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Feeding preferences and performance of an aquatic lepidopteran on macrophytes: plant hosts as food and habitat

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Abstract Although host preferences in phytophagous insects may be generated by several factors, few studies have simultaneously examined several potential host choice determinants. In this study we tested the impact of the following potential host choice determinants on host preference of the semi-aquatic lepidopteran *Munroessa gyralis* (Pyrallidae): growth on different host plants; protein content, polyphenolic content, toughness, and chemical extracts of different host plants; prior feeding experience; and predation pressure on the caterpillar by fishes. Two water lilies, *Brasenia schreberi* and *Nymphaea odorata*, were preferred in cafeteria-style feeding experiments over 14 other species of vascular plants. The most preferred water lily (*Brasenia*) also afforded the fastest growth relative to three other species on which growth was measured. Feeding preferences across species were unrelated to protein content, polyphenolic content, or toughness. Domiciles constructed by caterpillars from leaf fragments were protective from field assemblages of fishes, but domiciles made from preferred or unpreferred host species conferred no significant protection from fish in the laboratory. Caterpillars responded positively to chemical cues of water lilies, and prior feeding experience increased preference for an otherwise unpreferred water lily (*Nuphar advena*) within the life-span of individual caterpillars. *M. gyralis* is a generalist herbivore exhibiting modest preference induction and preferences for and among members of the family Nymphaeaceae. Our results suggest that relative growth

rates, chemical cues, and previous feeding experience are important factors determining feeding preference. Protein content, polyphenolic content, and toughness appear less important, and the importance of fish predators remains in question. As pupation seems to occur exclusively on *Nymphaea*, we suggest that host use may be restricted due to life-stage-specific developmental constraints that are not apparent from the results of growth or preference assays. It is currently unknown how often specific life-stages may restrict host use, but our work suggests this as a potentially important area of inquiry.

Keywords Aquatic macrophyte · Aquatic lepidopteran · Herbivory · Preference induction · Water lily

Introduction

Plant–herbivore interactions have been studied from an overwhelmingly terrestrial perspective (Rosenthal and Jansen 1979; Crawley 1983; Rosenthal and Berenbaum 1992). Recent meta-analyses, however, demonstrate that freshwater macrophytes are subject to rates of herbivory as great or greater than those for terrestrial plants (Cyr and Pace 1993; Lodge et al. 1998). Thus, herbivory on freshwater macrophytes offers a new opportunity for experimental and comparative analyses of plant–herbivore interactions. Having many plant and herbivore taxa divergent from or evolutionary distant from terrestrial organisms, the freshwater system may offer novel insights into plant–herbivore dynamics. Aquatic and semi-aquatic insects, for example, are important herbivores of aquatic macrophytes (Wallace and O’Hop 1985; Juliano 1988; Otto and Wallace 1989; Kouki 1991, 1993; Newman 1991; Setälä and Makela 1991; Cronin et al. 1998; Lodge et al. 1998) and offer a convenient point of comparison to extensively studied terrestrial insect herbivores.

Insects are the most prolific group of animals known; 40% are herbivorous and about 90% of those feed on

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three or fewer families of host plants (Bernays 1989). Factors in host choice (or feeding preference) are numerous, and include ultimate (evolutionary) factors such as optimum nutritive value (Newman et al. 1996; Cronin 1998), prime mating rendezvous (Colwell 1986), or enemy-free space (Price et al. 1980; Damman 1987; Bernays and Graham 1988), as well as more proximate (behavioral) factors like secondary chemistry (Shultz 1988; Newman et al. 1996), toughness, and previous feeding experience (Papaj and Prokopy 1989; Howard et al. 1996; Solarz and Newman 1996). Among aquatic insects, Newman (1991) notes that macrophyte herbivores related phylogenetically to terrestrial groups (i.e., secondarily aquatic insects like Chrysomelids, Curculionid beetles, aquatic lepidopterans, and dipterans) are relatively more often specialists, while primarily aquatic insects (e.g., caddisflies, mayflies) are often generalists.

Like many other lepidopteran larvae, *Munroessa gyralis* (Hulst) uses its host for both habitat and food. Early descriptive studies (McGaha 1952, 1954) suggested that the aquatic lepidopteran *M. gyralis* is a specialist found only on plants of the genus *Nymphaea*. However, our observations in Michigan and other work in South Carolina (Stoops et al. 1998) seem to indicate that *M. gyralis* is a generalist (*sensu* Newman 1991); i.e., eating from more than four host species in at least three families. Using observations, field experiments, and several laboratory assays we attempted to answer two questions: (1) what is the food/host preference of *M. gyralis* and (2) which plant traits govern host preference?

Materials and methods

Natural history of *M. gyralis*

The semi-aquatic larvae of the lepidopteran *M. gyralis* (Hulst) are small (2–30 mm, 0.5–30 mg wet mass), white, gill-less caterpillars. They are commonly found on water lilies in Michigan (USA) lakes as well as in parts of Canada (Monroe 1972, personal observations). The adult female moths lay eggs directly into the water column and the first or second instars migrate to the surface via macrophyte petioles (McGaha 1954). Instar numbers of field-caught caterpillars are unknown; however, extremely small caterpillars (<0.8 mg wet mass) can be commonly found on leaves of floating macrophytes. *M. gyralis* caterpillars have a variety of behaviors, including leaf mining, petiole boring, and domicile building (McGaha 1954, personal observation). Caterpillars use domiciles or “mobile homes” by securing themselves (using silk) to a small piece of leaf material (usually cut by the caterpillar). Early instars are often found leaf mining or feeding exposed on the underside of floating leaves. Later instars cut domiciles and migrate (commonly on the dry side of the leaf) to the base of the leaf just above the petiole where they begin the petiole stage of larval development. Additional feeding and subsequent pupation occur in the petioles of *Nymphaea* spp. (McGaha 1954, personal observation). Adult moths are sexually dimorphic in size and coloration, with the females being larger and drab and males being smaller with variable and distinct wing patterns (Monroe 1972).

