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## Ecology and Behavior

# Herbivore Damage and Prior Egg Deposition on Host Plants Influence the Oviposition of the Generalist Moth *Trichoplusia ni* (Lepidoptera: Noctuidae)

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### Abstract

Female insects have the difficult task of locating host plants that maximize the survival and success of their offspring. In this study, the oviposition preferences of the cabbage looper moth, *Trichoplusia ni* (Hübner), for soybean plants, *Glycine max* (L.), under various treatments—undamaged, mechanically damaged, damaged by *T. ni* or *Spodoptera frugiperda* (Smith) larvae or by *Bemisia tabaci* (Gennadius) adults, egg-free plants, and plants previously oviposited by conspecific or heterospecific females (*S. frugiperda*)—were investigated using two-choice tests. Additionally, the volatile compounds emitted by the plants under the different treatments were identified by gas chromatography–mass spectrometry. Our results showed that females showed no preferences for undamaged or mechanically damaged plants. However, they oviposited more often on undamaged plants than on those previously damaged by *T. ni, S. frugiperda*, or *B. tabaci.* In contrast, females preferred to oviposit on plants previously oviposited by conspecific and heterospecific females than on egg-free plants. Plants damaged by conspecific or heterospecific larvae emitted methyl salicylate, indole, and octyl butyrate, compounds not released by undamaged or mechanically damaged plants. Whitefly damage induced the release of higher quantities of Z(3)-hexenyl acetate, (R)-(+)-limonene, and (E)-β-ocimene compared to plants damaged by larvae and suppressed the emission of linalool. Egg deposition by conspecific and heterospecific moths induced the emission of (R)-(+)-limonene, octyl butyrate, and geranyl acetone but suppressed the release of linalool. This study showed that a generalist moth species can discriminate between plants of different quality, and suggests that females use volatile compounds as cues during this process.

**Key words:** *Glycine max, Spodoptera frugiperda, Bemisia tabaci*, host-finding behavior, volatile compound

Female holometabolous insects have the difficult task of locating host plants that ensure the survival and success of their offspring (Renwick 1989, Landolt and Phillips 1997, Thompson 1998, Bernays 2001, Gripenberg et al. 2010). During this search process, females are stimulated by external and internal factors that influence their choice and acceptance of host plants (Visser 1986, Bell 1990, Schoonhoven et al. 2005). Moreover, the degree of diet specialization of insects may affect the host-finding behavior of ovipositing females (Schäpers et al. 2015). A number of studies have shown that females with a broad diet breadth (generalists) have relatively more difficulty in discriminating between good and bad host plants (Levins and Macarthur 1969, Janz and Nylin 1997). This may occur because generalist insects have a limited neuronal capacity for processing sensorial information, making them less efficient in discriminating between host plants (Bernays 2001, Cunningham 2012).

During the host-finding process, females may use chemical cues (e.g., volatile and nonvolatile compounds) as well as physical cues (e.g., color, size) originating from both the habitat and from the plants to determine acceptable host plants (Renwick 1989, Schoonhoven et al. 2005).

The cabbage looper moth, *Trichoplusia ni* (Hübner), is considered a generalist species. Although it prefers cruciferous plants (Janmaat and Myers 2003), it can attack >160 species distributed across 36 plant families (Sutherland and Greene 1984). Relatively little is known concerning the host-finding behavior of this moth species (Khan et al. 1987; Landolt 1989, 1993, 2001; Landolt and Molina 1996). For instance, Khan et al. (1987) reported that females were attracted to extracts from a susceptible soybean variety, whereas extracts from a resistant variety repelled them. Landolt (1989) found that mated females were attracted to intact potted

cabbage, soybean, tomato, and celery plants in wind tunnel assays (Landolt 1989). Later, this same author reported that females were more attracted to mechanically damaged cotton plants or plants damaged by conspecific larvae than to undamaged plants, whereas cabbage plants that were mechanically damaged or infested by *T. ni* larvae were less attractive to females than undamaged plants (Landolt 1993).

In this work, we extend previous studies concerning the host-finding behavior of *T. ni*, focusing specifically on the oviposition behavior of this generalist moth. Specifically, using two-choice tests, we investigated whether *T. ni* females can discriminate between conspecific host plants with differences in quality. Undamaged soybean plants were considered as good quality hosts, while plants damaged by conspecific or heterospecific [*Spodoptera frugiperda* (Smith)] larvae, plants damaged by a sucking insect [*Bemisia tabaci* (Gennadius)], or plants previously oviposited by conspecific or heterospecific females (*S. frugiperda*) were considered as poor quality hosts. We hypothesized that females would not discriminate between the two categories of host plants. Additionally, we identified the compounds emitted by soybean plants from different treatments using gas chromatography–mass spectrometry.

## Materials and Methods

### Biological Material
Larvae of both moth species (*T. ni* and *S. frugiperda*) were reared with an artificial diet (Rojas et al. 2003). Insects were maintained at $26 \pm 2°C$, $70 \pm 5\%$ relative humidity, with a photoperiod of 14:10 (L:D) h. To obtain mated females of *T. ni* and *S. frugiperda*, 10 females and 15 males were kept together in cages (30 by 30 by 30 cm) from the first night of emergence. The moths were fed with a 10% sucrose solution dispensed on cotton wool in a Petri dish. After the assays, some females were dissected to determine their mating status by evaluating the presence of spermatophores in the bursa copulatrix. Five-day-old naïve females were used in all experiments. The whiteflies used to infest soybean plants were obtained from infested tomato plants. Some adults and nymphs were taken for taxonomic identification.

Soybean plants, *Glycine max* (L.) variety Cristalina, were used in the assays and for sampling the volatile compounds. Experimental seedlings were used at 15–20 d after planting, when they were ∼30 cm high (five to eight folioles). Single seeds were sown in plastic containers (12 cm height by 10 cm diameter) containing sterile soil under natural light conditions (12 h light:12 h darkness), temperature (24–34 °C), and relative humidity (60–70%). The plants were placed in screened cages to protect them from insect damage and were not fertilized.

### Bioassays
The assays were performed in pyramidal screen cages (75 by 60 by 60 cm) using two-choice tests. The cages were placed outdoors under a covered area with circulating air currents at a temperature of 24–30°C and a relative humidity of 60–70%. Two plants, one undamaged and one damaged or oviposited, were positioned at opposite corners of the cage, ∼40 cm apart. The position of each plant was randomly determined in each cage. The cages were separated from each other by 1 m. The trials began at 19:00 h. In each trial, a gravid moth was released on a platform 15 cm high placed at the entrance of the cage. The number of eggs deposited on the abaxial and adaxial surfaces of leaves were recorded 12 h later. Plants and females were used once and then discarded.

