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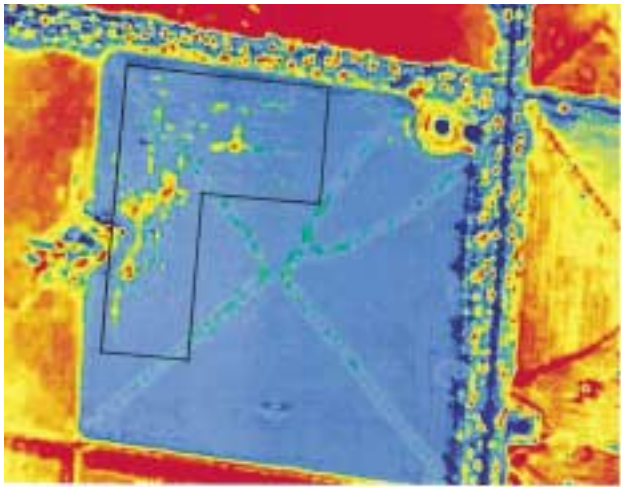

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A heated air quarantine disinfestation treatment against Queensland fruit fly (Diptera: Tephritidae) for tomatoes

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Abstract. A circulated heated-air treatment at 92% RH to achieve and maintain a minimum fruit core temperature of 44°C for 2 h is shown to disinfest tomatoes against Queensland fruit fly, *Bactrocera tryoni* (Froggatt) for market access quarantine purposes. The efficacy of the treatment exceeded 99.99%, tested at the 95% confidence level. An estimated 78 439 eggs were used for large-scale trials, as the stage of the pest most tolerant of heat at the treatment temperature.

Additional keywords: *Bactrocera tryoni*, vapour heat.

Introduction

Tomatoes (*Lycopersicon esculentum* Mill.), grown in Queensland in winter months, are exported to parts of Australia and other countries unable to produce field-grown tomatoes at that time. Because tomatoes are a host of tephritid fruit flies, quarantine disinfestation treatments are a condition of entry to many of these markets. Currently, disinfestation can be done with an insecticide such as dimethoate applied as a dip (Swaine *et al*. 1984) or as a packing line flood-spray treatment (Heather *et al*. 1987). This provides economical, efficient, logistically simple disinfestations, but leaves chemical residues. Although these residues provide ongoing quarantine security and are below the approved maximum residue limit of 0.01 mg/kg (Anon. 1988), consumer preference for no pesticide residues is expected to cause insecticide treatments to be phased out. Residue-free pesticide treatments available for fruit include physical methods using heat, cold or irradiation (Heather 1994; Heather *et al*. 1997). Cold sensitivity of tomatoes and susceptibility to hot water damage (R. Jordan pers. comm.), together with the need for irradiation approvals makes hot-air heating the most suitable non-chemical disinfestation treatment for tomatoes at present.

Four significant fruit fly pests of tomatoes are recorded from commercial production areas in eastern Australia (May 1953). Queensland fruit fly, *Bactrocera tryoni* (Froggatt), and the closely related *B. neohumeralis* (Perkins) are of most concern to quarantine authorities. Cucumber fly, *B. cucumis* (French), is frequently the most common fruit fly infesting commercial field-grown tomatoes (Heather unpublished data), but this species is of less concern to quarantine authorities of markets in temperate regions because its habitat range is limited to coastal areas north of latitude 29°S. There is 1 published record from tomatoes for the Solanum fruit fly, *B. cacuminata* (Hering), but this is not a recognised economic pest species. There is, in addition, 1 record of a further species, *B. kraussi* (Hardy), but its habitat range is limited to localities well north of commercial tomato production areas and it also was categorised by May (1963) as a species of no economic importance. In Australia, disinfestation research results for *B. tryoni* have been accepted as applicable to all Australian fruit flies because of expected similarity of response to treatments. For example, when *B. curcumas* was tested in heat-disinfestation trials on cucurbits, it was equally or less tolerant than *B. trigoni* (Corcoran *et al*. 1993). However, it is the international status of *B. tryoni* as an international quarantine pest and its wide climatic distribution range that make it the primary target for mandatory disinfestation treatments.