Barnes and Benjamin (from McGaha 1954) and McGaha (1952, 1954) identified and studied this moth under the name *Nymphula serralinealis*. The life history was carefully studied by McGaha (1954) in Cheboygan County, Michigan (USA). McGaha (1952) stated that *M. gyralis* larvae were most often found on *Nymphaea* spp. (in Michigan, USA); however, in Manitoba (Canada)

Table 1 Species specific wet mass (mg) conversion factors for 4.84-cm² squares of Gray Lake macrophytes

Macrophyte	Wet mass (mg)	SE	<i>n</i>
<i>Brasenia schreberi</i>	192	11	35
<i>Nymphaea odorata</i>	222	15	35
<i>Nuphar advena</i>	230	12	35
<i>Potamogeton amplifolius</i>	90	2	35

it was reared on a water lily identified as *Nuphar advena* (Monroe 1972), and in South Carolina (USA) it was found on six species of host plant belonging to four different families (Nymphaeaceae, Potamogetonaceae, Hydrocharitaceae, and Onagraceae) (Stoops et al. 1998).

Field sites and collections

Most macrophytes and all caterpillars were collected from Gray Lake in Cass County, Michigan (41.81°N, 86.08°W). Approximately 80% of Gray Lake was covered by the following floating-leaved macrophytes used in this study: *Brasenia schreberi*, *Nymphaea odorata*, *Nuphar advena*, and *Potamogeton amplifolius* (identified using Voss 1972, 1985). Hereafter these four species will be listed by their generic name only. Other lakes and wetlands located within 20 km of Gray Lake were sources of additional macrophytes used in feeding assays. *M. gyralis* caterpillars were always collected and transported in association with *Nymphaea* leaves.

Measurement of field density

To estimate field density of caterpillars during May–July 1996, samples of macrophyte leaves (*n*=10 leaves for each of the four above-mentioned species) were taken from Gray Lake every 2 weeks. Leaves were digitized for area (cm²) and dissected for larvae and pupae.

Performance on diets of different macrophytes

To compare growth on different monospecific diets, early instar caterpillars collected in June 1996 from *Nymphaea* were housed and fed one of four macrophytes ad libitum. Eighty caterpillars were placed in individual cups with moist paper towel and a 4.8 cm² leaf portion of either *Brasenia*, *Nymphaea*, *Nuphar*, or *Potamogeton*. A fifth treatment consisted of caterpillars that received no food (starvation controls, *n*=20). Every 2–3 days plant squares and paper towels were replaced, and percent consumption was visually estimated on each date. The visual estimation method is similar to that used by Cronin et al. (1998); visual estimates by a “calibrated” eye allowed rapid accurate measurement of leaf damage (visual estimate vs digitized measure $r^2=0.917$, $n=31$ representative values >0 and <100%). Every 8–10 days, the caterpillars were weighed. The experiment was carried out for 43 days or until ~2/3 of the caterpillars in each treatment had died. Statistical analysis was limited to the first 24 days because increased mortality between days 24 and 34 reduced sample sizes greatly. In addition, field observations indicated that surviving caterpillars of the same cohort were either pupae or adults after 24–29 days. All percent consumption data from experiments comparing the four Gray Lake macrophytes, were converted to wet mass prior to statistical analysis (conversion factors in Table 1). Prior to analysis, consumption for individual caterpillars was converted to mass-specific consumption rates to correct for differences in larval sizes. Consumption and growth data were analyzed using repeated measures ANOVA for the first 24 days, with multiple comparison analyses performed for day 24 (Tukey HSD, SYSTAT 8.0/SPSS).

For the first 3 days of no-choice feeding (when the caterpillars were of similar size) we also compared feeding rates among the plant species using ANOVA and Tukey HSD.

Choice feeding preference assay (16 choices)

To test caterpillar preference among 16 macrophyte species we conducted a cafeteria style assay on 24–25 June 1996. Tested macrophyte species were, *Brasenia schreberi*, *Nymphaea odorata*, *Salix babylonica*, *Nuphar advena*, *Potamogeton amplifolius* (floating leaves), *Iris versicolor*, *Typha latifolia*, *Polygonum* sp. (floating), *Polygonum* sp. (emergent), *Impatiens* sp., *Stachys* sp., *Convolvulus* sp., *Peltandra* sp., *Sagittaria* sp., *Acer saccharum*, and *Quercus rubra*. Methods followed Cronin et al. (1998); in brief, leaves were cut into squares (4.8 cm², one square per species) and arranged in randomized 4×4 grids on moist paper towel in closed plastic containers. For each plant species, 10–16 leaves were used to make the squares. Individual caterpillars (mid to late instars >9.5 mg) were allowed to feed on a grid for 72 h ($n=20$). Percent consumption of each square was then estimated (after eye calibration with a digitizer, as described in a previous section). Percent of leaf area consumed per caterpillar was compared using a nonparametric Friedman rank test (Conover 1980). Because the area of leaf squares did not change during the assay, no controls for autogenic area changes were necessary (Peterson and Renaud 1989).

Feeding preferences of early versus late instars (9 choices)

To compare plant choices of large (>22 mg) and small (<10 mg) caterpillars we performed a 72-h feeding assay in which we offered 15 individuals in each of the two size classes a 3×3 randomized grid of nine macrophyte species. Tested species were a subset of the 16 from the former preference assay. Consumption was quantified visually as in other assays. Comparisons between the size classes were made qualitatively after Friedman rank tests were performed within each size class.

Influence of previous feeding experience (4 choices)

To determine the effect of previous diet on plant choice, caterpillars raised for 24 days on single foster hosts of *Brasenia*, *Nymphaea*, *Potamogeton*, or *Nuphar* were offered a 4-choice assay using the same four macrophyte species. Methods were the same as in the other choice assays. Thirteen to seventeen caterpillars from each of the foster hosts were tested and feeding was terminated after 72 h. Percent consumption was quantified visually (as in previous experiments) and converted to wet mass (mg) according to species-specific wet masses of the individual macrophytes (Table 1). Friedman tests were used to determine preference within foster-host groups. Across foster hosts, differences in feeding preference were compared qualitatively as no appropriate statistical test was known for this purpose.

Plant tissue measurements

Protein concentration, polyphenolic concentration, and toughness were measured for each of the 16 macrophytes used in the choice feeding preference assay. For each of the three tissue characteristics, we tested for significant correlations with the results of the choice feeding preference assay (Pearson Correlation with Bonferroni correction, SYSTAT 8.0/SPSS).

Portions of 10–16 leaves for each of the 16 macrophyte species (used in the preference assay) were pooled and frozen at -70°C (21 days) for analysis of protein and polyphenolic analysis. Protein content was analyzed using the methods of Duffy and Hay (1991) using bovine serum albumin as the standard. Davis (1988) determined this method reliable for measures of relative protein concentration.

