Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*

REBECCA E. FORKNER[1], ROBERT J. MARQUIS[1] and JOHN T. LILL[2]   [1]Department of Biology, University of Missouri–St Louis, Missouri, U.S.A. and [2]Department of Biological Sciences, George Washington University, Washington, U.S.A.

Abstract. 1. Community level oak–tannin–insect patterns have been largely unexplored since Paul Feeny's ground-breaking research. Two hypotheses were tested for *Quercus velutina* and *Q. alba* in the Missouri Ozarks: abundance and richness of leaf-chewing herbivores are negatively correlated with foliar condensed tannin concentrations and variation in condensed tannin concentrations explains variation in herbivore community structure.

2. In 2001, foliar condensed tannins in the understorey and canopy of these two oak species were quantified simultaneously with censuses of herbivores in May, during leaf expansion, and in June and August, when leaves were fully expanded. Thirty-eight of the 134 species encountered had densities sufficient to be analysed individually ($n = 10$). Of those, *Acronicta increta* (Noctuidae) and *Attelabus* sp. (Curculionidae), both oak specialists, were negatively correlated with condensed tannins in the canopy of *Q. alba*. One additional specialist, *Chionodes pereyra* (Gelechiidae), was marginally negatively correlated with condensed tannins in the understorey of *Q. velutina*. Understorey species richness of May *Q. velutina* herbivores was negatively correlated with condensed tannins, as were total canopy insect density and species richness of August herbivores on *Q. alba*.

3. Principal component analysis (PCA) of insect abundances indicated that understorey and canopy *Q. velutina* and *Q. alba* had different communities of leaf-chewing insects. Furthermore, condensed tannin levels contributed significantly to variation in PCA scores for *Q. velutina*, explaining 25% of the total variation.

4. Overall, these results indicate that specialists were more likely than generalists both to correlate negatively with condensed tannins and to occur in lower tannin habitats; abundance and richness of both early and late season fauna correlated negatively with tannins; and species were more likely to correlate negatively with condensed tannins when feeding on *Q. alba* than on *Q. velutina* and when feeding in the canopy than in the understorey. Future studies of tannin–insect interactions should manipulate leaf quality in combination with manipulations of other factors that likely influence community structure.

Key words. Canopy, community ecology, condensed tannins, insect–plant interactions, plant chemistry, polyphenolics, *Quercus*.

Correspondence: Rebecca E. Forkner, Department of Biology, 8001 Natural Bridge Road, University of Missouri–St Louis, St Louis, MO 63121, U.S.A. E-mail: biorfork@jinx.umsl.edu

Introduction

Oak (*Quercus*)–tannin–insect interactions have played a key role in the development of plant anti-herbivore defence theory (Feeny, 1975, 1976; Rhoades & Cates, 1976; Bryant *et al.*, 1983; Coley *et al.*, 1985; Schultz, 1988; Herms & Mattson, 1992; Jones & Hartley, 1999). Feeny (1970) documented a decline in insect abundance on *Quercus robur* over the course of the season as foliar tannin concentrations increased. In combination with an extensive literature review and laboratory bioassays using *Operophtera brumata*, these data led him to conclude that tannins 'act as a *broad spectrum* defensive mechanism against herbivores' and that seasonally increasing tannin levels were responsible for the concentration of herbivory in the spring. Feeny (1970) also argued that these changes in leaf quality were more important in determining densities of herbivores than were other factors, such as bird predation. Since Feeny's ground-breaking work, research has focused almost exclusively on the physiological effects of tannins on insects, while community level impacts largely have been undocumented. Studies in both oak and non-oak systems have shown that tannins have detrimental effects on insects: they deter feeding (Bettolo *et al.*, 1986), reduce growth and survivorship (Rossiter *et al.*, 1988; Karowe, 1989; Kause *et al.*, 1999; Kopper *et al.*, 2002), correlate negatively with pupal mass (Mutikainen *et al.*, 2000; Lill & Marquis, 2001), cause lethal deformities (Barbenhenn & Martin, 1994), and increase parasitism rates (Faeth & Bultman, 1986). In some cases, however, depending on the host plant, insect species, and environmental conditions, response to tannins is positive (see citations in Schultz, 1989; Panzuto *et al.*, 2002). This inconsistent support (Schultz, 1989; Clausen *et al.*, 1992; Hamilton *et al.*, 2001) for the hypothesis that phenolics are defensive agents (Loomis, 1932; Feeny, 1976; Bryant *et al.*, 1983; Coley *et al.*, 1985; Schultz, 1988; Herms & Mattson, 1992) has led some to challenge the importance of folivorous insects for the evolution and diversification of polyphenolics in plant tissue (Jermy, 1984; Beart *et al.*, 1985; Matsuki, 1996; Ayers *et al.*, 1997; Bi *et al.*, 1997; Close & McArthur, 2002). Although the *raison d'être* for polyphenolics may have been their antioxidant capacity (Grace & Logan, 2000; Close & McArthur, 2002), this does not preclude a contemporary role for tannins as anti-herbivore compounds (Hagerman & Butler, 1991).

Insects may vary in their responses to tannins based on their level of adaptation to polyphenolics (Barbenhenn *et al.*, 2003b). Frequently cited tannin adaptations include alkaline gut pH (Appel & Schultz, 1992; Appel, 1993), high levels of surfactants (Martin & Martin, 1984), and high levels of antioxidants, such as ascorbate (Barbenhenn *et al.*, 2001, 2003a), which help maintain tannins in a reduced state. Defensive compounds, while having a negative impact on non-adapted species (Berenbaum, 1983; Karowe, 1989), may show little detrimental effect on specialists (Schultz, 1989), except at the highest concentrations. To date, bioassays and field studies of the impacts of phenolics on insects have been restricted to generalist herbivores (citations in Schultz, 1989 and herein) or specialists that do not feed on oaks (Steinly & Berenbaum, 1985; Bi *et al.*, 1997). With the exception of two studies (Faeth & Bultman, 1986; Mauffette & Oechel, 1989), the impacts of tannins on specialist oak feeders remain untested. If tannins contribute to resistance against oak herbivores, then negative correlations between insects and tannins should exist in naturally occurring *Quercus* communities and polyphagous species should be more sensitive than specialists to variation in tannin concentrations.

An additional part of the complexity of tannin–insect interactions derives from the fact that tannins are a heterogeneous collection of compounds. Broadly defined, tannins are water soluble, high molecular weight polymers of phenolic constituents that have the ability to bind proteins (Bate-Smith & Swain, 1962). Within this definition, tannins may be classified into two functional groups: condensed tannins, which are polymers of flavon-3,4-diols, and hydrolysable tannins, which consist of galloyl esters attached to glucose (Haslam, 1981). Even within these groups, there is great variation in oxygenation patterns, degree of polymerisation, stereochemistry, number, length, and identity of side chains, and amount of cross-linkage among side chains (Clausen *et al.*, 1992). Recent studies show that even congeneric species may have largely different tannin structure (Ayers *et al.*, 1997). Because the impact of a tannin relates to its chemical structure (Zucker, 1983; Bettolo *et al.*, 1986; Clausen *et al.*, 1990; Ayers *et al.*, 1997; Kraus *et al.*, 2003), it can be predicted that the impact of tannins on insects will vary among plant species.

Furthermore, tannin concentrations and identities may vary greatly within plants (Schultz *et al.*, 1982), within populations (Hunter *et al.*, 1996; Laitinen *et al.*, 2000), and temporally both over the course of leaf development (Macauley & Fox, 1980; Schultz *et al.*, 1982; Riipi *et al.*, 2002) and across years (Laitinen *et al.*, 2000; Covelo & Gallardo, 2001). Other studies have shown induction of tannins in oaks (Hunter & Schultz, 1993, 1995; Wold & Marquis, 1997), decreased concentration with increasing nutrient availability to plants (Hunter & Schultz, 1995; Forkner & Hunter, 2000), and increases in response to increased light levels (Mole *et al.*, 1988; Dudt & Shure, 1994). Other factors that can modify tannin–insect interactions include leaf age (Coley, 1983; Kause *et al.*, 1999; Haukioja *et al.*, 2002), foliar nutrients or water (Bernays & Woodhead, 1982; Barbenhenn *et al.*, 2003b), temperature (Stamp & Yang, 1996; Buse *et al.*, 1998; Dury *et al.*, 1998), and presence of other species, such as pathogens (Taper *et al.*, 1986; Schultz *et al.*, 1992; Faeth & Hammon, 1996). These additional variables may explain why in the high light, rapid growth conditions of light gap environments, herbivory was higher on oaks with higher tannin levels (Hunter & Forkner, 1999). In combination with modifying factors, large temporal and spatial variation in tannin concentrations may explain why tannins may be negatively correlated with herbivory in some years but not others (e.g. Faeth, 1985). Haukioja (2003) argues that the impacts of secondary compounds on insects should be interpreted within the context of variation in additional plant quality

factors relevant to insect growth. Based on these studies, it is predicted that insect herbivores emerging and feeding early in the season, when leaves have high nutrient and water levels and low tannin concentrations, will be less negatively affected by tannins than late feeding species, which feed on tough leaves with high tannins and low nutrients and water. It is also predicted that tannin impact will vary among insects specialising on certain plant strata (canopy vs. understorey or lower vs. upper canopy; Fortin & Mauffette, 2002) when variation in light and temperature generate differences in tannins between strata (Le Corff & Marquis, 1999).

