Why Oviposit There? Fitness Consequences of a Gall Midge Choosing the Plant's Youngest Leaf

G.A.S.M. GANEHIARACHCHI,[1] KIRK M. ANDERSON,[2] JASON HARMON,[2] AND MARION O. HARRIS[2,3]

Environ. Entomol. 42(1): 123–130 (2013); DOI: http://dx.doi.org/10.1603/EN12213

ABSTRACT For animals that lay eggs, a longstanding question is, why do females choose particular oviposition sites? For insects that lay eggs on plants there are three hypotheses: maximizing suitable habitat for juveniles, maximizing female lifespan, and maximizing egg survival. We investigated the function of the oviposition-site choice behavior of a gall midge, the Hessian fly, *Mayetiola destructor* (Say). In spite of living less than a day and having hundreds of eggs, the ovipositing female is choosy about the placement of eggs. Choosiness makes sense. The tiny gall-making neonate larva has limited movement and strict requirements for colonization. We examined whether offspring benefit from the Hessian fly female's preference for the plant's youngest leaf. To do this we restricted the female's access to the first, second, or third leaf of a seedling (wheat *Triticum aestivum* L.) plant. Being placed on older leaves did not impact egg survival or larval survival during migration to attack sites at the base of the plant, but did have negative impacts on egg-to-adult survival (reduced by 48%) and reproductive potential (reduced by 30–45%). These negative impacts appear to come from larvae having to search harder to find the limited number of reactive plant cells that can be reprogrammed to form the gall nutritive tissue. We propose that the ability of larvae to find these reactive cells in spite of being placed on an older leaf is important because it creates leeway for female behavior to evolve in the face of other selection pressures, e.g., attack by egg parasitoids.

KEY WORDS behavior, Cecidomyiidae, insect–plant interactions, preference, performance

For the hundreds of thousands of insect species that lay eggs on plants (Strong et al. 1984), important contributions to offspring survival and phenotype come from the behavior of the adult female. Many species show nonrandom oviposition behavior (Bernays and Chapman 1994), selecting a narrow range of plant species as well as choosing particular sites within each plant. A long-standing question is, what is the function of these choices?

A recent review of oviposition-site choice across a range of animals, including fish, reptiles, birds, and insects, examined six hypotheses that seek to explain oviposition-site choice (Refsnider and Janzen 2010). Three are relevant to phytophagous insects. The first hypothesis is that the female selects sites that maximize survival during the egg stage, most commonly by minimizing impacts of predation or conspecific competition. The second hypothesis is that, in choosing particular sites, the female maximizes her own survival. This hypothesis is most relevant for iteroparous species with multiple breeding periods, which need to find other things other than oviposition sites to extend adult lifespan and continue to produce eggs, e.g., adult food and multiple mates (Courtney 1981, Thornhill and Alcock 1983, Forister et al. 2009). The third hypothesis is that sites are chosen to place juveniles in suitable habitat, e.g., habitat that maximizes finding food for growth and development locations or habitat that minimize threats from predators. Refsnider and Janzen (2010) ended with a plea for more studies linking oviposition decisions to fitness, as well as more studies on the third hypothesis, this being an "underappreciated driver of oviposition-site choice."

Selection pressure to optimize oviposition-site choice is likely to be strong for species that have neonate larvae that cannot move to a better location if the ovipositing female makes a poor choice (Jaenike 1978, Thompson 1988). The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is an example of such an insect, having a soft-bodied neonate larva that is tiny (≈460 μm in length, Harris et al. 2010) and slow-moving, crawling at a rate of 2.5 cm/h (McColloch and Yuasa 1917). Finding a good location for offspring is made more difficult by the Hessian fly's gall-making habit (Harris et al. 2003, 2006). This means that, instead of simply taking food from the plant, the larva manipulates the plant to become susceptible by reprogramming the plant's "reactive" cells to become a gall nutritive tissue (Weis et al. 1988, Bronner 1992, Rohfritsch 1992, Stone and Schönrogge 2003). Finding the plant's "reactive" cells is not trivial. Only a tiny

[1] Department of Zoology, University of Kelaniya, Kelaniya 11600, Sri Lanka.
[2] Department of Entomology, North Dakota State University, Fargo, ND 58108.
[3] Corresponding author: Marion Harris, Department of Entomology, North Dakota State University, NDSU Department 7650, PO Box 6050, Fargo, ND 58105 (e-mail: marion.harris@ndsu.edu).

fraction of the millions of cells that comprise the plant can be forced to create the nutritive tissue (Weis et al. 1988, Bronner 1992, Rohfritsch 1992). For the Hessian fly, these cells are the undifferentiated epidermal cells found in zones of cell elongation at the base of the plant, near the apical meristem (Harris et al. 2006). Once these reactive cells are found, the larva uses its tiny mandibles (1 μm in length by 0.1 μm in diameter, Hatchett et al. 1990) to apply secreted salivary effector proteins (Stuart et al. 2012). Effectors are common features of plant parasites (e.g., fungi, bacteria, oomycetes, nematodes, mites, and insects) and are introduced to the plant to interfere with a variety of functions, including plant defense (Hogenhout et al. 2009).

