White-Tailed Deer Alter Specialist and Generalist Insect Herbivory Through Plant Traits

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ABSTRACT Within a plant species, leaf traits can vary across environmental, genetic, spatial, and temporal gradients, even showing drastic differences within individuals. Herbivory can also induce variation in leaf morphology, defensive structure, and chemistry including nutritional content. Indirect effects of prior insect herbivory on later herbivores have been well documented, but the induction of trait changes after vertebrate herbivory has been little explored. Here, we examined how browsing of spicebush (*Lindera benzoin* L.), a dominant understory shrub in eastern mesic forests, by white-tailed deer (*Odocoileus virginianus* L.) altered plant quality and subsequent foliar herbivory by insects. Browsing history explained ≈10% of overall leaf trait variation; regenerated leaves had greater water content and specific leaf area ($P = 0.009$), but were lower in nitrogen and greater in carbon ($P < 0.001$), than leaves on unbrowsed plants. However, browsing did not shift terpene chemistry as revealed by GC-MS. In the lab, caterpillars of the specialist spicebush swallowtail (*Papilio troilus* L.) preferred ($P = 0.02$) and grew 20% faster ($P = 0.02$) on foliage from browsed plants; whereas total herbivory in the field, including generalist insect herbivory, was twice as high on unbrowsed plants ($P = 0.016$). These results suggest that the ecological impacts of deer in forest understories can have cascading impacts on arthropod communities by changing the suitability of host-plants to insect herbivores.

KEY WORDS indirect interactions, induced defenses, leaf traits, terpenes, vertebrate herbivory

Leaf traits often determine host-plant suitability for foliar herbivores. Preference and performance of herbivorous insects, for example, can hinge on leaf nutritional content, especially the relative concentration of carbon and nitrogen (Mattson 1980, Awmack and Leather 2002, Behmer 2009). Other leaf traits can also influence suitability, including leaf morphological traits like toughness, thickness, and trichome density (Agrawal and Fishbein 2006); chemical defenses (Broadway and Duffey 1988); and chemical signaling to higher trophic levels (Kessler and Baldwin 2001).

Given the overwhelming importance of plant tissue traits in mediating herbivory, a major challenge for understanding insect community structure is the spatial and temporal heterogeneity of plant tissue quality. Spatially, soil conditions (Wright et al. 2010) and light environment (Osier and Jennings 2007, Niesenbaum and Kluger 2006, Mooney et al. 2009) can alter plant nutrient content and defensive compounds, resulting in substantial variation in plant palatability in space. Even variation among leaves on an individual plant can affect insect herbivore growth and survival (Roslin et al. 2006) and oviposition choice (Kessler and Baldwin 2002). Temporally, the seasonality of leaf quality (Schultz et al. 1982) and ontogenetic development of a plant (Boege and Marquis 2005) can also have significant impacts on insect preference and performance.

One key source of leaf trait variation that varies both spatially and temporally is induction, or changes initiated after herbivory (reviewed in Karban 2011). Typically, induction of defensive chemicals reduces future susceptibility of the plant, resulting in detrimental effects on subsequent herbivores, and is thus a net benefit to plant fitness (Agrawal 1998). In other cases, induction of nutrients toward a wound can actually increase future herbivory by making leaf tissue more palatable (Kaplan et al. 2010). Importantly, evidence suggests that these induced traits can have asymmetric impacts on different herbivores, leading to trait-mediated indirect interactions among herbivores co-occurring on the same host plant (Van Zandt and Agrawal 2004, Ohgushi 2005).

These types of indirect interactions via induced defenses often are studied among insects co-occurring on a host-plant, but indirect interactions among herbivores need not co-occur in space or time. For instance, vertebrate herbivores can have large effects on plant abundance and community composition (McShea et al. 2007), and studies have shown subsequent impacts on the distribution and performance of plant-

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associated insects, including beaver browsing on cottonwood (Martinsen et al. 1998), moose browsing on willows (Den Herder et al. 2004), and deer browsing on a variety of woody plants (Barrett and Stiling 2007). These indirect effects often have focused on chemical defenses (e.g., higher concentrations of toxins), but nutritional changes because of regrowing tissue might be an important component of indirect effects mediated by induction.

