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Effects of Different Combinations of Sterile and Fertile *Thaumatotibia leucotreta* on Fruit Infestation and Population Growth Rate

Michael M. Githae[1] | Candice A. Coombes[1] | Reyard Mutamiswa[1,2] | Sean D. Moore[1,3] | Martin P. Hill[1]

[1]Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa | [2]Tugwi Mukosi Multidisciplinary Research Institute, Midlands State University, Gweru, Zimbabwe | [3]Citrus Research International, Gqeberha, South Africa

Correspondence: Michael M. Githae (michaelmathenge7@gmail.com)

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ABSTRACT

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae) is a key phytosanitary pest of citrus in South Africa. The pest damages fruit by burrowing and feeding on the pulp, thus causing yield losses and export rejections. There are several *T. leucotreta* management strategies, such as orchard sanitation, mating disruption, attract-and-kill, the use of biocontrol agents, and the sterile insect technique. A laboratory cage study was conducted to examine the impact of different combinations of treated (sterile) and untreated (fertile) *T. leucotreta* adults on fruit damage and reproduction rate. *Thaumatotibia leucotreta* adults were grouped into different combinations of treated (T) and untreated (U) male (M) and female (F): UM × UF (control), TM × UF, UM × TF, TM × TF, and UM × UF × TM × TF. These combinations were released into cages to mate and oviposit on the Navel oranges. Each treatment was replicated three times, and the experiment was repeated three times. After 4 weeks, the number of damaged fruit and larval entries per fruit per cage were recorded. Damaged fruit per treatment were then incubated until all emerging F1 progeny were collected and outcrossed with fertile *T. leucotreta*. Fecundity and fertility per treatment were recorded. Cages with sterile *T. leucotreta* had significantly fewer damaged fruit, larval entries, and emerged F1 adults compared to the control, except for UM × UF × TM × TF treatments. Similarly, control cages and UM × UF × TM × TF treatments had significantly higher fecundity and fertility compared to other treatments involving sterile *T. leucotreta*. The TM × UF combination exhibited the lowest rate of increase per generation (< 0.57× from the parental to F1 generation), demonstrating a reduction in the fertile population. The results demonstrated that the release of sterile *T. leucotreta* leads to sterile-fertile matings rather than fertile-fertile matings, thereby aiding in pest suppression in the *T. leucotreta* SIT programme.

1 | Introduction

The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is a key agricultural pest endemic to sub-Saharan Africa (Bloem et al. 2007; Hofmeyr et al. 2015; Hattingh et al. 2020). The pest is polyphagous with a wide host range of cultivated plants including economically important crops such as macadamia, peppers, pomegranates, stone fruit, and most citrus cultivars (EPPO 2013, EFSA 2024), as well as wild plant species (Kirkman 2007; EPPO 2013, EFSA 2024). *Thaumatotibia leucotreta* is a key pest of citrus in southern Africa, particularly Navel oranges (Grout and Moore 2015, EFSA 2024). South Africa is the second-largest exporter of citrus in the world after Spain (CGA-SA 2023). As a result, strict quarantine and import protocols have been implemented by export markets such as the USA, People's Republic of China, South Korea, and the European Union, to prevent live specimens of this phytosanitary pest from entering these regions (USDA 2010, SA-DAFF 2015, EU 2017, EFSA 2024). The damage inflicted by *T. leucotreta* involves larval penetration of the fruit and pulp feeding, resulting in fruit drop (Grout and Moore 2015; Moore 2022). Fruit abscission occurs within three to 5 weeks after infestation (Grout and Moore 2015). If left uncontrolled, or where the effective biocontrol complex is disrupted, crop losses can be significant (Newton 1998; Moore 2002).

Enhancing control methods is a vital strategy for effectively managing pest populations below economic injury levels, particularly when it comes to *T. leucotreta* in citrus orchards as any fruit bearing brown larval penetration holes/spots with frass are regarded as damaged and unsuitable for export (Moore and Hattingh 2012; Moore 2022). Several methods are used to control *T. leucotreta* in South Africa, such as orchard sanitation, chemical control, behavioural control, biological control, sterile insect technique (SIT), and post-harvest control (Moore 2022; Githae et al. 2024, 2025). However, none of these methods is effective alone, highlighting the need for an integrated pest management strategy (Moore 2022). Since 2007, the SIT has been practised in Western Cape, Eastern Cape and Northern Cape Provinces in South Africa (Hofmeyr et al. 2005, 2015; Boersma 2021). This method has proven effective in reducing the mean number of trapped wild *T. leucotreta* males and the damaged fruit in these citrus-growing areas (see Boersma 2021). In South Africa, *T. leucotreta* are reared, and adults are sterilised at X Sterile Insect Technique (XSIT) (Pty) Ltd. in Citrusdal, Western Cape Province, before being transported in a cold, immobilised state, using refrigerated trucks, to release sites (Barnes et al. 2015; Boersma & Carpenter, 2016; XSIT, 2025). During this immobilised state, the cold treatment can negatively impact their fitness, reducing their ability to compete successfully for mating with their wild counterparts in the orchards (Nepgen et al. 2015). However, for SIT to be successful, the treated male moths must compete successfully with fertile wild males for wild females, to induce sterility in the fertile population (Boersma 2021). In the *T. leucotreta* SIT programme, both sterile males and females are released in the citrus orchards and sterile females have been shown to act as a positive sperm sink for the fertile *T. leucotreta* (Van Steenderen 2017, unpublished; XSIT 2025).

Regular monitoring of the mating dynamics and compatibility between untreated and treated insects is essential. This is because there is a possibility that genetic changes occurring in laboratory-reared colonies can lead to alterations in reproductive mechanisms *viz*, pheromone emission, detection, and mating behaviour as suggested by Whitten and Mahon (2005). For instance, research conducted by Aigbedion-Atalor, Upfold, et al. 2024, Aigbedion-Atalor, Heiduk, et al. 2024 showed that *T. leucotreta* males can mate with females from geographically different populations in South Africa. Conversely, Hibino and Iwahashi (1991) demonstrated that female melon flies, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), on Okinawa Island were less inclined to mate with treated males compared to fertile males. Similarly, Weldon (2005) discovered that mass-reared male Queensland fruit flies, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), modified their calling and mating behaviour due to unnatural selection pressures in a laboratory environment, although resistance to treated insects by wild individuals is exceedingly rare and has not been documented as significant in any SIT programme (Whitten and Mahon 2005). Male-only SIT releases, such as those in Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) programmes through a genetic sexing strain, are considered preferable because they are feasible and economical from a production standpoint and prevent fruit damage caused by treated females through 'blind stings' (Rendón et al. 2004; Hofmeyr et al. 2015).

