Foliage of Oaks Grown Under Elevated CO_2 Reduces Performance of *Antheraea polyphemus* (Lepidoptera: Saturniidae)

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ABSTRACT To understand how the increase in atmospheric CO_2 from human activity may affect leaf damage by forest insects, we examined host plant preference and larval performance of a generalist herbivore, *Antheraea polyphemus* Cram., that consumed foliage developed under ambient or elevated CO_2. Larvae were fed leaves from *Quercus alba* L. and *Quercus velutina* Lam. grown under ambient or plus 200 μl/liter CO_2 using free air carbon dioxide enrichment (FACE). Lower digestibility of foliage, greater protein precipitation capacity in frass, and lower nitrogen concentration of larvae indicate that growth under elevated CO_2 reduced the food quality of oak leaves for caterpillars. Consuming leaves of either oak species grown under elevated CO_2 slowed the rate of development of *A. polyphemus* larvae. When given a choice, *A. polyphemus* larvae preferred *Q. velutina* leaves grown under ambient CO_2; feeding on foliage of this species grown under elevated CO_2 led to reduced consumption, slower growth, and greater mortality. Larvae compensated for the lower digestibility of *Q. alba* leaves grown under elevated CO_2 by increasing the efficiency of conversion of ingested food into larval mass. Despite equivalent consumption rates, larvae grew larger when they consumed *Q. alba* leaves grown under elevated compared with ambient CO_2. Reduced consumption, slower growth rates, and increased mortality of insect larvae may explain lower total leaf damage observed previously in plots in this forest exposed to elevated CO_2. By subtly altering aspects of leaf chemistry, the ever-increasing concentration of CO_2 in the atmosphere will change the trophic dynamics in forest ecosystems.

KEY WORDS arthropod, global change, feeding efficiency, herbivory, leaf nitrogen, phenolic content

If unabated, human activities will double the concentration of CO_2 in the atmosphere during the 21st century (IPCC 2001) and by stimulating the rate of photosynthesis may directly increase forest productivity (Bazzaz 1990, Bazzaz and Miao 1993, Ceulemans and Mousseau 1994, Curtis 1996, Drake et al. 1997, Curtis and Wang 1998, Norby et al. 1999) if other factors (e.g., nitrogen) are not limiting (Reich et al. 2006). By influencing the relationship between plants and herbivores, future increases in atmospheric CO_2 may also indirectly affect forest productivity. Insect herbivory typically removes 2–15% of net primary production in temperate deciduous forests (Whittaker 1970, Ohmart et al. 1983, Cyr and Pace 1993), and the removal of photosynthetically active leaf area by herbivorous insects alters carbon exchange by trees (Schowalter et al. 1986). Currently, there is no consensus about how elevated CO_2 will affect herbivory in native ecosystems (Lindroth et al. 2001, Stiling et al. 2003, Hamilton et al. 2004, Sanders et al. 2004, Agrell et al. 2005, Knepp et al. 2005).

Elevated CO_2 affects plant–insect interactions by altering the chemical composition and physical properties of foliage (Strain and Bazzaz 1983, Lincoln et al. 1993, Lindroth 1996a, b, Bezemer and Jones 1998, Peñuelas and Estiarte 1998). Ostensibly, the stimulation of photosynthesis by CO_2 increases levels of nitrogen-free metabolites such as carbohydrates and phenolics (Lincoln et al. 1993, Poorter et al. 1997, Peñuelas and Estiarte 1998, Veteli et al. 2002), decreases N content, and increases the C:N ratio of foliage (Lincoln et al. 1993, Cotrufo et al. 1998, Zvereva and Kozlov 2006). In laboratory experiments, leaf-chewing insects often consume more foliar tissue from plants grown under high CO_2, presumably to compensate for low foliar N (Lincoln et al. 1986, 1993, Fajer et al. 1989, Johnson and Lincoln 1990, Lindroth et al. 1993, 1995, Williams et al. 1994, Kinney et al. 1997, Agrell et al. 2000). This direct relationship between leaf quality and the amount of tissue removed is considerably more complex in the field where other factors affecting the population size of insect herbivores come into play. For example, the amount of leaf damage may be modulated by changes in herbivore mor-

