



Know-how for Horticulture™

**Disinfestation
treatments for
additional varieties of
tomatoes for New
Zealand using
dimethoate**

R Corcoran
Queensland Horticulture
Institute

Project Number: VG98139

VG98139

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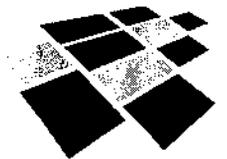
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FINAL REPORT

Disinfestation Treatments for Additional Varieties of Tomatoes for New Zealand Using Dimethoate

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Corcoran, R.J. *et al*



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The purpose of the report is to communicate results to Horticulture Australia at the conclusion of the project.

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Contents

Contents.....	3
List of Figures.....	4
<i>Media Summary</i>	5
<i>Technical Summary</i>	6
<i>Introduction</i>	7
<i>Materials and Methods</i>	8
Part I: Studies on the Effects of Fruit Ripeness and Cool Storage	8
Fruit	8
Test Insects.....	8
Infestation	8
Treatment	9
Post-treatment	9
Residue testing	9
Part II: Dimethoate Treatment of Fruit	10
Fruit	10
Insecticide	10
Insects	10
Infestation	10
Treatment	11
Post treatment.....	11
Measurement of Infestation Variability Due to Cage Infestation	12
<i>Results</i>	<i>12</i>
Part I: Studies on the Effects of Fruit Ripeness and Cool Storage	12
Cool storage trial	12
Ripeness trial.....	13
Part II: Dimethoate Treatment of Fruit	14
Dip Treatment	14
Spray Treatment	15
Measurement of Infestation Variability Due to Cage Infestation	17

<i>Discussion</i>	18
<i>Recommendations</i>	19
<i>References</i>	20

List of Tables

Table 1. Effect of 1 min dip in dimethoate at 400 mg/L, with and without post-treatment cool storage at 10°C for 5 days, and cool storage alone on survival of eggs of <i>B. tryoni</i> in tomatoes (cv Floradade).....	12
Table 2. Dimethoate residues recovered from tomato fruit (cv "Floradade" held at 10oC and 26 oC after treatment with a dimethoate dip at 400 mg/L.....	13
Table 3. Effect of stage of maturity of tomatoes on mortality of <i>B. tryoni</i> eggs when treated with a dimethoate dip at 400 mg/L for 1 min. "green" fruit were green to 1/4 colour; "ripe" fruit were at colour to full colour (Bagshaw <i>et al.</i> 1997).....	13
Table 4. Dimethoate residues recovered from tomatoes (cv Floradade) treated at two stages of ripeness. Fruit dipped in dimethoate solution at 400 mg/L.	14
Table 5. Effect of a dimethoate dip at 400 mg/L for 1 minute on eggs and larvae of Queensland fruit fly in tomatoes..	15
Table 6. Effect of dimethoate spray treatments on eggs and larvae of Queensland fruit fly, <i>Bactrocera tryoni</i> , in tomatoes (var "Daniella").....	16
Table 7. Effect of dimethoate spray treatments on eggs and larvae of Queensland fruit fly, <i>Bactrocera tryoni</i> , in tomatoes (var "Redcoat").....	16
Table 8. Effect of dimethoate spray treatments on eggs and larvae of Queensland fruit fly, <i>Bactrocera tryoni</i> , in tomatoes (var "Tracer").....	17
Table 9. Effect of dimethoate spray treatments on eggs and larvae of Queensland fruit fly, <i>Bactrocera tryoni</i> , in tomatoes (var "Guardian").....	17

List of Figures

Figure 1. Cage infestation of tomatoes. Tomatoes set up in rows (left), female flies ovipositing in punctured tomato (right).....	11
Figure 2. Tomatoes being spray treated on conveyor system. Tomatoes under spray nozzles (left), tomatoes drying on rollers after treatment (right).....	11
Figure 3. Holding tomatoes after treatment to allow excess liquid to drain away.....	12

Media Summary

Currently tomatoes are approved for export to New Zealand subject to a quarantine disinfection treatment with the insecticide dimethoate. However, this approval extends to only five cultivars. Previously New Zealand has required extensive research on each cultivar. Recent negotiations resulted in an agreement on research methodology that is simpler and less costly making it feasible to undertake research to extend the approval to include other varieties. The tomato industry identified four other varieties of tomatoes, "Daniella", "Tracer", "Redcoat" and "Guardian", with potential for export to New Zealand.

Results of the research showed that:

- ◆ Dimethoate is equally effective against fruit fly eggs in green and ripe fruit.
- ◆ Cold storage of tomatoes after dimethoate treatment did not diminish the effect of the dimethoate. Cool storage alone caused significant mortality to fruit fly eggs.
- ◆ Dipping in a 400 mg/L solution of dimethoate for 1 minute produced a mortality of at least 99.996% for eggs and larvae of Queensland fruit fly.
- ◆ Spraying with a 400mg/L solution of dimethoate + Agral 600 at the rate of 24L/m²/min produced a mortality of at least 99.953% for eggs and larvae of Queensland fruit fly.
- ◆ Therefore both spray and dip treatments can provide a high level of quarantine security against fruit flies in all varieties.

Technical Summary

Currently tomatoes are approved for export to New Zealand subject to a quarantine disinfection treatment with the insecticide dimethoate. However, this approval extends to only five cultivars. Previously New Zealand has required extensive research on each cultivar. Recent negotiations resulted in an agreement on research methodology which acknowledges previous research to develop quarantine treatments and treats any further research as an extension of an existing process. Research methods under this arrangement are simpler and less costly making it feasible to undertake research to extend the approval to include other varieties. The tomato industry identified four other varieties of tomatoes, "Daniella", "Tracer", "Redcoat" and "Guardian", with potential for export to New Zealand.

