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Suitability of false codling moth eggs from different sterile to fertile moth ratios in the sterile insect technique programme, to parasitism by *Trichogrammatoidea cryptophlebiae*

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ABSTRACT

The sterile insect technique (SIT) and augmentative releases of egg parasitoids, *Trichogrammatoidea cryptophlebiae* have been employed to manage false codling moth (FCM), *Thaumatotibia leucotreta* in South Africa. To enhance FCM control, simultaneous releases of sterile moths and egg parasitoids are conducted. It is, therefore, necessary to determine the compatibility and combined values of these approaches. A laboratory study was conducted to explore the susceptibility of FCM eggs resulting from various pairings of sterile and fertile moths to parasitism by egg parasitoids. The ratios of sterile to fertile FCM used in the study were 0:1, 10:1, 20:1, 40:1, and 60:1. The fitness of the egg parasitoids emerging from these ratios was assessed using a flight chamber test. Sterile male and female FCM treated with 150 Gy of gamma irradiation were crossbred with fertile counterparts. The resulting eggs were then exposed to egg parasitoids for parasitism. The study evaluated the parasitism rates of newly laid (24 h), 48 h and 72 h old eggs. Overall, eggs from all ratios were suitable for egg parasitoid development and acceptable for oviposition. Significantly higher proportions of parasitised eggs were recorded between the control (0:1) and ratios 40:1 and 60:1 with 48 h old eggs. Additionally, a higher number of egg parasitoids emerged across the ratios, with a higher proportion of female-to-male parasitoids recorded. These findings indicate that egg parasitoids can successfully accept and hatch from FCM eggs from different sterile to fertile moth ratios. This suggests the potential for achieving a synergistic suppressive effect by combining SIT and augmentative releases of egg parasitoids for improved FCM control. Nevertheless, further studies are necessary to investigate the combined releases of sterile moths and parasitoids under field conditions.

1. Introduction

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae), commonly known as the false codling moth (FCM), is endemic in sub-Saharan Africa. It exhibits an extensive host range in southern Africa, including citrus, particularly Navel oranges (Stofberg, 1954; Economides, 1979; Yahia et al., 2011; Maniania et al., 2017; Moore, 2021; Moore and Manrakhan, 2022). FCM exhibits a multivoltine life cycle with four to six overlapping generations per annum in South Africa (Stofberg, 1954). Females typically deposit about 100–250 eggs on fruit, dependent mainly on temperature, relative humidity, and food quality (Daiber, 1980; Adom et al., 2021). After hatching, the newly emerged neonates penetrate the fruit, where they undergo five larval instars. Upon completing their development, larvae exit the fruit and create cocoons, either within the topsoil or leaf litter (Stofberg, 1954; Georgala, 1969; Moore, 2022). FCM commonly leads to premature fruit drop prior to harvest (Georgala, 1969). Severe infestations can have significant impacts, causing notable crop losses and potential rejections at packing houses (Hofmeyr et al., 2005). However, over the last two decades, control measures in the field have improved to the extent that severe levels of infestation are now rare (Moore et al., 2016, 2017; Moore, 2022).

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FCM has been declared as a key phytosanitary pest in many export regions, due to its endemism in the sub-Saharan region (Hattingh et al., 2020) and zero-tolerance policy in certain export markets. The growing global demand for fresh citrus fruit has heightened export market concerns about the potential introduction of this pest (Yahia et al., 2011; Moore, 2021). This is despite FCM having been shown to be a poor disperser and coloniser (Moore et al., 2024), having established in only one region, outside of its endemic range, Israel (Wysoki, 1986), despite large volumes of fresh produce being shipped intercontinentally for many decades. Given that South Africa is the second-largest exporter of citrus, with approximately 75% of its citrus crop destined for export (Citrus Growers Association, 2023), it is crucial to ensure that exemplary control measures are undertaken before shipping (Moore, 2021). Consequently, the demand for high-quality fruit in the export market has translated into a need for new, efficient, and effective Integrated Pest Management (IPM) strategies. These IPM approaches employ a harmonious combination of both chemical and ecologically sound techniques to manage and suppress pest populations to keep them below economic injury thresholds and to ensure they remain at acceptable phytosanitary levels (Malan et al., 2018). The use of several different control strategies together helps compensate for the drawbacks associated with any one strategy used alone, with monitoring being a focal point in helping to decide which control method to use (Urquhart, 1999; Carpenter and Tan, 2000; Malan et al., 2018).