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tality, oviposition preference, and fecundity (Brooks and Whittaker 1999, Wu et al. 2006), as well as by changes in interactions with higher trophic levels, such as increased exposure or susceptibility to predators (Sanders et al. 2004) and parasitoids (Stiling et al. 1999). Moreover, where multiple food sources are available, elevated CO_2 does not seem to increase overall leaf consumption by insects (Peeters 2002, Agrell et al. 2005, 2006). Laboratory experiments do not therefore produce results identical to those obtained in field experiments.

In large plots in a southeastern pine forest exposed to free-air CO_2 enrichment (FACE), insect damage to foliage of understory hardwoods was reduced under elevated CO_2 (Hamilton et al. 2004, Knepp et al. 2005). Reduced leaf damage corresponded to significant reductions in leaf nitrogen content and higher C:N ratio, as well as a trend in greater specific leaf area for hardwood tree species grown under elevated CO_2 (Knepp et al. 2005). In addition, there was a strong increase in total phenolic precipitation capacity, a measure of tannin biological activity (Hagerman 1987) for *Quercus alba* grown under elevated CO_2. These changes in leaf chemistry may contribute to a reduction in herbivore damage by altering insect consumption and performance. In light of these changes in leaf quality, the objective of this study was to determine if feeding on foliage grown under elevated CO_2 affects consumption and performance of *Antheraea polyphemus*, a leaf-chewing generalist lepidopteran representative of the most abundant feeding guild in this community (Hamilton et al. 2004).

Materials and Methods

Study Organisms. Experiments were conducted on leaves from trees established naturally in the understory of an unmanaged 17-yr-old loblolly pine plantation at the Forest Atmosphere Carbon Transfer and Storage (FACTS-1) research site in the Piedmont region of North Carolina (35°97′ N, 79°09′ W). *Quercus alba* (white oak) and *Q. velutina* (black oak) were chosen for this study because of their contrasting responses to elevated CO_2 (Hamilton et al. 2004, Knepp et al. 2005) and because they were each present in at least two of the elevated CO_2 plots. Both oak species had lower leaf N content and less leaf damage, but only white oak had a large increase in total leaf phenolics when grown under elevated compared with ambient CO_2 (Knepp et al. 2005). *Antheraea polyphemus* (Lepidoptera, Saturniidae) was selected as a representative herbivore because it commonly feeds externally on oaks throughout most of the United States (Tuskes et al. 1996) and because its large size permitted easy handling and accurate measurement of growth rate. Eggs were obtained from a private colony of reared females and wild males (B. Oehlke, New Jersey and Prince Edward Island, Canada), and newly hatched larvae were separated from egg masses and distributed across treatments to minimize potential maternal or population effects.

Fumigation with CO_2 was initiated in August 1996 in three 30-m-diameter plots using the FACE system to raise the atmospheric concentration by ≈200 μl/liter above current levels (≈577 μl/liter at 1 m; Hendrey and Kimball 1994, Lewin et al. 1994, Hendrey et al. 1999). This level was chosen to represent the CO_2 concentration predicted for 2050 (IPCC 2001). Three additional fully instrumented control plots received ambient air (CO_2: 386 ± 27 μl/liter). The leaf area index of the pine and hardwood canopy was ≈6 (DeLucia et al. 2002), and trees in the understory received on average ≈6% of full irradiance (Singsaas et al. 2000).

Performance Experiment. To identify potential mechanisms underlying the reduction in herbivore damage on leaves grown under elevated CO_2 (Hamilton et al. 2004, Knepp et al. 2005), consumption, growth, food processing efficiency, and mortality of larvae on leaves grown under ambient and elevated atmospheric CO_2 were examined with leaves from subcanopy trees (6–15 m). Assays were performed under laboratory conditions during June and July 2003 on leaves harvested from experimental field plots. Average air temperature during the performance experiments and subsequent preference experiments was 22–24°C, and photoperiod was ≈15 h.

