ASSESSMENT OF DICYPHUS HESPERUS (KNIGHT) FOR THE BIOLOGICAL CONTROL OF BEMISIA TABACI (GENNADIUS) IN GREENHOUSE TOMATO

By

PRITIKA PANDEY

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To my family
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Florida produces about 34% of the fresh market tomatoes grown in the United States. *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is one of the most devastating pests of tomato globally. *Dicyphus hesperus* Knight (Hemiptera: Miridae) has been evaluated for control of greenhouse whitefly *Trialeurodes vaporariorum* Westwood in temperate environments and has potential as a biocontrol agent for *B. tabaci*. The major objective of this research is to assess the predatory potential of *D. hesperus* on *B. tabaci* under Florida’s subtropical condition. An experiment was designed to determine the average number of sweetpotato whitefly eggs or nymphs consumed by female *D. hesperus* adults per day and during her lifetime on tomato plants. *Dicyphus hesperus* from a colony and a commercial source, Beneficial Insectary, showed 7% reduction on *B. tabaci* eggs population. Mated and unmated female *D. hesperus* did not show any significant difference in their consumption rate when fed on *B. tabaci* eggs and nymphs. Average number of eggs consumed by *D. hesperus* was 10 per day and 83 over their lifetime, with adult survival of 15 days whereas average nymphs consumed was 93 per day and 835 over their lifetime with survival of 25 days.
The average consumption rate of *D. hesperus* on sweetpotato whitefly eggs, early instar nymphs (1<sup>st</sup> and 2<sup>nd</sup>) and later nymphs differed significantly with consumption higher on earlier and later nymphs than on eggs. In addition, we studied the development of *D. hesperus* on mullein (*Verbascum thapsus*) and tomato with and without *Ephestia kuehniella* Zeller eggs as a dietary supplement. The average time for nymphal development on tomato was 24.5 days, on mullein with *E. kuehniella* was 22.5 days, and on mullein without *E. kuehniella* was 25.8 days. The adult longevity and survival percentage of *D. hesperus* was 30.36 d and 91.96% on mullein with *Ephestia kuehniella* eggs, 22.44 d and 75% on mullein without *E. kuehniella* eggs and 26.86 d and 87.5% on tomato with *Ephestia kuehniella* eggs. In greenhouse experiments, the population of *D. hesperus* showed a greater increase on tomato provided with *E. kuehniella* eggs as a supplement prey source than on mullein with supplemental prey.
CHAPTER 1
INTRODUCTION TO BEMISIA TABACI

Introduction

The United States is one of the leading producers of fresh market tomatoes in the world with annual production of about 1.14 million metric tons. Florida contributes about 38% of total tomato production in the United States with recent annual revenue averaging $365,774,000 from 2015-2018 (USDA 2018). In 2017, about 29,000 acres of tomato were planted in Florida with production of about 426739.7 metric tons (USDA 2018). Insect pests are a major problem in Florida. Among them, *Bemisia tabaci* Gennadius MEAM1, the sweetpotato whitefly, is the most devastating.

*Bemisia tabaci* is one of the most serious pests of agriculture globally (Thompson 2011). It causes devastating economic losses to agriculture all over the world, primarily in tropical and sub-tropical regions, but also in protected agriculture in temperate regions (Oliveira et al. 2001). It is recorded as having about 600 different host plant species (Mound and Halsey 1978, Secker et al. 1998). Most host plants belong to the families Fabaceae, Asteraceae, Malvaceae, Solanaceae and Euphorbiaceae (Mound and Halsey 1978). Some vegetable crops produced in greenhouses, including tomato, pepper, beans, eggplant and cucumber are attacked by *B. tabaci* (Cock et al. 1986). It is now globally distributed on all continents except Antarctica (Martin 1999, Martin et al. 2000).

*Bemisia tabaci* was first recorded by Gennadius in Greece on tobacco (*Nicotiana* sp.) in 1889. Gennadius named the species *Aleurodes tabaci*, and placed it in the family Aleyrodidae, order Hemiptera, sub-order Sternorrhyncha (Martin 1987). The first New World *Bemisia tabaci* was found in the United States on sweetpotato
(Ipomoea batatas Lam) in 1894 and described as *Aleyrodes inconspicua* Quaintance with the common name, sweetpotato whitefly (Quaintance 1890). In 1914, the genus *Bemisia* was first described (Quaintance and Baker 1914) and in 1936, Takashaki replaced the name *Aleurodes tabaci* with *Bemisia tabaci* (Takahashi et al. 1936).

*Bemisia tabaci* has been described as a complex species that constitutes a large number of genetically variable populations, which are referred to as biotypes (Brown et al. 1995). *Bemisia tabaci* is currently considered a species complex composed of 28 morphologically similar species that can be differentiated by mitochondrial cytochrome oxidase (mtCOI) (Barro et al. 2011). The two most devastating biotypes are B (Costa and Brown 1991) and Q (Guirao et al. 1997, Rosell et al. 1997). Biotype B was determined to have originated from the Middle East-Asia Minor region (Costa and Brown 1991), and has become one of the most damaging pests in tropical and subtropical regions (Oliveira et al. 2001, De Barro et al. 2011). The Q biotype was determined to have originated from the Mediterranean region (Rosell et al. 1997). The B biotype of *Bemisia tabaci* became established in Florida in the late 1980s and by the early 1990s had caused devastating damage to agricultural crops in warm agricultural regions of the United States from California to Florida. Perring and co-workers defined this species as *Bemisia argentifolii* (Perring et al. 1992), but this designation has not been widely accepted. After molecular and genetic studies, DeBarro and co-workers described it as *B. tabaci* Middle East Asia Minor (MEAM1). *Bemisia tabaci* biotype B and *B. argentifolii* are now formally named as *Bemisia tabaci* Middle East Asia Minor (MEAM1), and the Q biotype is formally named *Bemisia tabaci* MED (Tay et al. 2012).
Feeding and Damage

*Bemisia tabaci* has opisthognathus piercing-sucking mouthparts (Gerling et al. 1990). *Bemisia tabaci* feeds on phloem sap and excretes honeydew, a sugar-rich excreta that provides habitat for saprophytic fungi (sooty mold) and reduces harvest quality (Gerling et al. 1990). It transmits over 150 different plant viruses from seven virus groups including Geminiviridae, Closteroviridae, Carlaviridae, Potyviridae, Nepoviridae, Luteoviruses and DNA-containing rod-shaped viruses. Of these, the Geminiviridae and Closteroviridae are the most devastating (Duffus 1987, Duffus et al. 1996). Among the Geminiviridae, *B. tabaci* transmits *Tomato yellow leaf curl virus* (TYLCV) and *Tomato yellow mottle virus* to tomato (Polston and Anderson 1997). *Bemisia tabaci* MEAM1 also induces irregular ripening in tomatoes, leaf silvering in squash, and a white streak in cole crops (Cohen et al. 1992).

*Bemisia tabaci* adults select suitable parts for feeding that vary according to host species. *Bemisia tabaci* is apparently attracted to UV light when predisposed to migrate and attracted to the yellow spectrum of light associated with most vegetation when predisposed to colonize plants (Mound 1962). *Bemisia tabaci* can identify suitable host plants only after landing on them and receiving gustatory information through probing (Janssen et al. 1989). They typically feed on the underside of the leaf. Younger leaves are more commonly exploited for feeding as well as for oviposition, presumably because younger leaves have higher nutritional quality. In addition, younger leaves may be easier to penetrate than older leaves (Lenteren and Noldus 1990).
Life Cycle

*Bemisia tabaci* has six life stages: egg, crawler (*1st* nymphal instar), three sessile instars (*2nd*, *3rd* and *4th* nymphal instars) and the adult. Reproduction in *B. tabaci* is usually sexual, but females can also reproduce by arrhenotokous parthenogenesis (Dobreanu and Manolache 1969). In this form of parthenogenesis, unmated females produce only male offspring and mated females produce both male and female offspring. *Bemisia tabaci* oviposit on younger leaves of tomato, eggplant and cotton (Arx et al. 1984) and lay eggs on the underside of leaves. They mostly prefer plant species with smooth or glabrous leaves (Mound 1965). The egg to adult cycle of *B. tabaci* takes 27.3 d on tomatoes at 25°C and 65% RH (Salas and Mendoza 1995). Under these conditions, lifetime fecundity was 194 ± 59.1 eggs per female on tomato, with an egg viability of 86.5%. On the basis of research conducted in Egypt on cotton plants, the number of eggs that *B. tabaci* can produce varies from 48-394, depending on temperature (Azab et al. 1972). During the course of their lifetime, females oviposit about 250 eggs at 28.5°C, 204 eggs at 22.7°C, and 61 eggs at 14.3°C (Azab et al. 1972).

Eggs are ovoid and initially cream colored but darken with age to a deep brown. Eggs are attached to the plant by a pedicel and are 0.21 mm in length and 0.096 mm in width, attached to a stomatal opening or slit produced by the female (Byrne and Bellows 1991). The pedicel facilitates the intake of water from leaves into the eggs. The incubation period of the egg is about 7-8 d on tomato and cotton at 25°C and 75% RH. The first instar emerges from the egg apex. Nymphs are classified into four nymphal instars and the *4th* nymphal instar is sometimes called a red-eyed nymph. Whitefly nymphs produce wax that surrounds them. The *1st* instar is active for the first few hours
after hatching (named the “crawler” stage) before it settles for the remainder of its nymphal development. The crawler has three-segmented legs and two-segmented antennae. These become one-segmented in 2nd and 3rd instars, which are immobile (Byrne and Bellows 1991). At 250°C and 75% RH the nymphal stages of 2nd and 3rd instar last for 6-7 d each, and the 4th instar lasts for 6-8 d on tomato (Salas and Mendoza 1995). Adults emerge from nymphal exuvia leaving a T-shaped exit hole in the integument. Generally, the size of B. tabaci varies from 0.85-1.2 mm. Females average 0.96 mm and males are a bit smaller at 0.82 mm (Byrne and Bellows 1991). Female adult longevity is about 19 d while male longevity is about 19.5 d on tomato plants (Salas and Mendoza 1995). The whitefly has a unique dorsal anus structure known as the vasiform orifice. It is present on the abdominal segment on males and eighth and ninth segments on females (Mound 1983).

