# EFFECT OF A SHORT HOT WATER TREATMENT ON THERMAL TOLERANCE OF QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* (FROGGATT) INFESTING *CAPSICUM ANNUUM* CULTIVARS

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## Summary

Capsicums and chillies (*Capsicum annuum*) grown on Australia's east coast are vulnerable to infestation by Queensland fruit fly (*Bactrocera tryoni* Froggatt) (Qfly). The fruit must be treated to ensure it is free of this pest before transport to fruit fly free markets interstate and internationally. Cold storage offers a relatively cheap and easily applied disinfestation option, but has not been recommended for *C. annuum* cultivars because the fruit is chilling sensitive. Recently it has been shown that a short hot water shower (HWS) can reduce chilling sensitivity, allowing fruit to be stored at 3°C. In this trial we examined whether a 60 second shower under 55°C water affected mortality of Qfly eggs and larvae in *C. annuum* cultivars stored at 3°C. Differences between the lifestages were observed, with 1st instar larvae being the most cold tolerant overall. The HWS alone caused significant mortality to all lifestages, the most mature larvae proving most susceptible. Treatment significantly reduced the time at 3°C required to induce 90% mortality in 1st, 2nd and 3rd instar larvae although eggs were less affected. No eggs or larvae survived more than 7 days at 3°C, regardless of treatment or variety. It is proposed that storing capsicums and chillies for 10 days at 3°C can provide a high level of quarantine security against Qfly, irrespective of whether the fruit is first subjected to a HWS treatment.

**Key words**: heat treatment, cold disinfestation, thermal tolerance, fruit fly, *Bactrocera tryoni,* capsicum, chilli

## INTRODUCTION

Capsicums (*Capsicum annuum* L.*)* are a major crop in Australia, over 60,000 tonnes being produced annually with a fresh market value >$230 million (Freshlogic 2012). While greenhouse production is increasing, the majority of capsicums (>80%) are still field grown. More than half the crop (~58%) is grown in Queensland, with large plantings around Bowen and Bundaberg and smaller summer plantings in the Lockyer Valley and the Granite Belt. Significant volumes are currently exported to New Zealand and traded domestically in Australia. Other existing and potential markets include Dubai, Singapore and Hong Kong. Chillies (*C. annuum* L.*)* are primarily grown for the domestic market, with production centred on Bundaberg in Queensland. Varieties range greatly in size and taste with 'red cayenne' and the smaller, hotter 'birds-eye' the most popular types grown.

Both capsicums and chillies are vulnerable to infestation by Queensland fruit fly (*Bactrocera tryoni* Froggatt) (Qfly). The pest is endemic in most production areas (Dominiak and Daniels 2011), so fruit must be treated before transport to fruit fly free areas such as Tasmania and South Australia. In the past, susceptible produce were usually flood sprayed with dimethoate insecticide in order to access these interstate markets and the international New Zealand market (Dominiak and Ekman 2013). However, in August 2008 a review of dimethoate by the Australian Pesticide and Veterinary Medicine Association (APVMA) led to suspension of postharvest use of this chemical on edible skinned crops including capsicums and chillies. Alternative quarantine methods are therefore urgently required.

Cold storage has been commonly used as a quarantine treatment against Qfly for many crops, including blueberries, stonefruit, citrus, cherries, avocadoes and grapes (Hill *et al*. 1988, Jessup *et al*. 1993, Jessup *et al*. 1998, Jessup 1994, Heather *et al*.1996). However, capsicums originated in the tropics and can be damaged by low temperatures. Capsicums are not recommended to be stored below 7°C and in practice are stored close to 12°C (Mitchell 1992). In contrast, cold disinfestation usually involves storage at 3°C or lower.