The objective of this study was to quantify plant-mediated indirect interactions between early season (April–May) vertebrate and later-season (June–July) insect herbivores on a shared host plant. We were especially interested in the potential impacts of white-tailed deer, an herbivore that has undergone a recent, dramatic increase in abundance (Côté et al. 2004). Here, we examined whether extensive deer browsing on a common understory shrub, spicebush (*Lindera benzoin* L.), indirectly altered insect communities by inducing changes in host plant tissue. We asked the following specific questions: 1) Does deer browsing induce chemical and physical changes to leaf tissue? 2) Do induced changes result in more or less herbivory by generalist chewing insect herbivores in the field? 3) Do deer-induced changes in leaf quality result in differential preference and performance by a specialist insect herbivore?

Materials and Methods

Study Area and Overview. Research was conducted at the Smithsonian Environmental Research Center (SERC), a 2650-acre research preserve located on the western shore of the Chesapeake Bay (38.8860° N, −76.5500° W), MD. Forests are primarily secondary mesic forest growth 75–120 yr of age after agricultural abandonment (Parker et al. 2010). The forest understory at SERC contains >200 native and non-native plant species, but spicebush (*L. benzoin*) is the most common understory shrub. Within a 16-ha mapped Forest Dynamics Plot at SERC, spicebush comprises 41% of all woody plant stems >1 cm in diamter at breast height, occurring at a density of 850 stems ha^{-1} (J.D.P., unpublished data). White-tailed deer (*Odocoileus virginiana*) occur in estimated densities of ≈4 km^{-2} on and around the SERC property (J.D.P., unpublished visual counts from helicopter surveys with infrared imaging), which is in agreement with estimated historical densities (McCabe and McCabe 1997). At SERC, deer browse spicebush primarily as spicebush flowers are senescing and leaves are flushing, leaving distinctive tearing marks on leaves and stems (E.M.L., unpublished data).

We conducted studies over two growing seasons to quantify the frequency and intensity of deer browse on spicebush, its impacts on leaf traits including terpene chemistry, and the response of specialist and generalist insects. In summer 2008, we sampled a 500-m^2 spicebush patch for browsing, as well as marked two separate 500-m transects of spicebush plants from which we sampled leaves for trait analysis, lab herbivory assays, and insect herbivory rates. In late winter of 2009, we fenced a separate set of spicebush plants to prevent browse by deer, and then, in the summer of 2009, we collected leaves from these and paired browsed plants for analysis of terpene chemistry. Finally, we used data gathered on browsing rates from a separate study in the summers of 2010–2011 to bolster our estimates of deer browse rates on spicebush. All studies were conducted within closed canopy secondary forest within 2-km of the geographic coordinates above (see previous paragraph).

Frequency and Intensity of Deer Browsing on Spicebush. To quantify the extent and intensity of deer browse on spicebush plants at SERC, and thus the potential for indirect impacts on insect herbivores, we conducted two surveys of spicebush in the forest understory. In June of 2008, we established a 50-m by 10-m grid with nodes spaced every 5 m. At each node, the nearest spicebush plant was measured for height of tallest growing stem and largest diameter of canopy. We then counted each live stem, noting which, if any, had evidence of deer browsing. The frequency of deer browsing on a plant was determined by dividing the number of stems having at least one leaf or meristem with evidence of deer herbivory by the total number of stems on that plant. To determine the spatial extent of this herbivory, in 2010, we recorded the same metric of deer browsing on all spicebush plants <2 m tall found in $N = 489$ 1-m^2 quadrats located systematically >15 m apart in a nearby 16-ha mapped Forest Dynamics Plot. In 2011, we revisited 186 of these spicebush plants and scored evidence of any browsing to assess whether browsing in 1 yr was predictive of browsing in the next year, which might suggest that deer were targeting particular plants.

Consequences of Browsing on Plant Leaf Traits. To determine whether deer browsing had indirect impacts on foliar insects, we quantified insect herbivory and leaf chemical and morphological traits on browsed versus unbrowsed plants. We first identified spicebush plants with evidence of current-year browse (freshly injured stems and leaves, typically 30–50% of growing stems), in early June 2008, along two transects ≈500 m in length located in closed secondary forest canopy. We found and tagged a total of 120 browsed plants along both transects combined. Selected plants were fully accessible to deer (≤2 m tall). We then found the nearest unbrowsed (lacking signs of browsing in the current season) spicebush plant, approximately matched for size, within 2–5 m of each of the tagged plants.