Management of Bemisia tabaci

Chemical Control

Historically insecticides used to manage B. tabaci included carbamates (for example: aldicarb, carbaryl, carbofuran), organophosphates (for example: bromophos, demeton, methyl parathion, mevinphos) and pyrethroid insecticides (for example: cypermethrin, fenpropathrin, fenvalerate). Among the most common modes of action used for chemical control of whitefly are sodium channel modulators (pyrethroid insecticides) and nicotinic acetylcholine receptor (NACHR) competitive modulators (neonicotinoids) (Freeman et al. 2016). Whitefly resistance to insecticides was first recorded in 1985 before the description of biotypes (Barro et al. 2011). Bemisia tabaci has been characterized by a high degree of resistance to many chemical insecticides including organophosphates, carbamates, pyrethroids, insect growth regulators and
chlorinated hydrocarbons (Cahill et al. 1996). Resistance is also detected on α-cypermethrin, bifentrin, pirimiphos-methyl, endosulfan, imidacloprid, thiamethoxam and alpha-cypermethrin (Roditakis et al. 2005, Wang et al. 2010).

Cultural Methods

Cultural methods of pest control rely on the manipulation of the crop environment to reduce damage by harmful insects. Cultural control can play a significant role in the management of B. tabaci. Cultural practices used to manage B. tabaci include repellent metallized plastic mulch, manipulation of planting time, employing a crop-free period, crop rotation, crop sanitation, and trap crops (Zitter and Simons 1980, Cock et al. 1986). Metallized plastic reflective mulch reflects UV light, which interferes with host location and orientation of whiteflies (Csizinszky et al. 1999). Buckwheat and clover are living mulches that have been evaluated to manage B. tabaci. These species provide habitat for predators that attack whiteflies (Frank and Liburd 2005). Living mulches such as the legumes Arachis pintoi (perennial peanuts) and Styzolobium deeringianum (mucuna) (Fabaceae), Drymaria cordata (“cinquillo”) (Caryophyllaceae), and Coriandrum sativum (coriander) (Umbelliferae) reduced numbers of whitefly and incidence of Tomato mottle virus (Hilje and Stansly 2008).

Host Plant Resistance

Reduction in pest population growth through the impact of host plant shape, size, chemistry, nutritional characteristics, or other characters, is known as host plant resistance. Breeding is used to incorporate resistance traits often found in wild species into commercially acceptable varieties (Berlinger 1986). Some varieties of Lycopersicon peruvianum (L) Mill, L. hirsutum and L. hirsutum F. glabratum are resistant to B. tabaci.
because of their glandular trichomes (Channarayappa et al. 1992). The toxins secreted by glandular hairs of tomatoes can confer resistance to *B. tabaci* (Kennedy and Yamamoto 1979). Densities of leaf trichomes, the existence of acyl sugars in the exudate of glandular trichomes and type of trichomes can alter the relationship between the whitefly and tomato (Simmons 1994). Several wild species of tomato show resistance against *B. tabaci*. *Lycopersicon pennellii* (Corr.) D'Arcy contains sugar esters in its glandular exudates (Goffreda et al. 1990). Plant hairs can provide a physical barrier against whiteflies (Duffey 1986). *Bemisia tabaci* prefers not to oviposit on very hairy leaves (Mound 1965). More whiteflies were found in pubescent and hirsute soybean than glabrous soybean (McAuslane et al. 1995). *Bemisia tabaci* prefers lightly pubescent varieties to densely pubescent varieties as the hairy varieties may cause a physical barrier and less hairy leaves provide more suitable microclimate with high relative humidity for the pest (Mound 1965).

**Biological Control**

Biological control uses natural enemies to reduce damage by pests. Natural enemies are predators, parasitoids and pathogens that reduce pest abundance, leading to reduced economic damage. There have been some successes in the biological control of whiteflies (Arnó et al. 2009). There are about 92 species of predatory insects and mites and more than 52 parasitoid species that are known to attack *B. tabaci* (Gerling et al. 2001).

Whitefly parasitoids: Parasitoids are host specific and live within or attached to the host’s body. They lay eggs on or in the host and can also kill the host by feeding on it (Emden and Service 2004). Primary parasitoids that are being employed against whiteflies include *Encarsia* spp. and *Eretmocerus* spp. (Hymenoptera: Aphelinidae)
(Gerling et al. 1980, De Barro and Coombs 2009), *Amitus* spp. (Hymenoptera: Platygasteridae) (Joyce et al. 1999), and *Metaphycus* spp. (Hymenoptera: Encyrtidae) (Polaszek et al. 1999). About 23 species of *Encarsia* and 10 species of *Eretmocerus* have been studied as parasitoids of whitefly (Gerling et al. 2001). Parasitoids *Encarsia formosa*, *Eretmocerus mundus*, and *Eretmocerus queenslandensis* are among the most effective natural enemies that are commercially available (Gerling et al. 2001).

**Whitefly predators:** Predators of *B. tabaci* include beetles (Coccinellidae), true bugs (Miridae, Anthocoridae), lacewings (Chrysopidae, Coniopterygidae), mites (Phytoseiidae) and spiders (Araneae) (Nordlund and Legaspi 1996, Li et al. 2011). Some coleopteran predators of whitefly are *Serangium parcesetosum* (Abboud and Ahmad 1998) found in India, *Clitostethus arcutatus* (Booth et al. 1996), and *Delphastus catalinae* (Hoelmer et al. 1993). Some Hemiptera such as *Macrolophus caliginosus* and *Dicyphus tamaninii* are effective predators of greenhouse whitefly (Barnadas et al. 1998, Lucas and Alomar 2002). The mirid, *Nesidiocoris tenuis* Reuter, is an omnivorous predator that has been evaluated for biological control of whiteflies in greenhouse-grown tomatoes (Calvo et al. 2012). *Serangium parcesetosum* larvae can consume about 89.2 nymphs of *B. tabaci* per day and 1119.1 nymphs during the course of larval development at 30°C on cotton plants (Sengonca et al. 2005). *Nephaspis oculata* is another predatory lady beetle introduced for whitefly control; females consume approximately 86 whitefly eggs per day, males consume approximately 79 eggs per day and a pair can consume about 184 eggs per day (Liu et al. 1997). Some effective predators that are commercially available for the control of *B. tabaci* are *Coccinella*
septempunctata, Neoseiulus californicus, Neoseiulus cucumeris, D. tamaninii, M. caliginosus, Dicyphus hesperus and Chrysoperla carnea (Gerling et al. 2001).

Fungal pathogens: Fungal pathogens for the management of B. tabaci include Beauveria bassiana, Paecilomyces amoenoroseus, P. fumosorosaeus, Verticillium lecanii and Metarhizium anisopliae. These entomopathogenic fungi are commercially available and can contribute to the control of B. tabaci (Faria and Wraight 2001). Natural epizootics of entomopathogenic fungi can also suppress B. tabaci populations through infection. The entomopathogenic fungus Lecanicillium muscarium is used as biocontrol agent to control B. tabaci (Cuthbertson et al. 2005).

Paecilomyces fumosorosaeus has been used to reduce whitefly populations in the field and greenhouse (Lacey and Kirk 1993, Castineiras 1995). Some fungi are highly effective for nymphal stages of whitefly such as B. bassiana (Vicentini et al. 2001, Quesada-Moraga et al. 2006) and P. amoenoroseus (Candido 1999). Isaria fumosorosaeus (Paecilomyces fumosorosaeus designated) is one of the most important fungal pathogens of whiteflies (Huang et al. 2010).

Dicyphus hesperus

Dicyphus hesperus (Hemiptera: Miridae; Subfamily: Bryocorinae; Tribe: Dicyphini) is an omnivorous predator native to North America (Henry et al. 1988). Dicyphus hesperus was first described on whiteflower leaf cup, Polymnia canadensis L. (Compositae) by Knight in 1941. It has since been described from many varieties of plants. Most of the insects of the Bryocorinae subfamily have a wide host range. They are well adapted to plants with sticky glandular hairs (Wheeler and Krimmel 2015). Dicyphus hesperus is mostly associated with the Solanaceae, Scrophulariaceae, Rosaceae, Lamiaceae, Ericaceae, and Hydrophyllaceae families (Cassis 1986).
*Dicyphus hesperus* is a zoophytophagous species that feeds on both plant tissue and prey. It consumes water from plants, and engages in extra-oral digestion of prey (Gillespie and McGregor 2000, Sinia et al. 2004). Consumption of prey is necessary for the completion of nymphal development on tomato plants. *Dicyphus hesperus* feeds by inserting its sucking mouthparts into prey and sucking out the inner contents. After consuming whitefly eggs or nymphs, only the chorion or integument remains, with a tiny hole where the mouthparts were inserted (McGregor et al. 1999).

**Biology**

*Dicyphus hesperus* requires 42 d to complete its life cycle from egg to adult at 20°C on tomato plants (Gillespie et al. 2004), passing through four nymphal stages. Wing development is initiated during the 3rd nymphal instar. Both males and females are about 3.5 mm long and lightly covered with pale-brown, erect long scales. Adult females insert eggs into plant stem tissue, veins and stalks with a sword-like ovipositor (Cohen 1995, 1998). When *Ephestia kuehniella* eggs are provided as a dietary supplement, the lifetime fecundity of *D. hesperus* is about 63.2 ± 10.2 eggs on tomato and 49.6 ± 7.8 eggs on mullein (*Verbascum thapsus* L., Scrophulariaceae) (Sanchez et al. 2004). Greenhouses with mullein as well as tomato had higher numbers of *D. hesperus* than greenhouses with tomato alone (Sanchez et al. 2003). *Dicyphus hesperus* fed with *E. kuehniella* eggs produces eggs that hatch in 18 d on tomato at 20°C and L16:D8.

The 1st instar lasts for 6-7 d then molts to 2nd instar, which lasts 4-5 d. The 3rd instar lasts 5-6 d and the final (4th) instar lasts for 9-10 d at 20°C. Total nymphal development takes about 25-27 d on tomato plants at 20°C (Sanchez et al. 2004). Adult *D. hesperus* are good fliers and will move throughout large areas to find prey. Nymphal stages of *D. hesperus* cannot fly but are fast walkers and will move up and down and between plants.
which are in contact with each other. *Dicyphus hesperus* seems more active at nighttime than in daylight (VanLaerhoven et al. 2006). *Dicyphus hesperus* can complete its life cycle on tomato if prey such as whitefly or mites is available (McGregor et al. 1999). *Dicyphus hesperus* can complete its life cycle when provided only with plant material on some plant species like mullein, sweet pepper (*Capsicum annuum* L.) and catnip. It cannot complete its lifecycle without prey on tomato, tobacco (*Nicotiana tabacum* L.), broadbean (*Vicia sativa* L.) and corn (*Zea mays* L.) (Sanchez et al. 2004). Lack of prey also decreases adult longevity and fecundity (Sanchez et al. 2004).