It may be possible to protect capsicums from chilling injury using a short hot water treatment. Such treatments can promote the formation of heat shock proteins, which maintain membrane integrity at low temperatures (Florissen *et al*., 1996) as well as controlling storage pathogens such as *Botrytis cinerea* and *Alternaria alternata* (Fallik *et al*. 1996). Gonzalez-Aguilar *et al.* (2000) claimed that a 4 minute dip in 53°C water reduced decay and chilling injury of capsicums following storage for up to 4 weeks at 8°C. Fallik *et al*. (1999) proposed brushing capsicums with 55°C water for 15 seconds to clean and prevent decay during transport and subsequent retail (2 weeks at 7°C + 3 days at 20°C). This method was patented and is now commercially applied in Israel. In Australia, Ekman and Pristijono (2010)

demonstrated excellent protection against chilling injury for both capsicums and chillies using a 30-60 second shower under 55°C water. This treatment could potentially be applied on packing lines using the drenching systems previously used for dimethoate. This suggests that cold storage could be a disinfestation option for capsicums and chillies, especially if combined with a short hot water shower (HWS). However, the effects of a HWS on Qfly cold tolerance were not known. While heat treatments are used as a quarantine treatment against Qfly in tropical fruit such as lychees, and mangoes (Jacobi *et al*. 1993, Heather et al. 1997), the treatment proposed here is too short to result in full mortality. Thermal conditioning at sub-lethal temperatures has been shown to increase heat tolerance of Qfly eggs, extending the time at 46°C needed to cause 99% mortality (Waddell *et al*. 2000). It therefore seemed possible that, just as a short heat treatment can protect capsicums from cold, Qfly larvae may also gain a similar protection, effectively reducing mortality.

In these trials we have used probit analysis to compare LD50 and LD90 values (Finney, 1971) of Qfly eggs and larvae in capsicums and chillies stored at 3°C for up to 14 days. Survival was evaluated with and without a pre-storage HWS .The results are presented in the context of developing a quarantine treatment for these products against Qfly.

## MATERIALS AND METHODS

### Fruit

Block type red capsicums and cayenne chillies (both *Capsicum annuum*) were grown inside a secure greenhouse at Narara, NSW, Australia (151°19'E, 33°23'S) over two summers; 2010-2011 and 2011-2012. Pairs of plants were grown in bags of cocopeat substrate supplied with a standard hydroponic nutrient solution in a run to waste system. No insecticides were used during fruit development, ensuring that the fruit was suitable for infestation by Qflies.

Each trial used 8-9kg of red cayenne chillies, 160 small capsicums or 100 large capsicums. Fruit were picked early in the morning and immediately taken to the Ourimbah laboratory (approx 15minute journey). They were then sorted to remove damaged or diseased fruit and stored at 20°C until infestation.

