Field Observations of Oviposition by a Specialist Herbivore on Plant Parts and Plant Species Unsuitable as Larval Food

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ABSTRACT Where a female places her eggs can have a major impact on the fitness of her offspring, especially for insects in which the winged adults are far more mobile than the neonates. Larvae of *Heliothis subflexa* (Guenee) (Lepidoptera: Noctuidae), a specialist moth phylogenetically nested within a generalist clade, feed only on fruit of some *Physalis* species. Field observations of the oviposition behavior of *H. subflexa* revealed that 1) females laid most of their eggs on leaves of the *Physalis* plant, despite the larvae's frugivorous diet, and 2) females laid nearly 20% of the eggs on nonhost plant species. Most eggs oviposited on nonhosts were placed close to the host plant—88% were within 15 cm of the *Physalis* plant. However, in a study of neonate movement, we found that a distance of 2 cm from the hatch site to the host plant significantly decreased the ability of neonates to establish on the host plant. The estimated fitness cost, quantified as reduced neonate survival, for females ovipositing on nonhosts is 8–17%. Many ecological and evolutionary factors could result in oviposition on less suitable host parts and on nonhosts. One possibility is that specialization on *Physalis* has recently evolved in *H. subflexa*, and females have not fully optimized their oviposition behavior. However, the fitness cost of oviposition on nonhosts may be balanced by fitness benefits of such behavior, such as faster decision-making and reduced predation.

KEY WORDS *Heliothis subflexa*, *Physalis*, oviposition mistakes, nonhost oviposition, neonate movement

The ability of adult females to choose the appropriate host plant species has been documented for many insect herbivores (Thompson and Pellmyr 1991). Most of this research has focused on female choice among plants within one or a few host species that vary in suitability for larval growth and survival, and on female choice among plant species that are taxonomically or phytochemically related to the host plant(s) (Courtney 1986, Waldvogel and Gould 1990). Few studies have examined oviposition behavior on nonhost plants that are phylogenetically and chemically unrelated to the host plant, but share a common habitat with the host plant (Chew and Robbins 1984, Mitter et al. 1991, Finch and Collier 2000).

Oviposition preference for specific parts of the host plant has been found in many specialist herbivores. For example, heliconiine butterflies (Benson 1978, Mitter and Brooks 1983), yucca moths (Aker and Udovic 1981, Pellmyr 2003), and fig wasps (Weiblen 2002) typically oviposit directly on the plant parts where the larvae will feed. Each of these particular herbivores are embedded within a phylogenetic clade showing a long history of host plant specialization. These phylogenetic histories suggest that the specialized oviposition patterns we see now evolved over a long evolutionary period. Examination of a specialist insect species that recently diverged from generalist ancestors could provide a glimpse into the evolution of specialized oviposition behavior and the forces shaping host plant specialization. *Heliothis subflexa* (Guenee) offers an excellent system for doing just that.

Heliothis subflexa is a specialist noctuid moth, phylogenetically nested within a group of generalists; thus larval host specialization appears to be a recently derived trait (Fang et al. 1997). *Heliothis subflexa* larvae feed solely on some species within the genus *Physalis* (Solanaceae) (groundcherry) (Bateman 2006). Feeding assays using both neonates and final instars on 30 or more nonhost plants have demonstrated that *H. subflexa* larvae do not survive on, and rarely even attempt to feed on, non-*Physalis* plants (F. Gould, unpublished data). Starting as neonates, larvae feed primarily on fruits and are almost never found on leaves. Larvae must feed on several fruit to develop to pupation, but usually can do so on one plant because there are usually many fruit available.

The genus *Physalis* includes ≈90 species with distributions ranging from local to cosmopolitan, and with life histories ranging from annual to perennial. Most of the species, including those used in this study,

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are patchily distributed in early succession natural areas and along crop rows in agricultural fields (Radford et al. 1968, unpublished data). Plants in the genus *Physalis* are characterized by a lantern-like inflated calyx that surrounds the fruit. *Heliothis subflexa* larvae find and enter *Physalis* fruits more quickly than the closely related generalist *Heliothis virescens.* In addition, *H. subflexa* larvae exhibit the specialized behavior of pulling their entire bodies into the inflated calyx of the fruit to take full advantage of the enemy-free space inside the calyx (Sisterson and Gould 1999, Oppenheim and Gould 2002). In contrast, *H. virescens* larvae often feed with part of their bodies outside the calyx (Oppenheim and Gould 2002). Like many heliothine larvae, *H. subflexa* neonates are able to spin silk to change location, but have limited mobility because of their small size (Zalucki et al. 2002).