Polyphenolic concentration was measured using methods similar to Cronin and Hay (1996). These methods are a modified Folin–Denis, which includes PVPP (polyvinyl-pyrrolidone) in one measurement per sample to correct for the accessory reactions of Folin–Denis reagents (Andersen and Todd 1968). We used methanol solutions of tannic acid solutions to build a standard curve. All protein and polyphenolic analyses were performed in triplicate.

We measured leaf toughness for each macrophyte using a homemade penetrometer. Pieces of leaves were flattened and secured between two pieces of wood with matching holes. A standard seamstress pin attached to the bottom of a plastic cup was inserted into the hole in the top block of wood where it came to rest on the upper surface of a leaf. Water was dripped into the cup at a constant rate using a burette until the pin fully penetrated the leaf. The mass of the cup, pin, and water was used as a measure of toughness (reported as g required to penetrate leaf). One to three measures were taken per leaf, and 12–20 leaves were measured for each species.

Choice feeding assay with chemical extracts

To test whether other chemical cues affected plant preference, we coated crude extracts from four macrophytes (*Brasenia*, *Nymphaea*, *Nuphar*, *Potamogeton*) onto undamaged leaves of *Salix babylonica* at natural wet mass concentrations (i.e., extract from 1 g of macrophyte was coated onto 1 g of *Salix*). *Salix* was used because caterpillars did not show high preference for this plant but still consumed it. Organisms used in this assay were collected from Gray Lake in July 1998 and shipped to the University of Colorado. To obtain crude extracts, we extracted a pooled sample of at least 20 leaves of each species twice in acetone for about 18 h. Extracts were concentrated with a rotavap at reduced pressure, then re-dissolved in acetone to make stock extract solutions for each macrophyte species (compounds from 2 g fresh tissue/ ml solution).

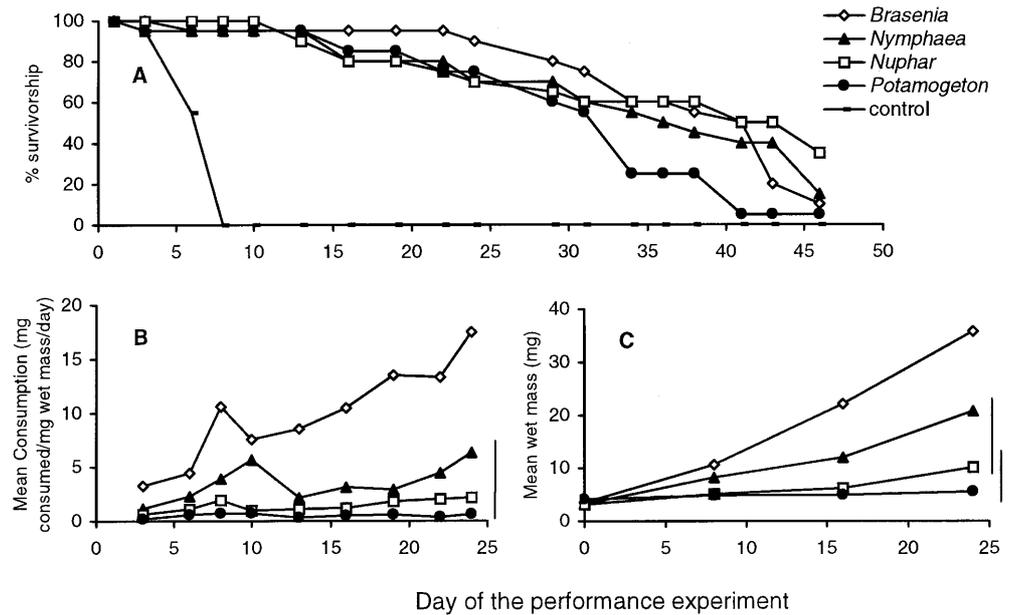
Twenty-nine pairs of early instar caterpillars were allowed to feed for 57 h on a cafeteria choice of four similar sized *Salix* leaves; each leaf was coated with one of the four macrophyte extracts. As in other choice experiments, leaves were randomly placed on a damp napkin in a 2×2 array inside a covered plastic tray to prevent desiccation. Consumption (mm²) for each leaf was estimated visually by an observer who did not know the identity of extract on the leaves. Consumption data were compared across the four extracts with a nonparametric Friedman rank test.

Predation assays with domiciled caterpillars

To determine whether domiciles made from leaf tissue afforded protection from predatory fish, a field feeding trial was performed in August 1996 with domiciled and naked caterpillars. Caterpillars with and without *Brasenia* domiciles were tossed onto the water surface at Gray Lake. Ten different open water spaces (areas free of macrophyte leaves and separated by >2 m) were used. Fish predators included various unidentified cyprinids and juvenile sunfish (*Lepomis* spp.). One naked caterpillar and one domiciled caterpillar were introduced to each open area and observed for 10 min. Results were recorded as consumed or not consumed and analyzed using Fisher's exact test.

To determine whether domiciles made from different leaf material afforded differential protection, caterpillars with *Brasenia* or *Potamogeton* domiciles were fed to adult bluegill (*Lepomis macrochirus* 115–140 mm TL) individually housed in laboratory aquaria. If a bluegill would first consume a palatable food item (waxworm), a pair of domiciled caterpillars and a control (naked caterpillar) were introduced. The status of each caterpillar was recorded at 5, 30, 60, and 120 min. Statistical analysis was performed with a nonparametric Pearson chi-square statistic which tests for an association between treatments (naked, *Potamogeton* domiciled, *Brasenia* domiciled) and final condition (eaten or uneaten).

Fig. 1 A Survivorship curve for 100 *Munroessa gyralis* (Hulst) caterpillars raised on one of four diets plus a starvation control in the laboratory. **B** Mean mass-specific consumption rate of caterpillars feeding during the experiment. **C** Mean wet mass of *M. gyralis* caterpillars over the time course of the experiment. In B and C, at day 24, points joined by a line are statistically indistinguishable (Tukey HSD $P < 0.05$)



Results

Natural history of *M. gyralis*

During casual observations at Gray Lake, *M. gyralis* caterpillars were found on the floating leaves of *Nymphaea*, *Brasenia*, *Nuphar*, and *Potamogeton*. Older leaves of *Nuphar advena* are often emergent and never had caterpillars. In addition, domicile building (disc cutting) damage was found on leaves of all four species. Small instars were abundant in early June and again in mid-July suggesting the presence of two distinct summer cohorts (personal observation 1996).

