INSECTICIDE SUBLETHAL DOSE EFFECTS ON REPRODUCTION OF THE WHITEFLY, *Bemisia tabaci* (HEMIPTERA: ALEYRODIDAE)

by

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(Under the Direction of DAVID G. RILEY)

ABSTRACT

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is a key pest of vegetable crops in Georgia. Currently, we suspect that insecticide resistance to the insecticides, cyantraniliprole and pyriproxyfen is reducing control levels by these products in the Southeastern USA. As insecticide efficacy/lethality declines, the whitefly population experiences more sublethal effects on reproduction. A common method to quantify these sublethal effects is life table analysis. We tested sublethal concentrations of cyantraniliprole and pyriproxyfen on the host settling behavior, oviposition, survival of different instars of nymphs, and adult emergence on a population of Georgia whiteflies, one relatively resistant to insecticides. We found reduced reproduction with the LC_{10} of both insecticides, but a significant stimulation of oviposition with the low rate of pyriproxyfen.

INDEX WORDS: sweetpotato whitefly, *Bemisia tabaci*, insecticide resistance, bioassay methods, insecticide resistance management, life table, whitefly reproduction

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B.S., Universidade Estadual Paulista “ Júlio de Mesquita Filho”, Brazil, 2021

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2023

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December 2023

DEDICATION

I dedicate this to my parents. Your encouragement and sacrifices have been the foundation of my academic journey.

ACKNOWLEDGEMENTS

I want to express my gratitude to my major professor, David Riley, and committee members for their support and guidance. Additionally, I extend my appreciation to the lab members, especially Paulo Cremonez, whose voluntary co-advisement, collaborative spirit, knowledge, and friendship significantly enriched my research experience.

I want to extend my genuine appreciation to my friends in both the United States and Brazil. Your friendship, encouragement, and occasional breaks we shared were of immense importance to me during this endeavor.

To my family for their belief in my abilities and their encouragement. Your love and support have been my rock throughout this journey, and I am deeply thankful for everything you have done for me.

In conclusion, this thesis would not have been possible without the contributions, guidance, and support of all those mentioned above, as well as countless others who have shaped my academic path. I am deeply grateful for each and every one of you.

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CHAPTER 1. INTRODUCTION

The state of Georgia, USA, has a wide range of crop commodities that can potentially be impacted by whiteflies. Row crops and vegetables combined farm gate value represents more than US$ 3.5 billion, equivalent to 29.5% of the total value of state commodities. Among the row crops grown in Georgia, cotton represents 31.1% of the total (USDA/NASS 2023). Georgia vegetables consists of over 15 major commodities representing 85% of its total value and dozens of smaller commodities that all combined for an annual farmgate value of about $ 1.34 billion in 2021, contributing significantly to the Georgia economy, and ranking it in the middle of all the State's agricultural income (Fig. 1.1).

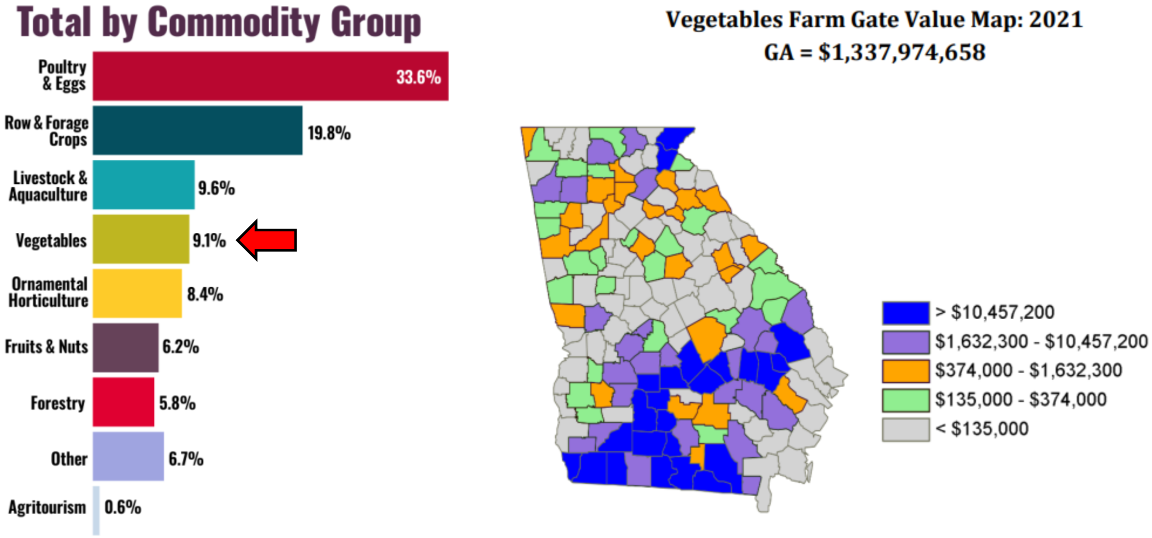

Figure 1.1. Georgia agricultural commodities (left) from UGA-CAES Ag Snapshots 2023 and (right) from the Georgia Farm Gate Value Report 2021 (Kane 2021).

Since vegetables are close in overall value to a row crop like cotton, but grown on one-tenth of the acreage, they are considered to be very high-value crops in which farmers are much less likely to tolerate damage caused by plant pests, like whiteflies. Partly because of this high-intensity pest management, vegetable crops are typically where we see some of the more frequent incidences of pesticide resistance and, when it does happen, very expensive crop failures can occur. The important thing to realize about vegetable pest management in Georgia is that it is dynamic, intense, and relies on insecticides, which often leads to pesticide resistance in important insect pests.

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a major pest of agricultural, horticultural, and ornamental crops (Perier et al. 2022). This pest can cause direct injuries by feeding on phloem, and indirect through the transmission of plant pathogenic viruses and excreting honeydew on leaves and fruits (Jones 2003, Horowitz et al. 2020, Abubakar et al. 2022, Cremonez et al. 2023). *B. tabaci* is highly polyphagous and over 1000 host plant species have been reported (Simmons et al. 2008, Abd-Rabou and Simmons 2010, Li et al. 2021). There are more than 1,500 species of whiteflies globally and among them, only a few species are considered serious pests (Nauen et al. 2014, Li et al. 2021). The sweetpotato whitefly, *B. tabaci*, and the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) are the most known species that cause damage to crops (Nauen et al. 2014, Li et al. 2021).

Infestations of *B. tabaci* can damage the plant in four ways. First, as a consequence of directly feeding on plant sap from the phloem, leaves turn yellow, and in large populations, physiological plant stress can cause leaf abscission, i.e., leaves may fall off the plant (Jimenez et al. 1991). Second, whitefly's excretion of honeydew forms a substrate on the leaves for the growth of sooty mold which attracts pestiferous ants, negatively affects plant respiration, and can greatly reduce photosynthesis (Byrne & Bellows 1991, Horowitz & Ishaaya 2014). Physiological disorders such as silvering leaves in squash (Jiménez et al. 1991) and irregular ripening in tomatoes (Schuster et al. 1990) are a third type of injury, and are caused during feeding by the injection of salivary fluid. Finally, one of the most devastating injuries caused by whiteflies is the transmission of the viral disease (Jones 2003). While feeding, whiteflies acquire plant viruses, and after moving and feeding on another plant they may transmit the viruses (Hogenhout et al. 2008). *Bemisia tabaci* has been described as a vector for more than 150 different viruses, which belong mostly to the *Begomovirus* genus (Byrne and Bellows 1991, Jones 2003, Krause-Sakate et al. 2020).

Among all the whitefly management tactics, the main one is the application of chemical insecticides (Palumbo et al. 2001, Riley and Srinivasan 2019, Li et al. 2021). Some of the most important insecticide groups for whitefly management in the USA are neonicotinoids, growth regulators such as pyriproxyfen, and diamides (Li et al. 2021, Palumbo et al., 2001). Horowitz et al. (2020) reviewed all insecticides currently used for the control of whiteflies worldwide and summarized the status of insecticide resistance for each group. When insecticides begin to lose efficacy due to resistance, these same toxins may still have sublethal effects that can directly impact whitefly management in the field.

Sublethal effects of insecticides on whiteflies are quite varied, but include the length of whitefly survival, developmental duration, and fecundity (Qu et al. 2017) as well as altered behaviors relative to host preference, feeding, and virus transmission (Liu et al. 2021). Furthermore, Ellsworth et al. (1997) found that pyriproxyfen has an impact on the viability of whiteflies eggs and Kerns & Stewart (2000) demonstrated the effects of sublethal doses of pyriproxyfen on cotton aphid reproduction and survival. A complete discussion on the toxicological response of insects, both lethal and sublethal, to toxins was summarized by Yu (2015).

The study of insecticide resistance is clearly of great commercial importance in formulating effective pest management strategies, but understanding what happens to the pest population as they start to survive treatment can also be critical. Acknowledging that there is an on-going selection caused by insecticide usage on the biological response of whiteflies is crucial for predicting the consequences of our principal commercial pest control activity, the use of pesticides. If we are able to predict insecticide resistance and sublethal effects on whitefly reproduction, we will be in a better position to promote sustainable whitefly management practices into the future.

Resistance denotes the capacity of pests to withstand or survive exposure to formerly effective insecticides (Yu 2015). The emergence of resistance reduces the efficacy of insecticides, leading to diminished pest control and heightened crop damage. Investigating resistance aids in identifying underlying mechanisms such as target site mutations or enhanced metabolic detoxification, thereby providing valuable insights for developing strategies to mitigate resistance development, with the focus on mortality of the pest.

At the same time, sublethal effects encompass the repercussions of insecticides on pests that do not cause immediate mortality, but instead impact various physiological, behavioral, and reproductive aspects (Wang et al. 2017). These effects can have substantial implications for pest populations and ecosystem dynamics. Sublethal doses may disrupt pest reproductive capabilities, alter behavior, or influence population growth rates. Understanding these effects is critical for predicting long-term population dynamics, evaluating the potential for resistance development, and designing integrated pest management and insecticide resistant management approaches

The goal of this research was to provide a better understanding of the sublethal effects of some common insecticides on whitefly reproduction and reproductive behavior. Frequently, the concern that most pest management researchers have about the potential risks associated with insecticide use is resistance to mortality. However, our aim was to understand what happens with the population dynamics as that pest population is selected for resistance to a given insecticidal toxin and continues to be exposed to the same toxin. It is expected that this knowledge could help in the development of strategies to minimize resistance development, optimize pest control, and foster sustainable agricultural practices. In this research we focused on two classes of insecticides, Insecticide Resistance Action Committee (IRAC 2023) groups #7, the juvenile hormone mimics, and # 28, the diamides.

Therefore, the primary objective of this research was assessing the impact of sublethal concentrations of cyantraniliprole (IRAC Group 28) and pyriproxyfen (IRAC Group 7C) on the reproductive capabilities of *Bemisia tabaci*. The subobjectives were to:

- Examine the mortality rates of nymphs exposed to cyantraniliprole and pyriproxyfen in controlled laboratory cultures, determining a sublethal concentration (LC_{10}) effects of these insecticides, in comparison to an untreated control, on various life table parameters (Chapter 3) and
- Investigate any changes in host preference behavior, particularly with regards to settling or selecting a plant to land on, when the cotton host plant is treated with sublethal doses of the aforementioned insecticides (Chapter 4).

The null hypothesis of this study was that *B. tabaci* exposed to sublethal doses of pyriproxyfen or cyantraniliprole would not result in observable changes in fertility, fecundity, net reproduction and preference for host settling and oviposition.

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CHAPTER 2. LITERATURE REVIEW

Whitefly host plant system in Georgia

The diverse cropping systems cultivated in Georgia contribute to a continuous supply of cultivated whitefly host plants throughout the year, which directly influences the population dynamics of whiteflies. Whiteflies in Georgia are known to transition from winter cole crops to spring cucurbits, then to cotton, followed by fall cucurbits, and eventually returning to winter cole crops (Sparks et al. 2018). The presence of a wide range of crops provides whiteflies with a constant availability of hosts for feeding and reproduction.

The rate of insect development is influenced by various factors, with host quality being a significant factor (Powell and Bellows 1992). According to the findings of Nava-Camberos et al. (2001), cantaloupe and cotton have been identified as highly suitable crops for the reproduction and population growth of whitefly. In the same study, pepper was observed to be an unsuitable host for this insect, and the development time remains relatively consistent across host plants, primarily influenced by temperature (Nava-Camberos et al. 2001). The important factor to remember is that host plants can vary greatly in their suitability for whitefly reproduction (Riley et al. 1995).