A question is, does the oviposition-site selection behavior of the Hessian fly help the larva find the reactive cells that are needed to create the nutritive tissue? Assistance is limited at best because of two features of the host plant. The first is that the reactive cells, being near the apical meristem at the base of the seedling plant, are sheltered within the encircling sheaths of older leaves (Kemp 1980) and therefore are not accessible to the female's ovipositor, which places eggs on leaf surfaces rather than piercing plant tissue. The second reason is that during the 3–4 d that lapse between the time when the egg is placed on the plant and the time when the larva ecloses from the egg (Gagné and Hatchett 1989), the focus of growth within the seedling wheat plant shifts from one leaf to another (Kemp 1980, Anderson and Harris 2006). This means that the location of reactive cells also shifts over time.

On a seedling wheat plant, the ovipositing Hessian fly invariably places eggs on the leaf blade rather than the leaf sheath and, among the plant's blades, has a strong preference for the plant's youngest leaf (Harris and Rose 1989). We tested whether this preference benefits offspring survival, growth, and reproductive potential. Oviposition site was manipulated by restricting the female's access to just one leaf of a three-leaf wheat seedling, i.e., the first, second, or third leaf. It was possible to manipulate female choice in this way because, over the course of 1–2 h, Hessian flies deprived of preferred oviposition sites eventually oviposit on less preferred sites (Harris and Rose 1989).

Materials and Methods

Plants and Insects. Studies were conducted over the course of a year. Wheat (*Triticum aestivum* L., susceptible 'Newton') seeds were planted in plastic conetainers (3.8 cm in diameter by 21 cm in height; Stuewe and Sons, Inc., Corvallis, OR) containing a potting mix (Sunshine SP 100; Sun Gro Horticulture Inc., Bellevue, WA). Seedlings were grown to the three-leaf stage under controlled conditions (20 ± 2°C, 60–70% RH, a photoperiod of 16:8 [L:D] h, and 590–710 micromoles/m^2/S light intensity) in a plant growth chamber (E-8 model; Conviron, Winnipeg, Canada). Seedlings were watered daily and fertilized every 7–10 d with Jack's Professional 20:20:20 (N:P:K) Fertilizer (J.R. Peters, Inc., Allentown, PA). Seeds were sown every other week for a continuous supply of plants.

The Hessian flies used in tests originated from a culture of the "Great Plains" biotype provided by S. Cambron and J. Stuart (United States Department of Agriculture [USDA], Purdue University, West Lafayette, IN). This culture is reared continuously on *T. aestivum* 'Reeder' in the North Dakota State University (NDSU)/USDA greenhouse complex in Fargo, ND. The "Great Plains" biotype is avirulent (i.e., dies on) on wheat lines that carry any of the following resistance genes: *H3*, *H5*, *H6*, *H7H8*, *H9*, *H13*, or *H26* (Harris et al. 2006, 2010, 2012).

Experiment 1: Oviposition-Site Choice. The experimental design was a randomized complete block. Four groups of plants (blocks) were planted 1 d apart. On each of the 4 d of the experiment, six individual females were tested for their choice of leaves. For each female, six plants in three-leaf stage were set up in a 2 by 3 array (≈5 cm apart) in a circular cage (30 cm in diameter by 40 cm in height). Cage walls were made from blue construction paper, with a ceiling of cotton mesh. Females had eclosed and mated earlier the same day (0600–0700 hours) and were introduced individually into a cage at 1000 hours, before the onset of oviposition, which continues uninterrupted until the female dies, usually by midafternoon (Harris and Rose 1989, 1991). Plants were removed after death of the female so that the total eggs per female represented her lifetime reproductive output. Eggs on each of the three leaves of the seedling were counted under the microscope. Oviposition on the three leaves was analyzed using analysis of variance (ANOVA) (JMP Statistical Software, version 9, SAS 2012), including a leaf variable for the three leaves available for the ovipositing female and a random blocking variable for the four groups of plants. The statistical model took into account that the six plants presented to each female might not be good independent measurements and it would very likely violate the assumption of independence in the ANOVA if we used the response from each plant separately. Thus, we summed, over all six plants presented to each female, the eggs found on the first, second, or third leaves. Thus, each of the 24 females had a single response for each leaf type. We ran the model by using either the proportion of eggs each female laid on each leaf type or the total number of eggs laid by each female on each leaf type. However, because both response variables showed the same pattern, we only present the proportional data. Means separations were performed by the Tukey–Kramer HSD test at $P < 0.05$ (JMP Statistical Software). Many papers have debated the best analysis of multiple choice preference tests such as the one we just described (reviewed in Bruzzone and Corley 2011). Therefore, we used a second analysis to help verify the results of the perhaps overly simple, but commonplace use of an ANOVA. To do this we used a multivariate method that calculates Hotelling's T^2 statistic based on normalized data. We followed the analytical method of Lockwood (1998) to help verify a statistical difference in eggs laid by leaf type across experimental units. Calculations were performed "by hand" in a Microsoft Excel spreadsheet.

Experiment 2: Offspring Survival, Movement, and Adult Size. The experimental design was a randomized complete block. For each of the 10 blocks of 12 wheat seedlings grown to the 3-leaf stage, four plants were assigned randomly to one of three treatments: 1) eggs on the first leaf, 2) eggs on the second leaf, and 3) eggs on the third leaf. To control where the female placed eggs, a single mated female that had not been exposed to plants was introduced into a small glass vial (1 cm in diameter by 6 cm in height) at 1300 hours. The vial then was inverted, placed over the chosen leaf, and held there until five to 15 eggs had been deposited on the adaxial surface of the leaf. After removing the vial and female, the number of eggs was counted under a microscope and recorded. Each female was only used to infest a single leaf. Infesting plants with eggs was carried out in a controlled temperature chamber (fluorescent lights, 20 ± 2°C, 70–80% RH). After being infested, plants were held for 3 d in a plant growth chamber (20°C, 60–70% RH, a photoperiod of 16:8 [L:D] h, and 590–710 micromoles/m^2/S light intensity; E-8 model, Conviron) and then were moved (at 1500 hours) to a higher humidity chamber for 48 h (20 ± 2°C, 70–80% RH, and a photoperiod of 16:8 [L:D] h). The elevated humidity improves larval survival during eclosion from the egg as well as during migration down the leaf blade to the bundled leaf sheaths (McColloch and Yuasa 1917). Plants then were returned to the lower humidity of the plant growth chamber and remained there until being sampled.

