Tri-trophic effects of plant defenses: chickadees consume caterpillars based on host leaf chemistry

Martina S. Müller, Scott R. McWilliams, David Podlesak, Jack R. Donaldson, Helen M. Bothwell and Richard L. Lindroth

Few studies have addressed how plant chemical defenses that directly affect herbivores in turn affect consumption patterns of vertebrates at higher trophic levels. We studied how variable foliar chemistry of trembling aspen (*Populus tremuloides* Michx.) affects the diet preferences of an avian insectivore feeding on an introduced herbivore, the gypsy moth (*Lymantria dispar* L.). Black-capped chickadees (*Poecile atricapilla*) were offered paired choices of gypsy moth caterpillars feeding on one of three genotypes of aspen that differed in chemical composition. Chickadees chose to eat caterpillars fed aspen foliage with low levels of both condensed tannins and phenolic glycosides, or caterpillars fed foliage with high levels of tannins and low levels of phenolic glycosides, over caterpillars fed foliage with low levels of condensed tannins and high levels of phenolic glycosides. In addition, diet choices of the birds were affected by their previous experience. These findings are consistent with the "extended phenotype" concept, in that genetically-based chemical traits in an ecologically dominant plant influence the feeding behavior of third trophic level organisms, whose efficacy as regulators of herbivore populations may in turn be modified.

M. S. Müller, S. R. McWilliams, D. Podlesak, Dept of Natural Resources Science, Univ. of Rhode Island, Kingston, RI 02881, USA (mmul8252@postoffice.uri.edu). Present address for DP: Dept of Biology, Univ. of Utah, Salt Lake City, UT 84112, USA. – J. R. Donaldson, Dept of Zoology, Univ. of Wisconsin, Madison, WI 53706, USA. – H. M. Bothwell and R. L. Lindroth, Dept of Entomology, Univ. of Wisconsin, Madison, WI 53706, USA.

Genetic, environmental, and developmental factors can cause intraspecific variation in plant secondary compounds (Bryant et al. 1991, Kennedy and Barbour 1992, Rosenthal and Berenbaum 1992, Osier and Lindroth 2001, O'Reilly-Wapstra et al. 2004, Donaldson et al. 2006). Studies of plant–herbivore interactions have demonstrated that such intraspecific variation affects the performance and preferences of insect and vertebrate herbivores (Jakubas et al. 1993a, 1993b, Hemming and Lindroth 1995, Lawler et al. 1998, Underwood and Rausher 2000, O'Reilly-Wapstra et al. 2004). However, few studies have focused on whether these effects of

plant chemistry on herbivores extend to higher trophic levels including vertebrate predators (Bairlein 1997).

Aspen is a dominant tree species in early-successional forests throughout much of northern and western North America. Aspen express significant variation in foliar chemistry, which affects their vulnerability to insect herbivory (Lindroth and Hwang 1996, Hwang and Lindroth 1997, Osier et al. 2000, Donaldson 2005). Whether intraspecific variation in plant chemistry in an ecologically dominant species such as aspen influences avian insectivores that prey on herbivorous insects is unknown, yet potentially important. In temperate

forests, insectivorous birds are major predators of herbivorous insects, especially during summer when insect larvae are abundant and birds are nesting (Holmes et al. 1979, 1986, Robinson and Holmes 1982, Marshall et al. 2002). If chemical composition of plants affects performance of both insect herbivores and avian predators, such tri-trophic effects could influence the population dynamics of both the vertebrate predator and the insect prey (Crawford and Jennings 1989, Kirk et al. 1996, Mols and Visser 2002).

We studied how differences in aspen foliar chemistry that have demonstrated effects on insect herbivores (Hwang and Lindroth 1997, Osier et al. 2000) in turn influence diet preferences of an avian insectivore. Aspen genotypes produce variable concentrations of secondary compounds, including condensed tannins and phenolic glycosides (Hemming and Lindroth 1995, Osier and Lindroth 2004). The growth, survival and reproduction of herbivorous insects such as gypsy moths raised on aspen is inversely related to foliar phenolic glycoside levels, but not related to tannin levels (Hemming and Lindroth 1995, Hwang and Lindroth 1997, Osier et al. 2000). Although many studies have demonstrated that plant secondary compounds can influence the chemical compositions of herbivores that consume them (Duffey 1980, Blum 1992, Nishida 2002), few have focused on whether such changes affect diet preferences and consumption rates of predators (Bowers 1980, Camara 1997, Sword 2001). Our goal was to determine whether quantitative variation in chemical composition of aspen affects feeding preferences at the third trophic level. We tested the following two related hypotheses: (1) black-capped chickadees (*Poecile atricapilla*) prefer caterpillars with lower concentrations of condensed tannins and phenolic glycosides, and (2) higher trophic level predators such as chickadees have the ability to learn so that their previous experience will influence their preferences for caterpillars fed foliage with certain levels of secondary compounds. This latter hypothesis is important to test because learned behaviors may influence the direct and indirect effects of variation in plant secondary compounds.

Methods

We conducted the following experiments using aspen trees grown in common gardens at the Univ. of Wisconsin in Madison and gypsy moth (*Lymantria dispar*) caterpillars reared in quarantine facilities in Madison. Aspen and caterpillars were then sent to the Univ. of Rhode Island, where bird feeding trials were conducted using chickadees captured in southern Rhode Island. We used aspen and gypsy moths for this experiment because previous work established that allelochemicals in aspen affect gypsy moth growth and

survival (Hwang and Lindroth 1997, Osier et al. 2000) as well as aspen defoliation rates (Donaldson and Lindroth, unpubl.). We used chickadees for this experiment because these birds are common insectivores in New England hardwood forests where gypsy moths may also be common (McManus et al. 1989, Smith 1993), and because during summer chickadees eat primarily lepidopteran larvae including gypsy moths (Smith and Lautenschlager 1981, Heinrich and Collins 1983, Smith 1993). Although we chose aspen, gypsy moths, and chickadees based in part on their documented ecological interactions, we conducted this aviary study primarily to determine whether quantitative variation in allelochemicals of aspen directly affects diet preferences of an insectivorous bird.

Aspen foliage

We selected three aspen genotypes that represent the range of foliar allelochemical concentrations typical of aspen populations in southern Wisconsin (Hwang and Lindroth 1997). The three genotypes, Wau-1 (hereafter G1), Wau-2 (hereafter G2), and Dan-2 (hereafter G3), were originally collected from Waushara and Dane counties, respectively, in south-central Wisconsin. Previous studies of these clones (Hwang and Lindroth 1997, 1998) indicated that G1 contains low levels of both phenolic glycosides and condensed tannins, G2 contains low levels of phenolic glycosides but high levels of condensed tannins, and G3 contains high levels of phenolic glycosides and low levels of condensed tannins. Aspen were originally propagated via root cuttings and are maintained in common gardens at the Univ. of Wisconsin, Madison. Leaves used in this study were from two sources. The G1 cuttings were 50–100 cm long mid-crown branches harvested from 8-year old common garden trees. For the other two genotypes, G2 and G3, clonally propagated, one-year-old potted trees (ca one meter tall) were harvested whole by cutting at the base. To minimize the tannin content of G3 and thereby maximize the allelochemical differences among clones (Osier and Lindroth 2001), G3 trees were fertilized with Osmocote (14-14-14, N-P-K+micronutrients) at a rate of 4.5 grams per liter soil media.