**Use of D. hesperus for Management of Insect Pests in Greenhouses**

In British Columbia, Canada, *D. hesperus* was first examined for its potential to control pests of greenhouse tomato plants, including greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) and two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae) with effective results (McGregor et al. 1999). Dicyphine mirids are most often described as predators of whiteflies (Gabarra et al. 1995, Alomar and Albajes 1996, Barnadas et al. 1998), and are considered effective for control of thrips (Thysanoptera: Thripidae), aphids (Hemiptera: Aphididae), leafhoppers (Hemiptera: Delphacidae), and other small insects (Salim and Heinrichs 1986, Chua and Mikil 1989, Braman and Beshear 1994, Gabarra et al. 1995).

Banker plants are non-crop plants that directly or indirectly provide habitat, food, and/or prey to the natural enemies that are released within a crop to control a pest (Frank 2010). The purpose of banker plants is to boost the population of natural enemies in an agricultural production system and sustain the population for long-term pest suppression (Huang et al. 2011). The mullein plant has been evaluated as a banker plant for *D. hesperus* (Sanchez et al. 2004). Studies have demonstrated that
mulein can be used to establish and increase populations of *D. hesperus* (Sanchez et al. 2004). Sanchez et al. (2003) demonstrated that mullein can serve as a reservoir for *D. hesperus* in commercial greenhouse production of tomato and help suppress populations of *T. vaporariorum*.

**Challenges to Managing Tomato Pests in Protected and Organic Agriculture**

Protected agriculture, consisting of greenhouses, screen houses and shade houses, is a small but growing sector of Florida’s agricultural economy (Hochmuth and Toro 2014). The use of high tunnels for vegetable production increased from zero in 2001 to 186.41 acres in 2013. Hochmuth and Toro (2014) estimate that intensive production of greenhouse tomatoes in Florida could yield 90.71 metric ton per acre with a gross income of $250,000 per acre.

*Bemisia tabaci* is one of the primary pests of tomato and other horticultural crops. Chemical pesticides can provide effective control of *B. tabaci* under certain circumstances, but frequent use of chemicals elevates production costs and can lead to the development of insecticide resistance. Insecticides may also have negative effects on natural enemies as well as agricultural workers and the environment. In addition, many insecticides are not registered for use in protected agriculture and are not compatible with the use of commercial pollinators. As the adoption of protected agriculture is increasing, the use of biological control methods may contribute to effective management of *B. tabaci* and other pests in protected structures. *Dicyphus hesperus* has been shown to be an effective predator of greenhouse whitefly, thrips and mites, with potential application for control of *B. tabaci*.

**Objectives**

1. Evaluate predation of *B. tabaci* by *D. hesperus*
a) Estimate the consumption rate of *B. tabaci* eggs by female *D. hesperus* directly from the insectary and from an established colony

b) Estimate the consumption rate of *B. tabaci* eggs and nymphs by mated vs. unmated female *D. hesperus*

c) Determine the consumption rate of *D. hesperus* on different life stages (eggs, earlier nymphs, later nymphs)

2. Determine the parameters for establishing a population of *D. hesperus* on banker plants in the greenhouse.

a) Evaluate the life cycle of *D. hesperus* on tomato and on mullein with and without *E. kuhniella* eggs under controlled conditions of relatively constant temperature

b) Determine the survival of *D. hesperus* on mullein plants with *E. kuhniella* eggs under actual greenhouse conditions

c) Evaluate the population development of *D. hesperus* on mullein with and without *E. kuhniella* eggs and on tomato with *E. kuhniella* eggs in the greenhouse
CHAPTER 2
EVALUATING THE PREDATORY CAPACITY OF DICYPHUS HESPERUS ON BEMISIA TABACI

Introduction

Tomato, Solanum lycopersicum L., is an economically important crop in Florida (USDA 2016). Approximately 29,000 acres of tomato was produced in Florida in 2017, yielding 426,739.7 metric tons. This comprised about 38% of the total tomato production in the United States and generated $365,774,000 from 2015-2018 (USDA 2018). In recent years, vegetable production in greenhouses has increased from zero in 2001 to 186.41 acres in 2013. Hochmuth and Toro (2014) estimated that intensive production of greenhouse tomatoes in Florida could yield 200,000 lb per acre with a gross income of $250,000 per acre.

The sweetpotato whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), is a severe pest of tomato widely distributed throughout the world. Bemisia tabaci causes direct damage to plants by phloem sap removal and indirect damage by transmitting viruses and producing honeydew that serves as substrate for sooty mold on plants, thereby reducing photosynthesis (Oliveira et al. 2001). Although chemical pesticides provide effective control of B. tabaci under certain circumstances, they may have deleterious effect on natural enemies as well as agricultural workers. The frequent use of chemicals elevates production costs and B. tabaci has also demonstrated the tendency to develop resistance to many classes of insecticides, including organophosphates pesticides (e.g., α- cypermethrin, bifenthrin, pirimiphos- methyl, endosulfan, pymetrozine, pyrethroids, cyclodienes, and alpha- cypermethrin) and neonicotinoid pesticides (e.g., thiamethoxam, imidacloprid) and insect growth regulators (Cahill et al. 1995, 1996; Roditakis et al. 2005 and Wang et al. 2010).
In addition, many insecticides are not registered for use in protected agriculture and are not compatible with the use of commercial pollinators. As the adoption of protected agriculture is increasing, the use of biological control methods may contribute to effective management of \textit{B. tabaci} and other pests in protected structures. As a result, an integrated approach with the use of biological control agent should be employed.

Many predators have been reported for the biocontrol of greenhouse whitefly \textit{Trialeurodes vaporariorum} (Westwood) and sweetpotato whitefly \textit{B. tabaci} (Nordlund and Legaspi 1996). Some mirid bugs native to the Mediterranean region, \textit{Macrolophus caliginosus} Wagner and \textit{Dicyphus tamaninii} Wagner, are commercially used as natural enemies for whitefly and thrips on tomato and cucumber plants (Barnadas et al. 1998, Albajes and Alomar 1999). Both the adult and nymph of \textit{M. caliginosus} and \textit{D. tamaninii} prey upon all the stages of whitefly and can significantly reduce greenhouse whitefly populations (Lucas and Alomar 2002). The predator \textit{Nesidiocoris tenuis} Reuter has demonstrated effective control of adult \textit{B. tabaci} on greenhouse tomato (Calvo et al. 2009).

The Dicyphine mirids are zoophytophagous generalist predators. They have been traditionally neglected for use for biological control of pests because they are facultative plant feeders. They feed on plants to acquire water, and this may produce economic damage (Castañé et al. 2011). However, the potential for economic loss due to feeding by the predator depends on the predator: prey ratio and the actual plant parts upon which they feed (Castañé et al. 2011). \textit{Dicyphus hesperus} Knight (Hemiptera: Miridae) is a widely distributed zoophytophagous true bug native to North America
The economic damage caused by *D. hesperus* is minor as they prefer plant leaves over fruits (McGregor et al. 2000). This predator has been evaluated for management of pests on greenhouse-grown tomato plants (Gillespie et al. 2007). Female adult *D. hesperus* effectively controlled greenhouse whitefly, *T. vaporariorum* Westwood and two-spotted spider mites, *Tetranychus urticae* Koch, on tomato plants, consuming on average 24 early instar nymphs and 43 older nymphs of whitefly per day and consuming 28 mites per day (McGregor et al. 1999).

*Dicyphus hesperus* released at a rate of one predator per plant reduced sweetpotato whitefly populations by 88.8% and potato psyllid populations by 90.1% on tomato plants in the greenhouse under a wide range of climatic conditions (Calvo et al. 2016). A predator: prey ratio of 0.5-1:10 is required for the adequate control of western flower thrips, *Frankliniella occidentalis* Pergande, populations in tomatoes (Shipp and Wang 2006).

The main objective of this research was to evaluate the effectiveness of *D. hesperus* for the biological control of *B. tabaci* (MEAM 1) and to develop baseline information about the number of predators needed to control this pest. To meet this goal, we determined the daily and lifetime consumption rate of *D. hesperus* on different stages of *B. tabaci*.

### Methods and Materials

#### Plants and Insects

Mullein seedlings (*Verbascum thapsus*) were purchased from Gloeckner and Company (550 Mamaroneck Avenue, Harrison, NY) and were transplanted into 10-cm-diameter pots in a greenhouse at the Gulf Coast Research and Education Center (GCREC), Balm, Florida. They were provided with 20:20:20 All Purpose nutrient mix
(Hummert International Company, 4500 Earth City Expressway, MO 63045) (10 g for 3 liters), at a rate of 125 ml water per plant every 15 d. Six-week-old mullein plants were placed in a Bug Dorm (60 × 60 × 60 cm, MegaView Science Co. Ltd., Taiwan), which were used to rear D. hesperus. A colony of D. hesperus was established from individuals originally purchased from Beneficial Insectary (Redding, CA) (Figure 2-1), and reared on mullein plants in a growth room (temperature 24-28°C; RH 48-55%; L: D 12:12) (Figure 2-2). Ephestia kuehniella Zeller eggs, purchased from Beneficial Insectary, were provided, attached to a 2-cm adhesive section of a yellow Post-It note (3M Corporation, St Paul, MN) (Figure 2-3). Approximately 0.25 g (0.1 g Ephestia/100 Dicyphus hesperus) was provided to each Bug Dorm every week as a protein source. This method for providing E. kuehniella eggs to D. hesperus was developed by Chris Daye of Beneficial Insectary. HOBO data loggers (Onset Technology Company, Pocasset, MA) were set up to record temperature and relative humidity.

A colony of B. tabaci MEAM1 established in the early 1990s from field populations in the Bradenton, Florida area and maintained on cotton (Gossypium hirsutum L.) plants was used as a prey source. Cotton plants (DPO935B2RF variety) were grown from seed and transplanted to 20-cm-diameter pots with Fafard (BWI-Apopka, Plymouth, FL 32768) soil potting mix in a greenhouse. Plants were watered as required and fertilized with a 20:20:20 nutrient mix every week. Whiteflies were reared in the growth room (temperature 24-28°C; RH 48-55%; L: D 12:12) with four to six cotton plants per rearing cage. Tomato plants (variety 'Lanai') supplied by the tomato breeding program at GCREC were grown from seed in 20-cm-diameter pots with Fafard
potting mix in a greenhouse. Plants were watered as required and fertilized with a 20:20:20 nutrient mix every week.

**Estimating the Consumption Rate of *Bemisia tabaci* Eggs by Female *Dicyphus hesperus* from Two Different Sources**

*Dicyphus hesperus* used in predation studies were from two sources, Beneficial Insectary (Redding, CA) and the *D. hesperus* colony that was established from *D. hesperus* sent from Beneficial Insectary and maintained in a growth room at GCREC. *Dicyphus hesperus* females were randomly selected (mating status unknown) from Beneficial Insectary and GCREC colony. Females from Beneficial Insectary were used the day after arrival and females from the colony were chosen according to their wing and abdomen color to confirm that they were not older than 72 h (Figure 2-4). The color of wings remains light green for 1 d and the color of abdomen remains bright green for 3 d and then started change to darker green up to 7 d.