Tomatoes are picked at mature green before being treated and held in cool storage for transport to New Zealand. There was some concern on the part of New Zealand scientists that the action of dimethoate would be different in green and ripe fruit. Comparison of mortality rates showed no measurable difference in efficacy when treatments were applied against *Bactrocera tryoni* (Froggatt) eggs in green and ripe fruit.

Before insecticide treatments were conducted, it was also necessary to determine if holding tomatoes in cold storage after dimethoate treatment could slow development of eggs and delay hatch to a point where the residual levels of dimethoate was reduced below the lethal level.

Tomatoes (cultivar "Floradade") were infested with eggs of Queensland fruit fly, *B. tryoni* and treated using a dimethoate dip at 400 mg/L for 1 minute. Treatment using dimethoate alone resulted in 0 survivors from 14 131 treated insects. The addition of a post-treatment cool storage treatment at 10°C for 5 days significantly enhanced treatment mortality based on undipped fruit. On the basis of these results we conducted research on dimethoate treatments of additional tomato cultivars using ripe fruit and without post-treatment cool storage. This ensured that testing was carried out under conditions most conducive to insect survival.

Insecticide treatments were conducted on each of the four additional varieties of tomatoes, "Daniella", "Redcoat", "Tracer" and "Guardian". Tomatoes were dipped in a 400 mg/L solution of dimethoate for 1 min. No survivors resulted from 185 020 treated eggs and larvae in 6 188 fruit. This represents a mortality rate of >99.996%. The dip treatment described here would provide an adequate level of quarantine security against fruit flies for tomatoes generally and for these varieties in particular.

Tomatoes of the four varieties were treated with a spray treatment of dimethoate plus Agral for 10 sec. Spray treatment resulted in consistently high mortality for eggs and larvae of Queensland fruit fly. In statistical terms, mean mortality across all varieties at the 95% confidence level (CL) was >99.996% for eggs and >99.953% for larvae. For technical reasons, the number of insects treated from the spray treatment in some of these experiments fell short of the nominated 30 000 larvae. When this treatment is evaluated taking into account pre-harvest controls for fruit flies and the historical record of its use, we believe that these would provide a treatment efficacy equivalent to 99.990% (95% CL) which is implied by the requirement to treat 30 000 insects.

During infestation for the confirmatory tests a measurement of infestation variability due to cage infestation was taken. Comparison of the estimated number with the actual number provides an indication of the inherent variability in the number of eggs oviposited during cage infestation.

Introduction

Many horticultural products are hosts for fruit flies (family Tephritidae) which are often considered high-risk quarantine pests by regulatory authorities. The presence of fruit flies in the main tropical and sub-tropical production areas of Australia, including Queensland, results in the imposition of quarantine barriers to the movement of fresh produce. These quarantine barriers greatly impede trade both within Australia and to overseas markets that are free of these pests. Postharvest disinfestation treatments are often required in order to overcome these quarantine barriers.

Tomatoes of the varieties "Floradade", "Hayslip", "Tristar", "Sunny" and "Duke" are approved for export to New Zealand subject to a fruit fly disinfestation treatment using dimethoate at 400 mg/L applied as either a spray or a dip. Newer tomato varieties with improved agronomic and marketing characteristics are now available. Further research using immersion (dip) and spray treatment of dimethoate against fruit flies in four additional tomato varieties was carried out. These varieties, "Daniella", "Redcoat", "Tracer" and "Guardian" are suitable for export and are representative of a range of new seed lines now available.

Areas of research identified by Ministry of Agriculture and Fisheries, New Zealand (MAFNZ) as requiring action relate to:

- ◆ assessment of the effects of cool storage of treated fruit on insect survival
- ◆ assessment of the effects of fruit ripeness on treatment efficacy
- ◆ Confirmatory testing of the dimethoate dip treatment against eggs and larvae of Queensland fruit fly, *Bactrocera tryoni*
- ◆ Confirmatory testing of the dimethoate spray against eggs and larvae of Queensland fruit fly, *Bactrocera tryoni*
- ◆ Infestation variability in fruit due to cage infestation.

Infestation variability in fruit due to cage infestation was examined specifically at the request of MAFNZ scientists in their response to previous research reports.

The agreed research protocol required that:

- ◆ Confirmatory trials were carried out on each additional variety
- ◆ Eggs and larvae (mix of second and third instars) were treated with a flood spray at 400mg/L for 10 sec (this also justified a dip application)
- ◆ Eggs to be treated 24h after infestation
- ◆ 30 000 eggs and 30 000 mature larvae to be treated
- ◆ Treatments to be carried out as 3 replicates of at least 10 000 insects or 4 replicates of at least 7 500
- ◆ Fruit to be infested using punctured fruit placed in cages of fruit flies.

The methodology used is in accordance with MAFNZ requirements as agreed to at a joint meeting between MAFNZ, AQIS, HortResearch, Crop and Food Research and QDPI representatives on 20 November 1998.

Materials and Methods

Part I: Studies on the Effects of Fruit Ripeness and Cool Storage

Fruit

Tomatoes of the variety "Floradade" were grown at the Department of Primary Industries Research Station at Bundaberg without the use of any insecticides. They were harvested at the colour break stage and sent to the Indooroopilly laboratory by overnight road transport. Some fruit were allowed to ripen naturally and some were held at 5°C until required. All fruit were allowed to warm to ambient (~24°C) before infestation.