Aspen foliage was harvested and shipped on three different days, corresponding to the beginning of the 3-day acclimation period, and on day 1 and day 4 of the 6-day preference trials. For each shipment, 4–6 trees or branches were cut from each aspen genotype, immediately placed in 25 l buckets with their bases in water, and taken to the laboratory. Within hours of harvest, the cut stem bases were sealed in plastic bags with water soaked paper towels, after which the branches and several cold packs were placed into shipping boxes lined with plastic bags. Packages arrived in Rhode Island less than 20 h

from harvest time. Upon arrival, cuttings were removed and stored in water filled flasks under ambient light and temperature for a maximum of 72 h.

Phytochemical analyses

Samples for phytochemical analyses were collected by haphazardly selecting eight to ten leaves from each of the 8 cuttings per genotype on the first harvest date. Samples were flash-frozen in liquid nitrogen and freeze-dried, a method that preserves the integrity of aspen allelochemicals (Lindroth and Koss 1996). The phenolic glycosides salicortin and tremulacin were quantified by high performance thin layer chromatography using purified aspen phenolic glycosides as standards (Lindroth et al. 1993). Total phenolic glycosides were calculated by adding percent dry weights of salicortin and tremulacin. Condensed tannin concentrations were quantified by the acid butanol method of Porter et al. (1986) using purified aspen tannins as standards. We used percent foliar nitrogen as an index of protein concentration. Leaf nitrogen was determined with an auto-analyzer (Leco FP-528) using glycine p-toluenesulfonate (Hach Company, Loveland, Co) as a standard. Chemical analyses of a subset of cuttings indicated that leaf chemistry did not change during shipment or over a four-day period after cuttings were taken.

Gypsy moth caterpillars

USDA-APHIS (Otis Air National Guard Base, Massachusetts) provided the gypsy moth egg masses for these experiments. Egg masses were surface sterilized for five minutes using a 1.9% bleach solution containing Tween 80 and hatched in a Percival environmental chamber set at 25°C with a photoperiod of 15:9 (light:dark). Larvae were reared under the same conditions on standard wheat germ diet obtained from ICN Biomedicals (Irvine, CA). At the beginning of the 2nd instar, numbers of caterpillars were reduced to ca 100 per 500 ml rearing dish. As caterpillars molted into the 3rd instar, they were further reduced to ca 25 larvae per rearing dish. We used a staggered hatching schedule and sorted the caterpillars to obtain nine approximately even-aged/sized cohorts to be used on each of the nine days of the feeding experiments. For each shipment, caterpillars were sorted into 3 age classes (late 3rd, molting 3rd, and early 4th instars), placed into 500 ml rearing dishes (ca 100 per dish), and shipped overnight express to Rhode Island, coincident with shipments of aspen foliage.

Upon arrival in Rhode Island, caterpillars were transferred to 2 l paper ice cream cartons (100 larvae per container) and fed wheat germ diet. All caterpillars used in the chickadee feeding trials were 4th instars. Caterpillars were maintained on the wheat germ diet

inside the lab at room temperature until one day prior to being offered to the chickadees. At 10:00–12:00 hrs on this day, 100 4th instar caterpillars were transferred to new cartons with haphazardly selected sprigs (ca 25 leaves) of one of the three aspen genotypes. All aspen genotypes were palatable to gypsy moths and caterpillars were allowed to feed on aspen foliage for ca 20 h. (Earlier trials showed that this was sufficient time to replace artificial diet with ingested leaf material in insect guts). The following morning caterpillars were offered to the birds in controlled feeding studies.

Capture and maintenance of birds

We used mistnets to capture 30 black-capped chickadees in Kingston, Rhode Island (41°10′N, 71°34′W) between May 23–28, 2002 (RI DEM permit no. 2002-04). Captured chickadees were housed individually in stainless-steel cages (51 × 36 × 21 cm) at constant temperature (21°C) and 13:11 (l:d) photoperiod. Prior to the experiments with gypsy moths, all birds were given ad libitum water and waxworms (*Galleria mellonella*). Chickadees ate 30–35 waxworms per day. All protocols were approved by URI IACUC (AN02-04-033).

Chickadee feeding trials

We used two different types of feeding trials to determine whether intraspecific variation in plant allelochemicals have "extended" effects on third trophic level diet selection. First, we designed "acclimation trials" to assess the effects of aspen genotype on chickadee body mass and food consumption. These trials were also used to establish three groups of birds with different prior experience. Second, we used "preference trials" to assess bird preferences for caterpillars fed the different aspen genotypes, as well as the influence of prior experience (during acclimation trials) on those preferences.

Acclimation trials

Three-day acclimation trials were conducted on 16–18 June 2002 (hereafter day 1–3) to evaluate the effects of each aspen genotype on the body mass and food intake of chickadees. Each of three groups (n = 10 birds per group) consisted of birds fed caterpillars that ate only one of the three aspen genotypes. At 08:00 hrs on day 1, we removed all waxworms, weighed each chickadee, and then offered each bird 25 caterpillars in each of two adjacent rearing dishes (50 total per bird) at the front of their cage. Presentation of two adjacent dishes during these acclimation trials allowed us to evaluate whether birds exhibited side preferences when given the same type of caterpillar in both dishes. (Such preferences

would complicate interpretation of the later preference trials with different types of caterpillars in each dish.) We also placed 4–6 leaves of the appropriate aspen genotype in each dish to ensure that caterpillars would continue feeding and contain freshly ingested leaf material when eaten by the birds. The inside rim of all rearing dishes was coated with a thick layer of petroleum jelly to prevent the caterpillars from dispersing. At 14:00 hrs, we added more aspen leaves to a dish if the caterpillars had eaten the original 4–6 leaves. At 20:00 hrs, we removed dishes from the cages and counted the number of caterpillars remaining.

We chose to initially offer each chickadee 50 caterpillars (25 per dish) per day based on the average daily food intake of a 12 g chickadee (ca 30 waxworms, each ca 0.125 g wet weight, or 3.75 g day^{-1}) and the average measured body mass of a fourth instar gypsy moth (0.2 ± 0.025 g wet weight). Thus, we were surprised to find four dead chickadees at 08:00 hrs on day 2 of the acclimation period. Because most chickadees had eaten all or most of the caterpillars offered on day 1 (indicating caterpillars were less digestible than waxworms), we offered each chickadee 60 caterpillars (30 per dish) at 09:00 hrs on day 2. However, by 14:00 hrs on day 2, two additional chickadees had died and most other chickadees had eaten all the caterpillars offered. Since it was apparent that chickadees could not survive when eating only these caterpillars, we added 24 waxworms (12 per dish) at 14:00 hrs in an effort to avoid further mortality. At 20:00 hrs on day 2, we found two more dead chickadees. Thus, at 20:00 hrs, we removed all dishes from the cages, counted the number of caterpillars remaining, and provided each bird with 10 waxworms (5 in each of two 90 mm × 25 mm tall petri dishes). On day 3, we offered each chickadee 30% more caterpillars (40 per dish) at 09:00 hrs and, at 15:00 hrs, we added 24 waxworms (12 per dish). We stopped the acclimation trial at 20:00 hrs on day 3 when we removed all dishes from the cages, counted the number of caterpillars remaining, and provided each bird with 10 waxworms.

We calculated the number of caterpillars eaten by each bird as the difference between the number of caterpillars offered and the number of caterpillars remaining in each dish plus those on the floor of each cage (selected but dropped by chickadees). We tested for differences in food intake over time and among aspen genotypes using repeated measures ANOVA (Systat).