Despite the apparent central role of tannins in oak herbivore resistance, the predominance of oaks in many North American, Central American, and European forests, and the importance of these interactions for plant defence theory, community-level patterns of oak–insect–tannin interactions are largely undocumented other than by the initial work of Feeny (1966, 1968, 1970). The few studies that have examined community patterns have been restricted to gall-forming insects (Abrahamson *et al.*, 2003), which can manipulate host plant chemistry (Cornell, 1983), or to non-oak plant species (Coley, 1983; Marquis *et al.*, 2001). Comprehensive faunal surveys of the leaf-chewing herbivores of *Quercus* in south-east Missouri Ozark forests provided an opportunity to look at the full range of species, family, and guild-specific tannin–herbivore patterns in a natural oak community. Although hydrolysable tannins may also negatively impact herbivores, as a first look at the patterns of interactions in this community, this study was limited to condensed tannins. To determine the relationship between condensed tannin concentrations and the densities of leaf-chewing herbivores, leaf tissue from individual trees of *Quercus alba* and *Q. velutina* (white oak and black oak respectively) was collected for spectrophotometric assays and censuses of insect densities were conducted in 2001. Specifically, the following predictions were tested: (1) negative correlations should exist between condensed tannin concentration and insect abundances, occurring more frequently for generalised feeders than for oak specialists; (2) significant correlations should occur more often later rather than earlier in the season because condensed tannin levels are lower and foliar nitrogen and water are higher at leaf flush; (3) insect species specialising on understorey foliage, where condensed tannin concentrations are consistently lower, should show negative correlations with tannins when they occur in the canopy, where levels of tannins are higher; moreover, there should be more specialists than generalists in the canopy; and (4) variation in tannin concentration should account significantly for variation in oak herbivore community structure.

Methods

Site description

This research was conducted as a part of a large-scale, multicollaborator, biodiversity research project called the Missouri Ozark Forest Ecosystem Project (MOFEP).

MOFEP research sites are located in south-eastern Missouri and encompass nearly 18 000 ha of Shannon, Reynolds, and Carter Counties, between $37°00'$ and $37°12'$N and $91°01'$ and $91°13'$W. This area is primarily upland oak–hickory and oak–pine communities and is 84% forested (Brookshire & Hauser, 1993; Xu *et al.*, 1997). *Quercus alba* (white oak) and *Q. velutina* (black oak), along with *Q. coccinea* (scarlet oak), dominate the canopy. *Quercus alba*, *Q. velutina*, *Cornus florida*, and *Sassafras albidum* constitute the majority of woody understorey species. Prior to MOFEP, no harvesting had occurred in the study sites since 1950, and most overstorey trees were 50–70 years old.

Insect surveys

During each census, both tops and bottoms of all leaves of marked canopy (15–20 m) branches or understorey saplings and low hanging branches (< 2.5 m) of subcanopy and canopy trees were searched and the number of individuals of each species of leaf-chewing herbivore encountered was recorded. A bucket truck was used for access to the canopy. Field assistants were trained in insect sight identification prior to censuses and were given lists of unidentified morphospecies previously encountered. More than a decade of collecting, photographing, and rearing oak herbivores by R. J. Marquis (Marquis *et al.*, 2004) made possible these *in situ* species identifications, which were based on larval morphology and natural history. All insects were left intact on the plant unless they could not be identified. These unknowns were collected, given a unique number, and reared in the laboratory for identification and photographing. Insects were observed throughout development until their identity was confirmed or they were classified as a species new to the inventory. In the analyses, only those species observed to feed on *Q. alba* or *Q. velutina* (confirmed through field observations or laboratory rearings) were included. To control for sampling effort, the number of leaves censused on marked trees and branches was counted in June. Herbivore censuses were conducted three times in 2001 – early May, late June, and late August–early September – to account for known seasonal changes in oak herbivore community composition (Marquis & Whelan, 1994; Marquis & Le Corff, 1997; R. E. Forkner, R. J. Marquis, J. T. Lill and J. Le Corff, unpubl. data). Three canopy trees (200–500 leaves per tree) of each oak species in 12 different forested stands ($n = 36$ trees/oak species) were censused. At 36 understorey locations, at least five saplings or low hanging branches of each oak species were censused, such that approximately the same amount of leaf area per site (1000–3000 leaves) was sampled in both the understorey and canopy. These sample sizes were based on a previous study (Marquis & Le Corff, 1997) indicating that they were adequate to describe leaf-chewing herbivore densities and species composition within a stand.

To determine if individual herbivore species showed differential distributions between strata (canopy vs. understorey) that were related to leaf quality, canopy stands

were paired with adjacent understorey census locations ($n = 12$ paired stands). Data from previous MOFEP canopy and understorey censuses (1997–2001; Marquis *et al.*, 2002) were summed across years for this analysis because individual species densities from any single year were often too low to determine if a preference between strata existed.

Sample collection

In the canopy, three to five leaves were collected from non-census branches of the same trees on which insect censuses were conducted ($n = 36$ trees/oak species). In the understorey, because there was concern about destructive leaf removal on census saplings that had few leaves, three to five leaves were collected from three near neighbours of census trees for each oak species in the 36 stands. Samples were collected at the same time as each of the three insect censuses to account for potential seasonal changes in concentrations of condensed tannins (Feeny, 1970; Schultz *et al.*, 1982). Leaves were flash-frozen in liquid nitrogen as they were collected, returned to the laboratory on dry ice, lyophilised, ground to a fine powder, and stored at $-80\,^{\circ}$C.

Chemical assays

Condensed tannins were assayed using a microscale modification of the acid–butanol technique (Rossiter *et al.*, 1988; Waterman & Mole, 1994). Standards were generated separately for each oak species by purifying aqueous extracts of pooled leaf tissue from all census dates with multiple washes of 95% ethanol followed by 70% aqueous acetone on Sephadex LH-20 in a Büchner funnel. Acetone was removed by rotary evaporation, and aqueous extracts were lyophilised to yield dry, purified oak tannin. Standards generated for *Q. alba* and *Q. velutina* had different absorbances at equal concentrations, indicating that the identity of the condensed tannins differed between oak species (J. C. Schultz, pers. comm.). Per cent dry mass foliar carbon and nitrogen were determined by microcombustion of dried leaf tissue (Perkin-Elmer Series II CHNS/O Analyzer 2400). A previous study of these same trees (Le Corff & Marquis, 1999) indicated that strata did not differ in %N. Therefore, because of limited time and funds, per cent carbon and nitrogen were determined only for canopy leaf samples. Contrast statements in an ANOVA for assays of a preliminary leaf collection in August 2000 indicated condensed tannin concentrations did not differ between understorey saplings and low-hanging branches of canopy and subcanopy trees ($F_{1,68} = 0.91$, $P = 0.3423$). Similarly, leaf morphology (area, toughness) differs more between understorey and canopy leaves on the same tree than between understorey leaves from saplings and low-hanging branches.

Statistical analyses

Ground leaf tissue from two canopy *Q. alba* trees in the June leaf collections consistently gave condensed tannin concentrations greater than 65% dry mass; however, because the values for per cent dry mass carbon did not agree with these values for condensed tannin concentrations, these two samples were excluded from the analysis. Additionally, one canopy *Q. velutina* sample from May was lost due to a grinding mill malfunction.

Repeated measures ANOVA was used to determine if the two oak species differed in total insect density, species richness, and per cent dry mass condensed tannins and nitrogen. Insect abundance and species richness were log transformed and per cent dry mass condensed tannins and nitrogen were arcsine square root transformed to normalise the residuals. Oak species was the between subjects effect and census date and census date × oak species were within subjects effects. For canopy data, because insects were censused on the same trees from which leaves were collected, ANOVAS and correlations were run using trees as sampling units. In the understorey, because it was not possible to collect leaves from the same trees on which insects were censused, stand level averages of condensed tannins were used. *Quercus velutina* did not occur in all stands so sample sizes for the understorey were $n = 36$ stands for *Q. alba* and $n = 33$ stands for *Q. velutina*. Because condensed tannin concentrations were not calculated on the same spatial scale for each strata, canopy and understorey analyses were run separately.

Because the abundances of individual species could not be transformed to meet the assumptions of parametric analyses, Spearman rank correlation was used to test for significant correlations between condensed tannin concentration and (1) species abundances (number of individuals per m^2 leaf area), (2) densities of insects in different guilds (free-feeders, leaf-miners, shelter-builders), and (3) densities of different families. Parametric correlation analysis was used to test for significant correlations between arcsine square root transformed per cent dry mass condensed tannins and log transformed (1) total insect abundance (number of insects per m^2 leaf area) and (2) species richness (number of herbivore species per m^2 leaf area). Analyses were run separately for *Q. velutina* and *Q. alba*, and the *P*-value for rejecting the null hypothesis was adjusted for multiple tests using Bonferroni's correction. Nonparametric Wilcoxon paired sample tests were used to test for significant differences in log transformed densities between strata. Because condensed tannin concentrations were lower in the understorey than in the canopy, species whose densities were significantly higher in the understorey than in the canopy were considered *tannin avoiders*.

Differences among *Q. velutina* and *Q. alba* trees in terms of insect community composition were characterised using principal component analysis (PCA). PCA is appropriate for indirect gradient analysis when species densities are linearly (monotonically) related to the latent environmental

variables (Jongman *et al.*, 1995), and a linear relationship between insect densities and tannin concentrations was expected. May, June, and August 2001 densities of the most abundant species, 18 species in the canopy and 27 species in the understorey, were summed for each tree or stand respectively, and the log transformations of yearly species abundances per m^2 leaf area were used in the PCA of community composition. Although these species represented approximately only 20% of the total species encountered in 2001, together they accounted for more than 80% of total insect abundance. Using the larger data set for paired canopy and understorey stands, an additional PCA was performed on the log transformed densities of the top 27 species from 1997 to 2001 to further elucidate differences between canopy and understorey communities. As with the ordination for the 2001 data, these 27 species represented a large proportion (85%) of the total number of insects encountered over the 5-year period. To classify species as generalists or specialists, information on host plant use was ascertained from the literature (Tietz, 1972; Covell, 1984; Marquis *et al.*, 2004). Insect species were classified as specialists if they feed only on species of *Quercus* and generalists if they feed on one or more additional genera.