Fully expanded, undamaged (<5% area removed) leaves were collected daily from two to three trees of each species from each CO_2 treatment ($n = 10$ trees per treatment). Because of the uneven distribution of trees among plots, leaf tissue from ambient and elevated CO_2 plots was supplied to larvae as a mixture of leaf punches from different trees and canopy positions. The punches (13–18 mm diameter) were taken from the entire leaf excluding the main vein and mixed within each treatment. Fresh leaf material was provided to the larvae daily, thus reducing the likelihood that preparing the leaf punches caused a significant change in leaf quality by inducing chemical defenses. Larvae were reared individually in 10-cm polystyrene petri dishes lined with 8-cm Whatman no. 1 filter paper moistened with distilled water. Although 80% of leaf matter is consumed in the final instar, we used early instars because they may be more sensitive to changes in foliar quality (Scriber and Slansky 1981, Fajer 1989, Williams et al. 1998).

Larval feeding trials (184 on black oak; 207 on white oak) lasted for 19–22 d (from hatch to third instar). Leaf disks were replaced daily and maintained in excess (≈50% of tissue consumed) based on consumption during the previous day (0–234 dry mg). The difference between the mass of disks (adjusted to dry mass based on aliquots) supplied to the larva, and the amount remaining was used to calculate the amount consumed. Frass was removed daily, combined every 2 d, dried, and weighed. Larvae were weighed every 2 d, and molting to third instar was recorded daily to determine the rate of development. A dry weight conversion was determined from the mean proportional dry weight of a subset of larvae from

each treatment and host species (≈100; data not shown).

Several variables describing different components of larval performance were calculated according to Waldbauer (1968). These variables were as follows: approximate digestibility (AD = [total mass of leaf tissue consumed − total mass of frass produced]/total mass of leaf tissue consumed), efficiency of conversion of digested food (ECD = larval mass gained/[mass of leaf tissue consumed − mass of frass produced]), and efficiency of conversion of ingested food (ECI = larval mass gained/mass of leaf tissue consumed). All indices were calculated on the basis of dry masses. When combined with the growth data, these indices provide a conceptual model for parsing how various aspects of larval nutrition affect growth rates. For example, relative growth rate (RGR) is approximately equal to the product of the relative consumption rate (RCR) and ECI. Similarly, ECI is approximately equal to the product of AD and ECD. These mathematical expressions are approximations because they are applied to variables calculated over discrete intervals rather than as derivatives.

Unambiguous interpretation of these performance ratios is not always possible because changes can arise by factors affecting either the numerator or the denominator. However, using the parameters described by Waldbauer (1968) permits comparison with earlier literature of lepidopteran performance (Scriber and Slansky 1981). Analysis of covariance (ANCOVA) also was used to compare growth and feeding data as suggested by Raubenheimer and Simpson (1992).

Preference Experiment. A feeding preference experiment was conducted to determine if larvae are capable of distinguishing between leaves developed under ambient and elevated CO_2. Immediately after hatching, 70–83 larvae were placed individually in the middle of a petri dish and offered weighed leaf disks from trees grown in elevated or ambient CO_2. Uneaten disks were dried to constant mass at 60°C and weighed. Leaf disks collected as described earlier were replaced daily for the duration of the experiment (5–7 d). Total consumption was calculated as in the performance experiment.

Elemental and Chemical Analyses. Several attributes that affect insect feeding and development were measured on leaves grown under ambient and elevated CO_2. Leaf tissue was dried at 60°C to constant mass and weighed to calculate specific leaf area (SLA; cm^2/g). The dried tissue was ground to a fine powder and analyzed for total C and N content per unit dry mass using an Elemental Combustion System (model 4010; Costech Analytical Technologies, Valencia, CA). Protein precipitation capacity of leaf tissue and frass was quantified by an assay developed by Hagerman (1987) and modified as in Knepp et al. (2005). This method is appropriate for comparing the relative quantity of potentially biologically active (protein-precipitating) phenolics between treatments (Mole and Waterman 1987). Previous research showed that phenolics affect both preference and performance of oak-feeding herbivores (Coviella and Trumble 1999, Hunter 2001).