Preference trials
We designed preference trials to determine the effect of both aspen genotype and previous feeding experience on bird preferences for caterpillars. Each bird participated in two consecutive 3-day preference trials. The two 3-day preference trials involved offering chickadees a paired choice between caterpillars fed the G1 aspen that had low phenolic glycoside and condensed tannin levels

versus caterpillars fed one of the other two aspen genotypes. We randomly selected half the birds from each of the three acclimation groups to be first offered G1 vs G2 while the other birds in each group were first offered the other paired-choice (i.e. G1 vs G3). After the first 3-day preference trial we then switched the location of the G1 dish and the caterpillar type in the other dish. Thus, birds initially offered G1 vs G2 were subsequently offered G1 vs G3, while birds initially offered G1 vs G3 were subsequently offered G1 vs G2. This new arrangement and paired-choice was maintained for the next 3-day preference trial.

From 09:00–13:00 hrs on each day of the six-day preference experiment the birds were simultaneously presented with two rearing dishes, each with 25 caterpillars that had fed for at least 20 h on leaves from one of the aspen genotypes. We also placed 4–6 leaves of the appropriate aspen genotype in each rearing dish to ensure that caterpillars would continue feeding and contain freshly ingested leaf material when eaten by the birds.

Each day at 13:00 h we counted the number of caterpillars remaining in each dish as well as the number of uneaten caterpillars on the floor of each cage. Bird preferences were assessed based on the number of caterpillars removed from rearing dishes and the number of caterpillars eaten. The total number of caterpillars eaten by each bird (both genotypes combined) was calculated as the difference between the number of caterpillars offered and the number of caterpillars remaining in each dish plus those that were uneaten on the cage floor. Each day, immediately after caterpillars were removed, we added 34 waxworms per cage (17 in each of 2 petri dishes). At 20:00 hrs, we removed petri dishes and counted the number of waxworms remaining. After the preference trials were completed, the birds were released near where they were captured.

We tested for differences in food intake and body mass of chickadees over time and between aspen genotypes using repeated measures ANOVA (Systat). We also used repeated measures ANOVA to analyze the effect of original genotype and order of choice on the total number of caterpillars removed, number of caterpillars removed of each genotype, and the proportion of the total number of caterpillars removed that were G1 caterpillars. For all statistical tests, $P < 0.05$ was deemed significant.

Results

Leaf chemistry

Leaf chemistry varied markedly among the three aspen genotypes (Fig. 1) and was representative of variation previously observed among aspen clones growing in south-central Wisconsin (Hemming and Lindroth 1995,
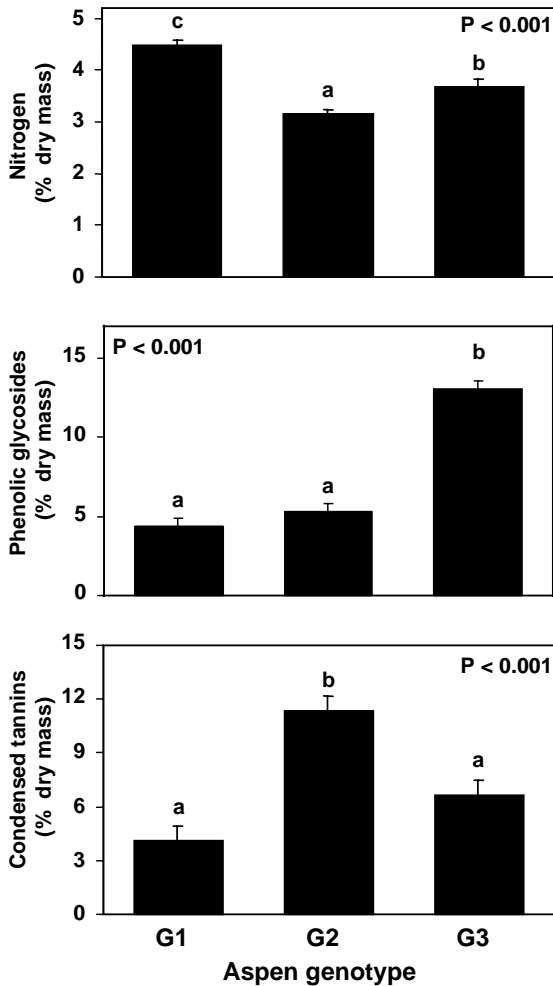
Fig. 1. Variation in foliar chemistry among 3 aspen genotypes; n = 8 cuttings per genotype. P- values indicate the significance of analysis of variance; bars bearing different letters are significantly different (Tukey post-hoc test, $\alpha = 0.05$). Vertical lines represent 1 SE.

Hwang and Lindroth 1997). Using N concentration as an index for protein concentration suggests that foliage from the G1 trees had almost 30% more protein than that of G2 and G3 trees. G3 foliage contained a high concentration of phenolic glycosides, whereas concentrations in G1 and G2 were quite low. G2 foliage contained a high concentration of condensed tannins, whereas concentrations in G1 and G3 were quite low. Total phenolics, calculated as the sum of % condensed tannins and % phenolic glycosides, comprised ca 9% of G1 leaves, 17% of G2 leaves, and 20% of G3 leaves. In summary, the three aspen genotypes provided an ideal set of contrasts in nutritional quality: G1 contained high levels of protein and low levels of chemical defenses, G2 contained moderate levels of protein, low phenolic glycoside and high condensed tannin concentrations, and G3 contained moderate levels of protein, high phenolic glycoside and moderate condensed tannin concentrations.

Acclimation trials: body mass and food intake of chickadees fed G1, G2 or G3 gypsy moth caterpillars

Although we estimated that 50 gypsy moth caterpillars per day would provide an adequate amount of food for chickadees, 9 birds died during the acclimation trial, as described in Methods. Body mass of birds that died (9.01 ± 0.18 g, range: 8.4–10.1 g) was on average lower than the body mass of birds that survived (10.10 ± 0.10 g, range: 9.2–11.0 g) (fate: $F_{1,24} = 37.93$, $P < 0.0001$), and was not significantly affected by type of caterpillar eaten (genotype: $F_{2,24} = 2.38$, $P = 0.114$; fate × genotype: $F_{2,24} = 0.60$, $P = 0.559$). Of the nine birds that died, five of the six birds fed G1 and G3 caterpillars had eaten >90% of caterpillars offered (one G3 bird ate few caterpillars on day 2), while birds fed G2 caterpillars ate 72–74% of caterpillars offered (Table 1). In contrast, surviving birds fed G2 and G3 caterpillars ate essentially all caterpillars offered while birds fed G1 caterpillars ate only 65–75% of those offered (Table 1). During the first three days of the experiment, surviving birds ate fewer gypsy moth caterpillars and more waxworms each successive day (Table 1). Given that we detected no effect of caterpillar treatment on the fate of the birds and that all birds ate most of the caterpillars offered, mortality of chickadees was likely the result of low digestibility or nutrient content of gypsy moth caterpillars, in general, rather than a direct toxic effect of aspen allelochemicals.

