BIOLOGY AND POPULATION DYNAMICS OF MELONWORM, *DIAPHANIA HYALINATA* L. (LEPIDOPTERA: CRAMBIDAE) ON FOUR CROPS OF CUCURBITS

By

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To my mom and dad

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Melonworm, *D. hyalinata* L., is a serious foliage-feeding pest of cucurbits that causes huge annual economic losses to cucurbit crops. To develop effective management strategies, its biology and population dynamics were studied on four crops of cucurbits: yellow squash, zucchini, cucumber, and watermelon in Homestead, FL, during 2014. Population densities of *D. hyalinata* ranged from a maximum of 6.58 ± 0.14 larvae per 2 leaves (± SE) during September 2014, when temperatures were relatively large, to a low population density in December 2014 (1.25 ± 0.04 larvae per 2 leaves; ± SE) when temperatures were relatively low. *D. hyalinata* distributions tended to be aggregated during crop-growing periods in May 2014, June-July 2014, and September 2014, when temperatures were relatively high, but had uniform distributions in December 2014, when temperatures were relatively low.

Studies on the oviposition and larval preferences of *D. hyalinata* on four cucurbit crops showed that yellow squash was the most preferred host and watermelon was least preferred. The preference level for yellow squash by melonworm did not differ greatly from zucchini. The preference of melonworm for different on-plant locations was also investigated and showed that the middle of the plant was most preferred for

oviposition. However, plant locations did not affect significantly for larval population and leaf defoliation. There were more small larvae than medium or large larvae in the field of cucurbit crops.

Survival of melonworm larvae was least when reared on yellow squash, but greatest on watermelon. However, larvae reared on watermelon required more time to develop than on other crops with development time shortest on yellow squash. A larger head capsule width occurred when larvae were reared on yellow squash than on watermelon. However, whole-body length, pupal weight, and pupal body dimensions showed little or no differences among melonworm larvae reared on the four host plant species.

CHAPTER 1
LITERATURE REVIEW

Importance

Cucurbit crops are widely grown in the USA, with a production of about 3.9 million metric tons on 157,370 hectares, and a value of $1.53 billion. Florida has been the leader in fresh-market production of cucumber, squash, and watermelon, which contribute nearly $247 million to the Florida economy (USDA 2014, Cantliffe et al. 2007). Cucurbitaceae family is only plant group with most species used as human food (Saade and Hernández 1994). Summer squash (*Cucurbita pepo* L.), butternut squash (*Cucurbita moschata* L.), cucumber (*Cucumis sativus* L.), cantaloupe (*Cucumis melo* L.), calabaza (*Cucurbita moschata* L.) and watermelon (*Citrullus lanatus*) are the most commonly grown cucurbit crops in Florida. Melonworm, *Diaphania hyalinata* L. (Lepidoptera: Crambidae), is the most serious insect problem with cucurbit production (Guillaume and Boissot 2001).

The melonworm is a serious pest of cucurbitaceae throughout the southeastern United States (Fulton 1947, Dupree et. al. 1955). The adult moth is active throughout the winter months in southern Florida and disperses throughout the southern and gulf coast states every summer (Reid et al. 1954, Reid & Cuthbert 1956). During the summer, melonworm also migrates into the Carolinas, Oklahoma, Nebraska, and other more northern states (Zehnder 2011).

Melonworm can feed throughout cucurbit plants causing primary and secondary damages resulting in yield reduction (Guillaume and Boissot 2001, Valles and Capinera 1992). Third through fifth instar melonworm larvae feed voraciously on the whole plant including fruit, leaves, and stalks; often removing leaf material while leaving veins and

veinlets intact (Valles and Capinera 1992). Melonworms feed mostly on leaves; however, once the leaf supply is exhausted, larvae begin to feed on the fruit surfaces, or even bore into the fruit (Capinera 2005). The pickleworm (*Diaphania nitidalis* (Stoll)), a closely related species, tunnels into flowers, buds, stalks, vines, and fruits; hence, it can also be destructive (Quaintance 1901, Dilbeck & Canerday 1968). Severe infestation by melonworms may result in total crop failure, leaving only skeletonized remains of plants. In general, squash and cucumber are more severely affected by melonworms than watermelon and pumpkins (Zehnder 2011). Melonworm foliage-feeding results in indirect yield reductions of about 23% (McSorley and Waddill 1982). In some instances, when infestation occurs at an early stage of its host crops, melonworm feeding on foliage may cause >70% yield loss in cucumber. In Florida, further decreases in yields (about 9 to 10%) have been noted from melonworms causing direct losses by feeding on flowers and fruits. In southern and central Florida, the common cucurbit weeds creeping cucumber, *Melothria pendula* L., and wild balsam apple, *Momordica charantia* L., serve as wild hosts of melonworm (Elsey et al. 1985).

Morphology

The melonworm adult is about 2-3 cm in length with a wingspan of 2.5-4.3 cm (Capinera 2005, Sorensen and Baker 2002). Wings are mostly white with a narrow brown band around the wing margin. The adults also have a brown head with white abdomen, which differs from *D. nitidalis*, which has a brown abdomen. On the tip of the abdomen, there are several bushy bristles. The wings of *D. nitidalis* are mostly brown, with flecks of yellow in the center.

Biology

The melonworm is active all year in Florida. Adult females deposit eggs singly or in clusters on flowers, leaf buds, and shoots of host plants (Zehnder 2011). The female moth generally oviposits at day in small clusters of two to six eggs. Individual eggs require 3-4 days for development and are oval, white, 0.6 mm wide, 0.7 mm long, and become dull yellow before larval eclosion (Capinera 2005). The melonworm life cycle from oviposition to adult emergence requires about 30 days with some variation because of temperature. Melonworms have five larval instars which require about 14 days to complete with mean stage durations of 2.2, 2.2, 2.0, 2.0 and 5.0 days for instars 1 through 5, respectively (Capinera 2005). The head capsule widths of instars 1 to 5 are 0.22, 0.37, 0.62, 1.04, and 1.64 mm, respectively (Smith et al. 1994). The newly eclosed first-instar melonworm larva is colorless, but it turns pale green by the second instar with two dark green stripes appearing on the latero-dorsal surface of the fifth instar. Melonworm larvae generally construct and remain within loose silken structures on the undersides of host leaves. Melonworms pupate within a loose silken cocoon on the host plant, often enclosed in a folded section of leaf. The pupa is 12-15 mm long, 2-3 mm wide, both ends are pointed, and is initially whitish, but it becomes brown with maturity. The pupal stage lasts for 9-10 days after which the adult moth emerges. Feeding by all larval stages of melonworm damages the host plants, but the later instars are more voracious and cause considerably more damage than earlier instars.

Within-field and Within-plant Distribution

Distribution of an insect within its host plant varies with the growth of the plants. Often, adults prefer younger leaves and buds for depositing eggs. Resulting larvae disperse throughout the plants as they progress through the 5 instars. However, as the

plant grows older, the larvae tend to remain on the same leaves; hence, older larvae tend to be most common on the older mature leaves. An example of this within-plant distribution is the silver leaf whitefly. The adult female oviposits on the young leaves at the top of plants. Older stages are observed on the older leaves at the middle and bottom of the host plants. Conversely, Seal (1997) found that melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae) abundance is large on the bottom of older leaves at the beginning of infestations with populations dispersing to the top leaves as plant growth progresses. Ali et al. (1989) found that most oviposition by fall armyworms *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) was in the middle to upper portion of plants and most commonly on the undersides of leaves.

Regarding within-field distributions, melon thrips infestation begins at the edge of the host crop field (Seal et al. 2000). Populations then move toward the center of the infested field as the season progresses. In another example, chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) first infests the edge of a host-plant field, then move toward the center following wind directions (Seal et al. 2005). Corn silk fly infestations also begin at the perimeter of corn field (Goyal et al. 2011). Hence, growers prefer to spray a 10-15-foot-wide area along the edge or perimeter of the fields. Mitchell & Fuxa (1987) working with *S. frugiperda* generally supported these studies by noting that as larvae change to later instars, they become less densely distributed and/or less aggregated because of factors such as diminishing numbers from mortality and dispersal. This information on within-plant and within-field distributions can help growers to focus control methods specifically on infested areas. Hence, growers can reduce the

cost of chemicals and delay the development of resistance. By targeting specific areas, the control methods can be applied more efficiently.

Effect of Temperature

Temperature is the most important abiotic factor regulating various biological parameters of an insect, such as the development rate, adult longevity, and reproduction. Effects of temperature on development of insects is exemplified by Ju et al. (2011), who investigated the development and fecundity of *Corythucha ciliate* (Say) (Hemiptera: Tingidae) at seven constant temperatures ranging from 16˚ – 36˚C. Developmental time generally decreased with increasing temperature with female longevity shortest at 33ºC, but fecundity was the largest at 30ºC. Elsey (1982) found that larvae of *D. nitidalis* exposed to -8ºC survived 73% longer within squash fruit compared to larvae at the same temperature in petri dishes without the fruit (0%). However at 0ºC, there was no significant difference in larval survival between fruit and Petri dishes without fruit. Pickleworm adult moths exposed to -8ºC for 1 h had 52% mortality, but at 0ºC for 21 h, there was only 35% mortality, but fecundity among the survivors was reduced to almost 0 (Elsey 1982). Insects also generally consume greater amounts of food at an optimum temperature than at suboptimum temperatures. Insects typically stop oviposition below 10ºC or above 35ºC. Dispersion behavior of insects is also influenced by temperature with dispersion often less below 25ºC than at or above 25ºC.

Management of Melonworm

Biological Control

To avoid reliance on insecticides, management of melonworm using natural enemies plays an important role in developing an Integrated Pest Management (IPM)

program. According to Merriam-Webster (2015), biological control is a method of controlling harmful insects, diseases, etc., in an environment (such as a garden or a lawn) by using other insects or natural substances. It also refers to an insect or natural substance used to control harmful insects, diseases, etc. Several egg, larval, and pupal parasitoids have been reported as biological control agents of *Diaphania spp*. Peña et al. (1987a) collected nine species of insect parasitoids in three families (Braconidae, Ichneumonidae and Tachinidae) from larvae of *Diaphania* spp. from cultivated and wild cucurbits in southern and central Florida. However, rates of parasitism in their study were low despite the diversity. Of the nine parasitoids species, only *Apanteles spp.* was found in significant numbers. The 2nd, 3rd and 4th instars were the most susceptible stages for parasitization by these parasitoids (Peña et al. 1987). Melonworm and pickleworm larvae are also susceptible to parasitism by *Cardiochiles diaphaniae* Marsh (Hymenoptera: Braconidae), with melonworm more susceptible than pickleworm (Smith et al. 1994). Parasitized melonworm and pickleworm larvae are characterized by loss of the two white dorsal stripes, cessation of feeding, and formation of a silken pre-pupal cell (Smith et al. 1994). Parasitism of later instar larvae can affect size (e.g., head capsule width) and growth rates of host insects (Vinson and Iwantsch 1980, Slansky Jr 1986). *Trichogramma exiguum* Pinto and Platner (Hymenoptera: Trichogrammatidae) was recorded to parasitize melonworm and pickleworm eggs at rates of 0 - 69%, and the wasp appeared to be density dependent on host egg populations (Elsey 1980). Fire ants (*Solanopsis spp.)* were found to prey on pupae of melonworm and pickleworm (Elsey 1980). The entomopathogenic nematode, *Steinernema carpocapsae,* is a potential biological control of melonworm and pickleworm, causing up to 100% infection

in Melonworm, but it also infects the parasitoid *Cardiochiles diaphaniae* (Shannag and Capinera 2000). In a study conducted by Shannag and Capinera (1995), *S. carpocapsae* infected 88% of melonworm larvae under laboratory conditions, and 53-55% in the field, suggesting potentially good control of the pest.

Biological Insecticides

Bio-pesticides, a contraction of 'biological pesticides', include several kinds of pest management intervention through predatory, parasitic, or chemical relationships. In the EU, bio-pesticides have been defined as "a form of pesticide based on micro-organisms or natural products" (European Commission 2008, Wikipedia 2015). According to EPA (2012) and Wikipedia (2015), they "include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs".

Various products derived from *Bacillus thuringiensis* are highly effective in controlling different lepidopterous pests especially in earlier instars. Xentari™ (*Bacillus thuringiensis, subspp aizawai*, Strain ABTS-1857) is effective in controlling worm pests of various vegetable crops. One pound of this product has a potency of 15.9 billion diamondback moth units. DiPel DF (*B. thuringiensis subspp kurstaki*; Strain) is another product from *B. thuringiensis* that is commonly used by commercial growers to control lepidopterous pests such as melonworms. One pound of DiPel has a potency of 14.5 billion cabbage looper units. Upon ingestion of *Bt*-treated plant leaf tissues, the Cry protein toxin found in *Bt* becomes inserted into the membrane of midgut cells followed by pore formation, cell lysis, and death of the insect (Schnepf et al. 1998). The effectiveness of *Bt* products is often comparable to new synthetic chemical insecticides

(DRS, personal communication). Because of frequent application of *Bt* insecticides, many insect pest species have become resistant to different *Bt* and Cry proteins. The Indian meal moth *Plodia interpunctella* (Lepidoptera: Pyralidae) developed resistance against various *Bt* strains causing a 100-fold decrease in susceptibility to the *Bt* pesticide DiPel (McGaughey and Johnson 1992, 1994). Similarly, diamondback moths showed more than a 100-fold increase in resistance against *Bt* (Ferré et al. 1991). Resistance development to *Bt*'s also has been documented in *Heliothis virescence* (Gould et al. 1995), *Spodoptera exigua*, *Ostrinia nubilalis* (Huang et al. 1997), *Helicoverpa armigera* (Akhurst et al. 2003) and *Diatraea saccharalis* (Wu 2008). Development of resistance against *Bt* has led to more in-depth studies of Cry protein modes of action and other biochemical and genetic bases of resistance, which could help in developing resistance management methods that include the use of *Bt*'s (Gould 1998). Recent studies have showed the effectiveness of *Bt*'s in controlling diamondback moths and other lepidopterous pests (Seal 1995). However, relatively few studies have applied *Bt*'s to melonworm control. Hence, additional research should focus on determining the effectiveness of *Bt*'s in managing melonworms. The use of *Bt*'s rotated with other chemical insecticides can potentially help in developing a strong management program for melonworms.

The Uses and Disadvantages of Chemical Control

Management of melonworm in commercial fields relies heavily on synthetic insecticides of different modes of action. In a preliminary field study, diamide insecticides (cyazypyr and rynaxypyr, IRAC Group 28) provided significant control of melonworm larvae when compared with the non-treated control. Diamide insecticides can be used as a soil drench or a foliar application. Most of the chemicals negatively

affect natural enemies of melonworms and other insects in the treated fields; hence, the ideal insecticide should also control the pest without harming its natural enemies. Frequent use of the pesticides may also cause other insect species to become pests in the absence of their natural enemies (secondary pest outbreaks). Repeated use of the same insecticide or different insecticides of the same mode of action may also lead to the development of insecticide resistance (Etienne et al. 1990, Paine 1992). To avoid the development of resistance in melonworms, insecticides of different mode of actions should be used in rotation.

Resistant Cultivars

The development of resistant cultivars is a promising approach for inclusion in integrated pest management programs (Wiseman 1994). Guillaume and Boissot (2001) found that two naturally occurring genotypes of *Cucumis spp.* (*C. pustulatus* HSD 200 and *C. metuliferus* CSP 15) were highly resistant to *D. hyalinata* and three genotypes of *C. melo* (Concombre Chien, Meloncillo, and 90625) were partly resistant. Ovipositional non-preference has been the only resistance mechanism identified against melonworm and pickleworm. Melonworms oviposit fewer eggs on glabrous mutants of cucumber and muskmelon than on plants with the normally pubescent foliage (Elsey & Wann 1982, Elsey 1985).

Justification and Objectives

Despite the economic importance of cucumber, squash, and watermelon on the southern Florida economy and the devastating effects of melonworms and/or pickleworms on these crops, there has been relatively little research specific to the biology and the pests in southern Florida. Hence, the present research focuses on the

seasonal abundance and spatial distribution, host preference and within plant-distribution, and host selection and growth response of melonworm in cucurbits.

CHAPTER 2
SEASONAL ABUNDANCE AND SPATIAL DISTRIBUTION OF MELONWORM, *DIAPHANIA HYALINATA* L. (LEPIDOPTERA: CRAMBIDAE) ON YELLOW SQUASH IN SOUTHERN FLORIDA

The melonworm, *Diaphania hyalinata* L. (Lepidoptera: Crambidae) is a serious tropical pest of cucurbitaceae throughout the southeastern United States (Fulton 1947, Dupree et. al. 1955). It overwinters in southern Florida and disperses throughout the southern and Gulf Coast state every summer (Reid et al. 1954, Reid & Cuthbert 1956). During the summer, it migrates into the Carolinas and up to the northern states and even to Oklahoma and Nebraska in the west (Zehnder 2011). The host range of melonworm is limited to cucurbits with most damage to yellow squash followed by zucchini and cucumber. The larval stage of melonworm feeds on cucurbit foliage with the later instars (3^{rd}-5^{th} instars) completely skeletonizing leaves. They generally remain on the underside of leaves and feed on them. In extreme conditions when population abundance is large, they can feed on the entire plant including fruit, leaves, stalks and vines, leaving only veins and veinlet of plants (Valles and Capinera 1992). Melonworm can cause serious damage to its host crops by significantly reducing yields (Guillaume and Boissot 2001). Melonworms feeding on foliage (indirect loss) may account for 23 % yield reduction (McSorley and Waddill 1982). Further yield loss (about 9 to 10 %) has been documented due to melonworms feeding on flowers and fruits (direct loss) in Florida.

To achieve effective control of melonworm, appropriate control techniques must be applied at the right time of their biology. Thus, knowledge about the biology of melonworm is key to a successful management program. Knowledge about seasonal abundance helps to indicate when melonworms will appear in the crop and the

abundance of its development stages at different phenological stages of a host crop. However, despite the economic damage this pest has inflicted to the cucurbit production industry, information on seasonal abundance and spatial distribution of this pest is lacking in the southern Florida agro ecosystem.

Variations in seasonal and annual abundance have been reported in many lepidopteran tropical insects (Braby 1995, Frith and Frith 1985). Temperature is an important environmental factor that regulates various biological parameters of an insect and has direct effect on its abundance (Elsey 1982; Ju et al. 2011). Peña et al. (1987b) reported that the larval populations of pickleworm, another closely related *Diaphania* species, were generally low during extreme hot summer and cold winters and that the population peaked during Fall. The fluctuation in seasonal abundance of pickleworm was due to change in temperature, which was further supported by Elsey (1982) with laboratory results.

Knowledge of insect spatial distribution is important for developing sampling methodology to understand population abundance in time and space (Brewer and Story 1987). Distribution indicates how the population of an area is arranged in response to various environmental factors such as food, temperature, habitat condition and other biotic and abiotic factors. Within a population, individuals can be spaced in different ways called dispersion patterns. Patterns of distribution can be categorized as clumped, random or uniform (Southwood 1978). The abundance of the insect population in a particular part of the plant or field determines its distribution pattern. Usually smaller populations result in random distribution and greater populations results in an aggregated pattern of distribution of insect in the field. Information on spatial distribution

of a pest insect also can be used in estimating number of samples required from an area to reliably estimate pest infestation to develop effective management programs. This information would help to minimize inappropriate use of insecticides in the field.

The two objectives of this study were to determine seasonal abundance and spatial distribution of melonworm in field-planted cucurbits.