### Oviposition of *T. ni* Females on Undamaged Plants Versus Mechanically Damaged Plants
In this experiment, we investigated whether females are able to discriminate between undamaged plants and mechanically damaged plants. Plants with mechanical damage were obtained by creating three holes (6 mm diameter) per foliole with a paper hole punch to roughly simulate the amount of leaf that larvae might remove. The mechanical damage was initiated 1 h before beginning the assays. In total, 30 replicates were performed.

### Oviposition of *T. ni* Females on Undamaged Plants Versus Plants Damaged by Conspecific or Heterospecific Larvae
In this experiment, we evaluated whether females were able to discriminate between undamaged plants and plants damaged by *T. ni* or *S. frugiperda* larvae. Feeding damage was caused by placing five third-instar *T. ni* or *S. frugiperda* larvae on a plant for ∼12 h; during this time, the larvae consumed between 25–30% of the leaves. Afterward, the larvae were removed from the plant, and any remaining larvae feces were carefully removed with a soft bristle brush. Plants were used in the bioassays 12 h later. In total, 30 replicates were performed.

### Oviposition of *T. ni* Females on Undamaged Plants Versus Plants Damaged by a Sucking Insect
In this experiment, we investigated whether females were able to discriminate between undamaged plants and plants damaged by *B. tabaci*. Fifteen-day-old seedlings were infested with 20 whiteflies, and the plants were evaluated 20 d later. The infested plants carried eggs, nymphs, and ∼100 adults, all of which were removed with a soft bristle brush 8 h before beginning the assays. In total, 20 replicates were performed.

### Oviposition of *T. ni* Females on Egg-Free Plants and Plants Previously Oviposited by Conspecific or Heterospecific Females
In this experiment, we evaluated whether females were able to discriminate between egg-free plants and plants previously oviposited by *T. ni* or *S. frugiperda* females. To obtain oviposition-treated plants, individual plants were placed into collapsible cages (61 by 34 by 34 cm, Live Monarch Foundation, Boca Raton, FL) with one mated *T. ni* female or three mated *S. frugiperda* females. The next morning, the plants were checked, and the number of eggs (*T. ni*) or masses (*S. frugiperda*) and their locations on the leaves were recorded. For *T. ni*, the egg location was useful to differentiate the eggs oviposited during the assays from those deposited previously. Plants oviposited by conspecific females had ∼50 eggs, while plants oviposited by heterospecific females had at least one egg mass. Oviposited plants were withdrawn from the cages and used 12 h later. In total, 26 and 25 replicates per treatment were performed to evaluate the effect of previous oviposition by conspecific and heterospecific females on the behavior of *T. ni*, respectively.

### Sampling and Identification of Volatile Compounds
Plant volatile compounds were sampled using the solid-phase microextraction (SPME) technique. Individual plants were enclosed in a glass container (34 cm height by 9 cm diameter) with a hole cap, which was sealed with aluminum foil. Volatiles were sampled with a polydimethylsiloxane/divinylbenzene film (thickness 65 μm, Supelco, Toluca, Mexico). Sampling was performed by inserting the

SPME needle through the aluminum foil into the headspace of the glass container, and volatiles were allowed to be adsorbed onto the fiber for 12 h. The volatiles emitted by plants damaged by larvae or oviposited were sampled 24 h after beginning the feeding or oviposition. However, insects were removed from host plants 12 h after feeding or ovipositing. Plants infested with whiteflies were sampled after the eggs, nymphs, and adults were removed. Mechanically damaged plants were sampled about 1 h after the damage was caused. The fiber was subsequently removed from the vial, and the volatiles desorbed inside the heated injection port of a gas chromatograph for 1 min.

Chemical analysis of the volatiles adsorbed on the SPME fibers were conducted in a Varian CP-3800 Gas Chromatograph coupled with a Varian Saturn 2200 (GC-MS) Mass Spectrometer. A nonpolar fused silica capillary column (DB5-MS, 30 m by 0.25 mm; Supelco, Toluca, Mexico) was used. The analysis was performed using an initial temperature of 50°C (for 2 min), increasing by 15°C/min to 280°C (for 10 min). Helium was used as the carrier gas. The temperature of the injector was 200°C. Ionization was by electronic impact at 70 eV. The compounds were identified by comparing the Kovats indices and mass spectra of the synthetic standards. Other compounds were tentatively identified based on comparisons with spectra from the NIST/EPA/NIH Mass Spectral library (version 2.0, 2002) and those described by Adams (2007). The standards were purchased from Sigma-Aldrich (Toluca, Mexico) and were 97–99% pure according to the supplier.

### Statistical Analysis

The number of eggs on the different treatments were transformed $(\ln(x+0.5))$ to normalize variations in the data and analyzed with unequal variance Welch's t-test. In some cases, we used Mann–Whitney test because the data were still not normally distributed despite the transformation. GC peak area units of the compounds were analyzed using the canonical discriminant analysis (a multivariate analysis) technique, which allows differentiation between two or more groups and takes into account the performance measures of the individuals of each group. Also, GC peak area units were analyzed by analysis of variance (ANOVA) followed by Tukey post hoc comparisons ($\alpha = 0.05$) to evaluate the difference in the emissions of individual compounds among treatments. Data were transformed $(\ln(x+0.5))$ prior to ANOVA to satisfy assumptions of normality and homogeneity of variances. All analyses were performed using R software (version 2.15.3).

## Results

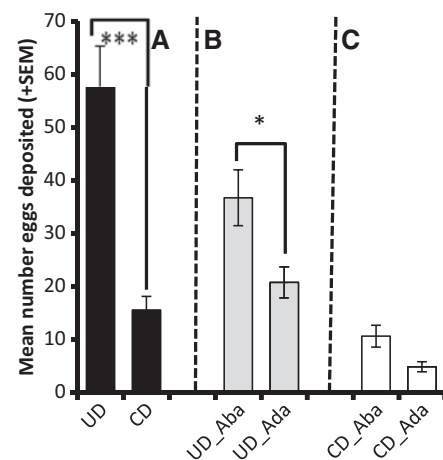### Oviposition of *T. ni* Females on Undamaged Plants Versus Mechanically Damaged Plants

Females showed no preference for undamaged or mechanically damaged plants ($t = -1.56$, df = 57, $P = 0.123$). However, moths oviposited more eggs on the abaxial surfaces than on the adaxial surfaces of leaves from undamaged plants ($t = 3.38$, df = 58, $P < 0.001$). In contrast, there was no difference in the number of eggs deposited on the abaxial and adaxial surfaces of leaves from mechanically damaged plants ($t = 1.69$, df = 55, $P = 0.096$; Fig. 1).

