The commercialisation of *Trichogrammatoidea cryptophlebiae* (Nagaraja) in South Africa: from mass-rearing to augmented field releases

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Abstract

Trichogrammatoidea cryptophlebiae (Nagaraja) is an endemic egg parasitoid from southern Africa that shows great potential as a biological control agent for tortricid pests in high-value export crops. This study aimed to develop an integrated framework for its use in pest management by addressing three critical aspects: optimising mass-rearing protocols in the laboratory, evaluating parasitism efficacy in the field across different crops and release strategies, and assessing non-target effects from agrochemical residues. In the laboratory, a series of controlled trials were conducted to identify key indicators of parasitoid quality, including parasitism rate, emergence success, sex ratio, and morphological traits such as hind tibia length and body length. Host egg age, female age, and foundress-to-host ratios significantly influenced reproductive output and fitness. These results were used to refine rearing methods to maximise the production of high-quality, fecund females suitable for release. Field trials in macadamia, grape, litchi, and pome fruit orchards revealed that the timing and intensity of parasitoid releases had a significant impact on parasitism rates. Both standard and late season releases consistently produced the highest levels of parasitism, likely due to better synchronisation with host egg availability and fewer environmental constraints. Increased release numbers also resulted in improved parasitism, indicating a positive density-dependent effect under natural conditions. To ensure that field releases are compatible with existing pest control practices, a comprehensive assessment of chemical residues on adult and pre-imaginal life stages was conducted. It was found that several commonly used insecticides and fungicides had significant sublethal effects, reducing emergence, skewing sex ratios, and impairing reproductive potential, even when direct mortality among adults was low. These findings were assigned toxicity classifications determined by the International Organisations for Biological Control (IOBC), providing practical guidance on chemical compatibility for growers. This research offers practical recommendations for the successful implementation of *T. cryptophlebiae* in integrated pest management systems. These recommendations include optimised rearing and quality control protocols, evidence-based release strategies tailored to pest phenology, and informed selection of agrochemicals to ensure parasitoid survival and efficacy. Together, these contributions enhance the viability of biological control in export-driven agriculture, promoting more sustainable and environmentally responsible pest management in South Africa.

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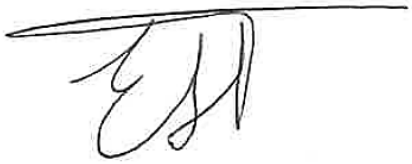
Declaration

I, Emma Jane Stirk, hereby declare that the following thesis has not been submitted to any other Academic institution other than Rhodes University, Grahamstown, South Africa and the work presented here is that of the Author.



Emma Jane Stirk

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List of Abbreviations

ANOVA	Analysis of Variance
CGA	Citrus Growers' Association
CM	*Cydia pomonella*
DAFF	Department of Agriculture, Forestry and Fisheries
FCM	*Thaumatotibia leucotreta*
FPEF	Fresh Produce Exporters' Forum
FRAC	Fungicide Resistance Action Committee
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
IPM	Integrated Pest Management
IRAC	Insecticide Resistance Action Committee
IOBC	International Organisation for Biological Control
LM	*Cryptophlebia peltastica*
LV	*Lobesia vanillana*
MNB	*Thaumatotibia batrachopa*
MRL	Maximum Residue Limit
PPECB	Perishable Products Export Control Board
QC	Quality Control
R	Statistical software "R"
SALGA	South African Litchi Growers' Association
SAMAC	South African Macadamia Growers' Association
SATI	South African Table Grape Industry
SE	Standard Error

Chapter 1: General Introduction & Literature Review

1.1. South African Agriculture

Although agriculture only makes up 2-3 % of South Africa's GDP, the country is largely reliant on its agricultural sector in terms of employment, food security, and promotion of rural livelihoods (Barrientos & Visser, 2013; Statistics South Africa, 2021). The exportation of produce from the country is also essential, as it generates considerable foreign exchange, which contributes meaningfully to the economy (Barrientos & Visser, 2013; National Treasury of South Africa, 2021). Agriculture generates approximately $3.4 billion from the export of horticultural produce (Wakelin & Taylor, 2024). Despite its status as a semi-arid water-stressed country (only receiving an average of 490 mm of rain annually), South Africa produces and exports a significant amount of produce throughout the year to countries in the European Union, the United States, China, the United Kingdom and others (Kyei & Hassan, 2019; Barrientos & Visser, 2013). However, due to water scarcity, crop production has been confined to specific regions of the country, with the majority of crop production being restricted to only 12 % of the country's land mass (National Treasury of South Africa, 2021). Farmers have taken advantage of these regions and the varying climatic conditions of South Africa, from arid to subtropical and tropical, to produce horticultural crops that are of high value or exportable (Charleston *et al.* 2004).

Over fifty percent of the produce grown in South Africa is exported, with fresh fruit being the dominant horticultural crop exported globally (Barrientos & Visser, 2013). The largest contributing horticultural tree crops to the export market include citrus, avocado, stone fruit, macadamia nuts, and grapes (Sihlobo, 2023). Small but notable exports come from the litchi and pome fruit industries, which are growing (Sihlobo, 2023). The success of crop production and exports in the global agricultural sector is dependent on several factors. These include weather, market access, and market demand, which all contribute to the quality and quantity of produce harvested. The South African export industry has developed significantly since 1997, as more markets have become accessible (Barrientos & Visser, 2013). The demand for South African produce has increased, and production of high-value exportable crops in the country has followed suit (Barrientos & Visser, 2013). There are several growers' associations in South Africa that assist producers with relevant market and trade information, which promotes the growth of these various industries.

1.1.1. South African Horticultural Associations

Fruit South Africa (Fruit SA) is a non-profit company that is registered to facilitate the growth of horticultural production in South Africa (Fruit South Africa, 2022b). Fruit SA represent the Fresh Produce Exporters Forum of South Africa (FPEF), the Citrus Growers Association (CGA), Hortgro, the South African Table Grape Industry (SATI), the South African Subtropical Growers Association (Subtrop) and Berries ZA (Fruit South Africa, 2022a). Fruit South Africa acts as an umbrella body for these groups to assist them through constructive engagement with the government, public institutions, and stakeholders on relevant producer information (Fruit South Africa, 2022b). The FPEF's relationship with the CGA, SATI, Hortgro, Subtrop and Berries ZA allows the non-profit organisation to be in a strong position in connecting exporters of produce with relevant information on market access and government regulations (Subramanian *et al.,* 2011; Fresh Produce Exporters' Forum, 2023). The following sections will discuss horticultural commodities of interest to this study.

1.1.2. South African Horticultural Commodities of Interest

1.1.2.1.Macadamia Nut Production and Export

The South African Macadamia Growers Association (SAMAC) states that South Africa is the leading producer of macadamia (*Macadamia integrifolia* (Maiden & Betche) (Proteales: Proteaceae)) nuts globally, with an estimated 81 302 established macadamia nut hectares (Southern African Macadamia Growers Association, 2025). Major production regions of macadamia nuts in South Africa occur in the Limpopo, Mpumalanga, and KwaZulu-Natal provinces, with smaller production areas being found in the Eastern Cape, Western Cape and North West provinces (Southern African Macadamia Growers Association, 2025). Numerous companies, including Green Farms Nut Co. and Mayo Macs SA, process macadamia nuts in the main production regions. Considering South Africa exports 98 % of its macadamia produce, this increase in hectares has allowed the country to surpass Australia and Kenya as the largest exporter of macadamia nuts to foreign markets. Macadamia annual yield in 2023 was recorded at 78 110 tonnes, increasing to 87 227 tonnes in 2024, and a forecasted crop of 93 433 tonnes for 2025 (Southern African Macadamia Growers Association, 2025). Exports during the 2023 production season generated R4.84 billion in industry turnover. The majority of macadamia nut export shipments went to countries that have a cultural association with the nut; these countries are in East Asia and China. Currently, the demand for macadamia nuts globally is not being met, which provides the South African macadamia industry with opportunities to grow further and develop (Southern African Macadamia Growers Association, 2025).

1.1.2.2.Litchi Production and Export

The South African Litchi Growers Association (SALGA) is the smallest of the three members forming part of the South African Subtropical Growers Association (Subtrop). The production of litchi (*Litchi chinensis* (Sonnerat) (Sapindales: Sapindoideae)) in South Africa is done on 1389 hectares, with Mpumalanga being the largest production area (904 ha), followed by Limpopo (426 ha) and KwaZulu-Natal (59 ha) (South African Litchi Growers' Association, 2024a). South Africa is a relatively new producer of litchis, and it does not rank among the top-producing and exporting countries globally (South African Litchi Growers' Association, 2022b). South African Litchi Growers' Association (2024b) has recorded an export tonnage of 5 086 for the 2022/2023 production season, and only 2 637 for the 2023/2024 production season, which represented 54 % and 60 % of the total tonnage, respectively of the total crop that was harvested in those two seasons. The remaining percentage of the crop that isn't exported is consumed domestically or processed (South African Litchi Growers' Association, 2024b).

1.1.2.3.Citrus Production and Export

South Africa is one of the leading exporters of *Citrus* (Linnaeus) (Sapindales: Rutaceae), commonly referred to as citrus, globally, having exported 2 384 000 tonnes in the 2023/2024 season, which is 67 % of the crop produced in that season (World Citrus Organisation, 2022). Although it is one of the leading exporters of citrus, it is not a leading producer, with under 10 % of citrus globally being grown in southern Africa (World Citrus Organisation, 2022). The major export regions for South African citrus in the 2021/2022 season were Europe, the Middle East, Southeast Asia, and the United Kingdom (Citrus Growers Association of Southern Africa, 2022a). South Africa has 97 128 hectares of various citrus varieties planted throughout the country, with the main production regions being Limpopo, Eastern Cape, and the Western Cape provinces (Citrus Growers Association of Southern Africa, 2022b). The Citrus Growers Association of Southern Africa (CGA) is working hard to increase market access for the increasing quantities of citrus being grown in the country (Citrus Growers Association of Southern Africa, 2022a).

1.1.2.4.Table Grape Production and Export

The quantities of table grapes (*Vitis vinifera* (Linnaeus) (Vitales: Vitaceae) exported from South Africa rank the country as the 5th largest exporter worldwide, with the majority of exports going to countries in the European Union, the United Kingdom and Canada (Ferreira,

2024). Table grapes in South Africa for the 2023/2024 and 2024/2025 production seasons were produced on 19 788 to 19 488 hectares, respectively, spread across the Western Cape, Limpopo, and Northern Cape provinces (Ferreira, 2024). The South African Table Grape Industry (SATI) reported export tonnages of 286 166 in the 2023/2024 production season and 333 673 in the 2024/2025 production season (Ferreira, 2024). This being a record high harvest in the 2024/2025 production season, despite an overall 2 % decrease in production hectares in South Africa during the season (Ferreira, 2024). The increased production and harvest are a result of the plantings of high-yielding varieties and limited weather-related damages which had been experienced in previous seasons.

1.1.2.5.Deciduous Fruit Production and Export

The South African deciduous fruit producers are represented by Hortgro, whose goal is to develop the industry through production, research, market access and communication. South Africa ranks as the top producer of deciduous fruit globally, exporting up to 50 % of its total production (Hortgro, 2021a). As of 2023, there are a recorded 54 271 hectares of deciduous fruit under production in South Africa, with pome fruit consisting of 38 006 hectares. Deciduous fruit production in South Africa is currently sitting at 2.1 million tonnes harvested in the 2022/2023 season (Hortgro, 2021a). In the 2023/2024 season, South Africa exported 754 975 tonnes of pome fruit, with apples exports recorded at 531 562 tonnes and pears at 223 413 tonnes exported.

All horticultural industries in South Africa have the support of their relevant associations for assistance in producing and exporting high quality produce. The production of all these crops does not come without its challenges. Pest management, especially in crops destined for export, is one of the biggest challenges that growers face (Subramanian *et al.,* 2011). As countries move towards a consumer and environmental health focus, the use of conventional pest management strategies cannot be relied upon as the sole control option for pest management (Fuchs & Kalfagianni, 2010; Mamasalievich *et al.,* 2023). Regulations and standards have been put in place by various countries, and exporters need to ensure they meet these standards if they wish to remain competitive in the market (Mamasalievich *et al.* 2023).

1.1.3. South African Export Regulations

Global regulatory frameworks have been put into place to govern the food safety and quality of exported produce to ensure the protection of human health and natural ecosystems (Fuchs & Kalfagianni, 2010). These regulations, if abided by, create opportunities for

producers in the form of gaining access to new markets and by maintaining a competitive export environment (Floretti & Shingal, 2014). In South Africa, with regard to the export of produce, there are regulations put in place by the Department of Agriculture, and by importing countries, on maximum residue limits (MRLs) permitted on produce (Cloete *et al.,* 2013; Fuchs & Kalfagianni, 2010). There are two organisations in South Africa to take note of; they are the Perishable Products Export Control Board (PPECB) and Global Good Agricultural Practices (GlobalGAP), which ensure, through audits and certification, that producers meet export regulations (Fresh Food Trade SA, 2023).

PPECB is a company based in South Africa that does regular audits of farming, packhouse, and shipping practices to ensure they are in line with the wants of the European Union and other export markets (Perishable Products Export Control Board, 2023). PPECB acts as a third-party auditor and assists South African producers through conducting stringent audits in-country, which therefore lowers the audit intensity when produce arrives in the European Union. The European Union has an agreement with PPECB, which requires the company to do work on its behalf (Perishable Products Export Control Board, 2023).

The organisation GlobalGAP has developed a set of standards for growers to receive certification (Fuchs & Kalfagianni, 2010). GlobalGAP certification is globally recognised as it promotes sustainable and safe farming practices to ensure the end product is safe for consumers. The aim of GlobalGAP is to establish one standard for good agricultural practices (Fuchs & Kalfagianni, 2010). Through achieving GlobalGAP certification, producers can access new markets and increase their profits through exportation (Baah-Annor *et al.,* 2014; Fuchs & Kalfagianni, 2010).

Achieving PPECB and GlobalGAP standards creates opportunities for South African producers to export their produce, however, there are also challenges that come along with it (Baah-Annor *et al.,* 2014). The standards imposed by PPECB and GlobalGAP, regarding pest management, require producers to limit the use of synthetic pesticides that are more persistent and have higher toxicity to non-target organisms (Hanford *et al.,* 2015). Any produce due for export needs to meet the maximum residue limits (MRLs) imposed by the country for which the produce is destined (Hejazi *et al.,* 2022). MRL legislation differs for countries globally and navigating this poses a challenge for producers, with the majority of standards making use of the European Union's MRL standards, as they tend to have the most stringent regulations (Hanford *et al.,* 2015). Producers then follow guidelines, generally developed by their growers'

association, to ensure they meet MRLs. The challenge in this is that MRLs reduce the amount of chemicals one can spray, and therefore adequate pest management requires supplementation in the form of other, more target-specific, lower-toxicity control methods that minimise harm to beneficial organisms and reduce environmental persistence. This is why integrated pest management (IPM) has become a focal point in all cropping industries (Hanford *et al.,* 2015; Fuchs & Kalfagianni, 2010).

The concept of IPM was developed to better incorporate multiple pest management tactics into a programme for effective pest control. IPM focuses on understanding crop-pest complexes, as well as the simultaneous use of biological, cultural, mechanical, and chemical pest control methods (Karlsson Green *et al.,* 2020). The development of pesticide resistance and MRL regulations has resulted in producers' increased adoption of biological control methods worldwide, and IPM principles have been used to guide the uptake of these softer pest management approaches. Thus, IPM has been encouraged and is supported by many governments and international organisations for the purposes of environmental health, human health, and climate change mitigation (Hanford *et al.,* 2015; Mamasalievich *et al.,* 2023).

DALRRD have recognised that the use of IPM strategies is important in maintaining and increasing the access of South African produce to foreign markets (Cloete *et al.,* 2013). DALRRD support the use of IPM strategies through Act 36 of 1947 (The Agricultural Pests Act), whereby they stipulate that biological products should be registered for use in the agricultural industry. Through the registration of biological control products, DALRRD encourages farmers to adopt IPM practices, which assists them in meeting MRL regulations and adapting to the changing foreign market demands (Cloete *et al.,* 2013). The South African biological control industry represents an eighth of the country's crop protection market (Du Toit, 2022). There is, however, an increase in demand for biological control options, and the industry in South Africa is expected to grow. Companies are allocating more resources to the research and development of biological technologies to be used in IPM practices, to provide producers with alternate options that will meet their pest management needs, while adhering to MRL regulations (Du Toit, 2022).

1.2. Integrated Pest Management (IPM)

1.2.1. The Principles of IPM

IPM has been practised for over 60 years (Deguine *et al.,* 2021). There are multiple definitions of IPM that have been developed, with varying focal points, but all emphasise

environmental health, human health, reduction of synthetic chemical usage in agriculture and integration of different pest management methods to form a holistic pest reduction strategy (Deguine *et al.,* 2021; Ehler, 2006; Vreysen *et al.,* 2007). Barzman *et al.* (2015) suggest that there are eight principles of IPM (Figure 1.1). These relate to the broad application of this pest management strategy, so that it can be implemented across all crop types (Gross & Gundermann, 2016).

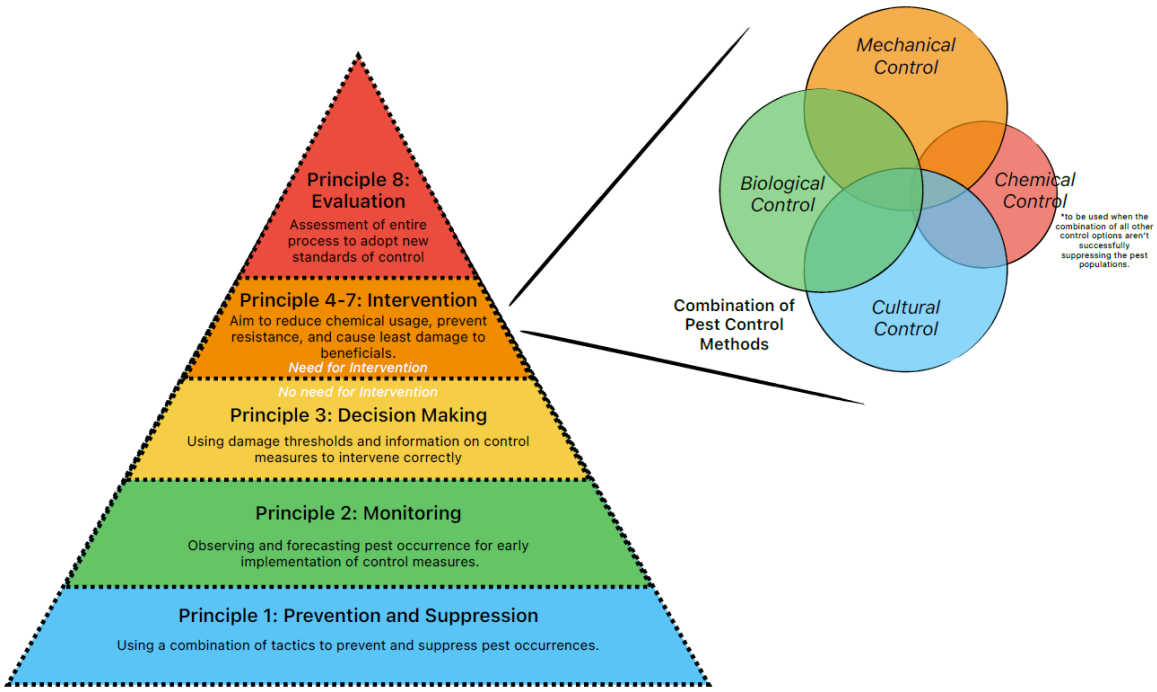

Figure 1.1. The eight principles of IPM (based on Barzman *et al*., 2015 principles of IPM).

1.2.1.1. Prevention and Suppression

The first principle in IPM practice is the prevention and suppression of pests in agriculture, through the incorporation of multiple biological, cultural, mechanical, and chemical control methods (Barzman *et al.*, 2015; Baker *et al.,* 2019; Dara, 2019). Prevention and suppression consider the reality that no pest will be eradicated from the environment, but rather maintained below economic thresholds that have been developed for individual pest species (Barzman *et al.,* 2015; Stenberg, 2023; Dara, 2019). Further work into developing economic thresholds for pest species needs to be done, as this is lacking in numerous crop varieties (Dara, 2019). Calvo-Garrido *et al.* (2014) demonstrated that through early applications of three natural products, *Candida sake,* a yeast combined with protective agent, Fungicover ®, Fungicover ® alone, and *Ulocladium oudemansii,* a naturally occurring soil

fungus, *Botrytis cinerea* on aborted samples of flowers, fruit and calyptra, can be effectively reduced. The biological fungicides were found to significantly reduce *B. cinerea* on aborted flowers and calyptras by 46-85 % through early season applications. The treatments proved to reduce sporulation of *B. cinerea* by 48 %, which has the potential to reduce infection by *B. cinerea* in vineyards and thereby prevent yield losses, through early suppression of *B. cinerea* (Calvo-Garrido *et al.,* 2014).

1.2.1.2. Monitoring

The second principle is monitoring, which involves the regular use of monitoring systems and forecasting of pest pressure, to determine whether action for control of the pest is required (Prasad & Prabhakar, 2012). This is directly related to the management of pests to levels below the economic threshold. Monitoring and forecasting systems for pest species vary according to the type of pest and the crop it infests. Further development of these systems across numerous pest species needs to be done to effectively implement IPM strategies (Barzman *et al.,* 2015; Nwilene *et al.,* 2008). Monitoring of pest populations can prove extremely useful in effectively timing the application of pest control measures for successful pest suppression, and this is where the third principle comes in (Baker *et al.,* 2019; Prasad & Prabhakar, 2012; Dara, 2019).

Monitoring of arthropod pests can be done in various ways, depending on the arthropod life cycle, plant damage symptoms and mechanisms available for monitoring the pest. Two commonly used monitoring methods are, scouting for eggs on the crop and making use of semiochemicals to trap pests on sticky foils or in toxic fluids (Gross & Gunderman, 2016; Prasad & Prabhakar, 2012). Semiochemicals are predominantly used to monitor the adult stages of a pest's life cycle. The information gained through this method of monitoring provides insight into the pest's population dynamics and is often used to make calculated decisions in the timing of control measures to achieve the most effective suppression (Gross & Gunderman, 2016; Prasad & Prabhakar, 2012). Countries within the European Union have assisted growers by developing practical forecasting and monitoring tools that can be used to make calculated decisions on pest pressure (Kudsk & Jenson, 2014). For example, Denmark has the lowest pesticide usage of all EU countries. This has been attributed to their extensive monitoring networks, which are linked to a farm advisory system that assists in making calculated and timely decisions for effective control of pest populations (Kudsk & Jenson, 2014).

1.2.1.3. Decision Making

The third principle of IPM is decision-making that is based on monitoring and intervention thresholds. Such decision-making processes allow for the design and development of long-term strategic IPM programmes that are locality specific (Barzman *et al.,* 2015; Dara, 2019). Decision making in IPM needs to consider the relationships that various control products have with each other and the impact that they have on the agroecosystem. There are numerous examples on how singular products are effective in controlling a pest, but there is a lack of research in the IPM sphere on the influence that products, namely biologicals, have on each other within an agricultural ecosystem (Deguine *et al.,* 2021; Stenberg, 2023). IPM is an interdisciplinary approach to pest management, and it needs to be researched as such. IPM strategies should ensure biological control methods and chemical programmes are compatible, to allow for satisfactory control of the pest complex on a crop (Barzman *et al.,* 2015; Stenberg, 2023; Thomas, 1999).

There are numerous accounts of research on symbiont relationships between biological, cultural, and chemical control methods, and examples demonstrating the importance of decision making in IPM are readily available. Tang *et al.* (2010) developed models assessing the optimum timing and rates of pesticide application and the release of natural enemies. The models considered the damaging effects of pesticides on natural enemy populations and indicated that the timing of chemical application prior to or post natural enemy release is highly important, as damage to the released natural enemy population will be lowered regardless of the rate of chemical application (Tang *et al.,* 2010). If optimal timing is achieved, it can result in satisfactory pest control and a decrease in pesticide usage.

1.2.1.4. Intervention

The fourth principle that Barzman *et al.* (2019) mention is the use of non-chemical control methods. This principle suggests that IPM strategies should be based on non-chemical control methods such as biological, cultural, and mechanical. Reliance on chemical control is still exceedingly high globally in most cropping systems (Deguine *et al.,* 2021; Stenberg, 2023). There are numerous non-chemical products available for pest management, with biological control methods gaining attention in recent years (Barzman *et al.,* 2015; Thomas, 1999). One form of biological control is the suppression of a pest through the release of a natural enemy such as a predator, pathogen, or parasitoid (Stenberg, 2023). The release of pathogens, predators and parasitoids has proved to be highly effective in reducing pest

populations (Dara, 2019). In order to gain satisfactory results when using non-chemical control options, it is often recommended that growers make use of a combination of biological pest management tools (Barzman *et al.,* 2015; Thomas, 1999).

One of the most successful adoptions of IPM practices came from the Cańete Valley in Peru, where insecticides decimated natural enemy populations in cotton fields. This resulted in the resurgence of pests that chemical insecticides could no longer control (Chavez-Dulanto *et al.,* 2020). The farmers' associations had the support of the government, and together they implemented an IPM programme that focused on the reintroduction of natural enemies, crop diversification schemes, good varietal selection, and a ban on the use of synthetic organic chemicals (Chavez-Dulanto *et al.,* 2020). As a result of using alternative control methods and good farming practices, farmers were able to gain control of their pest issues and maintain them below economic thresholds (Chavez-Dulanto *et al.,* 2020).

1.2.1.5. Pesticide Selection

The fifth principle of IPM (Barzman *et al.* 2015) is the judicious selection of pesticides if satisfactory control through alternative control options is not achieved. IPM is an integrated approach that allows for chemical insecticide usage, but only as a supplementary option (Deguine *et al.,* 2019). Unfortunately, many farmers view IPM as a chemical-based strategy that uses biological control options as a supplement when chemicals are no longer effective, or when residue limits are of concern (Dara, 2019; Deguine *et al.,* 2021). This is a challenging aspect in the adoption of IPM practices globally. Farmers have been compelled to adopt IPM practices as a result of changing market trends and consumer demands, rather than making the decision to pursue IPM independently to achieve more holistic pest management. This transition into IPM has resulted in chemical control being the base management strategy, and biological options being supplementary (Deguine *et al.,* 2019), which, in effect, does not constitute IPM. The selection of a chemical for supplementary control should leave natural enemy populations undisturbed; if this is ignored, pest resurgences may arise (Barzman *et al.,* 2015; Nwilene *et al.,* 2008; Baker *et al.,* 2020; Deguine *et al.,* 2021).

Zang *et al.* (2021) highlighted detrimental and suitable chemical insecticides that can be used in crops with the augmented releases of *Trichogramma* species in China. Chemical insecticides that proved lethal for three *Trichogramma* species were chlorfenapyr, cypermethrin, and fipronil. Some biopesticides, such as avermectin and spinosad, reportedly did as much harm as the chemical insecticides. Insecticides that proved safer for use in conjunction with *Trichogramma* releases were azadirachtin, *Bacillus thuringiensis* (*Bt*),

chlorfluazuron, and tebufenozide (Zang *et al.,* 2021). It is extremely important in any IPM programme that makes use of chemicals to understand how the chemical will impact the agroecosystem. The incorrect choice of an insecticide, applied at the wrong time, could result in pest resurgences or secondary pest infestations, which would increase the cost of pest control (Tang *et al.,* 2010).

1.2.1.6. Reduction in Chemical Pesticide Usage

In line with principle 5, principle 6 of IPM puts focus on the reduction of chemical usage as a tool to minimise human and environmental health risks (Barzman *et al.,* 2015). Chemical usage can be reduced through decreased application frequency, precision targeted applications and the use of resistant cultivars (Barzman *et al.,* 2015). Chemical control has its place in IPM programmes, but it can no longer be solely relied on, due to the risk of insecticide resistance, access to export markets, MRL regulations and legislation regarding human and environmental health (Nwilene *et al.,* 2008). Principles 4 and 5 of IPM assist in achieving the 6th principle, which is to reduce chemical usage.

The adoption of IPM practices in numerous cropping industries has been able to reduce chemical insecticide use significantly. The International Rice Research Institute (IRRI) was able to attain 50-80 % reductions in chemical pesticide usage, without noticeable yield losses (Bottrell & Schoenly, 2012). The adoption of IPM practices has the ability to reduce the costs associated with pesticide use in agriculture, and the ability in the long term to increase crop yields through the improved stability of IPM programmes (Deguine *et al.,* 2021). Waddington & White (2014) conducted a review of over 500 IPM programmes across the globe and reported a 13-19 % increase in crop yields and profits (van de Fliert, 2014). With a reduction in pesticide usage and the consumer demand for organic and sustainable produce, there is an opportunity to capitalise on the organic markets while still maintaining high crop yields when using biological control options.

1.2.1.7. Chemical Resistance

Principle 7 relates to principles 4, 5 and 6, as it focuses on anti-resistance strategies, which coincide with chemical application and its overuse (Barzman *et al.,* 2015). Chemical pesticides have been used indiscriminately for decades, and this has led to the development of insecticide resistance in many pest species (Deguine *et al.,* 2021; Thomas, 1999). Insecticide resistance is the reduced susceptibility of an insect to chemical applications through either behavioural, penetration and metabolic resistance pathways (Georghiou, 1994). The genes from the surviving individuals that were able to adapt are passed on from parent to offspring

within a pest population, allowing for the continuation of the pest's presence (Georghiou, 1994). Principles 4, 5 and 6 encourage the reduction of pesticide usage, which in turn reduces the risk of build-up of insecticide resistance in pest populations (Barzman *et al.,* 2015; Dara, 2019). Insecticide resistance is one of the forces driving the increased adoption of IPM practices. There are a limited number of chemical modes of action available for rotation on crops, and the lack of diverse chemical controls could lead to resistance issues. The fear of inducing resistance in pests and of losing important active ingredients has forced agricultural stakeholders to consider alternative methods of control to achieve better resistance management (Baker *et al.,* 2019).

Bemisia tabaci (Gennagius) (Hemiptera: Aleyrodidae) is a whitefly that is considered a major agricultural pest to field and vegetable crops worldwide (Horowitz *et al.,* 2020). It is a pest that, through the overuse of chemical insecticides, has developed resistance in numerous countries, including China, Brazil, Greece, India, Israel, Pakistan, Spain, Turkey, and the United States of America (Horowitz *et al.,* 2020). Horowitz *et al.* (2020) have identified means to control this pest through further understanding its genetic and molecular makeup, and have indicated that the use of IPM and insecticide resistance management strategies is able to reduce the development of insecticide resistance in this species. IPM-based insecticide resistance strategies include the selection of biological insecticides, insecticide rotation (so as not to rely on one mode of action to suppress the pest throughout the cropping season), and the use of other cultural and mechanical non-chemical methods. Horowitz *et al.* (2020) also highlight the importance of implementing chemical resistance strategies for the control of whiteflies, suggesting the Insecticide Resistance Action Committee (IRAC) as a source for further education to farmers facing this challenge. Non-chemical methods of whitefly management are increasing, with options of natural enemies, *Amblyseius swirskii* and *Eretmocerus* spp. and pathogenic fungi, *Lecanicillium lecanii* and *Beauveria bassiana,* being used (Horowitz *et al.,* 2020).

1.2.1.8. Evaluation

The last principle, principle 8, is the evaluation of the IPM strategies used for pest control. Farmers are encouraged to engage with the IPM methods they have implemented to see if improvements can be made to their pest management strategy. The use of IPM strategies is personalised to the farmer's agroecosystem. The practice of IPM strategies cannot be generalised for an area or crop type, as it is highly specific to the individual farmers' requirements (Barzman *et al.,* 2015; Deguine *et al.,* 2021). The efficacy of an IPM programme

is highly dependent on the interaction between biological and chemical products, the timing of applications, and the presence of pests' specific life stages that are being targeted. IPM requires a holistic approach that considers the agroecosystem's role in suppressing and preventing pests (Deguine *et al.,* 2021). This is where biological control fits in. As an ecosystem service, it should lead in the implementation of IPM practices, since the use of biocontrol is applicable to all eight principles of IPM described by Barzman *et al.* (2015).

1.2.2. Biological Control in IPM

Biological control, simply put, is the use of a living organism, such as predators, parasitoids, pathogens, or viruses, to suppress pest populations in more sustainable ways (Tang *et al.,* 2010; Cook, 1988). Biological control has been implemented in various cropping systems for decades and has achieved great success. Over the years, there have been numerous types of biological control methods developed for several reasons: animal and human protection, invasive plant management, and agricultural pest management. There are four different types of biological control that have been recognised over the years for the management of pest species in agriculture: natural, conservation, classical, and augmentative biological control (van Lenteren *et al.,* 2018). These four types of biological control all have the role of plant protection against herbivorous pest species, but are used in different circumstances.

Natural biological control requires no human intervention or disturbance to the ecosystem, allowing natural enemies, climatic conditions, and host plants to provide the service of controlling a pest (van Lenteren *et al.,* 2018). Pereira *et al.* (2018) demonstrated how these natural elements in agroecosystems can sufficiently control the soybean looper, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae). Climatic conditions regulate insect life cycles and population dynamics through air temperature, rainfall, and photoperiod. *Chrysodeixis includens* was found to have a complex of fungi that regulated the population of this pest. For all other pest species, in their native range, there are predators, parasitoids and pathogens that occur naturally to control the pest sufficiently in undisturbed areas (Pereira *et al.,* 2018).

Conservation biological control requires human involvement in the form of protecting and stimulating the performance of natural enemies, through manipulating ecosystems to increase the natural enemy diversity and function in controlling a pest (Wyckhuys *et al.,* 2013; Snyder, 2019). Harris *et al.* (2022) conducted a study on a fruit fly parasitoid, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), for the management of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), which aimed to enhance the species

populations through conservation biological control methods. They implemented augmentoria, or mesh-covered containers, into which growers can place sanitised fruit. These prevented emergent pests from exiting the fruit but allowed for parasitoids to emerge into the environment. The authors also investigated the suitability of wild tobacco plantings as a refuge for the parasitoid and the planting of floral resources to provide the natural enemy with a carbohydrate source (Harris *et al.,* 2022). The results indicated that 90 % of parasitoids were able to escape the netting, which could drastically increase the presence of the parasitoid in the agroecosystem. The use of tobacco as a refuge for the parasitoid proved successful (Harris *et al.,* 2022). The parasitoid was present in the tobacco plantings throughout the year (parasitising 31 % of flies associated with the plant in winter and 60 % in the spring), and they were able to move successfully between the tobacco and the target crop (Harris *et al.,* 2022). The introduction of floral resources as a carbohydrate source did not benefit the parasitoid. The longevity of the parasitoid did not increase and therefore did not contribute to pest control (Harris *et al.,* 2022). However, the provision of food resources in the form of honey water was beneficial, significantly increasing the life span of the parasitoids and showing potential as a tool for improving conservation biocontrol (Harris *et al.,* 2022). This demonstrates how multiple tactics can be used in conjunction to enhance natural enemy populations.

Classical biological control is the collection of a natural enemy from its native country/range for release in areas where its host is considered an invasive species. Classical biological control was the first form of biological control to be widely practised. A great example of classical biological control comes from the introduction of *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae) into citrus orchards in California for the control of the citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) (Kairo *et al.,* 2013). *Planococcus citri* was causing significant damage to citrus orchards in California in the 1890s and proved unmanageable through insecticide use (Bartlett, 1978). *Cryptolaemus montrouzieri* was discovered to be an effective natural enemy against *P. citri* in Australia and was subsequently introduced into Californian citrus orchards in 1891-1892. *Cryptolaemus montrouzieri* effectively controlled *P. citri* and was therefore produced in insectaries for continuous mass releases (Bartlett, 1978). Since its first introduction into a foreign country, *C. monotrouzieri* has been released in 64 different countries for the control of more than 16 pest species from the Pseudococcidae, Coccidae and Monophlebidae families (Kairo *et al.,* 2013).

Augmentation biological control is the use of a natural enemy that is mass-reared and released for pest control or to re-establish a population (Parrilli, 2021; Perez-Alvarez *et al.,*

2019). There are two forms of augmentation biological control, inundative and inoculative. Inundative biological control is the release of natural enemies to control a pest, exclusively through the released natural enemy, whereas inoculative biological control is the periodic or seasonal release of natural enemies to re-establish a balance that has been disrupted by other control methods (Parrilli, 2021; Van Driesche & Bellows Jr., 1996).

Inoculative and inundative augmented releases have been practised widely using numerous predator, pathogen, and parasitoid species. Several species from the family Trichogrammatidae, parasitoid wasps, have been commonly used throughout the world for the control of lepidopteran pests in agriculture. Six hundred and twenty species in the family Trichogrammatidae have been described worldwide (Zang *et al.,* 2021). Inoculative and inundative biological control practices have been used to release Trichogrammatidae. The preferred method of release is dependent on the requirements necessary to achieve control of the target pest species. Sharma *et al.* (2020) demonstrate how inundative releases of *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) and *Trichogramma japonicum* (Ashmead) (Hymenoptera: Trichogrammatidae) can control the stem borers, *Chilo infuscatellus* (Snellen) (Lepidoptera: Crambidae) and *Scirpophaga excerptalis* (Walker) (Lepidoptera: Crambidae) in sugarcane fields. Sharma *et al.* (2020) did eight releases at 10-day intervals, whereby they released 50 000 *T. chilonis* and 50 000 *T. japonicum* per hectare. The aim of these weekly releases was for the released *Trichogramma* spp., rather than their progeny, to control the pest population (Eilenberg *et al.,* 2001). When doing inundative releases of natural enemies, there will always be a "residual effect" where some of the progeny of the released organism will continue to control the pest population, even though it is not the main purpose of inundative releases (Eilenberg *et al.,* 2001). The study done by Sharma *et al.* (2020) indicated that overwhelming releases of these two parasitoid wasps, for control of their respective host species, were effective in reducing the percentage incidences of the pests in sugarcane fields. When conducting inundative biological control releases, it is highly important to consider the generational nature of the target pest.

Kuhar *et al.* (2002) demonstrated how inoculative biological control is able to reduce pest pressure through one release of *Trichogramma ostriniae* (Pang & Chen) (Hymenoptera: Trichogrammatidae) against the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). 72 000 female *T. ostriniae* were released per hectare, once during the season, to re-establish the population in the corn fields (Kuhar *et al.,* 2002). They indicated that the progeny from one release of *T. ostriniae* were able to proliferate and provide continuous

control of *O. nubilalis* throughout the season, without additional releases being required (Eilenberg *et al.,* 2001; Kuhar *et al.,* 2002). The mortality of *O. nubilalis* that was the result of *T. ostriniae* parasitism ranged from 61-92 % throughout the season (Kuhar *et al.,* 2002). Inoculative releases require the correct timing of a single release to decrease the pest pressure before it is exceedingly high. If timing is incorrect, additional releases may be required to ensure the population is re-established. In addition to the correct timing of releases, it is important to avoid unnecessary disturbances that have the ability to significantly reduce the population size of parasitoids, predators, and pathogens in the field (Kuhar *et al.,* 2002).

1.3.*Trichogrammatoidea* spp.

Trichogrammatoidea is a genus of egg parasitoids that is globally distributed and adapted to almost every terrestrial habitat (Malik, 2000; Wahner, 2008). This genus is able to parasitise over 300 species across eight insect orders, predominantly lepidopterans (Wahner, 2008). Egg parasitoids in the family Trichogrammatidae have been commercially recognised as efficient biological control agents for various pests on varying crop types across different agroecosystems worldwide (Smith, 1996; Pratissoli *et al*., 2004; Wahner, 2008; Samara *et al*., 2011).

One of the common species of *Trichogrammatoidea* in South Africa is *Trichogrammatoidea cryptophlebiae* (Nagaraja) (Hymenoptera: Trichogrammatidae). This species is an egg parasitoid of several lepidopteran pests of economic importance across a variety of crops grown in the country. *Trichogrammatoidea cryptophlebiae* parasitises lepidopterans from the Tortricidae, but their relative efficacy against their tortricid hosts is not known (Newton, 1988a, 1988b, 1989; Newton and Odendaal, 1990; Moore and Fourie, 1999; Moore and Richards, 2000, 2001, 2002; Moore *et al*., 2015). In order to determine the effectiveness of *T. cryptophlebiae* against various lepidopteran pests on different crops, the biology of the wasp must be elucidated in order to rear it for both laboratory and field trials successfully.

1.3.1. *Trichogrammatoidea cryptophlebiae* morphology

Trichogrammatoidea cryptophlebiae is a minute parasitic wasp that parasitises eggs of various economically important Lepidoptera within the Tortricidae family. The male adults are orange-yellow in colour with a body length that ranges between 0.44 mm and 0.50 mm and a width of 0.15 mm and 0.18 mm (Nagaraja, 1979). Female adults are more yellow than males and have a body length between 0.40 mm and 0.55 mm, and a width of between 0.18 mm and 0.20 mm (Nagaraja, 1979). The male body parts are composed of a light fuscous pronotum and

mesoscutum, a dark fuscous abdomen, an antenna with a flagellum (1.5 x the length of scape) and normal club segments with 21-24 hairs (Nagaraja, 1979). The forewings are wide (width < 0.5 x the length), remigium with long setae, infuscate at the base in line with stigma and have long fringe setae. The hind wings have fringe hairs as long as those of the forewings (Nagaraja, 1979). The genitalia are narrow (width 0.33 x the length), distinctly tapered from base to apex, and have an indistinct dorsal connecting membrane (Nagaraja, 1979). Chelate structure is far below the level of gonoforceps, the median cleft is a quarter length of the genitalia. They have medium ventral projection; lateral tubercles are indistinct, and the aedeagus has apodemes of equal length (about 0.6 x the hind tibia) (Nagaraja, 1979). Female adults have an abdomen with dark fuscous anterior, two terga, an antenna with a flagellum (> 0.5 x the length of scape), club segments as long as scape (width 0.4 x the length) and a pedicel (0.5 x the length of scape) (Nagaraja, 1979). The forewings have fringe setae (0.33 x the width), and the hind wings have fringe setae longer than those of the forewing. Their ovipositor is as long as the hind tibia, and the width of the ovipositor plate is half its length (Nagaraja, 1979).

1.3.2. *Trichogrammatoidea cryptophlebiae* biology

There has been success in rearing *T. cryptophlebiae* in South Africa on *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) eggs, which has allowed the life cycle in the laboratory to be understood. The developmental time for *T. cryptophlebiae* from egg to eclosing adult is 10 days at a temperature of 25°C, 60-65 % relative humidity and a 14:10 L:D photophase in the laboratory (Newton and Odendaal, 1990). It is not known how many eggs a *Trichogrammatoidea cryptophlebiae* female adult can lay in her 5-day life span. The larval stage is 4 days, where the entirety of the host egg contents is consumed before pupation, which is noticed by a change in egg colour to black (Figure 1.2). After 6 days in the pupal stage, *T. cryptophlebiae* adults will then emerge by chewing through the host egg shell (Scholler *et. al*., 2006, Sani *et. al.*, 2016; Zang *et al.,* 2021). Having a shorter developmental period than their host species, *T. cryptophlebiae* is a suitable control option for several pest species as it proliferates readily (Newton, 1988a, 1988b).

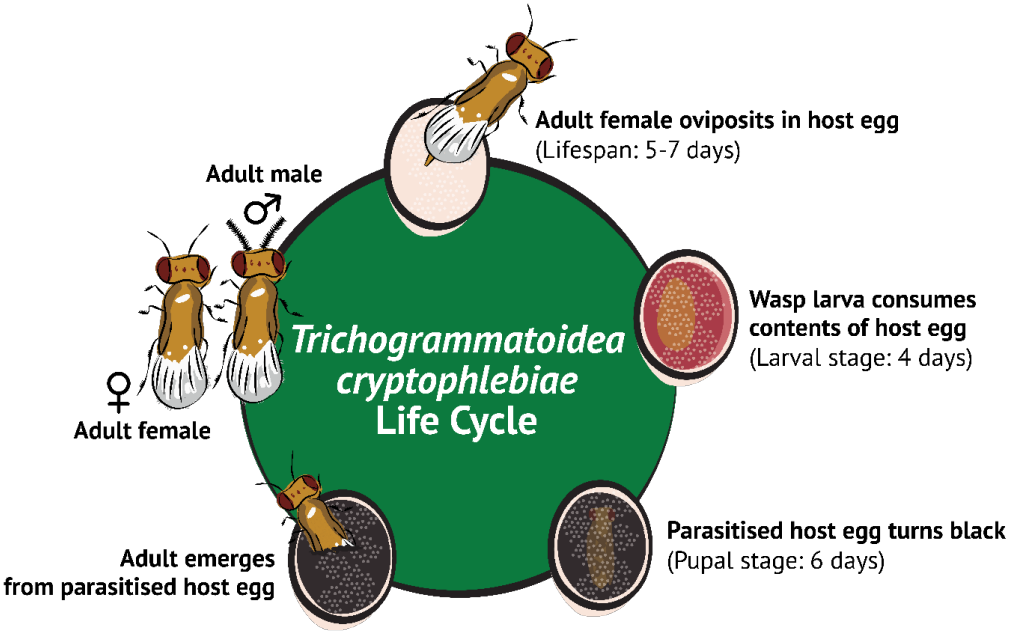

Figure 1.2. A typical life cycle of an insectary-reared *Trichogrammatoidea cryptophlebiae* egg parasitoid reared on *Thaumatotibia leucotreta* eggs.

The insectary mass-rearing of *T. cryptophlebiae* for commercial release requires that high-quality parasitoids be produced. The quality of the parasitoids is influenced by several factors in the wasp's biology (Gou *et al.,* 2017). Temperature in combination with humidity and photoperiod will influence the quality and quantity of wasps produced, as their development and behaviour can be impaired (Scholler *et al.,* 2006; Kalyebi *et al.,* 2006). High temperatures and humidity will result in an increased rate of development of the parasitoid, and the inverse is true for lower temperatures and humidity. This can have detrimental effects on the wasp's eclosion, fitness, longevity, and fecundity (Scholler *et al.,* 2006; Zang *et al.,* 2021). Malik (2000) investigated the biological parameters of *Trichogrammatoidea bactrae* (Nagaraja) (Hymenoptera: Trichogrammatidae), reared on the pink bollworm, *Pectinophora gossypiella* (Saunders, 1844) (Lepidoptera: Gelechiidae), under various temperature conditions. They found that larval development time from egg to adult takes about 36 days longer at temperatures between 13 °C and 18 °C, compared to temperatures between 23 °C and 28 °C, suggesting that *T. bactrae* develops faster at higher temperatures than at lower temperatures. Similar observations were made by Naranjo (1993), who observed that *T. bactrae* larval development period takes about 8 to 10 days at temperatures between 20 °C and 31 °C. At temperatures of 38 °C, development ceased (Naranjo, 1993). Not only does temperature affect the developmental period, but it can also influence the fecundity and

longevity of the parasitoid. Baitha & Ram (1997) conducted a study to determine the effect of cold storage (at 12 ± 2 °C for up to 6 days) on the biology of *Trichogrammatoidea* sp.nr. *armigera* (Nagaraja) (Hymenoptera: Trichogrammatidae) reared on the rice moth, *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae) eggs. After cold storage, the eggs were removed and placed under the environmental conditions, 25 ± 1 °C and 30 ± 5 % RH, to resume development. They found that adult emergence was not affected (mean of 62 % emergence). However, female longevity and fecundity were significantly reduced, from 3.4 days to 1.6 days and from 25.2 eggs laid to 9.8 eggs laid per female (Baitha & Ram, 1997). Low temperatures combined with shortened light exposure through photoperiod can lead to the induction of diapause in some *Trichogrammatidae* species. *Trichogrammatidae* will enter diapause during the larval or pupal stages of their life cycle, and as the photoperiod and temperatures increase, they will resume their development (Reznik *et al.,* 2010). There are no reports of *T. cryptophlebiae* entering diapause. The ideal environmental factors for *T. cryptophlebiae* have been determined as 25°C, 60-65 % relative humidity and a 14:10 L:D photophase (Newton and Odendaal, 1990; Moore & Fourie, 1999; Moore & Richards, 2000; Kaspi *et al.,* 2020).

An important consideration when rearing *T. cryptophlebiae*, is to consider the quality of the host-egg on which the wasp will proliferate (Cherif *et al.,* 2021). A high-quality host is preferential as the nutrient content relates directly to the wasp's longevity, fitness and fecundity (Grenier & De Clecq, 2003; Cherif *et al.,* 2021). There are numerous reports of research conducted on artificial diets for parasitoid larvae to develop in vitro, which have been successful. This research has demonstrated the impact of different components of the artificial diets on the wasp's longevity, fitness and fecundity (Cherif *et al.,* 2021). Interestingly, Norlund *et al.* (1997) showed that *T. minutum* females that were reared on an artificial diet showed higher size, longevity and parasitism rates in comparison to the females reared on irradiated bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) eggs.

The host egg not only has an impact on the quality of the emergent wasp, but it will also affect the acceptability by the female parasitoid to oviposit (Carpenter *et al.,* 2004). Female *T. cryptophlebiae* can determine if host eggs are suitable for the development of their offspring through a series of checks they perform. The female, through antennal drumming, will detect vibrations in the host egg to determine if parasitism has already occurred, as well as to determine the stage of the host egg's development (Wang *et al.,* 2016; Zang *et al.,* 2021). If the egg has not been parasitised and is not at the black head stage of its development, the female

will measure the egg through a series of backwards and forwards movements over its surface. If it is a suitable size to support her offspring, she will probe the egg with her ovipositor and, through several abdominal contractions, she will release her egg into the host egg (Wang *et al.,* 2016; Zang *et al.,* 2021). Carpenter *et al.* (2004) conducted a study where they investigated the acceptability and suitability of *T. leucotreta* (Meyrick) (Lepidoptera: Tortricidae) eggs to parasitism by *T. cryptophlebiae*. They found that 60 % parasitoids emerged from *T. leucotreta* eggs that were 24 hours or less in age when given to the parasitoids, and the percentage emergence declined on eggs of 48 hours and 72 hours of age to less than 20 % adult emergence. This suggests that *T. cryptophlebiae* prefers to parasitise *T. leucotreta* eggs that are laid in less than 24 hours. Studies conducted by Naranjo (1993) and Kaspi *et al.* (2020) also support this observation. The female *T. cryptophlebiae* biology will also have an impact on parasitism levels seen in the host egg, as she has the highest oviposition rate in the first 48 hours of emergence, with this declining as she ages (Saour, 2009). Considering these two factors of egg age and female age indicates that the provision of high-quality and newly laid host eggs should be given to *T. cryptophlebiae* females immediately after their emergence to maximise their parasitism capabilities (Kaspi, 2020; Carpenter *et al.,* 2004).

In a mass-rearing system, competition due to high-density conditions can have an influence on the quality and the quantity of parasitoids produced (Martel & Boivin, 2004). High-density conditions are generally used for the mass-rearing of trichogrammatid species, to maximise their commercial purposes. High-density conditions are known to affect the quality of the individual through phenotypic traits (Zboralski *et al.,* 2016). The most frequent effects are size reduction, decreased emergence rates and male-biased sex ratio, which will all negatively impact the success of the parasitoid in controlling its intended target pest in the field (Zboralski *et al.,* 2016). To avoid this in a mass-rearing system, factors such as female to male ratios, host egg to female wasp ratios, and food supply should be investigated to reduce competition to achieve high-quality parasitoids in high numbers whilst minimising negative traits in the parasitoid (Mawela *et al.,* 2013; Zboralski *et al.,* 2016).

Parasitic Hymenoptera can determine the sex ratio of their offspring at oviposition via haplodiploidy (Luck *et al.* 2001). It is not known if *T. cryptophlebiae* has this ability, but it tends to have a naturally higher female to male sex ratio of roughly one male to two females (Newton, 1988a; Newton, 1989). Luck *et al.* (2001) suggest that there may be two reasons for the sex ratio determination of hymenopteran parasitoids, which are the Local Mate Theory and

Host-Quality-Dependent sex allocation. The Local Mate Theory suggests that females determine the sex ratio of their offspring to be male-biased when there is an occurrence of more female parasitoids in their vicinity (Luck *et al.,* 2001). The Host-Quality-Dependent sex allocation is determined by the quality and size of the host egg, with higher quality hosts being selected for female allocation, as they require more nutrients than males (Luck *et al.,* 2001). It is important to understand how *T. cryptophlebiae* wasps determine their sex allocation in a mass-rearing setup to ensure that the population that is released in the field is always female-dominant.

The number of host eggs to female wasps has been suggested as one female *T. cryptophlebiae* wasp to four or five *T. leucotreta* eggs by Newton (1988a). Shifting the ratios of host egg to parasitoid female can increase parasitism as competition for oviposition is decreased (Mawela *et al.,* 2013; Zboralski *et al.,* 2016). Not only will adjustments in the female-to-host egg ratio increase parasitism, but it will also ensure that the correct sex ratio of one male to two females is maintained. In a mass-rearing system of *Trichogrammatidae* species intended for field releases, a higher male sex ratio should be avoided. An additional biological trait of *T. cryptophlebiae* that can influence sex ratios is self- or conspecific-superparasitism. Superparasitism is the ability of a single or multiple females to oviposit more than one egg per host egg, therefore limiting the resources that the offspring have in which to develop (DaSilva *et al.* 2016; Wang *et al.,* 2016). Superparasitism can affect physiological, morphological and behavioural characteristics of these parasitoids, all of which have a negative impact on the mass-rearing system and efficacy of the parasitoid in the field (DaSilva *et al.* 2016; Martel & Boivin, 2004). The sharing of nutrients in a single host egg will firstly have a negative influence on the developing pre-imaginal stages. The time taken from egg to emerging adult is often lengthened and often the survivorship of parasitoids in superparasitized eggs is low. The survivorship of males is generally higher in eggs that have been superparasitized (Suzuki *et al.*, 1984). Adults that have emerged from superparasitized host eggs will also suffer negative traits, such as a reduction in size, which is directly correlated to fitness and fecundity abilities, with decreased locomotion and the inability to mate (DaSilva *et al.* 2016).