Body mass of chickadees that survived declined on average 12% during the first day when the birds were fed 50 gypsy moth caterpillars and no waxworms (Fig. 2); body mass increased during the second and third days when the birds were offered 60 and 80 gypsy moth caterpillars, respectively, and were supplemented with waxworms. The changes in body mass during these three days were significant (time effect: $F_{3,54} = 145.26$, $p < 0.0001$) and were consistent for birds fed G1, G2, or G3 caterpillars (genotype: $F_{2,18} = 0.91$, $p = 0.42$; time × genotype: $F_{6,54} = 1.70$, $p = 0.138$). During the 6-day preference trial, body mass of all chickadees continued to gradually increase (time: $F_{6,108} = 20.81$, $p < 0.0001$). Body mass during these six days did not differ significantly among the three groups, although there was a trend for birds originally fed G1 caterpillars to be heavier (genotype: $F_{2,18} = 1.00$, $p = 0.39$; time × genotype: $F_{12,108} = 1.44$, $p = 0.16$).

Table 1. Proportion ($\pm$SE) of gypsy moth caterpillars and waxworms eaten by black-capped chickadees during the three-day acclimation trial. Each group of chickadees was offered caterpillars that were fed leaves from one genotype of aspen (G1, G2, G3). Nine of the 30 chickadees were unable to maintain body mass and died during the acclimation period despite eating the majority of caterpillars and waxworms offered.

Type of caterpillar	Fate	n	Proportion of caterpillars eaten			Proportion of waxworms eaten	
			Day1[a]	Day2[b]	Day3[c]	Day2	Day3
G1	survived	6	0.65 ± 0.14	0.76 ± 0.10	0.55 ± 0.06	0.83 ± 0.07	0.92 ± 0.04
	died	4	0.91 ± 0.10	0.98 ± 0.03		0.88 ± 0.12	
G2	survived	7	0.91 ± 0.05	0.95 ± 0.02	0.44 ± 0.07	0.77 ± 0.06	0.96 ± 0.02
	died	3	0.72 ± 0.28	0.74 ± 0.17		1.00 ± 0.00	
G3	survived	8	0.95 ± 0.02	0.94 ± 0.03	0.45 ± 0.09	0.82 ± 0.03	0.95 ± 0.02
	died	2	0.99 ± 0.01	0.67 ± 0.02		1.00 ± 0.00	

Statistical comparisons[d]:	F-value	p-value			F-value	p-value
Genotype	$F_{2,24}=0.877$	$p=0.425$			$F_{2,18}=0.084$	$p=0.919$
Fate	$F_{1,24}=3.922$	$p=0.059$			–	–
Fate $\times$ genotype	$F_{2,24}=8.647$	$p=0.001$			–	–
Time	$F_{1,24}=8.603$	$p=0.007$			$F_{1,18}=16.373$	$p=0.001$
Time $\times$ genotype	$F_{2,24}=18.125$	$p<0.0001$			$F_{2,18}=0.704$	$p=0.508$

[a] birds were offered 50 caterpillars and 0 waxworms
[b] birds were offered 60 caterpillars at 09:00 hrs and then 34 waxworms at 14:00 hrs and 10 more at 20:00 hrs
[c] birds were offered 80 caterpillars at 09:00 hrs and then 34 waxworms at 15:00 hrs and 10 more at 20:00 hrs
[d] repeated measures ANOVA comparing the proportion of caterpillars and proportion of waxworms eaten across the 3-day acclimation period. Only birds that survived were used for the analysis of the proportion of waxworms eaten over time.
"Genotype" refers to the type of aspen fed to gypsy moth caterpillars prior to being eaten by chickadees
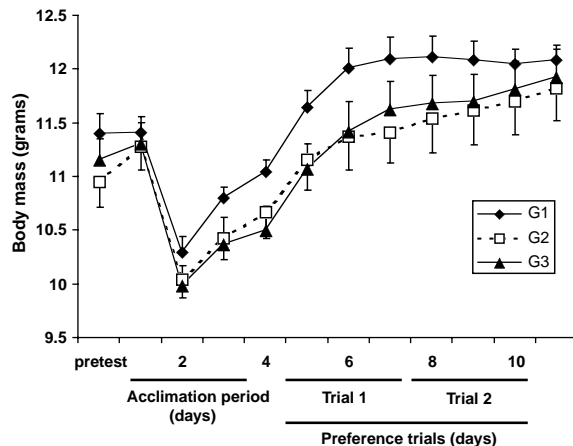

Fig. 2. Body mass of black-capped chickadees during the pretest period, the 3-day acclimation period, and the two 3-days preference trials. Each of the three lines denotes a group of chickadees (n = 6 GI birds, n = 7 G2 birds, n = 8 G3 birds) that was first offered 4th instar gypsy moth caterpillars that were fed leaves from one genotype of aspen (acclimation period) and then which participated in two 3-day preference trials. Because chickadees were unable to maintain body mass when fed only gypsy moth caterpillars on day 1, birds were supplemented with waxworms on the 2nd and 3rd days of the acclimation period (Table 1) which allowed birds to regain body mass. During each preference trial chickadees were offered a paired-choice of capterpillar (G1 vs G2 or G1 vs G3) from 09:00–13:00 hrs each day and then waxworms for the remainder of each day. During the pretest day and on day 4, chickadees were offered only waxworms.

Preference trials: selection of gypsy moth caterpillars by chickadees

In general, chickadees showed strong preferences for certain types of caterpillars although the demonstrated preference depended on two factors related to previous experience: (a) the type of caterpillar they were offered during the acclimation period (hereafter "original genotype"), and (b) the order in which they were offered the specific paired choices (hereafter "genochoice"). We use the term "genochoice 3/2" as shorthand for birds given the G1/G3 choice during trial 1 and the G1/G2 choice during trial 2, and "genochoice 2/3" for birds given the G1/G2 choice during trial 1 and the G1/G3 choice during trial 2.

Proportion of caterpillars eaten by birds indicates preferences

Since G1 caterpillars were always offered during preference trials, the proportion of G1 caterpillars removed by birds indicates whether birds preferred caterpillars fed a certain aspen genotype. When all independent variables were considered, the paired-choice offered to birds (i.e. G1 vs G2, or G1 vs G3) was the only significant factor affecting the proportion of G1 caterpillars removed by birds (original genotype: $F_{2,30}=0.093$, p = 0.912; genochoice: $F_{1,30}=0.328$, p = 0.571; paired-choice: $F_{1,30}=18.181$, p < 0.0001; original genotype $\times$ genochoice: $F_{2,30}=0.489$, p = 0.618; original genotype $\times$ paired-choice: $F_{2,30}=0.222$, p = 0.802; genochoice $\times$ paired-choice: $F_{1,30}=3.194$, p = 0.084). In general, birds consistently removed proportionately more G1 caterpillars when offered the G1 vs G3 choice, whereas birds

removed proportionately fewer G1 caterpillars when offered the G1 vs G2 choice (Fig. 3). This indicates that chickadees clearly preferred G1 caterpillars over G3 caterpillars, and that their preference for G2 over G1 caterpillars was weak given that chickadees removed almost equal proportions of each caterpillar type when offered the G2 vs G1 paired-choice (Fig. 3). In summary, chickadee preferences were in the following order from the most to least preferred type of caterpillar: G2 ≈ G1 > G3.