Three days after eclosion of larvae from eggs, two of the four plants assigned to each leaf treatment were sampled destructively between 0900 and 1000 hours to score survival during the egg and migratory stages, as well as the location of individual larvae. This time interval was chosen because larvae have had time to migrate to the base of the plant (this taking ≈6–14 h, McColloch and Yuasa 1917) and also have had ≈2.5 d to search for attack sites. Under the microscope, the following data were recorded: 1) number of unhatched eggs on the leaf surface, 2) number of larvae at the base of the plant, and 3) the leaf that the larva was found on. Hessian fly larvae always feed on the abaxial leaf surface within 2 cm of the base of the leaf, with the head facing toward the leaf base (McColloch and Yuasa 1917, Harris et al. 2006).

The other two plants assigned to each leaf treatment were held in the plant growth chamber for 20 d. By this time, Hessian fly larvae have completed feeding, formed a puparia, and pupated (Gagné and Hatchett 1989). Each plant was removed from the soil and soil was washed away from the roots. Leaves and roots of each plant were trimmed so that a 5-cm section of the base of the plant and 2 cm of roots remained. This section of the plant contained all pupae, which were still located at the site where they had fed. This trimmed plant then was placed in a glass vial (3 cm in diameter by 8 cm in length). A 2-cm-deep layer of moist potting mix was placed around the roots to ensure that the plant remained viable. This soil layer then was covered with a 1 cm layer of moist white sand, with this making it easier to find the adults that emerged from the plant. Each vial was sealed with a plastic lid having an inset of a fine mesh (1.5 cm in diameter) to allow air exchange. Vials were held in a high humidity chamber (20 ± 2°C, 70–80% RH, and a photoperiod of 16:8 [L:D] h) and checked daily (0900–1000 hours) for adults. Adults were removed using an aspirator and placed in a labeled vial containing 70% ethanol. Wing length was recorded using the method of Bergh et al. 1990). Each measurement (one wing per fly) was made to the nearest 0.01 mm by using an eyepiece micrometer (20×). The start point of the measure was the axillary sclerite and the end point was the radial sector vein. Adult emergence was recorded for 7 wk after emergence of the first adult.

Data were tested for normal distribution and homogeneity of variance (O'Brien's test, JMP Statistical Software). Survival was analyzed using ANOVA (JMP Statistical Software). Survival during the egg stage was calculated as hatched eggs per plant divided by total oviposited eggs per plant. Survival during migration was calculated as the number of larvae found at the plant base divided by the total larvae that hatched from eggs. Egg-to-adult survival was calculated as the number of adults that emerged from the plant divided by the total eggs per plant. The statistical model included a leaf variable for the three leaves and a blocking variable for the 10 groups of plants. Adult wing lengths were analyzed using ANOVA (JMP Statistical Software). The statistical model included a leaf variable for the three leaves and a blocking variable for the 10 groups of plants. Means were separated using the Tukey–Kramer HSD test at $P < 0.05$. To determine if oviposition-site choice influenced where larvae ended up attacking the plant, we used the same model structure in a nominal logistic model (JMP Statistical Software). Here response variables were if the larva 1) remained to attack the adjacent younger leaf, 2) moved within the bundled leaf sheaths to attack a younger leaf, or 3) moved within the bundled leaf sheaths to attack an older leaf.

Experiment 3: Oviposition Choice, Feeding Choice, and Larval Growth. Design was a randomized complete block design with three blocks completed over a 2-mo period. Methods were identical to those described for experiment 2, again with three leaf treatments achieved by restricting access of ovipositing females to either the first, second, or third leaf. However, here plants were sampled 10 d after the day of larval eclosion (0900–1000 hours) to determine if larvae had remained in the place where the female laid her eggs or moved to attack at a different location. Ten days was chosen because this is when larvae have almost completed feeding (Gagné and Hatchett 1989). Each larva was scored as having fed on the third or fourth leaf (no other locations were found) and then measured for body length (to the nearest 0.01 mm) using a Nikon SMZ800 microscope (Nikon, Tokyo, Japan) fitted with an eyepiece micrometer (Fryer Company, Minneapolis, MN).