Small instars were usually found feeding on the undersides of floating *Nymphaea* or *Brasenia* leaves while larger instars cut or scavenged plant material from various macrophytes (including fragments of submerged species) for domicile construction and feeding. Domiciled caterpillars were usually attached with silk to the top or bottom of *Nymphaea* leaves. The largest instars and pupae were always found in *Nymphaea* petioles or midveins. Our observations are generally consistent with McGaha (1952, 1954); however he never reported finding *M. gyralis* on any hosts other than *Nymphaea* spp.

Measurement of field density

In systematic collections of leaves of *Brasenia*, *Nymphaea*, *Nuphar*, and *Potamogeton*, overall densities fell throughout the summer on a per cm^2 basis; however corrections were not made for the increase in populations of leaves. High variability in the number of caterpillars per leaf also made it difficult to detect trends in the caterpillar population using our sampling scheme. Highest caterpillar densities on a species of macrophyte were found on *Nymphaea* leaves on 30 May 1996 (mean of 5/leaf and

0.018/ cm^2 ; $n=10$ leaves). On one single *Nymphaea* leaf, 45 early instar caterpillars were found on 3 June, corresponding to a mass-specific density of 2.41 caterpillars per gram of macrophyte. No caterpillars were found on *Nuphar* during collections, and ranges of mean density (no. per cm^2) on the three other macrophytes throughout the summer were as follows: *Nymphaea* 0.0013–0.018, *Brasenia* 0–0.011, *Potamogeton* 0–0.003. On a per cm^2 basis, *Brasenia* had the highest mean density for three of the five collections.

Performance on diets of different macrophytes

The 80 caterpillars raised on diets of *Brasenia*, *Nymphaea*, *Nuphar*, and *Potamogeton* exhibited different levels of consumption and growth on their foster hosts (Fig. 1). All starvation "control" caterpillars died within 8 days. No caterpillars pupated in this experiment, and more than 2/3 of the caterpillars died by day 43 (Fig. 1A). Analyses of growth and consumption were limited to the first 24 days due to sample size constraints.

Analysis of mass specific consumption values from days 3–24 indicates significant differences in consumption between the four macrophyte treatments (rmANOVA between treatment effect, $F=56.7$, $df=3,51$, $P < 0.00001$). Caterpillars feeding on *Brasenia* had a greater mass-specific consumption rate on day 24 than caterpillars eating any other macrophyte (Fig. 1B, $P < 0.007$, Tukey HSD).

Analysis of wet mass from days 0–24 indicated significant differences in mass between the four treatments (rmANOVA between treatment effect, $F=15.4$, $df=3,57$, $P < 0.00001$) as well as growth rates (rmANOVA, treatment \times time interaction, $F=5.17$, $P < 0.00001$). At day 24, *Brasenia*-raised caterpillars were significantly larger than caterpillars grown on the other diets (Tukey HSD

$P < 0.0045$), and *Nymphaea*-raised caterpillars were significantly larger than those raised on *Potamogeton* ($P < 0.0065$) (Fig. 1C).

Day 3 consumption data were analyzed as a no-choice feeding assay for comparison with the choice feeding assays. At the beginning of the feeding trial caterpillars were of similar mass (ANOVA $P = 0.950$) and feeding rates for the first 3 days are not confounded by size-specific consumption differences. Caterpillars eating *Brasenia* consumed more tissue than those feeding on the other three macrophytes (Tukey HSD $P < 0.001$), and caterpillars eating *Nymphaea* consumed significantly more plant tissue than caterpillars feeding on *Potamogeton* ($P = 0.012$). Consumption of *Nymphaea* and *Nuphar* did not differ significantly ($P = 0.084$). These patterns are the same as those for mass specific consumption at day 24 (Fig. 1B).

Choice feeding preference assay (16 choices)

M. gyalis caterpillars, all of which had been collected from *Nymphaea*, ate from 9 of the 16 macrophytes in the 16-choice assay and exhibited preferences among the plants consumed (nonparametric Friedman rank test, $P < 0.01$, Fig. 2 A). The order of preference was *Brasenia* > *Nymphaea* > *Salix*, *Nuphar*, *Potamogeton* > everything else (multiple comparisons $\alpha = 0.01$).

Feeding preferences of early versus late instars (9 choices)

The 9-choice assays with early and late instars yielded overall preferences similar to the 16-choice feeding assay (Fig. 2). Although large caterpillars consumed approximately 45% more macrophyte than small instars, qualitatively similar preference patterns were displayed (Fig. 2B, C). Friedman rank tests indicated large instars preferred *Nymphaea* over all other macrophytes (Fig. 2C) while small instars did not show significant preferences between *Nymphaea* and *Brasenia* (Fig. 2B). *Nymphaea* was never ranked lower or tied in preference ranking with any macrophyte other than *Brasenia* in any of these choice assays.

Influence of previous feeding experience (4 choices)

Caterpillars raised on foster host diets for 24 days showed somewhat different preferences when subjected to a 4-choice assay (Fig. 3). *Brasenia* (Fig. 3A) and *Potamogeton*-raised (Fig. 3D) caterpillars exhibited similar patterns of preference (i.e., *Nymphaea* and *Brasenia* > *Nuphar* and *Potamogeton*), but caterpillars raised on *Nymphaea* preferred *Nymphaea* over *Brasenia* (Fig. 3B). Caterpillars raised on *Nuphar* exhibited no clear (statistical) preferences and were the only caterpillars which ate substantial amounts of *Nuphar* (Fig. 3C).

