Influence of Holding Temperature and Irradiation on Field Performance of Mass-Reared Thaumatotibia leucotreta (Lepidoptera: Tortricidae)

Authors: Boersma, Nevill, and Carpenter, James E.

Source: Florida Entomologist, 99(sp1) : 215-221

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.099.sp126

Influence of holding temperature and irradiation on field performance of mass-reared *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae)

Nevill Boersma[1,*] *and James E. Carpenter*[2]

Abstract

The sterile insect technique (SIT) is as an important component to the area-wide integrated management of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), which was successfully implemented in the Western Cape region of South Africa and subsequently expanded to citrus areas in the Eastern Cape region of South Africa. This integrated control program, which transports sterile moths from a rearing facility in Citrusdal, South Africa to orchards in both the Western and Eastern Cape, must continuously examine production, handling, processing, transport, and release protocols to ensure the delivery of high-quality sterile moths. While the use of cold temperature to immobilize moths is standard protocol for SIT programs to increase the density of moths for purposes of collecting, handling, irradiation, transport and release, some concern has been raised that rapid chilling and long cold temperature storage of moths may negatively impact field performance of some insectary-reared insects. We conducted trials to examine the effect of irradiation with 150 Gy of gamma rays and cold temperature storage on the performance of *T. leucotreta* moths released in citrus orchards. The radiation treatment did not significantly affect the performance of *T. leucotreta* moths released in citrus orchards. However, compared with moths held at room temperature, moths that were rapidly chilled were less likely to be captured in pheromone traps and less likely to disperse as great distances following release in citrus orchards. Additional research is needed to identify an alternative to rapid chilling and cold temperature storage that does not impair mating competitiveness and dispersal of irradiated *T. Leucotreta* adults. Procedural changes that will maintain or enhance sterile *T. leucotreta* moth quality and performance in the field, while allowing for the cost-effective handling and processing of the sterile moths, need to be considered.

Key Words: sterile insect technique; competitiveness; false codling moth; chilling; handling; quality control

Resumen

La técnica del insecto estéril (TIE) es un componente importante del programa de manejo integrado en zonas extensas para el gusano falso de la manzana, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), que se implementó con éxito en la región del Cabo Occidental y posteriormente se amplió a las áreas productoras de cítricos en la región del Cabo Oriental de Sudáfrica. Este programa de control integrado, en el cual se transportan polillas estériles de una instalación de cría en Citrusdal, Sudáfrica hacia huertos en los Cabos Occidental y Oriental, debe revisar continuamente los protocolos de producción, procesamiento, transporte y liberación, para asegurar la entrega de polillas estériles de alta calidad. El uso de bajas temperaturas para inmovilizar las polillas es un protocolo estándar en programas de TIE para procesar altas densidades de polillas durante la recolección, manipulación, irradiación, transporte y liberación. Existe preocupación porque el enfriamiento rápido y el almacenamiento de las polillas en condiciones de frio por periodos largos, pueden influir negativamente rendimiento en el campo de los insectos liberados. Realizamos ensayos para examinar el efecto de la irradiación (con rayos gamma de 150 Gy) y la temperatura de almacenamiento en frío en el rendimiento de las polillas *T. leucotreta* liberadas en huertos de cítricos. El tratamiento de radiación no afectó significativamente el rendimiento de las polillas *T. leucotreta* liberadas en huertos de cítricos. Sin embargo, en comparación con las polillas mantenidas a temperatura ambiente, las que se enfriaron rápidamente fueron menos propensas a ser capturadas en trampas de feromonas y a dispersarse a largas distancias después de su liberación en huertos de cítricos. Se requiere más investigación para identificar una alternativa al enfriamiento rápido y almacenamiento en frío que no perjudique la competitividad de apareamiento y la dispersión de los adultos irradiados de *T. leucotreta*. Se debe analizar el costo-beneficio de estos cambios en los procedimientos que permitan mantener o mejorar la calidad de polillas estériles de *T. leucotreta* y su rendimiento en el campo.

Palabras Clave: técnica del insecto estéril; competitividad; polilla falsa de la manzana; enfriamiento; manejo; control de calidad

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is indigenous to sub-Saharan Africa, the Ethiopian Region and many African islands (CIBC 1984) where it infests many commercial and wild fruit-bearing plant species (Economides 1979). The pest was unknown in the Western Cape Province of South Africa until the end of the 1960s. Subsequently the geographical range of *T. leucotreta* expanded and by the late 1970's it was well established on commercial citrus in the Western Cape of South Africa (Hofmeyr et al. 2015). Because *T. leucotreta* is a key pest of citrus in the Western Cape and a phytosanitary pest of concern to many citrus importing countries, including the United States, and because conventional control tactics were not fully adequate to protect the crop, investigations were

[1]Xsit, PO Box 422, Citrusdal, 7340 South Africa
[2]U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS-CPMRU), Tifton, GA 31794, USA
*Corresponding author; E-mail: nb@xsit.co.za

initiated to evaluate the sterile insect technique (SIT) as a component of area-wide integrated pest management (AW-IPM) (Klassen 2005; Carpenter et al. 2007; Vreysen et al. 2007). Results from a series of trials (Bloem et al. 2003; Carpenter et al. 2004; Hofmeyr et al. 2005, 2015) convinced the citrus industry in South Africa to commercialize the SIT for *T. leucotreta* control in 2007, and for this purpose X Sterile Insect Technique (Pty) Ltd [Xsit] was established.