For each given plant, the number of larvae found on the third leaf and the fourth leaf are probably not independent, therefore, we calculated the difference

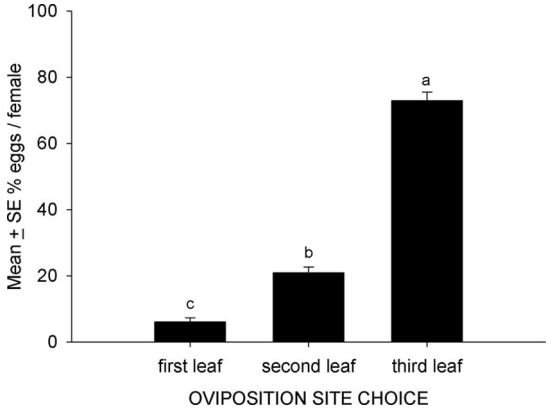

Fig. 1. Oviposition-site choice by Hessian fly females ($n = 24$) given a choice between the three leaves of a wheat seedling. The first leaf is the oldest leaf and the third the youngest. Means accompanied by a different letter are significantly different at $P < 0.05$ (Tukey–Kramer HSD test).

in the number of larvae found on the two leaves for each plant in the experiment. We then used that data to see if oviposition site influenced this difference in where larvae were found. To look for effects of oviposition site and feeding site on larval length, we used the same procedure as above for treatments where females laid eggs on the first or second leaf. Because no larvae were found to attack the third leaf when the eggs were laid on the youngest (third) leaf, we asked an additional question, which is if the larval length varies by oviposition location for only those larvae attacking the fourth leaf. Data for all of these analyses were tested for normal distribution and homogeneity of variance (O'Brien's test, JMP Statistical Software) and analyzed using ANOVA (JMP). The statistical model included an oviposition site variable and a blocking term. For the second analysis, means were separated using the Tukey–Kramer HSD test at $P < 0.05$. One additional analysis was performed to test if the distribution in differences in larval length had a mean different than zero by using a *t*-test (JMP Statistical Software). This allowed us to determine whether larvae found attacking the fourth leaf were larger than those attacking the third leaf after accounting for plant-to-plant differences.

Results

Oviposition-Site Choice. The 24 females tested for oviposition-site choice placed 204.25 ± 17.33 (mean ± SEM) eggs on the six plants they were given before dying (range, 81–367 eggs per female). Females laid their eggs differently across the three leaf types (Fig. 1; $F_{2, 66} = 333.11$, $P < 0.0001$), a result we confirmed using a more complicated multivariate analysis ($T^2_{2, 22} = 553.8$, $P < 0.0001$) that helps to account for potential problems that arise from the assumption of independence among data points. The majority of eggs were placed on the youngest leaf (Tukey–Kramer, $P < 0.05$). Percentages of eggs placed on the youngest leaf ranged from 35 to 95%. However, the vast majority of females (90%) placed ≥60% of their eggs on the youngest leaf.

A second analysis compared the effects of leaf type ($F_{2, 138} = 86.57$, $P < 0.0001$) and leaf surface ($F_{1, 138} = 181.15$, $P < 0.0001$), as well as the leaf type × surface interaction ($F_{2, 138} = 69.68$, $P < 0.0001$). The interaction was associated with a stronger preference for the adaxial surface when oviposition occurred on the second and third leaves (94.56 ± 1.38% and 94.48 ± 0.06% of total eggs per leaf type, respectively) rather than the first leaf, where the preference was less pronounced (89.26 ± 3.64% of total eggs per leaf type).

Offspring Placement, Survival, and Growth. Survival was not influenced by oviposition-site choice during the egg stage (Table 1; survival from egg placement to larval eclosion, $F_{2, 30} = 0.15$, $P = 0.87$) or during migration to attack sites at the plant base (Table 1; $F_{2, 30} = 0.63$, $P = 0.54$). Egg-to-adult survival was influenced by oviposition-site choice (Table 1; $F_{2, 30} = 7.63$, $P = 0.0013$), being almost twice that observed for offspring placed on the first or second leaf. There were no blocking effects.

Within-plant selection of oviposition site had significant effects on adult wing length (Fig. 2). For males that emerged from eggs on the first, second, and third leaf, the younger the leaf, the longer the adult wing length ($F_{2,25} = 37.09$, $P < 0.001$). A similar pattern was observed for females ($F_{2,35} = 47.14$, $P < 0.001$). There were no blocking effects.

Larval Movement and Growth on Different Leaves. Three days after initial attack at the base of the plant, placement of eggs by the adult female influenced how far larvae moved to find attack sites at the base of the plant. For eggs placed on the first, second, or third leaf, percentages of larvae moving one or more leaves were 100% ($n = 130/130$), 46% ($n = 66/144$), and 0% ($n = 0/119$) ($\chi^2_2 = 346.18$, $P < 0.0001$). When movement occurred ($n = 196$ larvae), it was always to a younger leaf. How far larvae moved was influenced by oviposition-site choice ($\chi^2_4 = 379.73$, $P = 0.0001$). Larvae placed

Table 1. Hessian fly survival after being placed in one of three plant locations by the ovipositing female

Hessian fly stage	Location of eggs		
	First leaf % survival (mean ± SE)	Second leaf % survival (mean ± SE)	Third leaf % survival (mean ± SE)
Egg stage	94.72 ± 2.29a	94.53 ± 2.95a	92.56 ± 3.13a
Migration to plant base	77.68 ± 7.00a	87.56 ± 4.17a	83.54 ± 8.01a
Egg-to-adult	30.22 ± 5.87b	30.69 ± 6.27b	57.51 ± 6.25a

Within each row, means followed by the same letter are not significantly different at $P < 0.05$ (Tukey–Kramer HSD test).