Materials and Methods

All studies were conducted at the Tropical Research and Education Center (TREC), Homestead, FL, under field conditions. Studies were conducted in 2014 using four plantings of yellow squash set at different sites. The plantings were established in 6 May, 27 June, 11 August and 18 November 2014. The soil type of all field plots was Krome gravelly loam (loamy-skeletal, carbonated hyper thermic lithic Udorthents), which consisted of 33% soil and 67% pebbles > 2mm. Yellow squash was planted in a 92 m x 10 m field comprised of 6 raised beds each measuring 92-m x 1-m. Centers of adjacent beds were separated by 0.91m. Each bed was divided into eight 11.5-m plots; hence, there were 48 plots. Granular fertilizer (N-P-K: 8-16-16) was applied during bed preparation at 908 kg per ha in a 10 cm wide band on each side of the raised bed 25 cm from the center of the bed. To control weeds, halo sulfuron methyl (Sandea®, Gowan Company LLC., Yuma, AR) was applied before planting at 55 ml per ha. For irrigation, one drip tape was placed on each side of the raised bed 30 cm from its center. Beds were subsequently covered with black-and-white plastic mulch, with the white side installed upward, for additional weed control and to maintain temperature and soil moisture in the beds. Three weeks after application of herbicide, seeds of yellow squash: cv. ‘Enterprise’ (Syngenta Seeds, Pasco, WA) were direct seeded in the center of each bed 40 cm apart within the row in 3 cm deep holes that were 0.91m in between

the adjacent rows. To study melonworm abundance in different seasons, crops were planted four times in a year using similar methods and cultural practices as described above. In each planting, liquid fertilizer (N-P-K: 4-0-8) was injected through irrigation drip lines beginning four weeks after planting and continued weekly at 236 liter per ha per wk for 5 weeks. No insecticides were used during the study. To prevent fungal diseases, chlorothalonil (Bravo Weather Stik®, Syngenta Crop Protection LLC, Greensboro, NC) at 1.75 liter per ha, and copper hydroxide (Kocide® 3000, DuPont Crop Protection, Wilmington, Delaware) at 0.8 liter per ha were applied weekly in rotation. The field was checked daily to record germination of seeds. Temperature and rainfall data were obtained from the Florida Automated Weather Network (FAWN), Homestead and used to compare with the abundance and distribution of melonworm larvae.

Seasonal Abundance of Melonworm

Abundance of melonworm was studied separately in each of four plantings to understand the time of their optimum population increase in each planting. Data of all plantings were then considered together to determine peak abundance of melonworm population in yellow squash along all sampling dates of four plantings. To obtain information on population abundance, sampling for melonworm was initiated (26 May, 18 July, 1 September and 9 December) two weeks after germination of yellow squash plants in each planting and continued for four times at weekly intervals. Five plants per plot were randomly selected and two leaves from each plant (10 leaves per plot) were collected. Thus, for the whole study consisting of 48 plots, 240 plants with 480 leaves were checked weekly in each planting. The sampled leaves from each plot were placed into separate plastic bag and labeled with plot and sample numbers and sampling date.

Immediately after collecting samples, all samples were transported to the IPM Laboratory, TREC, Homestead, and the number of larvae on each sample was recorded. To understand the age composition of larvae in each sample, they were visually divided into small (1st and 2nd instar), medium (3rd and 4th instar) and large (5th instar) based on the size and color.

Spatial Distribution of Melonworm

The distribution of melonworm was studied in the same field that was used for seasonal abundance. Plot design, sample collection and sample preparation were same as described in the previous study. Spatial distribution of melon worm was determined using two sized plots: 10 m^2 (48 plots) and 40 m^2 (12 plots). Analyses were carried out on each sampling date from each of the four cropping seasons.

Statistical Analysis

Data on seasonal abundance were square root transformed (x+0.25) before analysis to normalize the error variance. Transformed data were analyzed by least-square analysis of variance (ANOVA, PROC MIXED, SAS Institute Inc. 2013). PROC MIXED was used to analyze due to the potential covariance structure associated with taking repeated measures through time on the same plots of plants. Season, larval size and their interaction were modeled in whole experiment. Sample date was substituted for season in the analysis by season. Post-hoc mean separation [Waller Duncan K-ratio test ($\alpha < 0.05$) using SAS, SAS Institute Inc. 2013] was used for variables where ANOVA indicated a significant effect of the variable on the model. Regression analyses (PROC REG, SAS Institute Inc. 2013) were performed to determine relationship of larval abundance with temperature and rainfall. All means were square root transformed

for statistical analysis; however, mean values in the figures below were back-transformed.

To assess melonworm spatial distributions, two indices of dispersion commonly used to study insect distribution were calculated (Southwood 1978): Taylor's power law (*b*) (Taylor 1961) and Iwao's patchiness regression (*β*) (Iwao 1968). In both of the models, when the slope (*b* and *β*) value is not significantly different from 1, it indicates a random distribution pattern; slope significantly > 1 indicates an aggregated distribution pattern; and slope significantly < 1 indicates a uniform distribution pattern ($P < 0.05$). Taylor's power law (Equation 2-1) and Iwao patchiness regression parameters (Equation 2-2) were calculated using the general linear regression models (Southwood 1978, SAS Institute 2013). Taylor's power law determines relationships between mean density of larvae (log $\bar{x}$) and variance (log s^2), and sampling factor (log *a*) Equation 2-1.

$$b = (\log s^2 - \log a) / \log \bar{x} \qquad (2\text{-}1)$$

Iwao patchiness regression relates the Lloyd (1967) mean crowding index [($s^2/\bar{x}$ $\bar{x}$) – 1], the sample mean ($\bar{x}$), and the index of contagion or tendency toward crowding (*α*) in Equation 2-2.

$$\beta = [(s^2/\bar{x}\ \bar{x}) - 1] + \bar{x}\bar{x}\ \bar{x} - \alpha \qquad (2\text{-}2)$$

To determine the within-field distributions for *D. hyalinata* using Taylor (*b*) and Iwao (*β*) indices, we first determined the goodness of fit of data to both linear models using regression coefficients (r^2) from each field test. Then a student's t-test ($p<0.05$) was used to determine if the slopes *b* and *β* were significantly different from 1.0. Taylor (*b*) and Iwao (*β*) tests can be checked to determine correlation values (r^2), which indicates the reliability of the test value.

Results

Seasonal Abundance

Abundances of small, medium and large melonworm larvae were significantly affected by season ($F_{3, 2292} = 354.75$, $P < 0.0001$), larval size ($F_{2, 2292} = 512.70$, $P < 0.0001$) and interaction of season and larval size ($F_{6, 2292} = 96.39$, $P < 0.0001$). Therefore, the data were divided for further analysis by the four seasons tested in the field.

Abundance of larvae by size and sampling dates by season

Abundances of small, medium and large melonworm larvae during crop planting season from 26 May to 16 June were significantly affected by sampling date ($F_{3, 564} = 4.19$, $P = 0.0060$), larval size ($F_{2, 564} = 264.45$, $P < 0.0001$) and interaction of sampling date and larval size ($F_{6, 564} = 9.80$, $P < 0.0001$). The number of small larvae dipped significantly at three and four weeks after germination from the initial sampling date, but then rebounded to the season high (2.2 ± 0.1; mean ± SE larvae per two leaves) five weeks after germination (Figure 2-1). However, the numbers of both middle and large larvae reached peaks of 0.7 ± 0.1 and 0.3 ± 0.0, respectively, three weeks after germination declining significantly at five weeks after germination for large larvae. The number of small larvae was significantly greater across all the sampling dates compared to other larval sizes.

Abundances of small, medium and large melonworm larvae during crop planting season from 18 July to 8 August were significantly affected by sampling date ($F_{3, 564} = 113.75$, $P < 0.0001$), larval size ($F_{2, 564} = 40.58$, $P < 0.0001$) and interaction of sampling date and larval size ($F_{6, 564} = 16.35$, $P < 0.0001$). As in the first season, small larvae reached their peak five weeks after germination (2.0 ± 0.1) (Figure 2-2). However,

medium (1.0 ± 0.1) and large larvae (2.4 ± 0.1) reached their peaks four and five weeks after germination, respectively. The number of small larvae was greater during two and three weeks after germination (18 and 25 June) but results did not differ significantly among larval sizes during four weeks after germination (1 August). During five weeks after germination (8 Aug), the number of large larvae was significantly greater.

Abundances of small, medium and large melonworm larvae during crop planting season from 1 to 22 September were significantly affected by sampling date ($F_{3, 564}$ = 60.88, $P < 0.0001$), larval size ($F_{2, 564}$ = 645.62, $P < 0.0001$) and interaction of sampling date and larval size ($F_{6, 564}$ = 66.42, $P < 0.0001$). The number of small larvae two weeks after germination (4.3 ± 0.2) was greater in the third planting than the other three seasons (max 1.5 ± 0.1) (Figure 2-3). The number of small and medium larvae was significantly greater (7.1 ± 0.3 and 2.4 ± 0.1, respectively) on 8 September, three weeks after germination than the other sample dates in the third season. As in the second season, the number of large sized larvae reached its peak (1.5 ± 0.1) five weeks after germination (22 September). The number of small larvae was significantly greater across all the sampling dates compared to other larval sizes.

Abundances of small, medium and large melonworm larvae during crop planting season from 9 to 30 December were significantly affected by sampling date ($F_{3, 564}$ = 18.74, $P < 0.0001$), larval size ($F_{2, 564}$ = 127.31, $P < 0.0001$) and interaction of sampling date and larval size ($F_{6, 564}$ = 20.10, $P < 0.0001$). The overall pattern for numbers of small, medium, and large larvae was different in the fourth than the other three seasons. While the pattern for small and medium sized larvae were very similar for the fourth and fifth seasons, large larvae peaked (0.3 ± 0.0) during the fourth week after germination in

the fourth season (Figure 2-4) compared to the fifth weeks during the second and third seasons. The number of small larvae was greatest at two (1.1 ± 0.1) and three weeks after germination. The peak of medium sized larvae (0.6 ± 0.1) occurred at 3 weeks after germination. The number of small larvae was significantly greater across all the sampling dates compared to other larval sizes.

Abundance of total larvae across sampling dates in four individual plantings

Abundance of total melonworm larvae was significantly affected by season ($F_{3, 11516} = 596.6$, $P < 0.0001$). During all the four crop seasons (May-June, July-August, September and December), abundance of total melonworm larvae was significantly affected by sampling dates ($F_{3, 2876} = 5.41$, $P = 0.001$; $F_{3, 2876} = 160.95$, $P < 0.0001$; $F_{3, 2876} = 53.22$, $P < 0.0001$ and $F_{3, 2876} = 18.31$, $P < 0.0001$, respectively). During May-June crop season, the number of larvae was significantly greater (2.9 ± 0.2) on 16 June, five weeks after germination (Figure 2-5). During July-August crop season, the number of larvae was significantly less at the beginning of season but reached peaks of 5.3 ± 0.2 on 8 August, five weeks after germination. During September crop season, the number of larvae was significantly less at the beginning of season but peaked (9.9 ± 0.3) during three weeks after germination (8 September). During December crop season, the peak of larvae (1.6 ± 0.1) occurred at three weeks after germination (December 16) but did not differ significantly from number of larvae during two weeks after germination (December 09).

Temperature and rainfall effects on larval abundance

There was a weak significant positive linear relationship ($r^2 = 0.23$, $P = 0.05$) between temperature and larval abundance. In the present study, temperature varied within a very short range ($26^o – 30^oC$) during the period of first three cropping seasons

(26 May – 22 September) (Figure 2-7). A sharp drop in temperature (to 12°C) was observed on 9 December and then increased to 23°C on 23 December (Figure 2-6). Abundance of melonworm larvae fluctuated regardless of temperature during the first three crop seasons (26 May – 22 September) but abundance of melonworm decreased following the sharp drop in temperature during the fourth cropping season (9 December – 30 December). During the present study, rainfall varied from 0 – 0.7 mm/day but average rainfall/day had no significant effect on melonworm larval abundance (r^2 = 0.0004, P = 0.94).

Spatial Distribution

Distribution during May-June 2014

At the beginning of the first season, two weeks after germination (26 May), the slope (b and β) values from the linear regression models were significantly > 1 (P < 0.05) for total larvae and larvae of each size indicating aggregated distributions in plots of both sizes (10 m^2 and 40 m^2) (Tables 2-1 and 2-2).. Three weeks after germination (2 June), the distribution of the larvae in the 10 m^2 plot based on two regression models (Taylor and Iwao) were not in agreement, and Iwao's patchiness regression model with greater r^2 value provided a better fit of the data. The distribution pattern of total larvae was aggregated and so was that of medium and large larvae for both plot sizes (10 m^2 and 40 m^2). For 10 m^2 plots, the two regression models were not in agreement with the distribution pattern of medium-sized larvae, but we chose the value with Iwao's patchiness regression, which is aggregated, based on results from other population. In contrast, both regression models were in agreement for small-sized larvae indicating a uniform distribution pattern during the sampling date three weeks after germination for both 10 m^2 and 40 m^2 plots (2 June). The distribution pattern of medium larvae was

found to be random on 16 June, five weeks after germination, which was supported by a greater r^2 value of Taylor's power law in 10 m^2 plots. Otherwise, all larval sizes on 9 and 16 June for both plot sizes (10 m^2 and 40 m^2) showed aggregated distributions.

Distribution during July-August 2014

During the second cropping season 18 July to 7 August, most of the population sampled showed aggregated distributions and a few showed uniform distributions in both plot sizes (Table 2-3 and 2-4). Large larvae on the first sampling date, two weeks after germination (July 18), were uniformly distributed in 40 m^2 plot. Medium larvae on the second and fourth sampling dates (July 24 and August 7) showed uniform type of distributions in both 10 m^2 and 40 m^2 plots. Also, large larvae on July 24, three weeks after germination, showed a uniform distribution in 10 m^2 plots. When all larvae were considered together, the distribution pattern was aggregated during the entire season from 18 July to 7 August in both plot sizes.

Distribution during September 2014

During the third cropping season from 1 to 22 September, most of the larval populations were aggregated except a few of them were uniformly distributed. Large larvae were uniformly distributed the third, fourth, and fifth weeks of the cropping season (8, 15 and 22 September) in 10 m^2 plots. However, in the case of 40 m^2 plots, large larvae were only uniform on 15 September, four weeks after germination. The small larvae were found to be uniformly distributed on 22 September, five weeks after germination. Apart from that, all the larval sizes showed aggregated distribution for the entire season from 1 to 22 September in both 10 m^2 and 40 m^2 plots (Table 2-5 and 2-6).

Distribution during December 2014

All larval sizes and total larvae were found to be aggregated on 9 and16 December in both 10 m^2 and 40 m^2 plots (Table 2-7 and 2-8). However, the population distribution for all larval sizes was uniform on 23 irrespective of larval sizes in 10 m^2 plots. Four weeks after germination (December 23), the population distribution was aggregated for all larval sizes except for large larvae (uniform) in 40 m^2. During the fourth crop season from 9 to 30 December, *D. hyalinata* showed both uniform and aggregated distribution in both plot sizes.

Discussion

The number of small, medium, and total melonworm larvae peaked during the cropping season of September with the largest numbers at the second sampling date on 8 September. Large larvae peaked during the cropping season from 18 July to 8 August with the largest value at 8 August. The smallest numbers of all sizes of melonworm larvae were observed during crop season in December. The number of small and total larvae was smallest on 30 December. The populations of medium and large larvae increased over the seasons with a peak during the cropping season of September. The population then began to fall after the middle of the third season and was lowest during December. The dramatic drop in temperature in mid-December with a similar great drop in the number of larvae suggests developmental and survivorship thresholds are relatively high for of *D. hyalinata*.

The population of small larvae was large compared to other sizes larvae throughout the year. The abundance of large size larvae was consistently low over the whole crop season except for a few sampling dates. Basically, larval abundance was directly affected by the initial population (Figure 2-2). Within each season, the

population of melonworm larvae declined over time. This decrease in population was probably due to mortality. Even though the populations of medium and large larvae were less than small larvae, the level of damage was still high, because larger larvae consume more food. Thus, despite the difference in number, each larval population had an equal chance of causing serious damage.

Fluctuation in the population levels of insects over the season in tropical areas is common and has been reported several times (Wallner 1987; Braby 1995; Novotny & Basset 1998; Zanuncio et al. 2002). Weather parameters such as fluctuation of temperature, rainfall, and relative humidity may have direct or indirect effect on the abundance of insect population (Wallner 1987; Zanuncio et al. 2002). In my studies, the abundance of melonworm appeared to be affected by temperature with populations dropping dramatically with similar drop mean air temperature. Similarly, Liu et al. in 2002 reported that the survivability of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) decreased rapidly above and below the temperature range of 12 to 28ºC. During summer, when the temperature was high and constant, the melonworm population was steady. Later in the season, the population level decreased (Figure 2-7). This decrease was preceded by the decrease in the temperature from about 27ºC to 16ºC.

The distribution of melonworm larvae was found to be aggregated during most of the season, particularly when densities were greatest, although there were several occasions when uniform or random distribution were observed. When distribution pattern of melonworms in four study seasons were compared, the distribution was aggregated during the first three seasons in 2014 when the population abundance was greatest. However, the distribution pattern was uniform during the later part of the year,

when the melonworm population was lowest. The distribution pattern of melonworm larvae was not significantly affected by the relatively small plot sizes used in this study. Much larger field sizes should be studied to evaluate population distributions in commercial sized fields. Further studies should be conducted to understand the distribution pattern of melonworm in different seasons with variable weather patterns and different densities, as distributions affect how the crop should be sampled.

Table 2-1. Taylor's power law and Iwao's patchiness regression parameters for distribution of *Diaphania hyalinata* larvae sampled in 10 m^2 plots May-Jun 2014.

Date	Larva	Taylor's power law			Iwao's patchiness regression		
		a	*b*	r^2	*α*	*β*	r^2
26 May	Small	-0.05	1.08 AGG	0.53	-0.07	1.18 AGG	0.64
	Medium	0.06	1.15 AGG	0.75	-0.20	1.53 AGG	0.65
	Large	0.70	2.00 AGG	1.00	-1.00	6.00 AGG	1.00
	Total	-0.04	1.14 AGG	0.54	-0.29	1.28 AGG	0.74
2 Jun	Small	-0.60	0.86 UNI	0.43	0.07	0.94 UNI	0.56
	Medium	-0.04	0.94 UNI	0.44	-0.14	1.37 AGG	0.44
	Large	0.09	1.17 AGG	0.74	-0.29	1.97 AGG	0.51
	Total	-0.11	0.93 UNI	0.20	-0.23	1.09 AGG	0.56
9 Jun	Small	-0.18	0.97 UNI	0.49	-0.31	1.07 AGG	0.82
	Medium	-0.01	1.02 AGG	0.65	-0.18	1.38 AGG	0.58
	Large	0.03	1.07 AGG	0.74	-0.15	1.50 AGG	0.49
	Total	-0.25	1.15 AGG	0.36	-0.42	1.10 AGG	0.78
16 Jun	Small	-0.02	1.23 AGG	0.60	-0.32	1.35 AGG	0.78
	Medium	-0.04	1.00 RAN	0.69	-0.05	1.08 AGG	0.51
	Large	0.48	1.70 AGG	0.93	-0.57	3.94 AGG	0.87
	Total	-0.14	1.38 AGG	0.63	-0.45	1.26 AGG	0.88

AGG, aggregated distribution; *b* and *β* significantly ($P \leq 0.05$) > 1. UNI, uniform distribution; *b* and *β* significantly < 1 ($P \leq 0.05$). RAN, random distribution; *b* and *β* not significantly different from 1 ($P > 0.05$).

Table 2-2. Taylor's power law and Iwao's patchiness regression parameters for distribution of *Diaphania hyalinata* larvae sampled in 40 m^2 plot May-Jun 2014.

Date	Larva	Taylor's power law			Iwao's patchiness regression		
		a	*b*	r^2	*α*	*β*	r^2
26 May	Small	0.14	1.15 AGG	0.36	0.48	1.11 AGG	0.28
	Medium	0.29	1.42 AGG	0.87	-0.51	2.99 AGG	0.63
	Large	0.58	1.28 AGG	1.00	0.58	5.21 AGG	1.00
	Total	-0.01	1.78 AGG	0.53	-0.86	1.85 AGG	0.60
2 Jun	Small	0.00	0.90 UNI	0.71	0.20	0.85 UNI	0.66
	Medium	0.11	1.22 AGG	0.68	-0.18	1.58 AGG	0.59
	Large	0.20	1.24 AGG	0.79	-0.11	2.04 AGG	0.36
	Total	-0.23	1.61 AGG	0.75	-0.78	1.35 AGG	0.91
9 Jun	Small	-0.07	1.53 AGG	0.70	-0.80	1.67 AGG	0.73
	Medium	0.10	1.39 AGG	0.82	-0.27	1.57 AGG	0.71
	Large	0.16	1.19 AGG	0.98	-0.12	1.87 AGG	0.84
	Total	-0.26	1.71 AGG	0.82	-0.71	1.31 AGG	0.91
16 Jun	Small	0.07	1.47 AGG	0.68	-0.45	1.58 AGG	0.75
	Medium	0.07	1.06 AGG	0.85	0.07	1.15 AGG	0.47
	Large	0.48	1.40 AGG	0.97	-0.40	5.87 AGG	0.90
	Total	0.07	1.32 AGG	0.49	-0.02	1.29 AGG	0.79

AGG, aggregated distribution; *b* and *β* significantly ($P \leq 0.05$) > 1. UNI, uniform distribution; *b* and *β* significantly < 1 ($P \leq 0.05$). RAN, random distribution; *b* and *β* not significantly different from 1 ($P > 0.05$).