For the cool storage trial fruit were infested at the 3/4 colour to full colour grade. For the ripeness trial, fruit allocated to the maturity stage "green" were infested at green to 1/4 colour; fruit allocated to maturity stage "ripe" were infested at coloured to full. Assessment of colour stage was made using the standard of Bagshaw *et al.* (1997).

Test Insects

Adults of *B. tryoni* were housed at the Queensland Horticulture Institute (QHI) laboratories of the Queensland Department of Primary Industries (QDPI) at Meiers Road, Indooroopilly, Brisbane. Adult flies were held in cubic cages with sides of 0.7 metres covered on the sides and top with nylon mesh of 2mm. Each cage contained sugar (sucrose) cubes and autolysed brewer's yeast as food, and water containers fitted with sponge wicks. The colony of *B. tryoni*, was held in a controlled environment room receiving both artificial and natural light with temperature and humidity maintained at 26°C and 70% RH. Artificial lighting was turned off before dusk and on after dawn so that flies experienced a natural dusk and dawn.

The colony used in these experiments had been in laboratory culture for 18 generations having been reared from peaches, guavas and mangoes collected during November to March 1997. Further wild flies were added to the colony in November 1997 from collections of feral peaches and papaws.

Culturing of the colony was carried out as described by Heather and Corcoran (1985), with one exception. Eggs were collected from adults using a plastic collection cup punctured using a 0.5 mm diameter pin as the oviposition receptacle, rather than a hollowed apple dome. The collection cup was coated internally with apple juice before being placed into the adult cage to stimulate oviposition activity.

Infestation

Fruit were punctured 10 times with a 0.5mm diameter entomological pin around the flower end and placed into cages containing approximately 20 000 adult *B. tryoni*. Flies were between 3 and 5 weeks old when used in these experiments ensuring maximum fertility and fecundity. Flies held under the conditions in our insectary are positively phototactic: insect density being greatest against the face of the cage closest to the light and diminishing with distance. Therefore, fruit for infestation were arranged in groups of six, parallel to the lighted face of the cage. One fruit from each row was randomly selected from each row as a control. After infestation, fruit were held in a controlled environment room at 26°C and 70% RH for 24-26 hours prior to treatment.

Treatment

Cool storage trial

Treatments were:

Dimethoate only. Fruit was fully immersed in a solution of dimethoate at 400 mg/L for 1 minute, allowed to air dry.

Dimethoate plus cool storage. Fruit were dipped in dimethoate as above, allowed to air dry then held in cartons at 10°C for 5 days.

Cool storage only. Fruit were held in cartons at 10°C for 5 days; no dimethoate treatment.

Ripeness trial

Fruit for treatment from both maturity stages were fully immersed in a solution of dimethoate at 400 mg/L for 1 minute, allowed to air dry.

Post-treatment

After treatment fruit were held in ventilated cages at 26°C over plastic boxes covered in gauze to collect any liquid produced during fruit breakdown. This procedure allowed any survivors to develop to the pupal stage. These boxes were placed over moistened vermiculite as a pupation medium. All treated and control fruit were held the same way in the same controlled environment room. Surviving puparia were recovered by sieving the vermiculite.

The number of insects treated was estimated from the number of pupae surviving in the untreated control fruit corresponding to each batch of treatment fruit.

Residue testing

Cool storage trial

Uninfested fruit were dipped in dimethoate as for the above treatments and allowed to air dry. One hour after dipping, a sample of 4 fruit was frozen at -17°C. One half of the remaining fruit were held at 26°C and the other half at 10°C. Samples of four fruit were taken from each group at 1, 3 and 5 days after treatment then frozen at -17°C. These fruit samples were subsequently analysed for whole fruit residues of dimethoate and omethoate using the methods of Swaine *et al.* (1984) and Heather *et al.* (1987)..

Ripeness trial

Uninfested fruit were dipped in dimethoate as for the above treatments and allowed to air dry. One hour after dipping a sample of 4 fruit at the green to 1/4 colour grade and 4 fruit from the coloured to full colour grade were frozen at -17°C. The remaining fruit from both ripeness grades were held at 26°C. At 1, 3 and 5 days after treatment a sample of 4 fruit was taken from each ripeness grade and frozen. These fruit samples were subsequently analysed for whole fruit residues of dimethoate and omethoate using the methods of Swaine *et al.* (1984) and Heather *et al.* (1987).

Part II: Dimethoate Treatment of Fruit

Fruit

Tomatoes were specially grown at QDPI research stations at Bundaberg and Bowen. Fruit were grown without the use of chemical insecticides to ensure that no residues likely to confound the results of future experiments would be present. Fruit were sprayed with the fungicide Mancozeb when required. The biological insecticide Dipel was used on occasion and also oils (DC Tron and Synetrol Horti-oil) to control white flies.

Fruit were harvested at colour break and were allowed to ripen naturally before infestation. Fruit for larval treatment were infested at about quarter colour while fruit for egg treatments were infested at half to full colour. It was necessary to infest fruit for larvae at an earlier stage since this fruit had to be held for *ca* 5 days to allow larval development and still be sound enough to handle and treat.

Insecticide

Commercial dimethoate was used in these experiments. The concentrate was analysed at the beginning of each season and found to contain 413 g/L active constituent in 1999 and 410 g/L in 2000. These figures were used to calculate the dimethoate component of the treatment solutions. After analysis, the concentrate was stored in a refrigerator when not in use.