The efficacy required of quarantine treatments varies from country to country and can range from 99.5 to 99.9968% mortality. Most markets require the efficacy to be proven in large-scale trials using parallel control samples to estimate the numbers treated. The purpose of trials reported here was to determine the heat-treatment parameters for a minimum efficacy of 99.99% at a confidence level (CL) of 95%. Typically, the efficacy of a disinfestation treatment is required to be demonstrated on the stage of the pest most tolerant of the treatment. For hot-air treatments, the most heat-tolerant stage of *B. tryoni* in fruit is the egg (Heard *et al*. 1992; Heather *et al*. 1997; Corcoran *et al*. 1998). We used heated-air treatment technology developed in Japan (referred to there as ‘vapour heat’) for the disinfestation of fruit flies (Sugimoto *et al*. 1983). This terminology was first used for a

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related process developed in USA in 1929 (Baker 1952; Hallman and Armstrong 1994).

Materials and methods

Infestation of test fruit

Insecticide-free tomatoes var. Flora Dade of commercial market maturity were infested in laboratory cages [as described by Swaine *et al*. (1984)] with *B. tryoni*, reared using the method of Heather and Corcoran (1985). Fruit were individually weighed and treated in weight ranges to minimise variation in infestation levels and in heating time between fruit within each of the 3 or 5 replicate samples depending on the purpose of the trial. Infestation time for each fruit was 10 min and all fruit in a sample were infested simultaneously, ensuring homogeneity of age of eggs in each treatment replicate cohort. After infestation, fruit were held at 26 ± 0.5°C and 70 ± 5% RH for development to the stage to be treated.

Stages to be treated were determined by age, from a study of development times at 26°C involving destructive sampling at regular intervals and identification of the larval stages using mouthpart characters (Anderson 1963). Development times at treatment were 32 h for mature eggs, 52 h for first instars, 82 h for second instars and 128 h for third instars. For stages other than eggs, these times ensured a preponderance of the stage to be tested. However, some overlapping of larval stages was unavoidable because of natural variation in development rates.

The number treated was estimated from the number of surviving pupae in a parallel control sample. For trials with small numbers of fruit for dose estimation, an untreated parallel sample of equal numbers of fruit was used. For large-scale trials, the number of fruit in the untreated sample was a minimum of one-fifth of the number of treated fruit.

Most tolerant stage

A test for the most tolerant in-fruit stage of the pest in tomatoes was done to satisfy those market authorities that might insist on it as a procedural requirement, although effectiveness of physical treatments can be accepted to be independent of the commodity (Armstrong and Couey 1989). Three replicated trials were conducted at the proposed fruit core temperature of 44°C to identify the most tolerant stage. To ensure survivors for comparison of the stages, the fruit core temperature was held for only 10 min.

The percentage mortalities observed for each insect stage were compared using analysis of variance for a randomised block design. On the basis of significance in this analysis, differences between stages were identified using least significant difference at $P = 0.05$. Diagnostic plots indicated that it was not necessary to apply an arcsin transformation to the data to stabilise the variances.

Dose estimation

Small-scale trials on the most tolerant stage were done using units of 50–60 fruit containing estimated totals of more than 5000 eggs at a series of treatment times at 10-min intervals, 10–130 min, with 3 untreated controls. A range of dose–response models (GenStat 2000) was fitted to the pupal mortalities using the probit, logit and complementary log–log scales to express the probability of mortality p as a linear model. It was assumed that the number of insects killed, Y, had a mean $E(Y) = np$ and variance var $(Y) = \Phi np(1–p)$, where n is the number of insects, p is the probability of mortality and Φ is the heterogeneity factor used to scale the variance and account for extra-binomial variation. The model which best fitted the observed data was selected and used to predict the treatment time required to achieve a minimum efficacy of 99.99%. Selection was based on residual deviance and examination of the fitted curves in the upper dose range.

Large-scale trials

A replicated large-scale trial was done on the most tolerant stage. For this large-scale trial a treatment time of 2 h was adopted. The trial was done on 5 replicate samples of >10000, although the number needed to demonstrate the required efficacy of 99.99% at the 95% confidence level is 29956 as a cumulative total with no survivors (Couey and Chew 1986). The additional samples were done to cover full and half chamber loadings as this factor could possibly affect efficacy due to slightly differing times to reach the required core temperature.

Treatment

This was done in a Sanshu EHK-1000B vapour heat treatment unit (Sanshu Sangyo, Kagoshima, Japan) with a treatment chamber volume of 1 m^3. A treatment temperature of 44°C measured at the fruit core was used in all trials. This temperature had been found to be the practical maximum likely to be tolerated by tomatoes without risk of unacceptable injury, provided that fruit are treated at or after first colour 'break' (R. Jordan pers. comm.).