Results

Variation in leaf quality

Understorey concentrations of condensed tannins were consistently lower than canopy concentrations in all censuses for *Q. velutina* and lower than canopy condensed tannins in June and August but equivalent in May for *Q. alba* (Fig. 1). Condensed tannin levels were lowest in May and increased throughout the season for *Q. velutina* (Fig. 1, census effect; canopy: $F_{2,66} = 203.96$, $P < 0.0001$, understorey: $F_{2,66} = 196.22$, $P < 0.0001$). *Quercus alba* had significantly higher condensed tannin levels than did *Q. velutina* in the
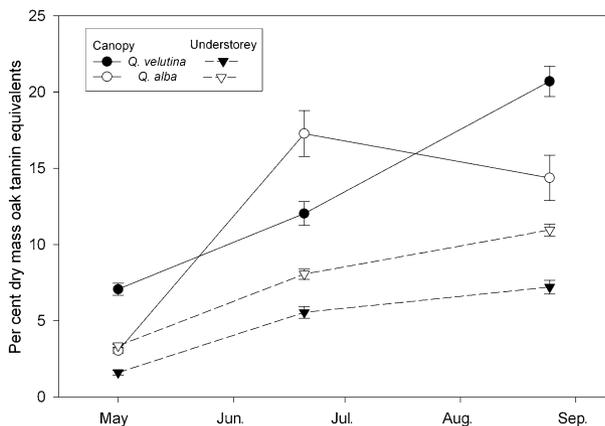

Fig. 1. Seasonal patterns of % dry mass foliar condensed tannins (mean ± SE), expressed as oak condensed tannin equivalents, for canopy and understorey *Quercus velutina* and *Quercus alba* in 2001.

understorey (oak effect; $F_{1,67} = 45.13$, $P < 0.0001$); however, there was a significant oak ($F_{1,67} = 11.28$, $P = 0.0013$) and census × oak species interaction effect ($F_{2,66} = 67.10$, $P < 0.0001$) for the canopy because condensed tannin levels in *Q. alba* were lower than *Q. velutina* condensed tannin levels in May and August, but higher in June (Fig. 1). There was some evidence that trees had consistent within-season tannins concentrations: May and June understorey and June and August canopy and understorey condensed tannin concentrations in *Q. velutina* were positively correlated (May–June: understorey, $P = 0.0027$; $r^2 = 0.26$; June–August: understorey, $P < 0.0001$, $r^2 = 0.42$; canopy, $P < 0.0001$, $r^2 = 0.49$). Only June and August canopy condensed tannin concentrations were positively correlated for *Q. alba* foliage ($P < 0.0001$, $r^2 = 0.58$). Per cent dry mass nitrogen ranged from 1.4 to 3.3% and from 1.5 to 3.6% in canopy foliage of *Q. alba* and *Q. velutina* respectively. Repeated measures ANOVA indicated that %N declined over the course of the season (census effect, $F_{2,64} = 250.81$, $P < 0.0001$), but did not differ significantly between oak species ($F_{1,65} = 0.33$, $P = 0.5691$).

Canopy condensed tannin concentrations were positively correlated with % dry mass C, but only in mid and late season (*Q. velutina*, June: $F_{1,35} = 11.59$, $r^2 = 0.23$, $P = 0.0017$; *Q. alba*, June: $F_{1,34} = 14.24$, $r^2 = 0.29$, $P = 0.0007$; August: $F_{1,35} = 29.46$, $r^2 = 0.46$, $P = 0.0001$). Canopy condensed tannin concentrations did not correlate with % dry mass N or carbon to nitrogen ratio in the leaves of *Q. alba*; however, May and June canopy condensed tannin levels in *Q. velutina* were negatively correlated with foliar % nitrogen ($F_{1,33} = 10.57$, $r^2 = 0.23$, $P = 0.0027$ and $F_{1,35} = 6.92$, $r^2 = 0.17$, $P = 0.0127$, respectively).

Leaf-chewing herbivore community

The majority of herbivores encountered in 2001 were small Lepidoptera ($n = 18\,179$ individuals from 134 species). Other leaf-chewers included sawfly larvae (Tenthredinidae), walking sticks (Phasmatidae), beetles (Chrysomelidae and Curculionidae), and katydids (Tettigoniidae). Early season (May) fauna consisted mainly of free-feeding noctuids, leaf-rolling species of Gelechioidea and Tortricidae, and a curculionid, *Attelabus* sp. Leaf-tying gelechiids, leaf-mining gracillariids, and web-building pyralids dominated early and mid-summer fauna (June and July). Limacodidae and Notodontidae fed predominantly in August. The relative proportions of each family varied more over the course of the season than between oak species or strata. Only one introduced species was encountered: the Asiatic oak weevil, *Crytepistomus castaneus* Roelofs. The gypsy moth (*Lymantria dispar*) has yet to establish in Missouri.

Total canopy insect abundance declined significantly in mid-summer for both oak species (census effect: $F_{2,69} = 99.40$, $P < 0.0001$ but did not differ between oak species ($F_{1,70} = 0.04$, $P = 0.8481$); however, there was a significant census–oak species interaction ($F_{2,69} = 20.03$, $P < 0.0001$) because canopy insect densities were higher on *Q. velutina* in May but higher on *Q. alba* in August (Fig. 2a). While
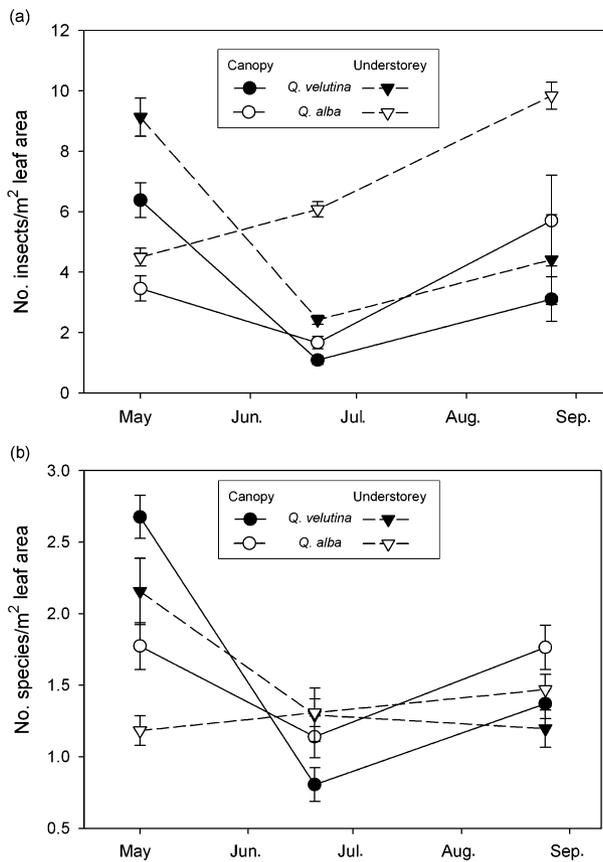
Fig. 2. Seasonal patterns of (a) total insect abundance and (b) insect species richness on canopy and understorey *Quercus velutina* and *Quercus alba* in 2001. Values are means ± SE.

understorey insect abundances on *Q. velutina* peaked in May and remained low for the remainder of the season, they increased linearly over the season for *Q. alba* (oak effect: $F_{1,67} = 18.52$, $P < 0.0001$; census effect: $F_{2,66} = 18.33$, $P < 0.0001$; census × oak effect: $F_{2,66} = 93.39$, $P < 0.0001$, Fig. 2a).

Canopy richness was highest in May, declined in mid-summer, and increased slightly in August (census effect: $F_{2,68} = 64.14$, $P < 0.0001$). Canopy species richness followed the same pattern as total insect abundance: number of insect species per m^2 leaf area was higher on *Q. velutina* in May but higher on *Q. alba* in August (Fig. 2b; oak effect: $F_{1,69} = 0.01$, $P = 0.9386$; census × oak species effect: $F_{2,68} = 18.69$, $P < 0.0001$). Understorey species richness remained relatively constant on *Q. alba* but declined after the May census on *Q. velutina* (census effect: $F_{2,66} = 11.34$, $P < 0.0001$; census × oak effect: $F_{2,66} = 37.76$, $P < 0.0001$; Fig. 2b).

Species, guild, and family correlations with condensed tannins

Within each census date, 10–12 canopy species and 18–26 understorey species had densities sufficiently high (≥ 10

individuals) to be analysed individually (see Table 1 for species' identities). In the canopy in May 2001, only one species was correlated with condensed tannin concentrations: the number of larvae of the curculionid *Attelabus* sp. on *Q. alba* was negatively correlated with condensed tannin levels. At the guild level, total canopy density of shelter-builders was marginally negatively correlated with condensed tannin levels in *Q. alba* ($\rho = -0.34$, $P = 0.0405$). There were no significant correlations at the family level for *Q. alba*, nor did any of the species feeding in the canopy on *Q. velutina* appear to be impacted by tannin concentrations. In the understorey, the leaf-roller *Chionodes pereyra* Clarke (Gelechiidae), was marginally negatively correlated with condensed tannin concentrations on *Q. alba* ($\rho = -0.4587$, $P = 0.0073$). Spearman rank correlations were not significant for individual species, families, or guilds in June for either oak species in either strata. In August, canopy abundance of *Acronicta increta* Grote (Noctuidae) on *Q. alba* was negatively correlated with condensed tannin levels ($\rho = -0.4978$, $P = 0.002$), as was the collective abundance of all Noctuidae ($\rho = -0.4978$, $P = 0.002$) and of free-feeders, which included Noctuidae, Notodontidae, Limacodidae, Tettigoniidae, Hesperiidae, Geometridae, and Curculionidae ($\rho = -0.5371$, $P = 0.0007$). Neither understorey nor canopy densities of species, families, or guilds on *Q. velutina* showed significant correlations with condensed tannin concentrations in August. Correlations of canopy densities of individual species, families, and guilds with per cent dry mass nitrogen were not significant for either oak species for any census date.