### Infestation and Larval Development

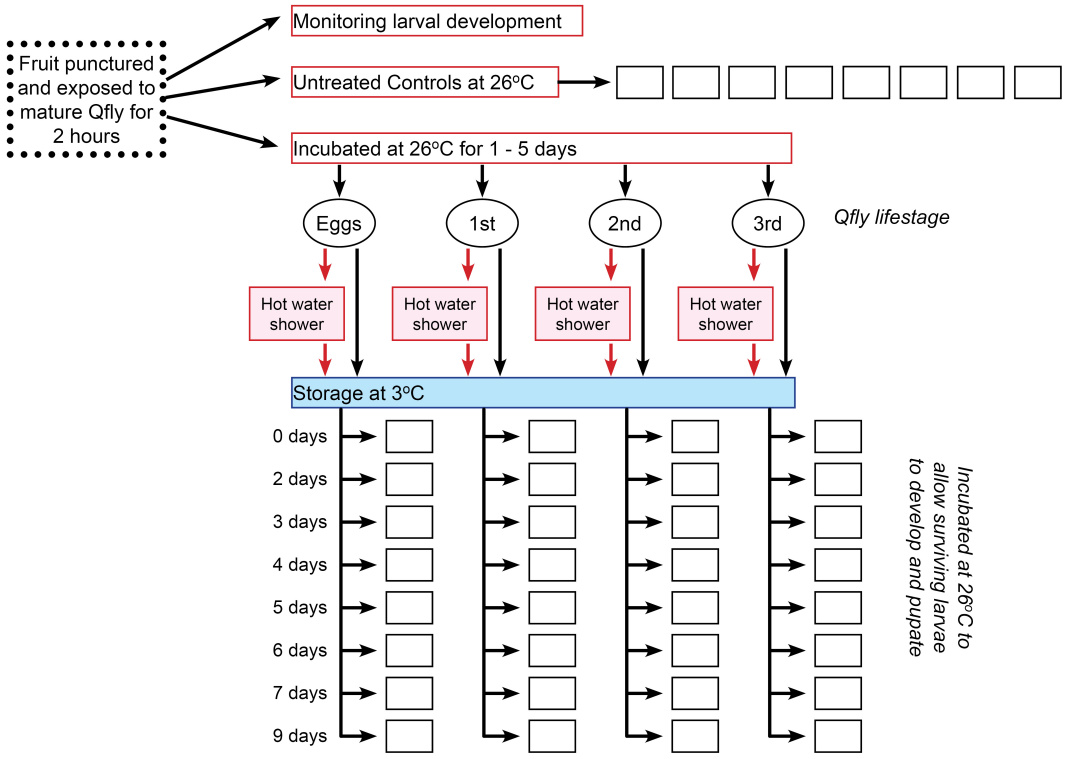
All infestations were conducted using the experimental Qfly colony located at the NSW Department of Primary Industries Laboratory at Ourimbah. The flies are kept at an optimum 26°C with 12 hour day / night cycling including 1 hour simulated dawn and dusk. Adult flies are fed a mixture of protein and sugar, becoming sexually mature after 2 weeks and disposed of after 4 weeks as vigour declines. The colony is replaced with wild stock at least every two years.

Fruit were infested by pricking with a fine pin board then placing on top of 4, 5 or 6 large cages holding mature (>2 weeks old) Qflies. Pricking the fruit in this way stimulates oviposition (Stange 1999) and facilitates oviposition directly into the fruit through the cage wire. Care was taken to ensure that all fruit had pricked areas in good contact with the cage so that flies could access all the capsicums and chillies used.

After 2 hours, the capsicums and chillies were removed from the cages. Chillies were weighed and sorted into 40 mesh bags, each containing 200g (±5g, approximately 10 fruit) randomly selected from all the cages used. Capsicums were randomised into four groups containing a minimum of either 16 or 24 fruit, with an additional fifth group (controls) containing up to 32 fruit. An additional sample was put aside to be used to monitor larval development. All fruit were incubated at 26°C.

Each day during the week following infestation at least 3 infested chillies or 2 infested capsicums were randomly selected, dissected and gently washed through a series of soil sieves to remove eggs and larvae. These were examined and counted under a binocular microscope (10-40 x magnification) to determine larval development stage from their mouthparts. Once >70% of larvae had reached the desired life stage treatment could commence. Experimental procedures are outlined in Figure 1.

Figure 1. Experimental procedure used to determine the most cold tolerant *Bactrocera tryoni* instar in capsicums and chillies with and without a short hot water treatment before cold storage. The procedure was replicated 3 times for chillies and 4 to 5 times for capsicums.




**Treatments**

At least 20% of all infested fruit were designated as untreated controls, used to estimate infestation rates in the fruit. Each "unit" (1 bag chillies or 2 to 3 capsicums) was placed individually on a layer of vermiculite inside a ventilated container and incubated at 26°C to allow normal development and pupation. At least 8 and up to 16 individual containers of control fruit were used for each replication. After 10 and again after 17 days, the vermiculite was sieved to remove pupae and these were counted to estimate average larvae per fruit (LPF).

