Foliar Chemistry and Gypsy Moth, *Lymantria dispar* (L.), Herbivory on Pure American Chestnut, *Castanea dentata* (Fam: Fagaceae), and a Disease-Resistant Hybrid

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ABSTRACT We investigated herbivore suitability, foliar chemistry, and seedling growth of blight-susceptible pure American chestnut, *Castanea dentata* (Marsh.) Borkh., and a blight-resistant Chinese chestnut, *Castanea mollisima* Blume × American chestnut hybrid, using supplemental fertilizer and ectomycorrhizal inoculation to affect nutrient availability and nutrient uptake, and the gypsy moth, *Lymantria dispar* (L.), to measure herbivore suitability. Gypsy moth performance was best on fertilized hybrid seedlings, and was lowest on untreated American chestnut seedlings. Foliar carbohydrates were greatest, and tannin levels were lowest, in mycorrhizae-inoculated American chestnut. Foliar nitrogen was also high in mycorrhizal American chestnut, and was equivalent to that found in fertilized seedlings of both species. American chestnut seedlings had greater height and diameter growth than hybrids, regardless of soil amendments. Our results suggest that blight resistance may exact a cost in plant growth and productivity for this chestnut hybrid, and may enhance plant suitability for a generalist herbivore. Additionally, enhanced gypsy moth performance on blight-resistant chestnut hybrids has implications with respect to the restoration of chestnut to eastern deciduous forests, because intense herbivore pressure could compromise seedling growth and survival, and play a role in sustaining potentially damaging gypsy moth populations. The implications of this work within the context of current theories addressing herbivore–plant relations are discussed.

KEY WORDS restoration forestry, defoliation, mycorrhiza, herbivore–plant relations

PLANT FOLIAR CHEMISTRY is influenced by nutrient availability, realized and potential nutrient uptake (Chapin 1991), and soil conditions (Kramer and Kozlowski 1979), and can influence plant structure (Karban and Myers 1989), plant fitness (Whitham and Mopper 1985, Agrawal 1998, 1999), herbivore feeding patterns (Faeth 1985), and herbivore survival (Hartley and Lawton 1987). Symbiotic mycorrhizal fungi enhance plant nutrient uptake (Smith and Read 1997), leading to changes in mineral composition of plant tissue (Pacovsky and Fuller 1988, Bolan 1991), and potentially affecting plant susceptibility to herbivory (Rhoades 1985, Schultz 1988, Jones and Coleman 1991). Although the relationship between herbivore susceptibility and foliar chemistry has been well characterized in many systems, including insects feeding on deciduous trees (Thorsteinson 1960, Mattson 1980, Scriber and Slansky 1981, Mattson and Scriber 1987), herbivore–plant–fungal interactions in the context of mycorrhizal symbioses are poorly understood (Jones and Last 1991). There is an increasing body of evidence, however, to suggest that plant herbivores interact in system-specific ways with both vesicular arbuscular mycorrhizae (Warnock et al. 1982, Bethlenfalvay and Dakessian 1984, Rabin and Pacovsky 1985, Gange and West 1994, Borowicz 1997) and ectomycorrhizae (Del Vecchio et al. 1993, Gehring and Witham 1994, Rieske 2001). Herbivory can affect the extent of mycorrhizal colonization (Bethlenfalvay and Dakessian 1984, Del Vecchio et al. 1993, Gehring and Witham 1994), and conversely, mycorrhizal colonization can affect herbivory through changes in plant carbon allocation and foliar chemistry (Rabin and Pacovsky 1985, Gange and West 1994, Borowicz 1997), or other undetermined mechanisms (Rieske 2001).

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), is a defoliating herbivore that is sensitive to changes in plant foliar chemistry (Barbosa and Greenblatt 1979, Barbosa et al. 1983). Although they are tannin-adapted insects (Schultz and Lechowicz 1986, Barbosa and Krischik 1987), gypsy moths are extremely polyphagous and can exploit >400 species of deciduous trees as hosts (Liebhold et al. 1995). The gypsy moth was introduced into North America in the late 1800s, and began expanding its geographic range during the same time period that American chestnut, *Castanea dentata* (Marsh.) Borkh. (Fam: Fagaceae),

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was devastated by the introduced chestnut blight fungus, *Cryphonectria (Endothia) parasitica* (Murrill) Barr (Ascomycetes: Diaporthales). Historically, American chestnut was a dominant component of the northern and central hardwood forests of eastern North America, and at one time was among the most abundant trees in portions of the Appalachians (Braun 1950, Sharpe et al. 1976). The accidental introduction of the chestnut blight fungus in 1904 virtually eliminated the American chestnut from its former range. In spite of the temporal segregation, Mosher (1915) demonstrated that the gypsy moth could exploit American chestnut as a host plant.