### Oviposition of *T. ni* Females on Undamaged Plants Versus Plants Damaged by Conspecific or Heterospecific Larvae

Moths preferred to oviposit on undamaged soybean plants rather than on plants damaged by *T. ni* larvae (W = 144.5, $P < 0.001$).
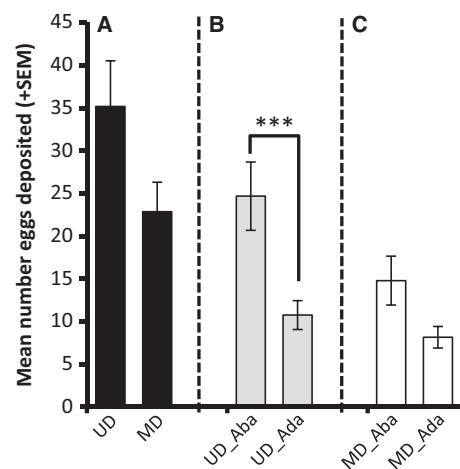


**Fig. 1.** Oviposition of *T. ni* females on undamaged soybean plants and mechanically damaged plants in two-choice tests. The data were log transformed $(\ln(x+0.5))$ and analyzed by Welch t test. (**A**) The mean number ($\pm$SEM) of eggs on undamaged plant and mechanically damaged plant, (**B**) eggs deposited on surfaces of undamaged plants, and (**C**) eggs deposited on surfaces of mechanically damaged plants. Data sets that are significant at different levels: ***($P < 0.001$). UD, undamaged plant; UD_Aba, abaxial surface of UD; UD_Ada, adaxial surface of UD; MD, mechanically damaged plant; MD_Aba, abaxial surface of MD; MD_Ada, adaxial surface of MD.



**Fig. 2.** Oviposition of *T. ni* females on undamaged soybean plants and damaged plants by conspecific larvae in two-choice tests. The data were log transformed $(\ln(x+0.5))$ and analyzed by Mann–Whitney test. (**A**) The mean number ($\pm$SEM) of eggs on undamaged plant and damaged plants by conspecific larvae, (**B**) eggs deposited on surfaces of undamaged plants, and (**C**) eggs deposited on surfaces of damaged plants by conspecific larvae. Data sets that are significant at different levels: *($P < 0.05$), ***($P < 0.001$). UD, undamaged plant; UD_Aba, abaxial surface of UD; UD_Ada, adaxial surface of UD; CD, plants damaged by conspecific; CD_Aba, abaxial surface of CD; CD_Ada, adaxial surface of CD.

Females oviposited more often on the abaxial surfaces than on the adaxial surfaces of leaves from undamaged plants (W = 604.5, $P = 0.02$), whereas there was no difference in the number of eggs oviposited on both surfaces of leaves on plants damaged by conspecific larvae (W = 575, $P = 0.06$; Fig. 2).

Similarly, females oviposited more often on undamaged soybean plants than on plants damaged by *S. frugiperda* larvae ($t = 4.17$, df = 57, $P < 0.001$; Fig. 3). However, in this case, moths deposited

more eggs on the abaxial surfaces than on the adaxial surfaces of leaves from both undamaged plants ($t = 2.57$, df $= 58$, $P = 0.013$) and plants damaged by heterospecific larvae ($t = 2.64$, df $= 56$, $P = 0.011$; Fig. 3B, C).
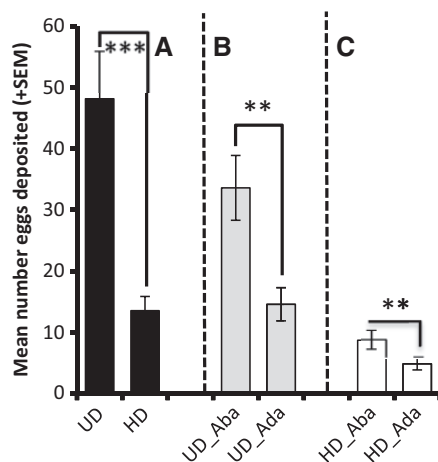
## Oviposition of *T. ni* Females on Undamaged Plants Versus Plants Damaged by a Sucking Insect

Females preferred to deposit more eggs on undamaged soybean plants than on plants infested by whiteflies ($t = 4.79$, df $= 38$, $P < 0.001$). More eggs were oviposited on the abaxial surfaces than on the adaxial surfaces of leaves from undamaged plants ($t = 4.12$, df $= 37$, $P < 0.001$), but females showed no preference for either surface of leaves on plants damaged by whiteflies ($t = -0.73$, df $= 38$, $P = 0.47$; Fig. 4).

## Oviposition of *T. ni* Females on Egg-Free Plants and Plants Previously Oviposited by Conspecific or Heterospecific Females

Moths deposited more often on soybean plants previously oviposited by conspecific females than on egg-free plants (W $= 163$, $P < 0.001$). Females showed no preference for ovipositing on either the abaxial or adaxial surfaces of leaves from egg-free plants ($t = 1.89$, df $= 49$, $P = 0.06$) or on plants previously oviposited by *T. ni* females ($t = 1.48$, df $= 50$, $P = 0.15$; Fig. 5).

Similarly, *T. ni* females preferred to oviposit on soybean plants previously oviposited by heterospecific females than on egg-free plants (W $= 163$, $P = 0.003$). However, females did not show a preference for ovipositing on either leaf surface from egg-free plants ($t = 0.41$, df $= 48$, $P = 0.68$). In contrast, moths oviposited more often on the abaxial surface than on the adaxial surface of leaves of plants previously oviposited by *S. frugiperda* females ($t = 2.81$, df $= 48$, $P = 0.007$; Fig. 6).

