INSECTICIDE BIOASSAYS FOR DETECTING RESISTANCE IN WHITEFLY

by

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(Under the Direction of DAVID G. RILEY)

ABSTRACT

The B-strain (MEAM1) of sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is a key pest of vegetable and agronomic crops in the Southeastern USA. Insecticide resistance is one of the major concerns for management of this pest in commercial crops. Four whitefly bioassay methods (tube, cup, petri dish, and clip cage) were evaluated under leaf and root drench application routes across five whitefly insecticides compared with a water check. The application route (root or leaf drench) made no significant difference on whitefly mortality. The clip-cage bioassay resulted in less overall whitefly mortality and the best separation of insecticide treatments and rates followed by the cup and tube methods, respectively. High rates of cyantraniliprole, dinotefuran and flupyradifurone insecticides resulted in the highest whitefly mortality. Interactions occurred between the methodology and insecticide treatment in both populations. Whole-plant bioassays in 2018 and 2020 showed that these same insecticides significantly reduced adult and egg numbers using a lab colony, but results were more variable when using the whole plant exposure method. Insecticide response provided greater separation when using the clip cage technique. Finally, a modified tube bioassay method was tested for obtaining LC_{50} values resulting in promising preliminary results.

INDEX WORDS: sweetpotato whitefly, *Bemisia tabaci,* whitefly, insecticide resistance, bioassay methods, dose response, insecticide resistance management (IRM), Hemiptera, Aleyrodidae

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DEDICATION

This work is dedicated to all the people in my life. I couldn’t have made it this far without you all.

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I would like to thank my advising professor and committee members who worked on this project with me. Without their guidance and encouragement, I would not have been able to complete this project and all the accompanying work. I would also like to thank my coworkers for all their efforts in keeping this lab running.

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CHAPTER 1. INTRODUCTION

The b-strain of sweetpotato whitefly, *Bemisia tabaci* Gennadius MEAM1, is one of the most devastating insect pests of vegetable, agronomic and ornamental crops globally (Thompson 2011). This whitefly is highly polyphagous with hundreds of crop- and weed-host plants in both open field and protected agriculture (Abd-Rabou and Simmons 2010, Boykin et al. 2013). Whiteflies have piercing sucking mouthparts (Gill 1990), and at high densities, whiteflies debilitate plants by extracting sap from the phloem. They can produce large quantities of honeydew, which serves as substrate for sooty mold, contaminating cotton, ornamental and other plants and reducing their marketability (Riley and Palumbo 1995, Schuster et al. 1996, Oliviera et al. 2001). The greatest damage caused by whiteflies to vegetable crops is through the transmission of viruses (Polston et al. 1997). *B. tabaci* transmits >300 types of virus, primarily in the Geminiviridae (Jones 2003, Adkins et al. 2011). *Tomato yellow leaf curl virus* (TYLCV) is the most damaging whitefly-transmitted virus of tomato in the United States and many other tomato-growing regions of the world (Riley and Srinivasan 2019). In cucurbits, *B. tabaci* transmits *Squash vein yellowing virus*, which causes watermelon vine decline, *Cucurbit leaf crumple virus*, and *Cucurbit yellow stunting disorder virus* (Adkins et al. 2011). In addition, sweetpotato whitefly is responsible for irregular ripening in tomatoes and silver-leafing of squash, plant disorders that are primarily associated with feeding by nymphs (Schuster et al. 1996).

Current agricultural practices rely heavily on the usage of insecticides for pest population reduction and disease prevention (Van Doorn et al. 2013). *Bemisia tabac*i has shown an impressive ability to adapt through insecticide resistance (Horowitz et al. 2020). Neonicotinoids have been used in the past to reduce field populations with great success; however, increased resistance has developed in the SE (Schuster et al. 2010) and SW United States (Castle and Prabhaker 2013). Additionally, resistance to pyrethroids is common (Smith et al. 2013). The recently developed diamides have had baseline susceptibility recorded in Florida as of 2011 (Caballero et al. 2013), but resistance has already begun to develop (Wang et al. 2018).

Reducing the possibility of insecticide resistance development is critical to the future of Georgia agriculture. Rotating the usage of different insecticides with differing modes of action is capable of stabilizing resistance development (Basit 2013). An important aspect of resistance management is to have a monitoring program established that can detect changes in a population's resistance to specific insecticide groups (Caballero et al. 2013). There is currently very little published data (Schuster et al 2008) for similar baselines for the state of Georgia which puts the state's agriculture sector at risk to allow resistance to go undetected with increased whitefly damage brought about by insecticide resistance. This research will be the baseline for future study into the state's whitefly population.

The goal of this research is to provide a better understanding of insecticide resistance and potential insecticide control of the sweetpotato whitefly. This involved standard insecticide bioassays, testing different bioassay techniques, and developing a more efficient bioassay technique for larger regional sampling. The goal of this last activity is to develop easy and efficient bioassay techniques to assess the mortality-dose response of whiteflies in commercial fields. The research objective and associated hypotheses tested in this thesis are organized by chapter as follows.

Chapter 3 – Comparison of Toxicological Bioassays for Whiteflies

Objective 1. Refine toxicological bioassays to quantify changes in mortality response of the whitefly population to both systemic and contact insecticides. This will include the University of Florida whitefly excised leaf bioassay protocol for nicotinic and diamide insecticides, a published Insecticide Resistance Action Committee cup method, the standard clip cage intact plant bioassays and a new UGA tube method. The emphasis will be to evaluate interactions between application method (main plot), bioassay method (subplot) and treatments (sub-subplot).

Hypotheses:

A) The main (drench vs foliar application), subplot (bioassay method) and sub-subplot (insecticide treatments) effects will result in significantly differing whitefly mortality.

B) Leaf and root drench applications will result in different mortalities across bioassay methods and across specific insecticide treatments (*i.e.*, significant main to sub and sub-subplot interactions).

C) The different bioassay methodologies will result in different mortalities across specific insecticide treatments (*i.e.*, significant sub and sub-subplot interaction).

Chapter 4 – Insecticide Control of Whiteflies

Objective 2. Evaluate the efficacy of select insecticides to reduce adult, egg, and nymph numbers on treated plants in a greenhouse setting.

Hypothesis:

A) Certain insecticides will provide significant control for adult and immature whiteflies when compared to a water-treated control.

One last chapter was planned that involved using a modified version of the tube methodology from chapter 3 to obtain mortality data on three lab colonies and obtain LC_{50} values for different insecticides. Experimentation was suspended due to the Covid-19 shutdown that occurred from March to June of 2020. Work on this study is expected to be published after the completion of this master’s thesis. Methodology and data collected so far is included in this document under the appendix section.

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CHAPTER 2. LITERATURE REVIEW

2.1 The biology of the sweetpotato whitefly

Whiteflies belong to order Hemiptera, family Aleyrodidae. Whiteflies, as their name suggests, are a powdery white winged insect between 2 and 3 mm long (Gill 1990). As a member of Hemiptera, they possess a needle-like mouthpart that is inserted into the phloem of plants during feeding (Van Lenteren and Noldus 1990). Whiteflies feed exclusively on the sap of their host plants and have a digestive system adapted to derive complete nutrition from the sugar-rich food source (Thompson 2011). In addition to nutrient extraction, whiteflies damage the host plant by vectoring plant pathogens (Polston and Anderson 1997). The excretion of sugar-rich honeydew on plant tissue feeds mold growth that covers leaves and reduces photosynthesis (Ernst 1994, Schuster et al. 1996).

Bemisia tabaci eggs are laid on the underside of leaves of their host plant. After seven days, the egg hatches into the first instar nymph. This first instar is often called a “crawler” and is set apart from later instars by its developed legs and ability to move about the plant. The crawler will find a suitable location to begin feeding, inserts its proboscis, and will not move until adulthood. The next two instars are immobile and lack appendages. The fourth, and final, juvenile instar is often referred to as the “pupa”. During this stage, the juvenile whitefly is developing the wings and sex organs that will mark it as a full-grown adult (Gill 1990, Salas 1995).

It takes approximately twenty days for the whitefly egg to reach maturity. This time may vary by temperature. *Bemisia tabaci* shows quickest development between 30° to 35°C with decreased time at cooler temperatures down to 17°C when development stops completely (Bonato 2007). From there, both male and female adults are winged and will fly off to mate and lay eggs on the same or other plants. Females can produce from 100 to 300 eggs during their lifetime. Whiteflies may reproduce parthenogenetically with unfertilized eggs developing into males and fertilized ones becoming females (Salas 1995, Byrne 1996).

Whiteflies feed by sticking their proboscis into the host plant until reaching the vascular bundle of the phloem (Van Lenteren and Noldus 1990). Their piercing-sucking mouthparts are comprised of three stylets, the maxillary stylet, and two mandibular stylets, that bundle together to create a needle capable of penetrating plant tissues. The two mandibular stylets come together to form a sheath around the maxillary stylet. This sheath provides structural support to the thinner maxillary stylet and allows the insect to guide the stylet into plant tissue. The maxillary stylet has two canals within it. The food canal is where phloem sap is taken into the digestive system. The lateral salivary canal is how salivary enzymes are injected into the plant in order to facilitate continued feeding (Czosnek 2002).

Bemisia tabaci is estimated to vector over 150 types of virus, primarily in the Geminiviridae (Jones 2003, Adkins et al. 2011). The circulation of fluids between the host plant and whitefly is what allows these insects to serve as vectors of numerous plant diseases like *Tomato yellow leaf curl virus* (TYLCV). Virus particles are acquired from infected plant sap and make their way into the midgut of the whitefly. Instead of being digested however, they penetrate the epithelial cells lining the insect's gut and enter the hemolymph. The virus then makes its way into the salivary glands and enters the next plant when enzymes are injected during feeding. In the case of TYLCV, the virus can make its way into the salivary glands within an hour of feeding on an infected host. The ability of adult *Bemisia tabaci* to fly facilitates speedy infection of otherwise healthy plants in a field or greenhouse with *B. tabaci* pressure (Czosnek 2002).

Taxonomically, *B. tabaci* is in a state of flux. There are currently 19 biotypes (named A through T) that are all morphologically similar but have different origins geographically with varying levels of fecundity and insecticide resistance. Of these biotypes, B and Q are the most relevant pests to crops (Horowitz et al. 2020). Biotype B originated in North Africa and spread to the Middle East before expanding out the rest of the world. Biotype Q was more recently discovered and originates from the Mediterranean and southern Europe. Biotype B is known for its high fecundity combined with insecticide resistance to outcompete populations of A in regions it has spread (Khan 2012). Biotype Q is more recently discovered and is capable of surviving insecticide treatments that would even knock out infestations of B. As a result, biotype Q is replacing B in areas that receive more intensive insecticide use (Wang et al. 2010). Because each biotype has such a large range of fecundity and host plant preference, it has been posited that *B. tabaci* is possibly a species complex with over 24 genetically distinct populations that happen to be morphologically indistinct (Khan 2012).

2.2 The history of insecticide control of whitely

Bemisia tabaci first appeared in North American greenhouses and fields on poinsettia grown in Florida in 1986. The species is believed to have spread further through the transportation of infested plants (Price 1991). Agricultural intensification and little tolerance for blemished products have resulted in higher usage of insecticides that put significant pressure on insects to adapt resistance (Dittrich and Ernst 1996). By the 90’s resistance to organochlorines, organophosphates, carbamates, and pyrethroids was apparent (Horowitz and Ishaaya 1996). The next generation of insecticides included the neonicotinoids and insect growth regulators (IGR) showed effectiveness at controlling populations previously resistant to organophosphates and pyrethroids (Denholm et al 1996, Horowitz and Ishaaya 1996, Singh et al 2013). Many of these newer insecticides, like IGRs, will not make an immediate impact on adult populations but will decrease their populations over the course of days to weeks due to their effects on immatures. The use of economic thresholds in timing insecticide applications was implored with examples from different regions and crops (Horowitz and Ishaaya 1996).

IGRs and neonicotinoids were highly effective on first use, but their overuse led to the rise in resistance to these as well (Palumbo et al. 2001). Successive use of the same insecticide will lead to resistance in *B. tabaci* (Prahbaker et al. 1996). Rotating applications of different modes of action should lower the chance of resistance development (Castle et al. 2002). Mixtures have the risk of creating resistance to both insecticides if used too often and should be used rarely for that reason and effects they may have on beneficial arthropods and plant health.

Insecticides can be categorized as mainly systemic or contact (Horowitz and Ishaaya 2016) relative to whitefly control. Examples of these include systemics like flupyradifurone (Sivanto) that penetrate plant tissues and enter the insect during feeding. Comparatively, a contact like bifenthrin (Brigade) must make physical contact with the whitefly and penetrate through its cuticle to be effective. A summary of the pesticides traditionally used to control whiteflies is summarized in Table 2 along with the insecticides' activity relative to being mainly as a contact toxin or systemically ingested toxin. This is important to determine the best bioassay method to use for a particular insecticide. Most of the insecticides used for the control of whiteflies in recent times have systemic activity (Horowitz et al. 2020).

Table 2.1. Pesticides typically used for the control of whiteflies in the USA in recent years, whether or not they are mainly a contact or systemic product relative to the crop and the reference cited.

IRAC group	Product common name	Example commercial name	C=contact S=systemic B=both	Reference cited*
1A	oxamyl	Vydate 2.4LV	B	Thomson (2001)
1B	naled	Dibrom 8	C	Thomson (2001)
3A	beta-cyfluthrin	Baythroid XL 1EC	C	Thomson (2001)
3A	bifenthrin	Brigade 2EC	C	Thomson (2001)
3A	fenpropathin	Danitol 2.4EC	C	Thomson (2001)
3A	lambda-cyhalothrin	Karate 2.08CS	C	Thomson (2001)
4A	acetamiprid	Assail 30SG	B	Thomson (2001)
4A	clothianidin	Belay 50WDG	S	Thomson (2001)
4A	dinotefuran	Venom 70SG	S	Thomson (2001)
4A	imidacloprid	Admire Pro 4.6F	B	Thomson (2001)
4A	thiamethoxam	Actara 25WDG	B	Thomson (2001)
4C	sulfoxaflor	Transform WG	S	Dow (2019)
4D	flupyradifurone	Sivanto Prime 1.67SL	S	Bayer (2019)

7C	pyriproxyphen	Knack 0.86EC	C	Thomson (2001)
9C	flonicamid	Beleaf 50SG	C	FMC (2019)
15	novaluron	Rimon 0.83EC	C	Chemtura (2020)
16	buprofezin	Courier 3.6SC	C	Thomson (2001)
23	spirotetramat	Movento 2SC	S	Bayer (2017)
23	spiromesifen	Oberon 2SC	S	Bayer (2010)
28	cyantraniliprole	Exirel 0.83SC	B	FMC (2018)
28	chlorantraniliprole	Coragen 1.67SC	B	FMC (2017)
un	azadirachtin	Neemix 4.5	C	Certis (2019)

* reference limited to product information, additional chemical information from Yu (2008).

Dittrich and Ernst (1996) reviewed the chemical control and subsequent resistance of *Bemisia tabaci* since 1970. They explain the reason whiteflies have become such a major pest is due to the intensification of growing practices in the field and the use of greenhouses. Ornamental greenhouses received the highest application of insecticides due to the low tolerance for feeding damage or blemishes like sooty mold. As a result, resistance was quickly developed which led to further development of resistance. Cotton was one of the most sprayed field crops for whiteflies and other pests. *Bemisia tabaci* has been reported to go under acceleration using DDT in Sudan. The stressor of the DDT did not necessarily kill the population due to resistance developed, but placed enough stress on their physiology to increase fecundity. *Bemisia tabaci* first appeared in North American greenhouses and fields on poinsettia grown in Florida in 1986. The species is believed to have spread further through the transportation of infested plants (Price 1991).

2.3 Insecticide bioassay methods for whitefly

Monitoring insecticide resistance in *Bemisia tabaci* has been critical to the management of this worldwide insect pest since the early 1980's (Prabhaker et al. 1985, Dittrich et al. 1990, Staetz et al. 1992, Denholm et al. 1996, Horowitz and Ishaaya 1996, Palumbo et al. 2001, Schuster et al. 2010, Caballero et al. 2013b). The appearance of biotype-B (MEAM1) of *B. tabaci* accelerated interest in understanding resistance because of reported associations between whitefly biotypes and insecticide resistance (Alon et al 2006) and biotype invasions into crop production areas (Ma et al. 2006). The bioassay methods used for monitoring resistance include treated vials for contact activity against adult whiteflies (Staetz et al. 1992), clip cage on plants or cuttings that were either directly drenched (Caballero et al. 2013a) or had insecticide mixtures applied systemically through the leaf petiole (Caballero et al. 2013a, 2015, Smith 2013), and similar bioassay application methods, but evaluated in a petri dish (Ma et al. 2007, Smith 2013, IRAC 2016) or a cup (IRAC 2009) container. Typically, only a single bioassay method was employed for a given insecticide resistance study.

2.4 Insecticide resistance management (IRM)

Control of *Bemisia tabaci* relies on the use of insecticides in most grower operations. Insecticides provide the most efficient and guaranteed reduction in pest insect populations of the tools available (Palumbo et al. 2001, Horowitz et al. 2011). Over reliance and repeated use of the same insecticides inevitably result in resistance to that insecticide within the pest population. The use of insecticides generates a selective pressure on the pest population that increases the frequency of genes conferring resistance within the pest species (Georghiou 1969, Denholm and Rowland 1992).

Resistance to insecticides can occur by changes in how the chemical binds to the target site. The active ingredient can fail to bind or only partially bind to receptor molecules and fail to trigger the proper response in the insect cells. It can become more difficult for the insecticide to enter the body by reducing the ability to penetrate cuticle or be absorbed through feeding. Mutations would also allow the insect to better break down or excrete the insecticide before it reaches the target site (Nkya 2012).