Efforts at developing blight-resistant chestnut varieties have met with some success (Griffin 2000). Hybridization with the inherently blight-resistant Chinese chestnut, *Castanea mollisima* Blume, followed by repeated selection of blight-resistant progeny and backcrossing with American chestnut, produces a blight-resistant product with many of the desirable growth and morphological characteristics of the American chestnut (Jaynes and Dierauf 1982, Griffin 2000). Intercrossing between blight-resistant backcross progeny ensures heritability of disease resistance. After three successive backcrosses and one intercross, the hybrid chestnuts will have received >93% of their genetic traits from American chestnut parents, and it is assumed that with the exception of blight-resistance, the backcross hybrids and the original American chestnuts will be identical (Burnham et al. 1986, Hebard 2001). The incidence of blight-resistance and disease susceptibility is not straightforward, however, and can be adversely affected by altitude (Griffin 2000), cold temperatures (Griffin et al. 1993), xeric conditions (Gao and Shain 1995), and vegetative competition (Griffin et al. 1991). Additionally, resistance to one stressing agent may affect susceptibility to a second stressor (Chapin 1991, Pell and Dann 1991), suggesting that blight-resistant chestnut could alter susceptibility to herbivory.

As progress toward development of a blight resistant hybrid chestnut continues and the prospects for the return of chestnut to the eastern North American landscape becomes more tangible, questions arise as to how these plants will interact with preexisting stresses. We expect that enhanced nutrient availability, whether because of increased nutrient uptake via fertilization or because of increased uptake potential from mycorrhizal colonization, will increase chestnut seedling productivity and defensive abilities, reducing herbivore performance (Rhoades 1985). However, our understanding of the herbivore–chestnut system is far from complete, and questions remain concerning the relative productivity and herbivore susceptibility of blight-resistant chestnut. Consequently, we investigated herbivore suitability, foliar chemistry, and growth of blight-susceptible pure American chestnut, and a blight-resistant Chinese × American hybrid, using supplemental fertilizer to affect nutrient availability and inoculation with *Pisolithus tinctorius,* a commonly occurring, commercially available ectomycorrhizal (ECM) fungus, to affect uptake potential.

Materials and Methods

Plant Material. Pure American chestnut seed originated from stands located near West Salem, WI. Chinese × American hybrids were open-pollinated second backcrosses from the American Chestnut Foundation breeding program (Meadowview, VA). Seeds were cold stratified in damp peat moss for 6 mo before planting in a peat-vermiculite potting mix in 6×25-cm plastic tree tubes (Stuewe and Sons, Corvallis, OR). Germinating seedlings were either: 1) inoculated with a commercially available ECM suspension containing spores of *P. tinctorius* (Persoon) (Basidiomycetes: Sclerodermatales) (Mycorrhizal Applications Inc., Grants Pass, OR) by irrigating 5-ml aliquots of spore concentrate, each containing 1×10^6 spores, into the rooting zone 14 d after seeding; 2) fertilized weekly with 25-ml aliquots of a 15 mM NH_4NO_3 solution; each fertilized seedling receiving a total of 73.5 mg of N during the trial; or 3) untreated control seedlings, watered with deionized water only throughout the study. Thus, there were two species (American chestnut and the Chinese × American hybrid) and three treatments (mycorrhizal, fertilized, and untreated controls). Seedlings were grown in a greenhouse (18–25°C, 60–90% RH, and 14:10 L:D) for 10 wk before experimental use, and irrigated four times weekly with deionized water. Because of differential seedling survival among species and among treatments, there were 66 seedlings available for herbivore feeding trials, 55 seedlings designated for phytochemical analysis, and 58 seedlings designated for plant growth measurements.