Populations of *B. tabaci* likely exist in low numbers during winter, possibly over-wintering on cole crops such as cabbage, kale, and collard greens. These particular crops cover approximately 4,300 acres throughout Georgia, accounting for roughly 7% of vegetable production in the state (Kane 2021). Typically, populations of *B. tabaci* are relatively low at the end of winter (Riley and Ciomperlik 1997). However, during mild winters, populations can still persist and reach pest status on certain crops (Sparks et al. 2018). As the cole crops are harvested and temperatures begin to rise in spring, these populations transition to cucurbit crops like melons (Riley and Ciomperlik 1997). With the arrival of warmer weather, cucurbits like cucumbers, melons, and squash become prime host plants for whiteflies. As a result, whitefly populations tend to proliferate on these crops. In Georgia, cucurbits hold a significant share of vegetable production, encompassing over 26% of the total vegetable crop acreage in the state (Kane 2021).

Towards the end of the spring's cucurbit season, whiteflies migrate in large numbers to cotton (Riley and Ciomperlik 1997, Sparks et al. 2018). Cotton fields offer an ideal habitat for whiteflies due to their extensive acreage, allowing for the support of large populations. The cultivation of cotton encompasses a significant area, with approximately 1.21 million acres dedicated to cotton production in Georgia (Kane 2021). Consequently, cotton becomes the most available host plant for whiteflies following the spring cucurbit season. Finally, as fall approaches, cucurbits regain their significance as hosts, completing the seasonal cycle (Sparks et al. 2018). By the end of July, whitefly populations move from defoliated cotton to fall vegetables such as cucumber, melons, peppers, and cabbage (Riley and Ciomperlik 1997). Understanding the dynamic nature of sweetpotato whitefly movement across different crops is crucial for implementing effective pest management strategies.

Due to the large host range, *B. tabaci* is considered a highly polyphagous pest, and has been reported to feed on over 1000 plant species from 74 families, including several important crops such as cotton, tomato, and soybean, as well as ornamental plants (Abd-Rabou and Simmons 2010), and non-cultivated weeds (Simmons et al., 2008; Kavalappara et al., 2022). Its host range enables its distribution around the world, and it is a cosmopolitan insect found in all continents except Antarctica (De Barro et al. 2011). In addition, the movement of fresh plant materials across countries and within the country contributes to the dissemination of whiteflies (Simmons et al. 2008).

Whiteflies can impact crops in different ways, direct or indirectly. Direct feeding on plant sap, weakens the plant, potentially causing defoliation, and even leading to plant death. The whitefly is unable to absorb a portion of the sap, resulting in its excretion in the form of high-carbohydrate honeydew, as noted by Perring et al. (2018). This secretion provides a substrate for saprophytic fungi growth, which can have negative effects on photosynthesis and fruit quality. The stickiness and discoloration significantly reduce the worth of agricultural produce, including ornamental plants, vegetables, and cotton. (Horowitz et al. 2011, Perring et al. 2018). Additionally, by direct feeding, the insects inject phytotoxins that induce physiological disorders such as silverleaf in cucurbits (Costa et al. 1993, LaTora et al. 2022) (Fig. 2.1), yellowing of leaves (Fig. 2.2) and irregular ripening in tomatoes (Schuster et al. 1990, Perring et al. 2018).

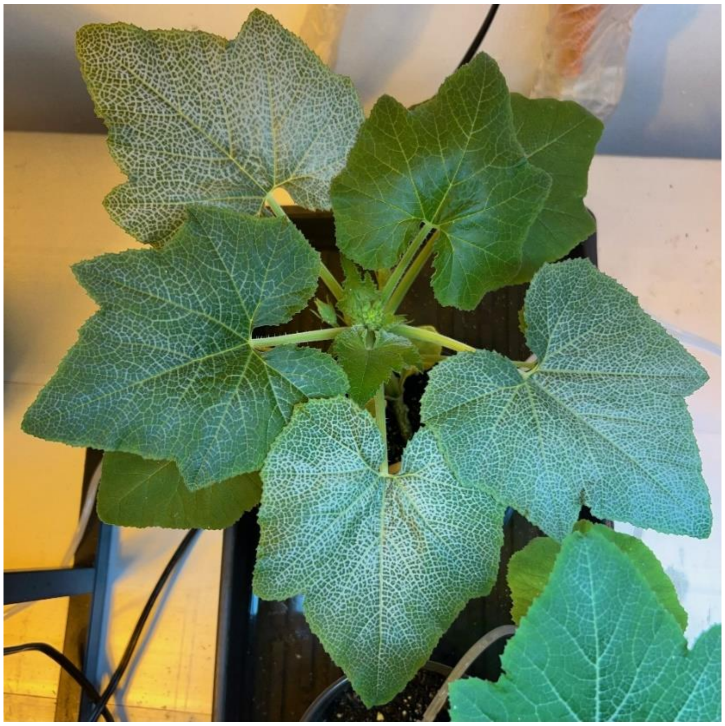

Figure 2.1 Silverleaf disorder in squash plant caused by *Bemisia tabaci*.

As an indirect injury, is the transmission of plant viruses. *B. tabaci* transmits more than 100 virus species (Jones 2003, Horowitz et al. 2020). Plant viruses are acquired by during their feeding process, and adult insects disperse and transmit the virus (Jones 2003). Whiteflies and their associated viruses pose a significant challenge in the management of various crops worldwide due to their behaviors and diverse host preferences (Krause-Sakate et al. 2020, Kavalappara et al. 2022).

Figure 2.2. Tomato yellow leaf curl virus transmitted by *Bemisia tabaci* (photo by D. Riley).

Biology and life history of *B. tabaci*

Bemisia tabaci was first documented more than a century ago in Greece, and initially classified as *Aleyrodes tabaci*, commonly known as the tobacco whitefly (Gennadius, 1889). Currently, it is accepted that the species complex is composed of 24 not morphologically distinguished or cryptic species (Brown et al. 2023, Cock 1986, De Barro et al. 2011). While there are more than 1,500 whitefly species (Hemiptera: Aleyrodidae), *B. tabaci* and the greenhouse whitefly *Trialeurodes vaporariorum* are the most known species, with *B. tabaci* being the most destructive (De Barro et al. 2011, Nauen et al. 2014).

The classification of the *B. tabaci* species complex continues to be debated within the scientific community, but, generally, *B. tabaci* is characterized as a group of cryptic species - populations that exhibit no discernible morphological differences but possess unique intrinsic traits that distinguish them from others. This term and the identifying characteristics of the various *B. tabaci* cryptic species (or biotypes) are currently under review (Perring 2001, de Moya et al. 2019, Brown et al. 2023). Nonetheless, the evolutionary path for the *B. tabaci* cryptic species complex involves genotypic alteration other than phenotypic features, which makes it only possible to determine the cryptic species by specific mitochondrial DNA markers (Frohlich et al. 1999, Boykin et al. 2007). Currently, the most prevalent cryptic species found are the global-spread Middle East-Asia Minor 1 (MEAM1, formerly known as B biotype), and the Mediterranean (MED, formerly known as Q biotype) (De Barro et al. 2011). MEAM1 is the most commonly identified cryptic species in the state of Georgia (Gautam et al. 2020, LaTora et al. 2022).

The life cycle of *B. tabaci* consists of egg, four nymphal instars (initiating with a first instar as a mobile crawler (Simmons 2002), followed by two sessile nymph instars, and a terminal sessile fourth instar nymph, functionally a pupa), and the adult. Usually, the eggs are laid on the underside of the leaves and are attached to its surface by a pedicel which is an extension of the egg chorion (Walker et al. 2010, Li et al. 2021). The first nymphal stage or crawler is able to move, although, its dispersal ability is limited to a few millimeters (Simmons 2002, Walker et al. 2010). After settling in one spot, the nymph will feed on the phloem and complete the reminiscent three juvenile instars. The last or fourth instar is the pupal stage, and the main characteristic is the enlarged red eyes spots and developing wing pads (Walker et al. 2010, Li et al. 2021). Adult females have a rounded abdomen and males have a more pointed abdomen (Simmons and Mahroof 2011, Li et al. 2021).

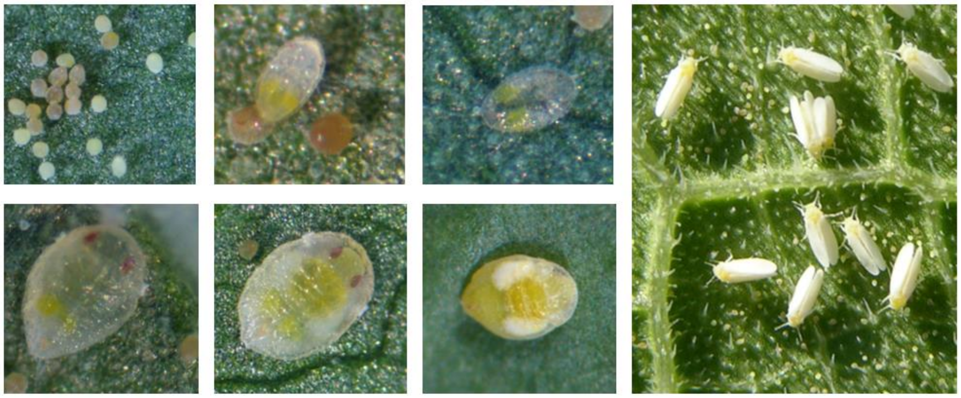

Figure 2.3. Life cycle stages of *Bemisia tabaci* (nymph photos M. Nagaoka and adult D. Riley)

The whitefly can reproduce by arrhenotokous parthenogenesis, in which unmated females produce unfertilized eggs that develop into males, and mated females produced fertilized eggs that develop into females, but haploid males can still result from unfertilized eggs in mated females (Byrne and Bellows 1991). Females can produce 150 to 200 eggs and the life cycle can be completed in less than three weeks in southern Georgia's summer temperatures (Sparks et al. 2018). The typical way that biological parameters of the insect life cycle are quantified is with life table analysis (Southwood 1978).

A study done on development and life table of whiteflies was summarized by Butler et al. (1983), where the authors reported temperate dependent development of eggs and eggs to adult stages. Von Arx et al. (1983) studied the development of *B. tabaci* on cotton at different constant temperatures. The fastest developmental rates for eggs and nymphs were observed at 33°C and 27 °C, respectively. Coudriet et al. (1985) reported a mean developmental time from egg to adult of 21.7 days on cotton at 26.7 °C. Powell and Bellows (1992) studied the developmental rates of *B.*

tabaci on cotton (Deltapine® 61) at four constant temperatures. Egg developmental periods varied from 8.75 days at 20°C to 3.92 days at 32°C. Mean overall developmental times varied from 28.6 days at 20°C to 17.7 days at 25.5°C. Riley et al. 1996 reported age specific life table data for *B. tabaci* (reported as *B. argentifolii*) for pepper, cotton, and melon host plants, clearly showing large effects of host plant on survivorship. Cantaloupe serves as a good host crop for whiteflies, while cotton demonstrates intermediate suitability, and pepper is a poor reproductive host, and development time is primarily affected by temperature (Nava-Camberos et al. 2001). At a temperature of 27°C, the duration of each nymphal stage of development is approximately five days (Chandi et al. 2021). However, the optimal temperature for *B. tabaci* reproduction may be between 28 and 33°C (Curnutte et al. 2014).

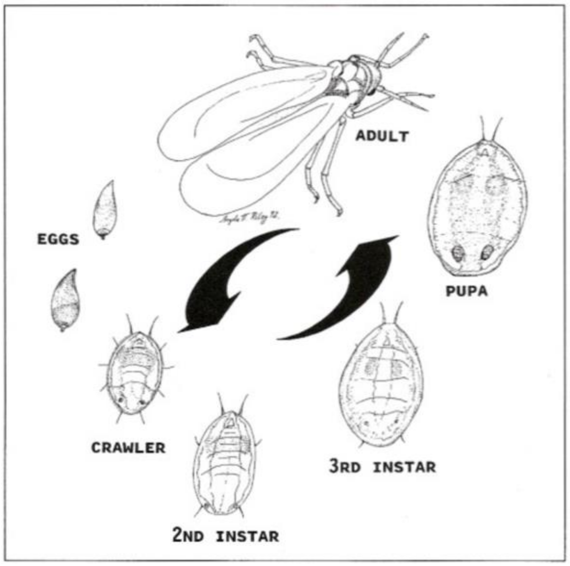

Figure 2.4. Illustration of a *Bemisia tabaci* life cycle from Riley and Sparks Jr. (1993)

Life table studies are undeniably valuable when it comes to examining the influence of different factors on development, population growth, and survival (Harcourt 1969, Southwood and Henderson 2021). They provide a comprehensive description of the life cycle of a population by quantifying the vital rates and demographic parameters of individuals within a given species

(Harcourt 1969, Southwood and Henderson 2021). These studies typically focus on key parameters such as survival rates, age-specific fecundity, and age-specific mortality (Harcourt 1969, Southwood and Henderson 2021).