Here we present results of experiments that quantified *H. subflexa* female oviposition choices and and the moth's decision-making process, and how those choices affect larval fitness. Because previous laboratory studies suggested a lack of oviposition preference (Benda 2007), we observed oviposition behavior in natural populations of *H. subflexa* in the field. We conducted observations in two different field settings: 1) *Physalis* plants were planted between rows of cotton, simulating an agricultural field where *Physalis* was a weed, and 2) *Physalis* were planted in small patches, representing a more natural distribution in a nonagricultural field. Assuming limited mobility of the highly specialist larvae, we tested two hypotheses: 1) *H. subflexa* females will place their eggs directly on the calyces of *Physalis* plants, nearest to the larval food, and 2) in an environment with high plant diversity, *H. subflexa* females will selectively oviposit only on *Physalis* plants. Based on results of the oviposition behavior studies, we examined how oviposition on nonhost plants affected the fitness of larvae.

Materials and Methods

Female Oviposition Patterns and Behavior. *Study Site and Plants. Physalis angulata*, *P. pubescens*, and *P. cordata* plants were started from seed in the greenhouse. All three *Physalis* species serve as relatively equitable hosts for *H. subflexa,* and are commonly used by *H. subflexa* for oviposition and larval feeding in the field (Bateman 2006, Benda 2007). Fertilizer and herbicide regimes can be found in Benda (2007). Field studies were conducted in 2004 and 2005 in a 0.26-h field in Clayton, NC. For both 2004 and 2005, daily wind, temperature, and precipitation data were collected <1.5 km from the field site by the North Carolina Department of Agriculture.

Oviposition Behavior in a Typical Agricultural Setting (2004). Five sets of two rows of *Physalis* were interspersed with 4–8 rows of cotton, for 21 rows in total. Rows were 1 m apart. Cotton, *Gossypium hirsutum* L. (FiberMax 991 Roundup Ready, Bayer, Research Triangle Park, NC) was planted on 20 May 2004 with 20 cm between adjacent plants. Rows to be used for Physalis were covered with 183-cm-wide Lumite black woven plastic groundcover (Lumite Inc., Gainesville, GA) to reduce weed competition and drought stress. In mid-July 2004, 6-wk-old *P. angulata, P. pubescens* and *P. cordata* plants (≈15–20 cm tall) were transplanted into this groundcover with 1 m between adjacent plants (Benda 2007).

The bare ground between rows of cotton and the groundcover (≈0.5 m wide) was quickly colonized by various weeds including crabgrass (*Digitaria* sp.) and nutsedge (*Cyperus* sp.), and less frequently, pigweed (*Amaranth* sp.) and evening primrose (*Oenothera* sp.). There were no solanaceous nonhosts in the field.

Oviposition Behavior in a Setting Simulating the Patchy Distribution of Wild Physalis Plants (2005). Sixty squares of Lumite groundcover (0.9 by 0.9 m) were placed 3.6 m apart throughout a plowed field (Benda 2007). In late May 2005, four 6-wk-old *P. angulata* plants were transplanted into each groundcover square. In late June 2005, 24 10-wk-old *P. pubescens* plants were transplanted individually 2.4 m apart in a plot alongside the patches of *P. angulata.* Lumite groundcover was not used for these plants because older plants are more tolerant of drought and weed competition than are seedlings. Weeds colonized the bare ground as in 2004. "Volunteer" *Physalis* were manually removed (Benda 2007).

Oviposition and Flight Behavior Observations. Based on preliminary observations, ovipositing female moths were observed in the field starting ≈1 h before sunset until 2 h after sunset, using a red-filtered flashlight as needed in dark conditions. Behavior observations were recorded in real time on a cassette recorder (Sony Corp., Tokyo, Japan) as follows: 1) occurrence and duration of each bout of activity including flying, ovipositing, resting, and feeding; 2) occurrence and duration of each bout of pre-oviposition behavior (alightment, abdomen curling) and oviposition; 3) location, path of movement, and oviposition choices of the female moth relative to plant species (*Physalis* and weed species) and plant part; and 4) distance from an egg laid on a nonhost plant to the nearest *Physalis* plant (2005 only).

Statistical Analysis. Heliothis subflexa oviposition data were combined across all *Physalis* species within each year because there were insufficient data to analyze moth behavior separately on each *Physalis* species. Data from 2004 and 2005 were combined when year was not a statistically significant factor in the analysis of variance (ANOVA). Observed moths were identified (2004 only) by collecting eggs as they were laid ($N = 39$ moths, mean 4.4 eggs collected per moth; range 1–10 eggs) and rearing them to fourth- to fifth-instar larvae. All larvae were identified as *H. subflexa* ($N = 221$) (Brazzel et al. 1953).