Effect of previous experience on bird preferences

Although original genotype did not affect the proportion of G1 caterpillars eaten by birds, it did influence the number of G1 caterpillars removed over the six days of the preference experiment ($F_{2,15}=3.99$, p$=0.041$; Fig. 4a). In general, birds that were originally fed only G2 caterpillars (hereafter "G2 birds") removed more G1 caterpillars than birds that were originally fed only G1 caterpillars ("G1 birds") and birds that were originally fed only G3 caterpillars ("G3 birds"). When G2 and G1 caterpillars were offered to birds, the G2 birds removed more G2 caterpillars than did G1 birds and G3 birds ($F_{2,15}=4.702$, p$=0.026$; Fig. 4b). When G3 and G1 caterpillars were offered to birds, the G2 birds in the genochoice 3/2 group removed more G3 caterpillars than did G1 birds, G3 birds, and G2 birds in the genochoice 2/3 group (original genotype: $F_{2,15}=2.31$, p$=0.133$; genochoice: $F_{1,15}=5.89$, p$=0.028$; original genotype × genochoice: $F_{2,15}=4.81$, p$=0.024$; Fig. 4c).

Genochoice also had a significant effect on the number and type of caterpillars eaten and removed during the preference experiments (Fig. 5). Genochoice 3/2 birds ate on average more caterpillars each day ($8.41 \pm$ SE $=1.02$) than the genochoice 2/3 birds ($4.88 \pm$ SE $=0.99$) regardless of original genotype (original genotype: $F_{2,15}=1.820$, P $=0.196$; genochoice: $F_{1,15}=$
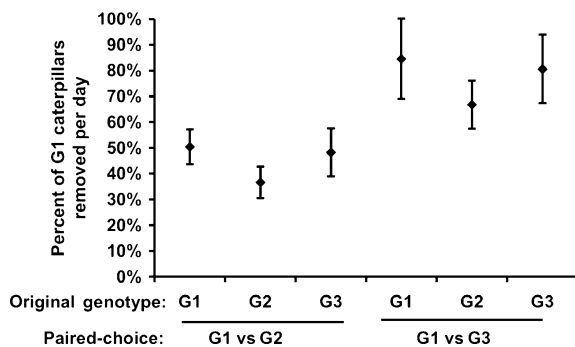

Fig. 3. Percent of G1 caterpillars removed by black-capped chickadees when offered a paired choice of either G1 vs G2 caterpillars Gl vs G3 caterpillars. Original genotype refers to the type of caterpillar (G1, G2 or G3) that each group of chickadee was offered during the 3-day acclimation period preceding the preference trials. These results suggest chickadees prefer G2 and G1 caterpillars over G3 caterpillars (see text for statistical analysis).
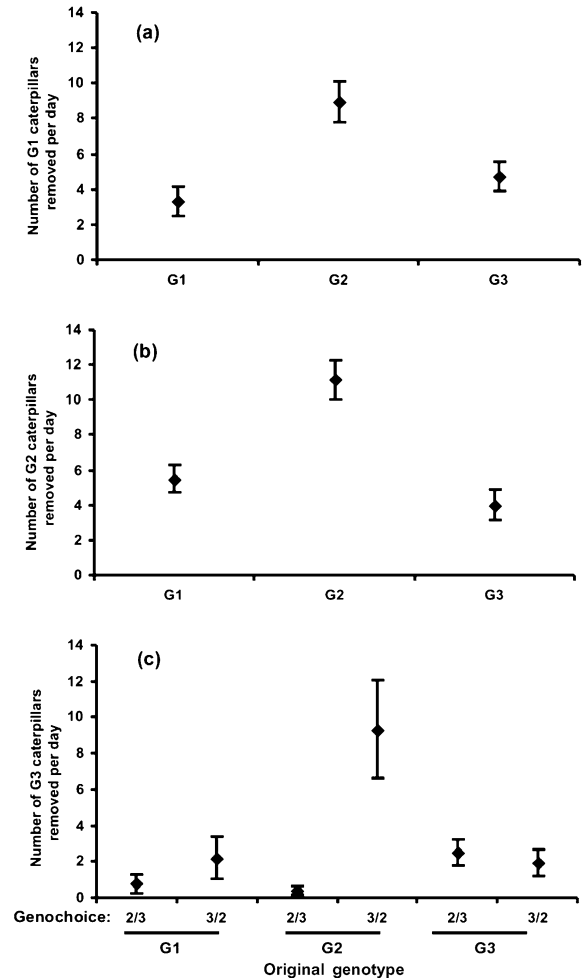

Fig. 4. Average number of gypsy moth caterpillars of certain types removed by black-capped chickadees when offered paired-choices of caterpillars during preference trials demonstrates that previous experience affects bird preferences. Chickadees that had been offered caterpillars fed G2 aspen during the 3-day acclimation period (original genotype G2) removed more G1 caterpillars during the six days of the preference experiment (a) and more G2 caterpillars when offered the G2 vs G1 paired-choice (b) than did chickadees that had been offered caterpillars fed G1 or G3 aspen during the 3-day acclimation period (original genotype G1 or G3, respectively). (c) Chickadees that had been offered caterpillars fed G2 aspen during the 3-day acclimation period also removed more G3 caterpillars when offered the G3 vs G1 paired-choice during the first preference trial (genochoice 3/2) than did chickadees in the other treatment groups.

7.48, P $=0.015$; time: $F_{5,75}=2.43$, p $=0.043$; all interaction terms had p >0.05). Birds usually ate $>85\%$ of caterpillars that they removed except for G2 birds in the genochoice 3/2 group, which ate only about half the caterpillars that they removed (Fig. 5). In general, chickadees that were offered G1 or G2 caterpillars during the acclimation period (original genotype G1 or G2 birds) removed more caterpillars if offered the G1 vs G3 paired-choice first rather than last during preference
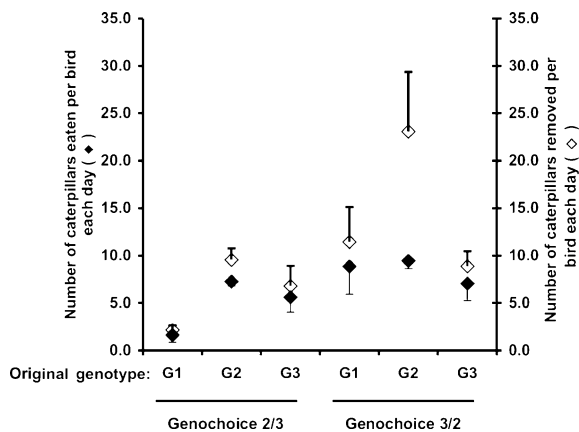
Fig. 5. Average number of gypsy moth caterpillars of certain types removed and then eaten by black-capped chickadees during preference trials was affected by the order in which the birds were offered each paired choice. Original genotype refers to the type of caterpillar (G1, G2 or G3) that each group of chickadees was offered during the 3-day acclimation period preceding the preference trials. During the two 3-day preference trials, half the chickadees in each original genotype group were first offered a paired choice of G1 vs G3 caterpillars for three days and then a paired choice of G1 vs G2 caterpillars for the final three days (denoted "genochoice 3/2") while the other chickadees were first offered a paired choice of G1 vs G2 caterpillars for three days and then a paired choice of G1 vs G3 caterpillars for the final three days (denoted "genochoice 2/3").

trials (genochoice effect: $F_{1,15} = 5.12$, $p = 0.039$ for G1 birds; $F_{1,15} = 9.053$, $p = 0.009$ for G2 birds; Fig. 5). These results clearly demonstrate that previous experience of birds influences their preferences for caterpillars fed particular aspen genotypes.