Hofmeyr et al. (2015) reported that during the first year of operation (2007-2008) the commercial program of Xsit released sterile *T. leucotreta* moths (males and females) over 1,500 ha of citrus orchards in Citrusdal, Western Cape, South Africa. This area was expanded to 3,000 ha in 2008–2009 and 4,000 ha in 2009–2010. During the first 3 years of operation, wild *T. leucotreta* male populations were reduced 3, 8, and 10-fold, pre-harvest crop losses decreased by 50, 80, and 93%, and post-harvest export fruit rejections decreased by 13, 25, and 38%, respectively in the SIT area compared to the non-SIT area (Hofmeyr et al. 2015). The citrus growers and industry have been very pleased with the SIT program, as evidenced by yearly growth of the program with respect to both the number of ha protected and the number of growers voluntarily participating in the program. In 2011 commercial releases of sterile *T. leucotreta* over 1,800 ha of citrus in the Sundays River Valley (Eastern Cape) near Port Elizabeth were initiated (Nepgen 2014), and currently releases are being made on more than 7,000 ha. Releases in Gamtoos valley (Eastern Cape) were initiated in 2014–2015 on part of a 1,700 ha planting of citrus, and the area treated is also expected to grow in the future.

Although Xsit has been successful in integrating SIT as an area-wide control tactic for *T. leucotreta* in South Africa, there have been many challenges to overcome in the process. One of the major hurdles has been to select the best methods and equipment to store, transport and release the irradiated *T. leucotreta* moths. Similar to other Lepidopteran SIT programs (Bloem et al. 2007), moths from the *T. leucotreta* insectary are chilled immediately after collection (Hofmeyr & Pretorius 2010), and held at a low temperature to immobilize the moths during packaging, irradiation, transport, and release in the orchards using all-terrain vehicles (ATV) and gyrocopters (Hofmeyr et al. 2015). Many factors can impact how a SIT program decides to handle and release sterile insects (Dowell et al. 2005), and these decisions can have a substantial impact on the quality and performance of the released sterile insects (Calkins & Parker 2005). For example, the use of rapid chilling and prolonged low temperature for handling, processing and shipping sterile insects prior to release is a convenience that comes at the expense of partial loss of field performance of laboratory-reared insects (Terblanche et al. 2008). Further, Stotter & Terblanche (2009) cautioned that the lack of rapid cold hardening found in *T. leucotreta* suggests that this species has limited capacity to adjust its thermal tolerance over short, daily timescales. Xsit management was concerned that the rapid chilling of *T. leucotreta* may reduce the performance of the released sterile moths in their program. Therefore, the objective of this study was to examine the effect of rapid chilling and cold temperature storage after irradiation with 150 Gy of gamma rays on the performance of *T. leucotreta* moths released in citrus orchards.

Materials and Methods

INSECT REARING HANDLING AND PROCESSING

The production of *T. leucotreta* was divided into the following phases: egg production, diet preparation and inoculation, larval rearing and pupal harvesting, moth emergence and collection, and moth irradiation.

Egg Production

Non-irradiated moths were contained in a stand-alone oviposition room of 150 m² to provide a constant supply of eggs for stocking the rearing facility. There were 6 rows with 72 egg pans (mating and oviposition containers) in a row. In each pan, approximately 4,000 moths with a 1:1 sex ratio were kept at 25 °C with a relative humidity of 70%. The inside bottom of each pan was lined with waxed paper (293 mm wide and 33 g per square meter) on which eggs were laid. A roll of waxed paper was fitted on a rolling mechanism for each pan, and the paper was pulled through the pan via 2 horizontal slits at each end of the bottom of the pan. Each day the waxed paper with eggs was rolled out while new paper from the roll was supplied to the bottom of the pan. The waxed paper was then cut into sheets with approximately 1,200 eggs per sheet (N. B. personal observations).

Diet Preparation and Inoculation

The dry ingredients of the insect diet used at Xsit were mixed in the following ratios: maize meal (84.11%), wheat germ (8.41%), brewer's yeast (4.21%), full fat milk powder (1.54%), Nipagin (0.84%), ascorbic acid (0.4%), canola oil (0.39%) and calco red dye (0.1%).

A volume of water equal to 60% of the total volume of the dry ingredients was added to form a coarse paste. This paste was then dispensed into 500 mL glass jars. The jars, containing 270 ± 10 g of diet, were then baked for 100 min at an oven temperature of 120 °C. After baking, the jars were held in a cooling chamber and then moved to the inoculation room. Egg sheets containing 1,200 ± 100 *T. leucotreta* eggs were dipped into a formaldehyde solution of 8% to prevent contamination of any bacteria and virus, and then placed into the glass jars on top of the diet. Diet jars inoculated with *T. leucotreta* eggs were placed on a cart and moved to the incubation rooms (N. B. personal observations).

Larval Rearing and Pupal Harvesting

The larval rearing room has a floor surface of 330 m^2. The rearing carts containing the inoculated diet jars were aligned according to egg collection date and parked in 13 separate rows, which represent the total life cycle of the larvae from inoculation until start of pupation. Temperature was maintained at 25 ± 1°C and the photoperiod was 12:12 h L:D. Humidity was not measured on a daily basis or controlled, but ranged between 40% and 60% RH (N. B. personal observations).

After 13 d the carts were moved to the pupation rooms, and the jars were opened and placed on their sides to facilitate larval exit from the jars. Corrugated cardboard pupation boards (570 × 530 mm, each with 16,500 holes) were placed beneath each layer of jars, and descending larvae entered the openings in the boards to pupate. The temperature was maintained at 25 ± 1 °C while humidity ranged between 30% and 50% RH. There were 15 pupation maturation rooms of 30 m^2 each. One room was available for each d of new production that entered from the larval rearing room. Larvae were allowed 7 d to exit the diet jars and pupate in the pupation boards, after which the pupation boards were collected and taken to the moth emergence room (N. B. personal observations).