Moth observations were analyzed using SAS version 8 (SAS Institute 1999). SAS programming details can be found in Benda (2007). A random statement was included in ANOVA analyses when needed to account for repeated measures on individual moths, such as when considering multiple eggs laid by a single moth (Moser 2004). P values were adjusted for multiple posthoc comparisons using a Bonferonni-correction.

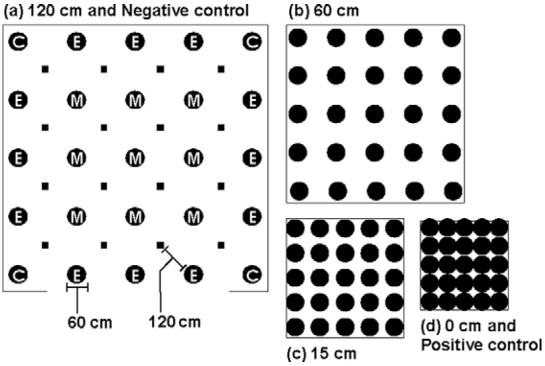

Fig. 1. Experimental design for host location by neonates. Diagrams a–d illustrate the layouts of the plots, where the distances from the each of the 16 egg sticks (solid squares; not shown in b–d) to the nearest *Physalis* plants was 120, 60, 15, and 2 cm, respectively. The diagram of plot (a), with 120 cm distance between the eggs and the nearest plant, is used to illustrate the spatial distribution of plants and experimental eggs. Each black circle denotes a single *Physalis* plant. The letter within each circle denotes the relative location of the plant in the plot (C, E, and M for Corner, Edge, and Middle, respectively). Plot size, plant spacing, and plant canopy (≈60 cm diameter) are represented proportionately.

A logistic regression was used to determine if the percentage of eggs placed on the reproductive parts of the *Physalis* plants differed by year, and a Pearson χ^2 was used to account for over-dispersion (i.e., error larger than the mean) across moths (Allison 2005).

The percentages of 1) alightments that led to abdomen curls, 2) abdomen curls that led to ovipositions, and 3) alightments that led to ovipositions were calculated. Effect of year on these factors was tested as described in the previous paragraph for percentage of eggs on reproductive parts. To account for two sets of alightments, abdomen curls, and oviposition observations, per moth (one on hosts and the other on nonhosts), a repeated statement with an unstructured covariance option was included in an ANOVA analysis. Estimate statements were used to compare these values on hosts and nonhosts and between years. Statistical significance was assessed with a Pearson χ^2 test.

Host Plant Location by Neonates. *Study Site and Plants.* The field was plowed 21 May 2005 and planted with *P. pubescens* 23 May in Apex, NC. *Physalis pubescens* was used in this experiment because it has a shrub-like architecture, with many leaves contacting the ground (Fig. 1). The 7.5-liter plastic pot holding each plant was sunk to just below ground level and covered with field soil so that the landscape immediately surrounding the plant resembled the rest of the field. The *P. pubescens* plants had a mean of 145 fruits (±112 SD, $N = 243$ plants; 95% had ≥20 fruits) and abundant flowers at the beginning of each replicate. The plowed ground around each planted *Physalis* repopulated with a diverse mix of grasses and forbs but no *Physalis.*

Experimental Eggs. Moths in laboratory colonies (Sheck et al. 2006) were allowed to lay eggs on cheesecloth for 18 h. A portion of this cloth with 50 eggs (±5 eggs) was sewn to a square of cotton terrycloth (2.5 cm^2) glued to the tip of a 30-cm bamboo stake, constituting an "egg stick". Egg sticks were held in an incubator at 25°C (±2°C) for 2 d and placed in the field >24 h before hatch (as indicated by the blackened head capsule visible inside many of the eggs).

Experimental Design. Plots of 25 6 wk-old plants each were laid out at four different densities (one plot per density treatment) so that the egg sticks would be 2, 15, 60, or 120 cm from the edge of the nearest plant canopy (Fig. 1), based on a *Physalis* canopy diameter of ≈60 cm. The experiment was replicated three times in 2005—on 11 June, 1 July, and 23 July.

Egg sticks were set in the ground, equidistant from plants in each treatment plot (Fig. 1a). A 251-ml Styrofoam cup on a wire stake shielded the eggs from direct sun while allowing dispersal. For the positive control (Fig. 1d), an egg stick and cup shield were placed directly in the canopy of each *Physalis* plant. Because there was an egg stick in each plant instead of one between each set of four plants as in the treatment plots, the number of eggs on the stick was adjusted, based on whether a plant was at a corner of the plot (labeled 'C' in Fig. 1a), on an edge ('E'), or in the middle inner portions ('M') of the plot. This adjustment was made so that each plant in the positive control would be proportionately exposed to a similar number of neonates as those in the treatment plots. In total, 800 eggs (16 egg sticks with 50 eggs each in the treatment plots, and 25 egg sticks with 12, 25 or 50 eggs in the positive control plot) were set out per plot in each replicate of the experiment.

