Seasonal Abundance and Spatial Distribution of Diaphania hyalinata (Lepidoptera: Crambidae) on Yellow Squash in South Florida

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Seasonal abundance and spatial distribution of *Diaphania hyalinata* (Lepidoptera: Crambidae) on yellow squash in south Florida

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### Abstract

Seasonal abundance and spatial distribution of melonworm, *Diaphania hyalinata* L. (Lepidoptera: Crambidae), on yellow squash were studied during 4 crop-growing periods in 2014 in Homestead, Florida. The abundance of *D. hyalinata* larvae ranged from a minimum during Dec 2014 (1.3 ± 0.0 larvae per 2 leaves) when temperatures were relatively low (15–20 °C), to a maximum in Sep 2014 (6.6 ± 0.1 larvae per 2 leaves) when temperatures were relatively high (24–26 °C). The abundance of small larvae (L1 + L2) was relatively greater, with a maximum of 7.1 ± 0.3 larvae per 2 leaves, as compared with medium-sized larvae (L3 + L4) (2.4 ± 0.1 per 2 leaves) and large larvae (L5) (2.4 ± 0.1 larvae per 2 leaves) throughout the year. The abundance of large larvae was consistently low over the entire year. *Diaphania hyalinata* distributions tended to be aggregated (b > 1) during the crop-growing periods during May, Jun–Jul, and Sep 2014, when the population densities were relatively high, but were uniform (b < 1) during Dec 2014, when the population densities were low. A weak but statistically significant positive linear relationship existed between temperature and larval abundance. The results from this study will help squash and cucurbit growers of south Florida in monitoring melonworm infestations in the field and in developing a knowledge-based management program.

### Keywords

- melonworm
- distribution pattern
- Taylor’s power law

The melonworm, *Diaphania hyalinata* L. (Lepidoptera: Crambidae), is a serious pest in the southeastern United States where it feeds on cucurbit foliage (Fulton 1947; Dupree et al. 1955). The distribution of melonworm ranges from the Gulf Coast states to the Carolinas and (on occasion) to northern states and west to Oklahoma and Nebraska (Reid et al. 1954; Reid & Cuthbert 1956; Zehnder 2011). The early instars of melonworm feed only on foliage of cucurbits. The late instars (3rd–5th instars) with wider head capsule are more voracious in nature than the early instars with narrower head capsule (Panthi et al. 2016) and can feed on the entire plant including fruit, leaves, stalks, and vines, often leaving only veins and veinlets of leaf tissue (Valles & Capinera 1992). Melonworm can cause serious damage to its host crops by significantly reducing yields (Guillaume & Boissot 2001). In southern Florida, investigators documented 23% indirect yield loss through foliage feeding (McSorley & Waddill 1982), and about 9 to 10% yield reduction through direct loss by damage to fruits (Capinera 2005).

The adults of melonworm most prefer yellow squash, zucchini, and cucumber for oviposition relative to other cucurbits, resulting in more larval feeding damage on these preferred crops (Panthi et al. 2016). To achieve effective control of melonworm, appropriate control methods need to be applied at the correct time. Thus, knowledge about the biology of melonworm is the key to a successful management program. Knowledge of seasonal abundance helps to determine when melonworms are likely to appear in the crop and reach action threshold levels. The abundance of these insects and their development stages may be associated with different phenological stages of a host crop.
However, despite the economic damage this pest has inflicted on the cucurbit production industry, information on seasonal abundance and spatial distribution of this pest is lacking for the southern Florida agroecosystem.

Variations in seasonal and annual abundance have been reported for many tropical lepidopterans (Frith & Frith 1985; Braby 1995). Temperature is an important environmental factor that regulates various biological parameters of insects and has a direct effect on their abundance (Elsey 1982a; Ju et al. 2011). Peña et al. (1987) reported that, in Florida, the larval population densities of pickleworm, a closely related Diaphania species, were generally low during extremely hot summers and cold winters, and that the population densities peaked during fall. The fluctuation in seasonal abundance of pickleworm was reported to be due to changes in temperature (Peña et al. 1987), and Elsey (1982b) had further supported this effect in laboratory studies.