To quantify how deer browsing altered host-plant quality, we measured a series of leaf traits on a subset of these paired plants in late June of 2008. We removed by hand and collected 10 fully expanded, terminal leaves from each of the browsed and unbrowsed plants from 30 haphazardly chosen pairs. Leaves from browsed plants included only those that grew after earlier browsing during the same growing season. Leaves were placed in plastic bags in a cooler, and then taken within 2 hr to the lab where we immediately measured leaf traits. We quantified leaf toughness as the grams of force required to pierce a fully expanded leaf below the inflorescence by using a force gauge penetrometer, type 516 (Chatillon, Largo, FL). We calculated specific leaf area (SLA) as the area of a leaf (centimeters squared as measured with a LP-80 handheld leaf area meter, LiCor, Lincoln, NE) divided by its dry mass (after 24 h in a drying oven at 60°C). We estimated percent water content as ([fresh mass − dry mass]/fresh mass)*100. We analyzed a subset of leaves from each plant for percentage carbon (C), nitrogen (N), and phosphorus (P). We first filled a plastic 2-ml centrifuge tube with dried leaf tissue and a stainless steel ball, and ran samples for 5 min in a ball mill grinder until samples were a fine powder. We calculated percent C and N by using a ≈2-mg sample of ground plant powder in an EAI CE-440 elemental analyzer (Exeter Analytics, Coventry, United Kingdom). We determined percent P by placing a known mass (≈2 mg) of dried, ground leaf material in a muffle furnace at 550°C for 2 hr (Miller 1998), followed by colorimetric analysis on a microplate spectrophotometer (PowerWave XS; Biotek, Winooski, VT) by using the ammonium molybdate method (Clesceri et al. 1998).

Measuring leaf quality for herbivores requires synthesizing the multivariate, collinear nature of many leaf traits (Cornelissen et al. 2003, Wright et al. 2004). Because we were interested in quantifying the overall multivariate change in leaf characteristics, although not assuming the functional response of insect herbivores, we used principal components analysis (PCA, Legendre and Legendre 1998) to identify independent axes of variance in leaf traits (Wright et al. 2004). These axes scores then were used independently as response variables in linear models, with browsing history as a fixed factor and with pair as a random factor to ask whether trait combinations differed by recent browsing. We also tested the amount of overall variance in leaf traits explained by current-year browsing by using redundancy analysis (Legendre and Legendre 1998), which constrains one axis of a PCA to align with the treatment of interest. All leaf trait values were standardized to a common scale (converted to z-scores) before analysis.

To assess changes in plant secondary chemistry induced by deer browsing, we used 1.22 m (4-ft) chicken wire fencing to exclude deer from 20 small (<2 m) spicebush plants in separate patches, distinct from those used in the leaf trait analysis. Caging preceded spring leaf flush and the onset of browsing, although some plants were in flower. In July of 2009, we collected ≈50 g of leaves from each protected plant and from a nearby, similarly-sized plant that had evidence of current-year browse. Immediately upon harvesting, we cut the leaves with shears directly into a vial filled with dichloromethane and kept samples cool until placing them in a freezer (−20°C). In February of 2010, we analyzed the leaf extract samples by using gas chromatography-mass spectrophotometry (GC-MS), using the methods of Fine et al. (2006). A known standard not naturally occurring in the plant tissue (tetradecane) was added as an internal control to each sample at a concentration of 1 μl/ml. Samples were analyzed using an Agilent 6890N GC and Agilent 5975 Inert MS detector (Agilent Technologies, Santa Clara, CA). Two microliters of sample solution were injected into a 250°C-inlet by using splitless injection. Samples were run on an HP-5MS column (30 m by 0.5 μm by 250 μm) programmed from 30 to 250°C at 10°C/min holding at the final temperature for 3 min at a constant flow of 1 ml/min. We compared observed peaks with databases of identified compounds in the National Institute of Standards and Technology's NIST11 library by using AMDIS software version 2.70 and with known concentrations of commercial terpene reference compounds (limonene, pinene, and caryophyllene oxide). The abundance of compounds was estimated by the area of detected peaks scaled by the tetradecane standard. We used PCA and randomization tests to partition variance in the abundance of detected compounds and test for differences between browsed and unbrowsed plants.