Even though releasing only treated males is typically considered sufficient in an SIT programme, due to the impracticality of sex-separating treated *T. leucotreta* in SIT programmes, dual-sex releases of both treated male and female *T. leucotreta* combinations are currently conducted (Marec and Vreysen 2019; Boersma 2021; XSIT, (X Sterile Insect Technique) 2025). Treated females can either create positive or negative sperm sinks, depending on whether they mate with untreated or treated males, respectively, thereby influencing the rate of population suppression (Calkins and Parker 2005; Van Steenderen 2017, unpublished). A positive sperm sink, where treated females attract wild fertile male sperm, is preferred. When this occurs, fruit infestation from treated and wild fertile *T. leucotreta* combinations might be reduced, hence improving the effectiveness of the technique (Moore 2022). A laboratory study by Moore 2021 demonstrated that treated males crossed with untreated female *T. leucotreta* and treated females crossed with untreated male *T. leucotreta* resulted in some fruit infestation. However, Van Steenderen (2017) demonstrated that the release of treated male and female *T. leucotreta* resulted in a significantly lower overall mean egg number and hatch than a male-only sterile release. However, the effect of UM × UF × TM × TF combination has not been investigated in controlled laboratory studies and this combination usually exists in the field after the release of sterile insects. Therefore, this study investigated whether this combination could affect the fruit damage and population growth rate compared to other different combinations of treated and untreated male and female *T. leucotreta* in laboratory cages. Our study hypothesised that the UM × UF × TM × TF combination reduces fruit damage and population growth rate compared to other combinations. The results obtained are discussed in the context of improving the effectiveness of SIT in the suppression of *T. leucotreta* through the release of different combinations of treated and untreated *T. leucotreta* in an area-wide sterile insect release programme in citrus orchards in South Africa.

2 | Materials and Methods

2.1 | Test Insects

Sterile and fertile *T. leucotreta* adult moths were sourced from XSIT. To render them sterile, the moths were placed into cardboard boxes and exposed to a dose of 180 Gy of gamma radiation using a 20 kCi 60Co source panoramic irradiator (Bloem et al. 2003). This dosage is used in the mass-rearing facility to sterilise moths for large-scale releases across South African citrus growing regions. Subsequently, the moths were transported to Rhodes University, Makhanda, South Africa (~840 km, 13–14 h) in a polystyrene cooler box with dry ice bricks, maintaining a temperature of 4°C–6°C. This controlled temperature was crucial in minimising the moths' activity and preventing mating while in transit (Nepgen et al. 2015). The moths used were approximately 48 h old upon arrival and kept in a refrigerator at a temperature of 5°C when not in use. They were immediately sorted by sex in a cold room using a stereomicroscope (Zeiss Microscopy, South Africa), at a magnification of 40× to prevent any unintended mating. Distinguishing characteristics included the presence of black tufts on the anal and hind tibiae in males, which are absent in females (Gilligan et al. 2011).

2.2 | Release of Treated *T. leucotreta* Into Laboratory Cages

Laboratory cage studies were conducted similarly to Hofmeyr et al. (2005), in a controlled environment (CE) room at 26°C ± 1°C, 70% ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. A total of 15 insect-rearing cages (40 cm × 40 cm × 60 cm) were used. The moths were released into the cages, each containing 35 ripe Washington Navel oranges, which were 8 cm in diameter and grown organically on a local farm in Sundays River Valley, Eastern Cape, South Africa. Before release into the cages, the treated and untreated *T. leucotreta* were sorted into different combinations (Table 1). This grouping was done 24 h prior to the actual release, with male and female *T. leucotreta* being released on opposite sides of the laboratory cage. The treatments were randomly assigned to the cages, with each treatment replicated three times, and the experiment repeated three times. Petri dishes with wet cotton wads were placed in each cage to provide water for the moths, and the wads were replaced daily until all the moths died in a cage. Throughout the experimental period, the insects were allowed to mate and oviposit without any disturbance. The laboratory experiment was conducted for 4 weeks since this is the period when the brown larval penetration holes on the fruits are easily visible. Thereafter, the fruits were thoroughly examined externally for any *T. leucotreta* damage signs (brown larval penetration holes/spots with frass). Any fruit with larval penetration holes/spots was regarded as damaged, and the total number of damaged fruits per cage was recorded. Similarly, the number of larval entries (penetration holes/spots) per fruit was recorded for each treatment.

2.3 | Determination of Fecundity and Fertility

The apparently damaged fruit was placed in individual 500 mL round plastic containers (8 cm × 10 cm) with mesh lids. For the provision of suitable pupating substrates for the larvae, wads of cotton wool were placed inside each container. Rapidly decaying fruit was cut open, and any larvae found were carefully transferred into diet jars (13 cm × 7 cm) (one diet jar per damaged fruit). The larvae were fed on *T. leucotreta* diet (see Moore et al. 2014) to enable them to complete their development under optimum conditions. Pupae collected from cotton wool, diet jars, and the cages were transferred to individual 90 mL clear round plastic containers (5 cm × 5 cm) and allowed to eclose.

After sexing, all the emerged adults (48 h) (F1 generation) were crossbred with untreated *T. leucotreta* adults (48 h) of the opposite sex sourced from XSIT. Each pair was placed in 90 mL clear round plastic containers with snap-on lids, perforated to hold a moistened cotton dental wick, and placed in the CE room for mating. Mating between the pairs was allowed to occur, followed by egg-laying on the smooth inner sides of the containers until the death of the females (~8–10 days). Thereafter, the containers were opened and the total number of eggs laid (fecundity) per container per treatment was counted and recorded under the stereo microscope (Zeiss Microscopy, South Africa), at a magnification of 40×. After 5 days, the number of neonates that hatched (fertility) was counted and recorded per container.

2.4 | Determination of F1 Sterility and Per Generation Rate of Increase

The percentage egg hatch (hatchability) per each combination was calculated as follows:

$$\text{Percentage egg hatch} = \frac{\text{Total number of F1 progeny}}{\text{Total number of eggs laid}} \times 100.$$

In these trials, it was assumed that treated females (TF) were completely sterile and as such, produced no F1 progeny (Bloem et al. 2003). The per generation rate of increase occurring from the parental generation (P1) to the filial generation (F1) in each cage was calculated by dividing the number of F1 male and female progeny produced by an untreated (UM × UF) mating by the number of P1 UM (10) and UF (10) released into the cages (Table 1). As a result, the reproductive rate obtained from each combination was used to project how the population growth rate can develop over three generations (Hofmeyr et al. 2005).

