Interactions between a fungal pathogen, foliar properties, and generalist herbivores

S.K. Kellogg[1], F.V. Hebard[2] & L.K. Rieske[1,*]

[1]*University of Kentucky, Department of Entomology, S-225 Ag. North, Lexington, KY 40546-0091 USA;* [2]*The American Chestnut Foundation Research Farm, 14005 Glenbrook Avenue, Meadowview, VA 24361-9703, USA*

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Abstract

American [*Castanea dentata* (Marsh) Borkh.] × Chinese [*Castanea mollissima* Blume] chestnut (Fagac, ae) hybrids are a novel system in which to study influences of phytopathogenic fungi and woody plant hybridization on herbivore susceptibility, as the hybrids are well characterized with regard to resistance to the chestnut blight fungus [*Cryphonectria parasita* (Murr) Barr (*Endothia*) Diaporthales: Valsaceae] and variability is present. We chose two groups of resistance-rated backcross chestnut that shared an F1 parent and had different American parents. Foliage from both backcross groups and the parent trees was sampled on three dates for use in feeding assays with gypsy moth larvae [*Lymantria dispar* (L.) [Lepidoptera: Lymantriidae], adult Japanese beetles [*Popillia japonica* Newman (Coleoptera: Scarabaeidae)], and fall webworm larvae [*Hyphantria cunea* Drury (Lepidoptera: Arctiidae)], respectively. Foliar analyses were performed concurrently and included carbohydrate, tannin, and nitrogen content, toughness, and density. Blight resistance had almost no effect on herbivore performance or foliar chemistry. When the parent trees and backcross groups were compared, however, significant differences in gypsy moth performance and Japanese beetle consumption were evident. There were no differences in fall webworm consumption. Most foliar characteristics measured differed among chestnut genotypes at some point in the season, and all varied seasonally. No clear pattern emerges with respect to the relationship among blight resistance, herbivore susceptibility, foliar properties, and plant genotype, and more research is needed to separate these effects.

Introduction

Interactions among stressing agents can influence host-plant performance and plant–community structure. In deciduous trees, both temporally and spatially segregated intraguild (Haukioja & Niemela, 1979; Bergelson et al., 1986; Hartley & Lawton, 1987; Neuvonen et al., 1988) and interguild interactions among herbivores have been characterized (Faeth, 1985, 1986; Rieske & Raffa, 1998; Foss & Rieske, 2004). A less-studied aspect of relations among stressing agents is the interaction between fungal pathogens and insect herbivores (but see Hatcher, 1995; Rostás et al., 2003).

Phytopathogenic fungal infection is a dynamic process, and when considered in the context of plant herbivory, is inherently complex. Neither plant nor herbivore response is static (Hatcher, 1995), and the cross-effects of these interactions are exceedingly complex (Rostás et al., 2003). Plant response to fungal infection ranges from physical alterations of the cell wall, either locally or distally, to production of pathogenesis-related proteins (Sticher et al., 1997). Some herbivores may be negatively affected by fungal-induced plant responses, whereas others may benefit from fungal predigestion of complex carbohydrates, or possibly through ingestion of the fungus itself, which may provide nutritional benefits (Mondy & Corio-Costet, 2000). Evidence of systemic effects of fungal pathogens on herbivore susceptibility of host plants is sparse, as most studies involve direct (Hatcher et al., 1994; Kok et al., 1996) or locally mediated effects (Tinney et al., 1998; Stout et al., 1999). Of particular interest are the systemic effects of a fungal pathogen on its host plant, with potential alterations in host-plant susceptibility to herbivory.

*Correspondence: E-mail: lrieske@uky.edu, University of Kentucky, Department of Entomology, S-225 Ag. North, Lexington, KY 40546-0091 USA.

Such interactions have been characterized to some extent in coniferous systems (Wallin & Raffa, 1999; Kopper et al., 2004), but are relatively unstudied in deciduous trees. We investigated such an interaction by examining several chestnuts, *Castanea* spp. (Fagaeae) that vary in resistance to the chestnut blight fungus, *Cryphonectria (Endothia) parasitica* (Murr.) Barr, (Diaporthalis: Valsaceae) for susceptibility to generalist herbivores.

The American chestnut [*C. dentata* (Marsh.) Borkh] was once a dominant overstory component of eastern US forests (Braun, 1950). It was effectively eliminated from the landscape within 50 years following the introduction of the blight fungus in 1904. Since the 1920s, many efforts have been made to develop an American-type chestnut that is resistant to the fungus (Burnham et al., 1986), with the goal of re-introducing a blight-resistant chestnut into eastern deciduous forests. Both Chinese (*C. mollissima* Blume) and Japanese (*C. crenata* Siebold and Zucc.) chestnuts are resistant (Roane et al., 1986) but lack the desirable silvicultural characteristics of the American chestnut. Current chestnut breeding programs focus on crossing Chinese with American chestnut, which is followed by repeated selection of blight-resistant progeny and back-crossing with American chestnut (Burnham et al., 1986). The product is a chestnut that is mostly American in genotype and phenotype, but possesses the blight resistance of the Chinese.

Several factors can negatively affect blight resistance, such as plant competition (Griffin et al., 1991), low temperatures (Griffin et al., 1993), and dry conditions (Gao & Shain, 1995). In addition, one stressor can influence a plant's response to a second stressor (Chapin, 1991); blight resistance may affect the tree's susceptibility to other stresses such as herbivory (Hatcher, 1995). Phytochemical differences among chestnut species that influence blight susceptibility, such as tannin solubility (Nienstaedt, 1953), may also influence susceptibility to herbivory.

Many Chinese × American chestnut hybrids are well characterized with respect to blight resistance, but little is known of their response to other stressors or how they will interact with existing forest species. Highly polyphagous insect herbivores such as the gypsy moth [*Lymantria dispar* (L.)], the Japanese beetle (*Popillia japonica* Newman), and the fall webworm (*Hyphantria cunea* Drury), will readily feed on American chestnut (Mosher, 1915; Tietz, 1952; Fleming, 1972), but differ in temporal and spatial interactions with their hosts. Each occurs in the eastern United States in areas of prospective chestnut restoration.

The gypsy moth is a univoltine, polyphagous defoliator that feeds by consuming entire leaves in early spring. Similarly, the Japanese beetle is a univoltine polyphage, but adults skeletonize leaf tissue in midsummer. Both are exotics whose introductions coincided with the demise of the American chestnut, but who have had ample exposure to chestnut in their native ranges. In contrast, the fall webworm is a middle-to-late-summer native, multivoltine polyphage that initially feeds by skeletonization and progresses to leaf consumption as the larvae develop.

