COMMUNITY ECOLOGY - ORIGINAL PAPER

Impact of plant architecture versus leaf quality on attack by leaf-tying caterpillars on five oak species

Robert J. Marquis · John T. Lill

Abstract Because shelter-building herbivorous insect species often consider structural features of their host plants in selecting construction sites, their probability of attack is likely to be a function of some combination of plant architectural traits and leaf quality factors. We tested the hypothesis that plant architecture, in the form of the number of touching leaves, influences interspecific variation in attack by leaf-tying caterpillars in five species of sympatric Missouri oaks (*Quercus*). We compared colonization on control branches, in which both architecture and leaf quality were potentially important, with colonization on experimental branches for which we controlled for the effects of architecture by creating equal numbers of artificial ties. Colonization of artificial ties was highly correlated with natural colonization on neighboring control branches, suggesting that leaf quality factors and not architecture influenced interspecific variation in attack by leaf-tying caterpillars. Of the leaf quality factors measured (water, protein-binding capacity, nitrogen, specific leaf area, pubescence, and toughness), nitrogen was the most explanatory. With the exception of white oak, natural leaf tie colonization was positively correlated with nitrogen availability (ratio of nitrogen to protein-binding capacity),

and negatively correlated with protein-binding capacity of leaf extracts. Both host plant species and subgenus oak influenced the community composition of leaf-tying caterpillars and the non-tying symbionts colonizing the ties. Host plant differences in leaf nitrogen content were positively correlated with pupal weight of one of two caterpillar species reared on all five host plant species. Thus, interspecific differences in nitrogen, nitrogen availability, and protein-binding capacity of leaf extracts are the best predictors at this time of interspecific differences in attack by leaf-tying caterpillars, in turn affecting their success on individual host plants in the laboratory.

Keywords Ecosystem engineering · Leaf-tying caterpillars · *Quercus* · Secondary compounds · Plant architecture

Introduction

Many herbivorous insect species manipulate portions of their host plants to make constructs. Leaves are tied together, folded over or rolled, or silk nests are constructed using adjoining leaves, twigs, and branches as anchoring bases. For such construct builders, the host plant is more than just a food source—it is also a potential source of escape from predators and parasites and a means of ameliorating the harshness of the environment (Fukui 2001; Marquis and Lill 2006; Lill and Marquis 2007).

A founding principle of plant-herbivore interactions is that plant quality factors (nutrition, secondary chemistry, and physical defenses) mediate these interactions (Fraenkel 1959; Ehrlich and Raven 1964). For those herbivores that build constructs, however, host plant choice is likely to depend on a combination of architectural traits, which

R. J. Marquis (✉) · J. T. Lill
Department of Biology, University of Missouri-St. Louis,
One University Boulevard, St. Louis, MO 63121-4499, USA
e-mail: robert_marquis@umsl.edu

Present Address:
J. T. Lill
Department of Biological Sciences,
George Washington University, 2023 G Street,
340 Lisner Hall, NW, Washington, DC 20052, USA

influence the ability of the insects to build the shelter, and plant quality factors, which determine success once feeding proceeds (Lawton 1983; Marquis et al. 2002; Wise and Abrahamson 2008). Potentially relevant traits include leaf flexibility, distance between leaves, distance between twigs, leaf size, leaf density, and branching angle (Marquis et al. 2002).

Leaf-tying caterpillars are examples of common shelter builders on Missouri oak trees. These caterpillars sew two or more mature leaves together with silk to form a leaf tie, analogous to a leaf sandwich (Carroll and Kearby 1978). Once constructed, these caterpillars live within the leaf tie, skeletonizing the two adjacent hidden leaf surfaces. In understory white oak (*Quercus alba*) saplings, plant architecture is an important determinant of attack rate by these caterpillars. Plants that have a greater percentage of touching leaves are more likely to be attacked by these leaf-tying caterpillars (Marquis et al. 2002). Manipulation of plant architecture (tying leaf bases together to decrease interleaf distance) increased attack by leaf-tying caterpillars and the damage they inflict (Marquis et al. 2002). Thus, traits that contribute to interleaf proximity are predicted to influence attack by these leaf-tying caterpillars in addition to any differences in plant quality factors (in oaks, these typically include leaf toughness, and water, nitrogen, and tannin content).

The overall goal of this study was to determine the relative importance of plant architecture versus plant quality for differences in attack and damage by the leaf-tying fauna on five sympatric oak species in Missouri. As far as we know, this is the first study to estimate the relative importance of plant architecture and plant quality factors for variation in herbivore attack. Besides the implications for defense theory, it is important to clarify which traits are relevant because these traits set the stage for leaf-tier attack and ecosystem engineering consequences of that attack for the associated arthropod community on the host plant (Lill and Marquis 2003). We took advantage of the fact that in our system, leaf-tying caterpillars quickly colonize leaves that are clipped together, and do so in high abundance (Fig. 1). Creating artificial leaf ties reduces the impact of variation in leaf architecture among plants, making leaves across each species equally available from the standpoint of architecture. We compared natural colonization by leaf tiers of unmanipulated leaves with leaves that were experimentally clipped together to control for both intra- and interspecific variation in plant architecture. We predicted that if architecture alone determined the probability of colonization, then colonization of artificial leaf ties would be equal across all plant species (Fig. 2a). As a result, there would be no correlation between natural colonization and colonization of artificial ties. In contrast, if architecture plays no role in differences in leaf-tier
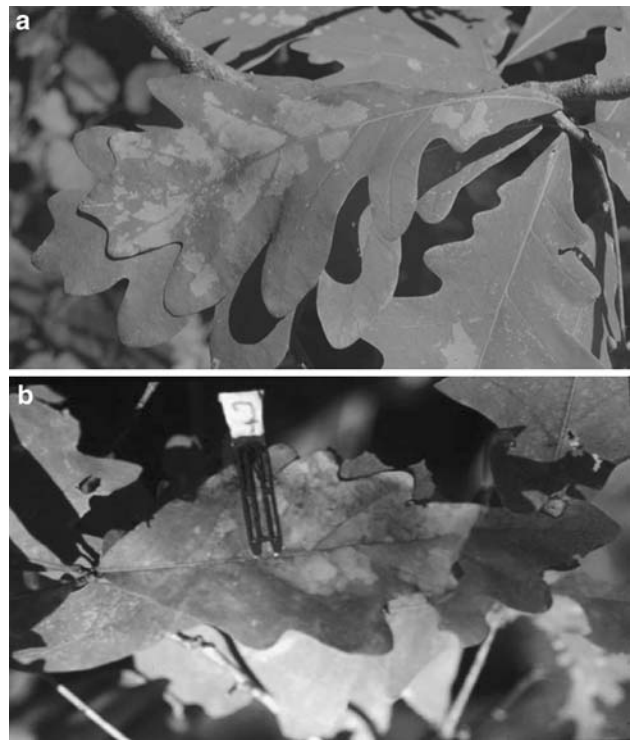

Fig. 1 **a** Natural versus **b** artificial leaf tie on *Quercus alba* at Cuivre River State Park, Troy, MO, USA

colonization among plant species, then colonization of artificial leaf ties would vary among plant species, and be determined only by differences in leaf quality (Fig. 2b). Lack of an architecture effect would result in a positive correlation between colonization of control leaves and artificial leaf ties. To determine the underlying mechanisms for differences in host plant use, we measured interspecific variation in plant architecture (number of touching leaves) and plant quality (water and nitrogen content, specific leaf area, leaf toughness, pubescence, and protein-binding capacity of leaf extracts as a measure of tannin defense). Finally, in laboratory feeding trials, we measured caterpillar survivorship and pupal weight as the realized impact of plant quality on caterpillar fitness components.