To evaluate nutrient processing by *A. polyphemus*, total C and N content of frass and carcasses was analyzed for a subsample of 18 randomly selected larvae from each treatment. The total protein precipitation capacity of the frass also was quantified to estimate the ability of *A. polyphemus* larvae to extract nutrients from leaves during digestion. For these measurements, frass was combined within treatments for the first 7 d of the experiment because of low sample mass.

Statistical Analyses. Because CO_2 has previously been shown to reduce insect damage and influence leaf chemistry of both oak species at this site (Hamilton et al. 2004, Knepp et al. 2005), the focus of this study was to investigate insect use of leaves developed under ambient and elevated CO_2. The unit of replication, therefore, is a larva, and only surviving larvae were included in the use analyses. Total consumption, relative consumption rate, larval mass gain, relative growth rate, and total frass produced were compared with an analysis of variance (ANOVA) with CO_2 treatment as the fixed effect (ANOVA, PROC MIXED; SAS version 8.1; SAS Institute, Cary, NC). Mortality and development time were analyzed by χ^2 test. One-way ANOVA was performed for variables describing feeding efficiency (AD, ECI, and ECD) using CO_2 treatment as a main effect. Initial dry mass rather than average dry mass was used to calculate relative growth rate (Schowalter et al. 1986) and relative consumption rate (RCR; Farrar et al. 1989). ANCOVA (PROC MIXED) models used use (total tissue mass consumed − total mass of frass) and mass gained as the dependent variables for analysis of AD and ECI, respectively, with amount of tissue consumed as the covariate; the model for ECD used mass gained as the dependent variable with use as the covariate as in Raubenheimer and Simpson (1992). The analyses were performed first with a model statement including an interaction term between the covariate and treatment to test for homogeneity of slopes ($P > 0.10$). Statistical analyses of larval performance and preference on *Q. alba* and *Q. velutina* foliage were carried out independently because these experiments were conducted at different times.

For the foliage preference experiment, the cumulative tissue consumed was compared among treatments by ANOVA with treatment as the fixed effect. The unit of replication for this experiment was tissue consumption by individual larvae. The foliage used for the preference experiments was the same pool of leaf disks used for the performance experiments.

The characteristics of foliage fed to larvae in these experiments (total C and N, C:N ratios, total protein precipitation capacity, water content, and SLA) were analyzed using ANOVA with treatment and collection date as fixed effects. Because the leaf disk samples were pooled from different trees and rings within a treatment, this analysis allows us only to make inferences about the composition of the leaves fed to the

Table 1. Effects of elevated CO_2 on growth, consumption, food processing efficiencies, and development time of *A. polyphemus*