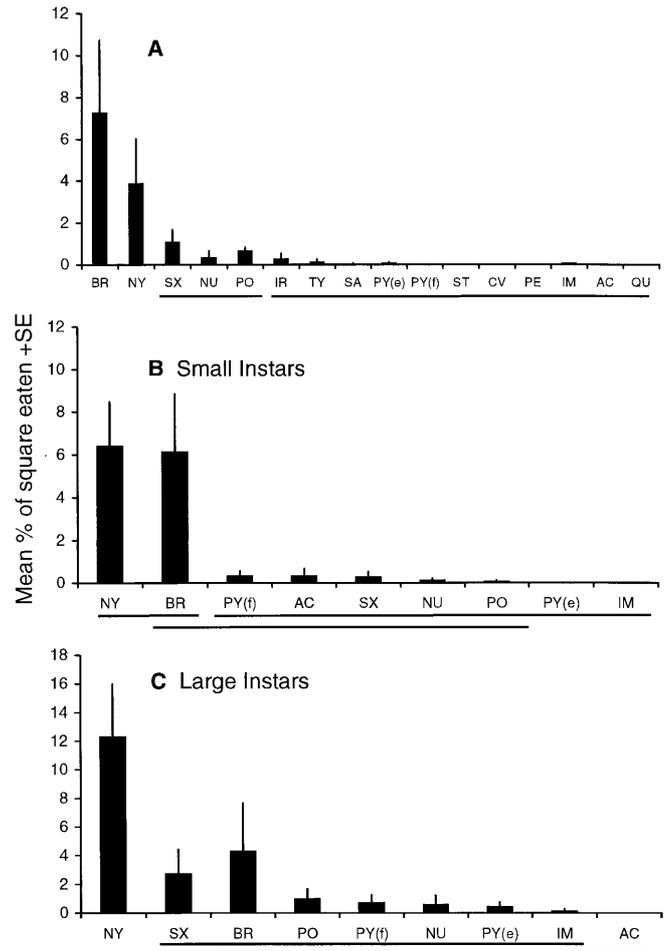


Fig. 2 Results of choice feeding assays with *M. gyalis* (Hulst). Bars represent the mean percent consumption + SE for each macrophyte offered in the assay. All assays lasted 72 h. **A** Sixteen-choice feeding assay with a range of medium to large instars (>9.5 mg) ($n = 15$). **B** Nine-choice feeding assay with small instars (2.0–9.1 mg) ($n = 14$). **C** Nine-choice feeding assay with large instars (22–131 mg) ($n = 14$). Macrophyte code: BR – *Brasenia schreberi*, NY – *Nymphaea odorata*, SX – *Salix babylonica*, NU – *Nuphar advena*, PO – *Potamogeton amplifolius* (floating leaves), IR – *Iris versicolor*, TY – *Typha latifolia*, PY(f) – floating *Polygonum* sp., PY(e) – emergent *Polygonum* sp., AC – *Acer saccharum*, IM – *Impatiens* sp., ST – *Stachys* sp., CV – *Convolvulus* sp., PE – *Peltandra* sp., SA – *Sagittaria* sp., QU – *Quercus rubra*. Consumption of macrophytes sharing an underline did not differ in a nonparametric Friedman rank test ($\alpha = 0.01$). Note the changes in macrophyte order from **A** to **C**. Bars indicate the mean amount of macrophyte eaten per caterpillar while the statistical analysis is indicating the differences between summed ranks. The fact that *Brasenia* (in **B**) is not statistically different than *Salix* and four other species is a result of using this nonparametric test

The fact that *Potamogeton* was tied with the other three macrophytes in our analysis (i.e., Fig. 3C) is an artifact of using a nonparametric rank test (see Fig. 2 legend). Only 4 of 14 caterpillars ate *Potamogeton* and none preferred it over all other macrophytes.

Further data exploration with the *Nuphar*-raised caterpillars indicated a bimodal distribution of *Nuphar* consumption. Eight of the 14 caterpillars raised on *Nuphar* ate more than 60 mg (or 2.5 mg/day by wet

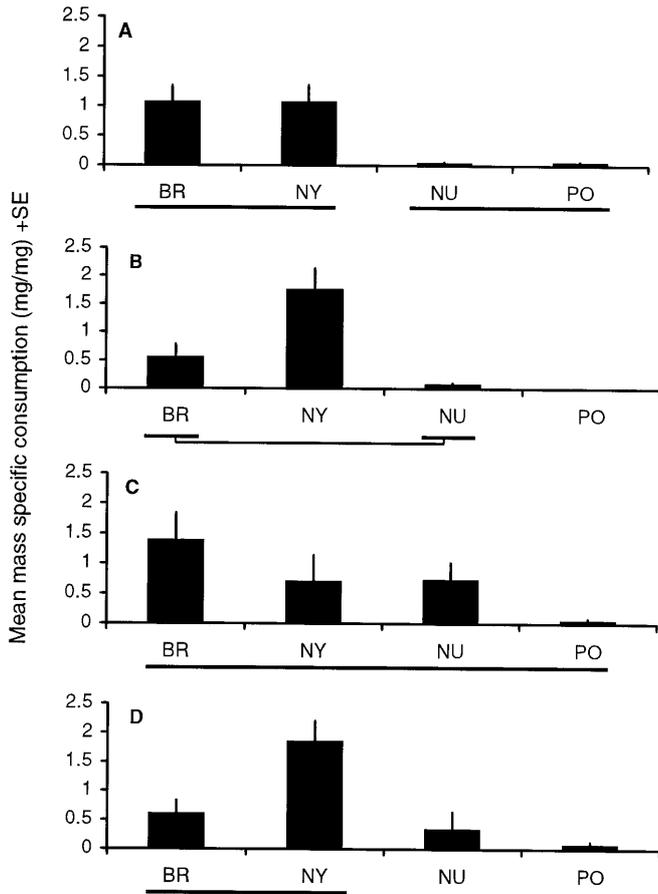


Fig. 3A–D Results of a 4-choice feeding preference assay to determine whether prior feeding experience of *M. gyralis* affected feeding preference. Bars indicate the mean size-specific consumption +SE for the 72-h assay. Four sets of caterpillars, raised individually on each of four hosts, were tested; **A** *Brasenia schreberi* ($n=17$), **B** *Nymphaea odorata* ($n=14$), **C** *Nuphar advena* ($n=14$), **D** *Potamogeton amplifolius* ($n=14$). Within each subfigure, consumption of macrophytes sharing an underline did not differ (Friedman rank tests $\alpha=0.05$)

mass) in 24 days of monoculture, and the average diet of these 8 consisted of 55% *Nuphar* in the choice assay (Fig. 4). Six caterpillars consumed less than 60 mg *Nuphar* in monoculture and had an average diet in the choice assay which included only 5% *Nuphar*. This difference in *Nuphar* consumption (mg and %) was statistically significant (ANOVA $P<0.05$). Five of the 14 caterpillars (36%) in this choice assay ate more *Nuphar* than both *Brasenia* and *Nymphaea*. This result stands in contrast to all of the other choice assays in which only 3 of 88 caterpillars (3%) ate more *Nuphar* than both *Brasenia* and *Nymphaea*.

