Evaluation of cold temperature and hot water dipping for postharvest disinfestation of citrus from Fuller's rose weevil (*Asynonychus cervinus* (Boheman)) eggs (Coleoptera: Curculionidae).

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Summary

Trials were carried out to determine the potential for postharvest disinfestation of citrus from Fuller's rose weevil (*Asynonychus cervinus* (Boheman)) (FRW) eggs by cold temperature storage or by hot water or hot fungicide dipping. Cold disinfestation at 1°C for 16 or 32 days did not significantly reduce the viability of FRW eggs. Although hot water dipping at 55°C for 2 minutes was sufficient to kill 100% of eggs in bare egg masses, dipping at 55°C for an extended period (3.6–4.4 minutes) was necessary to kill 99% of FRW eggs under the calyces of navel oranges. Hot dipping for 4 minutes or more at this temperature caused severe damage to the fruit predisposing it to *Penicillium* breakdown during storage. Hot dipping shows potential as a disinfestation technique to control FRW eggs on citrus fruit but further tests are required to more accurately determine the time required to kill FRW eggs whilst minimizing damage to different citrus fruits.

Introduction

Fuller's rose weevil, *Asynonychus cervinus* (Boheman) (FRW), is a widely distributed minor pest of citrus in many areas of the world. Since 1985, it has become prominent as a quarantine pest of citrus exported to Japan (Haney *et al.* 1987), because egg masses laid under the calyces of fruit are not removed during processing. Japanese quarantine authorities require fruit with viable FRW eggs to be fumigated with methyl bromide, which is toxic to some citrus cultivars, especially lemons.

Trial shipments of Australian navel oranges to Japan were found to be infested with viable FRW eggs in July 1987, despite fumigation with ethylene dibromide for fruit fly control prior to shipment. This finding was the impetus for a research program to develop ways of controlling FRW in citrus and/or disinfesting fruit.

This paper reports studies of the effect of the postharvest treatments of cold disinfestation and hot water dipping on the viability of FRW eggs. Cold disinfestation is an accepted treatment for citrus to control Queensland fruit fly (*Bactrocera tryoni* (Froggat)) and Mediterranean fruit fly (*Ceratitis capitata* (Weideman)), while hot water dips, with or without fungicide, have the potential to improve control of postharvest rots (Wild 1989).

During this study the proportion of fruit infested with egg masses (16.8%) was slightly lower than that on Valencia oranges (21.9%) in field surveys. The average number of egg masses per twenty fruit was also slightly less than that observed for Valencia oranges (3.7% compared with 6.15% for Valencias) (Madge *et al.* 1991). These differences, however, are unlikely to be important. As Japan will only accept fruit which is free from viable FRW egg masses, these results support the need for satisfactory pre- and postharvest measures for FRW in navel and Valencia oranges intended for export to Japan.

Materials and methods

Three postharvest trials were conducted to determine the effects of treatment on the viability of FRW eggs. The treatments investigated in these trials were: 1. Cold disinfestation, 2. Hot water or hot fungicide disinfestation, and 3. Optimal time and temperature of hot water dipping to give maximum disinfestation. A fourth trial was conducted to determine the effects of hot water treatments on fruit quality.

Trial 1. Effect of cold storage on FRW egg viability.

An experiment established to test the effect of cold disinfestation on the quality of oranges was used to determine the effect of cold treatment on the viability of FRW eggs. Eight cartons of Washington navel oranges, with approximately 80 fruit per carton, intended for export to Japan, were sampled from each of 15 different citrus groves in the Sunraysia district near Mildura. The cartons of fruit were stored according to two times × temperature and two control treatments as follows: Treatment A: 16 days storage at 1°C followed by 28 days at 10°C; Control A: 44 days at 10°C; Treatment B: 32 days storage at 1°C followed by 28 days at 10°C; and Control B: 60 days at 10°C. The trial was designed to test the effect of 16 or 32 days at 1°C on the viability of FRW eggs and the quality of fruit. The two controls were used to distinguish between damage caused by 1°C and damage caused by long term cool storage. Two cartons from each grove were subjected to each treatment. The fruit were stored in the cool-room in a completely randomized design. After storage samples of 50 fruit were removed from each carton and the FRW infestation and viability of eggs were determined. The proportion of oranges with one or more egg masses per fruit was determined by removal of the calyx from each fruit. Egg masses were removed and stored in petri dishes for 7 days at 20°C, after which egg viability (proportion of egg masses in which some eggs had hatched) was determined by observation of neonate larvae.

Trial 2. Effect of hot water or fungicide on FRW egg viability.