Herbivore Feeding Assays. Herbivore feeding assays were conducted by allowing caterpillars to feed on foliage for the duration of the fourth stadium. Gypsy moth caterpillars (USDA-ARS Laboratory, Otis AFB, Cape Cod, MA) were held in growth chambers with a 15:9 (L:D) photoperiod at 23°C, in the University of Kentucky Forest Entomology Quarantine Facility (voucher specimens are on file at the University of Kentucky). Newly molted caterpillars previously fed a wheat germ-based artificial diet (Southland Products, Lake Village, AR) were starved for 24 h before use in assays.

Whole leaves were excised with the petiole intact, surface-sterilized in 0.1% sodium hypochlorite solution, individually weighed, and placed in florists' water picks in 7×21-cm clear plastic rearing boxes. Caterpillars were placed individually in boxes with foliage and monitored at 12-h intervals for the duration of the stadium. At 2–3-d intervals, leaves were replaced to ensure freshness, using foliage from seedlings in the same treatment. Seedlings were retired as their leaves were consumed. Immediately after molting, insects were removed from the foliage and frozen, and plant tissue, insect cadavers, and waste material were oven-dried at 60°C for 5 d and weighed. Relative growth rate (RGR = caterpillar biomass gained (mg) $\cdot$ (initial caterpillar wt (mg))$^{-1}$ $\cdot$ (time (d))$^{-1}$), relative consumption rate (RCR = amount of leaf material consumed (mg) $\cdot$ (initial caterpillar (mg))$^{-1}$ $\cdot$ (time (d))$^{-1}$),

Table 1. Partial MANOVA results for herbivore performance, foliar chemistry, and seedling growth on ECM-inoculated, fertilized, and untreated control American chestnut and Chinese × American hybrid grown from seed

Factor	Pillai's Trace $F_{df}/Pr > F$		
	Herbivore performance	Foliar chemistry	Seedling growth
Chestnut species	$F_{3, 57} = 6.67/0.0006$	$F_{3, 47} = 4.71/0.0008$	$F_{2, 51} = 4.71/0.01$
Soil amendment	$F_{6, 116} = 2.56/0.02$	$F_{6, 96} = 3.30/0.006$	$F_{4, 104} = 0.73/0.58$
Species × amendment	$F_{6, 114} = 1.27/0.27$	$F_{6, 96} = 1.56/0.17$	$F_{4, 102} = 0.42/0.79$

and length of caterpillar stadium (duration of fourth instar in d) were calculated (Slansky and Scriber 1985) as measures of caterpillar performance and foliar suitability.

Phytochemical Analysis. Concurrent with the herbivore feeding trial, seedlings were assessed for foliar chemical characteristics. Leaves from each harvested seedling were removed at the base, flash frozen in liquid nitrogen, and immediately ground into a fine powder and stored at –80°C. Tissue was then freeze-dried (VirTis Freezemobile 12SL, VirTis Co., Gradiner, NY) for ≈36 h and stored at –80°C before analysis of foliar total nonstructural carbohydrates, nitrogen, and tannins. Carbohydrate analysis was conducted with an anthrone/thiourea reagent (Quarmby and Allen 1989), nitrogen was analyzed using a Leco TN-300 nitrogen determinator (Leco Corp., St. Joseph, MI), and tannin levels were analyzed using a radial diffusion assay (Hagerman 1987), with a protein-based agar as the substrate for tannic acid binding.

Plant Growth. We determined seedling height by measuring the length from a predetermined basal stem mark at the root collar to the tip of the leading branch, and determined basal stem diameter by averaging two perpendicular basal measurements at the predetermined point near the root collar. After harvest, mycorrhiza-inoculated seedlings were assessed for colonization by visually examining root tips under 20× magnification for the presence or absence of the ECM hyphal mantle.

Statistical Analysis. Multivariate analysis of variance (MANOVA; SAS Institute 1997) was used to assess

herbivore performance, foliar chemistry, and seedling productivity using tree species and soil amendments as main effects. Data were tested for normality before analysis. Gypsy moth RGR, RCR, and development time were used as herbivore response variables, with initial caterpillar weight as a covariate (Raubenheimer and Simpson 1992). Foliar carbohydrates, nitrogen, and tannins were used as the phytochemical response variables. Seedling height and basal stem diameter were our measures of seedling growth. Because our sample size was small, Pillai's Trace F statistic was used to assess overall significance of the multivariate models, which were followed by univariate analyses of individual response variables using a fixed effects model. A least squares means procedure was used to analyze pairwise comparisons. We also used canonical correlation analysis (CANCORR, SAS Institute 1997), which assesses the relationship between the best linear combination of response variables describing insect performance and correlates it with the best linear combination of response variables describing plant foliar chemistry. In addition, the relationship of each individual insect performance parameter to each of the foliar components was determined using linear regression analysis.