Among the available methods of FCM control, not one is sufficiently effective to be used as a stand-alone tactic when pest pressure is high. Therefore, it is imperative that FCM control be based on a multidisciplinary approach, focusing on pest suppression from the start of the season and curbing population increase during the season (Moore and Hattingh, 2012; Moore, 2022). Thus, the demand for a long-term solution for the management of FCM necessitated the citrus industry to initiate a sterile insect technique (SIT) research programme in Citrusdal, Western Cape Province, South Africa in 2002 (Barnes et al., 2015). This was conducted in several phases, where Bloem et al. (2003) determined that a radiation dose of 150 Gy completely sterilised female FCM, while male FCM were partially sterilised Charnov et al. (1981). This aids the males in maintaining their competitiveness in the field for pairing with fertile females, against their fertile wild male counterparts. However, full sterility is inherited by the F1 generation of an irradiated male-wild female pairing. Additionally, Hofmeyr et al. (2005) determined an ideal release ratio of 10:1, which is still in use. This was followed by the commercialisation of the technique in 2007 (Hofmeyr et al., 2016) as an Area-Wide Integrated Pest Management (AW-IPM) strategy, in which other orchard-specific techniques can be incorporated, all aiding in the suppression of FCM populations in citrus orchards (Moore and Hattingh, 2012; Malan et al., 2018). The technique is host-specific and environmentally benign to natural enemies; therefore, it is compatible with the application of augmentative biological control (Mannion et al., 1994; Moore, 2002; Bloem et al., 2007; Horrocks et al., 2020).

Augmentative releases of egg parasitoids and sterile insects as biological control agents have been shown to significantly reduce pest damage to citrus (Newton and Odendaal, 1990; Bloem et al., 1998, 2007; Carpenter et al., 2004; Kaspi and Parrella, 2006; Horrocks et al., 2020). Despite little empirical supporting data, some models predict mutual complementation (Barclay, 1987) or a mutual synergistic suppressive effect (Knipling, 1998) when the two techniques are used together. The mobility and enhanced host-searching capacity of egg parasitoids and sterile insects improve the efficacy of locating their target hosts in their natural habitats. Egg parasitoids and sterile insects can reach areas that other techniques, such as chemical sprays, are unable to reach (Pérez-Staples et al., 2021) and are complementary because they act on two different stages of the pest life cycle. As such more effort needs to be directed toward the control of the egg stage before the emergence of the larvae, which is the developmental stage of FCM responsible for crop damage (Newton and Odendaal, 1990; Moore, 2002; Schäfer and Herz, 2020; Masry and El-Wakeil, 2020). *Trichogrammatoidea cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae) is a naturally occurring FCM egg parasitoid found in citrus orchards (Newton and Odendaal, 1990; Moore, 2002). However, they can be commercially purchased to boost their numbers in citrus orchards, thereby improving their efficacy Citrus Growers' Association (2023). This parasitoid has been shown to reduce FCM infestation of fruit in citrus orchards by up to 60% under optimum conditions (Newton and Odendaal, 1990; Moore, 2002). Commercial mass-rearing and augmentation of FCM egg parasitoids in South Africa have been conducted since 1982, when it began in the Western Cape Province, achieving a decrease in infestation by 20% against FCM (Schwartz et al., 1982). For a long time, mass-rearing and production of egg parasitoids was conducted by Cederberg Insectary in Citrusdal in the Western Cape Province and at Zebediela Estate in Limpopo Province (Moore et al., 2014). Currently, there are two insectaries commercially rearing the parasitoid, Vitalbugs in Limpopo Province, and River Bioscience in the Eastern Cape Province (Moore, 2021).

These insectaries may not be sufficient to supply all citrus orchards in the country. However, conservation of naturally occurring parasitoids is also feasible, and with SIT being practiced in major citrus-producing areas across the country, these two techniques could be compatible. This combination could enhance the efficacy of the parasitoid in areas where it is augmented and could aid in the proliferation of egg parasitoids in areas where augmentative release is not possible. In a similar situation in the control of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), the combined release of sterile insects and *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) provided improved control (Nagy, 1973). Bloem et al. (1998) showed that an additive suppressive effect could be achieved when FCM sterile male moths are released at a 10:1 (sterile to wild) overflooding ratio, together with *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae) in field-cages, when compared with cages containing wild moths that received sterile moths or parasitoids only. However, achieving this overflooding ratio in a commercial field may be more challenging than in an insulated and protected cage environment. Nevertheless, some field trials using FCM SIT programmes have indicated that higher ratios may be more efficacious than achieved by 10:1 (Hofmeyr and Hofmeyr, 2009, 2010; Moore, 2011). Carpenter et al. (2004) determined the suitability of the FCM eggs from a ratio of 10:1 to egg parasitoids. However, ratios higher than 10:1 are often achieved under field conditions (unpublished data), necessitating the need to determine the acceptability and suitability of FCM eggs to egg parasitoids, from different sterile to fertile moth ratios, higher than 10:1.

Therefore, this study investigated the parasitism levels of FCM eggs as affected by different sterile to fertile FCM ratios and FCM egg ages. In particular, the study investigated (i) the effect of different FCM egg ages from different sterile to fertile ratios higher than 10:1 on parasitism by the egg parasitoid, and (ii) the effect of different FCM egg ages from different sterile to fertile ratios on the fitness of emerging egg parasitoids. The results obtained are discussed in the context of improving FCM pest suppression through the combined release of egg parasitoids and sterile FCM in an area-wide sterile insect release programme in citrus orchards.