Knowledge of insect spatial distribution is important for developing sampling methods to understand population abundance in time and space (Brewer & Story 1987). Within a population, individuals are distributed in measurable patterns. Patterns of distribution are usually categorized as clumped, random, or uniform (Southwood 1978a). Spatial distribution of an insect is affected by various environmental factors such as food, temperature, habitat condition, and other biotic and abiotic factors. Among the most important factors affecting the spatial distribution is the density of insects in the field. Often, insects are randomly distributed at low population densities and aggregated at high population densities in the field.

Information on spatial distribution of pest insects can be used in estimating the number of samples required from an area to reliably estimate pest infestation levels to develop effective management programs. Thus, considering the lack of information on distribution patterns of melonworm in south Florida vegetable production, we determined the within-field distribution of this pest to help develop a knowledge-based management program. This information could help to minimize inappropriate use of insecticides in the field. The specific objectives of this study were to determine i) the seasonal abundance of melonworm and ii) its spatial distribution in field-planted yellow squash.

Materials and Methods

All studies were conducted in the research fields of the Tropical Research and Education Center, University of Florida, Homestead, Florida (25.50°N, 80.49°W). Studies were conducted in 2014 using 4 plantings (cropping seasons) of yellow squash at different sites. The plantings were established on 6 May, 28 Jun, 11 Aug, and 18 Nov 2014. The soil type of all field plots was Krome gravelly loam (loamy skeletal, calcic, hyperthermic Lithic Udorthents) that consisted of 33% soil and 67% pebbles >2 mm (Li 2001; Nobel et al. 1996). Yellow squash was divided into eight 11.5 m plots; hence, there were 48 plots. Each of the 4 cropping seasons.

Spatial distribution of melonworm was studied in the same field that was used for seasonal abundance. Plot design, sample collection, and sample preparation were the same as described in the previous section. Spatial distribution of melonworm was determined using 2 plot sizes to represent small and medium-sized areas: 10 m² (48 plots) and 40 m² (12 plots). Analyses were carried out on each sampling date from each of the 4 cropping seasons.

SEASONAL ABUNDANCE OF MELONWORM

The abundance of melonworm was studied separately in each of 4 plantings to determine the time of population increase in each planting. Data from all plantings were then considered together to determine peak abundance of the melonworm population in yellow squash along all sampling dates of the 4 plantings. To obtain information on population abundance, sampling for melonworm was initiated (26 May, 18 Jul, 1 Sep, and 9 Dec) 2 wk after germination of yellow squash seeds in each planting and continued 4 times at weekly intervals. Five intact plants per plot were randomly selected each week, and 2 leaves from each plant (10 leaves per plot) were collected. Thus, for the entire 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 bags and labeled with the plot number, sample number, and sampling date. Immediately after collection, all samples were transported to the Integrated Pest Management Laboratory at the Tropical Research and Education Center, Homestead, and the number of larvae from each sample was recorded. To understand the age composition of larvae in each sample, they were visually divided into small (1st instar or L1, and 2nd instar or L2), medium (3rd instar or L3, and 4th instar or L4), and large (5th instar or L5) based on the size and color.

STATISTICAL ANALYSES

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 using SAS® software; SAS Institute Inc. 2013). PROC MIXED was used due to the potential covariance structure associated with taking repeated measures over time on the same plots of plants. Season, larval size, and their interaction were analyzed for the entire experiment.

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Sample date was substituted for the season in the analysis by season. Post hoc means separation (Waller–Duncan K-ratio test [α < 0.05] using SAS® software; SAS Institute Inc. 2013) was used for variables when ANOVA indicated a significant effect of the variable on the model. Regression analyses (PROC REG using SAS® software; SAS Institute Inc. 2013) were performed to determine relationships of larval abundance with temperature and rainfall. For presentation purposes, mean values were used in the figures.