Total insect abundance and richness correlations with condensed tannins

Species richness on understorey *Q. velutina* in May was negatively correlated with condensed tannin concentrations (Table 2). Both total canopy insect abundance and species richness on *Q. velutina* were marginally negatively correlated with condensed tannin levels in June (Table 2), but no significant correlations occurred in May or August (Table 2, Fig. 3). Total insect abundance and species richness on canopy leaves of *Q. alba* were significantly negatively correlated with condensed tannins in August, but no significant correlations occurred in May or June, nor were there any significant understorey correlations (Table 2).

Community-level patterns

Eigenvalues for the first four axes of the PCA of abundances of the top 18 most abundant species in the canopy in 2001 were 2.96, 2.26, 1.85, and 1.61 respectively for *Q. velutina*, and they explained a total of 51% of the variance in species abundances. Eigenvalues for the first four axes for *Q. alba* were 3.24, 2.15, 1.90, and 1.52 respectively, and they explained a total of 49% of the variance in species abundances. Stepwise regression of canopy May, June, and

Table 1. Wilcoxon signed rank test results for differences in species densities of leaf-chewing herbivores of *Quercus velutina* and *Quercus alba* between canopy and understorey strata, based on summed densities for paired stands from 1997 to 2001. Corrected *P*-value for rejection of the null hypothesis was $P = 0.0012$. Negative *Z*-values indicate higher abundance in the canopy and positive values indicate higher abundance in the understorey. Significant values are in boldface and marginally significant values italicised.

Species†	Season‡	Guild§	*Quercus velutina*		*Quercus alba*		Id**
			Z	P	Z	P	
Specialists							
Acronicta increta (Noctuidae)	2,3	Free	−0.165	0.8690	2.916	*0.0035*	Ai
Arogalea cristifasciella (Gelechiidae)	2,3	Shelter	−0.758	0.4483	−0.029	0.9770	Ac
Attelabus sp. (Curculionidae)	1	Free	−1.418	0.1563	−3.089	*0.0020*	A
Bucculatrix nr. *albertiella* (Bucculatracidae)	2,3	Shelter	1.683	0.0924	3.955	**<0.0001**	Ba
Catacola amica (Noctuidae)	1	Free	−0.936	0.3492	−0.789	0.4301	Ca
Chionodes fuscomaculella (Gelechiidae)	2,3	Shelter	−0.561	0.5750	−3.089	*0.0020*	Cf
Chionodes pereyra (Gelechiidae)	1	Shelter	0.758	0.4483	0.319	0.7498	Cp
Chionodes sp. (Gelechiidae)	1	Shelter	3.066	*0.0022*	3.378	**0.0007**	C
Crytepistomus castaneus (Curculionidae)	2,3	Free	−2.209	*0.0272*	0.606	0.5444	Cc
Dichomeris georgiella (Gelechiidae)	1	Shelter	−1.232	0.2179	−0.586	0.5578	Dg
Erynnis sp. (Hesperiidae)	1,2,3	Free	3.462	**0.0005**	3.262	**0.0011**	Ej
Hyperstrotia secta (Noctuidae)	2,3	Free	0.956	0.3390	1.761	0.0783	Hs
Phoberia atomaris (Noctuidae)	1	Free	0.259	0.7957	0.034	0.9726	Pa
Phyllonorycter fitchella (Gracillariidae)	2,3	Mine	NA¶	NA¶	4.128	**<0.0001**	Pf
Phyllonorycter sp. (Gracillariidae)	2,3	Mine	−0.627	0.5306	NA¶	NA¶	Ph
Pococera expandens (Pyralidae)	2,3	Shelter	2.664	*0.0077*	2.858	*0.0043*	Pe
Pseudotelphusa sp. (Gelechiidae)	2,3	Shelter	1.715	0.0864	3.724	**0.0002**	P
Stigmella sp. (Nepticulidae)	2,3	Mine	−3.924	**<0.0001**	−2.512	*0.0120*	S
Telphusa latifasciella (Gelechiidae)	1	Shelter	−0.824	0.4098	2.107	*0.0351*	Tl
Trypanisma prudens (Gelechiidae)	2,3	Shelter	1.723	0.0849	3.551	**0.0004**	Tp
Generalists							
Archips argyrospila (Tortricidae)	1	Shelter	−0.396	0.6921	−0.076	0.9395	Aa
Argyrotaenia quercifoliana (Tortricidae)	1	Shelter	−1.879	0.0601	1.011	0.3123	Aq
Diapheromera femorata (Heteronemiidae)	1,2,3	Free	2.736	*0.0062*	3.378	**0.0007**	Df
Dichomeris ligulella (Gelechiidae)	1	Shelter	0.066	0.9474	1.705	0.0883	Dl
Himella intractata (Noctuidae)	1	Free	−2.951	*0.0032*	−0.146	0.8838	Hi
Hypagyrtis esther (Geometridae)	1	Free	−0.185	0.8533	1.858	0.0632	He
Isa textula (Limacodidae)	3	Free	−3.885	**0.0001**	0.068	0.3854	It
Lithophane antennata (Noctuidae)	1	Free	−0.758	0.4567	−0.087	0.9310	La
Lochmaeus manteo (Notodontidae)	3	Free	1.815	0.0695	0.779	0.4357	Lm
Machimia tentoriferella (Oecophoridae)	2,3	Shelter	−3.463	**0.0005**	−0.029	0.9770	Mt
Morrisonia confusa (Noctuidae)	1,2,3	Free	1.286	0.1985	2.165	*0.0304*	Mc
Oligocentria lignicolor (Notodontidae)	3	Free	2.982	*0.0029*	1.668	0.0954	Ol
Psilocorsis quercicella (Oecophoridae)	2,3	Shelter	−1.154	0.2485	−2.165	*0.0304*	Pq
Psilocorsis reflexella (Oecophoridae)	2,3	Shelter	−1.549	0.1213	−4.128	**<0.0001**	Pr
Spargonothis pettitana (Tortricidae)	1	Shelter	0.297	0.7667	−0.318	0.7508	Sp
Tischeria citrinipennella (Tischeriidae)	2	Mine	−2.023	*0.0431*	3.204	*0.0014*	Tc
Unknown							
Morphospecies no. 23 (Tortricidae)	1	Shelter	−3.001	0.0027	−1.819	0.0690	s23
Morphospecies no. 29 (Tenthredinidae)	1	Free	−0.714	0.4752	1.819	0.0690	s29
Morphospecies no. 74 (Curculionidae)	1,2,3	Free	1.585	0.1129	2.817	*0.0048*	s74
Morphospecies no. 78 (Chrysomelidae)	1,2,3	Free	0.637	0.5244	−0.318	0.7508	s78
Morphospecies no. 265 (Tenthredinidae)	3	Free	1.517	0.1294	2.860	*0.0042*	s265

†S = Specialist, only found on *Quercus*; G = generalist, found on *Quercus* and one or more additional genera; Unknown = host plant preferences are undetermined.

‡Larvae present in 1 = May, 2 = June, 3 = August.

§Free = species feeding openly on leaf surface; Shelter = shelter-building species, including leaf-rollers, leaf-tiers, leaf-folders, and web-builders; Mine = leaf-miners.

¶*Phyllonorycter fitchella* is unique to *Q. alba*, and *Phyllonorycter* sp. is unique to *Q. velutina*.

**Id, refers to text symbols used in Figs 4 and 5 presenting results of PCA.

Table 2. Pearson's correlation coefficients for correlations of log-transformed total insect abundance (number of insects/m² leaf area) and species richness (number of species/m² leaf area) with per cent dry mass oak condensed tannins. Corrected *P*-value for rejection of the null hypothesis was $P = 0.0167$. Significant values are in boldface and marginally significant values italicised.

	Quercus velutina						*Quercus alba*					
	Canopy			Understorey			Canopy			Understorey		
	Rho	*P*	*N*†	Rho	*P*	*N*	Rho	*P*	*N*	Rho	*P*	*N*
Total insect abundance												
May	0.0092	0.9581	604	−0.3178	0.0715	2670	−0.2798	0.0983	398	0.0285	0.8689	2214
June	−0.3459	*0.0388*	104	−0.1258	0.4853	753	−0.1636	0.3551	188	−0.0252	0.8841	3651
August	0.2292	0. 1767	297	0.0861	0.6340	949	**−0.4718**	**0.0037**	705	0.2481	0.1446	5646
Species richness												
May	−0.1607	0.3564	32	**−0.4904**	**0.0038**	54	−0.1179	0.4931	30	0.2900	0.0862	59
June	−0.3549	*0.0337*	24	−0.2709	0.1273	43	−0. 1923	0.2728	29	−0.0268	0.8769	52
August	0.1343	0.4417	30	0.1218	0.4997	50	**−0.4039**	**0.0145**	34	0.2207	0.1958	64

†*N*, total number of insects or species encountered.

August condensed tannin concentrations and % dry mass N with scores for principal component axes 1 and 2 from this analysis showed that condensed tannins explained variation in community structure among canopy *Q. velutina* trees. While no variables entered the model for axis 1, in the stepwise regression for principal component axis 2, August condensed tannin concentrations were significantly negatively correlated with PCA scores ($r^2 = 0.15$, $F = 6.01$, $P < 0.0197$) and May condensed tannin concentrations were positively correlated

($r^2 = 0.10$, $F = 4.21$, $P = 0.0485$; Fig. 4). Condensed tannin concentrations did not correlate with principal component axis 1 or 2 scores for canopy insect abundances for *Q. alba*.