2. Materials and methods

2.1. Test insects

2.1.1. Sterile and fertile FCM moths

Adult sterile and fertile FCM were obtained from X Sterile Insect Technique (XSIT) (Pty) Ltd., Citrusdal, South Africa. Adult moths were sterilised by exposure to 180 Gy of gamma radiation at XSIT before being transported by road to Rhodes University, Makhanda, South Africa (~840 km, 13–14 h) in a cooler box containing dry ice bricks (4–6 °C). This low temperature minimised insect movement, preventing them from mating while in transit (Nepgen et al., 2015). On arrival, the moths were approximately 48 h old. The male and female moths were immediately sexed and separated to prevent mating. Adult males were distinguished from females by the presence of black tufts of fine hairs in the anal region and hind tibiae, which are absent in females (Gilligan et al., 2011).

2.1.2. Parasitoids

Egg parasitoids (*T. cryptophlebiae*) were provided by River Bioscience (Pty) Ltd. (Gqeberha, South Africa). The facility uses surplus unrequired FCM eggs from XSIT, which serve as a suitable host material for the production of egg parasitoids. FCM eggs laid on wax egg sheets are sorted and exposed to egg parasitoids for 24 h to ensure the continuity of the colony. Eight parasitised wax egg sheets were transported to Rhodes University (~125 km, 2 h) in a cooler box with ice blocks. On arrival, they were immediately placed in a controlled environment (CE) room at 26 ± 1 °C, a 14:10 (L:D) h photoperiod, and 65 ± 5% relative humidity (RH) for 48 h. Subsequently, they were allowed to mate for 24 h, after which they were sexed and used in subsequent experiments; approximately 50 female egg parasitoids were exposed to FCM eggs at different ratios (Table 1). A fresh batch of egg parasitoids was supplied for each repetition of the experiment when needed.

2.2. Wax egg sheet preparation

The laboratory trials were conducted as described by Carpenter et al. (2004). Sterilised FCM adults were sorted according to sex. Sterile and fertile FCM were placed inside mesh domes (each 30 cm in diameter by 15 cm height) that were placed on top of wax oviposition sheets (32 cm by 15 cm) placed on top of a Styrofoam board in a CE room. The CE room conditions were maintained at 26 ± 1 °C, a 14:10 (L:D) h photoperiod, and 65 ± 5% relative humidity (RH). Insect pins were used to secure the domes on top of wax egg sheets. Three replicates were set up for the different ratios of sterile to fertile moths: 0:1 (control), 10:1, 20:1, 40:1, and 60:1. The number of FCM used per treatment was as per Table 1. FCM were allowed to mate and oviposit on wax egg sheets under the aforementioned conditions for four days. The first wax egg sheet was collected after 12 h and disposed to ensure that the eggs used in subsequent experiments came from matings with different sterile to fertile FCM ratios. Thereafter, three more wax egg sheets were collected at 24 h intervals. Newly laid (24 h), 48 and 72 h old eggs were evaluated. Wax egg sheets containing 200 FCM eggs from each ratio treatment were placed inside three meshed plastic containers (5 cm diameter by 3 cm height). Wax egg sheets collected on days 1 and 2 were incubated at 26 ± 1 °C, a 14:10 (L:D) h photoperiod, and 65 ± 5% RH to allow for egg development (Carpenter et al., 2004). Thereafter, wax egg sheets, together with those collected on the third day, were exposed to egg parasitoids.

2.3. Exposure to egg parasitoids

Parasitised FCM wax egg sheets from River Bioscience (Pty) Ltd. were placed in large glass containers and kept at 26 ± 1 °C, a 14:10 (L:D) h photoperiod, and 65 ± 5% RH for 12 h before the emergence of egg parasitoids. To ensure that only egg parasitoids that emerged within 24 h were used in the trial, adult egg parasitoids were collected from the container every 12 h and transferred to plastic Petri dishes. A mixture of honey and water (2:1) was used as food for egg parasitoids. To ensure mating success, egg parasitoids were kept together for 24 h. Thereafter, using a fine camel hair paintbrush, 50 female egg parasitoids were transferred to each plastic container, with approximately 200 FCM eggs on the wax egg sheet, and were allowed 24 h to parasitise the FCM eggs, after which they were removed. The wax egg sheets were then incubated under the aforementioned conditions for seven days to enable the completion of parasitoid development. After the emergence of egg parasitoids, data were collected on the number of parasitised eggs, the number of parasitised eggs that produced two or more egg parasitoids (superparasitised), the number and sex of the emerging wasps, the number of egg parasitoids that died before emergence for each treatment, and host egg age. The experiment was conducted three times, requiring a total of 15 fertile and 390 sterile males and 15 fertile and 390 sterile females, each time.

Table 1
Experiment design: egg parasitoids were randomly assigned to three plastic containers per treatment containing 200 FCM eggs of different ages laid from different ratios, to examine the acceptability and suitability of these eggs to parasitism by egg parasitoids.