A natural infestation of *H. subflexa* was monitored using pheromone traps (Benda 2007). To calculate the baseline infestation rate of wild *H. subflexa,* the 120-cm treatment plot was considered a negative control (with no egg sticks) in the second and third replicates, because no larvae established in this plot in the first replicate. The number of larvae found in the negative control plot in each replicate was subtracted from the total found in each of the other plots.

To reduce predation by ants, Amdro Fire Ant Bait Yard Treatment, a bait insecticide for ants, was broadcast in the plots 8 d before the second and third replicates (50 kg/h, active ingredient: hydramethylnon, 0.036% by weight, Ambrands, Atlanta, GA).

Data Collection and Statistical Analysis. Preliminary experiments in the lab showed that ≈95% of the larvae died after 2 d when provided a moist cotton wick and a sprig of crabgrass. Therefore, egg sticks (with 2 d old, mature eggs) were placed in the field in the morning and kept there for 3 d. After 3 d, all the fruit from each plant were removed and the total number of larvae found was recorded.

The total number of larvae in each plot was analyzed as a square-root transformed percentage of the total found in the positive control. To determine if the percentage of larvae in each plot was affected by 1) replicate and 2) distance of eggs from the host plant (i.e., plot density treatment), a factorial ANOVA analysis using SAS was conducted. Distance (or plot density) was treated as class variable. Means were compared using a Tukey adjusted *t*-test of LS means.

Relative Fitness of *H. subflexa* Females. Based on results of the oviposition studies, it was clear that *H. subflexa* females were not laying all of their eggs on plants that were suitable for survival of larvae and that movement of larvae from unsuitable to suitable plants resulted in a high level of mortality. To estimate how much of a decrease in fitness was caused by oviposition on nonhosts, a general approach was used that has been established in the evolutionary biology literature (Fisher 1958, Levins 1968, Roughgarden 1979) and that was used in previous work (Groot et al. 2006). The basic approach is to set the fitness of an individual (or genotype or species) with an optimally adapted trait to 1.0. The fitness of other individuals is assessed relative to that of the optimally adapted individual. In this case, the trait is oviposition and the optimally adapted individuals are those that lay all of their eggs on the host plant. The fitness of individuals that lay some of their eggs on nonhosts is determined by the fraction of their eggs laid on nonhosts and the survival of those eggs compared with eggs laid on a host plant.

Results from the experiments described above provided values for three parameters needed to assess fitness of wild *H. subflexa* females that laid eggs on nonhosts:

1) The mean distance from where eggs were oviposited by wild *H. subflexa* on nonhosts to the nearest host plant.

2) The survival of eggs artificially placed at this mean distance compared with eggs placed on the host plant.

3) The percentage of eggs oviposited by wild *H. subflexa* on nonhost plants.

In this assessment, the fitness level of individuals that moved from a nonhost to a host and survived for the duration of the experiments was considered equal to that of individuals deposited directly on the host plant. The basic equation for fitness of the wild *H. subflexa* females relative to the hypothetical *H. subflexa* female that laid all of its eggs on host plants is as follows:

$$W_{Hs} = 1.0 - (P_{NH}) \times (1-S_{NH})$$

Where $W_{Hs}$is the fitness of wild *H. subflexa* females with typical oviposition behavior, P_{NH} is the proportion of eggs laid on nonhosts, and S_{NH} is the proportion of survival (i.e., proportion of neonates established on the host) of eggs laid on nonhosts compared with survival of eggs laid on hosts.

Results

Female Oviposition Patterns and Behavior. *Within-plant Distribution of Oviposition on Physalis.* We observed 297 ovipositions by *H. subflexa* (*N* = 66 moths) on *Physalis* (Table 1). The *Physalis* plants all had abundant (≥20) flowers and calyx-enclosed fruits (hereafter referred to as fruit for simplicity) throughout the period of observations. Considering plant parts used for oviposition as either reproductive (flowers, flower buds, and fruit) or vegetative (stems and leaves), moths oviposited 19.5 ± 4.4% and 44.8 ± 7.6% (mean ± SE) of eggs on reproductive plant parts in 2004 and 2005, respectively. There was a significant difference between years ($\chi^2 = 12.84, P = 0.0003$), due primarily to differences in the percentage of eggs on the fruit and leaves (Fig. 2).