Due to the ease at which *B. tabaci* develops insecticide resistance, management programs are necessary to reduce the chance that any insecticide formulation becomes ineffective on the species. IRM should always be included in any effective Integrated Pest Management (IPM) program. Part of doing so involves using insecticides according to their labels and selecting them based on what Mode of Action (MoA) is known to be effective for the local population. Of course, non-chemical control methods should always be considered first since they do not contribute to resistance development (Sparks and Nauen 2015, Horowitz et al. 2020). These include reflective mulches and host plant resistance to the plant virus to reduce the impact of whiteflies (Riley and Srinivasan 2019).

Switching between insecticides with different MoAs is thought to limit the development of resistance to any one chemical. Repeated use of similar insecticides creates selective pressure for changes in the single target site that increase in frequency within the population. Since chemicals in different IRAC groups have different binding sites and cause different changes within the insect body, it becomes harder for a mechanism to develop that can protect two target sites from two different molecules (Georghiou 1969, Denholm and Rowland 1992, Sparks and Nauen 2015). Though unlikely to happen, cross-resistance has occurred within the sweetpotato whitefly before between neonicotinoids and pymetrozine (Gorman et al. 2010, Nauen et al. 2013). The rotation of insecticides should be timed so that successive generations are not treated with the same MoA. For *B. tabaci*, that means a new insecticide should be used every 30 days and the same insecticide should only be reused at least 60 days (two generations) after its first use (Horowitz et al. 2020).

Mixing multiple insecticides with different MoA has been a tactic used for whitefly control (Horowitz and Ishaaya 1996, Denholm et al. 1998, Castle et al. 2002). Much like with rotations, it is thought to be rare for a pest population to develop a mechanism for resistance of two different chemicals that target different parts of the body. Cross-resistance is a major concern of this technique also (Denholm and Rowland 1992, Horowitz and Ishaaya 1996). The use of pyrethroids and OPs together was common in the 1990s. Control failure has resulted due to overreliance on the combination and development of resistance to both groups (Denholm et al. 1998, Dennehy and Williams 1997). Labels and research must be consulted before mixing any insecticides as not all combinations will be effective (Cloyd 2010, South and Hastings 2018). Despite previous failure, this method is still used extensively by growers (Horowitz et al. 2020).

Non-chemical methods for population control include exclusion of whiteflies from the crop through screens, reflective mulch, and cover cropping (Horowitz et al. 2011, Greer and Dole 2003, Hilje and Stansley 2008) and the use of natural enemies (Naranjo and Ellsworth 2009) to keep populations below damaging levels. These methods do not rely on any chemical to control and are able to reduce whitefly numbers without contributing to resistance development. In addition, these methods have a lower environmental impact than insecticides and should always be considered in an IPM approach to pest control (Sparks and Nauen 2015, Horowitz et al. 2020).

Natural enemies include entomopathogenic fungi, parasitoids, and predators. Generalist predators such as mites and mirids will not only limit whitefly numbers, but they will feed on other pest species such as aphids (Nomikou et al. 2001, Alomar et al. 2006). Predators are often present already within the crop system but are also killed as a side effect of broad-spectrum insecticide sprays and no longer available for further whitefly control. Their populations can be sustained by strategic application of insecticides as well as maintenance of the surrounding field site to continue a reservoir population (Heinz and Nelson 1996, Naranjo et al. 2004, Vandervoet et al. 2008, Naranjo and Ellsworth 2009). Parasitoids are also present within the natural environment but are specific to which pest species they target. Still, several species are available for their use on *B. tabaci* (Foltyn and Gerling 1985, Van Lenteren et al. 1997).

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CHAPTER 3: COMPARISON OF TOXICOLOGICAL BIOASSAYS FOR ADULT WHITEFLIES[1]

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Simple Summary: Insecticides are commonly used to manage whiteflies in many crops including vegetables, but frequent use can cause these pests to become resistant to insecticides. Resistance can lead to control failure and severe crop damage, thus the need for insecticide efficacy testing and insecticide resistance monitoring. A study was conducted to determine if any current methods of toxicity assays are better than others for testing whitefly adults for insecticide resistance and efficacy for better information to make effective pest control decisions.

Abstract: Two *Bemisia tabaci* populations from Georgia and Florida, USA, were tested for their response to insecticides across different toxicological bioassay methods. Five insecticides in four Insecticide Resistance Action Committee (IRAC) groups (imidacloprid (4A), dinotefuran (4A), flupyradifurone (4D), pyriproxyfen (7C) and cyantraniliprole (28)), were evaluated against a water check. The routes of application to the plant used were either leaf drench or (systemic) root drench. The four different whitefly bioassay methodologies tested were two published IRAC methods, a clip cage method, and a new tube method. A split–split experimental design was used to assess any interactions between application route, bioassay method and insecticide treatment. Application route had no significant effect on efficacy. However, bioassay method affected overall whitefly mortality, with the dish method having reduced mortality compared to other methods, except for the clip cage method. High rates of cyantraniliprole, dinotefuran and flupyradifurone insecticides resulted in the highest incidence of adult whitefly mortality. Significant interactions relative to percent adult mortality were found between the insecticide and bioassay method for both populations assayed. The clip cage method was more sensitive in terms of dose mortality response followed by the cup and tube methods. The dish method was the least responsive to insecticide dose. Other interactions are discussed.

Keywords: *Bemisia tabaci*; bioassay; insecticide resistance management; IRM; toxicology

3.1. Introduction

Bemisia tabaci (Gennadius) is a major pest of concern for vegetable growers in the southeastern United States. This diminutive hemipteran feeds on tomatoes, cucurbits, Cole crops, beans, peppers, and numerous other crops including cotton and ornamentals [1]. Whiteflies transmits at least 300 known plant diseases [2]. Through the use of toxicological bioassays, the highly prevalent biotype-B (Middle East-Asia Minor 1; MEAM1) was reported as expressing resistance to carbamates, organophosphates, pyrethroids, and imidacloprid from 1990 to 2013 [3-8]. Resistance to growth regulators like pyriproxyfen were documented within the southwestern USA [9] and by 2011, baseline susceptibilities to the new anthranilic diamides were also being established [10]. By 2007, there was bioassay evidence that more insecticide resistant populations were displacing the less resistant whitefly populations in China [11]. In Florida, imidacloprid was being over used to control whitefly-transmitted plant virus, so effective insecticide resistance management programs had to be developed [12]. Clip cage bioassay data formed the basis of this program which continued on to add diamides, such as chlorantraniliprole and cyantraniliprole, to the long-standing neonic bioassay surveys [13–17]. Increased resistance to cyantraniliprole in the Q biotype (Mediterranean; MED) was reported in 2018 through extensive toxicological tests [18], so the use of bioassays for whitefly insecticide resistance management continues to be of worldwide importance [19].

There is very limited information of the status of whitefly insecticide resistance in Georgia, USA [20]. The majority of insecticide resistance management (IRM) information for the southeast has been generated in Florida [12-17]. In the fall of 2017 in Georgia, there were record breaking whitefly populations and extensive vegetable crop damage following a population increase in cotton [21]. This event renewed interest in regional management of whiteflies and highlighted the need for insecticide efficacy testing and insecticide resistance monitoring for this pest. The first step for this new state monitoring program was to determine which whitefly bioassay method would provide the cleanest insecticide response data for a large-scale resistance monitoring program in Georgia.

The bioassay methods previously used for monitoring resistance include treated vials for contact activity against adult whiteflies [22], clip cage on plants or cuttings that were either directly drenched [13] or had insecticide mixtures applied systemically through the leaf petiole [13–16], and similar bioassay application methods, but evaluated in a petri dish [11, 17, 23] or a cup [24] container. This diversity in methodology reflects in part the various physical pathways of the toxicant to the whitefly, *i.e.*, whether the insecticide exhibits cuticular contact toxicity, toxicity through ingestion, or a combination of both. Because whiteflies are phloem feeders [25] and most insecticides used against whiteflies are currently systemic [19], most methods allowed the insecticide to be imbibed by the plant before bio-assaying. There is a need for comparison studies to evaluate the consistency between bioassay methods which allow for whitefly feeding needed further investigation relative to representative insecticides used against whiteflies.

Insecticides can be categorized as mainly systemic or contact relative to whitefly control. Examples of these include systemics such as cyantraniliprole and dinotefuran and mostly contacts such as the growth regulator buprofezin or the pyrethroid insecticide bifenthrin. Further, the action of insecticides can be quick knockdown, *e.g.*, imidacloprid, or slow acting, *e.g.*, insect growth regulators such as pyriproxyfen [26]. One bioassay method to compare diverse insecticide response based on a single collection of adults is a difficult goal. It was hypothesized that assessing mortality of adults along with effects on oviposition and egg hatch could provide such a multipurpose method. Thus, the objective of this study was to compare different types of methodologies for assessing adult mortality, oviposition, and nymph emergence. The null hypotheses tested were that (1) application route (drench vs. foliar application), (2) whitefly bioassay method and (3) insecticide treatment would not result in significantly differing whitefly mortality, and there would not be significant interactions between each of these factors.

3.2. Materials and methods

Laboratory whitefly colonies of *B. tabaci* used in this study were from a field collection on cotton in Georgia during a summer outbreak in 2017 at the Coastal Plain Experiment Station at Tifton, Georgia and from a whitefly population at the Gulf Coast Research and Education Center at Wimauma, Florida in the summer of 2018. Both colonies were maintained on cotton plants in individual rearing rooms until assays for this study were initiated during the spring of 2019. Adult *B. tabaci* were aspirated into pipettes from leaves of colony plants before being introduced to bioassay arenas. All plant material used in the bioassays were either whole plant or excised leaves from untreated cotton ST4946GLB2 seed grown at 30°C, 70% relative humidity, and 16:8 day: night cycle in growth chambers (Percival model E-36L2, Perry, IA, USA). The cotton plants in these tests were grown to the two-expanded-true-leaf stage before using. Initial whitefly mortality counts were taken one hour after introduction of adults to the bioassay arenas. This was done to assess whitefly mortality due to handling. This initial mortality was subtracted from later mortality counts because they did not reflect the effects of treatment on the insects. Adult whiteflies were counted as dead if they met the following criteria: lack of discernible movement, obvious desiccation, and/or resting on a surface with contact made by any part of the body other than the tarsi. Whiteflies that moved or were in contact with the plant tissue in what could be described in typical feeding posture were marked as live. Counts of living and dead whiteflies were taken every 24 h and testing was concluded after 72 h elapsed. Cotton leaves used for each test were placed in labeled Ziplock bags and frozen for later egg and nymph counts. Counts were made on the whole underside of leaves with a dissecting microscope at 20 x magnification.

The experimental unit in this study was the bioassay arena. Treatments were arranged in a split-split plot design with the main treatments as the application route, *i.e.*, leaf drench or systemic root treatment. The sub-treatments were the four assay methodologies used, and sub-sub-treatments were the insecticide treatments with two rates, the active ingredient at a high labeled rate and 1/10 that concentration. Specific methodologies were described in the following sections. The split-split experiment was employed to detect interactions between treatment levels.

3.2.1. Application Route (Main Plot)

Leaf dip treatments were performed by dipping cotton leaves into the sub-sub-treatment for 30 seconds. Afterward, they were air-dried under ventilation for one hour to reduce the possibility of drowning introduced whiteflies on surface water droplets. Systemic treatments were administered by filling the 20-ml scintillation vials used for the clip cage and tube methods with the sub-sub-treatments. The roots of cotton plants used in these tests were trimmed to ~5-cm length before placing in the vials with continual access to the insecticide for 24 h before introducing whiteflies. The reservoir space in the IRAC 008 method [24] was filled with insecticide solution or water and the leaf petiole was placed inside, allowing for continued uptake into the leaf inside of the top cup. Only the IRAC 015 method [23] with the petri dish did not provide a way for continual uptake into the leaf. Excised leaves instead had their petioles placed inside of the designated sub-sub-treatment and allowed to uptake the solution for 24 h before the introduction of whiteflies to the arena.

3.2.2. Bioassay Method (Subplot)

Clip cages were constructed of foam pipe insulation meant for 25.4 mm pipe by FrostKing (Thermwell, Mahwah, NJ, USA) sliced into rings with mesh screening glued on top to create a bioassay arena that could be placed over treated leaves and allow viewing of the whiteflies within (Figure 1). Aspirated adults were cooled for 5 min at 2°C to prevent escape during introduction to the arena. Whiteflies were tapped out of the pipettes and into the clip cage before securing the clip cage to the underside of the cotton leaf using another foam ring and paperclips. Plants used in this methodology had at least one fully mature leaf and was placed in a 20-ml scintillation vial with roots washed of soil, and the vial was filled with tap water. Plants used in a leaf drench treatment had the leaf designated for whitefly feeding dipped in treatment solution, while those used in root drench had their 20-ml vial filled with 15 ml of treatment solution.

The IRAC 008 method or the "cup" method used two clear, plastic cups as the arena [23]. The two cups (Fisher Science Education™ Plastic Cups, Waltham, MA, USA) were stacked inside one another forming a space between the bottom of the two that was filled with either water or the insecticide solution that was being tested (Figure 1). A hole ~ 2 mm in diameter was made on the bottom of the top cup so that the petiole of an excised leaf could fit through and attain water from the reservoir without allowing the whiteflies in the top cup to fall in. Adults were tapped into the arena from the pipette and screening was placed over the mouth of the cup to prevent escape.

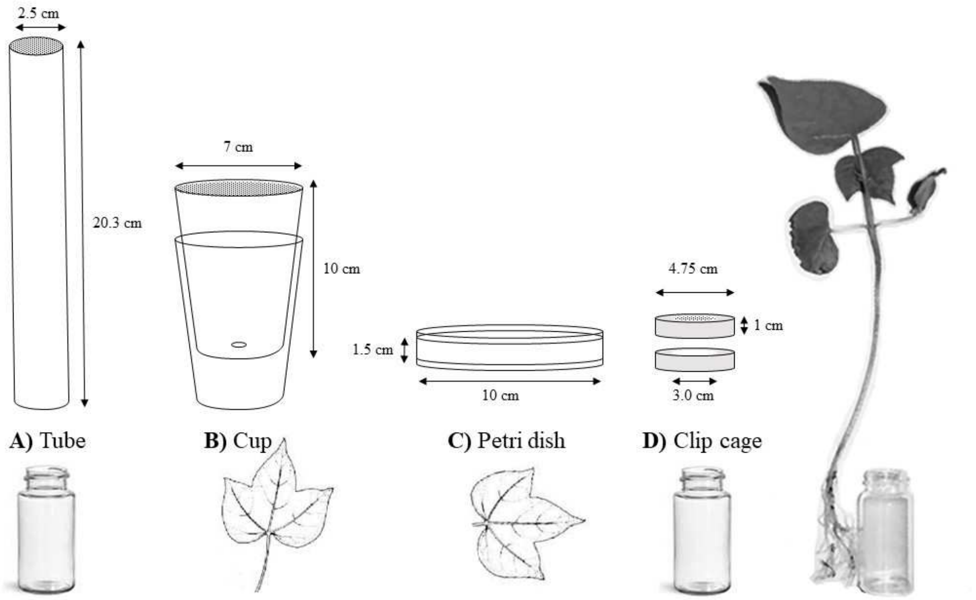

Figure 1. Bioassay methods for testing whitefly adult mortality: A) tube, and D) clip cage methods used a cotton seedling, B) cup and C) petri dish methods used an excised cotton leaf.

The IRAC 015 method required an agar gel to be made and pressing the treated leaf into the gel so that the underside was exposed out of the gel on which the whiteflies to feed [23]. Agar solution was poured into a 100 mm by 15mm disposable polystyrene petri dish (VWR™ 2020, Radnor, PA) with multiple ~0.5 mm diameter ventilation holes punched into the top lid for air flow (Figure 1). Care was taken to time the gel so that it was not so hot as to damage the leaf, but also not completely solidified to the point the leaf could not be pressed into it, *i.e.* still in liquid state. If not pressed into the gel enough, the leaf would fail to stay hydrated during the experiment and wither to the point that the adults would not feed on it. Leaves used for the systemic testing had their petioles placed in the treatment solution and could imbibe for 24 h before the beginning of testing. This was the only incidence in which systemic treatments were given before testing began and was done because the methodology did not provide for continuous systemic treatment.

The UGA tube method was created for testing insecticide response over an entire whitefly life cycle, *i.e*., adults, eggs, nymphal stages, and adult emergence. This method involved placing cotton seedling into 20-ml scintillation vials with water or insecticide as described earlier in the clip cage explanation. Due to size constraints, the plant selected with this method was the same age, but with only one true leaf fully developed. All cotyledons or budding second leaf were removed from the plant. Cotton balls were fitted around the stem in the mouth of the vial and Parafilm™ (Fisher Scientific, Waltham, MA, USA) was stretched around the cotton ball plug to lock the stem of the plant in place as well as create a waterproof seal from the vial and the plant above. A clear plastic tube 2.86 cm in diameter and 20.3 cm in length produced by ClearTec Packaging (Park Hill, MO, USA) was placed over the plant using a paper sleeve or a laboratory spatula to position the curled cotton leaf inside the tube (Figure 1). Ventilation with nylon chiffon screening was made in the side of the tube to reduce humidity and the possibility of condensation forming which trapped whiteflies inside the tube arena. Adults would stick to the plastic wall if they were wet or drowned in condensation droplets.