Availability and quality of adult nutrients are the last factors that can impact the success of a rearing system. *Trichogrammatoidea* and *Trichogramma* adults are fed a carbohydrate source of either soaked cotton wicks, filter paper, or smears of either a honey or varying sucrose solutions, as these mimic their natural food sources of honeydew and nectar in the field

(Wäckers, 2003; Perera & Hemachandra, 2014). Feeding trichogrammatid species the correct nutrition can increase their longevity and fecundity. Different species may benefit from different carbohydrate sources, as each source will vary in its makeup of mono-, di-, and tri-saccharides (Cherif *et al.,* 2021). Adults require the nutrients for energy, which allows them to mate and oviposit eggs continually. Without a suitable source of nutrients for adults, levels of parasitism could be lower than optimal (Wäckers, 2003; Perera & Hemachandra, 2014).

1.3.3. *Trichogrammatoidea cryptophlebiae* ecology

Trichogrammatoidea cryptophlebiae has numerous interactions within its field environment that have a direct impact on its ability to be a successful biological control agent. The wasps interact with the predators, their hosts, environmental conditions and agricultural practices (Pereira *et al.,* 2004; Newton, 1988; Zang *et al.,* 2021). In the field, there are predators of *T. cryptophlebiae,* mainly of the larval and pupal stages, as these are sessile life stages. Predators include ants and assassin bugs that feed on the eggs containing *T. cryptophlebiae*, thus reducing the population size in the field, leading to a reduction in the proliferation of the parasitoid (Pereira *et al.,* 2004; Heinz & Nelson, 1996).

The proliferation of *T. cryptophlebiae* is also affected by its ability to disperse in its intended target area (Hegazi *et al.,* 2012). Being minute parasitoids, they are only capable of dispersing short distances in the field, and this can be influenced both positively and negatively by the wind, as the wind distributes the wasp in the direction it is moving. Wind can negatively impact the efficacy of the parasitoid through the displacement of the wasp from its intended target area, thereby reducing the level of parasitism for long-term control (Zang *et al.,* 2021; Zhou *et al.,* 2019; Hegazi *et al.,* 2012). In the same way that the wind can negatively impact the efficacy of the parasitoid, it can also have a positive impact by means of even dispersal within the target area, thereby aiding the proliferation and long-term control of the parasitoid (Zhou *et al.,* 2019). When releasing the wasp in the field, consideration of wind direction should be taken to avoid the displacement of the wasp out of the target area (Zhou *et al.,* 2019; Zang *et al.,* 2021).

The varying densities of host eggs against which *T. cryptophlebiae* are released can influence the released population's efficacy (Newton, 1988). There is an inverse density-dependent relationship between host egg density and *T. cryptophlebiae* parasitism. In circumstances where the host egg densities are high, the likelihood of *T. cryptophlebiae* leaving

unparasitized eggs on fruit is increased (Newton, 1988). This results in the fruit still being infested despite the parasitoid having provided a sufficient level of control to other surrounding host eggs (Newton, 1988). The reason for this occurrence is the parasitoid's host-searching behaviour. Mated females use both chemical and visual cues in a sequence to locate host eggs for oviposition (Gingras, 2001; Wang *et al.,* 2016; Zang *et al.,* 2021). First, they detect semiochemicals that indicate the host's presence. From there, they narrow down the search for their host by detecting female oviposition pheromones. Once in the vicinity of the female host pheromone, they will rely on sight to locate the host egg, where she will thereafter parasitise the egg if it is suitable for her offspring to develop in (Gingras, 2001; Wang *et al.,* 2016; Zang *et al.,* 2021). The ability of *T. cryptophlebiae* females to locate host eggs can be disrupted by agricultural practices.

Agricultural practices influence the efficacy of *T. cryptophlebiae*. *Trichogrammatoidea cryptophlebiae* populations can be decimated by the application of a synthetic chemical insecticide, as they are extremely sensitive (Zang *et al.,* 2021; Costa *et al.,* 2022). The International Organisation for Biological Control (IOBC) have developed toxicity classifications that can be assigned to plant protection products. The IOBC toxicity classifications are determined based on the plant protection products impact on the relevant natural enemies they are tested against (Table 1.1.). Chemical insecticides are not the only management practice that can cause disturbances to the ability of *T. cryptophlebiae* to suppress its host (Table 1.1.). The use of semiochemicals for mating disruption can affect the ability of the parasitoid to locate host eggs (Zang *et al.,* 2021; Kelly *et al.,* 2014). Mating disruption makes use of a female pheromone released in a large plume, blanketing the area of focus, and this can result in *T. cryptophlebiae* being unsuccessful at locating its host and therefore at suppressing the pest (Zang *et al.,* 2021; Kelly *et al.,* 2014). Cultural practices, such as orchard sanitisation, can remove the parasitoid, thereby preventing the proliferation and establishment of a population as parasitised eggs on sanitised fruit are removed from the environment (Harris *et al.,* 2022). When doing augmented releases of *T. cryptophlebiae,* it is important to incorporate this parasitoid into a pest control management programme that will support the ecological behaviour of the wasp to allow for effective pest suppression (Zang *et al.,* 2021).

Table 1.1. The active ingredients, their mode of action and the duration of harmful activity on adult parasitoids and pre-imaginal development of trichogrammatid species.

Active Ingredient	Species	Adult				Pre-imaginal		Reference
		Mode of Action	Predicted Safety Interval	IOBC Classification	Persistency Ranking	Classification	Emergence (%)	
Azadirachtin	*Trichogramma mwanzai; Trichogramma lutea*	Disrupts insect growth	1-2 days	Harmless	Short lived	Harmless	80-90	Momanyi *et al.* 2012
Bifenthrin	*Trichogramma mwanzai; Trichogramma lutea*	Affects nervous system	4 weeks	Moderately harmful	Persistent	Moderately harmful	20-40	Momanyi *et al.* 2012
Lambda-cyhalothrin	*Trichogramma mwanzai; Trichogramma lutea*	Affects nervous system	4 weeks	Moderately harmful	Persistent	Slightly harmful	40-60	Momanyi *et al.* 2012
Pymetrozine	*Trichogramma chilonis*	Affects nervous system	10-14 days	Moderately harmful	Slightly persistent	Harmless	70-85	Ko *et al.* 2015
Methoxyfenozide	*Trichogramma nr. brassicae*	Insect growth regulator	1-2 days	Harmless	Short lived	Harmless	90-95	Hewa-Kapuge *et al.* 2003
Chlorantraniliprole	*Trichogramma brassicae; Trichogramma evanescens*	Affects muscle contraction	5-7 days	Harmless	Short lived	Harmless	75-88	Ashtari 2022

Acetamiprid	*Trichogramma exiguum; Trichogramma pretiosum*	Affects nervous system	2-3 days	Slightly Harmful	Short lived	Harmless	73-90	Ambrose 2003; Moura *et al*. 2006
Novaluron	*Trichogramma pretiosum;*	Insect growth regulator	1-2 days	Harmless	Short lived	Harmless	52-81	Carvalho *et al.* 2010
Pyrethrum	*Trichogramma pretiosum*	Affects nervous system	3-5 days	Harmful	Short lived	Moderately harmful	20-40	Paiva *et al.* 2020
Abamectin	*Trichogramma pretiosum*	Affects nervous system	5 – 15 days	Harmful	Slightly persistent	Moderately harmful	0-40	Khan & Ruberson 2017
Emamectin Benzoate	*Trichogramma chilonis; Trichogramma japonicum*	Affects nervous system	5 – 15 days	Slightly harmful	Moderatley Persistent	Slightly harmful	47-61	Sattar *et al.* 2011; Uma *et al.,* 2014
Flubendiamide	*Trichogramma chilonis*	Affects nervous system	< 5 days	Harmless	Short lived	Harmless	60-80	Sattar *et al.* 2011
Imidacloprid	*Trichogramma ostriniae*	Affects nervous system	15 – 20 days	Harmless	Moderately persistent	Harmless	70-80	Tai *et al.* 2022
Indoxacarb	*Trichogramma chilonis; Trichogramma japonicum*	Affects nervous system	< 5 days	Slightly harmful	Slightly persistent	Slightly harmful	53-66	Sattar *et al.* 2011; Uma *et al.,* 2014

Lufenuron	*Trichogramma chilonis*	Disrupts insect growth	< 5 days	Slightly harmful	Short lived	Harmless	19-76	Sattar *et al.* 2011
Neem Oil	*Trichogramma chilonis*	Non-specific	< 5 days	Harmless	Short lived	Harmless	53-78	Sattar *et al.* 2011
Spinosad	*Trichogramma chilonis; Trichogramma japonicum*	Affects nervous system	16 – 30 days	Harmful	Moderately persistent	Slightly harmful	37-40	Sattar *et al.* 2011; Uma *et al.,* 2014

1.3.4. *Trichogrammatoidea cryptophlebiae* host range

Studies in the Limpopo, Eastern Cape, and Western Cape provinces of South Africa have determined effective control of *T. cryptophlebiae* hosts through augmented releases in the field (Newton, 1988a and b; Newton, 1989; Newton and Odendaal, 1990; Moore and Fourie, 1999; Moore and Richards, 2000, 2001, 2002; Moore *et al*., 2015). *Trichogrammatoidea cryptophlebiae* is a biological control agent that kills its host at the egg stage, thus preventing crop damage from the destructive larval stage of the pest (Wahner, 2008; Sani *et al*., 2016). Trichogrammatid species have the flexibility to survive and thrive in a number of environmental conditions, indicating that *T. cryptophlebiae* could be suitable for augmented releases throughout South Africa's varying climatic ranges.

South Africa's climatic conditions vary throughout the country, and in conjunction with this, the life cycle of pest species varies as well (Foerster *et al*., 2014; dos Santos Carvalho *et al*., 2017; Kalyebi *et al*., 2006). Studies done in Israel by Kaspi *et al.* (2020) on *Trichogrammatoidea cryptophlebiae* host range demonstrated that they parasitise several agricultural pest species from the lepidopteran family Tortricidae, several of which occur in South Africa (Table 1.2). Fale codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), Macadamia nut borer, *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae), Codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), Litchi moth, *Cryptophlebiae peltastica* (Meyrick) (Lepidoptera: Tortricidae) and Vanillana vine moth, *Lobesia vanillana* (de Joannis) (Lepidoptera: Tortricidae) are all pests in South Africa that *T. cryptophlebiae* has been reported to parasitise in field and laboratory settings. Field trials into the efficacy of *T. cryptophlebiae* for augmented releases against these pests in varying crops need to be investigated further.

Table 1.2.Lepidopteran species of the Tortricidae family reported as hosts for the egg parasitoid *Trichogrammatoidea cryptophlebiae*.

Crop	Country	Host Insect	Test *	Results **	Reference
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Catling & Aschenborn, 1978
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Schwartz, 1980
Macadamia	Malawi	*Thaumatotibia batrachopa*, Macadamia nutborer	F	A	La Croix & Thindwa, 1986
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Van den berg *et al*., 1987
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Newton, 1988[a]
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Newton, 1988[b]
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Newton, 1989
Litchi	South Africa	*Cryptophlebia peltastica*, Litchi moth	F	A	Newton & Crause, 1990
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Newton & Odendaal 1990
Macadamia	Malawi	*Thaumatotibia leucotreta*, False codling moth *Thaumatotibia batrachopa*, Macadamia nutborer	F	A	Chambers *et al.*, 1995
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Moore & Fourie, 1999
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Moore & Richards, 2000
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Moore & Richards, 2001
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Moore & Richards, 2002
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Carpenter *et al.*, 2004
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Moore *et al.*, 2015

Macadamia	Australia	*Cryptophlebia ombrodelta*, Macadamia nutborer	F	A	Queensland Government
N/A	Israel	*Thaumatotibia leucotreta*, False codling moth	L	A	Kaspi *et al.*, 2020
N/A		*Cydia pomonella*, Codling moth	L	A	
N/A		*Lobesia botrana*, European grapevine moth	L	A	
N/A		*Epiblema strenuana*, Stem-galling moth	L	B	
Citrus	South Africa	*Thaumatotibia leucotreta*, False codling moth	F	A	Moore, 2021

*** F, Field; L, Laboratory**

**** A, Very Promising; B, Not Satisfactory**

1.3.4.1. *Thaumatotibia leucotreta*

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae) is one of the most economically important pests in South Africa, due to its extensive range of host plants (de Jager, 2013). It is hosted by various vegetables, cereal crops, and fruit, where it can cause damage. The European Commission for Plant Health has declared *T. leucotreta* an A2 phytosanitary pest and has put regulations in place on some imported goods that are hosts to *T. leucotreta* (Adom *et al*., 2020). The South African citrus industry is put under pressure to abide by these phytosanitary pest regulations, which increases the necessity to suppress *T. leucotreta* populations in orchards across the country (Adom *et al*., 2020). There is extensive research on all aspects of *T. leucotreta*, one of them being its life cycle. Having a good understanding of the *T. leucotreta* life cycle allows one to develop and implement various methods of control across all the different stages of their development.

The development of *Thaumatotibia leucotreta* takes 30-174 days to complete, depending on weather conditions (Adom *et al*., 2020). *Thaumatotibia leucotreta* has between two and 10 overlapping generations per year, and females can lay over 800 eggs (de Jager, 2013). The eggs are therefore a critical stage at which suppression of this pest can begin. Suppressing *T. leucotreta* at the egg stage not only decreases the population numbers of the next generation but also prevents the larval stage from causing any damage to the fruit. This, in turn, reduces the percentage of yield lost to this pest and reduces phytosanitary risk in

exported fruit (de Jager, 2013). This is where *T. cryptophlebiae*, as an egg parasitoid, could be of great importance to IPM programmes, targeting the egg stage.

Trichogrammatoidea cryptophlebiae not only occurs naturally in citrus orchards but can also be supplemented commercially from insectary-reared parasitoid cultures. *Trichogrammatoidea cryptophlebiae* has proven to be the most efficient biological control agent against the egg stage of *T. leucotreta* (Moore and Hattingh, 2012). Reductions of up to 60 % in *T. leucotreta* infestations have been recorded with augmented releases of *T. cryptophlebiae* in citrus orchards in the Sundays River Valley (Newton and Odendaal, 1990; Moore and Hattingh, 2004 & 2012). Furthermore, where parasitism was not disrupted by harsh chemical sprays, egg parasitism from naturally occurring parasitoids reached between 80 % and 100 %. This resulted in a 67 % reduction in *T. leucotreta* infestation in Navel oranges, from December through to harvest, to a total elimination of *T. leucotreta* infestations by harvest (Moore and Hattingh, 2012). The positive results achieved in the above-mentioned studies provide justification for further research to be done on the efficacy of augmented releases of *T. cryptophlebiae* on citrus varieties in South Africa on a commercial scale.

1.3.4.2. *Thaumatotibia batrachopa*

Thaumatotibia batrachopa (Meyrick) (Lepidoptera: Tortricidae) is an economically important pest that is known to have two commercially relevant host plants in South Africa, macadamia and litchi (Timm *et al*., 2006). *Thaumatotibia batrachopa* infestations are most commonly found in macadamia crops, where they cause significant damage. In Malawi, *T. batrachopa* has caused crop losses of up to 20 % (Rentel, 2013). The larval form of *T. batrachopa* feeds on the inside of the green husk. In some instances, where the nut is no older than 15-18 weeks, the larva will bore through the hard shell to feed on the kernel. It is estimated that 20 % of yields are lost to *T. batrachopa*, as they cause the nut to abort or prevent it from gaining enough nutrients to mature fully (Schoeman, 2009).

Due to difficulties in rearing *T. batrachopa* in the laboratory, there are aspects of this tortricid's life cycle that are still unknown. The appearance of the moth's life stages is, however, known. In a study done by La Croix and Thindwa (1986) in Malawi, they report that a single female *T. batrachopa* can lay up to 300 eggs in her lifetime. The larvae that hatch from these eggs have the ability to damage more than a single nut during their development. The nuts that the larvae feed on are often shed by the tree before harvest, which means the percentage of crop yield lost due to this pest could be higher than the reports of 20 %.

Controlling *T. batrachopa* before it reaches the larval stage is important for ensuring optimal crop yield (de Villiers & Schoeman, 2021).

A potential IPM mechanism for the prevention of *T. batrachopa* in its egg stage is *T. cryptophlebiae*. *Trichogrammatoidea cryptophlebiae* has been shown to give effective biological control of *T. batrachopa* in macadamia nuts in Malawi (La Croix & Thindwa, 1986; Chambers *et al*., 1995). *Trichogrammatoidea cryptophlebiae* has also been seen as effective in lychee, logans, and macadamias in Australia in controlling their Macadamia Nut Borer *Cryptophlebiae ombrodelta* (Lower, 1898) (Tortricidae) (Unknown, 2022). Parasitism levels as high as 90 % on *C. ombrodelta* eggs are fairly common (BioResources, 2023). In South Africa, no research has been conducted on the efficacy of using *T. cryptophlebiae* as a biological control agent against *T. batrachopa* in macadamia orchards. Conducting research into *T. cryptophlebiae* as a control mechanism against *T. batrachopa* in South Africa could benefit this growing industry if the parasitoid proves to be effective in suppressing the pest.

1.3.4.3. *Cydia pomonella*

Cydia pomonella (Linnaeus) (Lepidoptera: Tortricidae) is a major pest of apples and pears worldwide (Pajac *et al*., 2011). It was first discovered in South Africa in 1892 (Wahner, 2008). Since its discovery, infestation rates in South Africa have been recorded as being up to 80 % in pome fruit orchards, where there are few control measures in place for this pest. Besides causing damage to apples and pears, *C. pomonella* infests quinces, walnuts, apricots, plums, and peaches, making this pest of high importance to these industries (Wahner, 2008).

Cydia pomonella has been reared in laboratories throughout the globe, which has allowed for a full understanding of its life cycle to be known. *Cydia pomonella* females are able to lay up to 150 eggs during their lifetime, either on the fruit or on nearby leaves (Pringle *et al.,* 2021). The eggs are near impossible to find with the naked eye, making scouting for them challenging. Once the larvae have hatched, they enter the fruit, either through the calyx or the side of the fruit (Brunner, 2018; Pringle *et al*., 2021). These entry points are also challenging to identify when scouting for infestation. As the larva develops in the fruit, frass will protrude from the entry point, making it slightly more noticeable. Controlling *C. pomonella* before the larval stage could significantly reduce the percentage of yield lost to this pest (Malik *et al*., 2002).

Trichogrammatoidea cryptophlebiae was reared by Wahner (2008) on *C. pomonella* eggs for trial conduction in pome fruit orchards, identifying *C. pomonella* as a physiological

host of this parasitoid wasp. Similarly, Kaspi *et al*. (2020) determined the parasitoid's host range potential and preferences, demonstrating *T. cryptophlebiae*'s efficacy in no-choice bioassays. They found that *T. cryptophlebiae* parasitises up to 47.1 % of *C. pomonella* eggs, and 79.4 % of these eggs develop into *T. cryptophlebiae* adults. Kaspi *et al*. (2020) thus demonstrated that there was potential for *T. cryptophlebiae* to provide effective control of *C. pomonella*. Wahner (2008) suggests that *T. lutea* is likely to be a more effective parasitoid against *C. pomonella* than *T. cryptophlebiae*. However, further, more targeted in field testing needs to be conducted in South Africa to determine the control potential of *T. cryptophlebiaae* on *C. pomonella*.

1.3.4.4. *Cryptophlebia peltastica*

Cryptophlebia peltastica (Meyrick) (Lepidoptera: Tortricidae) is a pest of macadamia, mango, quince, and litchis in South Africa (Rentel, 2013). 58 % of litchis produced in South Africa are exported to Europe (South African Litchi Growers' Association, 2022b; Grove & de Beer, 2017). This makes *C. peltastica* a pest of high economic importance, and to avoid this pest receiving a phytosanitary status. Infestation levels of *C. peltastica* have been recorded as high as 15 % in some parts of South Africa and up to 20 % in Mauritius (Rentel, 2013; Manrakhan *et al*., 2008).

Cryptophlebia peltastica cultures have been established in laboratories on artificial diets, however their life history in the field has not been studied in detail. The female *C. peltastica* lays her eggs singly on the fruit during its ripening stage of development (Newton & Crause, 1990; Manrakhan *et al*., 2008). The larval stage causes damage to the litchi fruit as they bore through the skin, where they feed on the fruit flesh. Infestation reports from the 2004/5 season were variable, ranging from 0 to 12 % (Grove & de Beer, 2017). Preventing the larval stage from hatching could significantly reduce the percentage of yield lost to this pest (Grove & de Beer, 2017).

The parasitoid, *T. cryptophlebiae,* parasitises *C. peltastica* eggs in South Africa (Newton and Crause, 1990). If done early in the season, augmentative releases of *T. cryptophlebiae* could provide a viable alternative to the chemical controls currently used during the different life stages of *C. peltastica* development (Newton and Crause, 1990). The efficacy of *T. cryptophlebiae* against *C. peltastica* in the field is not known, and further research into this needs to be conducted.

1.3.4.5. *Lobesia vanillana*

Lobesia vanillana (de Joannis) (Lepidoptera: Tortricidae) is considered to be a sporadic minor pest in South Africa and causes similar damage to its relative, *Lobesia botrana* (Denis & Schiffermüller), which originates from Europe and feeds predominantly on grapes (Lepidoptera: Tortricidae) (Du Preez, 2019). *Lobesia vanillana* has only recently been recorded as a pest in South Africa and has only been identified as a potential pest on two crops, wine grapes and macadamias, both of which are important to the agricultural industries in the country (Morland, 2015; Enslin, 2023). Morland (2015) also recorded *L. vanillana* from a couple of damaged citrus fruits in the Western Cape. This infestation could therefore be secondary, not indicating a pest status on citrus. Authors speculate that *L. vanillana* has only recently been identified as a damaging pest, due to the shift away from the use of broad-spectrum insecticides in favour of IPM methods that target specific species. This has allowed *L. vanillana* populations to increase and for their damage to surpass economic thresholds in some regions of South Africa (Du Preez, 2019). An effective form of control against *L. vanillana* is necessary to keep this pest suppressed.

There are a few accounts of *L. vanillana* in the literature. It has, however, been determined as a polyphagous insect. Brown *et al.* (2014) were able to successfully rear it on Rutaceae, Anacardiaceae, Solanaceae, Icacinaceae and six other plant families. A few observations have been made on its life cycle by Du Preez (2019). Du Preez (2019) observed a four-to-five-week developmental period with three to four flight peaks occurring in a growing season. The eggs of *L. vanillana* are laid on grape berries, where the entire life cycle of this pest will then occur (Du Preez, 2019). The larvae hatch and start causing damage by feeding on the grape berries, which eventually develop bunch rot. This damage is synonymous with the damage caused by *L. botrana* (Du Preez, 2019).

Kaspi *et al.* (2020) showed that *T. cryptophlebiae* parasitises *L. botrana* eggs in a study where they investigated *T. cryptophlebiae* host range potential and preferences in Israel. They found that *T. cryptophlebiae* parasitises up to 100 % of *L. botrana* eggs, with some superparasitism, and with 76.5 % of these eggs developing into *T. cryptophlebiae* adults. *Lobesia botrana* and *L. vanillana* are close relatives. Therefore, it is highly likely that *T. cryptophlebiae* will be able to parasitise the eggs of *L. vanillana*. Research into determining this possibility would be highly valuable as a solution to the control of *L. vanillana* at the egg stage of its life cycle.

1.4. Justification

This literature review has identified areas in which *T. cryptophlebiae*'s biology, as it relates to mass-rearing, that need to be developed to ensure the biological integrity of this wasp from mass-reared cultures that are intended for augmented releases is maintained. Prior work on other trichogrammatid species has provided a guideline on the necessary knowledge required to ensure that *T. cryptophlebiae*'s biological integrity is maintained under mass-rearing systems.

This literature review has also identified key lepidopteran pest species in the family Tortricidae that impact the production of fruit crops of economic importance in the South African agricultural industry. Most, if not all, these pest species reportedly act as hosts to the egg parasitoid *T. cryptophlebiae*. Prior work in citrus has demonstrated that *T. cryptophlebiae* has the potential to provide area-wide pest population suppression when used as an augmentative biological control agent. However, further work is required to determine the efficacy of augmented releases of *T. cryptophlebiae* on the aforementioned pests and their crops.

1.5. Aims And Objectives

There were two aims of this research project: firstly, to develop our knowledge on *T. cryptophlebiae* biology under mass-rearing conditions, and secondly, to determine whether this parasitoid can be used on a commercial scale, as an effective biological control agent against *Thaumatotibia batrachopa*, *Cryptophlebia peltastica*, *Thaumatotibia leucotreta*, *Cydia pomonella* and *Lobesia vanillana.*

Although research has already been undertaken in citrus, there are limited data available on the use of *T. cryptophlebiae* in other fruit and nut crops. The focus will be to validate efficacy against these pests in other target crops, such as macadamias, litchis, pome fruit, stone fruit, and grapes, on a commercial scale. This will involve identification and collaboration with farmers and other industry stakeholders throughout the different fruit and nut growing regions of South Africa. Investigations into various aspects of using *T. cryptophlebiae* in a successful commercial biological control programme will also be conducted. Studies will focus on developing a mass-rearing strategy that ensures the parasitoid's biological integrity remains intact, establishing a guideline for the timing of applications in different crops, and on integration with other sustainable pest management practices. This project aimed to achieve the following three objectives:

A. The development of a suite of biological information on *T. cryptophlebiae* to ensure the biological integrity of this parasitoid is maintained in mass-rearing systems and allows for the optimisation of these systems.
B. The validation of the field efficacy of *T. cryptophlebiae* against the above identified tortricid pests in economically important fruit crops in South Africa.
C. The development of integration guidelines of *T. cryptophlebiae* into the field through determining the non-target effects of other chemical pest management practices on all life stages of this parasitoid.

Chapter 2: Mass-rearing of *Trichogrammatoidea cryptophlebiae*

2.1. Introduction

The process of mass-rearing beneficial insects differs from one species to the next because of differences in the biology and ecology of different insects, as well as the intended purpose of that insect (Leppla *et al.,* 2023). Globally, over 350 species of natural enemies have been mass-reared in large enough quantities to be made commercially available (van Lenteren, 2003; van Lenteren *et al.*, 2018). Each of these species is produced with the specific intention of controlling an intended target pest, whether it be through predation or parasitism, and the mass-rearing process must ensure the biological integrity of the species is kept through monitoring for quality assurance of biological parameters (van Lenteren, 2003). For decades, natural enemies from the genus *Trichogramma* have been mass-reared in biofactories in Asia and Europe, where their biological integrity has been successfully maintained. In South Africa, the species *Trichogrammatoidea cryptophlebiae* has been successfully reared, over many decades, by several research facilities and companies on *T. leucotreta* eggs for trials and commercial releases (Newton, 1988a and b; Newton, 1989; Newton & Odendaal, 1990; Moore & Fourie, 1999; Moore and Richards, 2000, 2001, 2002; Moore *et al*., 2015).

Trichogrammatoidea cryptophlebiae is a generalist egg parasitoid that has been reported to have successfully parasitised and emerged from species in the Grapholitini tribe, and one of the closely related Olethreutini tribe of the Tortricidae family (Newton, 1988a and b; Newton, 1989; Newton & Odendaal, 1990; Moore & Fourie, 1999; Moore and Richards, 2000, 2001, 2002; Moore *et al*., 2015). The species *T. cryptophlebiae* has been reported parasitising *T. leucotreta, C. pomonella, C. ombrodelta, C. peltastica, Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae), *T. batrachopa* and *L. botrana* (Kaspi *et al.,* 2020; BioResources, 2023; Newton & Crause, 1990; Chambers *et al*., 1995). The broad host range suggests that species other than *T. leucotreta* could also be used as successful hosts for this egg parasitoid to be reared on in a mass-rearing system. Several aspects of the interactions between the various hosts and *T. cryptophlebiae* need to be investigated, as the interactions they have with each other can directly impact parasitism levels and the fitness of the emergent adults.

2.1.1. *Trichogrammatoidea cryptophlebiae*-Host Interactions

Egg parasitoids, in general, interact with their idiobiont hosts at two points. The first is the adult-host egg interaction, and the second is the pre-imaginal-host interaction. Adult female egg parasitoid species are known to inspect the host egg to ensure it is suitable for her offspring to develop successfully. Female parasitoids may accept different hosts at varying levels for oviposition, based on host egg features, such as size, chorion thickness, and nutritional qualities (Pehlivan, 2021; Para, 2010; Saour, 2009). Each potential lepidopteran host of *T. cryptophlebiae* will differ in their overall egg morphology, which can influence the biology and ecology of the emergent wasps (Pehlivan, 2021). This factor is of great importance to mass-rearing, as it can impact the production capacity of a mass-rearing system if *T. cryptophlebiae* were to accept different hosts at different levels.

Adult female parasitoids of some trichogrammatid species, based on their interaction with the host egg and their surroundings, can make decisions regarding the sex ratio of their emerging offspring (Para, 2010). Firstly, if the host egg is small, ovipositing females can choose to oviposit an unfertilized egg, resulting in male development (Cherif *et al.*, 2021). Males require fewer nutrients to develop, as they are generally smaller than females, develop faster, and have a shorter life span (Suzuki *et al.*, 1984). The second possibility is that, if the host egg is of a suitable size, *T. cryptophlebiae* can super-parasitise it (Kaspi *et al.,* 2020). Superparasitism often results in increased male emergence, as the sharing of an egg limits the space and nutrients available for the development of multiple wasp larvae (Suzuki *et al.*, 1984). Not only does superparasitism increase the possibility of males emerging, but it can also result in lower emergence, as the pre-imaginal stages must compete for resources in a nutritionally restricted area, which results in unsuccessful development (Suzuki *et al.*, 1984; Para, 2010).

Trichogrammatoidea cryptophlebiae and all other egg parasitoids have nutritional constraints imposed on them by the host egg during their pre-imaginal stages of development (Martel *et al.*, 2011). The host and the egg parasitoid have a unique ecological interaction as specific characteristics of the host can pre-determine characteristics of the emergent parasitoid. The host egg has limited nutritional content available to the developing egg parasitoid, which can impact aspects of the emergent wasp's fitness and developmental success (i.e., emergence). The volume, size, and age of the host egg are characteristics that can limit the nutritional availability

of the developing egg parasitoid (Pehlivan, 2021). Numerous studies have been conducted on the developmental success and fitness traits of egg parasitoids that have developed in natural hosts of varying sizes and volumes, factitious hosts, artificial hosts, and ageing host eggs (Martel *et al.*, 2011; Kishani Farahani *et al.,* 2016; Heslin *et al.,* 2005). It has been determined that younger eggs are of a higher quality and produce offspring that have increased fecundity, longevity, and flight ability (Carpenter *et al.*, 2004; Cherif *et al.*, 2021; Tian *et al.*, 2017; Kishani Farahani *et al.,* 2016). As the host egg ages, the nutritional content changes, which can be unsuitable for the wasp to make full use of for development, resulting in the emergent parasitoid having lower fitness characteristics or unsuccessful development. Substitutions of natural hosts for either artificial or factitious hosts have shown how nutrient elements in the host egg can have an impact on the development of egg parasitoids and their fitness parameters (Norlund *et al.,* 1997; Cherif *et al.*, 2021; Zang *et al.*, 2021; Heslin *et al.*, 2005). There are cases where factitious hosts have produced egg parasitoids of higher fitness than the natural host, and this could be due to various reasons, one of which could be the host egg size (Norlund *et al.,* 1997; Zang *et al.*, 2021). Cherif *et al.* (2021) and Martel *et al.* (2011) report that host egg size directly influences the body size of the emergent female wasp, which determines the parasitoid's fecundity. Martel *et al.* (2011) reported a higher fecundity for females and males of *Trichogramma euproctidis* (Hymenoptera: Trichogrammatidae) that developed in larger hosts, even though the nutritional qualities of the three host egg species used in the research were similar (Martel *et al.*, 2011).

The interactions between *T. cryptophlebiae* female parasitoids and pre-imaginal stages with the host egg species must be thoroughly understood to determine the best possible rearing methods for mass production and to optimise the mass-rearing system continually. The host species selected for use in a mass-rearing system should indicate high levels of acceptance by the female parasitoid, should support the emergence of fit wasps suitable for field releases, and should ensure the production of offspring of a desirable sex ratio before wasps are further exposed to the influences of a mass-rearing system. Mass-rearing systems are generally overcrowded environments, and competition is prevalent among adult egg parasitoids (Para, 2010). The influences of competition in these mass-rearing systems must be elucidated to implement quality control measures that maintain the egg parasitoids' biological integrity.

2.1.2. Influences of Mass-Rearing Systems on *Trichogrammatoidea cryptophlebiae*-Host Interaction

Competition for mates and oviposition is a high occurrence in mass-rearing systems, and this can be caused by skewed sex ratios in the population (Luck *et al.,* 2001). The sex ratio can be skewed at varying levels, with different outcomes arising from these. In the case of a population being male-dominant, mating competition can lead to low parasitism levels as males bombard females, decreasing their time to actively parasitise eggs (Luck *et al.,* 2001). If the population is too heavily female-dominant, three outcomes are possible. Firstly, the frequency of mating may be decreased as the population lacks males, and due to many egg parasitoids being haplodiploid, this could result in the following generations' sex ratio being male-dominant (Luck *et al*., 2001; Martel & Boivin, 2004). Secondly, several trichogrammatid females can decide whether they oviposit fertilised eggs, producing females, or unfertilised eggs, producing males, and they make this decision based on their current surroundings (Luck *et al.* 2001; Martel & Boivin, 2004). The adult female acts to ensure her genetics have the highest probability of surviving through to the following generations. This suggests that if a female encounters a high number of other females in her vicinity, she will opt to oviposit unfertilized eggs as she has determined that the highest probability for her genetics to be passed on is through the production of males (Luck *et al.* 2001; Suzuki *et al.*, 1984). Thirdly, trichogrammatids can determine the availability of host eggs to them. This allows parasitoids to allocate offspring per host egg appropriately, and host numbers are conserved in the immediate environment of the parasitoid. If they determine that there is a limited number of host eggs, there may be a higher occurrence of superparasitism (DaSilva *et al.,* 2016; Martel & Boivin, 2004). Superparasitism can lead to more males being oviposited, but it can also reduce the overall fitness of the population (Suzuki *et al.*, 1984). Sex ratios can be challenging to manage in a mass-reared population from one generation to the next. One method to manage sex ratios, and therefore reduce competition for resources, is to ensure the correct ratio of females to host eggs is consistently maintained. The sex ratio of the population will balance itself if there is an adequate number of eggs available per female (Mawela *et al.,* 2013; Zboralski *et al.,* 2016). Newton (1988a) mentions that *T. cryptophlebiae* had been reared at a one-female to four or five-host egg ratio, resulting in a one-male to two-female sex ratio.

Managing the influences of a mass-rearing system on the interactions between *T. cryptophlebiae* and its host is crucial to ensuring the parasitoid population's biological integrity

and the consistent production of the parasitoid for commercial sale. Mass-rearing systems are complex, and implementing them requires constant research that allows for processes to be optimised to ensure production consistency and product quality (Para, 2010).

2.1.3. Optimisation of a Mass-Rearing System for *Trichogrammatoidea cryptophlebiae*

The mass-rearing of *T. cryptophlebiae* requires the simultaneous production of it and its host, both of which must be produced consistently to meet the necessary production requirements (Para, 2010; Cherif *et al.,* 2021). A mass-rearing system can be optimised at two points: the production and use of host eggs and the product shipment, which ensures production consistency and timely delivery of the product to customers (Para, 2010; Cherif *et al.,* 2021).

The supply of host eggs can be inconsistent, directly influencing the production capacity of the parasitoid (Para, 2010). To reduce the occurrence of inconsistent host egg production, options have been researched that indicate that stockpiling host eggs for later use in the mass production of trichogrammatids is possible (Para, 2010; Cherif *et al.,* 2021; Zang *et al.*, 2021). Stockpiling has been done through cold storage methods, ranging from a standard fridge to liquid nitrogen immersion (St-Onge *et al.*, 2014; Zang *et al.*, 2021; Drooz, 1981). St-Onge *et al.* (2014) suggest that the thicker the chorion, the more suitable the host egg is for freezing, allowing for extended storage periods. Different methods have been used for other species, and the most suitable one must be determined for the host eggs used to rear *T. cryptophlebiae*.

Another way to ensure consistent production of the wasp is to investigate using an artificial diet to rear the wasps (Para, 2010). There have been several studies on the use of artificial diets, which have proven to be less than ideal for trichogrammatid mass production and require further research (Cherif *et al.,* 2021; Norlund *et al.,* 1997; Heslin *et al.*, 2005; Notarte & Merritt, 2001). These artificial diets have shown low parasitism and emergence and a higher occurrence of deformities in adult wasps (Cherif *et al.,* 2021). A different option that has been researched is ultraviolet (UV) sterilisation of host eggs (Cherif *et al*., 2021; St-Onge *et al*., 2014). Sterilisation of host eggs has been successful for some species, removing larval development concerns during cold storage at temperatures above zero, which is a commonly used method of host egg preservation (Cherif *et al.,* 2021; St-Onge *et al.*, 2014). The sterilisation of the host eggs also provides more time for their provision to parasitoids, as the larvae in the eggs will no longer reach the blackhead stage of development, which is when *T. cryptophlebiae* and other parasitoids will

stop parasitising the eggs (Cherif *et al.,* 2021; St-Onge *et al.*, 2014). Sterilisation of the host eggs will also allow for timely shipment to customers, as there is no need to wait for the eclosion of the larvae from the host egg before sending the product out to customers (Cherif *et al.,* 2021). Sterilisation of the host eggs can lead to reduced levels of parasitism and emergence, but for some species, the trade-off is justifiable (Cherif *et al.,* 2021).

Trichogrammatoidea cryptophlebiae has an average developmental time of 10 days from oviposition to emergence at a temperature of 25°C ± 1, relative humidity of 60 % ± 5, and a photoperiod of 16:8 Light: Dark (L:D), and increased temperatures can increase the rate of their development (Newton & Odendaal, 1990). This is essential information to know regarding the shipment of the parasitoid to the customer. The shipment of the parasitoid from the insectary to customers' needs to be done promptly to ensure the customer has ample time to release the wasp into the field. Insectaries that rear trichogrammatids from colder regions of the world have induced diapause of these species, allowing them to stockpile the parasitoid until they are required (Reznik *et al.,* 2010; Para, 2010). Unfortunately, diapause in neotropical, tropical, and subtropical species still needs to be researched to determine if these trichogrammatids can be induced into diapause (Para, 2010). Although research into diapause still needs to be undertaken, other cold storage methods have been developed that allow the emergence of trichogrammatids to be delayed, which aids in the time constraints of product shipments around the country (Baitha & Ram, 1997; Para, 2010; Stinner *et al.*, 1974). Stinner *et al.* (1974) have reported delaying the development of *T. pretiosum* for up to 10 days with no detrimental effects to the parasitoid's fitness. Developing a methodology to delay the development of *T. cryptophlebiae* will allow for the short-term stockpiling of the parasitoid, which will aid in addressing product shipment time constraints and disruptions caused to production if they were to arise.

The aims of this chapter were to:

A. Develop the understanding of *T. cryptophlebiae* biology to develop indicators of quality control.
B. Determine the influences of competition on *T. cryptophlebiae* to implement the most suitable ratio of host eggs to female wasps in a mass-rearing system.
C. Determine which host, *T. leucotreta, C. peltastica, C. pomonella,* or *L. vanillana*, is most suitable for rearing *T. cryptophlebiae*.

2.2. Materials and methods

2.2.1. Source of Insects

Trichogrammatoidea cryptophlebiae was sourced from River Bioscience's production culture, originally sourced from the Sundays River Valley in 2022. The parasitoid was reared at a temperature of 25°C ± 1, a relative humidity of 60 % ± 5, and a photoperiod of 16:8 Light: Dark (L:D). *Trichogrammatoidea cryptophlebiae* was reared on 180 gamma-ray irradiated *T. leucotreta* eggs sourced from XSIT's *T. leucotreta* mass-rearing facility in Citrusdal. The parasitoid culture did not have new genetic material from the field introduced to it during the duration of these trials, as there was no reduction in the performance of the insect to suggest inbreeding depression.

Thaumatotibia leucotreta, L. vanillana, and *C. peltastica* eggs were sourced from River Bioscience's research cultures where they are all reared on an extruded maize-based diet that was sourced from XSIT (Boersma, 2021). *Thaumatotibia leucotreta* species are reared at a temperature of 26°C ± 1, relative humidity of 50 % ± 5, and a photoperiod of 12:12 L: D. *Lobesia vanillana* is reared at a temperature of 24° ± 1, relative humidity of 50 % ± 5, and a photoperiod of 12:12 L: D. *Cryptophlebiae peltastica* is reared at a temperature of 25° ± 1, relative humidity of 70 % ± 5, and a photoperiod of 12:12 L: D. *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) eggs were sourced from River Bioscience's production culture where the species was reared on a soya flour-based diet, at a temperature of 25° ± 1, relative humidity of 65 % ± 5, and a photoperiod of 12:12 L: D. *Cydia pomonella* eggs were sourced from Hortgro's culture in Stellenbosch, Western Cape, South Africa and from River Bioscience's production culture where they were reared at 26˚C, relative humidity of 65 % ± 5, and a photoperiod of 16:8 L: D on a wheat-based diet. Due to difficulties rearing *Thaumatotibia batrachopa*, no trials have been conducted in the laboratory.

For all tests, *T. cryptophlebiae* was provided with a modified version of Perera & Hemachandra's (2014) carbohydrate source, consisting of filter paper soaked in a 20 % sugar solution for one hour. The excess sugar solution was allowed to drain off the filter paper, and this was done to prevent *T. cryptophlebiae* adults from becoming stuck in the carbohydrate source.

2.2.2. Indicators of Fitness for Quality Control

Adult Size Variation

One hundred males and 100 females were randomly selected from the River Bioscience culture. For each individual, measurements of their head capsule, body length, forewing, and hind

tibia were taken. This was done to determine the variation in the size of male and female wasps. Individual wasps were mounted on a glass slide with a 0.01 mm ruler, with a drop of 70 % ethanol and a cover slip placed on top, and images were captured under a compound light microscope (Zeiss Primo Star) with a mounted camera (Zeiss Axiocam ERc 5s). The magnification of the microscope used was set to 4x/0.10, and the images were used for the measurements. Head capsule width was measured from the lateral margins of the compound eyes at their widest point. Body length was measured from the posterior margin of the head capsule to the posterior tip of the abdomen. Forewing measurements were done by measuring the central vein on their wing, and hind tibia measurements were done from the femur joint to the tarsal joint. The measurements were then taken using ImageJ 1.54d software, where every sample had the ruler calibrated to ensure measurements were to the nearest 0.01 mm.

Haplodiploidy Confirmation

One hundred single-parasitised eggs were separated from parasitised egg sheets containing numerous eggs and placed individually into glass vials for wasps to emerge. This was done to ensure no females were mated and only virgin females were used for the haplodiploidy testing. After parasitoid emergence, they were sexed, and all males were discarded, and only 30 virgin females were kept for haplodiploidy testing.

The 30 virgin female *T. cryptophlebiae* were individually provided with an excess of 24-hour-old *T. leucotreta* eggs on a piece of wax paper in their vials. After 24 h, the eggs were removed from the female parasitoid vial and placed into petri dishes to develop.

After 16 days at 25°C ± 1, relative humidity of 60 % ± 5, and a photoperiod of 16:8 L: D. The number of parasitised eggs and offspring were counted and sexed using a dissecting microscope, laminate counting grid, tally counter, and soft forceps, to determine if virgin-unmated female *T. cryptophlebiae* only produce male offspring.

Flight Testing

Three hundred *T. cryptophlebiae* parasitised eggs were placed into flight chambers as described by Forsse *et al.* (1992). The flight chambers were made from polyvinyl chloride (PVC) cylinders. The PVC flight chambers were 30 cm high and 10 cm in diameter (Figure 2.1). A Petri dish was fitted to the bottom of the chamber where the parasitised eggs with emerged wasps were

placed. The chamber's top was fitted with a second blacked-out petri dish, blocking any light. The top petri dish had a hole cut out to fit a small vial that contained irradiated *T. leucotreta* eggs, a carbohydrate source, and to allow light to pass into the chamber. This was done to attract the parasitoids to the top of the flight chamber. At 4 cm from the base of the flight chamber, a 0.5 cm strip of glue on a piece of plastic was fitted into the chamber. This was done to prevent walkers from reaching the top of the flight chamber. Every hour for 8 h, the vial at the top of the chamber, containing *T. cryptophlebiae* adults, was removed and replaced with another empty vial. Upon removal, vials were placed into the freezer to kill any *T. cryptophlebiae* adults contained therein. Once dead, each wasp was sexed, and the length of the forewing and hind tibia were measured to determine if there was a relationship between these features and flight ability for both male and female *T. cryptophlebiae*. The glue strip in the flight chamber was removed, and the males and females were counted to determine the percentage of "walkers" in the population. The parasitoids' forewings and hind tibia were measured according to the earlier description.

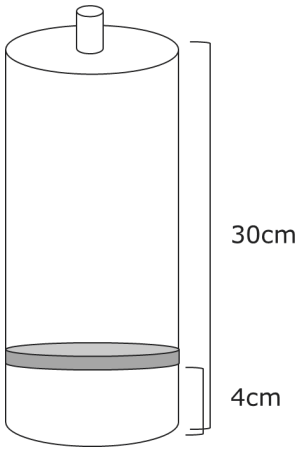

Figure 2.1. Diagram of the flight chamber design.

Fertility Testing

A newly mated female *T. cryptophlebiae* with no exposure to host eggs was placed in a glass vial containing a surplus of 24-hour *T. leucotreta* eggs on a piece of wax paper, following the experimental design of Thomson and Hoffman (2002). The *T. cryptophlebiae* female adults were easily distinguished from the males through the presence of long setae on the male antennae and the absence of setae on the female antennae. To handle the parasitoids to place them into the

vials, petri dishes were placed on ice to immobilise the parasitoids. Individual *T. cryptophlebiae* adults were carefully picked up using a modified paintbrush and placed into the glass vials.

After 24 h, the eggs were removed from the female parasitoid's vial, and a new set of 24-hour-old *T. leucotreta* eggs were provided to the same female, and the previous set of eggs were placed in a petri dish to develop. The removal and replacement of *T. leucotreta* eggs into a vial with a single female happened every 24 h. Carbohydrate sources were replenished every 48 h.

After 120 h, the female *T. cryptophlebiae* was removed and placed in a vial and frozen in a -20°C standard freezer to measure her head capsule, body length, forewing, and hind tibia lengths after she died. This was done for 150 females over 5 separate trials.

The recently parasitized host eggs were placed in a petri dish where they were left to develop for 16 days at a temperature of 25°C ± 1, relative humidity of 60 % ± 5, and a photoperiod of 16:8 L: D. The number of parasitized eggs, emergence holes, superparasitism, unsuccessful emergence, and sex ratio were counted to determine the fecundity of the female, and her sex allocation. The head capsule, body length, forewing length and hind tibia length were measured to determine if there was a relationship between parasitism and those four features of the parasitoid. The parasitoid measurements were done according to the earlier description.

2.2.3. Influence of Mass Production

Competition Testing

An unlimited number of eggs were provided to a single mating pair of *T. cryptophlebiae* for 48 h. The number of parasitised eggs from thirty separate mating pairs was counted, and the average number of parasitised eggs across three replicates of this trial was determined to determine the correct number of eggs to provide *T. cryptophlebiae* mating pairs for the following trials.

The average number of irradiated *T. leucotreta* eggs that *T. cryptophlebiae* could parasitise in 48 h was determined as 42. Host eggs were then provided to mating pairs of *T. cryptophlebiae* at a 42:1 ratio in 30 ml glass vials containing a carbohydrate source (Table 2.1.).

Table 2.1. The number of pairs of *Trichogrammatoidea cryptophlebiae* exposed to a set number of host eggs.

Number of Pairs of *Trichogrammatoidea cryptophlebiae*	**Number of Host Eggs**
1	42
2	84
3	126
4	168
5	210
6	252
7	294
8	336
9	378
10	420

The 42:1 ratio (host egg: female parasitoid) was selected to determine if increasing the number of mating pairs exposed to a host egg affected parasitism, emergence, unsuccessful emergence, superparasitism, and sex ratio, which is expected in a mass-rearing setup. The pairs of parasitoids were left for 48 h to parasitise host eggs and then removed. The host eggs were then left to develop and emerge for 16 days at 25°C ± 1, a relative humidity of 60 % ± 5, and a photoperiod of 16:8 (L:D). The eggs were then counted for parasitism, emergence, unsuccessful emergence, superparasitism, and the sex ratio of the emergent parasitoids was determined.

Foundress Trial

Different host patch sizes were selected to ensure competition for oviposition occurred, and the host patch size selection was based on data collected from previous trials According to the fertility trial results, the average number of eggs parasitised by female *T. cryptophlebiae* in 24, 48 and 120 h were 17, 48 and 51 respectively. Therefore, host patch sizes of 20, 40, and 60 were selected. The selected host patch numbers were exposed to 1, 3, 5, 7, and 9 *T. cryptophlebiae*

foundresses (females) for a 24 h period. The number of foundresses was selected to ensure no repetition in the ratio of host eggs to females and to demonstrate how varying foundresses can impact the sex ratio of their offspring. The *T. cryptophlebiae* foundresses were allowed to mate for 24 h post-emergence before being placed in the respective treatments (Tables 2.2.).

After 24 h of opportunity to parasitise host eggs, the host patches were removed and left to develop and emerge for 14 days at 25°C ± 1, a relative humidity of 60 % ± 5, and a photoperiod of 16:8 (L: D). The eggs were then counted for parasitism, emergence, unsuccessful emergence, superparasitism, and the sex ratio of the emergent parasitoids was determined for each combination of sex ratios.

Table 2.2. The combinations of foundress numbers and differing host patch sizes of *Thaumatotibia leucotreta* eggs that were used in the Foundress trial.

Number of Foundress	Host Patch Size
1	20
1	40
1	60
3	20
3	40
3	60
5	20
5	40
5	60
7	20
7	40
7	60
9	20
9	40
9	60

2.2.4. Host Specificity Testing

No-choice Testing

Ten sets of 100 eggs from each species, *T. leucotreta*, *C. peltastica*, *L. vanillana*, C. *pomonella,* and *H. armigera,* were counted under a dissecting microscope and placed into glass vials. *Helicoverpa armigera* was included in the study as a negative to control, to confirm that *T. cryptophlebiae* is likely to only parasitise eggs of lepidopteran species from the Tortricidae family.

A male and female pair of *T. cryptophlebiae* adults were placed into the glass vials with the carbohydrate source and respective host eggs. This was done by placing a petri dish of newly emerged *T. cryptophlebiae* in a fridge set at 10 °C for 1 h to reduce movement. The *T. cryptophlebiae* adults are easily sexed through the presence of setae on the male antennae and the absence of setae on the female antennae. The *T. cryptophlebiae* adults were carefully picked up using a modified paintbrush and placed into the glass vials. The glass vials were closed using cotton wool to prevent the *T. cryptophlebiae* from escaping.

The host eggs were exposed to *T. cryptophlebiae* adults for 48 h, and the females were examined to determine if they were still alive or had died during the 48 h. The host egg cards were removed from the glass vials and placed into Petri dishes, allowing the larvae to hatch. The parasitoids developed in the host eggs for 16 days at a temperature of 25°C ± 1, a relative humidity of 60 % ± 5, and a photoperiod of 16:8 L: D. Hatched larvae were removed from the Petri dishes. The Petri dishes were sealed with parafilm to prevent *T. cryptophlebiae* adults from escaping. Thirty eggs per host had their lengths (2a) and widths (2b) measured using a dissecting microscope and ocular micrometre to calculate the defined ellipse (axbxπ) (Kaspi *et al.,* 2020).

$$a\ (mm) = \frac{Length\ (2a)}{2}$$

$$b\ (mm) = \frac{Width\ (2b)}{2}$$

$$Ellipse\ (mm^2) = a\ x\ b\ x\ \pi$$

The number of parasitised eggs, emergence holes, superparasitism, and sex ratio were determined under a dissecting microscope using a laminate counting grid, tally counter, and soft

forceps. The forewing and tibia measurements were done according to the description used in the flight and fecundity tests.

Choice tests

Ten sets of 50 eggs from the respective tortricid cultures (Table 2.3.) were paired and placed into a glass vial. A single-mated adult female *T. cryptophlebiae* was placed into the glass vial for 120 minutes to parasitise eggs (Kaspi *et al.,* 2020). The glass vial was closed with cotton wool to prevent the female *T. cryptophlebiae* from escaping. After 120 minutes, the eggs were removed from the vial containing the female and placed into a Petri dish for 16 days, where the eggs were allowed to develop at a temperature of 25°C ± 1, a relative humidity of 60 % ± 5, and a photoperiod of 16:8 L: D. Thirty samples of each tortricid egg pairing were taken.

Helicoverpa armigera was excluded from the choice tests as no parasitism was found in the no-choice trials.

The number of parasitised eggs and emergence holes was counted under a dissecting microscope using a laminate counting grid, tally counter, soft forceps, and ocular micrometre.

Table 2.3. The pairings of tortricid eggs are to be used in choice trials. (Abbreviations indicate species names, CM - *C. pomonella*, LV - *L. vanillana*, LM - *C. peltastica*, FCM Non-irradiated - *T. leucotreta* non-irradiated eggs, FCM Irradiated - *T. leucotreta* irradiated eggs).