**Distinguishing Mated and Unmated Females for Predation Study**

Individual 4th instar *D. hesperus* nymphs were placed in small round clear plastic containers (diameter 15 cm) with one mullein leaf and *E. kuehniella* eggs. When these individuals became adults, females were either kept in isolation to prevent mating, or were confined with males for 3 d to allow mating. Mated females and unmated females were used for egg predation and nymph predation experiments.

**Estimating the Consumption Rate of *Bemisia tabaci* Eggs by Female *Dicyphus hesperus***

Lanai tomato plants, 6-7 weeks old, were used as a substrate for sweetpotato whitefly oviposition for predation studies. Sweetpotato whitefly adults were aspirated in groups of seven to 10 mating pairs into a glass eye dropper and tapped into a clip cage, which was then attached to a leaflet on a mid-stratum leaf, where they were allowed 24
h to lay eggs on leaves (Figure 2-5). After 24 h, leaves were removed from the tomato plant with a razor blade and all the sweetpotato whitefly eggs on the leaves were counted under the microscope. This was the “pre-count”. The stem of the leaf was then placed in a plastic vial (9 cm long with 3 cm diameter) filled with 60 ml water to maintain leaf turgidity. Parafilm was placed over the top of the vial around the base of the leaf to prevent spillage. The vial and tomato leaf were then placed in a clear Plexiglass cylinder (30 cm tall with 13 cm diameter) with an organdy top (Figure 2-6). Individual *D. hesperus* females were then placed in each cylinder, which served as the arena for the predation studies. Ten arenas, labeled 1-10, were set up for each trial of the experiment. *Dicyphus hesperus* females were confined in arenas 1-5. The remaining arenas, 6-10, were used as an untreated control to test for the disappearance of *B. tabaci* eggs due to non-predator related factors, such as experimental handling.

Leaves were examined under a microscope after 24 h. The total number of eggs present and eggs damaged due to *D. hesperus* feeding was recorded from the leaves that were confined with female *D. hesperus*. Eggs that had been fed upon by *D. hesperus* consisted of an empty, damaged chorion and were clearly identifiable under the microscope (Figure 2-7a). The total number of eggs present and missing in the controls were also counted to compare with the leaves confined with *D. hesperus*. The untreated control was added because preliminary studies revealed that leaves confined with *D. hesperus* sometimes had fewer post-count eggs even after damaged ones were taken into account. *Dicyphus hesperus* is not thought to consume whole eggs as they have piercing-sucking mouthparts, so it is possible that they dislodged the egg from the leaf in the process of feeding or locomotion. *Dicyphus hesperus* apparently engages in
extra-oral digestion (Cohen 1995). If this results in partial dissolution of the egg chorion, it could also help explain the disappearance of eggs. The untreated control was added to determine if there were fewer post-count eggs because eggs were being dislodged by *D. hesperus* or were falling off the plant for reasons unrelated to the activity of the predator, such as experimenter handling.

Each female was provided with a new leaf containing 24-h-old sweetpotato whitefly eggs each day until her death. The experiment was carried out using 34 females randomly selected (mating status unknown) from Beneficial Insectary from June 21 to August 4, 2016 and 22 females from the GCREC colony from August 17 to September 21, 2016. Comparing egg predation by mated and unmated female *D. hesperus* was carried out using 21 mated and 21 unmated female *D. hesperus* from the GCREC colony from October 2 to November 19, 2016. In each trial, the number of damaged eggs and missing eggs was recorded for each 24-h period of the female’s life.

Thus, the egg consumption study was carried out using females that had been handled in three different ways: 1) females that had arrived the day previously from Beneficial Insectary; 2) females that had been reared in a colony at GCREC and that were 72 h old or younger; and 3) females from the GCREC colony that were 72 h old or younger, and that were divided into mated or unmated groups.

**Estimating the Consumption Rate of *Bemisia tabaci* Nymphs by Female *Dicryphus hesperus***

The predation rate of *D. hesperus* on *B. tabaci* nymphs was evaluated following the same methods as used for the egg predation experiment in which mated and unmated *D. hesperus* females were used. Clip cages were set up on leaves and whiteflies were allowed to oviposit for 24 h. After 24 h the clip cages were removed and
the adults were removed from the plants. Those plants with sweetpotato whitefly eggs were then kept in a Bug Dorm of dimensions 60 × 60 × 60 cm for 9-10 d to allow the eggs to hatch into nymphs. The numbers of 1st and 2nd instar nymphs on leaves were counted and those leaves were then confined with female D. hesperus for 24 h using the same methodology as described for eggs. After 24 h the leaves were removed and the post-count was carried out. Damaged nymphs fed upon by D. hesperus consisted of an empty, damaged integument and were clearly identifiable under the microscope (Figure 2-7b). Each female D. hesperus was given a new leaf with nymphs until she died. This experiment was performed with 20 mated and 20 unmated females from November 23 to December 28, 2016.

**Predation Behavior of Dicyphus hesperus on Different Life Stages of Bemisia tabaci**

The predation behavior of D. hesperus on different sweetpotato whitefly nymphal stages was evaluated. The study was conducted in the growth room at 27°C. We placed clip cages on 12 tomato leaflets on mid-stratum leaves from five tomato plants and five to seven pairs of whiteflies were confined on those leaflets for oviposition. Whiteflies were removed from those leaflets after 24 h. The eggs that had been oviposited were allowed to develop to 3rd and 4th instar for 14 d. Five days after the first whiteflies were placed, we confined whiteflies for 24 h on different leaflets and allowed them to grow for 9 d in order to produce 1st and 2nd instar nymphs. To produce eggs for the study, whitefly adults were confined on the leaf for 1 d, then removed, and the plants were used immediately for the experiment. Thus, 2 weeks after the first infestation, eggs and early and late instar B. tabaci nymphs were available. The leaves were then placed in a plastic vial (9 cm long with 3 cm diameter) filled with 60 ml water to maintain leaf
turgidity. The top of the vial was covered with Parafilm wrapped around the base of the leaf to prevent spillage. The vial and tomato leaf were placed in a clear Plexiglass cylinder (30 cm tall with 13 cm diameter) with an organdy top and a single mated 3-4 d-old female *D. hesperus* from the GCREC colony was confined on a leaf. Thus the experiment evaluated predation of whiteflies of different ages in a no-choice situation. After 24 h, we counted the number of live and damaged eggs and nymphs. There were four replications (females) per trial of the experiment and the trial was repeated four times for a total of 16 replications (females) of each of the three whitefly-age treatments. Comparison of predation among *B. tabaci* eggs, earlier nymphs and later nymphs was done on the basis of the number of damaged individuals of different life stages of *B. tabaci*.

**Statistical Analysis**

Predation behavior of *D. hesperus* was evaluated using two sources: *D. hesperus* directly brought from Beneficial Insectary and *D. hesperus* from the colony. Average number of *B. tabaci* eggs consumed per day over the entire adult lifetime was calculated for the 34 females directly from Beneficial Insectary and for the 21 females (less than 72 h old) from the GCREC colony. Statistical comparisons could not be made between the females from the two sources because the experiments were done at different times.

A *t*-test run in *R* (R-studio 2013) was applied to compare 21 mated and 21 unmated female *D. hesperus* for their average daily consumption and lifetime consumption on both *B. tabaci* eggs and nymphs. A regression analysis was run to determine the correlation between the number of eggs/nymphs provided and the number of eggs/nymphs consumed by *D. hesperus*. 33
Predation of female *D. hesperus* on *B. tabaci* eggs, earlier nymphs and later nymphs was evaluated. The number of damaged life stages (eggs, 1\(^{\text{st}}\) / 2\(^{\text{nd}}\) and 3\(^{\text{rd}}\) /4\(^{\text{th}}\) instars) of *B. tabaci* was recorded separately. Predation of different life stages of *B. tabaci* was compared using PROC GLM in SAS (SAS institute 2001).

**Results**

**Predation of *Bemisia tabaci* Eggs by *Dicyphus hesperus* from Two Sources**

For both predator sources, the number of intact *B. tabaci* eggs on tomato leaves after 24 h was lower in the treatment provided with female *D. hesperus* than in the control without the predator (Beneficial Insectary: \( t = 7.2; \) df = 344; \( P < 0.0001 \) and GCREC colony: \( t = 5.45; \) df = 322; \( P < 0.0001 \)). However, the total reduction in *B. tabaci* egg population on treatment (Insectary) was 13.03\%, including 6.91\% with visible damage and 6.13\% which were missing compared to the control with 5.6\% missing. The reduction in *B. tabaci* egg population by the colony *D. hesperus* was 13.43\%, including 7.14\% with visible damage and 6.29\% missing compared to the control treatment with 6.97\% missing. The average consumption was 2.7 ±0.2 eggs per day and 14.1 ±1.7 over the lifetime by *D. hesperus* from Beneficial Insectary and 3.3 ± 0.9 per day and 25.5 ±7.1 over the lifetime by *D. hesperus* from GCREC colony. Female *D. hesperus* from Beneficial Insectary survived on average 5 ±0.4 d (Figure 2-8 a) whereas the average survival for colony *D. hesperus* was 7.4 ±0. 4 d (Figure 2-8 b). *Dicyphus hesperus* apparently engages in extra-oral digestion (Cohen 1995). If this results in partial dissolution of the egg chorion, it could also help explain the disappearance of eggs. The untreated control was added to determine if there were fewer post-count eggs because eggs were being dislodged by *D. hesperus* or were falling off the plant for
reasons unrelated to the activity of the predator, such as experimenter handling. The missing data from treatment and control were compared and found to be very similar. This suggests that the missing data were due to handling or egg hatching not by predator activity. So, the control was not used for further experiments.

**Predation of *Bemisia tabaci* Eggs and Nymphs by Mated and Unmated Female *Dicyphus hesperus***

The daily consumption rate of mated and unmated female *D. hesperus* on *B. tabaci* eggs did not differ significantly ($F = 1.29; \text{df} = 1,40; P = 0.2054$) but lifetime consumption rate of mated *D. hesperus* females on eggs was significantly higher than that of unmated *D. hesperus* ($t = 5.37; \text{df} = 40; P < 0.0001$) (Figure 2-9a). Daily nymph consumption ($t = 0.43; \text{df} = 40; P = 0.6684$) as well as lifetime nymph consumption ($t = 0.57; \text{df} = 1,40; P = 0.5708$) by mated and unmated female *D. hesperus* did not differ significantly (Figure 2-9b). On average, female *D. hesperus* consumed eight times more nymphs than eggs of *B. tabaci* in a 24-h period and 10 times more nymphs than eggs in their lifetime.