3.2.3. Insecticide Treatment (Sub-subplot)

Two sets of water-treated control treatments were prepared along with 10 insecticide treatments. Five insecticide products commonly used for whitefly control were chosen: pyriproxyfen (Knack 0.86EC, IRAC Group 7C, Valent Corporation, Walnut Creek, CA, USA), dinotefuran (Venom 70SG, IRAC Group 4A, Valent Corporation, Walnut Creek, CA, USA), cyantraniliprole (Exirel 0.83SC, IRAC Group 28, FMC, Philadelphia, PA, USA), flupyradifurone (Sivanto 200SL Bayer Crop Science, Philadelphia, PA, USA), and imidacloprid (Admire Pro 4.6F (Bayer Crop Science, Research Triangle Park, NC, USA). Two concentrations of each product were used during testing: the maximum label rate for vegetables according to the Georgia Pest Management Handbook [27] and one tenth that rate. These were pyriproxyfen 82.4 mg a.i. and 8.24 mg a.i., imidacloprid 99.2 mg a.i. and 9.92 mg a.i., dinotefuran 210 mg a.i. and 21 mg a.i., cyantraniliprole 105.4 mg a.i. and 10.54 mg a.i., and flupyradifurone 188 mg a.i. and 18.8 mg a.i. per liter, respectively. Two untreated checks were used in these experiments to measure variation within a treatment.

3.2.4. Data Analysis

Each split-split plot experiment had four replicates. The experiment was repeated twice using the whitefly populations, the Georgia and Florida laboratory colonies. All corrected percent mortality data were run through SAS Enterprise [28] using the Proc GLIMMIX procedure considering reading hour as a repeated measure. Interactions between treatment levels were measured as well as splicing each treatment into their 24-h observation points. Doing so allowed us to analyze how mortality changed over time. The 72-h live adult and total oviposition data were subjected to individual analysis of variance by using Proc GLM in SAS [28] using a spilt-split plot design with insect counts fitted to a negative binomial distribution. Significant interactions were reported and means graphed with standard errors for describing interaction effects. Main, sub and sub-subplot means separation was tested with Tukey's (for repeated measures data) and LSD (for non-repeated measures data such as egg counts) tests ($p < 0.05$) following a significant split-split-plot level effect ($p < 0.05$).

3.3 Results

The different whitefly populations used in these tests provided experimental replication, but without a major difference between these populations in terms of overall insecticide susceptibility. The overall average % mortalities for the Florida and Georgia populations by 24, 48 and 72-hour reading were 26 ± 1.3, 49 ± 1.7, 58 ± 1.8, and 36 ± 1.6, 57 ± 1.9, 68 ± 1.8, respectively. Thus, the Georgia population tended to be more susceptible by ~10%. Significant treatment effects on percent mortality tended to be the same across both populations with the only exception of the bioassay by insecticide interaction (Table 1).

Table 1. Effects of treatments and their interactions on adult mortality (percent dead corrected by subtracting dead adults at the time of loading the arenas) for Florida and Georgia whitefly populations, Coastal Plain Exp. Station, Tifton, GA, 2019.

Treatment	Florida population			Georgia population		
	DF[1]	F	*P*>F	DF	F	*P*>F
Application Route	1, 26.5	0.29	0.598	1, 23.7	0.27	0.606
Bioassay	3, 26.6	3.18	0.040	3, 23.9	8.29	<0.001
Insecticide	11, 296	41.5	<0.001	11, 354	39.1	<0.001
Hour	2, 915	222	<0.001	2, 798	338	<0.001

Application*Bioassay	3, 26.5	0.82	0.492	3, 23.2	1.95	0.149
Application*Insecticide	11, 315	1.88	0.041	11, 404	5.97	<0.001
Application*Bioassay*Insecticide	33,315	1.15	0.269	33, 399	1.12	0.307
Bioassay*Insecticide	33, 313	1.20	0.213	33, 394	2.69	<0.001
Hour*Application	2, 911	0.03	0.969	2, 760	1.08	0.339
Hour*Bioassay	6, 914	2.21	0.040	6, 769	3.96	<0.001
Hour*Insecticide	22, 913	2.75	<0.001	22, 766	2.91	<0.001

[1] Proc Glimmix degrees of freedom (numerator, denominator), results fitted to a normal distribution of data.

3.3.1. Application Route (Main Plot)

The application route, leaf vs root drench, did not significantly affect percent adult mortality regardless of the bioassay method or the insecticide tested for either whitefly population (Table 1). Application route did not affect oviposition counts in the Florida or Georgia population (Table 2). Differences in nymph hatch were not observed between the two application routes (Table 3). This confirmed that either application route was equally effective for assessing adult mortality using the diverse active ingredients in this study. This suggested that data generated from air dried leaf dips are comparable to 24 h systemic applications. Since these insecticides will usually enter the whitefly through ingestion of vascular fluid, it is likely that the insecticides used in this study could penetrate plant leaf tissues. There were a few significant interactions with one or the other whitefly population, but not both, described in the section on interactions.

Table 2. Effects of treatments and their interactions on egg numbers for a Florida and Georgia whitefly population, Coastal Plain Exp. Station, Tifton, GA, 2019.

Treatment	Florida population			Georgia population		
	DF[1]	F	*P*>F	DF	F	*P*>F
Application Route	1, 2	0.16	0.729	1, 3	3.74	0.149
Bioassay	3, 6	3.53	0.089	3, 9	2.39	0.136
Insecticide	11, 220	6.34	<0.001	11, 264	3.12	<0.001
Application*Bioassay	3, 6	0.25	0.859	3, 9	0.38	0.773
Application*Insecticide	11, 220	0.97	0.474	11, 264	1.74	0.065
Application*Bioassay*Insecticide	33, 220	0.95	0.554	33, 264	0.58	0.968
Bioassay*Insecticide	33, 220	1.44	0.065	33, 264	0.77	0.817

[1] Proc GLM degrees of freedom (numerator, denominator), results fitted to a normal distribution of data.

Table 3. Effects of treatments and their interactions on small nymph numbers for a Florida and Georgia whitefly population, Coastal Plain Exp. Station, Tifton, GA, 2019.

Treatment	Florida population			Georgia population		
	DF[1]	F	*P*>F	DF	F	*P*>F
Application Route	1, 2	1.30	0.372	1, 3	4.12	0.135
Bioassay	3, 6	0.820	0.529	3, 9	0.78	0.533
Insecticide	11, 220	1.16	0.317	11, 264	0.91	0.527
Application*Bioassay	3, 6	1.46	0.316	3, 9	0.78	0.533
Application*Insecticide	11, 220	0.85	0.595	11, 264	0.79	0.646
Application*Bioassay*Insecticide	33, 220	0.72	0.866	33, 264	0.73	0.860
Bioassay*Insecticide	33, 220	0.87	0.673	33, 264	0.97	0.526

[1] Proc GLM degrees of freedom (numerator, denominator), results fitted to a normal distribution of data.

3.3.2. Bioassay Method (Subplot)

The bioassay method significantly affected adult mortality for both populations (Table 1), but not oviposition (Table 2) and nymph hatch (Table 3). Among the four methodology types, the dish method exhibited reduced mortality compared to the tube method for both populations (Figure 2A, B). Interestingly, the dish method also had the fewest eggs laid on the test leaf tissue

(Figure 3). The clip cage and cup methods resulted in the largest egg count overall while the tube method resulted in an intermediate count (Figure 3). There were several significant interactions between bioassay method and other treatment levels discussed in the section on interactions.

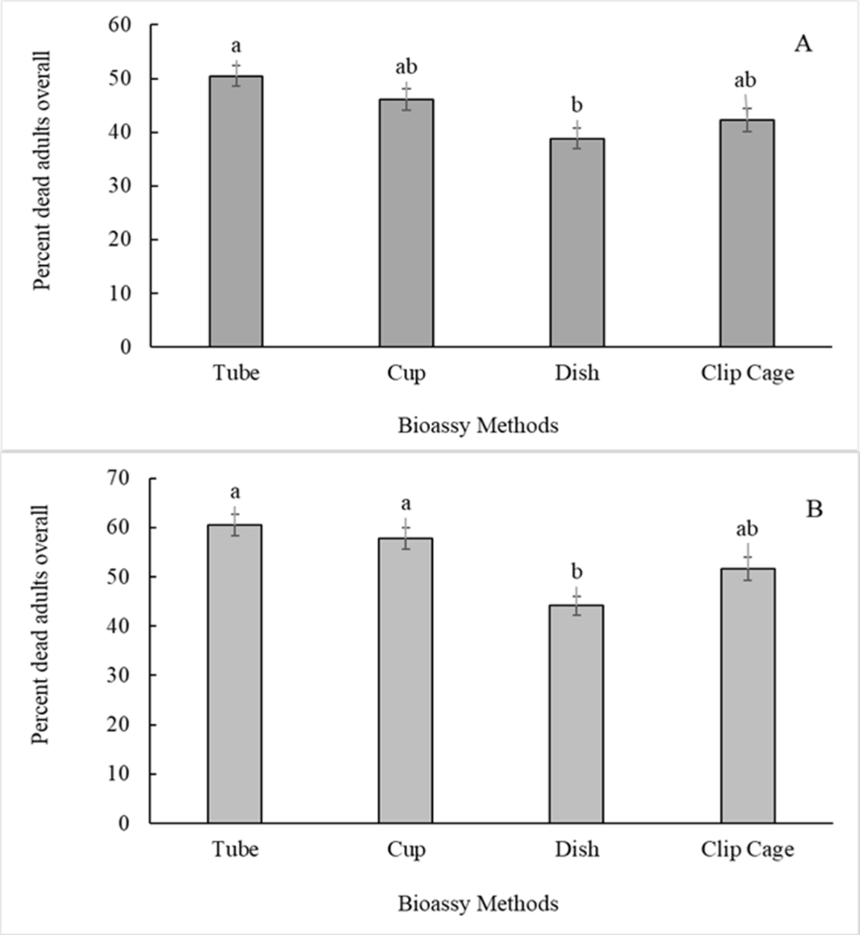

Figure 2. Percent adult mortality of the Florida (A) and Georgia (B) whitefly populations for tube, cup, petri dish, and clip cage bioassay methods. Bioassay means followed by the same letter are not significantly different, Tukey's test $p < 0.05$.

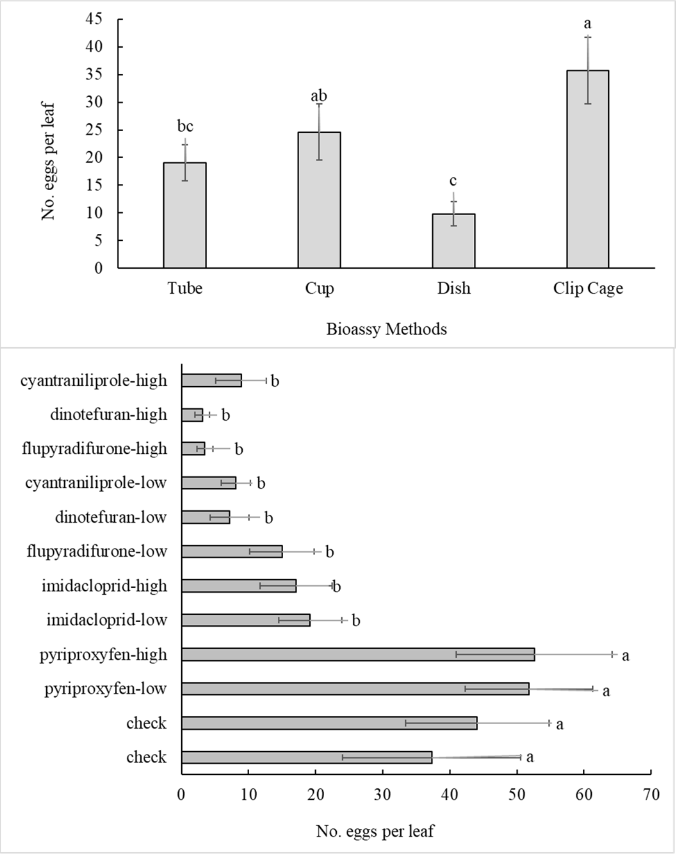

Figure 3. Total number of eggs observed on bioassay leaves over both whitefly populations combined across the tube, cup, petri dish, and clip cage methods (A) and insecticides (B). Means within treatment with the same letter are not significantly different, LSD $p < 0.05$.

3.3.3. Insecticide treatment (Sub-subplot)

There were significant insecticide treatments effects for both populations in terms of percent dead (Table 1) and number of eggs laid (Table 2). There was a lack of significant insecticide effects on nymph hatch (Table 3). This was likely due to the lack of sensitivity of these methods to detect nymph hatch with the short turn-around time from oviposition to nymph assessment. Adequate assessment of treatment effects whitefly nymph mortality was not indicated with this methodology and will likely need pre-infested leaves as a starting point. However, assessment of adult mortality was clearly achievable with all the methods tested at 24 and 48 hours (Figure 4A-D) with a couple of caveats. There was a significant amount of initial mortality in the checks that has to be accounted, averaging approximately 15% overall, even with eliminating initial mortality as a consequence of loading the arenas (assumed to be mechanical injury unrelated to the bioassay treatments). Second, the difference in mortality due to rates was not significant for three of the five insecticides tested (Figure 4A).

Sensitivity of a bioassay methodology to rates is a desirable attribute if monitoring dose mortality response is the goal. The most efficacious insecticide treatments were the high rates of flupyradifurone, cyantraniliprole and dinotefuran, each of which had significantly higher mortality than the high rates of imidacloprid and pyriproxyfen as well as the water check, overall (Figure 4E, F). Flupyradifurone had a significant rate response overall for both the Florida and Georgia populations (Figure 4E, F, respectively). Cyantraniliprole had a significant rate response in at least one population (Figure 4E, F). Significant interactions between bioassay method and insecticide treatment discussed in the section on interactions revealed sensitivity to rates.

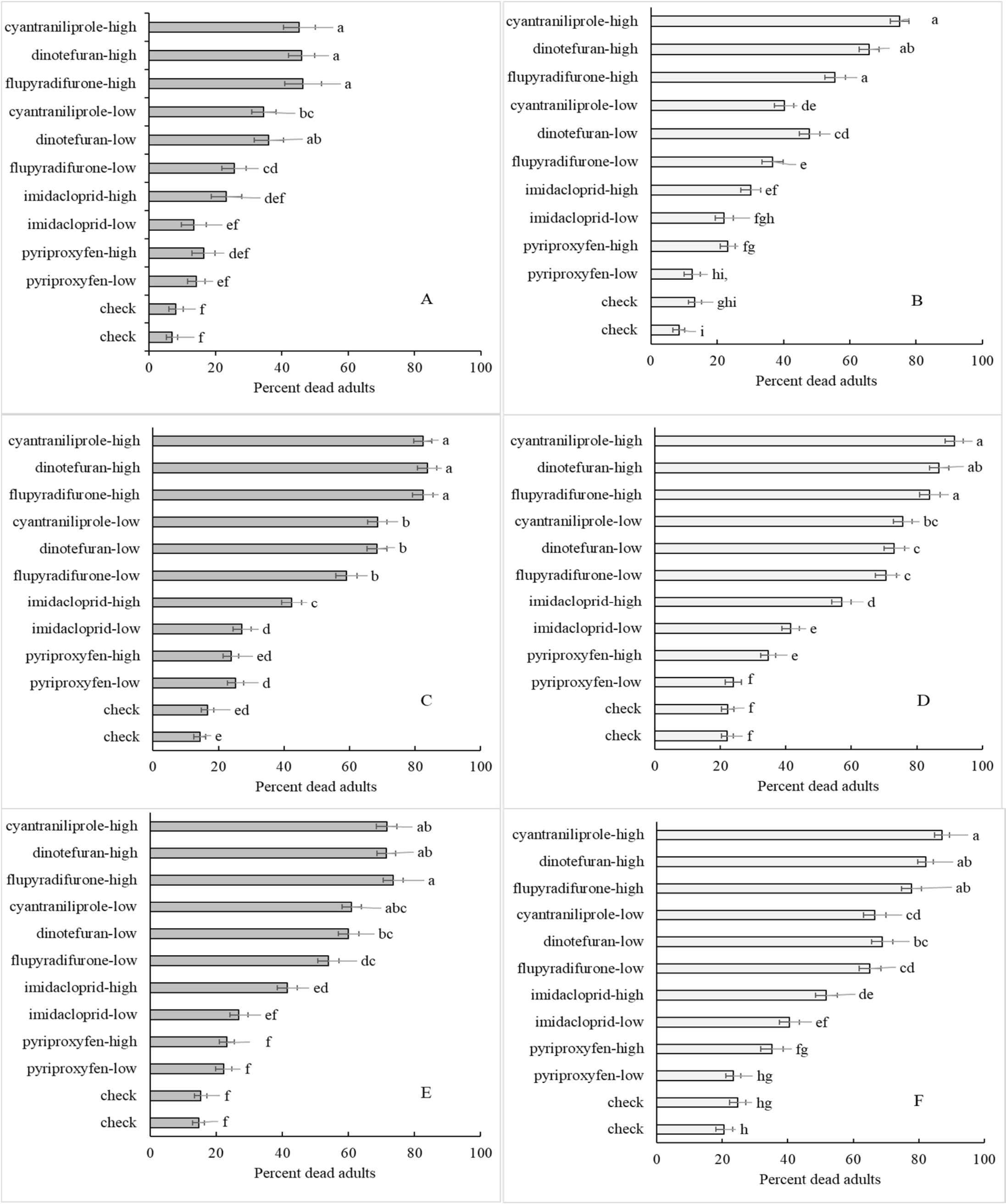

Figure 4. Percent adult mortality of the Florida (A, C, E) and Georgia (B, D, F) whitefly populations across insecticide treatments at 24 h (A, B), 48 h (C, D) and overall (E, F) (means with the same letter not significantly different, LSD 24 h and 48 h, Tukey's test overall, $p < 0.05$).

Neither concentrations of pyriproxyfen had significant differences in mortality or egg counts from both controls, though it did display a slightly raised mortality in adults. Pyriproxyfen, being an insect growth regulator (IGR), was not expected to show any mortality in adults and only affect nymph development. As previously mentioned, this methodology did not serve to assess this activity. To properly test the effectiveness of an IGR would require maintaining nymphs and observing mortality or growth rates over more time than the three days allotted for these bioassays.