Eigenvalues for the first four axes for PCA of the top 27 most abundant species in the understorey of *Q. velutina* were 4.80, 3.14, 2.78, and 2.08 respectively, and they explained a total of 49% of the variance in species abundances. Eigenvalues for the first four axes for *Q. alba* were 5.63, 4.71, 2.42, and 1.93 respectively, and they explained a
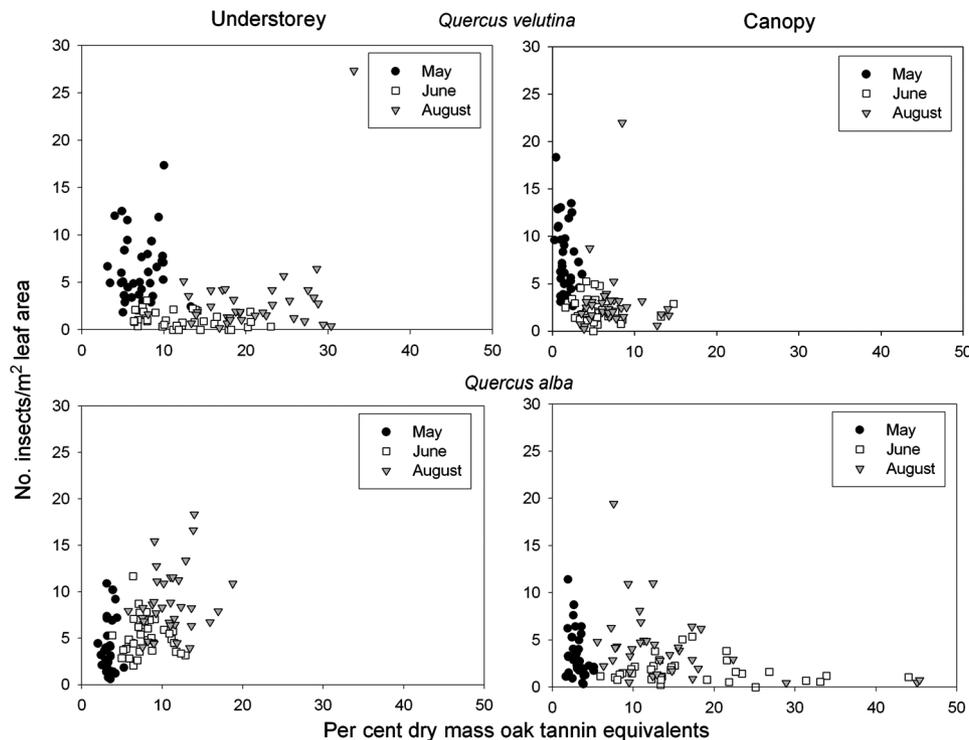

Fig. 3. Relationship between total insect abundance and concentrations of canopy foliar condensed tannins for canopy and understorey *Quercus velutina* and *Quercus alba* in May, June, and August of 2001.

total of 57% of the variance in species abundances. In the stepwise multiple regression for principal component axis 1 for *Q. velutina*, May condensed tannin concentrations were significantly positively correlated with PCA scores ($r^2 = 0.19$, $F = 7.36$, $P = 0.0108$, Fig. 4). No variables were significant in the stepwise multiple regression for axis 2 for *Q. velutina* or for axis 1 or 2 for *Q. alba*.

Eight species feeding on *Q. alba* were significantly more abundant in the understorey than in the canopy (Table 1). An additional five species, including *Acronicta increta*, were marginally more abundant in the understorey. On *Q. velutina*, one species, the skipper *Erynnis juvenalis* Fabricius, was more abundant in the understorey, and three additional species, *Diapheromera femorata* Say, *Chionodes* sp., and *Pococera expandens* Walker were marginally so. Of these understorey specialists, only *Acronicta increta* was negatively correlated with condensed tannin concentrations in 2001 (Table 1). On *Q. alba*, *Psilocorsis reflexella* Clemens was significantly more abundant in the canopy, as were
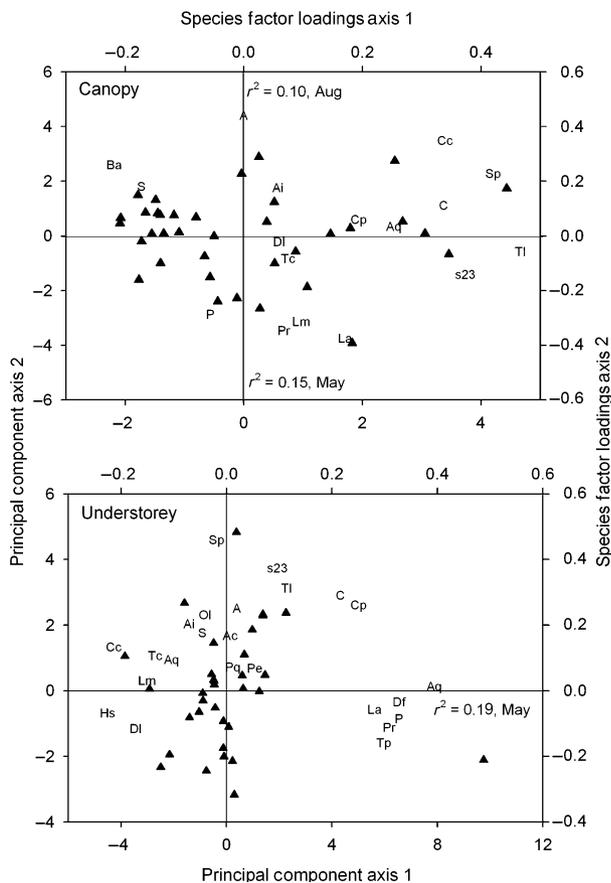
Isa textula Herrich-Schäffer, *Machimia tentoriferella* Clemens, and *Stigmella* sp. on *Q. velutina*. Three additional species were marginally more abundant in the canopy (Table 1). Of these canopy specialists, only *Attelabus* was negatively correlated with condensed tannin concentrations in 2001.

PCA of abundances of the top 27 most abundant insect species in paired canopy and understorey locations from 1997 to 2001 indicated that the four different species by strata environments constituted different herbivore communities. *Quercus alba* and *Q. velutina* separated largely on densities of the leaf-miner *Phyllonorycter fitchella*, the free-feeding *Acronicta increta* (higher on *Q. alba*), and spring leaf-rollers (higher on *Q. velutina*). Canopy and understorey communities differed in densities of mid-summer leaf-tying Oecophoridae (higher in the canopy) and in densities of species present throughout the growing season – *Diapheromera femorata*, *Erynnis juvenalis*, and *Morrisonia confusa* Hübner (higher in the understorey, Fig. 5). Eigenvalues for the first four axes were 7.8, 4.8, 2.3, and 2.1 respectively, and they explained a total of 63% of the variance in species abundances. Factor loadings for each species on axes 1 and 2 are presented in Fig. 5 (for visual clarity, arrows are not shown).

Discussion

As predicted, significant negative correlations of condensed tannins with leaf-chewing herbivore densities occurred; however, specialists, rather than generalists, were more likely to show correlations. There were no significant positive correlations between insect densities and tannin concentrations. Analyses indicated that the densities of two oak specialists, *Acronicta increta* and *Attelabus*, were negatively correlated with condensed tannin concentrations, and a third specialist, *Chionodes pereyra*, showed a similar trend. Although all three species feed exclusively on oaks, they have different life histories and feeding strategies. *Chionodes pereyra* is a leaf-rolling gelechiid that completes larval development 2–3 weeks after leaf flush. *Attelabus* belongs to a subfamily of weevils, the adults of which cut arcs from intact leaves and form them into solid, cylindrical rolls that provision offspring (one larva per leaf-roll). *Attelabus* adults are sometimes encountered later in the season, but larval leaf-rolls are only found in the spring at this study site. *Acronicta increta*, on the other hand, is a free-feeding noctuid, whose larvae, present from June to August, rest curled in a semicircle on the underside of an oak leaf during the day. *Acronicta increta* has two generations per season in this study area, and the larvae require more than a month to develop. Despite differences in phenology and feeding strategies, all three species are highly abundant in this system. In fact, *A. increta* and *Attelabus*, because of their high abundances, were responsible for driving the patterns that occurred at the family and guild levels. The densities of all spring shelter builders in the canopy showed a weak negative correlation with condensed tannins; however, this
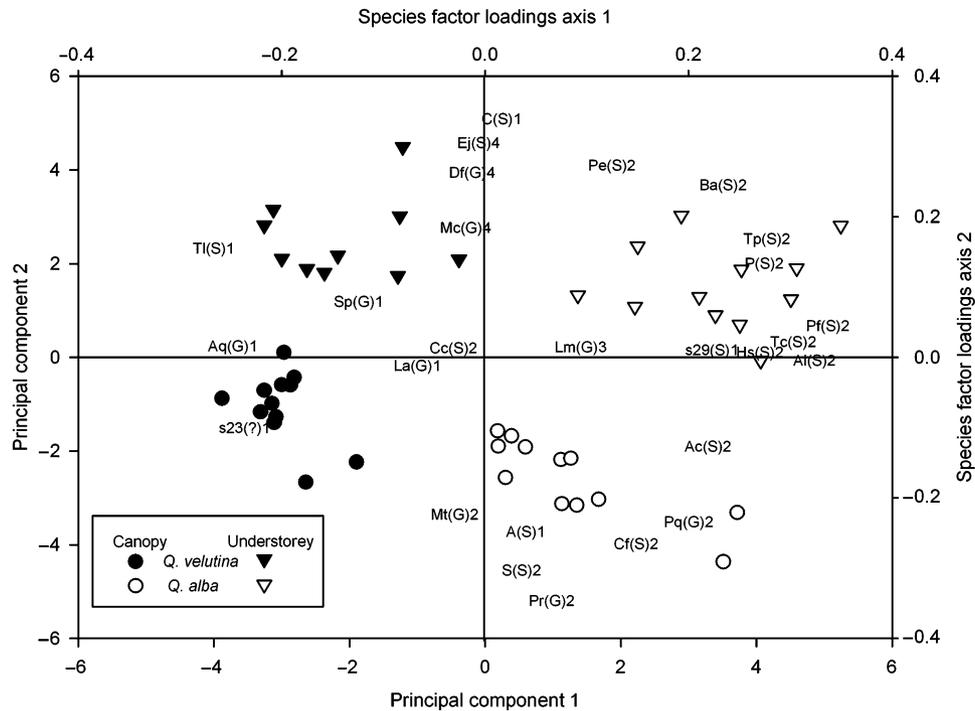