Results

Examination of seedling roots for the hyphal mantle indicative of colonization revealed no detectable colonization by ECM fungi. Despite the lack of observable colonization, mycorrhizal inoculation did appear to affect several seedling and herbivore performance

Table 2. Herbivore performance, foliar chemistry, and seedling growth (least squares mean (SE)) on ECM-inoculated, fertilized, and untreated control American chestnut and Chinese × American hybrid grown from seed

Parameter	American chestnut			Chinese × American hybrid		
	ECM-inoculated	Fertilized	Control	ECM-inoculated	Fertilized	Control
Herbivore growth	N = 7	N = 8	N = 10	N = 12	N = 13	N = 16
RGR[a]	0.15 (0.03)ab	0.12 (0.03)b	0.11 (0.02)b	0.18 (0.23)ab	0.21 (0.02)a	0.14 (0.02)b
RCR[b]	4.84 (0.71)a	4.19 (0.64)ab	2.97 (0.57)bc	4.08 (0.52)ab	2.53 (0.51)c	3.25 (0.48)abc
Development time[c]	9.42 (0.65)ab	9.20 (0.59)b	11.02 (0.53)a	8.03 (0.48)bc	7.35 (0.47)c	8.78 (0.44)b
Foliar chemistry	N = 9	N = 8	N = 8	N = 10	N = 10	N = 10
Carbohydrates (%)	10.44 (0.35)a	9.49 (0.38)abc	10.32 (0.38)a	9.73 (0.34)ab	8.51 (0.34)d	8.64 (0.34)cd
Nitrogen (%)	1.93 (0.07)a	1.86 (0.07)ab	1.72 (0.07)b	1.74 (0.07)b	1.98 (0.07)a	1.71 (0.07)b
Tannins (TAE)[d]	6.81 (0.56)c	7.55 (0.59)bc	7.99 (0.59)bc	8.79 (0.53)ab	7.47 (0.53)bc	8.76 (0.53)ab
Seedling growth	N = 8	N = 10	N = 7	N = 12	N = 7	N = 14
Height (cm)	23.55 (2.33)ab	26.52 (2.09)a	24.43 (2.50)ab	20.67 (1.91)b	24.01 (2.50)ab	19.07 (1.76)b
Diameter (cm)	2.91 (0.24)a	3.22 (0.22)a	3.11 (0.26)a	2.55 (0.20)b	2.76 (0.26)ab	2.25 (0.18)b

[a] RGR = relative growth rate $(mg)(mg)^{-1}(d)^{-1}$.
[b] RCR = relative consumption rate $(mg)(mg)^{-1}(d)^{-1}$.
[c] Development time = length of fourth larval stadium (d).
[d] TAE = tannic acid equivalents.

Table 3.   Herbivore performance, foliar chemistry, and seedling growth (least squares mean (SE)) on American chestnut and Chinese × American hybrid grown from seed

Parameter	American chestnut	Hybrid	$F_{df}/Pr < F$
Herbivore growth	N = 25	N = 41	
RGR[a]	0.13 (0.02)	0.18 (0.01)	$F_{1, 59} = 6.58/0.01$
RCR[b]	3.99 (0.38)	3.29 (0.29)	$F_{1, 59} = 1.94/0.17$
Development[c]	9.88 (0.35)	8.05 (0.27)	$F_{1, 59} = 13.13/0.0006$
Foliar chemistry	N = 25	N = 30	
Carbohydrates (%)	10.08 (0.21)	8.96 (0.19)	$F_{1, 49} = 15.51/0.0003$
Nitrogen (%)	1.84 (0.04)	1.81 (0.04)	$F_{1, 49} = 0.30/0.59$
Tannins (TAE)[d]	7.45 (0.33)	8.33 (0.30)	$F_{1, 49} = 4.09/0.05$
Seedling growth	N = 25	N = 33	
Height (cm)	24.83 (1.33)	21.25 (1.20)	$F_{1, 52} = 6.00/0.02$
Diameter (cm)	3.08 (0.14)	2.52 (0.12)	$F_{1, 52} = 11.81/0.001$