3.3.4. Interactions

The effect of time on mortality was significant and contributed to several significant interactions (Tables 1 and 2, respectively). A longer insecticide exposure interval increased the likelihood for lethal effects in the adult (Figure 5). In the Georgia population, there were significant interactions between hour and three factors, application route, bioassay method and insecticide treatment (Table 1). In the Florida population, only the hour x insecticide interaction was significant (Table 1). There was an hour x insecticide interaction which, based on the individual mean separation data, was that the differences in whitefly mortality between rates diminished for the most effective insecticides over time (Figure 4A, B, C, D). Also, the check mortality tripled from 24 h to 72 h, raising mortality rates in the less effective insecticides along with it. The average percent mortalities over both populations for the checks in tube, cup, dish and clip cage methods at 24 and 48 h were: 10.2 ± 1.6, 4.4 ± 1.9, 8.6 ± 3.6, 6.0 ± 2.7 and 15.0 ± 2.2, 12.7 ± 2.6, 18.2 ± 6.2, 21.8 ± 7.0, respectively. This explains in part how the mortality percentages increased with low rates of insecticide over time (Figure 4A, B, C, D). This interaction suggests that shorter bioassay intervals might better detect insecticide rate responses across all methods.

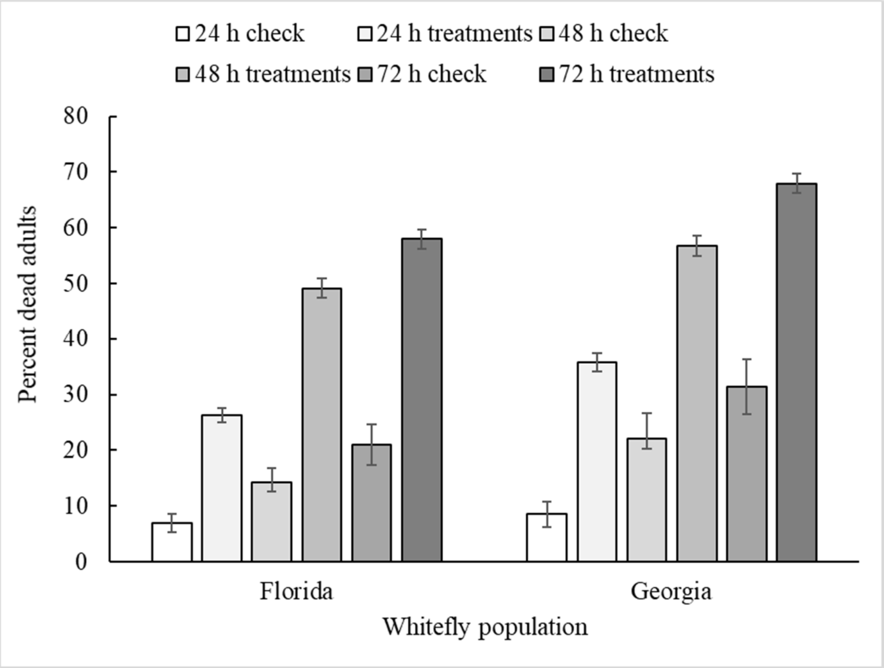

Figure 5. Mean % whitefly adult mortality (±se) for the untreated check and the average over all insecticide treatments at 24, 48 and 72 h for the two test populations.

The significant hour x bioassay method interaction for percent mortality was difficult to discern, but appears to be related to differences in bioassay response between hour readings (Figure 6). For example, the dish method had a clear stair-step change in response over time; this was similar to that seen in the overall mean in Figure 5. However, the tube and clip cage methods had the greatest change in response between 24 h and 48 h and much less change between 48 h and 72 h, suggesting that for these methods, waiting to 72 h does not provide as much additional response information as for the dish method. The average percent mortality over both populations for the checks in tube, cup, dish and clip cage methods at 72 h were: 26.8 ± 3.6, 25.2

± 5.9, 23.8 ± 6.1, 29.2 ± 6.8, respectively, which were all well above the acceptable amount of check mortality for a toxicological bioassay.

Unrelated to the reading time, there were significant interactions between the bioassay method and insecticide treatment for both populations in terms of percent dead (Table 1). In the Georgia population, there was also a significant application route x insecticide interaction for percent dead (Table 1). These interactions were important in that they related to how a particular method or application route might introduce bias into the response data. The range of Tukey's mean separation categories for each method was a-d (tube), a-e (cup), a-d or a-c (dish) and a-f or a-e (clip cage) (Table 4). A greater range suggested more sensitivity across treatments, so with this assumption, the clip cage method provided the most and the dish method the least sensitivity. As an example, the clip cage and cup methods separated out the high rates of the three top insecticides from the high rate of imidacloprid, whereas the dish and tube methods did not. The tube and the cup methods ranked treatments similarly (Table 4), whereas the dish had an odd response for the low rate of flupyradifurone (Table 4). The interaction between the application route and insecticides related to check mortality and the range of response for each route, *i.e.*, 14.6±2.9 % and 26.7+4.1 % dead in the check and 81.8+3.8 % and 92.7+2.2 % dead in the best insecticide treatment, cyantraniliprole, for the root drench and leaf dip routes, respectively. Root drench treatments tended to produce drier test leaves which in turn reduced mortality in the checks. Finally, there were no significant interactions in either population for egg numbers (Table 2) or nymphs (Table 3).

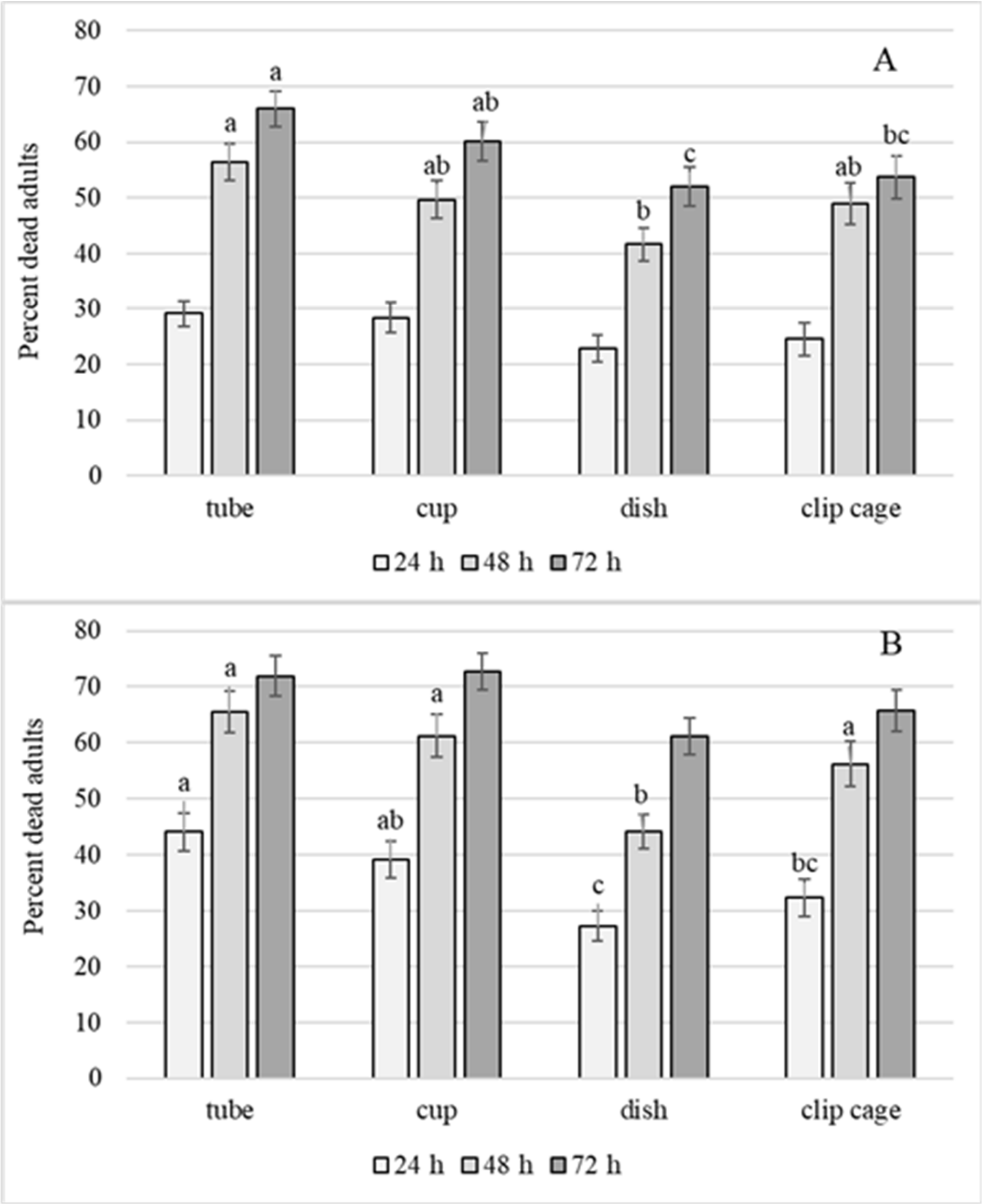

Figure 6. Mean % adult mortality (±se) of Florida (A) and Georgia (B) populations for tube, cup, petri dish, and clip cage methods at 24 h, 48 h and 72 h. Means within hour reading labeled with the same or no letter were not significantly different (LSD, P<0.05).

Table 4. Percent adult mortality of the Florida and Georgia whitefly populations across insecticide treatments by tube, cup, petri dish, and clip cage bioassay methods, respectively. Coastal Plain Exp. Station, Tifton, GA, USA, 2019.

Method	Insecticide treatment	Florida population	Georgia population
Tube	Check	25.3±3.15d	13.8±2.84d
	Check	13.5±1.91d	21.6±2.89d
	pyriproxyfen-low	20.7±4.22d	28.4±5.40d
	pyriproxyfen-high	21.7±3.27d	23.2±3.5d
	imidacloprid-low	32.9±4.66cd	53.6±6.62c
	imidacloprid-high	55.4±6.42bc	65.5±6.02bc
	flupyradifurone-low	64.3±5.73ab	78.0±5.76abc
	dinotefuran-low	73.7±5.02ab	85.0±4.51abc
	cyantraniliprole-low	69.0±5.31ab	79.3±5.26abc
	flupyradifurone-high	82.1±3.94a	87.7±4.31ab
	dinotefuran-high	77.2±4.73ab	95.8±1.56a
	cyantraniliprole-high	70.7±6.29ab	94.4±2.12a
Cup	Check	9.26±3.00e	19.6±3.88e
	Check	14.5±3.29de	23.3±3.18e
	pyriproxyfen-low	21.7±5.21cde	17.3±4.29e
	pyriproxyfen-high	18.1±3.57cde	45.6±7.66cde
	imidacloprid-low	26.9±4.98cde	44.5±5.93de
	imidacloprid-high	42.4±5.53bcd	56.8±6.40bcd
	flupyradifurone-low	50.5±5.72bc	73.4±6.10abc

	dinotefuran-low	67.1±5.75ab	77.4±4.91ab
	cyantraniliprole-low	65.4±5.33ab	70.5±7.01abcd
	flupyradifurone-high	77.7±5.54a	85.9±4.42a
	dinotefuran-high	81.1±3.99a	89.3±3.23a
	cyantraniliprole-high	77.1±4.44a	90.5±3.60a
Petri dish	Check	8.97±2.52d	25.8±5.74c
	Check	13.2±3.61d	30.0±7.03bc
	pyriproxyfen-low	25.6±5.16cd	24.5±4.75c
	pyriproxyfen-high	27.2±5.84cd	36.3±5.94bc
	imidacloprid-low	14.6±3.01d	32.7±5.47bc
	imidacloprid-high	31.6±5.09bcd	38.5±5.88abc
	flupyradifurone-low	55.7±6.22abc	57.3±7.52ab
	dinotefuran-low	48.4±5.54abc	48.4±6.69abc
	cyantraniliprole-low	54.6±5.73abc	49.1±5.87abc
	flupyradifurone-high	62.4±5.60ab	58.6±7.09ab
	dinotefuran-high	61.2±5.24a	58.2±4.76ab
	cyantraniliprole-high	65.8±7.04a	69.7±6.31a
Clip cage	Check	15.4±4.51d	23.6±7.01e
	Check	19.8±5.52cd	23.8±5.00e
	pyriproxyfen-low	21.2±5.49cd	23.1±3.63e
	pyriproxyfen-high	26.0±5.19bcd	35.7±8.74cde
	imidacloprid-low	33.3±7.89bcd	31.4±5.14de
	imidacloprid-high	36.5±6.22bcd	46.0±7.53cde
	flupyradifurone-low	45.7±7.21abcd	51.7±6.65cde

dinotefuran-low	50.9±7.23abc	64.1±6.82bc
cyantraniliprole-low	53.8±6.34ab	67.1±8.10bcd
flupyradifurone-high	71.9±7.96a	79.0±5.79ab
dinotefuran-high	66.1±7.88a	85.2±4.80ab
cyantraniliprole-high	72.6±6.71a	94.4±3.10a

[1] Means within method followed by the same lower case letter are not significantly different, Tukey's $p < 0.05$.

3.4 Discussion

Monitoring insecticide resistance in *B. tabaci* has been critical to the management of this worldwide insect pest since the early 1980's [3, 29]. Early concerns about whitefly toxicological bioassays where centered around contact toxicity as in glass vial bioassays [30] versus ingestion toxicity of systemic insecticides in leaf dip or membrane feeding studies [31]. For imidacloprid, a simple systemic treatment of leaves using a water pic was developed early on [5] which became a standard for testing systemic insecticides (13-16). The bioassay methods used for assessing adult mortality typically used clip cages on plants or excised leaves that were either directly drenched [13] or had insecticide mixtures applied systemically through the leaf petiole [16, 17]. Bioassays using treated leaves in a petri dish [17, 24] or a cup [23] container have also been used. The amount of systemic insecticide absorbed by leaf petiole treatments can be high, skewing response data up compared to the mortality measured in a field application [32], but it was not clear in the literature whether systemic (root or petiole) or leaf dip would also change insecticide response values. Our results suggest that either route, through the root/petiole or directly through the leaf itself provides similar adult mortality response.

In this study, we obtained similar results between the Florida and Georgia whitefly populations which give us some confidence that the interactions observed were predictive for what might be expected for these bioassays. The similarity in response between leaf and root drenches for these insecticides was reassuring for comparisons between these types of bioassays. There are relatively few whitefly toxicological bioassay studies where multiple methods were compared in the same project, mainly to assess either adult or immature whitefly response [14, 33]. Sain et al. [34] found that there was an advantage of using an intact leaf compared to multiple methods using leaf disk or detached leaf bioassays. Our results were similar in that the one detached leaf bioassay used, the dish method, provided the poorest dose response data for whiteflies. The reassuring result from our comparison is that the various methods of assessing whitefly response through bioassays is surprisingly similar, just reflecting a bit better resolution with some methods, such as the clip cage method. This means that the various bioassays employed in the literature for whitefly insecticide response are quite comparable.

3.5 Conclusions

Whitefly toxicological bioassays are critical in the development and maintenance of insecticide resistance management programs for the whitefly, *Bemisia tabaci*. We found that there is a fair amount of consistency between the various bioassay methods currently used for whiteflies, just small differences in the sensitivity to insecticide rate response. Clip cage bioassays with an intact leaf provided greater sensitivity to insecticide treatment than the petri dish method with the excised leaf. In these studies, high rates of cyantraniliprole, dinotefuran and flupyradifurone insecticides resulted in the highest incidence of adult whitefly mortality.

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Conflicts of Interest: The authors declare no conflict of interest**.**

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CHAPTER 4: WHOLE-PLANT INSECTICIDE BIOASSAYS FOR WHITEFLIES[2]

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[2] Sparks, T.C and D.G. Riley. To be submitted to *Journal of Economic Entomology*.

Abstract: Two *Bemisia tabaci* populations from Georgia and Florida, USA were tested for their response to insecticides using a whole plant bioassay method in 2018 and 2020. Six insecticides from four Insecticide Resistance Action Committee (IRAC) groups (Knack – pyriproxyfen (7C), Admire – imidacloprid (4A), Venom – dinotefuran (4A), Coragen – chlorantraniliprole (28), Exirel – cyantraniliprole(28), and Sivanto – flupyradifurone(4D) were tested at a high and low dosage. Results showed that all insecticides were capable of significantly reducing adult numbers on infested plants as well as reducing egg counts in the Florida population. However, control was more variable and less dramatic in the Georgia population with only a few active ingredients reducing adult and nymph numbers and none reducing the amount of eggs.

Key Words: *Bemisia tabaci*, bioassay, insecticide efficacy

4.1 Introduction

The B-strain of sweetpotato whitefly, *Bemisia tabaci* Gennadius MEAM1, is a worldwide pest of produce and ornamental crops (Abd-Rabou and Simmons 2010, Thompson 2011). Whiteflies weaken plants by feeding on phloem sap and removing nutrients from their hosts; they can produce large quantities of honeydew, which serves as substrate for sooty mold, contaminating cotton, ornamental and other plants and thereby reducing their marketability (Schuster et al. 1996). Their feeding habits and dispersal ability makes adult whiteflies excellent vectors of plant diseases that can cause economic ruin to many vegetable and field crops (Ernst. 1994, Schuster et al. 1996, Thompson 2011, Riley and Srinivasan 2019).

Current agricultural practices rely heavily on the usage of insecticides for pest population reduction and disease prevention (Horowitz and Ishaaya 1996, Castle et al. 2002, Van Doorn et al. 2013). *Bemisia tabac*i has shown an impressive ability to adapt through insecticide resistance (Dittrich et al. 1990, Basit et al. 2013, Horowitz et al. 2020). Neonicotinoids (group 4A) have been used in the past to reduce field populations with great success; however, increased resistance has developed in the SE (Schuster et al. 2010, Caballero et al. 2013b) and SW United States (Prabhaker et al. 1997, Castle and Prabhaker 2013). Additionally, resistance to pyrethroids (group 3A) is common (Alon et al. 2006, Smith and Giurcanu 2013). Resistance to growth regulators like pyriproxyfen (group 7C) has been reported (Ma et al. 2010). The most recently developed anthranilic diamides (group 28) have even started to show reduced efficacy in the field as early as 2015 (Caballero et al. 2015, Wang et al. 2018).