Table 1. **Number of night-time observations of oviposition by *Heliothis subflexa* females**

	Year	
	2004	2005
Nights of observation	12	10
Total moths observed	42	24
Total min. of observation	164	86
Total ovipositions observed	208	173
Total ovipositions on *Physalis*	160	137
Total ovipositions on non-hosts	48	36

Wild *Heliothis subflexa* females were observed while ovipositing in the field from sunset to 2 h post-sunset. Only females observed ≥1 min. are included.

Oviposition on Nonhost Plants. In total, 42 and 24 ovipositing moths were observed for more than one min. in 2004 and 2005, respectively. Of these moths, 45 and 63% laid eggs on nonhosts in 2004 and 2005, respectively. In 2004, the number of eggs laid per moth during the observation period was 5.0 ± 3.7 (±SE) and the mean percentage of eggs laid on nonhosts was 18.4 ± 4.1% (±SE). In 2005 the number of eggs laid per moth was 7.2 ± 7.0 (±SE) and the percent on nonhosts was 19.6 ± 3.0%.

Heliothis subflexa females oviposited on several different plant species, including crabgrass (*Digitaria* sp., Poaceae), nutsedge (*Cyperus* sp., Cyperaceae), evening primrose (*Oenethera* sp., Onagraceae), cotton (*Gossypium hirsutum*, Malvaceae), buckwheat (*Polygonum* sp., Polygonaceae), and marigold (*Tagetes* sp., Asteraceae). No test for the effect of plant species on likelihood of oviposition was performed (see Benda 2007 for oviposition frequencies on the different plant species). In both 2004 and 2005, the majority (83% in 2004, and 77% in 2005) of eggs laid on nonhosts were oviposited on crabgrass and nutsedge.

The mean (±SE) distance between nonhost oviposition sites and the nearest *Physalis* plant in 2005 was

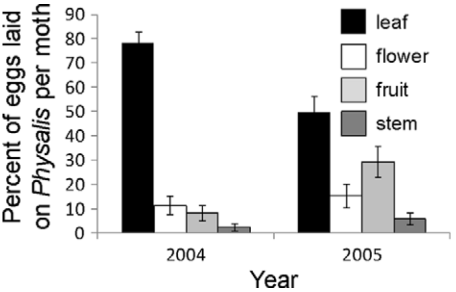

Fig. 2. Mean (±SE) percentage of eggs oviposited on the four main parts of the *Physalis* plant by wild *H. subflexa* females in the field in 2004 and 2005 (*N* = 160 and 137 total ovipositions, respectively).

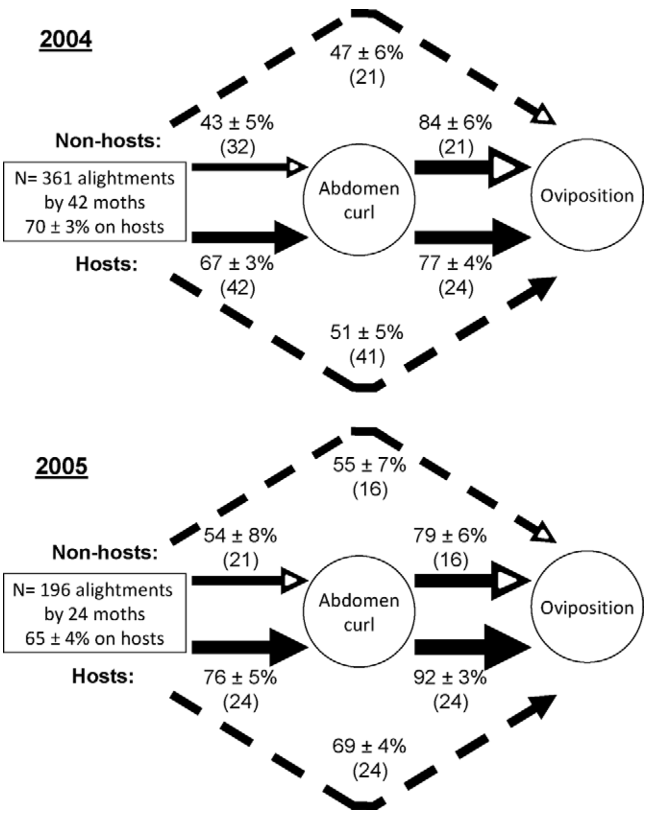

Fig. 3. Mean percentage (±SE) of preoviposition behaviors (alightment, abdomen curl) leading to oviposition per female *H. subflexa* on host and nonhost plants in 2004 ($N = 361$ alightments by 42 moths) and 2005 ($N = 196$ alightments by 24 moths). Thickness of arrows is proportional to the mean. White-tipped arrows trace the behavioral transitions on nonhosts, while black-tipped arrows trace the transitions on hosts. Dashed arrows represent the percentage of alightments that resulted in oviposition (including abdomen curl), whereas solid lines represent the separate transitions from alightment to abdomen curl and then from abdomen curl to oviposition.

