Testing insecticides against *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) using a tomato plant bioassay

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Summary

A bioassay technique was developed to test the efficacy of insecticides against potato moth (*Phthorimaea operculella* (Zeller)) on tomatoes. The technique tested efficacy against both larvae in mines and neonate larvae that had not yet penetrated the leaf, and explained the failure of some insecticides to control *P. operculella* infestations in commercial tomato crops. Neonate larvae placed on leaves of potted plants several days before treatment provided larvae for testing of insecticides against larvae in mines; other neonates were placed on leaves after treatment to test efficacy against larvae yet to penetrate the leaf. The plants were sprayed with the candidate insecticides, held for 5–7 days, and larval mortality assessed. Chlorfenapyr (100, 200 g a.i. ha^{-1}) and abamectin (8.1 g a.i. ha^{-1}) were effective against neonate larvae and larvae in mines. Sulprofos (720 g a.i. ha^{-1}), methomyl (450 g a.i. ha^{-1}) and spinosad (96 g a.i. ha^{-1}) were effective against neonate larvae but not against larvae in mines. Methamidophos (1102 g a.i. ha^{-1}), endosulfan (700 g a.i. ha^{-1}) and *Bacillus thuringiensis kurstaki* (1000 g ha^{-1}) had some effect against exposed larvae but little against larvae in mines. Thiodicarb (525 g a.i. ha^{-1}), azinphos-ethyl (440 g a.i. ha^{-1}), imidacloprid (59.5 g a.i. ha^{-1}), hexaflumuron (50 g a.i. ha^{-1}), methoxyfenozide (300 g a.i. ha^{-1}) and tebufenozide (200 g a.i. ha^{-1}) were ineffective. A field trial using chlorfenapyr (25, 50, 100, 150 and 200 g a.i. ha^{-1}) and methamidophos (1102 g a.i. ha^{-1}) validated the bioassay technique, with chlorfenapyr effective in reducing the numbers of larvae in mines in leaves.

Introduction

The potato moth, (*Phthorimaea operculella* (Zeller)), commonly called leafminer by tomato growers, is an important pest of tomatoes in Queensland. The larvae mine in the leaves and in the fruit, where their feeding results in loss of the fruit. It also is an important pest of potatoes. *P. operculella* has seriously affected tomato production since the late 1980s (Hall 1996, Stirling *et al.* 1996, Kay and Hall 1998) and it has been very difficult to control with insecticides in the field. Grower experience and field trials (Kay 1993) showed insecticides previously reported as effective, such as azinphos-ethyl, methamidophos, methomyl (Smith 1978) and sulprofos (Hargreaves and Cooper 1979), apparently no longer gave control. There are several possible reasons for this apparent lack of control: resistance developed by *P. operculella* to the insecticides; very large pest populations overwhelming the insecticidal effectiveness; and larval avoidance of the insecticides because of their cryptic habits or because of poor application of and coverage by the insecticides.

It is important to determine whether the old and new insecticides are effective against *P. operculella* on tomatoes in the absence of confounding factors. Field trials are expensive, and the results from them may be confounded by high pest pressure and difficulties in obtaining good coverage, so a bioassay technique may be more suitable. Bioassays commonly used to test insecticides against lepidopteran insects often involve measuring the mortality of larvae placed on excised leaves that have been dipped in insecticides (e.g. McPherson *et al.* 2003) or that have been sprayed in the field (e.g. Adamczyk *et al.* 1999), or of larvae placed on treated leaf discs (e.g. Reed and Smith 2001). However these techniques may not be suitable for use with larvae that mine in leaves, as does *P. operculella*, so a bioassay method was developed using potted tomato plants that allowed testing under almost natural conditions.

This paper describes the bioassay method, a field trial that validates the method, and reports on the effectiveness on *P. operculella* of 14 insecticides using the bioassay technique.

Materials and methods

All insecticides used in the bioassays and the field trial are described in Table 1.