Differences in insect phenology and feeding modes may influence the ability of these herbivores to exploit their chestnut hosts, whose foliar characteristics vary with genotype (Rieske et al., 2003), and may also vary seasonally (Feeny, 1970; Faeth, 1986). Assessing these chestnut hybrids for their interactions with other organisms prior to their introduction is a crucial step in understanding their potential impact on forest dynamics, and is also a unique system for studying host-mediated interactions between fungal pathogens and insect herbivores. We assessed the relationship of chestnut blight resistance to herbivore suitability to determine the relationship between blight resistance or host species, herbivore resistance, and foliar characteristics.

Materials and methods

Plant material

Foliage was obtained from trees at The American Chestnut Foundation nursery in Meadowview, VA. Sampled trees were planted in 1999 from seed collected in 1998. Trees were irrigated as needed, and were fertilized throughout the first and second growing seasons with 30-10-10 (NPK) at a rate of approximately 9.75 ml per tree every 2 weeks. During the second and third years, they were fertilized with ammonium nitrate, diammonium phosphate, and potash twice annually (May and June). In June 2002, each tree was inoculated with two virulent *C. parasitica* strains. Using a 4-mm cork borer, an agar disc containing the highly pathogenic, virulent isolate Ep155, and a less pathogenic, but still virulent isolate SG2-3 were placed in stem wounds of each tree (Kubisiak et al., 1997). After 5 months, each tree was rated on a scale of 1–5 for blight resistance (1 = highly resistant, 5 = highly susceptible) based on mean canker size.

Two groups of backcross trees were used, each with a common Chinese × American (F1) parent, but with a different American chestnut parent (designated AM1 and AM2) (Figure 1). The pedigrees for the American parents indicate that they were open-pollinated American chestnut trees with some natural resistance to the blight fungus.

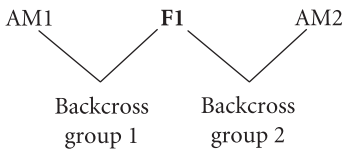

Figure 1 Origin of *Castanea* genotypes utilized to assess herbivore suitability and foliar characteristics.

Similarly, the F1 parent is an open-pollinated American chestnut, but growing in proximity to Chinese chestnut and with strong morphological, molecular, and resistance ranking data indicating Chinese chestnut parentage. There were 24 trees in group 1 (F1 (♀) × AM1) and 18 trees in group 2 (AM2 (♀) × F1), plus the three parent trees, for a total of 45 trees. The backcross trees had resistance ratings of 3–5 (averaging 3.8 for both crosses), while the two American parents were rated 5 (highly susceptible) and the F1 parent was rated 2. All experimental trees had stem cankers at the point of inoculation. Otherwise, the highly resistant F1 was virtually asymptomatic, whereas the highly susceptible American chestnut parents experienced periodic top dieback resulting from fungal infection. No foliar symptoms were present on any experimental trees at the time of our collections.

Two branches from the south side of each tree near breast height were removed, placed in water on ice, and transported back to the laboratory. One branch from each sampled tree was designated for herbivore feeding trials, and the second adjacent branch was designated for analysis of foliar properties. Because only single specimens of the three parent trees were available, additional branches of each respective tree served as replicates (six branches from each parent tree). Foliage was obtained on three dates (10 June, 8 July, and 29 July, 2003) for each of the three herbivore-feeding assays. Foliar properties were analyzed concurrently with each assay. One day following collection of plant material, herbivore assays were set up and foliage was processed for analysis.

Herbivore assays

To assess chestnut suitability to generalist herbivores, we measured foliar consumption by gypsy moth and fall webworm caterpillars, and adult Japanese beetles. For gypsy moths only, we also assessed growth rate, development time, and final larval weight. For all assays, insects were kept in growth chambers with a L15:D9 photoperiod at 23 °C. Each assay coincided temporally with active feeding of natural populations. Replication for all feeding assays except the Japanese beetle three-choice test (n = 30) was the same: n = 9 each for AM1, AM2, and F1, n = 72 for group 1, and n = 54 for group 2. For each of the parent trees, the replication resulted from the three branches sampled from each tree, each of which served as an experimental unit.

Gypsy moth assay. Laboratory-reared larvae (Otis Air Force Base, MA, USA) were allowed to feed on chestnut foliage for the duration of the fourth stadium in the University of Kentucky Forest Entomology Quarantine Facility. Leaves were excised, weighed, and placed individually in florists' water picks in 21 × 7 cm clear plastic rearing boxes. Newly molted larvae previously fed a wheat germ-based artificial diet were starved for 24 h, weighed, and placed individually in rearing boxes. Assays were monitored at 4–6 h intervals during daylight hours, and leaves were replaced as necessary. Immediately after molting, caterpillars were weighed and frozen, and the assays were terminated. Cadavers, waste, and leaf material were dried for 5 days at 60 °C and re-weighed. Relative growth rate [RGR = [final larval weight (mg)-initial larval weight (mg)]/initial larval weight (mg)/time (days)], relative consumption rate [RCR = leaf area consumed (mg)/initial larval weight (mg)/time (days)] (Scriber & Slansky, 1981), length of stadium (days), and final larval weight (mg) were assessed to determine performance and foliar suitability.

Japanese beetle assay. Adult Japanese beetles were field collected at the University of Kentucky Princeton Experimental Research Farm using traps baited with Spectracide® floral lures. Beetles were starved for 24 h prior to use in either a no-choice acceptability or three-choice preference test. Whole excised leaves were measured for leaf area using an electronic leaf area meter (LI-3100, LiCor, Lincoln, NE), weighed, and placed individually in water picks. For the no-choice test, three beetles were placed in a 21 × 7 cm clear plastic rearing box with a single leaf. For the three-choice test, beetles were placed in groups of nine in 17 × 12 cm clear plastic rearing boxes with three leaves: one from a backcross tree and one from each of its parent trees. Therefore, three resistance levels were used in each replicate: resistant (F1), susceptible (AM1 or AM2), and intermediate (group 1 or group 2, respectively). In both tests, beetles were held in a growth chamber and allowed to feed for 48 h. Beetles were then removed and the leaf area re-measured. Leaves were dried for 5 days at 60 °C, re-weighed, and amount of leaf material consumed was determined. Within each replicate of the three-choice test, percent of the total leaf area consumed was calculated for each leaf choice.

Fall webworm assay. Fall webworm tents were collected from roadside infestations in the Daniel Boone National Forest (Pulaski Co., KY). Third instar larvae were used in a no-choice acceptability test. Excised leaves were measured for leaf area and weight, and placed individually in water picks in 21 × 7 cm clear plastic rearing boxes. Because of concern over potential pesticide residue, sampled foliage was double-rinsed in deionized water. Caterpillars were starved for 24 h, and because they feed gregariously, were placed in groups of nine on chestnut foliage. After 48 h, caterpillars and silk were removed. Leaf area and weight were re-measured, and leaves were then dried for 5 days

at 60 °C and re-weighed. The amount of leaf material consumed was determined.