## Plant Volatile Compound Profiles

In total, 16 volatile compounds were identified from the different plant treatments. Undamaged soybean plants emitted only 7 compounds, while plants previously oviposited by conspecific or heterospecific females released 9 volatile compounds. Mechanically damaged plants emitted 13 compounds, and plants damaged by conspecific or heterospecific larvae emitted 14 compounds; plants damaged by whiteflies released 11 compounds (Table 1). The statistical analysis of the volatiles emitted by soybean plants showed a significant difference among treatments ($F = 3.34$; df $= 96,108$;
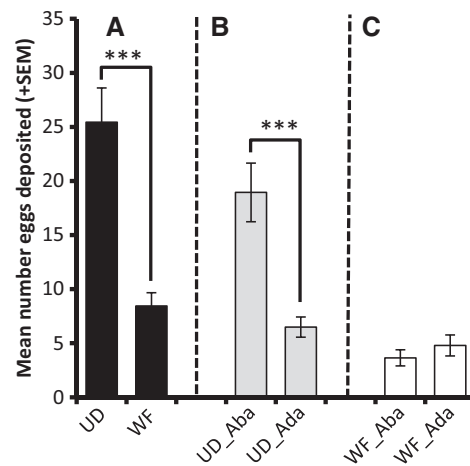
**Fig. 4.** Oviposition of *T. ni* females on undamaged soybean plants and damaged plants by whiteflies in two-choice tests. The data were log transformed ($\ln(x + 0.5)$) and analyzed by Welch *t* test. (**A**) The mean number ($\pm$SEM) of eggs on undamaged plant and damaged plants by whiteflies, (**B**) eggs deposited on surfaces of undamaged plants, and (**C**) eggs deposited on surfaces of damaged plants by whiteflies. Data sets that are significant at different levels: ***($P < 0.001$). UD, undamaged plant; UD_Aba, abaxial surface of UD; UD_Ada, adaxial surface of UD; WF, whitefly damage plant; WF_Aba, abaxial surface of WF; WF_Ada, adaxial surface of WF.
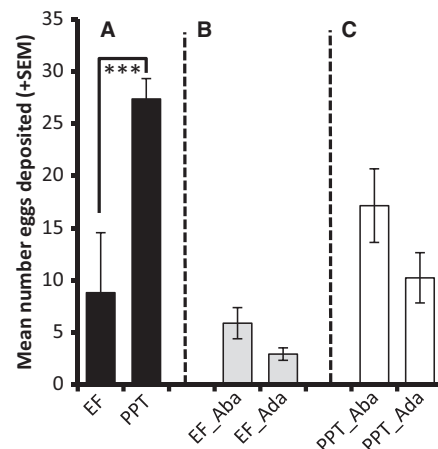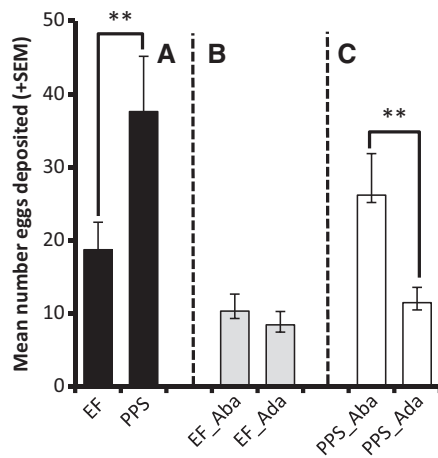
**Fig. 3.** Oviposition of *T. ni* females on undamaged soybean plants and damaged plants by heterospecific larvae in two-choice tests. The data were log transformed ($\ln(x + 0.5)$) and analyzed by Welch *t* test. (**A**) The mean number ($\pm$SEM) of eggs on undamaged plant and damaged plants by heterospecific larvae, (**B**) eggs deposited on surfaces of undamaged plants, and (**C**) eggs deposited on surfaces of damaged plants by heterospecific larvae. Data sets that are significant at different levels: **($P < 0.01$), ***($P < 0.001$). UD, undamaged plant; UD_Aba, abaxial surface of UD; UD_Ada, adaxial surface of UD; HD, plants damaged by heterospecific; HD_Aba, abaxial surface of HD; HD_Ada, adaxial surface of HD.

**Fig. 5.** Oviposition of *T. ni* females on egg-free soybean plants and previously oviposited plants by conspecific females in two-choice tests. The data were log transformed ($\ln(x + 0.5)$). (**A**) The mean number ($\pm$SEM) of eggs on egg-free plants and plants previously oviposited (Mann–Whitney test), (**B**) eggs deposited on surfaces of egg-free plants, and (**C**) eggs deposited on soybean plants previously oviposited. Data sets that are significant at different levels: ***($P < 0.001$). EF, egg-free plant; EF_Aba, abaxial surface of EF; EF_Ada, adaxial surface of EF; PPT, plant previously laid by *T. ni* females; PPT_Aba, abaxial surface of PPT; PPT_Ada, adaxial surface of PPT.

Fig. 6. Oviposition of *T. ni* females on egg-free soybean plants and previously oviposited plants by heterospecific females in two-choice tests. The data were log transformed (ln($x$ + 0.5)). (**A**) The mean number (±SEM) of eggs on egg-free plants and plants previously oviposited (Mann–Whitney test), (**B**) eggs deposited on surfaces of egg-free plants, and (**C**) eggs deposited on soybean plants previously oviposited. Data sets that are significant at different levels: **($P$ < 0.01$). EF, egg-free plant; EF_Aba, abaxial surface of EF; EF_Ada, adaxial surface of EF; PPS, plant previously laid by *S. frugiperda* females; PPS_Aba, abaxial surface of PPS; PPS_Ada, adaxial surface of PPS.

$P$ < 0.001). The canonical discriminant characteristic values of the testing data from the volatiles is shown in Table 2. Eigenvalues indicate that the first discriminant function has the highest canonical correlation (0.991) and explains *ca* 70% of the total variance. In accordance with these values, the first two functions are the most discriminating factors and provide a large contribution to the discrimination (86.57%). The Wilks' λ values of the first two discriminant functions are 0.000 and 0.00011, which indicate that the model has good discriminant power. In contrast, significance values less than 0.001 and $F$ tests indicate that there is a highly significant difference between the groups' centroids (Fig. 7).

Undamaged soybean plants, mechanically damaged plants, and previously oviposited plants clustered together in the first group, with decanal as the most important compound. A second group included plants damaged by conspecific and heterospecific larvae, with methyl salicylate, indole, and α-farnesene as the most important compounds. Plants infested by whiteflies were placed in a third group, with mesitylene, (Z)-3-hexenyl acetate, and (R)-(+)-limonene as the most important compounds (Fig. 7).