2.5 | Statistical Analysis

Data collected from the experiment were checked for homogeneity of variance (F test, Levene's test), and the determination of residual deviations for non-normality was done using the Shapiro–Wilk test (Shapiro and Wilk 1965). The test revealed that the data were not normally distributed. Due

TABLE 1 | Randomly assigned treatments in 15 insect-rearing cages to examine the effect of different combinations of treated and untreated *T. leucotreta* on fruit damage and population growth. UM, untreated male; UF, untreated female; TM, treated males; TF, treated females. A combination of 10:10 was used between treated and untreated *T. leucotreta* males and females.

Treatment	1	2	3	4	5
Combination	UM × UF	TM × UF	UM × TF	TM × TF	UM × UF × TM × TF
Untreated	10 10	10	10		10 10
Treated		10	10	10 10	10 10

to overdispersion of the data, negative binomial generalised linear mixed model analysis, as an extension of the Poisson distribution and a log link function to accommodate for count data with a significant proportion of zero values, was employed to analyse the data with different combinations as the sources of variation ($p \leq 0.05$), following a recommendation by O'Hara and Kotze (2010). Analysis of deviance (log-likelihood ratio statistic) was utilised to evaluate the goodness of fit of the Poisson regression model, which has a distribution akin to that of chi-squared (χ^2). The best fitting models were chosen based on the lowest Akaike Information Criterion (AIC). The dependent variables in the statistical model comprised the number of larval entries, the number of damaged fruits, and the number of F1 *T. leucotreta* adults emerging from the damaged fruit from the different treatments. Additionally, the model was used to analyse data on the number of hatched eggs laid by the F1 moths (resulting from damaged fruit and crossed with fertile adults of the opposite sex), with F1 adult sex and cage treatment as the sources of variation. Similarly, the model was applied to analyse the number of F1 males and females that were fathered by a fertile male, with the cage treatment as the source of variation. Differences among treatment means were separated using the Tukey–Kramer statistic ($p \leq 0.05$) for multiple comparisons when the statistical model indicated significant treatment effects. All analyses were conducted using R version 4.2.2 (R Core Team 2023).

3 | Results

3.1 | Fruit Damage, Larval Entries, and F1 Progeny

The different combinations of sterile to fertile *T. leucotreta* significantly affected the mean number of damaged fruit ($\chi^2 = 109.31$; df = 4; $p < 0.05$) (Figure 1), the mean number of larval entries ($\chi^2 = 59.90$; df = 4; $p < 0.05$) (Figure 2) and the mean number of emerged F1 *T. leucotreta* adults ($\chi^2 = 48.70$; df = 4; $p < 0.05$) (Figure 3). The highest mean number of damaged fruits was recorded in the control cages (33.89 ± 2.32), while the lowest was recorded in treatment TM × TF (4.67 ± 0.90 of damaged fruit). There was a significant decrease of 14.11 ± 2.04 and 10.89 ± 1.66 of damaged fruit between the control and TM × UF, UM × TF and TM × TF combinations respectively. The mean number of larval entries in the control cages (390.22 ± 134.62 larval entries per treatment replicate) was significantly higher than combinations TM × TF (0.01 ± 0.01 larval entries per treatment replicate), TM × UF (59.22 ± 20.57 larval entries per treatment replicate), and UM × TF (67.89 ± 23.55 larval entries per treatment replicate) (Figure 2). Likewise, the control cages exhibited a significantly higher mean number of emerged F1 adults (84.89 ± 32.15) relative to combinations TM × TF (0.00 ± 0.00 F1 adults per replicate), TM × UF (11.67 ± 4.54 F1 adults per replicate) and UM × TF (12.22 ± 4.75 F1 adults per replicate) (Figure 3). However, no significant differences in the mean number of damaged fruits, mean number of larval entries, and emerged F1 adults were recorded between the control cages and combination UM × UF × TM × TF (Figures 1–3). Furthermore, among cages receiving different combinations of treated and untreated *T. leucotreta*, significantly fewer damaged fruit and larval entries were recorded in cage treatment TM × TF. Conversely, cages receiving treatment UM × UF × TM × TF showed a significantly higher mean number of damaged fruit (29.56 ± 3.82), the mean number of larval entries (294.22 ± 101.54 per treatment replicate), and the emerged F1 adults (63.67 ± 24.15) than the cages receiving treatments TM × UF, UM × TF, and TM × TF (Figure 1; 2; 3).

3.2 | Fecundity and Fertility

The mean fecundity of F1 moths emerging from the damaged fruit and crossbred with fertile *T. leucotreta* of the opposite sex was significantly influenced by the different combinations of treated and untreated *T. leucotreta* ($\chi^2 = 37.18$; df = 4; $p < 0.05$), the sex of emerged F1 *T. leucotreta* adults ($\chi^2 = 38.65$; df = 1; $p < 0.05$), and the interaction between the different combinations of treated and untreated *T. leucotreta* and the sex of the emerged F1 *T. leucotreta* adults ($\chi^2 = 14.54$; df = 3; $p < 0.05$) (Table 2). Similarly, fertility was significantly affected by the different combinations of treated and untreated *T. leucotreta* ($\chi^2 = 66.05$; df = 4; $p < 0.05$), the sex of emerged F1 *T. leucotreta*

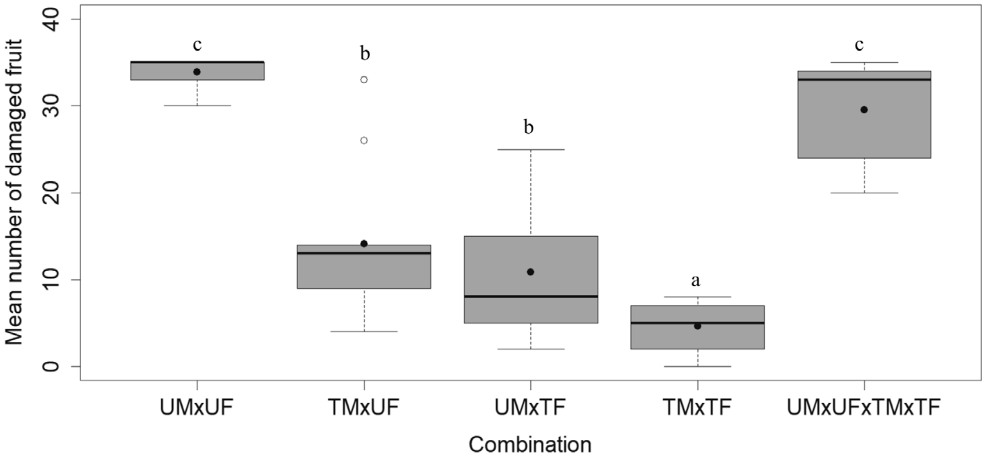

FIGURE 1 | The mean number of damaged fruits from different cages receiving different combinations of treated and untreated *T. leucotreta*. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots indicate the mean number of damaged fruit per treatment, while the white dots represent the outliers.