[a] RGR = relative growth rate $(mg)(mg)^{-1}(d)^{-1}$.
[b] RCR = relative consumption rate $(mg)(mg)^{-1}(d)^{-1}$.
[c] Development = length of fourth larval stadium (d).
[d] TAE = tannic acid equivalents.

parameters. In the multivariate analysis (Table 1), herbivore performance varied with tree species and with soil amendment. Caterpillar RGR was weakly affected by species and treatment (Table 2, $F = 1.91$; $df = 6, 59$; $P = 0.09$). Larvae-fed fertilized hybrid seedlings grew faster than larvae-fed hybrid controls or larvae-fed fertilized or control American chestnut. Caterpillar consumption was also weakly affected by species and soil amendments ($F = 1.86$; $df = 6, 59$; $P = 0.10$), and was lowest for those fed foliage from fertilized hybrid seedlings. Development time was most strongly affected ($F = 6.93$; $df = 6, 59$; $P < 0.0001$), and was most rapid for caterpillars reared on fertilized hybrid tissue, and slowest for those fed tissue from untreated American chestnut seedlings. Herbivore response differed between species, regardless of soil amendments (Table 3). Caterpillars fed hybrid foliage grew larger and developed more rapidly than caterpillars fed foliage from pure American chestnut, although the consumption rate was unaffected by tree species. Herbivore performance also varied based on soil amendments across species (Table 4). Caterpillars fed foliage from ECM-inoculated seedlings ate mar-

ginally more than their counterparts fed foliage from fertilized and untreated control seedlings. Caterpillars fed foliage from untreated control seedlings consumed the least and had the slowest development.

Plant foliar chemistry varied with tree species and with soil amendment (Table 1). Foliar carbohydrates were strongly affected by species and treatment (Table 2, $F = 5.46$; $df = 5, 49$; $P = 0.0005$). Fertilized hybrids had the lowest carbohydrate levels, which were equivalent to carbohydrate concentrations in the hybrid control seedlings. Within species, fertilized seedlings had the lowest foliar carbohydrates. Foliar nitrogen was similarly affected by species and treatment (Table 2, $F = 2.97$; $df = 5, 49$; $P = 0.02$). Nitrogen levels of ECM-inoculated American chestnut seedlings were equivalent to fertilized American chestnut and fertilized hybrid seedlings. ECM-inoculated hybrids and untreated control seedlings of both species had the lowest levels of foliar nitrogen. Foliar tannin concentrations were only weakly affected by species and seedling treatment (Table 2, $F = 2.07$; $df = 5, 49$; $P = 0.08$). Tannin levels were lowest in ECM-inoculated American chestnut seedlings, and highest in foliage from the ECM-inoculated and control hybrid seedlings. Seedling foliar chemistry varied between species, independent of treatments (Table 3). Foliar carbohydrates were higher, and foliar tannins were lower, in American chestnut foliage than in foliage from hybrids. There was no difference in foliar nitrogen between species. We also saw differences in foliar chemistry based on seedling treatments, independent of tree species (Table 4). ECM-inoculated seedlings contained higher levels of foliar carbohydrates than did fertilized and untreated control seedlings. Fertilized seedlings had higher levels of foliar nitrogen than did control seedlings, but there was no difference in foliar tannins across seedling treatments.

Seedling growth varied with chestnut species, but not with seedling treatment (Table 1). Seedling height was only weakly affected by species and treatment (Table 2, $F = 1.92$; $df = 5, 52$; $P = 0.10$). Fertilized American chestnuts had the greatest height growth, and grew taller than the ECM-inoculated and control

Table 4.   Herbivore performance, foliar chemistry, and seedling growth (least squares mean (SE)) on ECM-inoculated, fertilized, and untreated control American chestnut and Chinese × American hybrid grown from seed