## Discussion

In this study, we found that the generalist moth *T. ni* is able to discriminate between undamaged host plants and herbivore-damaged plants and between egg-free plants and plants previously oviposited by conspecific or heterospecific females. Thus, the results do not support our hypothesis that females would not discriminate between the two categories of host plants. Other studies, however, have shown that generalist females have difficulty discriminating between host plants of different quality. For example, Janz and Nylin (1997) found that two generalist butterfly species did not discriminate between good- and poor-quality host plants.

Our results indicate that females showed no preference for undamaged or mechanically damaged plants. This confirms the result obtained in a previous study, which reported that *T. ni* females did not discriminate between undamaged cotton plants and

mechanically damaged plants. However, *T. ni* females also showed no preference for uncut or cut cabbage plants (Landolt 1993). In contrast, *Mamestra brassicae* (L.) females oviposited more often on mechanically damaged cabbage plants compared with undamaged plants; however, they preferred to oviposit on undamaged tomato plants rather than on damaged tomato plants (Rojas 1999). Our results suggest that insect damage is essential in eliciting the cues that females use to discriminate between herbivore-damaged plants and undamaged plants. A number of studies have shown that plants recognize elicitors in insect oral secretions (Howe and Jander 2008) that activate its defensive responses. An alternative explanation is that the mechanical damage caused to soybean plants did not mimic the damage caused by insects; therefore, cues recognized by *T. ni* females were not elicited. Larvae feeding, for example, involves the action of specialized mandibles that remove similarly sized pieces of leaf tissue in a highly choreographed and foreseeable manner (Howe and Jander 2008).

The fact that insect damage is necessary to elicit the cues used by females for discriminating host plants is supported by our results, which show that females prefer to oviposit on undamaged plants than on plants damaged by conspecific or heterospecific larvae. Our results are in agreement with those of Landolt (1993), who reported that *T. ni* females more often oviposited on undamaged cotton plants than on plants infested with conspecific larvae. However, there was no difference between the number of eggs deposited on undamaged cabbage plants and plants infested with *T. ni* larvae (Landolt 1993). Landolt (1993) suggested that herbivore-induced plant volatile compounds mediate the host location and host acceptance by *T. ni*, but it was unclear whether the lack of preference for herbivore-damaged plants was due to the presence of larvae, frass, or plant compounds induced by feeding larvae because the larvae and its products remained on the plants used during the bioassays. Previously, it has been shown that oviposition of *T. ni* females on cabbage plants is deterred by larvae frass (Renwick and Radke 1980). By removing the larvae and frass before the assays, our study showed that these cues are not involved in the nonpreference of soybean plants damaged by conspecific larvae. Additionally, we found that *T. ni* females are able to discriminate between undamaged plants, plants damaged by heterospecific larvae, and plants damaged by whiteflies. Thus, our results suggest that egg-laying females use cues derived from plants to discriminate between undamaged and herbivore-damaged plants. However, we do not know whether females use the same cues for avoiding plants damaged by conspecific or heterospecific larvae. The volatile profile emitted by plants damaged by *T. ni* and *S. frugiperda* is quite similar, but it is slightly different in plants damaged by sucking insects. For instance, plants damaged by conspecific or heterospecific larvae emitted methyl salicylate, indole, and octyl butyrate, which are not released by undamaged or mechanically damaged plants. Plants damaged by whiteflies released higher quantities of Z(3)-hexenyl acetate, (R)-(+)-limonene, and (E)-β-ocimene than plants damaged by larvae and suppressed the emission of linalool.

Generally, *T. ni* females oviposited on the abaxial surfaces of plants without damage. However, when plants were damaged by *T. ni* or whiteflies, the eggs were laid indistinctly on the abaxial or adaxial surfaces, or preferring the abaxial surface when plants were damaged by *S. frugiperda* larvae, suggesting that herbivory affect the characteristics of the abaxial surface of soybean leaves. Several physical and chemical characteristics of leaves can affect the oviposition of insect females (Eigenbrode and Espelie 1995, Müller and Riederer 2005). Also, some studies have shown that leaf characteristics are affected by herbivory (Dutton et al. 2002). For instance, the