Materials and methods

This study was conducted in the North Wildlands Area of Cuivre River State Park (Troy, MO, USA). The North Wildlands Area is an unmanaged, secondary forest, dominated by white and black oak and hickories in the canopy, and oak saplings, *Cornus florida*, *Sassafras albidum*, and *Rhus aromatica* in the understory. The park itself is in the Lincoln Hills geographic region, a northern extension of the Ozark Plateau, and is surrounded by agricultural lands.
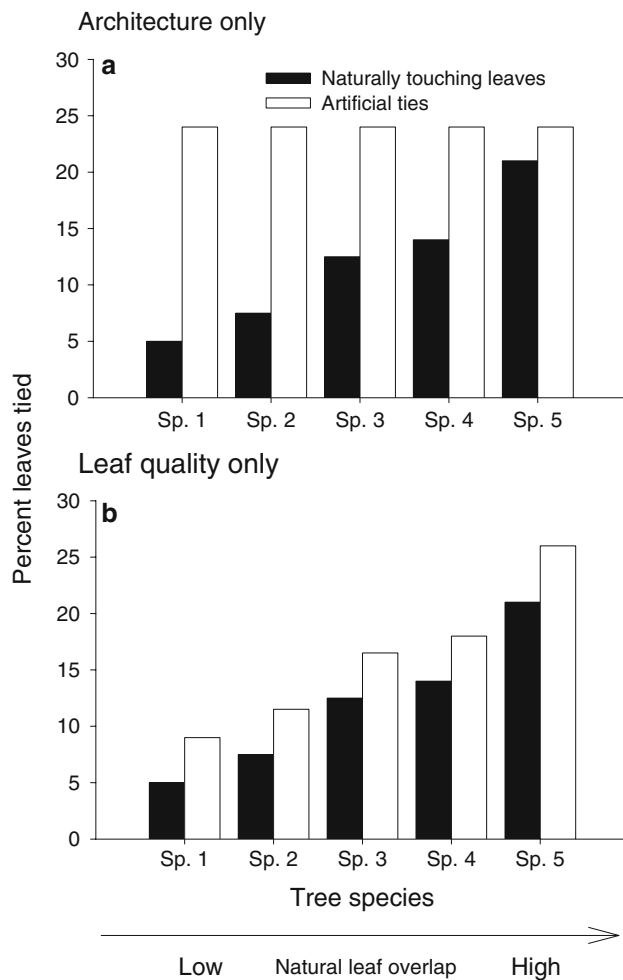
Architecture only

Fig. 2 Hypothesized colonization patterns for natural leaves and leaves clipped together (artificial ties) to equalize differences in plant architecture among tree species. **a** Patterns of colonization if only architectural differences among plant species influence the level of colonization. Hypothetical species are ranked based on the amount of colonization to unmanipulated plants. **b** Patterns of colonization if only leaf quality differences among plant species influence the level of colonization

Leaf ties and herbivore censuses

During the first week of July 1997, ten small, understory trees (<3 m) of each of five oak species (*Quercus alba, Quercus muehlenbergii,* and *Quercus stellata* of the white oak *Leucobalanus* subgenus and *Quercus velutina* and *Quercus rubra* of the red oak *Erythrobalanus* subgenus) were located and marked. On each tree, ten pairs of leaves that had <10% damage were "tied" together with labeled, spring-loaded hair clips (Sally's Beauty Supply, Denton, TX). Only leaves immediately adjacent to one another were clipped together to avoid placing stress on leaf petioles. Twenty additional solitary leaves with less than 10% damage also were marked with a clip to control for the presence of the clip ("control clips"). A branch or section of the

canopy of each experimental tree was left unmanipulated as an additional control to record the density of naturally occurring leaf ties. The number of leaves on the control branches was counted at this time.

Leaf ties were censused 3 times during the season: 23 July, 2–3 September, and 1–2 October. During each census, clips were removed, the number and species of each occupant was recorded, and the ties were re-clipped. Occupants were categorized as tiers (caterpillars that create/maintain the leaf shelter using silk) and non-tiers. Non-tiers included other lepidopteran caterpillars, spiders, leaf-feeding beetles, predaceous beetles, centipedes, phasmids, and predaceous bugs (nabids, reduviids, and pentatomids). During the first census, any missing ties were replaced with new clips, again using leaves with <10% damage. Early leaf abscission of marked leaves (tied and non-tied) was also noted during each census. At the final census, all marked leaves were collected, pressed, and then allowed to dry.

We used non-metric multidimensional scaling (NMDS; PC-ORD; McCune and Mefford 1999; McCune and Grace 2002), a non-parametric ordination analysis, to test for differences in arthropod community composition among the two oak subgenera (*Erythrobalanus* vs. *Leucobalanus*), and among species within subgenera, testing separately the leaf-tier community, the non-leaf-tying herbivores, and non-herbivorous arthropods. Only arthropod species that were encountered 10 or more times (*n* = 7 total species) were included in these ordinations. Data were relativized by the abundance of the most common species, with the Sørenson index used as the distance measure. Initial analysis was first done in the slow-thorough mode producing a starting configuration for a single final run with the real data. Final dimensionality was determined based on stabilization of stress (McCune and Grace 2002); when a stable solution was found (which occurred for the leaf-tier community only), we tested whether host plant subgenera and species within subgenera differed significantly in composition using the multi-response permutation procedure (McCune and Mefford 1999), with a Bonferroni correction for multiple comparisons. Finally, we tested for a plant species effect on estimated species richness of non-tying leaf symbionts found within ties using rarefaction (Coleman curves), calculated with the software EstimateS version 8.0.0 (Colwell 2006).

Leaf damage

For each oak species, 50–100 undamaged leaves spanning the range of leaf sizes encountered were collected and pressed. The length and width of each leaf was measured and leaf area determined by scanning and estimation using SigmaScan Pro (SPSS, Chicago, IL). Leaf area was then regressed on the product of length × width to obtain a

species-specific regression equation that predicted the undamaged leaf area associated with a damaged leaf of known length and width.