Impacts of Deer Browsing on Foliar Insect Herbivory. To quantify how patterns of insect herbivory changed after deer browsing, we collected all leaves along a major branch ($n = 20$–40 leaves from branch originating in a main stem) from 25 pairs of previously marked plants (not used in the trait collections). We flattened the leaves in a plant press until dry, and then photographed each sheet of leaves with a scale bar. These leaves then were scanned and analyzed using image processing software (SigmaScan, Systat Software, San Jose, CA) to quantify herbivory as the area of leaf missing. The percent of leaf area missing was analyzed using a linear mixed effects model with browsing as a fixed factor, the area of the intact leaf as a random covariate and with error nested by leaf within plant within pair.

Impacts of Deer Browsing on a Specialist Insect Herbivore. We also examined whether deer browsing would affect an herbivore that specializes on spicebush. The spicebush swallowtail butterfly, *Papilio troilus* L. (Lepidoptera: Papilionidae), is found commonly in deciduous lowland forests in eastern North America, where it feeds primarily on spicebush and the confamilial sassafras (*Sassafras albidum* Nutt. Nees) in the Lauraceae (Wagner 2005). At SERC, spicebush swallowtails are uni- to bivoltine, with adults first appearing in flight in late June and caterpillars appearing shortly thereafter, where they feed on the leaves of the plant on which they were laid. Sassafras is relatively uncommon at SERC (J.D.P. et al., unpublished data), and thus most *P. troilus* caterpillars are found on spicebush.

To determine the potential impact of deer browsing on *P. troilus*, we examined whether *P. troilus* exhibited preference and performance differences between browsed versus unbrowsed spicebush plant tissues. In early July of 2008, we collected first- to third-instars from shelters on spicebush plants outside of the experimental transect areas and brought them to the lab. Before use in feeding assays, we kept individual caterpillars in plastic containers (six oz deli containers, Solo Cup Co., Lake Forest, IL) with moistened filter paper and fresh leaves (field-collected from unbrowsed spicebush plants) changed every other day.

To test the preference of caterpillars for browsed versus unbrowsed spicebush leaves, we conducted choice feeding assays in a growth chamber (constant temperature of 25°C, with a photoperiod of 16:8 [L:D] h). Before each assay, we starved caterpillars for $\approx$12 h and recorded their fresh mass to the nearest milligram. Then, we offered each individual ($n = 30$) one fully expanded, entire leaf from each of a browsed and unbrowsed plant in a pair and allowed it to feed ad libitum for 24 h. Before and after the trial, we recorded the area of each leaf with the leaf area meter. We used analysis of covariance to test for the influence of browsing (fixed effect) on the amount of leaf area consumed, using the initial area of the leaf and initial mass of the caterpillar as covariates.

We estimated the performance of *P. troilus* caterpillars on deer-browsed and unbrowsed spicebush plants by randomly assigning caterpillars to be reared solely on leaves from browsed ($n = 18$) or unbrowsed ($n = 17$) spicebush plants. Before beginning the trial, we recorded the mass and instar of caterpillars we had collected in the field and then placed each in a deli container with moistened filter paper and one or two fully expanded leaves selected from previously marked plant pairs not used in the trait or herbivory trials. Leaves were collected from 20 to 30 plants at a time and mixed so that caterpillars experienced leaves from multiple host plants, but only one browsing history, across their development. Caterpillars were kept in a growth chamber that we set at conditions as described above. Every second or third day, we changed leaves and recorded the instar and mass of the caterpillar. Relative growth rate for each caterpillar was calculated as $[\ln(\text{mass}_2/\text{mass}_1)/(\text{time}_2 - \text{time}_1)]$, which gives an estimate of growth in d^{-1}. Because these field-collected caterpillars entered the experiment at different points (from first to third instars at the beginning of the trial), we took the mean of the relative growth rate values across the rearing trial for each caterpillar as one measure of performance. We recorded pupal mass on the second day after pupation as a second measure of performance. We used linear models to analyze each performance metric separately with browsing history of the plant as the fixed factor; in the pupal mass analysis the initial mass of the caterpillar was used as a random covariate.