Moth Emergence and Collection

There were 2 moth emergence rooms with a total floor space of 326 m^2 and a total 146 emergence cabinets positioned in 13 rows. Fifty pupation boards were placed in 1 cabinet which had a dimension of 673 (w) × 860 (l) × 1678 mm (h). Cabinets were divided into light and dark compartments by a metal panel containing small slits through which emerged moths could pass. Temperature was maintained at 25

± 1 °C. Pupation boards were placed in the dark compartments, and as adult moths emerged from the pupation boards, they were able to move through the metal panel holes and enter the light compartment where they were collected in an air-stream and transferred to the collection room at 12 m/s. Entering the moth collection room the moth-spassed through a plenum box, bringing the air-stream speed down from 12 m/s to 3 m/s. This allowed moths to have a "soft landing" in the collection troughs (Hofmeyr & Pretorius 2010). There were 13 collection troughs, 1 for each row of cabinets. Moths were cooled down to 9 ± 1 °C in order to keep them inactive. Collection troughs were emptied once the amount of moths in the pan reached a depth of approximately 2 cm. Moths were placed in baskets and then stored at 6 ± 1 °C. When moth temperature reached 8 ± 1 °C, moths were placed into cardboard boxes (130 × 130 mm). A scale extraction unit was used to remove loose scales that may have resulted from moving the moths while filling the boxes. Boxes were individually weighed and contained 14,000 ± 200 moths per cardboard box (N. B. personal observations).

Moth Irradiation

The rearing facility has a panoramic Co^{60} source for irradiation purposes. The source allowed the placement of 8 canisters, with each canister able to hold 4 cardboard boxes. Moths were irradiated with 150 Gy to ensure sterility (Bloem et al. 2003). Packaging was conducted in a refrigerated room maintained at 4 °C, while the moth temperature was an estimated ± 6 °C to ensure that the insects remained inactive (N. B. personal observations).

Xsit Moth Release Protocols

After irradiation the moths were kept in the distribution room in separate cages for Eastern and Western Cape deliveries. Ambient temperature was kept at 4–6 °C, while moth temperature were in the range, 6–8 °C (moth temperatures tend to be higher in boxes). Moths were not dispatched if their temperature was above 8 °C, as this would have resulted in increased activity during transport, which may have led to damaged and less effective moths (N.B. personal observations). Moths dispatched to the Eastern Cape were loaded into a refrigerated truck as soon as temperatures were within the required range. The transport temperature of the moths was maintained at 6 to 8 °C to ensure that the cold chain was undisturbed. Moths released in the Western Cape were treated similarly. When moths reached the airfield they were packed into the release mechanism (hopper) of a gyrocopter, which released them at the calibrated speed of 120 km/h above orchards. The release coordinator verified that the moths were slightly active (between 10–12 °C) before they were placed in the hopper. The hopper was designed to accommodate 1,000,000 moths. Two thousand moths were released per ha per week (N.B personal observations).

Experimental design

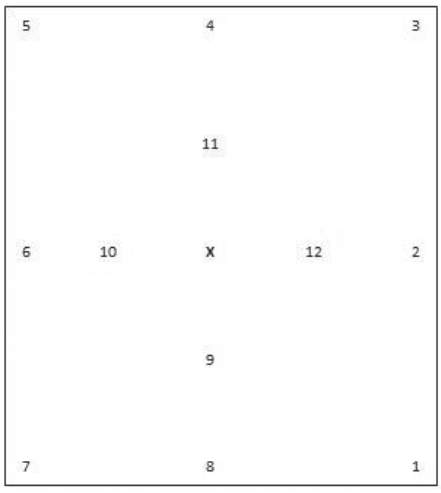
Laboratory-reared *T. leucotreta* used for our experiments were obtained from the Xsit rearing facility, which has the capacity to produce 50 million moths per week. The colony was initiated from wild moths collected in Citrusdal, South Africa, in 2006, and was last augmented with wild individuals to avoid inbreeding depression or genetic divergence from wild populations in 2009.

Orchard for Release and Recapture Trials

A 5 ha 'navel' orange orchard located 1,500 m from the rearing facility was used. Twelve pheromone traps (Chempac, Suider Paarl, South Africa) baited with synthetic pheromone (Z-8-dodecenyl acetate, E-8-dodecenyl acetate and E-7-dodecenyl acetate) (Chempack FCM Lure, Paarl, South Africa) were deployed in the orchard around a centrally located moth release site (Fig. 1). Distances from the release site to the different traps varied from 30 to 89 m.

Trial 1 (2013–2014)

Moths irradiated with 150 Gy and non-irradiated moths handled at 2 temperature treatments were compared in this trial. For the first temperature treatment, 100 male and 100 female moths were collected from the air-stream collection system and irradiated. At the same time another 100 male and 100 female moths (non-irradiated) were collected in the same way and all 4 groups of moths were kept in the insectary distribution room at a room temperature of 3–4 °C for 2 h. For the second temperature treatment, male and female moths were also collected from the air-stream collection system. One hundred males and 100 females were irradiated and kept at ambient temperature (25 ± 1 °C). Additionally, 100 non-irradiated males and 100 non-irradiated females were kept at room temperature (25 ± 1 °C) along with the irradiated moths. For each temperature treatment, the outcomes from 100 irradiated males and 100 irradiated females were compared to those of 100 non-irradiated males and 100 non-irradiated females. Although only males respond to the synthetic pheromone, females were also released to simulate the commercial releases of sterile insects. Each of the 4 moth types (irradiated warm, irradiated cold, non-irradiated warm, and non-irradiated cold) were colored with different colors of fluorescent powder (DayGlo Color Corp., Cleveland, Ohio, USA) to enable identification of differently marked males when captured in traps. After coloring, the moths were held at 25 °C for 30 min to allow the cold moths to recover from the chilled state, whereafter they were taken to the release site (travel time ± 5 min). Moths were released once a week at the same time, 11:00 AM, for 4 weeks from 23 Dec 2013 to 13 Jan 2014. The number of each male type recaptured each week per trap was recorded. The total distance from the release site at which males from each treatment were recaptured was calculated as $\sum_{1\text{-}12}$ (n × td), where n = the number of males captured in a trap and td = the distance of each trap to the release site. The mean distance at which males were recaptured from the release site was calculated each week for each male type as the total distance flown by all the males divided by the total number of males recaptured for each group.