The number of eggs damaged increased as the number of eggs provided to female *D. hesperus* increased up to certain level where the damage averaged 20 eggs per day, and then the regression line leveled off ($R^2 = 0.168$) (Figure 2-10). During most 24-h exposure periods, female *D. hesperus* damaged only up to 20 eggs, however, on a few days, a few predators consumed up to 100-120 eggs per day (Figure 2-11). The consumption of nymphs increased as the number of nymphs provided to *D. hesperus* increased ($R^2 = 0.44$) (Figure 2-12). On most days, female *D. hesperus* fed on 50 to 100 nymphs, however, on several days female predators fed on up to 300 nymphs per day (Figure 2-13). Predation rate increases as the number of available prey increases, and
then levels off (Figure 2-9; Figure 2-11). The predation over lifetime was also analyzed and there was no pattern seen in the damaged eggs and nymphs with the age of female *D. hesperus* although, the consumption was low at the near the end of the lifespan (Figure 2-15). The survival of mated and unmated *D. hesperus* fed on whitefly eggs and nymphs was not significantly different (*t* = 1.017; *df* = 40; *P* = 0.32 and *t* = 1.04; *df* = 40; *P* = 0.31) respectively. Predators had 100% survival up to 6th day when fed on eggs and all were dead by day 15. All *D. hesperus* provided with whitefly nymphs survived for at least 9 days and all predators were dead by day 25 (Figure 2-14).

The daily consumption and lifetime consumption of *D. hesperus* of *B. tabaci* eggs, earlier nymphs and later nymphs were significantly different (*F* = 209.89; *df* = 2,769; *P* < 0.01) and (*F* = 142.06; *df* = 2,774; *P* < 0.001) respectively (Figure 2-16). The consumption rate on earlier and later whitefly nymphs was not significantly different. The survival % of predators fed on whitefly early and later nymphs was greater than on eggs (Figure 2-17). One hundred percent of *D. hesperus* fed on older nymphs survived for 10 d. Thirty percent of *D. hesperus* fed on older nymphs survived for 31 d. One hundred percent survived for 9 d and 10% survived for 26 d when fed on earlier instar nymphs. One hundred percent survived for 4 d and 10% survived for 27 d when provided with whitefly eggs only (Figure 2-17).

**Discussion**

The female *D. hesperus* from the GCREC colony ate more during the course of their lifetime as they lived longer. The lifetime consumption of Insectary *D. hesperus* was low as they were short- lived (Figure 2-8). This could be because of the age of predator when used in the predation study. The age of females from the GCREC colony were known as we choose young females, based on their abdomen color, so they likely
had a longer remaining lifespan than the females of unknown age from Beneficial Insectary.

The average consumption rate of both mated and unmated female *D. hesperus* on *B. tabaci* eggs was about 10 eggs per day when an average of 94 eggs (ranging from 0-120 eggs) (Figure 2-11) were provided and the average consumption of *B. tabaci* nymphs was about 92 nymphs per day when an average of 246 nymphs (ranging from 10-300 nymphs) were provided to daily them (Figure 2-13). This rate of feeding is substantially higher than that shown by other hemipteran predators, such as *M. caliginosus* and *D. tamaninii*, which fed on only five and 13 *B. tabaci* nymphs respectively when 40 *B. tabaci* nymphs were provided (Barnadas et al. 1998). The reason for higher consumption rate of *D. hesperus* in our study could be because higher number of prey nymphs were provided to the predator in comparison to the studies using *M. caliginosus* and *D. tamaninii*. *Dicyphus hesperus* are known as potential predators of greenhouse whitefly and have been reported consuming 23.7 ± 6.5 younger nymphs per day and 43.2 ± 15.8 older nymphs per day. However, the number of nymphs provided to them was not recorded (McGregor et al. 1999). Our research supports that *D. hesperus* can be a potential predator for *B. tabaci* control. The predators live longer when fed on whitefly nymphs than on eggs. This could be because of size differences between eggs and nymphs; nymphs are bigger and likely easier to locate.

The major concern of using omnivorous mirid predator for biological control is their ability to damage plants, as the predator needs the plant for water sources and also to survive in the case of prey scarcity (Naranjo and Gibson 1996). Some
zoophytophagous predators can show direct damage to plants but in the case of *D. hesperus* the feeding damage on tomato fruit is related to prey availability under greenhouse conditions; if there is sufficient prey available for the predator, plant damage will be minimal (Shipp and Wang 2006). Other research also supports the use of *D. hesperus* as a predator and did not record any negative impact on tomato fruit (Gillespie et al. 2007). Overall, all the results suggest that the release of *D. hesperus* would be more effective if the pest population is high on tomato crops. It has considerable potential to improve the biological control of *B. tabaci* in the greenhouse. These studies provide baseline data to support the use of *D. hesperus* for sweetpotato whitefly management in greenhouse tomatoes in Florida. However, the potential of *D. hesperus* needs to be evaluated in commercial greenhouse before it can be recommended as a biological control agent for *B. tabaci*.
Figure 2-1. *Dicyphus hesperus* from Beneficial Insectary company (Redding, CA) used for predation study and also for establishing the GCREC colony. Photo courtesy of author.
Figure 2-2. Colony of *D. hesperus* maintained on mullein plants in growth room (temperature 25-28°C, RH 48-55% and L:D 12:12) Photo courtesy of author.
Figure 2-3. Provision of *D. hesperus* with approximately 0.25 g *Ephestia kuehniella* Zeller eggs (0.1g *Ephestia*/100 *D. hesperus*) as a supplemental prey source Photo courtesy of author.
Figure 2-4. Young female *D. hesperus* (2-3 d) with green abdomen used for the experiment to determine daily consumption and lifetime consumption of *B. tabaci* eggs and nymphs. Photo courtesy of author.
Figure 2-5. Tomato plant with clip cages holding 7-10 pairs of whiteflies confined for 24 h to lay eggs for use in predation study in the growth room. Photo courtesy of author.
Figure 2-6. Experiment set up in the growth room where tomato leaves with *B. tabaci* eggs/nymphs were confined with a female *D. hesperus* for 24 h with continuous re-provision until her death. Photo courtesy of author.
Figure 2-7. Damage to whitefly eggs (A) and nymphs (B) by the predator *D. hesperus*. Photo courtesy of author.
Figure 2-8. Survival percentage of female *D. hesperus* from Beneficial Insectary (A) and from GCREC colony (B) when fed on *B. tabaci* eggs on tomato leaves.
Figure 2-9. Average number of *B. tabaci* eggs and nymphs consumed by mated and unmated female *D. hesperus* (21 replication in each treatment) daily and over their lifetimes on tomato plants in an experiment conducted in the growth room of GCREC under controlled conditions. Errors bars indicate 1 SEM. The letters (Uppercase letter for daily consumption and lowercase for lifetime consumption) on the top of bars indicate significant difference. Bars within a variable topped by the same letters do not differ significantly (*t*-test; $\alpha < 0.05$).
Figure 2-10. Scatter plot of the number of *B. tabaci* eggs provided on tomato leaves to female *D. hesperus* (pre-count) and number damaged in a 24-h period. *Dicynhus hesperus* female studied = 42, Predation days (number of days all 42 females were provided with eggs) (n) = 343.
Figure 2-11. Number of 24-h periods where consumption of whitefly eggs on tomato leaves by *D. hesperus* females fell into damage frequencies shown on the x axis. The experiment was conducted in the growth room of GCREC.
Figure 2-12. Scatter plot of the number of *B. tabaci* nymphs provided on tomato leaves to female *D. hesperus* (pre-count) and number damaged in a 24-h period. *Dicyphus hesperus* females studied = 42, Predation days (number of days all 42 females were provided with nymphs) (n) = 480.

\[ y = -0.0004x^2 + 0.5846x - 20.795 \]

\[ R^2 = 0.4404 \]
Figure 2-13. Number of 24-h periods where consumption of whitefly nymphs on tomato leaves by *D. hesperus* females fell into damage frequencies shown on the x axis. The experiment was conducted in the growth room of GCREC.
Figure 2-14. Survival percentage of mated and unmated female *D. hesperus* (A) when fed on *B. tabaci* eggs and (B) when fed on *B. tabaci* nymphs on tomato leaves.
Figure 2-15. Patterns of damaged (A) eggs and (B) nymphs by *D. hesperus* throughout their lifetime.
Figure 2-16. Average number of *B. tabaci* eggs, earlier nymphs and later nymphs consumed by female *D. hesperus* daily and over their lifetimes on tomato plants in an experiment conducted in the growth room of Gainesville under controlled conditions. Data shown are means ± SE and the letters A and B indicate significant differences within treatments for daily consumption and a and b for lifetime consumption of *D. hesperus* (Tukeys, α < 0.05). The experiment was performed in Gainesville growth room. N = 16 number of *D. hesperus* females.
Figure 2-17. Survival percentage of female *D. hesperus* when fed on *B. tabaci* eggs, earlier nymphs and later nymphs on tomato leaves. The experiment was performed in Gainesville growth room. N = 16 number of *D. hesperus* females.
CHAPTER 3
ESTABLISHMENT OF *DICYPHUS HESPERUS* ON MULLEIN AND TOMATO UNDER CONTROLLED AND GREENHOUSE CONDITIONS

Introduction

The tomato, *Solanum lycopersicum* L., has been cultivated in the United States for decades, both outdoors and under glass, for fresh market consumption and for processing. Florida is a leading producer of tomato and contributes about 34% of the total U.S. production with recent annual revenue averaging $424,118,000 (USDA 2017). In 2016, about 30,000 acres of tomato were planted in Florida with production of about 815,360,000 lb (USDA 2017). Protected agriculture, consisting of greenhouses, screen houses and shade houses, is a small but growing sector of Florida’s agricultural economy (Hochmuth and Toro 2014). The use of high tunnels for vegetable production increased from zero in 2001 to 186.41 acres in 2013. Hochmuth and Toro (2014), estimated that intensive production of greenhouse tomatoes in Florida could yield 200,000 lb per acre with a gross income of $250,000 per acre. Arthropod pests are a primary constraint on the production of greenhouse tomatoes in Florida. One of the most serious pests limiting tomato production in south Florida is *Bemisia tabaci* (Gennadius) MEAM1, the sweetpotato whitefly.

Commercial agricultural yield losses due to whitefly have been reported all over the world, primarily in tropical and sub-tropical regions, but also in protected agriculture in temperate regions (Oliveira et al. 2001). The sweetpotato whitefly transmits *Tomato yellow leaf curl virus* (TYLCV) and also causes irregular ripening of tomato (Jones 2003). Chemical insecticides can provide effective control of *B. tabaci* under certain circumstances but frequent use of chemicals elevates production costs. It is also a very challenging pest to control by chemicals due to its high reproduction rate and potential
to develop resistance to insecticides, as well as the side effects on beneficial organisms used in IPM programs. The rapid build-up of insecticide resistance in this pest requires the development of alternative management approaches (Castle et al. 2010).