Tortricid 1	**Tortricid 2**
CM	FCM Non-irradiated
CM	FCM Irradiated
CM	LM
CM	LV
LM	FCM Non-irradiated
LM	FCM Irradiated
LM	LV
LV	FCM Non-irradiated
LV	FCM Irradiated
FCM Non-irradiated	FCM Irradiated

2.2.5. Statistical analysis

Indicators of fitness for quality control

Adult Size Variation:

The four measurements of adult size variation, namely: body length, head capsule width, forewing length and hind tibia length, were analysed using a generalised linear model (GLM). To do this, each measurement was specified as a continuous numeric response variable and insect sex (male versus female) was specified as a categorical fixed effect variable to test for differences in adult size variation measurements between males and females. The GLM models for each measurement were specified assuming a Gaussian error distribution and an identity-link function. Hypothesis testing was performed using a null-modelling approach and a Wald's test ($P < 0.05$). All models were fitted in R version 4.5.0 (R Core Team, 2025).

Flight

A generalised linear mixed-effects model (GLMM) with a negative binomial distribution and log-link was used to evaluate the effects of time, sex, and their interaction on the number of wasps reaching the top of the flight chamber. Time (hours), sex, and their interaction were included as fixed effects, with replicate as a random effect to account for repeated measures. Model selection was based on Wald's test comparing the full model to a null model containing time (hours) and sex, but no interaction term. Pairwise comparisons across time were adjusted for multiple testing using the Bonferroni method.

To assess whether female body length, head capsule width, forewing length and hind tibial length changed over time, a GLMM with a Gaussian error distribution and identity-link function was fitted. The model included hour as a continuous fixed effect and replicate ID as a random intercept to account for repeated measures within replicates. Model fit was evaluated by comparing the full model to a reduced null model (excluding the fixed effect of hour) using a Wald's test. Residual diagnostics and convergence checks were performed to ensure model assumptions were met. Marginal effects and confidence intervals were extracted for visualisation of the predicted trend over time. All models were fitted in R version 4.5.0 (R Core Team, 2025).

Haplodiploidy:

Haplodiploidy was assessed by comparing the number of female and male progeny produced by an unmated female using a GLM. To do this, the number of male and female F_1 produced per female was specified as a continuous numeric response variable and insect sex (male versus female) was specified as a categorical fixed effect. The GLM model was specified assuming a negative binomial error distribution and a log-link function. Hypothesis testing was performed using a null-modelling approach and a Wald's test ($P < 0.05$). All models were fitted in R version 4.5.0 (R Core Team, 2025).

Fertility

To assess overall parasitism and associated reproductive parameters of *T. cryptophlebiae*, data were collected at five time points (24, 48, 72, 96, and 120 h) for each replicate and sample. For each time point, the number of parasitised eggs, emerged offspring, unsuccessful emergence events, superparasitism events, and resulting sex ratio were recorded.

To account for the repeated measures within each replicate and sample unit, data were summed across all five time points per replicate and sample number. This allowed for the calculation of total parasitism, total emergence, total unsuccessful emergence, total superparasitism, and mean sex ratio per replicate. Subsequently, overall means and standard errors (SE) for each metric were calculated across all replicates.

Parasitism was assessed by comparing the number of eggs parasitised per female over time using a GLMM with negative binomial error distribution and log-link function. Percentage of emergence, unsuccessful emergence, superparasitism and sex ratio per female were assessed using a linear mixed-effects model (LMM) with a Gaussian error distribution and an identity-link function. To do this, the number of eggs parasitised, emergence percentage, unsuccessful emergence percentage, superparasitism percentage and sex ratio per female were specified as continuous numeric response variables, and time (in hours) (ranging from 24-120 h) was specified as a numeric fixed effect. Replicate was included as a random intercept term to account for repeated measurements taken from the same replicate over time (Bolker *et al*., 2009). Two models were compared: a null model, including only random intercepts, and an additive model including time (hours) as a fixed effect. Model selection was based on Wald's test. *Post hoc* pairwise comparisons between time points were conducted using Bonferroni correction.

The correlation between body size measurements and female parasitism was assessed using a GLMM. To do this, the number of eggs parasitised per female was specified as a continuous numeric response variable, and each of the four body size measurements (head capsule width, body length, forewing length, and hind tibia length) was specified as a numeric fixed effect in separate models. The GLMM model was specified, assuming a negative binomial error distribution and a log-link function. Replicate was included as a random intercept term to account for potential experimental variation between replicates (Bolker *et al*., 2009). Hypothesis testing was performed using a null-modelling approach and a Wald's test ($P < 0.05$). All models were fitted in R version 4.5.0 (R Core Team, 2025).

Influence of Mass Production

Competition

Parasitism, emergence, unsuccessful emergence, superparasitism rates and sex ratio, were analysed to determine if the number of mating pairs influenced these response variables differently. Parasitism percentage, emergence percentage, unsuccessful emergence percentage, superparasitism percentage and sex ratio were modelled using a GLMM with a Gaussian distribution and an identity-link function. Parasitism percentage was calculated as a proportion of the host egg number multiplied by 100. Emergence percentage, unsuccessful emergence percentage, and superparasitism percentage were calculated as proportions of parasitism multiplied by 100. Sex ratio was calculated as the number of male offspring divided by the total number of offspring produced by the various numbers of mating pairs. The number of mating pairs per vial was included as a continuous fixed effect. Replicate was included as a random effect to account for repeated measures within replicates. Two models were compared: a null model with only the random effect, and a full model including mating pair density as a fixed effect. Model comparison was performed using a Wald's test. Estimated marginal means were obtained across different mating pair levels with Bonferroni-adjusted p-values to correct for multiple comparisons. All models were fitted in R version 4.5.0 (R Core Team, 2025).

Foundress

The effect of foundress number and available host eggs on parasitism, unsuccessful emergence, and superparasitism rate was analysed using a negative binomial GLMM with a log-link function, including replicate as a random effect. The number of host eggs available was used

as an offset for parasitism, and the number of parasitised eggs was used as an offset for unsuccessful emergence and superparasitism.

Sex ratio was modelled using a GLMM with binomial error distribution and logit-link, where the number of males and females per replicate formed the response. Sex ratio was calculated as the proportion of male offspring (males/total emerged offspring) for each replicate, and not as a rate, as the other response variables were. Replicates with zero emerged offspring were excluded from the analysis to avoid divisions by 0.

Emergence was calculated as the proportion of parasitised eggs from which parasitoids successfully emerged, expressed as a percentage. A GLMM with a Gaussian error distribution and identity-link function was fitted, including replicate as a random effect. A different GLMM needed to be done for emergence due to a negative binomial distribution being unable to handle emergence that exceed the number of parasitised eggs.

Two models were compared for all response variables, an additive model including only main effects of foundress number and host egg number and a multiplicative model including the main effects and their interaction. Model comparison was conducted using a Wald's test. Estimated marginal means were calculated, and a Bonferroni correction was applied for multiple comparisons. Visualisation of predicted marginal means with 95 % confidence intervals was generated. All models were fitted in R version 4.5.0 (R Core Team, 2025).

Host Specificity Testing

No-choice

Parasitism, emergence, unsuccessful emergence, and superparasitism rates across different host species were analysed using a GLMM. Host species were treated as a categorical fixed effect with four levels (*T. leucotreta, L. vanillana, C. pomonella,* and *C. peltastica*), and replicate was included as a random effect to account for non-independence among repeated observations. For parasitism, a negative binomial distribution with a log-link function was used to model parasitism counts, with the total number of host eggs included as an offset to model parasitism rate per egg. For emergence, unsuccessful emergence, and superparasitism rates, a negative binomial distribution with a log-link function was used to model emergence counts, incorporating the number of parasitised eggs as an offset (only replicates with successful parasitism events

(parasitism > 0) were included to avoid division by zero in the model offset). Two models were fitted, a null model including only the random effect and a full model including host species as a fixed effect. Model comparison was performed using a Wald's test. *Post hoc* pairwise comparisons between host species were performed using estimated marginal means and Bonferroni-adjusted p-values.

Sex ratio (calculated as the proportion of male wasps relative to the total number of emerged wasps) was analysed across different host species using a GLMM. Sex ratio data were modelled using a binomial distribution with a logit-link function. The host species was included as a fixed effect, with FCM set as the reference category. Replicate was included as a random effect to account for repeated measures. A null model including only the random effect and a full model including host species as a fixed effect. Model comparison was conducted using a Wald's test. *Post hoc* pairwise comparisons were performed between host species using estimated marginal means with Bonferroni-adjusted p-values.

To assess whether the ellipse size differed among host species eggs, a GLMM with a Gamma distribution and log-link was used. The response variable was the ellipse area (mm^2). The host species was included as a fixed effect, and the replicate was included as a random effect to account for variation across experimental units. A Wald's test was used to compare the full model (with host species) to the null model. Pairwise comparisons were corrected using the Bonferroni method.

Morphological trait data were analysed to determine whether forewing length and hind tibia length of *T. cryptophlebiae* offspring emerging from different host species, *T. leucotreta, L. vanillana, C. pomonella,* and *C. peltastica*, varied between sexes and host species. A unique replicate identifier was created by combining the replicate number, the sample number, and the sex. For each morphological trait (forewing length and hind tibia length), GLMMs were fitted. Host species and sex were included as fixed effects, and their interaction was incorporated to test whether the effect of host species differed between sexes. Replicate was included as a random effect to account for the non-independence of observations. Two models were compared for each trait: a null model, including only additive effects of host and sex (no interaction), and a full model, including host, sex, and their interaction. Models were compared using Wald's test. Estimated

marginal means were calculated, and significant differences among groups were evaluated with Bonferroni-adjusted *Post hoc* tests. All models were fitted in R version 4.5.0 (R Core Team, 2025).

Choice

To assess whether parasitism preference differed across species combinations and within species pairings, LMMs and non-parametric tests were conducted. To do this, the parasitism difference for each replicate was calculated as the number of eggs parasitised by species A minus the number parasitised by species B. Positive values indicated a preference for species A, while negative values indicated a preference for species B. An LMM was fitted to test the effect of species combination on parasitism difference, with species combination as a fixed effect and replicate as a random effect. Model significance was assessed by comparing the full model to a null model containing only the random effect using Wald's Test ($P < 0.05$).

Preference within each species combination was further assessed using Wilcoxon signed-rank tests, comparing parasitism differences against zero for each species pair. This non-parametric approach was selected due to the non-normality of the data. Model assumptions were verified through inspection of residual plots, QQ-plots, and assessments of homoscedasticity. All models were fitted in R version 4.5.0 (R Core Team, 2025).

2.3. Results

2.3.1. Indicators of Fitness for Quality Control

Adult Size Variation

There was strong statistical support for a difference in body length between males and females ($\chi^2 = 0.262$, df = 1, $P < 0.001$). On average, male body length (0.610 ± 0.007) was approximately 0.072 mm longer than females (0.537 ± 0.008) ($\beta_{body} = 0.072$) (Figure 2.2. A).

There was strong statistical support for a difference in head capsule width between males and females ($\chi^2 = 0.014$, df = 1, $P < 0.001$). On average, male head capsule width (0.248 ± 0.002) was approximately 0.017 mm longer than females (0.231 ± 0.002) ($\beta_{head\ capsule} = 0.004$) (Figure 2.2. B).

There was strong statistical support for a difference in forewing length between males and females ($\chi^2 = 0.016$, df = 1, $P < 0.001$). On average, male forewing length (0.450 ± 0.002) was approximately 0.17 mm longer than females (0.432 ± 0.003) ($\beta_{forewing} = 0.017$) (Figure 2.2. C).

There was strong statistical support for a difference in tibia length between males and females ($\chi^2 = 0.001$, df = 1, $P = 0.014$). On average, male tibia length (0.132 ± 0.001) was approximately 0.004 mm longer than females (0.128 ± 0.001) ($\beta_{tibia} = 0.004$) (Figure 2.2. D).

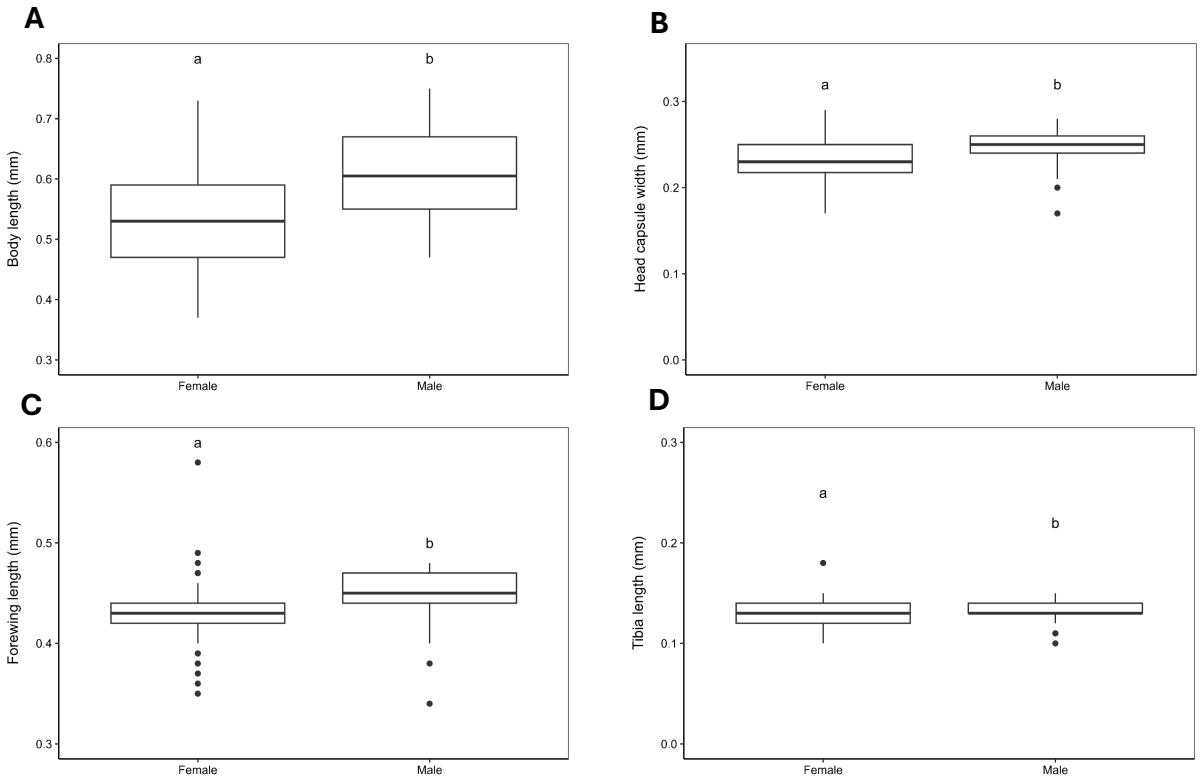

Figure 2.2. Body length (A), head capsule width (B), forewing length (C), and hind tibial length (D) (mm) for female and male *Trichogrammatoidea cryptophlebiae*. Different lowercase letters indicate significant differences between sexes ($P < 0.05$).

Haplodiploidy

There was strong statistical support for a difference in the number of female and male progeny produced per unmated female ($\chi^2 = 264.07$, df = 1, $P < 0.001$). This result was driven by no female progeny being produced, while each female produced approximately 26 ± 2 male progeny, (Figure 2.3.).

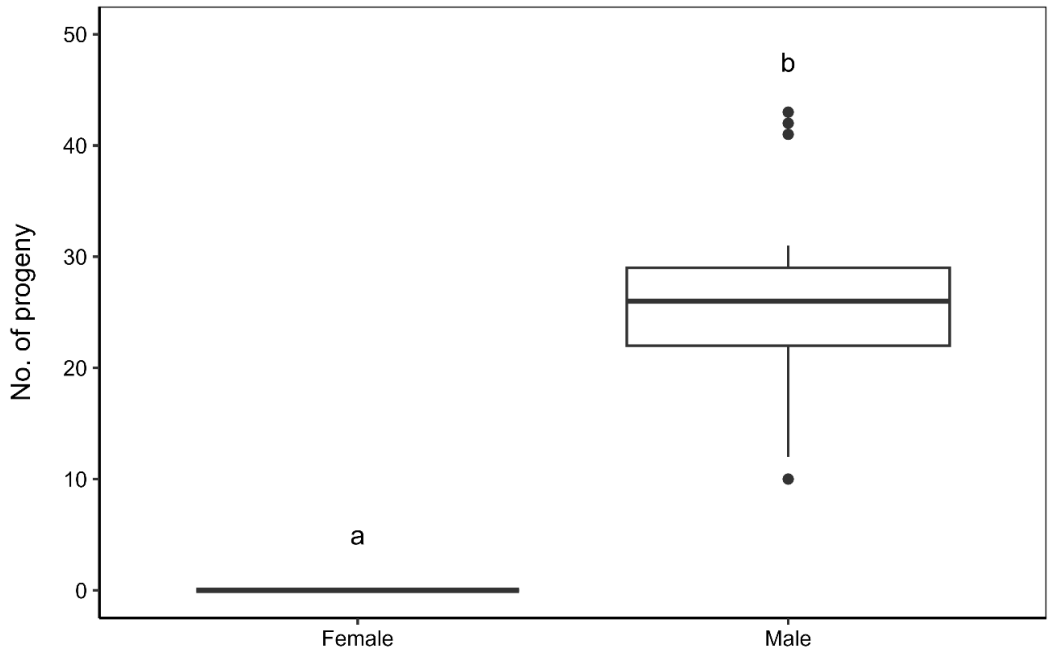

Figure 2.3. Number of female and male progeny produced per unmated female *Trichogrammatoidea cryptophlebiae.* Different lowercase letters indicate significant differences between sexes ($P < 0.05$).

Flight

Across all replicates, more wasps were recovered at the top of the chamber than at the bottom, but a large percentage were unaccounted for, potentially remaining in intermediate regions of the flight chamber. There was no significant difference in the percentage of males and females found entrapped at the bottom of the chamber ($t = 0.31$, df = 11, $P = 0.765$). However, there were significantly more females than males located at the top of the chamber ($t = -12.47$, df = 11, $P < 0.001$), with a mean difference of 64.3 %. These results indicate that female wasps were significantly more likely to fly to the top, suggesting sex-based differences in flight capacity or motivation (Table 2.4.).

Table 2.4. Summary of the total percentage of wasps placed in the flight chamber that were found at the top, entrapped at the bottom, or unaccounted for. The average percentage of the total number of females and males located at the top and bottom of the flight chamber is also indicated.

	Top of Chamber (%)	Bottom of Chamber (%)	Unaccounted (%)
Total	30.3 ± 3.7	15.6 ± 2.5	45.9 ± 4.8
Female	82.5 ± 2.6 a	49 ± 3.4 a	
Male	18.2 ± 2.6 b	51.0 ± 3.4 a	

* The letters indicate a significant difference between males and females at the two locations in the flight chamber.

The number of wasps reaching the top was not significantly influenced by the interaction between time and sex ($\chi^2 = 0.353$, df = 1, $P = 0.555$). The number of wasps reaching the top was significantly influenced by sex ($\chi^2 = 22.342$, df = 1, $P < 0.001$) and marginally by time ($\chi^2 = 3.261$, df = 1, $P = 0.071$). Time showed a weak negative trend ($P = 0.071$), but no interaction effect was recorded ($P = 0.553$). *Post hoc* comparisons revealed no significant differences between individual time points within either sex after correction for multiple testing. All time points were grouped together, indicating consistent flight performance across the recorded period (Figure 2.4.).

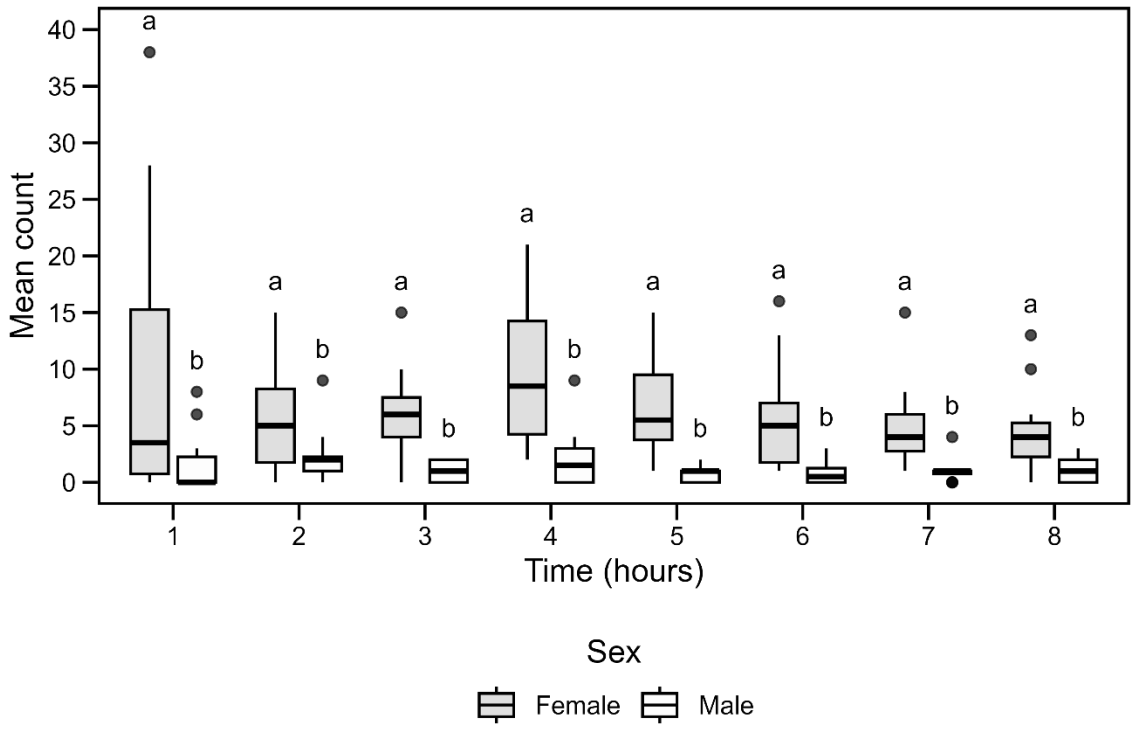

Figure 2.4. Mean count of females and males to reach the top of a flight chamber over an 8 h period. Letters of significance show differences between females and males at the relevant time points ($P > 0.05$).

Body length significantly decreased over time ($\chi^2 = 4.63$, df = 1, $P = 0.031$). On average, body length declined by 0.0015 mm per hour (Estimate = -0.0015). Marginal effects showed a steady linear decrease in predicted body length from 0.41 mm to 0.40 mm over the 8 h period (Figure 2.5. A).

Female head capsule width also decreased significantly over time ($\chi^2 = 7.63$, df = 1, $P = 0.0058$). On average, head capsule width declined by 0.0010 mm per hour (Estimate = -0.00102). Marginal predictions indicated a consistent decline from 0.218 mm to 0.211 mm across the 8 h period (Figure 2.5. B).

Forewing length did not differ significantly over time in females ($\chi^2 = 2.40$, df = 1, p = 0.12), although, on average, it declined by 0.0011 mm per hour (Estimate = -0.0011). (Figure 2.5. C).

Hind tibia length in females declined significantly over time ($\chi^2 = 6.96$, df = 1, $P = 0.008$). On average, hind tibia length declined by 0.0007 mm per hour (SE = 0.00026), suggesting a small but statistically significant decrease (Figure 2.5. D).

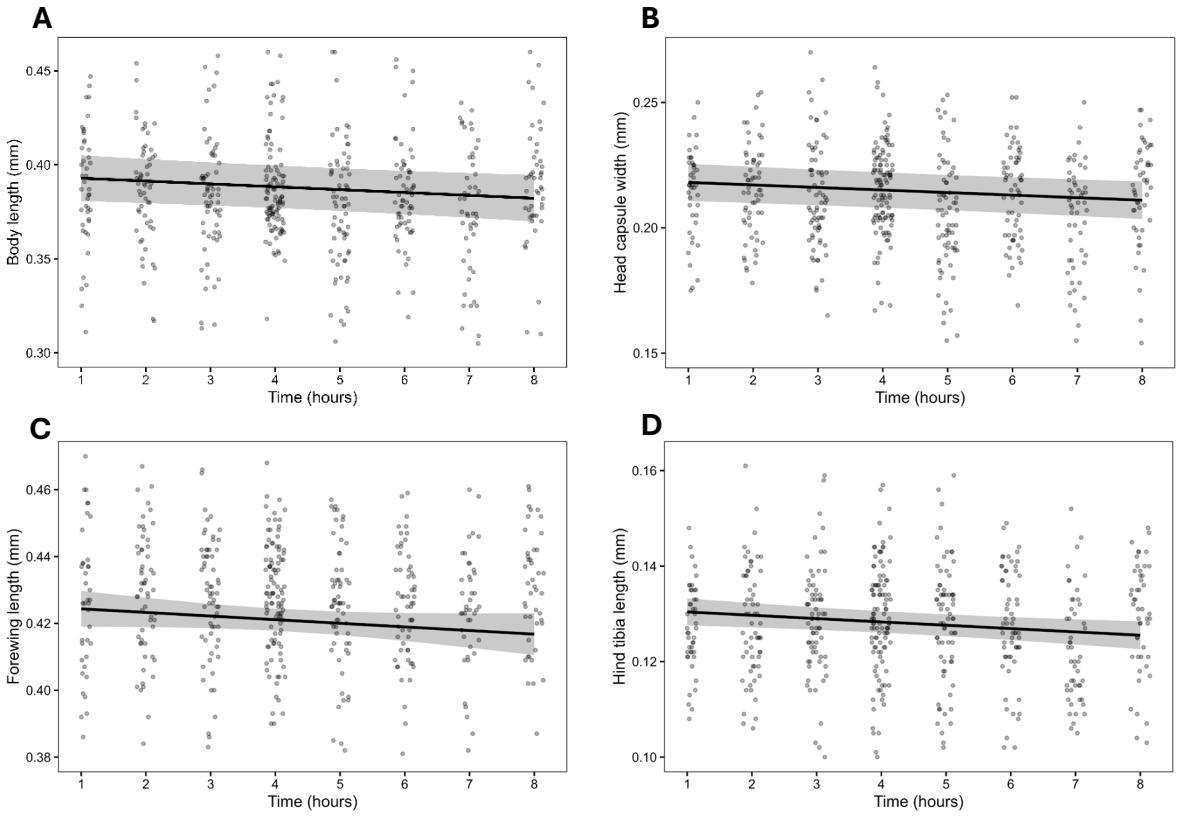

Figure 2.5. Correlations between the hours females reach the top of the flight chamber and body length (A), head capsule width (B), forewing length (C) and hind tibia length (D) (mm). The solid black line indicates the marginal mean value, and the grey shaded areas indicate the 95 % confidence interval of the mean expectation value.

Fertility

Across all replicates, the mean total number of parasitised eggs was 50.725 ± 1.064 SE per replicate. Of these, an average of 44.725 ± 0.948 SE individuals successfully emerged, while 7.604 ± 0.289 SE were recorded as unsuccessful emergence events. Superparasitism occurred at a mean of 1.604 ± 0.118 SE instances per replicate. The mean sex ratio was 0.710, indicating a male-biased sex production pattern by females across the trial. Variation among replicates was minimal, suggesting consistency in reproductive output under the tested conditions (Table 2.5.).

Table 2.5. The mean average parasitised eggs, emerged and unsuccessfully emerged offspring, superparasitized eggs and sex ratio production of single, once mated females in various segments of a 120-hour period.

Time (hours)	Parasitized	Emerged	Unsuccessful Emergence	Superparasitised	Sex Ratio
24	16.933 ± 0.411	16.624 ± 0.398	1.785 ± 0.127	1.478 ± 0.107	0.549 ± 0.022
48	10.517 ± 0.249	8.910 ± 0.244	1.655 ± 0.125	0.034 ± 0.015	0.632 ± 0.020
72	9.181 ± 0.264	7.825 ± 0.241	1.392 ± 0.114	0.035 ± 0.015	0.716 ± 0.020
96	8.085 ± 0.239	7.141 ± 0.222	0.972 ± 0.083	0.042 ± 0.02	0.799 ± 0.020
120	7.547 ± 0.236	5.482 ± 0.230	2.086 ± 0.145	0.022 ± 0.012	0.883 ± 0.022
Total Average	50.725 ± 1.064	44.725 ± 0.948	7.604 ± 0.289	1.604 ± 0.118	0.710

There was strong statistical support for an effect of time (hours) on female parasitism rates (X_2 = 381.46, df = 1, $P < 0.001$). On average, for each additional 24 h a female is alive, she parasitises approximately 19 % fewer eggs in the following 24 h time period, suggesting female age affects parasitism capacity (βtime = -0.0086). All pairwise comparisons between time points were statistically significant (adjusted $P < 0.0001$) (Figure 2.6. A).

Emergence percentage significantly declined with increasing time after mating (χ^2 = 112.15, df = 1, $P < 0.001$). On average, for each additional 24 h a female was alive, the emergence percentage decreased by approximately 4.9 % (βtime = -0.2051). Mean emergence was highest at 24 h (96.5 ± 1.08 %) and declined steadily to 76.9 ± 1.11 % by 120 h, indicating an effect of female age on offspring successfully developing. All pairwise comparisons between time points were statistically significant (adjusted $P < 0.0001$) (Figure 2.6. B).

Superparasitism percentage significantly declined with increasing time after mating (χ^2 = 130.61, df = 1, $P < 0.001$). On average, for each additional 24 h a female was alive, superparasitism declined by approximately 30% relative to its initial level at 24 h (βtime = -0.0007445). Mean

superparasitism was highest at 24 h (5.89 ± 0.37 %) and declined to 1.26 ± 0.38 % by 120 h, indicating that younger, therefore inexperienced, females are more likely to superparasitise (Kishani *et al*., 2014). All pairwise comparisons between time points were statistically significant (adjusted $P < 0.0001$) (Figure 2.6. C).

Unsuccessful emergence percentage significantly increased with increasing time after mating ($\chi^2 = 50.93$, df = 1, $P < 0.001$). On average, for each additional 24 h a female was alive, the percentage of unsuccessful emergence increased by approximately 2.9 % relative to its starting level at 24 h (βtime = 0.00120). Mean unsuccessful emergence was lowest at 24 h (10.1 ± 0.96 %) and increased steadily to 21.6 ± 0.99 % by 120 h, indicating an effect of female age on offspring successfully developing. All pairwise comparisons between time points were statistically significant (adjusted $P < 0.0001$) (Figure 2.6. D).

Sex ratio (proportion male) significantly increased with increasing time after mating ($\chi^2 = 209.71$, df = 1, $P < 0.001$). On average, for each additional 24 h a female was alive, the proportion of male offspring increased by approximately 8.7 % relative to the starting level at 24 hours (βtime = 0.00348). The mean sex ratio was lowest at 24 h (0.549 ± 0.022) and increased steadily to 0.883 ± 0.022 by 120 h, indicating that females can deplete their sperm stores (Durocher-Granger *et al*., 2011). All pairwise comparisons between time points were statistically significant (adjusted $P < 0.0001$) (Figure 2.6. E).

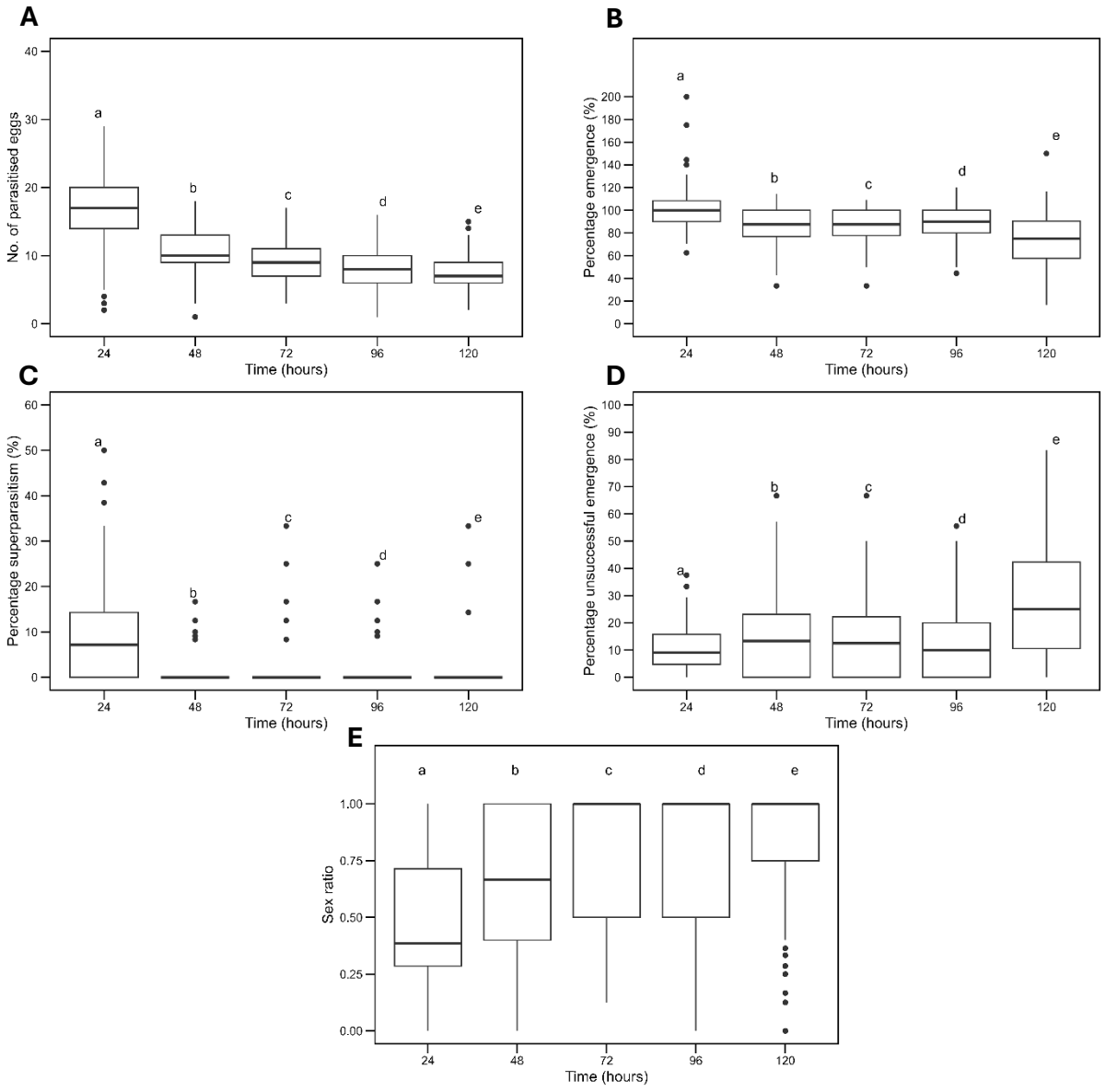

Figure 2.6. Number of parasitised eggs (A), percentage emergence (B), percentage superparasitism (C), percentage unsuccessful emergence (D), and sex ratio (E) produced per individual, once-mated female *Trichogrammatoidea cryptophlebiae* at 24 h time intervals (24-120 h). A sex ratio equal to 1 indicates only male production. Different letters denote statistically significant differences ($P < 0.05$).

There was strong statistical support for a correlation between body length and the number of eggs parasitised per female ($\chi^2 = 23.04$, df = 1, $P < 0.001$). On average, the number of eggs parasitised per female increased by 4.7 % for each additional 0.1 mm in body length ($\beta_{body\_length} = 4.65$) (Figure 2.7. A).

There was strong statistical support for a correlation between head capsule width and the number of eggs parasitised per female ($\chi^2 = 7.39$, df = 1, $P = 0.006$). On average, the number of eggs parasitised per female increased by 4.3 % for each additional 0.1 mm in head capsule width ($\beta_{head\_capsule} = 4.28$) (Figure 2.7. B).

There was strong statistical support for a correlation between forewing length and the number of eggs parasitised per female ($\chi^2 = 11.33$, df = 1, $P < 0.001$). On average, the number of eggs parasitised per female increased by 5.0 % for each additional 0.1 mm in forewing length ($\beta_{forewing} = 4.85$) (Figure 2.7. C).

There was strong statistical support for a correlation between forewing length and the number of eggs parasitised per female ($\chi^2 = 10.14$, df = 1, $P < 0.001$). On average, the number of eggs parasitised per female increased by 11.9 % for each additional 0.1 mm in hind tibia length ($\beta_{hind\_tibia} = 11.27$) (Figure 2.7. D).

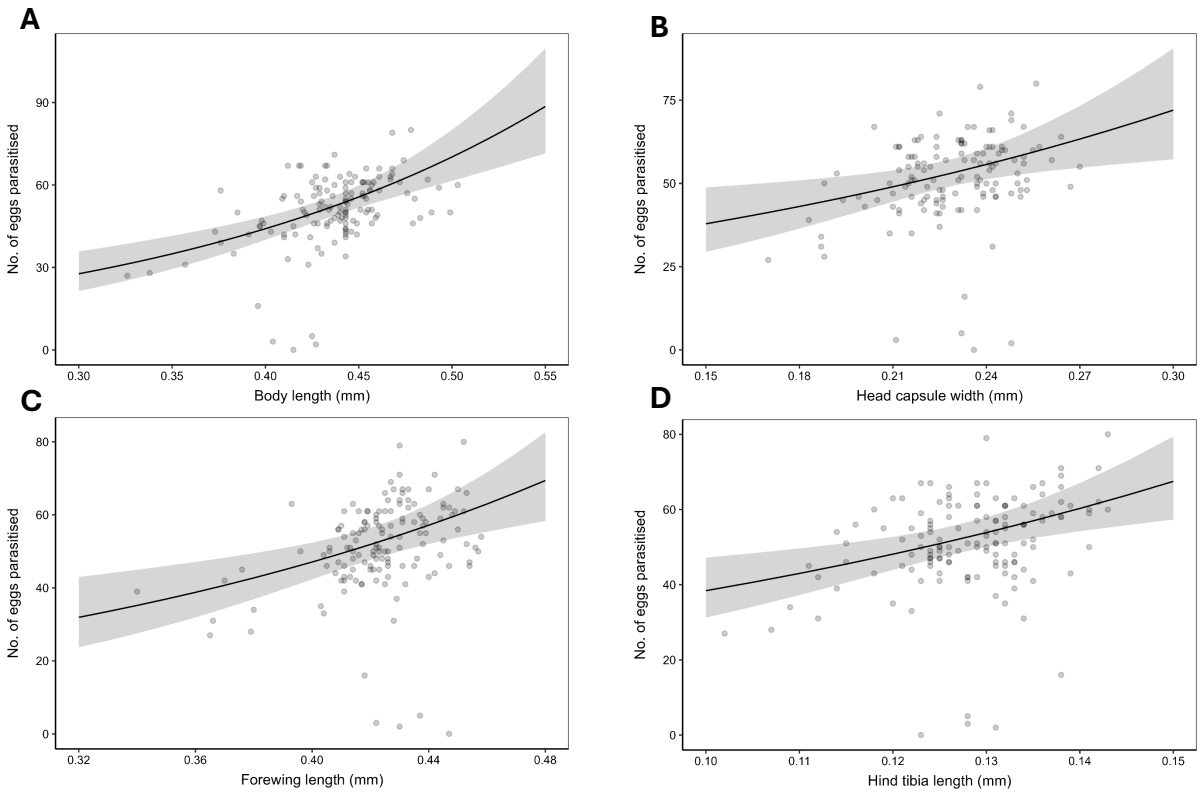

Figure 2.7. Correlation between the number of parasitised eggs per female with body length (A), head capsule width (B), forewing length (C), and hind tibia length (D). The solid black line indicates the marginal mean value, and the grey shaded areas indicate the 95 % confidence interval of the mean expectation value.

2.3.2. Influence of Mass Production

Competition

Percentage parasitism did not significantly differ with increasing numbers of mating pairs per vial ($\chi^2 = 0.28$, df = 1, $P = 0.594$). The estimated effect size was small and negative ($\beta_{pairs} = -0.00095$), indicating a negligible decrease in parasitism of less than 0.1 % per additional mating pair. The mean percentage parasitism remained consistent across treatments, ranging from 54.6 % ± 0.95 at 10 mating pairs to 55.4 % ± 0.95 at 1 mating pair, indicating no competition amongst females for oviposition. No pairwise comparisons between treatments were statistically significant after Bonferroni correction (adjusted $P = 1.000$ for all comparisons) (Figure 2.8. A).

Emergence success (proportion of emergence holes from parasitised eggs) did not vary significantly with increasing numbers of mating pairs per vial ($\chi^2 = 0.30$, df = 1, $P = 0.582$). The

effect size was small (β_{pairs} = 0.00104), equating to an increase of approximately 0.1 % in emergence for each additional mating pair. The mean percentage emergence remained consistently high across treatments, ranging from 99.5 % ± 1.32 at 1 mating pair to 100.4 % ± 1.32 at 10 mating pairs, indicating no effect of increased mating pairs on offspring developmental success. No pairwise comparisons between mating pair levels were statistically significant after Bonferroni adjustment (adjusted P = 1.000 for all comparisons) (Figure 2.8. B).

Superparasitism showed a marginally non-significant decline with increasing numbers of mating pairs per vial (χ^2 = 2.90, df = 1, P = 0.088). On average, each additional mating pair was associated with a decrease of approximately 0.27 % in superparasitism (β_{pairs} = -0.00275). The mean percentage superparasitism dropped from 7.8 % ± 1.12 at one mating pair to 5.3 % ± 1.12 at 10 mating pairs, but no statistical significance indicates that superparasitism remained consistent across mating pairs. However, no pairwise comparisons between treatments were statistically significant after Bonferroni correction (adjusted P = 1.000 for all comparisons) (Figure 2.8. C).

Unsuccessful emergence (proportion of parasitised eggs from which no adult emerged) did not significantly vary with increasing numbers of mating pairs per vial (χ^2 = 0.43, df = 1, P = 0.512). The effect size was negligible (β_{pairs} = 0.00044), equating to an increase of less than 0.05 % in unsuccessful emergence per additional mating pair. The mean percentage of unsuccessful emergence ranged from 5.6 % ± 0.51 at one mating pair to 6.0 % ± 0.51 at 10 mating pairs, indicating no effect of increased mating pairs on offspring developmental success. No pairwise differences between treatments were statistically significant after Bonferroni adjustment (adjusted P = 1.000 for all comparisons) (Figure 2.8. D).

Sex ratio (proportion male) was not significantly affected by the number of mating pairs per vial (χ^2 = 2.39, df = 1, P = 0.122). Although the estimated slope was negative (β_{pairs} = -0.00542), this represented only a slight decline in the proportion of male offspring per additional mating pair. The mean sex ratio marginally decreased from 0.575 ± 0.057 at one mating pair to 0.526 ± 0.057 at 10 mating pairs, indicating no effect of increased mating pairs on sex ratio production. No pairwise comparisons between treatments were statistically significant after Bonferroni adjustment (adjusted P = 1.000 for all comparisons) (Figure 2.8. E).

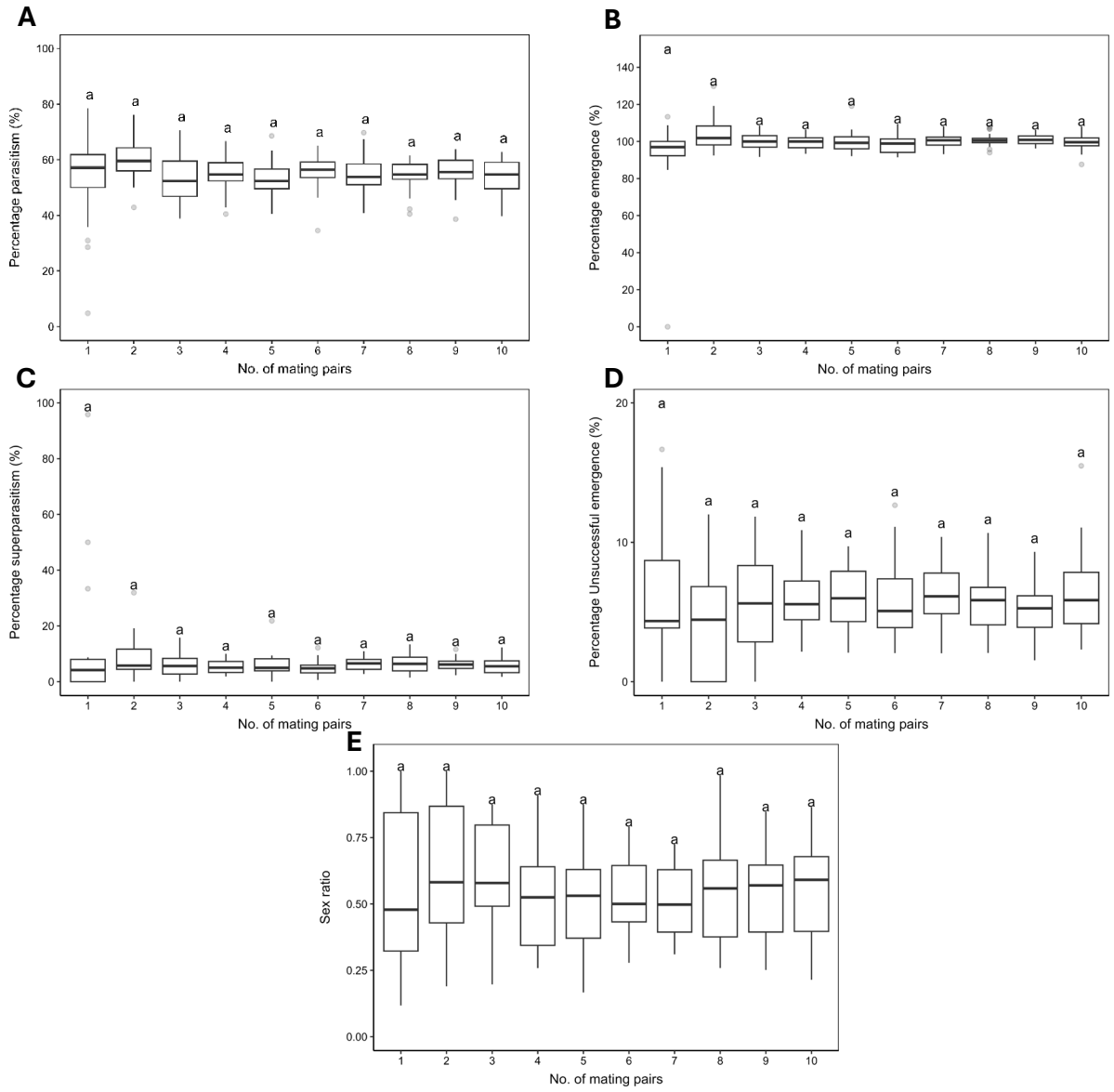

Figure 2.8. Boxplot of percentage parasitism (A), percentage emergence (B), percentage superparasitism (C), percentage unsuccessful emergence (D), and sex ratio (E) of different numbers of mating pairs per vial (1–10) *Trichogrammatoidea cryptophlebiae* when provided equal proportions of host eggs (1 mating pair to 42 host eggs). Letters above error bars denote statistically significant differences between groups ($P > 0.05$).

Foundress

All pairwise comparisons across host densities within each foundress level were significant at $P < 0.001$ after Bonferroni correction, except 40 vs 60 eggs for foundress 1 (Table 2.6.). Pairwise

comparisons across foundress levels within each host egg number varied in significance, indicating parasitism saturation at differing foundress levels. Parasitism saturated at different foundress numbers in the various host egg densities. At 20 eggs, parasitism saturated at three foundresses, at 40 eggs it was at foundresses 3 and 5; at 60 eggs it was foundresses 5 and 7, before a decline in parasitism was detected (Table 2.6.).

Table 2.6. The average number of host eggs parasitised by foundresses of 1, 3, 5, 7, and 9, at different host egg densities, 20, 40, and 60. Letters indicate statistical significance within the foundress number across the host egg densities.

	Foundress Number				
Number of Host Eggs	**1**	**3**	**5**	**7**	**9**
20	12.76 ± 0.423 a AB	13.42 ± 0.502 a A	11.82 ± 0.475 a AB	11.32 ± 0.498 a B	8.96 ± 0.513 a C
40	18.76 ± 0.759 b A	26.16 ± 0.653 b BC	26.82 ± 0.659 b B	24.46 ± 0.795 b B	23.52 ± 0.819 b BC
60	19.82 ± 1.011 b A	34.7 ± 1.109 c B	38.78 ± 1.133 c C	37.82 ± 1.084 c BC	35.12 ± 1.124 c B

* The lower-case letters indicate a significant difference between number of host eggs parasitised, within foundress number (down the columns), and upper-case letter indicate a significant difference between parasitism across foundress level within host egg number (across rows).

The interaction model, including both foundress number and host egg number, was significantly better than the additive model ($\chi^2 = 99.18$, df = 1, $P < 0.001$). Foundress number had a significant negative effect on parasitism ($\beta_{foundress} = -0.099$, $P < 0.001$), indicating parasitism decreased as the number of foundresses increased when egg numbers were low. Host egg number also had a significant positive effect ($\beta_{host\ egg\ number} = 0.030$, $P < 0.001$), and the significant positive interaction term ($\beta_{interaction} = 0.007$, $P < 0.001$) suggests that the negative impact of more foundresses on parasitism was buffered when more host eggs were available. Mean parasitism increased from 12.8 ± 0.42 eggs (1 foundress, 20 eggs) to 38.8 ± 1.13 eggs (5 foundresses, 60 eggs). Most pairwise comparisons were statistically significant after Bonferroni adjustment ($P < 0.05$) (Figure 2.9. A).

The interaction model significantly improved model fit over the additive model ($\chi^2 = 13.54$, df = 1, $P < 0.001$). Foundress number had a significant negative effect on emergence percentage ($\beta_{foundress} = -2.12$, $P < 0.001$), host egg number also had a significant negative effect ($\beta_{host\ egg\ number} = -0.14$, $P = 0.033$), and the interaction term was positive ($\beta_{interaction} = 0.042$, $P < 0.001$), indicating that emergence percentage decreased more with increasing foundress numbers when fewer host eggs were available. Mean emergence was highest for one foundress at 60 eggs (102.6 ± 2.2 %) and lowest for nine foundresses at 20 eggs (96.2 ± 2.2 %). No *post hoc* comparisons were statistically significant (adjusted $P > 0.05$) (Figure 2.9. B).

The model with interaction was marginally better than the additive model ($\chi^2 = 3.67$, df = 1, $P = 0.056$). Foundress number had a significant positive effect on superparasitism ($\beta_{foundress} = 0.102$, $P < 0.001$), while host egg number had a significant negative effect ($\beta_{host\ egg\ number} = -0.015$, $P < 0.001$). The interaction term was marginally non-significant ($\beta_{interaction} = 0.00095$, $P = 0.062$), suggesting the rate of superparasitism increased more steeply with more foundresses when fewer host eggs were available. Mean superparasitism rose from 10.9 ± 1.68 % (one foundress, 20 eggs) to 34.1 ± 2.75 % (nine foundresses, 20 eggs), and most *post hoc* comparisons were significant ($P < 0.05$) (Figure 2.9. C).

The interaction between foundress number and host egg number did not significantly improve model fit compared to the additive model ($\chi^2 = 0.013$, df = 1, $P = 0.91$). Foundress number had a significant positive effect on the proportion of offspring that failed to emerge ($\beta_{foundress} = 0.112$, $P < 0.001$), while host egg number had a significant negative effect ($\beta_{host\ egg\ number} = -0.0096$, $P = 0.007$). The interaction term was not significant ($\beta_{interaction} = 0.000064$, $P = 0.91$), indicating that the effect of more foundresses on unsuccessful emergence did not vary across egg densities. Mean unsuccessful emergence ranged from 9.98 ± 1.89 % (one foundress, 20 eggs) to 23.8 ± 2.28 % (seven foundresses, 20 eggs). Many pairwise comparisons between foundress and egg number combinations were statistically significant after Bonferroni correction ($P < 0.05$) (Figure 2.9. D).

The interaction between foundress number and host egg number did not significantly improve model fit compared to the additive model ($\chi^2 = 2.85$, df = 1, $P = 0.091$), and so the additive model was retained. Increasing foundress number was associated with a significant decrease in the proportion of male offspring ($\beta_{foundress} = -0.095$, $P < 0.001$), and higher egg densities were also

associated with fewer males ($\beta_{\text{host egg number}}$ = -0.0074, P = 0.001). The interaction term was marginally non-significant ($\beta_{\text{interaction}}$ = 0.00066, P = 0.093). Mean sex ratio declined from 0.637 ± 0.049 (one foundress, 20 eggs) to 0.457 ± 0.024 (nine foundresses, 40 eggs). Many *post hoc* comparisons between foundress-egg combinations were significant after Bonferroni correction (P < 0.05), indicating consistent changes in sex ratio across treatments (Figure 2.9. E).