Trap	Distance from X (release site)
1	89
2	60
3	89
4	66
5	89
6	60
7	89
8	66
9	33
10	30
11	33
12	30

Fig. 1. Diagram of a 5-ha navel orange orchard showing the central release site (X) for *Thaumatotibia leucotreta* moths and the pheromone trap placement. The distance (m) from the center release site to each trap is listed at the right of the diagram.

Data were analyzed using factorial analyses of variance (Statistica 10; Statsoft Inc., Tulsa, Oklahoma, USA) with the number of males recaptured, the mean distance of recaptured moths from the release site, and the total distance of recaptured moths from the release site as the dependent variables. Release date, radiation treatment, and temperature at which moths were held prior to release were sources of variation. All interactions were included in the statistical models to test the null hypotheses of independent effects of the different sources of variation. For all analyses of variance, when significant differences were indicated, means were separated by the Tukey HSD statistic at $P = 0.05$.

Trial 2 (2014)

During 13 Jan 2014 to 7 Apr 2014, a second trial similar to the above in all but 1 respect was conducted: all moths were treated with 150 Gy. The reason for conducting trial 2 was to include more replicates over a greater time frame to study the effect of temperature treatment on irradiated moths, similar to current handling protocols. Non-irradiated moths were excluded as they presented a risk of supplementing the wild population. Data were subjected to analysis of variance (Statistica 10; Statsoft Inc., Tulsa, Oklahoma, USA) with the number of males recaptured, the distance recaptured from the release site, and the total distance flown from the release site as the dependent variables. Release date and temperature at which moths were held prior to release were sources of variation. For all analyses of variance, when significant differences were indicated, means were separated by the Tukey HSD statistic at $P = 0.05$.

Results

TRIAL 1

Handling temperature (chilled vs not chilled) did not significantly interact with irradiation with regards to any of the dependent variables affecting field performance (i.e., the number of males recaptured ($F = 0.158$; df = 1,12; $P = 0.703$), the mean distance flown by males before being captured ($F = 0.011$; df = 1,12; $P = 0.920$), or the total distance flown by males before being captured ($F = 0.170$; df = 1,12; $P = 0.688$)). Handling temperature also did not significantly interact with the release date with regards to the number of males recaptured ($F = 0.726$; df = 3,8; $P = 0.565$) or the total distance flown by males before being captured ($F = 0.530$; df = 3,8; $P = 0.674$). However, the interaction between handling temperature and release date almost reached the level of significance with regards to the mean distance flown by males before being captured ($F = 4.014$; df = 3,8; $P = 0.051$). Further, the irradiation treatment did not significantly interact with the release date with regards to any of the dependant variables measuring field performance (i.e., the number of males recaptured ($F = 0.321$; df = 3,8; $P = 0.810$), the mean distance flown by males before being captured ($F = 0.124$; df = 3,8; $P = 0.943$), or the total distance flown by males before being captured ($F = 0.439$; df = 3,8; $P = 0.732$)).

Irradiation of males did not significantly influence any of the dependent variables measuring field performance (i.e., the number of males recaptured ($F = 0.487$; df = 1,14; $P = 0.497$), the mean distance flown by males before being captured ($F = 0.873$; df = 1,14; $P = 0.366$), or the total distance flown by males before being captured ($F = 0.167$; df = 1,14; $P = 0.689$)). Similarly handling temperature did not significantly influence the mean distance flown by released moths ($F = 0.303$; df = 1,14; $P = 0.591$). However, male moths that had been collected in the air-stream and deposited into the chilled collection room were significantly ($F = 13.32$; df = 1,14; $P = 0.0026$) less likely to be recaptured following release in the orchard compared to male moths that had been held at a warm temperature after collection from the air-stream (Fig. 2). Male moths collected in the air-stream and deposited into the chilled collection room also flew a significantly ($F = 7.47$; df = 1,14; $P = 0.016$) shorter total distance before being recaptured following release in the orchard compared to male moths that had been held at a warm temperature (Fig. 3).

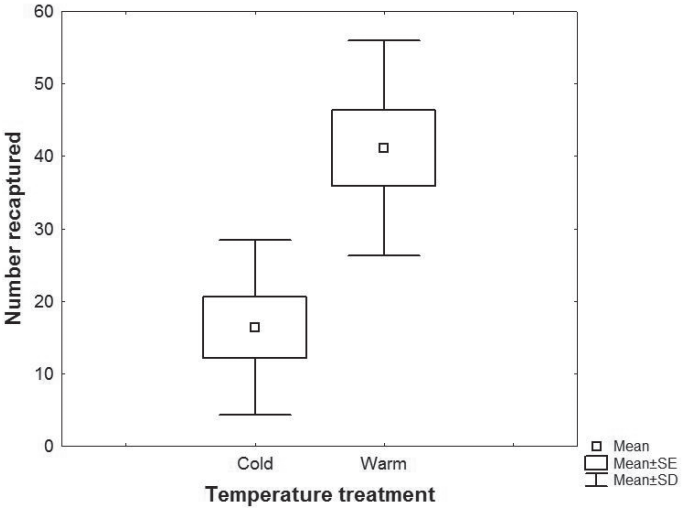


Fig. 2. Comparison of an insectary air-stream collection system and post-collection chilling (8 ± 1 °C) to manual collection without chilling (25 ± 1 °C) on the mean number of male *Thaumatotibia leucotreta* moths recaptured in pheromone traps after release in a citrus orchard during Dec 2013 and Jan 2014.