Fig. 4. Principal component ordination for canopy and understorey 2001 species abundances on *Quercus velutina*. Eigenvalues for principal component axes are given in the Results. Upper and right axis scales are for insect species eigenvector scores and indicate principal component axis loadings. r^2 values are given for significant correlations between PCA axis scores and condensed tannin concentrations. Letters indicate species' identities (see Table 1).

Fig. 5. Principal component ordination for species abundances in paired canopy and understorey stands from 1997 to 2001. Eigenvalues for principal component axes are given in the Results. Upper and right axis scales are for insect species eigenvector scores and indicate principal component axis loadings. Letters indicate species' identities (see Table 1). Parenthetical letters indicate phagy: S = specialist, G = generalist, ? = unknown. Numbers following species initials indicate seasonality of larvae: 1 = present in May, 2 = present in June, 3 = present in August, 4 = present in May through August.

relationship disappeared when *Attelabus*, which accounted for one-third of all shelter builders and 25% of all individuals counted in May, was excluded from the analysis. Likewise, the negative correlation of total canopy density of August free-feeders with condensed tannins disappeared when *A. increta*, which accounted for more than one-third of all late season insects on *Q. alba* in 2001, was excluded. Because larval *Attelabus* do not choose among leaves, but develop within shelters constructed by adult weevils, future studies in this system should examine the relationship between condensed tannins and oviposition or successful leaf-roll construction in *Attelabus*. Similarly, studies should examine the relationship between condensed tannins and oviposition, as well as growth rates or survival within leaf-rolls, for *C. pereyra*. For *A. increta*, whose larvae develop more slowly and may move among leaves or trees, studies should examine the distribution of larval instars in relation to foliar condensed tannin concentrations.

Contrary to prediction, negative correlations between condensed tannin levels and insect abundances were not more likely to occur late in the season. Sufficient empirical data exist to suggest that the general seasonal pattern of leaf quality for *Quercus* species is an increase in condensed tannins from low levels at bud break to high late season concentrations and a decrease in per cent dry mass nitrogen as leaves mature (Feeny & Bostock, 1968; Feeny, 1970; Faeth, 1985; Scalbert & Haslam, 1987; Mauffette & Oechel,

1989; Le Corff & Marquis, 1999). The data presented here are consistent with these studies of foliage quality. None-theless, negative correlations of individual species densities with condensed tannin levels did occur for understorey *Q. velutina* and canopy *Q. alba* in May when tannin concentrations were less than 5% of leaf dry mass. Two-thirds of those species feeding in May construct leaf-rolls, and work by Sagers (1992) suggests that this behaviour may decrease foliar tannin concentrations. Correlations of insect densities with tannins were performed at the tree level for the canopy and at the stand level for the understorey, rather than at the level of individual leaves. Therefore, it is possible that condensed tannin concentrations for the leaf on which the leaf-rolling insect developed were lower than estimates for the entire tree or stand.

While condensed tannins appeared to influence the abundance of a few specialist species at both low and high concentrations, significant negative correlations between overall insect abundance and condensed tannin concentrations were found only in August and only for *Q. alba* (Table 2). Seasonal patterns of insect abundance also did not parallel seasonal changes or differences between *Q. velutina* and *Q. alba* in condensed tannin levels (Figs 1 and 2). Feeny (1970) documented a concomitant decline in total insect abundance over the course of the season with increasing tannin concentrations, whereas total herbivore density in this system increased in August relative to mid-season densities for both oak

species in the canopy, and increased 67% on *Q. alba* in the understorey (Fig. 3). These increases occurred despite the fact that understorey concentrations of condensed tannins were approximately 50% higher on *Q. alba* than on *Q. velutina* throughout the season and increased more than 200% from May to August. A previous study of oak herbivores demonstrated that the presence of one leaf-tying species, *Pseudotelphusa* sp., which creates leaf-ties that subsequent species preferentially colonise, can increase species richness by as much as 38% (Lill & Marquis, 2003) and densities of late-season leaf-tiers as much as 300% on saplings of *Q. alba* (J. T. Lill & R. J. Marquis, unpubl. data). *Pseudotelphusa* is not only more abundant on *Q. alba* but also has significantly higher abundances in the understorey (Table 1), and, thus, may be driving the seasonal increases in density on *Q. alba*. Furthermore, densities of spring leaf rollers in the canopy were higher on *Q. velutina* despite the fact that condensed tannin levels were also higher on *Q. velutina* at that time. Leaves of *Q. velutina* are on average 60% larger than *Q. alba* leaves in the understorey and 20% larger in the canopy (Marquis & Le Corff, 1997). *Quercus velutina* leaves may simply provide more resources for larvae developing within leaf-rolls or enable them to make more secure structures (greater number of whorls).

There was no support for the hypothesis that densities of species that commonly feed in the understorey where tannin concentrations are lower would show negative correlations with condensed tannins when they were found feeding in the high tannin habitat of the canopy. None of the species that were higher in abundance in the understorey of *Q. alba* showed significant correlations with condensed tannin levels when feeding in the canopy. *Acronicta increta*, which was negatively correlated with condensed tannin levels when feeding in the canopy, did show a marginally higher abundance in the understorey. On the other hand, *Attelabus* had significantly higher abundance in the canopy of *Q. alba*. Generalist species did not appear to be more abundant in the understorey. In fact, the reverse appeared to be the case: the proportion of specialist species having significantly greater abundances in the understorey was 30%, whereas the proportion of generalists having greater abundances in the understorey was only 6% (Table 1). Of particular note, however, is that those species that develop slowly over the course of the entire growing season, such as *Diapheromera femorata* and *Erynnis* sp., did appear to be more abundant in the understorey where condensed tannin concentrations were lower (Table 1 and Fig. 5). Because laboratory feeding trials were not performed, it is not possible to determine if differential distributions between strata were related directly to feeding preferences for higher quality understorey foliage or the result of differences in abiotic or biotic factors between habitats. Future investigations should incorporate bioassays for larval preference and performance on canopy and understorey foliage (Fortin & Mauffette, 2002) or field studies of differences in parasitism or predation pressure between strata.

Variation in condensed tannin concentrations did contribute significantly to variation in community structure. Condensed tannin concentrations were significantly negatively correlated with species richness in May for *Q. velutina* and in August for *Q. alba* (Table 2). May condensed tannin concentrations contributed significantly to variation in understorey and canopy community composition for *Q. velutina*. August condensed tannin levels also contributed to variation in the canopy community of *Q. velutina*. The amount of variation in community structure or species richness explained by condensed tannin levels was on the order of 25%. Thus, while condensed tannin concentrations did explain some variation, other environmental factors, such as leaf toughness and desiccation-related microenvironmental factors (e.g. humidity, temperature), or species interactions (e.g. engineering effects of *Pseudotelphusa*, predation, competition) are also likely to play a role. Additionally, in this study, only condensed tannins were assayed. Hydrolysable tannins and total phenolics were not measured. Therefore, the importance of tannins to leaf-chewing herbivores may have been underestimated. At least one study (Lill & Marquis, 2001) found that hydrolysable tannins but not condensed tannins were negatively correlated with pupal mass in *Psilocorsis quercicella*, a common summer species in this system. Studies that examine hydrolysable tannins may reveal additional contributions of tannins to community structure. Given that many biotic and abiotic factors are likely to be correlated with condensed tannin concentrations, it cannot be certain that variation in tannin concentrations was ultimately responsible for the patterns observed. Factorial experiments that vary putative leaf quality factors and examine the impacts on community structure or multivariate studies that examine additional relevant variables are needed.

Temporal variation in herbivore abundance and richness can complicate attempts to find patterns in insect–tannin interactions. Low population densities can preclude testing for correlations with secondary chemistry or reduce the power of statistical inference; the majority (70%) of individual species encountered in 2001 occurred at densities too low to be analysed individually. Furthermore, the 134 species encountered in 2001 represent only half of all species to have been encountered in 10 years of censusing herbivore densities in this system (R. E. Forkner, R. J. Marquis, J. T. Lill and J. Le Corff, unpubl. data). Because May species occurred at higher densities than late season species, the impact of tannins on late season species may have been more difficult to detect. On the whole, the number of species whose densities were negatively correlated with condensed tannin concentrations was low (three species, or 8% of species whose densities were sufficiently high to run statistical tests). Those species that did show negative correlations, however, were some of the most abundant herbivores, representing a significant portion of the total herbivore pressure on *Quercus* in this system in 2001.