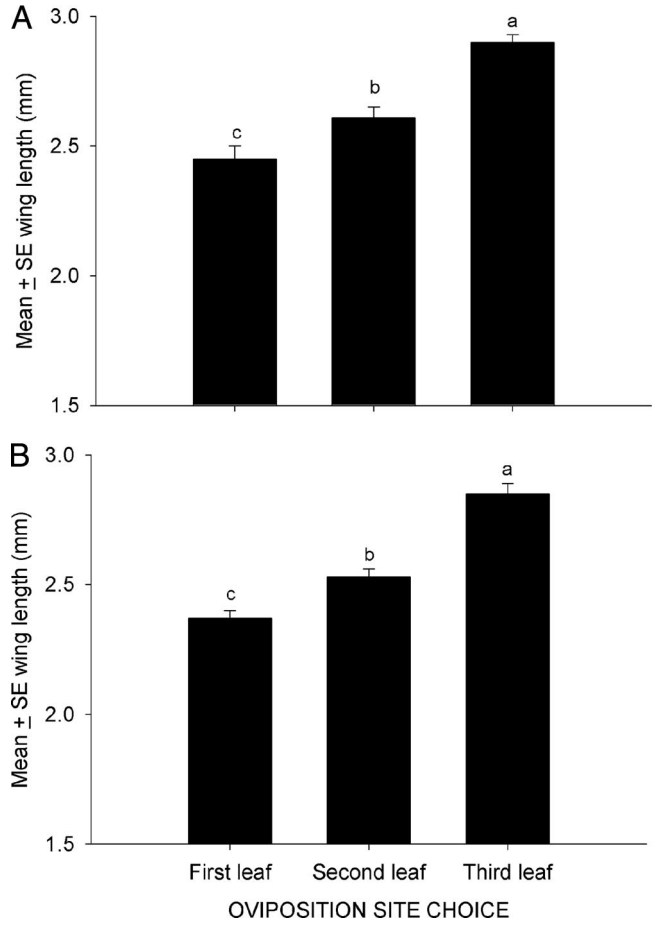

Fig. 2. Influence of oviposition-site choice by Hessian fly females on wing lengths of male (A) and female (B) offspring placed as eggs on the first, second, or third leaf of a wheat seedling. Means accompanied by a different letter are significantly different at $P < 0.05$ (Tukey–Kramer HSD test).

as an egg on the first (oldest) leaf of the seedling moved the greatest distance, with 72% moving one leaf away to attack the third leaf and the remainder (18%) moving two leaves away to attack the fourth leaf. For larvae placed on the second leaf, 54% remained in that location (i.e., they simply moved from the adaxial surface of the second leaf to attack the abaxial surface of the third leaf) and the remainder (46%) moved one leaf away to attack the fourth leaf. For larvae placed on the third leaf, 100% remained in that location (i.e., they simply moved from the adaxial surface of the third leaf to attack the abaxial surface of the fourth leaf).

Ten days after initial attack at the base of the plant, placement of eggs by the adult female influenced where larvae were found at the end of the feeding stage (Fig. 3A). Because Hessian fly larvae become sessile during the first instar once a feeding site is accepted and lose the creeping pads that are necessary for walking upon molting to the second instar (Gagné and Hatchett 1989), the larva's location at 10 d indicates where the larva fed throughout its feeding period. The difference between the number of larvae found feeding on the third leaf and the number of larvae found feeding on the fourth leaf of that same plant depended on where eggs had been placed (Mean ± SEM: for eggs on first leaf, 1.83 ± 0.48 more larvae found on third leaf compared with the fourth leaf; for eggs on second leaf, 0.83 ± 0.60 more larvae found on third leaf; for eggs on third leaf, 9.00 ± 0.26 more larvae found on fourth leaf; $F_{2,13} = 430.1$, $P < 0.0001$). None of the larvae eclosing from eggs placed on the first leaf remained in the location where oviposition occurred (Fig. 3A). Instead larvae moved either one leaf away to attack the third leaf (67%) or two leaves away to attack the fourth leaf (33%). Larvae eclosing on the second leaf either moved between two adjacent surfaces (56% moved from the adaxial surface of the second leaf to the abaxial surface of the third leaf) or moved one leaf away to attack the fourth leaf (44%). All larvae eclosing on the third leaf stayed in this location, simply moving from the adaxial surface of the third leaf to the abaxial surface of the fourth leaf. Across the three oviposition-site treatments, no larva moved to settle a leaf on an older leaf.

The original intent of the experiment scoring larvae at 10 d was to investigate the interactive effects of

oviposition site and feeding site on larval growth (Fig. 3B). However, although larvae placed as eggs on the first and second leaves distributed themselves between the third and fourth leaves, larvae that were oviposited on the third leaf were found only on the fourth leaf. This negated the three oviposition sites × two feeding sites analysis and made us ask different questions of the data. The first question looked at differences, within a single plant, in the size of larvae attacking the third leaf compared with those found attacking the fourth leaf. We found that this difference in larval size between the third and fourth leaf was similar and not significantly different for eggs oviposited on the first or second leaf (for eggs on the first leaf, larvae were on average 0.29 ± 0.07 mm longer when found attacking the fourth leaf compared with the third leaf; for eggs on the second leaf, larvae were on average 0.30 ± 0.06 mm longer on the fourth leaf; $F_{1,8} = 0.04, P = 0.85$). Because there was no difference across the two treatments, we combined the data and found that the larvae on the fourth leaf were significantly larger than larvae found on the third leaf. Thus, the distribution of values for the size of larvae on the fourth leaf minus the size of larvae on the third leaf of the same plant was significantly greater than zero ($t_{11} = 6.72, P < 0.0001$).

When looking at just those larvae found attacking the fourth leaf (Fig. 3B, black bars), oviposition site influenced larval size ($F_{2,13} = 21.58, P < 0.0001$), with larvae oviposited on the third leaf significantly larger than those oviposited on the second leaf, and those significantly larger than the larvae oviposited on the first leaf ($P < 0.05$).