The mean distance released male moths flew before being recaptured was significantly ($F = 4.199$; df = 3,12; $P = 0.03$) influenced by the date the moths were released in the orchard (Fig. 4). Males released on 23 Dec 2013 and 30 Dec 2013 flew just over 59 m from the release site when captured in the pheromone traps. The mean distance flown was reduced to 48.2 m for moths released on 6 Jan 2014 and further reduced to 39.6 m for moths released on 13 Jan 2014. A similar trend was observed for the total distance flown by captured males, with a significant influence due to release date ($F = 10.664$; df = 3,12; $P = 0.001$) (Fig. 5). However, the release date did not significantly affect the number of moths recaptured ($F = 2.01$; df = 3,12; $P = 0.166$).

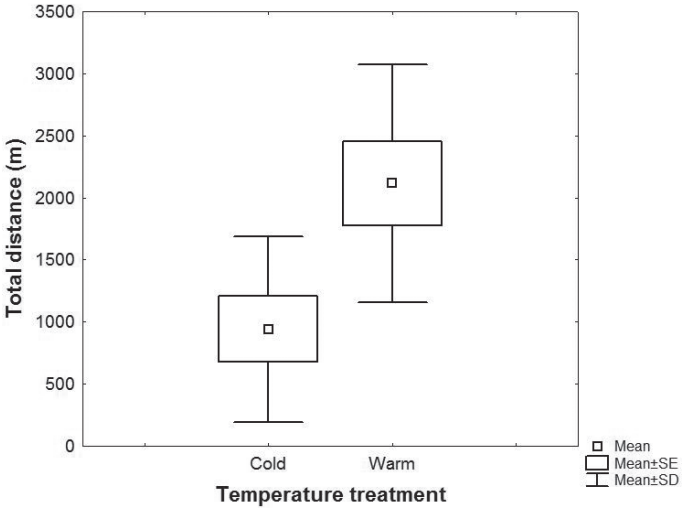


Fig. 3. Comparison of an insectary air-stream collection system and post-collection chilling (8 ± 1 °C) to manual collection without chilling (25 ± 1 °C) on the total distance (m) flown by male *Thaumatotibia leucotreta* moths recaptured in pheromone traps after release in a citrus orchard during Dec 2013 and Jan 2014.

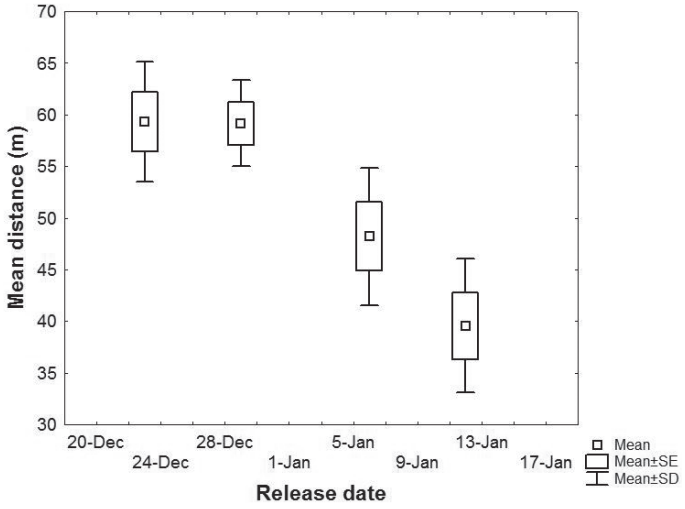


Fig. 4. Influence of release date on the mean distance (m) flown by male *Thaumatotibia leucotreta* moths recaptured in pheromone traps after release in a citrus orchard during Dec 2013 and Jan 2014.

TRIAL 2

The date that moths were released in the orchard did not significantly influence any of the dependent variables affecting field performance (i.e., the number of males recaptured (F = 0.443; df = 9,10; P = 0.882), the mean distance flown by males before being captured (F = 1.02; df = 9,10; P = 0.485), or the total distance flown by males before being captured (F = 0.446; df = 9,10; P = 0.880)). Also, the mean distance released male moths flew before being recaptured was not significantly influenced by the handling and holding temperatures of the moths (F = 2.94; df = 1,18; P = 0.103). Even though the mean (± SD) distance for male moths held at a warm temperature was greater (49.6 ± 11.3 m) than the mean (± SD) distance flown by male moths collected in the insectary air-stream and deposited into the chilled collection room (38.5 ± 17.2 m). The high variability in the data seemed to prevent the analysis to reveal the difference to be statistically significant.

However, handling temperature did significantly (F = 16.73; df = 1,18; P = 0.001) influence the number of released moths that were recaptured (Fig. 6). The mean (± SD) number of male moths captured in traps was greater when moths had been held at a warm temperature after collection (22.8 ± 14.1) than when male moths were collected in the insectary air-stream and deposited into the chilled collection room (4.2 ± 2.9). Likewise, the moth handling temperature significantly (F = 15.44; df = 1,18; P = 0.001) influenced the total distance flown by released moths (Fig. 7). The mean (± SD) total distance flown was greater when moths had been held at a warm temperature (1107.0 ± 747.2 m) than when male moths were collected in the insectary air-stream and deposited into the chilled collection room (166.8 ± 119.1 m).

