

**Oviposition Preference of the Leaf Mining Fly, *Scaptomyza nigrita*
(Drosophilidae) on its Native Host Plant, *Cardamine cordifolia* (Brassicaceae)**

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Abstract

I tested the oviposition preference of the leaf miner *Scaptomyza nigrita* of and within its native host plant *Cardamine cordifolia*. Gravid female *S. nigrita* were used in cafeteria-style choice tests to quantitatively evaluate feeding and oviposition preference between *C. cordifolia* from shaded and sun exposed habitats. Choice tests were conducted in two parts using whole plants and detached leaves. In addition, preference was examined within plants looking at leaf position. Gravid females chose to feed and oviposit on shade plants significantly more frequently than on sun plants in both experiments. Females also feed and oviposited more on lower (basal) leaves than on upper leaves (cauline).

Key words: *Cardamine cordifolia* – *Scaptomyza nigrita* – oviposit – stipple – choice test

Introduction

Many insects specialize on host plant lineages to varying degrees. For example, most herbivorous insects restrict their feeding to one or a few plant families (Thompson 1988). The link between preference of ovipositing female insects and larval performance and survival on the host is a key component in understanding the evolution of plant/insect interactions (Thompson 1988), and host specialization in particular. Oviposition preference of females and the relationship between larval performance and survival varies among and within species, ranging from strong to weak, and can correlate with local environment (Thompson 1988). Female *Scaptomyza* flies are phytophagous, and use their ovipositors to cut through the lower epidermis of the host plant leaf and consume the exudates (Collinge and Louda 1989; Whiteman et al 2010). Subsets of these punctures are used for oviposition (Whiteman et al 2010).

The larvae of the vinegar fly, *Scaptomyza nigrita*, mine the leaves of the host plant *Cardamine cordifolia*, throughout western North America eating the internal tissues of the leaf (Collinge and Louda 1989b). Herbivory of the host plant is habitat-dependent, and is significantly higher in *C. cordifolia* individuals in sunny habitats relative to those growing in consistently shaded habitats (Louda & Rodman 1996). Persistent herbivory by *S. nigrita* is suggested to influence the distribution of *C. cordifolia* (Louda & Rodman 1996). In addition, a greater concentration of herbivory and mines are observed on lower and midstem leaves (Louda & Rodman 1989). This could be due to the fact that the lowest leaves are the oldest and first available to ovipositing females or because lower leaves tend to have lower glucosinolate levels (Collinge & Louda 1989 a,b). These hypotheses are not mutually exclusive. While previous field experiments showed conflicting results with respect to a mechanism driving the sun vs. shade

distribution of herbivory in *C. cordifolia* there were no data on female behavior *per se*, specifically whether females actually prefer sun vs. shaded plants. Moreover, no preference experiments had been performed on whether adults prefer and larvae perform better on older rather than younger leaves. Thus, I examined the differences *between* host plant individuals of sun vs. shade of *S. nigrita* preference and the as well as the differences *between* leaves *within* individual plants. This was conducted using cafeteria style, choice tests on gravid adult female flies, using whole plants and detached leaves of *C. cordifolia*.

Methods

Experimental Design

Part 1 (Whole plant) - Wild *Cardamine cordifolia* plants were collected from two habitat types, heavily shaded by spruce (*Picea engelmanni*) trees, and direct sunlight. Wild female *S. nigrita* were collected from the same sites. Individual *C. cordifolia* were transplanted into small pots and examined for pre-existing herbivory or leaf minor damage which was recorded (number of stipples and eggs of *S. nigrita*). Four *C. cordifolia* plants were placed into the mesh cages (60x40x38cm), two plants from shaded and two from sun exposed habitats. The plants were randomly assigned to the four corners of the cage. Two Petri dishes were placed in the cages, each containing a piece of folded, unbleached paper towel, one moistened with water, and the other with sucrose solution in order to keep females alive for the duration of the experiment. Four adult female *S. nigrita* individuals were then placed in the cage for 24 hours and then plants were removed and feeding punctures and eggs counted. This process was replicated 8 times.