Table 2-3. Taylor's power law and Iwao's patchiness regression parameters for distribution of *Diaphania hyalinata* larvae sampled in 10 m^2 plot Jul-Aug 2014.

Date	Larva	Taylor's power law			Iwao's patchiness regression		
		a	*b*	r^2	*α*	*β*	r^2
18 Jul	Small	0.46	1.59 AGG	0.88	0.54	2.46 AGG	0.51
	Medium	0.21	1.44 AGG	0.8	-0.81	4.21 AGG	0.68
	Large	-0.69	0	0	0.2	0	0
	Total	0.4	1.55 AGG	0.84	0.33	2.38 AGG	0.47
24 Jul	Small	-0.03	1.27 AGG	0.64	-0.32	1.4 AGG	0.71
	Medium	-0.07	0.82 UNI	0.52	0.21	0.79 UNI	0.2
	Large	-0.069	0.89 UNI	0.73	0.06	0.9 UNI	0.62
	Total	0.002	1.01 AGG	0.37	-0.02	1.09 AGG	0.72
30 Jul	Small	0.1	1.39 AGG	0.84	-0.26	1.58 AGG	0.81
	Medium	0.02	1.17 AGG	0.72	-0.13	1.3 AGG	0.77
	Large	0.08	1.19 AGG	0.65	-0.14	1.56 AGG	0.7
	Total	-0.01	0.95 UNI	0.53	0.0004	1.06 AGG	0.91
7 Aug	Small	0.02	1.34 AGG	0.54	0.18	1.18 AGG	0.61
	Medium	-0.089	0.8 UNI	0.32	0.15	0.89 UNI	0.29
	Large	-0.08	1.24 AGG	0.47	-0.22	1.19 AGG	0.69
	Total	0.14	0.82 UNI	0.08	0.09	1.07 AGG	0.67

AGG, aggregated distribution; *b* and *β* significantly ($P \leq 0.05$) > 1. UNI, uniform distribution; *b* and *β* significantly < 1 ($P \leq 0.05$). RAN, random distribution; *b* and *β* not significantly different from 1 ($P > 0.05$).

Table 2-4. Taylor's power law and Iwao's patchiness regression parameters for distribution of *Diaphania hyalinata* larvae sampled in 40 m^2 plot Jul-Aug 2014.

Date	Larva	Taylor's power law			Iwao's patchiness regression		
		a	*b*	r^2	*α*	*β*	r^2
18 Jul	Small	0.48	1.39 AGG	0.85	0.97	2.09 AGG	0.56
	Medium	0.27	1.25 AGG	0.86	-0.49	5.44 AGG	0.47
	Large	-0.1	0.92 UNI	1	0.05	-0.05 UNI	1
	Total	0.44	1.43 AGG	0.77	0.85	2.01 AGG	0.39
24 Jul	Small	0.05	1.55 AGG	0.9	-0.66	1.77 AGG	0.91
	Medium	-0.035	0.82 UNI	0.75	0.35	0.56 UNI	0.25
	Large	0.01	1.23 AGG	0.94	-0.21	1.23 AGG	0.88
	Total	-0.04	1.28 AGG	0.76	-0.49	1.27 AGG	0.92
30 Jul	Small	0.23	1.53 AGG	0.93	-0.15	1.81 AGG	0.89
	Medium	0.14	1.2 AGG	0.85	-0.18	1.63 AGG	0.79
	Large	0.21	1.44 AGG	0.86	-0.67	2.49 AGG	0.82
	Total	0.05	1.25 AGG	0.67	-0.32	1.33 AGG	0.85
7 Aug	Small	0.13	1.27 AGG	0.81	0.11	1.26 AGG	0.84
	Medium	0.015	0.83 UNI	0.57	0.35	0.72 UNI	0.4
	Large	-0.04	1.44 AGG	0.81	-0.32	1.29AGG	0.88
	Total	0.04	1.16 AGG	0.49	0.27	1.04 AGG	0.89

AGG, aggregated distribution; *b* and *β* significantly ($P \leq 0.05$) > 1. UNI, uniform distribution; *b* and *β* significantly < 1 ($P \leq 0.05$). RAN, random distribution; *b* and *β* not significantly different from 1 ($P > 0.05$).

Table 2-5. Taylor's power law and Iwao's patchiness regression parameters for distribution of *Diaphania hyalinata* larvae sampled in 10 m^2 plot Sep 2014.

Date	Larva	Taylor's power law			Iwao's patchiness regression		
		a	*b*	r^2	*α*	*β*	r^2
1 Sep	Small	-0.67	2.18 AGG	0.6	-1.83	1.55 AGG	0.81
	Medium	-0.01	1.01 AGG	0.72	-0.05	1.15 AGG	0.63
	Large	0	0	0	0	0	0
	Total	-0.77	2.19 AGG	0.41	-1.48	1.41 AGG	0.79
8 Sep	Small	-0.85	2.09 AGG	49	-1.06	1.24 AGG	0.85
	Medium	-0.04	1.03 AGG	0.4	0.18	1.02 AGG	0.61
	Large	-0.04	0.93 UNI	0.63	0.001	1.17 AGG	0.3
	Total	-0.64	1.82 AGG	0.39	-0.5	1.14 AGG	0.87
15 Sep	Small	-0.53	1.84 AGG	0.46	-0.88	1.29 AGG	0.82
	Medium	0.001	1.35 AGG	0.5	-0.99	1.82 AGG	0.59
	Large	-0.11	0.64 UNI	0.18	0.43	0.53 UNI	0.06
	Total	-0.96	2.34 AGG	0.46	-2.3	1.54 AGG	0.68
22 Sep	Small	-0.11	0.92 UNI	0.22	-0.24	1.17 AGG	0.48
	Medium	-0.02	1.19 AGG	0.51	0.04	1.14 AGG	0.55
	Large	-0.04	0.91 UNI	0.41	0.13	0.99 UNI	0.49
	Total	-0.21	1.46 AGG	0.41	-0.16	1.15 AGG	0.88

AGG, aggregated distribution; *b* and *β* significantly ($P \leq 0.05$) > 1. UNI, uniform distribution; *b* and *β* significantly < 1 ($P \leq 0.05$). RAN, random distribution; *b* and *β* not significantly different from 1 ($P > 0.05$).

Table 2-6. Taylor's power law and Iwao's patchiness regression parameters for distribution of *Diaphania hyalinata* larvae sampled in 40 m^2 plot Sep 2014.

Date	Larva	Taylor's power law			Iwao's patchiness regression		
		a	*b*	r^2	*α*	*β*	r^2
1 Sep	Small	-1.19	3.28 AGG	0.69	-3.52	2.04 AGG	0.77
	Medium	0.12	1.02 AGG	0.62	0.41	1.03 AGG	0.12
	Large	0	0	0	0	0	0
	Total	-1.19	3.09 AGG	0.68	-2.39	1.66 AGG	0.84
8 Sep	Small	-0.18	1.57 AGG	0.28	0.46	1.12 AGG	0.65
	Medium	0.05	1.13 AGG	0.59	0.07	1.11 AGG	0.84
	Large	0.08	1.05 AGG	0.82	0.17	1.15 AGG	0.18
	Total	-0.71	2.04 AGG	0.53	-0.23	1.15 AGG	0.85
15 Sep	Small	-0.51	2.12 AGG	0.63	-1.29	1.49 AGG	0.91
	Medium	0.006	1.79 AGG	0.49	-1.07	2.05 AGG	0.44
	Large	-0.07	0.69 UNI	0.59	0.57	0.21 UNI	0.02
	Total	-0.85	2.46 AGG	0.49	-1.6	1.5 AGG	0.7
22 Sep	Small	0.41	0.12 UNI	0.0012	3.18	-0.19 UNI	0.005
	Medium	0.081	1.39 AGG	0.44	0.22	1.21 AGG	0.44
	Large	0.09	0.96 UNI	0.56	0.24	1.02 AGG	0.59
	Total	0.07	1.33 AGG	0.21	0.42	1.15 AGG	0.63

AGG, aggregated distribution; *b* and *β* significantly ($P \leq 0.05$) > 1. UNI, uniform distribution; *b* and *β* significantly < 1 ($P \leq 0.05$). RAN, random distribution; *b* and *β* not significantly different from 1 ($P > 0.05$).

Table 2-7. Taylor's power law and Iwao's patchiness regression parameters for distribution of *Diaphania hyalinata* larvae sampled in 10 m^2 plot Dec 2014.

Date	Larva	Taylor's power law			Iwao's patchiness regression		
		a	*b*	r^2	*α*	*β*	r^2
9 Dec	Small	0.15	1.05 AGG	0.51	0.73	0.96 UNI	0.24
	Medium	0.28	1.45 AGG	0.86	-0.55	3.09 AGG	0.74
	Large	0.2	1.29 AGG	1	-0.16	1.83 AGG	1
	Total	0.08	1.18 AGG	0.57	0.41	1.11 AGG	0.37
16 Dec	Small	-0.03	1.1 AGG	0.53	-0.27	1.37 AGG	0.41
	Medium	0.06	1.13 AGG	0.58	-0.21	1.75 AGG	0.39
	Large	0.08	1.11 AGG	0.74	-0.09	1.62 AGG	0.38
	Total	-0.08	1.29 AGG	0.29	-0.74	1.63 AGG	0.37
23 Dec	Small	0.02	0.99 UNI	0.58	0.15	1.08 AGG	0.27
	Medium	-0.11	0.83 UNI	0.55	0.07	0.82 UNI	0.25
	Large	-0.16	0.77 UNI	0.66	0.05	0.66 UNI	15
	Total	-0.27	0.93 UNI	0.33	0.16	0.95 UNI	0.42
30 Dec	Small	-0.24	0.63 UNI	0.44	0.15	0.38 UNI	0.04
	Medium	-0.05	0.91 UNI	0.67	0.01	1.05 AGG	0.24
	Large	0.05	1.07 AGG	0.7	-0.01	1.32 AGG	0.22
	Total	-0.16	0.64 UNI	0.29	0.33	0.45 UNI	0.07

AGG, aggregated distribution; *b* and *β* significantly ($P \leq 0.05$) > 1. UNI, uniform distribution; *b* and *β* significantly < 1 ($P \leq 0.05$). RAN, random distribution; *b* and *β* not significantly different from 1 ($P > 0.05$).

Table 2-8. Taylor's power law and Iwao's patchiness regression parameters for distribution of *Diaphania hyalinata* larvae sampled in 40 m^2 plot Dec 2014.

Date	Larva	Taylor's power law			Iwao's patchiness regression		
		a	*b*	r^2	*α*	*β*	r^2
9 Dec	Small	0.23	1.09 AGG	0.95	0.58	1.13 AGG	0.78
	Medium	0.29	1.25 AGG	0.85	-0.06	2.86 AGG	0.6
	Large	0.33	1.26 AGG	0.98	-0.18	4.1 AGG	0.93
	Total	0.17	1.26 AGG	0.87	0.23	1.31 AGG	0.73
16 Dec	Small	0.02	1.29 AGG	0.66	-0.43	1.58 AGG	0.48
	Medium	0.22	1.55 AGG	0.89	-0.51	2.28 AGG	0.78
	Large	0.1	1.08 AGG	0.93	-0.04	1.91 AGG	0.34
	Total	-0.03	1.48 AGG	0.15	-0.41	1.45 AGG	0.2
23 Dec	Small	0.11	1.15 AGG	0.53	0.05	1.33 AGG	0.23
	Medium	-0.02	1.02 AGG	0.91	-0.19	1.28 AGG	0.61
	Large	-0.1	0.89 UNI	0.83	0.05	0.49 UNI	0.07
	Total	0.01	1.16 AGG	0.44	-0.07	1.16 AGG	0.45
30 Dec	Small	-0.14	0.88 UNI	0.87	-0.04	0.67 UNI	0.3
	Medium	0.03	1.03 AGG	0.66	0	1.18 AGG	0.18
	Large	-0.01	0.91 UNI	0.76	0.4	-0.24 UNI	0
	Total	-0.15	0.65 UNI	0.35	0.11	0.61 UNI	0.21

AGG, aggregated distribution; *b* and *β* significantly ($P \leq 0.05$) > 1. UNI, uniform distribution; *b* and *β* significantly < 1 ($P \leq 0.05$). RAN, random distribution; *b* and *β* not significantly different from 1 ($P > 0.05$).

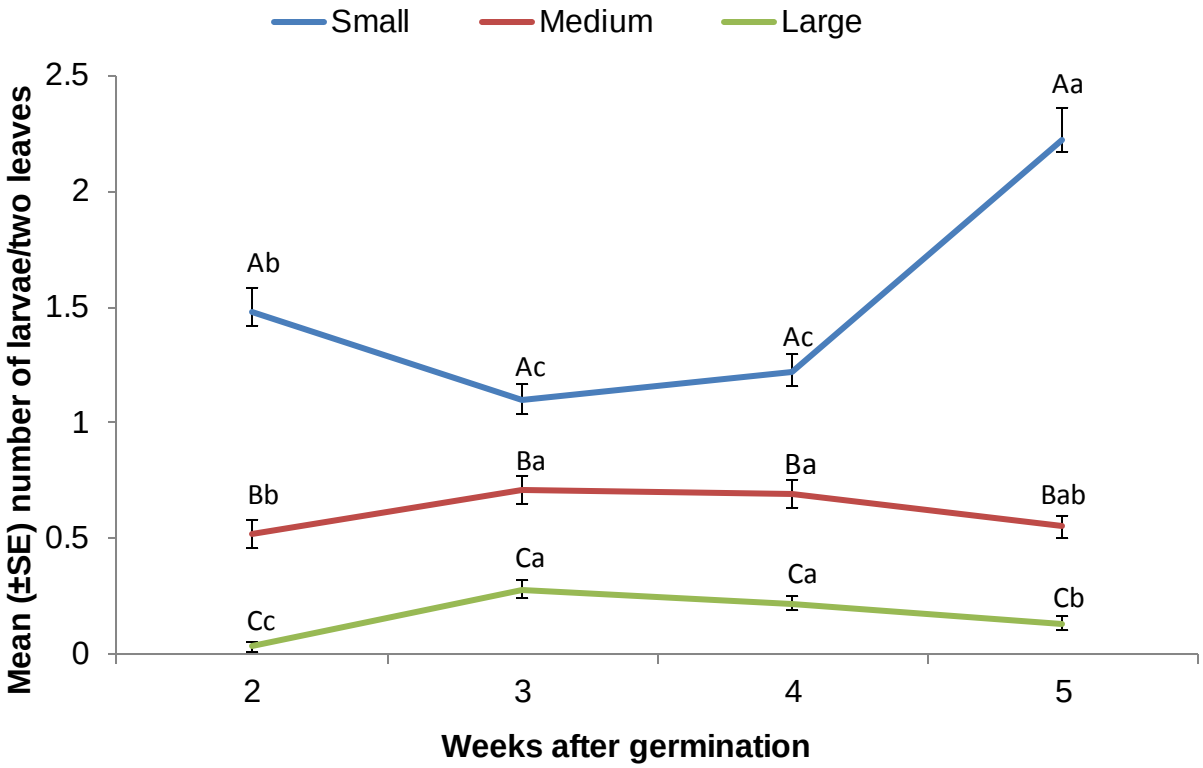

Figure 2-1. Weekly abundance (mean ± SE per two leaves) of small, medium, large and total *Diaphania hyalinata* larvae on yellow squash from 26 May through 16 June 2014. Means within columns (i.e. across larval sizes) for each sampling date followed by the same capital letter are not significantly different (P = 0.05). Means in the same line (i.e. across sampling dates) followed by the same small letter are not significantly different (P = 0.05) using analysis of variance and Waller-Duncan K-ratio procedure. Bars above and below means represent standard error.

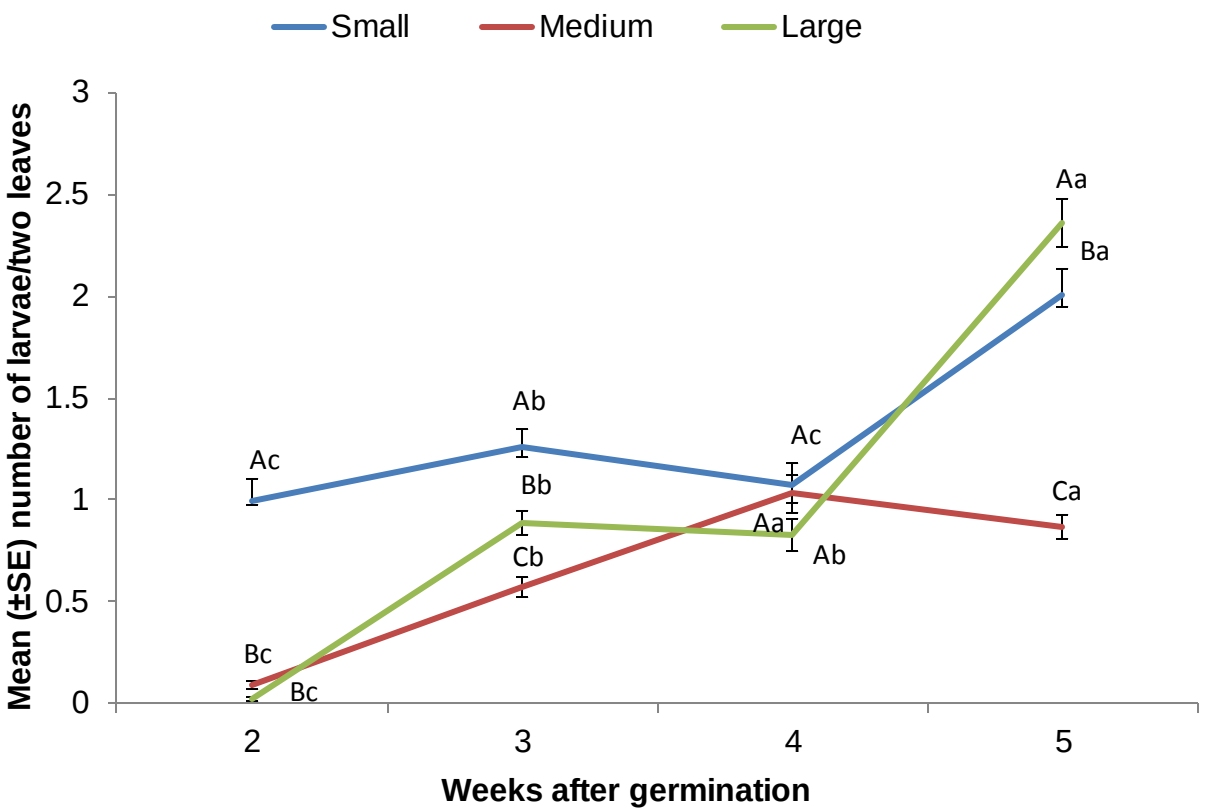

Figure 2-2. Weekly abundance (mean ± SE per two leaves) of small, medium, large and total *Diaphania hyalinata* larvae on yellow squash from 18 July through 8 August 2014. Means within columns (i.e. across larval sizes) for each sampling date followed by the same capital letter are not significantly different ($P = 0.05$). Means in the same line (i.e. across sampling dates) followed by the same small letter are not significantly different ($P = 0.05$) using analysis of variance and Waller-Duncan *K*-ratio procedure. Bars above and below means represent standard error.