Discussion

Diet preferences

Black-capped chickadees in our study discriminated between caterpillars that had eaten aspen foliage with different levels of protein and secondary compounds. Chickadees consistently preferred caterpillars fed foliage with lower concentrations of condensed tannins and phenolic glycosides (G1) or caterpillars fed foliage with more condensed tannins but low levels of phenolic glycosides (G2) over caterpillars fed foliage with high levels of phenolic glycosides (G3). Chickadees may have consistently avoided eating G3 caterpillars because they could detect the phenolic glycosides and so avoid any negative physiological effects of these compounds. Birds that detect secondary compounds in insect prey can quickly learn to avoid such prey (Bernays et al. 1989, Bairlein 1997). For example, Brower (1969) found that captive naïve blue jays (Cyanocitta cristata) readily ate Monarch butterfly caterpillars (Danaus plexippus) that did not contain cardenolides. However, when jays were offered Monarch butterfly caterpillars with cardenolides

from milkweed plants, the birds immediately rejected all Monarch caterpillars offered to them regardless of whether they contained cardenolides. In contrast, other bird species such as black-backed oriole (Icterus galbula) and black-headed grosbeak (Pheucticus melanocephalus) eat monarch caterpillars despite the presence of cardenolides (Brower 1988). These effects of plant metabolites on bird preferences for insect prey have implications for the evolution of aposematic coloration in butterflies (Jeffords et al. 1979; Bowers et al. 1985) and for the possible control of insect populations by bird predation (Campbell et al. 1983).

Previous studies have shown that condensed tannins and phenolic glycosides can negatively affect the performance of vertebrates and thus influence their diet choices. Condensed tannins can reduce the metabolizable energy of a food (Koenig 1991) by binding with dietary proteins (Martin and Martin 1982, Robbins 1993) and thus causing negative energy and protein balance in birds (Fleck and Tomback 1996, Dixon et al. 1997). Condensed tannins are apparently not absorbed from the digestive tract of birds (Jimenez-Ramsey et al. 1994) so that the primary negative effect of eating foods with condensed tannins is reduced digestibility and protein availability-for which birds may compensate by eating more over time (Bernays et al. 1989, Robbins 1993). Low molecular weight phenolics have been shown to increase protein loss in hares, ruminants, and herbivorous birds (Palo 1985, Sinclair et al. 1988, Jakubas et al. 1993a). Moreover, detoxification of the absorbed phenolic compound(s) may be costly in terms of losses of energy, endogenous materials, or water (Jakubas et al. 1993b, Dearing et al. 2001). Other studies have demonstrated that concentrations of simple phenolics, including phenolic glycosides, can influence diet choices in herbivorous vertebrates (Buchsbaum et al. 1984, Tahvanainen et al. 1985, Gauthier and Bedard 1990, Milton et al. 1994, Bairlein 1997, Pass and Foley 2000, Bailey et al. 2005, Diner and Lindroth, unpubl.). Our results along with just a few others (reviewed by Bairlein 1997) demonstrate that phenolic glycosides can influence diet choices in insectivorous songbirds.

In this study, condensed tannins had little if any impact on chickadee diet preferences, whereas phenolic glycosides were clearly avoided. Interestingly, this pattern is identical to that exhibited by several species of Lepidoptera that feed on aspen: performance is affected little by condensed tannins but strongly by phenolic glycosides (Hemming and Lindroth 1995, Hwang and Lindroth 1998, Osier and Lindroth 2004). This study suggests that as with insects, adult songbirds can circumvent the potential negative consequences of consumption of condensed tannins by increasing intake, although studies on nestling songbirds indicate that growth may be reduced by diets high in tannins (Perrins 1976, Bairlein 1997).

Effects of previous experience

Diet preferences of higher vertebrates may be substantially affected by learning from previous experience with foods (Pietrewicz and Richards 1985). Such effects of learning on diet preferences have been demonstrated for mammals offered diet choices with tannins (Villalba et al. 2004) and for jays offered caterpillars with or without cardenolides (Brower 1969). We observed two patterns in which previous experience affected bird preferences for caterpillars. First, the type of caterpillar eaten during the 3-day acclimation period affected the birds' subsequent choices during the preference trials. For example, birds originally fed G2 caterpillars during the acclimation period removed more G1 and G2 caterpillars during preference trials than did birds originally fed G1 or G3 caterpillars. This pattern of bird preferences is not consistent with the simple hypothesis that birds prefer familiar foods that they have recently eaten. Rather, this pattern of bird preferences is consistent with the hypothesis that condensed tannins (most abundant in G2 caterpillars) directly reduce nutrient availability which required birds to then eat more of the preferred caterpillars (G1, G2) when available during preference trials. Secondly, the order of choices offered during the preference trials also affected the birds' preferences. For example, birds originally fed G2 caterpillars during the acclimation period removed but did not eat (Fig. 5) many more caterpillars when offered the G1 vs G3 paired-choice during preference trials (Fig. 4c). Such a pattern is consistent with the hypothesis that birds presented with a novel diet choice must initially sample prey extensively. What is clear from these results is that learning and previous experience likely affect diet preferences of birds especially when their insect prey eat plants with various types and amounts of secondary compounds.

Tri-trophic interactions between plants, insect herbivores, and avian insectivores

Environmental conditions can differentially influence production of secondary compounds by plant genotypes. For example, increases in atmospheric carbon dioxide directly affect aspen productivity (Lindroth et al. 2001, McDonald et al. 2002) and the production of secondary compounds in certain aspen genotypes (Mansfield et al. 1999, Lindroth et al. 2001). Such changes in foliar quality may affect animals, such as predators and parasitoids, at higher trophic levels (Holton et al. 2003). If environmental conditions alter production of secondary compounds, then our results would predict shifts in the predator–prey–host interactions among birds, insects, and plants.

We have shown that differences in production of secondary compounds in aspen genotypes affect bird preferences for gypsy moth caterpillars and thus the likelihood of a bird preying on such caterpillars. These results have interesting implications for the hypothesis that caterpillars that grow slowly on plants containing more secondary compounds will suffer higher predation rates because they are more vulnerable for a longer period of time (slow growth–high mortality hypothesis, Clancy and Price 1987). Feeding trials with aspen genotypes containing varying concentrations of phenolic glycosides have clearly demonstrated that these compounds significantly slow lepidopteran growth rates (Hwang and Lindroth 1997, 1998, Osier and Lindroth 2001, 2004). We have shown that birds avoid eating caterpillars that have fed on aspen foliage with high levels of phenolic glycosides. Contrary to the predictions of the slow growth – high mortality hypothesis, these preferences would result in lower relative predation rates on such caterpillars, which in turn could reduce the selective advantage for the plant to produce phenolic glycosides.

If secondary compounds in the insect's host plant influence the intensity of bird predation on insect prey (Rowell-Rahier et al. 1995), several important ecological ramifications may occur. A reduction in predation pressure on herbivorous insects can have detrimental effects on plant demographics, particularly in areas where certain plant species (e.g. aspen) are ecologically dominant and extensive defoliation by insects is common (Marquis and Whelan 1994, Greenberg et al. 2000, Strong et al. 2000). Whether reduced likelihood of bird predation affects insect population dynamics depends on the strength of the ecological interaction between these taxa (Holmes et al. 1979, 1986, Robinson and Holmes 1982, Wiens et al. 1991, Marshall et al. 2002). Our results suggest that black-capped chickadees can not survive if eating only gypsy moth caterpillars, so their ability to regulate gypsy moth populations is limited (Whelan et al. 1989). However, if diet preferences of other insectivorous songbirds are similarly affected by secondary compounds, then these other bird species either individually or collectively may produce different functional and numerical responses between bird predator and insect prey (Crawford and Jennings 1989) that could alter the efficacy of regulation of prey populations (reviewed by Kirk et al. 1996, Mols and Visser 2002, Singer and Stireman 2005).