To assess melonworm spatial distributions, an index of dispersion was calculated (Southwood 1978b) using Taylor’s power law ($b$) (Taylor 1961). In this model, when the slope ($b$) value is not significantly different from 1, it indicates a random distribution pattern; when the slope is significantly $>1$, it indicates an aggregated distribution pattern; and when the slope is significantly $<1$, it indicates a uniform distribution pattern ($P < 0.05$). Taylor’s power law index of dispersion (Equation 1) was calculated using the general linear regression model (Southwood 1978a; SAS Institute Inc. 2013). Taylor’s power law determines relationships between the mean density of larvae (log $\bar{x}$) and variance (log $s^2$), and sampling factor log $a$ (Equation 1).

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

To determine the within-field distributions for *D. hyalinata* using Taylor’s index ($b$), we first determined the goodness of fit of data to a linear model using regression coefficients ($r^2$) from each field test. Then a Student’s $t$-test ($P < 0.05$) was used to determine if the slope ($b$) was significantly different from 1. Taylor’s index ($b$) can be checked to determine regression values ($r^2$), which indicate the reliability of the test value.

### Results

#### Seasonal Abundance

Abundances of small (L1 + L2), medium (L3 + L4), and large (L5) melonworm larvae were significantly affected by crop season ($F_{3, 2,292} = 354.75; P < 0.001$), larval sizes ($F_{2, 2,292} = 512.70; P < 0.001$), and interaction of crop seasons and larval sizes ($F_{6, 2,292} = 96.39; P < 0.001$). Therefore, the data were divided for further analysis by the 4 crop seasons tested in the field.

#### Abundance of Larvae by Size and Sampling Dates Within and Between the Four Plantings

Abundances of small (L1 + L2), medium (L3 + L4), and large (L5) melonworm larvae during the 1st planting (6 May) showed significant differences among sampling dates from 26 May to 16 Jun ($F_{3, 564} = 4.19; P = 0.006$), larval sizes ($F_{2, 564} = 264.45; P < 0.001$), and interaction of sampling dates and larval sizes ($F_{6, 564} = 9.80; P < 0.001$) (Fig. 1a). The number of small larvae dipped significantly 3 wk (mean ± SE: 1.2 ± 0.2 larvae per 2 leaves) and 4 wk (1.3 ± 0.2 larvae per 2 leaves) after germination, but then rebounded to the season high (2.2 ± 0.1 larvae per 2 leaves) 5 wk after germination. However, the numbers of both medium...
and large larvae reached peaks of 0.7 ± 0.1 and 0.3 ± 0.0 larvae per 2 leaves, respectively, 3 wk after germination, declining significantly 5 wk after germination for large larvae. The numbers of small larvae were significantly greater across all the sampling dates, compared with the numbers of medium and large larvae.

Abundances of small, medium, and large melonworm larvae during the 2nd planting (28 Jun) were significantly different among sampling dates from 18 Jul to 8 Aug \( F_{1,144} = 113.75; P < 0.001 \), larval sizes \( F_{1,144} = 40.58; P < 0.001 \), and interaction of sampling dates and larval sizes \( F_{1,144} = 16.35; P < 0.001 \) (Fig. 1b). Small larvae reached their peak (2.0 ± 0.1 larvae per 2 leaves) 5 wk after germination. However, medium (1.0 ± 0.1 per 2 leaves) and large larvae (2.4 ± 0.1 larvae per 2 leaves) reached their peaks 4 and 5 wk after germination, respectively. The numbers of small larvae were greater than those of medium and large larvae 2 and 3 wk after germination (18 and 25 Jun), but results did not differ significantly among larval sizes after 4 wk (1 Aug). Five wk after germination (8 Aug), the number of large larvae was significantly greater across all the sampling dates as compared with the other larval sizes.

The abundance of small, medium, and large melonworm larvae during the 3rd planting (11 Aug) was significantly affected by sampling dates from 1 to 22 Sep \( F_{1,144} = 60.88; P < 0.001 \), larval sizes \( F_{1,144} = 645.62; P < 0.001 \), and interaction of sampling dates and larval sizes \( F_{1,144} = 66.42; P < 0.001 \) (Fig. 1c). The number of small larvae 2 wk after germination (4.3 ± 0.2 per 2 leaves; Fig. 1c) was twice as large in the 3rd planting as the other 3 plantings (max. 1.5 ± 0.1 larvae per 2 leaves; Fig. 1a, b, d). The numbers of small and medium larvae were significantly greater (7.1 ± 0.3 and 2.4 ± 0.1 larvae per 2 leaves, respectively) 3 wk after germination than on the other sampling dates during the 3rd planting. The number of large larvae reached its peak (1.5 ± 0.1 per 2 leaves) 5 wk after germination. The number of small larvae was significantly greater across all the sampling dates as compared with the other larval sizes.