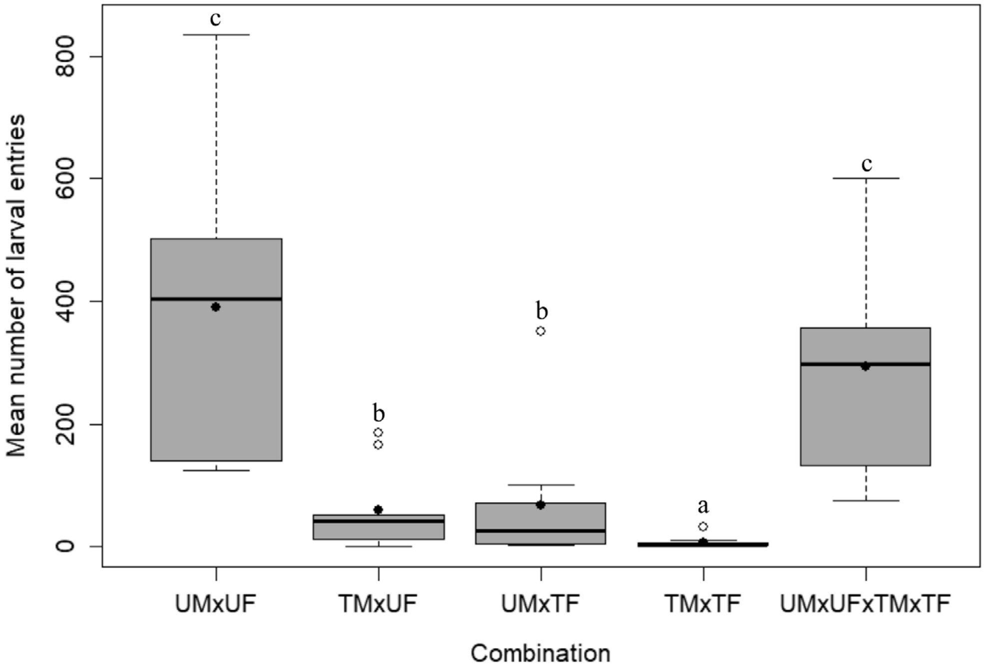

FIGURE 2 | The mean number of larval entries from different cages receiving different combinations of treated and untreated *T. leucotreta*. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots indicate the mean number of larval entries per treatment, while the white dots represent the outliers.

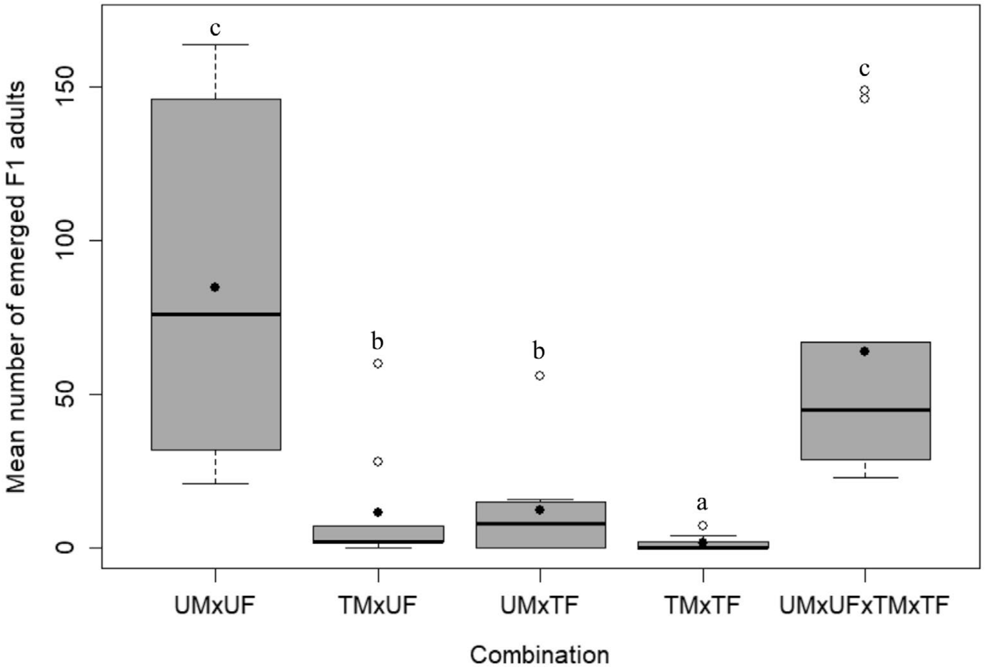

FIGURE 3 | The mean number of emerged F1 adults from different cages receiving different combinations of treated and untreated *T. leucotreta*. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots indicate the mean number of emerged F1 adults per treatment, while the white dots represent the outliers.

adults ($\chi^2 = 10.86$; df = 1; $p < 0.05$), and the interaction between the different combinations of treated and untreated *T. leucotreta* and the sex of the emerged F1 *T. leucotreta* adults ($\chi^2 = 7.78$; df = 3; $p = 0.05$). There was a significant reduction in fecundity and fertility between controls and TM × UF, UM × TF, and TM × TF from F1 females and F1 males' crosses. However, no significant differences in fecundity and fertility were recorded between controls and treatments UM × UF × TM × TF from crosses involving F1 females and F1 males (Table 2). Similarly, significantly fewer eggs were laid when crosses involved F1 males compared to crosses involving F1 females in cages receiving different combinations of treated and untreated *T. leucotreta*, except in combinations TM × TF and UM × UF × TM × TF.

TABLE 2 | Effect of different combinations of treated and untreated *T. leucotreta* released per cage on the mean fecundity and fertility of F1 moths emerging from damaged fruit and crossbred with fertile *T. leucotreta* of the opposite sex.