Plant tissue measurements

Among plant species, polyphenolic concentrations ranged from 0 to 8.2% (dry mass), protein concentrations ranged from 5% to 26%, and toughness measures ranged from

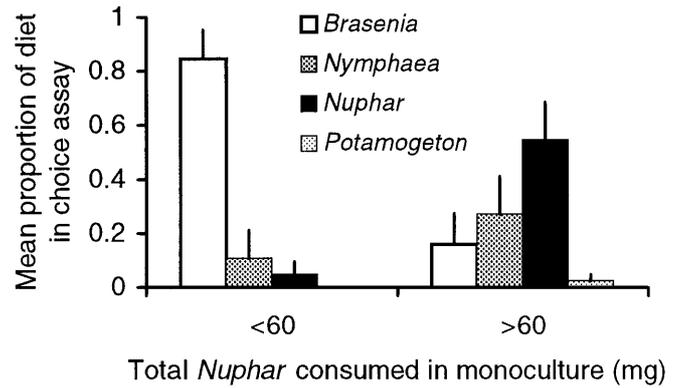


Fig. 4 Average diet composition (+ SE) of *Nuphar*-raised caterpillars in a four-choice assay

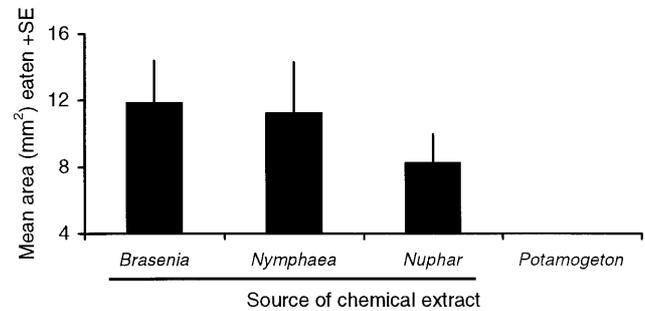


Fig. 5 Mean consumption (mm² + SE) by *M. gyralis* (Hulst) of *Salix* leaves coated with extracts from four Gray Lake macrophytes. Bars connected by underlining are not significantly different (Friedman rank test $\alpha=0.01$)

10.4 to 84.6 (grams required to penetrate leaf). None of these three tissue characteristics were related to caterpillar plant preference in the 16-choice assay: polyphenolic concentration (Pearson correlation coefficient = 0.038, $P=0.89$); protein concentration (−0.072, $P=0.792$); toughness (0.146, $P=0.590$). In addition, there was no obvious pattern between performance on the four Gray Lake macrophytes and any of the plant traits.

Choice feeding assay with chemical extracts

Results of the feeding assay of *Munroessa* among *Salix* leaves coated with crude extracts of the four Gray Lake macrophytes yielded overall significant differences in amount eaten (Friedman rank test $P<0.01$): *Brasenia* = *Nymphaea* = *Nuphar* > *Potamogeton* ($\alpha=0.01$, $n=29$) (Fig. 5).

Predation assays with domiciled caterpillars

In the field assay, *Brasenia*-domiciled caterpillars gained a significant amount of protection from natural assemblages of predatory fish; 8 of 10 naked caterpillars were consumed while none of the domiciled caterpillars were

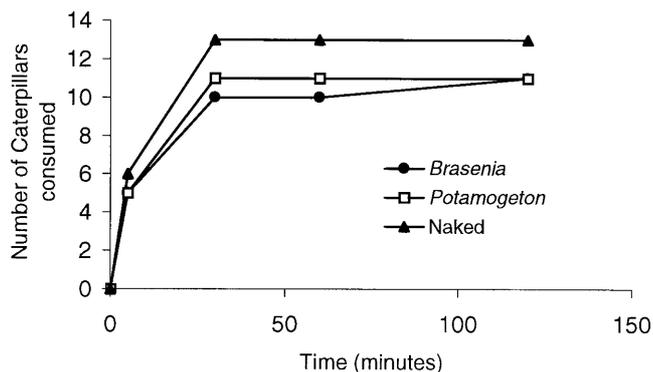


Fig. 6 Time course of caterpillar (*M. gyralis*) consumption by adult bluegill (*Lepomis macrochirus*) in a laboratory assay. Differences in survival at 30 min showed no association with treatment (Pearson chi-square statistic $P > 0.1$)

eaten (Fishers Exact Test $P < 0.001$). In laboratory assays with adult bluegill, caterpillars with *Brasenlia* and *Potamogeton* domiciles were consumed at a frequency nearly equal that of the naked caterpillars (Fig. 6). Observations indicated that bluegill were able to consume domiciled caterpillars without swallowing the domiciles. We analyzed the 30 min data, because differences in survival between treatments was greatest at this observation time, and found no association between treatment and final outcome with a nonparametric Pearson chi-square statistic ($P > 0.1$). Domiciled caterpillars in this assay gained no significant protection from adult bluegill.

Discussion

Host preferences of herbivores can be influenced by proximate cues of intrinsic plant properties (e.g., secondary compounds, toughness, nutritional value) (Lodge 1991; Newman 1991; Rosenthal and Berenbaum 1992; Lodge et al. 1998), extrinsic factors such as the presence of predators (Duffy and Hay 1991, 1994), prior experience of the herbivore, (Papaj and Prokopy 1989; Solarz and Newman 1996; this study), host use of the mother (Fox and Savalli 2000), or degree of satiation (Cronin and Hay 1996).

Proximate cross-host feeding decisions of *M. gyralis* were influenced by chemical cues and prior feeding experience, and were not clearly related to protein content, phenolic concentration, or toughness. Ultimate reasons for host decisions may be related to optimal nutritive value, assuming relative growth and nutritive value are related. Developmental ties (i.e., pupation sites) are quite possibly important, but the importance of predation pressure from fish (on domiciled caterpillars) remains unresolved. *M. gyralis* is a generalist herbivore exhibiting modestly inducible preference changes and strong preferences both for and among members of the family Nymphaeaceae.

Small, relatively immobile herbivores are often associated with noxious hosts, may be less deterred by host compounds than are larger generalist consumers, and may even specialize on noxious hosts (Hay et al. 1987;

Duffy and Hay 1991, 1994; Cronin et al. 1998). These small herbivores may avoid detection by predators by being relatively immobile and experience less detection or incidental consumption by living on noxious plants that are rarely visited by larger consumers. Our observations of *Munroessa* generally fit this pattern: the caterpillars have limited mobility, are susceptible to predators when not associated with vegetation (or domiciles), and prefer hosts of the family Nymphaeaceae which tend to be avoided by, and chemically defended from, large mobile generalists (Bolser and Hay 1998; Cronin 1998; Cronin et al., unpublished data).

