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Quarantine disinfestation of capsicums against Queensland fruit fly (Diptera : Tephritidae) with dimethoate

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Summary. A postharvest dimethoate treatment at 400 mg/L applied through a packing-line spray system achieved >99.99% efficacy as a quarantine disinfestation method against Queensland fruit fly, *Bactrocera tryoni* (Froggatt) infesting capsicums (peppers), *Capsicum annuum* L. There were no survivors in confirmatory tests on fruit containing 77 130 eggs, the most tolerant life stage. The spray system thoroughly wetted fruit at a delivery rate of 9.2 L/min.m^2 for a minimum time of 1 min.

Introduction

Capsicums (peppers), *Capsicum annuum* L., are a host of Queensland fruit fly, *Bactrocera tryoni* (Froggatt), which is endemic to regions where capsicums are grown commercially in Queensland. Export to parts of Australia and other countries where this pest does not occur would normally require a quarantine disinfestation treatment. Formerly, disinfestation of capsicums was done by fumigation with ethylene dibromide (EDB), but approval for use of this chemical as a quarantine treatment was withdrawn by all overseas markets resulting in a need for an alternative fruit fly quarantine treatment. Insecticides have advantages as postharvest treatments that include low cost and logistical flexibility (Heather 1994).

Postharvest dips and sprays using the insecticide dimethoate have an excellent performance record over 15 years as a quarantine disinfestation treatment for tomatoes and cucurbits within Australia and as exports to New Zealand. Treatment with dimethoate was developed initially as a 400 mg/L dip for use on tomatoes which can suffer phytotoxic effects from EDB fumigation (Swaine *et al.* 1984) and subsequently, as a packing-line flood spray (Heather *et al.* 1987). For capsicums, only a packing-line spray application was developed because of the perception that if they were dipped their hollow centres might take up insecticide solution through any holes, caused by larvae of *Helicoverpa armigera* (Hubner). The efficacy required of a quarantine treatment against fruit flies by Australia and New Zealand is usually mortality of 99.99% at the 95% confidence level (CL) demonstrated as no survivors from tests on 30 000 test insects (Couey and Chew 1986).

Our study was intended to determine whether the quarantine treatment efficacy required by Australia and New Zealand against *B. tryoni*, could be achieved for capsicums, as it is for tomatoes, by a packing-line spray application of dimethoate at the routine concentration of 400 mg/L.

Materials and methods

Test insects

Bactrocera tryoni used to infest fruit in these trials were maintained as described by Heather and Corcoran (1985) at the Queensland Department of Primary Industries, Entomology Laboratory at Indooroopilly, Brisbane.

Test fruit

Capsicums, *Capsicum annuum* L. used in trials were representative of the normal commercial size range (6–10 cm diameter) and were infested at the green to half-coloured stage. Fruit were obtained from registered organic growers to ensure freedom from insecticides that could inhibit fruit fly infestation.

Insecticide

A commercial emulsifiable concentrate formulation of dimethoate (400 g/L) was used, with the exact concentration of active ingredient determined by analysis immediately before the start of the trials. This value was used to prepare subsequent working dilutions.

Procedure

The rates of development of juvenile life stages of *B. tryoni* in capsicums were first determined. Fruit containing known life

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stages were then dipped in dimethoate solutions to compare the tolerance of each to the insecticide. This was followed by confirmatory tests on the life stage (eggs) shown to be the most tolerant to the treatment, to demonstrate the efficacy of dimethoate at 400 mg/L, the concentration used routinely in Australia for other produce.

Infestation

Capsicums were exposed to infestation for 10–20 min in cages of flies (about 25 000, sex ratio 1:1 and adult age 3–6 weeks). They were first punctured 10 times to a depth of 5 mm with a 0.25-mm-diameter steel pin, evenly distributed around the crown at the stem end. This procedure ensured an even distribution of eggs within the fruit, reduced the possibility of localised fruit collapse and helped to equalise numbers of eggs between fruits. Fruit were held at 26°C and 70% RH after infestation for development of the eggs to the life stages required for testing.

For life stage comparison tests, half of the fruit were left untreated, and for confirmatory tests 1 in 6 were left untreated. Fruit to remain untreated as controls were selected without conscious bias from each row of fruit in the cage of infesting flies, to overcome differing fly density due to light orientation. This gave parallel samples for estimation of numbers present in fruit at the time of treatment. Extra fruit were infested and destructively sampled at the time of treatment to ensure that fruit to be treated had the intended life stage present.

Life stage comparison tests

Dipping was used as the method of application for these tests as only small numbers of fruit were used and it was impracticable to treat them in the large packing-line spray system. Fruit were infested on a different day for each life stage so that fruit containing eggs or first, second or third instars, could be dipped concurrently. Dipping was done by wholly immersing a lidded wire cage containing the fruit in a large stainless steel vat containing a dimethoate solution of 4 mg/L, for 1 min. This low insecticide concentration ensured mortality of less than 100% in all stages, thus enabling meaningful comparisons. Treatments were replicated 6 times.