	Mean fecundity ± SE		Mean fertility ± SE (%)	
Cage treatment	F_1 female	F_1 male	F_1 female	F_1 male
Control (UM × UF)	176.70 ± 8.96e	145.90 ± 7.62e	77.00 ± 2.24c	73.6 ± 2.21c
TM × UF	140.20 ± 15.21 cd	64.60 ± 8.35b	65.10 ± 4.08b	49.60 ± 3.73b
UM × TF	144.00 ± 50.81 cd	57.00 ± 19.53b	62.10 ± 9.96b	35.90 ± 7.32b
TM × TF	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
UM × UF × TM × TF	159.30 ± 9.41e	99.40 ± 6.62e	83.50 ± 33.02c	55.60 ± 2.15c

TABLE 3 | Effect of different combinations of treated and untreated *T. leucotreta* on the mean number of fertile F1 female and male moths emerging from fruit removed from the cages, and the reproductive rate of increase for the P1-F1 generation.

	Mean ± SE fertile moths (progeny of non-treated males)		P1-F1 reproductive rate of increase	
Combinations	F_1 male	F_1 female	Male	Female
Control (UM × UF)	43.56 ± 14.40c	41.33 ± 15.32c	4.35×	4.13×
TM × UF	5.89 ± 2.37b	5.78 ± 2.67b	0.58×	0.57×
UM × TF	6.56 ± 2.31b	5.67 ± 2.23b	0.65×	0.56×
TM × TF	0.00 ± 0.00a	0.00 ± 0.00a	0.00×	0.00×
UM × UF × TM × TF	32.56 ± 10.81c	31.11 ± 11.57c	3.25×	3.11×

3.3 | F1 Sterility and Per Generation Rate of Increase

The different combinations of treated and untreated *T. leucotreta* per cage had a significant impact on the mean number of fertile (= progeny of unsterile males) F1 male *T. leucotreta* ($\chi^2 = 59.29$; df = 4; $p < 0.05$) and the mean number of fertile F1 female *T. leucotreta* ($\chi^2 = 52.09$; df = 4; $p < 0.05$) emerging from the damaged fruit in the different cages (Table 3). Control cages yielded significantly more F1 males and F1 females than other cages receiving different combinations of treated and untreated *T. leucotreta*, except for cages receiving treatment UM × UF × TM × TF. Among the cages receiving different combinations of treated and untreated *T. leucotreta*, treatment UM × UF × TM × TF produced significantly higher numbers of F1 males and females than treatments TM × UF, UM × TF, and TM × TF. Except for treatment UM × UF × TM × TF, the other cage treatments with different combinations of treated and untreated *T. leucotreta* exhibited a lower rate of reproductive increase than the control cages (Table 3). The control cages exhibited the highest per generation rate of reproductive increase, with a mean rate of increase exceeding 4 for both males (4.35×) and females (4.13×) from P1 to the F1 generation. Consequently, the mean per generation rate of reproductive increase for both males and females was 4.24×. No surviving progeny was produced from the TM × TF combination. Therefore, no per generation rate of increase could be determined (Table 3). However, treatment TM × UF had a lower mean number of fertile F1 males and females, as well as a lower per generation rate of reproductive increase from the P1 to the F1 generation. This treatment resulted in a mean rate of increase that was < 1 for both males (0.58×) and females (0.57×). Consequently, the mean per generation rate of reproductive increase for both males and females was (0.57×), a value resulting in a slight decline from P1 to the F1 generation (Figure 4).

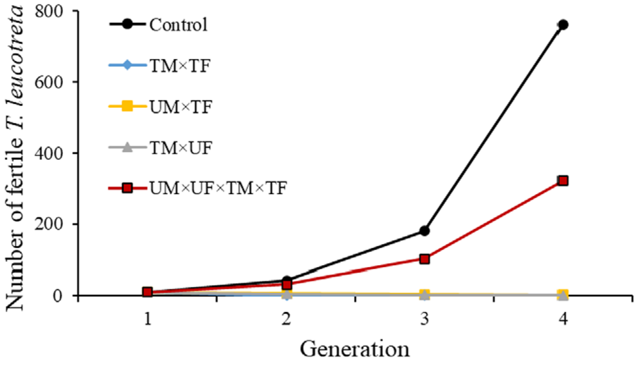

FIGURE 4 | Comparison of the projected rise in the count of fertile *T. leucotreta* between a control population and treatment populations exposed to releases of both treated and untreated *T. leucotreta*. In the control population, the initial generation began with 10 pairs of untreated *T. leucotreta* (ratio of 1:1), showing a reproductive rate of 4.24× per generation. Similarly, other treatments receiving treated *T. leucotreta* had a lower per generation rate of increase than the control population. The treatment population TM × UF (= with the lowest per generation rate of increase) initiated generation 1 with the release of 10 pairs of both treated and untreated *T. leucotreta* at the start of each of the three generations (ratio of 1:1), resulting in a reduced reproductive rate of 0.57× per generation. [Colour figure can be viewed at wileyonlinelibrary.com]

4 | Discussion

The efficacy of insect management strategies like mating disruption, chemical sterilisation, and sterile insect techniques increasingly relies on the understanding of insect mating preferences (Azrag et al. 2021; Pérez-Staples et al. 2021; Aigbedion-Atalor, Upfold, et al. 2024; Aigbedion-Atalor, Heiduk, et al. 2024). Moreover, the success of SIT programmes hinges on the ability of treated male populations to compete with fertile wild males to copulate with the fertile wild females, thereby reducing the pest population and serving as an effective autocidal control (Parker and Mehta 2007; Draz et al. 2016; Woods et al. 2016). In this study, we examined the mating capabilities of *T. leucotreta* under different combinations of treated and untreated moths and the impact on fruit damage and population growth. Our findings revealed that any cage treatment involving treated *T. leucotreta* resulted in reduced fruit damage and larval entries compared to the control treatment. This outcome may be attributed to the mating competitiveness of the released untreated *T. leucotreta*, which appeared to outcompete treated counterparts, resulting in more mating events and, thus, a greater transfer of fertile spermatophores between the untreated insects (Parker and Mehta 2007; Woods et al. 2016). Consequently, this led to increased fruit damage, larval entries, and ultimately, a higher number of emerged F1 adults from the cages. Despite this, the per generation rate of increase over several generations indicated that the presence of sterile *T. leucotreta* in the UM × UF × TM × TF combination, even under the recommended minimum ratio of 10:1 (sterile: wild) could have a suppressant effect on the fertile wild population.