Bioassays

Phthorimaea operculella larvae used in the bioassays were from a laboratory colony maintained on potato tubers. The colony originated from larvae collected from a commercial tomato crop and it was refreshed sporadically with field-collected larvae from tomatoes or potatoes.

Tomato plants, variety Floradade, grown in a standard peat-sand potting mix in 150 mm pots, were used in the bioassays.

Similar methods were used in each bioassay. Ten neonate (<12 h old) *P. operculella* larvae were placed on leaflets (one larva per leaflet) of each tomato plant. Leaflets were selected to distribute the larvae as widely as possible on the plant, and leaflets with a larva were tagged with red cotton tied around the petiolule so they could be found easily. These plants were then held in a constant temperature room at 24°C with a 13:11 L:D photoperiod for a number of days to provide larvae of a known age (termed 1, 2, or 4 d old larvae) in mines for testing. One and 4 d old larvae were used in bioassays 1 – 6, and

Table 1. The insecticides used in the bioassays and field trial.

Insecticide	Trade Name	Concentration of active ingredient
abamectin	Vertimec®	18 g L^{-1}
azinphos-ethyl	Gusathion® A	400 g L^{-1}
Bacillus thuringiensis kurstaki	DiPel Forté® DF	32000 IU mg^{-1}
chlorfenapyr	Secure® 360 SC	360 g L^{-1}
endosulfan	Thiodan®	350 g L^{-1}
hexaflumuron	Consult™	250 g L^{-1}
imidacloprid	Confidor® 200 SC	200 g L^{-1}
methamidophos	Nitofol®	580 g L^{-1}
methomyl	Lannate® - L	225 g L^{-1}
methoxyfenozide	Prodigy™ 240 SC	240 g L^{-1}
spinosad	Success™ Naturalyte	120 g L^{-1}
sulprofos	Helothion® EC	720 g L^{-1}
tebufenozide	Mimic®	200 g L^{-1}
thiodicarb	Larvin® 375	375 g L^{-1}

2 d old larvae in bioassays 7 – 13. On the day of testing several infested plants and a similar number of uninfested plants were sprayed in each treatment. Treatments were applied with a motorized knapsack sprayer fitted with a boom and four Albuz brown hollow cone nozzles operated at 690 kPa in the equivalent of 1000 L ha^{-1} of water over a measured area of ground onto which the potted plants had been placed. A control treatment for each larval age, sprayed with water only, was included in each bioassay. Several hours after spraying, when the plants had dried, 10 neonate larvae were placed on the upper surface of leaflets (one larva per leaflet) of each previously un-infested plant, which was designed to test the insecticides against larvae (termed 0 d old larvae) that had to move over and mine into treated leaves. Usually three or four plants, or replicates, (i.e. 30 or 40 larvae) were used for each treatment although only two plants were used in bioassay 1. Usually all replicates were treated at the same time but in bioassays 8, 10, 12 and 13 each replicate was treated separately in a randomized block design. After treatment, plants were held in a plastic-roofed semi-open-sided plant-house at ambient temperatures with a daily range of 12–35°C. The plants, and particularly the tagged leaflets, were examined carefully 5 d after spraying in bioassays 1 – 6 and 7 d after spraying in bioassays 7 – 13 and the number of live larvae were counted. It was assumed that larvae not found had died.

Field trial

The field trial was conducted on trellised tomatoes, variety Tornado, grown using standard commercial fertilizer and irrigation practices. The plants were sprayed regularly with chlorothalonil (Bravo®), mancozeb (Dithane DF®) and copper hydroxide (Kocide Blue®) for disease control, and weekly with sulprofos to control heliothis, (*Helicoverpa* spp.). The final sulprofos spray was applied 7 d before the trial started. The trial was a randomized block design with seven treatments and four replicates, with plots of one row by 10 m. Treatment rows were separated by unsprayed guard rows and plots along a row were separated by 2 m of unsprayed plants. Treatments were applied to each vertical surface of the trellised row in the equivalent of 1000 L ha^{-1} of water with a motorized knapsack sprayer fitted with a boom and four Albuz brown hollow cone nozzles operated at 690 kPa. Treatments were applied on Day 0 and Day 7. The trial was sampled on Day -1, Day 4, Day 11 and Day 14. On each sample date 10 compound leaves were collected in an unbiased manner (i.e. they were not checked for damage before collection) from each plot, five from each side of the row. Leaves were returned to the laboratory, examined over light, and the number of leaves with mines and the number of live small (<3 mm long), medium (3 – 7 mm) and large (>7 mm) larvae in mines recorded.