Discussion

Reliable production and delivery of high quality insects that perform competitively in the field are critical to the success of AW-IPM programs that have an SIT component (Calkins & Parker 2005; Vreysen et al. 2009). To ensure quality and success of the SIT component, it is crucial to assess potential quality degradation during each step of production, handling, processing and release of the sterile insects (Huettel 1976; Calkins & Parker 2005; Simmons et al. 2010). In control programs for lepidopteran pests, desirable performance of sterile insects released in the field would include adequate dispersal throughout the wild insect habitat, competitive pheromone-mediated flight toward the wild insects, and post-release longevity to maintain adequate over-flooding ratios between releases. The effect of different production, handling, processing, and release protocols on these performance attributes can be compared using release/recapture trials in which marked moths are captured within an array of pheromone-baited traps over time (Bloem et al. 2004; Carpenter et al. 2013).

The implementation of area-wide releases between 2007 and 2010 of sterile *T. leucotreta* as a component of a commercial pest management program (Xsit) in Citrusdal, South Africa resulted in progressively better control compared to conventional pest management practices. Over the 3-year period, wild male *T. leucotreta* populations in the SIT area steadily decreased, and pre-harvest crop losses due to *T. leucotreta* infestation in the SIT area dropped progressively, compared with areas using conventional pest management practices. The reduction in pre-harvest crop losses from the 2 treatment regimens resulted in

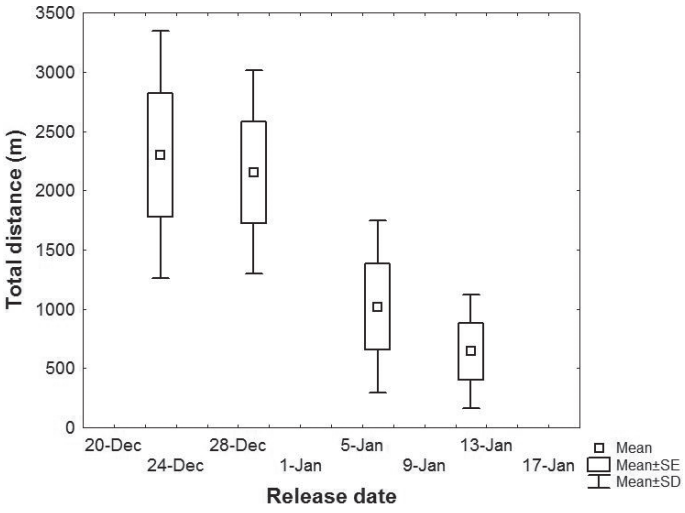


Fig. 5. Influence of release date on the total distance (m) flown by male *Thaumatotibia leucotreta* moths recaptured in pheromone traps after release in a citrus orchard during Dec 2013 and Jan 2014.

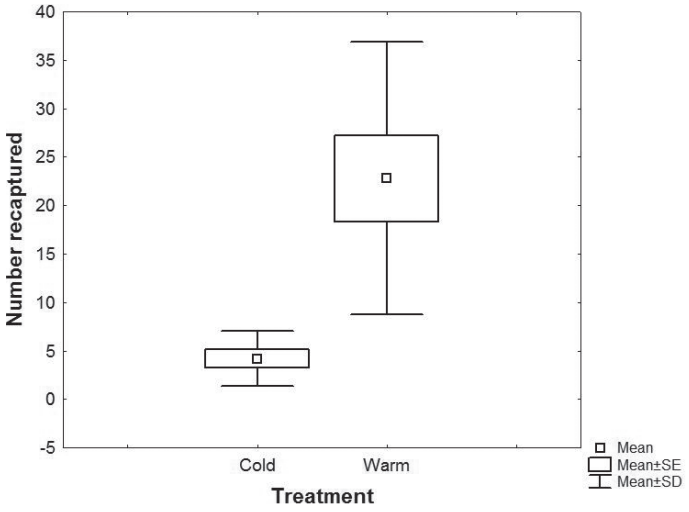


Fig. 6. Comparison of an insectary air-stream collection system and post-collection chilling (8 ± 1 °C) to manual collection without chilling (25 ± 1 °C) on the mean number of male *Thaumatotibia leucotreta* moths recaptured in pheromone traps after release in a citrus orchard during Jan 13 and Apr 7, 2014.

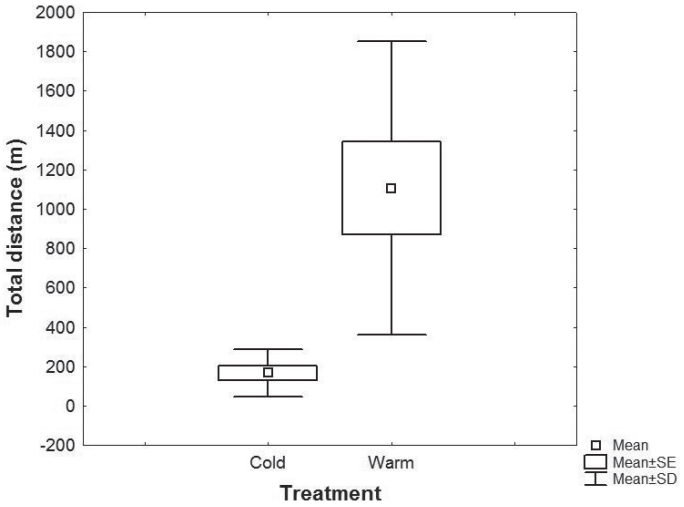


Fig. 7. Comparison of an insectary air-stream collection system and post-collection chilling (8 ± 1 °C) to manual collection without chilling (25 ± 1 °C) on the total distance (m) flown by male *Thaumatotibia leucotreta* moths recaptured in pheromone traps after release in a citrus orchard during Jan 13 and Apr 7, 2014.