Insects

Adults of *B. tryoni* were housed at the Queensland Horticulture Institute (QHI) of the Department of Primary Industries (QDPI) laboratories, 80 Meiers Rd, Indooroopilly, Brisbane. Adult flies were held in cubic cages with sides of 0.7 metres covered on the sides and top with nylon mesh of 2 mm. Each cage contained sugar (sucrose) cubes and autolysed brewer's yeast as food and water containers fitted with sponge wicks. Adults were held in a controlled environment room with natural and controlled lighting and with temperature and humidity maintained at 26°C and 70% RH. Cages contained approximately 20 000 flies. These were cultured on a carrot-based, semi-artificial diet as described by Heather and Corcoran (1985).

The colony used during the 1999 season was established in January 1997 (supplemented March 1999) and was used for experiments from the 3rd generation until the 8th generation. Regular quality control tests for the colony used in 1999 showed that fertility based on egg hatch was 91%, pupal emergence was 92% and flight ability was 93% (mean values). In the 2000 season a new colony established in November 1999 was used from the 5th to the 10th generation. Quality control data for 2000 was: egg hatch 90%, pupal emergence 93% and flight ability 89% (mean values).

Infestation

During the 1999 season, fruit were punctured 10 times with a size 5 entomological pin (0.5mm diameter) around the flower end of the fruit. During the 2000 season fruit were punctured 15 times with a micro-pin. This change was requested by MAFNZ scientists who had concerns about dimethoate uptake through the pin holes. Puncturing of fruit ensured an even distribution of eggs within the fruit and more uniform infestation levels between fruit. Fruit was placed in rows inside cages containing approximately 20 000 adults of *B. tryoni* (approximately 1:1 ratio of males and females) at maximum fecundity (3-6 weeks after eclosion). Female flies were allowed to oviposit for 5 to 20 minutes depending on observed activity.

One fruit from each row (usually of 6) were allocated to an untreated (control) group. This gave parallel samples for estimation of numbers present in fruit at time of treatment. To ensure that treated fruit containing larvae had the intended life stage present, extra fruit were infested from which all immature insects were examined at the time of treatment. Fruit were held at 26°C and

70% RH for about 4-5 days to allow development to the larval stage required. Fruit for egg treatments were held under the same conditions for 24 hours.

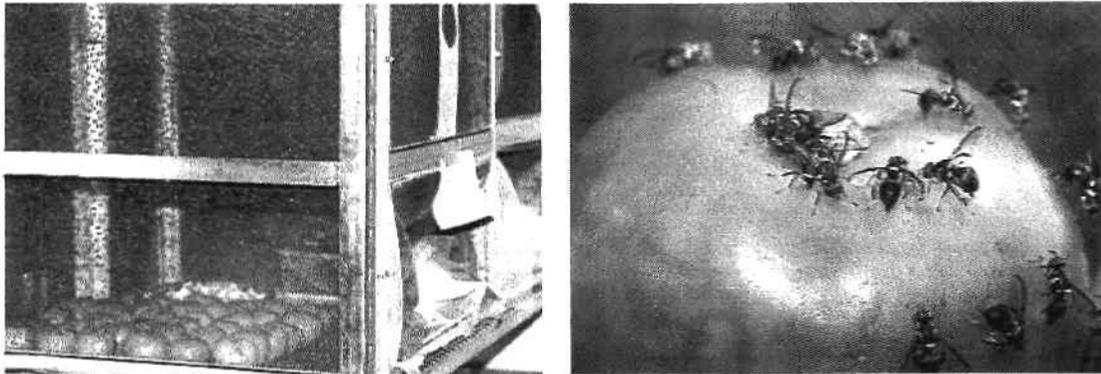


Figure 1. Cage infestation of tomatoes. Tomatoes set up in rows (left), female flies ovipositing in punctured tomato (right)

Treatment

Dip

Fruit were treated with an immersion dip for 1 minute using dimethoate solution at 400 mg/L. No wetting agent was used in these dip treatments. Fruit for dipping were placed in a stainless steel cage that was lowered into a stainless steel tank containing the dip solution. Fruit remained submerged for 1 minute then the cage was raised and allowed to drain. Fruit were removed from the cage and allowed to air dry. Dip solutions were sampled before and after treatment and analysed for dimethoate.

Spray

Tomatoes were treated on a commercial packing line module that would form part of a typical tomato grading and packing system (George and Courtier, Brisbane). The module consisted of a variable speed conveyor of brushes with overhead sprayers. The number of active spray nozzles was also adjustable. The concentration of dimethoate solution used for all treatments was 400 mg/L with Agral 600 being added at the rate of 0.5ml/L. The spray-line was calibrated to deliver 24L/m²/min. Fruit remained under the dimethoate spray (nominally 400 mg/L) for 10 sec and remained wet for at least a further 1 min as they passed along the conveyor. Spray solutions were sampled before and after treatment and were analysed for dimethoate.

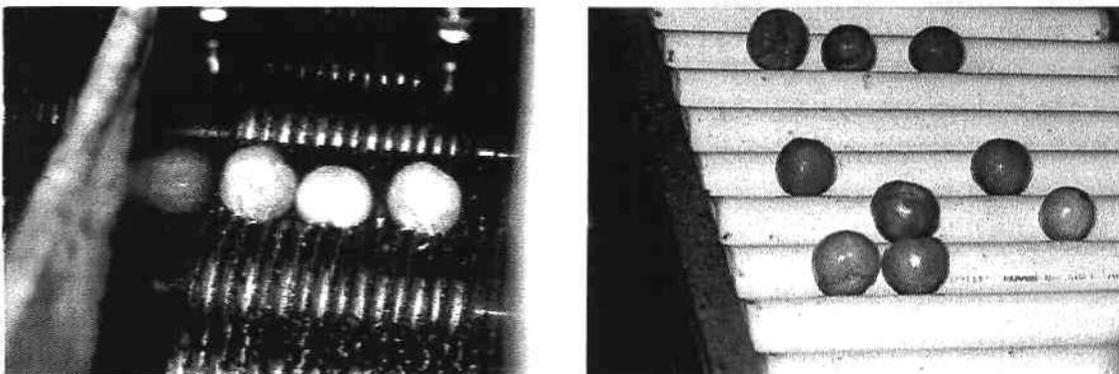


Figure 2. Tomatoes being spray treated on conveyor system. Tomatoes under spray nozzles (left), tomatoes drying on rollers after treatment (right)