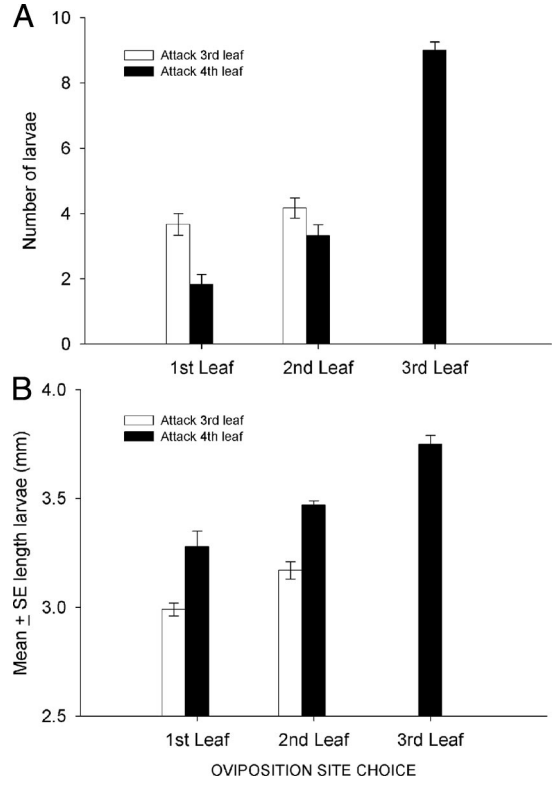

Fig. 3. Influence of oviposition-site choice by Hessian fly females on (A) where larval offspring were found feeding and (B) body length of larvae 10 d after attack of the grass seedling commenced.

Discussion

Hessian fly females exhibited oviposition-site choice by placing almost three-quarters of their eggs on the youngest leaf (Fig. 1). Choice of site within the leaf was even more specific, 95% of eggs being placed on the adaxial leaf surface. The significance of the Hessian fly's preference for the youngest leaf is suggested by its consistency across geographically widespread populations (70–75% of all eggs on the youngest leaf), specifically this population and another North American population tested decades ago (Harris and Rose 1989) and a third population from New Zealand (Kanno and Harris 2000, Harris et al. 2001). Nevertheless, the allocation of eggs to the youngest leaf is not so rigid that 100% of eggs are placed on the youngest leaf. Moreover, females varied in how choosy they were, with proportions of lifetime eggs allocated to the youngest leaf ranging from 35 to 95%.

One benefit of the female's preference for the youngest leaf is improved offspring survival. Egg-to-adult survival (Table 1) was almost twice as much for eggs placed on the third leaf versus eggs placed on the two older leaves. Negative impacts of being placed on older leaves did not occur during the egg stage or during migration from egg-laying sites to the plant base (Table 1), when survival was uniformly high across the three leaf treatments. Eggs can be harmed by oviposition-site choice when the female chooses a location that is more likely to be visited by predators (Schellhorn and Andow 1999) or when the location has its own direct defense against eggs (Doss et al. 2000, Hilker and Meiners 2011). For the latter, eggs can be killed or ejected from the plant through responses such as induced neoplasms, programmed cell death, and production of toxins. Such effects on Hessian fly eggs have not been observed either for host or nonhost plants (reviewed in Harris et al. 2003).

Another benefit of the female's preference for the youngest leaf is improved growth. Evidence for this comes from measures of larvae (Fig. 3B) and adults (Fig. 2), sizes being significantly greater when eggs are placed on the youngest leaf. For Hessian flies, size translates into reproductive potential because adults do not feed (Bergh et al. 1990). For the female, size determines the number of mature eggs carried at the time of adult eclosion. These eggs represent the female's full potential because gall midges are semelparous as well as being pro-ovigenic and cannot extend their lifespan by feeding or resorbing eggs (Rosenheim et al. 2007, 2008). For the male, size determines the number of females that can be inseminated (Bergh et al. 1990).

Using Hessian fly mean wing lengths across the three oviposition-site treatments (Fig. 2) and the relationships between adult size and reproductive po-

tential that were generated by Bergh et al. (1990), we can estimate the impact of oviposition-site selection on the female's offspring, which in the Hessian fly are usually unisexual (Stuart et al. 2012), being either all male or all female. From the relationship generated for female Hessian flies by Bergh et al. (1990, number of eggs = $-309 + 212 \times$ wing length), we estimate that that eggs placed on the first, second, or third leaf produce female offspring with 144, 190, or 269 eggs, respectively. From the relationship generated for male Hessian flies (Bergh et al. 1990, the number of eggs the male is able to fertilize = $-11{,}307 + 5{,}921 \times$ wing length), we estimate that that eggs placed on the first, second or third leaf produce males able to fertilize 3,259; 4,147; or 5,686 eggs, respectively, with these eggs representing the lifetime fecundity of 15, 19, or 30 females. These estimates indicate that, regardless of whether the offspring is male or female, eggs placed by the female on the first or second leaf have, on average, 50–75% of the reproductive potential of eggs placed on the youngest leaf.