Foliar characteristics. Using the second branch taken from each tree, three alternating leaves from the branch apex were used to measure cumulative leaf area, fresh weight, and toughness. Toughness, or the amount of force required to puncture leaf tissue, was determined with a force gauge fitted with a pointed cone attachment (Mark 10, Hicksville, NY, USA. Leaves were dried for 5 days at 60 °C, re-weighed, and their density calculated.

For phytochemical analysis, the first three remaining leaves at the branch apex were excised, flash-frozen in liquid nitrogen, ground into powder, freeze-dried (VirTis Freezemobile 12SL, Gradiner, NY, USA) for 36 h, and stored at −80 °C until analysis. Soluble carbohydrate content was determined with a colorimetric assay using an anthrone/thiourea reagent (Quarmby & Allen, 1989). Tannin levels were measured using a radial diffusion assay (Hagerman, 1987), and nitrogen content was analyzed with a Leco TN-300 nitrogen determinator (Leco Corp., St. Joseph, MI, USA).

Statistical analysis

Gypsy moth assay.

Blight resistance: Results from the gypsy moth assay were first analyzed using only backcross trees from groups 1 (F1 × AM1) and 2 (AM2 × F1), with blight resistance rating as the main effect and blocking by American parent. Multivariate analysis of variance (MANOVA, SAS Institute, 1997) was used to analyze gypsy moth performance using relative consumption rate (RCR), relative growth rate (RGR), and development time as dependent variables and initial larval weight as a covariate. The Wilks lambda test statistic was used to determine significance of the multivariate model, followed by univariate analysis of each dependent variable. We also conducted a univariate analysis of covariance using final larval weight as the dependent variable, and initial larval weight as the covariate. We again used blight resistance rating as the main effect, and blocked by American parent. Differences were determined by Tukey's HSD.

Chestnut genotype: A second analysis of the gypsy moth assay was conducted whereby chestnut genotype ('type') was used as the main effect, with the five genotypes consisting of group 1 (n = 24), group 2 (n = 18), AM1 (n = 3), AM2 (n = 3), and F1 (n = 3). Blight resistance rating was included as a covariate in the analysis using genotype as the main effect, but dropped when it had no significant effect. Again, a MANOVA was used to analyze gypsy moth performance using RCR, RGR, and development time as dependent variables and initial larval weight as a covariate. The significance of the multivariate model was determined by the Wilks lambda test statistic. We then used PROC MIXED (SAS Institute, 1997) to fit a nested linear model based on genotype, specifying 'tree' as a random effect. This approach tests for differences between genotypes using a nested structure that involves variance within species, trees, and branches. The experimental variance for the unreplicated groups (AM1, AM2, and F1) was then derived from the fully replicated groups, which implicitly assumed that this estimate of the variance was appropriate for the error associated with the groups containing a single experimental unit. Finally, we conducted a univariate analysis of covariance using final larval weight as the dependent variable and initial larval weight as the covariate, and chestnut genotype as the main effect (Raubenheimer & Simpson, 1992). Differences were determined by Tukey's HSD.

Japanese beetle assay. Analysis of variance (SAS Institute, 1997) was used to assess Japanese beetle consumption, first using blight-resistance level as the main effect, and then using chestnut genotype as the main effect. To determine Japanese beetle preference in the three-choice test, an analysis of variance was used with only resistance level as the main effect.

Fall webworm assay. Similar to the previous assay, we used analysis of variance to assess fall webworm consumption, first using blight-resistance level as the main effect, and secondly using genotype as the main effect.

Foliar characteristics. MANOVA was also used to analyze foliar properties with toughness, density, carbohydrates, nitrogen, and tannins as dependent variables. Again, blight-resistance rating was first used as the main effect, blocked by American parent and including only backcross trees. A second analysis was performed with genotype as the main effect, where blight resistance rating was first included as a covariate, but dropped after finding no significance. A repeated measures analysis with Satterthwaite's degrees of freedom was used to examine seasonal changes in foliar properties, and to characterize any interactions between sampling date and chestnut genotype. The Wilks lambda test statistic was used to determine significance of the overall models before performing univariate analyses for each dependent variable.

To investigate the relationship between foliar characteristics (toughness, density, carbohydrates, nitrogen, and tannins) and herbivore performance (gypsy moth RGR, RCR, and development time), we performed a multivariate canonical correlation analysis (CANCORR, SAS Institute, 1997) and used the standardized canonical coefficients (canonical weights) to assess relationships among variables. Pearson's correlations were performed to relate

herbivore consumption (Japanese beetle, fall webworm) to foliar characteristics. All assumptions were checked prior to each analysis, and data were transformed when necessary. Least squares means were used for all pairwise comparisons.

To further relate herbivore suitability to foliar characteristics and address issues associated with replication, we performed a cluster analysis (MODECLUS, SAS Institute, 1997) using a non-parametric clustering approach to group trees based on foliar properties. With this approach, each observation begins in its own cluster, and clusters are joined based on the greatest estimated density. To generate the clusters, we used the foliar characteristics of carbohydrate, tannin, and nitrogen concentrations, as well as leaf toughness. A principal components analysis was then performed on the cluster data to determine which foliar characteristics contributed most to herbivore performance. Analysis of variance and Tukey's HSD were then used to discern differences in insect herbivory based on the clusters.

Results

Herbivore assays

There was no effect of blight resistance on gypsy moth performance in the multivariate analysis ($F_{6,64} = 0.74$, $P = 0.62$). Although blight resistance did not affect RCR, RGR, or development time (Table 1), gypsy moth initial larval weight influenced development time ($F_{1,34} = 8.48$, $P = 0.006$). Similarly, final caterpillar weight was not affected by blight resistance (Table 1), but was influenced by initial larval weight ($F_{2,34} = 9.85$, $P = 0.004$). There were no significant differences in the leaf area consumed based on blight-resistance rating by either the Japanese beetle or the fall webworm.

Gypsy moth assay. There was a strong effect of chestnut genotype on gypsy moth caterpillar performance in the multivariate analysis ($F_{12,106.1} = 4.22$, $P<0.0001$). Gypsy moth RGR was greater on AM2 than on all other genotypes (Table 2). Caterpillar RCR on AM1 was significantly higher than either group 1 or group 2, but did not differ from the other two parent trees. Caterpillar development was slowest on F1 but the difference was not significant. Final gypsy moth caterpillar weight was also significantly affected by chestnut genotype. Final caterpillar weight was greatest for caterpillars fed foliage from backcross group 2, which differed significantly from the final weight of caterpillars fed group 1 foliage. There were no other differences in final caterpillar weight (Table 2).