Trial 2a. Effect of hot water and fungicide on bare egg masses. Adult FRW were collected from the field and placed individually in plastic disposable cups with fresh citrus leaves and a 10 mm square of plastic. The weevils were kept under natural light conditions at a constant temperature of 21°C. Approximately 80% of the weevils laid egg masses under the plastic square. Eggs adhered to the plastic squares and were removed with no mechanical damage.

Three replicates of four bare egg masses of between 1 and 8 days old on plastic squares were surface sterilized by immersion in 0.8% NaOCl for 3 minutes, followed by washing in sterile distilled water and subjected to one of the following treatments for 1 minute: 1. Thiabendazole (TBZ) (500 ppm) at 20°C; 2. TBZ (500 ppm) at 55°C; 3. Water at 20°C; 4. Water at 55°C; and 5. Chlorine (200 ppm CaOCl) at 20°C. The egg masses were then incubated at 27°C and 85% relative humidity in a completely randomized design and inspected for larval hatch after 20 and 44 days. The presence or absence of neonate larvae was recorded.

Trial 2b. Effect of water at different temperatures and exposure times on bare egg masses. Bare egg masses were subjected to one of the following water/temperature treatments: 1. 20°C for 1 minute; 2. 55°C for 1 minute; 3. 55°C for 2 minutes; 4. 55°C for 5 minutes; 5. 57°C for 1 minute; 6. 57°C for 2 minutes; and 7. 57°C for 5 minutes. Three replicates of four egg masses were treated, stored and assessed as in Trial 2a.

Trial 2c. Effect of water at different temperatures and exposure times on egg masses under calyces of fruit. Adult FRW were collected from the field and enclosed with mature navel oranges with intact calyces in insect cages at a ratio of approximately three weevils per fruit. After 8 days, the oranges were inspected for FRW egg masses under the calyces and six replicates of 10 oranges with egg masses were subjected to postharvest treatments as in Trial 2a.

Post treatment handling
After dipping, the fruit were processed in a standard citrus processing line with TBZ (1000 ppm) as the in-line fungicide followed by "Citruseal"® wax. The fruit were cold disinfested in the cool-room at 1°C for 16 days and then stored in a completely randomized design for 32 days at 10°C. After storage, egg masses were removed from the fruit, and incubated at 27°C and 85% relative humidity. The eggs were inspected for the presence of hatched neonate larvae at weekly intervals.

Trial 3. Effect of temperature and time of disinfestation with hot water or fungicide on FRW egg viability.

Trial 3a. The effect of temperature and exposure times on egg masses on fruit. Navel oranges with FRW egg masses laid in vitro, as described in Trial 2c, were subjected to the following treatments: A. Water at 20°C for 1 minute; B. Water at 53°C for 2 minutes; C. Water at 53°C for 5 minutes; D. Water at 55°C for 1 minute; E. Water at 55°C for 2 minutes; F. Water at 55°C for 5 minutes; G. Water at 57°C for 2 minutes; and H. TBZ (500 ppm) at 55°C for 2 minutes. Ten oranges were subjected to each treatment and the experiment was replicated by repeating the experiment three times. The fruit were treated and stored as previously described. Egg masses were removed from the fruit after storage, eggs counted and then stored at 27°C and 85% relative humidity. After 28 days the proportion of hatched larvae was determined (percentage egg hatch).

Trial 3b. Determination of LT95 and LT99 of water dipping at 55°C. Four replicates of 10 fruit were dipped in hot water at 55°C for 1, 2, 3, 4 or 5 minutes. The egg masses were removed from the fruit, the number of eggs counted and the per cent hatch determined after 28 days incubation. Standard methods of probit analysis were not adequate due to significant lack of fit and overdispersion of binomial observation. Consequently a logit analysis was undertaken to determine the time of dipping at 55°C that would kill 95% and 99% of the FRW eggs under the calyces of citrus fruit. The method of analysis employed a Generalized Linear Model with overdispersed binomial error and logit function, fitting time as a categorical factor. Estimates of the time required to kill 95% or 99% of eggs were obtained by linear interpolation of observed kill rates on the logit scale. Standard errors and confidence intervals were derived from the inverse observed information matrix using the delta method.

Trial 4. Effect of hot water dips on quality of oranges.

Trial 4a. Effect of dipping for different periods at 55°C on quality of orange fruit. Five replicates of 10 Washington navel oranges were subjected to hot water at 55°C for 1, 2, 3, 4, 5, 8, 10, 12, 15 or 20 minutes. After treatment fruit were processed using TBZ (100 ppm) as the in-line fungicide and cold disinfested for 16 days at 1°C prior to storage for 32 days at 10°C. The fruit were processed and stored in a completely randomized manner. The incidence of *Penicillium* rot (percentage surface area affected) and the proportion of sound fruit were recorded.