We discovered that the Hessian fly larva also makes choices. Thus, instead of being doomed to accept a bad choice made by its mother, a larva can leave that location and move in hopes of finding a better location within the plant. This sounds like a simple task but is complicated by the architecture of grass seedlings (Kemp 1980), which places the reactive sites required by larvae at the center of a labyrinth of encircling leaf sheaths. A female that places her egg on the youngest leaf saves her offspring from the added effort of having to find reactive cells by moving through this labyrinth. Instead the larva simply crawls down the leaf where it was placed as an egg to the plant base where reactive cells can be contacted by shifting from one adjacent surface to another. As can be seen in Fig. 3A, this is what all larvae eclosing on the youngest leaf did, i.e., they stayed in the location chosen by the female, simply shifting from the adaxial surface of the third leaf to the adjacent abaxial surface of the fourth leaf. In contrast, offspring of females that placed their eggs on the plant's oldest leaf must move away from that location and find younger leaves (Fig. 3A). Two-thirds of the larvae that survived moved to attack the next younger leaf (the third leaf), with the rest moving to attack the plant's youngest leaf (the fourth leaf). Offspring of females that placed their eggs on the second oldest leaf were intermediate in their movement (Fig. 3A), a little over half remaining in the location chosen by the female and the rest moving to attack the adjacent fourth leaf. We conclude from this that Hessian fly larvae have some way of determining whether epidermal cells can be manipulated to create nutritive cells. If not, the behavioral response of the larva is to continue moving, testing other cells at intervals to determine if the site is acceptable and movement can stop. We doubt that larvae follow directional cues to find the required reactive cells. Presumably some of the larvae that move end up dying because they never find reactive cells, this being the reason that egg to adult survival was reduced for offspring placed on older leaves (Table 1).

We propose that the Hessian fly larva's contribution toward finding "reactive" cells is important because it creates leeway for female behavior to evolve in the face of other selection pressures. If larvae were not able to move from a "bad" site chosen by the female to find a better site, the ovipositing female would be wasting her eggs if they were placed anywhere other than the plant's youngest leaves. But because larvae can move to find better attack sites (at least to some degree), this frees oviposition-site choice from being limited to the youngest leaf. This variable oviposition site selection in turn may benefit offspring by hindering discovery by natural enemies. The Hessian fly egg is the stage most at risk of predation. The adult has too short of a lifespan and spends too much time flying to be targeted by predators, although web-building spiders are a hazard for females (Barnes 1956). Larva and pupa are not vulnerable because they are sheltered within the encircling leaf sheaths. In contrast the Hessian fly egg sits exposed on leaf surfaces for 3–4 d and is attacked by a large number of parasitoids throughout its geographic range (Hill 1926, Barnes 1956, Gagné 1989, Withers and Harris 2005). In North Dakota it is not uncommon for 90% of the fall generation to be attacked by egg parasitoids (M.O.H. and K.M.A., unpublished data). An experiment to test the impact of oviposition-site choice could include the three treatments used in the current study, as well as a fourth treatment having a natural distribution of eggs over the three leaves, i.e., 5% on the first leaf, 20% on the second leaf, and 70% on the third leaf (Fig. 1). We predict that overall parasitism is lower for the naturally-occurring distribution of eggs than for a distribution of eggs that places all eggs on the youngest leaf. Escape from attack by natural enemies has been proposed as an important selection pressure for the evolution of oviposition-site choice (Bernays and Graham 1988, Mayhew 1997) but has been studied very little relative to selection pressures from plants and competitors.

Acknowledgments

We thank Emilie Amiot and Francois Bousselin (Ecole Superieure D'Agriculture D'Angiers, Angers, France) for initiating studies on the function of oviposition-site selection, Jeff Stuart (Purdue University) and Sue Cambron (USDA-ARS, Purdue University) for supplying us with Hessian fly, and Imelda Miller for patiently measuring thousands of Hessian fly wings. Hessian fly voucher specimens have been deposited in the North Dakota State University Insect Reference Collection, Fargo, ND. Research was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grants 2005-35302-16254, 2009-35302-05262, and 2011-67013-30211.

References Cited

Anderson, K. G., and M. O. Harris. 2006. Does *R* gene resistance to Hessian fly allow wheat seedlings to escape larval-induced growth deficits? J. Econ. Entomol. 99: 1842–1853.

Barnes, H. F. 1956. Gall midges of economic importance to cereal crops. Crosby Lockwood, London, United Kingdom.

Bergh, J. C., M. O. Harris, and S. Rose. 1990. Temporal pattern of emergence and reproductive behavior of the Hessian fly (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 83: 998–1004.

Bernays, E. A., and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman & Hall, New York.

Bernays, E. A., and M. Graham. 1988. On the evolution of host specificity in phytophagous arthropods. Ecology 69: 886–892.

Bronner, R. 1992. The role of the nutritive tissue in the nutrition of cynipids and cecidomyiids, pp. 118–141. *In* J. Shorthouse and O. Rohfritsch (eds.), Biology of insect-induced galls. Oxford University Press, New York.

Bruzzone, O. A., and J. C. Corley. 2011. Which is the best experimental design in animal choice tests? Anim. Behav. 82: 161–169.

Courtney, S. P. 1981. Coevolution of Pierid butterflies and their cruciferous food plants III. *Anthocharis cardamines* (L.) survival, development, and oviposition on different hostplants. Oecologia 51: 91–96.

Doss, R. P., J. E. Oliver, W. M. Proebsting, S. W. Potter, S. R. Kuy, S. L. Clement, R. T. Williamson, J. R. Carney, and E. D. Devilbiss. 2000. Bruchins-insect derived plant regulators that stimulate neoplasm formation. Proc. Natl. Acad. Sci. U.S.A. 97: 6218–6223.

Forister, M. L., C. C. Nice, J. A. Fordyce, and Z. Gompert. 2009. Host range evolution is not driven by the optimization of larval performance: the case of *Lycaeides melissa* (Lepidoptera: Lycaenidae) and the colonization of alfalfa. Oecologia 160: 551–561.