The abundance of small, medium, and large melonworm larvae during the 4th planting (18 Nov) varied significantly among sampling dates from 9 to 30 December \( F_{1,144} = 18.74; P < 0.001 \), larval sizes \( F_{1,144} = 127.31; P < 0.001 \), and interaction of sampling dates and larval sizes \( F_{1,144} = 20.10; P < 0.001 \) (Fig. 1d). The mean number of small larvae was greatest (1.1 ± 0.1 larvae per 2 leaves) on the 1st sampling date (2 wk after germination). In the same planting, mean numbers of medium and large larvae (0.6 ± 0.1 and 0.3 ± 0.0 larvae per 2 leaves, respectively) peaked 3 and 4 wk after germination, respectively. The numbers of small larvae were significantly greater across all the sampling dates as compared with the other larval sizes.

The number of small larvae was significantly greater across all the sampling dates, compared with the other larval sizes.

ABUNDANCE OF TOTAL MELONWORM LARVAE ACROSS SAMPLING DATES IN FOUR INDIVIDUAL CROP SEASONS

The abundance of total melonworm larvae was significantly affected by crop seasons (May—Jun: \( F_{3,34} = 5.41; P = 0.001 \); Jul—Aug: \( F_{3,34} = 160.95; P < 0.001 \); Sep: \( F_{3,34} = 53.22; P < 0.001 \); and Dec: \( F_{3,34} = 18.31; P < 0.001 \)). During the May—Jun and July—Aug crop seasons, the numbers of larvae reached peaks (2.9 ± 0.2 and 5.3 ± 0.2 larvae per 2 leaves, respectively) 5 wk after germination (Fig. 2). During the Sep and Dec crop seasons, the number of larvae peaked (9.9 ± 0.3 and 1.6 ± 0.1/2 leaves, respectively) 3 wk after germination (Fig. 2). However, in the Dec crop season, the peak did not differ significantly from the number of larvae 2 wk after germination, and then significantly declined on the following 2 sampling dates to the lowest abundance of the year (Fig. 2).

TEMPERATURE AND RAINFALL EFFECTS ON LARVAL ABUNDANCE

A weak but statistically significant positive linear relationship \( r = 0.48, P = 0.05 \); \( r^2 = 0.23, P = 0.05 \) existed between temperature and larval abundance. During the study, the temperature varied within a very small range (23–29 °C) during the first 3 plantings (26 May—22 Sep) (Fig. 3). A sharp drop in temperature to 16 °C was observed on 9 Dec, after which the temperature increased to 21 °C on 23 Dec. The abundance of melonworm larvae fluctuated regardless of temperature during the first 3 plantings (26 May—22 Sep), but it decreased markedly following the sharp drop in temperature during the 4th planting (9–30 Dec). Rainfall varied from 0 to 18.3 mm/d, but average rainfall per d had no significant effect on melonworm larval abundance \( r = −0.0, P = 0.94; r^2 = 0.0004, P = 0.94 \) (Fig. 3).

SPATIAL DISTRIBUTION

Melonworm larvae predominantly showed an aggregated pattern of distribution (slope value significantly >1) irrespective of planting time, sampling date, larval size, and plot size (Table 1). Out of 16 sampling dates in 4 plantings, small larvae (L1 + L2) were distributed in an aggregated pattern on 11 sampling dates and in a uniform pattern (b
random distribution on 1 sampling date in 10 m² on 10 sampling dates, a uniform distribution on 5 sampling dates, and a distribution of small larvae did not vary (aggregated distribution on 13 sampling dates, the distribution of large larvae was uniform. When the distribution across all larval sizes was considered, melonworm larvae were distributed in an aggregated pattern on 11 and 15 dates, the distribution of large larvae was uniform. The distribution patterns of medium (L3 + L4) and large larvae (L5) were similar to those of small larvae on all sampling dates of 4 plantings in both plot sizes. Medium larvae showed an aggregated distribution on 10 sampling dates, a uniform distribution on 5 sampling dates, and a random distribution on 1 sampling date in 10 m² plot; in the larger plot size (40 m²), medium larvae showed an aggregated distribution on 14 sampling dates and a uniform distribution on 2 sampling dates. Large larvae showed an aggregated distribution on 9 and 10 sampling dates in 10 and 40 m² plots, respectively; on the remainder of the sampling dates, the distribution of large larvae was uniform.