Insecticide control of *B. tabaci*

The management of *B. tabaci* populations presents substantial challenges. Factors such as their rapid population growth, development of resistance to insecticides, and their habitat preferences contribute to the difficulties encountered in effectively controlling these pests (Horowitz et al. 2011). The primary approach to manage whiteflies remains the use of insecticides (Horowitz et al. 2020). Horowitz et al. (2020) suggested that using an Integrated Pest Management (IPM) approach is crucial to avoid pesticide’s detrimental effects on the agroecosystem, the selection pressure for resistance, and failures in pest control. In specific agricultural systems, over-reliance on insecticides as a primary control method often leads to the frequent emergence of resistance. Several crucial technical principles support successful resistance management in chemical control of whiteflies, as pointed by Horowitz et al. (2020). Firstly, the use of insecticides should be strictly adhered to label recommendations, ensuring that the right recommended concentrations are applied, and preventing both ineffective control and undue harm to non-target organisms. Secondly, the selection of insecticides should be informed by local efficacy and selectivity, as emphasized in IPM strategies. This ensures that the chosen insecticides are both effective and minimize harm to the agroecosystem in the local context. Thirdly, it is vital to rotate insecticides with different Modes of Action (MoAs) by employing a 'MoA treatment windows' approach (Horowitz et al. 2020, Sparks et al. 2021). This rotation delays the selection of resistant population of pests from developing resistance to a single MoA and encourages the survival of susceptible populations. Lastly, non-chemical control methods should also be incorporated into pest management strategies, in line with the principles of IPM. These methods, including biological control and crop rotation, can supplement chemical control and reduce reliance on insecticides. These principles are all integral to the successful execution of Insect Resistance Management (IRM) strategies.

Beyond the fore mentioned IPM considerations, understanding the underlying resistance mechanisms of whiteflies is crucial for developing specific IRM practices. A clear comprehension of these mechanisms can help avoid issues of cross-resistance, which could otherwise jeopardize carefully developed IRM strategies. This is particularly important for *B. tabaci* management, as there are reports of cross-resistance to main insecticide compounds such as imidacloprid, bifenthrin, fenvalerate, cyantraniliprole, and pyriproxyfen (Basit 2019, Wang et al. 2020). Furthermore, understanding that pests might develop resistance to insecticides with similar MoAs can guide the selection and rotation of insecticides. Effective IRM also necessitates educational and communication efforts, as advocated by the Insecticide Resistance Action Committee (IRAC) (Sparks et al. 2021). These efforts can ensure that everyone involved in pest management, from farmers to policymakers, understands the importance of resistance management and the strategies to implement it.

At present, resistant whitefly populations have been documented in numerous countries, exhibiting resistance to a wide range of 40 active ingredients, including more recent insecticides (Basit 2019). The extensive utilization of insecticides in agriculture fosters the development of insecticide-resistant populations, driven by an enduring selection pressure. Currently, whiteflies have exhibited resistance to older active ingredients, but also include relatively new insecticides (Basit 2019, Horowitz et al. 2020). Resistance mechanisms in *B. tabaci* include increased levels of detoxification enzymes, which drive metabolic resistance, as well as point mutations that lead to target-site resistance, similar to the mechanisms found in other species (Horowitz et al. 2020).

Widespread resistance to pyrethroids has been observed in whiteflies, although the scope and features of this resistance, including cross-resistance, exhibit notable variations among countries and cropping systems, as summarized by Horowitz et al., 2020. Similarly, whitefly resistance to organophosphates and carbamates have become firmly established in multiple countries (Ahmad and Khan 2017, Naveen et al. 2017, Horowitz et al. 2020). Similarly, cases have been reported for insect growth regulators, including pyriproxyfen and buprofezin (Horowitz et al. 2005, Ma et al. 2010, Horowitz and Ishaaya 2014, Roy et al. 2019).

Despite the advent of newer techniques and more precise instruments, careful observation for unique symptomology often provides the crucial first signal for an interesting new insecticidal chemistry (Sparks and Lorsbach 2023). Noticing and analyzing peculiarities in the physiological responses or behavioral changes in pests exposed to a novel compound can lead to the discovery of a new class of insecticides. Throughout this last three-decade period, bioactive natural products have consistently served as the primary sources of new classes of insecticides. These resources, with their vast complexity and diversity of chemical structures, provide a rich wellspring for the discovery of molecules with novel modes of action (Sparks et al. 2023). By studying how these molecules interact with biological systems, scientists can formulate hypotheses about their bioactivity and use these as the starting point for designing and synthesizing new insecticides.

Following the 1980s, first-in-class insecticides became more common, reflecting a growing interest in molecules with improved selectivity (Sparks & Lorsbach, 2023). Rather than broad-spectrum insecticides, which can harm non-target organisms and lead to widespread resistance, there has been a shift towards insecticides that target specific pests or even specific physiological processes within these pests. This trend lines up with the principles of *B. tabaci* IPM (Ellsworth and Martinez-Carrillo 2001), which advocates for the least disruptive means of whitefly control, while achieving effective chemical use and aligning with complementing strategies of sampling and pest avoidance (Fig. 2.5). As such, while the discovery of new molecules and new classes of insecticides remains crucial, the focus has been on achieving more selective and sustainable control of pests like *B. tabaci*.

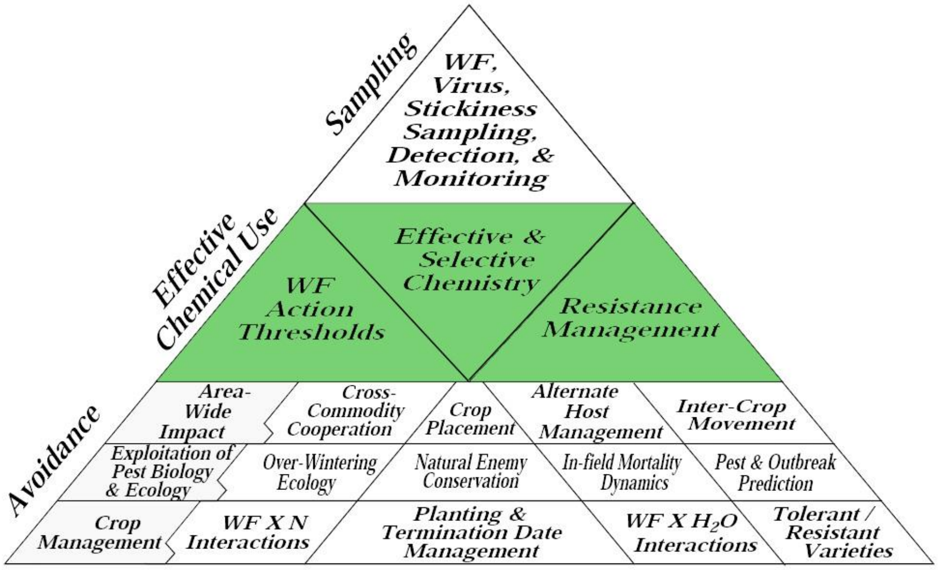

Figure 2.5. Conceptual diagram of *Bemisia tabaci* IPM, highlighting effective chemical strategy (Ellsworth and Martinez-Carrillo 2001).

Despite the successful integrated programs like Ellsworth and Martinez-Carrillo (2001), the push to employ insecticides with different MoAs from the conventional neurotoxins remains a key strategy in the IPM of *B. tabaci*. By leveraging insecticides that act differently than conventionally used neurotoxic compounds, such as those affecting growth regulation and muscle contraction processes, the potential for cross-resistance might be substantially reduced. Furthermore, these alternatives often have more specific targets, reducing their impact on non-target organisms and contributing to a more sustainable and environmentally-friendly pest control strategy.

Pyriproxyfen is a juvenile hormone mimic (IRAC group 7C) (IRAC 2023). Juvenile hormones, along with ecdysteroids, play vital roles in reproduction, molting and metamorphosis (Dhadialla et al. 1998, Hiruma and Kaneko 2013). The major source of juvenile hormones (JHs) in insects is the corpora allata, which is a specialized endocrine gland (Hiruma and Kaneko 2013). JH III is the most common homolog, it is present in most insect groups (Goodman and Granger 2005), and it is found in nymphs and eggs of *B. tabaci* (Gelman et al. 2007). Thus, the insect growth regulator pyriproxyfen has effects on egg viability through direct application or transovarial transmission, which leads to the disruption of embryogenesis (Ishaaya and Horowitz 1992, Ishaaya and Horowitz 1995, Devine et al. 1999, Horowitz et al. 2003, Sullivan and Goh 2008). Additionally, pyriproxyfen impacts the nymph stage by suppressing the emergence of adults, leading to mortality during the pupae (Ishaaya and Horowitz 1992, Ishaaya and Horowitz 1995). As an indirect impact on the larval stage, pyriproxyfen lead to a significant decrease in honeydew secretion, and resulting in suppression of adult formation (Ishaaya and Horowitz 1992).

Diamide insecticides, introduced to the market approximately two decades ago, are among the most recent classes of chemicals used to combat whiteflies (Nauen 2006, Jeanguenat 2013). However, even within this relatively new chemical class, resistance has also been reported (Yao et al. 2017, Wang et al. 2018, Wang et al. 2019). According to Wang et al. (2019), elevated levels of cyantraniliprole resistance could arise due to the upregulation of metabolic enzymes. Diamides target ryanodine receptors, which are a class of non-voltage-gated calcium channels, found in neuromuscular tissues (Lahm et al. 2009, IRAC 2023, Jeanguenat 2013, Horowitz et al. 2020). By activating these receptors, the intracellular calcium stores are released, resulting in contraction, paralysis, and rapid feeding cessation (Sattelle et al. 2008, Lahm et al. 2009, IRAC 2023, Cameron et al. 2014). The downregulation of IP3R (KY290442) has been previously associated with resistance to diamides in whiteflies (Guo et al. 2017).

Sublethal effects of insecticide on whitefly reproduction

When it comes to insecticides and their effects on insects, life table studies can be particularly informative. Insecticides are designed to kill or control insect populations, and their use can have both lethal and sub-lethal effects on target insects. Lethal effects are relatively straightforward to measure, as they result in mortality. However, insufficient exposure to lethal doses of insecticides can result in sublethal effects, which refers to the non-lethal consequences of exposure to insecticides, and may affect various aspects of physiology, behavior, and reproductive capacity (Moriarty 1969, Desneux et al. 2007, He et al. 2011).

Sublethal doses of insecticides can indeed have significant impacts on populations in three main ways. Firstly, they can indirectly affect the survival of individuals, potentially leading to a decline in their overall population size; sublethal doses can impair the reproductive ability of individuals, resulting in reduced fertility or decreased reproductive success (Moriarty 1969). This can lead to fewer offspring being produced, further impacting population numbers. Lastly, sublethal doses of insecticides can influence the genetic composition of future generations, potentially altering the genetic diversity and population adaptation over time (Moriarty 1969).

The complexity of pest insect responses to insecticides encompasses not only the primary and direct lethal effect (mortality), but also a wide range of sublethal effects (Guedes et al. 2016). These sublethal impacts can trigger intricate behavioral changes, such as both stimulus-dependent and independent responses, causing varied alterations from irritability to repellence depending on pesticide exposure levels (Desneux et al. 2007, He et al. 2011, Guedes et al. 2016). Moreover, these behavioral changes can significantly influence population dynamics and density-dependent relationships (Desneux et al. 2007, Guedes et al. 2016). The concept of hormesis, a population-level response where low doses of a toxin stimulate, and high doses inhibit, is a crucial aspect of sublethal effects. Furthermore, subtle modifications in behavior following sublethal exposure can lead to significant population-level implications, possibly resulting in pest outbreaks or influencing biological invasion dynamics (Guedes et al. 2016). Hence, acknowledging the role of sublethal effects expands our understanding of pesticide impacts and can provide novel insights into more effective and nuanced pest management strategies (Guedes et al. 2016).

Exposure to sublethal concentrations (LC_{10}) of cyantraniliprole was reported to have a significant effect on the life cycle of *B. tabaci*. It caused a lengthening of the developmental duration and a decrease in the survival rate of nymph stages, pseudo pupae, and adults. Additionally, females exposed to cyantraniliprole exhibited a significant reduction in oviposition duration and fecundity (Wang et al. 2017). Furthermore, it was observed that the exposure to sublethal levels of flonicamid had notable effects on the adult population of whiteflies. This pesticide elongated the duration of their development while diminishing the overall count of adult whiteflies. In a complicated twist, it did enhance their fecundity, but negatively affected their fertility (Abbas et al. 2022). The sublethal effects of novel insecticides, such as chlorogenic acid, β-asarone, and afidopyropen, have also been investigated for *B. tabaci* (Zhou et al. 2021, Wang et al. 2022, Wang et al. 2023). Additionally, exposure to sublethal doses of imidacloprid was found to potentially decrease the egg fertilization rate, interfere with the mating behavior, reduce development, and alter the sex ratio within the subsequent generation of *B. tabaci* (He et al. 2011).