The results of the performance experiment and the preference assays indicate that *M. gyralis* preferred to eat the macrophytes that afforded the best growth. However, without measurements of assimilation efficiency we cannot rule out the converse: caterpillars grew best on what they ate the most. Regardless, when raised in monoculture the caterpillars grew fastest on *Brasenlia* and grew significantly faster on *Nymphaea* than on *Potamogeton*.

The fact that no caterpillars pupated in our experiments could indicate that caterpillars require a mixed diet to complete the life cycle, although mixed diets are generally not necessary for complete development among lepidopterans (Bernays and Minkenberg 1997). Alternatively, it is possible that laboratory conditions failed in other ways to simulate the field conditions necessary to pupate. Several of the caterpillars eating *Nymphaea* and *Brasenlia* attained wet masses of 40–68 mg before dying or losing weight. Most large caterpillars that were found in the field fell within this range of sizes (personal observation). McGaha (1954) noted that the life history of this caterpillar involves a “petiole period” in which large caterpillars live and feed inside the petioles of *Nymphaea* (however, “in a few instances” pupae were found outside petioles in underwater chambers). This is consistent with our observations in Gray Lake: the largest caterpillars and all pupated caterpillars were found inside *Nymphaea* petioles or midveins. As no *Nymphaea* petioles were available in this experiment, perhaps the caterpillars could not complete their normal feeding and pupation cycle. *Nymphaea* petioles may provide nutrients essential for pupation or an essential specialized habitat.

Most of the choice feeding experiments indicated the same preference theme: *Brasenlia* and *Nymphaea* are preferred food plants for *M. gyralis* (Figs. 2, 3A, B, D). Larger instars seemed to prefer *Nymphaea* relative to smaller instars (Fig. 2B, C), consistent with the field pattern of pupation exclusively on *Nymphaea*, as well as the potential importance of prior feeding experience (i.e., larger caterpillars are probably more experienced with *Nymphaea* as they were all collected from *Nymphaea* in the field). Preference for *Brasenlia* indicated by Fig. 2A makes this interpretation less clear, as the caterpillars feeding in the 16-choice assay were a range of medium to large caterpillars (> 9.5 mg). In all choice experiments, *Brasenlia* and *Nymphaea* were preferred relative to the other Gray Lake floating/emergent macrophytes. This preference trend is consistent with the no-choice feeding

results for the first 3 days of the performance experiment with the exception that *Nuphar* and *Nymphaea* were not statistically distinguishable.

Across multiple macrophyte species, feeding preferences were not correlated with phenolic concentration, protein concentration, or toughness. However, we do not know whether these traits are important in making feeding decisions within-species. Additionally, it is possible that some unmeasured plant trait (e.g., polyunsaturated fatty acids) may be involved in feeding preferences. Other studies have noted a lack of correlation between host use and secondary chemistry (Smiley and Wisdom 1985; Cronin 1998). In terrestrial and marine systems it has been concluded in some cases that food considered chemically or nutritionally disadvantageous may offer advantages such as enemy-free space (Damman 1987; Bernays 1989; Duffy and Hay 1991; Fox and Eisenbach 1992).

Results from our examination of prior feeding experience indicated the same basic preferences by caterpillars raised on *Nymphaea*, *Brasenia*, and *Potamogeton* (Fig. 3A, B, D). *Nymphaea*-raised caterpillars preferred *Nymphaea*, but in the two other assays *Nymphaea* shared the number one ranking with *Brasenia*. The choice assay results following forced-feeding of *Nuphar* for 24 days indicated that normal preferences were slightly altered (Fig. 3C). The caterpillars that had been raised on *Nuphar* in monoculture ate more *Nuphar* than caterpillars in any other treatment group, and did not prefer either *Nymphaea* or *Brasenia* over *Nuphar*. In addition, caterpillars that ate larger amounts of *Nuphar* in monoculture ate more *Nuphar* when offered a choice (Fig. 4). We interpret this as an induced feeding preference because available lines of experimental and observational evidence indicate that Gray Lake caterpillars do not otherwise prefer to eat *Nuphar*. Although a subset of the *Nuphar*-raised caterpillars displayed induced feeding preferences, the decision to eat or not eat *Nuphar* in the first 24 days of the performance assay represents an element of individual plasticity not well understood (see Jermy 1987) and may indicate potential for variable host use. The fact that all experimental caterpillars were collected from *Nymphaea* yet many preferred *Brasenia* in the laboratory indicates that prior experience is not the main determinant of diet.

It could be argued that the preference-switching behavior of *M. gyralis* is actually habituation to deterrent chemicals (Dethier 1988; Papaj and Prokopy 1989), but the results of our chemical extract assay suggest otherwise. Results of the extract assay indicate that caterpillars respond positively to chemical cues of all water lilies (no significant aversion to *Nuphar* acetone extracts) and reject chemical cues of otherwise unacceptable *Potamogeton*. The relatively high orientation towards *Nuphar* extracts suggests that *Nuphar* has an acceptable "biochemical profile" (sensu Jermy 1984), such that the reasons for its rejection by most caterpillars results from other characteristics. It is possible that the induced feeding preference exhibited by *M. gyralis* is actually habituation based on some other unknown plant trait or some other compound not extractable with acetone (i.e., salts, polar

amino acids, sugars, highly lipophilic compounds). Preferences for rearing hosts have been shown for many terrestrial lepidopterans (Saxena and Schoonhoven 1982; De Boer and Hanson 1984, and references therein; Jermy 1987) and our results may suggest a future area of comparison between aquatic and terrestrial lepidopterans.

In light of these results we suggest that species within the genus *Nuphar* may be acceptable hosts, while remaining unpreferred in sympatry with *Nymphaea* and *Brasenia*. Two observational lines of evidence support this contention. The first is the indication by Monroe (1972) that *M. gyralis* "has been reared in Manitoba (Canada) on a yellow waterlily identified as *Nuphar advena*." In addition, when caterpillars were displaced to ponds lacking *Nymphaea* and *Brasenia* (Kellogg Biological Station ponds) they exhibited normal feeding/domicile construction behaviors on floating leaves of *Nuphar* spp. (personal observation). Unfortunately, pupation was not reported or observed in either case, so any conclusions about the host suitability of *Nuphar* are speculative.