We conducted a series of insecticide efficacy tests using whole plant bioassays to determine the relative efficacy of insecticides in southern Georgia in the fall of 2018 and summer of 2020. The objective of this study was to determine if whole plant bioassays could be used to evaluate insecticides in terms of controlling whitefly adults, reduction of oviposition and disruption of nymph development. The hypothesis tested was that the effectiveness of the tested insecticides for whitefly adult, egg and nymph control would be similar across test whitefly populations. Our expectations were that pyriproxyfen would not cause mortality in adults, but disrupt oviposition and nymph development for both populations and the other insecticides would show adult mortality, but variable effects on egg and nymph counts.

4.2 Materials and methods

Laboratory whitefly colonies used in this study were from a field collection in Georgia during a summer outbreak in 2017 at the Coastal Plain Experiment Station at Tifton, GA and the other from a lab colony at the Gulf Coast Research and Education Center at Wimauma, FL in the summer of 2018. The population from Florida had been in colony since the 90's and never been exposed to insecticides during that time. The expectation was that this colony would be susceptible to most insecticides used on it. The whole plant bioassays consisted of clean, two-true leaf cotton seedlings being introduced into indoor rearing rooms with heavily whitefly-infested plants for 72 h before conducting the whole plant treatments on greenhouse benches. The idea was to better control environmental conditions and limit the introduction of naturally occurring whitefly populations on plants so that specific whitefly populations could be characterized in terms of their response to insecticides.

All plant material used in the bioassays were whole plants from untreated cotton ST4946GLB2 seed grown at 30°C, 70% relative humidity, and 16:8 day: night cycle in growth chambers (Percival model E-36L2, Perry, IA, USA). The cotton plants in these tests were grown to the two-expanded-true-leaf stage before using. Cotton plants were held in a 2 x 2.5 m whitefly colony room for 72 hours. Once the plants were infested, plants were moved to greenhouse benches so that individual plants could be treated. In the 2018 experiment, each plant was treated on Sep 28th and again on Oct 1st. Plants were only sprayed once in the 2020 experiment on July 15th. We used a one-liter spray volume to treat plants at a rate equivalent to the 120 gallons per acre estimated equivalent spray volume used in field settings. A single spray was performed for each treatment with a hand-held compressed CO_2 sprayer (Model A3S, R&D Sprayers/Bell Spray Inc., Opelousas, LA) with single- TX-18 hollow cone nozzle. Each plant was sprayed until runoff. Six insecticides were tested at their maximum allowable field rate: Knack (pyriproxyfen) 0.8ml/L, Admire Pro (imidacloprid) 0.18ml/L, Venom (dinotefuran) 0.3g/L, Coragen (chlorantraniliprole) 0.4ml/L, Exirel (cyantraniliprole) 1.06ml/L, and Sivanto Prime (flupyradifurone) 0.94ml/L. A lower dose treatment was tested for each insecticide at 1/10th these rates.

4.2.1. Whole Plant Bioassay

In the 2018 Florida population experiments, adults were scouted once the day before treatment and twice 5 and 10 days after insecticide application. The Georgia population was only scouted once, 7 days after treatment. Scouting involved slowly turning over the most mature leaf on the plant and counting all adult whiteflies present and flying away from the leaf movement. Scouted leaves were then excised and placed within labelled plastic bags and frozen for later egg and nymph counting under dissecting scope at 20X magnification. In 2020, the most mature leaf on each plant had all egg and nymphs on its underside counted with desk-mounted magnifying glass and marked with ink to identify for post-treatment scouting and excision for egg and nymph counts.

4.2.2. Clip Cage Bioassay

The 2020 experiment using the GA population included the use of a clip cage to test adult mortality. 24 hours after treatment, clip cages were placed over a true leaf on each plant. Clip cages were constructed of foam pipe insulation meant for 25.4mm pipe by FrostKing (Thermwell, Mahwah, NJ) sliced into rings with mesh screening glued on top to create a bioassay arena that could be placed over treated leaves and allow viewing of the whiteflies within. Aspirated adults were cooled for five minutes at 2°C to prevent escape during introduction to the arena. Whiteflies were tapped out of the pipettes and into the clip cage before securing the clip cage to the underside of the cotton leaf using another foam ring and paperclips. Our clip cage methodology is described with figures in Sparks et al. 2020 published in the journal *Insects*. Adults were left inside clip cages for 24 hours before live and dead counts were taken.

4.2.3. Statistical Analysis

Adult, egg, and nymph counts, and clip cage mortality were analyzed using Proc GLM in SAS Enterprise (SAS Institute, Cary, NC). Repeats of the 2018 experiment were held congruently with each experiment occurring on separate tables within the green house. Plant location on the table was randomized in 2018 and 2020. Data collected from both populations were subjected to individual analysis of variance. Data was not transformed to match a normal distribution. Significant differences were reported and means graphed with standard errors for describing treatment effects. Insecticide treatment means separation was tested with LSD by date (for non-repeated measures data) tests ($p < 0.05$).

4.3 Results and discussion

4.3.1 Whole Plant Bioassays

Adult counts were significantly reduced compared to the control for both 2018 experiments that used the Florida population (Fig. 1, 2). (F = 6.97, df = 12, 123, P < 0.001, and F = 8.33, df = 12, 123, P < 0.001, respectively) In contrast, the 2020 Georgia population only had significantly reduced adult numbers in the high dosages of Venom and Sivanto (Fig. 3) (F = 1.89, df = 13, 120, P = 0.037). Both 2018 tests were similar to one another in actual values with little change in the significant differences between treatments. The 2020 experiment with the Georgia population was more variable resulting in less separation from the untreated check. There appeared to be high natural variation with respect to infestation levels from the colonies. Contributing to this variation was the ability of adults to disperse between plants before and after treatment. This variation was magnified in the egg and nymph counts (Fig. 4, 5, 6, 7) (F = 5.03, df = 13, 39, *P* < 0.001, F = 2.35, df = 13, 118, *P* = 0.008, F = 3.14, df = 13, 39, *P* < 0.01, and F = 3.24, df = 13, 118, *P* < 0.001, respectively).

4.3.2 Clip Cage Bioassay

Each insecticide treatment had a higher mortality than the control except for the high dosage of Knack in the 2020 clip cage (Fig. 8) (F = 3.17, df = 13, 50, P < 0.01). Oddly, the lower dosage of Knack had significantly higher adult mortality than the high dosage. The active ingredient in Knack is an insect growth regulator and should not act as an adulticide. Just as unexpected, Venom, Coragen, and Exirel also tended to have increased mortality with the lower dose, but none were statistically significant. The lower dose of Knack, both dosages of Venom, and the high dose of Sivanto showed significant control for adults. The trend appears in the adult counts for the Georgia population with lower adult presence for Venom and the higher dosage of

Sivanto. Knack and the low dose of Sivanto did not seem to affect live adult presence on plant leaves, but low amounts of Exirel reduced presence without having apparent lethality.

4.3.3. Eggs and Nymph Counts

Egg counts were significantly reduced for all insecticides except for the high doses of Knack and Admire and the low dose of Exirel in 2018 (Fig. 4). In contrast, the egg counts in 2020 were not statistically different with some insecticide treatments resulting in more eggs per leaf than the control (Fig. 5).

The presence of nymphs was low in 2018 with the low doses of Knack and Venom failing to produce a single one. The only treatments to average more than 5 nymphs per leaf were both dosages of Admire and the high dose of Coragen. Nymphs were far more numerous in 2020 with lowest numbers averaging at 18.9 ± 4.0 per leaf on the low Exirel treatment. The high dosage of Admire and both concentrations of Venom and Exirel showed marked decreases in eggs hatching to nymphs. It's interesting to note that while the higher concentration of Admire resulted in statistically higher nymph counts in 2018 and then lower ones in 2020, the actual numbers are similar for both years with an average of 30.5 ± 10.9 in 2018 and 27.5 ± 7.4 in 2020. The same can be said for that treatment's egg counts with 41.8 ± 14.0 in 2018 and 41.9 ± 11.6 in 2020. It would seem the two populations responded similarly to the higher dose of Admire, but there was too much variability between treatments to recommend this method for nymph mortality assays.

4.4 Conclusions

This whole-plant-pre-infestation experimental design for whitefly efficacy was thought to be a reasonable way to assess whitefly adult, egg and nymph response to insecticides, but the technique has some deficiencies along with its general utility. For example, Knack, being the only insect growth regulator in the experiment reduced adult numbers in the FL population and showed significant adult mortality for the low dosage only in the clip cage experiment on the GA population. Egg and nymph numbers were not reduced in the GA population and only the low dosage resulted in lower egg numbers in the FL population. Nymphs were extremely low on plants treated with Knack in 2018, as expected, but are other factors affecting this? The IRAC 4A insecticides, Admire and Venom, reduced adult numbers in the Florida colony population, but only Venom reduced adult presence for the GA population. Results from the clip cage indicate that both insecticides have lethality (% dead) to adults with the lower concentration of Admire not having significant difference from the control. Venom significantly reduced egg counts in the FL population and the low dose of Admire, but not the high, reduced egg numbers. Admire was responsible for heavy increases in nymph presence in the 2018 experiments on the FL population and the high dose reduced nymphs in the GA population. Venom significantly reduced nymphs for the GA population. Exirel and Coragen make up the group 28 diamides used for this research. Both reduced adult numbers in the FL population but not in the GA population. Neither were able to demonstrate lethality for adults in the clip cage. Both active ingredients reduced egg counts in the Florida population but not the GA population. The high dosage of Coragen increased nymph numbers in the FL population while both Exirel concentrations reduced nymphs in the GA population. Both rates of Sivanto Prime controlled adult populations in the 2018 experiments and only the higher dose was significant for the 2020 GA population. The clip cage experiment confirmed dose dependence with only the higher concentration producing significant mortality. Egg counts were significantly reduced in the FL population but not the GA one. Nymphs were not significantly reduced for either population.

4.5 Acknowledgments

I would like to acknowledge the assistance of undergraduate student workers in the Vegetable Entomology Research Laboratory, Charles Gruver Tech III, and internal reviewers at the University of Georgia Tifton Campus. This work was funded in part by University of Georgia Coastal Plain Experiment Station, USDA Project Number: 6080-22000-027-18-S entitled, “Managing Whiteflies and Whitefly-transmitted Viruses in Vegetable Crops in the Southeastern U.S” and the Georgia Commodity Commission for Vegetables. This study was conducted using the facilities of the Georgia Agricultural Experiment Stations.

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Figure Captions

Figure 4.1. Average number of adult whiteflies on a single leaf in the first experiment of 2018 using the Florida population. Bars with the same letter are not significantly different, $P < 0.05$.

Figure 4.2. Average number of adult whiteflies on a single leaf in the second experiment of 2018 using the Florida population. Bars with the same letter are not significantly different, $P < 0.05$.

Figure 4.3. Average number of adult whiteflies on a single leaf for the 2020 experiment using the Georgia population. Bars with the same letter are not significantly different, $P < 0.05$.

Figure 4.4. Average number of whitefly eggs on a single leaf in the Florida population. Reported values are combined from both repeats of the 2018 experiment. Bars with the same letter are not significantly different, $P < 0.05$.

Figure 4.5. Average number of whitefly eggs on a single leaf in the Georgia population. Bars with the same letter are not significantly different, $P < 0.05$.

Figure 4.6. Average number of whitefly nymphs on a single leaf in the Florida population. Reported values are combined from both repeats of the 2018 experiment. Bars with the same letter are not significantly different, $P < 0.05$.

Figure 4.7. Average number of whitefly nymphs on a single leaf in the Georgia population. Bars with the same letter are not significantly different, $P < 0.05$.

Figure 4.8. Percent mortality of adult whiteflies in the Georgia population clip cage experiment in 2020. Bars with the same letter are not significantly different, $P < 0.05$.

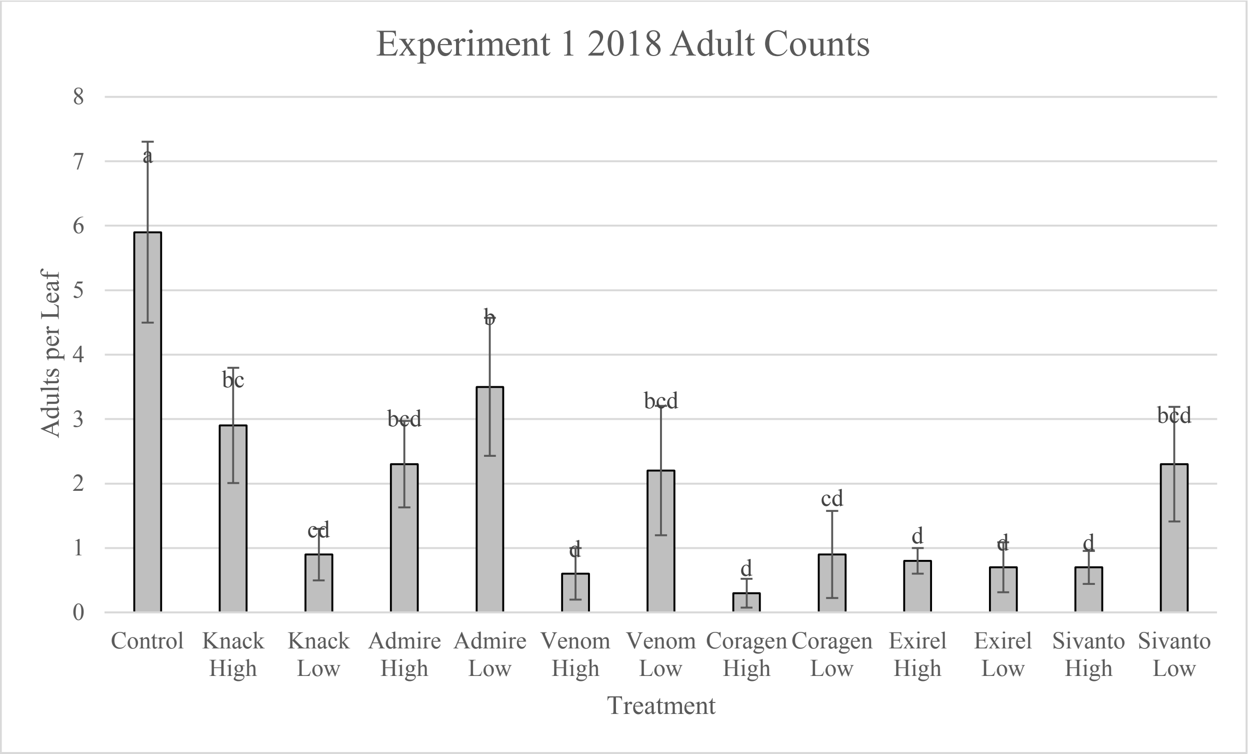

Figure 4.1. Average number of adult whiteflies on a single leaf in the first experiment of 2018 using the Florida population. Bars with the same letter are not significantly different, $P < 0.05$.

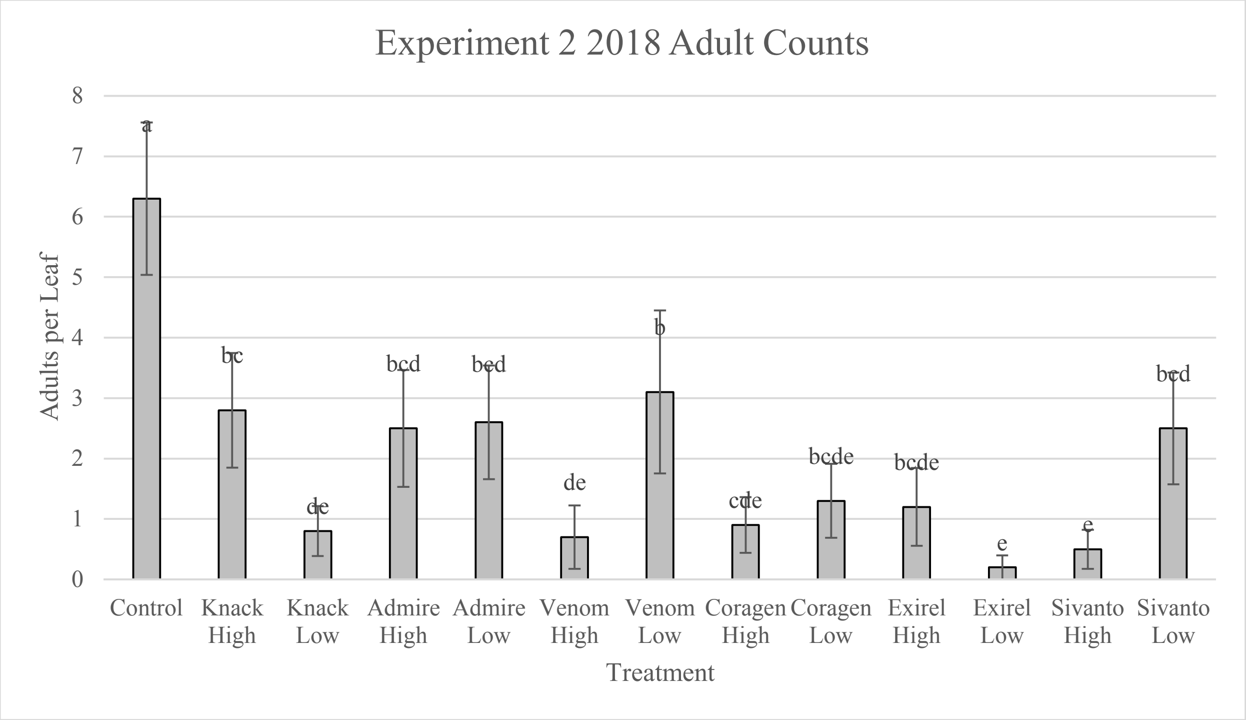

Figure 4.2. Average number of adult whiteflies on a single leaf in the second experiment of 2018 using the Florida population. Bars with the same letter are not significantly different, $P < 0.05$.