Japanese beetles strongly preferred both American parents to the other chestnut genotypes, consuming almost three times as much leaf area. Consumption by the fall webworm did not differ among chestnut genotypes (Table 2).

In the three-choice preference test, the Japanese beetle showed a strong preference for blight resistant (F1) and susceptible (AM1 and AM2) parent trees over intermediate backcross groups ($F_{2,84} = 8.09$, $P = 0.0006$). The percentage of the total leaf area consumed was similar between resistant (F1) (38.6% ± 5.3) and susceptible (AM1 and AM2) (44.6% ± 5.4) trees, and much higher than the intermediate chestnuts (16.8% ± 3.8).

Foliar characteristics

Multivariate analysis of variance showed no overall effect of blight resistance on foliar characteristics on 10 June ($F_{10,68} = 0.65$, $P = 0.76$) or 8 July ($F_{10,58} = 1.35$, $P = 0.23$). However, blight resistance did influence foliar characteristics

Table 1 Consumption and growth [least squares mean (SEM)] of herbivores on foliage of two *Castanea dentata* × F1 hybrid crosses varying in resistance to the chestnut blight fungus. Means within rows followed by the same letter do not differ

	Resistance rating[a]				
Parameter	3 (n = 54)	4 (n = 51)	5 (n = 39)	F (d.f.)	P
Gypsy moth performance					
RGR[b]	0.30 (0.02)a	0.34 (0.02)a	0.34 (0.03)a	1.24 (2,34)	0.30
RCR[c]	3.20 (0.28)a	3.30 (0.28)a	3.73 (0.43)a	0.55 (2,34)	0.58
Development time[d]	6.92 (0.11)a	6.78 (0.12)a	6.61 (0.17)a	1.09 (2,34)	0.35
Final larval weight (mg)	5.52 (0.17)a	5.88 (0.17)a	5.88 (0.26)a	1.25 (2,34)	0.30
Herbivore consumption[e]					
Japanese beetle	4.57 (0.75)a	4.14 (0.80)a	4.20 (1.17)a	0.08 (2,35)	0.92
Fall webworm	9.63 (1.83)a	9.21 (1.96)a	10.12 (2.86)a	0.04 (2,35)	0.97

[a]Resistance rating: 1, highly resistant through 5, highly susceptible. See text for explanation.
[b]RGR = relative growth rate (mg) $(mg)^{-1}$ $(days)^{-1}$.
[c]RCR = relative consumption rate (mg) $(mg)^{-1}$ $(days)^{-1}$.
[d]Length of fourth larval stadium (days).
[e]Consumption: leaf area consumed (cm^2).

Table 2 Herbivore suitability [least squares mean (SEM)] of *Castanea dentata* ('AM1' and 'AM2'), a *Castanea dentata* × *Castanea mollissima* F1 hybrid, and two groups of backcross trees (groups 1 and 2). Means within rows followed by the same letter do not differ

	Chestnut genotype[a]						
Parameter	F1 (n = 9)	Group 1 (n = 72)	Group 2 (n = 54)	AM1 (n = 9)	AM2 (n = 9)	F (d.f)	P
Gypsy moth performance							
RGR[b]	0.29 (0.04)b	0.33 (0.01)b	0.32 (0.02)b	0.31 (0.04)b	0.55 (0.04)a	8.53 (4,42)	<0.01
RCR[c]	3.89 (0.60)ab	3.43 (0.23)b	3.25 (0.24)b	5.21 (0.60)a	3.86 (0.61)ab	2.48 (4,42)	0.03
Development time[d]	7.32 (0.28)a	6.89 (0.11)a	6.73 (0.12)a	7.10 (0.28)a	6.87 (0.29)a	1.15 (4,42)	0.34
Final larval weight (mg)	5.23 (0.38)ab	5.38 (0.14)b	6.09 (0.15)a	5.32 (0.38)ab	5.89 (0.38)ab	3.42 (4,42)	0.02
Herbivore consumption[e]							
Japanese beetle	4.90 (1.98)b	4.56 (0.75)b	4.12 (0.81)b	15.06 (1.98)a	12.46 (1.98)a	10.03 (4,42)	0.01
Fall webworm	4.41 (4.10)a	9.14 (1.55)a	9.97 (1.67)a	6.80 (4.10)a	9.42 (4.10)a	1.41 (4,43)	0.80

[a]Chestnut genotype: a *Castanea dentata* × *Castanea mollissima* F1 hybrid, two groups of backcross chestnut (groups 1 and 2), and two American chestnut (AM1 and AM2). See Figure 1 and text for explanation.
[b]RGR = relative growth rate (mg) $(mg)^{-1}$ $(days)^{-1}$.
[c]RCR = relative consumption rate (mg) $(mg)^{-1}$ $(days)^{-1}$.
[d]Length of fourth larval stadium (days).
[e]Consumption: leaf area consumed (cm^2).

on 29 July ($F_{10,64} = 2.47$, $P = 0.01$). Univariate analyses for this sample date revealed that only tannin content was impacted by resistance rating ($F_{2,38} = 3.54$, $P = 0.04$), with the least resistant trees rated 5 having higher tannin content than the more resistant trees rated 3 or 4.

For the 10 June assay, the multivariate analysis revealed a strong effect of chestnut genotype on foliar characteristics ($F_{9,99.9} = 3.84$, $P<0.0001$), and significant differences were present for all foliar characteristics measured in the subsequent univariate analyses (Figure 2). Leaf toughness