The remaining infested fruit were randomly allocated to groups designated as 'eggs', '1st instar', '2nd instar' or '3rd instar'. Once larvae had reached the required stage (generally 1, 2, 3 and 5 days after infestation for eggs, 1st, 2nd and 3rd instars respectively) the fruit in that group were either placed directly into cold storage at 3°C or subjected to a short HWS.

The HWS was constructed using a hot water bath, a pump and a perforated header tank with water delivery area 14cm x 24cm. The pump had sufficient flow rate (4.45L.min$^{-1}$) to maintain a minimum of 3cm head in the fully foam insulated header tank. The rear and sides of the unit were covered with craftboard to provide additional temperature insulation. Water temperature was controlled to within 0.1°C of setpoint with a digital temperature controller (Thermoline Scientific, WiseCircu fuzzy control system). The temperature controller also circulated the water within the main tank. Temperatures in the header tank and at the bottom of the shower drop were measured independently and found to be within 0.1°C of the main tank.

Fruit were exposed to the HWS at 55°C for 60 seconds. Capsicums were rotated half way through treatment to ensure their whole surface was exposed to the water, while groups of chillies were moved continuously under the centre of the flow. After treatment the fruit were roughly dried with paper towel then air-dried with a fan for several minutes. They were then placed into covered tubs and transferred to 3°C.

Samples were removed from cold storage after 0 days (HWS treated fruit only), 2, 3, 4, 5, 6, 7, 9, and 11 days and placed individually in ventilated plastic tubs

lined with vermiculite. They were then incubated at 26°C to allow surviving larvae to develop and pupate. Pupae were counted after 10 and 17 days to estimate mortality.

Each replication of the trial involved a single infestation event at least 1 week apart. Additional replications were conducted to ensure that a total of at least 1,500 insects were treated for each product + life stage + storage time combination. This would provide sufficient data for confidence in the overall conclusions;

Capsicums, cold only- 5 replications
Capsicums, HWS + cold - 4 replications
Chillies, cold only - 3 replications
Chillies, HWS + cold - 3 replications

**Statistical Methods**

The mean number of larvae per capsicum per gram of chillies was estimated using the pupae recovered from the untreated controls. This was used to calculate the percentage mortality for each treatment and life stage combination.

Qfly survival data was further analysed separately for each fruit type and treatment. GenStat (VSNI 2011) code was prepared using probit analysis methodology, which specifically included analysis of replicates over time. The robustness of the model was increased by including the observed variability in mean Qfly pupae per fruit from one infestation event to the next.

Significant heterogeneity was identified using a $c^2$ test of residual deviance. When differences between infestation events were significant (5% level), the variance of the estimated parameters was scaled by the corresponding heterogeneity factor equal to the residual mean deviance (Finney 1971). A range of lethal number of days in cold storage plus the associated 95% confidence intervals were calculated using the method described by Robertson and Preisler (2007) (LD50 LD90, LD99).

Initial analysis of the results suggested that 1st instar larvae were the most cold tolerant. To test this hypothesis the relative resistance of eggs, 2nd and 3rd instar larvae to cold storage was compared to the resistance of 1st instars for each vegetable and treatment combination. If the upper 95% confidence limit of the relative resistance ratio was <1 (Robertson and Preisler 2007), we concluded there was a significant difference in cold susceptibility between the instars.

## RESULTS

**Infestation rates and data distribution**

The number of LPF varied between infestation dates. Mean LPF in individual capsicums ranged from 21 ± 2 to 344 ± 37 while mean pupae recovered from a 200g bag of chillies ranged from 102 ± 10 to 1582 ± 141. The estimated total number of insects treated for each life stage and storage time combination was 1,767 and 1,662 for capsicums with and without the HWS respectively and 1,981 and 2,837 for chillies with and without the HWS respectively.