Post treatment

After treated fruit had dried they were placed on gauzed plastic crisper boxes to allow surviving insects to develop and to allow excess liquid from fruit breakdown to drain away. These crispers were held in screened cages at 26°C and 70% RH containing moistened vermiculite as a pupation

medium. The pupation medium was sieved at least twice during the 8-21 day holding period to recover surviving pupae. Control fruit, representing 1/3 to 1/5 of the treated sample were treated identically but were not dipped or sprayed. Surviving pupae from both treated and control were counted, the numbers recovered from the controls were used to estimate the number that were treated.



Figure 3. Holding tomatoes after treatment to allow excess liquid to drain away

Measurement of Infestation Variability Due to Cage Infestation

During infestation for the confirmatory tests, a sub-sample of fruit from each trial of the varieties “Daniella” and “Tracer”, were set aside. These fruit were allocated to either “treated” or “control” as described under “Treatment” (above), but were not treated. Fruit were infested in an identical manner to the fruit for treatment using fruit flies from one of the same cages. After infestation each batch of fruit was held separately as “treated” and “control” so that pupae could be recovered and counted.

Actual numbers from those allocated to the “treated” batches were determined by counting recovered pupae. Estimates of numbers for the same batches were calculated from the corresponding controls as described in “Infestation” (above). Comparison of the estimated number with the actual number provides an indication of the inherent variability in the number of eggs oviposited during cage infestation.

Results

Part I: Studies on the Effects of Fruit Ripeness and Cool Storage

Cool storage trial

Treatment of tomatoes (cv “Floradade”) infested with *ca* 24 h old eggs of *B. tryoni* with a dimethoate dip at 400 mg/L for 1 min resulted in 0 pupal survival from an estimated 14 131 treated insects (Table 1). Addition of a cool storage treatment at 10°C for 5 days did not diminish the effectiveness of the dimethoate dip treatment (Table 1). Cool storage treatment at 10°C for 5 days alone caused significant mortality (99.610%) to eggs of *B. tryoni* in tomatoes (Table 1).

Table 1. Effect of 1 min dip in dimethoate at 400 mg/L, with and without post-treatment cool storage at 10°C for 5 days, and cool storage alone on survival of eggs of *B. tryoni* in tomatoes (cv Floradade).

Treatment	No. of Fruit Treated	No. insects treated ¹	Pupal Survival	Mortality (%)	Mortality 95% CL (%) ²
Dimethoate only	225	14 131	0	100	99.978
Dimethoate + cool storage	228	28 842	0	100	99.989
Cool storage only	205	32 793	128	99.610	NA

¹ Estimated from numbers surviving in untreated controls

² Lower 95% confidence limit of the true population mortality, calculated using the method of Couey and Chew (1986)

Residue determinations in held at 10 and 25°C after treatment showed that residue levels immediately after treatment (day 0) were similar in both treatments. The rate of breakdown of dimethoate was slowed in the fruit held at 10°C. In all samples the residues were below the maximum residue limit of 2 mg/kg (Table 2).

Table 2. Dimethoate residues recovered from tomato fruit (cv "Floradade" held at 10°C and 26°C after treatment with a dimethoate dip at 400 mg/L

Storage 10°C						
Run Number	Lab Number	Day	Date Analysed	Result (mg/kg)		
				Omethoate	Dimethoate	Total Dimethoate*
30	772/99/Ra	control	20-Jul-99	NDR	NDR	NDR
31	772/99/Rb	control	20-Jul-99	NDR	NDR	NDR
32	773/99/Ra	0	20-Jul-99	NDR	0.39	0.39
33	773/99/Rb	0	20-Jul-99	NDR	0.43	0.43
34	774/99/Ra	1	20-Jul-99	NDR	0.58	0.58
35	774/99/Rb	1	20-Jul-99	NDR	0.55	0.55
37	775/99/Ra	3	21-Jul-99	0.01	0.64	0.64
38	775/99/Rb	3	21-Jul-99	0.01	0.54	0.54
39	776/99/Ra	5	21-Jul-99	0.01	0.40	0.40
40	776/99/Rb	5	21-Jul-99	0.01	0.40	0.40

Storage 26°C						
Run Number	Lab Number	Day	Date Analysed	Result (mg/kg)		
				Omethoate	Dimethoate	Total Dimethoate*
30	772/99/Ra	control	20-Jul-99	NDR	NDR	NDR
31	772/99/Rb	control	20-Jul-99	NDR	NDR	NDR
32	773/99/Ra	0	20-Jul-99	NDR	0.39	0.39
33	773/99/Rb	0	20-Jul-99	NDR	0.43	0.43
41	777/99/Ra	1	21-Jul-99	0.01	0.39	0.39
42	777/99/Rb	1	21-Jul-99	NDR	0.43	0.43
43	778/99/Ra	3	21-Jul-99	NDR	0.35	0.35
44	778/99/Rb	3	21-Jul-99	0.01	0.33	0.33
45	779/99/Ra	5	21-Jul-99	0.02	0.27	0.27
46	779/99/Rb	5	21-Jul-99	0.02	0.27	0.27

* Dimethoate residues defined as sum of omethoate and dimethoate, expressed as dimethoate
 NDR no detectable residues. Limit of Quantitation Omethoate 0.01mg/kg Dimethoate 0.03mg/kg

Ripeness trial

Treatment of eggs of *B. tryoni* in "green" and "ripe" tomatoes with a dimethoate dip at 400 mg/L for 1 min resulted in no survivors from an estimated 14 800 and 14 584 treated eggs respectively (Table 3).