Additionally, the findings from the UM × UF × TM × TF combination, exhibiting higher levels of fruit damage, larval entries, and emerged F1 adults than other treatments apart from the UM × UF (control), could be attributed to factors other than just the sterilisation with the 180 Gy gamma radiation. According to Nepgen et al. (2015), both males and females experienced decreased flight ability due to irradiation, wing injuries during transportation, chilling, and seasonal effects. This is particularly noticeable in summer relative to winter conditions, owing to the drastic temperature change from chilled to warmer environmental conditions upon release or during preparation for releases. This did greatly affect the spermatophore production and transfer between the treated and untreated *T. leucotreta*, but could also have compromised mobility, visual ability, and pheromone detection by treated male *T. leucotreta*, due to irradiation, thereby affecting transferal of spermatophores to the female *T. leucotreta* (Calkins and Parker 2005). Similarly, Mutika et al. (2001) demonstrated that treated adult *Glossina pallidipes* (Austen) (Diptera: Glossinidae) males transferred significantly less sperm to females than their untreated male counterparts. This could be due to exposure of the adult *G. pallidipes* to chilling temperature affecting their copulation ability, hence low sperm transfer (Mutika et al. 2002).

The findings are congruent with those reported by Woods et al. (2016) on the Australian light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), indicating that releases of sterile males alone have the potential to enhance mating competitiveness of the treated moths as compared to the dual-sex moth releases. Conversely, Van Steenderen (2017, unpublished) and Hight et al. (2005) demonstrated that dual-sex releases are more effective than male-only releases, with the former showing greater suppression of *T. leucotreta* through reduced fruit damage and larval entries, and the latter showing improved control of *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae). However, Moore (2021) reported findings consistent with ours, showing significantly reduced fruit damage, larval entries, and F1 *T. leucotreta* adults in all cage treatments that included either treated males or females, compared with the control treatments. This is important, considering that SIT is an AW-IPM strategy and can be combined with other control methods, such as mating disruption (Barnes et al. 2015). Sterile females in dual-sex releases attract wild males (positive sperm sink), thereby aiding in the pest suppression (Van Steenderen 2017, unpublished). Our findings corroborate those of Aigbedion-Atalor, Upfold, et al. (2024), Aigbedion-Atalor, Heiduk, et al. 2024, which showed that female *T. leucotreta* can mate with males from geographically different populations of *T. leucotreta* in South Africa. This is significant because SIT can be implemented in different regions where *T. leucotreta* occurs, thereby enhancing pest control. The SIT programme works best in inverse-density pest populations, and the inclusion of biological control agents such as *Trichogrammatidae cryptophlebiae* (Nagaraja) (Hymenoptera: Trichogrammatidae) (Githae et al. 2024), orchard sanitation, and the use of biopesticides (Moore 2022), can help lower the wild pest populations, thereby improving the effectiveness of the SIT programme.

Various combinations of treated and untreated *T. leucotreta* significantly impacted the mean number of emerging F1 males and F1 females from the different cage treatments compared to the control cages, where a higher number of F1 males than F1 females were recorded. This can be attributed to the radiation dosage employed, as *T. leucotreta* males exhibit radiotolerance, resulting in partially sterile *T. leucotreta* males (Marec et al. 2021). When these partially sterile males copulate with fertile/untreated females, the radiation-induced detrimental effects are inherited by the F1 generation, leading to reduced egg hatch rates and the resultant offspring being both fully sterile and predominantly male (Marec et al. 2021). Our findings are consistent with those reported by Moore (2021), indicating a decrease in the F1 adults from P1 to F1 across various combinations of treated and untreated *T. leucotreta*. Similarly, our results align with the findings of Bloem et al. (2003) demonstrating the effects of F1 sterility resulting from irradiation of P1 males with specific radiation dosages to the F1 generation, where decreased F1 fecundity and fertility, increased F1 mortality during development, and a significant shift in the F1 sex ratio in favour of males in *T.* leucotreta.

The findings from this study suggest that employing various combinations of treated and untreated *T. leucotreta* adults can effectively mitigate fruit damage and reduce the number of emerging F1 offspring under laboratory conditions. These findings align with those reported by Hofmeyr et al. (2005), where fruit damage and the emergence of F1 adults were reduced in the study. However, accurately predicting the impact of releasing treated and untreated *T. leucotreta* into a wild population requires assessing its efficacy over several generations (Hofmeyr et al. 2005). Based on our model, derived from our laboratory data, the treatment receiving treated and untreated

T. leucotreta treatment experienced a decline, while the control treatment saw a growth of more than four-fold per generation. This outcome is similar to the findings reported by Hofmeyr et al. (2005), which demonstrated that the treatment receiving treated *T. leucotreta* at an overflooding ratio of 10T:1U decreased in number, while the control treatment increased in number by more than nine-fold in four generations. This is important in the SIT programme, as it demonstrates that if releases of sterile insects are maintained over time, the wild population is drastically reduced, resulting in pest suppression (Boersma 2021). The treatment combination TM × TF did not produce any surviving F1 progeny as the treated females are known to be radiosensitive, thereby resulting in completely sterile females (Bloem et al. 2003). However, Moore (2021) demonstrated that the TM × TF combination resulted in egg oviposition on the fruit but none of the eggs hatched. However, contrary to the findings of Bloem et al. (2003) and Moore (2021), some percentage of fruit damage was reported in the TM × TF combination. This could be attributed to an insufficient irradiation dose enough to render the female *T. leucotreta* fully sterile (Marec et al. 2021). However, the F1 mortality in the TM × TF combination could be attributed to the debilitating effects passed on to the progeny by the sterilised *T. leucotreta*, thus affecting their development. Hence, no F1 progeny was produced (Bloem et al. 2003). As a result, this is against the principles of XSIT, where no progeny is supposed to be produced from this combination and needs to be rectified by conducting proper quality control assessment. This is to ensure that the moths receive adequate dosage, and the females achieve complete sterility, with males being partially sterile without affecting their mating competitiveness in the orchards (Bloem et al. 2003; Boersma 2021).