Sublethal doses of insecticides can disrupt normal behavioral patterns and functions, affecting aspects such as feeding, mating, navigation, and communication (Haynes 1988). Sublethal effects of imidacloprid described by He et al. (2011), highlights its potential impact on the reproductive aspects and behavior of whitefly reproduction. When exposed to LC_{40}, there is a significant decrease in the number of females in the F1 generation, indicating that imidacloprid has negative impacts on fertilization rates in exposed females or potentially interfere with mating behavior (He et al. 2011). Furthermore, sublethal concentrations of imidacloprid and bifenthrin have been found to impair the phloem feeding behavior of *B. tabaci* (He et al. 2013).

In summary, life table studies offer a comprehensive description of population dynamics by quantifying vital growth rates and demographic parameters. They can be utilized to investigate the sub-lethal effects of insecticides on insects, including impacts on survival, reproduction, and overall population growth. Additional information on the sublethal effects of insecticides on reproductive behavior can improve our understanding of the overall sublethal insecticide effects on whitefly populations.

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CHAPTER 3

DETERMINATION OF SUBLETHAL CONCENTRATION EFFECTS OF PYRIPROXYFEN AND CYANTRANILIPROLE ON *Bemisia tabaci* REPRODUCTION[1]

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[1] Nagaoka, M.M., Cremonez, P.S.G., Simmons, A.M., and Riley, D.G. To be submitted to JEE for publication, December, 2023

ABSTRACT

The sweetpotato whitefly *Bemisia tabaci* is an important pest of vegetable crops and cotton in Georgia. Aside from the expected mortality from insecticides, their sublethal action on development could impair reproduction of infesting populations. This study aimed to investigate the effects of sublethal concentrations of pyriproxyfen and cyantraniliprole on *B. tabaci*, a major agricultural pest, by examining its life table parameters, including net reproduction (R_o) in treated and untreated situations. A 24-h cohort of eggs were established on cotton leaves using clip cages, allowed to develop for 12 days, and then the leaf was dipped in pre-selected concentrations of each insecticide, where mainly 2nd instar nymphs were present. Nymph mortality was counted 48 h after treatment. From these results, the sublethal concentrations (LC_{10}) were estimated. For the life table analysis, nymphs of 2nd instar were exposed to $LC_{10,}$ and resultant newly emerged couples were isolated in a small clip cage and moved every two days on the leaf surface. The number of eggs was counted daily until the female died. The sublethal concentration of cyantraniliprole and pyriproxyfen caused a reduction of 25% and 42% in the mean number of eggs per day, respectively. The R_o was 36% and 45% lower, respectively, when compared to the untreated check.

Keywords: whitefly, life table, insecticide resistance, sublethal effects

Introduction

The primary method of control for *B. tabaci* is through regular insecticide applications (Schuster et al. 2010, Caballero et al. 2013, Horowitz et al. 2020). However, it is essential to understand that insect responses to insecticides extend beyond mortality and encompass a diverse array of sublethal effects (Guedes et al. 2016). These sublethal impacts can lead to behavioral changes, such as stimulus-dependent and independent responses, causing varied alterations of insect reproductive behavior from irritability to repellence depending on the level of pesticide exposure (Desneux et al. 2007, Guedes et al. 2016, He et al. 2011). These behavioral changes can significantly influence population dynamics and density-dependent relationships (Desneux et al. 2007, Guedes et al. 2016). Even subtle changes in behavior following sublethal exposure can have significant population-level implications, potentially leading to pest outbreaks or influencing biological invasion dynamics (Guedes et al. 2016). Understanding sublethal effects expands our comprehension of pesticide impacts and can provide valuable insights into more effective and nuanced pest management strategies for whitefly (He et al. 2011).

Sublethal doses of cyantraniliprole are known to negatively affect pest development, longevity, fecundity, and predation activity (Amarasekare et al. 2015, Mills et al. 2015, Jiang et al. 2020). Moreover, sublethal doses of insecticides can disrupt normal behavioral patterns and functions, affecting aspects such as feeding, mating, navigation, and communication (Haynes 1988). For example, sublethal concentrations of imidacloprid and bifenthrin have been found to impair the feeding behavior of whiteflies (He et al. 2013).

Life table studies are valuable tools for understanding the sublethal effects of insecticides on insect development. Insecticides are designed to control or eliminate insect populations, but their impact can extend beyond outright mortality to include sublethal effects. Sublethal effects refer to non-lethal consequences resulting from exposure to insecticides, affecting various aspects of physiology, behavior, and reproductive capacity (Desneux et al. 2007, He et al. 2011, Moriarty 1969). As an insect population is selected for resistance to an insecticide, that insecticide's effect on that resistance population becomes sublethal.

In this study, life table studies were used to describe the effects of two insecticides on a moderately insecticide susceptible whitefly population from Georgia. The reason for looking at sublethal effects was because insecticide resistance means that standard insecticide treatments are not causing high mortality and the effects on demographic parameters are sublethal effects. We used a bioassay determined lethal concentration (LC) that would only cause 10% mortality (LC_{10} or 90% survival). These studies examined the sublethal effects on survival by life stage, reproduction, and overall population growth as measured by net production (R_o). By quantifying these sublethal effects, we aimed to better predict what potentially can happen to a whitefly population as resistance to the insecticide develops. The hypothesis tested was that LC_{10} concentrations of cyantraniliprole and pyriproxyfen would significantly reduce the reproductive potential of whiteflies, specifically its population increase as measured by R_o, compared with an untreated check.

Material and Methods

Dose response analysis. Laboratory whitefly colonies of *B. tabaci* used in this study were from a moderately cyantraniliprole-susceptible population collected from Georgia (Perier et al. 2023). The colony was maintained on cotton plants in individual rearing rooms (30 ± 2°C, 50% RH, 18:6 light: dark photo-phase) until assays for this study were initiated during the summer of 2022. The experimental unit in this study was the bioassay arena or the area of leaf enclosed by the clip cage (Figure 3.2).

Untreated cotton plants (cultivar ST4946GLB2) were utilized as the plant material in the bioassays. These cotton plants were grown in growth chambers (Percival model E-36L2, Perry, IA, USA) under specific conditions, including a temperature of 30°C, a relative humidity of 70%, and a 16:8 day-night cycle. Prior to being used in the study, the cotton plants were grown until they reached the two-expanded-true-leaf stage and were then transplanted into pots measuring 8.9 cm in diameter and 8.3 cm in height. All the plants used in this bioassay were approximately the same size with at least one of the terminal leaves having a width of 4 cm.

To determine the dose response, 25 pairs of adult *B. tabaci* were aspirated from the leaves of the colony plants using clear glass eyedroppers (De Marchi and Smith 2021). Subsequently, these adults were then introduced into the bioassay arenas, which were clip cages. The clip cages were crafted from vials with a diameter of 2.54 cm. The lower portion was removed to create an opening to facilitate air circulation and diminish humidity within the clip cages, and a small piece of breathable fabric was positioned at this opening. This assembly was affixed to a hair clip, providing practical and flexible forceps to open and close the clip cage as needed. A supplementary small piece of cardboard was added to ensure the confinement of the whiteflies within the arena. After 48 hours of infestation, the adult whiteflies were removed, and the infested plants were kept under controlled conditions to allow the nymphs to develop.

Approximately 12 days after the infestation, when the majority of nymphs had reached the second instar stage, the plants were treated with insecticides through a leaf dip method (Sparks et al. 2020). The concentrations tested spanned a range from 0% to 200% of the maximum labeled rate at 100 gallons per acre (MLR) for each respective insecticide, encompassing values of 0, 0.01, 0.1, 1, 10, 50, 100, and 200% MLR. The serial dilution method was employed for preparing these concentrations. The treatments were arranged in a randomized complete block design. After a 48-

hour period following the treatment, the mortality of the nymphs was recorded. The nymphs were considered dead or alive based on their visual aspect relative to their level of desiccation (Figure 3.1)

Life table analysis. In order to calibrate a method for life table analysis of whiteflies on cotton, we obtained the original raw data from Riley and Tan (2002) and calculated whitefly eggs, nymphs, pupae and emerged female adults per day for 342 whitefly mating pairs to estimate an overall net reproduction (R_o). The original method from that paper consisted of collecting fourth instar *B. tabaci* nymphs of each population on a leaf piece cut from infested cotton leaves and placed into gelatin capsules (No. 00, Eli Lily and Company Indianapolis, IN) until adults emerged. Sexual determination of adults in the capsules was made under 40x magnification using morphological characteristics (Gupta, 1970) and then one resistant female (or male) and one susceptible male (or female) were placed into a gelatin capsule on a fully expanded leaf of a pre-square cotton plant and sealed with a sticky clay (yellow Hand Tax®, Super Glue Corp., Hollis, NY). These micro-cages were moved to a new leaf location daily to keep track of daily oviposition and survival of each life stage.

The whiteflies used to determine the life table parameters in the current study were reared on prevenient treated plants with a LC_{10} rate of either pyriproxyfen or cyantraniliprole, and compared with an untreated plant. Cotton plants were infested with approximately 50 adults. The adults were removed 48 hours after infestation and the plants were kept under controlled conditions for nymphal development. Once the nymphs had developed to a majority of 2nd instar nymphs, the plants were treated with the LC_{10} through leaf dip methodology and were then monitored for adult emergence. The newly emerged adult whiteflies were used in life table study. They were paired and placed in a clip cage. The clip cages were crafted from microcentrifuge tubes of 1.5 mL, with the lower portion removed to create an opening. To facilitate air circulation and diminish humidity within the clip cages, a small piece of breathable fabric was positioned at this opening. This assembly was affixed to a hair clip, providing the flexibility to open and close the clip cage as needed. A supplementary small piece of cardboard was added to ensure the confinement of the whiteflies within the arena.

The clip cage was moved every 48 hours. In other words, the clip cage along with the remaining live adults, were moved to a new location on the leaf so that we could track whitefly development based on 48 h increments. This process continued until the end of the individual run, when females perished. To track each cluster of eggs, the eggs were circled with a marker and numbered. To determine the mortality of adult whiteflies, specific criteria were used. Adult whiteflies were considered dead if they exhibited a lack of discernible movement, showed obvious desiccation, or were found resting on a surface with contact made by any part of the body other than the tarsi. Counts of living and dead growth stages of whiteflies were conducted every 48 h, and the counts for each cluster of whiteflies concluded once all female adults had deceased.

Every 48 h, cotton leaves on the underside, where the clip cages were attached, were inspected using a 10x magnifying glass to count the eggs and nymphs. The counts of emerged adults were based on the typical "T" split observed in the pupal case, indicating normal emergence. All adults from each cohort were collected and identified to male or female as previously described. The numbers of individuals of each life stage (eggs, crawlers, 2nd instar, 3rd instar, pupa and average sex ratio (ASR) or the number of adult females/total adults) were recorded, so that we could calculate the net reproduction.

The life table was constructed following the methodology presented by Southwood and Henderson, 2021. The parameters used for the calculations where:

x: days

L_x: number of surviving individuals at a specific age

d_x: number of dying during the age interval x

e_x: expectation of life

The net reproduction (R_0), was calculated with the following formula proposed by Southwood and Henderson (2021):

$$R_0 = \int_0^\infty l_x m_x d_x = \sum l_x m_x$$

Statistical analysis. Dose response data for nymphs were analyzed using PROC PROBIT in SAS® Enterprise Guide v. 8.3 (SAS Institute Inc., Cary, NC) ((SAS) S.A.S. Institute. 2020) to determine the lethal concentration for 10% of the population. Mortality was recorded as percent mortality and corrected using Abbot's formula before being log-transformed (Abbott 1925).

Individual life table parameters were analyzed using PROC GLIMMIX for repeated measures over days and overall analyses of variance of means and sums over days using PROC GLM. The treatment means separation test used in this study was LSD following the significant treatment effect ($P \leq 0.05$). All means in graphs and figures were reported with standard error bars.

Results

Dose response analysis. Dose-response analysis identified the sublethal (LC_{10}) and median lethal (LC_{50}) concentrations of the tested insecticides for *B. tabaci* second-instar nymphs. The calculated LC_{10} value, the concentration resulting in 10% population mortality, was 0.012 (0.001 – 0.047) mg a.i. L^{-1} for cyantraniliprole, and 0.250 (0.002 – 1.990) mg a.i. L^{-1} for pyriproxyfen. The LC_{50}, the concentration inducing 50% population mortality, was estimated as

0.883 (0.310 – 1.950) mg a.i. L^{-1} for cyantraniliprole, and 4.800 (0.096 – 15.230) for pyriproxyfen (Table 3.2). The completed dose-response curves, along with the fiducial limit distribution are depicted in Figure 3.3.