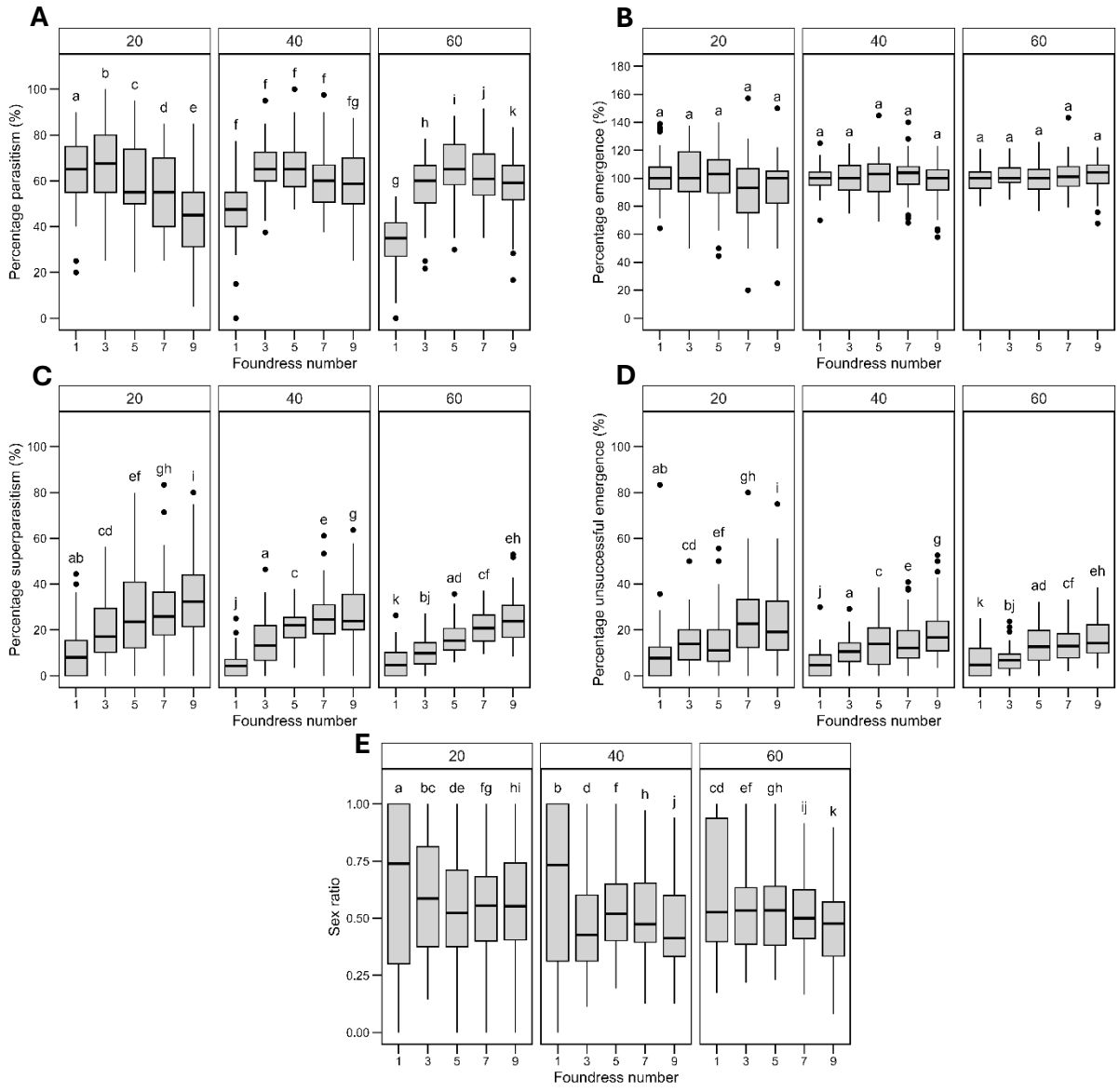

Figure 2.9. The percentage parasitism (A), emergence (B), superparasitism (C), unsuccessful emergence (D) and sex ratio (E) produced by foundress numbers, 1, 3, 5, 7, and 9, across three levels of host egg numbers, 20, 40, and 60 eggs. Letters above error bars denote statistically significant differences between groups (P > 0.05).

2.3.4. Host Specificity Testing

No-choice

Parasitism differed significantly among host species in the no-choice assay (χ^2 = 228.05, *df* = 4, *P* < 0.001), with the inclusion of host species significantly improving model fit over the null model. Relative to FCM (β = -1.43, SE = 0.18, *P* < 0.001), parasitism was significantly lower on LM (β = -1.07, SE = 0.15, *P* < 0.001) and CM (β = -0.36, SE = 0.15, *P* = 0.013), but not significantly different on LV (β = -0.25, SE = 0.15, *P* = 0.085). Parasitism on BW was effectively zero, and model estimates for this species were unstable, leading to its exclusion from Figure 2.9. (β = -23.04, SE = 3433.62, *P* = 0.995). *Post hoc* comparisons using Bonferroni correction confirmed that parasitism on LM (8.20 ± 0.19 %) and CM (16.70 ± 0.18 %) was significantly lower than on FCM (23.92 ± 0.18 %) and LV (18.63 ± 0.18 %) (*P* < 0.001). No significant difference in parasitism was recorded between FCM and LV (*P* = 0.511) or between CM and LV (*P* = 1.000) (Figure 2.10. A).

Emergence proportion differed significantly across host species (χ^2 = 76.05, df = 3, *P* < 0.001), with the inclusion of host species as a fixed effect significantly improving model fit over the null model. Relative to FCM (β = -0.17, SE = 0.05, *P* < 0.001), emergence was significantly higher on CM (β = 0.31, SE = 0.04, *P* < 0.001) and LM (β = 0.32, SE = 0.06, *P* < 0.001) but did not differ significantly on LV (β = -0.03, SE = 0.05, *P* = 0.590). *Post hoc* comparisons using Bonferroni correction confirmed that emergence on LM (137.3 ± 0.06 %) and CM (133.3 ± 0.05 %) was significantly higher than on FCM (98.5 ± 0.05 %) and LV (95.5 ± 0.05 %) (*P* < 0.001). No significant difference in emergence was recorded between FCM and LV (*P* = 1.000) or between CM and LM (*P* = 1.000) (Figure 2.10. B).

Superparasitism proportion differed significantly across host species (χ^2 = 232.52, df = 3, *P* < 0.001), with the inclusion of host species as a fixed effect significantly improving model fit over the null model. Relative to FCM (β = -3.39, SE = 0.17, *P* < 0.001), superparasitism was significantly higher on CM (β = 2.34, SE = 0.16, *P* < 0.001) and LM (β = 2.27, SE = 0.18, *P* < 0.001), and significantly lower on LV (β = -0.78, SE = 0.30, *P* = 0.009). *Post hoc* comparisons using Bonferroni correction confirmed that superparasitism on CM (34.8 ± 0.16 %) and LM (32.4 ± 0.18 %) was significantly higher than on FCM (3.4 ± 0.17 %) and LV (1.5 ± 0.27 %) (*P* < 0.001).

No significant difference in superparasitism was recorded between CM and LM ($P = 1.000$), while LV differed significantly from all other host species ($P < 0.001$) (Figure 2.10. C).

Unsuccessful emergence proportion differed significantly across host species ($\chi^2 = 16.36$, df = 3, $P < 0.001$), with the inclusion of host species as a fixed effect significantly improving model fit over the null model. Relative to FCM ($\beta = -1.71$, SE = 0.13, $P < 0.001$), unsuccessful emergence was significantly higher on CM ($\beta = 0.37$, SE = 0.11, $P = 0.001$) but did not differ significantly on LM ($\beta = -0.15$, SE = 0.16, $P = 0.330$) or LV ($\beta = 0.00$, SE = 0.12, $P = 0.979$). *Post hoc* comparisons with Bonferroni correction confirmed that unsuccessful emergence was significantly higher on CM (26.1 ± 2.9 %) than on FCM (18.1 ± 2.4 %), LM (15.6 ± 2.6 %), and LV (18.2 ± 2.5 %) ($P = 0.0072$; $P = 0.0052$; $P = 0.0164$, respectively). No significant differences were detected between FCM, LV, and LM ($P = 1.000$). Only CM differed significantly from all other host species (Figure 2.10. D).

Sex ratio (proportion of male offspring) differed significantly across host species ($\chi^2 = 61.25$, df = 3, $P < 0.001$), with the inclusion of host species as a fixed effect significantly improving model fit over the null model. Relative to FCM ($\beta = 0.61$, SE = 0.19, $P = 0.002$), sex ratio was significantly lower on CM ($\beta = -0.37$, SE = 0.10, $P < 0.001$) and LM ($\beta = -0.85$, SE = 0.12, $P < 0.001$), but did not differ significantly on LV ($\beta = 0.00$, SE = 0.11, $P = 0.987$). *Post hoc* comparisons with Bonferroni correction confirmed that the sex ratio was significantly lower on LM (0.439 ± 0.028) and CM (0.560 ± 0.026) than on FCM (0.647 ± 0.024) and LV (0.648 ± 0.025) ($P < 0.001$; $P = 0.0013$; $P = 0.0004$; $P = 0.0038$, respectively). No significant difference was detected between FCM and LV ($P = 1.000$). Only LM differed significantly from all other host species (Figure 2.10. E).

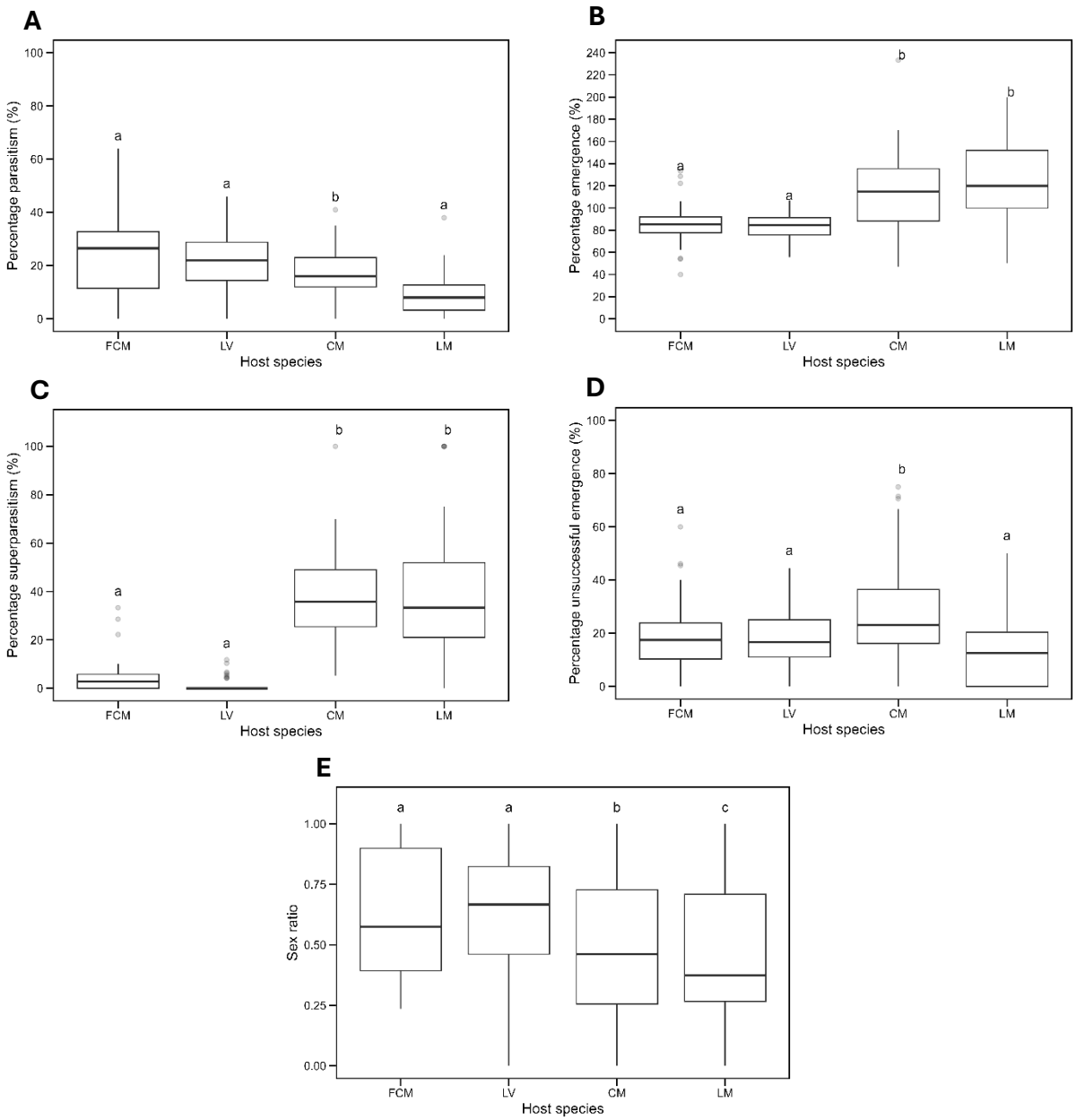

Figure 2.10. Boxplots of percentage parasitism (A), emergence (B), superparasitism (C), unsuccessful emergence (D) and sex ratio (E) by *Trichogrammatoidea cryptophlebiae* across host species FCM, CM, LM, and LV. Different letters above the boxes indicate significant differences ($P < 0.05$). Abbreviations indicate species names, FCM - *T. leucotreta,* LV - *L. vanillana*, CM - *C. pomonella*, and LM - *C. peltastica*.

Ellipse size differed significantly among host species (χ^2 = 1585.7, df = 4, P < 0.001). Ellipse size for CM was the largest (mean = 0.66 mm²), followed by LM (0.43 mm²), FCM (0.27 mm²), LV (0.20 mm²), and BW (0.19 mm²). BW and LV showed no significant differences in ellipse size. FCM, CM, and LM differed significantly in ellipse size (Figure 2.11.).

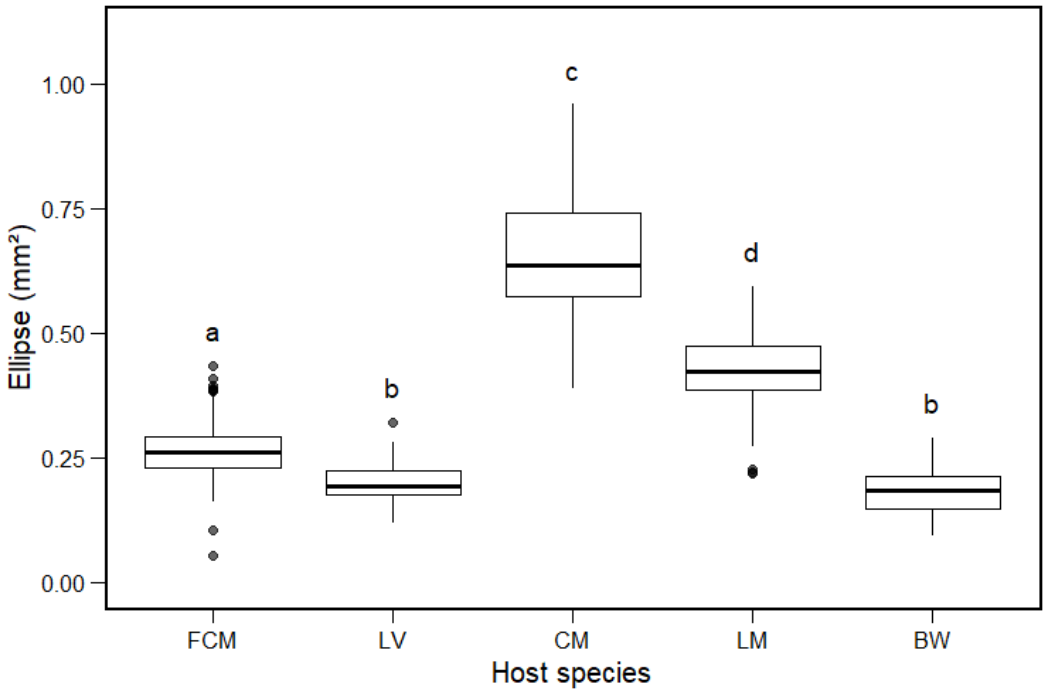

Figure 2.11. Ellipse size of potential host eggs offered to *Trichogrammatoidea cryptophlebiae* in no-choice testing ($P < 0.05$). Abbreviations indicate species names, FCM - *T. leucotreta,* LV - *L. vanillana*, CM - *C. pomonella*, LM - *C. peltastica,* and BW – *H. armigera*.

Hind tibia length also differed significantly across host species and sexes (χ^2 = 588.83, df = 3, $P < 0.001$), with the host-sex interaction again significantly improving model fit. Relative to females reared on CM (β = 0.129, SE = 0.0007), hind tibia length was significantly reduced on LV (β = -0.0154, SE = 0.0008, $P < 0.001$), slightly increased on LM (β = 0.0017, SE = 0.0008, P = 0.050), and showed no difference on FCM (β = -0.0002, SE = 0.0008, P = 0.793). The main effect of sex was non-significant (β = -0.0002, SE = 0.0009, P = 0.805), but a significant host-sex interaction was detected on LM (β = 0.0087, SE = 0.0012, $P < 0.001$), indicating that male tibia length was considerably longer than females only on this host. *Post hoc* comparisons confirmed that male hind tibia length was longest on LM (0.139 ± 0.0009 mm), while the shortest values

were recorded on LV for both sexes (Females = 0.113 ± 0.0008 mm; Males = 0.114 ± 0.0006 mm) (Figure 2.12. A).

Forewing length differed significantly across host species and sexes ($\chi^2 = 128.46$, df = 3, $P < 0.001$), with the inclusion of a host-sex interaction significantly improving model fit over the null model. Relative to females reared on CM ($\beta = 0.434$, SE = 0.0018), forewing length was significantly shorter on FCM ($\beta = -0.0063$, SE = 0.0023, $P = 0.006$), LM ($\beta = -0.0049$, SE = 0.0026, $P = 0.054$), and especially LV ($\beta = -0.0496$, SE = 0.0025, $P < 0.001$). Males had significantly longer forewings overall ($\beta = 0.0084$, SE = 0.0023, $P < 0.001$), with the greatest difference in sex being recorded on LM ($\beta = 0.0094$, SE = 0.0012, $P < 0.01$). *Post hoc* comparisons with Bonferroni correction showed that male forewing length was greatest on LM (0.447 ± 0.0024 mm) and FCM (0.445 ± 0.0014 mm), followed by CM (0.443 ± 0.0015 mm), and was smallest on LV (0.392 ± 0.0016 mm). Female forewing length followed a similar pattern, with the longest forewings on CM (0.434 ± 0.0018 mm) and shortest on LV (0.385 ± 0.0022 mm) (Figure 2.12. B).

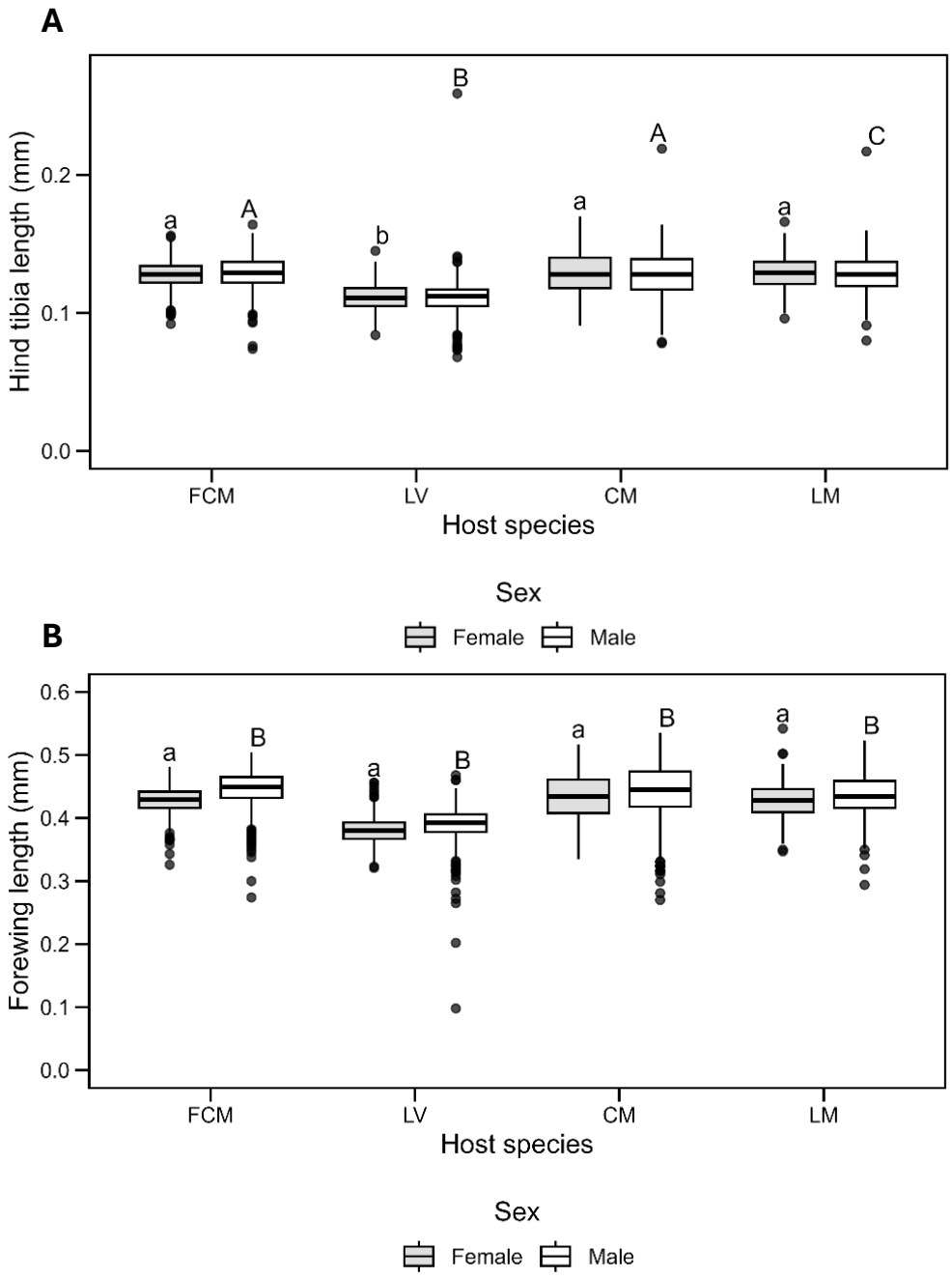

Figure 2.12. Male and female hind tibial (A) and forewing (B) measurements (mm) of *Trichogrammatoidea cryptophlebiae* offspring that emerged from FCM, LV, CM, and LM eggs. Different letters indicate statistically significant differences between sexes emerging from different hosts ($P < 0.05$). Abbreviations indicate species names, FCM - *T. leucotreta,* LV - *L. vanillana*, CM - *C. pomonella*, and LM - *C. peltastica*.

Choice

Parasitism preference differed significantly among host combinations in the choice trial assay (χ^2 = 22.01, df = 9, P = 0.009), with the inclusion of species combination significantly improving model fit over the null model. Relative to the CM vs FCM Non-irradiated (β = -1.36, SE = 1.57, P = 0.395), parasitism differences were significantly more negative in LM vs FCM Irradiated (β = -4.34, SE = 1.78, P = 0.015) and LV vs FCM Irradiated (β = -4.12, SE = 1.78, P = 0.021), indicating stronger preferences for FCM Irradiated in those combinations. No significant differences were detected for any other combinations (all $P > 0.05$). Wilcoxon signed-rank tests corroborated these findings. Significant differences in parasitism were recorded for CM vs FCM Irradiated ($P = 0.003$), LM vs FCM Non-irradiated ($P = 0.044$), LM vs FCM Irradiated ($P < 0.001$), LV vs FCM Non-irradiated ($P = 0.017$), and LV vs FCM Irradiated ($P = 0.003$). In each case, the more heavily parasitized host was FCM Irradiated or FCM Non-irradiated, suggesting consistent preference patterns (Figure 2.13. & Table 2.7.).

Table 2.7. The mean difference in the number of parasitised host eggs by *Trichogrammatoidea cryptophlebiae* across host species combinations. Abbreviations indicate species names, FCM Non-irradiated - *T. leucotreta* non-irradiated eggs, FCM Irradiated - *T. leucotreta* irradiated eggs, LV - *L. vanillana*, CM - *C. pomonella*, and LM - *C. peltastica*.

Species Combination	Mean Difference (± SE)	Preferred Species
CM vs FCM Non-irradiated	1.36 ± 1.57	No clear preference
CM vs FCM Irradiated	4.08 ± 1.57	FCM Irradiated
CM vs LM	0.36 ± 1.57	No clear preference
CM vs LV	0.98 ± 1.57	No clear preference
FCM Non-irradiated vs FCM Irradiated	1.46 ± 1.57	No clear preference
LM vs FCM Non-irradiated	2.62 ± 1.57	FCM Non-irradiated
LM vs FCM Irradiated	5.70 ± 1.57	FCM Irradiated
LM vs LV	1.40 ± 1.57	No clear preference
LV vs FCM Non-irradiated	4.40 ± 1.57	FCM Non-irradiated
LV vs FCM Irradiated	5.48 ± 1.57	FCM Irradiated

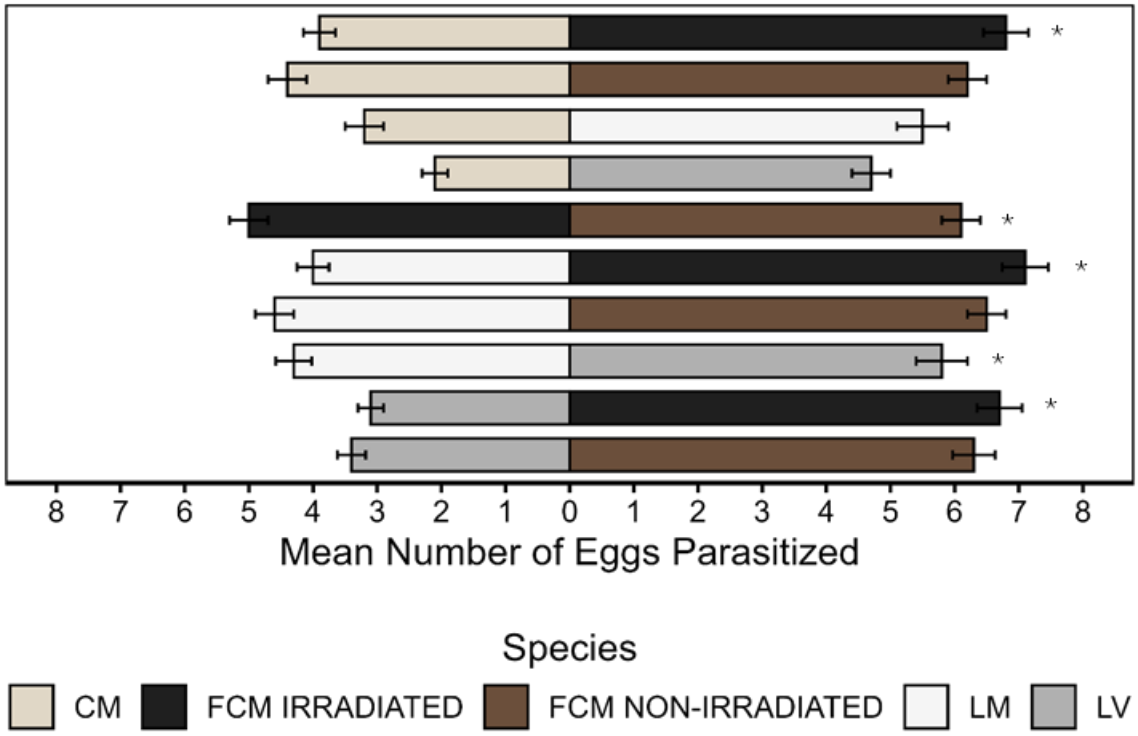

Figure 2.13. Mean number of eggs parasitised by Trichogrammatoidea cryptophlebiae in different paired host choice combinations. The stars indicate a significant preference for one species over the other ($P > 0.05$). Abbreviations indicate species names FCM Non-irradiated - *T. leucotreta* non-irradiated eggs, FCM Irradiated - *T. leucotreta* irradiated eggs, LV - *L. vanillana*, CM - *C. pomonella*, and LM - *C. peltastica*.

2.4. Discussion

There are two points of interaction that *T. cryptophlebiae* has with its idioboint hosts, the adult-host egg interaction and the pre-imaginal-host interaction. To obtain a complete understanding of the biology of *T. cryptophlebiae* throughout its life cycle, we need to understand how factors such as competition for oviposition and host species may influence rates of parasitism, emergence, unsuccessful emergence, superparasitism, sex ratio production and adult fitness. Through understanding the responses of the adult and pre-imaginal stages of *T. cryptophlebiae* with its hosts and immediate environment, we can make informed decisions on how to manage mass-reared cultures that will ensure the biological integrity of the egg parasitoid is maintained.

2.4.1 Indicators of Fitness for Quality Control

Maintaining the biological integrity of *T. cryptophlebiae* in a mass-rearing system requires the establishment of baseline fitness parameters that can be regularly monitored. These benchmarks help identify and correct any conditions that may degrade parasitoid performance (Van Lenteren *et al*., 2003). This section aimed to define such parameters by evaluating several body measurements as indicators of biological fitness, including flight ability and fertility. Previous studies in trichogrammatid species have shown that such morphological traits can serve as practical proxies for fitness, avoiding the need for time-consuming full-scale bioassays (Morales-Ramos *et al*., 2023).

To assess size variation, adult males and females from a mass-reared culture were randomly selected and measured. Contrary to what is reported for most trichogrammatids, including *T. cryptophlebiae*, where females are typically larger (Nagaraja, 1979), males in this study were larger than females across all measured traits (body length, head capsule width, forewing length, and hind tibia length). One plausible explanation is that the mass-rearing environment selected for larger males over generations. In such environments, intense competition among males for mating opportunities could favour those with larger body size, leading to this reversal in sexual size dimorphism (Damiens & Boivin, 2005). Importantly, female sizes aligned with those reported for wild populations, suggesting that their biological quality has not deteriorated under rearing conditions.

Body size in trichogrammatids has been positively correlated with fitness traits (Durocher-Granger *et al*., 2011). Larger females tend to have higher initial egg loads, greater sperm storage capacity, increased fecundity, and higher oviposition rates. Likewise, larger males show enhanced sperm production and transfer (Durocher-Granger *et al*., 2011). Forewing length, particularly when proportional to body size, is an indicator of dispersal capacity, which is essential for effective host location and mating. However, not all studies agree on the predictive power of these measurements of size in the field (Souza & Del Bianco Faria, 2022). The current chapter used both flight and fertility trials to test whether these associations also apply to *T. cryptophlebiae*.

In the flight trials, significantly more females reached the top of the flight chamber than males. This is likely due to the females' innate drive to locate host eggs, in this case, *T. leucotreta* irradiated eggs placed in the vial at the chamber's top (Zang *et al*., 2021). In contrast, males

appeared to remain near the bottom, presumably waiting to mate with emerging females. Two prior attempts at flight assessment using light, or light with a food source as an attractant, failed to motivate *T. cryptophlebiae* to ascend. However, the inclusion of *T. leucotreta* eggs proved effective, albeit with a female bias. Consequently, only female measurements were analysed for correlations between morphology and flight ability.

Forewing length did not differ significantly among females reaching the top between hours 1 and 8, though a slight decline was recorded. This stability may reflect consistent rearing quality or insufficient time resolution to detect variation. A more refined experimental design, with more frequent sampling, could clarify whether the forewing length predicts flight capacity. Nonetheless, the current data provide a reasonable basis for suggesting such a link. Interestingly, while forewing length remained stable, body length, head capsule width, and hind tibia length showed significant declines across time points. This may imply that smaller wasps take longer to reach the top chamber. The recorded decrease in head capsule width is particularly notable and may relate to host-searching ability, potentially suggesting that individuals with wider head capsules are more efficient at locating hosts (Zang *et al*., 2021). This hypothesis warrants further investigation, ideally with improved chamber designs that minimise wasp escape and allow more accurate time tracking. Future flight trials should incorporate more frequent intervals and possibly entrap wasps upon arrival using non-deterrent adhesives such as entomological glue (Githae *et al*., 2024). Such glue could also be used at the chamber's base to trap walkers, as opposed to double-sided tape, which would allow for measurements of walkers to be taken and compared to flyers.

The fertility trial revealed a clear age-related decline in parasitism. Approximately half of a female's total egg load was laid in the first 48 hours of the 120-hour trial, which has direct relevance to rearing practices (Tabebordbar *et al*., 2022). Emergence per 24-hour interval showed that female age may modestly affect offspring viability, as unsuccessful emergence increased slightly over time. Though typically seen as a pre-imaginal interaction, maternal age may influence the quality of her eggs enough to affect emergence (Tabebordbar *et al*., 2022). Superparasitism was highest during the first 24 hours and declined over time, possibly due to the initial inexperience of the female. Literature suggests that some trichogrammatids undergo a brief learning phase during early oviposition, which can lead to superparasitism as the female learns how to make use of her reproductive organs (Kishani *et al*., 2014). This may also explain emergence rates exceeding

100 % in some cases. Sex ratios shifted from female-biased at hour 24 to entirely male-biased by hour 120, consistent with sperm depletion in haplodiploid species (Luck *et al*., 2001; Martel & Boivin, 2004).

Morphological traits showed positive relationships with fertility. Larger females, indicated by greater body length and hind tibia length, exhibited higher parasitism rates and produced more female offspring. This suggests that larger individuals may require fewer matings, increasing their field efficacy (Durocher-Granger *et al*., 2011). Since hind tibia length equals ovipositor length in *T. cryptophlebiae* (Nagaraja, 1979), confirming this in the mass-reared strain would improve quality control protocols. While the fertility implications of head capsule width and forewing length remain unclear, hind tibia length continues to be a standard proxy for reproductive potential.

2.4.2 Influence of Mass Production

Trichogrammatid species have long been mass-reared, and it is well established that rearing conditions can impact parasitism rates and sex ratios through effects on oviposition competition and female interactions with their environment. Understanding how competition affects *T. cryptophlebiae* is essential for optimising mass-rearing protocols.

The foundress trial aimed to determine the optimal number of host eggs per female under conditions of potential oviposition competition. The fertility trial established that a single female oviposits an average of ~17 eggs over 24 hours when host eggs are abundant. The foundress trial produced similar estimates (~12-20 eggs), but when host eggs were limited (e.g., 20 eggs per female), parasitism plateaued at ~60 %, suggesting females conserve hosts for long-term availability (Okuyama, 2024; Mills & Lacan, 2004). This pattern held across all egg densities (20, 40, and 60), where mean parasitism rarely exceeded 60-65 %. These findings indicate that rearing facilities should provide approximately ~40 % more eggs than a female's estimated daily oviposition capacity if exposure is limited to 24 hours.

Host limitation can lead to increased competition and reduced parasitism. This was evident across all densities, where more foundresses led to lower parasitism. Additionally, superparasitism increased with more foundresses (Liu *et al*., 2023), likely as females attempted to maximise offspring production under constrained conditions. However, superparasitism may reduce offspring fitness, particularly size, which can negatively impact fertility (Luck *et al*., 2001). High superparasitism levels can also result in incomplete development and failed emergence, especially

when multiple larvae share limited host resources. Although overall emergence remained high, unsuccessful emergence rose with foundress number across all egg densities, mirroring trends in superparasitism. This supports the idea that intra-host competition compromises larval development and emergence success (Luck *et al*., 2001).

Interestingly, while the literature often reports increased male offspring production under high female density (Liu *et al*., 2023), the opposite was recorded here. More foundresses led to increased female production. A likely explanation is the greater probability of selecting mated females at higher foundress numbers. Despite providing 24 hours for mating before trials, unmated females were occasionally included, as evidenced by cases of all-male broods. Increased foundress numbers reduced the chance of selecting unmated individuals, improving overall sex ratios.

In contrast, the mating pair trial, which maintained a 1:42 female-to-host egg ratio across treatments, showed no significant differences in parasitism, emergence, unsuccessful emergence, superparasitism, or sex ratio. This suggests that when sufficient eggs are provided, competition is minimised, supporting the idea that maintaining optimal egg-to-female ratios can prevent performance issues. Like in the foundress trial, females rarely parasitised more than ~60% of available eggs, even with more prolonged exposure (48 hours). This further reinforces the concept of host conservation. Mass-rearing protocols should account for this behavioural ceiling and adjust egg input accordingly. Sex ratio management is another challenge in rearing systems. Female-biased ratios can be maintained by providing adequate egg densities to prevent female crowding, which has been linked to increased male production (DaSilva *et al*., 2016; Martel & Boivin, 2004).

Overall, trials like these offer critical insight into the dynamics of mass-reared cultures. They identify production bottlenecks and allow timely corrections, especially when paired with consistent quality control data. It is important to remember that host identity can further influence system performance, a topic addressed in the next section.

2.4.3 Host Specificity Testing

Parasitism levels reflect host acceptance for oviposition. In no-choice trials, *T. leucotreta, L. vanillana*, and *C. pomonella* were parasitised at similar rates, consistent with Kaspi *et al*. (2020), who also found comparable parasitism between *T. leucotreta* and *C. pomonella*. In contrast, *C. peltastica* eggs were parasitised at significantly lower levels, suggesting structural or physiological barriers to oviposition.

One possibility is that *C. peltastica* eggs have a thicker or harder chorion, requiring more time for drilling and reducing oviposition success within the 48-hour test period (Pak *et al*., 1990). Successful parasitism may depend on females with longer and wider ovipositors, which are more capable of penetrating tough chorions (Grenier *et al*., 2001). Because trichogrammatid sensory receptors are located along the ovipositor, if penetration fails, females may not assess host suitability at all (Leralec *et al.*, 1996). It would be valuable to investigate ovipositor morphology and egg chorion thickness further across confirmed hosts (*T. leucotreta, L. vanillana, C. pomonella, C. peltastica*). If ovipositor traits strongly predict success across hosts, rearing programmes can select for females with these traits.

Host egg development time may also play a role, as it alters egg structure and nutrition. Under optimal rearing conditions, *T. leucotreta* eggs hatch in ~3 days, *L. vanillana* and *C. pomonella* in ~6, and *C. peltastica* in ~4. However, these timelines do not clearly explain parasitism patterns, as *T. leucotreta* did not show the lowest parasitism. Environmental conditions optimised for *T. cryptophlebiae*, especially based on its interaction with *T. leucotreta*, may have unintentionally hindered parasitism on other hosts. Their development rates under trial conditions may have influenced chorion hardening, desiccation, or nutritional loss, contributing to variable parasitism rates.

Host egg size also influenced superparasitism. Larger egg ellipses in *C. pomonella* and *C. peltastica* correlated with higher superparasitism, including emergence rates exceeding 100 %. Conversely, *L. vanillana*, which had the smallest eggs, showed almost no superparasitism. This has implications for rearing: high superparasitism can yield more offspring per egg, but it also brings risks. These risks include increased unsuccessful emergence and skewed sex ratios. Male production is typically favoured under crowded pre-imaginal conditions (Suzuki *et al*., 1984; Para, 2010). However, in this study, unsuccessful emergence was highest in *C. pomonella*, likely due to poorer egg nutrition linked to adult diet (Martel *et al*., 2011). This is plausible since *T. leucotreta, L. vanillana*, and *C. peltastica* were reared on the same diet, distinct from that of *C. pomonella*, and unsuccessful emergence levels correspond to this.

Sex ratios were male-biased across all hosts, which deviates from expectations for trichogrammatids (Luck *et al*., 2001). As the rearing colony typically produces female-biased broods, this could reflect an artefact of the trial design. Placing a single mating pair with host eggs

may have led to repeated copulation, blocking the female's spermatheca and impeding fertilisation (Luck *et al*., 2001). Given that host egg quality and quantity were sufficient, and crowding was not a factor, this design issue likely explains the male-biased results. Interestingly, *C. pomonella* and *C. peltastica*, which had higher superparasitism, also produced significantly more female offspring. This contradicts prior findings but could relate to sex-specific size differences recorded in this study. If males are larger than females in this colony, superparasitism may select for smaller, more space-efficient females.

Host egg size also affected offspring size. *Lobesia vanillana*, the smallest host, produced the smallest offspring, particularly in hind tibia length, which suggests reduced fertility. Iranipour *et al.* (2010) showed that fitness differed in *T. brassicae* when reared on differing hosts, with the parasitoids having life table parameters that were significantly different when emerging from different hosts. Further studies could look at rearing the F_2 generations of *T. cryptophlebiae* offspring on the various hosts to test if these expected fitness trade-offs are apparent. Earlier sections of this chapter link larger body size to enhanced fertility and flight, and this information indicates that taking these measurements may be a justifiable way to determine the fitness of the parasitoid. However, forewing length was consistent across hosts, possibly reflecting developmental constraints (Martel *et al*., 2011). Despite significant differences in host egg size, offspring size was not dramatically different, likely due to phenological plasticity and spatial limitations imposed by host egg dimensions. Superparasitism may further homogenise offspring size due to competition.

In choice trials, *T. leucotreta* irradiated eggs were typically preferred over other hosts. This, however, is different to what Carpenter *et al.* (2004) found, where *T. cryptophlebiae* preferred non-irradiated *T. leucotreta* eggs, on which they had been reared for several generations. These contradicting findings suggest pre-imaginal conditioning from rearing history, although Kaspi *et al*. (2020) found *T. cryptophlebiae* still favoured *T. leucotreta* even after being reared on *L. botrana* for 10 generations. This suggests a genuine host preference hierarchy (Bjorksten & Hoffmann, 1998). Parasitism levels did not differ significantly between irradiated and non-irradiated *T. leucotreta* eggs, though irradiated eggs were marginally preferred. Irradiation may soften the chorion, inhibit host defences, and reduce developmental cues, thereby lowering resistance (Xu *et al*., 2016).

From a rearing perspective, *T. leucotreta* remains the most suitable host due to high parasitism, low superparasitism, high emergence, and consistent offspring size. Future research should assess how offspring from different hosts compare in fitness and whether ovipositor morphology influences parasitism success across hosts. Identifying a cost-effective factitious host with similar benefits remains a worthwhile goal. The confirmation of *L. vanillana* and *C. peltastica* as physiological hosts also opens the door to field trials for their biological control. While choice trials suggest clear preferences, it is important to remember that *T. cryptophlebiae* is a generalist egg parasitoid and may exploit multiple tortricid hosts opportunistically in natural systems.

Chapter 3: Field applications of *Trichogrammatoidea cryptophlebiae* against four economically important tortricid pests in South Africa

3.1. Introduction

The use of natural enemies for pest management in agriculture has been practised worldwide for decades and provides an ecologically sustainable approach to pest management (Van Lenteren, 2012; Smith, 1996). Among these, trichogrammatid egg parasitoids are some of the most widely studied and utilised biological control agents. Although technological advances have enabled the mass production of trichogrammatids in laboratory settings, field trials are still essential. These trials are crucial for establishing effective pest management strategies and encouraging the wider use of trichogrammatids, as the adoption of biological control agents often encounters significant challenges (Zang *et al*., 2021). These challenges typically arise during the research, development, and implementation phases and are often rooted in the complex ecological interactions among the natural enemy, its environment, and the target pest (Pereira *et al.*, 2004; Newton, 1988; Zang *et al*., 2021). To ensure the success of biological control programs, it is important to identify and address these obstacles through targeted, system-specific research.

This chapter examines these challenges in the context of *T. cryptophlebiae*, an egg parasitoid indigenous to southern Africa. Although *T. cryptophlebiae* has been commercialised in South Africa to suppress *Thaumatotibia leucotreta* in citrus, its broader deployment has historically been limited by operational constraints (Smith, 1996; Schwartz, 1980). Host rearing challenges in early insectaries restricted access to field-ready material and hindered progress in understanding its full potential across various crops (Charleston *et al.*, 2004). Nonetheless, the species has been recorded parasitising tortricid pests in citrus, macadamia, litchi, grapes, and pome fruit crops, where pests such as *Cydia pomonella*, *Thaumatotibia batrachopa*, *Cryptophlebia peltastica*, *T. leucotreta* and *Lobesia vanillana* occur. These overlapping pest complexes suggest that *T. cryptophlebiae* could be valuable beyond citrus.

Although the augmentation of *T. cryptophlebiae* for *T. leucotreta* control was first proposed in 1939, commercial implementation only began in the 1970s and has continued since (Ripley *et al*., 1939; Charleston *et al.*, 2004). Several South African companies supply *T.*

cryptophlebiae commercially under various trade names. With these agents now more readily available, renewed research is needed to optimise deployment in other high-value crops. By exploring its ecological requirements and release conditions, this chapter aims to develop practical guidelines for using *T. cryptophlebiae* in macadamia, litchi, grape, and pome fruit systems.

The success of egg parasitoids in the field depends heavily on environmental conditions (Foerster *et al*., 2014; dos Santos Carvalho *et al*., 2017; Kalyebi *et al*., 2006). Trichogrammatids are highly sensitive to temperature, humidity, and wind. Extreme weather, such as high temperatures, heavy rainfall, or dry, windy conditions, can inhibit adult activity, reduce longevity, and limit host-searching behaviour. Rain can physically displace parasitoids or prevent them from emerging, while high temperatures may shorten adult lifespan (Pereira *et al.,* 2004; Newton, 1988; Zang *et al.,* 2021). Within orchard systems, microclimates under dense foliage tend to favour parasitoid persistence due to increased humidity and protection from desiccation, whereas exposed or sunlit areas may be detrimental (Zang *et al.,* 2021; Smith, 1996). These environmental pressures must be considered carefully when scheduling releases.

Synchronisation with host egg availability is another critical factor (Van Lenteren, 2012; Smith, 1996). Adult trichogrammatids often live less than a week, and their effectiveness hinges on their emergence coinciding with periods when pest eggs are present and still at a susceptible stage (Zang *et al.,* 2021; Van Lenteren, 2012; Smith, 1996). Releasing parasitoids too early risks their death before host eggs are available, while releasing too late may miss the optimal parasitism window. This timing is challenging for pests with short or variable oviposition periods (Zang *et al.,* 2021; Van Lenteren, 2012; Smith, 1996). In South Africa, tortricid populations typically rise from early November, while natural peaks in *T. cryptophlebiae* activity are observed between December and January (Moore *et al*., 2015; Catling & Aschenborn, 1974). Augmentative programmes may benefit from initiating releases in September or October to bridge this mismatch and provide pre-emptive pest population suppression.

Appropriate release rates are essential for ensuring that a sufficient proportion of host eggs are parasitised before they hatch (Newton, 1988; Sani *et al.*, 2016). These rates, generally expressed as numbers of parasitoids per hectare, must be tailored to pest pressure and crop structure (Gingras, 2001; Sani *et al.*, 2016). Denser canopies often necessitate higher rates or more frequent

releases to ensure adequate penetration and coverage (Hegazi *et al.,* 2012). Inadequate release rates or limited spatial distribution can result in uneven field coverage, where parasitoids fail to reach interior zones, leaving hotspots of pest survival (Gingras, 2001; Van den Berg *et al*., 1987). Trichogrammatids are weak dispersers, with *T. cryptophlebiae* capable of flying only roughly 60 meters without wind assistance (Moore *et al*., 2015). Therefore, evenly spaced release points are critical (Hegazi *et al.,* 2012; Zhou *et al.,* 2019). Research in citrus has shown that releasing 25,000 wasps per hectare at 15-30 locations monthly over four months increased parasitism of *T. leucotreta* eggs by 40 % relative to natural levels of parasitism (Moore *et al*., 2015). Similar studies are needed to establish other crops' optimal rates and deployment strategies.

Predation also plays a significant role in reducing parasitoid efficacy. Once trichogrammatids oviposit into host eggs, their larvae remain inside the egg during development, where they are vulnerable to generalist predators such as ants, spiders, and beetles (Pereira *et al.,* 2004; Heinz & Nelson, 1996; Wahner, 2008). Predation at this stage can dramatically reduce emergence success and establishment of parasitoid populations (Pereira *et al.,* 2004). In addition, interactions with other natural enemies, including intraguild predation and hyperparasitism, may further limit success and require careful monitoring (Heinz & Nelson, 1996; Zang *et al.,* 2021).

The physical structure of the crop and the spatial distribution of pest eggs influence parasitoid foraging efficiency (Gingras, 2001; Zang *et al.,* 2021; Newton, 1988). In tall or highly stratified crops, pest eggs may be deposited in patterns that make host location complex for parasitoids to navigate efficiently (Gingras, 2001; Newton, 1988; Newton & Crause, 1990). This vertical mismatch can create refuge for pest development (Gingras, 2001; Zang *et al.,* 2021). Furthermore, uneven fruit and flower development can lead to asynchronous oviposition by pests, complicating release synchronisation (Gingras, 2001; Zang *et al.,* 2021). To address this, crop phenology and pest distribution must be mapped and considered during release planning to ensure parasitoids are placed where they are most likely to encounter host eggs (Gingras, 2001; Zang *et al.,* 2021).

Understanding the biology and ecological limits of *T. cryptophlebiae* is essential for maximising its potential as a biological control agent (Zang *et al.,* 2021). Its limited dispersal capacity highlights the need for spatially strategic releases, while its brief lifespan demands precise

timing with pest activity (Hegazi *et al.,* 2012). Agricultural monitoring practices such as pheromone trapping and egg scouting can assist in aligning release schedules with pest population dynamics (Zang *et al.,* 2021; Kelly *et al.,* 2014). Ultimately, developing crop- and pest-specific release guidelines is necessary to avoid ineffective application or wastage due to over- or under-release.

This chapter seeks to identify the ecological and operational constraints affecting *T. cryptophlebiae* and to propose practical recommendations for its successful integration into IPM programmes. This work supports the broader commercialisation of this indigenous parasitoid by synthesising research and adapting it to the requirements of macadamia, litchi, pome fruit, and grape systems. It promotes more sustainable, biologically based pest control across a range of South African crops.

3.2. Materials and methods

3.2.1. Source of Insects

Trichogrammatoidea cryptophlebiae were sourced from River Bioscience's production culture, where they were reared at a temperature of 25°C ± 1, a relative humidity of 60 % ± 5, and a photoperiod of 16:8 Light: Dark (L: D). *Trichogrammatoidea cryptophlebiae* were reared from 180 gamma-ray irradiated *T. leucotreta* eggs sourced from XSIT's *T. leucotreta* mass-rearing facility in Citrusdal.

3.2.2. Site Selection

Trichogrammatoidea cryptophlebiae were released in four crops: macadamia, wine grapes, litchis, and pome fruit. Five trial sites per crop, with the exception of litchis, which only had three sites, were selected at various locations throughout South Africa, and an additional, or sixth site, was added in the second season for macadamias (Table 3.1.). The varieties and ages of each crop at the different locations were used to set up the trial design. Crop varieties within each site were purposefully selected to be the same to allow for accurate comparisons to be determined between treatments and farms (Appendix: Table A.1.). Select varieties tend to be more susceptible to pest damage, and mismatched varieties within sites could result in unreliable conclusions due to plant phenology.

Table 3.1. The province and region of the selected sites in South Africa for each crop used in the trials.

Crop	Site Name	Province	Region
Macadamia	Goodwoods	Eastern Cape	Port Alfred
	Leppan Farming	Western Cape	Hoekwil
	Toorbos 1	Western Cape	Hoekwil
	Toorbos 2	Western Cape	Hoekwil
	Juanita	Mpumalanga	White River
	Myee	Kwa-Zulu Natal	Gingindlovu
Wine Grape	Zandvliet	Western Cape	Ashton
	Boskloof	Western Cape	Robertson
	Goedgeloof 1	Western Cape	Worcester
	Goedgeloof 2	Western Cape	Worcester
	Goedgeloof 3	Western Cape	Worcester
Litchi	Tomahawk 1	Mpumalanga	Komatipoort
	Tomahawk 2	Mpumalanga	Komatipoort
	Tomahawk 3	Mpumalanga	Komatipoort
Pome Fruit	JGS 1	Eastern Cape	Misgund
	JGS 2	Eastern Cape	Misgund
	Waboom 1	Western Cape	Herold
	Waboom 2	Western Cape	Herold
	Waboom 3	Western Cape	Herold

3.2.3. Field Augmented Releases of *Trichogrammatoidea cryptophlebiae* and evaluation of efficacy

Trichogrammatoidea cryptophlebiae were released at various rates and timings in macadamia orchards for *T. batrachopa, T. leucotreta* and *C. peltastica*, wine grape vineyards for *L. vanillana* and *T. leucotreta*, in litchi orchards against *C. peltastica* and *T. leucotreta* (Table 3.2.), and in pome fruit against *C. pomonella* (Table 3.3.). Release rates were selected based on work done by Moore *et al.* (2015) in citrus to control *T. leucotreta.* The timings of wasp releases differed to determine the most effective time to release the wasp to achieve the highest level of control of

the targeted tortricid pests. Early releases started in September, standard releases began in October, and late releases started in November. In litchis, the decision to do three early-season releases for the differing rates of parasitoid releases was due to the short length of the crop's growing season in comparison to the other crops included in these trials.

The parasitoid was released once a month from the start to the end of the respective release period for each crop. Releases ended in macadamia orchards in March, with the crop being harvested from May to July. March was selected as the last month of releases, as nuts are no longer at risk of damage, as the shell has fully hardened. Releases in wine grapes ended in January, with the crop being harvested from January to early February; releases in litchis ended in December, with the crop being harvested at the end of December.

Table 3.2. The monthly release rates of *Trichogrammatoidea cryptophlebiae* at different timings in macadamias, wine grapes, and litchis, with the total wasps released during the crop season.

Treatments	**Macadamia**		**Wine Grapes**		**Litchis**	
Monthly Release Rate (wasps/ha)	**Timing of Release**	**Total Release for the season (wasps/ha)**	**Timing of Release**	**Total Release for the season (wasps/ha)**	**Timing of Release**	**Total Release for the season (wasps/ha)**
0 (Control)	-	0	-	0	-	0
12,500	Standard	75,000	Standard	50,000	Early	37,500
25,000	Early	175,000	Early	125,000	Early	75,000
25,000	Standard	150,000	Standard	100,000	Standard	50,000
25,000	Late	125,000	Late	75,000	Late	25,000
50,000	Standard	300,000	Standard	200,000	Early	150,000

*Early – Releases of the parasitoid began in September; Standard – Releases of the parasitoid began in October; Late – Releases of the parasitoid began in November.

Releases of *T. cryptophlebiae* in pome fruit orchards were done twice in the season for four consecutive weeks during the oviposition period of *C. pomonella* (Mills *et al.,* 2000) (Table 3.3.). The farmers provided the historical oviposition periods to determine the appropriate release periods.

Table 3.3. Weekly releases of *Trichogrammatoidea cryptophlebiae* in pome fruit orchards during the *Cydia pomonella* oviposition period.

Weekly Release Rate (wasps/ha)	Timing of the 1st set of Releases	Timing of the 2nd set of Releases	Total Release for the season (wasps/ha)
0 (Control)	16th October	1st January	0
12,500	23rd October	8th January	100,000
25,000	30th October	15th January	200,000
50,000	6th November	22nd January	400,000

*release dates indicate the date that all sets of releases and of all rates took place.