Trial 4b. Effect of dipping at 55°C or 60°C on quality of orange fruit. A second trial, consisting of six replicates of 10 fruit, was subjected to the following treatments: A. Water at 20°C for 1 minute; B. Water at 55°C for 2 minutes; C. 5 minutes; or D. 7 minutes; or E. Water at 60°C for 2 minutes; F. 5 minutes; or G. 7 minutes. The fruit was processed and stored as above. After storage the proportion of sound fruit and the incidence of mould were recorded. This trial was repeated (Trial 4c.)

Analysis of data.
The data in the cold treatment trial (Trial 1), the hot dipping trial (Trial 3) and the fruit quality trials (Trial 4a, b and c) were analysed using Analysis of Variance. The data from Trial 2a, b and c could not be analysed due to the nature of the results.

Table 1. The incidence of FRW egg masses on export navel oranges and the proportion of egg hatch after cold temperature treatment at 1°C for 16 or 32 days or storage at 10°C for 44 or 60 days.

Grower number	Egg masses per 20 fruit	% fruit with egg masses	% egg masses hatching in treatment: Cold treatment at 1°C		Control - Storage at 10°C	
			16 days	32 days	44 days	60 days
1	5.2	19.5	16.0	17.9	4.5	16.0
2	5.0	20.8	16.5	23.1	26.0	32.0
3	1.0	7.6	0.0	0.0	0.0	0.0
4	0.0	0.6	0.0	43.8	0.0	50.0
5	5.3	30.0	16.5	18.0	20.0	19.0
6	4.2	24.1	16.5	23.8	15.0	24.5
7	3.4	14.4	21.0	18.9	11.0	7.5
8	2.9	22.8	22.0	18.4	27.5	17.5
9	1.9	7.4	7.5	16.9	0.0	33.5
10	2.3	14.9	14.0	21.0	28.5	16.5
11	5.9	22.7	12.5	24.6	30.0	37.0
12	13.6	41.9	13.5	12.0	6.0	20.0
13	0.1	1.0	0.0	0.0	0.0	50.0
14	1.1	11.5	10.0	100.0	0.0	0.0
15	3.2	13.0	0.0	33.0	38.0	0.0
Mean	3.7	16.8	11.1	24.7	13.8	21.6
LSD (P=0.05)	13.7					

Results and discussion.

The effect of cold treatment on FRW eggs.
In Trial 1, there was no significant difference ($P<0.05$) in egg survival in citrus stored at 1°C or 10°C or for 16 or 32 days at 1°C. Cold disinfestation for 16 or 32 days at 1°C had no significant effect on egg survival of FRW (Table 1). Haney *et al.* (1988) found that cold disinfestation at 1°C for 42 days significantly reduced FRW egg hatch on grapefruit but the reduction was not sufficient to provide an acceptable level of quarantine control.

The effect of temperature and exposure times of dipping on FRW eggs.
In Trial 2a, all egg masses in all treatments had neonate larvae present after storage for 44 days. TBZ or chlorine had no additional effect on the mortality of the eggs. It appears that the benefits gained in the control of postharvest rots by heating fungicides (Wild 1986) does not extend to the control of FRW eggs.

In Trial 2b, only treatments 1 and 3 had viable eggs present. One hundred percent of egg masses in treatments 1 and 3 had neonate larvae present after incubation compared to no egg mass having neonate larvae hatch in any other treatment. This indicates that hot water dipping for a minimum of 2 minutes at a minimum temperature of 55°C was required to kill 100% of eggs in bare egg masses. This supports work done by Soderstrom and Brandl (1986) who found that egg masses laid on wax paper were killed by hot water dipping at 55°C for a minimum of 2 minutes.

Egg masses under fruit calyces in Trial 2c were less susceptible to high temperature. Egg masses removed from fruit in all treatments gave rise to neonate larvae during incubation. In Trial 3a, 5 minutes at 55°C was required to provide 100% mortality (Figure 1).

The effect of hot dipping on fruit quality.
Hot water dipping resulted in damage to the rind which led to fungal breakdown, predominantly *Penicillium* spp., to occur during storage (Table 2). Dipping at higher temperatures or for an extended period of