Life table analysis. Life table analysis reflected the sublethal effects of pyriproxyfen and cyantraniliprole on *B. tabaci* reproduction (Table 3.3). Metrics examined included counts of eggs, nymphs, and adults, and the net reproduction rate (R_0). Notably, the R_0 decreased by 36% in pyriproxyfen-treated and 45% in cyantraniliprole-treated whiteflies.

The effects of the sublethal insecticides over the offspring development was observed and are described in Table 3.3. Pyriproxyfen-treated resulting nymph counts decreased on days two, four, and eight. Adult counts followed suit, dropping on days two, eight, and 16. However, the average sex ratio of females to overall (ASR), percentage of hatch and developmental time displayed no significant variation across treatments throughout the study period. The survivorship was higher until day ten. Net reproduction was lower on day two, four, and ten (Table 3.3).

The cyantraniliprole-treated whiteflies exhibited lower average egg counts on days two and four after emergence compared to the untreated control. Furthermore, they exhibited fewer nymphs on days two and four, reduced adult counts on day two, but a notable increase in emergence by day 16. Meanwhile, developmental time was significantly shortened on days two and four. The ASR and % of hatch remained consistently unchanged across days. The total number of female births by age $L_x m_x$ was lower on day two, four for both insecticides and on day 10 for pyriproxyfen (Table 3.3).

In the earlier study, the population's mean lifespan was approximately 33 days, as indicated in Figure 3.4. However, it's noteworthy that most individuals within this population lived and laid eggs for approximately 20 days, consistent with our own findings. The net reproduction in the previous study was 13.3, 52% lower than our findings. Remarkably, the count peaks closely resembled those observed in the unexposed population of our study two decades later.

Egg count peaks varied across treatments (Fig. 3.5). Unexposed whiteflies presented a peak oviposition 4-6 days post adult emergence. In contrast, both sublethal cyantraniliprole and pyriproxyfen-treated populations peaked later, between 6-8 days. Despite being significantly lower than the untreated whiteflies from days two through 10, the egg count in the pyriproxyfen-treated group remained relatively stable throughout the study. Unlike other treatments, there was no pronounced increase in egg-laying during the young adult phase nor a noticeable decrease as the adults aged, particularly between 10- and 12-days post-emergence. The cyantraniliprole-treated whiteflies exhibited lower average egg counts on days two and four after emergence compared to the untreated control. Furthermore, they exhibited fewer nymphs on days two and four, reduced adult counts on day two, but an increase by day 16. Meanwhile, developmental time was significantly shortened on days two and four.

Discussion

The use of sublethal pyriproxyfen and cyantraniliprole treatments of the *B. tabaci* populations demonstrably reduced the net reproduction rate (R_0), thus even without the immediate impact of mortality, these products may still reduce whitefly reproduction as efficacy fades. The decreased R_0 signified a potentially 45% suppressed population growth in the case of cyantraniliprole treatment. Such shifts in reproductive capacity may have significant consequences for whitefly outbreaks in agricultural systems, possibly allowing for a more “buffered” whitefly population response in the field, i.e., as efficacy wanes, you don’t have immediate high levels of reproduction of resistant individuals. Perhaps, bioassays could better predict what happens to a whitefly population as an insecticide loses its ability to cause mortality. The ability to predict *B.*

tabaci response to insecticides in field through laboratory bioassays, including the ones evaluated in this study, have been recently reported (Cremonez et al. 2023). The precision and accuracy of maximum label rate products were successfully estimated in a tandem lab-field setup. Furthermore, while insecticides play a significant role, natural mortality factors, such as predation of immatures, dislodgment, and environmental variables, have also been observed to critically influence *B. tabaci* population dynamics (Naranjo and Ellsworth 2005).

Sublethal concentrations of cyantraniliprole had effects on whitefly development, especially the reduced nymph counts and early surge in adult numbers. Research has shown that insect development and reproduction are influenced by insecticide exposure, and sublethal doses can have an effect on many developmental and reproductive parameters of insects (Desneux et al. 2007, Wang et al. 2017). Exposure to the sublethal concentrations of cyantraniliprole decreased survival rate, fecundity, duration of oviposition and egg hatching rate (Wang et al. 2017). Moreover, cyantraniliprole caused negative impacts in the feeding behavior of *Myzus persicae* (Jacobson and Kennedy 2014).

Shortened developmental time was observed in the cyantraniliprole-treated group, and it may have implications for population dynamics. Physiological adaptations to stresses during the immature stage can lead to negative effects on fecundity and decreased longevity in the adult stage (Guo et al. 2013). In summary, sublethal concentrations of cyantraniliprole exhibited significant adverse effects on whitefly development and reproductive parameters, highlighting the potential ecological consequences of insecticide exposure on insect populations. Additional investigation is required to evaluate its influence on population dynamics.

Sublethal pyriproxyfen doses definitely impacted the offspring's development, given the observed changes in nymph and adult counts from pyriproxyfen-treated whiteflies. The application of sublethal concentrations of pyriproxyfen in the first instar of *Spodoptera exigua* caused a reduction in the number of eggs laid once insects reached the adult stage (Moadeli et al. 2014). These effects on offspring probably have long-term consequences on overall population health and dynamics.

The consistency of ASR across the treatments indicates that the sublethal insecticides did not significantly affect gender bias in our study. Apart from the lethal effect (mortality), sublethal effects of insecticides over whiteflies have been reported to disrupt reproduction by decreasing fecundity and fertility (Ishaaya and Horowitz 1992, He et al. 2013, Esmaeily et al. 2014, Shahbaz et al. 2019). In one study, *B. tabaci* treated with sublethal doses of buprofezin and imidacloprid showed no evident change in the sex ratio (between 0.50 – 0.60) of the offspring (Sohrabi et al. 2011). There are possible ecological or genetic factors that could sustain this uniformity despite insecticide treatments. One possibility is that the insecticides used in our study, pyriproxyfen and cyantraniliprole, did not directly affect the formation of gametes and, thus, does not impair the eventual gender outcome balance. Therefore, a possible effect over the sex ratio would be related to indirect effects of these chemistries; along the germline cell formation, sublethal pyriproxyfen affected the disposition of spermatocytes and spermatic bundle formation in stink bugs (Hemiptera: Pentatomidae) (Cremonez et al. 2017, Cremonez et al. 2023). More intrinsically, sublethal insect growth regulators such as lufenuron (15) and buprofezin (16) affect parental gonadal formation and anatomical structure, with effects on the germ line cell and ovarian structure resulting in reduced reproduction rate, but no effect over the progeny sex ratio (Cremonez et al. 2019).

These observed sublethal effects could be integrated into an effective insecticide resistance management strategy that takes into consideration sublethal effects. However, it is important to acknowledge that using insecticides at sublethal concentrations will likely enhance the emergence of resistant insect populations. This resistance from sublethal exposure can result from two primary mechanisms: hormesis and the triggering of detoxification enzymes (Guedes et al. 2016). Sublethal doses delay the insurgence of single gene resistance, but can increase the chances of polygenic resistance (Gressel 2011). Given the potential for selecting resistant individuals in whitefly populations exposed frequently to sublethal doses, it becomes paramount to further identify effective insecticide rotations for cyantraniliprole and pyriproxyfen on *B. tabaci*. Future studies should encompass both the lethal and sublethal impacts of these compounds.

The exposure of *B. tabaci* to sublethal concentrations of pyriproxyfen and cyantraniliprole significantly altered reproductive rates, potentially suppressing population growth over time. Cyantraniliprole, in particular, has shown significant adverse effects on whitefly development and reproduction, while pyriproxyfen impact offspring development. Integrating these findings could enhance insecticide resistance management strategies for whiteflies in agricultural systems.

Acknowledgments

We acknowledge the research assistance of the staff at the Coastal Plain Experiment Station and the assistance of the internal reviewers at the USDA-ARS, U.S. Vegetable Laboratory, and the University of Georgia Tifton Campus. This work was funded in part by a USDA-ARS project (6080-22000-027-18-S) entitled "Managing Whiteflies and Whitefly-transmitted Viruses in Vegetable Crops in the Southeastern U.S." Studies that contributed to our understanding of this subject matter were conducted using the facilities of the Georgia Agricultural Experiment Stations. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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Table 3.1. Concentrations of pyriproxyfen (Knack®) and cyantraniliprole (Exirel®) used in *Bemisia tabaci* dose-response bioassay

Relative to MLR	Pyriproxyfen			Cyantraniliprole		
	Product concentration		A.I. concentration	Product concentration		A.I. concentration
%	mL L^{-1}	µL in 200 mL	mg L^{-1} or ppm	mL L^{-1}	µL in 200 mL	mg L^{-1} or ppm
200	3.9	780	401.9418	6.38	1276	198000
100	1.56	312	160.7767	3.19	638	99000
50	0.78	156	80.3884	1.595	319	49500
10	0.39	78	40.1942	0.319	63.8	9900
1	0.078	15.6	8.0388	0.0319	6.38	990
0.1	0.0078	1.56	0.8039	0.00319	0.638	99
0.01	0.00078	0.156	0.0804	0.000319	0.0638	10
0	0	0	0.0000	0	0	0

*MLR: maximum labeled rate per 100 gallons per acre.

Table 3.2. Sublethal (LC10) and median lethal (LC50) of cyantraniliprole and pyriproxyfen for *Bemisia tabaci* second instar nymphs.

Chemical	**LC_{10} (CI 95%)** **mg a.i. L^{-1}**	**LC_{50} (CI 95%)** **mg a.i. L^{-1}**	**Slope** **(± SE)**	**Probit fit line**		
				χ^2	**n**	***p***
Cyantraniliprole Exirel® 0.83 SC	0.012 (0.001 – 0.047)	0.883 (0.310 – 1.950)	0.686 (± 0.040)	296.39	40	<0.001
Pyriproxyfen Knack® 0.86 EC	0.250 (0.002 – 1.990)	4.800 (0.096 – 15.230)	1.262 (± 0.227)	30.96	40	<0.001

Note: CI = confidence interval; SE = standard error; *df* = degrees of freedom; *p* = slope p-value.

Table 3.3. Life table parameters of *Bemisia tabaci* individuals generated from sublethal insecticide-treated female.

Day	Treat	Eggs	Nymphs	Adults	% hatch	ASR	DT	L_x	$L_x m_x$	R_0
2	Untreated check	24.84 a	13.00 a	7.91 a	58.56 a	0.60 a	18.16 a	0.29 b	4.14 a	
	Cyantraniliprole LC_{10}	8.40 b	4.53 b	3.28 b	53.68 a	0.66 a	11.99 b	0.28 c	1.50 b	
	Pyriproxyfen LC_{10}	8.47 b	4.97 b	2.19 b	53.12 a	0.49 a	19.12 a	0.38 a	1.20 b	
4	Untreated check	31.03 a	17.75 a	10.13 a	57.77 a	0.58 a	17.50 ab	0.29 b	5.29 a	
	Cyantraniliprole LC_{10}	17.57 b	9.87 b	7.60 a	51.98 a	0.55 a	14.93 b	0.28 c	2.65 b	
	Pyriproxyfen LC_{10}	11.35 b	6.53 b	5.36 a	56.71 a	0.54 a	18.80 a	0.38 a	2.23 b	
6	Untreated check	31.00 a	14.68 a	10.01 a	48.70 a	0.50 a	16.16 a	0.29 b	4.48 a	
	Cyantraniliprole LC_{10}	25.66 ab	16.66 a	10.30 a	64.09 a	0.57 a	13.60 a	0.28 c	3.80 a	
	Pyriproxyfen LC_{10}	18.00 b	9.39 a	4.88 a	45.77 a	0.66 a	16.50 a	0.37 a	3.40 a	
8	Untreated check	30.08 a	16.91 a	13.09 a	55.80 a	0.54 a	15.16 a	0.29 b	4.81 a	
	Cyantraniliprole LC_{10}	28.00 a	18.20 a	11.41 a	55.76 a	0.62 a	13.20 a	0.28 b	4.92 a	
	Pyriproxyfen LC_{10}	17.80 b	6.86 b	4.15 b	45.25 a	0.64 a	17.50 a	0.36 a	3.73 a	
10	Untreated check	24.16 a	14.35 a	8.66 a	51.61 a	0.60 a	15.00 a	0.29 ab	4.08 a	
	Cyantraniliprole LC_{10}	21.53 a	14.35 a	8.46 a	51.53 a	0.53 a	10.92 a	0.24 b	2.70 ab	

	Pyriproxyfen LC_{10}	12.85 b	5.50 a	2.50 a	37.23 a	0.44 a	14.80 a	0.30 a	1.67 b	
	Untreated check	13.56 a	7.05 a	3.13 a	49.51 a	0.58 a	12.72 a	0.23 a	1.81 a	
12	Cyantraniliprole LC_{10}	16.50 a	8.50 a	4.22 a	39.98 a	0.67 a	11.42 a	0.15 b	1.31 a	
	Pyriproxyfen LC_{10}	12.93 a	5.03 a	2.11 a	19.01 a	0.57 a	16.67 a	0.22ab	1.35 a	
	Untreated check	21.00 a	7.00 a	5.03 a	38.46 a	0.48 a	18.58 a	0.10 a	1.14 a	
14	Cyantraniliprole LC_{10}	14.50 a	13.00 a	6.09 a	48.50 a	0.54 a	9.65 a	0.12 a	0.69 a	
	Pyriproxyfen LC_{10}	24.00 a	18.75 a	8.52 a	59.17 a	0.53 a	18.52 a	0.12 a	1.96 a	
	Untreated check	17.25 a	9.50 a	3.75a b	52.11 a	0.60 a	8.50 a	0.08 a	0.61 a	
16	Cyantraniliprole LC_{10}	38.00 a	16.00 a	17.00 a	42.11 a	0.47 a	20.00 a	0.01 a	0.34 a	
	Pyriproxyfen LC_{10}	17.20 a	10.50 a	2.00 b	32.85 a	0.28 a	17.50 a	0.17 a	0.66 a	
	Untreated check	23.25 a	-	-	-	-	-	0.04 a	0.32 a	25.25 a
18	Cyantraniliprole LC_{10}	6.00 a	-	-	-	-	-	0.01 a	0.00 a	16.05 b
	Pyriproxyfen LC_{10}	23.39 a	-	-	-	-	-	0.18 a	0.85 a	13.81 b

Note: ASR = average sex ratio of females/total; DT = developmental time (days); L_x = survivorship of individuals in day x; $L_x m_x$ = net reproduction at day x; R_0 = average net reproduction.