Gagné, R. J. 1989. The Plant-Feeding Gall Midges of North America. Cornell University Press, Ithaca, NY.

Gagné, R. J., and J. H. Hatchett. 1989. Instars of the Hessian fly (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 82: 73–79.

Harris, M. O., and S. Rose. 1989. Temporal changes in the egglaying behavior of the Hessian fly, *Mayetiola destructor.* Entomol. Exp. Appl. 53: 17–29.

Harris, M. O., and S. Rose. 1991. Factors influencing the onset of egglaying in a cecidomyiid fly, *Mayetiola destructor.* Physiol. Entomol. 161: 183–190.

Harris, M. O., M. Sandanayake, and W. Griffin. 2001. Oviposition preferences of the Hessian fly and their consequences for the survival and reproductive potential of offspring. Ecol. Entomol. 26: 1–14.

Harris, M. O., J. J. Stuart, M. Mohan, S. Nair, R. J. Lamb, and O. Rohfritsch. 2003. Grass and gall midges: plant defense and insect adaptation. Annu. Rev. Entomol. 48: 549–577.

Harris, M. O., T. P. Freeman, O. Rohfritsch, K. G. Anderson, S. A. Payne, and J. A. Moore. 2006. Virulent Hessian fly (Diptera: Cecidomyiidae) larvae induce a nutritive tissue during compatible interactions with wheat. Ann. Entomol. Soc. Am. 99: 305–316.

Harris, M. O., T. P. Freeman, K. M. Anderson, J. A. Moore, S. A. Payne, K. M. Anderson, and O. Rohfritsch. 2010. *H* gene-mediated resistance to Hessian fly exhibits features of penetration resistance to fungi. Phytopathology 100: 279–289.

Harris, M. O., T. P. Freeman, K. M. Anderson, J. P. Harmon, J. A. Moore, S. A. Payne, O. Rohfritsch, and J. J. Stuart. 2012. Hessian fly *Avirulence* gene loss-of-function defeats plant resistance without compromising the larva's ability to induce a gall tissue. Entomol. Exp. Appl. 145: 238–249.

Hatchett, J. H., G. L. Kreitner, and R. J. Elzinga. 1990. Larval mouthparts and feeding mechanism of the Hessian fly (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 83: 1137–1147.

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

Hill, C. C. 1926. *Platygaster hiemalis* Forbes, a parasite of the Hessian fly. J. Agric. Res. 32: 261–275.

Hogenhout, S. A., R.A.L. Van der Hoorn, R. Terauchi, and S. Kamoun. 2009. Emerging concepts in effector biology of plant-associated organisms. Mol. Plant Microbe Interact. 22: 115–122.

Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. Theor. Popul. Biol. 14: 350–356.

Kanno, H., and M. O. Harris. 2000. Both chemical and physical features of grass leaves influence host selection by the Hessian fly. J. Chem. Ecol. 26: 2335–2354.

Kemp, D. R. 1980. The location and size of the extension zone of emerging wheat leaves. New Phytol. 84: 729–737.

Lockwood, J. R. 1998. On the statistical analysis of multiple-choice feeding preference experiments. Oecologia 116: 475–481.

Mayhew, P. J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. Oikos 79: 417–428.

McColloch, J. W., and H. Yuasa. 1917. Notes on the migration of the Hessian by larvae. Anim. Behav. 7: 307–323.

Refsnider, J. M., and F. J. Janzen. 2010. Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. Annu. Rev. Ecol. Evol. Syst. 41: 39–57.

Rohfritsch, O. 1992. Patterns in gall development, pp. 102–117. *In* J. Shorthouse and O. Rohfritsch (eds.), Biology of insect-induced galls. Oxford University Press, New York.

Rosenheim, J. A., S. J. Jepsen, C. E. Matthews, D. A. Smith, and M. R. Rosenheim. 2007. Portrait of an ephemeral adult stage: egg maturation, oviposition, and longevity of the gall midge *Rhopalomyia californica* (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 100: 549–561.

Rosenheim, J. A., S. J. Jepsen, C. E. Matthews, D. A. Smith, and M. R. Rosenheim. 2008. Time limitation, egg limitation, the cost of oviposition and lifetime reproduction by an insect in nature. Am. Nat. 172: 486–496.

Schellhorn, N. A., and D. A. Andow. 1999. Cannibalism and interspecific predation: role of oviposition behavior. Ecol. Appl. 9: 418–428.

Stone, G. N., and K. Schönrogge. 2003. The adaptive significance of insect gall morphology. Trends Ecol. Evol. 18: 512–522.

Strong, D. R., J. H. Lawton, and T.R.E. Southwood. 1984. Insects on plants. Community patterns and mechanisms. Blackwell, Oxford, United Kingdom.

Stuart, J. J., M. Chen, R. Shukle, and M. O. Harris. 2012. Gall midges (Hessian fly) as plant pathogens. Annu. Rev. Phytopathol. 50: 339–357.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol. Exp. Appl. 47: 3–14.

Thornhill, R., and J. Alcock. 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, MA.

Weis, A. E., R. Walton, and C. C. Crego. 1988. Reactive plant tissue sites and the population biology of gall makers. Annu. Rev. Entomol. 33: 467–486.

Withers, T. M., and M. O. Harris. 2005. Influence of plant species on the foraging behaviour of *Platygaster hiemalis*, a parasitoid of the Hessian fly, *Mayetiola destructor.* N. Z. Plant Prot. 58: 197–201.

Received 20 July 2012; accepted 3 December 2012.