8.2 ± 2.5 cm ($N = 40$ eggs laid by 21 moths). However, as the distance from *Physalis* plants increased, the number of eggs laid on nonhosts decreased precipitously ($y = 20.672–11.6174x$, where x is the log of distance and y is the number of expected eggs, $R^2 = 0.7083$, two tailed $P < 0.0001$). Indeed, 88% of all eggs laid on nonhosts were placed within 15 cm of the nearest *Physalis* plant.

The number of alightments on *Physalis* host plants (5.7 ± 0.1, $N = 66$ moths) was greater than on nonhost plants (3.3 ± 0.1, $N = 66$) ($\chi^2 = 831.36$, df = 62, $P = <0.0001$), with no effect of year. Figure 3 presents behavioral transition probabilities for alightments leading to abdomen curls and then to oviposition. In both 2004 and 2005, the percentage of alightments on hosts that led to abdomen curls was significantly greater on host plants than on nonhost plants ($\chi^2 = 18.95$, df = 115, $P = <0.0001$), with no effect of year nor interaction of year and plant type (host versus nonhost). There was no effect of year or plant type on the percentage of abdomen curls that led to oviposition, but there was a significant interactive effect of year and plant type ($\chi^2 = 5.97$, df = 98, $P = 0.0145$). Most importantly, the percentage of alightments that led to ovipositions on hosts (57.4 ± 4.3, $N = 65$) was significantly greater on hosts than on nonhosts (49.8 ± 5.4, $N = 37$) ($\chi^2 = 5.04$, df = 98, $P = 0.0248$) and not affected by year nor the interaction of year and plant type.

Climate Factors. Average, minimum, and maximum daily temperatures during the period of observation in 2005 (24 ± 2°C, 22 ± 3°C, and 31 ± 3°C, respectively) were significantly higher than in 2004 (23 ± 2°C, 19 ± 3°C, and 28 ± 2°C, respectively). All other variables were not significantly different between years and so data were pooled. Relative humidity was 80 ± 6%, rainfall was 0.2 ± 0.5 cm, and average and maximum wind speeds were 6 ± 2 km/h and 12 ± 3 km/h, respectively.

Host plant Location by Neonates. In the positive control plots, the total number of neonates that established on *Physalis* fruits (out of 800 eggs placed within the *Physalis* canopy) was 20, 40, and 50 larvae for replicates 1, 2, and 3, respectively. Replicate and distance from the host plant had significant overall effects on the percentage of larvae (square-root transformed) established in the treatment plots relative to the positive control (effect of replicate: $F = 8.36$; df =

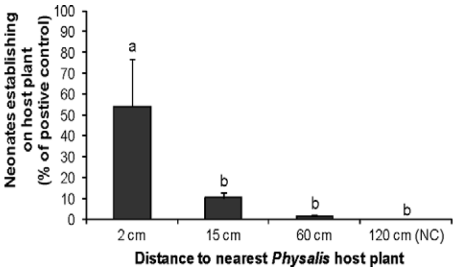

Fig. 4. Percentage of *H. subflexa* first-instar larvae that established on *Physalis* from eggs placed 2, 15, and 60 cm from the host plant (800 eggs and 25 *Physalis* plants per plot in each replicate). Percentage is relative to the number of larvae that established on *Physalis* in the positive control (where eggs were placed in the *Physalis* canopy). Histogram bars capped with different letters are significantly different ($P < 0.05$) using a Tukey-adjusted *t*-test of least squares means. NC = negative control.

4, 2; $P = 0.0373$; effect of distance: $F = 14.74$; df = 4, 3; $P = 0.0125$, $N = 25$ *Physalis* plants). The percentage of larvae that established in the smallest plot (2 cm to the nearest plant) was significantly greater than the percentage in the larger plots (15, 60, and 120 cm to the nearest plant). The percentages in these larger plots were not significantly different from each other using a Tukey-adjusted *t*-test of LS means ($P = 0.0461$, 0.0115 and 0.0492, respectively) (Fig. 4). A higher total number of larvae established in *Physalis* fruit in the second two replicates, where bait insecticide was applied to reduce ant numbers.

Relative Fitness of *H. subflexa* Females. A mean of 4.6% of the eggs placed within the *Physalis* canopy (positive control plots) were recovered as larvae in *Physalis* fruit (i.e., established on the host). This value was considered the absolute fitness of eggs laid on the host plant. We set this value to a relative fitness of 1.0 by dividing 1.0 by 0.046 (=21.74). Survival values of all other treatments also were multiplied by 21.74 to obtain their fitness relative to that of eggs laid directly on the host plant. All means in this section are ± SE.