FIGURE CAPTIONS

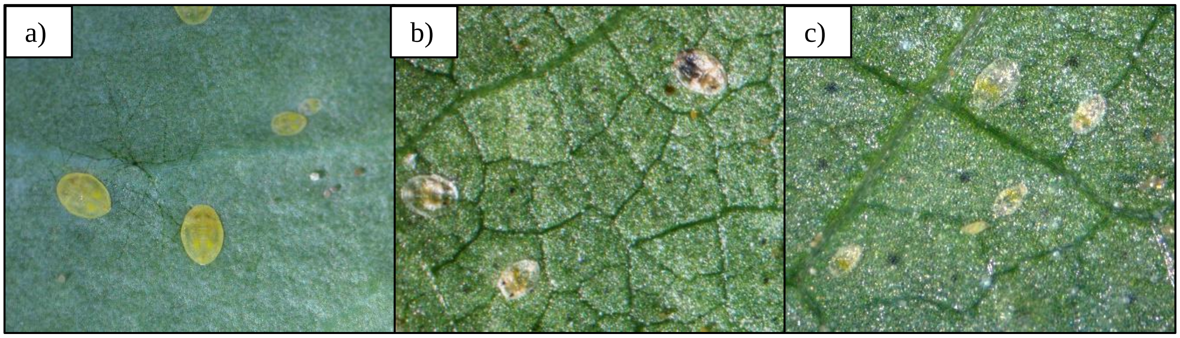
Figure 3.1 Aspect of *Bemisia tabaci* nymphs a) alive, untreated; b) dead, treated with cyantraniliprole; c) dead, treated with pyriproxyfen.

Figure 3.2. *Bemisia tabaci* life table experimental set up in growth chambers.

Figure 3.3. Dose-response curves of insecticides applied on *Bemisia tabaci* second-instar nymphs. a) Dose-response for cyantraniliprole; b) Dose-response for pyriproxyfen.

Figure 3.4. Mean number of Bemisia tabaci developmental stages by day (n=342), based on total raw data from Riley and Tan (2002).

Figure 3.5. Mean number of *Bemisia tabaci* developmental stages on an untreated check (a) (n=12), or exposed to sublethal concentrations (LC_{10}) of cyantraniliprole (b) (n=14) or pyriproxyfen (c) (n=15).



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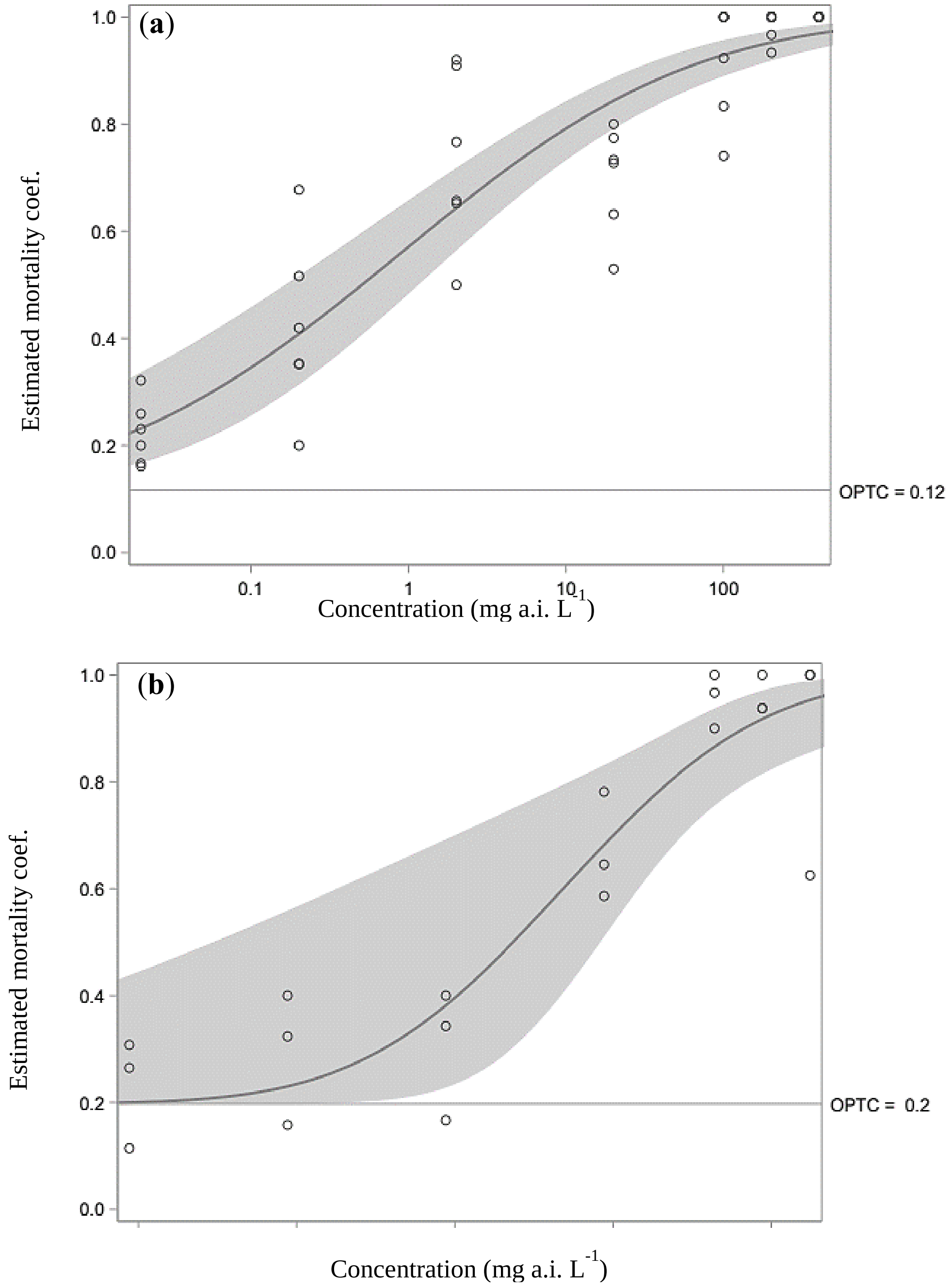

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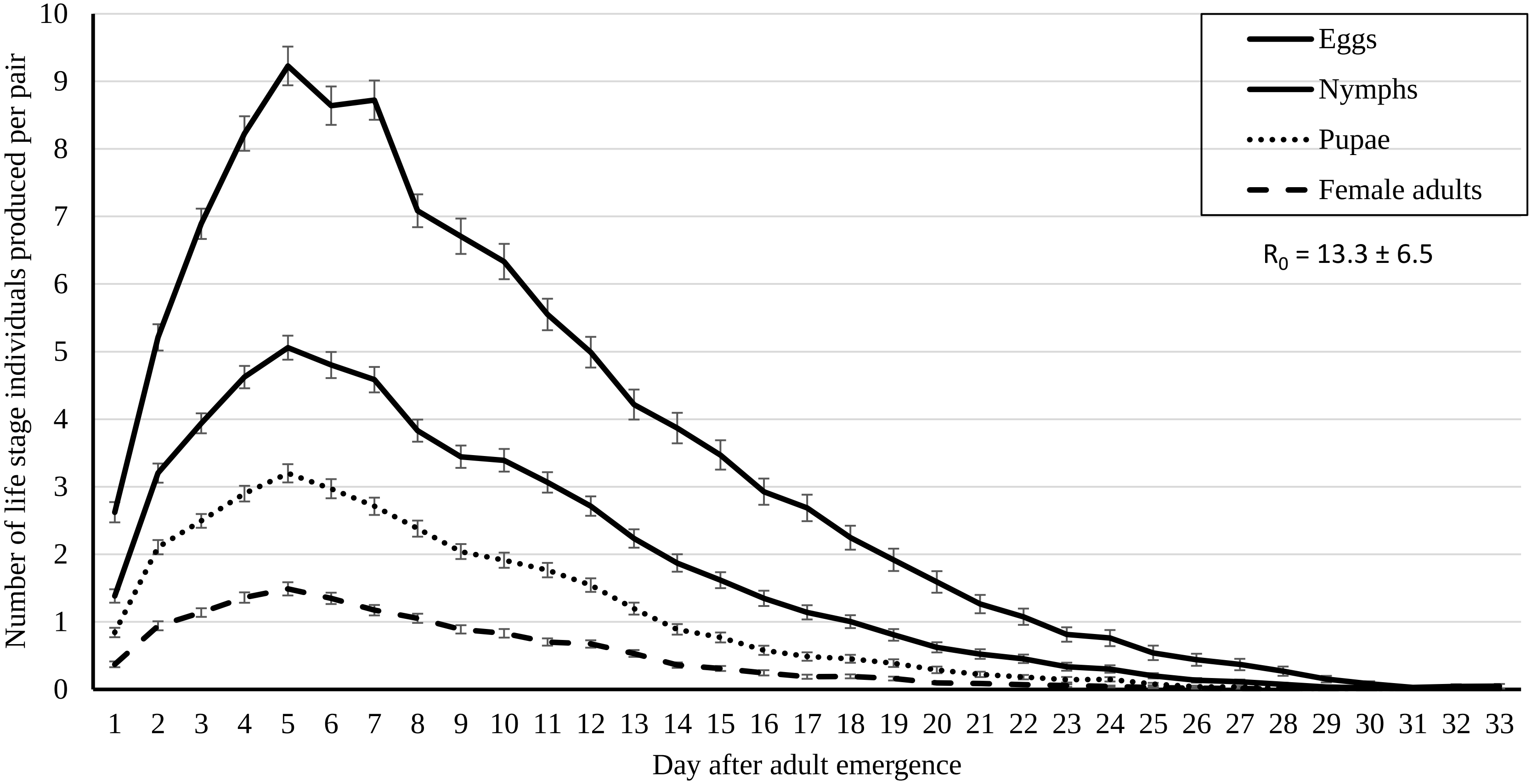