The length, width, and area of all experimental leaves (tied and control clip) were measured and the percent leaf area missing was calculated by subtracting the observed leaf area from the estimated undamaged leaf area. We estimated damage due to skeletonization, which was not included in the leaf-chewing damage estimate, by marking skeletonized portions of the leaf onto clear acetate paper, and then measuring the area of the marked areas with a leaf area meter. Percent damage was then analyzed with a two-way ANOVA to test for the effects of treatment (tied vs. control clips), oak species, and the treatment × species interaction. Differences in early abscission between artificially tied and control leaves by tree species, tree, and treatment were tested using a Friedman's non-parametric ANOVA.

Plant traits

Traits measured on census trees

Three leaves were collected from each census tree for analysis of nutritional content/defensive chemistry at the onset of the experiment (first week of July 1997) and during the second and third field censuses (September and October). Leaves were stored on ice and returned to the lab where they were weighed fresh, freeze-dried, and then weighed dry to determine water content. Dried leaves were stored at −80°C and then ground in a leaf sample mill. The nitrogen content of each leaf was determined by microcombustion (Perkin-Elmer CHNS/O analyzer 2400; Perkin-Elmer, Norwalk, CT). Protein-binding capacity of each leaf was determined for the first and third censuses only using the Hagerman radial diffusion assay (Hagerman 1987), with each leaf extract run on three different plates [55-μl aliquots per well, as in Wold and Marquis (1997)], and tannic acid (T-8406; Sigma, St. Louis, MO) as the standard. Additionally, a one-time estimate of the leaf specific area of each leaf collected at the start of the experiment was made by recording the dried mass of a fixed leaf area (five hole punches of 6 mm diameter each) taken from each leaf. Measures of leaf chemistry (water content, nitrogen, and protein-binding capacity) were analyzed using repeated measures ANOVA to test for the effects of census and species. In addition, species-level means for each trait were correlated with the number of naturally occurring leaf ties on control branches using Pearson product-moment correlation.

Traits measured at the species level

In late May 2000, after completion of leaf expansion but before any natural ties had appeared, the number of touch-ing leaves of all leaves of eight saplings per species (except $n = 28$ for *Q. alba*; data from Marquis et al. 2002) was recorded for each tree species. Censusing was conducted on days with little or no wind. In addition, pubescence, and toughness were measured at the species level on each of three leaves for four understory individuals per species. Pubescence was quantified by counting trichomes under a dissecting scope in a 20-mm^2 vision field in one spot per leaf midway along the main vein and midway between the main vein and the leaf edge. Toughness was measured by six punches per leaf with a fruit toughness tester (1.4 mm diameter; Chatillon, New York) as the force (g/mm^2) required to punch a hole through the leaf lamina. Area of each sampled leaf via a leaf area meter and its petiole length were also measured to account for differences in number of touching leaves among oak species. ANOVA (residuals based on untransformed data were normally distributed) was used to test for species effects on each of these traits (type III sums of squares used for proportion of touching leaves as sample size was not equal per species), except for pubescence, which was tested with a Kruskal–Wallis non-parametric ANOVA.

Feeding trials

Feeding trials were conducted in the laboratory to determine realized effects of potential differences in leaf quality among the five oak species on caterpillar fitness components (survival and pupal weight). Caterpillar species tested were those most commonly available at the time. All are primarily oak feeders. Sprigs of leaves (3–4 leaves and associated twigs from understory plants under closed canopy) were collected in the field and immediately placed in water-filled aquapics. Each sprig was enclosed in an inverted 32-oz clear plastic food container containing a hole in the lid to accommodate the protruding aquapic. Within 6 h of collecting the sprigs, single wild-caught leaf-tying caterpillars (first and second instars) were allowed to colonize a pair of clipped leaves on the sprig. Caterpillars were then reared at 23°C under a 14:10-h day:night light regime until they either died or pupated. Sprigs were replaced every 3–4 days, prior to showing any signs of wilting. Pupae were weighed within 3 days of pupation. The source of all leaves and caterpillars was Cuivre River State Park.

Two sets of feeding trials were conducted. In 1998, five different groups of caterpillars (five runs) were begun on the following dates: 23 June, 3 July, 10 July, 16 July, and 6 August. Five species of caterpillars, *Psilocorsis cryptolechiella* (Cham.), *Psilocorsis quercicella* Clems., and *Psilocorsis reflexella* (Pack.) (all Oecophoridae), and *Pseudotelphusa* sp. and *Chionodes fuscomaculella* (Chamb.) (both Gelechiidae), were tested in the different

runs, their relative abundance determined by their availability in the field. For each run, species were distributed evenly among host plants. Total numbers of caterpillars reared on each of the five oak species were: *Q. alba* (24), *Q. muehlenbergii* (34), *Q. rubra* (26), *Q. stellata* (25), and *Q. velutina* (26). Numbers of each species of caterpillar on each species of oak were not sufficient to analyze the effects of host plant on individual species survivorship and pupal weight. Instead, the weight of each pupa was first standardized by the mean for its species, and an ANOVA was run for the effect of host plant on relativized pupal weight, collectively analyzing the pooled results from all five runs and all five caterpillar species. Pupae were not sexed.

The second feeding trial was begun on 15 June 2001. Two species of caterpillars were tested, *Pseudotelphusa* sp. and *Arogalea cristifaciella* (Cham.) (Gelechiidae), with the numbers of caterpillars distributed approximately evenly among host plants for each run. Sample sizes for *Arogalea* and *Pseudotelphusa* were *Q. alba* (7, 27), *Q. muehlenbergii* (6, 7), *Q. rubra* (8, 8), *Q. stellata* (9, 9), and *Q. velutina* (9, 9), respectively. Pupae were sexed upon pupation. Results were analyzed by ANOVA for each species separately to determine the effects of host plant and caterpillar gender on final pupal weight. For both feeding trials, oak species level means for various plant traits were correlated (Pearson product-moment) with relativized (trial 1) and actual pupal weights (trial 2).

Results

Variation in plant architecture

Prior to colonization by leaf tiers, there was considerable intraspecific variation in the number of touching leaves on each species (*Q. alba*, 4–36%; *Q. muehlenbergii,* 12–32%; *Q. rubra,* 4–44%; *Q. stellata*, 16–48%; *Q. velutina*, 8–36%). There was also significant variation among species in the mean proportion of touching leaves ($F_{4,55} = 4.2$, $P = 0.0049$). *Q. alba* and *Q. stellata* had the lowest values, while *Q. rubra* averaged 10% more touching leaves (Table 1). At the species level, proportion of touching leaves was positively correlated with leaf petiole length ($r = 0.99$, $P = 0.0009$) but not with average leaf area ($P = 0.17$).