fewer shipments of export citrus rejected for *T. leucotreta* from the SIT area (Hofmeyr et al. 2015). It also was noted that progressively larger numbers of released male moths were recaptured in the monitoring traps, and the over-flooding ratio of sterile to wild males increased steadily from 2:1 to 12:1 to 54:1 during this period. Hofmeyr et al. (2015) suggest that the increase in the recapture of sterile males and the increase in the over-flooding ratio may have been influenced by several factors including: a) increased rearing efficiency which resulted in better quality of released moths and therefore improved mating competitiveness and longevity; b) improvements made to the sterile moth release program, especially changing over to aerial releases, and a rapid response with additional releases of sterile moths in a 'hot spot' (an area with a higher population of wild moths than the average *T. leucotreta* infested areas); and c) reduced competition with pheromone traps from a declining population of wild females. Trap catches of wild male moths of 13.0 moths per trap per week prior to the sterile moth releases in 2006, continued to decline in 2012, 2013 and 2014, to 2.0, 0.4 and 0.1 moths per trap per week, while fruit infestation by *T. leucotreta* decreased from 2.6% in 2010/11 to 0.1% in 2013 (Barnes et al. 2015).

The successful implementation of the SIT as an integral component to the area-wide integrated management of *T. leucotreta* in the Western Cape convinced the citrus industry and the program management of Xsit to initiate commercial releases of sterile *T. leucotreta* in 1,800 ha of citrus in the Sundays River valley (Eastern Cape) near Port Elizabeth in 2011. Since then, this area has expanded to 7,000 ha (Nepgen 2014). Releases in Gamtoos valley (Eastern Cape) were initiated in 2014/15 in 1,700 ha and here the area treated is also expected to expand in the future. This program's expansion and the need to transport sterile moths to orchards in both the Western and Eastern Cape has challenged the Xsit management to continuously examine production, handling, processing, transport, and release protocols to insure the delivery of high-quality sterile moths.

The use of cold temperature to immobilize moths is a standard handling protocol for SIT programs to increase the density of moths for purposes of collecting, handling, irradiation, transport and release (Dowell et al. 2005). However, some concern has been raised that while chilled, immobilized insects are less likely to damage themselves, the rapid chilling and long storage of insects may negatively impact field performance of laboratory-reared insects (Terblanche et al. 2008), especially species like *T. leucotreta,* which may have limited capacity to adjust their thermal tolerance over short, daily time scales (Stotter & Terblanche 2009). Nepgen et al. (2015) conducted a study to examine the effects of transportation of irradiated *T. leucotreta* between Citrusdal in the Western Cape, where the moths were reared and irradiated, and Addo in the Eastern Cape, where the moths would be released. One of the findings of their study was a negative effect of irradiation and chilling on flight ability of both male and female moths when flight tests were conducted indoors at 25 °C. They suggested that adverse effects of long-distance transport on moth quality should be addressed by first defining exact temperature thresholds (both high and low temperature) at various quality-control points from production to release.

In our study, which compared moths held at room temperature with moths rapidly chilled, the radiation treatment (150 Gy) did not significantly influence the number of moths recaptured, or the dispersal in the orchard following release. However, with regards to chilling the moths, the results from our trials are congruent with the findings of Nepgen et al. (2015). Although the chilling of moths in order to collect and package at high density protects them from damaging themselves, this convenience in handling may come with a cost to field performance. Compared to moths held at room temperature, moths collected in the insectary air-stream and rapidly chilled were less likely to be captured in pheromone traps, and those moths that were captured had dispersed a shorter distance following release in citrus orchard.

Many sterile insect release programs have evaluated the field performance and competitiveness of colony insects by using pheromone traps to conduct release-recapture tests (Henneberry & Keaveny 1985, Lance et al. 1988, Bloem et al. 2004, Carpenter et al. 2013). The use of field release-recapture bioassays can be a powerful tool in detecting moth fitness differences (Bloem et al. 2004), but daily variation in meteorological factors within the field may significantly influence the bioassay. In trials examining the effects of larval rearing strategies and radiation dose for *Cydia pomonella*, Bloem et al. (2004) not only found significant daily variation in recapture of released moths (probably due to temperature fluctuations), but also found significant interactions between larval rearing strategy and dose of radiation with respect to recapture day after release. Carpenter et al. (2013) also found the mean number of male *C. pomonella* moths recaptured in both field cage and orchard releases varied significantly by recapture day, but found no interaction between recapture day and moth treatment. In our present study, handling temperature for *T. leucotreta* did not significantly interact with the release date with regards to the number of males recaptured or the total distance flown by males before being captured. However, the total distance flown and the mean distance released male moths flew before being recaptured was influenced by the date the moths were released in the orchard. This might be explained by the fact that the average temperature for the latter part of December (21.7 °C) were nearly 2 °C warmer than the first part in January (19.5 °C) which may influence the dispersion and flight ability of the adult males. Carpenter et al. (2014) surmised that because of the influence of meteorological factors, release-recapture bioassays are better predictors of the daily performance of released males in the field than laboratory flight bioassay, but because of the controlled climatic conditions, laboratory flight bioassays may be more sensitive in detecting daily fluctuations in the quality of moths caused by factors within the mass-rearing facility. Therefore, both laboratory and field bioassays may be required to provide feedback on quality and performance of mass-reared moths in a SIT program. Nevertheless, protocol changes can only be made if there are viable, cost-effective alternatives available. Continuous quality monitoring and additional research are required to identify procedural changes that would maintain or enhance *T. leucotreta* moth quality and performance.

