

# Cold disinfestation of *Ceratitidis capitata* (Diptera: Tephritidae) in oranges using a larval endpoint

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## Introduction

Post-harvest, cold disinfestation treatments are required for exports of citrus from South Africa to Japan in order to control the Mediterranean fruit fly (MFF) *Ceratitidis capitata* (Wiedemann) and Natal fruit fly *C. rosa* Karsch. The treatment that is currently in use for oranges (*Citrus sinensis* (L.) Osbeck) requires the fruit pulp to be held at  $-0.6^{\circ}\text{C}$ ,  $\pm 0.6^{\circ}\text{C}$  (indicating an upper temperature threshold of  $0^{\circ}\text{C}$ ) for 12 d (Anonymous 2010) and was based on research in 1969 using a larval endpoint. However, more recently published research involving citrus has used successful pupariation as an endpoint (Hill et al. 1988; Jessup et al. 1993, De Lima et al. 2007); a less labor-intensive method. The advantage of using a larval endpoint is that an immediate decision can be taken when fruit are found to contain live larvae after a cold treatment, whereas fruit with surviving larvae after a cold treatment based on pupariation should be held for several days to wait for pupariation before a decision to reject or accept the fruit can be made. This results in expensive logistical delays. In order to reduce the risk of chilling injury with the current treatment of  $-0.6^{\circ}\text{C}$  for 12 d, further research was conducted with MFF in oranges at temperatures above  $0^{\circ}\text{C}$ , again using a larval endpoint.

## Materials and methods

The research was conducted at Citrus Research International facilities in Nelspruit, Mpumalanga, South Africa. Earlier research had shown that the 2<sup>nd</sup> instar MFF larvae were most tolerant to cold so this life stage was exposed to the cold treatment. Before inoculation, the calyces of small Valencia oranges were removed and a 5 mm-diameter hole was drilled  $\pm 30$  mm deep into each fruit beneath the calyx (Fig.1). A mixture of Torula yeast and water was inserted into the hole before 40 eggs in water were deposited using an autopipette (Fig. 2). The holes were sealed with cotton wool and molten wax. Three replicates were used with 2 500 fruit being inoculated in each. Each fruit was placed in a small brown paper bag, then in a crate. These were held at  $26^{\circ}\text{C}$  for 6 d until the larvae had developed into the 2<sup>nd</sup> instar. Five hundred fruit were then cut and the number of larvae and their survival recorded. The remaining 2 000 fruit were then moved to the cold room. The cold treatment was deemed to have begun when half the probes had reached the required temperature or below. After 15 d 23 h of cold treatment the fruit were moved to a room at  $26^{\circ}\text{C}$  to allow the pulp to reach ambient temperature before they were cut and larval numbers (Fig. 3) and



Fig. 1. Drilling holes beneath the calyx



Fig. 2. Inoculating with an autopipette

mortality determined. The first such trial was conducted in 2006 at a mean temperature of  $1.5^{\circ}\text{C}$  for 16 d but resulted in some survivors. The trial was then repeated in 2007 at a mean temperature of  $1.0^{\circ}\text{C}$  for 16 d.

## Results



Fig. 3. Dead larvae typically darkened by cold treatment

In the first experiment (Fig. 4), 17 657 larvae were used in the controls with 6.7% mortality. A total of 62 492 larvae were treated at a mean temperature of  $1.523^{\circ}\text{C}$  but 3 survivors were found in the third replicate. In the repeated experiment (Fig. 5), 21 801 larvae were used in the controls with 8.1% mortality and 71 756 larvae were treated at a mean temperature of  $1.043^{\circ}\text{C}$  with no survivors. This exceeded the Japanese requirement of

99.99% mortality at the 95% confidence level. The mean hourly maximum of probe readings was  $1.371^{\circ}\text{C}$ .

## Conclusion

Taking into consideration the practicalities of implementing cold treatment under commercial fruit handling conditions, where cold rooms are usually set  $1^{\circ}\text{C}$  below an upper threshold temperature, the suggested treatment schedule resulting from this research to guarantee freedom from live MFF larvae is: the storage of citrus for 16 d at temperatures at or below  $1.4^{\circ}\text{C}$  with the treatment starting when all temperature probes reach  $1^{\circ}\text{C}$  or below.