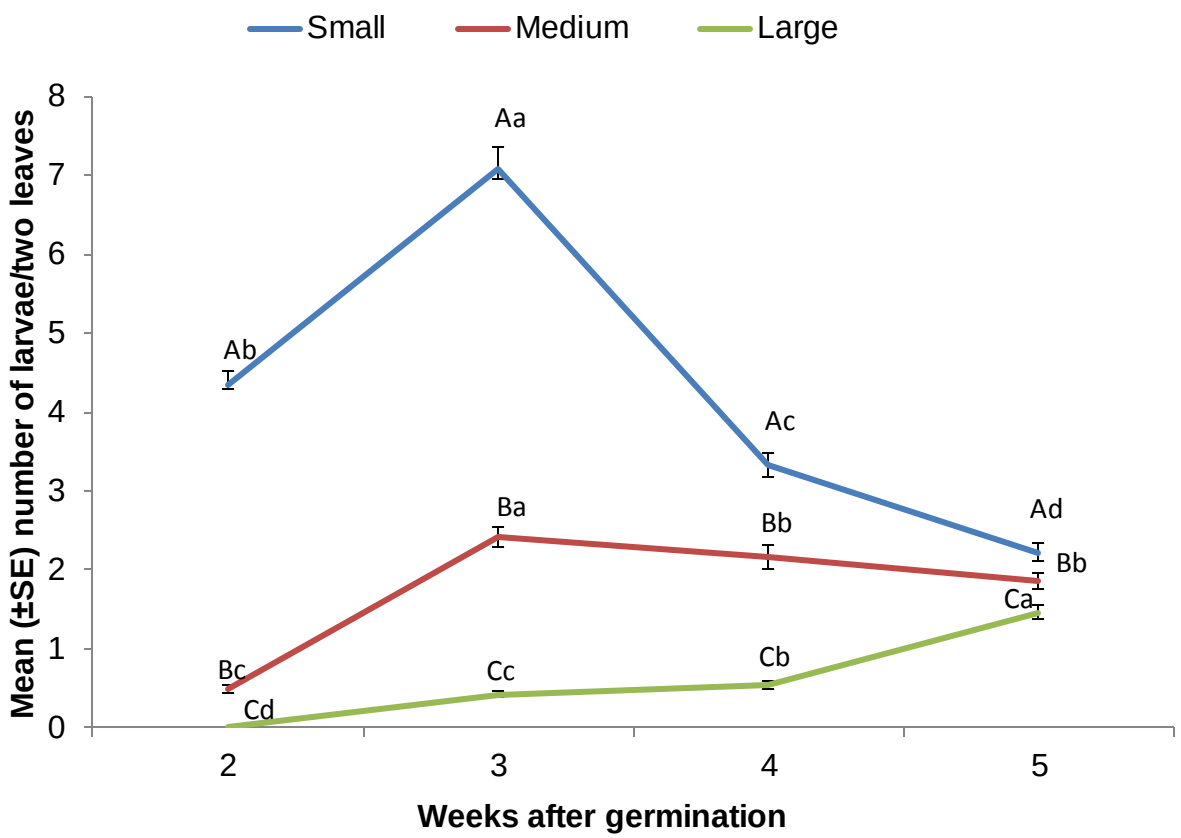

Figure 2-3. Weekly abundance (mean ± SE per two leaves) of small, medium, large and total *Diaphania hyalinata* larvae on yellow squash from 1 September through 22 September 2014. Means within columns (i.e. across larval sizes) for each sampling date followed by the same capital letter are not significantly different ($P = 0.05$). Means in the same line (i.e. across sampling dates) followed by the same small letter are not significantly different ($P = 0.05$) using analysis of variance and Waller-Duncan *K*-ratio procedure. Bars above and below means represent standard error.

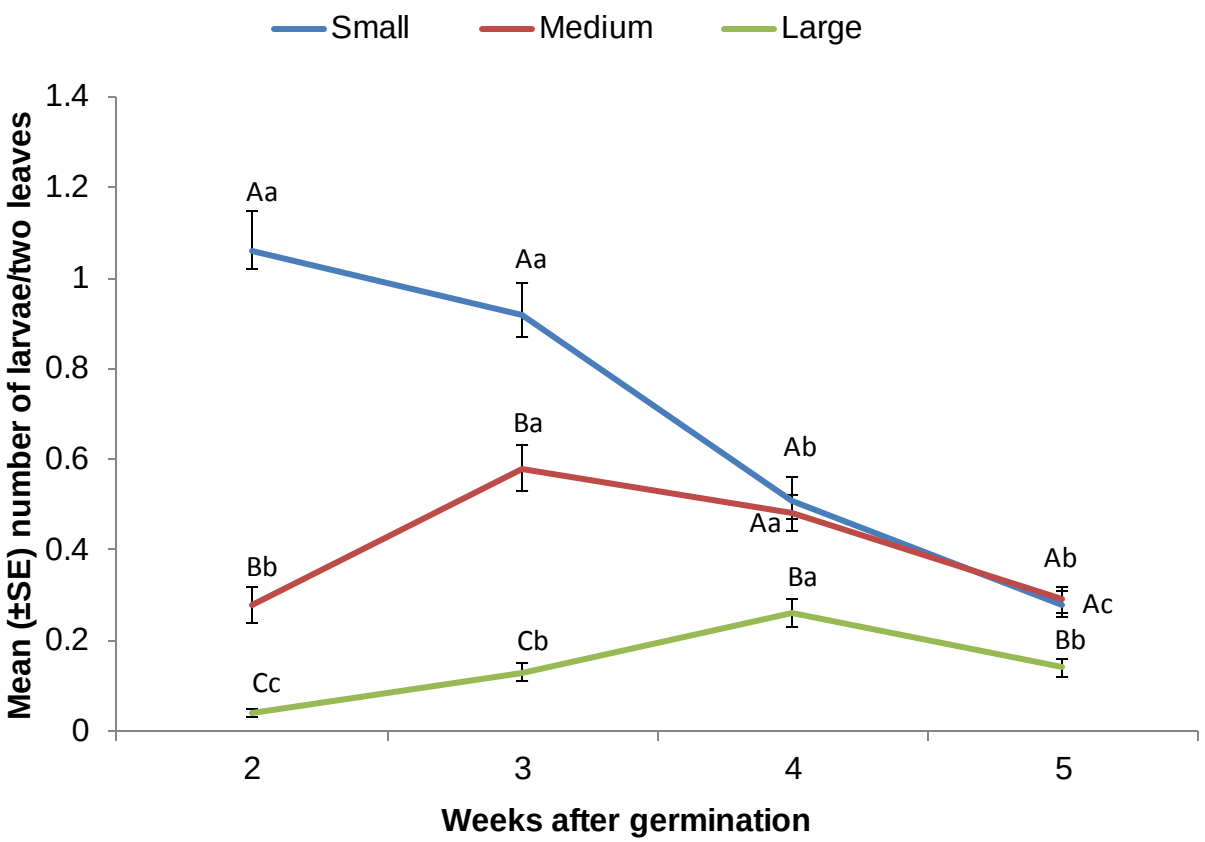

Figure 2-4. Weekly abundance (mean ± SE per two leaves) of small, medium, large and total *Diaphania hyalinata* larvae on yellow squash from 9 December through 30 December 2014. Means within columns (i.e. across larval sizes) for each sampling date followed by the same capital letter are not significantly different (*P* = 0.05). Means in the same line (i.e. across sampling dates) followed by the same small letter are not significantly different (*P* = 0.05) using analysis of variance and Waller-Duncan *K*-ratio procedure. Bars above and below means represent standard error.

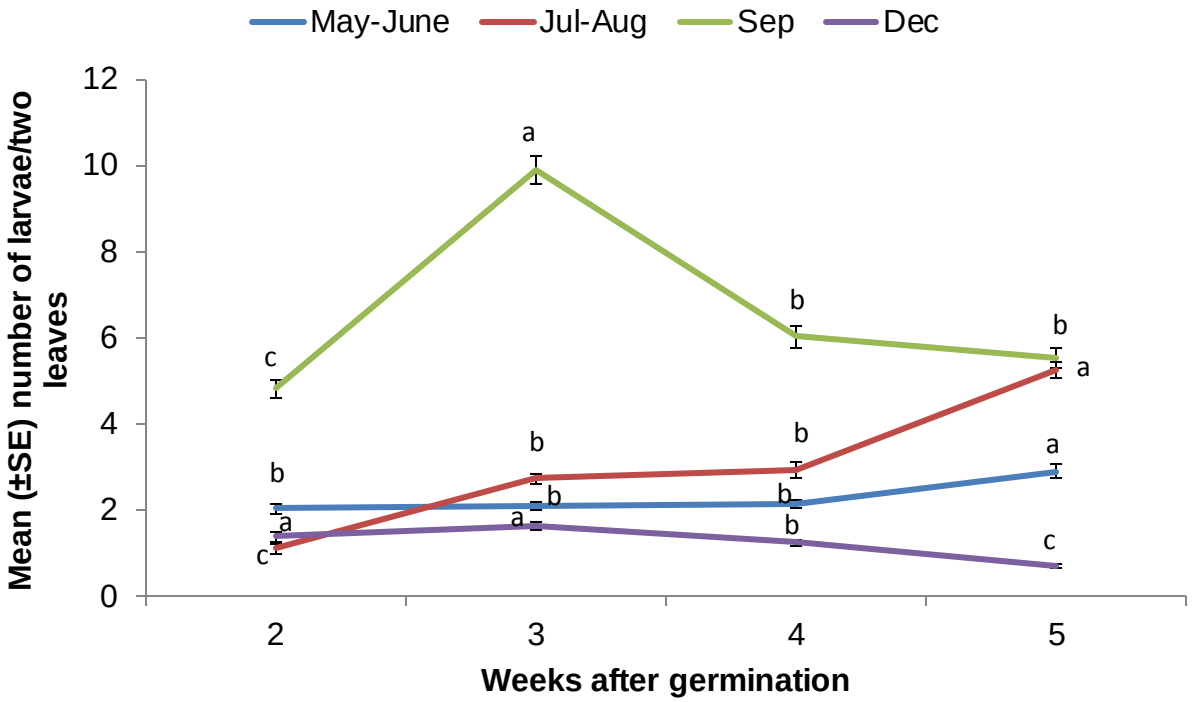

Figure 2-5. Weekly abundance (mean ± SE per two leaves) of total *Diaphania hyalinata* larvae on yellow squash during four seasons from 26 May through 30 December 2014. Means in the same line (i.e. across sampling dates) followed by the same letter are not significantly different (*P* = 0.05) using analysis of variance and Waller-Duncan *K*-ratio procedure. Bars above and below means represent standard error.

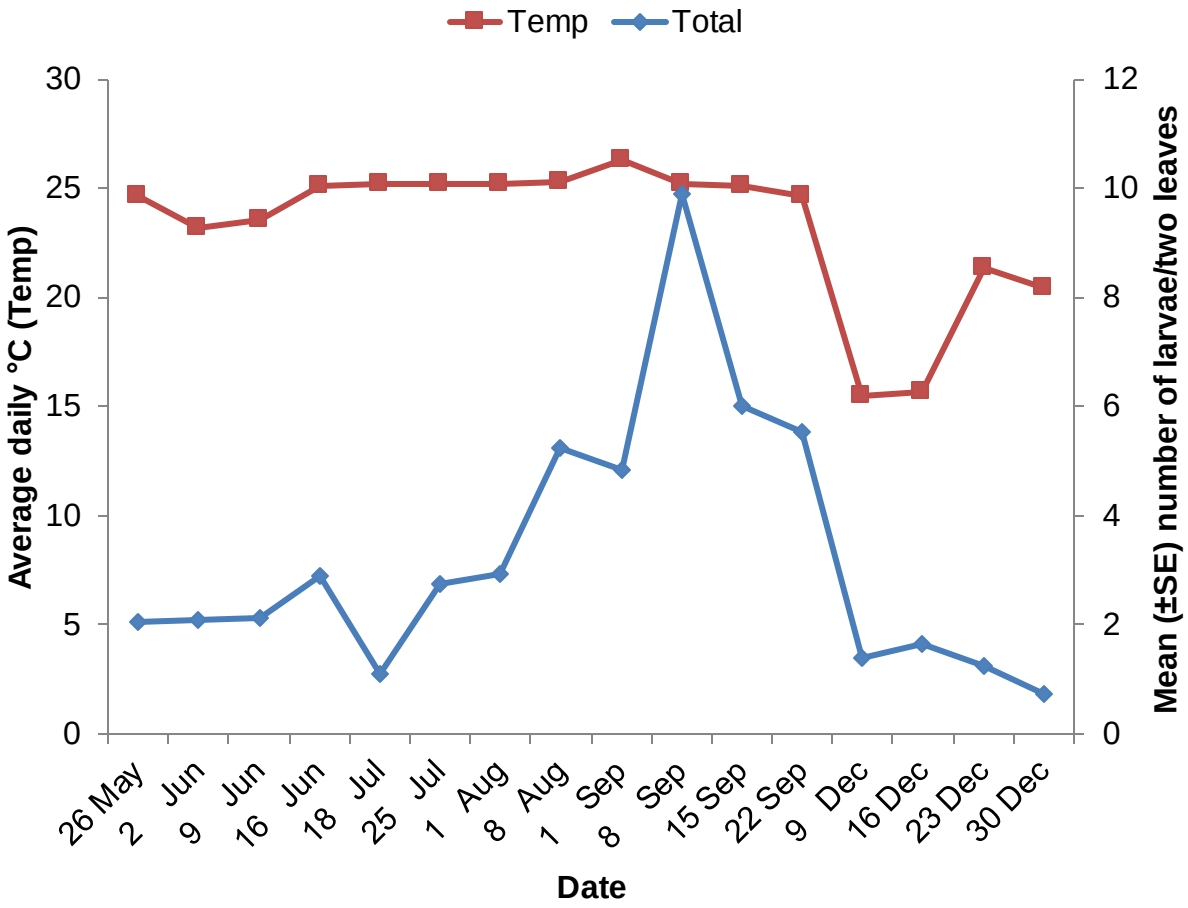

Figure 2-6. Comparison of average daily temperature (°C) and mean abundance (±SE) of total *Diaphania* hyalinata larvae during the four cropping seasons (26 May – 30 December, 2014) of yellow squash over one year of time. Data on temperature was obtained from FAWN, Homestead, Florida.

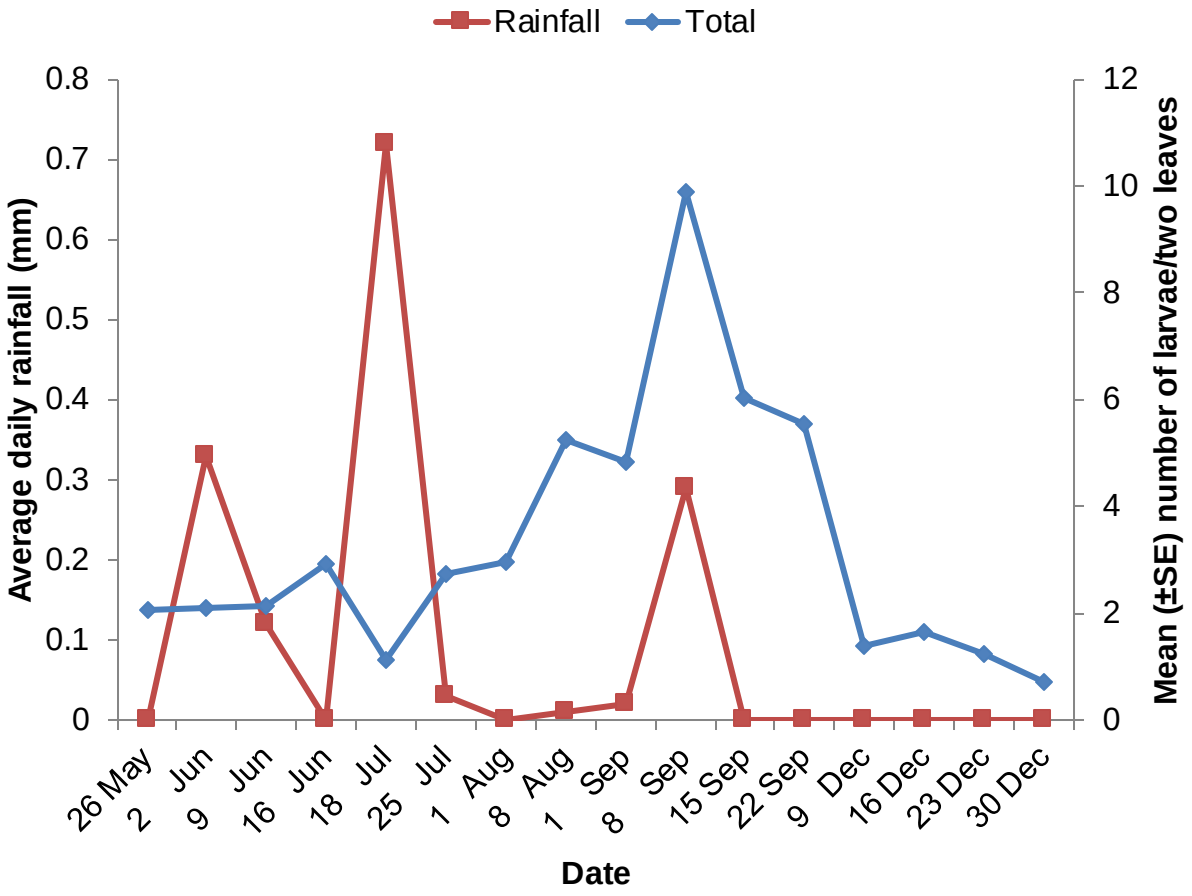

Figure 2-7. Comparison of average daily rainfall (mm) and mean abundance (±SE) of total *Diaphania* hyalinata larvae during the four cropping seasons (26 May to 30 December, 2014) of yellow squash over one year of time. Data on rainfall was obtained from FAWN, Homestead, Florida.

CHAPTER 3
HOST PREFERENCE AND WITHIN-PLANT DISTRIBUTION OF MELONWORM, *DIAPHANIA HYALINATA* L. (LEPIDOPTERA, CRAMBIDAE) ON FOUR CROPS OF CUCURBITS (YELLOW SQUASH, ZUCCHINI, CUCUMBER, AND WATERMELON)

The melonworm, *Diaphania hyalinata* L. (Lepidoptera: Crambidae) is a serious tropical pest of Cucurbitaceae throughout the southeastern United States (Fulton 1947, Dupree et. al. 1955). It overwinters in southern Florida and disperses throughout the southern and Gulf Coast states every summer (Reid et al. 1954, Reid & Cuthbert 1956). During the summer, it migrates into the Carolinas and up to the northern states and even to Oklahoma and Nebraska in the west (Zehnder 2011). The host range of melonworm is limited to cucurbits, with most damage to yellow squash followed by zucchini and cucumber. The larval stage of melonworm feeds on cucurbit foliage with the later instars (3^{rd}-5^{th} instars) more voracious in nature. They generally remain on the underside of leaves and feed on them. In extreme conditions, when population abundance is high, they can feed on the entire plant including fruit, leaves, stalks and vines, leaving only veins and veinlets of plants (Valles and Capinera 1992). Melonworm can cause serious damage to its host crops by significantly reducing yields (Guillaume and Boissot 2001). Melonworms feeding on foliage (indirect loss) may account for 23 % yield reduction (McSorley and Waddill 1982). Further yield loss (about 9 to 10 %) has been documented due to melonworms feeding on flowers and fruits (direct loss) in Florida. The primary method of control against larval stages of melonworm followed by most of the farmers is chemical insecticides. Because the insecticides are frequently used, there have been environmental problems with insects developing resistance, and thus are more difficult to control. So instead of relying solely on insecticides, effective

control strategies are needed that address issues with insecticide resistance and management costs.

To develop effective control strategies, knowledge about the biology of a pest and its interactions with the plant are needed. Host ovipositional preference by female adults is an important insect characteristic, which can be used in developing management programs. Deposition of small or large numbers of eggs depends on the level of preference to a plant species by the adult female (Thompson and Pellmyr 1991). Several factors affect the host preference and the ovipositional behavior of adult females including ecological and behavioral factors (Balagawi et al. 2005), secondary substances such as attractants and deterrents (Barros and Zucoloto 1999), and nutrient levels in host plants (Bartlet et al. 1994). Host selection by adult females is very important because it determines the survival of their progeny (Renwick 1989; Konstantopoulou et al. 2002). Pickleworm moths (*Diaphania nitidalis*) deposited more eggs on leaves of yellow squash than muskmelon and cucumber partly because of highly polar, non-volatile compounds and whole leaf volatiles associated with squash leaves (Peterson et al. 1994; Peterson and Elsey 1995). Along with the ovipositional behavior, within-plant distributions of different immature stages of an insect can affect their feeding. Information about within-plant distribution of melonworms in the field is very limited. Knowledge of the abundance of melonworms on different plant parts can be an important consideration in pest management programs. Information on preference levels of immature insects and their population distributions within plants can help in developing proper sampling methods. To develop a knowledge-based effective management program, I performed the following studies:

- Determined the host-preference levels of adult melonworm moths and their immature stages on four crops of cucurbits in the field. I hypothesized that the female adult melonworm moths would generally prefer yellow squash followed by zucchini, then cucumber, than watermelon.