For all treatments, an air temperature of 45 ± 0.1°C was used, at a RH of 92 ± 1% once the treatment temperature was reached. Treatment temperature was measured with platinum resistance probes inserted to the fruit core in 10 individual fruits for each load in the chamber. The heating time and temperature were recorded on a continuous chart from commencement of heating up to the time the last sensor fruit reached the required core temperature and subsequently until the treatment was complete. Time before the target temperature was attained by the last sensor approximated 2 h making a total treatment time of about 4 h. Fruits were then hydro-cooled to 30°C with tap water at ambient temperature (about 25°C), which normally took an additional 30 min.

Results

Most tolerant stage

Results from the trial to determine the most tolerant stage are presented in Table 1. Mean pupal mortality following treatment was 28.5% for eggs, 47.4% for first instars, 64.6% for second instars and 92.5% for third instars (Table 2). The stages differed significantly in response to the heat treatment, with eggs significantly more tolerant than all other larval stages, excepting first instars, at $P = 0.05$. Although no significant difference was found between the tolerance of eggs and first instars, the mean mortality of eggs was 18.9% lower than that of the first instars so further testing was performed on eggs. These results agreed with findings in previous trials on *B. tryoni* (Heard *et al*. 1992; Heather *et al*. 1997) that mature eggs were the most tolerant in-fruit stage for use in dose-estimation and large-scale trials.

Dose estimation

Comparison of the dose–response models indicated that the complementary log–log scale was better than either the probit or logit scales and that a better fit to the data was achieved with no log transformation of the dose. In the small-scale trial using a range of treatment times, no survival to pupae occurred from eggs in fruit treated at 44°C core temperature held for 110 min. The complementary log–log model (Fig. 1) predicted that a treatment time of 102.9 min, with 95% fiducial limits of 94.62–114.1 min, was necessary to achieve an efficacy of 99.99%. For practical application and to provide additional security, the treatment time for the large-scale trials was increased beyond the upper fiducial limit of 114.1 min to 2 h. Also, this was done because the

Table 1. Survival to pupae and adults in trials to determine the most tolerant in-fruit stage of *Bactrocera tryoni* in tomatoes treated at a fruit core temperature of 44°C for 10 min before hydro-cooling with water at ambient temperature

Replicate	No. of fruit		No. of pupal survivors		No. of adult survivors	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
			Treated at egg (32 h) stage			
1	32	32	3800	2694	3397	2127
2	31	31	3964	2832	3429	2371
3	31	31	2868	2073	2274	1640
			Treated at first instar stage			
1	30	30	4307	1662	2782	1356
2	32	32	4065	2831	3719	2540
3	29	29	3116	1542	2374	1356
			Treated at second instar stage			
1	25	25	1383	473	971	410
2	32	32	4436	885	3889	788
3	30	30	5279	2745	4267	1451
			Treated at third instar stage			
1	24	24	1957	150	1859	121
2	24	24	2728	199	2427	113
3	32	32	3274	247	2781	172

estimate of treatment time was made on samples of less than 8000 test insects but was to be used in a large-scale trial on >30000, where the chance of a survivor would be greater.

Large-scale trials

An estimated total of 78439 eggs was treated in 5 replications of fruit, each containing >10000 eggs, without any survivors to the pupal stage (Table 3). This gives 95% confidence that the mortality is 99.9961% or higher and exceeds a minimum required efficacy of 99.99% at the 95% confidence level (Couey and Chew 1986).

Discussion

Our high-humidity heat treatment at 44°C successfully disinfested tomatoes against *B. tryoni*. Three replicates of >10000 insects for a total of >30000 without survivors is typically required by countries requiring a minimum efficacy of 99.99% for a quarantine disinfestation treatment and our result exceeded this requirement. In-depth physiological studies on the effects of heat treatment on fruit quality are to be published separately (R. Jordan pers. comm.). However, in the course of large-scale tests, incidental observations on disinfested fruit, held for more than 2 weeks at 26°C to confirm the absence of survivors, did not identify any treatment-related injury.

Studies of this nature are largely governed by procedural requirements imposed by authorities regulating potential

Table 2. Mean pupal mortality of Queensland fruit fly after heat treatment of developmental stages in tomatoes at 44°C fruit core temperature for 10 min

Means followed by the same letter are not significantly different at $P = 0.05$

Stage treated	Mean pupal mortality (%)
Egg (32 h)	28.5a
First instar	47.4ab
Second instar	64.6b
Third instar	92.5c
l.s.d. ($P = 0.05$)	24.5