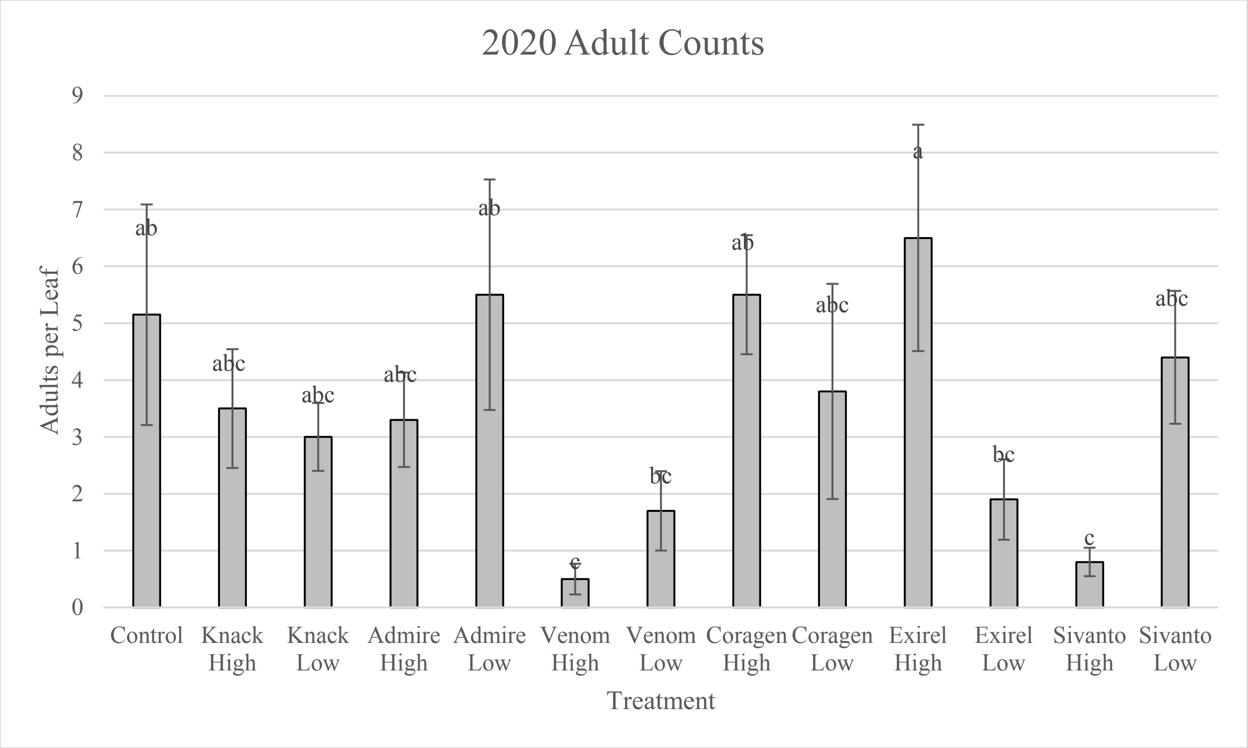

Figure 4.3. Average number of adult whiteflies on a single leaf for the 2020 experiment using the Georgia population. Bars with the same letter are not significantly different, $P < 0.05$.

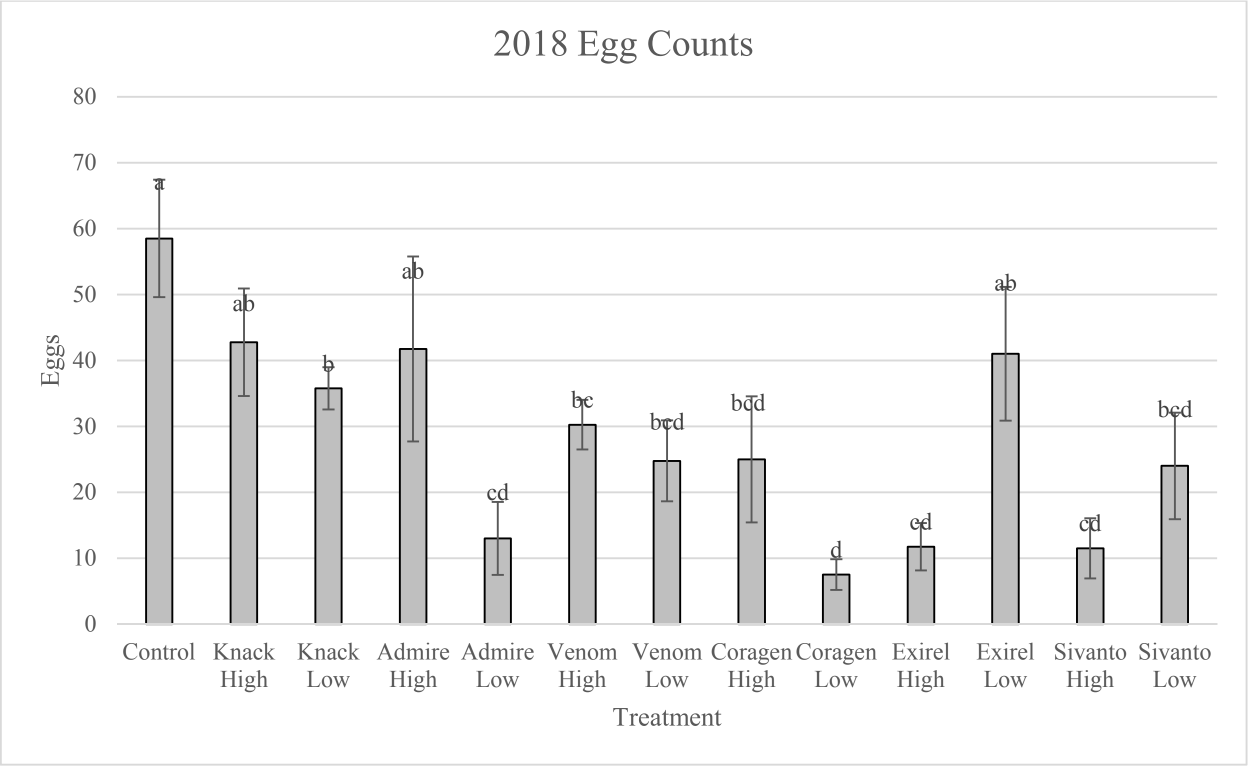

Figure 4.4. Average number of whitefly eggs on a single leaf in the Florida population. Reported values are combined from both repeats of the 2018 experiment. Bars with the same letter are not significantly different, *P* < 0.05.

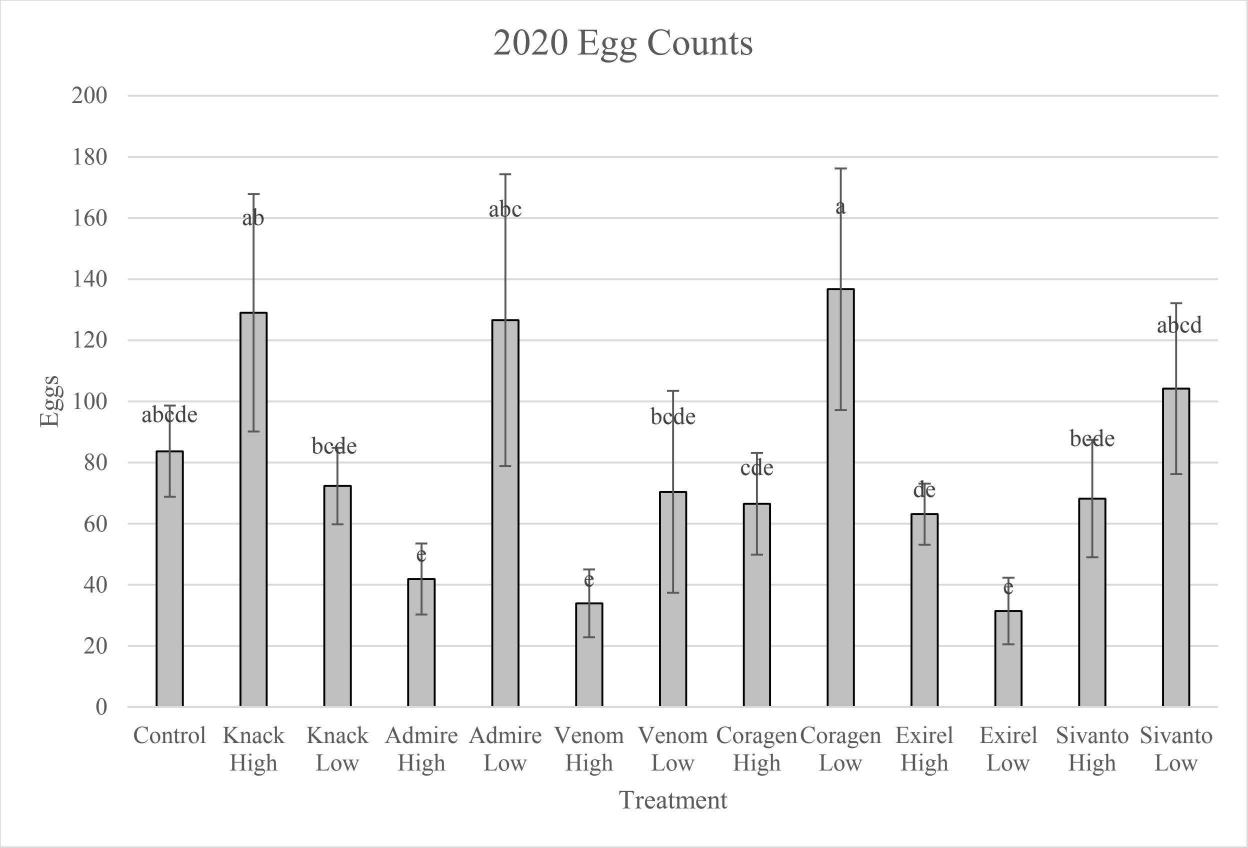

Figure 4.5. Average number of whitefly eggs on a single leaf in the Georgia population. Bars with the same letter are not significantly different, $P < 0.05$.

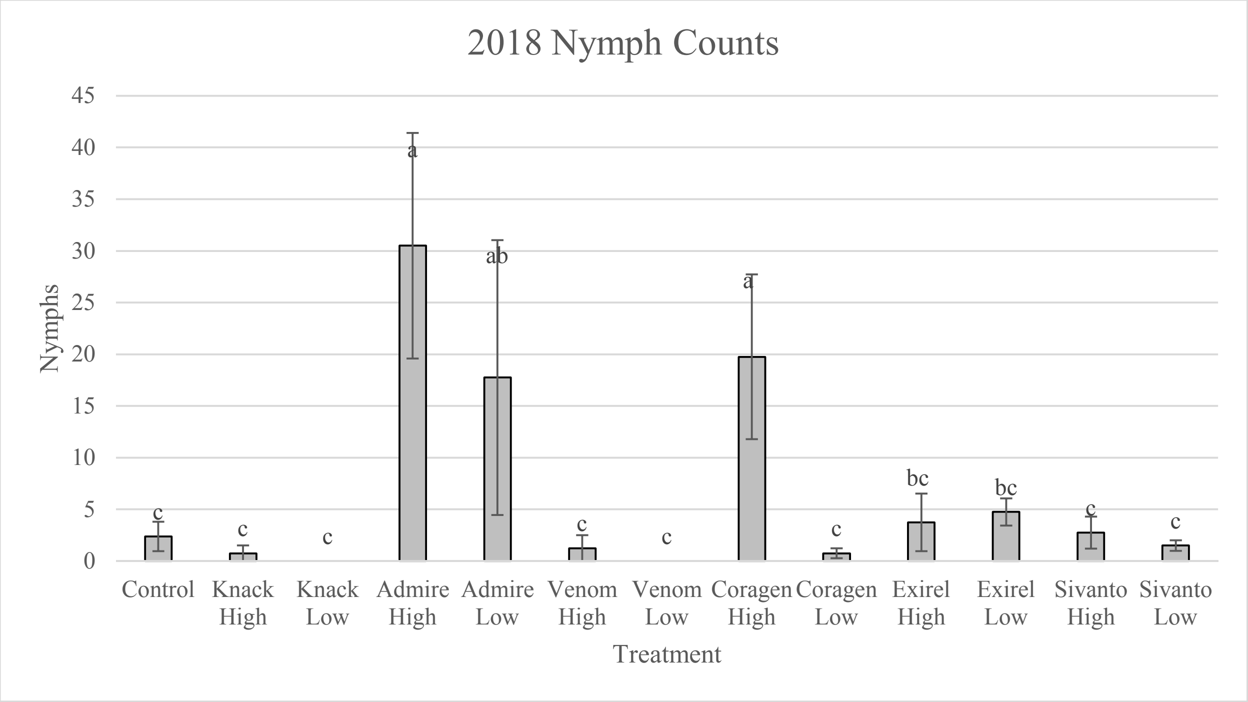

Figure 4.6. Average number of whitefly nymphs on a single leaf in the Florida population. Reported values are combined from both repeats of the 2018 experiment. Bars with the same letter are not significantly different, $P < 0.05$.

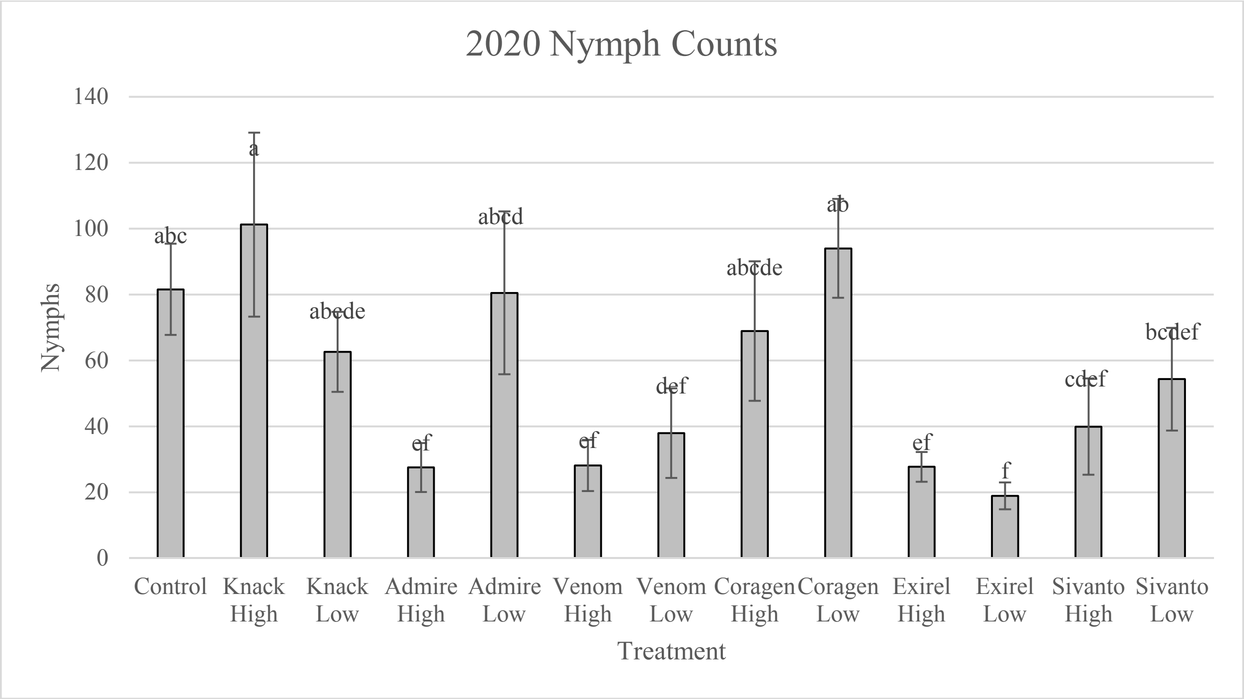

Figure 4.7. Average number of whitefly nymphs on a single leaf in the Georgia population. Bars with the same letter are not significantly different, $P < 0.05$.