Treatment	Ratio S: F	Number of sterile FCM		Number of fertile FCM	
		S male	S female	F male	F female
A	0:1	0	0	1	1
B	10:1	10	10	1	1
C	20:1	20	20	1	1
D	40:1	40	40	1	1
E	60:1	60	60	1	1

S = sterile moths; F = fertile moths. Three replicates per treatment were used.

2.4. Parasitoid quality

Parasitised FCM wax egg sheets were kept in a CE room under the aforementioned conditions until wasp emergence. Upon emergence, the quality of the egg parasitoids was assessed using a flight test. From the time of emergence of the first egg parasitoids, an interval of 48 h was given to ensure the complete emergence of all the egg parasitoids from the host eggs. The flight chamber test was conducted using a protocol and flight chamber setup originally proposed by Dutton and Bigler (1995) and modified by Prezotti et al. (2002). The flight chamber was a PVC cylinder (11 cm in diameter and 18 cm high), where the top of the cylinder was covered with a clear Petri dish (13 cm diameter) and sprayed with entomological glue (composed of polybutene and synthetic silica) (EntomoAlex-gr, Italy) to trap flying parasitoids ('flyers'). The interior of the cylinder was painted black, and the bottom was sealed with a flexible black plastic sheet to attract insects toward the light at the top of the flight chamber. Twenty-four hours before the start of the experiment, entomological glue was spread on the walls of the cylinder (3.5 cm from the bottom) to serve as a trap for walkers. The number of egg parasitoids trapped in the adhesive ring ('walkers'), the Petri dish ('flyers'), and the number of deformed individuals were recorded. The flyers were sexed to determine the sex ratio for each treatment. The experiment was conducted thrice.

2.5. Statistical analysis

Data collected from the experiment were checked for homogeneity of variance (F test, Levene's test) and normal distribution of residuals (Shapiro-Wilk test). As parametric assumptions were not met, non-parametric tests were used. The data were analysed using a negative binomial generalised linear model analysis as an extension of the Poisson distribution to allow for count data with a significant proportion of zero values, with ratios and egg age as the sources of variation, as recommended by O'Hara and Kotze (2010). Analysis of deviance (log-likelihood ratio statistic) was used to assess the goodness of fit of the Poisson regression model, which has a distribution similar to that of chi-squared (χ^2). The dependent variables used in the statistical model included the number of parasitised eggs, the number of super parasitised eggs, the number of emerged egg parasitoids, the sex ratio of emerged egg parasitoids, the number of flying male and female egg parasitoids, and the number of walking and flying egg parasitoids. Tukey's post-hoc comparisons were conducted at $P < 0.05$, where the statistical model indicated significant treatment effects and significant interactions. All statistical analyses were conducted using the R software version 4.3.1 (R Core Team, 2022).

3. Results

3.1. Rate of parasitism

The proportion of parasitised FCM eggs was significantly influenced by the different ratios ($\chi^2 = 64.15$; $df = 4$; $P < 0.05$) (Fig. 1A). Similarly, the egg age had a significant effect on the proportion of parasitised FCM eggs, irrespective of the ratio ($\chi^2 = 41.53$; $df = 2$; $P < 0.05$). There was a significant difference in the proportion of parasitised FCM eggs between the control (0:1) and 40:1 as well as control and 60:1 at egg age 48 h (Fig. 1A). However, no significant differences were recorded between 0:1 and other ratios at egg ages 24 and 72 h. At the ratio 0:1, the proportion of parasitised FCM eggs at egg age of 72 h was significantly less than FCM eggs parasitised at ages of 24 and 48 h. This trend was similar for ratios 20:1, 40:1 and 60:1. Conversely, the proportion of FCM eggs parasitised at a ratio 10:1 at egg ages 48 and 72 h was significantly different from FCM eggs parasitised at the egg age of 24 h. No significant interaction was recorded between the different ratios and egg ages ($\chi^2 = 11.61$; $df = 8$; $P = 0.17$).

The proportion of superparasitised FCM eggs was found to be significantly affected across the different ratios ($\chi^2 = 12.21$; $df = 4$; $P < 0.05$) (Fig. 1B). Similarly, different egg ages also had a significant effect on the proportion of superparasitised eggs across the ratios ($\chi^2 = 51.56$; $df = 2$; $P < 0.05$). The proportion of superparasitised FCM eggs was significantly higher between ratio 0:1 and other release ratios, at the egg age of 24 h (Fig. 1B). However, there was no significant difference in superparasitised eggs between 0:1 and other ratios at the egg ages of 48 and 72 h. No significant interaction was recorded between the different ratios and the egg ages ($\chi^2 = 11.91$; $df = 8$; $P = 0.16$).

The proportion of emerged egg parasitoids was significantly influenced by the different ratios ($\chi^2 = 45.75$; $df = 4$; $P < 0.05$). Similarly, egg age also had a significant effect on the proportion of emerged egg parasitoids across all the different ratios ($\chi^2 = 57.33$; $df = 2$; $P < 0.05$). Notably, there was a significant difference in the proportion of emerged egg parasitoids between 0:1 and 40:1when exposed to 72 h old eggs (Fig. 2A). In addition, at the egg ages of 24 and 48 h, the proportion of emerged egg parasitoids was significantly different from those at the egg age of 72 h, regardless of the ratios. The interaction between the different ratios and egg ages did not have a statistically significant impact on the proportion of emerging egg parasitoids ($\chi^2 = 14.69$; $df = 8$; $P = 0.07$).