We conducted statistical analyses in R version 2.12 (R Development Core Team 2010).

Results

White-tailed deer browsing of spicebush is frequent and widespread at SERC. In the small-scale gridded survey, a majority (53 ± 2.2%, mean ± SE) of spicebush plants <2 m tall showed evidence of current year browsing by deer. In the survey across 16 ha, 890 spicebush plants were recorded in 265 unique meters-squared quadrats; of these, browsing of spicebush was recorded on 30.3% ($N = 270$) of plant individuals and in 40% ($N = 106$) of quadrats. Moreover, browsing on 186 of these spicebush plants in 2010 was not predictive of browsing in 2011 (χ^2 test, $P = 0.97$). Thus, we attribute any trait differences in browsed plants to current-year deer browsing history rather than browsing in previous years.

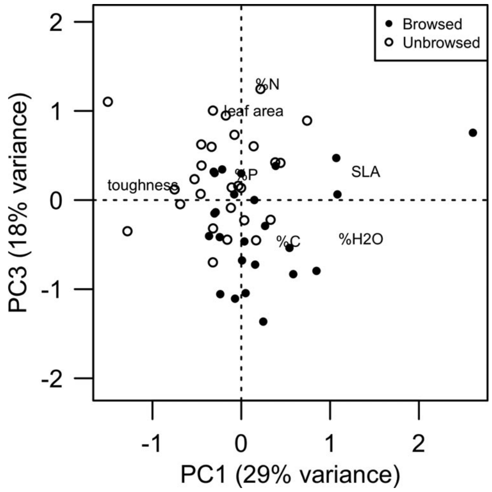

Fig. 1. Principal components analysis of leaf traits shows shift in traits of spicebush leaves after browsing by deer. Filled circles represent browsed plants, open circles represent unbrowsed plants, plotted according to their PCA scores of two PCA axes (PC1 and PC3, explaining 29 and 18% of leaf trait variance, respectively). Variables are plotted according to their mean value in the two PCA axes: leaf area; SLA, specific leaf area; toughness, leaf toughness; percentage H_2O, percent water; percentage C, percent carbon; percentage N, percent nitrogen; percentage P, percent phosphorus. Browsed and unbrowsed plants differed significantly in PC1 and PC3 scores, although not in PC2 scores (18% variance explained, not shown).

Deer browsing caused a significant shift in multivariate space of leaf traits, explaining $\approx$10% of observed variation in seven variables (constrained axis eigenvalue = 0.683, permutation test $P = 0.005$). Principal components analysis determined independent axes of variance, with the first three explaining a combined 64% of variance in leaf traits. The first axis separated plants with a high percentage of H_2O and SLA from plants with tough leaves (Fig. 1); the second axis separated plants with high percentage P from those with larger leaves (greater leaf area); the third axis separated plants with greater percentage N from those with high percentage C (Fig. 1). In independent linear models (*t*-tests), browsed plants were significantly higher along the water-SLA axis (Fig. 1; $F_{1,50} = 7.3885$, $P = 0.009$). Plants did not differ significantly along the size-phosphorus axis (PC2) despite a trend toward greater percentage P in browsed leaves ($F_{1,50} = 2.6688$, $P = 0.11$). Plants differed significantly along the nitrogen-carbon axis, with unbrowsed plants having greater percentage N and browsed plants having greater percentage C (Fig. 1; $F_{1,50} = 12.464$, $P < 0.001$).

Using GC-MS, we detected 12 identifiable terpene compounds in spicebush leaves, including monoter-

Table 1. Mean standardized fraction of GC-MS peak area of distinct terpene compounds of spicebush leaves from plants browsed (n = 17) or unbrowsed (n = 19) by white-tailed deer

