most hosts but bred well only on a few. Squash silverleaf was associated with feeding of B-type colonies (Table 3). Vein-clearing phytotoxicity was also noted for five B-type colonies in *S.nigrum*, *N.tobacum* cvs. White Burley and Samson and *Beloperone gutta* but was most severe in *L.japonica* where vein yellowing resulted. All colonies, with the exception of ABA and NI, were able to transmit many of the viruses tested. ABA insects had a narrow host range but efficiently transmitted the virus AGMV from the same geographic area. Two viruses, ICMV and ACMV could not be transmitted back to the original host of the virus, cassava. However ACMV-N could be transmitted readily to alternate hosts *Datura* and *Nicotiana*; ACMV-K to *Datura* and ICMV could be transmitted to *Phaseolus vulgaris*.

TABLE 3. Results of virus transmission tests and bioassay for phytotoxic disorder (SSL) for *B. tabaci* colonies together with esterase patterns, movement and host range observations

Country	F	C	Ar	Ar	G	MN	AN	AB	N	S	SA	Is	Cy	T	Y	Y	P	I
Host	N	C	P	Pu	C	C	W	A	I	C	P	C	C	C	C	W	C	W
CLCV	+					+				+					+	+	+	
SLCV	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+
BCMoV	+	+	+	+		+	+			+					+	+	+	+
SGMV-H	+	+	+	+		+	+				+	+	+		+	+	+	+
SGMV-CR	+	+	+	+		+	+				+	+	+		+	+	+	+
TYLCV	+	+	+			+	+	–	+	+	+	+	+	+	+	+	+	+
TLCV	+	+	+			+	+	–	–		+	+	+	+	+	+	+	+
WCSV	+	+	+	+		+	+	–	–	+	+	+	+	+	+	+	+	+
ACMV-N	+					+	+	–				+			–	–	–	
ACMV-K	+		–															
BLV	+						+					+					+	
SYVV	+					+												
AGMV	+	+	+	+		–	+	+	–	+	+	+	+	–	–	–	+	+
ICMV	+						+											
HYVMV	–	–				–	–			–		–	–		–	–	–	
PYVV	–		–			–	–	–							–	–	–	
AbMV	–		–				–							–				
SSL	+	+	+	–	–	–	+	–	–	–	+	+	+	–	+	+	–	–
Esterase	B	B	B	A	G	D	B	E	J	L	B	B	B	M	B	B	K	H
Movement	4	4	4	3	3	3	4	1	2	3	4	4	4	3	4	4	3	3
Host range	4	4	4	3	3	3	4	1	2	3	4	3	4	3	3	3	3	3

1-4 Higher numbers denote a greater colony activity or host range; Blank = result pending

The variegation on the ornamental plants *Abutilon pictum* var. *thompsonii*, *Lonicera japonica* var. *aureo-reticulata* and *Pseuderantheum* sp. are caused by three different geminiviruses, respectively, Abutilon mosaic, honeysuckle yellow vein mosaic and pseuderantheum yellow vein viruses. These three geminiviruses were not transmitted by any *B. tabaci* colonies tested, although they could be transmitted by grafting. SiGMV in Sida (a closely related geminivirus to AbMV) however, was readily transmissible to important crop plants such as french bean, tobacco and tomato, giving very severe symptoms.

DISCUSSION

There was such a large variation in size between males and females in one colony compared to those in some other colonies and the behaviour varied to such an extent that the taxonomic features of colonies were studied in detail to confirm the species. The electron-microscopy study of fourth instar *B. tabaci* nymphs showed that all the colonies had features typical of the species. However as some of the taxonomic features varied depending on the surface characters of the host plant, other methods will be desirable for identifying new material in future. The colonies could be divided into groups using isoenzyme patterns (Table 3). The most common pattern was the B-type. The preliminary tests using a fingerprinting technique (RAPD, randomly amplified polymorphic DNA's) to look for polymorphisms appears to agree closely with the grouping using enzyme patterns (Markham *et al.*, 1992). The important correlation was between type-B pattern and the ability to induce the toxin related symptom, silver-leaf. Two other behavioral characteristics were also associated with the B-type. These were the features of movement such as the tendency to collect on the underside of young leaves in large numbers and to "swarm" towards a light source at high population densities within the culture cage. Such features could be significant in the field and contribute to the spread of the pest and associated viruses as population numbers increase or crops mature.

The transmission of a virus is a complex of interactions between the virus, the vector and the plant. The initial tests were to establish if transmission of viruses, not previously encountered by a population, were possible. Although not all tests have been completed, it appears that most colonies that feed on both donor and test host will acquire and transmit to and from those hosts. Conversely the colony, ABA, survived poorly on most test plants and only transmitted a virus found in the same locality and on the favoured food host, but that virus was transmitted very efficiently. Another colony, NI, would tolerate many of the hosts which ABA did not, but did not colonise these hosts and transmitted poorly. The wide host range of the B-type colonies make these populations the most dangerous agriculturally. For example one B-type colony from Florida was tested fully and transmitted every virus except those found in ornamental plants. No *B. tabaci* population could acquire and transmit the viruses from the three ornamental plants (including *L. japonica* var *aureo-reticulata*) although the plants contained infectious virus, as indicated by grafting tests. It could be that these viruses have been propagated vegetatively for so many generations that they have lost the ability to be transmitted or some change of the virus location in the plant has occurred that prevents the insects acquiring the particles. Two viruses, ACMV and AGMV, could be readily transmitted to *D. stramonium* but neither virus could then be re-acquired by the whiteflies. Elucidating these changes could lead to means of controlling other geminiviruses. Other results indicate that the recognition between virus and vector may depend on various receptors because AGMV was transmitted by all colonies except NI, TC, YC and YW. Overall the NI colony was a poor vector whereas TC, YC and YW could efficiently transmit several other viruses. ACMV isolates were in general less efficiently transmitted than other viruses and could only be transmitted into hosts other than cassava.

Reports suggest that the B-type has been responsible for introducing viruses not only into new areas but into new crops (Brown & Bird, 1992). However it is not clear if the B-type replaces indigenous populations or produces hybrids. It has been clearly shown that the indiscriminate use of insecticides has resulted in populations becoming resistant (Byrne & Devonshire, 1992). Cropping practices such as irrigation, overlapping crops and the large scale use of single cultivars, replacing local land races have all contributed to the increase in the whitefly problem. The increasing use of protection for crops in southern Europe has enabled *B. tabaci* to establish itself on several crops undercover and to spread outside as the season progresses. The possibility exists that, due to effects of global warming this pest will establish itself further north in Europe.

It is clear from these studies that the interactions between virus, vector and host plant need careful study to evaluate the risk to European agriculture of this pest and the viruses it transmits.

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