The emerging parasitoid female-to-male sex ratio displayed some variation across different ratios and FCM egg ages (Fig. 2B). In the ratio of 0:1, parasitoid females-to-male sex ratio decreased as the egg age increased. Similarly, this was the trend at the ratio of 10:1. Conversely, at ratio of 60:1, parasitoid females-to-males sex ratio marginally increased with an increase in egg age. Fluctuations in the sex ratio were recorded across egg ages at ratios 20:1 and 40:1, such that more females than males emerged at egg ages 24 and 48 h, respectively. Subsequently, at the ratio of 60:1, more female to male parasitoid emerged at the egg age of 72 h. This differed from the ratio of 40:1, where more males to females emerged at the same egg age.

3.2. Parasitoid quality

The proportion of flying egg parasitoids that emerged from FCM eggs was significantly affected by the varying ratios ($\chi^2 = 20.94$; $df = 4$; $P < 0.05$). Additionally, the different ages of FCM eggs also had a significant influence on the proportion of flying egg parasitoids ($\chi^2 = 146.13$; $df = 2$; $P < 0.05$). Notably, there was a significant difference in the proportion of flying egg parasitoids between ratios 10:1 and 40:1, as well as

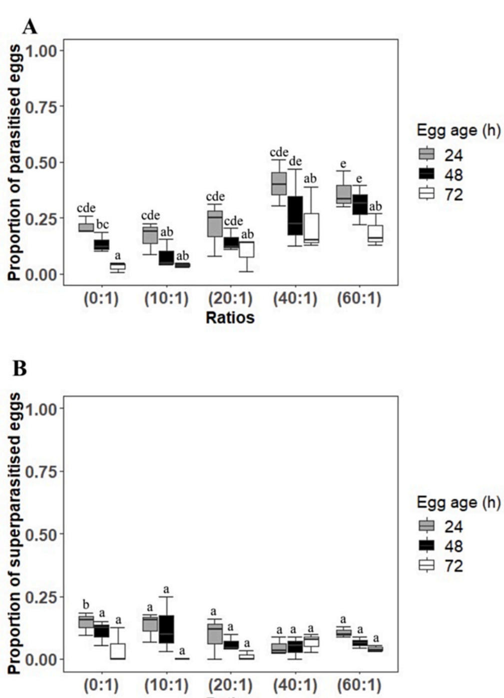

Fig. 1. The proportion of parasitised **A**, and superparasitised eggs **B** as affected by different ratios (sterile to fertile FCM) and age of the FCM eggs. Boxplots show median values (solid lines), and whiskers show the range of the data. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

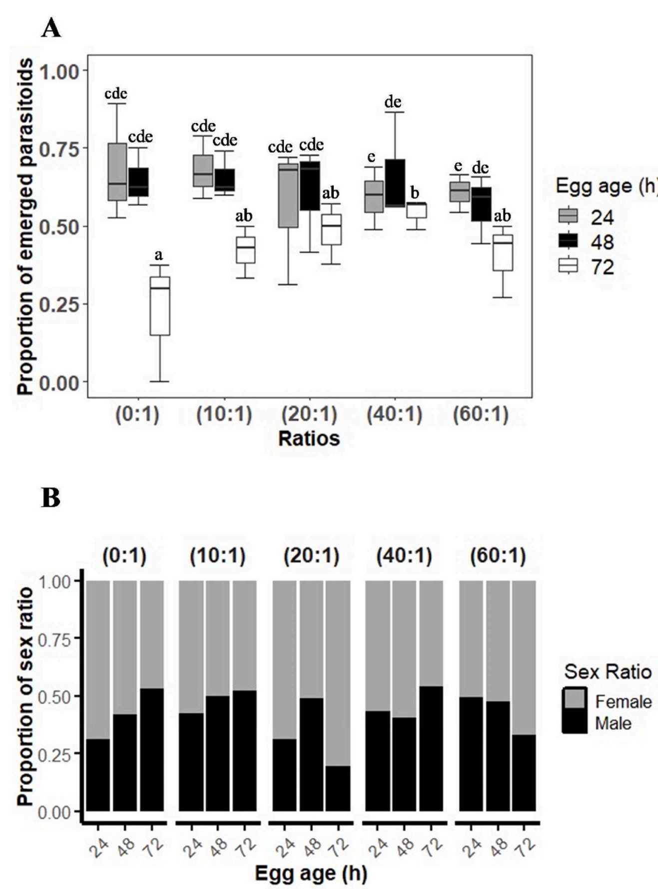

Fig. 2. The proportion of egg parasitoid emergence **A**, and the sex ratio of emerged egg parasitoids **B** as affected by different ratios (sterile to fertile FCM) and age of the FCM eggs. Boxplots shows median values (solid lines), and whiskers show the range of the data. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

between 20:1 and 40:1 at the egg age of 48 h. Additionally, at the egg ages of 24 and 48 h, the proportion of flying egg parasitoids was significantly different from the proportion of flying egg parasitoids at the egg age of 72 h across all ratios (Fig. 3A). Moreover, the proportion of flying egg parasitoids was significantly different across the three egg ages at ratio 20:1. The interaction between different ratios and FCM egg ages did not yield any significant differences ($\chi^2 = 6.62$; $df = 8$; $P = 0.58$).