1) Of the eggs laid by wild females on nonhost plants, eggs were placed 8.2 ± 2.5 cm from the host plant ($N = 40$ eggs).

2) Mean survival of eggs (i.e., proportion of neonates established on the host) that were artificially placed 15 cm from the host plant was 0.125 ± 0.043 (relative to the positive control, $N = 3$ replicates). Mean survival of eggs placed 2 cm from the host plant was 0.566 ± 0.254 (relative to the positive control, $N = 3$ replicates).

3) Wild moths laid 19.2 ± 3.0% of the eggs on nonhosts (calculated across years, $N = 65$ moths). Because moths laid eggs an average of 8.2 cm from the host plant, with 88% of all nonhost ovipositions within 15 cm of the plant, the fitness was calculated using 1) relative survival at 15 cm from the host plant (0.125), and 2) relative survival at 2 cm from the host plant (0.566). Because the survival-by-distance curve appears nonlinear, using survival values at both 2 cm and 15 cm provide the range of possible fitness values.

If all 19.2% eggs that were oviposited on nonhosts (P_{NH}) were 15 cm away from a host plant, the relative survival (S_{NH}) would be 0.125. Based on equation 1, the overall fitness of wild females would then be: $W_{Hs} = 1.0 - (0.192) \times (1-0.125) = 0.832$. If eggs were 2 cm away from a host plant, the relative survival (S_{NH}) would be 0.566. Overall fitness of females would then be: $W_{Hs} = 1.0 - (0.192) \times (1-0.566) = 0.916$.

Discussion

Heliothis subflexa larvae are highly specialized phytophagous insects, feeding only on fruits of the genus *Physalis.* Given these feeding constraints and low larval mobility, we hypothesized that oviposition behavior also would be specialized, with females placing the eggs very close to the larval food. Instead, we found that nearly 20% of the eggs were placed on nonhost plants. Because neonate survival was much lower when eggs were placed away from the host plant, the fitness cost of these eggs on nonhosts was substantial. Nevertheless, 3.5-fold more ovipositions were on *Physalis* than on nonhost plants. In addition, 88% of the eggs laid on nonhosts were oviposited within 15 cm of the host plant, and significantly more alightments on *Physalis* were followed by abdomen curls or probes than on nonhost plants. Collectively, these observations showed that females recognized and responded positively to the presence of their host plant.

Despite the fact that *H. subflexa* larvae feed only on the reproductive parts of the *Physalis* plant, and despite the enemy-free space the fruits provide (Sisterson and Gould 1999), *H. subflexa* females oviposited most of their eggs on unsuitable nonfruit plant parts. Although *H. subflexa* females differentiate and recognize their host plants from nonhosts, they do not preferentially oviposit on reproductive parts of the *Physalis* plant. This may be because of surface area availability, as calyxes represent a much smaller proportion of the plant surface area compared with leaves and stem combined. *Heliothis virescens,* a highly polyphagous moth closely related to *H. subflexa,* preferentially oviposits on the tobacco inflorescences, the most suitable larval food source on the plant (Lingren et al. 1977; personal observation). However, this does not happen when *H. virescens* and other heliothines that have been examined (all polyphagous) oviposit on cotton, another host plant (Fitt and Boyan 1991 and references therein, Jallow et al. 2001). Nuessly and Sterling (1994) found that higher rates of predation on *Helicoverpa zea* eggs occurred in the upper regions of the cotton plant, where *H. zea* females prefer to oviposit. Thus, both the generalists and the recently evolved specialist, *H. subflexa,* oviposit most of their eggs on suboptimal plant parts. Interestingly, a similar pattern has been observed in another specialist herbivore, *Crocidolomia pavonana* (F.), which feeds on cabbage, *Brassica oleracea* L.; females oviposit on nonpreferred plant parts and the neonates wander to the optimal feeding site on the plant (Takeuchi et al. 2009). Further studies quantifying fitness effects of oviposition on nonreproductive *Physalis* parts by *H. subflexa* are needed.

Most cases of eggs being laid on nonhosts involve plant species that are hosts of other races of the same insect species (Thompson 1988, Scriber et al. 1991); less suitable hosts (e.g., Jaenike 1978, Janz 2003); or taxonomically related to the host species (Chew 1977, Renwick and Chew 1994). Chew (1977) did report two noncrucifer ovipositions (of 224 observed ovipositions) by crucifer specialists, highlighting the rarity of this event, at least for the butterflies she studied. The intercropping literature provides examples of oligophagous moths laying 20–30% of their eggs on nonhosts that are phylogenetically distant from the host plants (Ampong et al. 1994, Zhang and Liu 2006). Polyphagous *Helicoverpa* spp. laid 56% of their eggs on phylogenetically distant nonhosts (Sequeira et al. 2001).