Variation in leaf quality

Plant species varied significantly in all leaf quality traits measured, both those measured at the plant level (specific leaf area, leaf water content, leaf nitrogen, and protein-binding capacity of leaf extracts: all $P < 0.0001$ except water $P = 0.0658$) and those measured at the species level (foliar pubescence and toughness: $P < 0.003$; Table 1). There was a significant effect of census on those traits measured over time (leaf water content, leaf nitrogen, and protein-binding capacity, $P < 0.001$), with all three variables

Table 1 Leaf quality and architectural traits (means $\pm$ SE) measured for each of the five *Quercus* species

Species	Specific leaf area (g/28.3 mm²)	Toughness (g/mm²)	Petiole length (mm)	% Leaves touching	% H₂O 23 July 1996	% H₂0 2 September 1996	% H₂0 1 October 1996
Quercus stellata	0.76 (0.10)[b]	206 (7) a	6.8 (0.6) a	22.9 (3.8) b	57.4 (1.1) a	54.3 (0.7) a	54.0 (0.7) a
Quercus alba	0.52 (0.07)[a]	193 (16) ab	5.2 (1.0) a	22.0 (1.1) ab	58.1(0.7) ab	54.7 (0.6) a	55.4 (0.5) ab
Quercus velutina	1.06 (0.16)[c]	184 (6) b	18.6 (2.6) c	28.8 (2.8) b	60.4 (0.7) b	57.2 (0.5) b	56.8 (0.6) b
Quercus rubra	0.85 (0.10)bc	211 (14) a	25.2 (3.6) d	33.1 (1.4) bc	59.6 (0.4) ab	56.8 (0.4) b	55.8 (0.6) ab
Quercus muehlenbergii	0.57 (0.07) a	125 (7) c	12.5 (2.7) b	24.7 (3.2) b	59.3 (0.6) ab	58.1 (0.7) b	55.8 (1.1) ab

Species	% Nitrogen 23 July 1996	% Nitrogen 2 September 1996	% Nitrogen 1 October 1996	PBC[a] 23 July 1996	PBC[a] 1 October 1996	Hairs[b] per 20 mm²	Leaf area (cm²)
Q. stellata	1.99 (0.06) ab	1.97 (0.04) b	1.81 (0.05) ab	7.24 (0.58) a	6.55 (0.50) a	32.7 (5.2) e	70.4 (2.1) b
Q. alba	1.93 (0.07) b	1.77 (0.05) a	1.68 (0.05) b	6.91 (0.42) a	4.86 (0.36) b	0.0 (0.0) a	59.2 (2.0) a
Q. velutina	1.94 (0.06) b	1.84 (0.04) ab	1.84 (0.04) ab	3.24 (0.23) b	3.52 (0.33) bc	5.5 (1.1) c	118.7 (4.1) d
Q. rubra	1.92 (0.02) b	1.86 (0.04) ab	1.81 (0.04) ab	2.96 (0.39) b	2.01 (0.13) c	1.4 (0.1) b	90.5 (3.1) c
Q. muehlenbergii	2.19 (0.04) a	2.18 (0.04) c	1.95 (0.05) a	3.35 (0.35) b	3.24 (0.46) c	19.9 (3.3) d	71.3 (2.3) b

[a] PBC measured in milligram tannic acid equivalents

[b] Underside of leaf

See text for sample sizes and sampling protocol. *Different letters* indicate means are significantly different at $P < 0.05$. *PBC* Bovine protein-binding capacity

generally declining with time (except for water content in *Q. muehlenbergii*, which remained similar between censuses 1 and 2, protein-binding capacity in *Q. muehlenbergii* and *Q. velutina,* which was similar between censuses 1 and 3, and nitrogen content in *Q. muehlenbergii* and *Q. stellata*, which remained similar between censuses 1 and 2; results not shown). Accordingly, the species × time interaction was significant for water ($P = 0.0004$) and protein-binding capacity ($P = 0.001$), and marginally significant for nitrogen ($P = 0.09$).

Natural tie density

The five oak species varied significantly in the density of leaf ties recorded on control branches (Fig. 3). The most heavily attacked species, *Q. alba*, had 5 times the density of tied leaves found on the least attacked species, *Q. stellata* ($F_{4,45} = 5.20$; $P = 0.016$).

There was a significant positive correlation between the maximum number of ties on control branches and colonization of experimental ties ($r = 0.967$, $P = 0.007$; Fig. 3). This result suggests that differences in plant architecture among the five species had little impact on colonization differences by leaf-tying caterpillars among the five oak species (scenario in Fig. 2b). Among species, the number of touching leaves was neither correlated with the density of leaf ties ($P = 0.7479$) nor with the total number of leaf-tying caterpillars found within those ties ($P = 0.5479$), contrary to our prediction based on the within-species comparison for *Q. alba*. In contrast, excluding *Q. alba*, average nitrogen availability (percent nitrogen/protein-binding capacity; Lill and Marquis 2001) in census 1 was positively correlated with leaf tie density observed in census 1 (Fig. 4a; $r = 0.9914$,

$P = 0.0087$, $n = 4$) and average nitrogen availability in census 3 showed a non-significant but positive trend with leaf tie density in census 3 (Fig. 4b; $r = 0.8596$, $P = 0.140$, $n = 4$). Again excluding *Q. alba*, mean protein-binding capacity in census 1 was negatively correlated with leaf tie density in census 1 (Fig. 4c; $r = -0.9841$, $P = 0.0159$), and a similar trend was found between mean protein-binding capacity in census 3 and leaf tie density in census 3 (Fig. 4d; $r = -0.8048$, $P = 0.1932$). Neither toughness ($P = 0.203$), specific leaf area ($P = 0.593$) nor pubescence ($P = 0.608$) was correlated with the number of natural ties.

Colonization and community structure of artificial ties

Artificial leaf ties were quickly colonized; 70% percent were occupied 2 weeks after creation, and 97% percent by 4 weeks. Fourteen species of leaf-tying caterpillars colonized these ties, in addition to 25 species of non-tying leaf-chewing insects, two species of sucking insects, and various non-herbivore species (mostly spiders and rove beetles). NMDS ordination found a stable three-axis solution for the seven most common species of the leaf-tier community (Fig. 5, Monte Carlo $P = 0.0392$, final stress = 14.58), but not for non-leaf-tier herbivores or non-herbivore arthropods. For the leaf-tier community, a single run based on the final configuration accounted for 87.8% of the original variation (axis 1 = 0.321, axis 2 = 0.368, and axis 3 = 0.188). A multi-response permutation procedure (MRPP) for five oak species demonstrated that leaf-tying caterpillar communities within ties of the two red oak species (*Q. velutina* and *Q. rubra*) were significantly different from those of the white oak *Leucobalanus* subgenus (*Q. alba*, *Q. muehlenbergii* and *Q. stellata*; $T = -2.40$, $A = 0.0244$, $P = 0.027$). Within