The adoption of biological control has increased over recent decades, as it is a more sustainable method of pest control, reducing the likelihood that arthropods will develop resistance against chemical insecticides. After the outbreaks of *B. tabaci* in the southern regions of the United States in 1992, the study of establishment and augmentation of natural enemies against this pest increased. Predators such as *Macrolophus caliginosus* Wagner, *Dicyphus taminii* Wagner and parasitoids such as *Encarsia formosa* Gahan and *Eretmocerus eremicus* Rose and Zolnerowich have been used successfully for whitefly control (Gerling et al. 2001). There are still some challenges for the use of biological control agents such as frequent mass release of natural enemies, crop damage by omnivorous predators, and plant structure hindering the efficiency of natural enemies. Parasitic Hymenoptera that have been used for whitefly management include *E. formosa* and *E. eremicus* (Arnó et al. 2011). Repeated releases of whitefly parasitoids are required for effective control, which is not always economically viable (Greenberg et al. 2002). *Encarsia formosa* has low efficiency for pest control on tomato in the greenhouse because of trichomes and exudates present on tomato leaves. Sticky honeydew produced by whitefly hinders the parasitoid’s movement and lowers pest foraging efficiency (Levin 1973). The predatory mirids *Macrolophus pygmaeus* Rambur and *Nesidiocoris tenuis* Rambur demonstrated efficacy against whitefly but their use has been constrained by impacts on non-target species and the potential risk of crop injury (Albajes et al. 2006, Van Lenteren et al. 2006).
*Dicyphus hesperus* Knight has been evaluated for the control of whitefly and the presence of the predator decreases the population of *B. tabaci* by about 88% (Calvo et al. 2016). In a previous study conducted in British Columbia, Canada, *D. hesperus* completed its development on the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), and two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae) and also controlled these pests (McGregor et al. 1999). Predatory mirids such as *D. hesperus* are well adapted to plants like tomato as they have pretarsal claws (curved, elongated) to remove plant exudates from their antennae, proboscis and body; this behavior allows them to attach to and live on sticky hosts. They have long, slender legs and walk on tiptoe to minimize contact with exudates (Wheeler and Krimmel 2015).

The need to release bio-control agents repeatedly can substantially increase the cost of control. Methods for establishing populations of biocontrol agents in the greenhouse with a limited number of releases can increase the viability of biocontrol as a management option for pests of protected structures (Huang et al. 2011). The banker plant system is an open-rearing system that supports augmentation and conservation of predators within a cropping system. Banker plants are non-crop plants that act as long-lasting rearing units for natural enemies and are infested with herbivores or provisioned with supplement food (Stacey 1977, Hansen 1983). The main purpose of banker plants is to maintain, and hopefully increase, the predator population even if the pest is not present (Bennison 1992). It is important to establish the population of *D. hesperus* even in the absence of prey for effective biocontrol of pests. The use of an alternative host
plant is sometimes necessary to help to preserve the predator and maintain their population density (Sanchez et al. 2003).

In previous studies, mullein, *Verbascum thapsus* L., was used as an alternative host to evaluate the growth response of *D. hesperus* on tomato plants. Whitefly populations were successfully controlled on tomato by *D. hesperus* in greenhouses with and without mullein plants, however, the abundance of predators was higher on the tomato plants with mullein plants than on tomato plants only (Sanchez et al. 2003). *Dicyphus hesperus* built up to higher populations on mullein than on pepper or eggplant when used as alternative host plants with tomato (Nguyen-Dang et al. 2016). In addition, the reproduction rate and longevity of *D. hesperus* was higher on mullein plants provided with *Ephestia kuehniella* Zeller eggs as supplemental prey sources than on mullein plants with no supplemental prey (Sanchez et al. 2004). *Ephestia kuehniella* eggs are produced by commercial insectaries and are frequently used as a dietary supplement to maintain certain predators, such as *D. hesperus* and *M. caliginosus* (Gillespie and Mcgregor 2000, Callebaut et al. 2004).

Based on the previous studies referenced above, I chose to investigate mullein plant as an alternative host plant for the establishment of *D. hesperus* in tomato crops. For the practical and effective use of *D. hesperus*, the predator must remain reproductively active throughout the cropping season. So, the main objectives of my research were to evaluate the establishment capacity of *D. hesperus* on different supplemental food sources so that it can control whitefly before pest populations reach unmanageable levels. In particular, I evaluated: 1) the survival and development of *D. hesperus* on mullein as a banker plant and on the main crop, tomato, with and without
E. kuhniella eggs as supplemental prey, under controlled conditions, and 2) the population development of D. hesperus under commercial greenhouse conditions.

Materials and Methods

Plants and Insects

Tomato plants (variety 'Lanai'), supplied by the tomato breeding program at the UF/IFAS Gulf Coast Research and Education Center (GCREC), Balm, FL, were grown from seed in 20-cm-diameter pots with Fafard 2 Mix (BWI-Apopka, Plymouth, FL 32768) soil potting mix in a greenhouse. Plants were watered as required and fertilized with Jacks 20:20:20 All Purpose nutrient mix (Hummert International Company, 4500 Earth City Expressway, MO 63045) (10 g for 3 liters) in 125 ml water every week. Mullein seedlings (Verbascum thapsus) were purchased from Gloeckner and Company (550 Mamaroneck Avenue, Harrison, NY) and were transplanted into 10-cm-diameter pots in a greenhouse at GCREC. Each mullein plant was provided with 20:20:20 water-soluble nutrient (10 g for 3 liters) in 125 ml water every 15 d.

A colony of D. hesperus, established from individuals originally purchased from Beneficial Insectary (Redding, CA), was reared in a growth room (temperature 24-28°C, RH 48-55 %; L: D 12:12) on 6-week-old mullein plants contained in a Bug Dorm (60 × 60 × 60 cm, MegaView Science Co. Ltd., Taiwan) (Figure 3-1). Ephestia kuehniella eggs, purchased from Beneficial Insectary, were provided attached to a 2 cm by 7.5 cm adhesive section of a yellow Post-It note (3M Corporation, St Paul, MN). Approximately 0.25 g of eggs (0.1 g Ephestia/100 D. hesperus) was provided to each Bug Dorm every week as a protein source (method developed by Chris Daye, Beneficial Insectary). HOBO data loggers (Onset Technology Company, Pocasset, MA) were set up to record temperature and relative humidity.
Life Cycle of *Dicyphus hesperus* on Tomato and Mullein With and Without *Ephiesta* eggs Under Growth Room

The development time of *D. hesperus* from 1st instar nymph to adult, survival of nymphs to adulthood, and adult longevity were evaluated on mullein and tomato plants with and without eggs of *E. kuehniella* as a dietary supplement. The four treatments evaluated were: 1) mullein with *E. kuehniella* eggs; 2) mullein without *E. kuehniella*; 3) tomato with *E. kuehniella*; and 4) tomato without *E. kuehniella*.

In order to produce individual *D. hesperus* 1st instar nymphs to track under the four treatments, five plants each of tomato and mullein of about 4 weeks old were kept separately in individual cages (35 x 35 x 60 cm) (Bio-Quip®, Compton, CA) in a growth room (temperature 24-28°C; RH 48-55%; L: D 12:12). Five pairs of male and female *D. hesperus* that had been adults for 1 week were released into each cage. Each cage was provided with 0.25 g *E. kuehniella* eggs on 2-cm adhesive strips every week. When a 1st instar nymph appeared on a mullein or tomato plant, it was removed and placed into a translucent round plastic box (5 cm height and 13 cm diameter) containing either a new tomato or mullein leaf, with or without *E. kuhniella* eggs, depending on the treatment. First instar nymphs produced by adults reared on tomato were used in the tomato treatments, and nymphs produced by adults reared on mullein were used in the mullein treatments. Leaf petioles were covered with wet cotton to maintain turgidity (Figure 3-2 and 3-3). Eight replicates (1st instar nymphs) were selected for each treatment. The trial was repeated three times for a total of 24 replicates for each of the four treatments. The four rearing substrate treatments were arranged in a randomized complete block design in a growth room under the same conditions as described above. Nymphs were observed every day and moved to a new leaf every 3-4 d. The date of
molting from one nymphal instar to the next was recorded according to the change in size and the presence of cast skin (Figure 3-4). Dates of molts were recorded until individuals reached the adult stage to calculate the days spent in each instar stage, and days required to complete development from nymph to adult under the four treatments. In order to determine treatment effects on adult longevity, adults were kept on the same treatment until they died.

Population Development of *Dicyphus hesperus* on Mullein Plants With *Ephestia* Eggs Under Greenhouse Conditions

Two experiments were conducted to determine the rate of population development of *D. hesperus* and the potential for establishment on mullein with an *Ephestia* supplement under actual greenhouse conditions. One trial was carried out in a commercial greenhouse in Wimauma, Florida and one trial was carried out in a greenhouse at GCREC (Table 3-1).

**Trial at Commercial Greenhouse, Wimauma**

Fifty-four gauze bags with bamboo sticks as support and binder clips to close the top were set up with one mullein plant (8-10 weeks old) in each cage in a 200 ft bay of a commercial greenhouse on June 14, 2016 (Figure 3-5). Cages were arranged in four blocks in the greenhouse. Thirteen cages were placed in the northeast and northwest corners of the bay, and fourteen cages were placed in the southeast and southwest corners of the bay. Five pairs of *D. hesperus* were placed in each cage provided with *Ephestia* eggs that were attached to 2-cm adhesive section of a yellow Post-It note every week. Two weeks after setup, starting on June 14, two cages were sampled randomly from each corner each week for 7 weeks, with the last sample taken August
16. *Dicyphus hesperus* of all stages were aspirated from each cage and kept in ethanol vials. Later, the number and life stage of each individual from each cage was recorded.

**Trial at GCREC Greenhouse**

Single 5-6 weeks old mullein plants in 15-cm-diameter pots were maintained in cages (35 x 35 x 60 cm) (Catalog #1466BV, Bio-Quip®, Compton, CA). Ninety cages were set up in the greenhouse on August 17, 2016, arranged on six greenhouse benches in groups of 15, corresponding to six replications of the experiment (Figure 3-6). Five pairs of male and female *D. hesperus* were placed on mullein in each cage. About 0.25 g *E. kuehniella* eggs were placed in each cage each week. Starting on September 1, one cage was sampled randomly from each block (bench) each week for 14 weeks, with the last sample taken December 2. *Dicyphus hesperus* of all stages were aspirated from each cage and kept in ethanol vials. Later, the number and life stage of each individual from each cage was recorded.