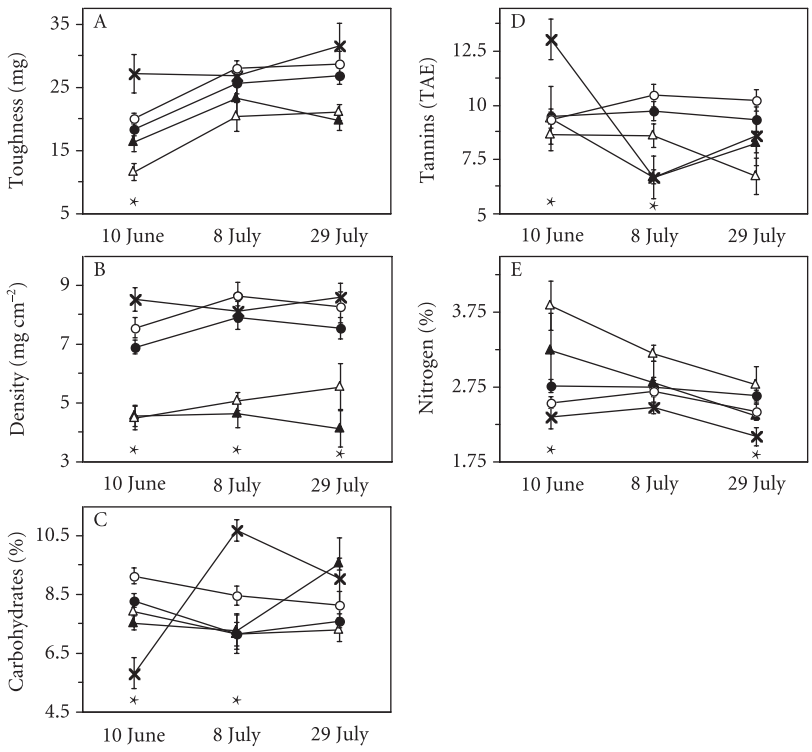

Figure 2 Foliar characteristics (mean ± SE) of two *Castanea dentata* parents, AM1 (▲) and AM2 (△), a *Castanea dentata* × *Castanea mollissima* F1 hybrid parent (×), and two groups of backcross trees, groups 1 (●) and 2 (○), sampled on three dates (10 June, 8 July, and 29 July, 2003). (A): toughness, (B): density, (C): carbohydrates, (D): tannins, and (E): nitrogen.

and density were highest for F1, intermediate for the backcross trees, and lowest for the American parents. Toughness of F1 was significantly higher than all other genotypes, and leaf density of the American parents was significantly lower than all other genotypes. Foliar carbohydrates were lowest in F1, intermediate in the American parents, and highest in the backcross trees. Group 2 trees had significantly higher and F1 had significantly lower carbohydrate content than all other genotypes. Tannins were equivalent across all species with the exception of F1, which was significantly greater. F1 also had the lowest nitrogen content and, along with group 2, was significantly different from AM1 and AM2, which contained the highest concentrations.

Foliage sampled on 8 July also showed a strong effect of chestnut genotype on foliar characteristics ($F_{20,123.7} = 2.41$, $P = 0.007$). No significant differences in toughness were present. As with the earlier sampling date, leaf density was lowest on the American parents (Figure 2). In contrast to the previous date, however, carbohydrate content was significantly higher in F1 than all other trees and tannin content significantly lower in F1 and AM1 than either backcross group. There were no significant differences in nitrogen content.

The effect of chestnut genotype persisted through the 29 July assay ($F_{20,133.6} = 2.09$, $P<0.01$), but with significant differences only in leaf density and nitrogen content. Consistent with the first two sampling dates, the American parents had the lowest leaf density, and foliar nitrogen was significantly lower in F1 than AM2 or group 1.

Seasonal changes were observed for all foliar characteristics measured (Figure 2). There was a significant effect of sampling date on toughness ($F_{2,46.2} = 16.27$, $P<0.0001$), with significant increases in group 1, group 2, and AM2. Leaf density did not differ significantly among sampling dates ($F_{2,46} = 1.09$, $P = 0.35$) nor did carbohydrates ($F_{2,44.7} = 1.25$, $P = 0.30$). Tannins were significantly different between sampling dates ($F_{2,46.1} = 5.60$, $P = 0.007$), with significant decreases in F1 and AM2. There was a significant effect of sampling date on nitrogen content ($F_{2,43.1} = 14.39$, $P<0.0001$), which decreased in all genotypes, but was significant only in AM2. A significant date* genotype interaction was present for carbohydrates ($F_{8,45} = 5.37$, $P<0.0001$) (Figure 2C), and tannins ($F_{8,46.4} = 3.88$, $P = 0.0014$) (Figure 2D), most likely due to the variability in the F1. Nitrogen was also impacted by a date* genotype interaction ($F_{8,43.9} = 2.44$, $P = 0.03$) (Figure 2E).

Correlation between herbivore suitability and foliar characteristics. The multivariate canonical correlation analysis used for the gypsy moth assay, which creates a linear combination of herbivore suitability variables (RGR, RCR, and development time) that most closely correlates with a linear combination of foliar characteristics (leaf toughness, density, carbohydrates, tannins, and nitrogen) revealed a highly significant correlation between herbivore suitability and foliar characteristics ($F_{15,351} = 3.77$, $P<0.0001$). Canonical weights revealed that gypsy moth RCR (0.63) and RGR (0.56) were negatively correlated with leaf toughness (0.63), density (0.21), carbohydrates (0.23), and tannins (0.21).

Pearson's correlations revealed that Japanese beetle consumption was weakly, but significantly inversely correlated with leaf toughness (0.20, $P = 0.012$), density (0.31, $P = 0.0001$), and tannin content (0.28, $P = 0.0006$). In contrast with the Japanese beetle but similar to the gypsy moth, area consumed by the fall webworm was significantly but weakly correlated with carbohydrate levels (-0.26, $P = 0.0011$). Fall webworm consumption was also weakly correlated with nitrogen content (0.21, $P = 0.013$).

In the cluster analysis of the data for the first herbivore assay using gypsy moth caterpillars, a cluster radius of 3 was used. This generated two useable clusters where the number of replicates per cluster was greater than three (Figure 3A). Cluster 1 ($n = 96$) contained no replicates of the AM1 parent, one replicate of the AM2 parent, three replicates of the F1 parent, 42 replicates of the group 1 backcross, and 50 replicates of the group 2 backcross. Cluster 2 ($n = 34$) contained six replicates of the AM1 parent, five replicates of the AM2 parent, none of the F1 parent, 15 replicates of the group 1 backcross, and eight replicates of the group 2 backcross. The remaining replicates were dropped because the number per cluster was <3. The principal components analysis showed that the characteristics making the greatest contribution to the clustering were foliar carbohydrates, toughness, and nitrogen, which had eigenvectors of 0.5702, 0.5944, and -0.5399, respectively (Figure 3A), and accounted for 50% of the variability. Gypsy moth RGR and RCR were significantly lower on those chestnut replicates grouped in to cluster 1 (Table 3). There was no difference in caterpillar development time or final weight based on our clustering.