**Effect of time and larval development stage on thermal tolerance**

In most cases, only 2-4 days at 3°C was sufficient to kill around 50% of all life stages in all tested varieties (Table 1). For all life stages other than 1st instars, 4-5 days at 3°C resulted in 90% mortality. No survivors were found when fruit was stored for more than 7 days at 3°C, regardless of life stage or cultivar.

Larval mortality for each life stage and storage time appeared to be independent of *C. annuum* variety, being similar for cayenne chillies and capsicums (Figure 2). Mortality of each life stage approximately conformed to a sigmoid mortality curve, approaching 100% after 7 days at 3°C.

It appeared from the results shown in Figure 2 that 1st instar larvae were more resistant to cold storage than other life stages. This was tested by calculating the relative resistance of 1st instar larvae to cold storage in comparison to other life stages, including the lower and upper 95% confidence limits (Table 2). In almost all cases, 1st instar larvae proved the most treatment tolerant. Examples where this was not the case include achieving 50% mortality of eggs in capsicums and 99% mortality of 2nd instars in chillies, but only at the upper 95% confidence interval in both cases. It was therefore concluded that 1st instar larvae were significantly more treatment tolerant.

**Table 1. Storage time at 3°C estimated to result in 50% (LD50), 90% (LD90) and 99% (LD99) mortality of eggs, 1st, 2nd and 3rd instar Qfly larvae in capsicums and cayenne chillies, with and without a hot water shower treatment (HWS), including the lower and upper 95% confidence limits (LCL, UCL).**

| | *Lifestage* | LD50 | | | LD90 | | | LD99 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Days* | *LCL* | *UCL* | *Days* | *LCL* | *UCL* | *Days* | *LCL* | *UCL* |
| Capsicum | eggs | 2.8 | 1.9 | 3.4 | 4.4 | 3.7 | 5.0 | 6.5 | 5.9 | 7.5 |
| | 1st | 3.6 | 2.9 | 4.1 | 6.9 | 6.1 | 8.1 | 11.9 | 9.9 | 15.9 |
| | 2nd | 2.5 | 1.8 | 3.0 | 4.0 | 3.4 | 4.5 | 5.8 | 5.4 | 6.4 |
| | 3rd | 2.4 | 1.7 | 2.8 | 4.2 | 3.6 | 4.6 | 6.6 | 6.1 | 7.4 |
| Capsicum + HWS | eggs | 1.3 | 0.9 | 1.7 | 2.8 | 2.3 | 3.2 | 5.3 | 4.9 | 5.8 |
| | 1st | 2.2 | 1.4 | 2.8 | 5.0 | 4.2 | 5.8 | 9.6 | 7.9 | 13.3 |
| | 2nd | 1.0 | 0.5 | 1.4 | 2.9 | 2.3 | 3.4 | 7.0 | 6.2 | 8.3 |
| | 3rd | 1.6 | 0.8 | 2.2 | 3.0 | 2.1 | 3.6 | 5.1 | 4.4 | 5.8 |
| Chillies | eggs | 2.2 | 1.4 | 2.8 | 3.3 | 2.6 | 3.9 | 4.8 | 4.2 | 5.3 |
| | 1st | 3.8 | 2.8 | 4.4 | 5.7 | 5.0 | 6.5 | 8.0 | 6.9 | 10.2 |
| | 2nd | 2.1 | 1.0 | 2.8 | 4.0 | 3.0 | 4.8 | 6.9 | 5.9 | 8.6 |
| | 3rd | 2.9 | 2.5 | 3.2 | 4.5 | 4.1 | 4.8 | 6.3 | 6.0 | 6.7 |
| Chillies + HWS | eggs | 2.5 | 1.6 | 3.1 | 4.6 | 3.9 | 5.1 | 7.4 | 6.6 | 8.9 |
| | 1st | 3.0 | 2.6 | 3.4 | 5.4 | 5.1 | 5.7 | 8.6 | 7.9 | 9.5 |
| | 2nd | 2.1 | 1.4 | 2.6 | 3.8 | 3.1 | 4.3 | 6.3 | 5.7 | 7.0 |
| | 3rd | 2.7 | 1.4 | 3.5 | 4.9 | 4.0 | 5.7 | 8.1 | 6.8 | 11.7 |

Figure 2. Mean mortality of eggs, 1st 2nd and 3rd instar larvae in cayenne chillies and capsicums following storage for up to 11 days at 3°C (n=3,4 or 5). Lines indicate the estimated mortality curve for 1st instar larvae (—) and all other lifestages (—).