**Population Development of *Dicyphus hesperus* on Mullein With and Without *Ephestia* eggs and on Tomato With *Ephestia* Eggs Under Greenhouse Conditions in Gainesville**

The population development of *D. hesperus* on three different combinations of plant and supplemental food was studied in the greenhouse in Gainesville, FL (Table 3-1). On August 14, 2017, 60 mullein plants and 30 tomato plants, 6-8 weeks old, were established individually in cages (35 x 35 x 60 cm) (Bio-Quip®, Compton, CA) for the following treatments: 1) mullein plant with *Ephestia* eggs, 2) mullein without *Ephestia* eggs, and 3) tomato plants with *Ephestia* eggs (Figure 3-7). Each treatment was replicated six times. Five pairs of 1-week-old *D. hesperus* males and females were introduced into each cage and treatments were arranged in a randomized complete block design with six blocks. After 3 weeks, one plant was randomly selected from each
of the three treatments in each of the six blocks. All nymphs and adults were collected carefully with the help of an aspirator from the cages and the plants and were counted. Additional plants were selected from each treatment in each block on the sixth, ninth, twelfth and fifteenth week after introduction of the mated adult D. hesperus. A fresh 6-week-old plant was placed in each cage on week 12 without removing the original plant. Experiment conditions were summarized (Table 3-1).

**Statistical Analysis**

Life history parameters of *D. hesperus* reared under four different treatments were compared with PROC GLIMMIX and TUKEY-HSD was used for mean separation (SAS Institute 2001). Nymphal survival was analyzed by using PROC LOGISTIC (SAS Institute 2001). Trial was set as a random factor in the analysis.

All the stages (1st, 2nd, 3rd, 4th and adult [male and female]) of *D. hesperus* were recorded separately for each of the 7 weeks from the commercial greenhouse data and 14 weeks from GCREC greenhouse data. Means of each life stage of *D. hesperus* were plotted to identify population trends. No statistical analysis was conducted.

The population development of *D. hesperus* in the Gainesville greenhouse experiment was compared among the three treatments with ANOVA using PROC MIXED in SAS (SAS Institute 2001). Population trends were plotted over 15 weeks. Mean separation was done by Tukey-Kramer in SAS.

**Results**

**Life Cycle of *Dicyphus hesperus* on Tomato and on Mullein With and Without *Ephestia* eggs Under Controlled Conditions**

There were significant differences in total nymphal development time among the treatments (F = 10.44; df = 2, 57; P < 0.0001) (Figure 3-8). The nymphal development
time of *D. hesperus* reared on mullein with *E. kuehniella* eggs and tomato with *E. kuehniella* eggs was shorter than on mullein without *E. kuehniella* eggs whereas *D. hesperus* did not reach the 3\textsuperscript{rd} instar on tomato without *E. kuehniella* eggs (Figure 3-8). The duration of the 1\textsuperscript{st} instar did not differ with and without *E. kuehniella* eggs on tomato treatments but was significantly shorter on mullein with *E. kuehniella* than without (F = 5.20; df = 3,90; P = 0.0023). The duration of the 2\textsuperscript{nd} instars did not differ among treatments (F = 1.92; df = 3,76; P = 0.133). The 3\textsuperscript{rd} instar development time was significantly shorter when reared on mullein with *E. kuehniella* eggs compared to mullein without *E. kuehniella* eggs and tomato with *E. kuehniella* eggs (F = 8.06; df = 2,56; P = 0.0008) and the development of 4\textsuperscript{th} instar differ among treatments (F = 23.50; df = 2,56; P < 0.0001) (Figure 3-8).

Survivorship of nymphs varied among the four treatments. The percentage of *D. hesperus* surviving on mullein and on tomato with *E. kuehniella* eggs was not significantly different but was significantly greater by 15% than the percentage surviving on mullein without *E. kuehniella* eggs (F = 1.31; df = 2,1; P = 0.5262) (Figure 3-9). Adult longevity of *D. hesperus* on mullein with and without *E. kuehniella* eggs and tomato with *E. kuehniella* eggs differed significantly and was longest on mullein with *E. kuehniella* eggs than on tomato with *E. kuehniella* eggs; both were longer than on mullein without *E. kuehniella* eggs (F = 39.75; df = 2,57; P < 0.0001) (Figure 3-9).

**Population Development of *D. Hesperus* on Mullein Plants With *Ephestia* Eggs Under Greenhouse Conditions**

In the commercial greenhouse in Wimauma, *D. hesperus* were reared on mullein with *E. kuehniella* eggs and population growth was studied for 7 weeks. The temperature recorded by HOBO in the bay throughout the 7-week duration of the
experiment ranged from 23.87 to 43.84°C (Figure 3-10 A). High in temperature has a negative effect on nymphal development (Gillespie et al. 2004) and about 37.6% of the time during 7 wk duration of my study, the temperature was above 27°C. The average number of 1st instar/2nd instar per sample (combined, as they were difficult to differentiate accurately) increased in the 3rd week and then declined at the end of 7th week whereas the average number of 3rd instar and 4th instar increased until the 4th week and then declined. The average number of adults increased until the 5th week and remained constant until the 7th week (Figure 3-11). Seven weeks after introducing five male and five female D. hesperus per plant, the average number per plant had increased 1.6-fold (Figure 3-11). The population initially increased 2 weeks after establishment when nymphs first began to emerge, and continued to increase up to the 5th week, after which the population decreased (Figure 3-11).

The population development of D. hesperus on mullein was observed in the GCREC greenhouse for 14 weeks (Figure 3-12). Temperature conditions over the 14-week experiment ranged from 15.37 to 36.13°C with 77.2% RH (Figure 3-10 B). About 37.6% of the time over 14 weeks, the temperature was over 27°C that could have negative impact on population growth of predator. The average number of 1st /2nd instar D. hesperus per sample (cage) reached up to 40 per sample during the 2nd and 3rd weeks and then decreased down to 10. Numbers of 3rd and 4th instars increased up to 15 in 4th week and again decreased to 6. The adult population maintained its density of approximately 15 per plant throughout the 14-week experiment. Total number of D. hesperus increased on 3rd to 4th weeks to 70 and then decreased to 35 and remained
constant (Figure 3-12). The number of *D. hesperus* had increased 3.5-fold per plant by the end of week 14 (Figure 3-12).

**Population Development of Dicyphus Hesperus on Mullein With And Without Ephestia Eggs and on Tomato With Ephestia Eggs Under Greenhouse Conditions**

The population development of *D. hesperus* was evaluated in a Gainesville greenhouse under three different treatments: mullein with and without *E. kuehniella* eggs, and on tomato with *E. kuehniella* eggs in the greenhouse. In previous trials, *D. hesperus* completed its life cycle on these three diets but not on tomato without a supplemental protein source (Figure 3-8). Therefore, tomato without *E. kuehniella* eggs was not evaluated in this trial. HOBO data loggers were set up to record the temperature, which averaged 25.3°C, ranging from 16.2 to 34.8°C over the 15-week experiment (Figure 3-10 C). About 26% of the time over 15 weeks the temperature was above 27°C, which may have had a negative impact on predator nymphal development.

The development of *D. hesperus* was significantly different on three treatments (*F* = 8.24; df = 2, 64; *P* = 0.0007) and the averaged totals of different instars of *D. hesperus* for 15 weeks were calculated (Figure 3-13). The experiment was started with five male and five female adult *D. hesperus* per plant. Populations increased 5-fold on tomato with *E. kuehniella* eggs, and 2.5-fold on mullein with *E. kuehniella* eggs but did not increase on mullein without *Ephestia* eggs by the end of 15 weeks (Figure 3-13). The population increased gradually on tomato provided with supplemental food of *E. kuehniella* eggs. The population remained constant on mullein with *E. kuehniella* eggs for a while then increased after the 09h week while on mullein without *E. kuehniella* eggs, the predator maintained its initial population density (Figure 3-13).
Discussion

The availability of alternative prey sources on the host plants in the agroecosystem is beneficial for *D. hesperus* as they provide additional nutrients that help in their development and survival (Sanchez et al. 2004). The development time of *D. hesperus* was shorter on mullein and tomato when provided with *E. kuehniella* eggs, similar to the results of previous research (Sanchez et al. 2004). We found that *D. hesperus* could not complete its life cycle on tomato plant without any prey sources which was also noted by Sanchez et al. (2004). Nymphal development duration was longer when the predator was reared on mullein without any supplemental food source. Longer development time of *D. hesperus* may be disadvantageous for biological control because whitefly reproduction capacity is high (Jones 2003). Adult longevity was longer on mullein and tomato with a prey source, which clearly indicates that *D. hesperus* needed supplemental food to enhance reproduction (McGregor et al. 2000). In my laboratory experiment, 92% of *D. hesperus* were able to complete their life cycle on mullein and 88% of *D. hesperus* on tomato plant with *E. kuehniella* eggs but only 75% completed their life cycle on mullein without a prey source (Figure 3-6). The faster growth of *D. hesperus* on mullein and tomato plant with prey source is likely due to the presence of more nutritious food (i.e., *E. kuehniella* eggs). Using tomato plant as a banker plant may contribute to the early establishment of *D. hesperus* in main crops when provided with supplemental food source and also help to maintain predator populations even after prey populations decrease. Tobacco plants have been used as banker plants for early establishment of *Macrolophus pygmaeus* in tomato greenhouses. The reproduction of predator was highest in the presence of banker plants and tomato plants than just with tomato plants (Bresch et al. 2014).
In the commercial greenhouse study, the population development of *D. hesperus* on mullein with *Ephestia* eggs was recorded and population trends plotted. We found that the population did not grow during the 7-week period. The nymphs of *D. hesperus* emerge 10-14 d after oviposition and complete their nymphal development in 24-32 d (Sanchez et al. 2003). Seven weeks may not have been sufficient time to allow the predator population to grow significantly.

In the GCREC greenhouse, the experiment was conducted for 14 weeks on mullein plants provided with supplemental food sources. The result showed that the population of the predator increased three-fold. The increase in the population in 4th week might be because of emergence of 1st instars. After the 4th week the population remained steady till the 14th week. Mullein can help maintain the predator population when *E. kuehniella* eggs are also provided. Although the predator can maintain their population on mullein only it would be more fruitful if a supplemental prey source was provided to increase the reproduction and predator population density. More the number of predators available, the greater the chances of pest population control.

In one of the previous studies, the development time of *D. hesperus* from Woody, California and Summerland, British Columbia decreased with increase in temperature up to 27°C. The mortality of *D. hesperus* nymphs increased in both populations when temperature increased from 27 to 35°C (Gillespie et al. 2004). In Florida greenhouses, however, 24-h average temperature sometimes reaches up to 40°C. An effect of extreme temperature on the mortality of nymphs and fitness of adult was likely to be a factor for low population growth of *D. hesperus*. In commercial greenhouse trial and in the GCREC greenhouse trial the temperature was above 27°C about 37.5% of the time.
and in Gainesville greenhouse trial, the temperature exceeded 27°C about 26% of the time.