The proportion of emerged flying female-to-male parasitoid sex ratio displayed some variation with regard to different ratios and FCM egg ages (Fig. 3B). At a ratio of 0:1, high proportion of females flying egg parasitoids were recorded. At the ratios of 10:1, 40:1 and 60:1, female-to-male sex ratio marginally decreased with an increase in egg age. However, at the ratio of 20:1, high proportion of flying males were recorded at the egg age of 72 h.

The proportion of walking egg parasitoids that emerged was significantly affected by the different ratios ($\chi^2 = 74.62$; $df = 4$; $P < 0.05$). (Fig. 4). Additionally, the different egg ages significantly affected the proportion of walking egg parasitoids emerging from the FCM eggs ($\chi^2 = 17.44$; $df = 2$; $P < 0.05$). There was a significant increase in the proportion of walking egg parasitoids between the ratio of 0:1 and 40:1 as well as 0:1 and 60:1 at egg ages of 24 and 72 h. The interaction between different ratios and FCM egg ages did not result in any significant differences ($\chi^2 = 13.20$; $df = 8$; $P = 0.11$).

4. Discussion

In a biological control programme incorporating egg parasitoids, the acceptance and suitability of host eggs by the parasitoids for their

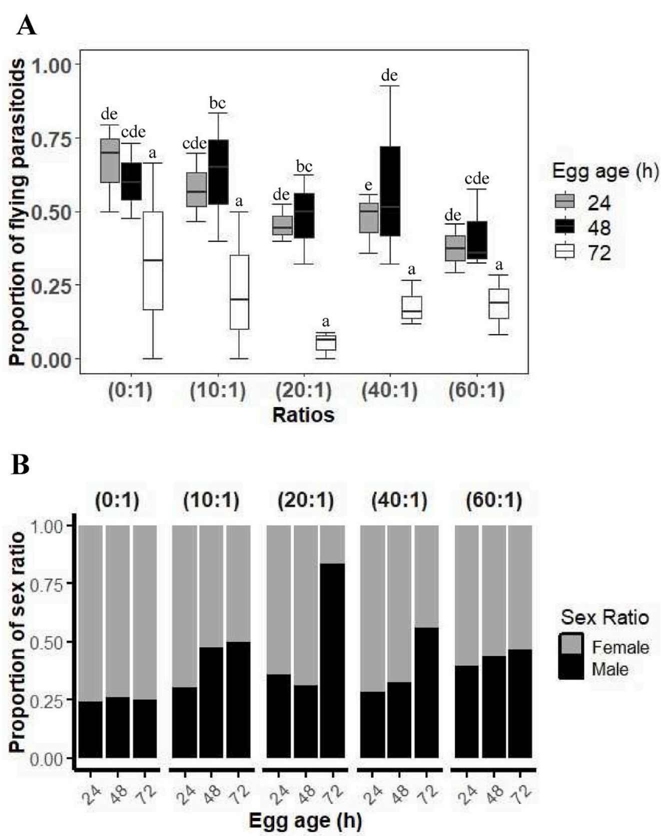

Fig. 3. The proportion of flying egg parasitoids **A**, and the sex ratio of emerged flying egg parasitoids **B** as affected by different ratios (sterile to fertile FCM) and age of the FCM eggs. Boxplots shows median values (solid lines), and whiskers show the range of the data. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

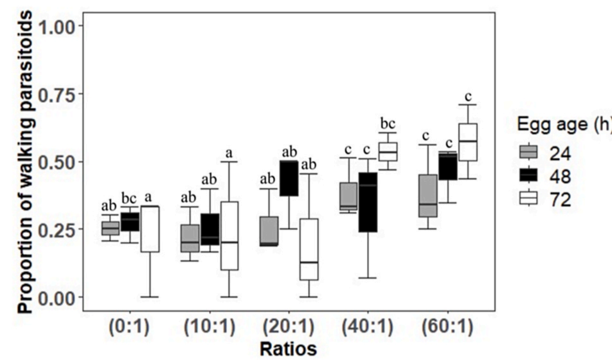

Fig. 4. The proportion of emerged walking egg parasitoids as affected by different ratios (sterile to fertile FCM) and age of the FCM eggs. Boxplots shows median values (solid lines), and whiskers show the range of the data. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

development is crucial for effective pest control (Carpenter et al., 2004; Kaspi et al., 2020). In this study, the parasitism levels of the egg parasitoid, *T. cryptophlebiae* against FCM eggs produced from different sterile to fertile ratios were determined. The results indicated that all FCM eggs laid from different ratios and exposed to female egg parasitoids were acceptable for oviposition, demonstrating their ability to support egg parasitoids development. This finding aligns with a study by Carpenter et al. (2004), which showed that egg parasitoids can successfully develop and emerge from eggs originating from different crosses in the field.