Table 3. Effect of stage of maturity of tomatoes on mortality of *B. tryoni* eggs when treated with a dimethoate dip at 400 mg/L for 1 min. "green" fruit were green to 1/4 colour; "ripe" fruit were at colour to full colour (Bagshaw *et al.* 1997).

Fruit maturity	No. of Fruit Treated	No. insects Treated ¹	Pupal Survival Control	Pupal Survival Treated	Mortality (%)	Mortality 95% CL (%) ²
Green	240	14 800	62/fruit	0	100	99.979
Ripe	239	14 584	61/fruit	0	100	99.979

¹ Estimated from numbers surviving in untreated controls

² Lower 95% confidence limit of the true population mortality, calculated using the method of Couey and Chew (1986).

Total dimethoate residues recovered from fruit treated at two ripeness stages ("green" and "ripe") were similar (Table 4).

Table 4. Dimethoate residues recovered from tomatoes (cv Floradade) treated at two stages of ripeness. Fruit dipped in dimethoate solution at 400 mg/L.

Treated as "Green"						
Run Number	Lab Number	Day	Date Analysed	Result (mg/kg)		
				Omethoate	Dimethoate	Total Dimethoate*
1	762/99/Ra	control	16-Jul-99	NDR	NDR	NDR
8	762/99/Rb	control	17-Jul-99	NDR	NDR	NDR
9	763/99/Ra	0	17-Jul-99	NDR	0.73	0.73
10	763/99/Rb	0	17-Jul-99	NDR	0.58	0.58
11	764/99/Ra	1	17-Jul-99	0.02	0.65	0.65
12	764/99/Rb	1	17-Jul-99	0.02	0.60	0.60
14	765/99/Ra	3	19-Jul-99	0.02	0.39	0.39
15	765/99/Rb	3	19-Jul-99	0.02	0.43	0.43
16	766/99/Ra	5	19-Jul-99	0.03	0.40	0.40
17	766/99/Rb	5	19-Jul-99	0.03	0.44	0.44

Treated as "Ripe"						
Run Number	Lab Number	Day	Date Analysed	Result (mg/kg)		
				Omethoate	Dimethoate	Total Dimethoate*
19	767/99/Rb	control	19-Jul-99	NDR	NDR	NDR
20	768/99/Ra	0	19-Jul-99	NDR	0.40	0.40
21	768/99/Rb	0	19-Jul-99	NDR	0.49	0.49
22	769/99/Ra	1	19-Jul-99	0.01	0.54	0.54
23	769/99/Rb	1	19-Jul-99	0.01	0.51	0.51
26	770/99/Ra	3	20-Jul-99	0.02	0.60	0.60
27	770/99/Rb	3	20-Jul-99	0.03	0.73	0.73
28	771/99/Ra	5	20-Jul-99	0.02	0.32	0.32
29	771/99/Rb	5	20-Jul-99	0.02	0.37	0.37

* Dimethoate residues defined as sum of omethoate and dimethoate, expressed as dimethoate
 NDR no detectable residues. Limit of Quantitation Omethoate 0.01mg/kg Dimethoate 0.03mg/kg

Part II: Dimethoate Treatment of Fruit

Dip Treatment

Dip treatments for 1 minute using 400mg/L dimethoate were fully effective against eggs and larvae of Queensland fruit fly in the four tomato varieties treated. Complete kill was achieved in every replicate and a total of 110 600 eggs and 74 420 larvae were treated without survival. This represents a mortality of $\geq 99.997\%$ (95% confidence) and $\geq 99.996\%$ (95% confidence) respectively (Table 5).

Analysis of the working dip solutions showed that the concentration of the dip solution was usually slightly below the nominal 400 mg/L. On one occasion an error occurred resulting in a lower than expected working concentration of 288/289 mg/L (Table 5).

Table 5. Effect of a dimethoate dip at 400 mg/L for 1 minute on eggs and larvae of Queensland fruit fly in tomatoes.

Variety	Stage/ Rep	No. Fruit	No. Insects Treated ¹	Pupal Survival	Mortality (%)	Mortality 95% CL (%) ²	Dimethoate Conc (mg/L) ³
Daniella	Egg 1	402	16 589	0	100	99.982	394/395
Redcoat	Egg 1	665	24 225	0	100	99.988	400/399
	Egg 2	680	11 572	0	100	99.974	370/370
Tracer	Egg 1	318	9 311	0	100	99.968	381/384
	Egg 2	600	24 072	0	100	99.988	288/289
Guardian	Egg 1	241	9 810	0	100	99.970	-
	Egg 2	841	15 021	0	100	99.988	387/388
Total		3 747	110 600	0	100	99.997	
Daniella	Larva 1	601	20 860	0	100	99.986	394/395
Redcoat	Larva 1	329	3 318	0	100	99.910	405/401
	Larva 2	483	7 916	0	100	99.962	390/393
Tracer	Larva 1	209	6 778	0	100	99.956	386/387
	Larva 2	659	29 625	0	100	99.950	366/371
Guardian	Larva 1	160	5 923	0	100	99.949	-
Total		2 441	74 420	0	100	99.996	

¹Estimated from numbers surviving in untreated controls

²Lower 95% confidence limit of the true population mortality, calculated using the method of Couey and Chew (1986)

³Concentration (mg/L) expressed as before/after treatment

There were no significant differences in concentration in samples taken before and after treatment. Instar checks showed that larval treatments consisted of a mixture of second and third instars with seconds predominating.