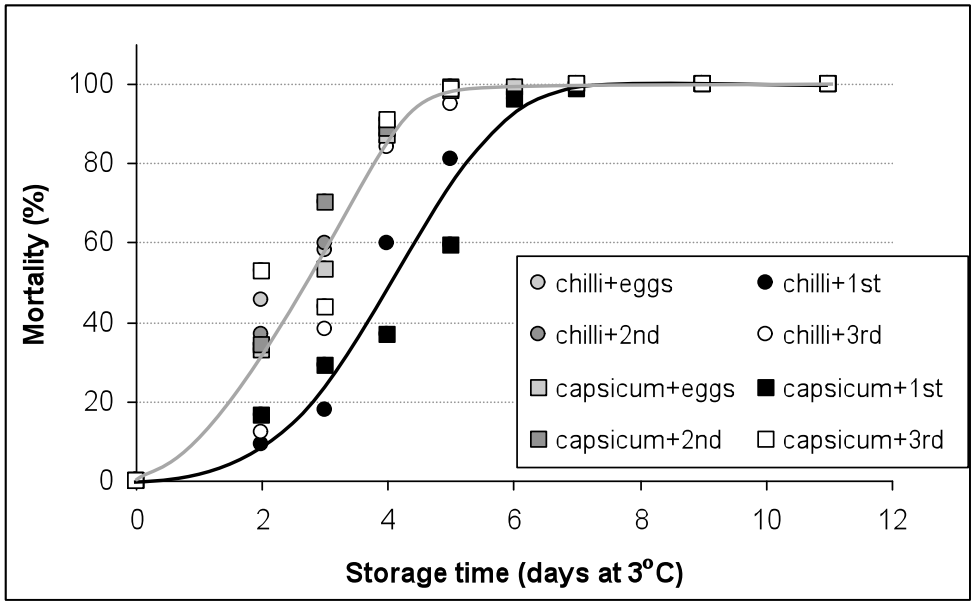


**Table 2. Relative resistance of 1st instar larvae subjected to storage at 3°C in comparison to other lifestages, including the lower and upper 95% confidence limits (LCL, UCL). Values are compared using the duration of cold treatment required to result in 50% (LD50), 90% (LD90) or 99% (LD99) mortality in either cayenne chillies or capsicums. UCL <1 indicates that less time was required to achieve the target mortality for eggs, 2nd or 3rd instar larvae compared to the 1st instar larvae.**

| *Lifestage* (compared to 1st instar) | | LD50 | | | LD90 | | | LD99 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *resist* | *LCL* | *UCL* | *resist* | *LCL* | *UCL* | *resist* | *LCL* | *UCL* |
| Capsicum | eggs | 0.57 | 0.40 | 0.82 | 0.59 | 0.47 | 0.73 | 0.60 | 0.49 | 0.73 |
| | 2nd | 0.55 | 0.35 | 0.86 | 0.71 | 0.56 | 0.89 | 0.87 | 0.69 | 1.09 |
| | 3rd | 0.77 | 0.61 | 0.96 | 0.78 | 0.68 | 0.89 | 0.79 | 0.68 | 0.90 |
| Cayenne Chilli | eggs | 0.76 | 0.57 | 1.02 | 0.63 | 0.52 | 0.76 | 0.54 | 0.42 | 0.69 |
| | 2nd | 0.69 | 0.52 | 0.92 | 0.57 | 0.48 | 0.69 | 0.49 | 0.39 | 0.63 |
| | 3rd | 0.65 | 0.48 | 0.87 | 0.60 | 0.50 | 0.72 | 0.57 | 0.44 | 0.73 |