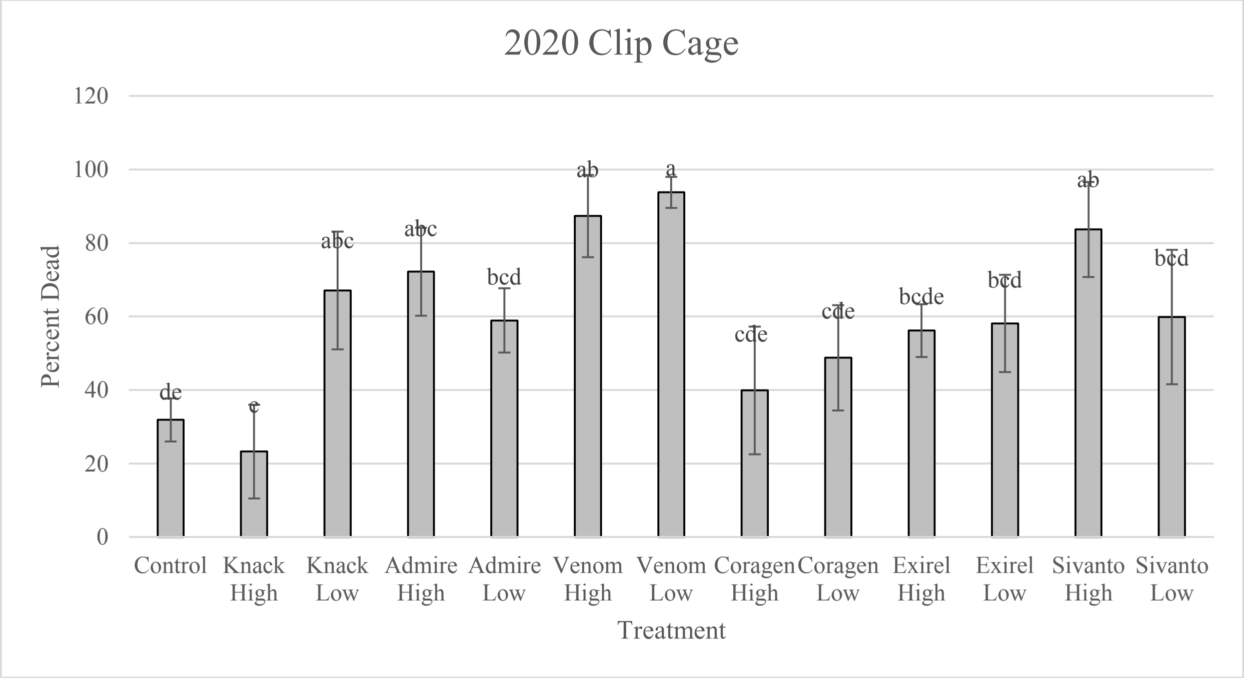

Figure 4.8. Percent mortality of adult whiteflies in the Georgia population clip cage experiment in 2020. Bars with the same letter are not significantly different, *P* < 0.05.

SUMMARY

The b-strain (MEAM1) of sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is a key pest of vegetable and agronomic crops in the Southeastern USA. Insecticide resistance is one of the major concerns for management of this pest in commercial crops. The goal of this research project was to provide an informed basis from which to launch a larger insecticide resistance management (IRM) program for whiteflies in Georgia. Part of this effort was to compare various insecticide response testing protocols, including lab bench bioassay methods for whitefly population screening of insecticide response and greenhouse whole plant bioassays for validating bioassay results. Bioassays would be the foundation of an insecticide response survey and assist investigations into the mechanisms of resistance. Thus, picking an effective bioassay method, whose strength and limitations were understood, would be important to the success of the long-term IRM project.

Using two different whitefly populations, one collected from Tifton, Georgia and one from Wimauma, Florida, we evaluated two different insecticide application routes, leaf vs root drench (main plots), four whitefly bioassays methods (subplots) and five whitefly insecticides compared with a water check (sub-subplots) in a split-split plot design so that we could quantify interactions. We obtained similar results between the Florida and Georgia whitefly populations which give us some confidence that the interactions observed were predictive for what might be expected for these bioassays. The similarity in response between leaf and root drenches for these insecticides was reassuring in that published results using root drench or leaf dip techniques appear to comparable based on the results of this experiment. There are relatively few whitefly toxicological bioassay studies where multiple methods were compared in the same project, mainly to assess either adult or immature whitefly response (Caballero et al. 2013, Chen et al. 2018). Sain et al. (2019) found that there was an advantage of using an intact leaf compared to multiple methods using leaf disk or detached leaf bioassays. Our results were similar in that the one detached leaf bioassay used, the dish method, provided the poorest dose response data for whiteflies. The reassuring result from our comparison is that the various methods of assessing whitefly response through bioassays is surprisingly similar, just reflecting better resolution with some methods, such as the clip cage method. This means that the various bioassays employed in the literature for whitefly insecticide response are also comparable. The high rates of cyantraniliprole (Exirel), dinotefuran (Venom) and flupyradifurone (Sivanto Prime) insecticides resulted in the highest whitefly mortality for the whitefly populations tested.

There were significant interactions between the bioassay method and insecticide treatment for both populations in terms of percent dead. In the Georgia population, there was also a significant application route x insecticide interaction for percent dead. These interactions were important in that they related to how a particular method or application route might introduce bias into the response data. The range of Tukey's mean separation categories for each method was a-d (tube), a-e (cup), a-d or a-c (dish) and a-f or a-e (clip cage). A greater range suggested more sensitivity across treatments, so with this assumption, the clip cage method provided the most and the dish method the least sensitivity. As an example, the clip cage and cup methods separated out the high rates of the three top insecticides from the high rate of imidacloprid, whereas the dish and tube methods did not. The tube and the cup methods ranked treatments similarly, whereas the dish had an odd response for the low rate of flupyradifurone.

The interaction between the application route and insecticides related to check mortality and the range of response for each route, *i.e.*, 14.6±2.9 % and 26.7+4.1 % dead in the check and 81.8+3.8 % and 92.7+2.2 % dead in the best insecticide treatment, cyantraniliprole, for the root drench and leaf dip routes, respectively. Root drench treatments tended to produce drier test leaves which in turn reduced mortality in the checks.

Whole-plant bioassays in 2018 and 2020 did show that the same insecticides tested significantly reduced adult and egg numbers in at least one test population and nymphs in the other, but whole plant infestations were variable. Unexpectantly, pyriproxyfen (Knack), being the only insect growth regulator targeting nymphs in the experiment, reduced adult numbers in the FL population and showed significant adult mortality for the low dosage only in the clip cage experiment on the GA population. Whereas egg and nymph numbers were not reduced in the GA population and only the low dosage resulted in lower egg numbers in the FL population. Nymphs were extremely low on plants treated with Knack in 2018, as expected, but the results should have been less variable. The IRAC 4a insecticides, imidacloprid (Admire) and dinotefuran (Venom), reduced adult numbers in the Florida colony population, but only Venom reduced adult presence for the GA population. Results from the clip cage indicate that both insecticides have lethality (% dead) to adults with the lower concentration of Admire not having significant difference from the control. Venom significantly reduced egg counts in the FL population and the low dose, but not the high dose, of Admire reduced egg numbers. Admire was responsible for heavy increases in nymph presence in the 2018 experiments on the FL population and the high dose reduced nymphs in the GA population. Venom significantly reduced nymphs for the GA population. Cyantraniliprole (Exirel) and chlorantraniliprole (Coragen) make up the group 28 diamides used for this research. Both reduced adult numbers in the FL population but not in the

GA population. Neither were able to demonstrate lethality for adults in the clip cage. Both active ingredients reduced egg counts in the Florida population, but not the GA population. The high dosage of Coragen increased nymph numbers in the FL population while both Exirel concentrations reduced nymphs in the GA population. Both rates of Sivanto Prime controlled adult populations in the 2018 experiments and only the higher dose was significant for the 2020 GA population. The clip cage experiment confirmed dose dependence with only the higher concentration producing significant mortality. Egg counts were significantly reduced in the FL population but not the GA one. Nymphs were not significantly reduced for either population. All in all, the clip cage technique improved the insecticide response data. For the whole plant bioassays to be more precise, we would need to have a more uniform whitefly infestion and increase the number of replicates to reduce variation.

In the Appendix we describe a modified tube bioassay method which was developed to collect adult whiteflies from field locations and determine LC_{50} for insecticides without necessitating keeping insects in colony. Even though the results were preliminary, we were able to measure LC_{50} values for Admire Pro (imidacloprid), Beleaf (flonicamid), Dibrom (naled), Exirel (cyantraniliprole), Sivanto Prime (flupyradifurone), Transform (sulfloxaflor), and Venom (dinotefuran). Check mortality using the tube method continues to be a problem. Getting clean LC_{50} values in a consistent manner using the tube collection technique, may take a combination of tube collection method with either the clip cage or cup method described in Chapter 3 to be effective. This work needs to continue before launching regional surveys of insecticide response for whiteflies in Georgia.

APPENDIX: A NEW METHOD FOR DETERMINING INSECTICIDE LC_{50} VALUES

Introduction

Monitoring insecticide resistance in sweetpotato whitefly, *Bemisia tabaci* Gennadius, has been critical to the management of this worldwide insect pest since the early 1980's (Prabhaker et al. 1985, Dittrich et al. 1990, Staetz et al. 1992, Denholm et al. 1996, Horowitz and Ishaaya 1996, Palumbo et al. 2001, Schuster et al. 2010, Caballero et al. 2013). The appearance of biotype-B (MEAM1) of *B. tabaci* accelerated interest in understanding resistance because of reported associations between whitefly biotypes and insecticide resistance (Alon et al 2006) and biotype invasions into crop production areas (Ma et al. 2006). The bioassay methods used for monitoring resistance include treated vials for contact activity against adult whiteflies (Staetz et al. 1992), clip cage on plants or cuttings that were either directly drenched (Caballero et al. 2013) or had insecticide mixtures applied systemically through the leaf petiole (Caballero et al. 2013, Smith 2013), and similar bioassay application methods, but evaluated in a petri dish (Ma et al. 2007, Smith 2013, IRAC 2016) or a cup (IRAC 2009) container. Typically, only a single bioassay method was employed for a given insecticide resistance study.

After comparing several whitefly bioassay methods for investigating insecticide efficacy (Chapter 3), we settled on a quick adult bioassay for conducting insecticide dose-response evaluations for multiple insecticides across multiple whitefly populations. We used a combination of the treated excised-leaf technique currently used by the University of Florida and the tube arena developed at the University of Georgia which facilitates rapid collection and easier sample manipulation in the tubes. Results for a lethal concentation 50 (LC_{50}) calculation can be attained within 24 hours for most products tested. We tested the technique for the major whitefly insecticides currently used in Georgia.

Materials and Methods

These tests were located at the University of Georgia's Vegetable Entomology Research Lab at the Coastal Plain Experiment Station (CPES) at Tifton, GA. Three populations of *Bemisia tabaci* were raised in colony. One was collected from the CPES, Tifton, GA in 2017 during the Summer breakout of 2017. Another population was collected from lab colony at the Gulf Coast Research and Education Center at Wimauma, FL in the summer of 2018. The third colony was started from a shipment of organic collards that arrived already infested. Both Georgia and Florida colonies were raised on untreated cotton grown at 30°C in Percival growth chambers. The organic collard colony has continued to be reared on untreated collard and mustard greens also grown under the same conditions as cotton.

Adult whiteflies were collected in this experiment using the tube method to mimic how collecting in the field on a large scale might be achieved. Whiteflies feeding on colony cotton were tapped into yellow funnels that emptied into a clear plastic tube 2.86 cm in diameter and 20.32 cm in length produced by ClearTec Packaging (ClearTec 2017, Park Hill, MO). Once at least ten adults were inside, ends of the tube were capped by nylon chiffon and a rubber band to allow for airflow without escape. Excised, treated cotton leaves were then introduced to the assay arena for the whiteflies to feed on for 24 hours.

Insecticides used in this study were mixed in serial dilutions of 500ml. Insecticide treatments consisted of Admire Pro (imidacloprid), Beleaf (flonicamid), Dibrom (naled), Exirel (cyantraniliprole), Sivanto Prime (flupyradifurone), Transform (sulfloxaflor), and Venom (dinotefuran). Whole, untreated cotton seedlings had their roots washed of soil media and placed into the serial dilutions to uptake insecticide. After 24 hours, the true leaves of the cotton seedlings were excised, and one leaf was placed inside each assay arena. For this study, the control was an excised leaf from a cotton plant that had been sitting in tap water for 24 hours.

After introducing treated leaves, the number of dead adults within the bioassay were counted as an initial mortality. For later statistical analysis, the initial mortality numbers are subtracted from the number dead and total whitefly count for each experimental unit. They are removed from counts as they do not represent effects of the treatment and are too difficult to simply remove from the arena without allowing others escape. Live and dead counts of whiteflies were taken after 24 hours. Probit analysis was used to construct LC_{50}s for each population (SAS Institute, Cary, NC).

Results and Discussion

This modified tube method was able to produce LC_{50}s for each insecticide tested and for each population (Table A.1). Dibrom treatments on the Florida and Georgia populations resulted in a LC_{50} value, but with no 95% fudicial limits, the result is likely not of much use, only to say that a very high amount of active ingredient was need to significantly kill whitefly adults.

The largest issue encountered in performing this experiment was the mortality present in the control. Generally, any bioassay where the control mortality was higher than 20% should be discarded. By that metric, only the Dibrom, Sivanto, and Venom treatments for all three colonies plus the Admire test on the Florida colony could be accepted to run Probit analysis. Graphing dose responses from there resulted in all Venom tests (Fig. A.4, A.8, A.11), the Florida Admire (Fig. A.5) and Sivanto (Fig A.7) trials being the only ones not previously rejected to produce a curve that resembles the sigmoidal shape expected for LC_{50} testing. Figures 5.1-11 are all graphs that resembled sigmoidal curves with many (Fig. A.1, A.2, A.3, A.6, A.9, A.10) being rejected for their control mortality being >20%. The high control mortality is an indicator that not all of the mortality seen in treatments is due to insecticide treatments and so the calculated LC_{50} values are not accurate enough to adequately describe that population's response to the insecticide. Future experiments with this methodology will have to be more careful with control mortality, being prepared to discard replicates where control mortality is over the 20% threshold.

Table A.1. Calculated LC_{50} values and 95% fudicial limits (in mg a.i./l) for select insecticides in the organic collard, Florida, and Georgia whitefly colonies.

Population	Insecticide	LC_{50}	95% Fudicial Limits
Collard	Admire	0.1889	0.0318 - 0.7682
	Beleaf	5666	1158 - 44420000
	Dibrom	7744	2818 - 27580
	Exirel	2.086	0.2859 - 9.229
	Sivanto	465.0	51.02 - 1199
	Transform	286.0	42.75 - 1396
	Venom	2891	475.2 - 62070
Florida	Admire	0.2327	0.02483 - 1.441
	Beleaf	4852	794.9 - 25290000
	Exirel	4.444	1.009 - 15.23
	Sivanto	3001	375.3 - 106800
	Transform	171.0	0.6040 - 2562
	Venom	408.6	73.37 - 4870
Georgia	Admire	0.1916	0.0109 - 2.067
	Beleaf	8464	176.5 - 1.494E10
	Exirel	1.041	0.1171 - 3.812
	Sivanto	39300000	6828 - 3.631E43
	Transform	313.6	14.26 - 3712
	Venom	21425	1866 - 4977000

Figure Captions

Figure A.1. Probit analysis for Admire in the organic collard colony.

Figure A.2. Probit analysis for Exirel in the organic collard colony.

Figure A.3. Probit analysis for Transform in the organic collard colony.

Figure A.4. Probit analysis for Venom in the organic collard colony.

Figure A.5. Probit analysis for Admire in the Florida colony.

Figure A.6. Probit analysis for Exirel in the Florida colony.

Figure A.7. Probit analysis for Sivanto in the Florida colony.

Figure A.8. Probit analysis for Venom in the Florida colony.

Figure A.9. Probit analysis for Admire in the Georgia colony.

Figure A.10. Probit analysis for Exirel in the Georgia colony.

Figure A.11. Probit analysis for Venom in the Georgia colony.

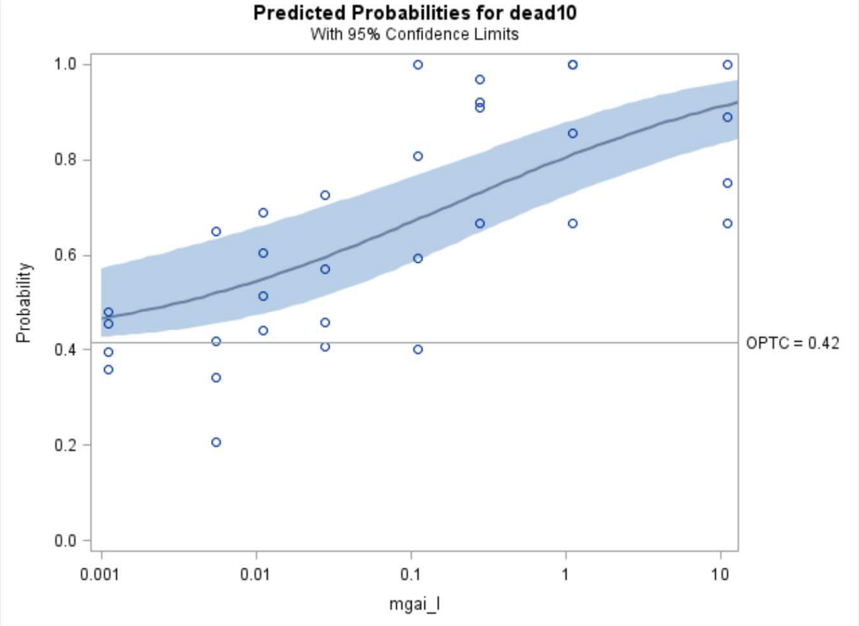

Fig. A.1. Probit analysis for Admire in the organic collard colony.

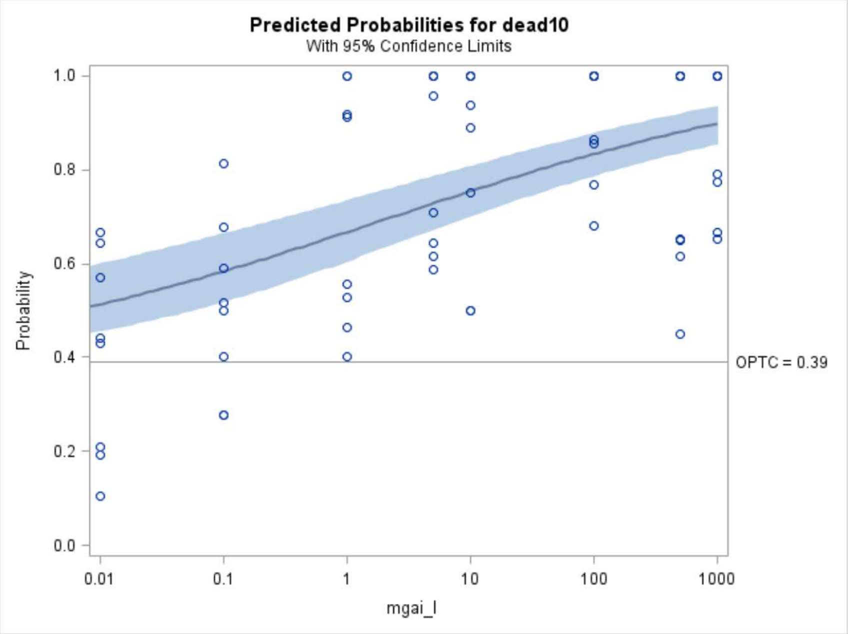

Fig. A.2. Probit analysis for Exirel in the organic collard colony.