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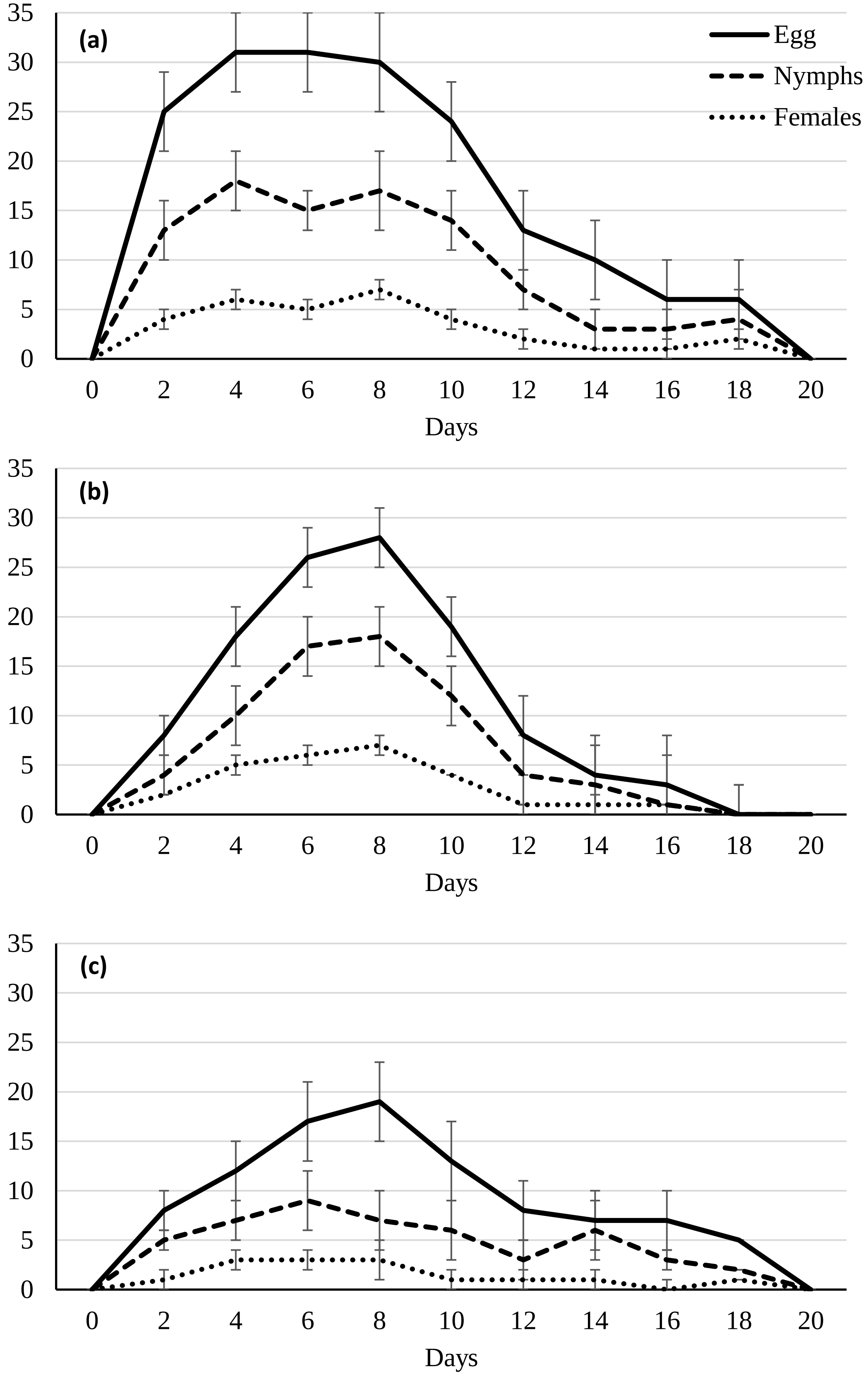

Figure 3.5. Mean number of *Bemisia tabaci* developmental stages on an untreated check (a) (n=12), or exposed to sublethal concentrations (LC_{10}) of cyantraniliprole (b) (n=14) or pyriproxyfen (c) (n=15).

CHAPTER 4

DETERMINATION OF SUBLETHAL EFFECTS OF PYRIPROXYFEN AND CYANTRANILIPROLE ON OVIPOSITIONAL BEHAVIOR OF *Bemisia tabaci*[2]

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[2] Nagaoka, M.M., Cremonez, P.S.G., Simmons, A.M., and Riley, D.G. To be submitted to JEE for publication, December, 2023

ABSTRACT

This research investigated the sublethal effects of two insecticides, cyantraniliprole and pyriproxyfen, on the settling and oviposition behavior of the sweetpotato whitefly, *Bemisia tabaci*. Three plants of cotton with one true leaf were treated with the sublethal concentrations (LC_{10}) of the above-mentioned insecticides and placed into a cage (0.6 x 0.6 m) for whitefly adult choice studies. A total of 100 whitefly adults (ca. 53±3.5% female) were released in the cage. Settling choice was recorded every 15 minutes, for one hour. For the ovipositional preference, the insects were removed after two days and the total number of eggs was recorded. There was no significant difference in settling choice. Plants treated with sublethal concentrations of pyriproxyfen had a significant higher number of eggs when compared to the other treatments.

Keywords: whitefly, behavior, insecticide resistance, sublethal effects

Introduction

Investigating host selection by phytophagous insects has been vital for the understanding of the population dynamics of insects (Scheirs and De Bruyn 2002) and, in particular, whiteflies (Jiao et al. 2012). The majority of studies concerning arthropod host selection revolve around the concept of optimal oviposition theory (Jaenike 1978, Scheirs and De Bruyn 2002, Jiao et al. 2012). The preference-performance hypothesis, suggests a correlation between the choice of where to lay eggs and the suitability of the host for the successful development of their offspring (Jaenike 1978). The decisions made by the female are aimed at maximizing the overall reproductive success of their offspring (Mayhew 1997). These decisions are frequently shaped by a combination of nutritional and defensive chemistry, and are subject to significant selective pressures (Bernays 1994, Mayhew 1997), but often, questions about the behavioral effects from sublethal doses of insecticides are neglected in these insect population dynamics discussions.

Consecutive applications of insecticides have the potential to modify the biochemical composition of cotton leaves, leading to a shift in preference, making the plant more attractive or repellent to adult whiteflies (Abdullah et al. 2006). Behavioral changes in *B. tabaci* oviposition have been detect on plants treated with fenvalerate and acephate (Abdullah et al. 2006). Moreover, tomato plants treated with label rate and sublethal concentrations of flupyradifurone had a negative impact on feeding behaviors of the *B. tabaci* MED, altering duration and frequency of probing actions (Liu et al. 2021). Exposure to sublethal concentrations of cyantraniliprole resulted in a suppression of feeding behavior of *Myzus persicae*, induced by a paralytic effect triggered by muscle contractions caused by the insecticide (Zeng et al. 2016). Moreover, whiteflies exposed to sublethal concentrations of imidacloprid and bifenthrin had a notable antifeedant response (He et al. 2013). Cyantraniliprole application significantly reduces the number of eggs that whiteflies lay on tomato plants (Libardi Miraldo et al. 2021).

Insects employ their olfactory systems as one of the mechanisms to locate suitable hosts (Visser 1988). Whiteflies appear to utilize olfactory cues for host plant selection, but their choice is guided by repulsion rather than attraction (Mrisho et al. 2021). We suspect that sublethal doses of insecticide affect this insect behavior. Furthermore, the findings might clarify interactions between *B. tabaci* and treated plants. Therefore, the hypothesis of this study was that the treatment of cotton plants with sublethal concentrations of cyantraniliprole and pyriproxyfen would affect the settling behavior or number of eggs laid by adult whiteflies.

Material and Methods

Laboratory whitefly colonies of *B. tabaci* used in this study were from a relatively insecticide-susceptible population collected from Georgia (Perier et al. 2023). The colony was maintained on cotton plants in individual rearing rooms until assays for this study were initiated during the fall of 2022. The insects were reared in mixed host plants (cucurbits and cotton).

Untreated cotton plants (cultivar ST 4946 GLB2) were utilized as the plant material in the bioassays. These cotton plants were grown in growth chambers (Percival model E-36L2, Perry, IA, USA) under specific conditions, including a temperature of 30°C, a relative humidity of 70%, and a 16:8 day-night cycle. Before being used in the study, the cotton plants were grown until they reached the two-expanded-true-leaf stage and were then transplanted into pots measuring 8.9 cm in diameter and 8.3 cm in height. Once the plants reached this stage, only one leaf was left, and it was treated with the LC_{10} of cyantraniliprole and pyriproxyfen through a leaf dip method. All the plants used in this bioassay were approximately the same size with at least one of the terminal leaves having a width of 4 cm.

For the host preference study, treated plants of cotton used as a single replicate were placed randomly into the bottom of a 0.6 x 0.6 x 0.6 m insect rearing cage (Specimen Handling Cage, Bioquip® Products, Rancho Dominguez, CA), the plants were placed in vertices on a triangle arrangement, equidistant to each other, and approximately 100 adults (53±3.5% female) were randomly selected of one of the whitefly populations and were released in the center of the cage (Fig. 4.1). The adults had been starved for 1 hour. Adults were counted every 15 min for one hour. Each cage was a block/replicate and the experiment was replicated 24 times.

The plants and insects used for ovipositional preference assessment originated from an identical source as those employed in the host preference experiment. The first series of bioassays encompassed three treatments: untreated check (UTC), as well as the application of the LC_{10} of pyriproxyfen and cyantraniliprole (figure 4.1). In the second series, we tested focused on differences between UTC and pyriproxyfen. Similarly, a third series focused on just the interplay between UTC and cyantraniliprole. The fourth and final series focused on just the differences between the LC_{10} treatments of pyriproxyfen and cyantraniliprole. For all of the tests, the adults were starved for 1 h prior to initiating the experiment and adult settling observations. After two days, all eggs were counted. Each cage was a block/replicate and the experiment was replicated 24 times.

In all experiments, a completely randomized design served as the framework with replication conducted over time. To confirm data normality, residual plots were assessed following analysis of variance, as per the guidelines set forth by Fernandez (1992). All statistical evaluations were executed in SAS® Enterprise Guide v. 8.3 (SAS Institute Inc., Cary, NC). For the preference experiment, the generated data were processed using PROC ANOVA, followed by a Tukey's HSD test for mean separation. For the oviposition experiment, the Student`s t-test was performed through PROC TTEST.

Results

There was no significant difference observed in the settling behavior of *B. tabaci* (Table 4.1) The choice of where to settle appeared to be random, and the insects exhibited minimal movement on the observed period. Thus, sublethal effects of pyriproxyfen and cyantraniliprole were not an important factor in whitefly adult host selection for simple adult settling behavior in this study. However, for the series of three treatments, the mean number of eggs was 51% higher in pyriproxyfen-treated plants ($F_{[2,71]} = 3.36$, $P = 0.040$) than the untreated check and 70% higher in comparison to cyantraniliprole (Table 4.2, figure 4.2).

Pyriproxyfen-treated plants had 35% more eggs when there was a choice with untreated plants ($P = 0.023$, t = 2.395) (Table 4.3, figure 4.3). Plants treated with cyantraniliprole presented no difference in the mean number of eggs when in combination with untreated plants ($P = 0.904$, t = 0.122) (Figure 4.3). However, when cyantraniliprole plants were combined with pyriproxyfen-treated plants, the mean number of eggs was 29% lower with cyantraniliprole ($P = 0.003$, t = 3.105) (Figure 4.3). Overall, combinations that had one plant treated with pyriproxyfen had a higher mean number of eggs, indicating a stimulation of oviposition (Figure 4.3).

Discussion

The lack of significant differences in whitefly settling behavior and their observed movement patterns show that the choice is not affected by sublethal doses, indicating that the treatments do not affect the olfactory-mediated orientation behaviors of whiteflies. Moreover, the treatments did not appear to have antifeedant properties based on our casual observation of settling behavior. However, to properly address this behavior it would be important to conduct more specific and comprehensive experiments based on feeding behavior, such as electropenetrography (EPG) studies. For instance, Civolani et al. (2014) employed EPG techniques with cyantraniliprole in *B. tabaci* MED biotype, concluding that the full rate of the diamide resulted in complete cessation of feeding on tomato. Other research techniques that might be needed for a clearer assessment of this behavior include testing for visual and olfactory cues (Johnston and Martini 2020). While many studies have delved into whitefly settling and feeding behaviors using varied techniques, the nuances of how sublethal insecticides impact these responses remain relatively unexplored. Our findings suggest that at least one insecticide, pyriproxyfen, aimed at managing *B. tabaci* might impact oviposition without discernible insect choice, suggesting a potential arrestant effect leading to greater opportunities for oviposition. Comprehensive research using the aforementioned techniques might help uncover the underlying mechanisms of our findings.

The results did show a significant increase in whitefly egg production on pyriproxyfen-treated plants compared to untreated plants and cyantraniliprole-treated plants. Pyriproxyfen can impact reproductive parameters of *E. heros*, disrupting the hormonal homeostasis in the insect (Cremonez et al. 2023). Pyriproxyfen, as a juvenile hormone analog, has shown a positive correlation with vitellogenesis (Edwards et al. 1993). As demonstrated by Cremonez et al. 2023, the ovarioles of newly emerged females of *E. heros* exhibited previtellogenic oocytes. Hence, treating the substrate with sublethal doses of pyriproxyfen may potentially trigger vitellogenesis in *B. tabaci*, leading to a subsequent increase in the egg count. Pioneering research on pyriproxyfen effects on *B. tabaci* revealed that this JH analog disrupts embryogenesis and negatively affect adult emergence (Ishaaya and Horowitz, 1992). While this indicates an impact on post-embryonic development, such effects are attributed with the usual rate of this insecticide. Therefore, it is important to account for the main effect of sublethal pyriproxyfen on oviposition stimuli. Ishaaya and Horowitz (1992) also documented the oviposition of infertile eggs, which aligns with our results. From this, one might infer that while sublethal concentrations of pyriproxyfen could stimulate oviposition, it would also detrimentally affect egg fertility and immature viability.