**Table 3. Mean % mortality and s.e. due to a 60 second shower under 55°C water only (no cold storage) for different Qfly lifestages in cayenne chillies and capsicums.**

| *Lifestage* | Mortality,± s.e. (%) due to HWS | |
|---|---|---|
| | *Cayenne chilli (n=3)* | *Capsicum (n=4)* |
| eggs | 39 ± 18 | 38 ± 16 |
| 1st | 45 ± 10 | 50 ± 17 |
| 2nd | 60 ± 5 | 61 ± 9 |
| 3rd | 79 ± 17 | 68 ± 5 |

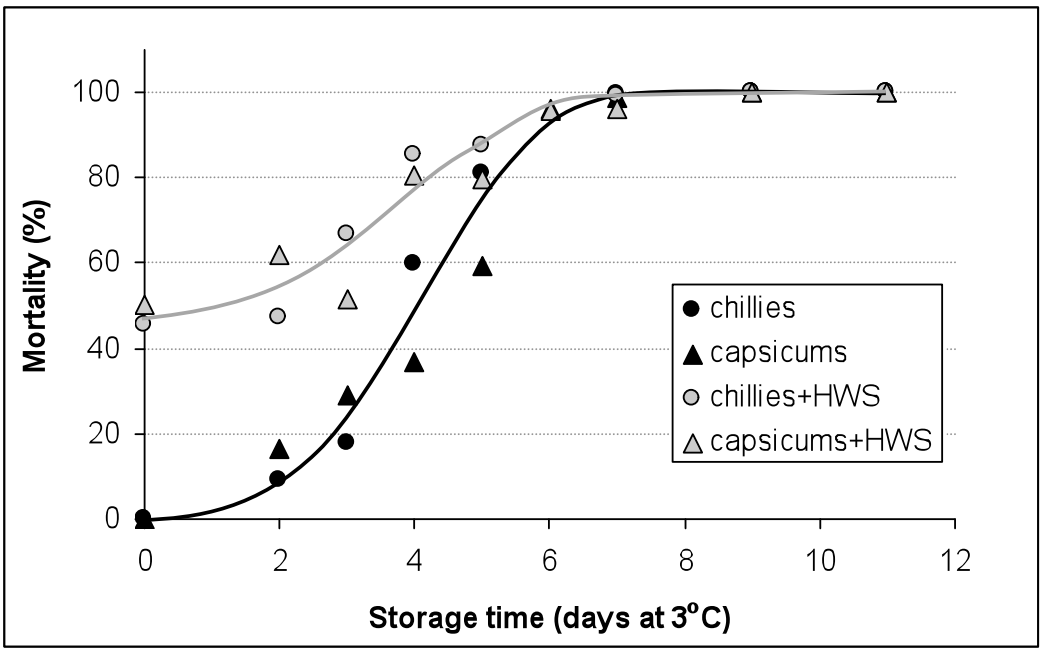
**Effect of a short hot water shower (HWS) on thermal tolerance**

The hot water shower (HWS) alone caused significant mortality, especially to older larvae (Table 3). The results suggest that in a batch of naturally infested fruit, where all ages of larvae could be present, the HWS would kill at least 50% of Qfly eggs and larvae.

At short storage periods, using the HWS resulted in higher mortality than cold storage alone. However, the effect was lost when storage time increased to 5 days or more, with small numbers of survivors regardless of HWS treatment (Figure 3). The HWS reduced the storage times required to reach LD50, LD90 or LD99 in capsicums by an average of 1.4 days. In the case of chillies the HWS increased the time required to reach LD50, LD90 or LD99 by an average of 0.4 days. In particular, it is notable that including the HWS reduced the LD99 storage time for 1st instar larvae in capsicum from 11.9 days to 9.6 days. This was not due to reduced survival at storage times >5days after HWS treatment, but rather to the highly variable nature of the data recorded for non-HWS treated fruit at short storage times. The improved consistency of results using the HWS meant that the data conformed more closely to calculated dose mortality curves.