To achieve the number of wasps released per treatment, the sizes of the cards with *T. cryptophlebiae* parasitised eggs were cut according to the dimensions provided (Table 3.4.). These card sizes were pre-determined by River Bioscience quality control data from their mass-reared *T. cryptophlebiae* culture. From all batches used in the field releases, 30 samples of *T. cryptophlebiae* were taken and placed into Petri dishes and allowed to develop at a temperature of 25°C ± 1, a relative humidity of 60% ± 5, and a photoperiod of 16:8 L:D to ensure the correct number of wasps were released across all the crops and treatments. The number of parasitised eggs was counted, and adults were sexed to determine the percentage of females in the population.

Trichogrammatoidea cryptophlebiae release cards were placed evenly throughout orchards at a total of 25 cards per hectare (See Appendix: Table A.2., A.3., A.4., and A.5. for each farm and the number of crops released calculated to the size of treatment blocks). Cards were stapled to the underside of leaves in the canopy of the trees and vines, to try to limit the direct impact of abiotic environmental conditions (i.e. sunlight, rain, chemical applications). Releases were done by dedicated personnel from each farm after training.

Table 3.4. The size of *Trichogrammatoidea cryptophlebiae* release cards per treatment.

Treatment (wasps/ha)	Timing of Release	Card Size (cm x cm)
Control	-	-
12,500	Standard	3 x 3
25,000	Early	3 x 6
25,000	Standard	3 x 6
25,000	Late	3 x 6
50,0000	Standard	6 x 6

Data were collected in macadamias, grapes and pome fruit fortnightly. In contrast, litchi data were collected weekly due to the short fruiting period of the tree, and grape trap data were collected weekly. On all crops, tortricid eggs, parasitised eggs, and damaged fruit were scouted for by randomly sampling 10 clusters or individual fruit on a set of 10 data trees centrally located in each trial block. Damaged nuts were not scouted for in macadamias on the tree. Due to the overlapping tortricid species in the various crops, the tortricid eggs could not be differentiated and were, therefore, just recorded as tortricid eggs. Parasitised eggs were differentiated as they appear black when the parasitoid larvae are in their pupal stage. No hatched tortricid or parasitised eggs were recorded.

Damaged apples, litchis, and grapes were removed from the tree/vine, as these crops do not abscise damaged fruit. The fruit was then dissected to determine if a tortricid species caused the damage. Damaged macadamia nuts are abscised from the tree. All nuts were collected from the orchard floor under ten data trees per treatment. The nuts were dissected to determine the percentage of tortricid damage seen. All larvae found in the damaged fruit/nuts were placed into Eppendorf tubes with ethanol and identified at the species level.

In all crops, the relevant tortricids were monitored (Table 3.5.) using Chempac Yellow Delta monitoring traps and Chempac pheromone lures. All sticky liners were replaced according to Chempac's recommendation of 8 weeks or when necessary. The pheromone lures were replaced according to the Chempac label recommendations (Appendix Table A.6.).

Table 3.5. The pheromone lures used to monitor the relevant tortricid pests occurring in each crop and the replacement intervals of the pheromone lures.

Crop	Tortricid monitored
Macadamia	*T. leucotreta*
	T. batrachopa
	C. peltastica
Wine Grape	*T. leucotreta*
	L. vanillana
Litchi	*T. leucotreta*
	T. batrachopa
	C. peltastica
Pome Fruit	*C. pomonella*

At three points during the various crops' growing season, five sentinel *T. leucotreta* egg sheets, irradiated with 180 gamma-ray, *T. leucotreta* measuring 5 cm x 5 cm, were sourced from XSIT and used to bait *T. cryptophlebiae* in orchards/vineyards. Irradiated *T. leucotreta* eggs were used to prevent the introduction of *T. leucotreta* larvae into the field. Baiting for *T. cryptophlebiae*, using the sentinel cards, was done pre-*T. cryptophlebiae* releases, mid-season of the *T. cryptophlebiae* releases, and close to crop harvesting. Baiting's were done to determine the presence of *T. cryptophlebiae* in the orchards at various stages of crop development and release periods. The sentinel bait cards were placed in the exact locations for each baiting, through stapling the card to the underside of a leaf in the canopy of three.

3.2.4. Statistical analysis

The field trials were only conducted over one growing season for the litchi, pome fruit and grape crop types due to sparse data being seen in this season due to trial design flaws or ecological reasons. The macadamia field trial was conducted over two growing seasons due to adequate data collection points available in the first season to justify the continuation of the trial into the second season. All crop data collection periods differed and are indicated in the results section.

Trichogrammatoidea cryptophlebiae ***quality control***

Quality control data from mass-reared batches of *T. cryptophlebiae* used for field releases were collected across both Season 1 and Season 2 to identify if release numbers were achieved. For each crop and release number, batch-specific quality control assessments were conducted. Specifically, the average number of parasitised eggs per batch and the percentage of female wasps (calculated as the proportion of females out of the total emerged wasps) were measured. These data were summarised across density levels (Low, Medium, High) for each release, with parasitism and emergence averaged using a weighted formula that accounted for the relative representation of host egg densities in the batches. In contrast, percentage female and percentage male values were averaged directly across all density levels without weighting. Mean values and associated standard errors (SEs) were then scaled to represent the three release numbers used in the field (12,500, 25,000, and 50,000 wasps/ha) to evaluate whether the number of parasitoids released was consistent with targets.

In-field parasitoid baiting with sentinel host eggs

Modelling was only possible for sentinel egg baiting from macadamia orchards. No parasitism was recorded on litchi, pome fruit orchards, and sentinel bait cards. In the grape vineyards, very sparse parasitism was recorded, making statistical analysis by these methods ecologically unreliable.

To assess the success of the sentinel egg baiting periods through parasitism by *T. cryptophlebiae* in macadamia orchards, a generalised linear mixed model (GLMM) with a negative binomial error distribution was fitted to the parasitism count data. The model included the baiting period (1 – start of the season, 2 – middle of the season, and 3 – end of the season), season (1 and 2), and their interaction as fixed effects, and farm as a random intercept to account for repeated sampling across farms. Model comparison using a Wald's test was conducted to evaluate the significance of including the interaction between baiting period and season. *Post hoc* comparisons of baiting periods within each season were performed on the response scale using Bonferroni-adjusted pairwise comparisons. Estimated marginal means and 95% confidence intervals are reported.

Trap counts

No modelling was possible for the grape and pome fruit orchards due to sparse trap catches of the target pests (*L. vanillana*, *T. leucotreta*, and *C. pomonella*, respectively) over the growing season. Modelling of the individuals caught would have been ecologically unreliable. In the macadamia and litchi orchards, *T. batrachopa* and *C. peltastica*, and *C. peltastica*, respectively, were able to be modelled using the approaches below. Modelling was done in the macadamia orchards for all farms combined for both seasons and their combined effect. Each farm was also individually modelled for the two seasons and the combined effect of the two seasons. Litchi orchards were only modelled for one season as a combined farm effect, not as individual farms.

To assess how *T. batrachopa* and *C. peltastica* trap catches per week over the growing season, for two growing seasons, might be affected by different parasitoid release timings and parasitoid release numbers, a GLMM approach was adopted (Bolker *et al*., 2009). Three different model sets were specified to assess the release timing and release number effects, as the full factorial combination of release number and timing treatments was not available, with the release number treatments only applied in a factorial manner within the 'standard' parasitoid release timing window.

Release timing

Weekly *T. batrachopa* and *C. peltastica* trap counts were modelled using a GLMM approach to assess the possible effect of parasitoid release timing on *T. cryptophlebiae* activity. Only data from trees that received one of the four release timing treatments (Control, Early, Standard, or Late) were included. For both *T. batrachopa* and *C. peltastica*, the number of individuals captured per trap per week was modelled as a function of release timing, specified as a categorical predictor with four levels, and trial week, a continuous predictor indicating the number of weeks since the start of each growing season. A saturated model for each species included a multiplicative interaction term between release timing and trial week to test the hypothesis that trap captures may vary between treatments over time. Season was included as a main effect and its interaction terms with timing and week to assess whether trap activity differed across years. Farm was included as a random intercept term to account for repeated trap sampling from the same site across time. *Thaumatotibia batrachopa* and *C. peltastica* models were specified using a negative binomial error distribution with a log-link function to account for overdispersion

in the count data. Season and its interactions were removed to model *C. peltastica* in the litchi orchards, as data was collected only from one season. Hypothesis testing of additive and multiplicative effects was performed using Wald's tests by comparing the saturated and simpler additive models.

Release number

To assess the possible effect of parasitoid release number on trap activity, weekly *T. batrachopa* and *C. peltastica* trap counts were modelled as a function of release number, trial week, and season using a GLMM approach. Only data from trees receiving the 'Standard' release timing treatment were included to isolate variation due to release number alone. Release number was treated as a continuous predictor, derived from four discrete release rates (0, 12,500, 25,000, and 50,000 wasps/ha), and was mean-centred and scaled to standard deviation = 1 to improve model convergence and interpretability. Trial week was specified as a continuous predictor representing weeks since the start of the growing season. A saturated model was constructed, including all two-way interaction terms between scaled release number, trial week, and season, to test the hypothesis that trap activity may vary with release number over time or between seasons. Farm was specified as a random intercept to account for repeated measures from the same sampling units. Models were fitted using a negative binomial error distribution with a log-link function to accommodate overdispersion in the count data. Hypothesis testing of additive and multiplicative effects was performed using Wald's tests by comparing the saturated model with the simpler additive model.

All models were specified using the 'glmmTMB' R package (Brooks *et al.*, 2017), and all data analyses were performed using R version 4.4.1 (R Core Team, 2025).

Fruit and nut damage

No modelling was possible for the grape, pome fruit, and litchi orchards because virtually no damaged fruit was recorded over the growing season. Modelling of the damage seen would have been ecologically unreliable. Modelling was done in the macadamia orchards for all farms combined for both seasons and their combined effect. Each farm was also individually modelled for the two seasons and the combined effect of the two seasons.

To assess how tortricid nut damage per week over the growing season, for two growing seasons, might be affected by different parasitoid release timings and parasitoid release number, a GLMM approach was adopted (Bolker *et al.,* 2009). Two model sets were specified to assess the release timing and release number effects, as the full factorial combination of release numbers and timing treatments was not available, with the release number treatments only applied in a factorial manner within the 'standard' parasitoid release timing window.

Release timing

To assess how tortricid nut damage varied in response to different parasitoid release timings over the growing seasons (Season 1 and Season 2), a GLMM approach was adopted. Only data from trees that received one of the four parasitoid release timing treatments, Control (no releases), Early, Standard, and Late, were included in this analysis. The proportion of damaged nuts per tree was modelled as a function of parasitoid release timing, specified as a categorical predictor with four levels, and trial week, a continuous predictor indicating the number of weeks since the start of each growing season. Nut damage was modelled as a binomial response using the number of damaged nuts per tree as the numerator and the total number of nuts as the denominator. A saturated model including multiplicative interaction terms between release timing, week, and season was specified to test the hypothesis that the effect of parasitoid release timing on nut damage may vary over time or differ between seasons. A random intercept was included for the farms to account for repeated sampling from the same data trees across weeks. Models were fitted using a binomial error distribution and logit-link function. Hypothesis testing of additive and multiplicative effects was performed using Wald's test by comparing the models.

Release number

A GLMM approach was adopted to assess the possible effect of parasitoid release number on tortricid nut damage. Only data from trees within the *standard* release timing treatment were included in this analysis, as this was the only timing category where all four release number levels (0, 12,500, 25,000, and 50,000 wasps per hectare) were applied in a factorial manner. The proportion of damaged nuts per tree was modelled as a binomial response, using the number of damaged nuts as the numerator and total nuts collected per tree as the denominator. The release number was included as a continuous predictor, derived from the four discrete levels. It was mean-centred and scaled (mean = 0, standard deviation = 1) to normalise the variable and improve model

convergence. Trial week, indicating the number of weeks since the start of each growing season, was included as a continuous predictor. A saturated model was specified that included all two-way interaction terms between release number, trial week, and season to test the hypothesis that the effectiveness of parasitoid release number may vary across time or between seasons. A random intercept was included for farms to account for repeated sampling of the same trees across weeks. Models were fitted using a binomial error distribution and a logit-link function. Hypothesis testing of additive and multiplicative effects was performed using Wald's tests by comparing the saturated and simpler additive models.

All models were specified using the 'glmmTMB' R package (Brooks *et al*., 2017), and all data analyses were performed using R version 4.4.1 (R Core Team, 2025).

Larval identifications

Larval identifications were only done for those collected from macadamia orchards, as no larvae from the pome fruit orchards, litchi orchards, and grape vineyards were found during the data collection periods from damaged fruit.

Larval identifications from each farm were assigned to their respective provinces to determine whether species of larvae (*T. batrachopa*, *C. peltastica*, *T. leucotreta*, and Unconfirmed) damaging nuts varied by province. To determine whether the relative proportions of identified larval categories (*T. batrachopa*, *C. peltastica*, *T. leucotreta*, and Unconfirmed) varied within each province, the raw counts were first converted to percentages based on the total number of larvae recorded per site over both seasons. Non-parametric tests were used to assess differences among parasitoid categories within each province. A Kruskal-Wallis test was applied to detect overall differences, followed by Dunn's *post hoc* tests with Bonferroni adjustment for multiple comparisons.

In-field parasitism

No modelling was possible for the grape, pome fruit, and litchi orchards due to virtually no recorded tortricid eggs over the growing season. Modelling of the eggs seen would have been ecologically unreliable. Modelling was done in the macadamia orchards for all farms combined for both seasons and their combined effect. Each farm was also individually modelled for the two seasons and the combined effect of the two seasons.

To assess how parasitism of tortricid eggs in macadamia orchards changed per week over the growing seasons, Season 1, Season 2, and the season effects combined, might be affected by different parasitoid release timings and parasitoid release number, a GLMM approach was adopted (Bolker *et al.,* 2009). Two model sets were specified to assess the release timing and release number effects, as the full factorial combination of release numbers and timing treatments was not available, with the release number treatments only applied in a factorial manner within the 'standard' parasitoid release timing window.

Release timing

To evaluate how *Trichogrammatoidea cryptophlebiae* parasitism of tortricid eggs in macadamia orchards varied across different parasitoid release timings over the two growing seasons (Season 1 and Season 2), a GLMM approach was employed (Bolker *et al*., 2009). The response variable was the number of parasitised eggs per cluster, modelled as a two-column binomial response comprising parasitised and non-parasitised eggs. The fixed effects included trial week (a continuous covariate representing weeks since parasitoid release began), season (a categorical predictor with two levels), and release timing (a four-level categorical variable: Control, Early, Standard, Late). To account for temporal dynamics, interaction terms between release timing and week and with season were specified. A random intercept was included for each unique data tree nested within farms to account for repeated measures across weeks and orchards. Models were fitted using a binomial error distribution and a logit-link. Two competing model structures were compared: an additive model including only main effects, and a complete multiplicative model including all two-way interactions between trial week, season, and timing. Hypothesis testing of additive and multiplicative effects was performed using Wald's test by comparing the models.

Release number

To assess how *T. cryptophlebiae* parasitism varied in response to different levels of parasitoid release number over the growing seasons (Season 1 and Season 2), a GLMM approach was adopted. Only data from the 'standard' parasitoid release timing treatments were included in this analysis, as the full factorial combination of release timings and release numbers was not implemented across all trials. The number of parasitised eggs per cluster of nuts was modelled as a function of parasitoid release number, specified as a continuous variable scaled from four discrete

levels (Control, 12,500, 25,000, and 50,000 wasps per hectare), and trial week, a continuous predictor indicating the number of weeks since parasitoid releases began. Parasitism was modelled as a count response using the number of parasitised eggs per cluster, with the total number of tortricid eggs included as a log-transformed offset to account for variation in egg availability. A saturated model including multiplicative interaction terms between release number, week, and season was specified to test the hypothesis that the effect of parasitoid release number on parasitism may change over time or vary between seasons. A random intercept was included for each tree (nested within farm) to account for repeated weekly sampling. Models were fitted using a Poisson error distribution and a log link function. Hypothesis testing of additive and multiplicative effects was performed using Wald's test by comparing the models.

All models were specified using the 'glmmTMB' R package (Brooks *et al*., 2017), and all data analyses were performed using R version 4.4.1 (R Core Team, 2025).

3.3. Results

3.3.1. Macadamia

Over two growing seasons, data were collected fortnightly for 28 weeks, starting in week 38 of the year, indicated in figures as week 0, through to week 12, indicated as week 26 in figures. Over both of those growing seasons, a total of 3,623 *T. batrachopa* (Season 1: 2055; Season 2: 1568), 313 *C. peltastica* (Season 1: 183; Season 2: 130), and 158 *T. leucotreta* (Season 1: 18; Season 2: 140) were captured in traps. Over both growing seasons, a total of 67,794 nuts were collected and dissected (Season 1: 22,806; Season 2: 44,988), with 5,784 being found to have been damaged by tortricids (Season 1: 3,082; Season 2: 2,702). Eight hundred seventy-one larvae were identified from the nuts collected (Season 1: 538; Season 2: 333), 770 were identified as *T. batrachopa* (Season 1: 481; Season 2: 289), 69 as *C. peltastica* (Season 1: 32; Season 2: 37), eight as *T. leucotreta* (Season 1: 3; Season 2: 5) and a remaining 24 (Season 1: 22; Season 2: 2) had uncertain identifications.

Trichogrammatoidea cryptophlebiae *release numbers*

Across both seasons, quality control sampling confirmed the delivery of parasitised eggs per hectare at scaled field release numbers of 12,500, 25,000, and 50,000 wasps/ha, with corresponding average percentages of female wasps recorded. In Season 1, two releases (Release 1 and Release 2) did not meet the minimum required threshold for field deployment due to low

parasitism levels (Table 3.6.). In Season 2, only one release (Release 6) failed to meet the minimum parasitism threshold (Table 3.7.). Overall, Season 2 demonstrated more consistent egg delivery and a significantly higher proportion of female wasps across all release events, suggesting improved rearing or handling protocols compared to Season 1.

Table 3.6. Mean (± SE) number of parasitised eggs per hectare and average percentage of female wasps recorded from quality control samples across parasitoid release events for macadamia orchards in Season 1. Values are presented for each release number and scaled to represent the three release numbers used in the field (12,500, 25,000, and 50,000 wasps/ha).

Release Number	12,500 wasps/ha	25,000 wasps/ha	50,000 wasps/ha	Percentage Female (%)
1 *	7740.62 ± 857.80	15481.25 ± 1715.59	30962.5 ± 3431.19	55.08 ± 12.75
2 *	5823.44 ± 1007.93	11646.88 ± 2015.86	23293.75 ± 4031.73	69.65 ± 6.11
3	19908.28 ± 469.39	39816.56 ± 938.78	79633.12 ± 1877.56	55.95 ± 2.88
4	18498.75 ± 565.87	36997.5 ± 1131.74	73995 ± 2263.49	40.67 ± 2.62
5	19395.94 ± 483.44	38791.88 ± 966.88	77583.75 ± 1933.76	72.03 ± 2.08
6	21103.13 ± 638.80	42206.25 ± 1277.60	84412.5 ± 2555.19	58.53 ± 1.78
7	19131.56 ± 547.39	38263.12 ± 1094.78	76526.25 ± 2189.55	70.77 ± 2.44
Total	111601.72 ± 1798.25	223203.4 ± 3596.49	446406.9 ± 7192.99	60.38 ± 4.28

**Asterisks (*) denote releases that did not meet the minimum number of parasitised eggs for field deployment.

Table 3.7. Mean (± SE) number of parasitised eggs per hectare and average percentage of female wasps recorded from quality control samples across parasitoid release events for macadamia orchards in Season 2. Values are presented for each release number and scaled to represent the three release numbers used in the field (12,500, 25,000, and 50,000 wasps/ha).

Release Number	12,500 wasps/ha	25,000 wasps/ha	50,000 wasps/ha	Percentage Female (%)
1	17440.78 ± 704.83	34881.56 ± 1409.65	69763.12 ± 2819.3	75.83 ± 1.18
2	13035.94 ± 456.39	26071.88 ± 912.79	52143.75 ± 1825.57	73.33 ± 1.1
3	17137.5 ± 576.34	34275 ± 1152.67	68550 ± 2305.34	71.97 ± 1.18
4	16524.38 ± 709.11	33048.75 ± 1418.22	66097.5 ± 2836.44	76.43 ± 1.33
5	16394.06 ± 1022.92	32788.12 ± 2045.84	65576.25 ± 4091.68	58.58 ± 1.59
6 *	8789.06 ± 755.77	17578.12 ± 1511.54	35156.25 ± 3023.07	73.88 ± 1.67
7	18604.69 ± 954.45	37209.38 ± 1908.9	74418.75 ± 3817.8	72.07 ± 1.37
Total	107926.4 ± 2017.08	215852.8 ± 4034.16	431705.6 ± 8068.3	71.73 ± 2.28

**Asterisks (*) denote releases that did not meet the minimum number of parasitised eggs for field deployment.

In-field parasitoid baiting with sentinel host eggs

A GLMM with a negative binomial distribution was used to assess the effects of baiting period and season on parasitism, including an interaction term and farm as a random effect. Model comparison revealed that the interaction between baiting period and season significantly improved model fit ($\chi^2 = 47.36$, df = 2, $p < 0.001$), indicating that the effect of baiting varied across seasons. In Season 1, no parasitism was recorded during the first baiting period (mean = 0.000), while baiting periods 2 and 3 resulted in higher parasitism (mean = 3.47 ± 2.19 SE and 2.40 ± 1.53 SE eggs, respectively) (Figure 3.1.). In Season 2, parasitism was again absent in baiting periods 1 and 2 (mean = 0.000 for both), and was only detected during baiting period 3, with a predicted mean of 0.77 ± 0.42 SE eggs. Due to the lack of parasitism in early periods, valid standard errors and confidence intervals could not be calculated for baiting periods 1 and 2 in Season 2, nor baiting period 1 in Season 1. These results indicate that baiting was only effective later in the season, with the strongest response recorded in the second baiting period of Season 1.

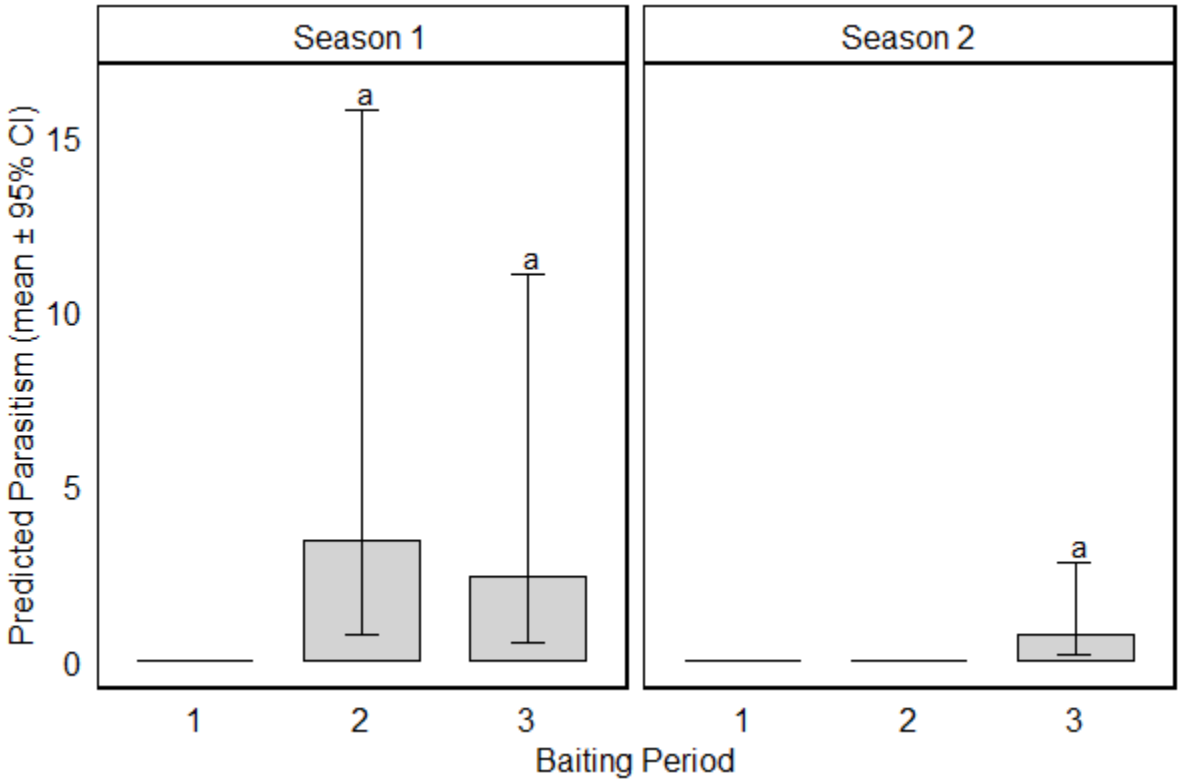

Figure 3.1. Predicted parasitism of baiting cards by *Trichogrammatoidea cryptophlebiae* at three periods of two macadamia growing seasons. Baiting one was done at the start of the season, baiting 2 in the middle and baiting three at the end. Letters indicate significant differences between baiting periods within each season ($P < 0.05$).

Trap counts

Release timing

Parasitism trends, as measured by LM trap counts across both growing seasons, showed strong statistical support for an additive effect of parasitoid release timing ($\chi^2 = 12.71$, df = 3, $P = 0.005$), with significantly higher trap counts under Early ($\beta = 1.18 \pm 0.38$, $P = 0.002$), Standard ($\beta = 1.06 \pm 0.39$, $P = 0.006$), and Late ($\beta = 1.28 \pm 0.39$, $P = 0.001$) releases compared to the Control (Figure 3.2. A). A strong main effect of time was also detected ($\chi^2 = 14.82$, df = 1, $P < 0.001$), with LM trap catches increasing by approximately 6.4 % per week ($\beta = 0.062 \pm 0.016$, $P < 0.001$). A multiplicative model including interactions between release timing, time, and season did not significantly improve model fit ($\chi^2 = 8.42$, df = 7, $P = 0.297$), suggesting that changes in LM catches over time were not dependent on timing treatment or season. Although the season effect

was not statistically significant ($\chi^2 = 3.43$, df = 1, $P = 0.064$), predicted LM trap catches tended to be slightly lower in Season 2 ($\beta = -0.46 \pm 0.25$, $P = 0.064$) (Figure 3.2. A). Across both seasons, LM trap catches were highest in the Late treatment, reaching ~0.92 per trap/week by Week 26 in Season 1 and ~0.58 in Season 2. Control plots remained consistently lower, not exceeding ~0.26 per trap/week.

For MNB trap catches, there was very strong support for a time effect across seasons ($\chi^2 = 94.25$, df = 1, $P < 0.001$), with counts increasing by approximately 10.3 % per week ($\beta = 0.098 \pm 0.010$, $P < 0.001$) from the start of the trial (Figure 3.2. B). A strong seasonal effect was also detected ($\chi^2 = 55.48$, df = 1, $P < 0.001$), with MNB counts significantly higher in Season 1 compared to Season 2 ($\beta = -1.08 \pm 0.14$, $P < 0.001$). However, there was no support for an additive effect of parasitoid release timing ($\chi^2 = 1.46$, df = 3, $P = 0.692$), nor a multiplicative interaction with time or season ($\chi^2 = 7.43$, df = 7, $P = 0.386$), indicating that MNB trap catches increased steadily over time regardless of release timing. By Week 26 in Season 1, MNB catches reached ~15.6 per trap/week in the Early release treatment and ~12.4 in the Late treatment. In Season 2, predicted trap catches were considerably lower, ranging from ~5.3 (Early) to ~4.2 (Late) per trap/week (Figure 3.2. B).

Together, these results demonstrate distinct trends in LM and MNB trap activity in response to parasitoid release timing. LM trap counts were strongly associated with release timing, with Early, Standard, and especially Late treatments producing significantly higher catches than the Control. However, this association does not imply a direct causal effect of parasitoid release on LM activity. The elevated LM catches in release treatments may instead reflect behavioural or environmental factors coinciding with release timing rather than a direct biological response. In contrast, MNB trap catches increased consistently over time and were more strongly driven by seasonal effects than release timing. These findings highlight differential behavioural responses between species, with LM showing more consistent and timing-sensitive patterns, and MNB being more influenced by broader seasonal conditions.

Release number

LM trap activity across both growing seasons showed strong statistical support for a main effect of parasitoid release number ($\chi^2 = 8.95$, df = 1, $P = 0.003$), with trap counts increasing significantly as release number increased ($\beta = 0.32 \pm 0.11$, $P = 0.003$) (Figure 3.3. A). Time (trial

week) also had a significant positive effect ($\chi^2 = 13.40$, df = 1, $P < 0.001$), indicating a steady increase in LM catches over the course of the season ($\beta = 0.052 \pm 0.014$, $P < 0.001$). No main effect of season was detected ($\chi^2 = 0.75$, df = 1, $P = 0.387$), and a multiplicative model including interactions with number, time, and season did not improve model fit ($\chi^2 = 1.49$, df = 3, $P = 0.684$), suggesting that LM trap catches were not dependent on season or changes in number over time. In Season 1, predicted LM trap catches rose from ~0.08 under the Control to ~0.66 under the highest release number by Week 26. In Season 2, trap catches were slightly lower overall, ranging from ~0.07 (Control) to ~0.55 (high release number) per trap/week (Figure 3.3. A).

MNB trap activity, by contrast, was primarily driven by time and seasonal effects. There was strong support for a time effect ($\chi^2 = 78.33$, df = 1, $P < 0.001$), with trap catches increasing by approximately 9.2 % per week ($\beta = 0.088 \pm 0.010$, $P < 0.001$) (Figure 3.3. B). A strong seasonal effect was also detected ($\chi^2 = 38.27$, df = 1, $P < 0.001$), with significantly higher MNB trap counts in Season 1 compared to Season 2 ($\beta = -0.88 \pm 0.14$, $P < 0.001$). However, there was no evidence for an additive effect of parasitoid release number on MNB catches ($\chi^2 = 2.03$, df = 1, $P = 0.154$), and the inclusion of interactions in a multiplicative model did not improve model fit ($\chi^2 = 0.54$, df = 3, $P = 0.911$). By Week 26, predicted MNB trap catches in Season 1 ranged from ~1.2 per trap/week under the Control to ~15.2 in the highest release number treatment, while in Season 2 they ranged from ~0.6 (Control) to ~6.3 (high release number) (Figure 3.3. B).

Together, these results indicate species-specific responses to parasitoid release number. LM trap catches were positively associated with increasing release number, suggesting a potential link between release density and local LM activity. However, this association does not imply a direct causal effect of parasitoid release on LM activity. The elevated LM catches in release treatments may instead reflect behavioural or environmental factors coinciding with release timing rather than a direct biological response. In contrast, MNB trap activity was primarily driven by time and season, with no detectable relationship with release number. These findings underscore contrasting behavioural dynamics between species, with LM showing release number responsive patterns, while seasonal trends more strongly governed MNB.

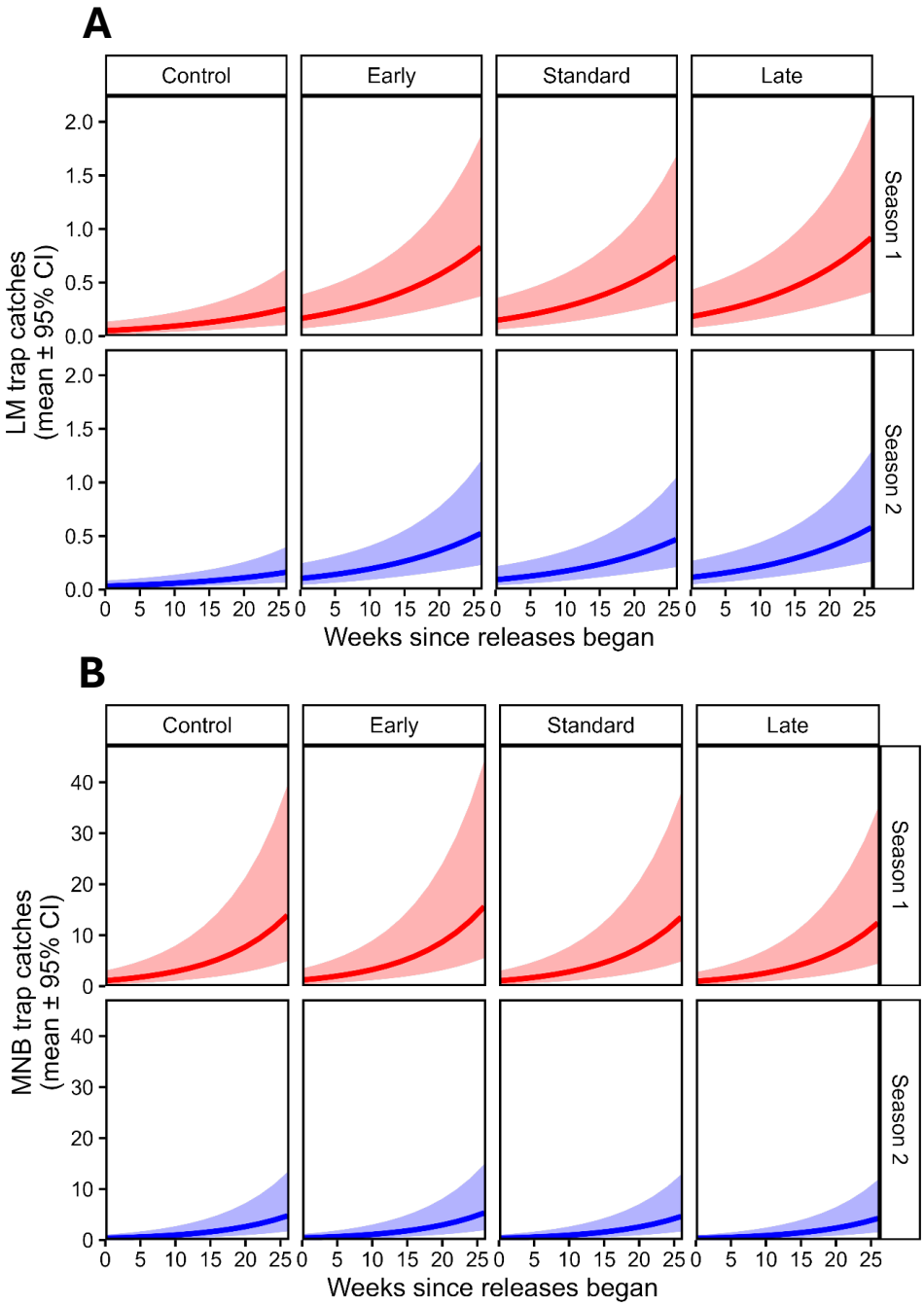

Figure 3.2. Marginal effect of *Trichogrammatoidea cryptophlebiae* releasing timing on (A) LM and (B) MNB trap catches in macadamia orchards over two growing seasons. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

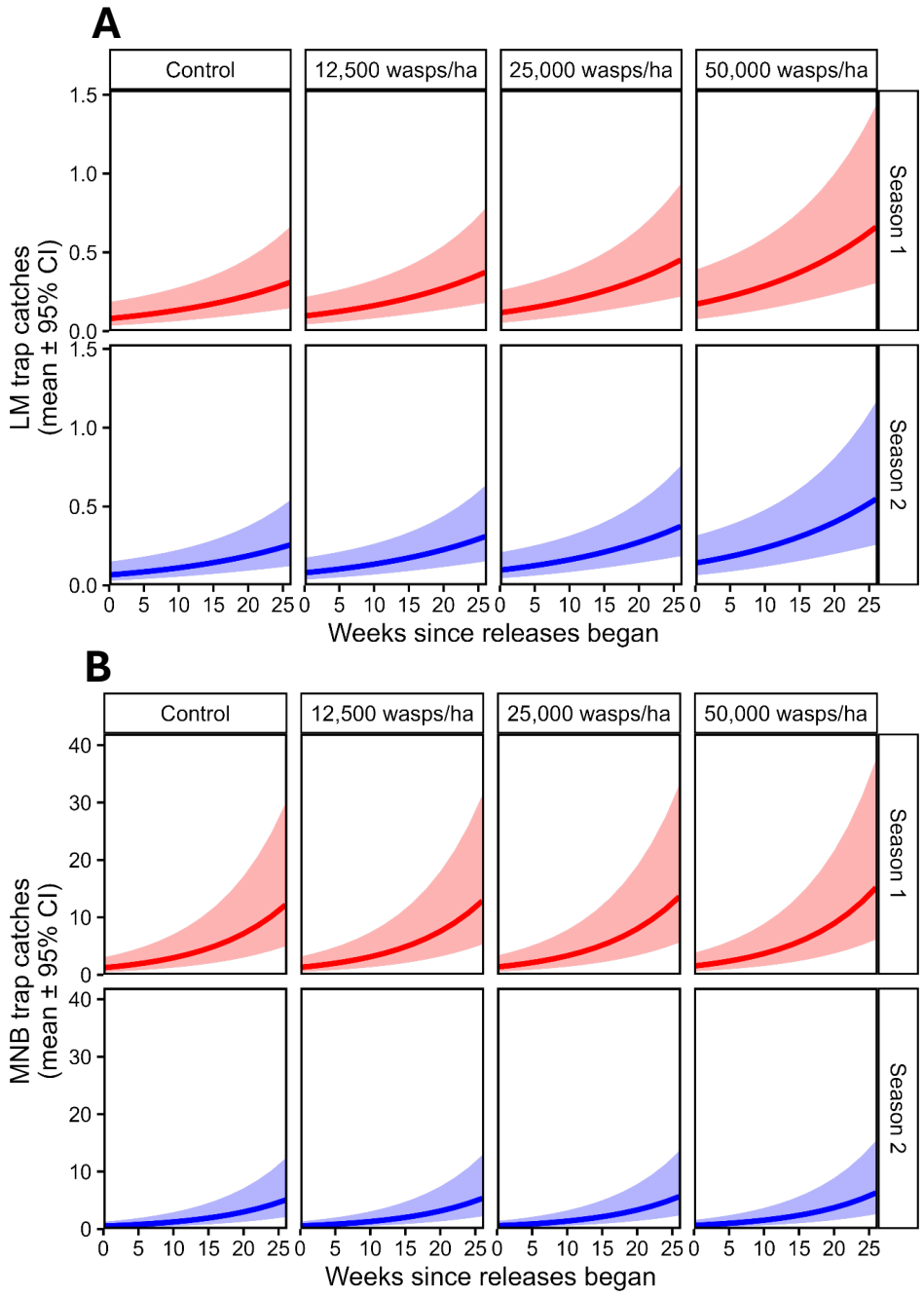

Figure 3.3. Marginal effect of *Trichogrammatoidea cryptophlebiae* releasing number on (A) LM and (B) MNB trap catches in macadamia orchards over two growing seasons. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

Nut damage

Release timing

There was strong statistical support for the full multiplicative model over the simpler additive model ($\chi^2 = 240.50$, df = 7, $P < 0.001$), indicating that the proportion of damaged nuts varied as a function of both season and the interaction between parasitoid release timing and time. While the main effect of time (trial week) was not significant overall ($\chi^2 = 0.27$, df = 1, $P = 0.606$), there was a highly significant interaction between trial week and season ($\chi^2 = 154.07$, df = 1, $P < 0.001$), suggesting that the temporal trajectory of nut damage differed markedly between seasons (Figure 3.4. A). There was also a strong main effect of parasitoid release timing ($\chi^2 = 35.63$, df = 3, $P < 0.001$) and highly significant interactions between timing and both season ($\chi^2 = 519.15$, df = 4, $P < 0.001$) and trial week ($\chi^2 = 51.95$, df = 3, $P < 0.001$), indicating that the effectiveness of different release timings changed over time and between seasons (Figure 3.4. A). In Season 1, predicted nut damage in the Control treatment remained relatively stable, averaging around 10.4 % across the trial period. The Late treatment showed the greatest decline in nut damage, falling from 20.3 % in Week 0 to 5.4 % by Week 26. Standard releases also led to notable reductions, decreasing from 15.3 % to 8.0 %, while the Early treatment had a limited impact, with nut damage remaining above 9.1 % throughout the season. In Season 2, overall nut damage was substantially lower. The Control plots began at just 1.2 % in Week 0 and gradually increased to 7.0 % by Week 26. In contrast, the Late and Standard treatments started higher at 4.0 % and 2.2 %, respectively, but rose more slowly, reaching 6.2 % and 7.1 % by Week 26. These results demonstrate that parasitoid release timing significantly influenced nut damage, particularly in Season 1, where Late and Standard treatments produced the most substantial reductions. The consistently lower levels of nut damage in Season 2 may reflect broader seasonal or environmental factors, such as lower pest pressure, cooler weather conditions, or differing pest phenology, that reduced overall infestation levels and masked treatment effects. This highlights the importance of seasonal context in shaping biological control outcomes, with greater treatment efficacy recorded under higher pest pressure.

Release number

There was strong statistical support for the full multiplicative model over the simpler additive model ($\chi^2 = 31.08$, df = 3, $P < 0.001$), indicating that the proportion of damaged nuts varied as a function of both season and the interaction between parasitoid release number and time. While the main effect of time (trial week) was not significant overall ($\chi^2 = 0.24$, df = 1, $P = 0.624$), there was a highly significant interaction between trial week and season ($\chi^2 = 22.19$, df = 1, $P < 0.001$), suggesting that the trajectory of nut damage over time differed between the two seasons (Figure 3.4. B). The main effect of release number was not significant on its own ($\chi^2 = 2.45$, df = 1, $P = 0.117$). Still, a significant interaction between release number and time ($\chi^2 = 4.66$, df = 1, $P = 0.031$) indicated that the impact of parasitoid release number on nut damage changed over the season. There was no significant interaction between season and release number ($\chi^2 = 2.02$, df = 1, $P = 0.155$), suggesting that the relative effect of increasing release number was consistent across years. In Season 1, predicted nut damage in the Control treatment remained relatively stable, averaging around 10.4 % across the trial period. In contrast, the 50,000 wasps/ha treatment steadily declined nut damage from 10.4 % in Week 0 to 5.4 % by Week 26. Moderate reductions were also seen at 25,000 wasps/ha, with nut damage decreasing from 12.4 % to 7.9 %, while the 12,500 wasps/ha treatment showed a smaller decline from 11.0 % to 9.1 % over the same period. In Season 2, nut damage was substantially lower overall. Control plots began with a predicted damage rate of 1.5 %, increasing to 7.0 % by Week 26. The most significant reductions were recorded in the 50,000 wasps/ha treatment, which started at 4.3 % and rose only slightly to 6.2 %. Similar trends were seen at 25,000 wasps/ha, with damage rising from 2.6 % to 7.1 %, and at 12,500 wasps/ha, increasing from 1.3 % to 5.4 % by the end of the season. These results demonstrate that increased parasitoid release number was associated with reduced nut damage, particularly in Season 1. While damage increased over time across all treatments in Season 2, overall rates remained much lower than in the previous season. The interaction between number and week suggests that the benefits of higher release densities accumulate over time, reinforcing the value of sustained release programmes in biological control efforts.

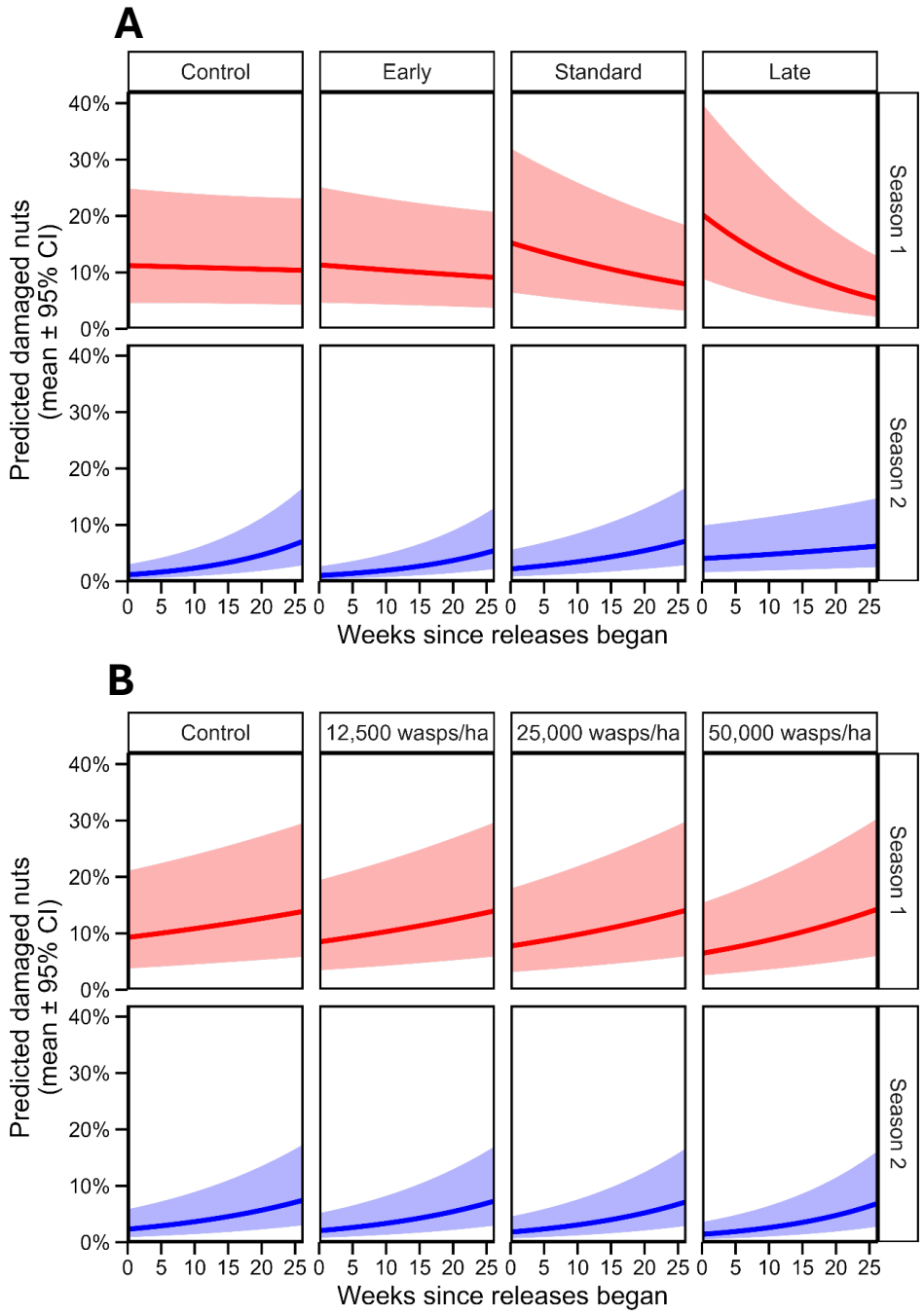

Figure 3.4. Marginal effect of *Trichogrammatoidea cryptophlebiae* releasing timing (A) and release number (B) on tortricid nut damage in macadamia orchards over two growing seasons. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

Larval identification

Within each province, the relative proportions of parasitoid identification categories (MNB, LM, FCM, and Unconfirmed) varied significantly. In all provinces, MNB was consistently and significantly more abundant than other categories across all provinces, whereas LM and FCM remained minor components, often statistically indistinct from Unconfirmed identifications (Table 3.8.). This data suggests the MNB is the tortricid causing significantly more damage to macadamia nut crops than the other tortricid species and unconfirmed identifications.

Table 3.8. The average percentage of larval species, MNB, LM, FCM, and unconfirmed, found in damaged nuts collected from three provinces, the Eastern Cape, Western Cape, and Mpumalanga.

Province	MNB (%)	LM (%)	FCM (%)	Unconfirmed (%)
Eastern Cape	83.33 ± 10.54 a	0.00 ± 0.00 b	0.00 ± 0.00 b	16.67 ± 10.54 c
Western Cape	87.92 ± 3.98 a	1.73 ± 0.81 b	2.46 ± 1.90 b	7.89 ± 3.72 b
Mpumalanga	81.34 ± 3.47 a	16.97 ± 3.39 ab	1.68 ± 0.76 bc	0.00 ± 0.00 c

* Letters indicate significant differences between larval species within each province ($P < 0.05$).

In-field parasitism

Release timing

Parasitism trends across the two growing seasons showed strong statistical support for the full multiplicative model over the simpler additive model ($\chi^2 = 24.39$, df = 7, $P < 0.001$), indicating that parasitism varied as a function of both season and its interactions with parasitoid release timing and week. Although the main effect of time (trial week) was not statistically significant overall ($\chi^2 = 1.02$, df = 1, $P = 0.312$), parasitism increased gradually over the trial, especially in Season 2. There was a significant main effect of release timing ($\chi^2 = 7.96$, df = 3, $P = 0.047$), and a strong interaction between timing and season ($\chi^2 = 11.34$, df = 3, $P = 0.010$), suggesting that the relative effectiveness of different timing strategies differed between years (Figure 3.5.A). There was also marginal support for an interaction between timing and week ($\chi^2 = 7.20$, df = 3, $P = 0.066$), primarily driven by a significant decline in parasitism over time in the Early release treatment (β

= -0.092 ± 0.043, P = 0.032). In contrast, parasitism levels in the Standard and Late release treatments remained stable across weeks (P > 0.58), indicating consistently high performance throughout the trial period (Figure 3.5.A). Predicted parasitism rates were consistently lowest in the Control plots across both seasons. In Season 1, parasitism in the Standard and Late treatments reached approximately 11-14 % by the end of the trial, while in Season 2, parasitism in the same treatments exceeded 28-36 %. These findings indicate a more favourable biological response in Season 2 and highlight the importance of seasonal variation in determining the success of augmentative parasitoid release strategies. Overall, the Standard and Late timing treatments delivered the highest parasitism levels and most consistent improvements over the Control, underscoring their potential value in integrated pest management programmes.

Release number

Parasitism rates were significantly influenced by parasitoid release number and its interaction with time. The full multiplicative model was supported over the simpler additive model (χ^2 = 7.85, df = 3, P = 0.049), indicating that parasitism was shaped not only by release number but also by its interactions with trial week and season (Figure 3.5.B). While the main effect of time (trial week) was not significant (χ^2 = 0.06, df = 1, P = 0.814), there was significant support for a main effect of parasitoid release number (χ^2 = 4.02, df = 1, P = 0.045), with higher release densities associated with increased parasitism. A significant interaction between release number and trial week (χ^2 = 5.49, df = 1, P = 0.019) indicated that the effect of increasing release number diminished over time (β = -0.027 ± 0.012, P = 0.019), suggesting that release number-dependent gains in parasitism may taper with prolonged exposure. Neither season nor its interactions with release number or time were statistically significant (all P > 0.19), implying that seasonal differences did not substantially alter the relationship between release number and parasitism rates. Back-transformed model predictions confirmed that parasitism increased with greater parasitoid release number in both seasons, although gains were more pronounced in Season 2. By Week 26, predicted parasitism under the highest release number treatment (50,000 wasps/ha) reached approximately 42.1 % in Season 2, compared to just 6.2 % in Season 1. These findings demonstrate that increased parasitoid release number substantially enhances biological control outcomes, particularly when environmental conditions are more favourable.

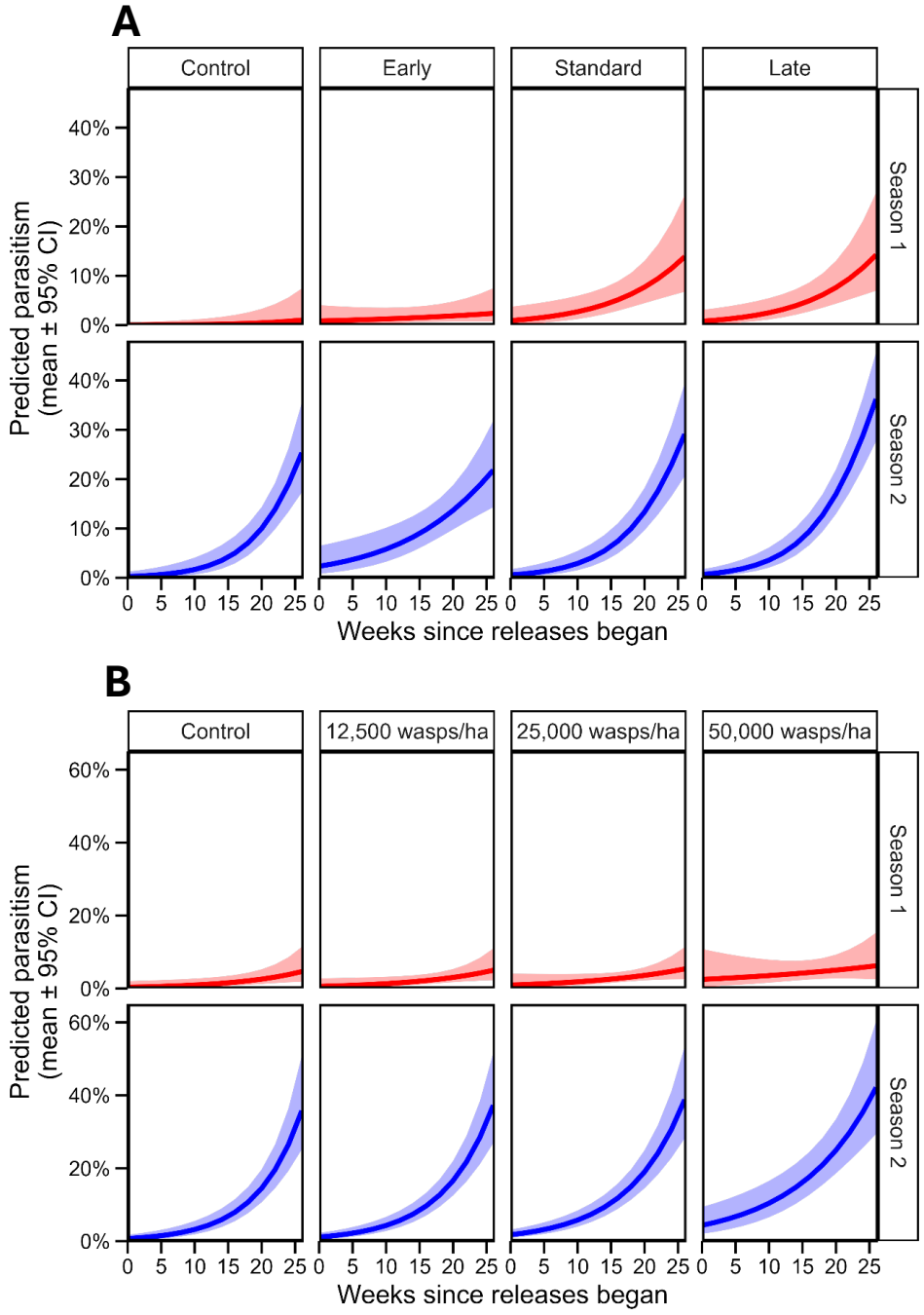

Figure 3.5. Marginal effect of *Trichogrammatoidea cryptophlebiae* releasing timing (A) and release number (B) on tortricid egg parasitism in macadamia orchards over two growing seasons. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

3.3.2. Grape

Over one growing season, data were collected fortnightly for 20 weeks, from week 40 of the year, indicated in figures as week 0, through to week 6, indicated as week 18 in figures. Over the growing season, a total of 0 *L. vanillana* and 16 *T. leucotreta* were captured in traps. Only 6 bunches of grapes were recorded to have damage, no larvae were found in those bunches. Zero tortricid eggs or parasitism were recorded either.