Analysis

In all bioassays the percentage mortality of *P. operculella* larvae was corrected for natural mortality as measured in the control treatments using Abbott's formula (Abbott 1925). The results were not used if the control mortality exceeded 20%. Analyses of variance were conducted on the $\sqrt{(x + 0.5)}$ transformed data from bioassays 8, 10, 12 and 13, and from the field trial (GenStat 2002).

Results

Bioassays

The corrected percentage mortality values (Table 2) show that sulprofos, methomyl, chlorfenapyr, abamectin and spinosad caused high levels (>70%) of mortality of 0 d old larvae, although the sulprofos, methomyl and spinosad results were a little variable. Thiodicarb, azinphos-ethyl, tebufenozide, hexaflumuron, imidacloprid, and methoxyfenozide killed few (<20%) 0 d old larvae, while the other insecticides used resulted in intermediate levels of mortality. Only chlorfenapyr and abamectin gave over 70% mortality of older larvae already in mines.

Table 2. Corrected percent mortality of larvae in the bioassays.

Bioassay number	Insecticide	Rate (g a.i. ha^{-1})	No. of larvae of each age	Corrected percent mortality of larvae of each age			
				0 d	1 d	2 d	4 d
1	methamidophos	1102	20	31.9	20.0	–	43.8
2	sulprofos	720	30	69.9	40.0	–	3.3
3	thiodicarb	525	30	19.6	0.0	–	18.6
3	azinphos-ethyl	440	30	4.1	0.0	–	7.4
4	*B. thuringiensis*	1000	30	33.4	29.7	–	9.8
4	*B. thuringiensis* + molasses	1000 + 5 L	30	36.8	29.7	–	13.8
5	endosulfan	700	30	33.4	*	–	*
5	methomyl	450	30	83.4	*	–	*
6	tebufenozide	200	30	14.8	3.9	–	0.0
7	sulprofos	720	40	90.3	–	22.9	–
7	tebufenozide	200	40	0.0	–	0.0	–
8	sulprofos	720	40	56.4	–	3.0	–
8	chlorfenapyr	100	40	92.3	–	75.8	–
8	chlorfenapyr	200	40	92.3	–	57.6	–
9	hexaflumuron	50	30	3.5	–	11.1	–
10	methomyl	450	40	67.6	–	7.9	–
10	sulprofos	720	40	70.3	–	2.6	–
10	methamidophos	1102	40	48.6	–	28.9	–
10	endosulfan	700	40	45.9	–	18.4	–
10	*B. thuringiensis*	1000	40	24.3	–	7.9	–
11	spinosad	96	30	61.9	–	*	–
11	abamectin	8.1	30	95.3	–	*	–
12	sulprofos	720	40	88.9	–	51.4	–
12	imidacloprid	60	40	5.6	–	0.0	–
12	abamectin	8.1	40	77.8	–	35.5	–
12	spinosad	96	40	75.0	–	23.8	–
12	methoxyfenozide	300	40	16.7	–	8.6	–
13	sulprofos	720	40	81.6	–	23.5	–
13	abamectin	8.1	40	86.8	–	61.8	–
13	abamectin	10.8	40	89.5	–	70.6	–
13	spinosad	96	40	73.7	–	29.4	–

* Control mortality >20%.