Part 1 Experimental Measurements – Prior to the addition of *S. nigrita*, total stem height, number of leaves, leaf width, leaf length (tip of leaf to the petiole) was quantified and recorded from each plant. The number of stipples (feeding punctures), and number of eggs was recorded

for each leaf. After exposure to *S. nigrita* for a 24 period, the plants were again examined and stipple number and number of eggs was recorded. .

Part 2 (Detached leaf) – Because plants from sun and shade differ in morphology, including overall height, number of leaves, petiole length, etc., I conducted a detached leaf assay to attempt to exclude variables other than those intrinsic to a leaf. Cages for detached leaf manipulations were made from clear, plastic food storage containers. Square vents (25cm²) were cut into the lids and covered with fine mesh. A 1.5cm thin layer of Phytoblend (Caisson Laboratories, North Logan, UT) plant agar was placed in the base of the container. Leaves from wild *C. cordifolia* were detached from the plant and placed at approximately 60 degree angles with the ~1cm of the petiole in the agar. Four leaves per replicate were used, and were taken from either the first or second lowest leaf. For each replicate shade (n=2) and sun (n=2). One female *S. nigrita* was placed in each replicate and left for 24 hours. This process was replicated independently 15 times, no flies were re-used.

Part 2 Experimental Measurements – Prior to experimentation individual leaves were measured for leaf width, leaf length, number of stipples, and number of eggs. After the treatment, the number of stipples and eggs were again quantified using a dissecting microscope.

Data collected were analyzed by means of ANOVA using JMP 8 (SAS Institute, Cary, NC). We used a general linear model in JMP in which log transformed (data were not normally distributed) egg number and stipple number per leaf were analyzed. Egg and stipple number were the dependent variable. Cage, light environment, leaf position, leaf area and initial number of eggs and stipples per leaf were the independent variable.

Results

Whole Plant: *Stipples*

The overall general linear model for stipple number was highly significant ($P < 0.0001$, $F_{17,335} = 8.05$), and the R^2 value for the entire model was 0.29. *S. nigrita* stippled the lower leaves with much greater frequency than upper leaves (Fig. 1; $P < 0.0001$, $F = 27.64$). *S. nigrita* chose plants from shaded habitats over those from sun exposed (Fig. 1; $F = 35.09$, $P < 0.0001$). The effect of leaf position on the number of stipples per leaf varied with the light environment ($P = 0.0027$, $F = 4.93$). Flies were more likely to stipple the lower leaves with greater frequency in plants from shaded habitats. Stipples on plants from sun exposed habitats had a more even distribution throughout the plant (Fig. 1). The difference in total stipple number varied by cage ($P < 0.0001$, $F = 5.72$). In addition, the cage effect on the number of stipples per leaf depended on light environment ($P = 0.0044$, $F = 3.01$). The initial number of stipples per leaf did not have a significant effect on the final number of stipples per leaf. Leaf area showed a significant effect on stipple number ($P = 0.0003$, $F_{1,325} = 13.38$) with an R^2 value of 0.03.

Eggs

The model for egg number was also highly significant ($P < 0.0001$, $F_{17,355} = 4.32$). The R^2 value of the overall model was 0.18. The number of eggs per leaf varied with leaf position ($P < 0.0001$, $F = 35.96$). As with stipples, eggs were more frequently oviposited on lower leaves, however, there was no significant difference in this distribution (leaf number) between sun and shade plants (Fig. 2). The number of eggs per leaf was significantly greater on plants from shaded light environment ($P = 0.0028$, $F = 9.07$), (Fig. 2). The effect of leaf position on the number of eggs per leaf was stronger for shade plants than sun plants ($P = 0.0061$, $F = 7.62$), (Fig. 3). Initial egg number did and initial egg number with respect to light environment did not have a significant effect on the final number of eggs. Cage number, and cage number by light environment, did not

have a significant effect on the number of eggs per leaf. Egg number with respect to leaf area was also significant ($P=0.02$, $F_{1,325}=5.43$) with an R^2 value of 0.02.