For the 8 July herbivore assay utilizing Japanese beetle adults, we used a cluster radius of 4 that generated three useable clusters (Figure 3B). Cluster 1 ($n = 39$) consisted of three replicates of AM1, six replicates of AM2, no replicates of F1, 18 replicates of backcross group 1, and 12 of group 2. Cluster 2 ($n = 45$) consisted of three replicates each of AM1 and AM2, nine of the F1 hybrid, nine of backcross group 1, and 21 of group 2. Finally, cluster 3 ($n = 39$) consisted of 18 replicates of backcross group 1 and 21 replicates of group 2. No parent material was included in cluster 3. Foliar carbohydrates, toughness, and nitrogen again made the greatest contribution to the clustering, with eigenvectors of 0.5734, 0.5530, and -0.54012 (Figure 3B), which accounted for > 41% of the variability. Japanese

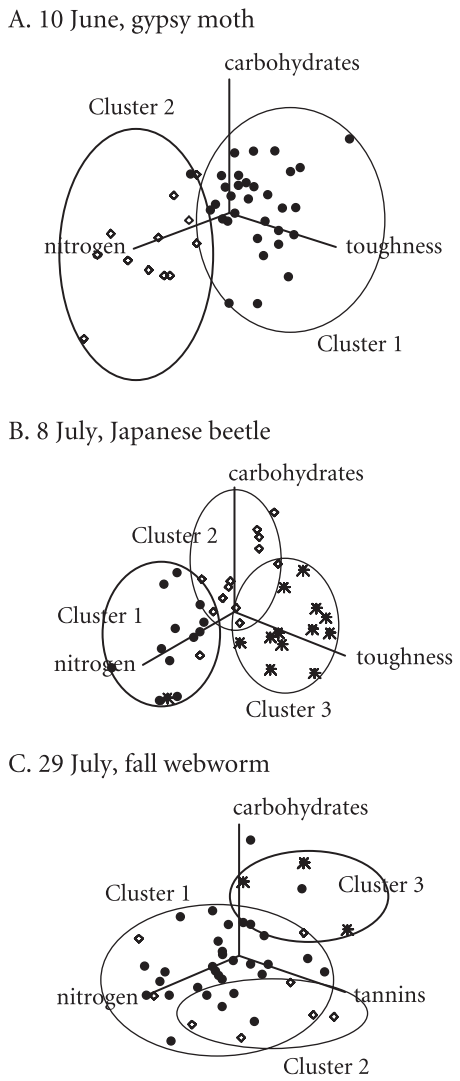

Figure 3 Cluster analysis of foliar characteristics of *Castanea dentata*, a *Castanea dentata* × *Castanea mollissima* F1 hybrid, and two groups of backcross trees on (A) 10 June, (B) 8 July, and (C) 29 July, corresponding to feeding trials of the gypsy moth, Japanese beetle, and fall webworm, respectively. Cluster 1 (●), cluster (◇), and cluster 3 (*). See Table 3 and text for explanation.

beetle consumption was significantly lower on cluster 3 replicates than on foliage from clusters 1 and 2, which did not differ (Table 3).

For the final herbivore assay using fall webworm larvae, a cluster radius of 5 was used to generate three useable clusters (Figure 3C). Cluster 1 (n = 99) consisted of nine replicates of AM1, eight replicates of AM2, six replicates of F1, 44 replicates of backcross group 1, and 32 of group 2. Cluster 2 (n = 27) contained no American parent material, but contained three replicates of the F1 parent, and 12 replicates each of backcross groups 1 and 2. Finally, cluster 3 (n = 12) contained no parental material, but consisted of three replicates of backcross group 1 and nine of group 2. The principal components analysis determined that the characteristics of foliar carbohydrates (eigenvector 0.4418), tannins (0.4976), and nitrogen (−0.6384) made the greatest contribution to the clustering (Figure 3C), and accounted for >48% of the data's variability. However, there were no differences in fall webworm caterpillar consumption based on our clustering (Table 3).

Discussion

Our study represents the first evaluation of differential resistance to insect herbivory on chestnut genotypes varying in resistance to the chestnut blight fungus. We found that gypsy moth caterpillar performance varied among chestnut genotypes, and during the caterpillar feeding trial there were significant differences in foliar characteristics. The blight-susceptible American chestnuts, which had the highest nitrogen and the lowest toughness and density, were better hosts than the F1 hybrid or the backcross groups we assayed. Caterpillar growth and consumption on the American chestnuts were among the highest, and development time was rapid. These results were corroborated by our cluster analysis, which grouped the majority of the American replicates in the cluster that generated greater caterpillar growth and consumption rates. Caterpillars fed foliage from the F1 hybrid performed poorly, demonstrating low growth, intermediate consumption, and slightly prolonged development. The F1 foliage was the lowest in nitrogen and carbohydrates during that assay period, and so was nutritionally the poorest of the genotypes tested. In addition, foliage of the F1 hybrid was well defended, containing the highest tannins, the greatest foliar toughness, and the highest density. The compensatory feeding that typically results in relatively high larval consumption of nutritionally poor foliage (Slansky & Feeny, 1977; Scriber & Slansky, 1981) may have been inhibited in our gypsy moth feeding trial by the physical characteristics of the foliage (increased toughness and density). Since gypsy moth larvae are tolerant of tannins (Montgomery, 1986; Barbosa & Krischik, 1987), and the alkaline midgut of late instar caterpillars inhibits their binding activity (Schultz & Lechowicz, 1986), it is unlikely that the elevated foliar tannins of the F1 hybrid negatively affected caterpillar performance.

The results of the gypsy moth feeding trial contrast with that of Rieske et al. (2003) in which gypsy moths fed foliage from an F1 hybrid had greater growth and more rapid development than those fed foliage from a pure American chestnut. Our results do concur with regard to foliar chemistry, with the F1 hybrids having higher tannin content and

Table 3 Consumption and growth [least square mean (SEM)] of herbivores on foliage of *Castanea dentata*, a *Castanea dentata* × *Castanea mollissima* F1 hybrid, and two groups of backcross trees using cluster analysis. Means within rows followed by the same letter do not differ

	Cluster[a]				
A. Gypsy moth	1 (n = 96)	2 (n = 34)	---	F	P
RGR[b]	0.31 (0.01) b	0.37 (0.02) a	---	10.0	0.002
RCR[c]	3.09 (0.13) b	4.31 (0.22) a	---	22.4	<0.01
Development time[d]	6.82 (0.10) a	6.80 (0.14) a	---	0.03	0.90
Final weight (mg)	6.08 (2.10) a	5.85 (3.50) a	---	0.34	0.50
B. Japanese beetle	1 (n = 39)	2 (n = 45)	3 (n = 39)		
Consumption[e]	6.4 (0.90) a	6.7 (0.90) a	3.2 (0.90) b	4.48	0.01
C. Fall webworm	1 (n = 99)	2 (n = 27)	3 (n = 12)		
Consumption[e]	8.9 (1.09) a	8.7 (1.50) a	6.7 (1.01) a	0.37	0.70

[a]See Figure 3 and text for explanation of clustering.
[b]RGR = relative growth rate (mg) $(mg)^{-1}$ $(days)^{-1}$.
[c]RCR = relative consumption rate (mg) $(mg)^{-1}$ $(days)^{-1}$.
[d]Length of fourth larval stadium (days).
[e]Consumption: leaf area consumed (cm^2).

lower carbohydrate levels than pure Americans. The two studies used different genotypes, and also differed in that Rieske et al. (2003) used blight-free greenhouse-grown plants grown from seed, whereas we used field-grown trees that had been inoculated with the blight fungus. The differing results demonstrate the complexity of these three-way interactions and the variability in the chestnut system, and suggest that plant age and fungal infection status will influence herbivore susceptibility.