**Table 1.** Identified compounds emitted by soybean plants under the different treatments

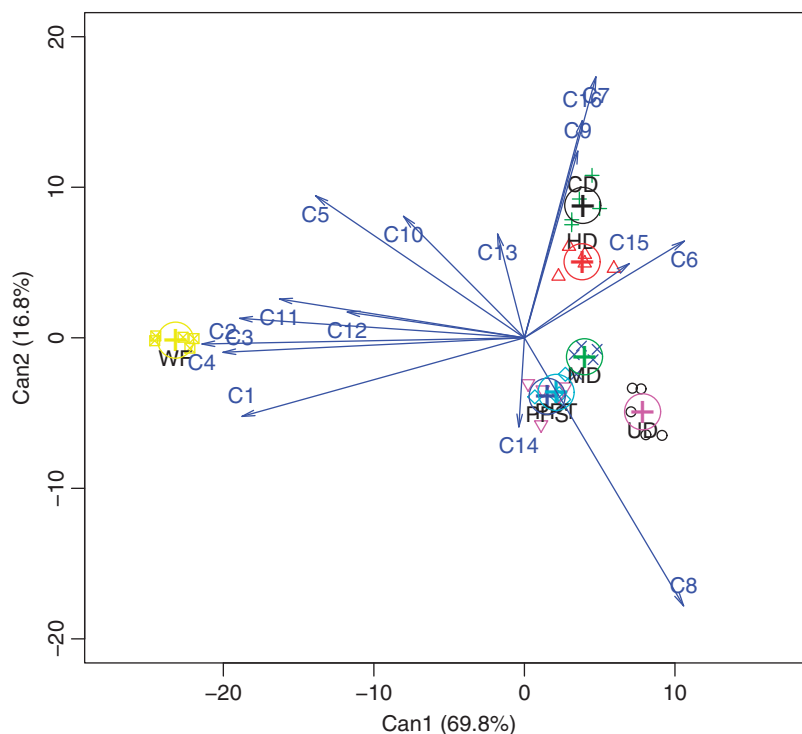| | Compound | RI[a] | Undamaged plants (UD)[b] | Mechanically damaged (MD) | T. ni caterpillar (CD) | S. frugiperda caterpillar (HD) | Whiteflies (WF) | Eggs T.ni (PPT) | Eggs S. frugiperda (PPS) | F | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C1 | *Cumene | 882.36 | 66571.4 ±8546.9bc | 63271.8 ±11587.6bc | 46051 ±2975.2 bc | 33070.4 ±9865.6c | 169674.6 ±10324.1a | 55533.4 ±8300.6bc | 74829.4 ±11943.1b | 13.705 | <0.001 |
| C2 | *Mesitylene | 969.84 | 250266.6 ±12313.6b | 227175 ±25365.9b | 200879.8 ±40051.2 b | 108189.8 ±33399.5b | 2244623.6 ±370830.2a | 221727.6 ±27154.2b | 191969 ±13984.3b | 41.668 | <0.001 |
| C3 | *(Z)-3-hexenyl acetate | 985.28 | n.d.[c] | 376368.4 ±222998.7b | 164505.2 ±39852.1b | 151540.4 ±34976.3 (b) | 1980132.6 ±387361.6a | n.d. | n.d. | 34.752 | <0.001 |
| C4 | *(R)-(+)-Limonene | 1016.7 | n.d. | 289643.2 ±30697.6b | 106661.6 ±23377.1b | 140807.2 ±37359.9b | 2867716.8 ±246544.2a | 186718.4 ±22812.4b | 194141.4 ±19210.2b | 161.21 | <0.001 |
| C5 | (E)-β-Ocimene | 1024.3 | n.d. | 198049.6 ±84439.6b | 1178173.8 ±453837.9 ab | 323367.6 ±219476.9b | 1786760.2 ±413602.7a | n.d. | n.d. | 15.976 | <0.001 |
| C6 | *Linalool | 1092.5 | 172551.8 ±24722.8ab | 273492 ±21941.6a | 192050 ±44166.4ab | 111947 ±16364.8b | | | | 76.882 | <0.001 |
| C7 | *Methyl salicylate | 1188.3 | n.d. | n.d. | 439594.2 ±128361.8a | 490205.6 ±113313.1a | | | | 33.358 | <0.001 |
| C8 | *Decanal | 1197.2 | 423078.6 ±38506.8a | 224271.4 ±27857.1b | n.d. | n.d. | | 237031 ±13392.1b | 234584.2 ±13670.9b | 263.11 | <0.001 |
| C9 | *Indole | 1308.4 | n.d. | n.d. | 1013835 ±353174.5a | 1559634 ±84251.8a | | n.d. | n.d. | 9.1043 | 0.0103 |
| C10 | Octyl butyrate | 1375.6 | n.d. | n.d. | 863591.8 ±270839.3a | 802707 ±158745.8a | 922216.2 ±297889.8a | 697127.8 ±81987.8a | 769858 ±57990.4a | 13.262 | <0.001 |
| C11 | *α-Copaene | 1387.5 | 135215.6 ±21627.6 (b) | 123687.4 ±18613.9b | 226373.4 ±15738.7b | 203187.6 ±23352.4b | 501677.2 ±126458.5a | 190818.2 ±4591.1b | 215399.2 ±8408b | 7.4517 | <0.001 |
| C12 | *β-Cariophyllene | 1439.5 | n.d. | 208152.8 ±83557.6a | n.d. | 155567.8 ±96240.1b | 348463 ±268802.5a | n.d. | n.d. | 8.805 | <0.001 |
| C13 | *Geranyl acetone | 1450.2 | n.d. | 363498.6 ±59889.1a | 319707.2 ±103316.8a | 790657 ±525955.5a | 387778.4 ±82888.3a | 175070.2 ±11021b | 216002 ±9281.2b | 4.9464 | <0.001 |
| C14 | <ar>Curcumene | 1490.4 | 153088 ±8426.5 (a) | 181357.2 ±24037.8a | 178819.8 ±110820.1b | n.d. | 155248.8 ±42908.4a | 18303 ±14462.7a | 288510.4 ±88598.2a | 5.1743 | <0.001 |
| C15 | *Pentadecane | 1500 | 198958.6 ±13088.9a | 174361.8 ±18308.5a | 211615.2 ±60747.3a | 199311.6 ±27129.6a | 126783.4 ±28119.6a | 168199.4 ±3725.2a | 138065 ±32038.3a | 0.8678 | 0.5305 |
| C16 | α-Farnesene | 1505.9 | n.d. | 814136.2 ±448578b | 12417219.2 ±2060936a | 7711718.6 ±6376889.7ab | n.d. | n.d. | n.d. | 12.33 | <0.001 |

[a] RI—Retention index relative to n-alkanes series. Compounds indicated with a (*) were identified by the comparison of synthetic standards.

[b] Data are GC peak area units (mean ± SE, n = 5). For each compound, different letters indicate significant differences among treatments.

[c] n.d.—not detected.

**Table 2.** Canonical discriminant characteristics for the volatile compounds emitted by experimental plants

| Function | Eigenvalue | Variance (%) | Cumulative (%) | Canonical correlation relation | Wilks' $\lambda$ | $F$ Testst | num DF | den DFn df | Significanceicance |
|---|---|---|---|---|---|---|---|---|---|
| 11 | 116.296 | 69.788 | 69.788 | 0.99147 | 0 | 64.611 | 36 | 103.761 | 0.000 |
| 22 | 27.977 | 16.788 | 86.577 | 0.96549 | 0.00011 | 38.19 | 25 | 90.658 | 0.000 |
| 33 | 15.28 | 9.169 | 95.747 | 0.93858 | 0.00329 | 26.454 | 16 | 77.014 | 0.000 |
| 44 | 5.474 | 3.285 | 99.032 | 0.84555 | 0.05358 | 16.41 | 9 | 63.428 | 0.000 |
| 55 | 1.424 | 0.854 | 99.887 | 0.58751 | 0.3469 | 9.421 | 4 | 54 | 0.000 |
| 66 | 0.189 | 0.113 | 100 | 0.15902 | 0.84098 | 5.295 | 1 | 28 | 0.029 |



**Fig. 7.** A plot showing the first two functions obtained from the canonic discriminant analysis of relative amounts of 16 volatile compounds released by undamaged plants (UD), mechanically damaged plants (MD), plants damaged by conspecific larvae (CD) or heterospecific larvae (HD), plants damaged by *B. tabaci* (WF), and plants previously oviposited by conspecific (PPT) or heterospecific females (PPS). The number of each compound correspond to that listed in Table 1.

amount of triterpene squalene increases in apple leaves after infestation of leaf miners (Dutton et al. 2002). The density of trichomes can increase in the surfaces of leaves from damage by insects (Valkama et al. 2005). In our case, the abaxial surface of soybean leaves has more trichomes in comparison to adaxial surface (G.G.C., unpublished data), but whether this characteristic explains the female preference for ovipositing on the abaxial surface or whether the density of trichomes, or other characteristics, changes after herbivory need to be investigated in future studies.