Trichogrammatoidea cryptophlebiae *release numbers*

Quality control assessments for parasitoid releases in grape vineyards during Season 1 revealed variable numbers across the five release events. Three of the five release events (Releases 1,2, 3) did not meet the minimum parasitism threshold required for field deployment (Table 3.9.). Notably, Release 3 had the lowest female proportion (33.61 ± 3.50%), suggesting potential issues with batch quality or sex ratio at that time (Table 3.9.). In contrast, Releases 4 and 5 met the required parasitism thresholds and delivered higher egg quantities with more balanced sex ratios (Table 3.9.). These results indicate that parasitoid production quality for vineyard releases was inconsistent during Season 1, leading to the total overall wasps due for release not meeting the requirement for each respective treatment.

Table 3.9. Mean (± SE) number of parasitised eggs per hectare and average percentage of female wasps recorded from quality control samples across parasitoid release events for grape vineyards in Season 1. Values are presented for each release number and scaled to represent the three release numbers used in the field (12,500, 25,000, and 50,000 wasps/ha).

Release Number	12,500 wasps/ha	25,000 wasps/ha	50,000 wasps/ha	Percentage Female (%)
1 *	7820.31 ± 469.09	15640.62 ± 938.17	31281.25 ± 1876.34	60.55 ± 11.07
2 *	9654.17 ± 614.7	19308.33 ± 1229.41	38616.67 ± 2458.81	66.2 ± 4.41
3 *	11344.69 ± 447.07	22689.38 ± 894.15	45378.75 ± 1788.3	33.61 ± 3.5
4	19312.5 ± 563.88	38625 ± 1127.76	77250 ± 2255.52	56.91 ± 2.29
5	13181.72 ± 433.49	26363.44 ± 866.98	52726.87 ± 1733.97	62.11 ± 2.93
Total *	61313.39 ± 1141.77	122626.8 ± 2283.55	245253.5 ± 4567.1	55.88 ± 5.76

**Asterisks (*) denote releases that did not meet the minimum required number of parasitised eggs for field deployment.

Trap counts

Over the 18-week monitoring period, a total of 570 trap inspections were conducted across five trial sites. Each site contained six treatments, with each treatment deploying six traps for *T. leucotreta* and six traps for *L. vanillana*, resulting in eighteen trap readings per trap across the trial duration. Only sixteen *T. leucotreta* individuals were captured across eleven readings, while no *L. vanillana* individuals were recorded in any of the trap inspections. Raw capture data are presented in Table 3.10.

Table 3.10. The raw capture data of *Thaumatotibia leucotreta* per trial week, parasitoid release treatment and timing of release.

Trial Week	Treatment	Timing	*T. leucotreta*
5	25,000 wasps/ha	Early	1
8	25,000 wasps/ha	Early	1
9	25,000 wasps/ha	Late	3
11	Control	n/a	1
13	50,000 wasps/ha	Standard	1
13	Control	n/a	1
14	25,000 wasps/ha	Early	1
14	25,000 wasps/ha	Standard	1
16	Control	n/a	1
16	25,000 wasps/ha	Late	4
18	50,000 wasps/ha	Standard	1

3.3.3. Litchi

Over one growing season, data were collected weekly for 10 weeks from week 41 of the year, indicated as week 0 in figures, through to week 50, indicated as week 9 in figures. Over the course of the growing season, a total of 53 *C. peltastica* and 0 *T. leucotreta* were captured in traps. Only 8 clusters of litchis were recorded to have damage, no larvae were found in those bunches. Thirty tortricid eggs were identified on the fruit, and 12 parasitised eggs.

Trichogrammatoidea cryptophlebiae *release numbers*

In litchi orchards during Season 1, quality control evaluations revealed moderate variability in parasitised egg delivery and female wasp proportions across six parasitoid release events. Four of the six release events (Releases 1, 2, 3, 4) did not meet the minimum threshold for field deployment (Table 3.11.). Release 4 delivered the lowest proportion of females (33.61 ± 3.50 %), indicating potential issues with batch sex ratio at the time (Table 3.11.). The final two releases (Releases 5 and 6) met deployment standards and provided higher egg outputs (Table 3.11.). These findings highlight inconsistent parasitoid production quality during Season 1 in litchi orchards, with two-thirds of releases failing to meet the numbers required for those respective releases and fluctuating sex ratios possibly affecting biological control efficacy. The total number of parasitoids required for the season was, however, met (Table 3.11.).

Table 3.11. Mean (± SE) number of parasitised eggs per hectare and average percentage of female wasps recorded from quality control samples across parasitoid release events for litchi orchards in Season 1. Values are presented for each release number and scaled to represent the three release numbers used in the field (12,500, 25,000, and 50,000 wasps/ha).

Release Number	12,500 wasps/ha	25,000 wasps/ha	50,000 wasps/ha	Percentage Female (%)
1 *	8212.5 ± 395.47	16425 ± 790.95	32850 ± 1581.89	67.9 ± 4.09
2 *	9654.17 ± 614.7	19308.33 ± 1229.41	38616.67 ± 2458.81	66.2 ± 4.41
3 *	8867.34 ± 431.32	17734.69 ± 862.64	35469.38 ± 1725.29	57.64 ± 3.16
4 *	11344.69 ± 447.07	22689.38 ± 894.15	45378.75 ± 1788.3	33.61 ± 3.5
5	20337.19 ± 690.7	40674.38 ± 1381.4	81348.75 ± 2762.81	64.57 ± 2.81
6	16782.19 ± 532.68	33564.38 ± 1065.37	67128.75 ± 2130.74	41.05 ± 2.72
Total	75198.08 ± 1296.52	150396.2 ± 2593.06	300792.3 ± 5186.13	55.16 ± 5.89

**Asterisks (*) denote releases that did not meet the minimum required number of parasitised eggs for field deployment.

Trap counts

Release timing

There was marginal statistical support for a time effect on LM trap catches ($\chi^2 = 3.77$, df = 1, $P = 0.052$), with LM catches decreasing by approximately 10% per week from the start of the season ($\beta_{week} = -0.102$), averaged across the four release timing treatments (Figure 3.6. A). However, there was no evidence for an additive effect of release timing on LM trap catches ($\chi^2 = 2.80$, df = 3, $P = 0.423$), nor a multiplicative interaction between release timing and week ($\chi^2 = 2.77$, df = 3, $P = 0.429$). This suggests that LM trap catches declined slightly over time, but the different release timings did not significantly influence this trend, either individually or in interaction with time. Across the season, mean weekly LM catches were consistently lowest under the Standard release timing, averaging ~0.33 moths per trap per week, and highest under the Control treatment (~0.69 moths/trap/week by week 6). While these differences were not statistically significant, the Standard treatment resulted in 20-30 % lower mean LM catches compared to other timings throughout the season (Figure 5. A).

Release number

There was strong statistical support for an effect of release number on LM trap catches ($\chi^2 = 7.03$, df = 1, $P = 0.008$), with LM trap catches decreasing by approximately 36 % per unit increase in scaled release number ($\beta_{number} = -0.448$), averaged across all weeks since the beginning of the growing season (Figure 3.6. B). However, there was no evidence for a time effect ($\chi^2 = 2.13$, df = 1, $P = 0.144$), nor for a multiplicative interaction between release number and week ($\chi^2 = 1.37$, df = 1, $P = 0.242$), suggesting that the effect of release number on LM catches remained consistent throughout the season. Mean weekly LM trap catches declined progressively with increasing wasp release number, from approximately 0.68 moths per trap per week in the Control (0 wasps/ha) to 0.24 in the 50,000 moths/ha treatment by week 6 (Figure 3.6. B). While not all differences were individually significant, a consistent and monotonic decline in LM captures was recorded with increasing parasitoid release number, indicating a potential suppressive effect of higher release rates on LM populations across the season.

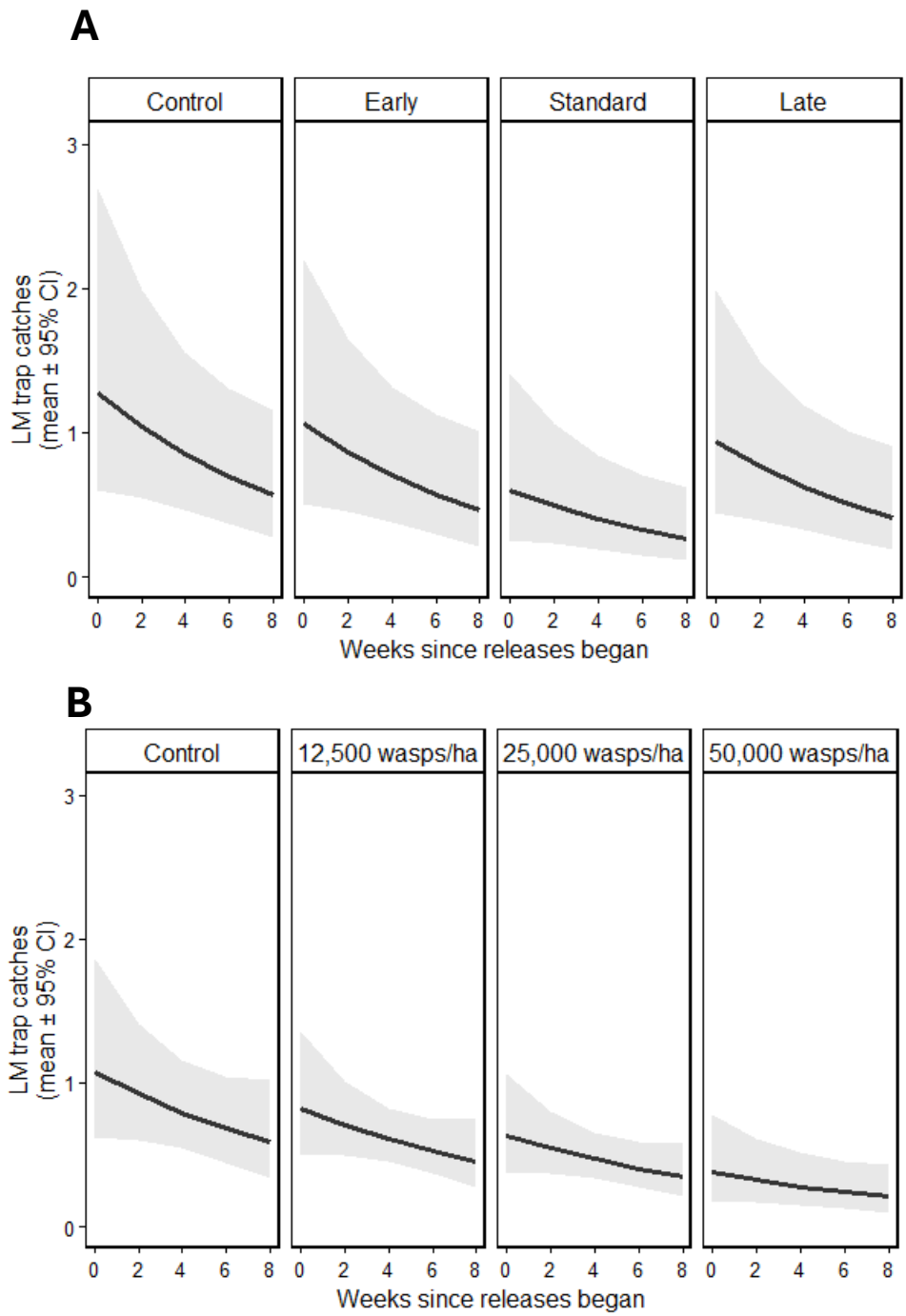

Figure 3.6. Marginal effect of *Trichogrammatoidea cryptophlebiae* releasing timing (A) and release number (B) on LM trap catches in Litchi orchards over one growing season. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

Fruit damage

Due to the extremely low incidence of fruit damage, tortricid egg oviposition, and parasitism recorded during the trial, statistical analysis was not possible. Instead, raw counts are presented in Table 3.12. below for descriptive purposes. Over a 10-week monitoring period, only 50 observations of damaged fruit, tortricid eggs, or parasitised eggs were recorded across all sampling points. This data scarcity likely reflects limitations in site selection, potentially due to naturally low pest pressure at the location or poor accessibility caused by tree architecture.

In particular, the height of the trial trees (approximately 8 m or more) may have restricted access to egg clusters. Given that *C. peltastica* oviposits higher in the canopy, it is plausible that many potential observations went undetected in the upper foliage, where sampling was not feasible (Newton & Crause, 1990). As a result, sampling efforts were confined mainly to the lower canopy, which may not have adequately captured pest or parasitoid activity.

Table 3.12. Raw count data of damaged fruit, tortricid eggs and parasitised eggs identified on clusters in Litchi orchards.

	Count
Damaged Fruit	8
Tortricid Eggs	30
Parasitised Eggs	12

3.3.4. Pome Fruit

Over one growing season, data were collected fortnightly for 28 weeks. Starting at week 38 of the year, indicated as week 0 in figures, and through to week 12 as week 26 in figures. Over the growing season, a total of 26 *C. pomonella* were captured in traps. Zero fruit were recorded to have damage, and zero tortricid or parasitised eggs were recorded either.

Trichogrammatoidea cryptophlebiae *release numbers*

Eight parasitoid release events were conducted in pome fruit orchards during Season 1, with quality control sampling used to assess delivery accuracy and wasp sex ratio. Of the eight releases, the first three (Releases 1, 2, 3) did not meet the minimum required parasitism levels for

field deployment (Table 3.13.). While the number of parasitised eggs increased consistently across later releases, female proportions varied, ranging from a low of 38.48 ± 3.97% in Release 4 to a high of 69.43 ± 2.26% in Release 6 (Table 3.13.). These results indicate an overall improvement in production consistency across the release period, though early batches underperformed in terms of parasitism thresholds. Variability in female proportion suggests a need for continued monitoring of sex ratios to ensure optimal biological control potential.

Table 3.13. Mean (± SE) number of parasitised eggs per hectare and average percentage of female wasps recorded from quality control samples across parasitoid release events for pome fruit orchards in Season 1. Values are presented for each release number and scaled to represent the three release numbers used in the field (12,500, 25,000, and 50,000 wasps/ha).

Release Number	12,500 wasps/ha	25,000 wasps/ha	50,000 wasps/ha	Percentage Female (%)
1 *	11152.08 ± 860.15	22304.17 ± 1720.3	44608.33 ± 3440.6	51.56 ± 4.18
2 *	8769.84 ± 702.76	17539.69 ± 1405.51	35079.38 ± 2811.02	56.71 ± 2.69
3 *	11966.72 ± 513.58	23933.44 ± 1027.17	47866.88 ± 2054.33	55.32 ± 3.67
4	13685.16 ± 532.88	27370.31 ± 1065.75	54740.62 ± 2131.5	38.48 ± 3.97
5	16604.53 ± 438.33	33209.06 ± 876.65	66418.13 ± 1753.31	68.94 ± 2.53
6	19959.84 ± 363.81	39919.69 ± 727.62	79839.38 ± 1455.24	69.43 ± 2.26
7	17789.53 ± 471.46	35579.06 ± 942.92	71158.12 ± 1885.83	66.12 ± 2.07
8	25968.28 ± 700.03	51936.56 ± 1400.07	103873.12 ± 2800.13	65.7 ± 2.71
Total	125895.98 ± 1678.77	251792 ± 3357.53	503584 ± 6715.06	59.03 ± 3.78

**Asterisks (*)* denote releases that did not meet the minimum required number of parasitised eggs for field deployment.

Trap counts

Over the 26-week monitoring period, a total of 260 trap inspections were conducted across 20 traps deployed at five trial sites. Each trap was inspected fortnightly, resulting in 12 readings per trap per treatment. Across all treatments and sites, only 26 adult *C. pomonella* individuals were captured, with moths recorded in just 16 readings. The raw capture data are presented in Table 3.14.

Table 3.14. The raw capture data of *Cydia pomonella* per trial week, parasitoid release treatment and pome fruit type.

Trial Week	Pome Fruit Type	Treatment	*C. pomonella*
10	Pear	Control	1
10	Pear	12,500 wasps/ha	1
10	Apple	Control	1
14	Apple	50,000 wasps/ha	3
16	Apple	50,000 wasps/ha	1
16	Apple	Control	1
18	Apple	50,000 wasps/ha	2
18	Pear	50,000 wasps/ha	1
20	Apple	50,000 wasps/ha	3
20	Apple	25,000 wasps/ha	2
20	Pear	Control	1
22	Apple	50,000 wasps/ha	3
22	Apple	25,000 wasps/ha	2
24	Apple	Control	1
24	Apple	50,000 wasps/ha	2
26	Apple	12,500 wasps/ha	1

3.4. Discussion

3.4.1. Macadamia

Quality control monitoring is foundational in effectively deploying *T. cryptophlebiae* within IPM programmes (Deguine *et al.*, 2021; Van Lenteren & Bigler, 2010). While the target is often framed in terms of parasitoids released per hectare, it is equally critical to ensure the biological quality of the parasitoids released. In particular, as high a proportion of females as possible is essential, as only females parasitise host eggs (Van Lenteren & Bigler, 2010). If either the release number or sex ratio is inconsistent, field efficacy can decline significantly (Smith, 1996). Moreover, excessive release densities beyond what the environment can support may lead to heightened competition among females for limited hosts, increasing the likelihood of

superparasitism and reducing overall impact (Zang *et al.*, 2021). These findings emphasise that greater numbers do not inherently lead to better control and instead highlight the need to optimise both quantity and biological quality across pest-crop systems (Moore *et al.*, 2015).

Routine quality control supports these goals by verifying that field releases align with expectations. By confirming release numbers and sex ratios, managers are better positioned to adjust strategies in real time, contributing to a more responsive and adaptive biological control programme (Zang *et al.,* 2021; Smith, 1996; Van Lenteren & Bigler, 2010). However, assessing quality under laboratory conditions alone may not fully reflect parasitoid performance in the field. Therefore, in this study, sentinel host egg cards were used to evaluate field emergence and fitness of *T. cryptophlebiae* (Hegazi *et al.*, 2012; Wahner, 2008). These cards were placed in orchards at various times throughout the season to detect wasp activity and parasitism as a proxy for successful establishment and host-finding behaviour.

While bait cards offered some insights, their reliability was variable. In many instances, they failed to detect parasitism even when it was known that wasps had been released. This could be due to poor synchronisation between parasitoid emergence and bait card placement, unattractive egg substrates, predation of the bait (particularly by snails), or suboptimal microclimatic conditions during exposure (Hegazi *et al.*, 2012; Wahner, 2008). Additionally, the positioning of cards, often limited to accessible lower-canopy branches, may have excluded microhabitats where parasitoids were more active. This suggests a better understanding of ideal bait card placement (within trees and across orchard zones) is needed. Nonetheless, in cases where parasitism was detected, it often coincided with known emergence peaks, confirming that the cards can be effective if well-timed.

Beyond quality and field fitness, the effectiveness of *T. cryptophlebiae* was also evaluated using trap catch data for *C. peltastica* and *T. batrachopa*. For *C. peltastica*, higher trap catches were associated with increased release number and later release timing. However, this likely reflects external factors, such as seasonal pest emergence or weather patterns, rather than a direct causal link with parasitoid activity (Schoeman, 2009; Smith *et al.*, 2022; Enslin, 2023). For *T. batrachopa*, no clear relationship with release variables was detected, and trap data suggested that week and season had a more substantial influence. This underscores that while trap catches can

indicate pest pressure trends, they cannot reliably serve as standalone indicators of parasitoid impact.

Interestingly, trap catches in Season 2 were significantly lower across both species, which may suggest legacy suppression from Season 1 releases. Repeated augmentative releases can reduce pest reproductive potential, leading to lower adult emergence in the following year (Bale *et al*., 2008). However, interpretation is complicated by inter-seasonal differences in rainfall, temperature, and orchard sanitation (Schoeman, 2009; La Croix & Thindwa, 1986; Chambers *et al.*, 1995). For example, residual nuts on trees following harvest, commonly seen in Season 1, are known to support overwintering tortricid populations (Schoeman, 2009). In Season 2, sanitation improvements, including more rigorous nut removal from trees and orchard floors, may have disrupted these overwintering sites, contributing to lower trap catches. Thus, it is suggested that a greater focus on cultural practices, alongside biological control, can aid in reducing carryover populations to the next cropping season and reduce overall pest activity.

Because of these complexities, crop damage was considered a more robust measure of parasitoid success. Nut damage levels decreased with higher parasitoid release numbers and with Standard and Late timing strategies. In Season 1, nut damage declined from over 20 % in some Late treatments to 5.4 % by Week 26, providing strong evidence of biological suppression. Early releases resulted in more moderate declines but maintained relatively stable damage levels throughout the season. This suggests a preventative effect, where early-season releases suppressed pest build-up before peak damage occurred (Moore *et al.*, 2002).

However, the effectiveness of early treatments may have been hindered by grower-applied insecticides. Early in the season, chemical programmes typically involve broad-spectrum and more persistent insecticides, which may negatively affect newly released parasitoids (Schoeman, 2009). Additionally, macadamia phenology in September, when Early treatments occurred, often coincides with flowering or match-head stages. Host egg availability is typically low during these stages, potentially making parasitoid foraging efforts less fruitful (Schoeman, 2009).

In contrast, Season 2 started with low baseline nut damage across all treatments (1 - 4 %), reducing the ability to detect treatment effects. Still, overall nut damage remained low throughout the season, which may indicate effective carryover suppression or improved orchard sanitation. The seasonal difference in initial pest pressure complicates interpretation but reinforces the value

of relative and absolute damage metrics (Peterson & Higley, 2001). For example, over 44,988 nuts were dissected in Season 2, double that of Season 1, due to higher nut drop and abscission rates. Therefore, expressing damage in absolute numbers (e.g., number of damaged nuts) is critical when comparing across seasons to avoid skewed results based on varying sampling intensities.

Larval identifications supported the trap and damage data. Across both seasons and all provinces, *T. batrachopa* was the dominant species recovered from damaged nuts, confirming it as the primary pest target of the biological control efforts (Smith *et al.*, 2022; Schoeman, 2009). *Cryptophlebia peltastica* and *T. leucotreta* were observed at low frequencies and were often inconsistently identified across the relevant growing areas during the trial period. In contrast, *T. batrachopa* was consistently the dominant tortricid found in the damaged nuts across all sites throughout the trial, indicating that it may pose the most significant threat among tortricid species damaging nuts during this time. It is important to note that macadamia orchards have overlapping tortricid populations that fluctuate throughout the growing season and vary in intensity depending on the geographic location of the orchards in South Africa (Schoeman, 2009; Enslin, 2023).

It is important to note that *T. cryptophlebiae* does not parasitise every egg within a cluster. The egg-laying patterns of these wasps can vary, leading to a selective foraging behaviour that results in some eggs being left unparasitised. This is particularly significant because, despite high rates of parasitism, the presence of these unparasitized eggs can still contribute to pest damage in crops (Newton, 1988b). Moreover, findings from Chapter 2 suggest that *T. cryptophlebia*e intentionally leaves certain host eggs unparasitized. This behaviour likely serves as a strategy to conserve hosts for future generations, enabling the wasps to ensure their offspring have sufficient resources over time. Consequently, while *T. cryptophlebiae* may not parasitise all tortricid eggs in proximity, this selective action plays a crucial role in moderating the overall levels of damaged nuts. However, it's essential to acknowledge a limitation in the trials conducted. The design focused on assessing parasitism rates per cluster rather than on an individual nut basis. This restriction hampers our ability to make direct comparisons between parasitism rates and tortricid egg levels in relation to nut damage (Chambers *et al*., 1995). Despite this limitation, the insights gained regarding parasitism rates are vital for strategising the optimal timing and numbers for releasing the parasitoids, ensuring more effective pest management in the future.

Standard and Late treatments achieved the highest parasitism, particularly in Season 2, where favourable conditions and grower cooperation likely enhanced parasitoid efficacy (Hegazi *et al.*, 2012; Kalyebi *et al.*, 2006). This highlights the importance of synchronising releases with pest egg availability and ensuring chemical programmes are compatible with biological control (Charleston *et al.*, 2004; Newton, 1989). Interestingly, parasitism in Early treatments began relatively high but declined over time, possibly due to reduced host availability following successful early suppression or mismatched phenology.

Higher release numbers, particularly the 50,000 wasps/ha treatment, led to consistently elevated parasitism rates and lower damage levels. While this effect was most pronounced in Season 1, benefits were still recorded in Season 2 despite low baseline damage. These results suggest that front-loading releases at higher densities may be the most effective strategy for pest suppression, especially when coupled with optimal timing and favourable conditions.

In conclusion, this study illustrates that successful biological control with *T. cryptophlebiae* hinges on more than just releasing large numbers of wasps. It requires an integrated approach involving quality control, field validation, strategic timing, grower cooperation, and environmental awareness. At the same time, trap counts and baiting offer practical context, but parasitism and nut damage remain the most informative metrics of biocontrol success. Future trials should refine methods to allow direct comparisons across these indicators, improving our ability to optimise and promote *T. cryptophlebiae* as a reliable IPM component in macadamia and other cropping systems.

3.4.2. Grape

Due to the low number of data points collected over the season, no reliable interpretations can be made. Considerations regarding the sporadic nature of *L. vanillana* make site selection challenging when trying to assess the efficacy of *T. cryptophlebiae* in suppressing their populations (Du Preez, 2019). The chemical application on grapes is also intense, limiting fungal growth when bunches of berries develop (Du Preez, 2019). Although most fungicides are not as detrimental to trichogrammatids, the vine architecture may provide few areas of refuge during the applications (Hegazi *et al.,* 2012). Future trials focusing on the suppression of *L. vanillana* through *T. cryptophlebiae* releases would need to be done in areas where the moth has been recorded before and would potentially need to run for several seasons due to their sporadic nature and the inability

to determine when they will be present in vineyards. Additionally, the focus on the suppression of *T. leucotreta* in grape vineyards would provide an indication of the ability of *T. cryptophlebiae* to manage tortricids occurring in vineyards successfully. Trap placement must also be considered, as hanging them on the wires supporting the vines was not ideal. Traps were consistently dirty and covered by leaves, preventing the pheromone from being picked up by the target pest.

3.4.3. Litchi

In litchi orchards, the selection of tree size is important to ensure the data collected is representative of the *C. peltastica* nature to oviposit eggs higher in the tree canopy, thereby influencing where the parasitoid will be searching for hosts (Newton & Crause, 1990; Grove & De Beer, 2017). The size of the trees also impacts the reliability of trap counts. If *C. peltastica* adults are more active in the tops of tree canopies, then this is where trap placement should be. The sites used for the trial had trees that were 9 m tall, making accessibility to the upper reaches not possible. Future trials should select for tree size that allows easy access to the upper reaches of the trees to improve data collection and statistical interpretability.

3.4.4. Pome Fruit

Due to the low number of data points collected over the season, no reliable interpretations can be made. Considerations regarding the chemical intensity during *C. pomonella* oviposition, when *T. cryptophlebiae* releases are assumed to be most effective, limits the parasitoid's potential success in suppressing this tortricid (Deguine *et al*., 2021). *Cydia pomonella* populations can be effectively monitored using degree day models, which help predict the moths' oviposition periods. Releasing parasitoids during these oviposition periods would be ideal, as the effectiveness of the wasps is likely to be higher when moth activity is at its peak (Mills *et al.,* 2000). Future trials should consider selecting sites that take a more integrated approach to pest management and where *C. pomonella* pressure is high.

3.5. Conclusion

These findings demonstrate that the success of *T. cryptophlebiae* as a biological control agent depends on more than just release quantity, it requires careful alignment with crop phenology, pest presence, and compatibility with existing pest management practices. While results in macadamia orchards highlight the potential of this parasitoid when supported by timely releases and high-quality individuals, trials in grapes, litchis, and pome fruit reveal important limitations related to host

availability, canopy structure, and chemical intensity. Moving forward, improved site selection, enhanced monitoring tools, and greater grower collaboration will be essential to optimising the integration of *T. cryptophlebiae* into diverse IPM systems.

Chapter 4: Non-target effects of chemical application on *Trichogrammatoidea cryptophlebiae*

4.1. Introduction

Macrobial biological control agents, such as predators and parasitoids, provide farmers with valuable ecosystem services, which can help them enhance their pest management programmes (Teder & Knapp, 2019). However, these natural enemies are often sensitive to the synthetic chemical pesticides that farmers use within their agricultural environment (Teder & Knapp, 2019). In recent years, there has been a greater focus on the development and implementation of alternative, sustainable, and non-chemical IPM practices that seek to conserve and promote natural enemy success (Deguine *et al.*, 2021). Despite the increasing uptake of IPM, synthetic pesticides still dominate the agricultural pest control market, due to their affordability, ease of use, and perceived efficacy (Deguine *et al.*, 2021; FAO, 2024; Thenoor *et al.*, 2024).

The mass production and release of macrobial biological control agents have been used for decades in global pest management, but their widespread adoption in commercial and conventional agriculture remains limited (van Lenteren *et al.*, 2021). Numerous obstacles must be addressed during the research and development phase of natural enemy production to guarantee the delivery of effective products to farmers for in-field use (van Lenteren, 2003; van Lenteren *et al.*, 2021). However, the provision of a high-quality product is only one component of a successful biological control programme. Farmers must also ensure that released natural enemies are compatible with other crop protection strategies used on the farm (van Lenteren, 2003; Thenoor *et al.*, 2024). One of the key barriers to biological control success is therefore the challenge of integrating natural enemies into pest management systems that rely heavily on chemical inputs (van Lenteren *et al*., 2021). Achieving this integration requires a better understanding of the non-target effects that different pesticides may have on beneficial organisms.

In agriculture, non-target effects typically refer to the unanticipated negative consequences that agrochemicals or other farming-related technologies exert on organisms or ecosystems that were not intended to be managed (Sanchez-Bayo, 2021). These unintended impacts can affect the biology and ecology of pests, benign species, and beneficial organisms alike (Wan *et al*., 2025). Insect parasitoids are particularly vulnerable to these effects due to their complex life histories and close associations with their hosts (Teder & Knapp, 2019). Pesticide applications may cause direct

mortality in natural enemies, or they may lead to sublethal physiological and behavioural effects that impair biological control (Sanchez-Bayo, 2021). While more selective, low-risk synthetic pesticides and biopesticide alternatives are now available and increasingly adopted, they are not necessarily free of risk. Although such products are often considered safer for beneficial insects compared to broad-spectrum pesticides, they may still exert harmful or unexpected effects (Schmidt-Jeffris, 2023; Lisi *et al.*, 2025). Moreover, the specificity and toxicity of these products are generally assessed using only a few model species within limited taxonomic groups, despite the fact that susceptibility can vary widely across species found in agroecosystems (Schmidt-Jeffris, 2023; Wan *et al.*, 2025).

Trichogrammatid species are among the most widely researched and applied natural enemies globally (van Lenteren *et al*., 2021). Substantial knowledge has been accumulated regarding the non-target effects of various agrochemicals on commonly used trichogrammatids such as *Trichogramma pretiosum* and *Trichogramma chilonis* (Rakes *et al.*, 2021; Khan, 2022; Salim *et al.*, 2025). However, there are relatively few studies investigating the non-target effects of relevant registered pesticides on agricultural biological control agents in South Africa. Hattingh *et al.* (2000) discuss the methodology for testing the residual impact of different pesticides on various natural enemies used in citrus, namely, *Aphytis linganensis* (Compere) (Hymenoptera: Aphelinidae) an ectoparasite of California red scale *Aonidiella aurantii* (Mask.) (Hermiptera: Diaspididae); *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae) an internal parasitoid of citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae); the predatory mites *Euseius citri* (Van der Merwe& Ryke) and *Euseius rubicolis* (Van der Merwe& Ryke)*;* and *T. cryptophlebiae.* Using this methodology up to 70 pesticides were tested against the different species, with 34 active ingredients being tested against *T. cryptophlebiae* (Grout *et al.,* 2011). Chemicals were rated for their harmful effects on the different species and the data shared with citrus industry stakeholders and chemical regulatory bodies (Hattingh *et al.,* 2000, Grout *et al.*, 2011). However, the residue assays focused solely on chemicals used in the citrus industry. Furthermore, much of the data is not publicly available and the information needs to be updated to reflect current pesticide usage, including new registered active ingredients that are commonly used in South African pest management. Therefore, both the residual and acute non-target effects of widely used agrochemicals still need to be confirmed for *T. cryptophlebiae.*

In Chapter 3, the mass release of *T. cryptophlebiae* in grape and pome fruit orchards resulted in little to no parasitism being observed over the trial season. These cropping systems, although compliant with international residue standards, still rely on conventional pesticides for pest and disease control in South Africa. During the experiments, parasitoid releases frequently coincided with chemical applications. Additionally, the simultaneous use of mating disruption and other pest control methods in these regions likely contributed to reduced pest pressure, as indicated by the consistently low numbers of adult moths captured in monitoring traps. While this low parasitism may reflect the limited availability of hosts due to intensive pest management, it could also suggest that chemical exposure negatively impacted the parasitoids' ability to function effectively.

This study, therefore, aimed to determine whether agrochemicals commonly used in the South African macadamia nut industry, and other fruit and tree crops, have a detrimental effect on *T. cryptophlebiae*. Specifically, it investigates both the acute and residual impacts of different chemical classes, including biopesticide products, on all life stages of the parasitoid. Generating such information is critical for improving IPM decision-making and timing, and for enhancing the efficacy of *T. cryptophlebiae* in cropping systems and geographic regions where host availability is higher, such as those presented in the macadamia field data discussed in Chapter 3.

4.2. Materials and Methods

A standard chemical spray programme for macadamias and those used by farmers from macadamia trial sites in Chapter 3 were received. The chemicals selected from these programmes were based on several factors: most commonly used, timing of their use in the growing season, their mode of action, and Insecticide Resistance Action Committee (IRAC) classification (Table 4.1.). The selected products are also registered for use in other crops and the product labels can be consulted for further information on good agricultural practices, crop usage and target pests (Act 36 of 1947).

Table 4.1. The chemicals selected for non-target chemical bioassays with *Trichogrammatoidea cryptophlebiae*, and their IRAC classification.

Active Ingredient	Product	IRAC Classification	Quantity of Product / L
Abamectin	Agrimec Gold	6	0.32 ml/ha
Acephate	Acephate	1B	0.75 g/L
Acetamiprid	Dominate	4A	0.5 g/L
Alpha-cypermethrin	Cyper 100 SC	3A	0.1 ml/L
Azoxystrobin & Difenoconazole	Amistar Top	FRAC 11 & 3	1 ml/ha
Beauveria bassiana (A)	Beauvitech	-	0.5 g/L
Beauveria bassiana (B)	Eco-BB	-	0.6 g/ha
Beta-cyfluthrin	Bulldock	3A	0.06 ml/ L
Chlorantraniliprole	Metro	28	0.2 ml/L
CrpeNPV	Multimax	-	0.08 ml/Ha
Cyprodinil	Chorus	FRAC 9	0.5 g/ha
Emamectin benzoate	Conflict Granule	6	0.075 g/L
Lambda-cyhalothrin	Karate Zeon	3A	0.05 ml/ L
Methoxyfenocide	Walker	18	0.6 ml/ L
Pymetrozine	Trivia	9B	0.4 g/L
Tau-fluvalinate	Klartan	3A	0.3 ml/ L
Thiamethoxam	Actara	4A	9 ml/tree

All selected chemicals are applied in macadamia orchards by means of spray applications, except for thiamethoxam, which has a systemic action and is applied as a root drench. For the dip assays (Section 4.2.1. and Section 4.2.2.), as described below, thiamethoxam was tested in the same manner at the recommended application rate. This was done to demonstrate how the incorrect application method can negatively impact *T. cryptophlebiae*.

4.2.1. Reproductive Potential

Wax paper cards, containing *T. leucotreta* non-irradiated eggs of 24 h old, were cut into 2 cm x 1 cm blocks and dipped into the respective chemical solutions. The chemicals were mixed according to the recommended application rates specified on the product labels for use in macadamias. It must be noted though that Acetamiprid is not registered for the use on macadamias, however the products composition is the same as others that are registered for use in macadamias. Acetamiprid was applied at the recommended rate as registered products. After dipping 10 egg blocks per chemical into the respective chemical solutions, they were allowed to dry completely before being placed into 30 ml glass vials that contained a carbohydrate source in the form of filter paper that had been soaked in a 20 % sugar solution. Two controls were used, a positive, where the egg blocks were dipped into reverse osmosis water, and a negative, where the egg blocks were not dipped at all. This was done to confirm that the dipping of the egg block into a liquid did not negatively impact the acceptance of the host eggs for parasitism by *T. cryptophlebiae* females. *Trichogrammatoidea cryptophlebiae* females that had been allowed to mate for 24 h were then placed individually into the glass vials containing the egg blocks treated with the respective chemicals. The vials were closed with cotton wool and placed into an environmental chamber set to 25°C ± 1, a relative humidity of 60% ± 5, and a photoperiod of 16:8 Light: Dark (L:D). After 24 h, the mortality of the females was recorded for each chemical, and the egg cards were removed and placed into petri dishes and sealed with parafilm.

Following a 16-day developmental period of the eggs at 25°C ± 1, a relative humidity of 60% ± 5, and a photoperiod of 16:8 Light: Dark (L:D), the following information was recorded. The number of parasitised eggs was recorded to determine if the reproductive potential of female *T. cryptophlebiae* differs when host eggs have been exposed to the respective chemicals used in macadamia orchards. The number of emergence holes, unsuccessful emergences, and sex ratios were recorded to determine if the chemical residues impact the development of the parasitoids or influence sex allocation by females.

4.2.2. Pre-Imaginal Effects

The same methodology as above was used for the dip assays, but rather for two stages of *T. cryptophlebiae* pre-imaginal development, the egg-larval stage, and the pupal stage. Ten egg blocks per development stage were dipped in each chemical and placed individually into 5 ml glass vials to develop. No filter paper was placed into the vials, as the longevity of the offspring

following their emergence was not recorded. The parasitoids were allowed to develop at 25°C ± 1, a relative humidity of 60% ± 5, and a photoperiod of 16:8 Light: Dark (L:D) until emergence. Following the emergence of the parasitoids, the number of emergence holes and unsuccessful emergences was counted. This was done to determine the impact of different chemical applications on the development of the parasitoid in its host egg.

Dip assays were repeated five times for both the reproductive potential and pre-imaginal effect trials.

Toxicity classifications based on those developed by the IOBC were assigned to female acute mortality and the emergence of offspring. The IOBC classifications have been widely adopted, despite several limitations, hence their inclusion (Mata *et al*., 2024). The IOBC classifications allow for easy interpretation of a chemical's status in relation to its impact on the select beneficial it is being evaluated against (Stark & Banks, 2024). In South Africa, Hattingh *et al.* (2000) have proposed a classification that has been adapted for more practical dissemination to farmers to help with questions surrounding the compatibility of pesticides and biological control organisms in the context of citrus.

4.2.3. Residual Effects

Residual trial methods described below were based on the methods used by Hattingh *et al.* (2000) whereby they evaluated the non-target effects of chemical products used in citrus on select beneficials, including *T. cryptophlebiae*.

Macadamia saplings, potted in 20 L bags, at around 2 m in height, were sprayed with the formulated chemical products listed in Table 4.1. at their recommended application rates with no adjuvants incorporated. Seedlings were sprayed individually with only one chemical using a 5-litre pressure sprayer. Thiamethoxam, the systemic, was applied as a root drench at the recommended dosage to a single potted sapling (Table 4.1.). They were sprayed to the point of runoff. The control sapling was sprayed with water only. Seedlings were then placed undercover, in a well-ventilated greenhouse that allowed natural light to pass through but prevented rain from landing on the plants.

Leaves that were fully expanded and had hardened off were picked to be used in the trials. Leaves were picked 0, 7, 14, and 21 days after spraying the seedlings. Leaves were picked from

the respective saplings and cut into 1 cm x 1 cm blocks to place into the 10 vials. The handling of the leaves was done carefully so as not to impact the residues remaining on the leaves. Each vial contained a piece of moistened filter paper, which was the carbohydrate source for parasitoids. A negative control was used, along with a positive control. The negative control had only moistened filter paper and no leaf in the vial, whereas the positive control used a leaf that had been sprayed with only water. Five males and five females were placed into each vial. The vials contained the leaf squares, and parasitoids were placed into an environmental chamber set to 25°C ± 1 °C, a relative humidity of 60 % ± 5 %, and a photoperiod of 16:8 Light: Dark (L: D). After 24 h, the mortality of the parasitoids was assessed under a dissecting microscope.

Two replicates of this trial were done, but more are needed to ensure reliable recommendations can be provided.

4.2.4. Statistical Analyses

Reproductive potential

To determine the effect of the chemical and survival of parasitoids on the number of parasitised host eggs, a generalised linear mixed model (GLMM) with a Poisson error distribution and a log-link function was used. Fixed effects included chemical treatment, parasitoid survival status (alive or dead) and their interaction, while replicate identity was included as a random intercept to account for within-replicate variation. Model fit was assessed by comparing the full model to a null model, which removed the interaction term, using a Wald's test. Estimated marginal means for each chemical treatment were obtained, and pairwise comparisons between parasitism levels between chemical treatments were adjusted using the Bonferroni method to control for multiple testing.

To evaluate whether the sex ratio of emerged parasitoids varied among chemical treatments and between survival statuses (alive vs. dead), we fitted a binomial GLMM with a logit-link. The response variable was the number of males relative to females that emerged per replicate. Fixed effects included chemical treatment and parasitoid survival status (dead or alive), and replicate was included as a random intercept to account for within-replicate variation. Model significance was assessed using a Wald's test comparing the full model to a null model containing only the random effect. Estimated marginal means were computed for each chemical treatment, and

Bonferroni-adjusted pairwise comparisons were used to identify significant differences in sex ratio.

Abbott's correction was used to correct female mortality based on the negative control (control that was not dipped) (Abbott, 1925). This allowed for IOBC classification to be assigned as follows:

- Class 1: *Harmless* (Corrected mortality < 30%) - Safe to natural enemies.
- Class 2: *Slightly harmful* (30-79%) - Acceptable risk; may be usable with care.
- Class 3: *Moderately harmful* (80-99%) - Not recommended; may suppress beneficial populations.
- Class 4: *Harmful* (> 99%) - Toxic; should not be used in conjunction with biological control agents.

No IOBC rating was assigned to thiamethoxam as it was not applied according to the product label and could lead to misinterpretation by users, thereby removing a product that is potentially compatible with *T. cryptophlebiae* when applied correctly.

Pre-imaginal development

For all stages of pre-imaginal development, including the fresh host eggs parasitised immediately after chemical exposure (reproductive potential), egg-larval stage, and pupal stage, the following model approach was taken to assess successful and unsuccessful emergence for each chemical.

To assess the impact of chemical treatments on parasitoid emergence, the percentage of successful emergence from parasitised eggs was analysed using a GLMM with a Gaussian error distribution and identity-link. Chemical treatment was included as a fixed effect, while replicate number (1 to 5) was included as a random intercept to account for variation between replicates. A model comparison was conducted using Wald's test between the full model, which included chemical treatment, and a reduced model containing only the random effect. The significance of chemical effects on emergence was evaluated through this comparison. *Post hoc* pairwise comparisons of estimated marginal means between treatments were performed with Bonferroni correction to adjust for multiple testing ($P > 0.05$).

To assess the impact of chemical treatments on parasitoid unsuccessful emergence, the proportion of unsuccessful emergence from parasitised eggs was analysed using a GLMM with a binomial error distribution and logit-link function. Chemical treatment was included as a fixed effect, while replicate number (1 to 5) was included as a random intercept to account for variation between replicates. A model comparison was conducted using Wald's test between the full model, which included chemical treatment, and a reduced model containing only the random effect. The significance of chemical effects on unsuccessful emergence was evaluated through this comparison. *Post hoc* pairwise comparisons of estimated marginal means between treatments were performed using Bonferroni correction to adjust for multiple testing ($P > 0.05$).

Abbott's correction was used to correct the emergence percentage based on the negative control (control that was not dipped) (Abbott, 1925). This allowed for IOBC classification to be assigned as follows:

- Class 1: Harmless (Emergence ≥ 70%) - Safe to natural enemies. Comparable to controls and suitable for use in biological control programmes.
- Class 2: Slightly harmful (Emergence 50-69%) - Acceptable risk; may be usable with care. Some reduction in emergence was observed.
- Class 3: Moderately harmful (Emergence 30-49%) - Not recommended; may suppress beneficial populations through reduced emergence.
- Class 4: Harmful (Emergence < 30%) - Toxic; should not be used in conjunction with biological control agents due to very low survival.

No IOBC rating was assigned to thiamethoxam as it was applied. No IOBC rating was assigned to thiamethoxam as it was not applied according to the product label and could lead to misinterpretation by users, thereby removing a product that is potentially compatible with *T. cryptophlebiae* when applied correctly.

Residual effects

To assess the impact of chemical residues on the survival of *T. cryptophlebiae* females and males, a binomial GLMM was fitted with a logit-link function. The model included fixed effects for chemical treatment, residue interval day treated as a numeric (0-, 7-, 14-, and 21-day post-application), and their interaction. Replicate number (1 to 5) was included as a random intercept

to account for repeated measurements within replicates. Survival was defined as the number of parasitoids (females or males) that remained alive after a 24 h exposure period, calculated as the total number introduced minus the number recorded dead. The response variable was modelled using a binomial error distribution with a logit-link function. *Post hoc* pairwise comparisons of estimated marginal means were performed to detect differences in survival among chemical treatments within each residue interval ($P > 0.05$).

No IOBC classification or persistency ratings were calculated due to only two replicates of the trial being insufficient to provide reliable ratings for practical use.

4.3. Results

4.3.1. Reproductive Potential

Parasitism rates by *T. cryptophlebiae* were significantly affected by chemical treatment and parasitoid viability ($\chi^2 = 1853.99$, df = 18, $P < 0.001$; $\chi^2 = 14.17$, df = 1, $P < 0.001$, respectively) (Figure 4.1.). Parasitoids that were recorded as alive after 24 h parasitised more eggs than those that were recorded as dead ($\beta = 0.145 \pm 0.039$ SE, $P < 0.001$), corresponding to an approximate 16 % increase in parasitism. *Post hoc* comparisons (Bonferroni-adjusted) revealed that several chemical treatments significantly reduced parasitism relative to the untreated Control (Dry), which had an estimated count of 15.50 ± 0.044 SE. The most toxic residues included Tau-fluvalinate (0.34 ± 0.262 SE, $P < 0.001$), Lambda-cyhalothrin (0.79 ± 0.171 SE, $P < 0.001$), Beta-cyfluthrin (1.53 ± 0.124 SE, $P < 0.001$), and Alpha-cypermethrin (1.29 ± 0.137 SE, $P < 0.001$), all of which differed significantly from the Control and formed distinct significance groups. Notably, for Alpha-cypermethrin, Lambda-cyhalothrin, Pymetrozine (2.37 ± 0.105 SE, $P < 0.001$), and Beta-cyfluthrin, parasitism remained low despite the majority of parasitoids being alive, suggesting that these chemicals may exert strong sublethal or behavioural effects that inhibit host location or oviposition. In contrast, parasitism following exposure to *Beauveria bassiana* (A: 15.52 ± 0.043 SE, $P = 1.000$; B: 16.23 ± 0.043 SE, $P = 1.000$), CrpeNPV (15.68 ± 0.043 SE, $P = 1.000$), Azoxystrobin and Difenoconazole (15.19 ± 0.043 SE, $P = 1.000$), and Chlorantraniliprole (14.73 ± 0.044 SE, $P = 1.000$) did not differ significantly from the control, indicating compatibility with parasitoid activity (Figure 4.1.). These results highlight that while several synthetic insecticides severely suppress parasitism via female mortality or repellent properties, select biopesticides and

fungicide mixtures appear benign, supporting their integration into parasitoid-based biological control programmes.

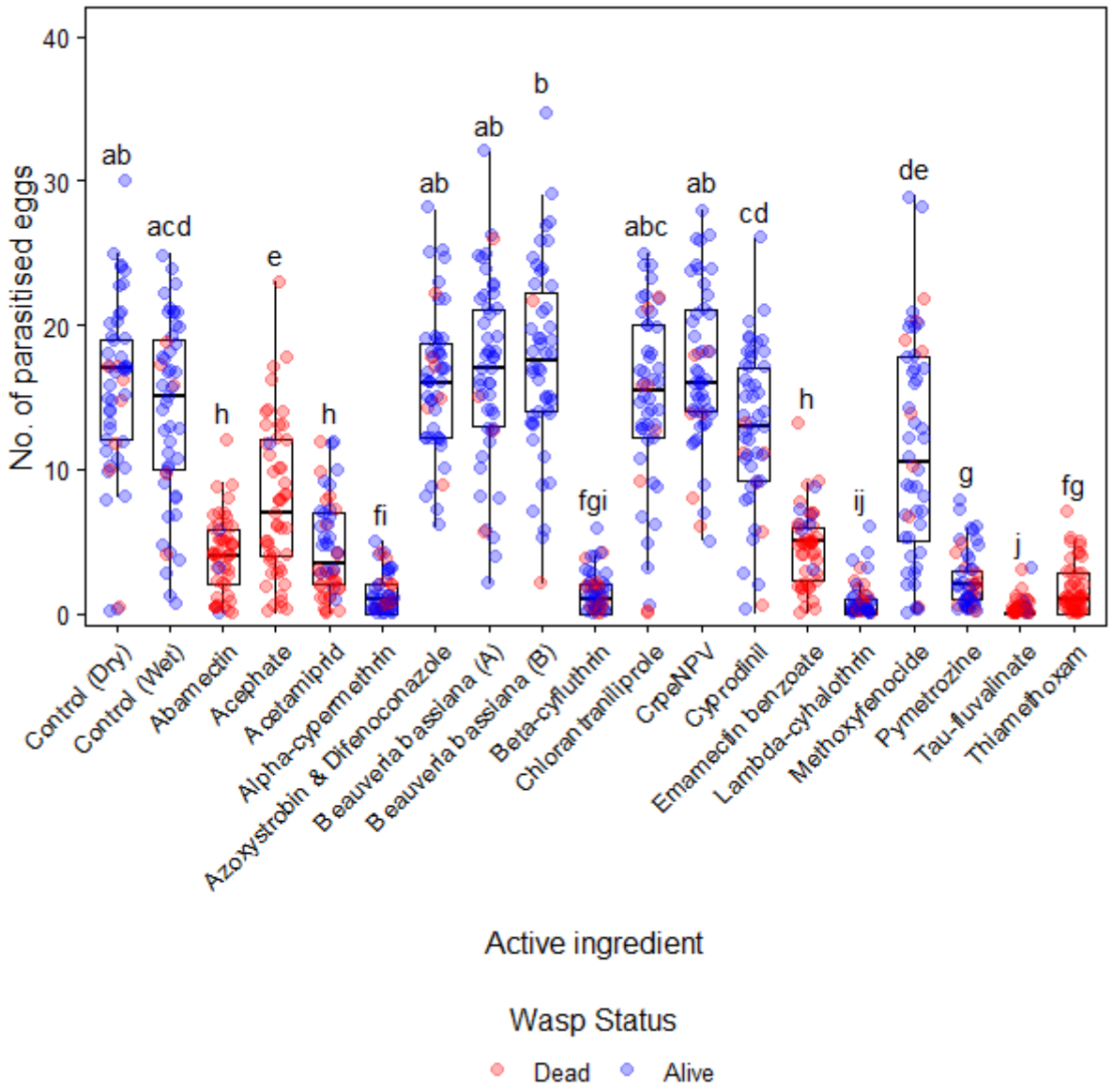

Figure 4.1. Number of parasitised host eggs per replicate across chemical treatments, separated by parasitoid survival status (alive vs. dead). Different letters indicate significant differences in parasitism level between chemical treatments (Bonferroni-adjusted pairwise comparisons of estimated marginal means).