- Investigated within-plant distributions of melonworm larvae on these cucurbits. Here, I hypothesized that abundance of different melonworm larval stages varied over time on different parts of the host plants.

Materials and Methods

Study Site

The study was conducted at the Tropical Research and Education Center (TREC), Homestead, FL. The soil type of field plots was Krome gravelly loam (loamy-skeletal, carbonated hyper thermic lithic Udorthents), which consists of 33% soil and 67% pebbles (> 2 mm diam.). The four crops of cucurbits used in this study were yellow squash cv. `Enterprise' (*Cucurbita pepo*) (Syngenta Seeds, Pasco, WA), zucchini cv. `Black beauty' (Cucurbita pepo) (Main Street Seed and Supply, Bay City, MI), watermelon cv. `Sugar baby' (*Citrullus lanatus*) (Main Street Seed and Supply, Bay City, MI), and cucumber cv. `Marketmore 76' (*Cucumis sativa*) (Main Street Seed and Supply, Bay City, MI). Seeds of these four cucurbits were planted at TREC research fields on May 2014.

Plot Design and Crop Management

The field used for the study was 84 m long and 6.3 m wide and included four beds each 84 m long by 0.9 m wide formed on 1.8 centers. When beds were prepared, granular fertilizer (N-P-K: 8-16-16) was applied at 908 kg per ha in a 10 cm wide band on each side of the raised bed with each band separated from the center of the bed by 25 cm. Additional liquid fertilizer (N-P-K: 4-0-8) was applied (236 liter per ha) weekly beginning three weeks after planting, and continuing for three weeks. For irrigation, a

drip tape was placed on the beds surface each side of the raised bed and 30 cm from its center. Then, beds were subsequently covered with black-and-white plastic mulch to control weeds. No insecticides were used, but the fungicides, chlorothalonil (Bravo Weather Stik®, Syngenta Crop Protection Co., Greensboro, NC) at 1.75 liter per ha and copper hydroxide (Kocide® 3000, DuPont Crop Protection, Wilmington, Delaware) at 0.8 liter per ha were applied weekly. To control weeds, halo sulfuron methyl (Sandea®, Gowan Co., Yuma, Az), was applied at 55 ml per ha before planting.

Each bed was further divided into eight 9.1-m-long plots with 1.5 m of non-planted space between the plots. Thus, the field was divided into 32 plots, or 4 blocks each having four plots. Each plot within a block was assigned randomly to a different cucurbit crop. Plots of specific crops were established by direct seeding 2-3 seeds in 4 cm diam and 4 cm deep 40 cm holes placed in a single row on each bed. The field was checked routinely to record germination. Plants were thinned within 10 days of germination by cutting stems with scissors leaving a single plant in each hole. Therefore, 20 – 22 plants remained in each plot following thinning. The middle two of the four rows were the experimental plots for collection of larvae, and the outer two rows were for sampling eggs. To preserve foliage for attraction of melonworm moths for oviposition and to protect the eggs to determine within plant distribution, plants in the outer two rows were frequently sprayed with a *Bt*-based insecticide (DiPel® DF Biological Insecticide, Valent BioSciences Co., Libertyville, IL) at 1.1 kg per ha to kill larvae. Then, leaves were collected from treated plants and used for counting eggs.

Sampling

Sampling for melonworms began 2 wk after crop germination and continued weekly. The three sampling dates were 27 May, 2 June and 10 June. Five plants per plot were

randomly selected. Each plant was visually divided into three equal sections to evaluate within plant distribution: top, middle and bottom. Leaves collected from top part correspond to tender unfolded but still expanding leaves, leaves from middle part were fully expanded mature leaves, and leaves from bottom part were older leaves which were still green but also showed some chlorosis. Two leaves from each section (i.e., 6 leaves per plant) were collected into separate plastic bags and labeled with plot and sample numbers and sampling date. Immediately after collecting samples, all samples were transported to the IPM Laboratory, TREC, Homestead. Leaves were thoroughly examined in the lab to record the number of eggs and larvae on each leaf. There were 4 replications per crop; thus, 20 plants per crop were checked per week. All larvae from each section of a plant were recorded into three size groups: small (1-5 mm), medium (5-15 mm), and large (15-25 mm). The small-size group corresponded to first and second instars, medium corresponded to third and fourth instars, and large size corresponded to fifth instar. The same plants were used for assessing percentage defoliation, which was determined by visual estimation. From the outer two rows, the same sampling technique was followed, but eggs rather than larvae were counted. The total number of eggs on each leaf was counted for each section of the four crops. Eggs and larvae were sampled on the same date and continued weekly until harvest. Objectives included documenting melonworm abundance on different cucurbit crops and understanding their within-plant distributions.

Statistical Analysis

Abundance data were square-root transformed to normalize the error variance; then, analyzed by one-way analysis of variance (ANOVA) (PROC MIXED, SAS Institute 2013). PROC MIXED was used to analyze due to the potential covariance structure

associated with taking repeated measures through time on the same plots of plants. Crop, date, within plant location and their interaction were modeled in study of ovipositional preference and leaf defoliation. Crop, date, within plant location, larval size and their interactions were modeled in larval preference study. Mean numbers of melonworm eggs and larvae, and percentage defoliation per date were compared using the Waller-Duncan K-ratio procedure ($\alpha<0.05$) (Waller and Duncan 1969, SAS Institute 2013). Although all data were transformed for statistical analysis, data were back-transformed for presentation in figures.

Results

Ovipositional Preference

Ovipositional preference of *Diaphania hyalinata* was significantly affected by sample date ($F_{2, 108} = 40.97$, $P < 0.0001$), crop ($F_{2, 108} = 23.04$, $P < 0.0001$), within plant location ($F_{2, 108} = 85.88$, $P < 0.0001$), and interactions between crop and within plant location ($F_{6, 108} = 3.79$, $P = 0.0018$), and between date and within plant location ($F_{4, 108} = 4.66$, $P = 0.0016$). The interactions date x crop and date x crop x within plant location during the May-June crop season did not significantly ($P = 0.09$) affect oviposition. Because the pattern of oviposition was the same at each of the three sample dates (Figure 3-1), oviposition was pooled across sample dates to evaluate differences among crops. The seasonal average was significantly affected by crop ($F_{3, 236} = 15.68$, $P < 0.0001$) with oviposition on yellow squash (5.4 ± 0.5; mean ± SE eggs per 6 leaves) cucumber (4.6 ± 0.4), zucchini (4.5 ± 0.6) significantly greater than watermelon (1.8 ± 0.2). Location within a plant (top, middle and bottom) significantly affected the oviposition preference of melonworms (Figure 3-2). Summarizing across crop, significantly greater numbers of eggs were found on leaves in the middle than on

the top or bottom of the plant. This ovipositional preference pattern was observed on all three sampling dates (2, 3 and 4 weeks after germination).

Larval Preference for Cucurbit Crops

Mean numbers of *D. hyalinata* L. larvae was significantly affected by date ($F_{2, 324}$ = 37.28, $P < 0.0001$), crop ($F_{3, 324}$ = 105.98, $P < 0.0001$), larval size ($F_{2, 324}$ = 155.47, $P < 0.0001$), and the interactions crop x date ($F_{6, 324}$ = 5.75, $P < 0.0001$), crop x larval size ($F_{6, 324}$ = 18.89, $P < 0.0001$), date x within plant location ($F_{4, 324}$ = 2.92, $P = 0.0212$), date x larval size ($F_{4, 324}$ = 8.45, $P < 0.0001$), location x size ($F_{4, 324}$ = 26.24, $P < 0.0001$), crop x date x within plant location ($F_{12, 324}$ = 3.02, $P = 0.0005$), crop x date x larval size ($F_{12, 324}$ = 5.16, $P < 0.0001$), date x within plant location x larval size ($F_{8, 324}$ = 2.14, $P = 0.032$), and crop x within plant location x larval size ($F_{12, 324}$ = 5.64, $P < 0.0001$). But larval abundance was not significantly affected by location, or the interactions crop x within plant location, and date x crop x within plant location x larval size ($P = 0.09$). Because the pattern of larval preference for each of the sample dates was the same, data were pooled across sample date for further analysis. For the seasonal average ($F_{3, 236}$ = 116.29, $P < 0.0001$), mean number of larvae per six leaves was significantly greater in zucchini (13.2 ± 0.9), yellow squash (12.4 ± 0.8), and cucumber (8.3 ± 0.9) than watermelon (0.7 ± 0.1) (Figure 3-3). When individual sampling dates were considered, data for all three dates showed mean numbers of larvae were significantly less on watermelon than on the other three crops during all three sampling dates. Mean number of larvae in zucchini never differed statistically from yellow squash. In weeks 2, 3, and the seasonal average, cucumber was significantly smaller than yellow squash or zucchini but significantly greater than watermelon.

Population Composition of Melonworm based on Larval Sizes

On each sample date and for the season average, significantly more small (4.8 ± 0.3) than large (0.6 ± 0.1) larvae were found on 6-leaf samples (Figure 3-4). Mean numbers of medium larvae (3.3 ± 0.3) were not significantly different than the other two sizes.

Mean Numbers of Melonworms among Four Cucurbit Crops based on Larval Sizes

Numbers of small larvae were significantly greater than large and medium larvae in all cucumber, watermelon, and yellow squash (Figure 3-5). Numbers of small and medium larvae were equivalent in zucchini. Numbers of medium larvae were significantly greater than large larvae except in watermelon.

Distribution of Three Larval Sizes in Three Parts of the Plant

The mean numbers of three larval sizes (small, medium and large) varied significantly on each of three plant parts (top, middle and bottom) (Figure 3-6). Small larvae were most abundant on the bottom of the plant, with the populations smaller in the middle, and lowest on the top; however, numbers of medium and large larvae each exhibited the opposite pattern. Large larvae were less common than small and medium larvae on all plant parts.

Defoliation by Melonworm Larvae

Percentage defoliation by melonworm larvae was significantly affected by date ($F_{2, 108} = 4.22$, $P = 0.0172$), crop ($F_{3, 108} = 50.27$, $P < 0.0001$), and interaction between date and within plant location. Defoliation was not significantly affected by location or the interactions crop x within plant location, date x crop, and date x crop x within plant location ($P = 0.09$). On each sampling date and seasonal average ($F_{3, 236} = 115.98$, $P <$

0.0001), percentage defoliation per six leaves was largest on yellow squash (23.2 ± 1.9) and zucchini (20.6 ± 1.5), and both were significantly different from cucumber (12.2 ± 1.4) and watermelon (0). Percentage defoliation of watermelon was significantly lower than the other three crops (Figure 3-7).

Discussion

The oviposition behavior of *D. hyalinata* females was affected by several factors including behavioral, ecological, and chemical factors (Thompson 1988, Thompson and Pellmyr 1991, Peterson et al. 1994). A female will typically deposit most of her eggs on the most preferred host plant and the fewest eggs on the least preferred host (Thompson 1988, Thompson and Pellmyr 1991). Similar results occurred in the present study, with more eggs generally oviposited on yellow squash, followed by cucumber, zucchini, and watermelon. Less preference for watermelon than the other three crops by adult moths may have resulted from chemical factors or poor growth and development of watermelon. The recommended planting dates for watermelon in southern Florida are December 15 – March 1, but during the current research, planting dates were May - June. The average temperature during the recommended planting dates was 20ºC (T_{max} = 31ºC and T_{min} = 0ºC), but during the May – June crop of the current research, average temperature was 24ºC (T_{max} = 33ºC and T_{min} = 15ºC) (FAWN 2014). As a result, watermelon growth and development was negatively affected, which may have deterred oviposition on that crop. Alternatively, the presence of the trichomes on yellow squash may have stimulated additional oviposition (Peterson et al. 1994, Peterson & Elsey 1995) and absence of trichomes with highly amphoteric compounds on watermelon leaves may have reduced the oviposition rates on watermelon (Elsey 1981). Oviposition preferences by melonworm adults directly influence the preference of larvae in choosing

host plants. Although larvae move from one plant to another due to shortage of food sources and progression of developmental stage, the initial choice of food is influenced by where the eggs are deposited.

The within-plant preference studies showed that numbers of small larvae were greater than other larval sizes, with highest observed on bottom part of the plant. This may have resulted from the ovipositional preference of melonworm adults with greater numbers of eggs oviposited on the middle than the other plant parts. After eclosing, small larvae start dispersing to search for food. Thus, the larval population on the bottom part of the plant is mostly composed small larvae instead of medium or large larvae. Later instars may have moved up towards the top resulting in greater numbers of medium and large larvae on the top compared to other plant parts.

Larval size is directly related to amount of food consumed which can be estimated by percentage defoliation. Later instars are much larger than earlier instar larvae and thus cause greater defoliation. Larger larvae (later instars) feed more voraciously than smaller larvae (earlier instars) and cause greater percentages of defoliation. Head capsule widths of fifth instars are around 7-8 times larger than those of first instars, and whole-body lengths of fifth instars are about 12-18 times longer than those of first instars (Table 4 -6 in Chapter 4). Although the populations of small larvae were large and mostly confined to the bottom of the plant, the smaller populations of large larvae in the top and middle parts may have caused more damage to host plants.

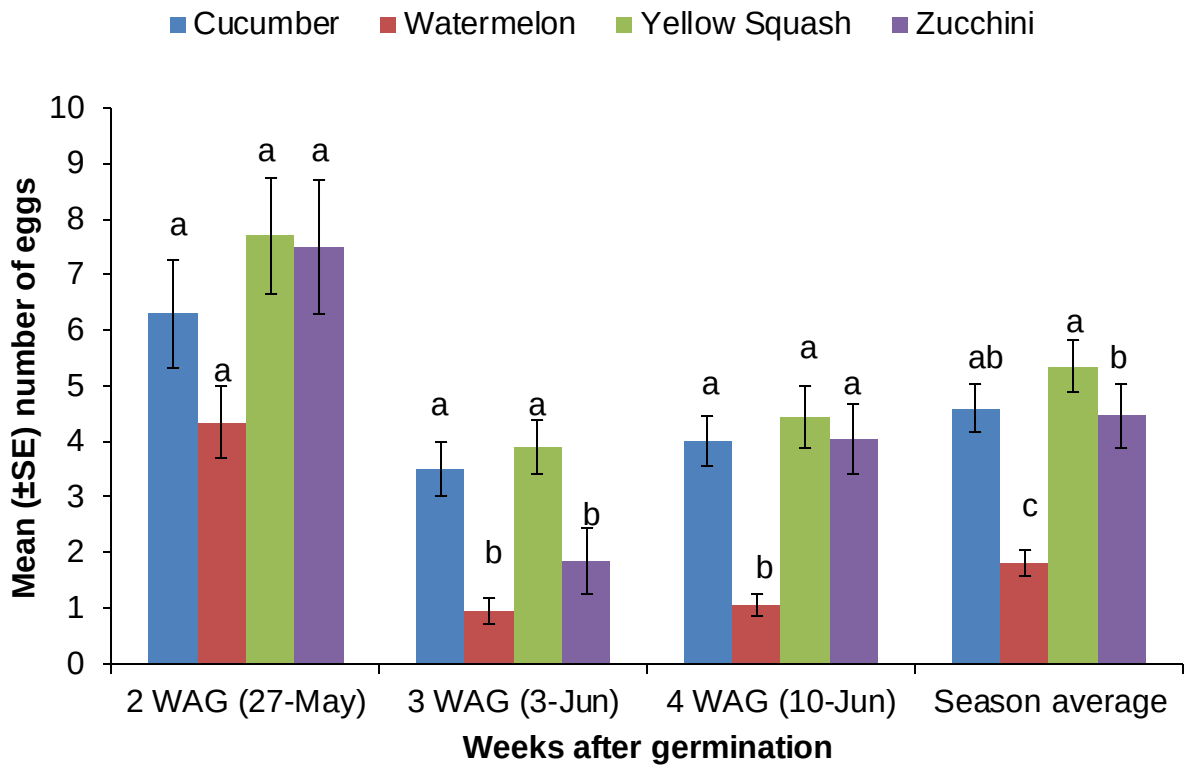

Figure 3-1. Mean ± SE number *Diaphania hyalinata* eggs per six-leaf sample across four cucurbit crops, sampled three times during May-June 2014. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan *K*-ratio procedure). Bars above and below means represent standard error. WAG = Weeks after germination.

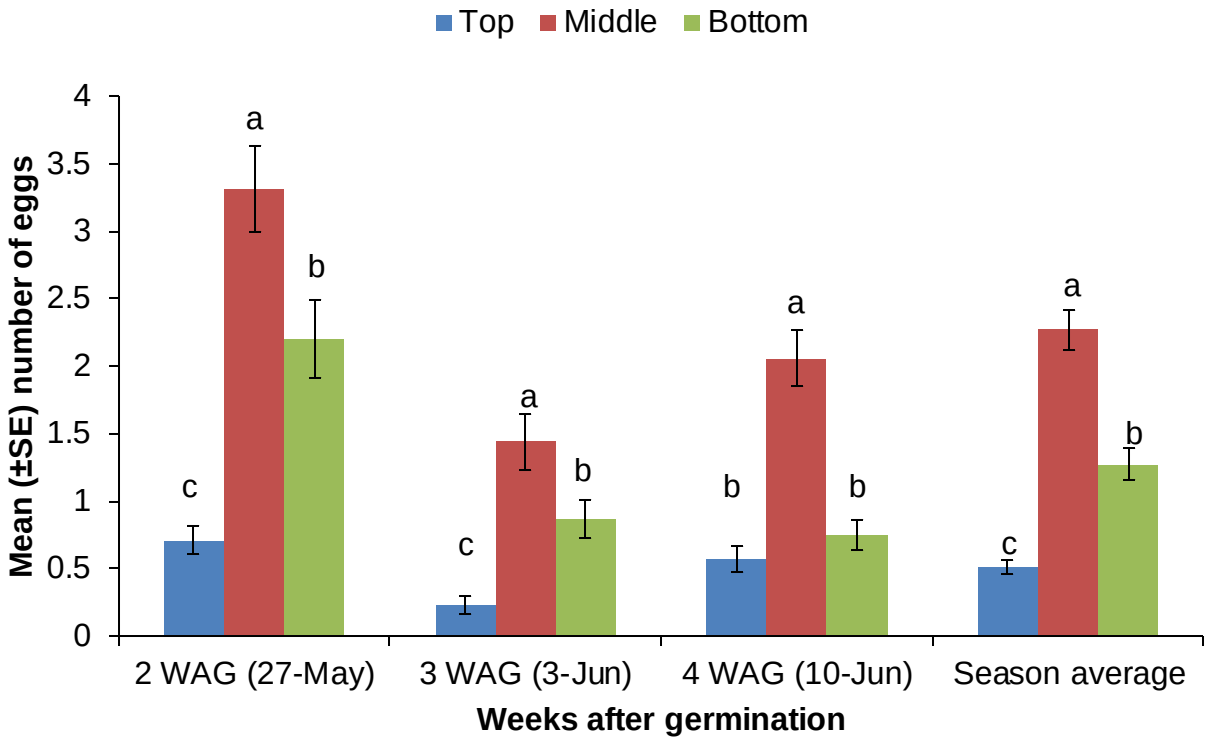

Figure 3-2. Mean ± SE number *Diaphania hyalinata* eggs per two-leaf sample of three plant parts across cucurbit crops, sampled three times during May-June 2014. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan *K*-ratio procedure). Bars above and below means represent standard error. WAG = Weeks after germination.