In summary, in support of Feeny's arguments that tannins have a defensive function, condensed tannin concentration was negatively correlated with individual species densities, total insect densities, and herbivore species richness. Negative correlations occurred even for species

adapted to feed on tannin-containing hosts and even at low concentrations. The community ordination analyses, together with correlations of richness and total abundance with condensed tannin levels, suggest that condensed tannins explain within-plant species variation in leaf-chewing herbivore abundance and community structure. On the other hand, condensed tannin concentrations did not correlate with individual densities for the large majority of herbivore species encountered, and the amount of variation explained was not on the order of that shown for biotic interactions with engineering species (Lill & Marquis, 2003). Thus, while these results provide some support for Feeny's original view that tannins play a role in structuring the herbivore fauna of *Quercus*, condensed tannins did not appear to act as '*broad spectrum* defensive mechanisms'. Experimental investigations that incorporate measures or manipulations of condensed tannins, other polyphenolics, and additional leaf nutritive qualities with manipulations of relevant biotic and abiotic community structuring forces are encouraged, as are studies of the physiological impact of tannins on specialist herbivores of *Quercus*.

Acknowledgements

References

Abrahamson, W.G., Hunter, M.D., Melika, G. & Price, P.W. (2003) Cynipid gall-wasp communities correlate with oak chemistry. *Journal of Chemical Ecology*, **29**, 209–223.

Appel, H. (1993) Phenolics in ecological interactions. *Journal of Chemical Ecology*, **19**, 1521–1522.

Appel, H.M. & Schultz, J.C. (1992) Activity of phenolics in insects: the role of oxidation. *Plant Polyphenols: Synthesis, Properties, Significance* (ed. by R. W. Hemingway and P. E. Laks), pp. 609–620. Plenum, New York.

Ayers, M.P., Clausen, T.P., McLean, S.F. Jr,, Redman, A.M. & Reichardt, P.B. (1997) Diversity of structure and antiherbivore activity in condensed tannins. *Ecology*, **78**, 1696–1712.

Barbenhenn, R.V., Bumgarner, S.L., Roosen, E.F. & Martin, M.M. (2001) Antioxidant defenses in caterpillars: role of the ascorbate-recycling system in the midgut lumen. *Journal of Insect Physiology*, **47**, 349–357.

Barbenhenn, R.V. & Martin, M.M. (1994) Tannin sensitivity in larvae of *Malacosoma disstira* (Lepidoptera): roles of the peritrophic envelope and midgut oxidation. *Journal of Chemical Ecology*, **20**, 1985–2001.

Barbenhenn, R.V., Poopat, U. & Spencer, B. (2003a) Semiquinone and ascorbyl radicals in the gut fluids of caterpillars measured with EPR spectrometry. *Insect Biochemistry and Molecular Biology*, **33**, 125–130.

Barbenhenn, R.V., Walker, A.C. & Uddin, F. (2003b) Antioxidants in the midgut fluids of a tannin-tolerant and a tannin-sensitive caterpillar: effects of seasonal changes in tree leaves. *Journal of Chemical Ecology*, **29**, 1099–1116.

Bate-Smith, E.C. & Swain, T. (1962) Flavonoid compounds. *Comparative Biochemistry*, Vol. III (ed. by M. Florkin and H. C. Mason). Academic Press, New York.

Beart, J.E., Lilley, T.H. & Haslam, E. (1985) Plant polyphenols – secondary metabolites and chemical defense: some observations. *Phytochemistry*, **24**, 33–38.

Berenbaum, M. (1983) Effects of tannins on growth and digestion in two species of papilionids. *Entomologia Experimentalis et Applicata*, **34**, 245–250.

Bernays, E.A. & Woodhead, S. (1982) Plant phenols utilized as nutrients by a phytophagous insect *Anacridium melanorhodon*. *Science*, **216**, 201–203.

Bettolo, G.B.M., Marta, M., Pomponi, M. & Bernays, E.A. (1986) Flavan oxygenation pattern and insect feeding deterrence. *Biochemical Systematics and Ecology*, **14**, 249–250.

Bi, J.L., Felton, G.W., Murphy, J.B., Howles, P.A., Dixon, R.A. & Lamb, C.J. (1997) Do phenolics confer resistance to specialists and generalist insect herbivores? *Journal of Agricultural and Food Chemistry*, **45**, 4500–4504.

Brookshire, B.L. & Hauser, C.H. (1993) The Missouri Ozark Forest Ecosystem Project: the effects of forest management on the forest ecosystem. *Proceedings of the 9th Central Hardwood Forest Conference; 1993 March 8–10, Purdue University, West Lafayette, IN. General Technical Report NC-161* (ed. by A. R. Gillespie, G. R. Parker, P. E. Pope and G. R. Rink), pp. 274–288. USDA Forest Service, North Central Forest Experiment Station, St Paul, Minnesota.

Bryant, J.P., Chapin, F.S. III & Klien, D.R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357–368.

Buse, A.J., Good, E.G., Dury, S. & Perrins, C.M. (1998) Effects of elevated temperature and carbon dioxide on the nutritional quality of leaves of oak (*Quercus robur* L.) as food for the Winter Moth (*Operophtera brumata* L.). *Functional Ecology*, **12**, 742–749.

Clausen, T.P., Provenza, F.D. & Burritt, E.A. (1990) Ecological implications of condensed tannin structure: a case study. *Journal of Chemical Ecology*, **16**, 2381–2392.

Clausen, T.P., Reichardt, P.B., Bryant, J.P. & Provenza, F.D. (1992) Condensed tannins in plant defense: a perspective on classical theories. *Plant Polyphenols: Synthesis, Properties, Significance* (ed. by R. W. Hemingway and P. E. Laks), pp. 639–651. Plenum, New York.

Close, D.C. & McArthur, C. (2002) Rethinking the role of many plant phenolics – protection from photodamage not herbivore? *Oikos*, **99**, 166–172.

Coley, P.D. (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs*, **53**, 209–233.

Coley, P.D., Bryant, J.P. & Chapin, F.S. III (1985) Resource availability and plant anti-herbivore defense. *Science*, **230**, 895–899.

Cornell, H.V. (1983) The secondary chemistry and complex morphology of galls formed by the. Cynipinae Hymenoptera: why and how? *American Midland Naturalist*, **110**, 225–234.

Covell, C.V. (1984) *A Field Guide to the Moths of Eastern North America*. Houghton Mifflin Company, Boston.

Covelo, F. & Gallardo, A. (2001) Temporal variation in total leaf phenolics concentration of *Quercus robur* in forested and harvested stands in northwestern Spain. *Canadian Journal of Botany*, **79**, 1262–1269.

Dudt, J.F. & Shure, D.J. (1994) The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, **75**, 86–98.

Dury, S.J., Good, J.E.G., Perrins, C.M., Buse, A. & Kaye, T. (1998) The effects of increasing CO_2 and temperature on oak leaf palatability and the implications for herbivorous insects. *Global Change Biology*, **4**, 55–61.

Faeth, S.H. (1985) Quantitative defense theory and patterns of feeding by oak insects. *Oecologia*, **68**, 34–40.

Faeth, S.H. & Bultman, T.L. (1986) Interacting effects of increased tannin levels on leaf-mining insects. *Entomologia Experimentalis et Applicata*, **40**, 297–300.

Faeth, S.H. & Hammon, K.E. (1996) Fungal endophytes and phytochemistry of oak foliage – determinants of oviposition preference of leafminers. *Oecologia*, **108**, 728–736.

Feeny, P. (1966) *Some effects on oak-feeding insects of seasonal changes in the nature of their food*. Oxford DPhil thesis, Radcliffe Science Library, Oxford.

Feeny, P. (1968) Effect of oak leaf tannins on larval growth of the winter moth *Operophtera brumata*. *Journal of Insect Physiology*, **14**, 805–807.

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.

Feeny, P. (1975) Biochemical coevolution between plants and their insect herbivores. *Coevolution of Animals and Plants* (ed. by L. E. Gilbert and P. H. Raven), pp. 3–19. University of Texas Press, Austin.

Feeny, P. (1976) Plant apparency and chemical defense. *Recent Advances in Phytochemistry*, **10**, 1–40.

Feeny, P. & Bostock, H. (1968) Seasonal changes in the tannin content of oak leaves. *Phytochemistry*, **7**, 871–880.

Forkner, R.E. & Hunter, M.D. (2000) What goes up must come down? Nutrient addition and predation pressure on oak herbivores. *Ecology*, **81**, 1588–1600.

Fortin, M. & Mauffette, Y. (2002) The suitability of leaves from different canopy layers for a generalist herbivore (Lepidoptera: Lasiocampidae) foraging on sugar maple. *Canadian Journal of Forest Research*, **32**, 379–382.

Grace, S.C. & Logan, B.A. (2000) Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Philosophical Transactions of the Royal Society of London B*, **355**, 1499–1510.

Hagerman, A.E. & Butler, L.G. (1991) Tannins and lignins. *Herbivores: Their Interactions with Secondary Plant Metabolites*, **Vol. 1**, 2nd edn (ed. by G. A. Rosenthal and M. Berenbaum), pp. 355–388. Academic Press, New York.

Hamilton, J.G., Zangerl, A.R., DeLucia, E.H. & Berenbaum, M. (2001) The carbon–nutrient balance hypothesis. Its rise and fall. *Ecology Letters*, **4**, 86–95.

Haslam, E. (1981) Vegetable tannins. *The Biochemistry of Plants*, **Vol. 7** (ed. by N. E. Tolbert), pp. 527–556. Academic Press, New York.

Haukioja, E. (2003) Putting the insect into the birch–insect interaction. *Oecologia*, **136**, 161–168.

Haukioja, E., Ossipov, V. & Lempa, K. (2002) Interactive effects of leaf maturation and phnolics on consumption and growth of a geometrid moth. *Entomologia Experimentalis et Applicata*, **104**, 125–136.

Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, **67**, 283–335.