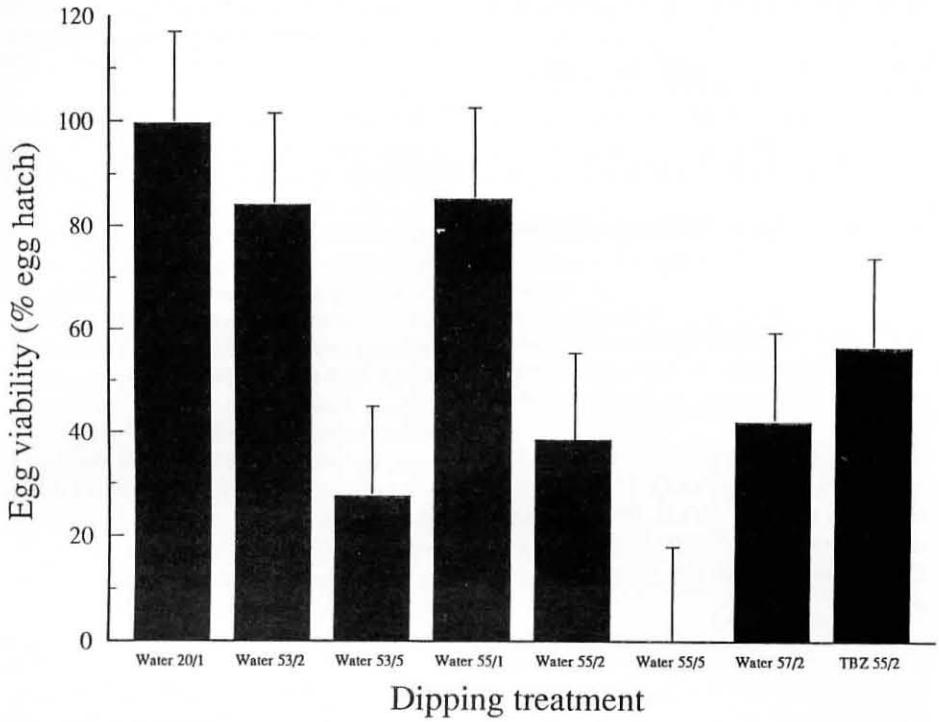

Figure 1. The viability of FRW eggs on oranges (percentage eggs hatched) after hot water and fungicide dipping at a range of temperatures and for a range of exposure times in Trial 3a. NB. 20/1 = dipping at 20°C for 1 minute.

Table 2. The effect of hot water treatment on the quality of navel oranges after cold disinfestation for 16 days at 1°C and storage for 32 days at 10°C in Trial 4.

Treatment Temperature (°C)	Time (min)	Incidence of Rot (% Surface area of orange)			Proportion of sound fruit (%)		
		Trial 4a	Trial 4b	Trial 4c	Trial 4a	Trial 4b	Trial 4c
Water 20	1	–[a]	0	2.7	–	90.0	78.1
55	1	29.4	1.3	–	42.0	82.5	
55	2	24.2	3.2	1.3	45.5	82.5	83.9
55	3	21.6	1 1.8	–	53.2	48.7	
55	4	5 1 .0	14.0	–	27.6	52.5	
55	5	64.0	18.5	3.8	15.9	41.2	78.3
55	6	62.2	–	11.0	23.3	–	58.7
55	7	77.8	–	–	7.4	–	–
55	10	70.8	–	–	35.3	–	–
55	12	99.6	–	–	0.0	–	–
55	15	52.4	–	–	27.2	–	–
55	20						
60	2	–	–	8.8	–	–	67.4
60	5	–	–	15.8	–	–	57.8
60	7	–	–	61.8	–	–	13.6
LSD		18.6	12.8	10.0	21.7	28.0	17.0

a. Treatment not included in Trial. Level of significance for LSD = 0.05

time also produced a brown scald on the fruit rind similar to cold scald (Tugwell and Gillespie 1989). This was difficult to quantify, however, as the scald predisposed the fruit to fungal infection which masked the extent of the scald. Hot dipping at 60°C caused severe damage to the fruit.

Fruit susceptibility to damage varies with variety and physiological state of the fruit. It may be possible to use a curing treatment to protect the fruit from damage during hot dipping. Further tests are required to determine the effect of variety or time of year on the response of the fruit to hot dipping at 55°C.

In general the incidence of fungal breakdown (per cent surface area affected) significantly increased ($P<0.05$) after 4 minutes at 55°C but was evident to some extent after 1 minute at 55°C. The proportion of sound, blemish free fruit decreased after 3–4 minutes at 55°C. It was estimated that approximately 4 minutes (confidence interval 3.6–4.4) of dipping at 55°C was required to kill 99% of FRW eggs under the calyces of fruit.

Recent Australian and American experience with Japanese citrus exports confirms FRW as a major quarantine impediment to the Japanese market (Haney *et al.* 1987, Madge *et al.* 1992). There is an immediate need for a field control program on FRW to minimize the threat to exports of Australian citrus to Japan. The results shown here confirm that cold disinfestation at temperatures and exposure times sufficient to control fruit flies would not be sufficient to control FRW eggs. Hot water dipping at 55°C will kill FRW eggs but the duration of dipping required to kill the eggs is greater for egg masses on fruit than it is for bare egg masses. The exposure time to the dip is crucial as extended periods of time at 55°C will cause damage to the fruit. Further tests are required to more accurately determine the time of dipping required to achieve 99% egg mortality with minimal damage to the fruit.

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