Detached Leaf: *Stipples/Eggs*

Stippling was significantly higher in leaves from shade plants ($P<0.0001$, $F=25.94$), and total egg number was also greater on shade leaves ($P=0.0499$, $F=4.06$), (Fig.5). The model showing stipple number with respect to leaf area was significant ($P=0.0003$, $F_{1,325}=13.38$), however the R^2 value was 0.04. The log transformed response of egg number to log transformed number of stipples per leaf showed an increase in egg number with an increase in stipple number ($P<0.0001$, $F_{1,58}=53.16$), (Fig.4). Leaf area did not show a significant effect stipple or egg number.

Discussion

Stipple number per leaf was shown to dependent on light environment, leaf position, and cage number. While stippling occurred on plants from both habitats, *S. nigrita* showed a significant preference for plants from shaded environments over those from exposed sun. This was also true of oviposition preference. These results are consistent with those found by Collinge and Louda (1988) in which sun plants were artificially shaded. They found that herbivory and oviposits by *S. nigrita* increased on the artificially shaded plants. These data suggest a chemical basis for preference of plants not exposed to direct sunlight; alternatively, they support the hypothesis that increased leaf miner damage to sun plants is due to a higher relative abundance of ovipositing flies (Collinge & Louda 1988). Leaf area of sun plants are generally much smaller than those of shade plants, however this difference does not provide strong evidence for itself as a measure of preference as leaf area in whole did not explain a significant portion of the variation. In detached leaf experiments the limited variation in leaf size

due to the selection of the first of second basal leaf may have decreased the importance of leaf area during selection. These data suggests that other factors are more strongly driving the preference for shade plants. The model measuring preference of stipple number using the variables described explained approximately 30% of the variation, and the model for egg number explains approximately 18%. While these model effects show an influence in preference, it provides evidence of other, potentially stronger influential factors. Future investigation is required and should consider leaf depth as an independent variable, the distance between upper and lower epidermises. In addition, chemical analysis of secondary metabolic compounds should be measured to consider stress differences between sun and shade habitats. Results from detached leaf experiments support the preference of shade plants while eliminating the influence of plant morphology. Further experimentation using either whole plants or detached leaves should also consider leaf turgidity as a possible factor in female preference.

S. nigrita also showed a greater preference for lower (basal) rather than upper (cauline) leaves in shade plants; however, this pattern was weaker in sun plants. This is also consistent with data collected by Collinge and Louda (1989) in which the number of oviposits were directly related to leaf size length as well as leaf position. The increase in herbivory and oviposition on these leaves is suggested to be due to a lower above ground concentration of mustard oils (Collinge and Louda 1989).

These lab based experiments support observation and data collected from the field in previous studies, and in some cases (shade vs. sun preference) provide counter intuitive results. *S. nigrita* have shown a preference for shade plants, whereas in the field they more frequently feed and oviposit on sun plants. These experiments eliminated temperature differences between sun and shade habitats. In light of the patterns seen that contradict those observed in the field

further investigation into the role of temperature, and micro-climate differences may provide insight into these observations.

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Figures

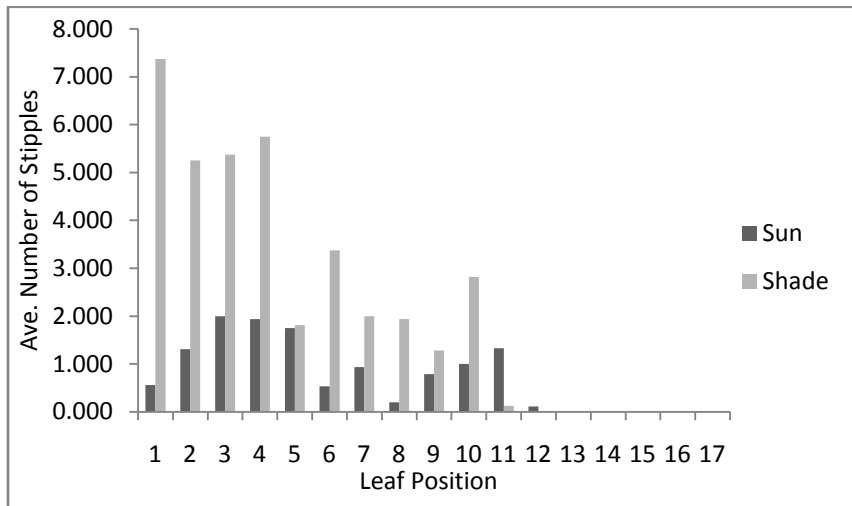


Figure 1: The average number of stipples per leaf across all leaves.