Hunter, M.D. & Forkner, R.E. (1999) Hurricane damage influences foliar polyphenolics and subsequent herbivory on surviving trees. *Ecology*, **80**, 2676–2683.

Hunter, M.D., Malcolm, S.B. & Hartley, S.E. (1996) Population level variation in plant secondary chemistry, and the population biology of herbivores. *Chemoecology*, **7**, 45–56.

Hunter, M.D. & Schultz, J.C. (1993) Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. *Oecologia*, **94**, 195–203.

Hunter, M.D. & Schultz, J.C. (1995) Fertilization mitigates chemical induction and herbivore response within damaged oak trees. *Ecology*, **76**, 1226–1232.

Jermy, T. (1984) Evolution of insect/host plant relationships. *American Naturalist*, **124**, 609–630.

Jones, C.G. & Hartley, S.E. (1999) A protein competition model of phenolic allocation. *Oikos*, **86**, 27–44.

Jongman, R.H.G., Ter Braak, C.J.F. & Van Tongeren, O.F.R. (1995) *Data Analysis in Community and Landscape Ecology*. Cambridge University Press, Cambridge.

Karowe, D.N. (1989) Differential effect of tannic acid on two tree-feeding Lepidoptera: implications for theories of plant–herbivore chemistry. *Oecologia*, **89**, 316–323.

Kause, A., Ossipov, V., Haukioja, E., Lempa, K., Hanhimaki, S. & Ossipova, S. (1999) Multiplicity of biochemical factors determining quality of growing birch leaves. *Oecologia*, **120**, 102–112.

Kopper, B.J., Jakobi, V.N., Osier, T.L. & Lindroth, R.L. (2002) Effects of Paper Birch condensed tannin on Whitemarked Tussock moth (Lepidoptera: Lymantriidae) performance. *Environmental Entomology*, **31**, 10–14.

Kraus, T.E.C., Yu, Z., Preston, C.M., Dahlgren, R.A. & Zasoski, R.J. (2003) Linking chemical reactivity and protein precipitation to structural characteristics of foliar tannins. *Journal of Chemical Ecology*, **29**, 703–730.

Laitinen, M., Julkunen-Tiitto, R. & Rousi, M. (2000) Variation in phenolic compounds within a birch (*Betula pendula*) population. *Journal of Chemical Ecology*, **26**, 1609–1622.

Le Corff, J. & Marquis, R.J. (1999) Differences between under-storey and canopy in herbivore community composition and leaf quality for two oak species in Missouri. *Ecological Entomology*, **24**, 46–58.

Lill, J.T. & Marquis, R.J. (2001) The effects of leaf quality on herbivore performance and attack from natural enemies. *Oecologia*, **126**, 418–428.

Lill, J.T. & Marquis, R.J. (2003) Ecosystem engineering by caterpillars increases biodiversity on white oak. *Ecology*, **84**, 682–690.

Loomis, W.E. (1932) Growth-differentiation balance vs. carbohy-drate–nitrogen ratio. *Proceedings of the American Society of Horticultural Science*, **29**, 240–245.

Macauley, B.J. & Fox, L.R. (1980) Variation in total phenols and condensed tannins in *Eucalyptus*: leaf phenology and insect grazing. *Australian Journal of Botany*, **5**, 31–35.

Marquis, R.J., Diniz, I.R. & Morais, H.C. (2001) Patterns and correlates of interspecific variation in foliar insect herbivory and pathogen attack in Brazilian cerrado. *Journal of Tropical Ecology*, **17**, 127–148.

Marquis, R.J., Forkner, R.E., Lill, J.T. & Le Corff, J. (2002) Impact of timber harvest on species accumulation curves for oak herbivore communities of the Missouri Ozarks. *Proceedings of the Second Missouri Ozark Forest Ecosystem Project Symposium: Post- Treatment Results of the Landscape Experiment. 2001 October 17–18, St Louis, MO. General Technical Report NC-227* (ed. by S. R. Shifley and J. M. Kabrick), pp. 184–1966. USDA Forest Service, North Central Forest Experiment Station, St Paul, Minnesota.

Marquis, R.J. & Le Corff, J. (1997) Estimating pre-treatment variation in the oak leaf-chewing insect fauna of the Missouri Ozark Forest Ecosystem Project (MOFEP). *Proceedings of the Missouri Ozark Forest Ecosystem Project Symposium: an Experimental Approach to Landscape Research. 1997 June 3–5, St Louis, MO. General Technical Report NC-193* (ed. by B. L. Brookshire and S. Shifley), pp. 332–346. USDA Forest Service, North Central Forest Experiment Station, St Paul, Minnesota.

Marquis, R.J. & Whelan, C.J. (1994) Insectivorous birds increase growth of white oak through consumption of leaf-chewing insects. *Ecology*, **75**, 2007–2014.

Marquis, R.J., Passoa, S., Whitfield, J.B., Le Corff, J., Lill, J.T. & Forkner, R.E. (2004) *An Illustrated Guide to the Immature Lepidopteran Fauna of Oaks in Missouri.* USDA Forest Service (in press).

Martin, J.S. & Martin, M.M. (1984) Surfactants: their role in preventing the precipitation of proteins by tannins in insects guts. *Oecologia*, **61**, 342–345.

Matsuki, M. (1996) Regulation of plant phenolic synthesis: from biochemistry to ecology and evolution. *Australian Journal of Botany*, **44**, 613–634.

Mauffette, Y. & Oechel, W.C. (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.

Mole, S., Ross, J.A.M. & Waterman, P.G. (1988) Light-induced variation in phenolic levels of rain-forest plants. I. Chemical changes. *Journal of Chemical Ecology*, **14**, 1–21.

Mutikainen, P., Walls, M., Ovaska, J., Keinanen, M., Julkunen-Tiitto, R. & Vapaavuori, E. (2000) Herbivore resistance in *Betula pendula*: effects of fertilization, defoliation, and genotype. *Ecology*, **81**, 49–65.

Panzuto, M., Mauffette, Y. & Albert, P.J. (2002) Developmental, gustatory, and behavioral responses of leafroller larvae, *Choristoneura rosaceana*, to tannin acid and glucose. *Journal of Chemical Ecology*, **28**, 145–160.

Rhoades, D.F. & Cates, R.G. (1976) Toward a general theory of plant anti-herbivore chemistry. *Recent Advances in Phytochemistry* (ed. by J. W. Wallace and R. L. Mansell), pp. 168–213. Plenum, New York.

Riipi, M., Ossipov, V., Lempa, K., Haukioja, E., Koricheva, J. & Ossipova, S. *et al.* (2002) Seasonal changes in birch leaf chemistry: are there trade-offs between leaf growth and accumulation of phenolics? *Oecologia*, **130**, 380–390.

Rossiter, M.C., Schultz, J.C. & Baldwin, I.T. (1988) Relationships among defoliation, *Quercus rubra* phenolics, and gypsy moth growth and reproduction. *Ecology*, **69**, 267–277.

Sagers, C. (1992) Manipulation of host plant quality: herbivores keep leaves in the dark. *Functional Ecology*, **6**, 741–743.

Scalbert, A. & Haslam, E. (1987) Polyphenols and chemical defense of the leaves of *Quercus robur*. *Phytochemistry*, **12**, 3191–3195.

Schultz, J.C. (1988) Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology*, **69**, 896–897.

Schultz, J.C. (1989) Tannin–insect interactions. *Chemistry and Significance of Condensed Tannins* (ed. by R. W. Hemingway and J. J. Karchesy), pp. 417–433. Plenum, New York.

Schultz, J.C., Hunter, M.D. & Appel, H.M. (1992) Antimicrobial activity in polyphenols mediates plant–herbivore interactions. *Basic Life Sciences*, **59**, 621–637.

Schultz, J.C., Nothnagle, P.J. & Baldwin, I.T. (1982) Seasonal and individual variation in leaf quality of two northern hardwoods tree species. *American Journal of Botany*, **69**, 753–759.

Stamp, N.E. & Yang, Y. (1996) Response of insect herbivores to multiple allelochemicals under different thermal regimes. *Ecology*, **77**, 1088–1102.

Steinly, B.A. & Berenbaum, M. (1985) Histopathological effects of tannins on the midgut epithelium of *Papilio polyxenes* and *Papilio glaucus*. *Entomologia Experimentalis et Applicata*, **39**, 3–9.

Taper, M.L., Zimmerman, E.M. & Case, T.J. (1986) Sources of mortality for a cynipid gall-wasp *Dryocosmus dubiosus* Hymenoptera: Cynipidae: the importance of the tannin–fungus interaction. *Oecologia*, **68**, 437–445.

Tietz, H.M. (1972) *An Index to the Described Life Histories, Early Stages and Hosts of the Macrolepidoptera of the Continental United States and Canada*, **Vol. 2**. Allyn Museum of Entomology, Sarasota, Florida.

Waterman, P.G. & Mole, S. (1994) *Analysis of Phenolics Plant Metabolites.* Blackwell Scientific, Oxford.

Wold, E.N. & Marquis, R.J. (1997) Induced defense in white oak: effects on herbivores and consequences for the plant. *Ecology*, **78**, 1356–1369.

Xu, M., Saunders, S.C. & Chen, J. (1997) Analysis of landscape structure in the southeastern Missouri Ozarks. *Proceedings of the Missouri Ozark Forest Ecosystem Project Symposium: an Experimental Approach to Landscape Research. 1997 June 3–5, St Louis, MO. General Technical Report NC-193* (ed. by B. L. Brookshire and S. Shifley), pp. 41–55. USDA Forest Service, North Central Forest Experiment Station, St Paul, Minnesota.

Zucker, W.V. (1983) Tannins: does structure determine function? An ecological perspective. *American Naturalist*, **121**, 335–365.