Domiciles made from preferred (*Brasenia*) and unpreferred (*Potamogeton*) macrophytes offered equivalently poor protection from adult bluegill in the lab; however further predation assays with other macrophytes should be performed. It would have been desirable to perform these assays with *Nuphar* and *Nymphaea*, but we could not generate enough domiciled caterpillars from those macrophytes for testing. Caterpillars feeding on *Potamogeton* or *Brasenia* in the performance experiment were found with domiciles ~4× more often than caterpillars feeding on the tougher *Nuphar* and *Nymphaea* (unpublished data), suggesting leaf toughness is an important constraint for caterpillars building domiciles. Similarly, Mueller and Dearing (1994) concluded that the aquatic lepidopteran *Parapoynx rugosalis* (also Pyralidae) avoids tough tissue when building domiciles. Although caterpillars in the field gained a substantial amount of protection from small fish by building *Brasenia* domiciles (see also Mueller and Dearing 1994), the importance of a domicile may extend beyond avoidance of fish predators. For example domiciles may be employed more importantly as protection from UV radiation, desiccation, or terrestrial predators as caterpillars migrate across leaf surfaces.

Although *M. gyralis* can be found eating from several different food sources in the laboratory or field (this study, Stoops et al. 1998), we found clear preferences for two water lilies. *Brasenia* may be preferred for maximal growth rates; and yet there may remain developmental or life history constraints which tie the caterpillars to *Nymphaea*. Larval diets may be constrained for the first cohort of the year, as *Nymphaea* emerges earlier in the spring than does *Brasenia* (personal observations). There also may be an important developmental constraint given *M. gyralis* was only found pupating in the midveins and petioles of *Nymphaea*. Ontogenetic changes in feeding niche (e.g., leaf mining to case-bearing) are common among terrestrial lepidopterans (Gaston et al. 1991), and others have hypothesized that concealed larvae and pupae experience physical protection from parasitoids

and other loss factors (i.e., desiccation, UV radiation, disease) (Hawkins and Lawton 1987; Mills 1993; Damman 1994; Connor and Taverner 1997). In addition, endophytic larvae may benefit from selective feeding and avoidance of toxins concentrated in particular tissues (Connor and Taverner 1997). We suggest that *M. gyralis* may be limited to pupation sites on *Nymphaea*, and that this developmental factor may restrict host use in Gray Lake. Although growth performance on *Brasenia* was good, *Brasenia* petioles are far too small for large caterpillars to use as pupation sites. In addition, it is likely that the lifespan of a "soft" *Brasenia* leaf is shorter than the lifespan of the more "robust" leaves of *Nymphaea*; making pupation (4–10 days, McGaha 1954) on *Brasenia* a risky venture. On the other hand, *Nuphar* petioles are large enough for pupation, with leaves as tough as *Nymphaea* leaves (unpublished data), and we believe the biochemical profile is acceptable (Fig. 5). However, older *Nuphar advena* leaves and petiole bases are emergent as opposed to the floating leaves and submerged petiole bases of *Nymphaea*. If pupation in an underwater environment allows *M. gyralis* to avoid terrestrial loss factors (e.g., desiccation, terrestrial parasitoids), *Nuphar* petioles may be unsuitable pupation sites. Additional studies should be performed testing the suitability of the petioles (and leaves) of the lilies as pupation sites. It is currently unknown whether or how commonly specialized life-history niches constrain host use among caterpillars, but our observations of this semi-aquatic caterpillar suggest that it may be a fruitful area of inquiry.

In light of the preference tendencies for two plants, an ontogenetic diet shift seems plausible. Preferences of large caterpillars for *Nymphaea* over *Brasenia* may support a hypothesis that large caterpillars are developmentally tied to *Nymphaea*, while the high growth on *Brasenia* would be advantageous for early instars assuming they are food-limited. However, this interpretation does not fit with all of our choice assay data (Fig. 2A). As well, large caterpillars collected from *Nymphaea* may be expected to show preferences for *Nymphaea* given the influence of prior experience. Additionally, observations indicate that *M. gyralis* is sometimes found in ponds lacking *Brasenia*, so we cannot conclude that *Brasenia* is essential or mixed diets are necessary or common. Bernays and Minkenberg (1997) found no significant increases in fecundity, growth, or survivorship when terrestrial lepidopterans fed on mixed diets versus monocultures. They hypothesized that the value of being a generalist (for lepidopterans) may lie in versatility for host use rather than diet mixing. In our system host versatility for early instars seems clearly advantageous as eggs are deposited in the water and young caterpillars may be forced to feed on the leaves belonging to the nearest available petioles. Tracking the progress of field enclosed caterpillars and performing mixed diet feeding experiments would be necessary to fully resolve this issue in our system.

Our results plus the references by Monroe (1972) and Stoops et al. (1998), seem to suggest an element of host

dynamism (Fox and Morrow 1981; Bernays and Graham 1988), such that an herbivorous insect species may be a localized specialist while remaining a generalist over its geographic range (Cronin et al. 1999). Monroe (1972) notes that *M. gyralis* was raised on *Nuphar advena* in Manitoba (Canada), and in South Carolina (USA), Stoops et al. (1998) observed *M. gyralis* feeding on several species outside of the family Nymphaeaceae. However, we do not know whether *M. gyralis* has ever been found at a site where members of the Nymphaeaceae were absent. In Michigan (USA), we have only found *M. gyralis* populations in systems with *Nymphaea* spp. ($n=6$ lakes; note: *Nuphar* and *Brasenia* did not occur in all of the lakes) and McGaha (1952, 1954) cites his observations of *M. gyralis* in association with *Nymphaea* spp. only.

Considering the observed variability in hosts and our results from the prior experience assay, where several *M. gyralis* individuals altered preferences towards *Nuphar*, it is possible that *M. gyralis* exhibits a level of host dynamism. Alternatively, *M. gyralis* may be a facultative generalist consuming any acceptable host species in sympatry with *Nymphaea* or members of the Nymphaeaceae.

The apparent tie to pupation on *Nymphaea* suggests that examination of developmental life-history constraints could be a fruitful area of inquiry in future studies of plant–insect interactions. If species-specific tissues, structures, or nutrients are necessary for proper development of particular life stages, plants providing high larval growth rates may not be suitable hosts if they provide overall poor development. Although plants conferring high growth may be preferred and utilized by certain food-limited larval stages, they may be avoided by other life-stages.

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