In conclusion, this study demonstrates that variation in chemical composition at the first trophic level can extend to influence consumption rates and preferences of vertebrate predators at the third trophic level. Given that concentrations of phenolic glycosides in aspen are largely genetically-determined (Osier and Lindroth 2004, Stevens and Lindroth 2005), these results are consistent with the "extended phenotype" concept (Whitham et al. 2003). Genetically-based traits in an ecologically dominant plant shape the feeding behavior

of vertebrate predators, which may influence their efficacy as regulators of herbivore populations.

References

Bailey, J. K., Deckert, R., Schweitzer, J. A. et al. 2005. Host plant genetics affect hidden ecological players: links among *Populus*, condensed tannins, and fungal endophyte infection. – Can. J. Bot. 83: 356–361.

Bairlein, F. 1997. Food choice in birds and insect chemical defenses. – Entomol. Gener. 21: 205–216.

Bernays, E. A., Cooper-Driver, G. and Bilgener, M. 1989. Herbivores and plant tannins. – Adv. Ecol. Res. 19: 263–302.

Blum, M. S. 1992. Ingested allelochemicals in insect wonderland: a menu of remarkable functions. – Am. Entomol. 38: 222–234.

Bowers, M. D. 1980. Unpalatability as a defensive strategy of *Euphydryas phaeton* (Lepidoptera: Nymphalidae). – Evolution 34: 586–600.

Bowers, M. D., Brown, L. L. and Wheye, D. 1985. Bird predation as a selective agent in a butterfly population. – Evolution 39: 93–103.

Brower, L. P. 1969. Ecological chemistry. – Sci. Am. 220: 22–30.

Brower, L. P. 1988. Avian predation on the monarch butterfly and its implications for mimicry theory. – Am. Nat. Suppl. 131: S4–S6.

Bryant, J. P., Kuropat, P. J., Reichardt, P.B. et al. 1991. Controls over the allocation of resources by woody plants to chemical antiherbivore defense. – In: Palo, R. T. and Robbins, C. T. (eds), Plant defenses against mammalian herbivores. CRC Press, pp. 83–102.

Buchsbaum, R., Valiela, I. and Teal, J. M. 1984. The role of phenolic compounds and other plant constituents in feeding by Canada geese in a coastal marsh. – Oecologia 63: 343–349.

Camara, M. D. 1997. Predator responses to sequestered plant toxins in buckeye caterpillars: are tritrophic interactions locally variable? – J. Chem. Ecol. 23: 2093–2106.

Campbell, R. W., Torgersen, T. R. and Srivastava, N. 1983. A suggested role for predaceous birds and ants in the population dynamics of the western spruce budworm. – For. Sci 29: 779–790.

Clancy, K. M. and Price, P. W. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. – Ecology 68: 733–737.

Crawford, H. S. and Jennings, D. T. 1989. Predation by birds on spruce budworm *Choristoneura fumiferana*: functional, numerical, and total response. – Ecology 70: 152–163.

Dearing, M. D., Mangione, A. M. and Karasov, W. H. 2001. Plant secondary compounds as diuretics: an overlooked consequence. – Am. Zool. 41: 890–901.

Dixon, M. D., Johnson, W. C. and Adkisson, C. S. 1997. Effects of caching on acorn tannin levels and blue jay dietary performance. – Condor 99: 756–764.

Donaldson, J. R. 2005. Benefits and costs of defense in aspen-insect interactions: causes and consequences of phytochemical variation. – PhD thesis, Univ. of Wisconsin, Madison, Wisconsin.

Donaldson, J. R., Stevens, M. T., Barnhill, H. R. et al. 2006. Developmental shifts in aspen (*Populus tremuloides* Michx.) leaf chemistry: ecological implications. – J. Chem. Ecol. (in press).

Duffey, S. S. 1980. Sequestration of plant natural products by insects. – Annu. Rev. Entomol. 25: 447–477.

Fleck, D. C. and Tomback, D. F. 1996. Tannin and protein in the diet of a food-hoarding granivore, the western scrub-jay. – Condor 98: 474–482.

Gauthier, G. and Bedard, J. 1990. The role of phenolic compounds and nutrients in determining food preference in greater snow geese. – Oecologia 84: 553–558.

Greenberg, R., Bichier, P. and Angon, A.C. 2000. The impact of avian insectivory on arthropods and leaf damage in some Guatemalan coffee plantations. – Ecology 81: 1750–1755.

Heinrich, B. and Collins, S. L. 1983. Caterpillar leaf damage and the game of hide-and-seek with birds. – Ecology 64: 592–602.

Hemming, J. D. C. and Lindroth, R. L. 1995. Intraspecific variation in aspen phytochemistry: Effects on performance of gypsy moths and forest tent caterpillars. – Oecologia 103: 79–88.

Holmes, R. T., Schultz, J. C. and Nothnagle, P. 1979. Bird predation on forest insects: an exclosure experiment. – Science 206: 462–463.

Holmes, R. T., Sherry, T. W. and Sturges, F. W. 1986. Bird community dynamics in a temperate deciduous forest: long-term trends at Hubbard Brook. – Ecol. Monogr. 56: 201–220.

Holton, M. K., Lindroth, R. L. and Nordheim, E. V. 2003. Foliar quality influences tree-herbivore-parasitoid interactions: effects of elevated CO_2, O_3, and genotype. – Oecologia 137: 233–244.

Hwang, S. and Lindroth, R. L. 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. – Oecologia 111: 99–108.

Hwang, S. and Lindroth, R. L. 1998. Consequences of clonal variation in aspen phytochemistry for late season folivores. – Ecoscience 5: 508–516.

Jakubas, W. J., Karasov, W. H. and Guglielmo, C. G. 1993a. Coniferyl benzoate in quaking aspen (*Populus tremuloides*): its effect on energy and nitrogen digestion and retention in ruffed grouse (*Bonasa umbellus*). – Phys. Zool. 66: 580–601.

Jakubas, W. J., Karasov, W. H. and Guglielmo, C. G. 1993b. Ruffed grouse tolerance and biotransformation of the plant secondary metabolite coniferyl benzoate. – Condor 95: 625–640.

Jeffords, M. R., Stemberg, J. R. and Waldbauer, G. P. 1979. Field demonstration of the survival value of pipe vine swallowtail and monarch color patterns. – Evolution 33: 257–286.

Jimenez-Ramsey, L. M., Rogler, J. C., Housley, T.L. et al. 1994. Absorption and distribution of ^{14}C-labeled condensed tannins and related sorghum phenolics in chickens. – J. Agr. Food Chem. 42: 963–967.

Kennedy, G. G. and Barbour, J. D. 1992. Resistance variation in natural and managed systems. – In: Fritz, R. S. and Simms, E. L. (eds), Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. Univ. of Chicago Press, pp. 13–41.

Kirk, D. A., Evenden, M. D. and Mineau, P. 1996. Past and current attempts to evaluate the role of birds as predators of insect pests in temperate agriculture. – Curr. Ornithol. 13: 175–269.

Koenig, W. D. 1991. The effects of tannins and lipids on digestion of acorns by acorn woodpeckers. – Auk 108: 79–88.