No pupae were recovered from either chillies or capsicums stored for more than 7 days at 3°C following a HWS. The cold storage time required for effective disinfestation was therefore unaffected by HWS treatment.

Figure 3. Mean mortality of 1st instar larvae in cayenne chillies and capsicums following a short hot water shower (HWS) treatment and storage for up to 11 days at 3°C (n=3 or 4). Data for other instars was similar.




## DISCUSSION

One issue encountered during these trials were the large variations observed in infestation rates from one infestation event to the next. Variability between infestation events is a common issue in conducting this type of research with fruit flies. While this variation reflects what almost certainly happens in the orchard, the reasons for such effects in a controlled laboratory environment are not well understood. Small variations in weather, colony fitness, fruit attributes or simply the way the fruit was placed on the cage for infestation may either stimulate or inhibit egg laying and larval survival. The issue is not unique to Qfly; Fallik et al. (2012) also reported significant variability in pupal recovery of Mediterranean fruit fly (*Ceratitis capitata*) from capsicums despite artificially infesting the fruit with a known volume of eggs to reduce this effect.

Despite this, 1st instar larvae proved significantly more cold tolerant than other lifestages. This is consistent with previous published research on Qfly (Jessup *et al*. 1993, 1998, Heather *et al*. 1996). Mortality of each lifestage was similar for the two cultivars of *C. annuum* tested.

Cold storage can provide a relatively cheap, easy and chemical free method to disinfest produce from insect pests of quarantine significance. However, use of this method can be limited by the cold tolerance of the product, especially for crops of tropical origin.

Heat treatments have been demonstrated to increase cold tolerance in many fruit, including avocado, mango, papaya and citrus (Florissen *et al*. 1996, Lurie, 1998, Schirra *et al*. 2011). Exposure to hot air before cold disinfestation delayed chilling injury development in persimmons (Dentener *et al*. 1997) while hot water dips can similarly protect cold treated citrus and avocadoes (Jessup 1994, Hofman et al. 2002, Schirra *et al*. 2004, Woolf et al. 2004, Bassal and El-Hamahmy 2011).

In this trial we examined the effect of a short HWS treatment (55°C for 60 seconds) on Qfly mortality, this treatment having been shown to provide excellent protection from chilling damage in capsicums and chillies (Fallik *et al.* 1996, Ekman and Pristijono 2010). The HWS treatment did not increase the cold tolerance of the larvae. In some cases, HWS significantly enhanced mortality, especially of more mature life stages after short storage times. This is consistent with the results of Jang *et al*. (2001), who claimed that a heat shock of 38°C did not increase survival of Mediterranean fruit fly in avocadoes. Fallik *et al*. (2012) demonstrated that 21 days at 1.5°C is sufficient to disinfest capsicums from all life stages

of *C. capitata*, regardless of whether or not fruit are subjected to a 15 second hot water rinse and brush at 55°C before treatment.

By comparing LD50 and LD90 values we found that 1st instars were the most cold tolerant life stage. Adding a HWS before storage did not increase cold tolerance, instead tending to reduce the time at 3°C needed to kill Qfly larvae, especially for more mature instars.

No survivors were found in any capsicums or chillies stored for >7 days at 3°C. We recommend that a quarantine treatment of 10 days at 3°C will provide a high level of quarantine security against Qfly in capsicums and chillies. Exposing the fruit to a short HWS before storage can protect them against potential chilling damage without compromising the effectiveness of the treatment. This protocol could prove useful in accessing interstate as well as international markets.

## ACKNOWLEDGMENTS

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