	Ambient CO_2	Elevated CO_2	F or χ^2	P
Performance of larvae fed black oak leaves				
TC (mg)	358.3 ± 15.66	260.4 ± 18.59	25.91	<0.01
RCR (mg mg^{-1} d^{-1})	14.7 ± 0.56	10.4 ± 0.67	4.93	<0.01
Larval mass gain (mg)	24.6 ± 1.34	18.2 ± 1.59	15.17	<0.01
RGR (mg mg^{-1} d^{-1})	1.0 ± 0.05	0.7 ± 0.06	3.82	<0.01
Frass (mg)	236.0 ± 11.76	192.2 ± 13.96	10.28	<0.01
AD (%)	35.7 ± 1.13	27.4 ± 1.35	22.06	<0.01
ECD (%)	21.0 ± 1.02	28.9 ± 1.21	24.27	<0.01
ECI (%)	7.1 ± 0.15	7.2 ± 0.18	0.26	0.61
Development time (d)	17.6 ± 0.15	18.0 ± 0.17	51.62	<0.01
Mortality (%)	32.6	52.1	7.26	0.01
Performance on white oak leaves				
TC (mg)	268.5 ± 5.53	272.6 ± 5.40	0.34	0.56
RCR (mg mg^{-1} d^{-1})	11.5 ± 0.32	11.9 ± 0.31	0.72	0.40
Larval mass gain (mg)	19.1 ± 0.69	22.7 ± 0.68	13.78	<0.01
RGR (mg mg^{-1} d^{-1})	0.8 ± 0.04	1.0 ± 0.04	11.96	<0.01
Frass (mg)	124.3 ± 4.50	170.3 ± 4.39	54.58	<0.01
AD (%)	54.7 ± 0.83	38.1 ± 0.81	206.13	<0.01
ECD (%)	14.3 ± 0.63	24.2 ± 0.61	125.44	<0.01
ECI (%)	7.6 ± 0.19	8.9 ± 0.19	24.71	<0.01
Development time (d)	14.4 ± 0.11	14.6 ± 0.09	26.37	<0.01
Mortality (%)	21.2	17.5	0.45	0.50

Analysis of covariance was performed for final larval mass and total consumption (TC) with initial larval mass as the covariate. Development time and mortality were analyzed using χ^2. All other measurements were analyzed using ANOVA. Least squared means ± SE calculated from pooled variance, except for mortality. For all measurements, degrees of freedom = 1 and n = 102 for black oak and n = 164 for white oak. All calculations are based on dry masses.

larvae and not, per se, the effect of CO_2 on leaf composition.

Total C and N content, C:N ratio, and total protein precipitation capacity in frass were analyzed with a repeated-measures ANOVA with treatment and date collected as the fixed effects; total C and N content and C:N ratio of larval carcasses were analyzed using ANOVA with treatment and date hatched as fixed effects.

Results

Growth under elevated CO_2 reduced approximate digestibility of foliage (AD) of both oak species for *A. polyphemus* larvae; caterpillars on foliage grown under elevated CO_2 irrespective of species experienced delayed development relative to growth on foliage grown under ambient conditions (Tables 1 and 2). Larvae converted digested leaves of black oak grown under elevated CO_2 7.9% more efficiently than foliage from ambient plots. Despite enhanced conversion efficiencies, larvae consumed 29% less leaf tissue, grew 30% more slowly, and experienced 20% greater mortality on foliage of black oak grown under elevated CO_2 (Tables 1 and 2). Caterpillars ingested equivalent amounts of leaf tissue and experienced comparable mortality on white oak foliage grown under ambient and elevated CO_2. As was the case with black oak, feeding efficiency was increased 14% for digested food and 1.3% for ingested food for foliage grown under elevated CO_2 (Tables 1 and 2).

Of the several leaf attributes measured in this experiment that potentially affect insect performance, only a small increase in carbon content of white oak leaves grown under elevated CO_2 could be resolved statistically (Table 3). The elemental composition and phenolic content of frass and larvae indicated that the capacity of *A. polyphemus* to extract nutrients during digestion was reduced by consuming leaves grown under elevated CO_2 (Table 4). Carbon content, nitrogen content, and total protein precipitation capacity of frass increased by 2, 6, and 14%, respectively, and C:N ratio decreased 4% in larvae that consumed white oak leaves grown under elevated CO_2. A similar trend of increased protein precipitation capacity in frass was observed for larvae fed black oak leaves grown under elevated CO_2 compared with ambient CO_2. Irrespective of host plant, *A. polyphemus* larvae that consumed leaves grown under elevated CO_2 contained less nitrogen than those that consumed leaves grown under ambient CO_2 (Table 5).

A. polyphemus preferred black oak foliage grown under ambient CO_2 (mean = 66.7 mg) over leaves grown at elevated CO_2 (60.5 mg; F = 6.38, P = 0.01). However, larvae presented with white oak foliage showed no preference as a function of CO_2 treatment (19.2 mg average consumption).