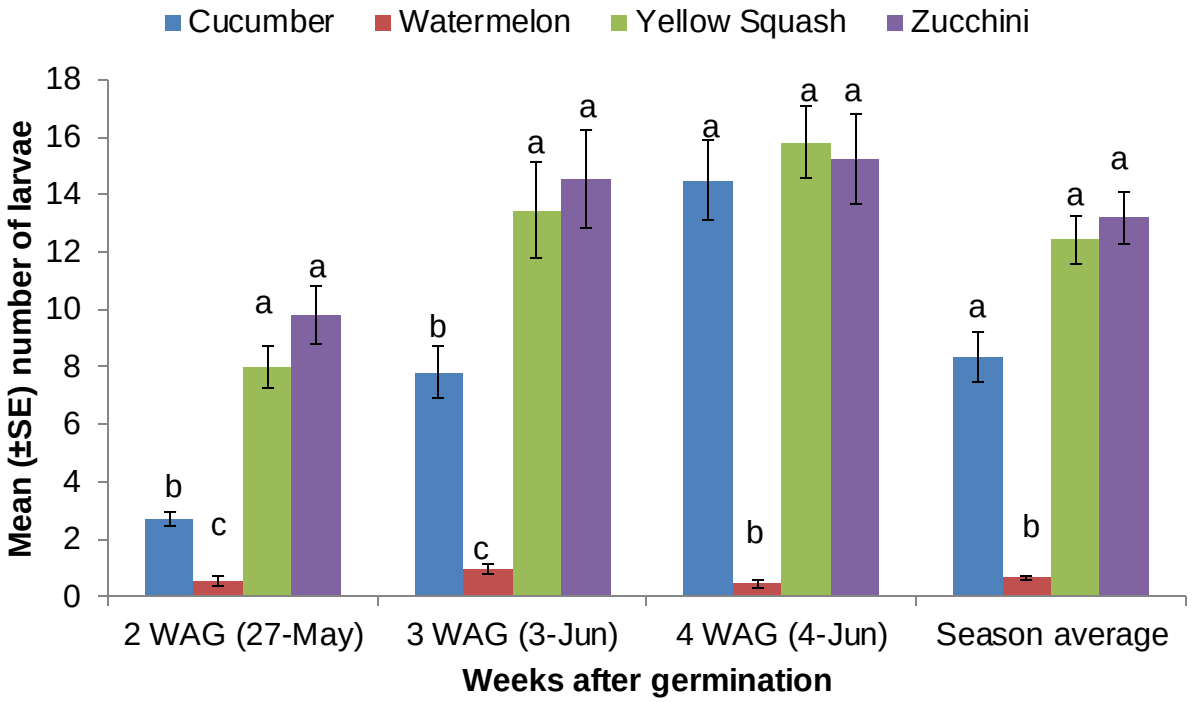

Figure 3-3. Mean ± SE number *Diaphania hyalinata* larvae per six-leaf sample across four cucurbit crops during May-June 2014. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error. WAG = Weeks after germination.

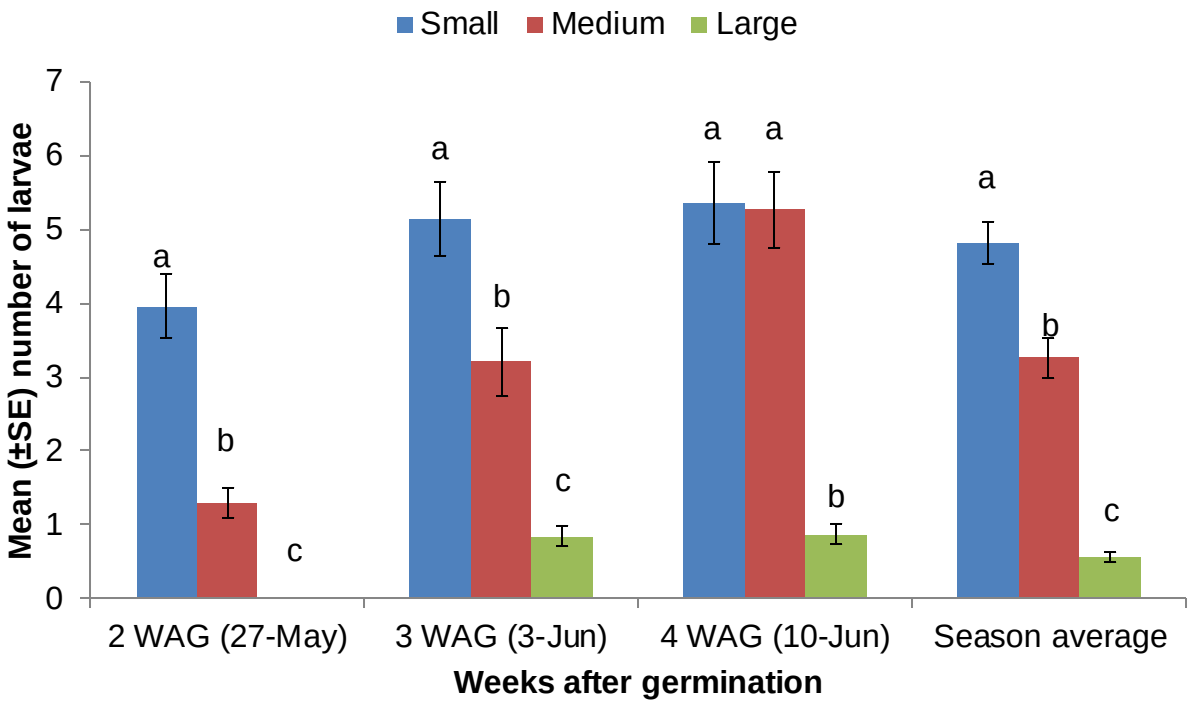

Figure 3-4. Mean ± SE number *Diaphania hyalinata* larvae by size per six-leaf sample across cucurbit crops during May-June 2014. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error. WAG = Weeks after germination.

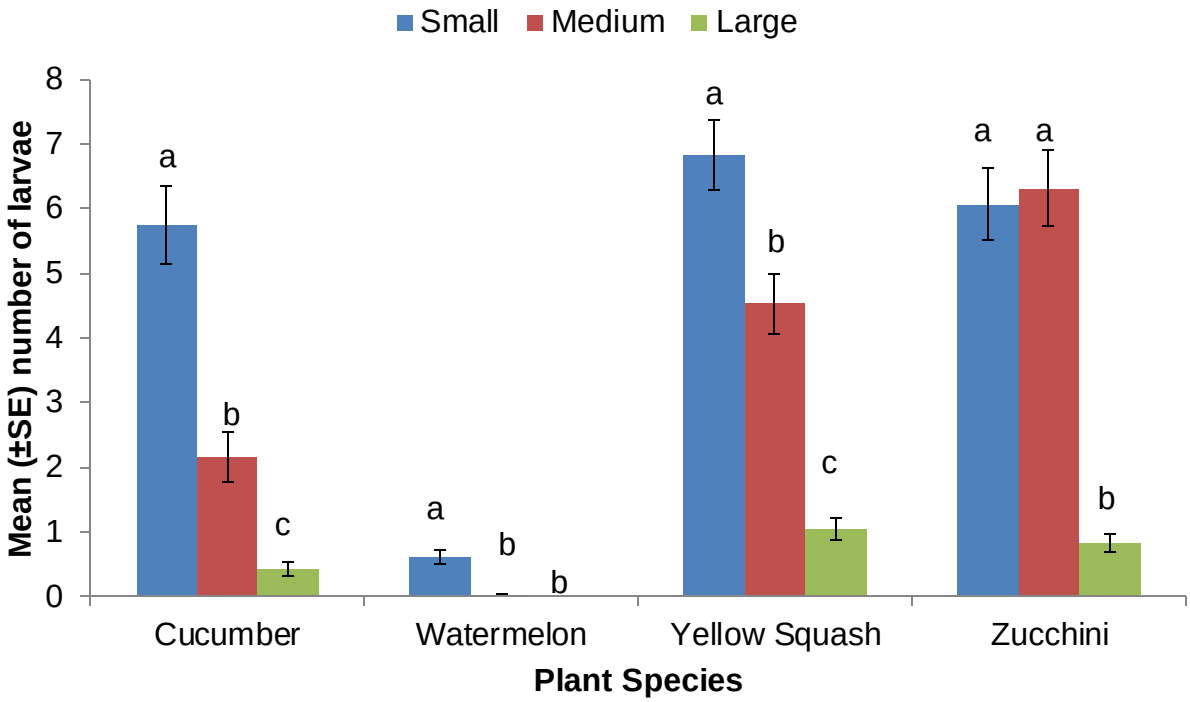

Figure 3-5. Mean ± SE number *Diaphania hyalinata* larvae by size per six-leaf sample across four cucurbit crops during May-June 2014. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error.

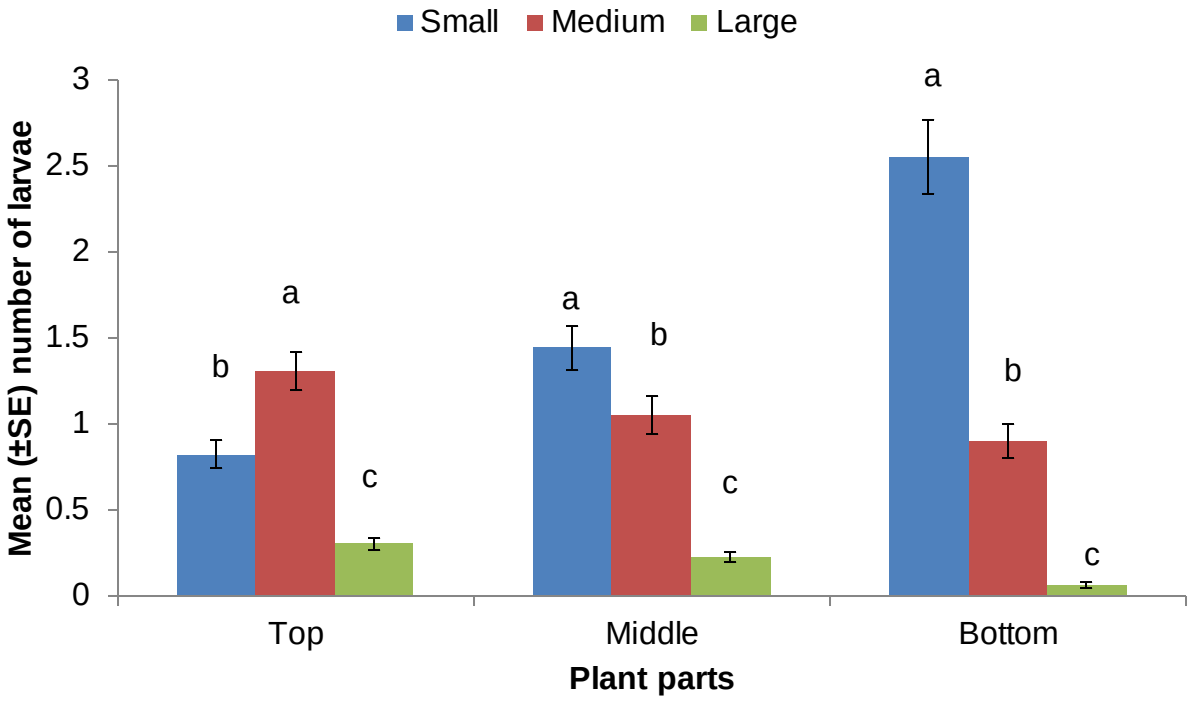

Figure 3-6. Mean ± SE number *Diaphania hyalinata* larvae per two-leaf sample by plant parts across cucurbit crops during May-June 2014. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error.

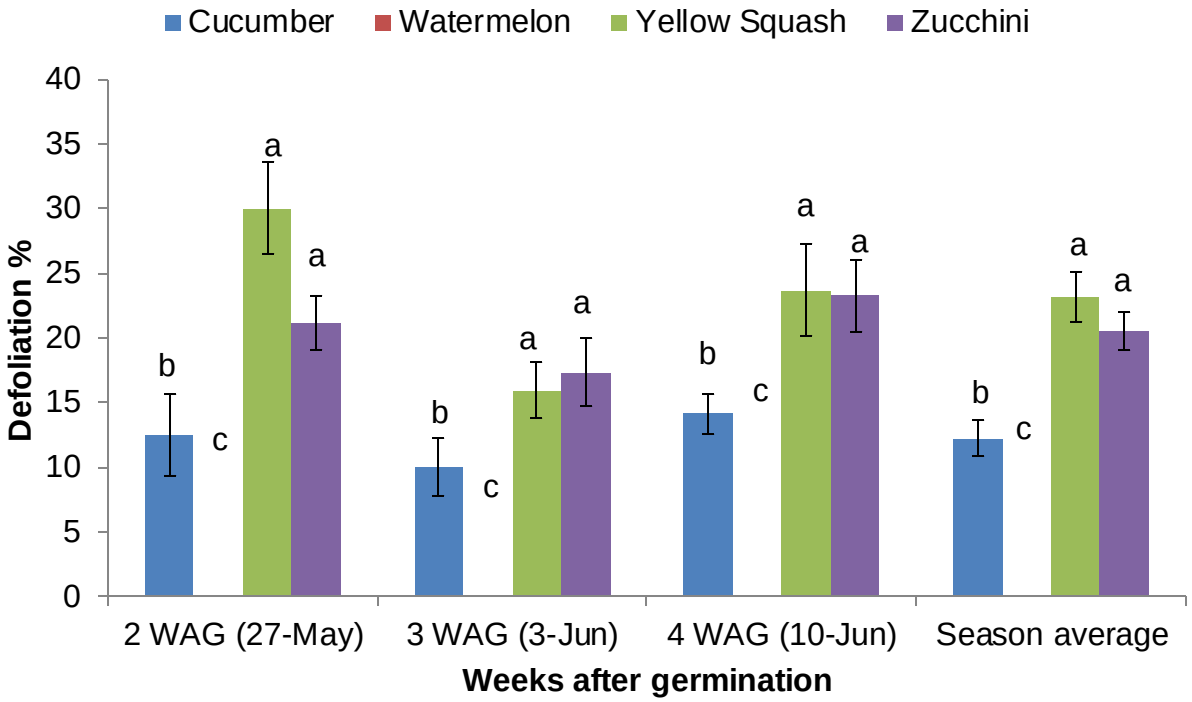

Figure 3-7. Percentage defoliation by *Diaphania hyalinata* larvae per six-leaf sample by cucurbit crops during May-June 2014. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error. WAG = Weeks after germination.

CHAPTER 4
HOST SELECTION AND GROWTH RESPONSE OF MELONWORM, *DIAPHANIA HYALINATA* L. (LEPIDOPTERA, CRAMBIDAE) ON FOUR CROPS OF CUCURBITS (YELLOW SQUASH, ZUCCHINI, CUCUMBER, AND WATERMELON) UNDER LABORATORY CONDITIONS

The ovipositional behavior of adult moths directly affects the development and survival of resulting larvae feeding on plants. The nutritional content and feeding deterrents of a plant species can lead to differences in the growth, development, and survival of melonworm larvae. This differentiation can be assessed by measuring head capsule widths of larvae and pupal dimensions (Dyar 1890, Caltagirone et al. 1983, Godin et al. 2002, Calvo and Molina 2008). Dyar (1890) reported that head capsule widths of Lepidopteran larval instars increase with the number of molts as the developmental period progresses. Factors influencing the size of larval head capsules also include growing season, generation, parasitism, sex, host plant species, diet, and temperature (Savopoulou-Soultani and Tzanakakis 1990, Goldson et al. 2001, Frouz et al. 2002). Hence, the present study was designed to compare the oviposition, feeding preference, survival, and developmental responses of *D. hyalinata* on four cucurbit species under laboratory conditions. First, we tested ovipositional preferences of *D. hyalinata* in a free-choice caged condition using four types of cucurbits. Second, we studied the survivorship and developmental periods from one stage to another from egg to adult. Third, the relative growth rates were compared by measuring body dimensions of the larval instars and pupae. Finally, larval herbivory was tested using four different cucurbit crops under choice and no-choice conditions.

Materials and Methods

Studies were conducted to determine duration and growth of individual development stages of melonworms on different cucurbits, which included yellow

squash, zucchini, cucumber, and watermelon. The study was conducted at the Tropical Research and Education Center (TREC), University of Florida, Homestead.

Plant Culture

All plants used in this study were grown in a greenhouse (11 m x 14 m) with partial shade and temperatures ranging from 20°C to 34°C with an average of 27°F. Relative humidity ranged from 71% to 95% with an average of 83%. The roof of the greenhouse was designed to block 60 – 70% of the sunlight to facilitate proper plant growth. Four cucurbit crops used in this experiment were yellow squash: cv. 'Enterprise' (Syngenta Seeds, Pasco, WA), zucchini: cv. 'Black Beauty' (Main Street Seed and Supply, Bay City, MI), watermelon: cv. 'Sugar Baby' (Main Street Seed and Supply, Bay City, MI) and cucumber: cv. 'Marketmore76' (Main Street Seed and Supply, Bay City, MI). All plants were grown by initially planting two seeds 10 cm deep in a one-quart plastic pot containing standard soil mixture (Farfard # 2, Premier Tech Horticulture, Quakertown, PA). Newly emerged seedlings were thinned to one per pot with the use of scissors. Plants were irrigated with 50 ml water two times per day. Granular fertilizer (N-P-K: 8-16-16) was applied on the soil surface of each pot at planting at 908 kg per ha. Beginning three weeks after germination, liquid fertilizer (N-P-K: 4-0-8) was also applied weekly at 236 liter per ha. To prevent infection by fungal pathogens, plants were sprayed and soil was drenched with chlorothalonil (1.75 liter per ha, Bravo weather stick®, Syngenta Crop Protection Co., Greensboro, NC) once a week, however; no insecticide was applied.

Insect Colony Maintenance

Initially, a melonworm colony was started by collecting 100 4th or 5th instar larvae from TREC research plots where squash and cucumber were grown for several

seasons. They were put in Petri dishes containing fresh cucurbit leaves. Cucurbit leaves were collected from healthily growing cucurbit crops in the greenhouse. Petri dishes including leaves were replaced every 24 hours with a fresh set to avoid fungal infection. The larvae were checked daily to collect fresh pupae, which were then placed in a different Petri dish provided with a moist filter paper to avoid desiccation. Adults emerging from these pupae were used in the experiment. The sole purpose of this was to get healthy adults to be used in the experiment.

Oviposition Preference

Ovipositional preference of melonworms was studied in the laboratory at 28 ± 1.5°C, 77 ± 5 % R.H. and 14:10 (L: D) in a 4-sided nylon mesh cage (61 cm x 61 cm x 183 cm). Three cage sides were covered with 200-mesh nylon cloth and one side with thin plastic sheet. On the front of the cage, there was zipper to open the cage, to facilitate handling insects and other contents within the box.

Four of the aforementioned cages were used to study oviposition behavior of melonworm provided free choice of four cucurbit host plants: yellow squash, zucchini, cucumber, and watermelon. Each cage served as a block (replicate), wherein one potted plant of each of the four crops was placed, resulting in a randomized complete block design. Twenty melonworm adults (unsexed) that were 24 + 4 h old were collected from the aforementioned laboratory colony and held at 7°C for 5 – 7 min to immobilize them. They were then placed in a Petri dish positioned in the center of each cage at equal distance from each plant pot. A distilled water and sugar solution was provided in vials as an adult food and moisture source within the cages. In 4-5 minutes, the previously chilled insects became active and started moving to the plants. Plants were removed 24 h after introducing the adult moths to carefully inspect the entire plant

to record the number of melonworm eggs. Plants were replaced every 24 h with fresh, non-infested plants from the greenhouse. At the same time, mortality of adults was recorded and confirmed by gently probing each adult with a fine insect pin. Adults that did not move in response to pin-probing was considered dead. The number of eggs was counted from each crop until all the moths in a cage were dead.

Egg Development Time and Percentage of Larvae Eclosion

A separate study was conducted to determine the melonworm egg development period. Twenty, 8-12 h old adults (about 10 males and 10 females) were placed in a cage with 2-leaf plants of each of four crops. Additional leaves had been removed and discarded from each plant. This method was adopted to concentrate egg deposition on a minimum number of leaves. The cage was left undisturbed for 4 h (20:00 – 24:00 EST), after which the adults were collected from the host plants. The leaves were checked carefully and the location of twenty eggs (0-4 h old) were marked adjacent to the eggs with a red marker. Additional eggs were discarded by removing them with an insect pin. Leaves containing eggs were placed in a Petri dish (9.5 cm diam.) with a moist filter paper at the bottom to avoid desiccation. The study was conducted in a laboratory using the same procedures described for the oviposition preference study. The eggs were checked at 4-h intervals to record the egg development period, which was confirmed by the eclosion of first instar larvae. Larvae were immediately removed from the Petri dishes to avoid cannibalism. The number of larvae removed from each Petri dish at each time period was also recorded to determine the percentage of larval eclosion. The study was replicated 5 times.