When the distribution across all larval sizes was considered, melonworm larvae were distributed in an aggregated pattern on 11 and 15 sampling dates in the 10 and 40 m² plots, respectively. Overall, the spatial pattern of distribution of melonworms was aggregated irrespective of larval sizes. Occasionally, a small population size led to a uniform pattern of distribution. Plantings, sampling dates, and plot sizes did not have any marked effect on the distribution pattern of melonworm larvae.

### Discussion

The abundance of melonworm larvae gradually increased from the 1st planting of yellow squash in May and peaked during the 3rd planting in Sep. The population then began to decline after the middle of the 3rd planting during Sep and was smallest during the end of the 4th planting during Dec 2014. The dramatic drop in temperature in mid-Dec, concurrent with a similar great drop in the number of larvae, suggests the importance of warm weather for a population increase of *Diaphania hyalinata* in yellow squash. The population of small size larvae (L1 + L2) was larger compared with larvae of other sizes throughout the year. The abundance of large size larvae (L5) was consistently low during the whole crop season except for a few sampling dates. The abundance of melonworm larvae in the 1st (May–Jun) and 2nd (Jul–Aug) seasons showed an increasing trend and that in the 3rd (Sep) and 4th (Dec) seasons showed a decreasing trend over time (Fig. 1). This decrease in abundance is due to mortality associated with biotic and abiotic environmental factors, presumably due to the activities of natural enemies (*Orius insidiosus* [Say] [Hemiptera: Anthocoridae], *Zelus longipes* [L.] [Hemiptera: Reduviidae], *Solenopsis invicta* Buren [Hymenoptera: Formicidae], microbial pathogens), degrading quality of hosts, and fluctuating patterns of weather.

During the study, the abundance of small size larvae was almost consistently greater in each season than that of medium and large size larvae. Though the populations of medium and large size larvae were smaller than the populations of small size larvae, the level of damage was high, because larger larvae consumed more leaf matter. Thus, despite the difference in numbers, each larval population has the potential to cause economic damage.

Fluctuation in the population levels of insects over the season has been reported by many authors (Wallner 1987; Novotný & Basset 1998; Zanuncio et al. 2001; Kakkar et al. 2011; Seal et al. 2013; Kumar et al. 2014). Weather changes such as fluctuations in temperature, rainfall, and relative humidity may have a direct or indirect effect on the abundance of insects (Zanuncio et al. 2001). In the current study, the abundance of melonworm larvae appeared to be affected by temperature, with populations dropping dramatically with the decrease in mean air temperature. During summer, when the temperatures were high and constant in the first 3 planting seasons, the melonworm population was mostly steady, peaking at the end of the 3rd crop season; however, the population level decreased in the 4th season (Fig. 3). This decrease was preceded by the decrease in the mean air temperature from about 27 to 16 °C during the Dec 2014 planting. Effects of temperature on the population abundance and growth of other insects were reported by various authors (Liu et al. 2002; Aysal & Kvan 2008; Al-Digair et al. 2012). For example, Liu et al. (2002) reported that the survival of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) decreased rapidly outside the temperature range of 8 to 28 °C.