Acknowledgments

This work was part of the FAO/IAEA Coordinated Research Project on Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control. We acknowledge the IAEA for providing funds to support this research. We acknowledge Hendrik Hofmeyr for proof reading the document. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References Cited

Barnes BN, Hofmeyr JH, Groenewald S, Conlong DE, Wohlfarter M. 2014. The sterile insect technique in agricultural crops in South Africa: A metamorphosis …. but will it fly? African Entomology 23(1): 1-18.

Bloem S, Carpenter JE, Hofmeyr JH. 2003. Radiation biology and inherited sterility in false codling moth (Lepidoptera: Tortricidae). Journal of Economic Entomology 96(6): 1724-1731.

Bloem S, Carpenter JE, Bloem KA, Tomlin L, Taggart S. 2004. Effect of rearing strategy and gamma radiation on field competitiveness of mass-reared codling moths (Lepidoptera: Tortricidae). Journal of Economic Entomology 97(6): 1891-1898.

Bloem S, Carpenter JE, McCluskey A, Fugger R, Arthur S, Wood S. 2007. Suppression of the codling moth *Cydia pomonella* in British Columbia, Canada using an area-wide integrated approach with an SIT component, pp. 591-601 *In* Vreysen MJB, Robinson AS, Hendrichs J [eds.], Area-Wide Control of Insect Pests. From Research to Field Implementation. Springer, Dordrecht, The Netherlands.

Calkins CO, Parker AG. 2005. Sterile insect quality, pp. 269-296 *In* Dyck VA, Hendrichs J, Robinson AS [eds.], Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Carpenter JE, Bloem S, Hofmeyr JH. 2004. Acceptability and suitability of eggs of false codling moth (Lepidoptera: Tortricidae) from irradiated parents to parasitism by *Trichogrammatoidea cryptophlebiae* (Hymenoptera: Trichogrammatidae). Biological Control 30: 351-359.

Carpenter JE, Bloem S, Hofmeyer H. 2007. Area-wide control tactics for the false codling moth *Thaumatotibia leucotreta* in South Africa: a potential invasive species, pp 351-360 *In* Vreysen MJB, Robinson AS, Hendrichs J [eds.], Area-Wide Control of Insect Pests. From Research to Field Implementation. Springer, Dordrecht, The Netherlands.

Carpenter JE, Blomefield T, Hight SD. 2013. Comparison of laboratory and field bioassays to examine lab-reared *Cydia pomonella* (Lepidoptera: Tortricidae) quality and field performance. Journal of Applied Entomology 137: 631–640.

Commonwealth Institute of Biological Control. 1984. Possibilities for the biological control of the false codling moth, *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae). Biocontrol News and Information 5: 217-220.

Dowell RV, Worley J, Gomes PJ. 2005. Sterile insect supply, emergence, and release, pp. 297-324 *In* Dyck VA, Hendrichs J, Robinson AS [eds.], Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Economides CV. 1979. False codling moth, a serious pest of citrus in Zambia. Farming in Zambia 12(2): 4.

Henneberry TJ, Keaveny DF. 1985. Suppression of pink bollworm by sterile moth releases. USDA-ARS. ARS-32.

Hofmeyr JH, Carpenter JE, Bloem S. 2005. Developing the sterile insect technique for *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae): influence of radiation dose and release ratio on fruit damage and population growth in field cages. Journal of Economic Entomology 98(6): 1924-1929.

Hofmeyr JH, Carpenter JE, Bloem S, Slabbert JP, Hofmeyr M, Groenewald SS. 2015. Development of the sterile insect technique to suppress false codling moth *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in citrus fruit: Research to implementation (part 1). African Entomology 23(1): 180-186.

Hofmeyr JH, Pretorius J. 2010. Development of a device to collect mass-reared false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), in a commercial insectary. African Entomology 18(2): 374-378.

Huettel MD. 1976. Monitoring the quality of mass-reared insects: A biological and behavioral perspective. Environmental Entomology 5: 807-814.

Lance DR, Odell TM, Mastro VC, Schwalbe CP. 1988. Temperature-mediated programming of activity rhythms in male gypsy moths (Lepidoptera: Lymantriidae): implications for the sterile male technique. Environmental Entomology 17: 649-653.

Nepgen ES. 2014. A study on the application technology of the sterile insect technique, with focus on false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a pest of citrus in South Africa. M.Sc. thesis, Rhodes University, South Africa.

Nepgen ES, Hill MP, Moore SD. 2015. The effect of long-distance transportation on the fitness of irradiated false codling moth (Lepidoptera: Tortricidae) for use in a sterile insect release program. Journal of Economic Entomology tov246 DOI: http://dx.doi.org/10.1093/jee/tov246

Simmons G, Suckling D, Carpenter J, Addison M, Dyck V, Vreysen M. 2010. Improved quality management to enhance the efficacy of the sterile insect technique for lepidopteran pests. Journal of Applied Entomology 134(3): 261-273.

Stotter RL, Terblanche JS. 2009. Low-temperature tolerance of false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in South Africa. Journal of Thermal Biology 34: 320-325.

Terblanche JS, Clusella-Trullas S, Deere JA, Chown SL. 2008. Thermal tolerance in a south-east African population of tsetse fly *Glossina pallidipes* (Diptera: Glossinidae): implications for forecasting climate change impacts. Journal of Insect Physiology 54: 114-127.

Vreysen MJB, Robinson AS, Hendrichs J [eds.], 2007. Area-Wide Control of Insect Pests. From Research to Field Implementation. Springer, Dordrecht, The Netherlands.