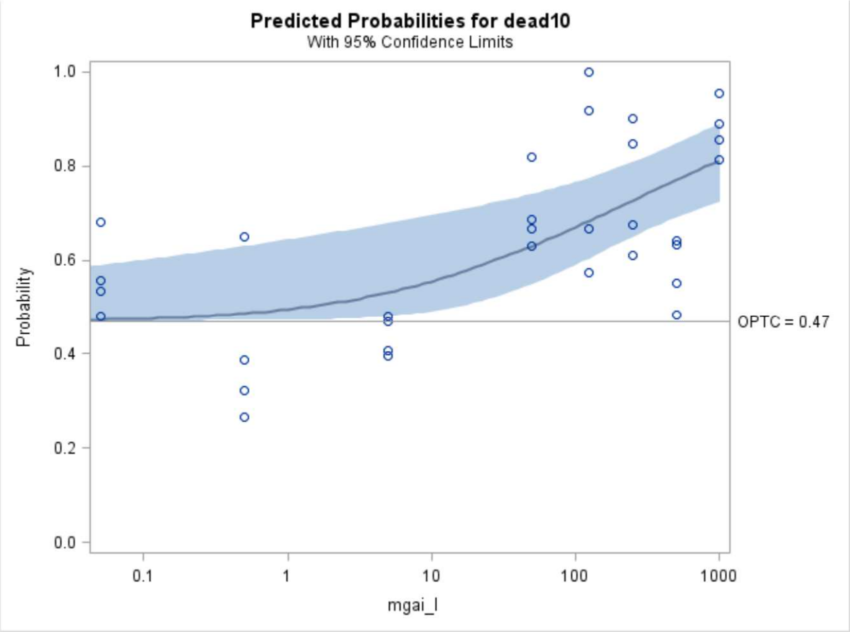

Fig. A.3. Probit analysis for Transform in the organic collard colony.

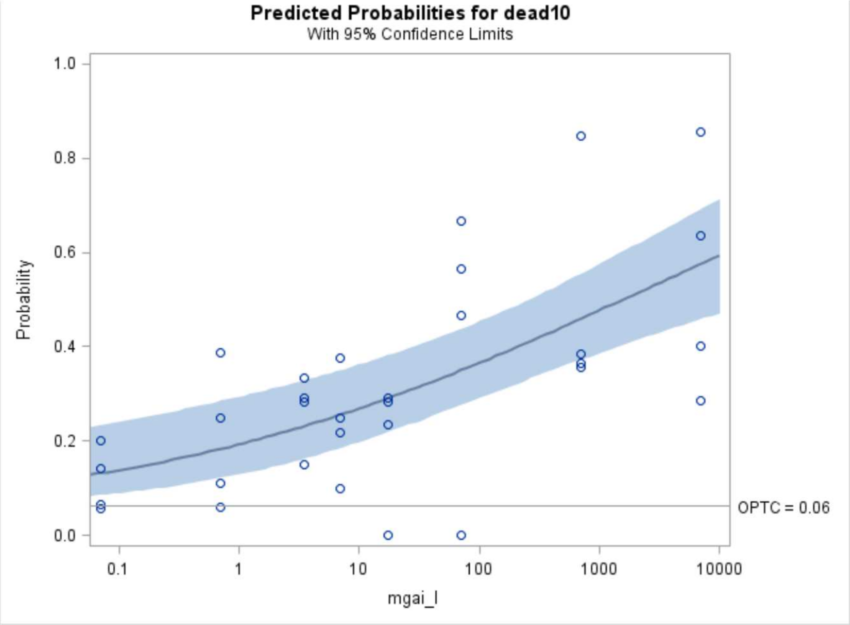

Fig. A.4. Probit analysis for Venom in the organic collard colony.

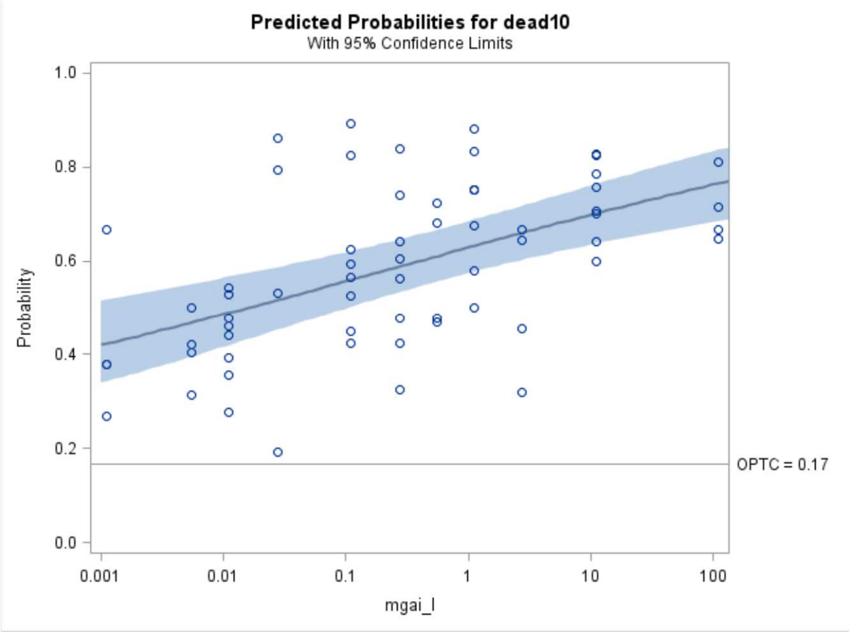

Fig. A.5. Probit analysis for Admire in the Florida colony.

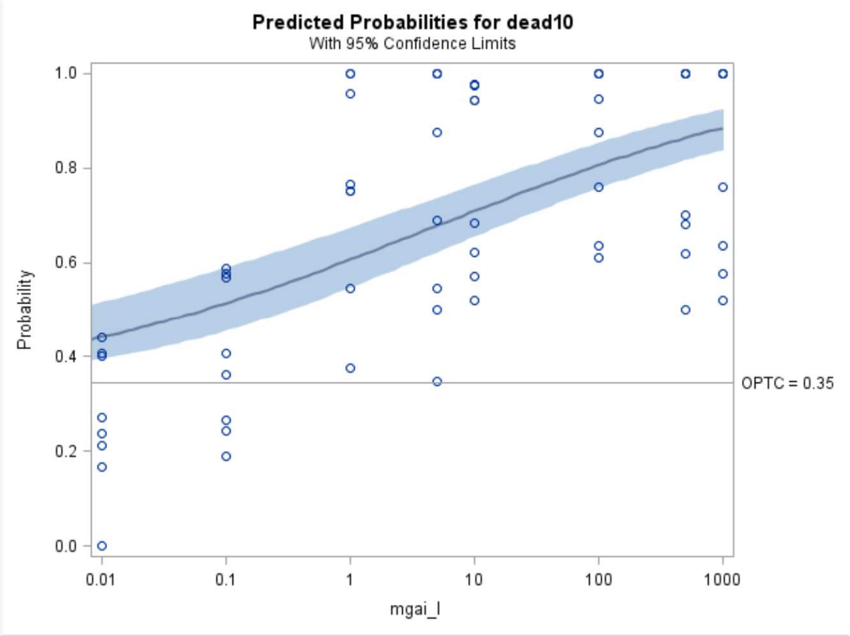

Fig. A.6. Probit analysis for Exirel in the Florida colony.

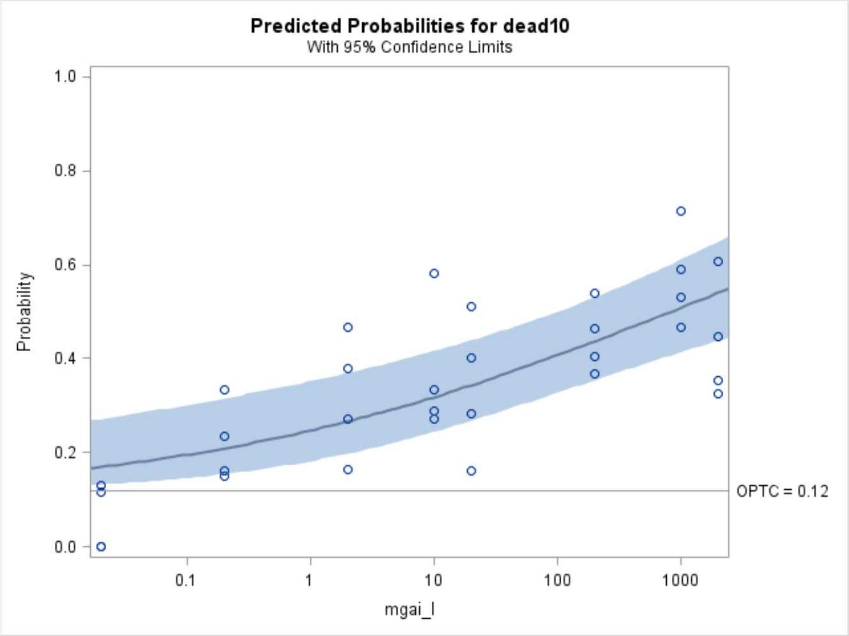

Fig. A.7. Probit analysis for Sivanto in the Florida colony.

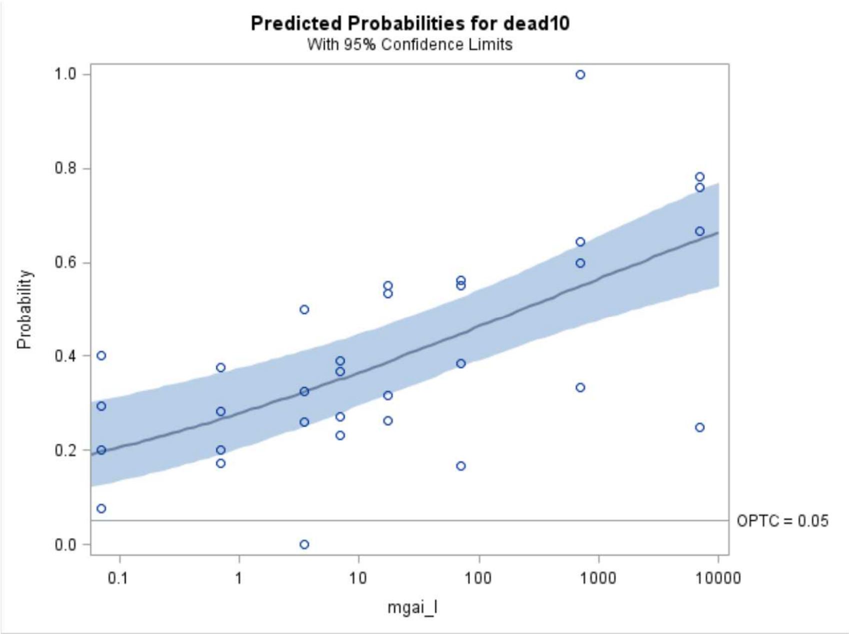

Fig. A.8. Probit analysis for Venom in the Florida colony.

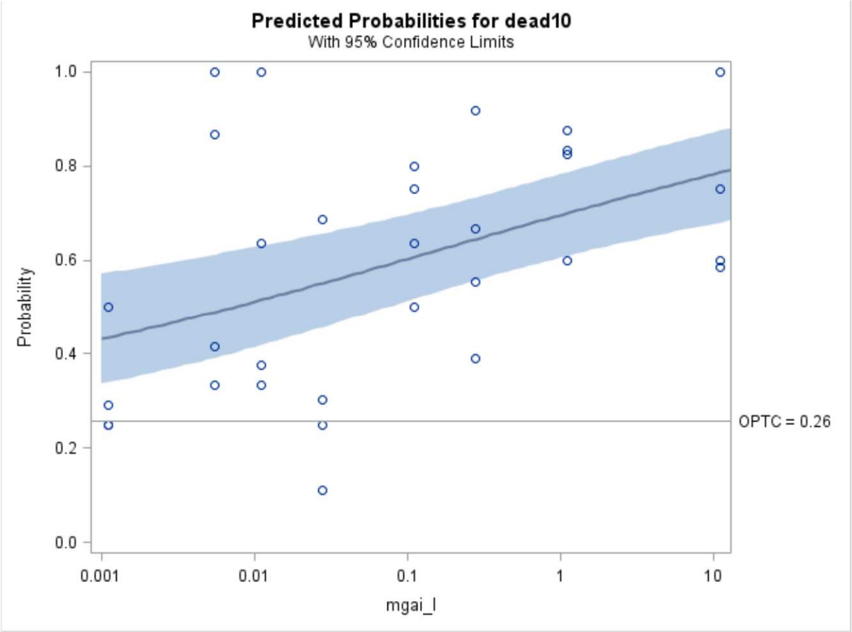

Fig. A.9. Probit analysis for Admire in the Georgia colony.

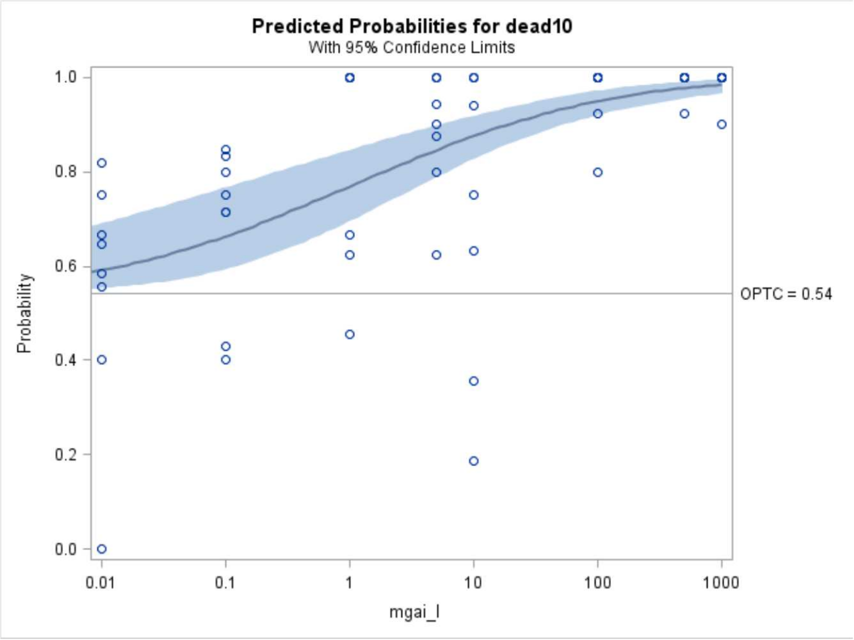

Fig. A.10. Probit analysis for Exirel in the Georgia colony.

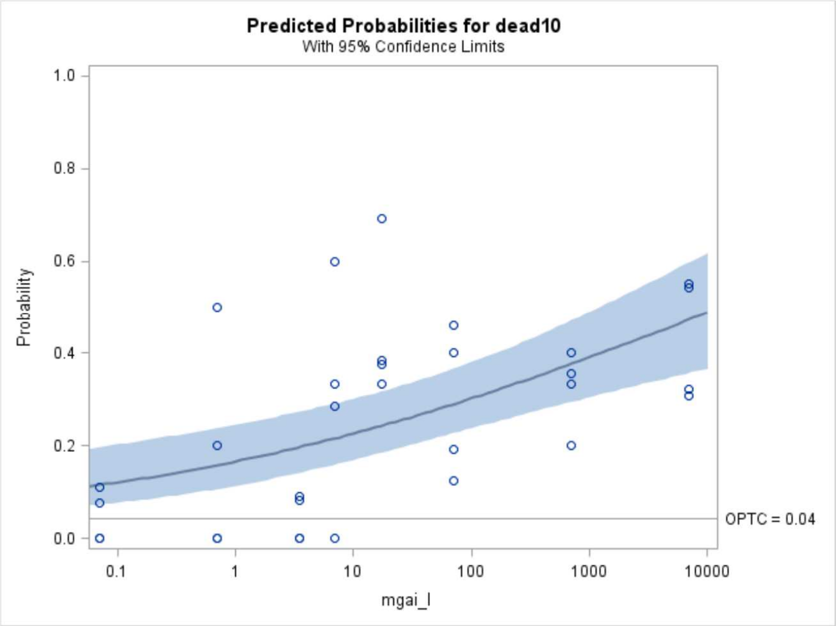

Fig. A.11. Probit analysis for Venom in the Georgia colony.

Tanner C. Sparks

SHORT BIOGRAPHY

Tanner has been a student at UGA since August 2013 where he began as a freshman in undergrad. He registered as an entomology major immediately and graduated with a BS in entomology in December 2016. During that time, he worked as a student worker at the UGA Blackfly lab and interned at the Georgia Natural History Museum. Tanner went back to school for his master's degree in January 2018 under the direction of David Riley at the Gulf Coast Experiment Station. There, he studied insecticide resistance in whiteflies. He is set to graduate December 2020.

Tanner is known for his interest in plants and animals. He collects insects for a hobby and cares for a collection of exotic house plants. Tanner keeps tarantulas and other exotic arthropods as pets which he uses for public outreach to teach people about entomology. In addition to those, he is involved in the reef keeping hobby and has two tanks of live corals. During the year between undergraduate and graduate school, he made use of his talents to work as the floral designer for an Athens floral shop and was the small animal expert at a pet store chain. In his free time, Tanner volunteers with the Tifton community garden contributing his entomological and gardening expertise.