Confirmatory tests

Three replicated confirmatory tests were conducted, each treating more than 10 000 eggs, the life stage most tolerant to the insecticide in the host fruit, as identified above. These tests used 400 mg/L dimethoate applied so that the fruit was wetted to the point of run-off by a multi-nozzle sprayer in a module of a commercial tomato grading and packing conveyor system (George and Courtier, Brisbane). The module consisted of a variable speed conveyor belt feeding fruit onto a system of brushes and rollers at 0.5 m/min, which gave a nominal spray exposure time of 1 min. The sprayer was positioned above the brushes. Eight spray nozzles were used covering a section of the conveyor, 90 by 25 cm. Because of difficulties in obtaining a full set of identical nozzles, the first of the 3 replicates used 4 nozzles with an aperture size of X4 (discharge of 18.2 L per hour at 250 kPa) and 4 nozzles with an aperture size of X2 (discharge of 9.1 L per hour at 250 kPa). The second and third tests used 8 nozzles with an aperture size of X4. The insecticide solution was recirculated, with recovery of excess solution from fruit via the conveyor brushes to a drain tray with filtered return to the reservoir. Because the spraying was completed in a few hours with a new solution for each replicate, no loss in concentration was likely (Noble 1985).

Recovery of survivors

Treated and untreated fruit for both the life stage comparison tests and confirmatory tests were held separately over gauzed plastic boxes in holding cages, with sawdust as a pupation medium. All fruit were held in a controlled environment room maintained at 26°C and 70% RH. When pupation was observed to be complete, pupae were sieved from the sawdust.

Residue monitoring

For residue monitoring, 10 duplicate fruit samples of 4 capsicums were taken from a batch of fruit, treated as for the confirmatory tests, and stored at 13 or 20°C. Samples were analysed as for tomatoes (Heather *et al.* 1987) at 1 h and 1, 2, 3, and 7 days after treatment to simulate minimum handling, transport and shop shelf times. The spray solution was analysed pre- and post-treatment.

Statistical analysis

For the life stage comparison tests, an arcsine transformation was applied to the data, which were then analysed using a single factor analysis of variance. Treatment means were compared using the protected least significant difference (l.s.d.) method. The confirmatory tests were judged against the requirement to demonstrate 99.99% mortality at the 95% confidence level (Couey and Chew 1986).

Results

Life stage comparison tests

Holding times before treatment, on the basis of development studies (Table 1) were: first instars, 56 h; second instars, 96 h; and third instars, 128 h. Eggs were held for 32 h, which represented 80% development at 26°C. Life stages differed significantly in response to the dimethoate dip ($F_{3,9} = 7.64$; $P<0.01$) (Table 2). Eggs were significantly more tolerant ($P<0.05$) than first and third instars. Significant differences also occurred between the larval stages: second and third instars were significantly more tolerant than first instars. Although no significant difference was found between the tolerance of eggs and second instars, the mean mortality of eggs was lower so the confirmatory tests were performed on eggs. Young eggs were not tested because the residual life of the insecticide far exceeds 32 h, the age at which eggs were treated.

Table 1. Development times of life stages of *Bactrocera tryoni* in capsicums at 26°C and 70% relative humidity

Life stage	Range	Modal range
Eggs	0–40 h	—
First instars	40–96 h	50–70 h
Second instars	70–140 h	95–110 h
Third instars	110–200 h	124–140 h
Pupae	10–16 days	—

Table 2. Determination of the most tolerant stage of *Bactrocera tryoni* in capsicums treated with a dimethoate dip at 4 mg/L for 1 min

Values followed by a different letter are significantly different at $P = 0.05$

Life stage treated	Estimated number of insects treated	Mean mortality	
		Transformed mean[A]	Equivalent mean (%)
Eggs	1 792	0.9014a	61.49
First instars	2 642	1.2689c	91.16
Second instars	1 924	0.9779ab	68.78
Third instars	3 332	1.0876b	78.42
Average s.e.m.		0.05489	

[A] Arcsine transformation applied to the data before analysis.

Confirmatory tests

No *B. tryoni* eggs survived to the pupal stage from a total of 77 130 treated in 3 replicates of 20 045, 15 195, and 41 890 estimated from numbers of pupae in controls (Table 3). This gives 95% confidence that the mortality is 99.9961% or higher and exceeds the minimum required efficacy of 99.99% at the 95% confidence level (Couey and Chew 1986). The application rate in each confirmatory test was dependent on nozzle size (Table 4).

Residue monitoring

Mean dimethoate residues in fruit (Table 5) were less than the Australian maximum residue limit (MRL) of 2 mg/kg (Anon. 1999) from the day of application so no withholding period would be necessary. The actual concentration of dimethoate in the spray solution was 374 mg/L pre-treatment and 378 mg/L post-treatment.