From this study, it is evident that both the different ratios of sterile to fertile moths and the egg age did influence the parasitism rate, with higher levels of parasitism occurring in the younger eggs compared to the older eggs. A significant proportion of FCM eggs were parasitised at the egg age of 24 and 48 h compared to 72 h, across the ratios, except for 10:1. Host fertility, host age and the contact time between host and parasitoid, play a key role in parasitism (Cossentine et al., 1996; Makee, 2006). Therefore, the decrease in parasitism rate with increasing egg age in the current study may be attributed to the declining suitability of host eggs as they age, given that more nutrients are used up by the developing FCM larva (El Sharkawy, 2011; Peñaflor et al., 2012), which aligns with the findings of Carpenter et al. (2004), indicating a similar trend of decreasing parasitism rate with increasing FCM egg age.

The results suggest that a combination of different ratios of sterile to fertile FCM and egg parasitoids could provide an additional suppressive effect against FCM, given the acceptability and suitability of the eggs for oviposition by the egg parasitoids. This observation corresponds with studies by Nagy (1973), Cossentine et al. (1996) and Botto and Glaz (2010), which found that combining the release of sterile insects with *Trichogramma* spp. egg parasitoids achieved a suppressive effect on *C. pomonella*. Similarly, Bloem et al. (1998) suggested that combining both control techniques yielded better results than using either method alone. The lack of feasible sexing methods in most SIT programmes for lepidopteran pests, results in various mating events, including those involving irradiated moths, which produce non-viable eggs (Marec and Vreysen, 2019). These non-viable eggs can support parasitoid build-up, by providing additional host material in the field (Nagy, 1973; Cossentine et al., 1996; Bloem et al., 2007; Marec and Vreysen, 2019). Moreover, the FCM eggs that are not parasitised by the egg parasitoids may either fail to hatch if they originate from a sterile FCM female or may develop into sterile F_1 adults if they originate from a sterile male-fertile female pairing, contributing to further suppression of FCM (Bloem et al., 2003). This is because the radiation dose used completely sterilises the female FCM, while the males are partially sterile, resulting in production of progeny that is both completely sterile (F1 or inherited sterility) and strongly biased towards males (Bloem et al., 2003).

Superparasitism was not greatly affected by the different ratios and the age of FCM eggs. A higher proportion of superparasitism was recorded in ratio 0:1 at an egg age of 24 h, but this proportion decreased significantly at the egg age of 48 and 72 h. This decrease in superparasitism can be attributed to the lower quality of host eggs, resulting from crosses between sterile males and females, which may be unsuitable for the development of two or more parasitoids compared to high-quality eggs from fertile moths (Carpenter et al., 2004; Moreira et al., 2009).

The different ratios and egg age did influence the oviposition and emergence of egg parasitoids, with the younger eggs being preferred and, therefore, more suitable for egg parasitoid development compared to the older eggs. This finding aligns with studies by Reznik and Umarova (1985, 1990), El Sharkawy (2011), Peñaflor et al. (2012) and Bari et al. (2016), which suggested that the proportion of emerging egg parasitoids decreases as the age of the host egg increases, due to competition for resources with the developing host embryo. The chemical composition of the host egg changes as nutrients are gradually taken up by the host embryo, and the variations in the physical characteristics of the chorion, which hardens as the egg ages (Peñaflor et al., 2012). Similar results were reported by Pizzol et al. (2012), showing higher emergence rates of *Trichogrammatoidea cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) obtained in 1-day-old host eggs compared to older hosts. However, these findings differ from Carpenter et al. (2004), who reported a significant reduction in the parasitoid emergence from 24 h old host eggs laid by sterile FCM compared to that from older eggs. This could be a result of the host egg being too young, resulting in low nutrient availability to support parasitoid development or leading to superparasitism, which ultimately results in parasitoid mortality (Reudler et al., 2007; Pizzol et al., 2012; Zhu et al., 2014). Nevertheless, the significant difference disappeared when the egg age was 48 h and 72 h. This could be attributed to the low preference of female parasitoids for host eggs laid from matings between sterile moths, resulting in a low level of parasitism and, hence, poor emergence of the parasitoids (Zhu et al., 2014).

There was variation in the female-to-male sex ratio of the emerging egg parasitoids, across different ratios and FCM egg ages. The different ratios did not affect the sex ratio of the egg parasitoids, as the egg age did, especially the young egg ages, where more females than males emerged. These results indicate that majority of host eggs from sterile moths and older than 72 h are least preferred for oviposition, leading to the observed sex bias. This is crucial since female egg parasitoids are responsible for parasitism and population build-up in the citrus orchards. This finding conforms with the findings of Godin and Boivin (2000), which show host acceptance, successful parasitoid development, and that the fecundity of emerging females declines with increasing host age. According to Schmidt (1994), the clutch size and the progeny sex ratio are influenced by the female parasitoids' assessment of host age, size, and nutrient availability. This concept is in line with Charnov's (1981) host quality model, which predicts that female parasitoids should produce a high proportion of males in low-quality host eggs, such as small and old host eggs and a high proportion of females in high-quality host eggs. The males are allocated to the least-preferred hosts (El Sharkawy, 2011).