The general pattern of distribution of melonworm larvae was aggregated. When the distribution patterns of melonworm in 4 crop-growing periods were compared, the distribution was aggregated during the first 3 plantings, when the populations were large and their

### Table 1. Index of dispersion (b) using Taylor’s power law for the distribution of *Diaphania hyalinata* larvae sampled in 10 m² and 40 m² plots during 4 crop seasons.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Small size larvae (L1 + L2)</th>
<th>Medium size larvae (L3 + L4)</th>
<th>Large size larvae (L5)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 m²</td>
<td>40 m²</td>
<td>10 m²</td>
<td>40 m²</td>
</tr>
<tr>
<td>20-May</td>
<td>1.08</td>
<td>1.15</td>
<td>1.15</td>
<td>1.42</td>
</tr>
<tr>
<td>2-Jun</td>
<td>0.86</td>
<td>0.90</td>
<td>0.94</td>
<td>1.22</td>
</tr>
<tr>
<td>9-Jun</td>
<td>0.97</td>
<td>1.53</td>
<td>1.02</td>
<td>1.39</td>
</tr>
<tr>
<td>16-Jun</td>
<td>1.23</td>
<td>1.47</td>
<td>1.00</td>
<td>1.06</td>
</tr>
<tr>
<td>18-Jul</td>
<td>1.59</td>
<td>1.39</td>
<td>1.44</td>
<td>1.25</td>
</tr>
<tr>
<td>24-Jul</td>
<td>1.27</td>
<td>1.55</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td>30-Jul</td>
<td>1.39</td>
<td>1.53</td>
<td>1.17</td>
<td>1.20</td>
</tr>
<tr>
<td>7-Aug</td>
<td>1.34</td>
<td>1.27</td>
<td>0.80</td>
<td>0.83</td>
</tr>
<tr>
<td>1-Sep</td>
<td>2.18</td>
<td>3.28</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>8-Sep</td>
<td>2.09</td>
<td>1.57</td>
<td>1.03</td>
<td>1.13</td>
</tr>
<tr>
<td>15-Sep</td>
<td>1.84</td>
<td>2.12</td>
<td>1.35</td>
<td>1.79</td>
</tr>
<tr>
<td>22-Sep</td>
<td>0.92</td>
<td>0.12</td>
<td>1.19</td>
<td>1.39</td>
</tr>
<tr>
<td>9-Dec</td>
<td>1.05</td>
<td>1.09</td>
<td>1.45</td>
<td>1.25</td>
</tr>
<tr>
<td>6-Dec</td>
<td>1.10</td>
<td>1.29</td>
<td>1.13</td>
<td>1.55</td>
</tr>
<tr>
<td>23-Dec</td>
<td>0.99</td>
<td>1.15</td>
<td>0.83</td>
<td>1.02</td>
</tr>
<tr>
<td>30-Dec</td>
<td>0.63</td>
<td>0.88</td>
<td>0.91</td>
<td>1.03</td>
</tr>
</tbody>
</table>

AGG, aggregated distribution, *b* is significantly >1 (*P* ≤ 0.05); UNI, uniform distribution, *b* is significantly <1 (*P* ≤ 0.05); RAN, random distribution, *b* is not significantly different from 1 (*P* > 0.05).
densities high. However, in the present study the distribution pattern was uniform during the later part of the year, when the populations were small and their densities low. Southwood (1978b) reported that when the population density in an area declines, the chances of an individual occurring in any sample unit are so low that the distribution becomes random.

The distribution pattern of melonworm larvae was not significantly affected by plot size in this study, but much larger field sizes should be studied to evaluate population distributions in commercial-sized fields. Further studies should be conducted using larger plots (0.2 ha) to understand the abundance and distribution pattern of melonworm in multi-year and multi-season settings with variable weather patterns and different population sizes. Additional studies over years would help to determine the within- and between-year variations of pest abundance and distribution that may exist.

The results of the present study demonstrate that melonworm infestation can occur throughout the cucurbit growing period, with high abundance during the warm planting seasons (>20 °C). However, small size larvae were more abundant than the medium and large size larvae in all planting seasons, and were susceptible to natural mortality factors. Thus, attention should be paid to detect the initiation of infestation and apply, for example, Bacillus thuringiensis Berliner (Bacillaceae)—based products to achieve high mortality of small size larvae with significant reduction of overall melonworm populations (Capinera 2008). At times of high infestation, as observed in this study, application of B. thuringiensis based products can be adopted to manage melonworm. This approach of melonworm management will be environmentally and economically sustainable.

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