Adult Japanese beetle consumption of the American chestnuts was greater than the F1 hybrid and the backcross groups in our no-choice feeding trial. In the three-choice test, however, Japanese beetles consumed greater amounts of both the American chestnuts and the F1 hybrid than the backcross groups. Again, this is corroborated by the cluster analysis, which grouped the American and hybrid parental replicates in the two clusters in which the beetles consumed the greatest leaf tissue. Foliar carbohydrates were highest in the F1 hybrid, but were not elevated in foliage from the American chestnuts at the time of the Japanese beetle feeding assays. Although foliar sugars are phagostimulatory to adult Japanese beetles (Ladd, 1986), we found no linear correlation between foliar carbohydrates and beetle consumption. Clearly, additional factors are at play in determining Japanese beetle preferences for chestnut foliage.

The fall webworm was the only polyphage we tested that did not discriminate among chestnuts. During the fall webworm feeding trial (29 July), there were few significant differences in foliar characteristics. Foliar nitrogen was significantly lower in the F1 hybrid, and leaf toughness and density were lowest for the backcross groups. Foliar nitrogen was positively correlated with fall webworm consumption, so it is surprising that these differences did not translate into significant differences in caterpillar consumption. However, total webworm consumption was low and variability was high, which may have obscured differences in caterpillar preferences.

Foliar characteristics changed over the course of the season, although not always in similar or predictable ways. The seasonal increase in leaf toughness and density, and decrease in foliar nitrogen, are consistent with previous studies (Feeny, 1970; Mauffette & Oechel, 1989). The most extreme changes in foliar chemistry occurred in the F1 parent. Foliar carbohydrates in the F1 hybrid nearly doubled between 10 June and 8 July, before reaching an intermediate level on 29 July. Concurrently, foliar tannins decreased markedly. These findings are consistent with other studies of deciduous trees (Faeth, 1985; Wold & Marquis, 1997; Adams & Rieske, 2003) in which foliar tannins have declined seasonally.

Herbivore susceptibility in some woody plant systems is influenced by plant age (Zanuncio et al., 2001; Lawrence et al., 2003), genotype (Peacock et al., 2002; Osier & Lindroth, 2004), and hybridization (Fritz et al., 1998). Our results corroborate the effects of genotype and hybridization, and suggest that in the chestnut system these factors may be more important than blight resistance in determining herbivore susceptibility. A genotype effect was evident in foliar properties from all dates, and in two of the herbivore-feeding

assays. However, our data do not cleanly fit current hypotheses addressing the effects of plant hybridization on herbivory. Depending on the herbivore parameters measured, the results of our gypsy moth and Japanese beetle feeding trials lend support to either the dominance hypothesis or the hybrid resistance hypothesis (Fritz et al., 1994) predicting the effects of plant hybridization on herbivore susceptibility.

Research on the effects of systemic fungal pathogen infection on herbivory are limited (but see Hatcher, 1995, and Rostás et al., 2003). In an annual plant system, Cardoza et al. (2002) showed that systemic fungal infection increased herbivore susceptibility; larvae of a noctuid moth preferred leaves from a plant infected with a stem fungus over leaves from a healthy plant of the same genotype. In contrast, Biere et al. (2004) demonstrated that resistance to systemic fungal infection coincided with resistance to herbivory; healthy plants that were resistant to fungal pathogens were also resistant to herbivory. However, some studies with herbaceous plants have shown no effects on herbivore resistance (Moran, 1998; Rostás & Hilker, 2002). Clearly, there is variability in herbivore responses among different systems and the herbivores within those systems.

We found no clear trends relating chestnut blight resistance to herbivore resistance across herbivore species. In our study, gypsy moth performance appeared inversely related to blight resistance; caterpillars performed poorest on the most blight-resistant F1 chestnut. In contrast, Japanese beetle consumption was greatest on blight-susceptible American chestnut. Fall webworm preference showed no pattern. The seasonal changes in foliar characteristics, coupled with differences in insect feeding modes, undoubtedly influenced our results. The extent to which blight resistance has an effect on herbivore resistance in the chestnut system of hybridization remains unclear.

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References

Adams AS & Rieske LK (2003) Prescribed fire affects white oak seedling phytochemistry: implications for insect herbivory. Forest Ecology and Management 176: 37–47.

Barbosa P & Krischik VA (1987) Influence of alkaloids on feeding preference of eastern deciduous forest trees by the gypsy moth *Lymantria dispar*. American Naturalist 130: 53–69.

Bergelson JM, Fowler S & Hartley S (1986) The effects of foliage damage on casebearing larvae, *Coleophora serratella*, feeding on birch. Ecological Entomology 11: 241–250.

Biere A, Marak HB & van Damme JMM (2004) Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? Oecologia 140: 430–441.

Braun EL (1950) Deciduous Forests of Eastern North America. Hafner Press, New York.

Burnham CR, Rutter PA & French DW (1986) Breeding blight-resistant chestnuts. Plant Breeding Reviews 4: 347–397.

Cardoza YJ, Alborn HT & Tumlinson JH (2002) *In vivo* volatile emissions from peanut plants induced by simultaneous infection and insect damage. Journal of Chemical Ecology 28: 161–174.

Chapin FS III (1991) Effects of multiple environmental stresses on nutrient availability and use. Response of Plants to Multiple Stresses (ed. by HA Mooney, WE Winner & EJ Pell), pp. 67–88. Academic Press, New York.

Faeth SH (1985) Host leaf selection by leaf miners: Interactions among three trophic levels. Ecology 66: 870–875.

Faeth SH (1986) Indirect interactions between temporally separated herbivores mediated by the host plant. Ecology 67: 479–494.

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.

Fleming WE (1972) Biology of the Japanese beetle. USDA Technical Bulletin 1449, Washington, D.C.

Foss LK & Rieske LK (2004) Stem galls affect oak foliage with potential consequences for herbivory. Ecological Entomology 29: 273–280.

Fritz RS, Nichols-Orians CM & Brunsfeld SJ (1994) Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics and variable responses in a diverse herbivore community. Oecologia 97: 106–117.

Fritz RS, Roche BM & Brunsfeld SJ (1998) Genetic variation in resistance of hybrid willows to herbivores. Oikos 83: 117–128.

Gao S & Shain L (1995) Effects of water stress on chestnut blight. Canadian Journal of Forest Research 25: 1030–1035.

Griffin GJ, Kahn MA & Griffin SL (1993) Superficial canker instability during winter and virulence of *Endothia parasitica* associated with managed forest clearcut and plantation American chestnut trees. Canadian Journal of Plant Pathology 15: 159–167.

Griffin GJ, Smith HC, Dietz A & Elkins JR (1991) Importance of hardwood competition to American chestnut survival, growth, and blight development in forest clearcuts. Canadian Journal of Botany 69: 1804–1809.