Table 3. Mortality of *Bactrocera tryoni* eggs infesting capsicums treated with dimethoate as a packing-line spray of 400 mg/L for a nominal 1 min

Number of eggs treated was estimated from numbers of pupae in untreated fruit

Pupal viability shown by adult numbers

No. of fruit treated	No. of fruit untreated	No. of pupae from untreated fruit	No. of adults from untreated fruit	Estimated no. of eggs treated	No. of pupal survivors
170	34	4009	2616	20 045	0
360	72	3039	2879	15 195	0
200	40	8378	5603	41 890	0
730	146	15 426	11 098	77 130	0

Table 4. Packing-line spray application rates for dimethoate (400 mg/L) in confirmatory tests

Run	Application rate
First confirmatory test	7.25 L/min.m^2 for 64 s
Second confirmatory test	9.20 L/min.m^2 for 65 s
Third confirmatory test	9.20 L/min.m^2 for 73 s

Discussion

Levels of security for treatments against fruit fly vary from country to country and, within Australia. Current levels for treatments for Japan require zero survivors from 30 000 insects tested, which gives 95% confidence that the mortality is 99.99% or higher and for USA, no survivors from 93 613 tested or 99.9968% mortality at the 95% confidence level (Couey and Chew 1986) is required. Historically, treatments for Australia and New Zealand have been tested at the 30 000 level. The results presented here (Table 3) exceed that level of security with zero survivors from 77 130 tested, which gives 95% confidence that the mortality is 99.9961% or higher (Couey and Chew 1986).

Quarantine security in consignments of commercial fruit is derived from a combination of the initial risk of infestation and the efficacy of the disinfestation treatment. Regardless of quarantine requirements, commercially produced capsicums must have a very low incidence of fruit fly to meet consumer's quality requirements. Therefore capsicums for export to markets requiring a quarantine disinfestation treatment should have infestation levels below the level of detection before treatment. When this is considered in conjunction with a treatment efficacy of >99.99%, there is an extremely high level of assurance that any quarantine risk is minimal. Our treatment is at least as efficacious on capsicums as the dimethoate packing-line flood treatment approved for tomatoes by Australian and New Zealand authorities, of zero survivors from 33 310 insects tested (Heather *et al.* 1987).

Table 5. Mean dimethoate residues in capsicums treated as for confirmatory tests and stored at 13°C or 20°C

Duplicate samples are in parentheses

Time after treatment (days)	Dimethoate residues (mg/kg)	
	13°C	20°C
0	0.69 (0.66, 0.72)	0.69 (0.66, 0.72)
1	0.91 (1.05, 0.78)	0.79 (0.75, 0.82)
2	0.83 (0.63, 1.04)	0.91 (1.00, 0.82)
3	0.79 (0.73, 0.85)	0.66 (0.62, 0.70)
7	0.72 (0.63, 0.81)	0.60 (0.65, 0.54)

Fruit fly eggs are present just below the surface at treatment and could be expected to be exposed to higher insecticide concentrations for a longer time than larvae. This gives added assurance that eggs, shown to be more tolerant (or not less tolerant) than other in-fruit stages, were the most appropriate life stage for testing the treatment. The decision to adopt a single dose approach to identify which, if any, life stage was more tolerant to the treatment in fruit was made on the basis of past experience (Corcoran *et al.* 1993). While a multiple dose experimental design could identify any differences in responses between life stages in a holistic way, this is only possible if the response lines can be shown to be within acceptable limits of parallelism. Typically, only single point pairwise comparisons are appropriate, such as at the LD50. These are equivalent to our single treatment design but are probably less precise as they are an estimate from a regression line whereas our data were actual mortalities, albeit estimated from survivors.

Maximum wetting of fruit has been found to be important although it is doubtful if a time of more than 1 min is advantageous (Swaine *et al.* 1984). In the confirmatory tests we changed the size of nozzles and hence the application rate after the first replicate when extra nozzles of size X4 became available. Although there were no survivors from the first replicate, any treatment to be recommended from this work would need to be at the higher rate of application since the lower rate of the first replicate was not tested on the full required complement of 30 000. Once fruit are thoroughly wetted any increase in the application rate (but not the concentration) should be irrelevant. It can be expected that, if residues on the fruit surface are not subsequently removed, maximum uptake of insecticide would occur from any fruit which had been wetted to the point of run-off.

Mean residues were below the Australian MRL for dimethoate on capsicums of 2 mg/kg (Anon. 1999) from the time of treatment. At the normal handling temperature of 13°C there was no marked fall in the level of dimethoate for the 7-day test period.

On the basis of our results we propose that a postharvest packing-line spray treatment with 400 mg/L dimethoate which results in fruit being kept thoroughly wetted for 1 min be accepted as an appropriate fruit fly quarantine disinfestation treatment for capsicums.

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