Chemical treatment had a significant effect on the proportion of male offspring among emerged parasitoids ($\chi^2 = 56.90$, df = 18, $P < 0.001$), while parasitoid survival status (dead or alive) had no significant influence ($\chi^2 = 0.63$, df = 1, $P = 0.43$) (Figure 4.2.). The full model, including chemical and survival effects, fit the data significantly better than a null model with only the random intercept ($\chi^2 = 81.49$, df = 19, $P < 0.001$). Estimated marginal means revealed that *Beauveria bassiana* (A) produced the most male-biased sex ratio (0.64 ± 0.04 SE, $P = 0.21$), although it was not statistically different from either control. In contrast, Acephate resulted in the most female-biased sex ratio (0.43 ± 0.03 SE, $P > 0.0009$), forming the only treatment statistically distinct from most other chemicals. Emamectin benzoate also yielded a relatively female-biased ratio, although differences were not statistically significant (0.40 ± 0.03 SE, $P = 0.26$). Most synthetic insecticides, fungicides, and microbial agents, including CrpeNPV (0.58 ± 0.04 SE, $P = 1.00$), Methoxyfenocide (0.58 ± 0.04 SE, $P = 1.00$), and Acetamiprid (0.61 ± 0.05 SE, $P = 1.00$), produced intermediate sex ratios not significantly different from controls. Control treatments themselves, Dry (0.57 ± 0.04 SE) and Wet (0.59 ± 0.04 SE, $P = 1.00$), did not differ from each other, indicating stable baseline sex ratios in untreated conditions (Figure 4.2.). These findings suggest that exposure to certain chemical treatments can alter offspring sex ratios in *T. cryptophlebiae*, but it is more likely that the mating status of females used, and their sex allocation behaviour, influenced sex ratio production more than chemical treatments themselves.

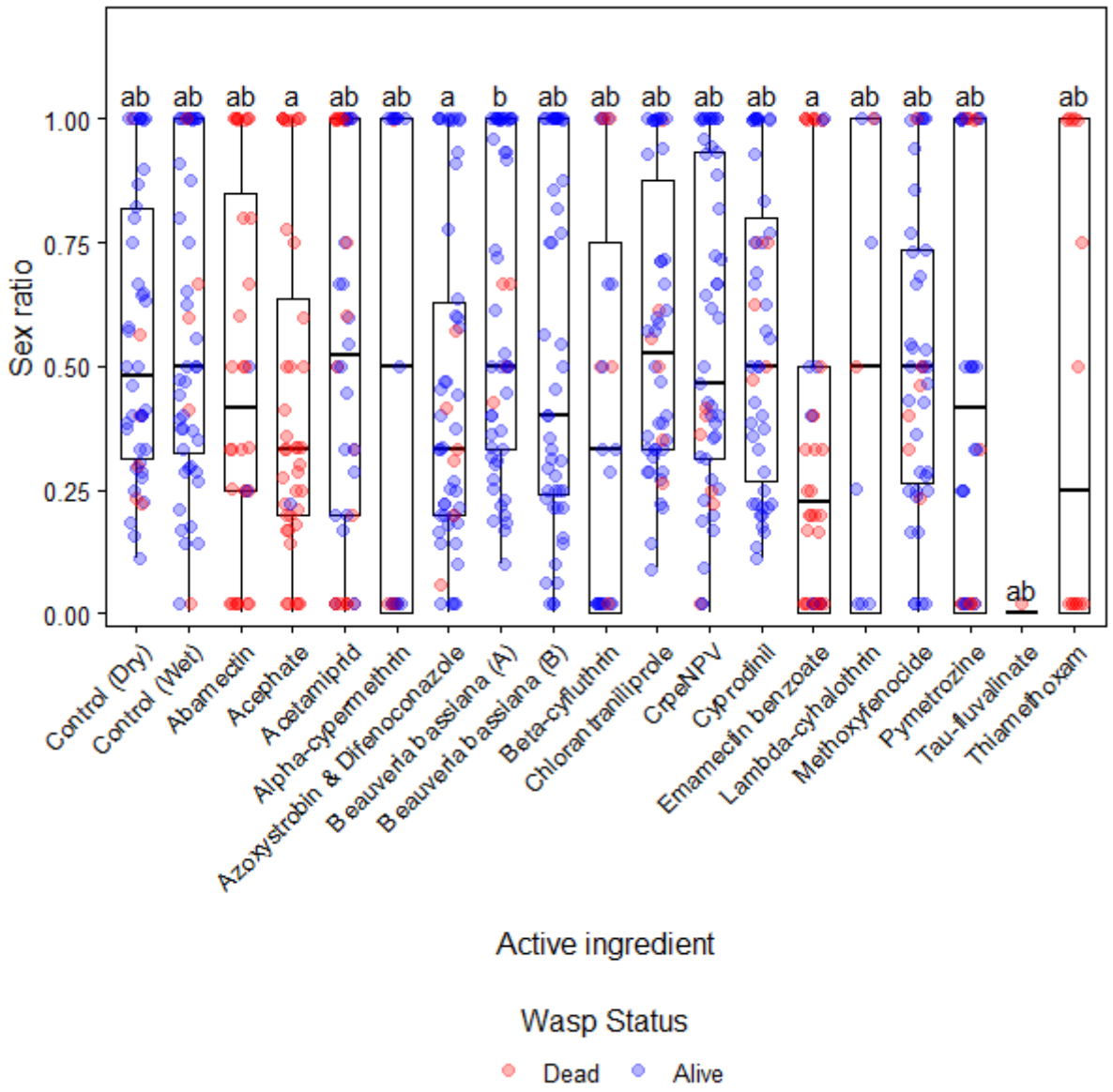

Figure 4.2. Sex ratio produced across chemical treatments and survival statuses of exposed individuals (alive vs. dead). Letters denote groups that are not significantly different between sex ratios produced females exposed to the different active ingredient (Bonferroni-adjusted pairwise comparisons of estimated marginal means).

IOBC Classifications

IOBC classifications were assigned to each chemical treatment based on both 24-hour female mortality of *T. cryptophlebiae* following exposure to residues of commonly used active ingredients in macadamia orchards. Table 4.2 presents toxicity classes based on corrected adult mortality, with most biological and reduced-risk products such as *B. bassiana*, CrpeNPV, and Azoxystrobin & Difenoconazole mixtures falling into Class 1 (Harmless). In contrast, Emamectin benzoate, Acephate and Abamectin were classified as Class 3 (Moderately harmful), respectively, due to high mortality.

Table 4.2. IOBC classifications assigned to 24-hour *Trichogrammatoidea cryptophlebiae* female mortality percentages for a selection of active ingredients commonly used in macadamia orchards.

Active Ingredient	Mortality (%)	IOBC Classification
Control (Dry)	0.00	1
Control (Wet)	0.00	1
Abamectin	93.00	3
Acephate	97.62	3
Acetamiprid	48.67	2
Alpha-cypermethrin	14.29	1
Azoxystrobin & Difenoconazole	0.00	1
Beauveria bassiana (A)	0.00	1
Beauveria bassiana (B)	0.00	1
Beta-cyfluthrin	20.67	1
Chlorantraniliprole	2.00	1
CrpeNPV	0.00	1
Cyprodinil	0.00	1
Emamectin benzoate	83.67	3
Lambda-cyhalothrin	23.81	1
Methoxyfenocide	2.00	1
Pymetrozine	20.67	1
Tau-fluvalinate	88.10	3

***** Class 1: *Harmless* (Corrected mortality < 30%) – Safe to natural enemies. Class 2: *Slightly harmful* (30–79%) – Acceptable risk; may be usable with care. Class 3: *Moderately harmful* (80–99%) – Not recommended; may suppress beneficial populations. Class 4: *Harmful* (> 99%) – Toxic; should not be used in conjunction with biological control agents.

4.3.2. Pre-Imaginal Effects

Successful Emergence

Chemical treatment had a significant effect on the percentage of emergence of offspring from host eggs that had been exposed prior to parasitism by *T. cryptophlebiae* ($\chi^2 = 162.42$, df = 18, $P < 0.001$) (Figure 4.3. A). The dry control group showed high emergence (94.7 % ± 5.30 SE), with similar values recorded in the wet control (99.1 % ± 5.14), *B. bassiana* isolates (A: 95.0 % ± 5.14; B: 91.9 % ± 5.14), CrpeNPV (95.7 % ± 5.14), Azoxystrobin & Difenoconazole (97.4 % ± 5.14), and Chlorantraniliprole (94.1 % ± 5.30). These treatments formed a high-performing group with no significant differences from the dry control. In contrast, emergence was significantly lower in Tau-fluvalinate (34.8 % ± 11.5), Lambda-cyhalothrin (56.5 % ± 8.53), Thiamethoxam (58.4 % ± 6.05), Abamectin (58.4 % ± 5.67), Emamectin benzoate (61.8% ± 5.30), and Alpha-cypermethrin (63.3 % ± 6.63) compared to the dry control (all $P < 0.05$, Bonferroni-adjusted). The highest emergence was recorded in Pymetrozine (121.4 % ± 5.81), which was significantly greater than the dry control ($P < 0.001$) (Figure 4.3. A).

Chemical treatment had a highly significant effect on the emergence percentage of offspring exposed during the egg-larval stage of development ($\chi^2 = 1333$, df = 18, $P < 0.001$) (Figure 4.3. B). The full model, including chemical as a fixed effect, provided a significantly better fit than the null model with replicate as a random effect alone. Control (Dry) and Control (Wet) treatments both supported high emergence rates (97.1 % ± 1.69 SE and 98.4 % ± 1.69 SE, respectively), statistically indistinguishable from several other treatments. In contrast, multiple insecticides significantly reduced emergence compared to the controls. These included Abamectin (57.0 % ± 1.69 SE, $P < 0.0001$), Emamectin benzoate (71.0 % ± 1.69 SE, $P < 0.0001$), Acetamiprid (74.3 % ± 1.69 SE, $P < 0.0001$), Alpha-cypermethrin (83.6 % ± 1.69 SE, $P < 0.0001$), Beta-cyfluthrin (84.2 % ± 1.69 SE, $P < 0.0001$), and Lambda-cyhalothrin (61.3 % ± 1.69 SE, $P < 0.0001$). Tau-fluvalinate and Thiamethoxam caused the most pronounced reductions in emergence, with estimated means of 57.7 % ± 1.69 SE and 16.1 % ± 1.69 SE, respectively (both $P < 0.0001$). In contrast, treatments such as *B. bassiana* (A) (95.5 % ± 1.69 SE), *B. bassiana* (B) (93.5 % ± 1.69 SE), CrpeNPV (96.7 % ± 1.69 SE), Chlorantraniliprole (97.1 % ± 1.69 SE), Cyprodinil (95.9 % ± 1.69 SE), Methoxyfenocide (96.1 % ± 1.73 SE), Pymetrozine (96.5 % ± 1.71 SE), and the fungicide Azoxystrobin & Difenoconazole (95.7 % ± 1.69 SE) all yielded emergence

rates comparable to the dry control (all $P > 0.05$) (Figure 4.3. B). These results suggest that while certain insecticides, particularly those in the avermectin, neonicotinoid, and pyrethroid classes, can significantly impair emergence, microbial biocontrol agents and several fungicides appear to be safe for use in integrated biological control programs.

Chemical treatment had a significant effect on the emergence percentage of offspring exposed during the pupal stage of development ($\chi^2 = 822.76$, df = 18, $P < 0.001$) (Figure 4.3. C). The full model with chemical effects fit the data significantly better than the null model with replicate ID as a random effect alone. Emergence in the dry control was high (73.5 % ± 1.83 SE), and not significantly different from a number of other treatments. Several chemicals significantly reduced emergence compared to the control. The most severe reduction was caused by Thiamethoxam (21.6 % ± 1.83 SE, $P < 0.0001$), followed by Tau-fluvalinate (36.3 % ± 1.83 SE, $P < 0.0001$), Lambda-cyhalothrin (47.1 % ± 1.83 SE, $P < 0.0001$), and Abamectin (48.7 % ± 1.83 SE, $P < 0.0001$). Other significantly lower treatments included Acetamiprid (52.5 % ± 1.83 SE, $P < 0.0001$), Beta-cyfluthrin (57.0 % ± 1.83 SE, $P < 0.0001$), Emamectin benzoate (62.6 % ± 1.83 SE, $P < 0.001$), and Alpha-cypermethrin (62.8 % ± 1.83 SE, $P < 0.001$). In contrast, treatments such as *B. bassiana* (A) (74.8 % ± 1.83 SE), *B. bassiana* (B) (70.2 % ± 1.83 SE), CrpeNPV (74.0 % ± 1.83 SE), Chlorantraniliprole (70.2 % ± 1.83 SE), Cyprodinil (67.3 % ± 1.83 SE), Methoxyfenocide (69.3 % ± 1.83 SE), Pymetrozine (70.0 % ± 1.83 SE), and Azoxystrobin & Difenoconazole (70.6 % ± 1.83 SE) yielded emergence rates comparable to the control (all $P > 0.05$) (Figure 4.3. C). These results suggest that certain insecticides, particularly neonicotinoids, avermectins, and pyrethroids, may impair parasitoid emergence at the pupal stage, whereas microbial agents and fungicides appear relatively safe for use in integrated pest management programmes.

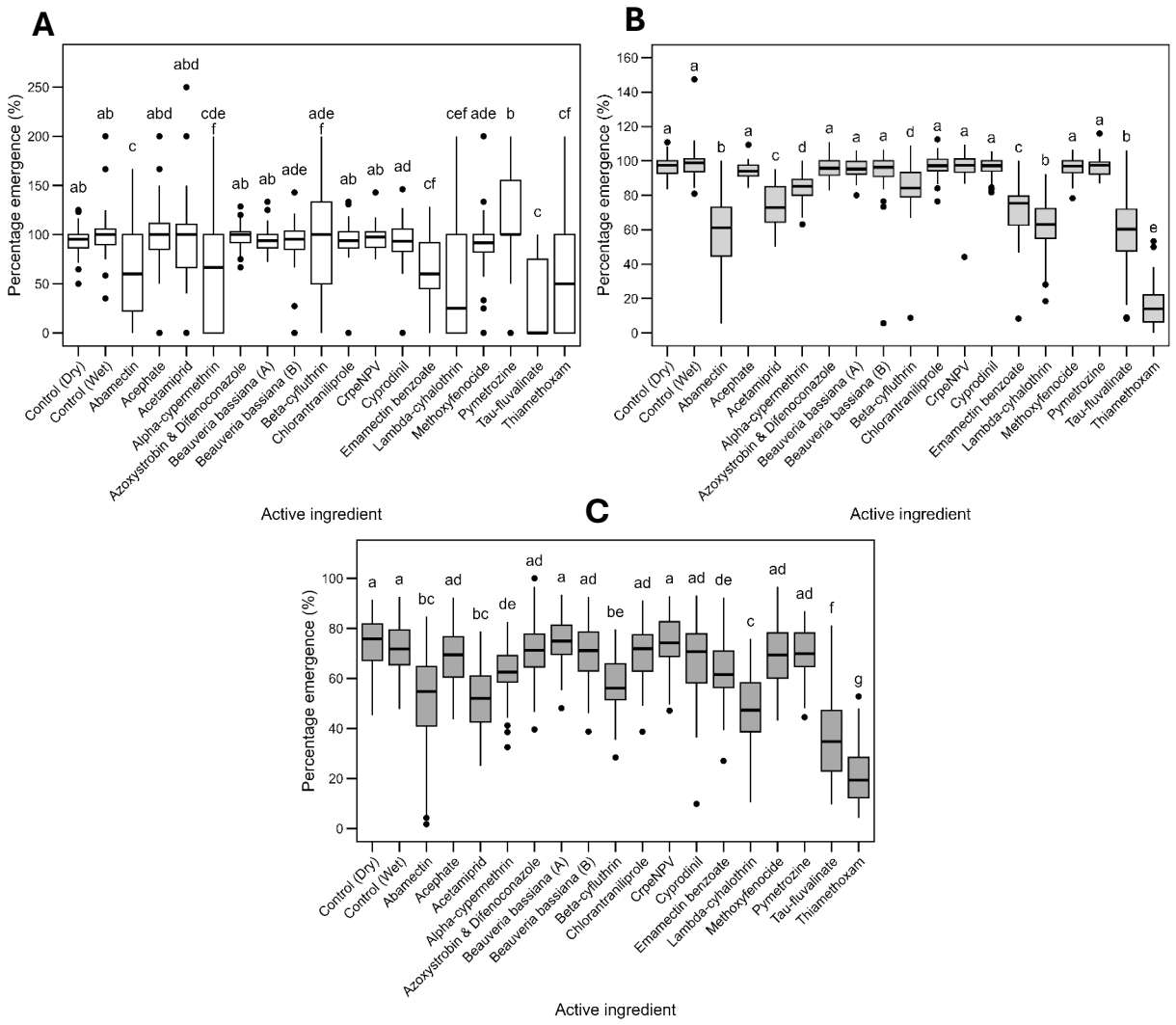

Figure 4.3. The emergence percentage of *Trichogrammatoidea cryptophlebiae* across chemical treatments when host and parasitoid were exposed at different points in their development. (A) host egg exposed to parasitoid for parasitism, (B) egg-larval stage, and (C) pupal stage of parasitoid development. Letters denote groups that are not significantly different (Bonferroni-adjusted pairwise comparisons of estimated marginal means).

Unsuccessful Emergence

Chemical treatment significantly affected the percentage of unsuccessful emergence of offspring from host eggs that had been exposed prior to parasitism by *T. cryptophlebiae* (χ^2 = 182.0, df = 18, $P < 0.001$) (Figure 4.4. A). The model including chemical treatment explained the data significantly better than a null model with only replicate as a random effect. The Control (Dry)

group exhibited a low unsuccessful emergence rate (11.3 % ± 3.74 SE), not significantly different from several other treatments. However, multiple chemicals significantly increased the proportion of unsuccessful emergence relative to this control. These included Tau-fluvalinate (67.6 % ± 8.13 SE, $P < 0.0001$), Thiamethoxam (49.3 % ± 4.27 SE, $P < 0.0001$), Lambda-cyhalothrin (43.4 % ± 6.03 SE, $P = 0.0010$), Emamectin benzoate (40.6 % ± 3.74 SE, $P < 0.0001$), Alpha-cypermethrin (40.2 % ± 4.68 SE, $P < 0.0001$), and Abamectin (33.6 % ± 4.00 SE, $P = 0.0072$). In contrast, treatments such as *B. bassiana* (A: 10.5 % ± 3.63 SE; B: 15.4 % ± 3.63 SE), CrpeNPV (10.3 % ± 3.63 SE), Chlorantraniliprole (12.0 % ± 3.74 SE), Cyprodinil (15.7 % ± 3.70 SE), Methoxyfenocide (17.6 % ± 3.82 SE), Pymetrozine (15.2 % ± 4.11 SE), and Azoxystrobin & Difenoconazole (12.2 % ± 3.63 SE) yielded non-emergence rates statistically comparable to the dry control (all $P > 0.05$) (Figure 4.4. A). These findings suggest that certain insecticides, particularly those in the pyrethroid, avermectin, and neonicotinoid classes, substantially impair successful parasitoid emergence. Meanwhile, most microbial agents and fungicides appear not to interfere with emergence success, supporting their potential compatibility in integrated biological control programmes.

Chemical treatment had a highly significant effect on the percentage of unsuccessful emergence of offspring exposed during the egg-larval stage of development ($\chi^2 = 12{,}534$, df = 18, $P < 0.001$) (Figure 4.4. B). The full model, including chemical treatment as a fixed effect, provided a significantly better fit than the null model with replicate identity as a random intercept alone. Control (Dry) and Control (Wet) treatments showed low unsuccessful emergence rates (11.1 % ± 1.1 SE and 9.8 % ± 1.1 SE, respectively) and were statistically indistinguishable from a range of other treatments. These included Azoxystrobin & Difenoconazole (10.3 % ± 1.0 SE, $P > 0.05$), *B. bassiana* (A) (11.8 % ± 1.1 SE, $P > 0.05$), *Beauveria bassiana* (B) (10.2 % ± 1.1 SE, $P > 0.05$), Chlorantraniliprole (10.8 % ± 1.1 SE, $P > 0.05$), CrpeNPV (9.1 % ± 1.0 SE, $P > 0.05$), Cyprodinil (10.4 % ± 1.1 SE, $P > 0.05$), Methoxyfenocide (10.1 % ± 1.1 SE, $P > 0.05$), and Pymetrozine (9.2 % ± 1.0 SE, $P > 0.05$). In contrast, several insecticide treatments caused significantly elevated levels of unsuccessful emergence compared to the controls. These included Abamectin (46.6 % ± 1.2 SE, $P < 0.0001$), Emamectin benzoate (46.2 % ± 1.2 SE, $P < 0.0001$), Acetamiprid (42.3 % ± 1.2 SE, $P < 0.0001$), Alpha-cypermethrin (29.3 % ± 1.2 SE, $P < 0.0001$), Beta-cyfluthrin (22.8 % ± 1.2 SE, $P < 0.0001$), and Lambda-cyhalothrin (58.1 % ± 1.2 SE, $P < 0.0001$). Tau-fluvalinate (46.0 % ± 1.3 SE, $P < 0.0001$) and Thiamethoxam (73.3 % ± 1.3 SE, $P < 0.0001$) caused the most

pronounced increases in unsuccessful emergence (Figure 4.4. B). These results indicate that several chemical treatments, particularly the neonicotinoids, pyrethroids, and avermectins, substantially impair the successful emergence of parasitoids. In contrast, many microbial agents and fungicides tested did not adversely affect parasitoid emergence success, highlighting their compatibility with biological control programmes.

Chemical treatment had a highly significant effect on the percentage of unsuccessful emergence of offspring exposed during the pupal stage of development ($\chi^2 = 8451.7$, df = 18, $P < 0.001$) (Figure 4.4. C). The full binomial GLMM with logit-link, including chemical treatment as a fixed effect and replicate identity as a random intercept, provided a significantly better fit than the null model, including only the random effect. Control (Dry) and Control (Wet) treatments exhibited relatively low unsuccessful emergence rates (31.2 % ± 1.45 SE and 32.3 % ± 1.41 SE, respectively) and were statistically indistinguishable from several other treatments. These included Azoxystrobin & Difenoconazole (32.7 % ± 1.51 SE, $P > 0.05$), *B. bassiana* (A) (28.6 % ± 1.33 SE, $P > 0.05$), *B. bassiana* (B) (33.8 % ± 1.72 SE, $P > 0.05$), Chlorantraniliprole (32.6 % ± 1.34 SE, $P > 0.05$), CrpeNPV (29.6 % ± 1.20 SE, $P > 0.05$), Cyprodinil (36.4 % ± 1.69 SE, $P > 0.05$), Methoxyfenocide (34.6 % ± 1.58 SE, $P > 0.05$), and Pymetrozine (34.5 % ± 1.26 SE, $P > 0.05$). In contrast, several insecticide treatments caused significantly higher unsuccessful emergence levels than the controls. These included Abamectin (53.3 % ± 3.01 SE, $P < 0.0001$), Emamectin benzoate (40.9 % ± 1.62 SE, $P < 0.0001$), Acetamiprid (51.2 % ± 1.51 SE, $P < 0.0001$), Alpha-cypermethrin (41.3 % ± 1.44 SE, $P < 0.0001$), Beta-cyfluthrin (46.4 % ± 1.58 SE, $P < 0.0001$), and Lambda-cyhalothrin (56.2 % ± 2.04 SE, $P < 0.0001$). The most severe effects were recorded in response to Tau-fluvalinate (65.7 % ± 2.36 SE, $P < 0.0001$) and Thiamethoxam (79.7 % ± 1.58 SE, $P < 0.0001$) (Figure 4.4. C). These findings suggest that certain insecticides, particularly neonicotinoids, pyrethroids, and avermectins, substantially impair the successful emergence of parasitoids. In contrast, most microbial agents and fungicides tested were not detrimental to emergence and may be more compatible with biological control-based integrated pest management programmes. Unsuccessful emergence was much higher at the pupal stage of treatment, even for control groups. This suggests the pupal stage is more sensitive to treatment and handling when compared to other pre-imaginal stages, egg and larval.

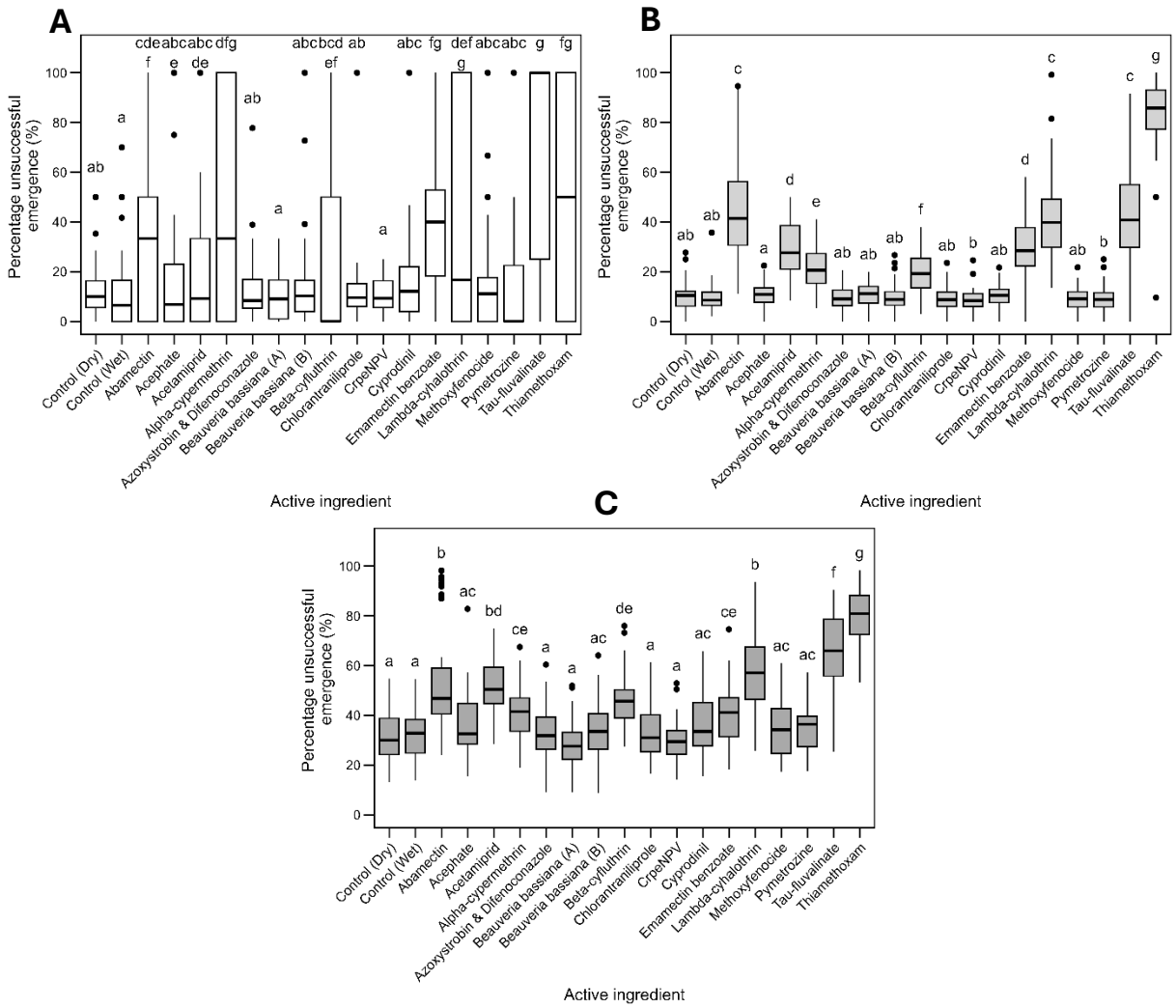

Figure 4.4. The percentage of unsuccessful emergence of *Trichogrammatoidea cryptophlebiae* across chemical treatments when host and parasitoid were exposed at different points in their development. (A) Host eggs are exposed to the parasitoid for parasitism, (B) egg-larval stage, and (C) pupal stage of parasitoid development. Letters denote groups that are not significantly different (Bonferroni-adjusted pairwise comparisons of estimated marginal means).

IOBC Classifications

IOBC classifications based on emergence percentages were used to assess the sublethal effects of chemical residues on *T. cryptophlebiae*. As shown in Table 4.3, most biological and reduced-risk products, including *B. bassiana*, CrpeNPV, and the fungicidal combination of Azoxystrobin & Difenoconazole, were classified as Class 1 (Harmless), with emergence rates

comparable to untreated controls. In contrast, conventional insecticides such as Abamectin, Lambda-cyhalothrin, and Tau-fluvalinate were classified as Class 3 (Moderately harmful), indicating substantial suppression of emergence. Several other treatments, including Acetamiprid, Emamectin benzoate, and Beta-cyfluthrin, fell into Class 2 (Slightly harmful), suggesting moderate sublethal effects that may impair development or reduce fitness.

Table 4.3. IOBC classifications assigned to *Trichogrammatoidea cryptophlebiae* emergence percentages for selecting active ingredients commonly used in macadamia orchards.

Active Ingredient	Emergence (%)	IOBC Classification
Control (Dry)	73.47 ± 1.56	1
Control (Wet)	71.93 ± 1.53	1
Abamectin	48.74 ± 3.18	3
Acephate	68.63 ± 1.63	2
Acetamiprid	52.46 ± 1.79	2
Alpha-cypermethrin	62.81 ± 1.48	2
Azoxystrobin & Difenoconazole	70.62 ± 1.77	1
Beauveria bassiana (A)	74.83 ± 1.43	1
Beauveria bassiana (B)	70.24 ± 1.71	1
Beta-cyfluthrin	57.00 ± 1.64	2
Chlorantraniliprole	70.15 ± 1.57	1
CrpeNPV	74.05 ± 1.40	1
Cyprodinil	67.26 ± 2.18	2
Emamectin benzoate	62.55 ± 1.83	2
Lambda-cyhalothrin	47.13 ± 2.06	3
Methoxyfenocide	69.27 ± 1.75	2
Pymetrozine	70.02 ± 1.39	1
Tau-fluvalinate	36.35 ± 2.60	3

*Class 1: Harmless (Emergence ≥ 70%) – Safe to natural enemies. Comparable to controls and suitable for use in biological control programs. Class 2: Slightly harmful (Emergence 50–69%) – Acceptable risk; may be usable with care. Some reduction in emergence was recorded. Class 3: Moderately harmful (Emergence 30–49%) – Not recommended; may suppress beneficial populations through reduced emergence. Class 4: Harmful (Emergence < 30%) – Toxic; should not be used in conjunction with biological control agents due to very low survival

4.3.3. Residual Effects

Chemical treatment, residue interval and their interaction had a highly significant effect on the mortality of adult female *T. cryptophlebiae* (χ^2 = 147.74, df = 18, P < 0.001) (Figure 4.5.). At day 0, Control (Leaf) and Control (No Leaf) treatments resulted in low female mortality (12.2 % ± 3.36 SE and 21.7 % ± 3.03 SE, respectively) and were statistically indistinguishable from several other treatments. These included Chlorantraniliprole (31.7 % ± 2.97 SE, P = 0.322), CrpeNPV (34.0 % ± 2.98 SE, P = 0.183), Cyprodinil (35.0 % ± 2.96 SE, P = 0.134), Methoxyfenocide (34.4 % ± 2.93 SE, P = 0.199), Emamectin benzoate (20.1 % ± 3.06 SE, P = 0.302), and *B. bassiana* (A) (20.4 % ± 3.08 SE, P = 0.329). In contrast, several insecticide treatments caused significantly elevated mortality compared to the controls. These included Acetamiprid (40.3 % ± 2.89 SE, P = 0.021), Pymetrozine (40.0 % ± 2.94 SE, P = 0.024), and Lambda-cyhalothrin (42.2 % ± 2.92 SE, P = 0.010). The most severe impact was caused by Acephate, which resulted in a mortality rate of 91.8 % ± 3.12 SE (P < 0.001). Alpha-cypermethrin (9.2 % ± 3.48 SE, P < 0.001) and *B. bassiana* (B) (5.1 % ± 3.57 SE, P < 0.001) also differed significantly from controls, though in the direction of reduced mortality. Female mortality declined across all treatments over time. By day 7, mortality from Acephate decreased to 69.1 % ± 3.12 SE, and further to 30.9 % ± 3.12 SE and 8.2 % ± 3.12 SE at days 14 and 21, respectively. Similar declines were recorded for Acetamiprid (from 40.3 % to 6.4 %), Pymetrozine (40.0 % to 2.1 %), and Lambda-cyhalothrin (42.2 % to 3.0 %) (Figure 4.5.). Many other treatments, including *Beauveria bassiana* (formulation A and B), CrpeNPV, Methoxyfenocide, and Chlorantraniliprole, remained consistently low throughout the residue interval, with mortality below 10 % by day 21 (Figure 4.5.). These findings indicate that while some insecticides pose high acute risks to adult females, their persistence is low with effects dissipating over time. In contrast, microbial agents and growth regulators exhibited minimal immediate or residual impact and may be suitable for integration into biological control programmes.

Chemical treatment, residue interval and their interaction had a highly significant effect on the mortality of adult male *T. cryptophlebiae* (χ^2 = 176.96, df = 18, P < 0.001) (Figure 4.5.). At day 0, male mortality was highest in the Acephate treatment (79.9 % ± 2.9 SE, P < 0.0001), which differed significantly from all other treatments. Moderate levels of mortality were recorded for Acetamiprid (32.0 % ± 2.9 SE, P = 0.0706), Thiamethoxam (35.4 % ± 2.9 SE, P = 0.0231), Methoxyfenocide (35.2 % ± 2.9 SE, P = 0.0260), and Lambda-cyhalothrin (33.5 % ± 2.9 SE, P =

0.0433), all of which were significantly greater than or marginally different from controls. Controls exhibited lower male mortality: Control (Leaf) (13.3 % ± 3.0 SE, $P = 0.0726$) and Control (No Leaf) (18.7 % ± 2.9 SE, $P = 0.6130$), statistically indistinguishable from several microbial and fungicidal treatments. These included *B. bassiana* (A) (18.0 % ± 3.0 SE, $P = 0.523$), *B. bassiana* (B) (5.1 % ± 3.1 SE, $P < 0.0001$), Beta-cyfluthrin (10.0 % ± 2.9 SE, $P = 0.0114$), and Azoxystrobin & Difenoconazole (10.2 % ± 2.9 SE, $P = 0.0154$), which consistently showed low impact on male survival. Male mortality declined significantly across all treatments with increasing residue interval. In Acephate, for instance, mortality decreased from 79.9 % at day 0 to 47.0 % at day 7, 17.0 % at day 14, and just 4.4 % by day 21. A similar decline was recorded for Acetamiprid (from 32.0 % to 4.7 %), Methoxyfenocide (35.2 % to 1.2 %), and Thiamethoxam (35.4 % to 1.5 %) (Figure 4.5.). Many microbial and IGR treatments, including *B. bassiana* (A and B), CrpeNPV, and Methoxyfenocide, maintained low mortality levels throughout all residue intervals, often under 10 % (Figure 4.5.). These findings demonstrate that some synthetic insecticides, particularly Acephate, Thiamethoxam, and Lambda-cyhalothrin, pose significant short-term risks to male parasitoid survival, but their toxicity declines substantially over time. In contrast, microbial agents, fungicides, and IGRs had minimal immediate or residual effects on male survival and may thus be better suited for integration into biological control-based IPM programmes.

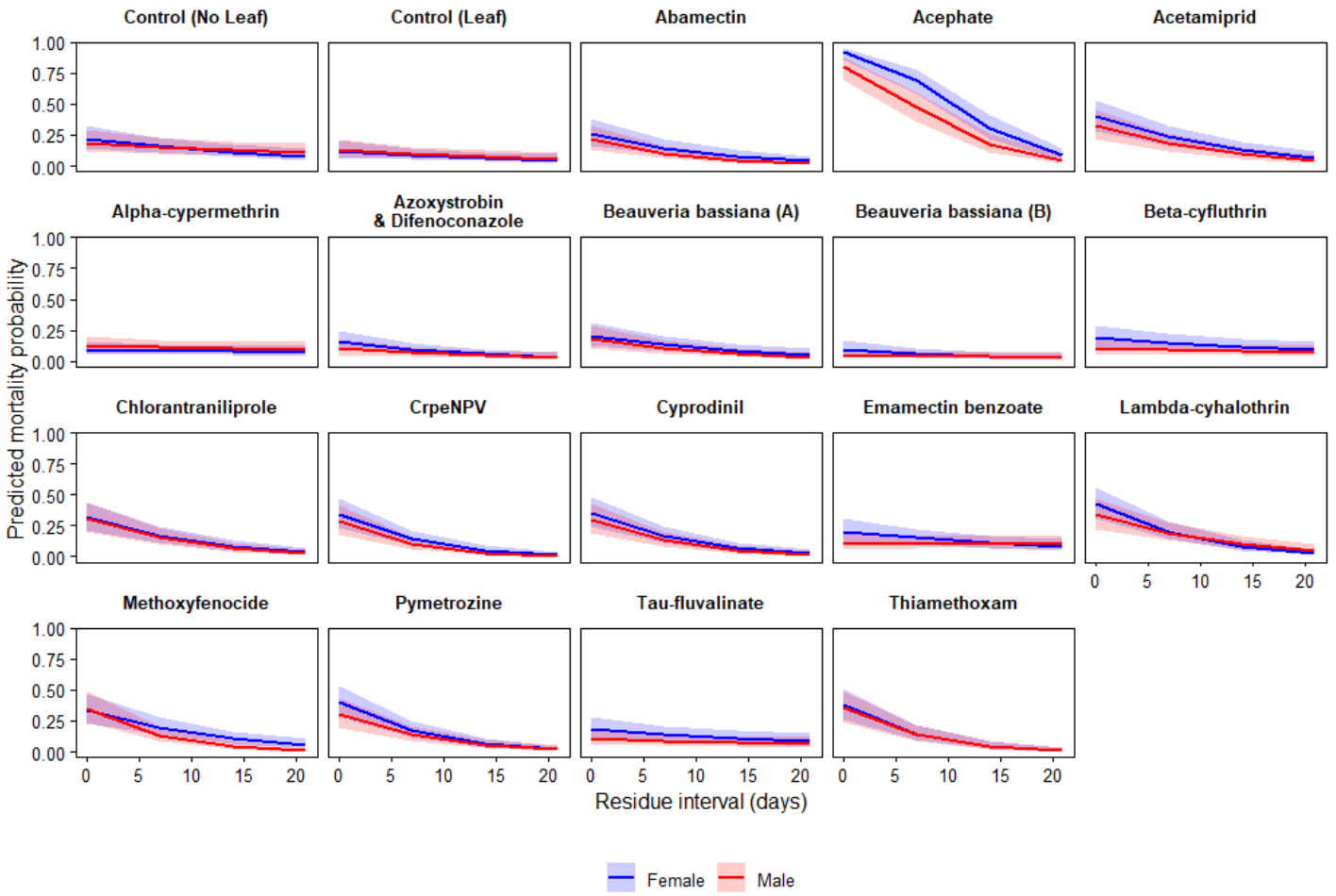

Figure 4.5. Predicted mortality probability of *Trichogrammatoidea cryptophlebiae* females and males across residue interval days (0-21) for each chemical treatment, based on binomial GLMM estimates with 95 % confidence intervals. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

4.4. Discussion

The non-target impact of various active ingredients used for pest management in macadamias had varying effects on the survival, reproductive potential, and pre-imaginal development of *T. cryptophlebiae,* which would influence its success as an effective biological control agent for the management of tortricid pests in this crop and others where the active ingredients are registered for use. Knowledge of each active ingredient's impact on *T. cryptophlebiae* at various life stages and its reproductive potential will allow growers to better integrate this parasitoid into IPM programmes (Rakes *et al.,* 2021; Zang *et al.,* 2021; Costa *et al.,* 2022).

The reproductive potential of *T. cryptophlebiae* was influenced by exposure to different chemical residues, with distinct impacts recorded on both parasitism rates and sex ratio of the offspring. In general, parasitoids that remained alive following chemical exposure parasitised more eggs. However, high parasitoid survival did not translate into higher parasitism in several cases. This was particularly evident in treatments involving synthetic pyrethroids and neonicotinoids, such as Tau-fluvalinate, Lambda-cyhalothrin, Beta-cyfluthrin, Alpha-cypermethrin, and Pymetrozine (Chenga *et al.,* 2018; Rakes *et al.,* 2021). Despite the presence of living wasps at the time of their survival assessment, parasitism under these treatments remained consistently low. This suggests that these chemicals may have had a deterrent effect on the parasitoid, potentially impairing their essential behavioural functions, such as host searching, egg recognition, or oviposition, likely through sublethal or neurotoxic effects that are not captured by survival assessments alone (Khan *et al.*, 2015). The products that resulted in parasitoid survival with corresponding low parasitism, if used with *T. cryptophlebiae* releases, would not be conducive to effective tortricid suppression, despite the female's survival ability (Khan *et al.*, 2015).

In contrast, several chemical treatments were more compatible with the reproductive activity of the parasitoids. Biopesticides such as *B. bassiana* (both formulations) and CrpeNPV, as well as fungicide combinations such as azoxystrobin and difenoconazole, supported parasitism rates comparable to the untreated controls (Zang *et al.,* 2021). Similarly, chlorantraniliprole, a diamide insecticide often regarded as soft on natural enemies, did not negatively affect parasitoid performance (Khan & Ruberson, 2017). These findings highlight the potential for incorporating

these softer chemical options into integrated pest management programmes that rely on parasitoid releases (Zang *et al.,* 2021).

Female survival and reproductive potential are not the only parameters of concern when attempting to identify guidelines for the safe and effective use of chemicals with the parasitoids' release in the field (Stark & Banks, 2024). Successful development and emergence of parasitoids from host eggs should also be assessed to determine which chemicals are compatible with the biological control species in question (Stark & Banks, 2024; Macfadyen *et al.*, 2013). The generation of offspring emerging from host eggs should also be female-biased, and identifying chemicals that influence the allocation of sexes, to be more male-biased, is important (Zang *et al.,* 2021).

The effect of chemical treatments on sex ratio determination appeared variable, with most chemicals, including synthetic insecticides and biologically based options, not substantially altering the proportion of male to female offspring. The differences recorded could be attributed to female mating. Whether parasitoids were found alive or dead at the time of assessment did not significantly influence sex ratios, suggesting that chemical exposure had minimal direct impact on this aspect of reproductive behaviour (Zang *et al.,* 2021).

The emergence of *T. cryptophlebiae* offspring was significantly affected by exposure to chemical residues during all stages of development. These findings highlight the importance of considering adult parasitoid survival and parasitism capacity and the latent impacts of chemical residues on immature stages developing within treated host eggs (Khan & Ruberson, 2017).

The highest emergence rates were recorded in the biopesticides and fungicide mixtures. These included *B. bassiana* (both formulations), CrpeNPV, Chlorantraniliprole, Methoxyfenocide, and the fungicidal combination of Azoxystrobin and Difenoconazole. These treatments were consistently associated with low rates of unsuccessful emergence, suggesting they do not interfere with the developmental success of the parasitoid. They may therefore be considered compatible with IPM strategies that use *T. cryptophlebiae* releases (Zang *et al.,* 2021). Pymetrozine can be used as an example to highlight the importance of considering multiple factors of parasitoid-chemical compatibility. Pymetrozine did not appear to disrupt the development of the parasitoid, which was viewed as a successful emergence, but it led to significantly lower levels of parasitism in comparison to the controls. This underscores that multiple factors need to be assessed when

determining chemical compatibility with a biological control agent, and this is one of the critiques that the IOBC toxicity classifications receive (Sterk *et al*., 2023; Thomson & Hoffmann, 2006; Stark & Banks, 2024)

In contrast, the range of synthetic insecticides was associated with significant reductions in emergence and elevated rates of unsuccessful emergence. This was especially true for neonicotinoids (Thiamethoxam and Acetamiprid), pyrethroids (Lambda-cyhalothrin, Tau-fluvalinate, Alpha-cypermethrin and Beta-cyfluthrin), and avermectins (Abamectin and Emamectin benzoate). These active ingredients impaired development across all life stages, causing a marked increase in the proportion of individuals that failed to complete emergence (Cheng *et al.,* 2018; Khan & Ruberson, 2017). Notably, Tau-fluvalinate caused some of the most severe developmental disruptions, with extremely low emergence and high unsuccessful emergence rates recorded under all exposure scenarios. It also showed the lowest levels of parasitism by females when their reproductive potential was assessed. Importantly, these effects were evident even when eggs were parasitised after chemical exposure, suggesting that residue persistence or transovarial effects may compromise the viability of the developing parasitoid (Khan & Ruberson, 2017). This finding indicates that sublethal developmental toxicity can occur even in the absence of direct contact with adult parasitoids, posing a hidden risk to population establishment in the field.

Whilst laboratory-based research on non-target effects of chemicals is necessary and beneficial, it can be misleading as they don't consider the degradation of the chemical in the field (Zang *et al.,* 2021). This is why residual toxicity testing in semi-field conditions was done to assist in determining the safety interval periods of the selected chemicals tested. However, the results provide only an insight because only two replications of the residual trial have been completed. Further testing to improve their reliability needs to be done before clear recommendations can be made.

The mortality of adult *T. cryptophlebiae* following exposure to chemical residues varied substantially across treatments and declined consistently with increasing residue interval days, as expected (de Paiva *et al.*, 2021). This pattern underscores the transient but sometimes severe impact of freshly applied pesticides and the importance of residue age in determining parasitoid

survival, and therefore, the interval periods of their releases with select chemical application (de Paiva *et al.*, 2021).

Among all treatments, Acephate posed the highest acute risk to female and male wasps. Mortality on day 0 was extreme and substantially higher than that recorded in any other treatment. However, mortality from Acephate declined steadily over time, with minimal residual effect remaining by day 21. This highlights its acute toxicity and suggests relatively short environmental persistence (de Paiva *et al.*, 2021). While this may reduce long-term risk in the field, its immediate application period poses a significant threat to adult wasps. It would severely disrupt biological control if releases occur too close to its application (de Paiva *et al.*, 2021).

Other insecticides, including Acetamiprid, Lambda-cyhalothrin, Thiamethoxam, and Pymetrozine, also caused moderate to high mortality immediately after application. However, like Acephate, their toxicity diminished substantially by 7 days post-application. These findings suggest that a minimum waiting period following application may be necessary before parasitoid releases to avoid mortality and disruption of the population establishing in the field (de Paiva *et al.*, 2021). In contrast, microbial biopesticides, such as *B. bassiana* (both formulations) and CrpeNPV, insect growth regulators like Methoxyfenocide, and fungicide combinations, such as Azoxystrobin and Difenoconazole, exhibited consistently low mortality across all time points for both sexes (Zang *et al.,* 2021). Mortality in these treatments remained low following the day 0 application, indicating minimal acute or residual toxicity, which is supported by the findings on the acute toxicity in reproductive potential assessment of *T. cryptophlebiae* females. These treatments were comparable to control conditions and appear fully compatible with adult parasitoid activity. Grout *et al.* (2011) unpublished work on the non-target effects of chemicals used in citrus on *T. cryptophlebiae* appears to reflect the same results seen here, except for Abamectin. Grout *et al*. (2011) classify Abamectin as slightly harmful, whereas it is classed as moderately harmful here. This difference may be due to the methods used to classify the products. The IOBC uses Abbott's Correction for mortality, whereas Grout *et al.* (2011) made use of a formula developed by Hattingh *et al.* (2000), which takes residual persistence into account. This again contributes to the discussion for a call to re-evaluate the IOBC classification system to some degree (Sterk *et al.,* 2023; Mata *et al.,* 2024; Thomson & Hoffmann, 2006)

There was little evidence of pronounced sex-specific vulnerability across most treatments. While slight differences were recorded, for instance, female mortality under Acephate was slightly higher than male mortality on day 0, both sexes followed the same general trend of declining mortality over time. This suggests that recommendations around residue safety intervals can reasonably apply to both male and female wasps (de Paiva *et al.*, 2021).

Together, the findings highlight the complex interplay between chemical exposure and the reproductive success of *T. cryptophlebiae*, underscoring that survival alone is not a sufficient indicator of compatibility (Mata *et al.*, 2024; Zang *et al.,* 2021). While some parasitoids may survive exposure to specific residues, their reproductive capacity, including parasitism activity, offspring emergence, and sex ratio balance, can still be compromised through lethal and sublethal effects (Stark & Banks, 2024; de Paiva *et al.*, 2021; Thomson & Hoffmann, 2006). Chemicals such as biopesticides and selective fungicides were generally harmless across multiple metrics, supporting their integration into IPM programmes (Macfadyen *et al.*, 2013; Zang *et al.,* 2021). In contrast, broad-spectrum insecticides often impaired reproductive traits, skewed sex ratios, or reduced emergence, even when adult mortality appeared low. Residue age further modulated toxicity, with synthetic insecticides becoming less harmful over time, suggesting that application timing is crucial for minimising non-target impacts (Thomson & Hoffmann, 2006; de Paiva *et al.*, 2021). The IOBC classifications based on adult mortality and emergence offer a practical tool for guiding chemical selection, enabling growers to avoid products that may suppress parasitoid populations (Thomson & Hoffmann, 2006; Sterk *et al.,* 2023; Zang *et al.,* 2021). Ultimately, to maximise the success of augmentative releases, careful selection and timing of chemical inputs, alongside additional research into effects on longevity and fertility, are essential for maintaining the efficacy of *T. cryptophlebiae* in tortricid suppression.

Chapter 5: General Discussion

5.1. Introduction

The objectives of this study were threefold. The first objective was to optimise the mass-rearing protocols and production system for *T. cryptophlebiae*, whilst also ensuring that the biological integrity of the parasitoids does not degrade over time. Through this, augmented releases could be conducted against an array of tortricid pests, with the knowledge that parasitoid quality was high, thus supporting in-field efficacy. The second objective was to investigate appropriate release timing and rates in different fruit and nut crops. The final objective was to assess farming pesticide practices and chemical non-target effects as a limiting factor to the success of this parasitoid as a biological control agent of tortricids. Results from these three key focus areas allowed for a fuller understanding of *T. cryptophlebiae* as a biological control agent from mass production and augmented field releases to its incorporation into IPM programmes across various crops to support the management of economically important tortricids that impact South African agricultural production and export markets.

5.2. Key Findings and Implications

This egg parasitoid, *T. cryptophlebiae*, native to southern Africa, has been proven to be an effective control option against *T. leucotreta* in citrus (Newton, 1988a, 1988b, 1989; Newton and Odendaal, 1990; Moore and Fourie, 1999; Moore and Richards, 2000, 2001, 2002; Moore *et al*., 2015), and is currently being mass-reared for augmented releases against this pest. Its potential for further use against other economically significant tortricids in various crops was realised through observations from laboratory trials and field assessments, which identified other tortricids as physiological hosts to *T. cryptophlebiae* (Newton, 1988a and b; Newton, 1989; Newton & Odendaal, 1990; Moore & Fourie, 1999; Moore and Richards, 2000, 2001, 2002; Moore *et al*., 2015; Kaspi *et al.,* 2020; BioResources, 2023; Newton & Crause, 1990; Chambers *et al*., 1995). The no-choice and choice trials conducted in this research are consistent with previous studies, confirming that *L. vanillana* and *C. peltastica* serve as physiological hosts for *T. cryptophlebiae,* as does *C. pomonella*, as established by Kaspi *et al*. (2021) and Wahner (2008). These findings led to the inclusion of these hosts in field research targeting *T. batrachopa*, *C. peltastica*, and *T. leucotreta* in macadamia orchards; *L. vanillana* and *T. leucotreta* in grape vineyards; *C. peltastica* and *T. leucotreta* in litchi orchards; and *C. pomonella* in pome fruit.

5.2.1. Production Constraints

Production constraints were faced during the season, with suboptimal numbers being released at the start of the first season in all crops. Constraints were faced regarding the availability of *T. cryptophlebiae* from the production system which prevented the appropriate integration of it into spray programmes. However, as there are limitations to the production of any product, optimising parasitoid production to allow a dynamic and flexible supply of *T. cryptophlebiae* is challenging. The production constraints faced may have impacted the field results recorded, but it is more beneficial to understand why these constraints were encountered, how they can be mitigated, and how they affect the product's reputation from the grower's perspective (Morales-Ramos *et al.*, 2023). This reflects the practical realities of the limitations in the production of this parasitoid, like many other beneficials.

The mass production of *T. cryptophlebiae,* like other parasitoids and natural enemies, requires stringent quality control measures to be put in place to ensure the biological integrity of the parasitoid is not lost over time (van Lenteren, 2003; van Lenteren *et al.*, 2018). The research done here provided a baseline for quality control metrics of this parasitoid, based on fertility and sex ratio production outcomes, from trials where mass-rearing environments were simulated. Mitigating issues regarding parasitism levels and sex ratio production in *T. cryptophlebiae* can be accomplished by ensuring a surplus of host eggs is available per female. If target parasitism levels are met, production numbers will be achieved, allowing for a consistent and reliable supply chain of this parasitoid.

Additionally, to ensure a consistent supply chain of the parasitoid, optimising its rearing system to allow for more flexibility and dynamic supply would further encourage the adoption of the product by growers, as it makes the ease of its incorporation into spray programmes more achievable. Further optimisation of the *T. cryptophlebiae* mass-rearing system should look at the ability of its host eggs to be stockpiled long term, as suggested by Para (2010), Cherif *et al.* (2021), and Zang *et al.* (2021) for several other trichogrammatid host species. The potential of stockpiling *T. cryptophlebiae* long-term through diapause induction or enhanced cold storage methods, as indicated by Reznik *et al.* (2010) and Para (2010), would improve the flexibility of supply of the production of this parasitoid. Further research addressing these areas of *T. cryptophlebiae* mass production should carefully consider the impact these optimisations may have on the parasitoids'

biological integrity, so as not to degrade their fitness from one generation to the next, as this has implications for field releases (van Lenteren, 2003; van Lenteren *et al.*, 2018).

5.2.2. Field Release Rates and Timing

The standard release rate for parasitoids, such as *T. cryptophlebiae*, against their target pests needs to be determined by crop due to differing plant phenology and pest behaviour. These factors can influence the location and level of parasitism by this parasitoid (Perez *et al.*, 2019). In the field releases of *T. cryptophlebiae* into the macadamias, higher release rates of 50,000 wasps per hectare done monthly resulted in the most consistent levels of parasitism and substantial reductions in nut damage. This release rate, however, is significantly different from that used in Australia for *C. ombrodelta* management by *T. cryptophlebiae*. Every week, 1000 parasitised eggs per hectare, divided across 24 cards, are placed into macadamia orchards for *C. ombrodelta* suppression (BioResources Pty Ltd, 2023). The behaviour and population pressure of *C. ombrodelta* may differ significantly from that of *T. batrachopa,* which would explain the difference in release rates, but it indicates the need to determine appropriate release rates of *T. cryptophlebiae* by target species and crops as pest and crop phenology do influence the ecological behaviour of the parasitoid (Perez *et al.*, 2019).