Parameter	ECM-inoculated	Fertilized	Control	$F_{df}/Pr < F$
Herbivore growth	N = 19	N = 21	N = 26	
RGR[a]	0.16 (0.02)a	0.16 (0.02)a	0.13 (0.02)a	$F_{2, 59} = 1.55/0.22$
RCR[b]	4.46 (0.43)a	3.36 (0.41)b	3.11 (0.37)b	$F_{2, 59} = 3.03/0.06$
Development[c]	8.72 (0.40)b	8.28 (0.38)b	9.90 (0.34)a	$F_{2, 59} = 9.41/0.0003$
Foliar chemistry	N = 19	N = 18	N = 18	
Carbohydrates (%)	10.09 (0.24)a	9.00 (0.25)b	9.47 (0.25)b	$F_{2, 49} = 4.90/0.01$
Nitrogen (%)	1.83 (0.05)ab	1.92 (0.05)a	1.71 (0.05)b	$F_{2, 49} = 4.65/0.01$
Tannins (TAE)[d]	7.80 (0.38)a	7.51 (0.39)a	8.37 (0.39)a	$F_{2, 49} = 1.35/0.26$
Seedling growth	N = 20	N = 17	N = 21	
Height (cm)	22.10 (1.51)a	25.27 (1.63)a	21.75 (1.53)a	$F_{2, 52} = 1.54/0.22$
Diameter (cm)	2.73 (0.16)a	2.99 (0.17)a	2.68 (0.16)a	$F_{2, 52} = 1.17/0.32$

[a] RGR = relative growth rate $(mg)(mg)^{-1}(d)^{-1}$.
[b] RCR = relative consumption rate $(mg)(mg)^{-1}(d)^{-1}$.
[c] Development = length of fourth larval stadium (d).
[d] TAE = tannic acid equivalents.

Table 5. Linear regression analysis of herbivore performance with chestnut seedling foliar components

Herbivore parameter	Variable	Parameter estimate (SE)	t-value	$Pr > |t|$	Overall model $F_{df}/Pr < F$
RGR[a]	intercept	0.16 (0.04)	3.75	<0.001	$F_{3,\ 613} = 5.61/0.0008$
	carbohydrate	−0.009 (0.003)	−3.30	0.001	
	nitrogen	0.04 (0.02)	2.71	0.007	
	tannin	0.001 (0.002)	0.72	0.47	
RCR[b]	intercept	1.97 (0.94)	2.10	0.04	$F_{3,\ 613} = 5.72/0.0007$
	carbohydrate	0.25 (0.06)	1.08	<0.001	
	nitrogen	−0.34 (0.34)	−0.99	0.32	
	tannin	−0.03 (0.04)	−0.60	0.55	
Development[c]	intercept	8.99 (1.00)	8.96	<0.001	$F_{3,\ 613} = 17.50/<0.0001$
	carbohydrate	0.35 (0.07)	5.37	<0.001	
	nitrogen	−1.89 (0.36)	−5.19	<0.001	
	tannin	−0.02 (0.05)	−0.32	0.75	

[a] RGR = relative growth rate $(mg)(mg)^{-1}(d)^{-1}$.
[b] RCR = relative consumption rate $(mg)(mg)^{-1}(d)^{-1}$.
[c] Development = length of fourth larval stadium (d).

hybrid seedlings, but there was no difference in height between seedling treatments within either species. Seedling stem diameter was strongly affected by species and seedling treatment (Table 2, $F = 3.11$; df = 5, 52; $P = 0.01$). Fertilized American chestnut seedlings also had the greatest stem diameter, and again, put on significantly more diameter growth than either the ECM-inoculated or the control hybrid seedlings. Stem diameter growth among seedling treatments of American chestnut was equivalent to that of the fertilized hybrid seedlings. Independent of seedling treatments, American chestnut grew larger than the hybrid with respect to both seedling height and stem diameter (Table 3). However, there were no significant differences in seedling growth based on treatments across species (Table 4).

The multivariate canonical correlation analysis, which generates a linear combination of foliar chemistry variables (carbohydrates, nitrogen, and tannins) that correlates most closely with a linear combination of insect growth variables (RGR, RCR, and development time), yielded a highly significant relationship between one combination of foliar chemistry variables and herbivore performance variables ($F = 7.83$; df = 9, 1839; $P < 0.0001$), which explained 10% of the variability in the data (squared canonical correlation = 0.1018). The linear combination of foliar response variables was strongly and positively correlated with nitrogen (0.55), and negatively correlated with carbohydrates (0.78). The corresponding linear combination of herbivore response variables was negatively correlated with insect development time (0.87) and consumption (0.46), and positively correlated with growth rate (0.51). These results can be interpreted to mean that high levels of foliar nitrogen and low levels of foliar carbohydrates are significantly related to rapid caterpillar development, low consumption rates, and high growth rates. The analysis of American chestnut alone yielded no significant canonical correlations ($F = 1.54$; df = 9, 489; $P = 0.13$). For the hybrid, however, there was a highly significant relationship between foliar chemistry and herbivore performance ($F = 6.11$; df = 9, 983.4; $P < 0.0001$), which again explained >10% of the variability