Fig. 3 Natural tie abundance on control branches compared to percent colonization (means ± SE) of artificial ties on those same trees. Compare to Fig. 2
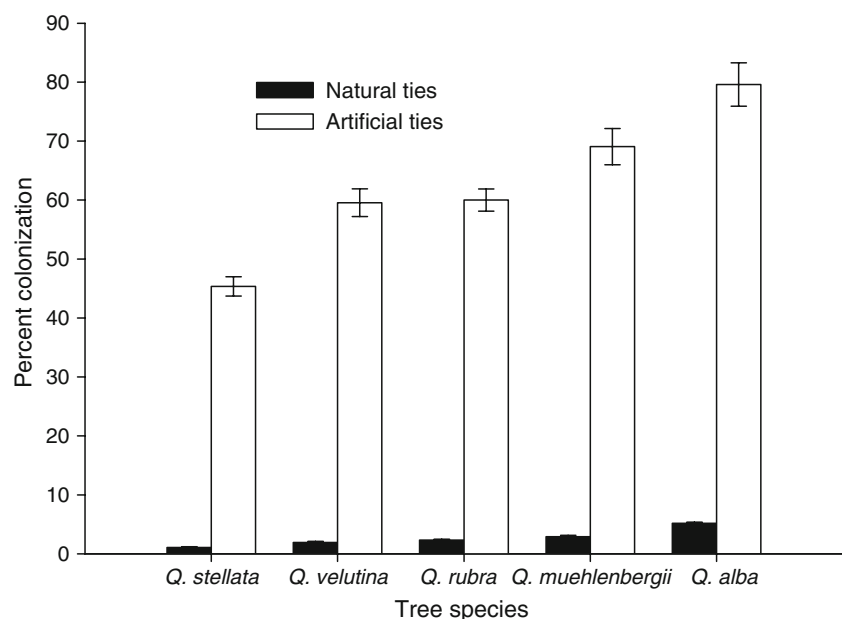
Fig. 4 Relationship between mean (±SE) leaf tie density (number of observed leaf ties per censused leaf recorded) on control branches and mean available nitrogen (±SE; total nitrogen/protein-binding capacity) for **a** census 1 (without *Q. alba*, r = 0.9935, P = 0.0087) and **b** census 3 (without *Q. alba*, r = 0.8595, P = 0.1404), and mean (±SE) leaf tie density and mean (±SE) protein-binding capacity for **c** census 1 (without *Q. alba*, r = −0.9841, P = 0.0159) and **d** census 3 (without *Q. alba*, r = −0.8048, P = 0.1952)
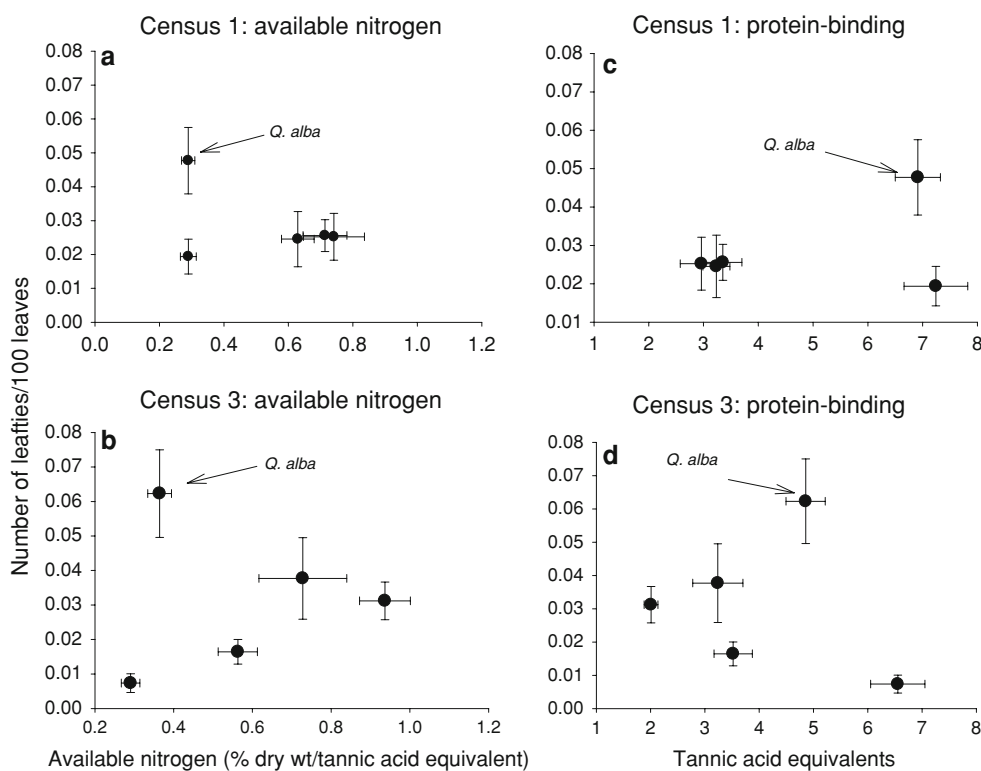
the red oak *Erythrobalanus* subgenus, the communities did not differ between *Q. velutina* and *Q. rubra* (P = 0.36), while within the white oak subgenus, communities were different between *Q. muehlenbergii* and *Q. stellata* (P = 0.002) and between *Q. muehlenbergii* and *Q. alba* (P = 0.006), but not between *Q. stellata* and *Q. alba* (P = 0.14; Fig. 5).

All seven of the common leaf-tying caterpillar species were found at least once on each of the five oak species, except *A. cristifaciella* and *Setiostoma xanthobasis* Zeller, both of which were absent from *Q. stellata*. Four of the seven most common leaf-tier species differed significantly (*Psilocorsis cryptolechiella*, $F_{4,44} = 5.48$, P = 0.011) or marginally so (*Pseudotelphusa* sp., $F_{4,44} = 2.57$, P = 0.0513; *Psilocorsis reflexella*, $F_{4,44} = 2.36$, P = 0.0679; and *Psilocorsis quercicella*, $F_{4,44} = 2.16$, P = 0.0897) in abundance on the five host tree species while the other three did not (ANOVA P ≥ 0.2623). The relative abundances of Gelechiidae (*Pseudotelphusa* sp. and *C. fuscomaculella*) versus Oecophoridae (*P. reflexella*, *P. cryptolechiella*, and *Antaeotricha humilis*) on each tree species contributed to the spread of communities along axis 1 (Fig. 5).