Studies that resolve where in the behavioral process the decision is made to accept or reject an oviposition site can help elucidate how insects perceive their host plants (e.g., Ramaswamy et al. 1987). In the current study, there was a consistently short distance between the nonhost plants on which females oviposited and the nearest host plant. In 2005, nonhosts as far from the host plant as 135 cm were available, but eggs were always laid within a 91-cm radius, with most ovipositions much closer. In addition, we found that once the moth curled her abdomen on a plant, the likelihood that she will lay an egg is the same regardless of whether she is on a host or nonhost plant, suggesting that contact of the ovipositor sensilla with the plant surface (a result of the abdomen curl) has no effect on the decision to oviposit. In contrast, Ramaswamy (1990) found that abdomen curls by *H. virescens* on cotton plants did result in oviposition more often than on screen, although an ablation study found no evidence that ovipositor receptors of *H. virescens* were involved in host-plant perception (Ramaswamy et al. 1987).

Plant volatiles have been shown, in many cases, to elicit oviposition on nonhosts as well as on artificial substrates such as cheesecloth and paper (Renwick and Chew 1994). *Physalis* volatiles elicit attraction (Tingle et al. 1990) and oviposition (Mitchell et al. 1990) in *H. subflexa* females. However, in both of these studies, 12–50% of the females were attracted to or oviposited on nonhosts. Similarly, in laboratory cage experiments conducted by our research group (Sheck and Gould 1995, Sisterson 1997, Benda 2007), some eggs were laid on or near nonhosts, regardless of whether contact with the whole plant was allowed. The results of the current study suggest that at least some of these 'lab artifact' ovipositions (Chew and Robbins 1984) reflect the natural tendency to oviposit on nonhosts. Moreover, the close proximity of all *H. subflexa* ovipositions to *Physalis* plants suggests that females perceive features of the host plant even during nonhost ovipositions.

Theory predicts that a fitness cost, or selective advantage, of 0.002 is sufficient to fix a monogenically inherited trait within 10,000 generations (Roughgarden 1979). Given that the fitness costs of nonhost oviposition estimated in this study are much larger than this, oviposition on nonhosts should be strongly selected against. Four larvae were found in the negative control plot in the third replicate of the study of larval host location, indicating a possible infestation by wild *H. subflexa*, *H. virescens*, or *Helicoverpa zea*. Early-instar larvae of these three species are indistinguishable (Brazzel et al. 1953). If some of the larvae found in the other plots were offspring of wild moths, rather than from the eggs we placed in the field, this could overestimate the effect of hatch distance from the host plant.

The literature on oviposition specialization provides several interesting hypotheses to explain the mechanistic and evolutionary underpinnings of what appear to be oviposition mistakes. Primary among mechanistic explanations is the idea that neural and other constraints on perception may limit the ability of the female to discriminate among some plants (Bernays 2001, Janz 2003). The evolution of oviposition behavior may not yet be optimized because of a constraint of genetic variation (Chew 1977). Perhaps the phylogenetically recent evolution of monophagy in *H. subflexa* has allowed, thus far, for development of only some of the fine-tuned behaviors found in deeper lineages of specialized lepidopterans. Environmental and physiological factors (such as plant apparency, previous experience, and egg load) may also lead to oviposition on plants unsuitable for larval development (Chew and Robbins 1984). A contrasting possibility is that the 'suboptimal' oviposition behavior is not really suboptimal. The fitness costs of oviposition on nonhosts that we found could be balanced by other fitness benefits of such behavior (Bernays and Graham 1988). For example, *Physalis* leaves sometimes respond to the presence of *H. subflexa* eggs by developing necrosis, neoplasms that decrease egg survival in the field, or both (Petzold 2009).

Variation among wild females in the field in both the percentage of eggs placed on different plant parts, and the percentage of eggs placed on nonhosts is difficult to explain. Contrary to the neural limitations hypothesis, we found that there were no relationships between either the rate of oviposition or the rate of movement and either 1) percentage of eggs placed on palatable plant parts or on nonhosts by individual moths, or 2) distance of nonhost ovipositions from the host plant (Benda 2007). These relationships would have suggested a trade-off between efficiency and ideal egg location. Longer per-moth observations or an additional factor such as moth age, time of night, or experience may better explain the observed variation.

Further studies of *H. subflexa* and other specialists recently derived from generalists will improve our understanding of how herbivores evolve optimal oviposition. Meanwhile, ongoing research on a host-plant response to *H. subflexa* eggs may demonstrate that nonhost plants offer a more benign environment for *H. subflexa* eggs than *Physalis* plants.

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