The analysed results from bioassays 8, 10, 12 and 13, expressed as numbers of live larvae, are shown in Table 3. Survival of 0 d old and 2 d old larvae in the untreated controls was high in each bioassay. In bioassay 8 there were significantly fewer ($P < 0.05$) live larvae in any chlorfenapyr treatment than in the controls, no differences ($P > 0.05$) between the two rates of chlorfenapyr in numbers of live larvae of the same age, and significantly more ($P < 0.05$) live 2 d old larvae than 0 d old larvae at each concentration. There were significantly fewer ($P < 0.05$) live 0 d old larvae in the sulprofos treatment than in the control, but more than in the chlorfenapyr treatment, but there was no significant difference ($P > 0.05$) between the sulprofos and control in numbers of live 2 d old larvae.

In bioassay 10 numbers of live 0 and 2 d old larvae in the *B. thuringiensis* treatments did not differ significantly ($P > 0.05$) from the controls. There were fewer ($P < 0.05$) live 0 d old larvae in the endosulfan treatment than in the control or the endosulfan 2 d old treatment, which did not differ ($P > 0.05$) from the control. There were significantly fewer ($P < 0.05$) live larvae of either age in the methamidophos treatment compared to the controls, and no significant difference ($P > 0.05$) between the two ages of larvae. Both methomyl and sulprofos had significantly fewer ($P < 0.05$) live 0 d old larvae than all other treatments, but numbers of live 2 d old larvae did not differ ($P > 0.05$) from the control.

In bioassay 12, in the abamectin and spinosad treatments there were significantly fewer ($P < 0.05$) live 0 d old larvae than in the control, but not 2 d old larvae. Numbers of live 0 d and 2 d old larvae in the imidacloprid and methoxyfenozide treatments did not differ significantly ($P > 0.05$) from the controls. There were fewer ($P < 0.05$) live 0 d and 2 d old larvae in the sulprofos treatment than in the controls.

There were significantly fewer ($P < 0.05$) live 0 d and 2 d old larvae in both abamectin treatments compared to the controls, but no differences ($P > 0.05$) between the two rates of abamectin, or between the two ages of larvae in bioassay 13. For both spinosad and sulprofos, there were significantly fewer ($P < 0.05$) live 0 d old larvae than in the control, but not 2 d old larvae.

Table 3. Mean number of live larvae in bioassays 8, 10, 12 and 13 conducted as randomised block experiments.

Insecticide treatment (g a.i. ha^{-1})	Larval age (d)	Mean number of live larvae [A]			
		Bioassay 8	Bioassay 10	Bioassay 12	Bioassay 13
untreated control (-)	0	9.75 e	9.25 de	8.99 e	9.49 b
untreated control (-)	2	8.23 e	9.49 e	8.72 de	8.48 b
abamectin (8.1)	0	–	–	1.74 ab	0.98 a
abamectin (8.1)	2	–	–	5.18 cd	2.66 a
abamectin (10.8)	0	–	–	–	0.77 a
abamectin (10.8)	2	–	–	–	2.20 a
B. thuringiensis (1000)	0	–	6.95 cd	–	–
B. thuringiensis (1000)	2	–	8.73 cde	–	–
chlorfenapyr (100)	0	0.50 a	–	–	–
chlorfenapyr (100)	2	1.95 bc	–	–	–
chlorfenapyr (200)	0	0.70 ab	–	–	–
chlorfenapyr (200)	2	1.94 cd	–	–	–
endosulfan (700)	0	–	4.82 b	–	–
endosulfan (700)	2	–	7.73 cde	–	–
imidacloprid (60)	0	–	–	8.49 de	–
imidacloprid (60)	2	–	–	8.99 e	–
methamidophos (1102)	0	–	4.72 b	–	–
methamidophos (1102)	2	–	6.68 bc	–	–
methomyl (450)	0	–	2.81 a	–	–
methomyl (450)	2	–	8.72 cde	–	–
methoxyfenozide (300)	0	–	–	7.49 de	–
methoxyfenozide (300)	2	–	–	7.96 de	–
spinosad (96)	0	–	–	2.24 ab	2.21 a
spinosad (96)	2	–	–	6.32 cde	5.95 b
sulprofos (720)	0	4.12 d	2.61 a	0.77 a	1.49 a
sulprofos (720)	2	7.96 e	9.25 de	3.62 bc	6.42 b

[A] Back-transformed means following $\sqrt{(x + 0.5)}$ transformation before analysis. For each bioassay means followed by the same letter are not significantly different ($P = 0.5$) (least significant difference test).