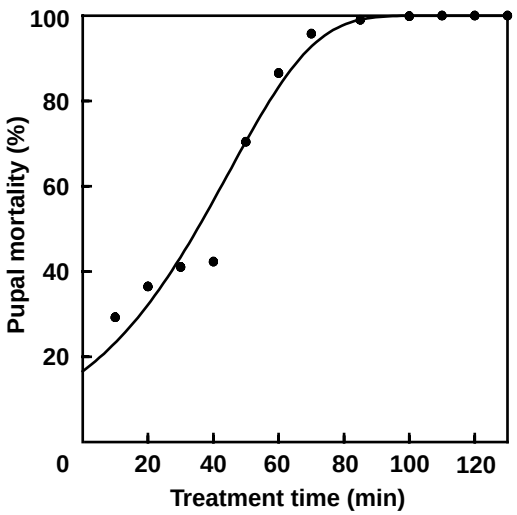

Figure 1. Pupal mortality (%) response of eggs of *Bactrocera tryoni* with time treated at 44°C:

$\ln[-\ln(1-p)] = -1.710 (\pm 0.161) + 0.03819 (\pm 0.00313)$ Time,

where p is the corrected proportion of pupal mortality. Standard errors of the coefficient estimates are shown in parentheses.

Table 3. Fruit fly survival in large-scale trials on tomatoes infested with mature eggs (32 h) of *Bactrocera tryoni* in fruit treated with air raised from 30 to 45°C to a core temperature of 44°C and held for 2 h after all sensor fruit reached 44°C then cooled to 30°C with ambient tap water

No. of fruit treated	No. of fruit untreated	Av. fruit size (g) (range)	Chamber load (kg)	No. of pupae from untreated	No. of adults from untreated	Estimated no. of eggs in treated[A]	No. of pupal survivors from treated
850	181	131 (100–150)	120	5910	4357	27754	0
130	26	207 (190–230)	120	2906	2534	14530	0
160	32	168 (150–190)	120	2216	2088	11080	0
205	41	218 (200–229)	60	2433	2158	12165	0
215	43	163 (100–199)	60	2582	2222	12910	0
1560	323		480	16047	13359	78439	0

[A]Estimated number treated is the number of pupae (untreated)/number of fruit (untreated) × number of fruit (treated).

import markets. Considerable emphasis is placed on the determination of the most tolerant stage to be used for prediction of the treatment to meet the disinfestation efficacy required. We compared the stages at the treatment temperature that was the practical maximum likely to be tolerated by tomatoes at commercial maturity (R. Jordan pers. comm.) because this would enable the shortest treatment time and enable maximum utilisation of treatment facilities. The levels of infestation in test fruit greatly exceeded normal fruit quality tolerance for infestation in commerce so, in practice, treatments would not be expected ever to meet such extreme pest survival risk conditions. It is the large-scale trials that provide the best estimate of efficacy and hence the quarantine security achievable with a disinfestation treatment.

Within limits, a time–temperature relationship is widely recognised for disinfestation treatments with heat. Our proposed temperature for use against *B. tryoni* in tomatoes is lower than the 47°C applied for 15 min on mangoes using the same equipment (Heather *et al*. 1996) and for other Tephritidae including *B. dorsalis*, 46.5°C for 10 min (Unahawutti *et al*. 1992), although this is compensated by the extended time of 2 h. Also, Sugimoto *et al*. (1983) found 43.5°C for 3 h to be effective against *B. dorsalis* in green peppers (capsicums) and Corcoran *et al*. (1993) found 45°C for 30 min adequate against *B. cucumis* in zucchini marrows. Given the difficulty of precise comparisons due to the low numbers of survivors at these limits, this is indicative of similar responses from at least some species within the genus *Bactrocera*. Comparisons with reported treatments against other *Bactrocera* and *Anastrepha* (Heather 1994) need to take into account the different equipment used, but they show a close similarity of relationship between times and temperatures.

Throughout the literature on fruit fly disinfestation treatments, based on in-fruit testing, eggs are predominantly the most tolerant in-fruit stage (Heard *et al*. 1992; Heather 1994; Heather *et al*. 1996). Consequently, we believe this to be the most appropriate basis for disinfestation treatments because it takes into account the effect the normal position of each stage within fruit has on the heat reaching the insect.

Our treatment offers a practical alternative to insecticide dipping as a disinfestation treatment to facilitate access to markets in fruit fly-free areas for Australian tomatoes grown where Queensland fruit fly is endemic. Commercial tomatoes in this category have a low initial risk of infestation because they are produced under pest management regimes with a strong emphasis on fruit fly control. They are then transported to markets at cool temperatures unfavourable to fruit fly survival so quarantine security should be enhanced by the time of arrival at the importing market (Armstrong and Couey 1989). Heated-air disinfestation could also assist in meeting ‘organically grown’ parameters in commerce.

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