Interestingly, the cabbage looper moths were able to discriminate between egg-free plants and plants previously oviposited by conspecific or heterospecific females. We found that they prefer to oviposit on plants previously oviposited by *T. ni* or *S. frugiperda* females. A number of studies have shown that insect egg deposition elicits both direct and indirect plant defenses (Little et al. 2007, Hilker and Meiners 2011, Kim et al. 2012). The plant responses to herbivorous insect egg deposition include the formation of neoplasms, leaf necrosis, ovicidal substances, changes in internal secondary metabolites, phytochemical changes in leaf surfaces, and changes in plant

volatiles (Hilker and Fatouros 2015). We did not observe that soybean leaves responded to *T. ni* or *S. frugiperda* eggs by forming necrotic tissue or neoplasms under the eggs; however, egg deposition changed the volatile profiles emitted by the plants. Egg deposition by conspecific and heterospecific moths on soybean plants induced the emission of (R)-(+)-limonene, octyl butyrate, and geranyl acetone but suppressed the release of linalool. Oviposition of the spotted stemborer females *Chilo partellus* Swinhoe on plants of signal grass, *Brachiaria brizantha* (A. Rich.), reduced the emission of Z(3)-hexenyl acetate (Bruce et al. 2010), while maize plants oviposited by *S. frugiperda* females suppressed the emission of linalool (Peñaflor et al. 2011).

To our knowledge, few studies have investigated the effects of prior egg deposition on the behaviors of egg-laying females (Bruce et al. 2010, Fatouros et al. 2012, Raitanen et al. 2013). For instance, *C. partellus* females oviposit more eggs on egg-free plants of *B. brizantha* than on plants previously oviposited by conspecific females (Bruce et al. 2010). A similar situation was observed for *Pieris brassicae* L., where gravid females prefer to oviposit on egg-free plants

of *Brassica nigra* L. over plants previously oviposited by conspecific females. However, they did not show a preference for egg-free cabbage plants or for plants previously oviposited by *M. brassicae* females (Fatouros et al. 2012). In contrast, *Pieris napi* (L.) females oviposited more eggs on host plants [*Rorippa sylvestris* (L.)] that already carried eggs than on egg-free plants (Raitanen et al. 2013). The fact that *T. ni* females oviposited on previously laid plants may increase the costs of resource competition, at least until the neonate larvae are able to disperse from the home plant. A previous study reported that small larvae (1–2 instar) were more aggregated than large larvae, but it was not clear if the decreased aggregation observed for large larvae was due to mortality or to the dispersion of small larvae (Trumble et al. 1987). Raitanen et al. (2013) suggested that the conspecific attraction, rather than avoidance, of *P. napi* females implies that the likely benefits of choosing previously oviposited plants during oviposition site selection compensate for the costs of competition in the wild.

We observed that, in some cases, *T. ni* females approached a plant, landed on it, and began to evaluate (by tapping, abdomen bending, and ovipositor dragging) the plant before egg deposition. In other cases, females bent their abdomen in the air, landed, and began to oviposit on plant with no previous evaluation. In this last case, we speculate that females may have used volatile compounds as cues for determining that a plant was suitable before landing, while in the first case, females may use additional cues such as leaf roughness and nonvolatile compounds to assess and accept a host plant. Although volatile compounds mediate female attraction to host plants (Schoonhoven et al. 2005), they may also be important during oviposition (Fatzinger and Merkel 1985, Renwick and Chew 1994, Witzgall et al. 2005). For example, citrus volatiles trigger a higher frequency of host visits, increasing oviposition by *Papilio polyxenes* L. females (Feeny et al. 1989). As discussed above, herbivore damage and egg deposition affected the volatile compounds emitted by experimental plants both qualitatively and quantitatively. Thus, our results suggest that volatiles may be used by females to discriminate between host plants. However, nonvolatile compounds or physical cues may also affect the behavior of females. For example, egg deposition of *P. brassicae* on *Arabidopsis thaliana* (L.) plants induced changes in the leaf epicuticular wax composition (Blenn et al. 2012).

In summary, our study extends the knowledge concerning the oviposition behavior of generalist herbivores. In particular, this study contributes to the literature by showing that generalist moth species can discriminate between plants of different quality. Our results also suggest that plant volatiles are important cues used by egg-laying females to discriminate between host plants. However, more studies are needed to examine the effect of a female's choice of oviposition host plant on her offspring and to confirm the roles of volatile compounds as well as the possible involvement of physical and nonvolatile cues during moth oviposition.

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## References Cited

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/mass spectrometry, p. 804.

Bell, J. W. 1990. Searching behavior patterns in insects. Annu. Rev. Entomol. 35: 447–467.

Bernays, E. A. 2001. Neural limitations in phytophagous insects: Implications for diet breadth and evolution of host affiliation. Annu. Rev. Entomol. 46: 703–727.

Blenn, B., M. Bandoly, A. Küffner, T. Otte, S. Geiselhardt, N. E. Fatouros, and M. Hilker. 2012. Insect egg deposition induces indirect defense and epicuticular wax changes in *Arabidopsis thaliana*. J. Chem. Ecol. 38: 882–892.

Bruce, T. J., C. A. Midega, M. A. Birkett, J. A. Pickett, and Z. R. Khan. 2010. Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. Biol. Lett. 6: 314–317.

Cunningham, J. P. 2012. Can mechanism help explain insect host choice? J. Evol. Biol. 25: 244–251.

Dutton, A., L. Mattiacci, R. Amadò, and S. Dorn. 2002. A novel function of the triterpene squalene in a tritrophic system. J. Chem. Ecol. 28: 103–116.

Eigenbrode, S. D., and K. E. Espelie. 1995. Effects of plant epicuticular lipids on insect herbivores. Annu. Rev. Entomol. 40: 171–194.

Fatouros, N. E., D. Lucas-Barbosa, B. T. Weldegergis, F. G. Pashalidou, J. J. A. van Loon, M. Dicke, J. A. Harvey, M. Gols, and M. E. Huigens. 2012. Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels.PLoS ONE 7: e43607.