ID	Chemical	RT[a]	Browsed (SE)	Unbrowsed (SE)	t-statistic	*P* value
1	5-Hepten-2-one, 6-methyl-	8.69	0.00806 (0.00183)	0.0108 (0.002)	−1.02	0.31
2	Eucalyptol	9.58	7.87e-05 (5.51e-05)	0 (0)	1.43	0.17
3	beta-linalool	10.58	0.000148 (0.000112)	0 (0)	1.32	0.21
4	citronellal	11.43	0.000603 (0.000298)	0.000132 (8.23e-05)	1.52	0.14
5	*carvol*	*12.93*	*1.85e-05 (1.85e-05)*	*0 (0)*	*1*	*0.33*
6	Caryophyllene	15.53	0.0114 (0.00154)	0.0106 (0.0016)	0.383	0.7
7	trans-nerolidol	17.08	0.00243 (0.000794)	0.00181 (0.000604)	0.619	0.54
8	Unidentified sesquiterpene	22.17	0.115 (0.0139)	0.132 (0.0166)	−0.769	0.45
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	20.1	0.0347 (0.00808)	0.038 (0.00836)	−0.285	0.78
10	Unidentified sesquiterpene	20.35	0.0048 (0.00266)	0.000424 (0.000424)	1.62	0.12
11	Unidentified sesquiterpene	20.55	0.00352 (0.00152)	0.00821 (0.00262)	−1.55	0.13
12	Unidentified sesquiterpene	21.76	9.76e-05 (9.76e-05)	0 (0)	1	0.33

[a] RT: retention time in GC-MS sample.

penes, and sesquiterpenes such as caryophyllene (Table 1). Three monoterpenes (eucalyptol, β-linalool, and citronellal) appeared only in plants with a history of current year browse, although not in sufficient occurrence and concentration to detect a significant difference. Analyzing the overall terpene profile in the PCA (Fig. 2), the majority of plants are clustered when plotted against the first two PC axes, which explain 50% of the variance in terpene chemistry. More browsed than unbrowsed plants appear to have divergent terpene profiles in these axes (Fig. 2), but the permutation test revealed little of the variance was explained by current-year browse (3.5% variance explained, $P = 0.21$).

In the field, there was significantly greater total insect folivory on unbrowsed plants (Fig. 3; $F_{1,1} = 6.762$, $P = 0.016$). Visual inspection of invertebrates on browsed and unbrowsed spicebush plants indicated that a diversity of potential herbivores including specialist and generalist caterpillars, leaf beetles, and orthopterans were the most common insect herbivores, likely to be responsible for the differential damage.

In contrast to patterns of total herbivory in the field, the specialist caterpillar *P. troilus* in the lab significantly preferred leaves from plants that had been browsed (Fig. 4a and $F_{1,1} = 5.5697$, $P = 0.02$). Rearing experiments demonstrated that swallowtail caterpillars also performed better on leaves from browsed plants, with a mean relative growth rate significantly greater for caterpillars reared on these leaves (Fig. 4b and $F_{1,31} = 5.947$, $P = 0.02$). Pupal mass did not differ by browsing treatment (Fig. 4c and $F_{1,16} = 0.2287$, $P = 0.63$).

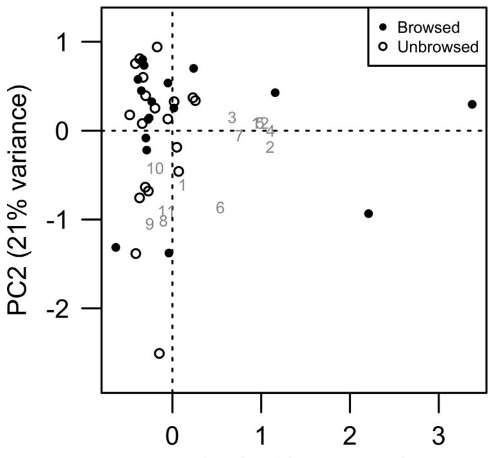

Fig. 2. Principal components analysis of abundance of 12 terpene compunds (gray numbers, see Table 1 for identity) detected in spicebush leaves by GC-MS. Filled circles represent browsed plants, open circles represent unbrowsed plants, plotted according to their PCA scores of two PCA axes (PC1 and PC2, explaining 29 and 21% of leaf terpene variance, respectively). Permutation tests show no significant effect of current-year browsing history on terpene chemistry ($P = 0.21$).