Discussion

Consuming leaves of two different oak species that developed under elevated CO_2 caused a consistent decrease in the approximate digestibility of foliage (AD) for *A. polyphemus* larvae and slightly slowed their rate of development (Tables 1 and 2). This reduced digestibility had different consequences for larvae depending on oak species. Black oak foliage grown under elevated CO_2 was consumed more slowly and larval growth was delayed despite increased conversion of digested food. Insofar as reduced growth rates

Table 2. Analysis of covariance of the food processing efficiencies: approximate digestibility (AD; use with total consumption as the covariate), efficiency of conversion of digested food (ECD; larval growth with use as the covariate), and efficiency of conversion of ingested food (ECI; larval growth with total consumption as the covariate)

	Ambient	Elevated	*F*	*P*
Performance of larvae fed black oak leaves				
AD				
Treatment			0.57	0.45
Total consumption			177.87	<0.01
Treatment × total consumption			2.89	0.09
ECD				
Treatment	21.6 ± 1.0	26.2 ± 1.3	6.68	0.01
Use			114.25	<0.01
ECI				
Treatment			1.95	0.17
Total consumption			1378.61	<0.01
Treatment × total consumption			8.86	<0.01
Performance of larvae fed white oak leaves				
AD				
Treatment	144.4 ± 1.8	101.8 ± 1.7	297.14	<0.01
Total consumption			101.09	<0.01
ECD				
Treatment	18.5 ± 0.8	26.2 ± 0.8	34.01	<0.01
Use			18.57	<0.01
ECI				
Treatment	20.7 ± 0.5	24.0 ± 0.5	22.9	<0.01
Total consumption			170.4	<0.01

For all measurements, degrees of freedom = 1 and $n = 102$ for black oak and $n = 164$ for white oak. Values are least squared means ± SE calculated from pooled variance. The analyses were first performed with a model statement including an interaction term between the covariate and treatment to test for homogeneity of slopes ($P > 0.10$). Nonsignificant interactions were omitted from the final model.

up to the third instar contribute to a reduction in pupal mass, consuming foliage developed under elevated CO_2 may lower fecundity (Tammaru et al. 1996, 2004). Consistent with reduced growth rates, lower final mass and longer development time, more *A. polyphemus* died on a diet of black oak foliage grown under elevated CO_2 (Table 1). This increased mortality may reflect a general trend for declines in insect populations under elevated CO_2 (Hamilton et al. 2004, Hall et al. 2005, Knepp et al. 2005). These results suggest that observed reductions in leaf damage in this understory community after exposure to elevated CO_2 are attributable at least in part in the alteration of the nutritional quality of plants (Hamilton et al. 2004, Knepp et al. 2005), a finding similar to that of other oak communities (Stiling et al. 2002, 2003, Hall et al. 2005).

Growth under elevated CO_2 alters a number of aspects of leaf chemistry that can potentially affect the palatability and nutritional quality to insect herbivores (Ceulemans and Mousseau 1994, Wilsey 1996, Buse et al. 1998, Dury et al. 1998, Stiling et al. 1999, Hall et al. 2005, Knepp et al. 2005). Larvae typically grow more slowly on nutritionally poor foliage associated with high CO_2 (Lindroth et al. 1993, Roth et al. 1997, 1998). Although our analysis of leaf composition revealed no significant differences in leaf quality in the parameters we measured, the elemental composition of *A. polyphemus* larvae in our study indicate that the ca-

Table 3. Elemental analyses and chemical composition of white and black oak leaf tissue used in the performance and food preference experiments