Field trial

There were no significant differences ($P > 0.05$) in numbers of mined leaves, or numbers of larvae on Day -1 (i.e. pre-treatment) in the field trial (Table 4). On Day 4, four days after the first treatment application, there were significantly fewer ($P < 0.05$) mined leaves in the chlorfenapyr 100, 150 and 200 g a.i., and methamidophos treatments than in the control, and significantly fewer ($P < 0.05$) small and total larvae in the chlorfenapyr 150 and 200 g a.i. treatments compared to the control. There were no significant differences ($P > 0.05$) between treatments in numbers of medium and large larvae.

On Day 11 (four days after the second treatment application) there were significantly fewer ($P < 0.05$) mined leaves in the chlorfenapyr 150 and 200 g a.i. treatments compared to the control, and in the chlorfenapyr 100, 150 and 200 g a.i. treatments compared to methamidophos. There were more ($P < 0.05$) small larvae in the methamidophos treatment than in all the other treatments, but only chlorfenapyr 150 g a.i. had significantly fewer ($P < 0.05$) than the control. Chlorfenapyr 25, 100, 150 and 200 g a.i. had significantly fewer ($P < 0.05$) medium larvae than the control, chlorfenapyr 50 g a.i. and methamidophos treatments, while the chlorfenapyr 100, 150 and 200 g a.i. treatments had significantly fewer ($P < 0.05$) large larvae than the control and methamidophos treatments. There were significantly fewer ($P < 0.05$) total larvae in the chlorfenapyr 25, 100, 150 and 200 g a.i. treatments than in the control, methamidophos and chlorfenapyr 50 g a.i. treatments.

On Day 14 there were no significant differences ($P > 0.05$) between treatments in numbers of mined leaves. Methamidophos had significantly more ($P < 0.05$) small larvae than the other treatments, while only chlorfenapyr 200 g a.i. had significantly fewer ($P < 0.05$) small and medium larvae than the control. Chlorfenapyr 100, 150 and 200 g a.i. had significantly fewer ($P < 0.05$) large larvae than the control or methamidophos treatments, and chlorfenapyr 150 and 200 g a.i. had fewer ($P < 0.05$) total larvae than the control.

Table 4. The number of mined leaves and the number of larvae in mines on each sample day in the field trial. Treatments were applied on Day 0 and Day 7.