In summary, our findings demonstrated that untreated *T. leucotreta* males and females per treatment resulted in a significantly higher mean number of damaged fruits, larval entries, and F1 adult emergence compared to combinations receiving treated and untreated *T. leucotreta* males. This is the normal situation that happens in the citrus orchards whenever *T. leucotreta* releases are conducted. Although the per generation rate of population growth was notably higher in both control cages and the UM × UF × TM × TF combination, the per generation rate of population growth results revealed that any combination receiving treated and untreated moths can have a suppressant effect over time on *T. leucotreta*, as it provides the opportunity for some fertile-sterile pairings, rather than fertile-fertile pairings. Our findings support those of Van Steenderen (2017, unpublished), which suggested that dual-sex releases improve the efficacy of SIT. Dual-sex releases of sterile *T. leucotreta* have been conducted since the technique's inception in 2007, as the separation of sterile males and females is not practically feasible (XSIT 2025). Additionally, our findings demonstrated that the sterile females are beneficial in an SIT *T. leucotreta* programme, as they do not cause any fruit damage and can aid in pest suppression. Nevertheless, as this study was conducted in a controlled environment, further investigation under semi-field and field conditions is needed. This is because SIT programmes under field conditions rely on treated *T. leucotreta* mating with wild fertile counterparts, and higher treated-to-wild ratios enhance this likelihood (Barnes et al. 2015; Githae et al. 2025). The findings from our study support the ongoing improvement of the efficacy and effectiveness of the SIT programme as a strategy for managing *T. leucotreta* in South Africa.

Author Contributions

Conceptualisation: M. M. G. Methodology: M. M. G. Data curation: M. M. G. Investigation: M. M. G. Funding acquisition: S. D. M. and M. P. H. Supervision: C. A. C., R. M., S. D. M., and M. P. H. Formal analysis: M. M. G. Writing – original draft: M. M. G. Writing – editing: M. M .G., C. A. C., R. M., S. D. M., and M. P. H. Validation: M. M. G., C. A. C., R. M., S. D. M. and M. P. H. Visualisation: M. M. G., C. A. C., R. M., S. D. M. and M. P. H.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Rhodes University repository at https://doi.org/10.21504/RUR.29245511.v1.

References

Aigbedion-Atalor, P., A. Heiduk, J. Upfold, et al. 2024. "Geographic Variation in Genetic Composition, Sexual Communication and Mating Compatibility of the False Codling Moth, *Thaumatotibia leucotreta* for Optimisation of Area-Wide Control." *Frontiers in Ecology and Evolution* 12: 1360395.

Aigbedion-Atalor, P., J. Upfold, C. Coombes, S. Moore, and M. Hill. 2024. "Sexual Selection in *Thaumatotibia leucotreta* (Meyrick) is Shaped by Contrasting Geographic Adaptations, but Does It Matter for Area-Wide Sex Pheromone Control Tools?" *Annals of Applied Biology* 185, no. 2: 202–212.

Azrag, A. G., S. Ndlela, A. M. Mkiga, and S. A. Mohamed. 2021. "Mating Frequency of Female False Codling Moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae): Trade-Off Between Fecundity and Longevity." *Journal of Insect Behavior* 34, no. 5: 319–333.

Barnes, B. N., J. H. Hofmeyr, S. Groenewald, D. E. Conlong, and M. Wohlfarter. 2015. "The Sterile Insect Technique in Agricultural Crops in South Africa: A Metamorphosis....But Will It Fly?" *African Entomology* 23, no. 1: 1–18.

Bloem, S., J. E. Carpenter, and J. H. Hofmeyr. 2003. "Radiation Biology and Inherited Sterility in False Codling Moth (Lepidoptera: Tortricidae)." *Journal of Economic Entomology* 96, no. 6: 1724–1731.

Bloem, S., J. E. Carpenter, and J. H. Hofmeyr. 2007. “Area-Wide Control Tactics for the False Codling Moth *Thaumatotibia leucotreta* in South Africa: A Potential Invasive Species.” In *Area-Wide Control of Insect Pests: From Research to Field Implementation*, edited by J. E. Carpenter, 351–359. Springer Netherlands.

Boersma, N. 2021. “The Suppression of the False Codling Moth in South Africa Using an AW-IPM Approach With a SIT Component.” In *Area-Wide Integrated Pest Management*, 93–109. CRC Press.

Calkins, C., and A. Parker. 2005. “Sterile Insect Quality.” In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, edited by V. A. Dyck, J. Hendrichs, and A. S. Robinson, 269–296. Springer.

CGA-SA, (Citrus Growers Association of South Africa). 2023. *Key Industry Statistics Export Season*, 50. CGA.

Draz, K. A., R. M. Tabikha, M. A. El-Aw, and H. F. Darwish. 2016. “Impact of Gamma Radiation Doses on Sperm Competitiveness, Fecundity and Morphometric Characters of Peach Fruit Fly *Bactrocera zonata* (Saunders) (Diptera: Tephiritidae).” *Journal of Radiation Research and Applied Sciences* 9, no. 3: 352–362.

EFSA, (European Food Safety Authority). 2024. “Pest Survey Card on *Thaumatotibia leucotreta*.” *EFSA Supporting Publications* EN-8487: 1–28. https://doi.org/10.2903/sp.efsa.2024.EN-8487.

EPPO, (European and Mediterranean Plant Protection Organisation). 2013. *Pest Risk Analysis for Thaumatotibia leucotreta*, 1–125. EPPO.

EU (European Union). 2017. *Official Journal of the European Union L184*, 1–124. Publications Office of the European Union.

Gilligan, T. M., M. E. Epstein, and K. M. Hoffman. 2011. “Discovery of False Codling Moth, *Thaumatotibia leucotreta* (Meyrick), in California (Lepidoptera: Tortricidae).” *Proceedings of the Entomological Society of Washington* 113, no. 4: 426–435.

Githae, M. M., C. A. Coombes, R. Mutamiswa, S. D. Moore, and M. P. Hill. 2024. “Suitability of False Codling Moth Eggs From Different Sterile to Fertile Moth Ratios in the Sterile Insect Technique Programme, to Parasitism by *Trichogrammatoidea cryptophlebiae*.” *Crop Protection* 182: 106744.

Githae, M. M., C. A. Coombes, R. Mutamiswa, S. D. Moore, and M. P. Hill. 2025. “Influence of Overflooding Ratios on Fruit Damage and Population Growth of *Thaumatotibia leucotreta* (Meyrick): Implications for the Sterile Insect Technique Program.” *Pest Management Science*. https://doi.org/10.1002/ps.70068.

Grout, T. G., and S. D. Moore. 2015. “Pests of Citrus.” In *Insects of Cultivated Plants and Natural Pastures in Southern Africa*, edited by G. L. Prinsloo and G. M. Uys, 447–501. Entomological Society of Southern Africa.

Hattingh, V., S. Moore, W. Kirkman, et al. 2020. “An Improved Systems Approach as a Phytosanitary Measure for *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in Export Citrus Fruit From South Africa.” *Journal of Economic Entomology* 113, no. 2: 700–711.