Fatzinger, C. W., and E. P. Merkel. 1985. Oviposition and feeding preferences of the southern pine coneworm (Lepidoptera: Pyralidae) for different host plants materials and observations on monoterpenes as an oviposition stimulant. J. Chem. Ecol. 11: 689–699.

Feeny, P., E. Städler, I. Åhman, and M. Carter. 1989. Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). J. Insect. Behav. 2: 803–827.

Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. Ecol. Lett. 13: 383–393.

Hilker, M., and N. E. Fatouros. 2015. Plant responses to insect egg deposition. Annu. Rev. Entomol. 60: 493–515.

Hilker, M., and T. Meiners. 2011. Plants and insect eggs: How do they affect each other? Phytochem 72: 1612–1623.

Howe, G. A., and G. Jander. 2008. Plant immunity to insect herbivores. Annu. Rev. Plant. Biol. 59: 41–66.

Janmaat, A. F., and J. Myers. 2003. Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*. Proc. R. Soc. Lond. B 270: 2263–2270.

Janz, N., and S. Nylin. 1997. The role of female search behaviour in determining host plant range in plant range in plant feeding insects: A test of the information processing hypothesis. Proc. R. Soc. Lond. B. 264: 701–707.

Khan, Z. R., l. A. Ciepiela, and D. M. Norris. 1987. Behavioral and physiological responses of cabbage looper, *Trichoplusia ni* (Hübner), to steam distillates from resistant versus susceptible soybean plants. J. Chem. Ecol. 13: 1903–1915.

Kim, J., J. F. Tooker, D. S. Luthe, C. M. De Moraes, and G. W. Felton. 2012. Insect eggs can enhance wound response in plants: A study system of tomato *Solanum lycopersicum* L. and *Helicoverpa zea* Boddie. PLoS ONE 7: e37420.

Landolt, P. J. 1989. Attraction of the cabbage looper to host plants and host plant odor in the laboratory. Entomol. Exp. Appl. 53: 117–124.

Landolt, P. J. 1993. Effect of host plant leaf damage on cabbage looper moth attraction and oviposition. Entomol. Exp. Appl. 67: 79–85.

Landolt, P. J. 2001. Moth experience and not plant injury affected female cabbage looper moth (Lepidoptera: Noctuidae) orientation to potato plants. Fla. Entomol. 84: 243–249.

Landolt, P. J., and O. Molina. 1996. Host-finding by cabbage looper moths (Lepidoptera: Noctuidae): Learning of host odor upon contact with host foliage. J. Insect. Behav. 9: 899–908.

Landolt, P. J., and T. W. Phillips. 1997. Host plant influences on sex pheromones behaviour of phytophagous insects. Annu. Rev. Entomol. 42: 371–391.

Levins, R., and R. Macarthur. 1969. An hypothesis to explain the incidence of monophagy. Ecology 50: 910–911.

Little, D., C. Gouhier-Darimont, F. Bruessow, and P. Reymond. 2007. Oviposition by pierid butterflies triggers defense responses in *Arabidopsis*. Plant Physiol. 143: 784–800.

Müller, C., and M. Riederer. 2005. Plant surface properties in chemical ecology. J. Chem. Ecol. 31: 2621–2651.

Peñaflor, M.F.G.V., M. Erb, C.A.M. Robert, L. A. Miranda, A. G. Werneburg, F.C.A. Dossi, T.C.J. Turlings, and J.M.S. Bento. 2011. Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize. Plant 234: 207–215.

Raitanen, J., J. T. Forsman, S. M. Kivelä, M. I. Mäenpää, and P. Välimäki. 2013. Attraction to conspecific eggs may guide oviposition site selection in a solitary insect. Behav. Ecol. 25: 110–116.

Renwick, J.A.A. 1989. Chemical ecology of oviposition in phytophagous insects. Experientia 45: 223–228.

Renwick, J.A.A., and C. D. Radke. 1980. An oviposition deterrent associated with frass from feeding larvae of the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). Environ. Entomol. 9: 318–320.

Renwick, J.A.A., and F. S. Chew. 1994. Oviposition behavior in Lepidoptera. Annu. Rev. Entomol. 39: 377–400.

Rojas, J. C. 1999. Influence of host plant damage on the host-finding behavior of *Mamestra brassicae* (Lepidoptera: Noctuidae). Environ. Entomol. 28: 588–593.

Rojas, J. C., A. Virgen, and L. Cruz-López. 2003. Chemical and tactile cues influencing oviposition of a generalist moth, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Environ. Entomol. 32: 1386–1392.

Schäpers, A., M. A. Carlsson, G. Gamberale-Stille, and N. Janz. 2015. The role of olfactory cues for the search behavior of a specialist and generalist butterfly". J. Insect. Behav. 28: 77–87.

Schoonhoven, L. M., J.J.A. Van Loon, and M. Dicke. 2005. Insect-Plant Biology. Oxford University press, New York.

Sutherland, D.W.S., and G. L. Greene. 1984. Suppression and management of cabbage looper populations: Cultivated and wild host plants, p. 1–13. *In* P. D. Lingren and G. L. Green, (eds.), Suppression and management of cabbage looper populations, technical bulletin. United States Department of Agriculture, Columbia, no. 1694.

Thompson, J. N. 1998. The evolution of diet breadth: monophagy and polyphagy in swallowtail butterflies. J. Evol. Biol. 11: 563–578.

Trumble, J. T., J. V. Edelson, and R. N. Story. 1987. Conformity and incongruity of selected dispersion indices in describing the spatial distribution of *Trichoplusia ni* (Hübner) in geographically separate cabbage plantings. Res. Popul. Ecol. 29: 155–166.

Valkama, E., J. Koricheva, V. Ossipov, S. Ossipova, E. Haukioja, and K. Pihlaja. 2005. Delayed induced responses of birch glandular trichomes and leaf surface lipophilic compounds to mechanical defoliation and simulated winter browsing. Oecologia 146: 385–393.

Visser, J. H. 1986. Host odor perception in phytophagous insects. Annu. Rev. Entomol. 31: 121–144.

Witzgall, P., M. Tasin, H. R. Buser, G. Wegner-Kiss, V.S.M. Mancebon, C. Ioriatti, A. C. Bäckman, M. Bengtsson, L. Lehmann, and W. Francke. 2005. New pheromone components of the grapevine moth *Lobesia botrana*. J. Chem. Ecol. 31: 2923–2932.