Spray Treatment

Packing line spray treatments using dimethoate caused consistently high mortality to eggs and larvae of *B. tryoni* in all tomato varieties (Tables 4-7). However, occasional pupal survivors occurred in all treatments. Treatment was most effective in the variety "Daniella" where mortality rates for both eggs and larvae were 99.985% and 99.988% (95%CL) from 79 308 and 96 599 treated insects respectively (Table 6).

The concentration of spray solutions was consistently within 5% of the nominal 400mg/L with one exception. On this occasion a lower than expected working concentration occurred due to operator error (Table 6). No significant differences in concentration were detected in samples taken before and after treatment.

Table 6. Effect of dimethoate spray treatments on eggs and larvae of Queensland fruit fly, *Bactrocera tryoni*, in tomatoes (var "Daniella").

Stage/ Rep	No Fruit	No Insects Treated ¹	Pupal Survival	Mortality (%)	Mortality 95% CL(%) ²	Dimethoate Conc (mg/L) ³
Egg 1	416	13 633	0	100		392/397
Egg 2	557	22 695	0	100		390/391
Egg 3	620	42 980	4	99.991		391/394
	1593	79 308	4	99.995	99.985	
Larva 1	666	38 392	2	99.995		399/397
Larva 2	627	28 793	4	99.986		347/363
Larva 3	658	29 414	0	100		390/391
	1951	96 599	6	99.994	99.988	

¹Estimated from numbers surviving in untreated controls

²Lower 95% confidence limit of the true population mortality, calculated using the method of Couey and Chew (1986)

³Concentration (mg/L) expressed as before/after treatment

Spray line treatment of the variety "Redcoat" produced mortalities of 99.986% (95% confidence) and 99.970% (95% CL) for eggs and larvae respectively from a total of 56 255 and 30 869 insects treated respectively (Table 7).

Table 7. Effect of dimethoate spray treatments on eggs and larvae of Queensland fruit fly, *Bactrocera tryoni*, in tomatoes (var "Redcoat").

Stage/ Rep	No. Fruit	No. Insects Treated ¹	Pupal Survival	Mortality (%)	Mortality 95% CL (%) ²	Dimethoate Conc (mg/L) ³
Egg 1	840	19 259	0	100		396/407
Egg 2	717	11 632	2	99.983		394/392
Egg 3	598	25 364	1	99.996		394/397
	2 155	56 255	3	99.995	99.986	
Larva 1	374	2 618	0	100		391/392
Larva 2	657	16 054	0	100		383/399
Larva 3	602	12 197	4	99.967		396/398
	1 633	30 869	4	99.987	99.970	

¹Estimated from numbers surviving in untreated controls

²Lower 95% confidence limit of the true population mortality, calculated using the method of Couey and Chew (1986)

³Concentration (mg/L) expressed as before/after treatment

Dimethoate spray treatment of the variety "Tracer" resulted in a total of 38 599 eggs and 15 150 larvae being treated with a mortality of 99.953% (95%CL) for eggs and 99.969% (95%CL) for larvae (Table 8).

Table 8. Effect of dimethoate spray treatments on eggs and larvae of Queensland fruit fly, *Bactrocera tryoni*, in tomatoes (var "Tracer").

Stage/ Rep	No. Fruit	No. Insects Treated ¹	Pupal Survival	Mortality (%)	Mortality 95% CL (%) ²	Dimethoate Conc (mg/L) ³
Egg 1	448	10 936	0	100		398/404
Egg 2	381	13 603	9	99.934		397/409
Egg 3	524	14 060	2	99.986		393/398
	1353	38 599	11	99.972	99.953	
Larva 1	548	15 150	1	99.993		398/401
	548	15 150	1	99.993	99.969	

¹Estimated from numbers surviving in untreated controls

²Lower 95% confidence limit of the true population mortality, calculated using the method of Couey and Chew (1986)

³Concentration (mg/L) expressed as before/after treatment

Spray line treatment of variety "Guardian" gave mortalities of 99.982% and 99.963% (95% CL) for eggs and larvae respectively. A total of 35 893 eggs and 8 162 larvae were treated.

Table 9. Effect of dimethoate spray treatments on eggs and larvae of Queensland fruit fly, *Bactrocera tryoni*, in tomatoes (var "Guardian").

Stage/ Rep	No. Fruit	No. Insects Treated ¹	Pupal Survival	Mortality (%)	Mortality 95% CL (%) ²	Dimethoate Conc (mg/L) ³
Egg 1	671	13 931	2	99.986		399/400
Egg 2	633	21 962	0	100		396/402
	1 304	35 893	2	99.994	99.982	
Larva 1	215	8 162	0	100		399/404
	215	8 162	0	100	99.963	

¹Estimated from numbers surviving in untreated controls

²Lower 95% confidence limit of the true population mortality, calculated using the method of Couey and Chew (1986)

³Concentration (mg/L) expressed as before/after treatment

Measurement of Infestation Variability Due to Cage Infestation

Comparison of estimated and actual numbers of pupae resulting from cage infestation showed significant variation in both directions when individual infestation events were examined. However, the tendency was for these variations to cancel each other out over the course of an experiment (Table 10).