Discussion

Widespread and intense deer browsing induced significant changes in spicebush leaf traits, including shifts in morphology and nutrient concentration. In the field we also observed greater total insect herbivory on unbrowsed plants, but a specialist insect

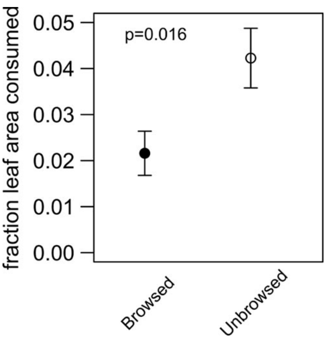

Fig. 3. Unbrowsed plants in the field sustain more leaf herbivory from chewing insects than browsed plants. Mean fraction consumed per leaf type is plotted as points for clarity, although the statistical model analyzed area consumed with total leaf area as a covariate. Bars indicate standard errors, and *P* value is of model estimate of fixed effect of browsing on amount of leaf tissue consumed.

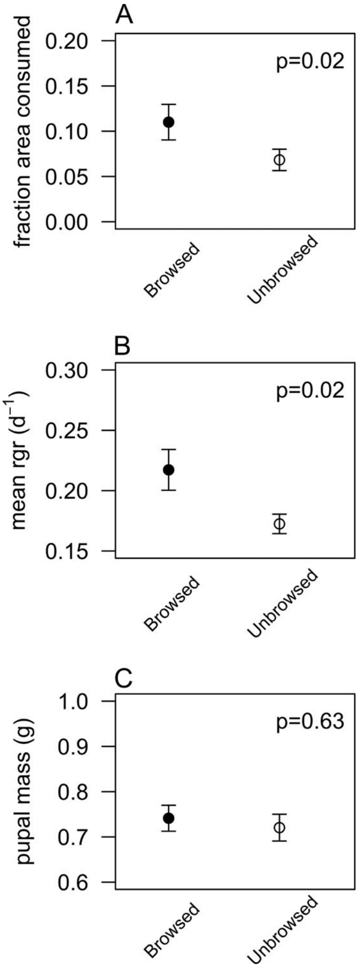

Fig. 4. Preference and performance of the specialist herbivore *P. troilus* (Lepidoptera: Papilionidae) on leaves collected from recently browsed versus unbrowsed spicebush (*L. benzoin*) plants. Third- or fourth-instar caterpillars preferentially consumed (A) and had greater relative growth rates (B) but not pupal mass (C) on regrown leaves from browsed versus unbrowsed plants. Bars indicate standard errors, and *P* value is from model estimate of fixed effect of browsing on amount of leaf tissue consumed. In (A), mean fraction consumed per leaf type is plotted as points for clarity, although the statistical model analyzed area consumed with total leaf area as a covariate. In (B) and (C), *P* values are of linear model estimates of browsing impact on relative growth rate and pupal mass, respectively.

showed preference for browsed regrowth in lab trials. Although previous studies have implicated induction in chemical defenses as predictors of future insect herbivory, we did not detect significant shifts in defensive terpene compounds.

The changes to forest plant communities caused by white-tailed deer have been addressed by a large body of literature (e.g., McShea and Rappole 2000, Côté et al. 2004), but the potential for deer browsing to be reflected in changes to insect communities is relatively unstudied (but see Barrett and Stiling 2007, Bressette et al. 2012). Spicebush is frequently and heavily browsed by white-tailed deer (Results, and Liang and Seagle 2002, Bressette et al. 2012), and spicebush itself supports a diversity of plant-associated insects and their avian predators (Niesenbaum 1992, Skoczylas et al. 2007). Thus, induced changes to spicebush leaves could have pervasive impacts on insect communities.

In our study, browsing altered spicebush leaf morphology and nutrient content. Leaves on browsed plants were thinner, less tough, and had higher water content compared with leaves from nearby unbrowsed plants—changes that are generally favorable to insect herbivory. However, leaves from browsed plants also had lower percentage N and greater percentage C—changes that can make leaves less attractive to herbivores.