Insecticide treatment (g a.i. ha^{-1})	Number of mined leaves[A]	Number of larvae in mines[A]			
		Small	Medium	Large	Total
Day -1					
untreated control (-)	5.11 a	0.61 a	5.85 a	10.39 a	16.97 a
chlorfenapyr (25)	6.19 a	0.61 a	3.70 a	10.06 a	14.87 a
chlorfenapyr (50)	6.95 a	2.18 a	8.86 a	12.17 a	23.61 a
chlorfenapyr (100)	5.94 a	2.60 a	9.42 a	7.28 a	19.84 a
chlorfenapyr (150)	6.61 a	2.14 a	5.95 a	12.68 a	21.59 a
chlorfenapyr (200)	6.45 a	0.90 a	5.16 a	12.32 a	18.95 a
methamidophos (1218)	6.70 a	1.08 a	8.09 a	14.17 a	23.80 a
Day 4					
untreated control (-)	6.99 c	14.66 c	7.01 a	3.26 a	26.17 c
chlorfenapyr (25)	5.15 abc	13.46 c	4.65 a	5.65 a	24.53 c
chlorfenapyr (50)	6.12 bc	11.41 c	2.06 a	2.60 a	16.85 bc
chlorfenapyr (100)	4.192 ab	9.04 bc	3.95 a	1.32 a	15.38 bc
chlorfenapyr (150)	3.67 a	1.77 a	0.78 a	1.19 a	4.84 a
chlorfenapyr (200)	4.67 ab	3.42 ab	2.36 a	3.78 a	10.15 ab
methamidophos (1218)	5.98 ab	10.64 c	4.52 a	2.92 a	19.05 bc
Day 11					
untreated control (-)	4.72 bc	4.60 b	11.93 c	6.24 c	23.28 de
chlorfenapyr (25)	4.19 abc	4.19 b	3.14 b	3.67 bc	11.22 c
chlorfenapyr (50)	4.74 bc	4.43 b	9.22 c	6.07 c	20.03 d
chlorfenapyr (100)	3.82 ab	1.54 ab	1.49 ab	1.68 ab	4.80 ab
chlorfenapyr (150)	2.87 a	0.00 a	0.90 a	0.70 a	1.68 a
chlorfenapyr (200)	2.64 a	1.80 ab	2.45 ab	1.49 a	6.12 bc
methamidophos (1218)	5.88 c	13.12 c	11.93 c	6.46 c	32.22 e
Day 14					
untreated control (-)	4.72 a	2.09 bc	5.85 bc	8.00 d	16.41 cd
chlorfenapyr (25)	3.17 a	2.78 c	6.03 bc	4.21 bcd	12.52 bc
chlorfenapyr (50)	4.21 a	2.99 c	9.98 cd	5.32 cd	18.39 cd
chlorfenapyr (100)	3.20 a	1.05 abc	3.41 ab	1.56 ab	6.21 abc
chlorfenapyr (150)	3.18 a	0.20 ab	2.41 ab	1.65 abc	4.24 ab
chlorfenapyr (200)	2.21 a	0.00 a	0.20 a	0.00 a	0.20 a
methamidophos (1218)	6.73 a	12.08 d	15.63 d	5.94 d	33.90 d

[A]Back-transformed means following $\sqrt{(x + 0.5)}$ transformation before analysis. For each column for each sample day means followed by the same letter are not significantly different (P = 0.5) (least significant difference test).

Discussion

The bioassay technique was successful and effective. There generally was very high recovery of 0 d old larvae and of larvae in mines in the control treatments, indicating that the handling of larvae in the bioassays had no adverse effects on the larvae, and that the lower recovery of larvae in the other treatments was due to the treatments applied rather than the bioassay technique. Larval cadavers sometimes were found on the leaves or in the mines in the leaves of insecticide treated plants, although not all larvae were accounted for. The bioassay method allowed testing of insecticides against neonate larvae that had to move over leaves and mine into them, as well as against larvae of a known age in mines in live plants, simulating the situation in the field. This would not be possible using excised leaves or leaf discs, and several attempted experiments placing larvae on excised leaves were unsuccessful, with rapid wilting of the leaves and high larval mortality (Kay unpublished data). The method is flexible enough to test larvae of any age in mines, with the only constraint being that mortality should be assessed before larvae leave the mines to pupate. One day old, 2 d old and 4 d old larvae were used in these bioassays, with 2 d old larvae eventually used as a standard age.

In these experiments the plants were held in a plant-house at ambient temperatures after treatment. It would be preferable to hold plants at a constant temperature or under a standard fluctuating temperature regime so that each bioassay is conducted under standard conditions. However the technique is robust enough to produce useful results when facilities to produce such conditions are not available.