Duration of Different Instars and Survival

Twenty first-instar larvae (0-2 h old) were collected from the larval eclosion study and placed in a Petri dish (13 cm diam.), each containing a leaf disc (2.22 cm diam.) of the same host plant species on which the eggs were deposited. The youngest fully expanded leaves were cut from the potted plants in greenhouse and leaf disks were made using a leaf cutter avoiding major leaf veins. The same procedure was repeated for all four host plant species and the whole experiment was replicated 5 times. The larvae were checked at 4 h intervals to observe molting to the next stage, which was confirmed by looking at the color and size of the head capsule (Smith et al. 1994). In addition, larval exuviae were also noted to confirm molting to the next stage. The presence of black dots on the dorsum of each segment identified the larvae as first instars. These black dots disappeared during second instar. Two lateral white stripes appeared in on the dorsum during the third instar and became more prominent and visible during fourth and fifth instars. Once a larva molted, it was taken out of the Petri dish and the time required to molt was recorded. To avoid fungal infection, Petri dishes were replaced with clean petri dishes, and host-plant leaves were replaced with fresh leaves on a daily basis. Then each of the larvae were moved into new Petri dishes with fresh leaves with the help of clean and sterilized paint brush. The procedure for determining developmental periods and percentage mortalities for the other instars was otherwise the same as for the first instar with the duration, mortality, and total numbers for each larval instar recorded.

Growth and Development Parameters

To identify the effects of cucurbit species on developmental rates of larvae, their growth was recorded every 2 d. Head capsule width and body length of larvae in the

same age group were measured every 2 d using a digital ocular microscope (Leica Application Suite, Leica Microsystems, Wetzlar, Germany). Larval body length varies with the amount of food consumed and stretching during each instar. Therefore, multiple measurements were taken for each larvae and average values were used for analysis. Pre-pupal and pupal weights were recorded for each treatment using an electronic balance (PB3002-S Delta Range, Mettler Toledo, Switzerland). Pupal dimensions were recorded for each treatment using a digital ocular microscope.

Choice and No-choice Feeding

This study was conducted using the previously described laboratory conditions. Two studies, one with choice feeding and another with no-choice feeding, were conducted to determine melonworm feeding preferences. Larvae used in this experiment were obtained from the above laboratory colony. All larvae were 5-d-old and belonged to the same cohort to maintain similarity in their growth and physiological development. For the choice study, twenty 14-cm-diameter petri dishes were used, and four leaf discs (3.9 sq cm), one from each crop, were randomly placed in each Petri dish. The younger fully expanded leaves collected from greenhouse were used to make leaf discs using leaf cutter. Leaf discs were arranged in a randomized complete block design with each Petri dish serving as a block with its four leaf discs as treatments. One 5 d old melonworm larva was placed in the center of each Petri dish, which was then covered with a lid. Petri dishes were placed in a laboratory as described above and left undisturbed for 17 h. To quantify amounts of larval feeding and their preference in each crop, all the leaf discs with melonworm feeding damage were removed after 17 h and scanned using a leaf area meter (LI-COR portable area meter LI 3000, Lambda

Instrument Co., Lincoln, Nebraska, USA). This instrument was used to quantify the amount of leaf area removed by larval herbivory.

For the no-choice study, 9 cm diam. Petri dishes were utilized with one leaf disc (3.9 sq cm) placed into each dish. Four dishes each with a single leaf disc of one of the four cucurbit crops represented a block and the whole experiment was replicated five times. The experimental procedure was otherwise as described for the choice study above.

Statistical Analysis

All sets of data were square root transformed to normalize the error variance. Analysis of variance (ANOVA) (PROC GLM, SAS Institute 2013) for a randomized complete block design was performed for oviposition choice experiments with cage as a blocking factor. Crop, life stages and their interaction were modeled in study of survival and duration of melonworm life stages using factorial ANOVA (PROC MIXED). One-way ANOVA (PROC GLM) for a completely randomized design was performed for head capsule width, whole body length, pre-pupal and pupal measurements, choice test, and no-choice test to test the differences between treatments. Mean values of oviposition, survival and duration of life stages, head capsule width and whole body length of larvae, pre-pupal and pupal measurements, and choice and no-choice tests were compared using Waller-Duncan K-ratio procedure ($\alpha<0.05$) (Waller and Duncan 1969, SAS Institute 2013). Means values in tables and figures were back-transformed.

Results

Oviposition Preference

The oviposition preference of *D. hyalinata* in laboratory studies differed significantly among the four cucurbit crops ($F_{3, 12} = 11.73$, $P = 0.0007$). A large number

of eggs was deposited on yellow squash (1335.5 ± 289.3 eggs per plant; mean ± SE) followed by zucchini (964.5 ± 124.4) and cucumber (869.3 ± 231.3) during entire lifetime of adult moth (Figure 4-1). Significantly fewer eggs were deposited on watermelon (183.0 ± 20.9) than the other crops. There was no significant difference for oviposition among yellow squash, zucchini and cucumber.

Survival on an Individual Crop

Survival of *D. hyalinata* across all life stages were significantly affected by crop ($F_{3, 128} = 8.48$, $P < 0.0001$), life stages ($F_{7, 128} = 9.34$, $P < 0.0001$) and interaction between crop and life stages ($F_{21, 128} = 2.33$, $P = 0.002$). Egg survival was significantly greater on yellow squash (89.46%) and cucumber (88.5%) than on either zucchini (74.5%) or watermelon (70.0%) (Table 4-1). Percentage larval survival in each of the first three instars (1st, 2nd and 3rd) averaged greater than 81% but did not differ significantly among the crops. Percentage survivals of 4th and 5th instar *D. hyalinata* were significantly affected by crop. Greatest 4th instar survival occurred on watermelon (96.2%) and the worst on yellow squash (65.8%). Percentage survival of 5th instar larvae was significantly greater on watermelon and zucchini than on either yellow squash or cucumber. Pre-pupal and pupal survivals were not significantly affected at $p = 0.05$ by larval host plant.

Summarizing survival across stages, plant species significantly affected the survival of *D. hyalinata* from 1st instar to pupa and 1st instar to adult. In each case, across-stage-survival was greatest on watermelon, followed by zucchini with the worst survival on yellow squash and cucumber (Figure 4-2).

Development Times

Development times of *D. hyalinata* across all life stages were significantly affected by crop ($F_{3, 128} = 3.97$, $P = 0.0096$), life stage ($F_{7, 128} = 36.41$, $P < 0.0001$), and interaction between crop and life stages ($F_{21, 128} = 1.85$, $P = 0.0197$). There was no significant difference ($p = 0.05$) among crops in egg, 1st instar, pre-pupae, or pupae development times (Table 4-2). Developmental times of *D. hyalinata* 2nd through 5th instars were significantly slower on watermelon than on the other crops. Developmental times for the 3rd and 4th instars were significantly slower on zucchini than yellow squash and cucumber. Overall development times of *D. hyalinata* from 1st instar to pupa and 1st instar to adult did not vary significantly among crops (Figure 4-3).

Head Capsule Width

Head capsule width (mm) of *D. hyalinata* larvae was significantly affected by crop ($F_{3, 128} = 20.83$, $P < 0.0001$), larval age ($F_{7, 128} = 806.19$, $P < 0.0001$) and interaction between crop and larval age ($F_{21, 128} = 3.69$, $P < 0.0001$). Head capsules were greater on larvae reared on yellow squash than on watermelon 5, 7, 9, 11 and 13 days after emergence (Table 4-3). Head capsule width of 1, 3, and 15 day-old larvae did not differ significantly among the cultivars. Head capsule widths of 5-day-old larvae reared on cucumber and 7-d-old larvae on zucchini were intermediate between those reared on yellow squash and watermelon. Larvae reared on zucchini had significantly larger head capsules than larvae on watermelon, 5 and 7 days after emergence. There was no significant difference between larvae reared on zucchini and watermelon 11 days after emergence.

Whole-body Length

Whole body length (mm) of *D. hyalinata* larvae was significantly affected by crop ($F_{3, 128} = 18.94$, $P < 0.0001$), larval age ($F_{7, 128} = 769.94$, $P < 0.0001$) and interaction between crop and larval age ($F_{21, 128} = 3.12$, $P < 0.0001$). Crop species had a significant effect on the whole-body length of *D. hyalinata* larvae ($F_{3, 128} = 18.94$, $P < 0.0001$). Cucurbit cultivars affected body length on 1, 5, 7, and 9 days after emergence (Table 4-4). Whole-body length of 1-day-old larvae reared on zucchini was significantly longer than that of yellow squash and watermelon. Whole-body lengths for 5, 7, and 9 day-old larvae reared on zucchini, yellow squash, and cucumbers were each significantly larger than watermelon. There was no significant difference among crop species in whole-body length for 3-, 11-, 13-, or 15-day-old larvae.

Measurement of Pre-pupae and Pupae

Pre-pupal weight of *D. hyalinata* was significantly affected by crop and was greater on watermelon and cucumber than on yellow squash ($F_{3, 36} = 4.75$, $P = 0.0069$). However, there was no significant difference in pupal weight, and pupal body length and width between *D. hyalinata* reared on yellow squash, zucchini, cucumber, or watermelon (Table 4-5).

Choice and No-choice Tests

Larval food plant species significantly affected the percentage defoliation by larvae of *D. hyalinata* in free-choice test ($F_{3, 76} = 5.64$, $P = 0.0015$) with significantly greater defoliation on yellow squash, zucchini, and cucumber than on watermelon (Figure 4-4). Larval food plant species also significantly affected percentage defoliation by larvae of *D. hyalinata* in a no-choice test ($F_{3, 16} = 12.23$, $P = 0.0002$) (Figure 4-5). Percentage defoliation was significantly greater on zucchini than on cucumber, which

was significantly greater than watermelon, which was significantly smaller than all three other crop species.

Discussion

Several factors affect the ovipositional behavior of *D. hyalinata* females. However, results showing survival, developmental times, larval growth, and food choice suggest that *D. hyalinata* females also chose host plant species resulting in the greatest fitness of their offspring. Of the four cucurbit crop species used in the present study, yellow squash, zucchini, and cucumber are the most common and suitable hosts of melonworm, whereas watermelon is minor host of melonworm (Capinera 2008). Large acreages of yellow squash, zucchini, and cucumber are grown in southern Florida, where melonworm is also prevalent. But the climate is not favorable for watermelon production in southern Florida; hence, the growth and development of watermelon was not as satisfactory as the other crop species, which may have affected the results. Only watermelon seemed to show relatively poor results in all parameters tested: oviposition preference was lowest, larval developmental time was longest, larval head capsule width was smallest, whole-body length of larvae was shortest, and percentage defoliation by larvae was the lowest among the four crops evaluated. For all these parameters, yellow squash, zucchini, and cucumber were each significantly different from watermelon, but were not significantly different among themselves. Herbivorous insects increase in abundance, growth rate, and reproduction when fed on nutrient rich diets (Auclair et al. 1957, Dixon 1970, Weibull 1987, Sandstrom and Pettersson 1994).

Diaphania hyalinata deposited more eggs on yellow squash, zucchini, and cucumber than on watermelon. The difference in egg deposition could result from biochemical differences among the host plants. Peterson et al. (1994) described how

chemical factors are involved in host plant selection for oviposition by *Diaphania nitidalis.* According to Peterson et al. 1994 and Peterson & Elsey 1995, females are stimulated to deposit more eggs on leaves of yellow squash, because of non-volatile, highly polar, amphoteric compounds with relatively low molecular weights. The greater number of eggs deposited on leaves of yellow squash than on watermelon may result from the lack of similar oviposition stimulants on watermelon leaves.

Unexpected results were obtained with melonworm larval survival. Although yellow squash was the most preferred ovipositional host, larval survival rates were lower on yellow squash than on cucumber and watermelon for 4th instars, and lower than on watermelon for 5th instars. The difference in survival may have resulted from nutrients, less resistance, or less defensive compounds in or on the leaves that are more favorable to larval survival in watermelon than in the other crop species.

In apparent contrast with survival results; however, developmental times were generally longer for larvae reared on watermelon leaves than on the other crops. The slower development of larvae on watermelon leaves also seemed to correspond with a smaller head capsule width for watermelon-fed larvae than for the other crops. Irrespective of same larval instar, same day old larvae were measured for head capsule width. This inspires ideas about the effects of food nutrients on the growth and development of larvae, which can be measured through head capsule width (Dyar 1890). Results of whole-body lengths of larvae show similar differences among crops as with head capsule widths with longer body length on larvae fed leaves of yellow squash, zucchini, and cucumber than watermelon leaves. In addition to the slower development of melonworm larvae on watermelon leaves than on the other crops, the

nutrient contents in watermelon leaves may have resulted in reduced head capsule widths (Savopoulou-Soultani and Tzanakakis 1990). In contrast, the greater pre-pupal weights of larvae fed watermelon and cucumber than yellow squash weaken the foregoing arguments. However, pre-pupal weights were the only variable measured supporting greater melonworm fitness when reared on watermelon compared to yellow squash, whereas several variables supported reduced melonworm fitness on watermelon compared to at least one of the other crops in the foregoing discussion.

Ovipositional preference by melonworm was noted by comparing numbers of eggs deposited per crop species in a choice test. Larval preferences in choice and no-choice tests were determined by noting the percentages of defoliation caused by melonworm larvae. The melonworm adult choice test found that yellow squash, zucchini, and cucumber were all preferred over watermelon. Larval choice and no-choice tests each showed that yellow squash, zucchini and cucumber were preferred over watermelon as hosts. Hence, all these tests showed that yellow squash and zucchini were preferred over watermelon. When combined with the foregoing results showing survival, developmental times, larval growth, it suggests that *D. hyalinata* females also chose host plant species resulting in the greatest fitness of their offspring, and the ovipositional behavior of melonworm females favors placement of larvae for their maximum food preference and fitness.

Table 4-1. Mean (± SE) percentage survival by stage of *Diaphania hyalinata* reared on leaf tissue of yellow squash, zucchini, cucumber, and watermelon in the laboratory.

Growth Stage	Plant Species				$F_{3,16}$	P
	Yellow Squash	Zucchini	Cucumber	Watermelon		
Egg	89 ± 4.6 a	74.5 ± 4.4 b	88.5 ± 3.7 a	70 ± 5.6 b	4.42	0.0095
1st instar	93 ± 2.5 a	95 ± 2.2 a	94 ± 1 a	91 ± 1.9 a	0.73	0.5501
2nd instar	82.5 ± 4.7 a	93.9 ± 4.0 a	92.5 ± 2.3 a	90.1 ± 3.2 a	1.93	0.1655
3rd instar	87.9 ± 10.5 a	96.5 ± 2.4 a	81.9 ± 7.0 a	92.5± 3.1 a	0.82	0.4995
4th instar	65.8 ± 6.4 c	72.1 ± 3.9 bc	80.3 ± 2.0 b	96.2 ± 2.4 a	9.39	0.0008
5th instar	44.8 ± 9.5 bc	65.1 ± 7.7 ab	32.3 ± 8.5 c	90.8 ± 3.3 a	6.34	0.0049
Pre-pupa	61.6 ± 13.2 a	72.9 ± 6.0 a	45.1 ± 15.8 a	92.6 ± 4.2 a	2.75	0.0767
Pupa	62 ± 18.5 a	49.8 ± 7.9 a	36.7 ± 15.2 a	91.9 ± 3.7 a	2.22	0.1259

Means within a row followed by the same letter did not differ significantly ($P = 0.05$) based on analysis of variance and Waller-Duncan K-ratio procedure.

Table 4-2. Mean (± SE) developmental time by stage of *Diaphania hyalinata* reared on leaf tissue of yellow squash, zucchini, cucumber, and watermelon in the laboratory.

Growth Stage	Plant Species				$F_{3,16}$	P
	Yellow Squash	Zucchini	Cucumber	Watermelon		
Egg	4.3 ± 0.1 a	4.7 ± 0.2 a	5.1 ± 0.4 a	4.2 ± 0.1 a	2.53	0.0726
1st instar	3.1 ± 0.1 a	3.0 ± 0.1 a	3.1 ± 0.0 a	3.3 ± 0.1 a	2.39	0.1065
2nd instar	2.1 ± 0.1 b	2.2 ± 0.1 b	2.1 ± 0.0 b	2.9 ± 0.1 a	15.84	<.0001
3rd instar	1.9 ± 0.1 b	1.4 ± 0.0 c	2.0 ± 0.0 b	2.5 ± 0.2 a	25.39	<.0001
4th instar	1.7 ± 0.1 ab	1.3 ± 0.1 c	1.6 ± 0.1 b	1.9 ± 0.0 a	11.73	0.0003
5th instar	2.0 ± 0.2 b	1.2 ± 0.1 b	1.8 ± 0.5 b	3.7 ± 0.2 a	7.49	0.0024
Pre-pupa	2.1 ± 0.1 a	1.9 ± 0.2 a	1.7 ± 0.5 a	2.4 ± 0.1 a	1.29	0.3118
Pupa	8.4 ± 2.1 a	10.6 ± 0.2a	6.2 ± 2.5 a	10.6 ± 0.2a	1.54	0.2435

Means within a row followed by the same letter did not differ significantly ($P = 0.05$) using analysis of variance and Waller-Duncan K-ratio procedure.

Table 4-3. Mean (± SE) head capsule width (mm) of *Diaphania hyalinata* larvae reared on leaf tissue of yellow squash, zucchini, cucumber, and watermelon in the laboratory.

No. days after eclosion	Plant Species				$F_{3,16}$	P
	Yellow Squash	Zucchini	Cucumber	Watermelon		
1	0.26 ± 0.016 a	0.28 ± 0.008 a	0.27 ± 0.014 a	0.27 ± 0.015 a	0.22	0.8782
3	0.29 ± 0.003 a	0.3 ± 0.003 a	0.34 ± 0.031 a	0.3 ± 0.011 a	2.08	0.1434
5	0.46 ± 0.027 a	0.48 ± 0.011 a	0.39 ± 0.022 b	0.31 ± 0.01 c	16.67	<.0001
7	0.72 ± 0.03 a	0.65 ± 0.021 b	0.72 ± 0.025 ab	0.46 ± 0.021 c	25.43	<.0001
9	1.23 ± 0.066 a	1.27 ± 0.094 a	1.15 ± 0.072 ab	0.94 ± 0.04 b	4.53	0.0176
11	2.06 ± 0.046 a	1.47 ± 0.082 b	1.76 ± 0.182 ab	1.44 ± 0.114 b	5.88	0.0066
13	2.04 ± 0.016 a	2.06 ± 0.003 a	2.05 ± 0.028 a	1.7 ± 0.174 b	3.91	0.0287
15	2.06 ± 0.018 a	2 ± 0.021 a	2.07 ± 0.062 a	2.05 ± 0.041 a	0.56	0.6499

Means within a row followed by the same letter did not differ significantly ($P = 0.05$) using analysis of variance and Waller-Duncan *K*-ratio procedure.

Table 4-4. Mean (± SE) whole-body length (mm) of *Diaphania hyalinata* larvae reared on leaf tissue of yellow squash, zucchini, cucumber, and watermelon in the laboratory.

No. days after eclosion	Plant Species				$F_{3,16}$	P
	Yellow Squash	Zucchini	Cucumber	Watermelon		
1	1.46 ± 0.059 b	2.02 ± 0.147 a	1.65 ± 0.066 ab	1.43 ± 0.156 b	5.06	0.0119
3	2.72 ± 0.199 a	2.98 ± 0.137 a	2.84 ± 0.194 a	3.14 ± 0.333 a	0.61	0.6203
5	4.72 ± 0.582 a	4.92 ± 0.262 a	3.86 ± 0.527 a	2.56 ± 0.068 b	7.22	0.0028
7	8.45 ± 1.094 a	8.51 ± 0.415 a	7.41 ± 0.187 a	4.52 ± 0.511 b	9.97	0.0006
9	14 ± 1.196 a	15.37 ± 1.363 a	14.98 ± 0.778 a	9.08 ± 0.281 b	9.39	0.0008
11	18.12 ± 0.348 a	18.08 ± 0.915 a	17.95 ± 0.719 a	16.29 ± 0.877 a	1.45	0.2661
13	26.16 ± 1.313 a	27.61 ± 0.563 a	28.92 ± 0.766 a	23.27 ± 0.661 a	2.5	0.0965
15	26.72 ± 1.247 a	24.32 ± 1.621 a	26.73 ± 0.719 a	26.56 ± 0.818 a	1.09	0.3817

Means within a row followed by the same letter did not differ significantly ($P = 0.05$) using analysis of variance and Waller-Duncan K-ratio procedure.