Both plant species and plant subgenus had a significant effect on the engineering impact of leaf ties on species richness of non-leaf-tying symbionts, species that do not make ties but subsequently inhabit them. The estimated number of species (mean ± 95% confidence intervals) of symbionts was significantly greater for the three species of the white
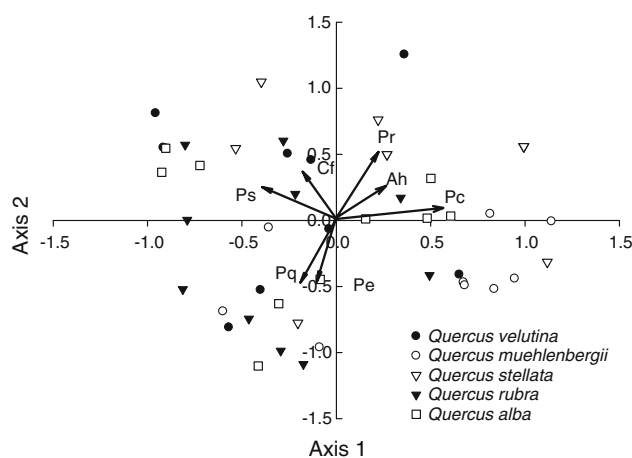

Fig. 5 Non-metric multidimensional scaling ordination of five oak species based on their leaf-tier communities. The leaf-tier communities on *Q. velutina* and *Q. rubra* of the red oak subgenus were significantly different from the leaf-tier communities on *Q. muehlenbergii*, *Q. stellata*, and *Q. alba*, representing the white oak subgenus (multi-response permutation procedure, P < 0.028). *Arrows* represent the weightings of the seven species of insects that served as the basis for the ordination. *Pseudotelphusa* sp. (*Ps*), *Chionodes fuscomaculella* (*Cf*), *Psilocorsis reflexella* (*Pr*), *Psilocorsis cryptolechiella* (*Pc*), *Psilocorsis quercicella* (*Pq*), *Antaeotricha humilis* (*Ah*), *Pococera expandens* (*Pe*)

oak subgenus (*Q. stellata*, 19.9 ± 0.5; *Q. muehlenbergii*, 19.9 ± 0.5; *Q. alba*, 21.9 ± 0.7) than for the two species of the red oak genus (*Q. rubra*, 15.9 ± 0.4; *Q. velutina*, 17.9 ± 0.6).

Leaf damage

Clipping leaves together, and its effect on the abundance and composition of herbivore communities within ties, had a significant effect on the amount of foliar damage. For all five oak species, skeletonization damage caused by leaf-tying caterpillars, was 3–7 times greater on tied leaves than on non-tied (control clip) leaves ($F_{1,910} = 285.04$, $P < 0.0001$; Fig. 6a). In addition there was significant variation among plant species in total skeletonization ($F_{4,20} = 3.50$, $P = 0.025$), as well as a significant species × treatment interaction ($F_{4,910} = 5.96$, $P < 0.0001$). Thus, species varied in the degree to which clipped leaves had increased skeletonization damage compared to controls.

Leaf area lost to leaf chewers (species that do not tie leaves) was also 50–100% greater for tied leaves than for control leaves for three of five tree species (overall treatment effect, $F_{1,910} = 35.43$, $P < 0.0001$; species × treatment interaction, $F_{4,910} = 2.46$, $P = 0.044$; Fig. 6b). *Q. stellata*, *Q. rubra*, and *Q. velutina* showed increased levels of leaf-chewing damage on tied leaves relative to controls, while *Q. alba* and *Q. muehlenburgii* did not.

Total damage (skeletonization and leaf-chewing damage combined) was also 75–100% greater in tied than in non-tied leaves ($F_{1,910} = 150.09$, $P < 0.0001$; Fig. 6c). In addition there was a significant oak species effect ($F_{4,20} = 3.58$, $P < 0.0233$) but no significant species × treatment interaction for total damage ($P = 0.310$).

Leaves were 2–9 times more likely to abscise early when in ties compared to non-tied leaves (tie median = 0.15, control median = 0.00; Friedman's rank ANOVA, $F_{1,83} = 144.3$, $P = 0.0001$; both tree and species effects were non-significant). This early abscission of tied leaves occurred despite the fact that a single control leaf bore the full weight of a clip while that load was shared by the two leaves of an experimental tie. *Q. stellata* and *Q. muehlenbergii* had the highest levels of abscission (Fig. 7). This early abscission was well in advance of natural leaf drop, which typically occurs mid- to late October in Missouri. If skeletonization damage drives the likelihood of early abscission, we would expect a correlation between the amount of this damage and abscission level. However, percent skeletonization damage at the tree level was not correlated with percent of ties abscised early ($P = 0.5842$), suggesting that the response is at the individual leaf level and not at the plant level.

Effect of host plant on caterpillar survival and pupal weight

In the 1998 trial, there was a significant effect of host plant species on relativized pupal weight ($F_{4,130} = 2.44$, $P = 0.048$), but not on survivorship (G-test, $df = 4$, $P = 0.118$). Caterpillars reared on *Q. muehlenbergii* were on average 20% heavier than those reared on the other four species (Fig. 8a).
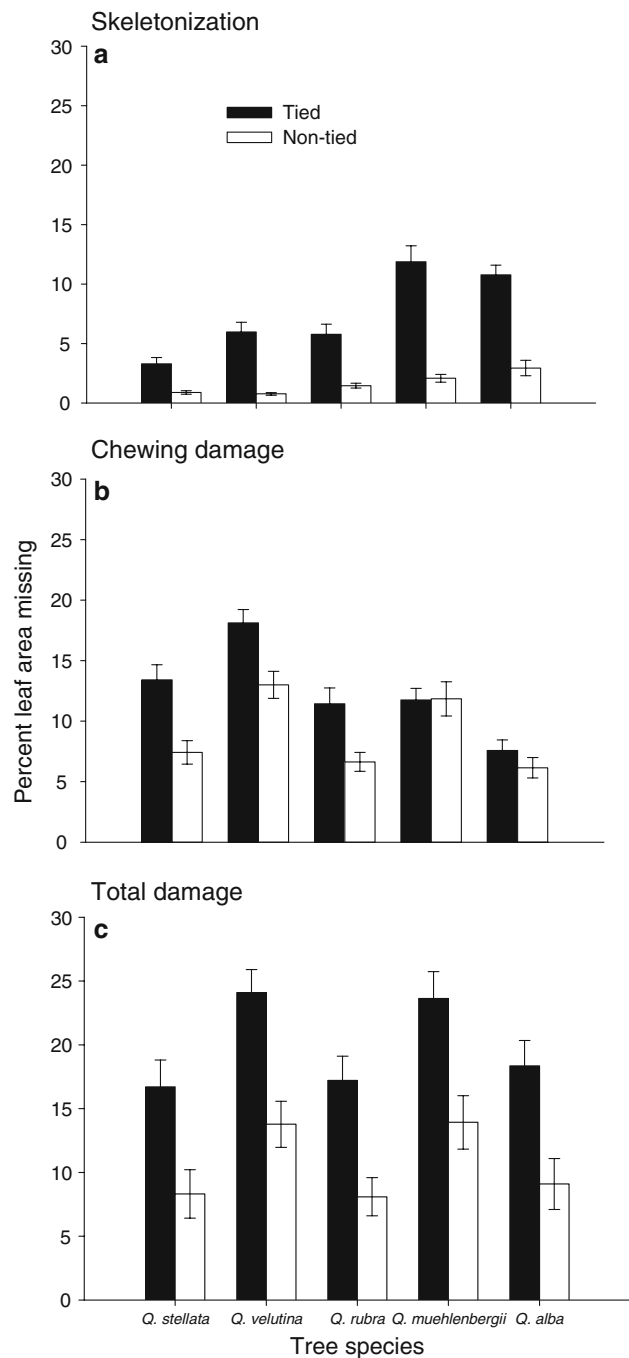