Not only is the rate of release important, but the timing of the first release of *T. cryptophlebiae* in the cropping season is of huge importance, as it allows for the targeted pest to be managed prior to its population reaching peak activity during the season. The timing of Early releases of *T. cryptophlebiae* in the macadamias indicated some suppression of tortricid populations, but their effectiveness may have been compromised by suboptimal conditions such as chemical applications (Tang *et al.*, 2010). Harsh chemical applications occur early in the macadamia production season for the management of heteropteran complexes, which could have impacted the establishment of parasitoids in the field (Schoeman, 2008). Additionally, the synchronisation of releases timed with pest presence should be considered in determining the ideal release timing of *T. cryptophlebiae,* as generally there is a lack of host eggs available during the flowering and initial nut development stages, but the presence of out-of-season nuts in orchards must also be taken into consideration as they could be a refuge for tortricids and carried across seasons (Schoeman, 2008). Nevertheless, the preventative advantages of these Early releases could be significantly enhanced if compatible chemicals are used in combination with the release of these

parasitoids during this period. Moore & Richards (2002) indicated this through their research on the development of an appropriate release rate and timing of *T. cryptophlebiae* in citrus, whereby early releases were beneficial over and above natural parasitism and prevented infestation by reducing pest population build-up.

The levels of parasitism seen in the macadamia field trials overall were positive but could likely benefit from better timing of releases around chemical applications that are compatible with the parasitoid. In the other crops assessed, litchis, wine grapes and pome fruit, results did not reflect the potential of *T. cryptophlebiae*, due to issues that arose in regard to site selection, which influenced pest pressure and representative sampling areas. This underscores the reality that environmental factors and farming practices can disrupt parasitoid activity and survival, necessitating an understanding of the non-target chemical impacts on the parasitoid to be evaluated.

5.2.3. Chemical Compatibility

It is important to note that producers did not modify their chemical applications to facilitate the establishment of parasitoids during the field trials, and releases often coincided with incompatible pesticide applications. This had an impact on the outcomes of the field research. However, they reflect the practical realities of on-farm practices that make harmonising chemical and biological control challenging. The non-target effect trials done on *T. cryptophlebiae* justify that chemical applications in the field would have had an impact on the parasitoids' activity in the field. Select biopesticides did, however, show suitability to be used in conjunction with parasitoid releases. However, for other trichogrammatid species, differing tolerances to chemicals have been recorded (Zang *et al.,* 2021), underscoring the need for species-specific guidelines in African IPM programmes. This clearly highlights the realistic focus of the approach taken by this research, which is to successfully integrate *T. cryptophlebiae* into chemical-dominated IPM programmes. The only way to determine the effectiveness of the parasitoid was to release it into the systems and monitor its impact. The results were positive and provided a definite indication that its use in IPM programmes is beneficial for tortricid management practices, specifically in macadamias. The continued optimisation of crop protection strategies involving *T. cryptophlebiae*, along with the management of other pest species, could enhance control levels. This is suggested by BioResources Pty Ltd (2023) in Australia, where *T. cryptophlebiae* is used to manage *C.*

ombrodelta. BioResources proposes that optimising the timing of releases in conjunction with chemical sprays benefits the establishment of the parasitoid in orchards, leading to improved suppression of host populations (Bright, 2022). The effectiveness of *T. cryptophlebiae* in suppressing *C. ombrodelta* in Australia has been remarkable, allowing growers to stop spraying for this specific pest in their macadamia orchards after January each season (Bright, 2022). The similarities in pest management in South African and Australian macadamias, and the success seen of *T. cryptophlebiae* against *C. ombrodelta,* should serve as an indication of the capabilities of this parasitoid in South African orchards against *T. batrachopa.*

5.3. IPM in the Legislative Environment

The production of horticultural crops in South Africa and worldwide requires strict adherence to safety standards designed to protect both human health and the environment. These standards were established by organisations such as the Department of Agriculture and international importing markets (Cloete *et al*., 2013; Fuchs & Kalfagianni, 2010). A major challenge for South African producers is complying with maximum residue limit (MRL) regulations, which differ across various markets and crops (Hejazi *et al*., 2022; Hanford *et al*., 2015). To ensure compliance, producers must adapt their pest management strategies according to the specific requirements of each export destination, with regular audits conducted by agencies such as the Perishable Products Export Control Board (PPECB) and GlobalGAP (Hanford *et al*., 2015). Fortunately, grower associations provide valuable assistance in navigating these regulations and play a crucial role in maintaining market access (Fresh Food Trade SA, 2023; Subramanian *et al*., 2011). Given agriculture's significant contribution to the national economy (Barrientos & Visser, 2013; Wakelin & Taylor, 2024), adherence to MRL standards is essential. However, the loss of key pesticide products has created an increasing need for viable alternatives, reshaping the agricultural pest management industry in South Africa.

Similar trends are reshaping pest management across Africa for these same reasons. Mulungu *et al.* (2024) contextualise the benefits of biological control based IPM by demonstrating the economic, social, and environmental benefits it can provide when approached from an ecologically centred standpoint. Mulungu *et al.* (2024) report that IPM management of the mango fruit fly in mangoes, in both Kenya and Uganda, lead to significant economic returns (with less input required), improvements in food security, and a reduction in poverty. This highlights the

benefits of IPM and should encourage its further adoption, as it presents a promising alternative to the reliance on chemical pesticides. IPM advocates for the strategic use of chemical, biological, cultural, and mechanical methods of pest management, with chemicals employed only when necessary (Deguine *et al*., 2021). Nevertheless, the reality is that most programmes continue to rely predominantly on chemical solutions. While the utilisation of biological control agents is on the rise, their integration into existing systems remains incomplete (Barzman *et al*., 2015; Deguine *et al*., 2021; Ehler, 2006; Vreysen *et al*., 2007). Shifting toward more biologically oriented programmes will require long-term knowledge sharing, relationship building, and dedicated research efforts (Deguine *et al*., 2021). This research aimed to facilitate that transition by examining how *T. cryptophlebiae* can be effectively integrated into chemically dominated IPM systems in South Africa.

5.4. Toward Practical Implementation

Insectaries that produce *T. cryptophlebiae* for commercial use now have an evidence-based guideline on how to optimise parasitism and sex ratios in a mass-rearing system that will allow for a consistent supply to the market. Baseline metrics are now available for which to compare generational changes in the parasitoid's fitness over time as mass-rearing continues. This will enable the guaranteed production and release of high-quality parasitoids, which are capable of effectively suppressing pest populations. The use of quality control metrics in mass-rearing systems of *T. cryptophlebiae* is an area where product assurance builds trust with producers using the parasitoid and can further encourage its adoption in IPM programmes in South Africa (van Lenteren *et al.*, 2021).

Furthermore, grower education on the augmentation of this parasitoid in the field can significantly improve *T. cryptophlebiae* adoption (Deguine *et al.,* 2021). The IOBC classifications developed in this research for *T. cryptophlebiae* offer a valuable framework for converting laboratory findings into practical guidance for growers. By categorising each product according to its effects on adult survival and emergence success, one is able to distinguish chemicals by their level of harm to *T. cryptophlebiae*. However, it is important to use these classifications with caution, as they can sometimes overestimate or underestimate the impact of chemicals on natural enemies when implemented in practice. Despite this, the IOBC classifications are still valuable, and for *T. cryptophlebiae* and the products tested, they should be considered alongside all tested

parameters to derive the most informed interpretation of the chemicals' effects. This can empower growers to make informed decisions about which products to avoid, and which can be safely employed before, during, or after parasitoid releases, and the waiting periods required (Barratt *et al.*, 2017). Such information is crucial for harmonising chemical and biological control strategies within an IPM framework. Minimum safety interval periods need to be developed, based on residual toxicity, to further enhance the releases of *T. cryptophlebiae*.

The rate and release timing of *T. cryptophlebiae* into respective crops needs to be developed further in order to make definitive recommendations, especially in litchi, grapes, and pome fruit. In Macadamias, release rates of 25,000 and 50,000 wasps/ha per month are suitable in suppressing *T. batrachopa* when released monthly. The overlap in confidence intervals of these two release rates demonstrates no significant difference in the level of control they offer, thus suggesting that 25,000 wasps/ha is most practical, as it is more cost-effective for growers and insectaries can provide the product for a larger hectarage. The timing of early-season releases is recommended, despite the potential conflict between parasitoids and pesticides, as early-season suppression is more beneficial to pest tortricid management purposes than delaying the releases (Moore & Richards, 2000; Moore & Richards, 2001; Moore & Richards, 2002). Growers can utilise the IOBC classification of active ingredients to guide early-season releases of *T. cryptophlebiae* in relation to harsh chemical sprays.

5.5. Conclusion

This study bridges critical gaps in the application of *T. cryptophlebiae,* from mass-rearing optimisation to field deployment under real-world constraints. The successful integration of *T. cryptophlebiae* into biological control programmes for managing tortricid pests in macadamia and other crops hinges on the convergence of biological integrity, operational precision, and chemical compatibility. The research presented here highlights the importance of maintaining the fitness of mass-reared parasitoids, aligning release strategies with pest phenology and environmental conditions, and wisely selecting insecticides based on IOBC classification and residue persistence, as critical components for achieving favourable field outcomes. Furthermore, the IOBC framework proves to be a valuable decision-support tool, enabling growers to make evidence-based choices when synchronising chemical and biological control methods. Collectively, these insights establish a solid foundation for advancing augmentative biological control using *T. cryptophlebiae* in

southern Africa and present clear, actionable strategies to optimise its performance within IPM systems. Tailoring strategic adjustments in monitoring, rearing, and field implementation to specific crop contexts will be crucial for scaling this approach and ensuring effective pest suppression while minimising reliance on disruptive chemical inputs.

5.6. Future research

Future research on *T. cryptophlebiae* should focus on further optimising its mass-rearing system through host and parasitoid stockpiling. Successfully achieving this would benefit both companies producing the product and growers, as it would increase production output, application flexibility and the area (hectarage) that can be serviced. This improvement would significantly contribute to the adoption of IPM in southern Africa by overcoming current product limitations.

Moreover, additional efficacy testing in the field should be conducted with different crops to confirm that *T. cryptophlebiae* is a suitable biological control option for tortricids in those settings. Similar trial designs can be employed, but careful attention must be given to site selection and cooperation with farmers.

It is also essential to develop safety interval release timing periods for *T. cryptophlebiae* following chemical applications. This should include the evaluation of additional active ingredients that not only pertain to macadamia pest management programs but also to other crops. Investigating the longevity of *T. cryptophlebiae* in relation to all active ingredients will provide crucial information that enhances the integration of this parasitoid into IPM programmes. This knowledge is invaluable for building producers' trust in *T. cryptophlebiae* as an effective tool for managing tortricids.

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Appendix

Table A. 1. The selected trial sites per crop specifying the site names, varieties, ages, and locations.

Crop	Site Name	Variety	Age (years)	Province	Location	
					Latitude	Longitude
Macadamia	Goodwoods	695, A4, Nelmak 2	20	Eastern Cape	33° 32’ 28’’ S	26° 38’ 39’’ E
	Juanita	695, A4, Nelmak 2	20	Mpumalanga	25° 19’ 03’’ S	30° 59’ 41’’ E
	Leppan Farming	695, A4, Nelmak 2	5	Western Cape	33° 57’ 18’’ S	22° 39’ 48’’ E
	Toorbos 1	695, A4, Nelmak 2	18	Western Cape	33° 56’ 22’’ S	22° 40’ 23’’ E
	Toorbos 2	695, A4, Nelmak 2	18	Western Cape	33° 56’ 45’’ S	22° 40’ 49’’ E
	Myee	695	16	Kwa-Zulu Natal	29° 01’ 16’’ S	31° 36’ 34’’ S
Wine Grape	Zandvliet	Shiraz	8/20	Western Cape	33° 51’ 44” S	20° 02’ 42” E
	Boskloof	Steen	10	Western Cape	33° 40’ 48” S	19° 29’ 44” E
	Goedgeloof 1	Hermitage	23	Western Cape	33° 30’ 06” S	19° 11’ 54” E
	Goedgeloof 2	Steen	16	Western Cape	33° 30’ 36” S	19° 11’ 54” E
	Goedgeloof 3	Sirah	3	Western Cape	33° 30’ 47” S	19° 11’ 54” E
Litchi	Tomahawk 1	Mauritius	33	Mpumalanga	25° 36’ 41” S	31° 36’ 58” E
	Tomahawk 2	Mauritius	16	Mpumalanga	25° 36’ 29” S	31° 37’ 53” E
	Tomahawk 3	Mauritius	37	Mpumalanga	25° 35’ 51” S	31° 37’ 57” E
Pome Fruit	JGS 1	Fuji	26	Eastern Cape	33° 46’ 32” S	23° 28’ 48” E
	JGS 2	Forelle	26	Eastern Cape	33° 46’ 19” S	23° 28’ 21” E
	Waboom 1	Kanzi	12/13	Western Cape	33° 52’ 22” S	22° 20’ 25” E
	Waboom 2	Rosy Glow	14	Western Cape	33° 52’ 38” S	22° 20’ 08” E
	Waboom 3	Forelle	10/38	Western Cape	33° 52’ 51” S	22° 20’ 26” E

Table A. 2. The size and number of *Trichogrammatoidea cryptophlebiae* release cards per treatment at all macadamia sites.

Site Name	Treatment (wasps/ha)	Timing of Release	Orchard size (ha)	Card Size (cm x cm)	No. of Cards
Goodwoods	Control	-	0.91	-	-
	12,500	Standard	0.92	3 x 3	23
	25,000	Early	1.00	3 x 6	25
	25,000	Standard	0.73	3 x 6	19
	25,000	Late	0.75	3 x 6	19
	50,0000	Standard	0.90	6 x 6	23
Juanita	Control	-	1	-	25
	12,500	Standard	1.12	3 x 3	28
	25,000	Early	1.1	3 x 6	28
	25,000	Standard	1.1	3 x 6	28
	25,000	Late	1.1	3 x 6	28
	50,0000	Standard	1.1	6 x 6	28
Leppan Farming	Control	-	1.27	-	-
	12,500	Standard	2.29	3 x 3	58
	25,000	Early	2.20	3 x 6	55
	25,000	Standard	2.41	3 x 6	61
	25,000	Late	2.64	3 x 6	66
	50,0000	Standard	2.00	6 x 6	50
Toorbos 1	Control	-	2.14	-	-
	12,500	Standard	2.20	3 x 3	55
	25,000	Early	2.10	3 x 6	53
	25,000	Standard	1.10	3 x 6	28
	25,000	Late	1.89	3 x 6	48
	50,0000	Standard	1.52	6 x 6	38
Toorbos 2	Control	-	1.28	-	-
	12,500	Standard	1.58	3 x 3	40
	25,000	Early	2.54	3 x 6	64
	25,000	Standard	4.51	3 x 6	113
	25,000	Late	2.87	3 x 6	72
	50,0000	Standard	1.54	6 x 6	39
Myee	Control	-	2.1	-	-

	12,500	Standard	2.85	3 x 3	72
	25,000	Early	3.25	3 x 6	82
	25,000	Standard	2	3 x 6	50
	25,000	Late	3.36	3 x 6	84
	50,0000	Standard	2.79	6 x 6	70

Table A. 3. The size and number of *Trichogrammatoidea cryptophlebiae* release cards per treatment at all wine grape sites.

Site Name	Treatment (wasps/ha)	Timing of Release	Vineyard size (ha)	Card Size (cm x cm)	No. of Cards
Zandvliet	Control	-	1.00	-	25
	12,500	Standard	1.00	3 x 3	25
	25,000	Early	1.00	3 x 6	25
	25,000	Standard	1.00	3 x 6	25
	25,000	Late	1.00	3 x 6	25
	50,0000	Standard	1.00	6 x 6	25
Boskloof	Control	-	1.00	-	25
	12,500	Standard	1.00	3 x 3	25
	25,000	Early	1.00	3 x 6	25
	25,000	Standard	1.00	3 x 6	25
	25,000	Late	1.00	3 x 6	25
	50,0000	Standard	1.00	6 x 6	25
Goedgeloof 1	Control	-	0.82	-	21
	12,500	Standard	0.80	3 x 3	20
	25,000	Early	0.75	3 x 6	19
	25,000	Standard	0.81	3 x 6	21
	25,000	Late	0.82	3 x 6	21
	50,0000	Standard	0.76	6 x 6	19
Goedgeloof 2	Control	-	1.00	-	25
	12,500	Standard	1.34	3 x 3	34
	25,000	Early	1.00	3 x 6	25
	25,000	Standard	0.84	3 x 6	21
	25,000	Late	0.88	3 x 6	22
	50,0000	Standard	1.10	6 x 6	28
Goedgeloof 3	Control	-	0.93	-	24
	12,500	Standard	1.00	3 x 3	25
	25,000	Early	0.70	3 x 6	18
	25,000	Standard	0.70	3 x 6	18
	25,000	Late	0.84	3 x 6	21
	50,0000	Standard	0.86	6 x 6	22

Table A. 4. The size and number of *Trichogrammatoidea cryptophlebiae* release cards per treatment at all litchi sites.

Site Name	Treatment (wasps/ha)	Timing of Release	Orchard size (ha)	Card Size (cm x cm)	No. of Cards
Tomahawk 1	Control	-	1.00	-	25
	12,500	Standard	1.00	3 x 3	25
	25,000	Early	1.00	3 x 6	25
	25,000	Standard	1.00	3 x 6	25
	25,000	Late	1.00	3 x 6	25
	50,0000	Standard	1.00	6 x 6	25
Tomahawk 2	Control	-	1.00	-	25
	12,500	Standard	1.00	3 x 3	25
	25,000	Early	1.00	3 x 6	25
	25,000	Standard	1.00	3 x 6	25
	25,000	Late	1.00	3 x 6	25
	50,0000	Standard	1.00	6 x 6	25
Tomahawk 3	Control	-	1.00	-	25
	12,500	Standard	1.00	3 x 3	25
	25,000	Early	1.00	3 x 6	25
	25,000	Standard	1.00	3 x 6	25
	25,000	Late	1.00	3 x 6	25
	50,0000	Standard	1.00	6 x 6	25

Table A. 5. The size and number of *Trichogrammatoidea cryptophlebiae* release cards per treatment at all pome fruit sites.

Site Name	Treatment (wasps/ha)	Orchard size (ha)	Card Size (cm x cm)	No. of Cards
JGS 1	Control	1.49	-	-
	12,500	2.54	3 x 3	64
	25,000	2.19	3 x 6	55
	50,000	3.59	6 x 6	90
JGS 2	Control	2.10	-	-
	12,500	1.42	3 x 3	40
	25,000	2.15	3 x 6	54
	50,000	2.10	6 x 6	53
Waboom 1	Control	2.34	-	-
	12,500	1.63	3 x 3	41
	25,000	1.53	3 x 6	39
	50,000	1.33	6 x 6	34
Waboom 2	Control	1.36	-	-
	12,500	1.00	3 x 3	25
	25,000	1.00	3 x 6	25
	50,000	1.00	6 x 6	25
Waboom 3	Control	1.54	-	-
	12,500	1.16	3 x 3	29
	25,000	1.00	3 x 6	25
	50,000	1.10	6 x 6	28

Table A. 6. The pheromone lures used to monitor the relevant tortricid pests occurring in each crop and the replacement intervals of the pheromone lures.

Crop	Tortricid monitored	Pheromone Lure	Active ingredients	Lure replacement intervals (weeks)
Macadamia	*T. leucotreta*	Chempac False Codling Moth Lure	Z-8-Dodecenyl Acetate E-8- Dodecenyl Acetate E-7- Dodecenyl Acetate 62,5g/kg	12
	T. batrachopa	Chempac MNB Lure	Z-8-Dodecenyl Acetate	6
	C. peltastica	Chempac MNB Lure	Z-8-Dodecenyl Acetate	6
Wine Grape	*T. leucotreta*	Chempac False Codling Moth Lure	Z-8-Dodecenyl Acetate E-8- Dodecenyl Acetate E-7- Dodecenyl Acetate 62,5g/kg	12
	L. vanillana	Chempac Carob Moth Lure	(7Z,9E)-7,9,11-Dodecatrienyl formate 1mg/capsule	6
Litchi	*T. leucotreta*	Chempac False Codling Moth Lure	Z-8-Dodecenyl Acetate E-8- Dodecenyl Acetate E-7- Dodecenyl Acetate 62,5g/kg	12
	T. batrachopa	Chempac MNB Lure	Z-8-Dodecenyl Acetate	6
	C. peltastica	Chempac MNB Lure	Z-8-Dodecenyl Acetate	6
Pome Fruit	*C. pomonella*	Biolure CM x10	E8-E10 Dodecadienol-1-ol 5.25 g/pheromone	6 to 8

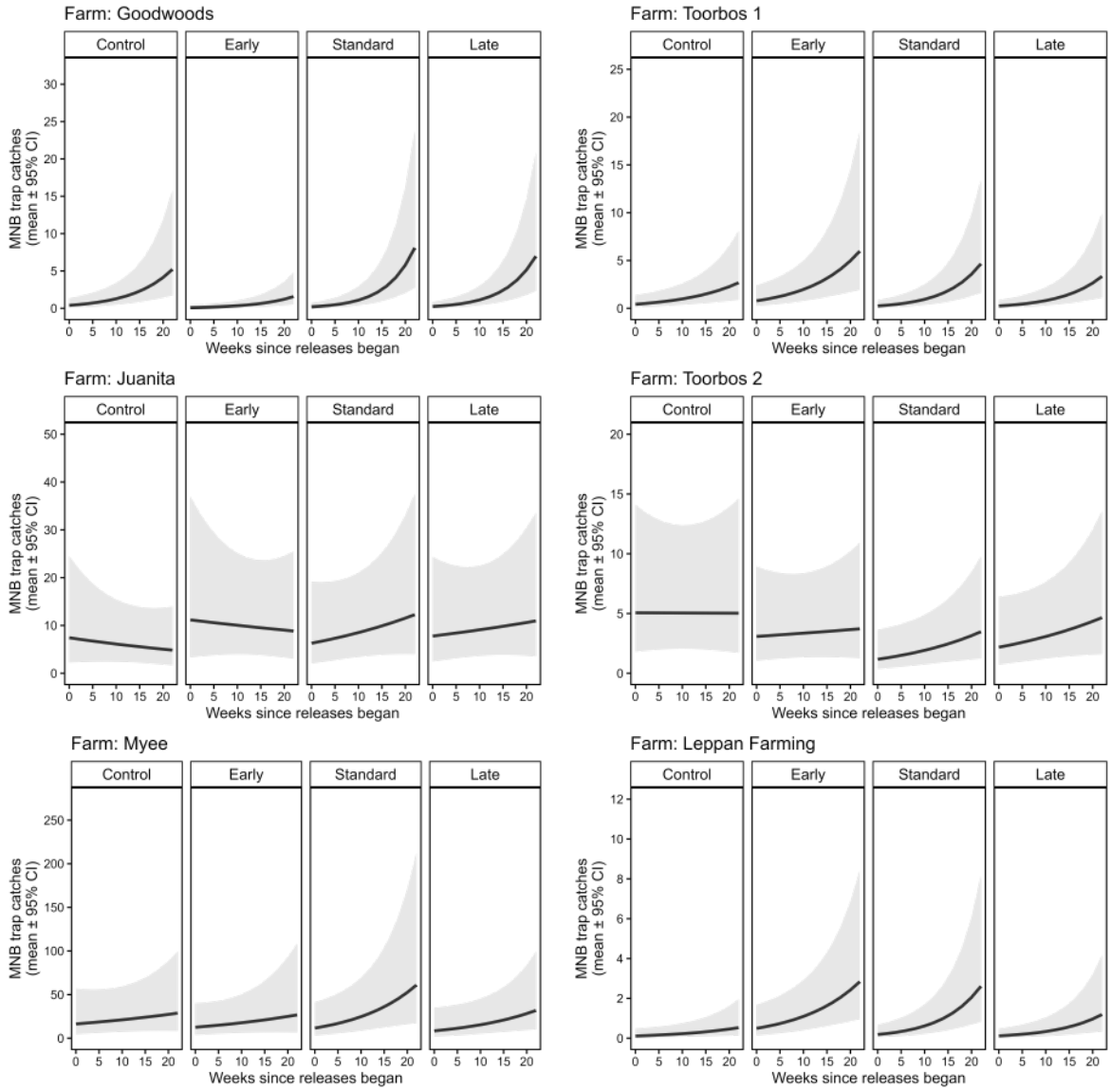

Figure A. 1. Marginal effect of *Trichogrammatoidea cryptophlebiae* release timing on MNB trap catches in macadamia orchards over two growing seasons per farm, Goodwoods, Toorbos 1, Toorbos 2, Leppan, Juanita, and Myee. Note differences in y-axis per farm done to show visual trends clearly. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

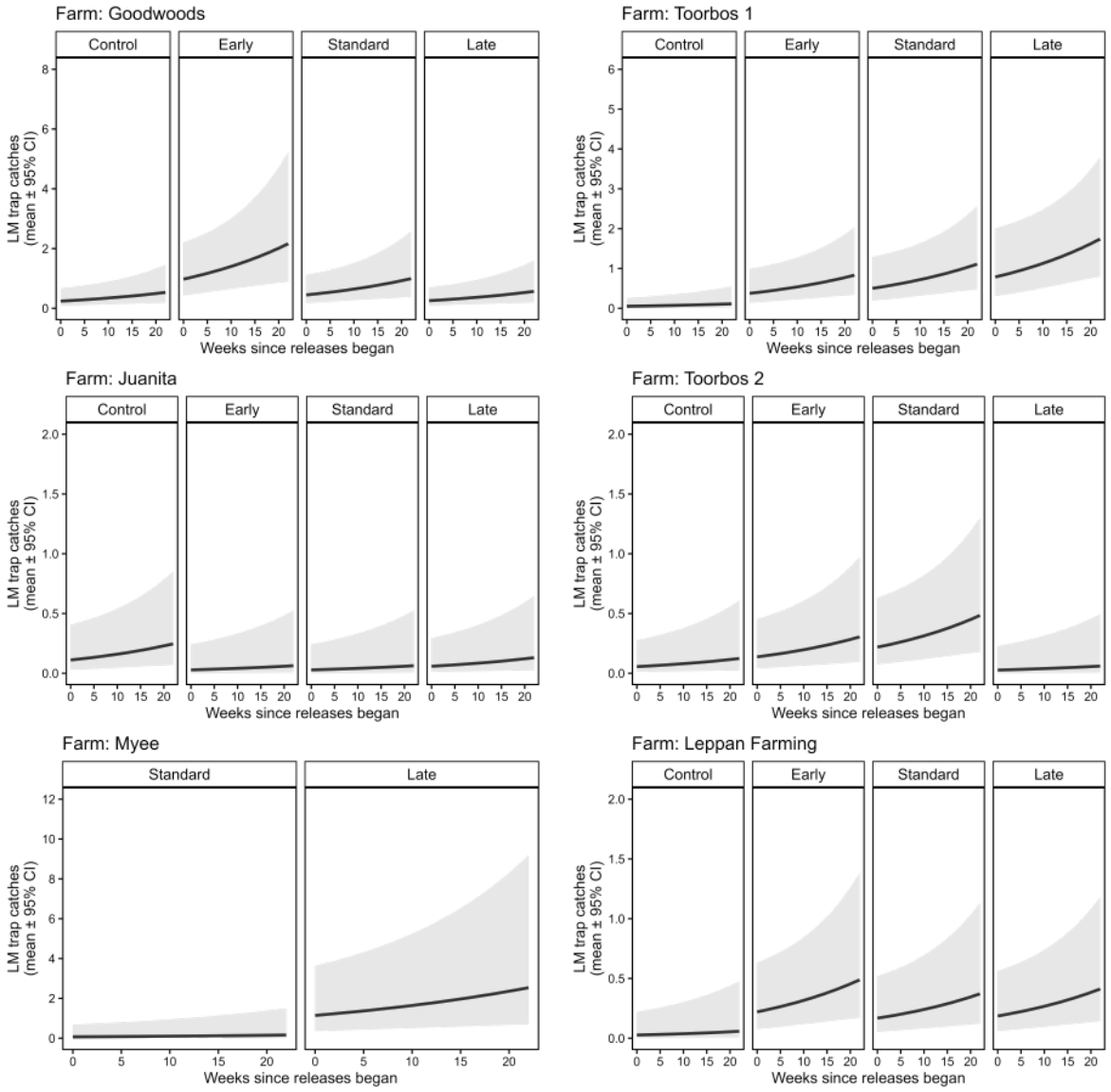

Figure A. 2. Marginal effect of *Trichogrammatoidea cryptophlebiae* release timing on LM trap catches in macadamia orchards over two growing seasons per farm, Goodwoods, Toorbos 1, Toorbos 2, Leppan, Juanita, and Myee. Note differences in y-axis per farm done to show visual trends clearly. Note LM trap catches at Myee for Control and Eraly treatments are not shown in the figure due to zero LM being captured through the season. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

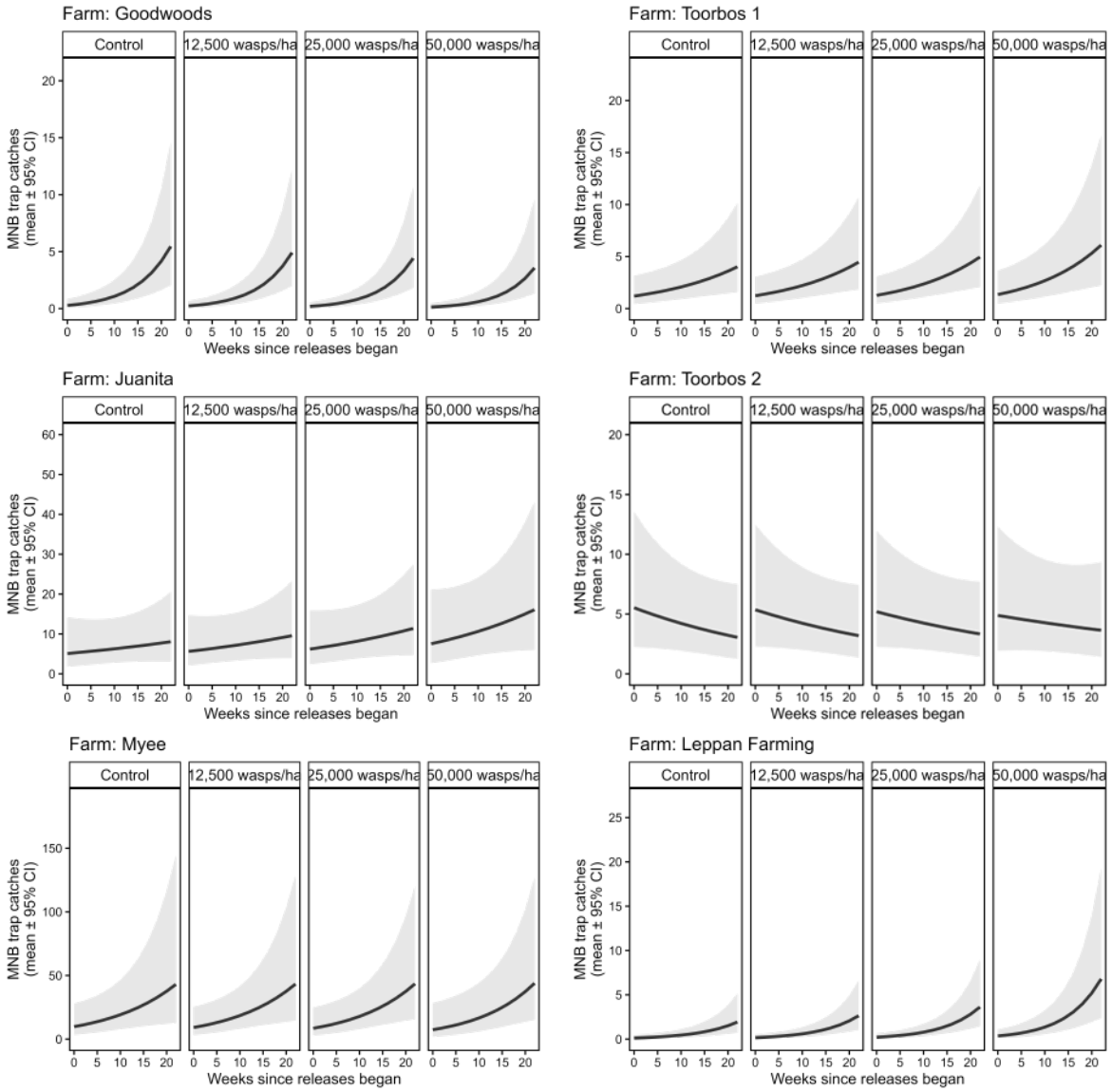

Figure A. 3. Marginal effect of *Trichogrammatoidea cryptophlebiae* release numbers on MNB trap catches in macadamia orchards over two growing seasons per farm, Goodwoods, Toorbos 1, Toorbos 2, Leppan, Juanita, and Myee. Note differences in y-axis per farm done to show visual trends clearly. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

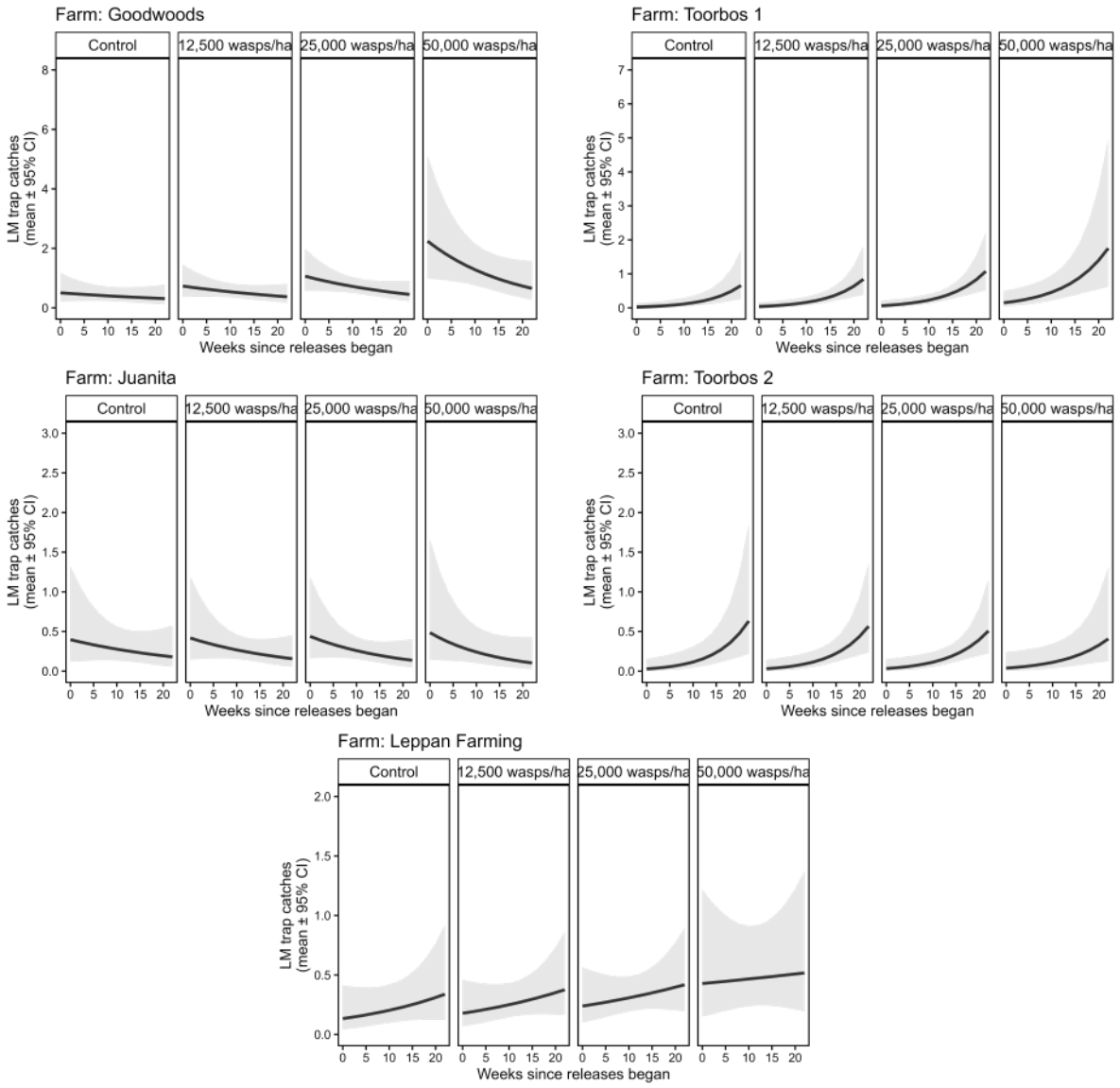

Figure A. 4. Marginal effect of *Trichogrammatoidea cryptophlebiae* release numbers on LM trap catches in macadamia orchards over two growing seasons per farm, Goodwoods, Toorbos 1, Toorbos 2, Leppan, Juanita, and Myee. Note differences in y-axis per farm done to show visual trends clearly. Note Myee is not reflected in this figure due to near zero LM being captured over the growing seasons making prediction unreliable. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

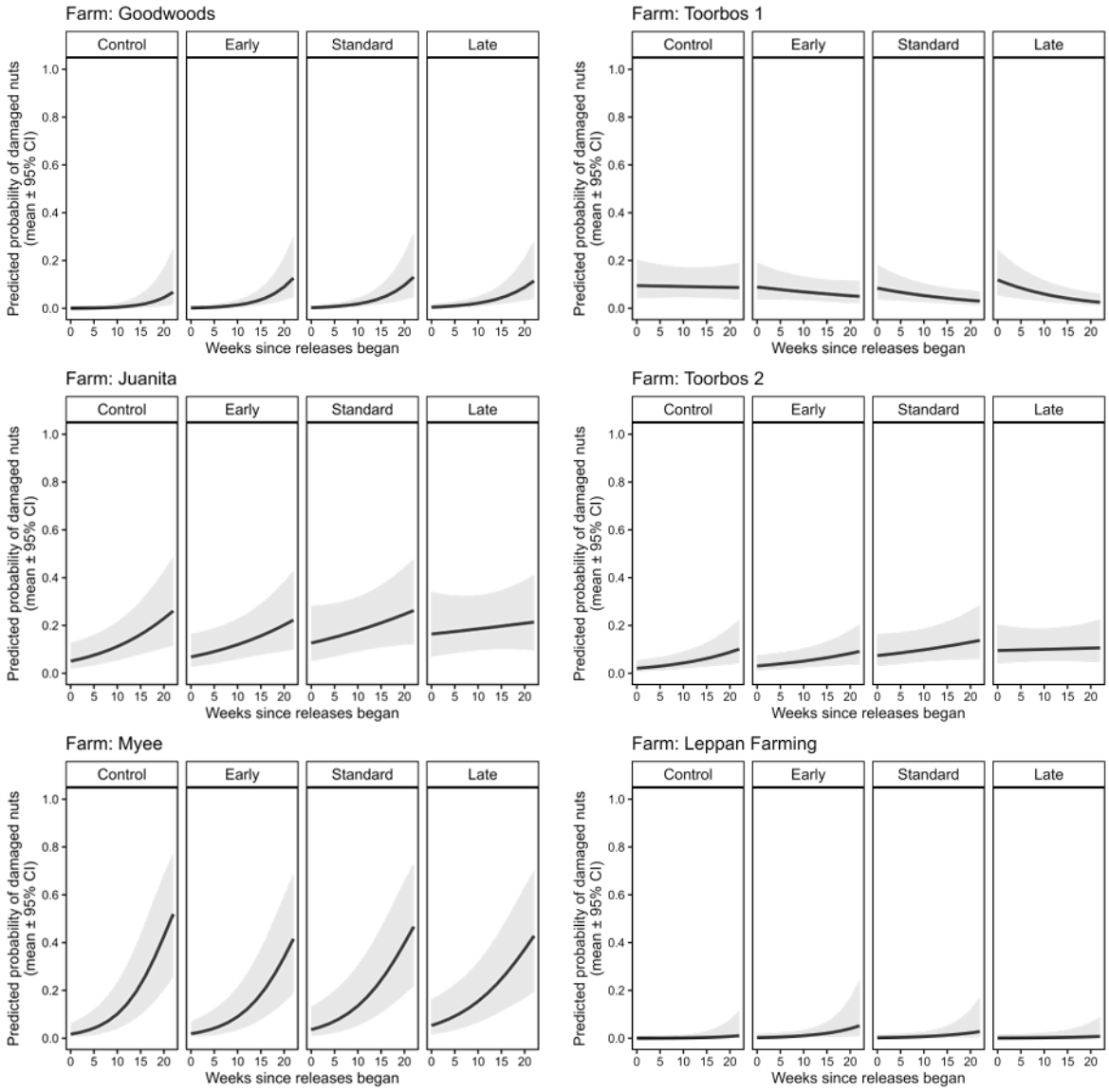

Figure A. 5. Marginal effect of *Trichogrammatoidea cryptophlebiae* release timing on tortricid nut damage in macadamia orchards over two growing seasons per farm, Goodwoods, Toorbos 1, Toorbos 2, Leppan, Juanita, and Myee. Note differences in y-axis per farm done to show visual trends clearly. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

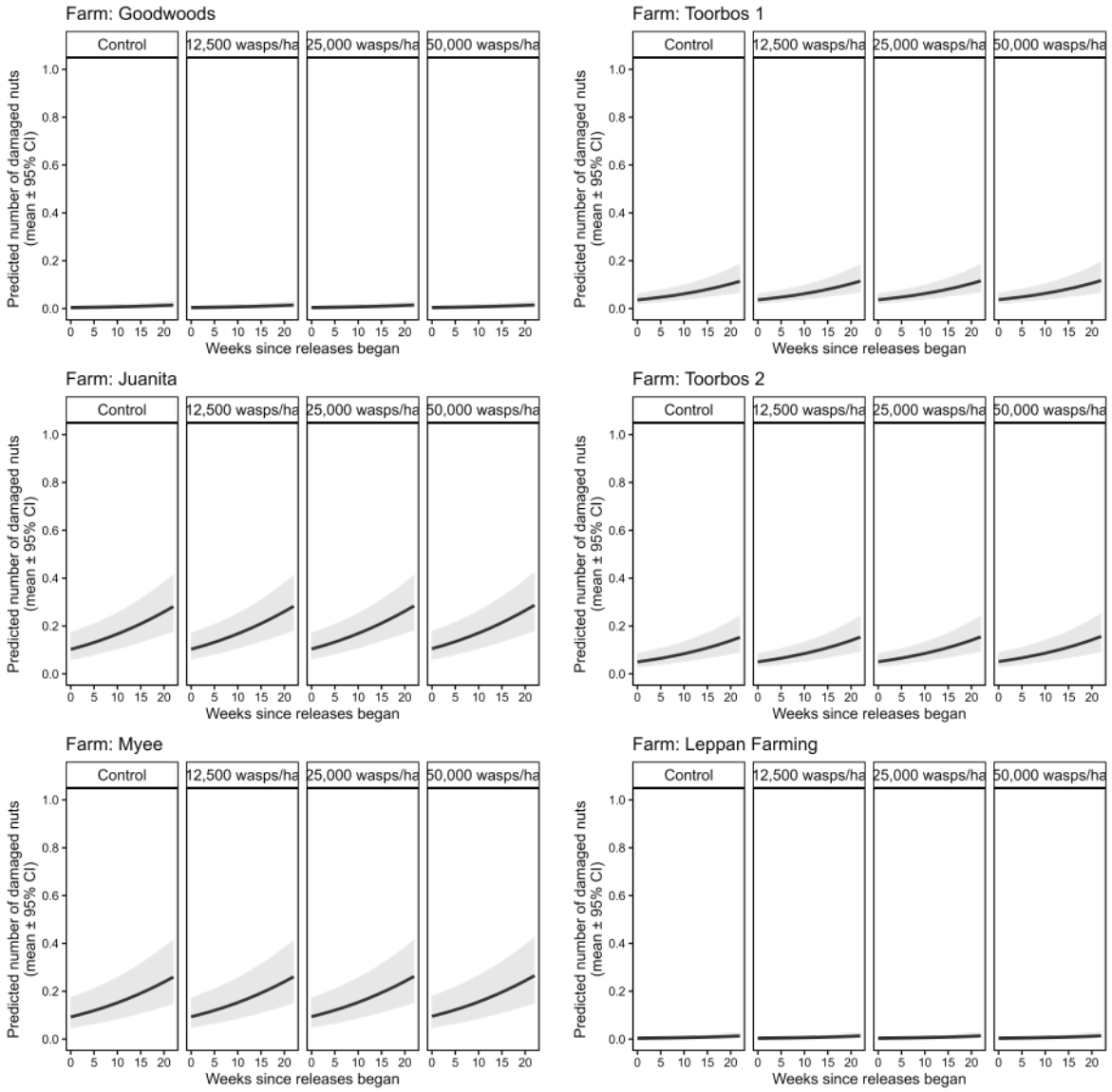

Figure A. 6. Marginal effect of *Trichogrammatoidea cryptophlebiae* release numbers on tortricid nut damage in macadamia orchards over two growing seasons per farm, Goodwoods, Toorbos 1, Toorbos 2, Leppan, Juanita, and Myee. Note differences in y-axis per farm done to show visual trends clearly. The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

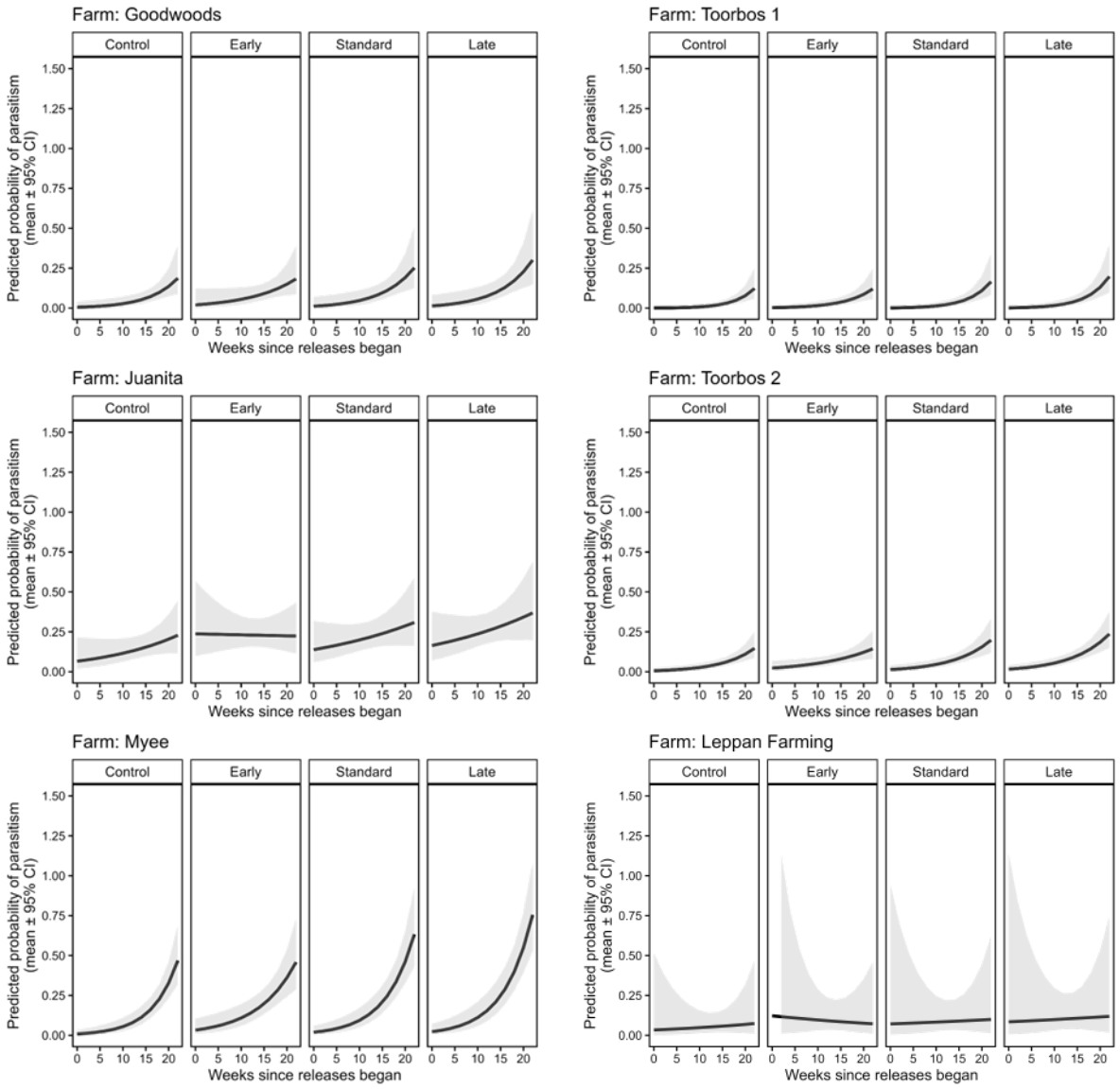

Figure A. 7. Marginal effect of *Trichogrammatoidea cryptophlebiae* release timing (Control, Early, Standard, and Late) on in-field parasitism levels of tortricid eggs in macadamia orchards for combined season 1 and 2 of the growing season per farm (Goodwoods, Juanita, Myee, Toorbos 1, Toorbos 2, and Leppan). The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.

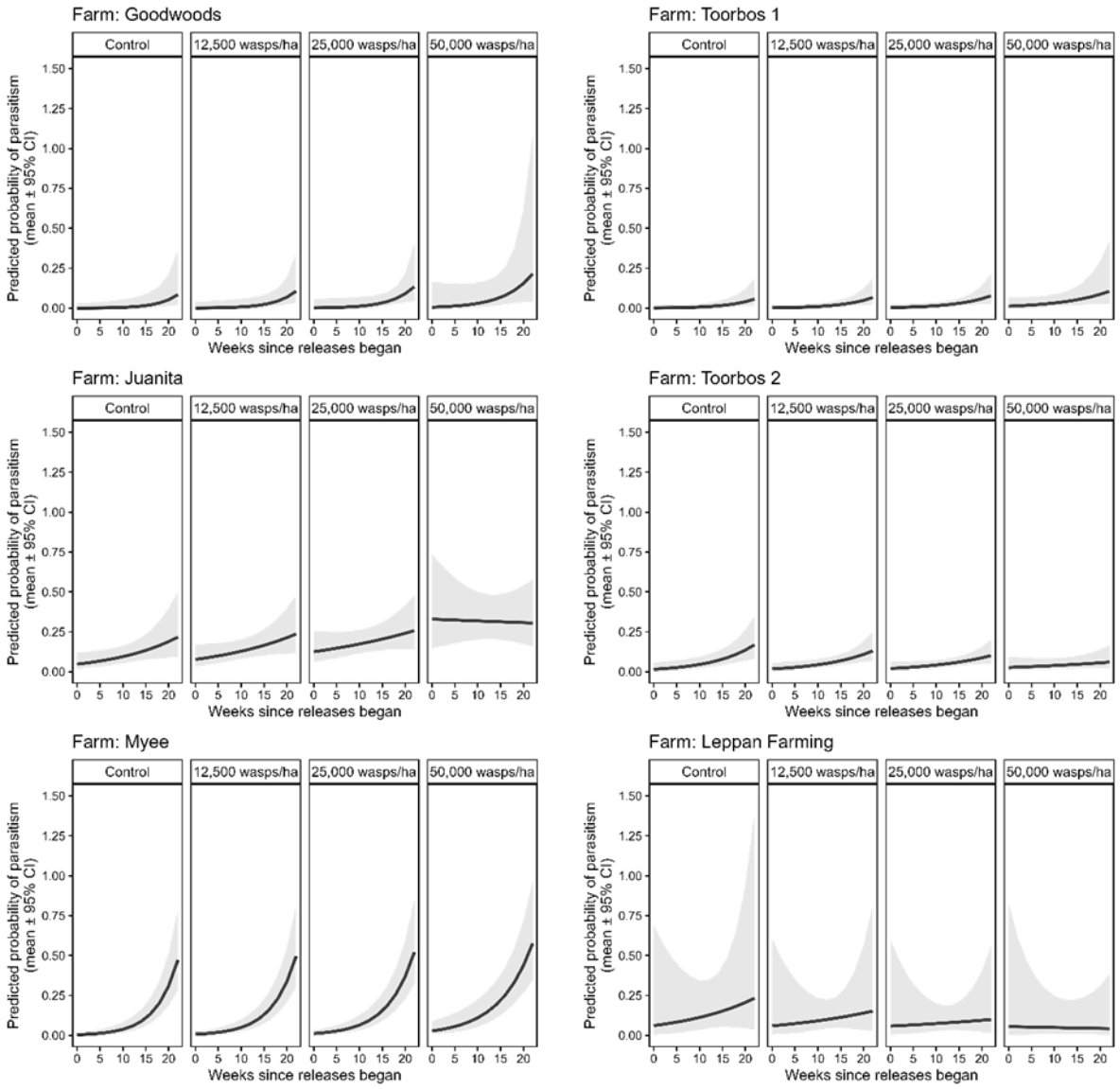

Figure A. 8. Marginal effect of *Trichogrammatoidea cryptophlebiae* release number (Control (0), 12,500, 25,000, and 50,000 wasps/ha) on in-field parasitism levels of tortricid eggs in macadamia orchards for combined season 1 and 2 of the growing season per farm (Goodwoods, Juanita, Myee, Toorbos 1, Toorbos 2, and Leppan). The solid lines indicate the marginal mean value, and the shaded areas indicate the 95 % confidence interval of the mean expectation value.