	N	Ambient CO_2	Elevated CO_2	*F*	*P*
Black oak foliage					
Carbon (%)	165	50.8 ± 0.19	50.9 ± 0.20	0.07	0.80
Nitrogen (%)	165	2.14 ± 0.02	2.10 ± 0.02	1.38	0.24
C:N ratio	165	24.2 ± 0.30	24.6 ± 0.31	0.77	0.38
SLA ($cm^2\ g^{-1}$)	165	227.5 ± 4.32	220.8 ± 4.53	1.13	0.29
Water (% fresh mass)	165	57.8 ± 2.6	55.8 ± 2.4	0.24	0.66
Total protein precipitation capacity (mg mg^{-1})	117	2.56 ± 0.13	2.82 ± 0.13	1.84	0.18
White oak foliage					
Carbon (%)	104	49.0 ± 0.44	49.5 ± 0.45	16.95	0.01
Nitrogen (%)	104	2.45 ± 0.03	2.43 ± 0.03	0.23	0.63
C:N ratio	104	19.97 ± 0.26	20.3 ± 0.27	0.70	0.41
SLA ($cm^2\ g^{-1}$)	104	284.2 ± 4.99	275.7 ± 5.20	1.63	0.20
Water (% fresh mass)	104	58.7 ± 1.0	57.8 ± 1.2	0.41	0.61
Total protein precipitation capacity (mg mg^{-1})	94	2.82 ± 0.39	3.09 ± 0.44	0.64	0.55

All measurements were analyzed using ANOVA. Values are least squared means ± SE calculated from pooled variance. The *F* statistic and probability level are designated as "*F*" and "*P*," respectively. All calculations are based on dry masses except water content. "Harvest date" and none of the interaction terms were statistically significant at $P < 0.05$.

Table 4. Elemental analyses and chemical composition of the frass from larvae fed leaves developed under ambient or elevated CO_2

	N	Ambient CO_2	Elevated CO_2	*F*	*P*
Frass of larvae fed black oak leaves					
Carbon (%)	221	49.9 ± 0.14	50.0 ± 0.15	0.15	0.70
Nitrogen (%)	221	1.89 ± 0.01	1.85 ± 0.02	1.15	0.28
C:N ratio	221	27.0 ± 0.23	27.7 ± 0.25	1.41	0.24
Total protein precipitation capacity (mg mg^{-1})	62	3.35 ± 0.16	3.66 ± 0.11	2.65	0.11
Frass of larvae fed white oak leaves					
Carbon (%)	186	47.1 ± 0.27	47.9 ± 0.27	4.54	0.03
Nitrogen (%)	186	1.80 ± 0.03	1.91 ± 0.03	7.92	<0.01
C:N ratio	186	26.6 ± 0.35	25.6 ± 0.35	4.10	0.04
Total protein precipitation capacity (mg mg^{-1})	113	4.16 ± 0.17	4.83 ± 0.20	6.39	<0.01

All measurements were analyzed using ANOVA. Values are least squared means ± SE calculated from pooled variance (SE = 0.0 is <0.05). For all measurements, degrees of freedom = 1. The *F* statistic and probability level are designated as "*F*" and "*P*," respectively.

pacity to extract nitrogen during digestion was reduced by consuming leaves grown under elevated CO_2 (Table 4). Nitrogen content of larvae was reduced 9.5% by feeding on black oak leaves and 7% by feeding on white oak leaves grown under elevated CO_2. Although there were only trends for increased protein-precipitating capacity in elevated CO_2 leaves of both species (Table 2), other evidence suggests that phenolics interfered with protein, and therefore nitrogen, assimilation. The frass of larvae fed white oak leaves from elevated CO_2 rings exhibited 16% greater protein-precipitating capacity and 6% more nitrogen than frass from larvae fed foliage from ambient rings (Table 4). A similar trend of increased protein precipitation capacity was observed in frass of larvae fed black oak leaves grown under elevated CO_2.

Although a number of studies have documented that insect herbivores, particularly lepidopteran larvae, prefer leaves grown under current ambient conditions to those grown under elevated CO_2 (Goverde and Erhardt 2003, Agrell et al. 2005, 2006), this is not always the case (Arnone et al. 1995, Traw et al. 1996, Diaz et al. 1998). Indeed, in this study, the larvae preferred black oak leaves grown under ambient CO_2, but there was no preference for white oak leaves as a function of treatment. Some of this variation may stem from differences in the induction of defensive compounds after herbivore damage under elevated CO_2.