Fig. 6 Damage (mean ± SE) by **a** leaf-tying caterpillars (*Skeletonization*), **b** non-leaf-tying caterpillars (*Chewing damage*), and **c** total damage on control versus artificially tied leaves

In the 2001 trial, caterpillars of *A. cristifaciella* reared on *Q. muehlenbergii* were again heavier by about 50% than those reared on the other four species ($F_{4,28} = 8.41$, $P = 0.0001$; Fig. 8b). There was no significant effect of host plant on *Pseudotelphusa* pupal weight, however ($P = 0.252$). There was also a significant gender effect for *A. cristifaciella* (females were heavier, $F_{1,28} = 16.26$,
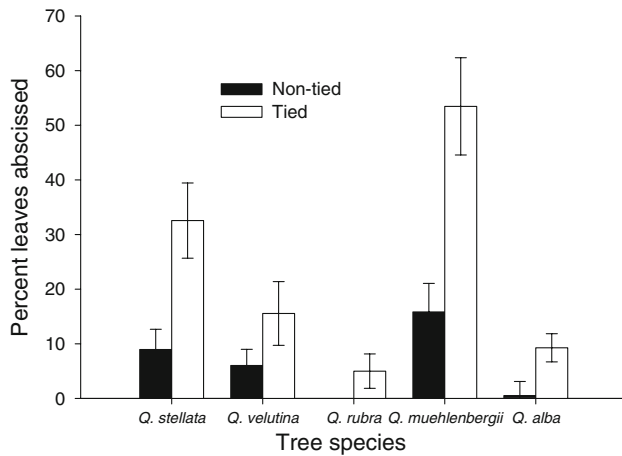
Fig. 7 Effect of leaf-tying on percent early abscission. Abscission of artificially tied leaves (two leaves clipped together with a hair clip) is compared with non-tied leaves (single leaf with clip)

$P = 0.0004$; Fig. 8c), but not for *Pseudotelphusa* sp. ($P = 0.5043$). The interaction between gender and host plant was non-significant for both species. In the feeding trial of 2001, pupal weight of *A. cristifaciella* females was positively correlated with interspecific variation in leaf nitrogen content ($r = 0.915$–0.966, $P = 0.0074$–0.029, depending on the date of leaf collection) and marginally negatively correlated with interspecific variation in toughness ($r = -0.82$, $P = 0.087$). None of the measured plant traits predicted pupal weight differences in *A. cristifaciella* males or in either gender of *Pseudotelphusa* sp. reared on the different host plant species.

Discussion

Individual saplings within each of the five species of oaks varied substantially in the number of touching leaves. There was also significant variation among species in this architectural trait (Table 1). In *Q. alba*, we have already shown that architectural variation among individuals likely accounts for a portion of the variation in attack by leaf-tying caterpillars (Marquis et al. 2002). A reasonable hypothesis would be that such variation might explain differences among plant species in the level of attack by leaf tiers (Fig. 2a). However, our data did not support this hypothesis.

When we made leaves equally accessible architecturally by clipping leaves together, colonization of these artificial leaf ties was highly correlated with the level of colonization of unmanipulated branches (Fig. 3). This result suggests that architecture, as manifested by the number of touching leaves, had little influence on interspecific variation in leaf-tier attack. In contrast, differences in available nitrogen and protein-binding capacity
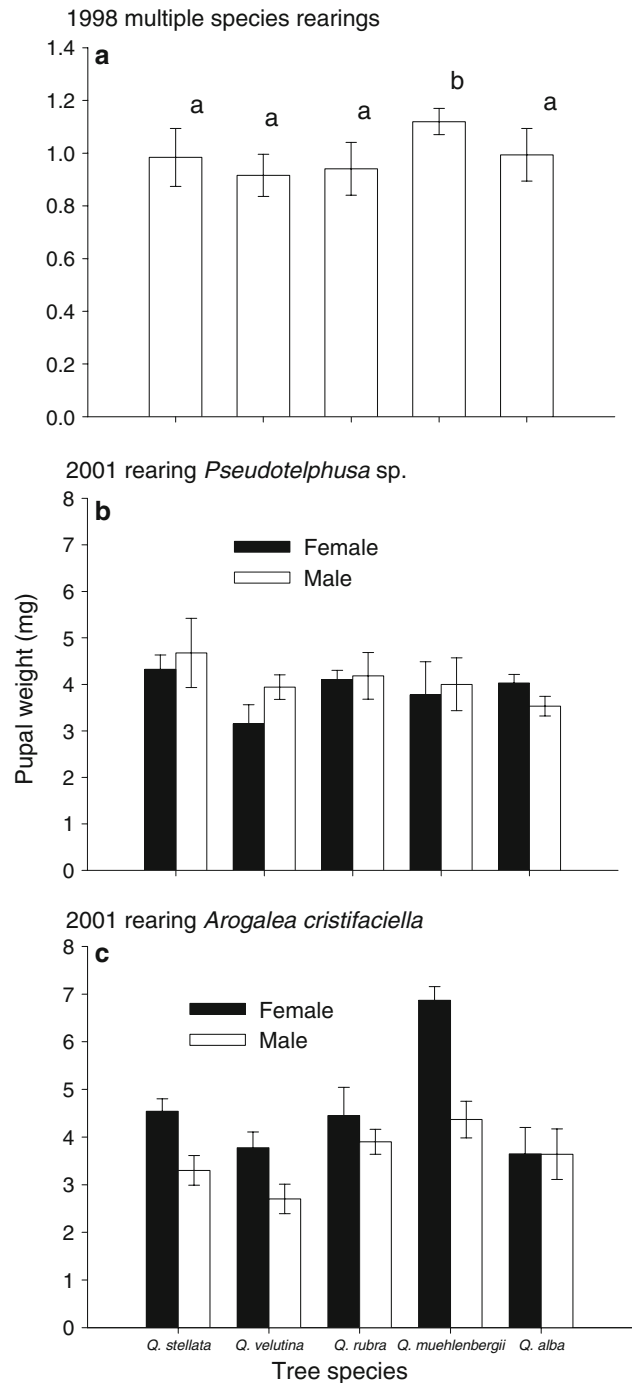

Fig. 8 Effect of host plant species on mean pupal weight (±SE) of leaf-tying caterpillars reared on leaves in the laboratory. **a** Rearings in 1998 of *Pseudotelphusa* sp., *Psilocorsis cryptolechiella*, *Psilocorsis quercicella*, and *Psilocorsis reflexella* and *Chionodes fuscomaculella,* after first standardizing for mean pupal weight by species. Means with *different letters* are significantly different at $P < 0.05$. **b** Trial in 2001 for effect of host plant species on *Arogalea cristifaciella*. **c** Trial in 2001 for effect of host plant species on *Pseudotelphusa* sp.

of leaf extracts were more strongly related with differences in natural colonization among four of the five oak species (Fig. 4). We are currently testing whether these

relationships hold true across eight species of oaks at the same study site.