Lawler, I. R., Foley, W.J. and Eschler, B. M. 1998. Intraspecific variation in *Eucalyptus* secondary metabolites determines

food intake by folivorous marsupials. – Oecologia 116: 160–169.

Lindroth, R. L. and Hwang, S. 1996. Clonal variation in foliar chemistry of quaking aspen (*Populus tremuloides* Michx.). – Biochem. Syst. Ecol. 24: 357–364.

Lindroth, R. L. and Koss, P. A. 1996. Preservation of Salicaceae leaves for phytochemical analyses: further assessment. – J. Chem. Ecol. 22: 765–771.

Lindroth, R. L., Kinney, K. K. and Platz, C. L. 1993. Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry and insect performance. – Ecology 74: 763–777.

Lindroth, R. L., Roth, S. and Nordheim, E. V. 2001. Genotypic variation in response of quaking aspen (*Populus tremuloides*) to atmospheric CO_2 enrichment. – Oecologia 126: 371–379.

Mansfield, J. L., Curtis, P. S. and Zak, D. R. 1999. Genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides*, Salicaceae) under elevated CO_2 and in high- and low-fertility soil. – Am. J. Bot. 86: 1154–1159.

Marquis, R. J. and Whelan, C. J. 1994. Insectivorous birds increase the growth of white oak through consumption of leaf-chewing insects. – Ecology 75: 2007–2014.

Marshall, M. R., Cooper, R. J., DeCecco, J. A. et al. 2002. Effects of experimentally reduced prey abundance on the breeding ecology of the red-eyed vireo. – Ecol. Appl. 12: 261–280.

Martin, J. S. and Martin, M. M. 1982. Tannin assays in ecological studies: lack of correlation between phenolics, proanthocyanidins, and protein-precipitating constituents in mature foliage of six oak species. – Oecologia 54: 205–211.

McDonald, E. P., Kruger, E. L., Riemenschneider, D. E. et al. 2002. Competitive status influences tree-growth responses to elevated CO_2 and O_3 in aggrading aspen stands. – Funct. Ecol. 16: 792–801.

McManus, M., Schneeberger, N., Reardon, R. 1989. Gypsy moth. USDA Forest Service Forest Insect and Disease Leaflet 162.

Milton, S. J., Dean, W. R. J. and Siegfried, W. R. 1994. Food selection in ostrich in southern Africa. – J. Wildl. Manage. 58: 234–248.

Mols, C. M. M. and Visser, M. E. 2002. Great tits can reduce caterpillar damage in apple orchards. – J. Appl. Ecol. 39: 888–899.

Nishida, R. 2002. Sequestration of defensive substances from plants by Lepidoptera. – Annu. Rev. Entomol. 47: 57–92.

O'Reilly-Wapstra, J. M., McArthur, C. and Potts, B. M. 2004. Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. – Funct. Ecol. 18: 677–684.

Osier, T. L. and Lindroth, R. L. 2001. Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. – J. Chem. Ecol. 27: 1289–1313.

Osier, T. L. and Lindroth, R. L. 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.

Osier, T. L., Hwang, S. and Lindroth, R. L. 2000. Effects of phytochemical variation in quaking aspen *Populus tremuloides* clones on gypsy moth *Lymantria dispar* performance in the field and laboratory. – Ecol. Entomol. 25: 197–207.

Palo, R. T. 1985. Chemical defense in birch: inhibition of digestibity in ruminants by phenolic extracts. – Oecologia 68: 10–14.

Pass, G. J. and Foley, W. J. 2000. Plant secondary metabolites as mammalian feeding deterrents: separating the effects of the taste of salicin from its post-ingestive consequences in the common brushtail possum (*Trichosurus vulpecula*). – J. Comp. Phys. 170: 185–192.

Perrins, C. M. 1976. Possible effects of qualitative changes in insect diet of avian predators. – Ibis 118: 580–584.

Pietrewicz, A. T. and Richards, J. B. 1985. Learning to forage: an ecological perspective. – In: Johnston, T. B. and Pietrewicz, A. T. (eds), Issues in the ecological study of learning. L. Erlbaum Associates, pp. 99–117.

Porter, L. J., Hrstich, L. N. and Chan, B. G. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. – Phytochemistry 25: 223–230.

Robbins, C. T. 1993. Wildlife feeding and nutrition, 2nd edition. – Academic Press.

Robinson, S. K. and Holmes, R. T. 1982. Foraging behavior of forest birds: the relationships among search tactics, diet, and habitat structure. – Ecology 63: 1918–1931.

Rosenthal, G. A. and Berenbaum, M. R. (eds). 1992. Herbivores: their interactions with secondary plant metabolites, 2nd edition. Vol. II. Ecological and evolutionary processes. – Academic Press.

Rowell-Rahier, M., Pasteels, J. M., Alonso-Mejia, A. et al. 1995. Relative unpalatability of leaf beetles with either biosynthesized or sequestered chemical defense. – Anim. Behav. 49: 709–714.

Sinclair, A. R. E., Krebs, C. J., Smith, J. N. M. et al. 1988. Population biology of snowshoe hares. III. Nutrition, plant secondary compounds and food limitation. – J. Anim. Ecol. 57: 787–806.

Singer, M. S. and Stireman, J. O. III. 2005. The tri-trophic niche concept and adaptive radiation of phytophagous insects. – Ecol. Lett. 8: 1247–1255.

Smith, S. M. 1993. Black-capped chickadee. – In: Poole, A., Stettenheim, P. and Gill, F. (eds), The birds of North America, No. 39. Acad. Nat. Sci. Am. Ornithol. Union.

Smith, H. R. and Lautenschlager, R. A. 1981. Gypsy moth predators. – In: Doane, C. C. and McManus, M. L. (eds), The gypsy moth: research toward integrated pest management. USDA For. Serv. Tech. Bull. 1584. Washington DC.

Stevens, M. L. and Lindroth, R. L. 2005. Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*). – Oecologia 145: 298–306.

Strong, A. M., Sherry, T. W. and Holmes, R. T. 2000. Bird predation on herbivorous insects indirect effects on sugar maple saplings. – Oecologia 125: 1689–1698.

Sword, G. A. 2001. Tasty on the outside, but toxic in the middle: grasshopper regurgitation and host plant-mediated toxicity to a vertebrate predator. – Oecologia 128: 416–421.

Tahvanainen, J., Helle, E., Julkunen-Tiitto, R. et al. 1985. Phenolic compounds of willow bark as deterrents against feeding by mountain hare. – Oecologia 65: 319–323.

Underwood, N. and Rausher, M. D. 2000. The effects of host-plant genotype on herbivore population dynamics. – Ecology 81: 1565–1576.

Villalba, J. J., Provenza, F. D. and Han, G. 2004. Experience influences diet mixing by herbivores: implications for plant biochemical diversity. – Oikos 107: 100–109.

Whelan, C. J., Holmes, R. T. and Smith, H. R. 1989. Bird predation on gypsy moth (Lepidoptera: Lymantriidae) larvae: an aviary study. – Environ. Entomol. 18: 43–45.

Whitham, T. G., Young, W. P., Martinsen, G. D. et al. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. – Ecology 84: 559–573.

Wiens, J. A., Cates, R. G., Rotenberry, J. T. et al. 1991. Arthropod dynamics on sagebrush (*Artemisia tridentata*): effects of plant chemistry and avian predation. – Ecol. Monogr. 61: 299–321.