Plant defense theory (reviewed in Stamp 2003) posits a fundamental tradeoff between allocation of resources to growth and to defense against herbivory. Thus, we might expect spicebush leaves to be more well-defended after browsing, or to be faster growing (more nutrient-rich, and thus more palatable) after browsing, but not both. Our findings of higher SLA and water content (Fig. 1) suggest a growth response by the plant, although leaves of browsed plants had less percentage N and more percentage C (Fig. 1). Because this observed higher percentage C could represent an investment in carbon-based defenses, we tested whether browsing induced higher levels of terpenes and terpenoids, carbon-based compounds found in high concentrations in spicebush (Tucker et al. 1994) that are known to deter deer browsing (Duncan et al. 2001). We hypothesized that plant terpene chemistry might also be important to the spicebush caterpillar *P. troilus* because adults use plant defenses as cues to finding a suitable host plant (Carter and Feeny 1999), and because *P. troilus* caterpillars exude terpenoids from their defensive osmeteria as fourth instars (Omura et al. 2006).

We did not see a strong difference in overall terpene profile among browsed versus unbrowsed plants, although certain browsed individuals appeared more chemically unique than others (Fig. 2). This divergence was based on the presence of three monoterpenoids and one unidentified sesquiterpene unique to plants that had been browsed; unbrowsed plants had no compounds that were not also found in at least one browsed plant (Table 1). These results suggest that deer herbivory may induce terpene synthesis in spicebush, such as that found in pines after defoliation by leaf-cutter ants (Barnola et al. 1994). However, detailed studies of the impact of Sitka black-tailed deer (*Odocoileus hemionus sitkensis* Merriam) on western redcedar (*Thuja plicata* Donn ex D.Don) could not isolate induction of terpenes in field (Vourc'h et al. 2002) or in greenhouse experiments (Vourc'h et al. 2003), suggesting that terpenoid induction by vertebrates may not be widespread. Our ability to document induced terpene chemistry, and particularly any within-plant tradeoffs between defensive terpene chemistry and leaf nutrient content, may be limited because our terpene measurements were conducted separately from our estimation of other leaf traits. Finally, the terpenoids that we identified in spicebush foliage contained no known compounds that overlap with *P. troilus* osmeterial secretions (Omura et al. 2006), suggesting de novo synthesis rather than sequestration of plant defensive compounds.

The reduced herbivory that we observed on leaves from browsed plants in the field (Fig. 3) is consistent with a decrease in leaf percentage N, as N is often limiting to insects and thus a strong predictor of insect preference (Mattson 1980). In contrast, Barrett and Stiling (2007) found that herbivory by Key deer (*Odocoileus virginianus clavium* Barbour & G.M. Allen) caused leaf percentage N to increase, with concomitant increases in insect herbivory. Although contradictory in the overall direction of the impact, both results suggest a strong influence of nutrient content as an induced trait that predicts future herbivory. Response to induced leaf trait changes also differed by a broad category of herbivore specialization. Although we did not identify the chewing herbivores responsible for the leaf injury observed in the field, contemporaneous visual surveys found a diversity of generalist herbivores (Orthoptera, Coleoptera, and Lepidoptera), in addition to the locally known specialist (*P. troilus*) and the oligophagous caterpillar, *Epimecis hortaria* F. (Lepidoptera: Geometridae). Thus, we consider the field herbivory to represent a generalized response to leaf traits induced by deer browse, implying that deer could have largely negative plant-mediated impacts on generalist insect herbivores of spicebush.

In contrast, the specialist *P. troilus* responded positively in terms of larval preference and growth rate performance to leaves from plants browsed by deer (Fig. 4). Faster larval development is hypothesized to lead to greater survival through escape from natural enemies (Benrey and Denno 1997), raising the possibility of facilitation of this and other similarly-responding specialists as part of an indirect interaction web triggered by deer herbivory on spicebush (Ohgushi 2005).

The mechanisms of the preference for, and better performance on, browsed plants by the specialist caterpillar are not clear. Leaves from browsed plants were thinner and had greater average water content, but less percentage N, than leaves from unbrowsed plants. We did not assess the broader suite of constitutive carbon-based molecules such as fiber, tannins, and phenolic acids, but these digestibility-reducing compounds could play a role in increasing available nitrogen even if total percentage N by mass is lower in browsed leaves.

In conclusion, we found that white-tailed deer herbivory induced significant changes in spicebush leaf chemistry that had opposing effects on insect herbivory in the field (a mixed group of specialists and generalists) versus a specialist caterpillar in the lab. Such induced changes suggest the potential for widespread but relatively unstudied indirect effects mediated by this keystone herbivore.

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