The five tree species varied significantly in natural density of leaf-tying caterpillars (Fig. 3). In addition, specific insect species showed greater abundances on individual plant species, suggesting differential host preferences. As a result, leaf-tier community composition varied among host plant species (Fig. 5). Our analysis also suggests that there is a phylogenetic signal of the two major subgenera on leaf-tier community composition, as the two red oak subgenus species as a group differed significantly from the three white subgenus oak species. In addition, there was an effect of host plant species and subgenus on estimated species richness of non-tying symbionts in leaf ties, indicating a phylogenetic signal underlying the ecosystem engineering impacts as well.

The five oak species also varied significantly in leaf quality traits that in other systems have been shown to be correlated with inter- and intraspecific variation in insect herbivore attack (Feeny 1970; Forkner et al. 2004). In this study, pupal weight of six leaf-tying caterpillar species was influenced by the host plant species upon which they were reared. Furthermore, pupal weight was positively correlated with leaf nitrogen concentration in the five host plant species, and appears negatively related to leaf toughness. These results, together with the correlations of protein availability and protein-binding capacity with natural leaf tie density (Fig. 4), suggest that leaf quality factors influence interspecific variation in attack by this community of leaf-tying caterpillars. At this time, it is unclear why *Q. alba* had a high level of attack by leaf-tying caterpillars, despite its low level of protein availability in leaf extracts (Fig. 7). Relative host plant abundance certainly must be considered. Although we do have not quantitative measures at this time, *Q. alba* dominates the canopy, and saplings of this tree species are very common in the understory. This high relative abundance may help explain the high relative abundance of leaf-tying caterpillars on *Q. alba* despite its relatively low leaf quality, compared to the other co-occurring species of *Quercus*.

When artificial leaf ties were provided, leaf-tier abundance increased, resulting in an increase in skeletonization damage by the leaf tiers (Fig. 5a). In addition, in three of five species (*Q. stellata*, *Q. rubra*, and *Q. velutina*), damage also increased by non-tying leaf-chewing herbivores (Fig. 5b). We suggest that these non-leaf-tying species seek out leaf ties as a refuge, and as a result increase the total amount of damage to the tie. Whether or not damage by non-leaf-tying species increases at the whole plant level with increased numbers of leaf ties is not clear at this time. Artificial ties may have simply attracted non-tiers from other parts of the plant, and in so doing concentrated their damage, which otherwise would have been more dispersed.

Experiments are needed in which the replicates are entire plants in order to test for these hypothesized indirect effects of leaf ties (ecosystem engineering: Jones et al. 1997; Lill and Marquis 2003).

Not only did overall damage double in all five plant species studied (Fig. 6) as a result of changing plant architecture (i.e., clipping leaves together), but early abscission occurred in four of the five *Quercus* species (Fig. 7). Thus, as a result of leaf-tier attack, plants lose leaf area directly due to consumption and then lose any remaining photosynthetic area represented by those leaves when they are abscised early. Early abscission was noted as early as the 1–2 September census. Given that natural leaf drop at the study sites occurs in mid- to late October, this means that abscission associated with leaf ties began 6–7 weeks early. For *Q. rubra*, the effect would be even greater, as leaf drop in this species is another 2 weeks after that of four other species at the study site (R. J. M., personal observation).

A hypothesis consistent with these results is that leaf quality factors determine the level of attack *among* plant species, and that architectural traits further contribute to colonization *within Quercus* species, as has been shown previously for *Q. alba* (Marquis et al. 2002). Under such a scenario, ovipositing moths might use host plant-specific chemical cues (e.g., plant volatiles) to discriminate among potential hosts in coarse-grained foraging, and architectural traits as fine-grained cues once high-quality hosts have been located. It is clear that further study will be needed to determine the role of architecture versus leaf quality in influencing intraspecific variation in leaf-tier attack for the other four species of oak. Moreover, any comprehensive explanation for observed preferences must also include the third trophic level (Singer and Stireman 2005). Experiments are planned to estimate the effects of host plant identity on parasitism levels for the common leaf-tying caterpillar species, which may help further resolve observed interspecific differences in levels of attack.

References

Carroll MR, Kearby WH (1978) Microlepidopterous leaf tiers (Lepidoptera: Gelichioidea) in central Missouri. J Kans Entomol Soc 51:457–471

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 18:586–608

Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51:565–581

Forkner RE, Marquis RJ, Lill JT (2004) Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*. Ecol Entomol 29:174–187

Fraenkel GS (1959) The raison d'etre of secondary plant substances. Science 129:1466–1470

Fukui A (2001) Indirect interactions mediated by leaf shelters in animal-plant communities. Popul Ecol 43:31–40

Hagerman AE (1987) Extraction of tannin from fresh and preserved leaves. J Chem Ecol 14:1561–1573

Jones CG, Lawton JH, Shashak M (1997) Positive and negative effects of organisms as ecosystem engineers. Ecology 78:1946–1957

Lawton JH (1983) Plant architecture and the diversity of phytophagous insects. Annu Rev Entomol 28:23–39

Lill JT, Marquis RJ (2001) The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia 126:418–428

Lill JT, Marquis RJ (2003) Ecosystem engineering by caterpillars increases insect herbivore density on white oak. Ecology 84:682–690

Lill JT, Marquis RJ (2007) Microhabitat manipulation: ecosystem engineering by shelter-building insects. In: Cuddington K, Byers JE, Wilson WG, Hastings A (eds) Ecosystem engineers: plant to protists. Elsevier, San Diego, pp 107–138

Marquis RJ, Lill JT (2006) Effects of herbivores as physical ecosystem engineers on plant-based trophic interaction webs. In: Ohgushi T, Craig TP, Price PW (eds) Indirect interaction webs: non-trophic linkages through induced plant traits. Columbia University Press, New York

Marquis RJ, Lill JT, Piccini A (2002) Effect of plant architecture on colonization and damage by leaf-tying caterpillars of *Quercus alba*. Oikos 99:531–537

McCune B, Grace JB (2002) Analysis of ecological communities. MJM Software Design, Gleneden Beach

McCune B, Mefford MJ (1999) PC-ORD. multivariate analysis of ecological data, version 4. MJM Software Design, Gleneden Beach

Singer MS, Stireman JO III (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. Ecol Lett 78:1356–1369

Wise MJ, Abrahamson WG (2008) Ducking as a means of resistance to herbivory in tall goldenrod, *Solidago altissima*. Ecology 89:3275–3281

Wold EN, Marquis RJ (1997) Induced defenses in white oak: effects on herbivores and consequences for the plant. Ecology 78:1356–1369