From the first development experiment conducted under controlled conditions, we found that *D. hesperus* can survive on mullein and tomato with prey and mullein without prey. So, I studied dynamics of *D. hesperus* on all three treatments in the greenhouse. Tomato plant with prey source (i.e. *E. kuehniella* eggs) serves as a best source for establishment of *D. hesperus* and mullein plant with *E. kuehniella* eggs also showed population growth but *D. hesperus* on mullein plant without *E. kuehniella* eggs could only maintain their population. Just to maintain the predator population, mullein can be good source but to increase the population of predators mullein plant alone will not be an option. This implies that mullein plants alone cannot be a banker plant system; it is the prey source provided to them that plays a vital role to establish predator population.

The lifecycle period of *D. hesperus* was shorter on mullein with *E. kuehniella* eggs than on tomato with *E. kuehniella* eggs but the population growth rate was twice as high on tomato with *E. kuehniella* eggs than on mullein with *E. kuehniella* eggs. This indicate that the fecundity could be higher on tomato than on mullein, similar to previous research where fecundity on tomato was 63 and on mullein was 49 (Sanchez et al. 2003). The other reason for increase in population on tomato plants supplemented with *E. kuehniella* eggs might be due to high nutrition from prey. From my study I conclude that *D. hesperus* do not need alternative host plants as long as tomato plants have supplemental prey sources such as *E. kuehniella* eggs but in the absence of prey they cannot build their population. It would be effective to use supplement source in the
development of predator on tomato crop also on banker plant. Tomato plant can be a good banker plant for earlier establishment of *D. hesperus* in the presence of pest or alternative prey. Mullein plants can also be used as alternative host plants to conserve predator populations when the main crop is not available. Those plants can also influence the performance of *D. hesperus* for the biological control of pest. It is important to learn more about the predator establishment on different plant species. Banker plant system has potential to improve biological control efficiency. Further research is needed to integrate adoption of biological control method with banker plant system.
Table 3-1. Different experiments conducted to evaluate the development of *D. hesperus* populations under greenhouse conditions.

<table>
<thead>
<tr>
<th>Location</th>
<th>Dates</th>
<th>Daily average Temp. (°C)</th>
<th>Replicates</th>
<th>Treatments</th>
<th>No. of Sampling times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial greenhouse, Wimauma</td>
<td>June 14 - Aug 16, 2016</td>
<td>24.56 – 32.43</td>
<td>8</td>
<td>Mullein plant with <em>E. kuehniella</em> eggs</td>
<td>7</td>
</tr>
<tr>
<td>GCREC greenhouse, Balm</td>
<td>Aug 17 - Dec 1, 2016</td>
<td>21.47 – 32.43</td>
<td>6</td>
<td>Mullein plant with <em>E. kuehniella</em> eggs</td>
<td>14</td>
</tr>
<tr>
<td>Research greenhouse, Gainesville</td>
<td>Aug 14 - Oct 27, 2017</td>
<td>22.18 – 31.03</td>
<td>6</td>
<td>Mullein with <em>E. kuehniella</em> eggs</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mullein without <em>E. kuehniella</em> eggs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tomato with <em>E. kuehniella</em> eggs</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3-1. Rearing of *D. hesperus* on mullein plants provided with *E. kuehniella* eggs in the growth room of GCREC. Photo courtesy of author.
Figure 3-2. Cage set-up for test of the effect of host plant and supplemental food on *D. hesperus* development. Five pairs of *D. hesperus* were kept in each cage and each cage represents one treatment (mullein plant with and without *E. kuehniella* eggs and tomato plants with and without *E. kuehniella* eggs). Experiment was conducted under controlled conditions in a growth room. Photo courtesy of author.
Figure 3-3. First instar *D. hesperus* was transferred to translucent round plastic box set up for development test. Each box represents one treatment (mullein plant with or without *E. kuehniella* eggs or tomato plants with or without *E. kuehniella* eggs). Mullein and tomato leaves were kept in each box (dependent on treatment) with wet cotton to maintain leaf turgidity. Experiment was conducted in the growth room. Photo courtesy of author.
Figure 3-4. Sizes of different life stages of *D. hesperus*. Photo courtesy of author.
Figure 3-5. Experiment set up with 54 bags of mullien plant each with five male and five female of *D. hesperus* provided with *E. kuehniella* eggs in the commercial greenhouse, Wimauma. Photo courtesy of Hugh A. Smith.
Figure 3-6. Survival of *D. hesperus* was evaluated on mullein plant with *E. kuehniella* eggs. Ninety cages of mullein plant, each with five pairs of *D. hesperus*, were set up in the greenhouse of GCREC. Photo courtesy of author.
Figure 3-7. Evaluation of best dietary source for the development of *D. hesperus* was performed in a greenhouse in Gainesville by setting up five pairs of *D. hesperus* in 90 cages (30 of each of the three treatments: 1) mullein plant with *E. kuehniella* eggs, 2) mullein without *E. kuehniella* eggs, and 3) tomato with *E. kuehniella* eggs). Photo courtesy of author.
Figure 3-8. Average number of days spent in each instar and total nymphal development time of *Dicyphus hesperus* in four treatments: mullein plant with *Ephestia* eggs, mullein plant without *Ephestia* eggs, tomato plant with *Ephestia* eggs and tomato plant without *Ephestia* egg conducted in the growth room. Data shown are means ± SE and the letters a, b and c indicate significant differences within instar and total nymphal development time of *D. hesperus* (Tukeys, $\alpha < 0.05$).
Figure 3-9. Adult longevity of *D. hesperus* reared from first instar nymphs on four treatments: mullein plant with *Ephestia* eggs, mullein plant without *Ephestia* eggs, tomato plant with *Ephestia* eggs and tomato plant without *Ephestia* eggs. Experiment conducted in the growth room of GCREC. Data shown are means ± SE and the letters A, B and C indicate significant differences within treatments for adult longevity and a, b and c for survival of *D. hesperus* (Tukeys, α < 0.05).
Figure 3-10. Average, minimum and maximum temperature of (A) commercial greenhouse (Wimauma) during the 7-wk duration, (B) GCREC greenhouse during the 15-wk duration and (C) Gainesville greenhouse over 15 weeks duration of experiments to determine establishment and population growth of *D. hesperus*. Four HOBO dataloggers, two in front and two in back side of greenhouse were set up and temperature was recorded over a 24-h period. Data shown are high and low temperatures averaged on a weekly basis.
Figure 3-11. Average number of different instars and total number of *D. hesperus* per sample over 7 weeks in a trial conducted in the commercial greenhouse in Wimauma, starting with five pairs of *D. hesperus* on each mullein plant provided with *Ephestia* eggs.
Figure 3-12. Average number of different instars and total number of *D. hesperus* per sample over 14 weeks in a trial conducted in the GCREC greenhouse, starting with five pairs of *D. hesperus* on each mullein plant provided with Ephestia eggs.
Figure 3-13. Average number of *D. hesperus* per sample on each treatment (Tomato with *Ephestia* eggs, Mullein with and without *Ephestia* eggs) over 15 weeks. The experiment was completed in the greenhouse in Gainesville and data shown are means ± SE. The letters a, b and c describe significant differences in population size of *D. hesperus* on three different treatment each week.
CHAPTER 4
SUMMARY

Sweetpotato whitefly, *Bemisia tabaci* Gennadius, is a major pest worldwide in subtropical and tropical agriculture and also in greenhouse production. It has a wide host range recorded in more than 600 species and transmits numerous viruses including Tomato leaf curl virus. The effective control measures used are chemical insecticides, cultural methods and host-plant resistance. *Bemisia tabaci* MEAM 1 has developed resistance against many chemicals that lead to develop a biological control method. *Dicyphus hesperus* was evaluated as an effective predator against greenhouse whitefly, thrips and mites under the greenhouse condition. The predation potential of *D. hesperus* on *B. tabaci* eggs, early nymphs and later nymphs was studied by recording daily consumption and lifetime consumption of female *D. hesperus* on sweetpotato whitefly eggs and nymphs under controlled conditions. Predation was significantly higher on nymphs than on eggs and the predator survived for longer time when fed on whitefly nymphs than on eggs. The consumption rate of predator depends on the pest population density; if the pest population was high in density the consumption rate was also high and vice versa.

The lifecycle of *D. hesperus* was studied on mullein with and without *Ephestia* eggs and on tomato with and without *Ephestia* eggs under controlled condition. There was a significant difference among the treatments; the nymphal development time was shorter on mullein and tomato with *Ephestia* eggs and longer on mullein without *Ephestia* eggs; the predator could not complete the lifecycle on tomato with *Ephestia* eggs. The establishment of *D. hesperus* was studied in the greenhouse on mullein with and without *Ephestia* eggs and on tomato with *Ephestia* eggs for 15 weeks. There was
a significant difference in population growth among treatments. *Dicyphus hesperus* population was more on tomato with Ephestia eggs than on mullein with *Ephestia* eggs and the predator just maintained their population on mullein without *Ephestia* eggs. The results indicate that the *D. hesperus* can establish in main crops when the pest is present or when provided with supplement prey source.

Overall, our results confirmed that *D. hesperus* can be effective control agent for *B. tabaci* in the greenhouse tomato if the pest density is high. The consumption rate was significantly influenced by the different sweetpotato life stages as they preferred nymphs to eggs. This data can be useful for further greenhouse study about the potential of predator to control whitefly such as number of *D. hesperus* needed to control pest population in the greenhouse. Additionally, the interaction of *D. hesperus* with other biological agents as well as with other bio-pesticides for the control of pest can be next step. Our results showed that predator establishment was better on tomato than on mullein provided with supplemental prey source, which is useful to understand the predator growth in the presence of actual pest. Therefore, *D. hesperus* can be potentially considered as an effective predator for the control of *B. tabaci* in the greenhouse.
LIST OF REFERENCES


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BIOGRAPHICAL SKETCH

Pritika Pandey was born in Nepal. She received her M.S. in entomology and nematology from University of Florida in August of 2018 and B.S. in agriculture from Institute of Agriculture and Animal Science, Tribhuwan University, Chitwan, Nepal. She showed her interest in entomology during her undergraduate program. After her B.S. graduation she worked as Livelihood officer in OXFAM International Government Organization in Nepal where she conducted IPM trainings and plant protection work. She was admitted to the University of Florida in 2016 and started her work under the supervision of Dr. Hugh A. Smith at Gulf Coast Research and Education Center GCREC- Balm, FL. During her master’s study, she received two travel awards from Graduate Student Council and GCREC travel grant. She wants to develop her career on researching insects and their ecology.