Table 10. Comparison of actual and estimated numbers of pupae resulting from cage infestation

Variety/Cage	Actual Number Recovered (pupae)	Estimated Number Based on Controls (pupae)
Daniella A	2 471	1 970
Daniella B	1 073	1 130
Daniella C	2 152	2 740
Daniella D	2 073	2 220
Daniella E	1 526	1 485
Daniella F	1 013	385
Total	10 308	9 930
Tracer A	527	355
Tracer B	1 726	1 940
Tracer C	688	530
Tracer D	655	1 320
Total	3 596	4 145
Grand Total	13 904	14 075

Discussion

Cool storage of tomatoes after dimethoate treatment, as often happens in practice, does not diminish the overall effectiveness of this treatment (Table 1). Results of the cool storage treatment alone show that even the relatively "soft" storage treatment used here (10°C for 5 days) can cause significant mortality. Therefore there is no reason to believe that the use of cool storage treatments to preserve fruit quality after disinfestation treatment would compromise quarantine security.

Experiments to determine treatment effects on fruit flies in fruit are usually carried out on ripe fruit. This is to ensure that effects of allelopathic chemicals, sometimes present in unripe fruit, do not confound results. A number of plants produce allelochemicals that are toxic to fruit flies that feed upon them (Chan and Tam 1985, Seo *et al.* 1983). These chemicals occur particularly in the immature fruit where their presence discourages interference with seed production and maturity. As fruit ripens, these chemicals degrade allowing activities by insects and other animals to aid seed dispersal.

Infestation of "green" and "ripe" tomatoes in this study was equally successful as measured by mean control survival (Table 2). Therefore there is no indication that green or colour break Floradade tomatoes are less suitable for the development of *B. tryoni* than those at the ripe stage, as measured by survival (pupal recovery). However, no data was collected on other parameters such as development time or pupal weight so there could have been other, non-lethal effects of ripeness that were not recorded. That is, insects in green fruit may have been under greater "stress" even though this was not ultimately lethal.

Dip treatments resulted in 0 survivors from 14 800 and 14 584 for green and ripe fruit respectively (Table 2). Therefore there is no reason to believe that fruit ripeness at treatment is a significant factor in determining disinfestation efficacy for dimethoate treatments of tomatoes.

The dip treatment tested against eggs and larvae of *B. tryoni* in tomatoes of the cultivars "Daniella", "Redcoat", "Tracer" and "Guardian" showed a high level of efficacy. During these trials, a total of 6 188 fruit containing 185 020 eggs and larvae were treated. No pupal survivors were recovered from any treatment (Table 3). Mortality at the 95% confidence level based on the method of Couey

and Chew (1986) was 99.997% for eggs and 99.996% for larvae. These mortality rates approximate "Probit 9", the highest treatment efficacy required in international trade: the result for eggs being slightly higher, larvae slightly lower. A treatment providing this level of efficacy would ensure that, on average, no survivors would result from a consignment containing ~30 000 insects (Couey and Chew 1986).

On one occasion ("Tracer", egg treatment, rep 2), an error occurred in the mixing of the dimethoate dip solution. This error was detected during the regular testing of dip solutions that were carried out on samples taken before and after treatment. The resulting treatment solution was only 288 mg/L, representing a reduction of about 30% on the nominal concentration of 400 mg/L (Table 3). However, even at this reduced concentration, no survivors resulted from 24 072 treated eggs. This result further demonstrates the robustness of the dip treatment.

Spray treatment with dimethoate plus Agral resulted in consistently high mortality for eggs and larvae of Queensland fruit fly in the varieties "Daniella", "Tracer", Redcoat" and "Guardian". However, there were a small number of survivors to the pupal stage. These experiments were carried out on "organically-grown" tomatoes. In practice, tomatoes grown in Queensland are subject to field control programs for fruit flies and other pests to ensure quality and marketability. This fact, together with a high standard of selection on the packing line, means that fruit fly infestations in commercial product would be at worst very low and probably non-existent in most cases. In the unlikely situation that 1 000 insects were present in a consignment, both the spray and the dip treatments reported here would provide adequate quarantine security. The additional effect of these agronomic practices further enhances the security of this treatment.

This treatment has been used for other tomato cultivars moving within Australia and to New Zealand over a *ca* fifteen-year period. There are no records of any live fruit flies being detected in any of these shipments.

Because of difficulties in growing fruit without the use of chemical insecticides, the number of insects tested for the spray treatment in these experiments fell short of the nominated 30 000 eggs and larvae. However, when this treatment is evaluated taking into account pre-harvest controls for fruit flies and the historical record of its use, we believe that these would provide a treatment efficacy equivalent to 99.99% (95% CL) which is implied by the requirement to treat 30 000 insects.

Experimental work to measure infestation variability due to cage infestation was conducted on request by MAFNZ scientists. Complete control of oviposition is not possible using the cage infestation method. However, alternative natural infestation methods can lead to even more serious inaccuracies and artificial infestation is not feasible when treating large numbers of insects nor when fruit is to be dipped. Comparison of actual numbers and estimated numbers of treated insects shows that significant variations can occur between batches (Table 8). However, as these batch (and cage) variations result in both underestimation and overestimation of the true number, within the experiment as a whole the tendency is for these to cancel each other out. Therefore within an experiment, where large numbers of fruit and insects are used, this method of estimation provides a good approximation of actual treated numbers.

Recommendations

This research should lead to the development of a commercial treatment protocol negotiated between AQIS and their counterparts in New Zealand (MAF).

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