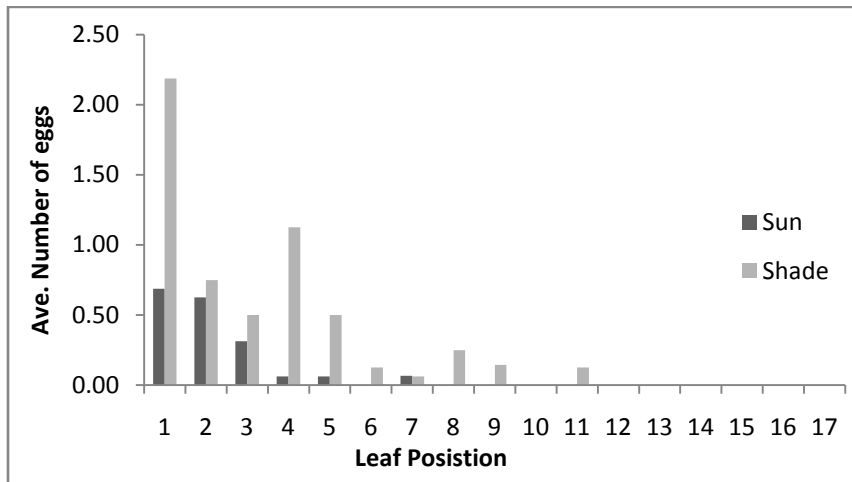


Figure 2: The average number of eggs per leaf across all leaves.

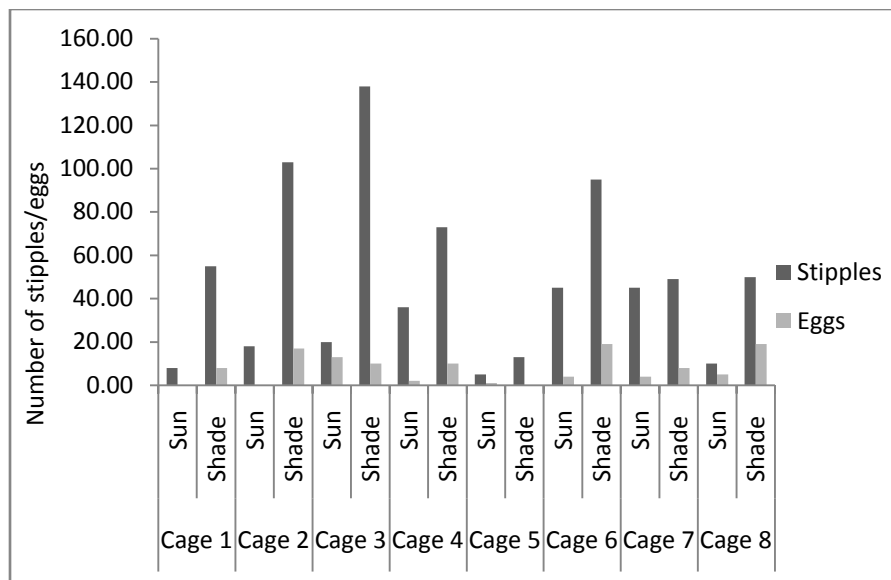


Figure 3: The sum stipple and egg number by cage separated by light environment.