Table 5. Elemental analyses of larvae fed either white oak or black oak leaf tissue developed under ambient or elevated CO_2

	Ambient CO_2	Elevated CO_2	*F*	*P*
Larvae fed black oak				
Carbon (%)	49.7 ± 1.23	45.8 ± 1.42	4.44	0.04
Nitrogen (%)	9.5 ± 0.26	8.6 ± 0.30	5.83	0.02
C:N ratio	5.2 ± 0.09	5.4 ± 0.10	0.96	0.33
Larvae fed white oak				
Carbon (%)	46.6 ± 0.67	46.0 ± 0.73	0.46	0.50
Nitrogen (%)	9.9 ± 0.21	9.2 ± 0.22	4.24	0.05
C:N ratio	4.8 ± 0.10	5.0 ± 0.11	2.68	0.11

ANOVA was performed for all measurements. Values are least squared means ± SE calculated from pooled variance. For all measurements, degrees of freedom = 1 and $n = 37$ for white oak and $n = 36$ for black oak. The *F* statistic and probability level are designated as "*F*" and "*P*," respectively.

That changes in phenolics underlie changes in insect responses to these oak hosts grown under elevated CO_2 is consistent with previous studies. Rossi et al. (2004) reported increased protein binding in *Q. myrtifolia* under elevated CO_2, and significant increases in phenolic compounds in foliage of other tree species under elevated CO_2 have been documented (Lindroth et al. 1993, 1997, Roth and Lindroth 1995, Traw et al. 1996, Kinney et al. 1997, Williams et al. 2000). Protein-binding capacity of *Q. rubra* is negatively correlated with gypsy moth pupal mass, egg mass, and fecundity (Rossiter et al. 1988). An increase in protein precipitation capacity may reflect an increase in the concentration of tannins; these compounds act as feeding deterrents and decrease protein use efficiency (Feeny 1970, Simpson and Raubenheimer 2001).

Elevated CO_2 seems to have reduced the food value of both oak species but to a greater extent for black oak, because several measures of larval performance were adversely affected by consumption of black oak–growth rate, final mass, development time, and mortality. That is not to say that feeding on white oaks under elevated CO_2 is without consequence for herbivores. Delays in development recorded in this study are similar those recorded in others (Lindroth et al. 1995, Goverde and Erhardt 2003). Longer development time may contribute to higher mortality by increasing the cumulative consumption of toxic allelochemicals and increasing exposure to pathogens, predators, and parasites (Price et al. 1980, Fajer 1989, Stiling et al. 1999, Fagan et al. 2002, Sanders et al. 2004). However, Kopper et al. (2002) suggested that prolonged development times may under some circumstances enable herbivores to compensate for reduced consumption and growth rates.

Based on results of earlier laboratory experiments, investigators predicted that compensatory feeding by herbivores on foliage with high C:N ratio will contribute to greater levels of herbivore damage when plants are exposed to elevated atmospheric CO_2 (Lincoln et al. 1986, Fajer et al. 1989, Johnson and Lincoln 1990). Predicting community responses, however, is difficult given the species-specific nature of plant responses to atmospheric change. As our study has shown, even sympatric congeners interact differently

with a single herbivore under ambient and elevated CO_2 environments. How different plant species respond to elevated CO_2 undoubtedly will cause differences in the responses of insect herbivores, and the rapid increase in CO_2 and corresponding changes in leaf chemistry will reshape the co-evolutionary interactions between plants and insects. By altering myriad aspects of leaf chemistry, elevated levels of CO_2 will fundamentally alter the relationship between plants and herbivores. How particular relationships will be altered, however, will depend on species-specific responses of plants to elevated CO_2 (Bazzaz 1990, Lindroth et al. 1993, Bezemer and Jones 1998, Peñuelas and Estiarte 1998), and in turn on species-specific behavioral and physiological responses of insect herbivores.

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