Acknowledgments

We acknowledge the research assistance of the staff at the Coastal Plain Experiment Station. This work was funded in part by a USDA-ARS project (6080-22000-027-18-S) entitled "Managing Whiteflies and Whitefly-transmitted Viruses in Vegetable Crops in the Southeastern U.S." Studies that contributed to our understanding of this subject matter were conducted using the facilities of the Georgia Agricultural Experiment Stations. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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Table 4.1. Mean number of adults of *Bemisia tabaci* per leaf over time.

Treatment	**15 min**	**30 min**	**45 min**	**60 min**
Untreated check	18.33 ± 3.44a	19.13 ± 3.33a	17.66 ± 3.23a	16.4 ± 3.11a
LC_{10} Cyantraniliprole	17.93 ± 3.05a	19.33 ± 2.81a	20.66 ± 3.06a	18.26 ± 3.23a
LC_{10} Pyriproxyfen	15.73 ± 2.75a	19 ± 2.73a	20.13 ± 3.68a	18.80 ± 3.11a

Note: means followed by the same letter in the column did not differ significantly, LSD test ($P < 0.05$). n = 23 replicates per treatment.

Table 4.2. Mean number of eggs of *Bemisia tabaci* per leaf by treatment plus confidence intervals.

Chemical	**Number of eggs**	**Lower 95% CI**	**Upper 95% CI**
Untreated check	96.42 ± 14.00 b	64.99	123.09
Cyantraniliprole LC_{10}	85.38 ± 12.28 b	61.35	112.30
Pyriproxyfen LC_{10}	145.78 ± 21.28 a	101.64	189.91

Note: means followed by the same letter in the column did not differ significantly, LSD test ($P < 0.05$), n=23

Table 4.3. Number of eggs of *Bemisia tabaci* per leaf by treatment in each combination.

Chemical	**Number of eggs**	**Lower 95% CI**	**Upper 95% CI**
Untreated check	58.50 ± 6.84 a	41.94	73.08
Cyantraniliprole LC_{10}	57.31 ± 7.21 a	43.91	72.68
Untreated check	154.94 ± 23.74 b	104.32	205.54
Pyriproxyfen LC_{10}	208.50 ± 32.04 a	140.19	276.80
Cyantraniliprole LC_{10}	111.21 ± 16.98 b	76.08	146.33
Pyriproxyfen LC_{10}	157.75 ± 19.52 a	117.37	198.12

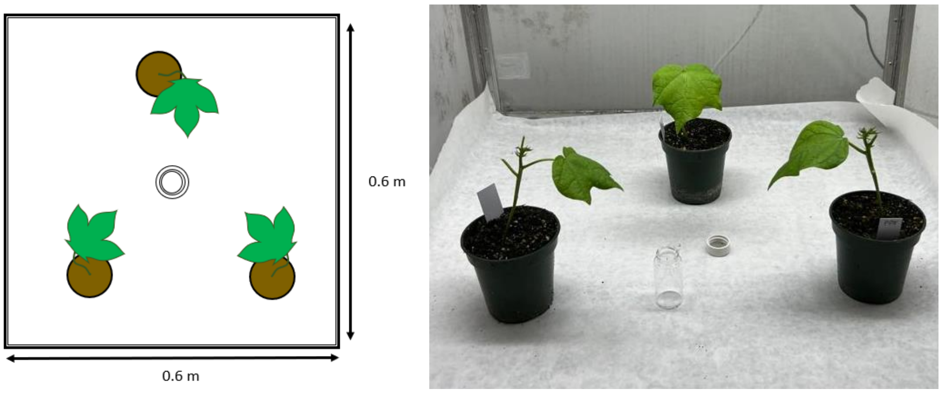
Note: means followed by the same letter in the column did not differ significantly, Student t test ($P < 0.05$), n=16.

FIGURE CAPTIONS

Figure 4.1. Experimental set up of the choice behavior test.

Figure 4.2. Number of eggs of *Bemisia tabaci* by treatment.

Figure 4.3. Number of eggs of *Bemisia tabaci* by treatment in each combination.



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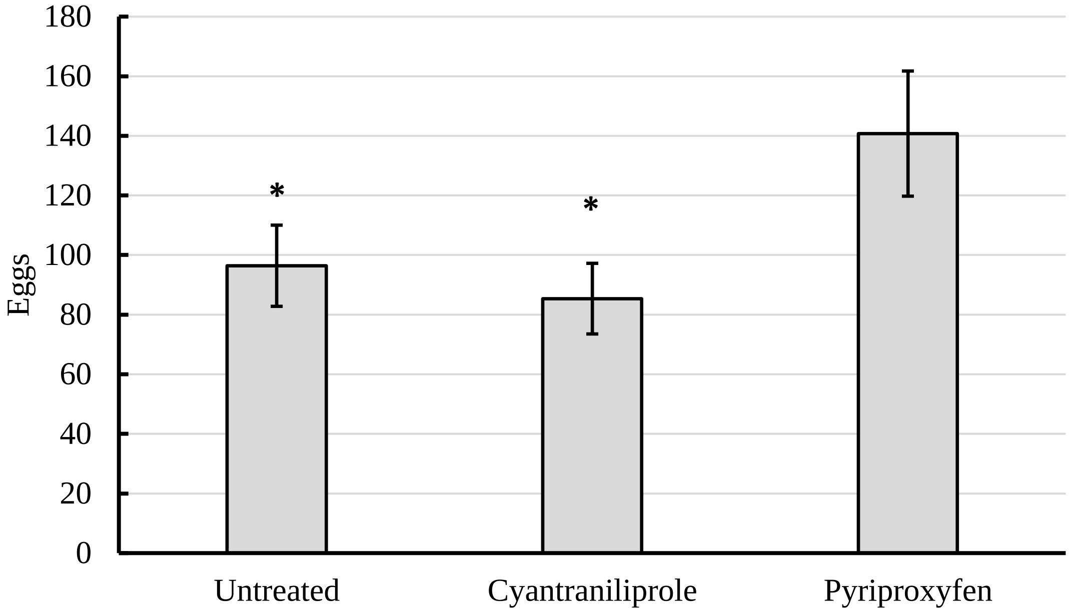

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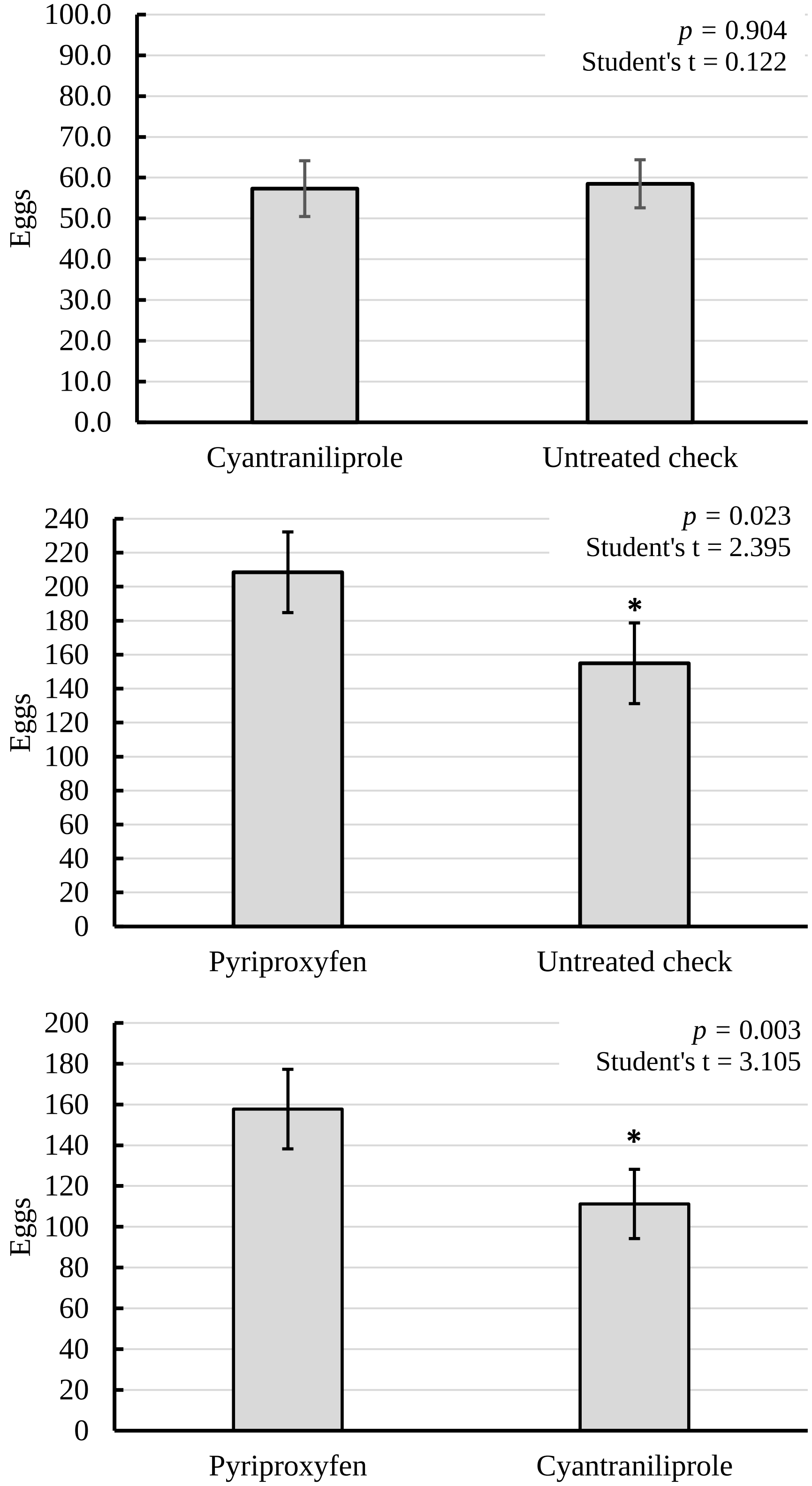

Figure 4.3. Number of eggs of *Bemisia tabaci* by treatment in each combination.

CHAPTER 5. OVERALL SUMMARY

The sweetpotato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a significant agricultural pest in Georgia, known for its resistance to commonly used insecticides. Traditional insecticides not only cause mortality, but at sublethal concentrations also affect the development and behavior of this pest. This study intended to assess the sublethal effects of pyriproxyfen and cyantraniliprole on *B. tabaci*, focusing on their impact on life table parameters, feeding preference, settling, and oviposition behavior.

Second instar nymphs exposed to sublethal concentrations of pyriproxyfen and cyantraniliprole resulted in a reduction in the average number of eggs per day when the insects reached the adult stage, with pyriproxyfen showing a greater impact (42% reduction) compared to an untreated check. The net reproduction rate was significantly lower in insects exposed to both insecticides, with pyriproxyfen causing a 45% reduction.

Settling choice was not significantly affected by the sublethal concentrations of the insecticides. However, plants treated with sublethal concentrations of pyriproxyfen had a significantly higher number of eggs compared to other treatments, indicating a preference for oviposition on these plants. We think that treating the substrate with sublethal doses of pyriproxyfen has the potential to stimulate vitellogenesis in *B. tabaci*, leading to an increased number of eggs being produced. However, this does not necessarily translate to a higher number of offspring, since the resulting eggs may not be viable.

Exposing *B. tabaci* to sublethal concentrations of pyriproxyfen and cyantraniliprole resulted in reductions in reproduction, suggesting that even non-lethal concentrations of these insecticides have the potential to limit population growth over time. Cyantraniliprole, in particular, exhibited substantial detrimental effects on both whitefly development and reproduction, while pyriproxyfen influenced the development of offspring.

The observed effects mentioned here offer additional information which can be integrated into an effective insecticide resistance management strategy. Nevertheless, it is crucial to recognize that the use of insecticides at sublethal concentrations can have implications for the emergence of resistant insect populations. This resistance resulting from sublethal exposure can be attributed to two primary mechanisms: hormesis and the activation of detoxification enzymes. Sublethal doses have been reported to delay the development of resistance associated with single genes, but simultaneously increasing the likelihood of polygenic resistance. Considering the potential for the selection of resistant individuals in whitefly populations that are frequently exposed to sublethal doses, it becomes imperative to develop effective rotations for insecticides like cyantraniliprole and pyriproxyfen against *B. tabaci*. Future research on insecticide resistance management in whiteflies should encompass a comprehensive examination of both the lethal and sublethal impacts of these compounds.

Currently, the use of insecticides is the main strategy to control *B. tabaci.* Nonetheless, it is necessary to adopt Integrated Pest Management (IPM) strategies to mitigate potential development of resistance due to selection pressure, and lapses in pest control. Incorporating these findings into pest management strategies could strengthen efforts to manage insecticide resistance.