Hagerman AE (1987) Radial diffusion method for determining tannin in plant extracts. Journal of Chemical Ecology 13: 437–449.

Hartley SE & Lawton JH (1987) Effects of different types of damage on the chemistry of birch foliage, and the responses of birch feeding insects. Oecologia 74: 432–437.

Hatcher PE (1995) Three–way interactions between plant pathogenic fungi, herbivorous insects and their host plants. Biological Reviews 70: 639–694.

Hatcher PE, Paul ND, Ayres PG & Whittaker JB (1994) The effect of a foliar disease (rust) on the development of *Gastrophysa viridula* (Coleoptera: Chrysomelidae). Ecological Entomology 19: 349–360.

Haukioja E & Niemela P (1979) Birch leaves as a resource for herbivores: Seasonal occurrence of increased resistance in foliage after mechanical damage of adjacent leaves. Oecologia 39: 151–159.

Kok LT, Abad RG & Baudoin ABAM (1996) Effects of *Pucinia carduorum* on musk thistle herbivores. Biological Control 6: 123–129.

Kopper BJ, Klepzig KD & Raffa KF (2004) Components of antagonism and mutualism in *Ips pini*-fungal interactions: relationship to a life history of colonizing stressed and dead trees. Environmental Entomology 33: 28–34.

Kubisiak TJ, Hebard FV, Nelson CD, Zhang J, Bernatzky R, Huang H, Anagnostakis SL & Doudrick RL (1997) Molecular mapping of resistance to blight in an interspecific cross in the genus *Castanea*. Phytopathology 87: 751–759.

Ladd TL (1986) Influence of sugars on the feeding response of Japanese beetles (Coleoptera: Scarabaeidae). Journal of Economic Entomology 79: 668–671.

Lawrence R, Potts BM & Whitham TG (2003) Relative importance of plant ontogeny, host genetic variation, and leaf age for a common herbivore. Ecology 84: 1171–1178.

Mauffette Y & Oechel WC (1989) Seasonal variation in leaf chemistry of the coast live oak *Quercus agrifolia* and implications for the California oak moth *Phryganidia californica*. Oecologia 79: 439–445.

Mondy N & Corio-Costet M-F (2000) The response of the grape berry moth (*Lobesia botrana*) to a dietary phytopathogenic fungus (*Botrytis cinerea*): the significance of fungus sterols. Journal of Insect Physiology 46: 1557–1564.

Montgomery ME (1986) Gypsy moth host plant relationships and population dynamics. Proceedings 18th IUFRO World Congress, Div. 2, Vol. II: Forest plants and forest protection. Lyubljana, Yugoslavia, pp. 743–754.

Moran P (1998) Plant-mediated interactions between insects and a fungal plant pathogen and the role of plant chemical responses to infection. Oecologia 115: 523–530.

Mosher FH (1915) Food plants of the gypsy moth in America. USDA Bulletin 250, Washington, D.C.

Neuvonen S, Hanhimaki S, Suomela J & Haukioja E (1988) Early season damage to birch foliage affects the performance of a late season herbivore. Journal of Applied Entomology 105: 182–189.

Nienstaedt H (1953) Tannin as a factor in the resistance of chestnut, *Castanea* spp., to the chestnut blight fungus, *Endothia parasitica*. Phytopathology 43: 32–38.

Osier TL & Lindroth RL (2004) Long-term effects of defoliation on quaking aspen in relation to genotype and nutrient availability: plant growth, phytochemistry and insect performance. Oecologia 139: 55–65.

Peacock L, Herrick S & Harris J (2002) Interactions between the willow beetle *Phratora vulgatissima* and different genotypes of *Salix viminalis*. Agricultural and Forest Entomology 4: 71–79.

Quarmby C & Allen SE (1989) Organic constituents. Chemical Analysis of Ecological Materials (ed. by SE Allen), pp. 160–201. Wiley, NY.

Raubenheimer D & Simpson SJ (1992) Analysis of covariance: an alternative to nutritional indices. Entomologia Experimentalis et Applicata 62: 221–231.

Rieske LK & Raffa KF (1998) Interactions among insect herbivore guilds: Influence of thrips bud injury on foliar chemistry and suitability to gypsy moths. Journal of Chemical Ecology 24: 501–523.

Rieske LK, Rhoades CC & Miller SP (2003) Foliar chemistry and gypsy moth, *Lymantria dispar* (L.), herbivory on pure American chestnut, *Castanea dentata* (Fam: Fagaceae) and a disease resistant hybrid. Environmental Entomology 32: 359–365.

Roane MK, Griffin GJ & Elkins JR (1986) Chestnut blight, other *Endothia* diseases and the genus *Endothia*. APS Press, St. Paul, MN.

Rostás M & Hilker M (2002) Asymmetric plant-mediated cross-effects between a herbivorous insect and a phytopathogenic fungus. Agricultural and Forest Entomology 4: 1–9.

Rostás M, Simon M & Hilker M (2003) Ecological cross-effects of induced plant responses towards herbivores and phytopathogenic fungi. Basic and Applied Ecology 4: 43–62.

SAS Institute (1997) SAS/STAT software: changes and enhancements through release. SAS Institute, Cary, NC.

Schultz JC & Lechowicz MJ (1986) Hostplant, larval age, and feeding behavior influence midgut pH in the gypsy moth (*Lymantria dispar*). Oecologia 71: 133–137.

Scriber JM & Slansky F Jr (1981) The nutritional ecology of immature insects. Annual Review of Entomology 26: 183–211.

Slansky F & Feeny P (1977) Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecology 47: 209–228.

Sticher L, Mauch-Mani B & Métraux JP (1997) System acquired resistance. Annual Review of Phytopathology 35: 235–270.

Stout MJ, Fidantsef AL, Duffey SS & Bostock RM (1999) Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. Physiological and Molecular Plant Pathology 54: 115–130.

Tietz HM (1952) The Lepidoptera of Pennsylvania. PA State College Agricultural Experiment Station, State College.

Tinney GW, Hatcher PE, Ayres PG, Paul ND & Whittaker JB (1998) Inter- and intra-species differences in plants as hosts to *Tyria jacobea*. Entomologia Experimentalis et Applicata 88: 137–145.

Wallin KF & Raffa KF (1999) Altered constitutive and inducible phloem monoterpenes following natural defoliation of jack pine: Implications to host mediated inter-guild interactions and plant defense theories. Journal of Chemical Ecology 25: 861–880.

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

Zanuncio JC, Guedes RNC, Zanuncio TV & Fabres AS (2001) Species richness and abundance of defoliating Lepidoptera associated with *Eucalyptus grandis* in Brazil and their response to plant age. Austral Ecology 26: 582–589.