Hibino, Y., and O. Iwahashi. 1991. “Appearance of Wild Females Unreceptive to Sterilised Males on Okinawa Island in the Eradication Programme of the Melon Fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae).” *Applied Entomology and Zoology* 26, no. 2: 265–270.

Hight, S. D., J. E. Carpenter, S. Bloem, and K. A. Bloem. 2005. “Developing a Sterile Insect Release Program for *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae): Effective Overflooding Ratios and Release-Recapture Field Studies.” *Environmental Entomology* 34, no. 4: 850–856.

Hofmeyr, J. H., J. E. Carpenter, and S. Bloem. 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, no. 6: 1924–1929.

Hofmeyr, J. H., M. Hofmeyr, J. E. Carpenter, S. Bloem, J. P. Slabbert, and S. S. Groenewald. 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, no. 1: 180–186.

Kirkman, W. 2007. “Understanding and Improving the Residual Efficacy of the *Cryptophlebia leucotreta* Granulovirus (Cryptogran). (MSc thesis, Rhodes University), pp. 1–115.”

Marec, F., S. Bloem, and J. E. Carpenter. 2021. “Inherited Sterility in Insects.” In *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management*, edited by V. A. Dyck, J. Hendrichs, and A. S. Robinson, 163–200. Springer.

Marec, F., and M. J. Vreysen. 2019. “Advances and Challenges of Using the Sterile Insect Technique for the Management of Pest Lepidoptera.” *Insects* 10, no. 11: 371.

Moore, S. D. 2002. “The Development and Evaluation of *Cryptophlebia leucotreta* Granulovirus (CrleGV) as a Biological Control Agent for the Management of False Codling Moth, *Cryptophlebia leucotreta*, on Citrus. (PhD thesis, Rhodes University), pp. 1–308”.

Moore, S. D. 2021. “Biological Control of a Phytosanitary Pest (*Thaumatotibia leucotreta*): A Case Study.” *International Journal of Environmental Research and Public Health* 18, no. 3: 1198.

Moore, S. D. 2022. “Moths and Butterflies: False Codling Moth.” *Citrus Research International IPM Production Guideline* 3, no. Part 9.4: 1–9.

Moore, S. D., and V. Hattingh. 2012. “A Review of Current Pre-Harvest Control Options for False Codling Moth in Citrus in Southern Africa.” *South African Fruit Journal* 11, no. 4: 82–85.

Moore, S. D., G. I. Richards, C. Chambers, and D. Hendry. 2014. “An Improved Larval Diet for Commercial Mass Rearing of the False Codling Moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae).” *African Entomology* 22, no. 1: 216–219.

Mutika, G. N., E. Opiyo, and A. S. Robinson. 2001. “Assessing Mating Performance of Male *Glossina pallidipes* (Diptera: Glossinidae) Using a Walk-In Field Cage.” *Bulletin of Entomological Research* 91, no. 4: 281–287.

Mutika, G. N., E. Opiyo, and A. S. Robinson. 2002. “Effect of Low-Temperature Treatment on the Quality of Male Adult *Glossina pallidipes* (Diptera: Glossinidae) in Relation to the Sterile Insect Technique.” *Entomological Science* 5: 209–214.

Nepgen, E. S., M. P. Hill, and S. D. Moore. 2015. “The Effect of Long-Distance Transportation on the Fitness of Irradiated False Codling Moth (Lepidoptera: Tortricidae) for Use in a Sterile Insect Release Programme.” *Journal of Economic Entomology* 108, no. 6: 2610–2619.

Newton, P. J. 1998. “False Codling Moth, *Cryptophlebia leucotreta* (Meyrick).” In *Citrus Pests in the Republic of South Africa*, edited by E. C. G. Bedford, M. A. van den Berg, and E. A. de Villiers, 192–200. Dynamic Ad.

O'Hara, R. B., and D. J. Kotze. 2010. “Do Not Log-Transform Count Data.” *Methods in Ecology and Evolution* 1: 118–122.

Parker, A., and K. Mehta. 2007. “Sterile Insect Technique: A Model for Dose Optimisation for Improved Sterile Insect Quality.” *Florida Entomologist* 90, no. 1: 88–95.

Pérez-Staples, D., F. Díaz-Fleischer, and P. Montoya. 2021. “The Sterile Insect Technique: Success and Perspectives in the Neotropics.” *Neotropical Entomology* 50, no. 2: 172–185.

R Core Team. 2023. *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing.

Rendón, P., D. McInnis, D. Lance, and J. Stewart. 2004. “Medfly (Diptera: Tephritidae) Genetic Sexing: Large-Scale Field Comparison of Males-Only and Bisexual Sterile Fly Releases in Guatemala.” *Journal of Economic Entomology* 97, no. 5: 1547–1553.

SA-DAFF, (South African Department of Agriculture Forestry and Fisheries). 2015. “Special Export Protocols/Programmes/Directives”.

Shapiro, S. S., and M. B. Wilk. 1965. “An Analysis of Variance Test for Normality (Complete Samples).” *Biometrika* 52, no. 3–4: 591–611.

USDA (United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine, Emergency and Domestic Programme). 2010. *New Pest Response Guidelines: False Codling Moth Thaumatotibia leucotreta*, 1–98.

Van Steenderen, C. Unpublished Manuscript. “Sterile Females in a False Codling Moth (Thaumatotibia Leucotreta Meyrick) (Lepidoptera: Tortricidae) Sterile Insect Technique (SIT) Programme: Beneficial or Detrimental?” Last modified 2017.

Weldon, C. W. 2005. “Mass-Rearing and Sterilisation Alter Mating Behaviour of Male Queensland Fruit Fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae).” *Australian Journal of Entomology* 44, no. 2: 158–163.

Whitten, M., and R. Mahon. 2005. “Misconceptions and Constraints.” In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, edited by V. A. Dyck, J. Hendrichs, and A. S. Robinson, 601–626. Springer.

Woods, B., D. McInnis, E. Steiner, et al. 2016. “Developing Field Cage Tests to Measure Mating Competitiveness of Sterile Light Brown Apple Moths (Lepidoptera: Tortricidae) in Western Australia.” *Florida Entomologist* 99, no. sp1: 138–145.

XSIT, (X Sterile Insect Technique). 2025. “The Control of *T. leucotreta* (FCM) on a Commercial Basis Through the Application of the Sterile Insect Technique (SIT).” https://xsit.co.za/our-product-2/.