According to Prezotti et al. (2002), to maintain the high-quality standards required for egg parasitoids, longevity, flight activity, and parasitism index need to be assessed. This approach is simpler than the evaluation of seven different biological variables in the laboratory, as recommended by the International Organization of Biological Control (IOBC Global Working Group: Quality Control of Mass-Reared Arthropods) (van Lenteren, 2003). In our flight tests conducted to assess the quality of the egg parasitoids emerging from FCM eggs produced from different ratios of sterile and fertile FCM, egg parasitoids were categorised into two groups: flyers and walkers, depending on their location after release in a flight test chamber, and egg parasitoids quality was assessed. The different ratios and egg ages significantly affected the proportion of flying and walking egg parasitoids after emergence. The majority of flying egg parasitoids emerged from young-age host eggs (24 and 48 h) compared to older host eggs (72 h). With the high emergence rate of flying egg parasitoids from FCM host eggs, the egg parasitoids can be effectively used as biological control agents in combination with SIT. Conversely, there was a significantly higher proportion of walking egg parasitoids emerging at the egg age of 72 h, in the ratios of 40:1 and 60:1 compared to 20:1, 10:1, and 0:1. Therefore, flight and walking are crucial locomotory aspects of trichogrammatid wasps, which aid in their dispersion in the field during foraging for food, hosts, and mates (Dutton and Bigler, 1995; Soares et al., 2012). Consequently, the emergence of more flying egg parasitoids will be beneficial, as they are more mobile and can search and parasitise more FCM eggs, even in areas not easily accessible by other control techniques, such as chemical sprays (Pérez-Staples et al., 2021). There was a variation in the proportion of emerging flying egg parasitoid sex ratio, across different ratios and FCM egg ages. At the ratio of 0:1, the proportion of flying female egg parasitoids was higher than that of male egg parasitoids in all egg ages. The trend was also evident in other ratios, with more egg parasitoids emerging at egg ages of 24 and 48 h compared to 72 h. This female-biased sex-ratio is beneficial in a biological control programme, as a few males can fertilize many females, contributing to parasitoid population development (King, 1987). Species with high emergence rate, high proportion of females, and high flight capacity can disperse faster in the field, locate hosts, and produce a high number of progeny in shorter periods, aiding in more effective pest suppression (Soares et al., 2012).

Currently, the benchmark ratio of sterile to fertile FCM used in the SIT programmes on citrus orchards is 10 sterile males to 1 fertile male (Hofmeyr et al., 2005, 2015). This ratio was shown to be effective in research trials conducted to determine what overflooding ratio to use (Hofmeyr et al., 2005, 2015). Results from these trials convinced the South African citrus industry to adopt the technique and expedite the commercial production of the sterile moths through the establishment of XSIT. However, to mitigate the negative impacts of FCM on the citrus industry in South Africa, it is essential to implement additional management strategies that are efficient, effective, and environmentally friendly. Therefore, the combination of high ratios of sterile to fertile FCM and the biological control agent, *T. cryptophlebiae*, can provide some additive suppressive effects. These products can also potentially be supplied at a reduced cost where simultaneous production occurs, as done by River Bioscience. This company uses the excess FCM from XSIT for mass rearing of egg parasitoids, productively using the FCM eggs that would otherwise be discarded. This innovative approach contributes to cost-effectiveness and sustainability in the control of FCM. For full integration of SIT and augmentative releases of egg parasitoids to be effective in pest management, it is crucial to ensure that egg parasitoids do not undermine the efficacy of SIT programme (Carpenter, 1993; Carpenter et al., 2004). This study indeed showed that the released sterile insects do not negatively affect the efficacy of the egg parasitoids. Therefore, the eggs produced from matings involving released sterile FCM can provide additional host material for egg parasitoid population build-up. Our finding suggests that high FCM ratios, greater than the current benchmark (10:1), can support the successful development of egg parasitoid populations and can be effectively combined for FCM control. However, further research is warranted to thoroughly examine the effectiveness of integrating these two strategies for FCM control under field conditions. Such studies will provide valuable insights and pave the way for more robust and efficient pest management practices in South Africa's citrus industry.

Submission declaration and verification

This work has not been previously published.

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CRediT authorship contribution statement

Michael M. Githae: Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Candice A. Coombes:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration. **Reyard Mutamiswa:** Writing – review & editing, Visualization, Supervision, Software, Formal analysis, Data curation. **Sean D. Moore:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration. **Martin P. Hill:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare they do not have any conflicts of interest.

Data availability

The data that has been used is confidential.

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