Table 4-5. Mean (± SE) length (mm), width (mm) and weight (mg) for pre-pupal and pupal *Diaphania hyalinata* reared on leaf tissue of yellow squash, zucchini, cucumber, and watermelon in the laboratory.

Dimensions	Plant Species				$F_{3,36}$	P
	Yellow Squash	Zucchini	Cucumber	Watermelon		
Pre-pupa wt	0.07 ± 0.006 b	0.08 ± 0.005 ab	0.09 ± 0.007 a	0.1 ± 0.005 a	4.75	0.0069
Pupa wt	0.06 ± 0.005 a	0.07 ± 0.003 a	0.06 ± 0.003 a	0.07 ± 0.005 a	1	0.4024
Pupa length	16.6 ± 0.559 a	17.24 ± 0.47 a	17.34 ± 0.279 a	18.06 ± 0.333 a	1.95	0.1392
Pupa width	3.83 ± 0.235 a	3.77 ± 0.093 a	3.71 ± 0.083 a	3.81 ± 0.073 a	0.12	0.947

Means within a row followed by the same letter did not differ significantly ($P = 0.05$) using analysis of variance and Waller-Duncan *K*-ratio procedure.

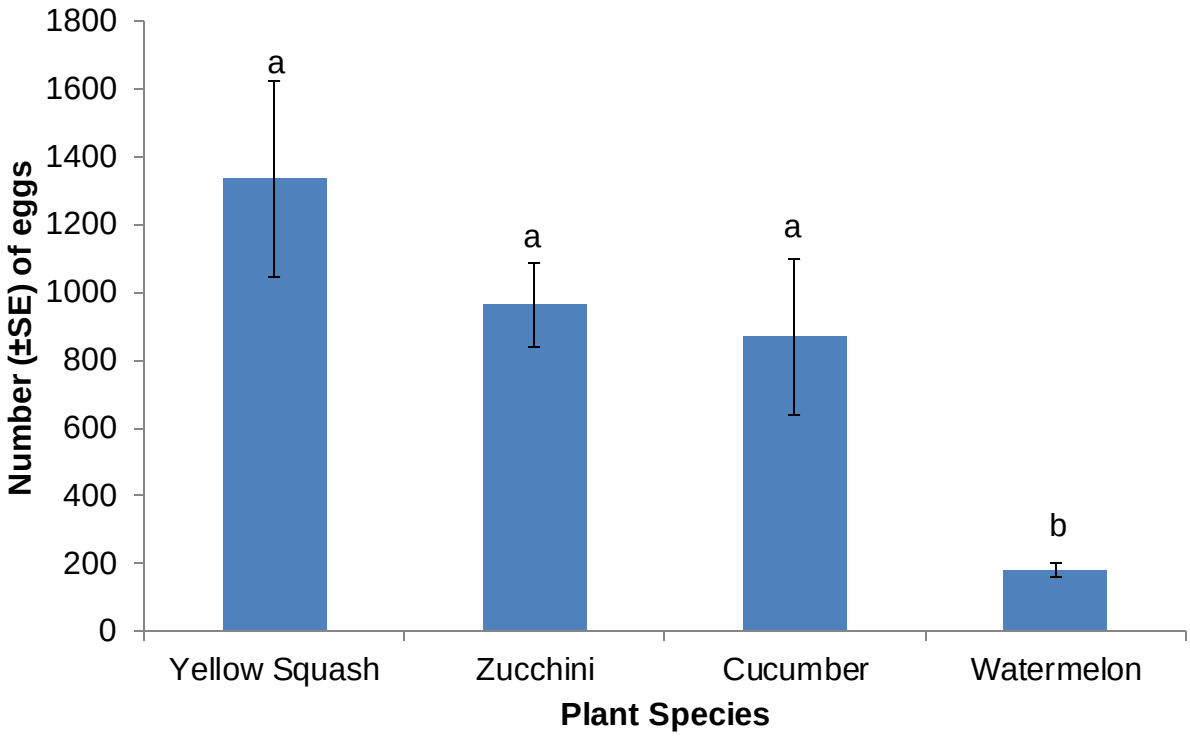

Figure 4-1. Mean oviposition (±SE) of *Diaphania hyalinata* on plants of yellow squash, zucchini, cucumber, and watermelon. Means sharing the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error.

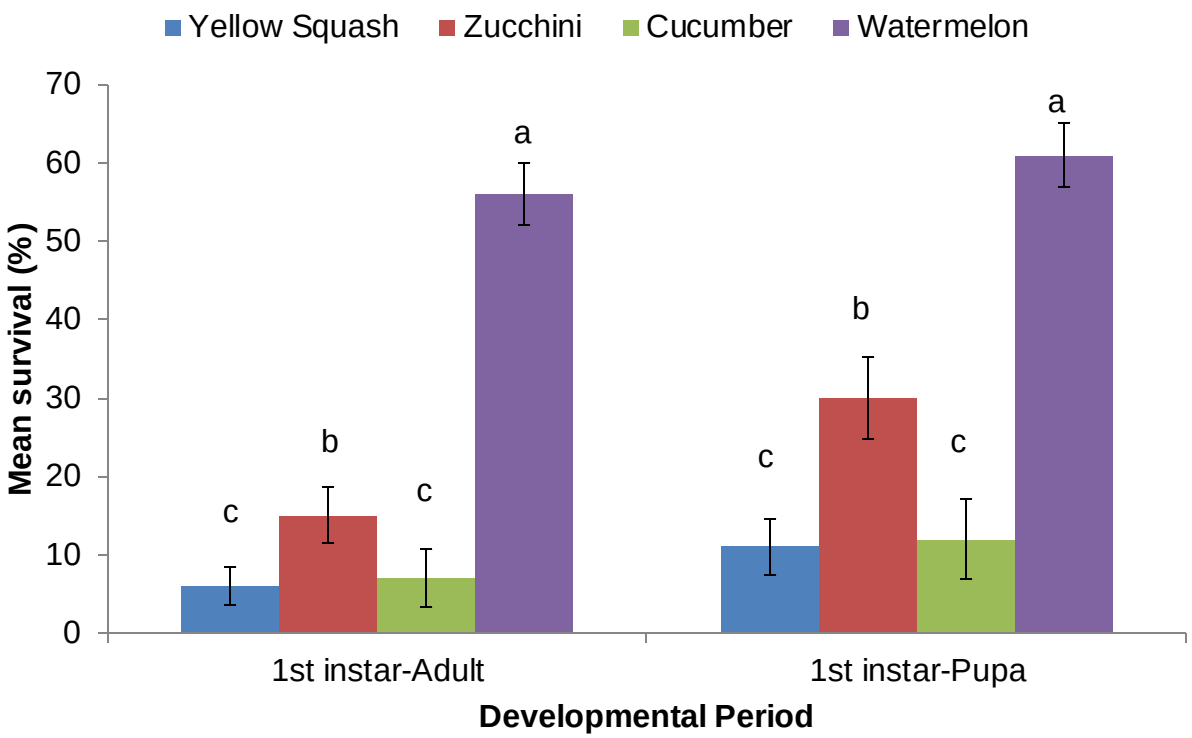

Figure 4-2. Mean (± SE) percentage survival of larvae of *Diaphania hyalinata* reared on leaf tissue of yellow squash, zucchini, cucumber, and watermelon. Means with the same letters did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error.

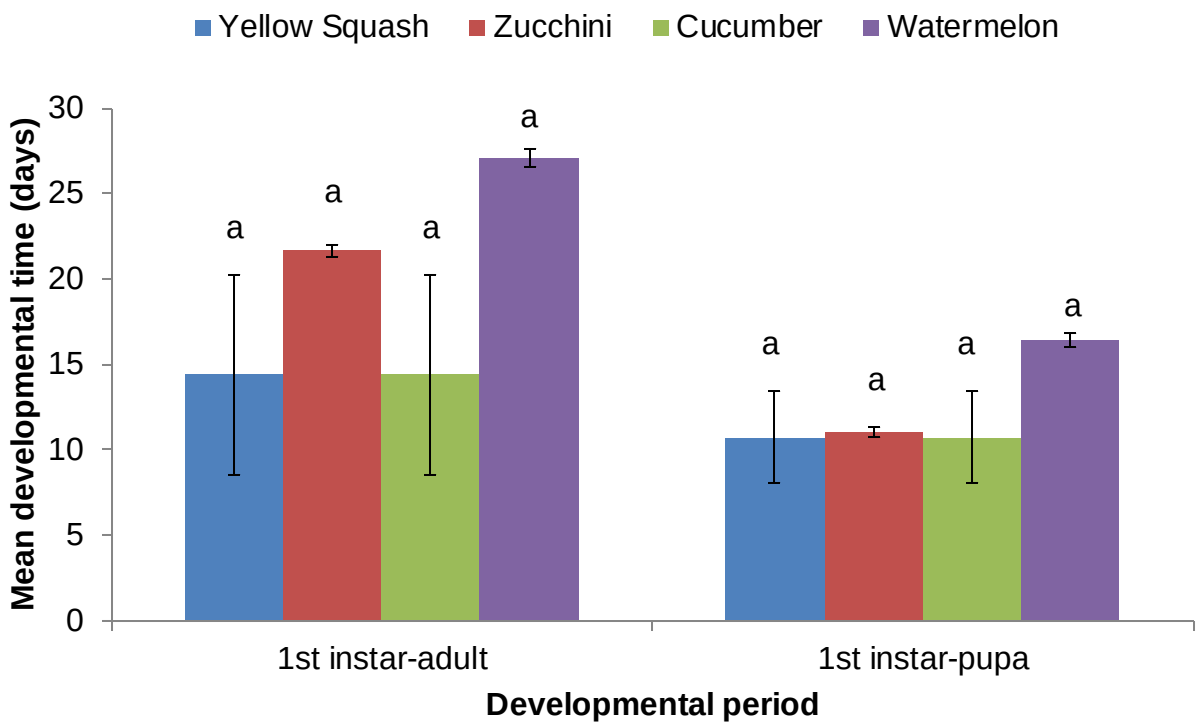

Figure 4-3. Mean (±SE) developmental time of *Diaphania hyalinata* larvae comparing plant species when larvae were reared on leaf tissue of yellow squash, zucchini, cucumber, and watermelon. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error.

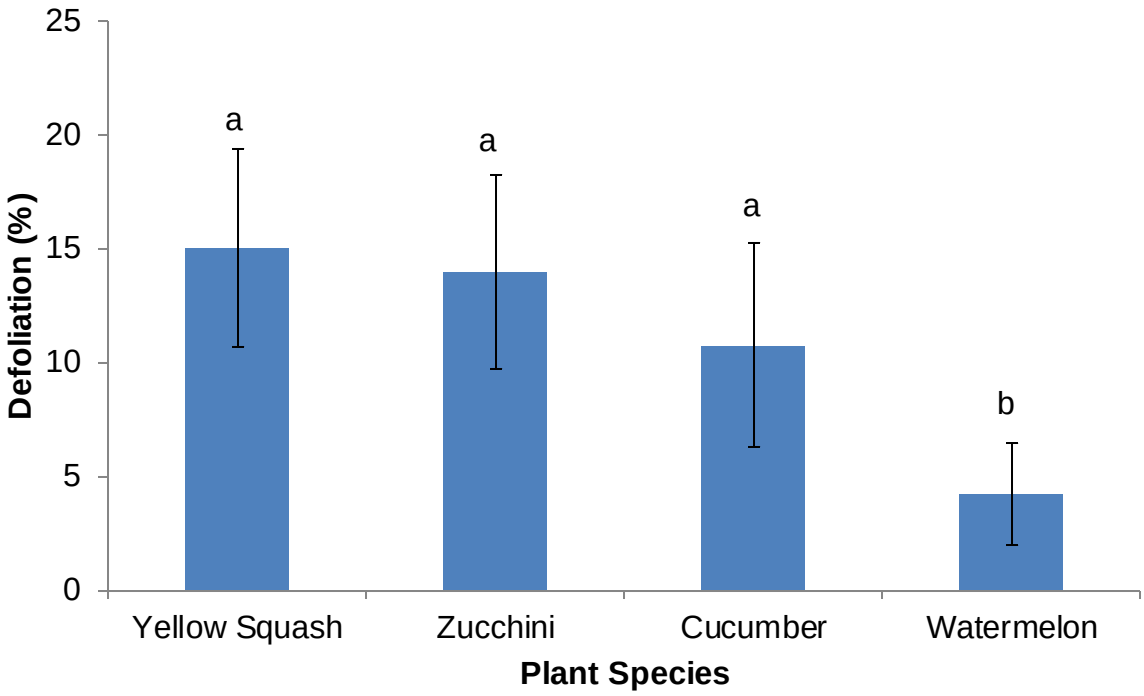

Figure 4-4. Mean (± SE) percentage defoliation by larvae of *Diaphania hyalinata* on leaf tissues of yellow squash, zucchini, cucumber, and watermelon in a free-choice test. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error.

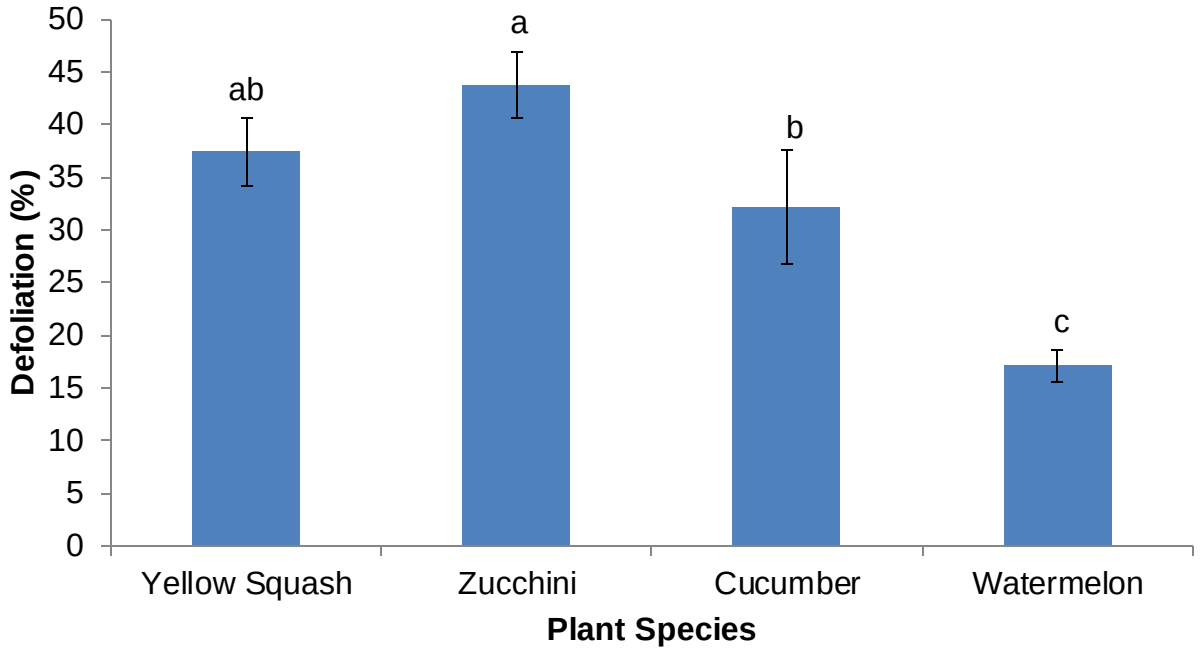

Figure 4-5. Mean (± SE) percentage defoliation of leaf tissue of yellow squash, zucchini, cucumber, and watermelon by larvae of *Diaphania hyalinata* in a no-choice test. Means with the same letter did not differ significantly ($P = 0.05$, Waller-Duncan K-ratio procedure). Bars above and below means represent standard error.

CHAPTER 5
CONCLUSIONS

Melonworm, *Diaphania hyalinata* L., is a serious tropical pest of Cucurbitaceae throughout the southeastern United States (Fulton 1947, Dupree et al.1955). They are foliage feeders on plants in the Cucurbitaceae, or cucurbits, and cause huge annual economic losses to crops in this family. Although management practices including chemical control, biological control, and culture practices have been established to control this pest, growers mainly use control with insecticides. Maximum use of insecticide often causes the development of insecticide resistance by target insects and negatively affects their natural enemies. An area with large acreage of cucurbit crops is southern Florida, where melonworms have become a serious problem causing major economic losses to cucurbits. To develop effective management strategies against melonworms, several studies have been conducted to assess seasonal abundance, spatial distribution, growth response, host-plant preference, and within-plant distribution of melonworms on four cucurbit crops.

The abundance of *D. hyalinata* varied seasonally with greater density in the warmer months than in the cooler months. Mean numbers of small larvae were greater than medium and large larvae on the average for the four crop seasons. Mean melonworm densities were affected by fluctuations of temperature, but not rainfall. *Diaphania hyalinata* exhibited aggregated distributions in the cucurbit field when mean densities were at their peak. However, when populations were depressed, as in the December study, *D. hylaninata* exhibited a uniform distribution. Results of this study can help in planning a program to monitor melonworm population densities throughout the year and in determining within-field distribution patterns.

Adult females deposited more eggs on yellow squash, zucchini, and cucumber, than on watermelon under both laboratory and field conditions. Overall, yellow squash was the most-preferred host and watermelon was least preferred. *Diaphania hyalinata* larvae preferred yellow squash most, followed by zucchini, then cucumber, with watermelon least preferred. Small *D. hyalinata* larvae were more common than medium and large larvae. Small larvae were most common at the bottom plant part. In free-choice tests under laboratory and field conditions, percentage defoliation was largest on yellow squash, followed by zucchini, then cucumber, and was lowest on watermelon. In no-choice tests, defoliation percentages were largest on zucchini, followed by yellow squash, then cucumber, with watermelon least preferred. In either choice or no-choice tests, watermelon was least defoliated by *D. hyalinata* larvae. Using these results, we were able to determine the most preferred and least preferred hosts, the plant location most populated by larval sizes, and the mean densities based on *D. hyalinata* larval sizes. The results can help in developing management strategies against melonworm in different cucurbit crops and also in determining on-plant and within-field locations for the most accurate sampling of melonworm larvae.

Despite yellow squash appearing as the most preferred host and watermelon least preferred, survival rates were lowest on yellow squash and greatest on watermelon. Although there were contrasting results in survival rates of melonworm larvae, there weren't any differences in development times among the four host-plant species. When examining the development of individual instars, larvae required more time to develop on watermelon than on the other crops. Rearing larvae on watermelons resulted in smaller head capsule widths for a given instar than yellow squash. The other

variables including whole body length, pupal weight, and pupal dimensions showed little or no differences among melonworms reared on the four host plant species. Preferences and growth responses of *D. hyalinata* were determined using different parameters such as egg deposition, survival, development time, head capsule width, whole-body length, pre-pupal weight, pupal weight and pupal body dimensions. These results provide basic information on the biology of *D. hyalinata* on different crops of cucurbits, which is useful for further research on the crop pest.

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BIOGRAPHICAL SKETCH

Babu Ram Panthi was born in Kanchanpur, Nepal in 1988. He graduated from Institute of Agriculture and Animal Sciences, Tribhuvan University, Nepal with a Bachelor of Science in agriculture in 2011. He volunteered as a research assistant at Nepal Agricultural Research Council, Nepal for a brief period in the entomology department. In May 2013, he joined the Department of Entomology and Nematology at the University of Florida as a master's student under the supervision of Dr. Dakshina R. Seal. He worked in Dr. Seal's lab at Tropical Research and Education Center, Homestead, Florida as graduate student, and graduated with a Master of Science in 2015. He studied biology and population dynamics of melonworm, *Diaphania hyalinata* L. (Lepidoptera: Crambidae), a serious pest of cucurbits in southern Florida.