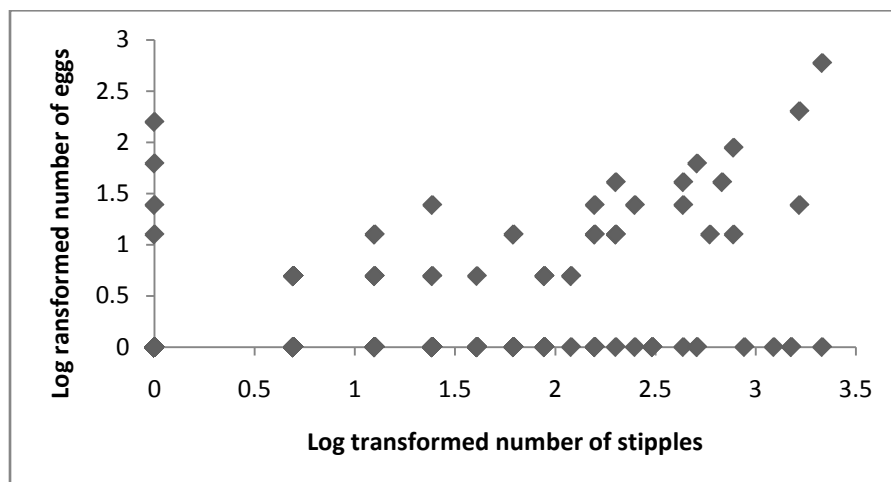


Figure 4: The number of eggs per leaf (log transformed) with respect to the number of eggs per leaf (log transformed).

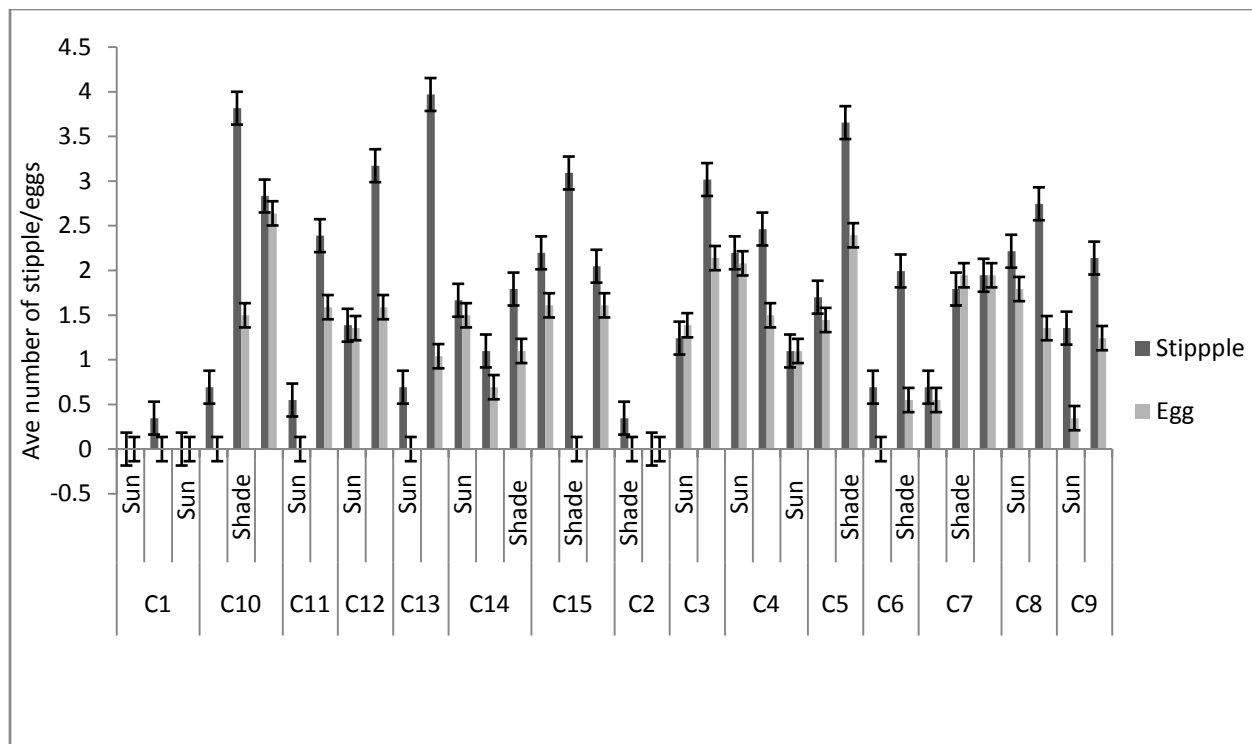


Figure 5: Average number of stipple and egg number (log transformed) relative to cage number (Cx) and light environment of the leaf with standard error.