The bioassay results differentiated between insecticides that were effective and those that were not. For example, thiodicarb, imidacloprid and azinphos-ethyl were not effective against 0 d old larvae (<20% mortality) while chlorfenapyr and abamectin (>70% mortality) were effective. Many of the insecticides tested showed intermediate levels of effectiveness against 0 d old larvae in the bioassays. The results also clearly showed the effectiveness of the insecticides against larvae in mines. Some insecticides, particularly sulprofos, were included in several bioassays and the results were reasonably consistent across the bioassays. These results indicate that insecticides that resulted in mortality of over 70% of 0 d old larvae and 50–60% of larvae in mines are likely to be reasonably effective against *P. operculella* in the field. Those with lower levels of mortality, particularly against larvae in mines, are likely to be ineffective in the field.

The field trial validated the bioassay results. Chlorfenapyr at 100 and 200 g a.i. ha^{-1} was effective against 0 d old and 2 d old larvae in bioassay 8, and its use at those rates, and at 150 g a.i., resulted in reduced numbers of larvae in the field trial. In comparison, methamidophos, which had only intermediate levels of effectiveness against 0 d old and 2 d old larvae in bioassays 1 and 10, was ineffective in the field trial.

Hence the bioassay technique is a useful tool that could be used to rapidly screen candidate insecticides against *P. operculella* in tomatoes, and in other host crops such as potatoes. Those insecticides that showed promising results in a bioassay could then be tested in field trials.

Of the insecticides tested in the bioassays only chlorfenapyr and abamectin

caused high mortality of both 0 d old larvae and larvae in mines. Chlorfenapyr was effective in the field trial, particularly at rates of 150 and 200 g a.i ha^{-1}, but not at 25 and 50 g a.i ha^{-1}. Abamectin is now registered for use at 10.8 g a.i ha^{-1} against *P. operculella* in tomatoes.

Azinphos-ethyl was completely ineffective in bioassay 3 despite previously being reported as effective in the field (Smith 1978), and it seems likely that *P. operculella* has developed resistance to it. Methamidophos, reported as effective by Smith (1978) and Hargreaves and Cooper (1979), killed less than 50% of 0 d old larvae and between 20% and 44% of larvae in mines in the bioassays, but it was ineffective in the field trial. Sulprofos, the most effective insecticide against *P. operculella* reported by Hargreaves and Cooper (1979), was effective against 0 d old larvae in the bioassays, but ineffective against larvae in mines. It had been used on the field trial crop before the trial but clearly was ineffective as shown by the number of mined leaves and larvae in mines in the pre-treatment count. Imidacloprid had almost no effect on *P. operculella*, while endosulfan and thiodicarb resulted in some mortality of 0 d old larvae but had little effect on larvae in mines, and are unlikely to be effective in the field. Methomyl, reported by Smith (1978) as effective, killed a high percentage of 0 d old larvae but had little effect on 2 d old larvae. Spinosad was reasonably effective against 0 d old larvae but had little effect on 2 d old larvae in mines, so may not be effective in the field. *B. thuringiensis,* alone or with molasses, was not particularly effective against 0 d old larvae or larvae in mines, although Broza and Sneh (1994) reported that it was effective against *P. operculella* in Israel.

The three insect growth regulators, tebufenozide, hexaflumuron and methoxyfenozide, were ineffective. It is possible that this bioassay technique is not a suitable method to assess the effectiveness of insect growth regulators. However the time between treatment and assessment (5 d or 7 d) should have been long enough at the temperatures experienced (approximate mean daily temperatures of 23.5°C) for the larvae to have moulted at least once. At assessment the larvae had developed and were apparently healthy and unaffected, indicating that the growth regulators had no effect.

The results help to explain why the insecticides such as methamidophos, sulprofos and methomyl fail to control *P. operculella* in commercial tomato crops. Insecticides that failed to kill a high percentage of larvae in mines in potted plants would not kill larvae in mines in a dense, trellised crop where good spray coverage is difficult to achieve, and the lack of control would be particularly evident when pest numbers were high.

Acknowledgements

I thank Aaron Goodman-Jones and Paul Garland for technical assistance, Cyanamid Australia for funding the field trial and for permission to publish the results, and Bernie Franzmann, Brendan Nolan and two anonymous referees for their helpful comments on earlier drafts of the manuscript.

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