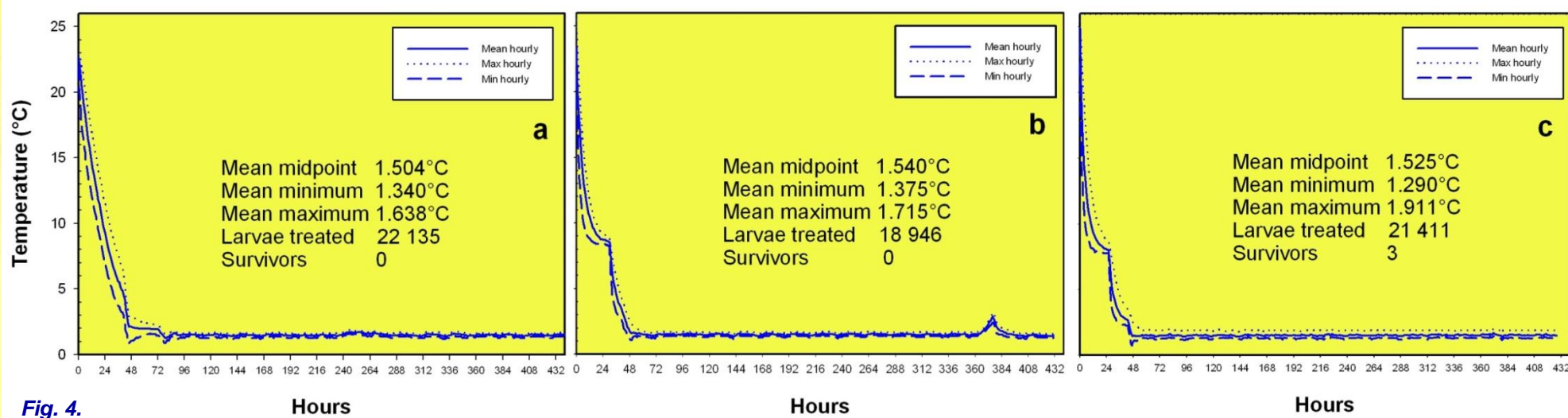


Fig. 4.

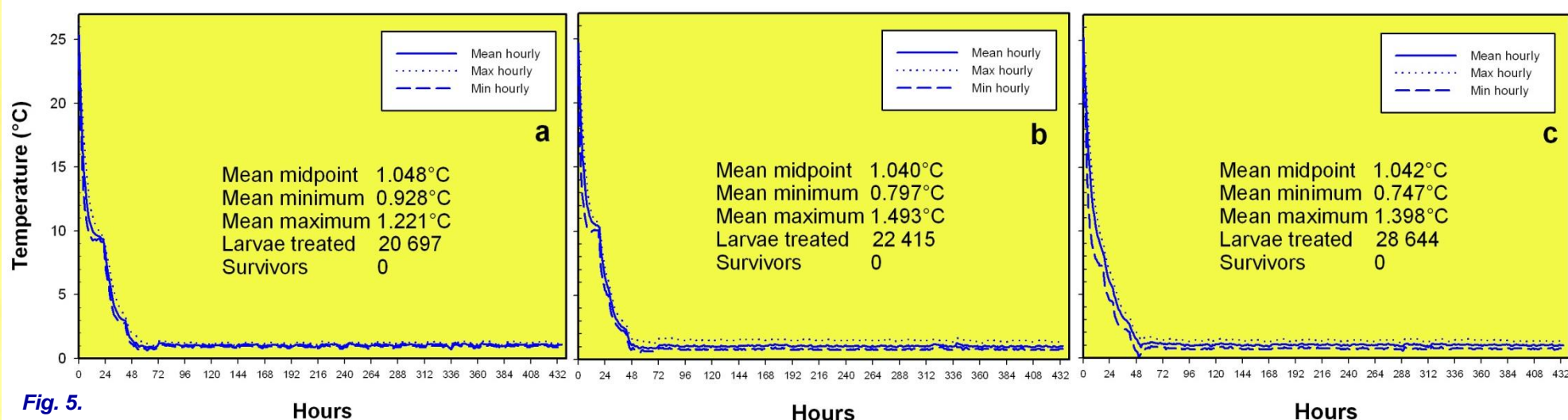


Fig. 5.

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