(squared canonical correlation = 0.1042). The relationship was strongly and positively correlated with nitrogen (0.90), and negatively correlated with carbohydrates (0.32) and tannins (0.53). The corresponding combination of herbivore response variables included a negative correlation with development time (0.84) and consumption (0.65), and a positive correlation with growth (0.41). Again, this suggests that high levels of foliar nitrogen and low levels of foliar carbohydrates (and also foliar tannins, in the case of the hybrid) is related to rapid caterpillar development, low consumption rates, and high growth rates. The univariate linear regression analysis corroborated these findings. Gypsy moth RGR was positively and significantly correlated with foliar nitrogen, and slightly but significantly negatively correlated with foliar carbohydrates (Table 5). There was no relationship between caterpillar RGR and foliar tannins. Gypsy moth consumption was positively and significantly associated with foliar carbohydrates, but there was no significant relationship between caterpillar consumption and foliar nitrogen and tannins. Lastly, caterpillar development time was positively correlated with foliar carbohydrates, and negatively correlated with foliar nitrogen, and again, was not affected by foliar tannins.

Discussion

Chestnut seedling growth, foliar chemistry, and susceptibility to gypsy moth herbivory were affected by species and by our soil amendments. American chestnut seedlings had greater height and diameter growth than the hybrids. However, the Chinese chestnut hybrid appeared more responsive to nutrient additions, with slight increases in height and diameter that were not evident in the fertilized American chestnuts.

Differences in seedling performance and foliar chemistry were not affected by mycorrhizal inoculation, and examination of seedling roots in our study did not show appreciable mycorrhizal colonization. However, seedling growth and herbivore performance on mycorrhizae-inoculated seedlings was often intermediate between the fertilized and untreated control

seedlings, which indicates that mycorrhizae may have been present but that colonization was too low for visual detection. Mycorrhizae appear to function as mutualists under nutrient-poor conditions, enhancing plant growth and defense through increased nutrient acquisition (Smith and Read 1997), and appear to function as carbon parasites under nutrient-rich conditions, drawing carbon away from plant tissues for fungal growth.

Foliage of American chestnut had higher concentrations of carbohydrates and lower concentrations of tannins, which, given equivalent levels of foliar nitrogen, suggest that it is a higher quality host for generalist herbivores than the hybrid. Herbivore performance was weakest on untreated American chestnut seedlings. Gypsy moths reared on foliage from untreated American chestnut had the lowest growth, low to moderate consumption, and the slowest development time. Hybrid foliar chemistry appeared strongly responsive to nutrient addition. Foliar nitrogen concentrations in the fertilized hybrids were among the highest measured. This may in turn explain why gypsy moth caterpillar performance was superior on the fertilized hybrid, because nitrogen is a limiting factor in the growth and development of herbivorous insects (Mattson 1980, Scriber and Slansky 1981). Decreased consumption with increased growth and rapid development suggests a significant increase in host plant quality with fertilization of the hybrids.

Our data fit with predictions of the growth–differentiation balance hypothesis, which postulates a physiological trade-off between plant growth and plant defense that exists when the production of secondary metabolites diverts resources away from the production of additional biomass (Loomis 1932, 1953; Lorio and Sommers 1986). The hypothesis predicts that environmental factors that limit plant growth more than photosynthesis (including nutrient deprivation) will cause the accumulation of carbon-based compounds for use in secondary metabolism (Herms and Mattson 1992). American chestnut seedlings exhibited increased height and diameter growth, and decreased herbivore performance, when compared with the hybrid. The lower nutritional quality and higher tannin concentration of the hybrid should provide a barrier to successful herbivory. However, increased nutrient acquisition in plants with carbon-based defenses can reduce overall defense, ultimately increasing herbivore performance (Herms and Mattson 1982). Our data support that view. In addition, our results suggest that partial blight resistance or some other trait of the hybrid seedlings may exact a cost in plant growth and productivity, as well as in herbivore susceptibility.

Enhanced performance of gypsy moth caterpillars on the Chinese × American hybrid implies that gypsy moth pressure may increase with the widespread planting of hybridized chestnut in eastern deciduous forest stands. Herbivore pressure on newly introduced hybrid chestnuts could compromise their growth and survival, and play a role in sustaining potentially damaging gypsy moth populations.

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