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Insect herbivory in an intact forest understory under experimental CO₂ enrichment

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Abstract Human-induced increases in atmospheric CO₂ concentration have the potential to alter the chemical composition of plant tissue, and thereby affect the amount of tissue consumed by herbivorous arthropods. At the Duke Forest free-air concentration enrichment (FACE) facility in North Carolina (FACTS-1 research facility), we measured the amount of leaf tissue damaged by insects and other herbivorous arthropods during two growing seasons in a deciduous forest understory continuously exposed to ambient (360 $\mu\text{l l}^{-1}$) and elevated ($\sim 560 \mu\text{l l}^{-1}$) CO₂ conditions. In 1999, there was a significant interaction between CO₂ and species such that winged elm (*Ulmus alata*) showed lower herbivory in elevated CO₂ plots, whereas red maple (*Acer rubra*) and sweetgum (*Liquidambar styraciflua*) did not. In 2000, our results did not achieve statistical significance but the magnitude of the result was consistent with the 1999 results. In 1999 and 2000, we found a decline (10–46%) in community-level herbivory in elevated CO₂ plots driven primarily by reductions in herbivory on elm. The major contribution to total leaf damage was from missing tissue (66% of the damaged tissue), with galls, skeletonized, and discolored tissue making smaller contributions. It is unclear whether the decline in leaf damage is a result of altered insect populations, altered feeding, or a combination. We were

not able to quantify insect populations, and our measurements did not resolve an effect of elevated CO₂ on leaf chemical composition (total nitrogen, carbon, C/N, sugars, phenolics, starch). Despite predictions from a large number of single-species studies that herbivory may increase under elevated CO₂, we have found a decrease in herbivory in a naturally established forest understory exposed to a full suite of insect herbivores and their predators.

Keywords Arthropod herbivores · Elevated carbon dioxide · FACE · Folivory

Introduction

Human activity is rapidly increasing the concentration of atmospheric CO₂. For the past 100,000 years, the level of atmospheric CO₂ was near or below 280 $\mu\text{l l}^{-1}$ (Houghton et al. 2001). Since the Industrial Revolution, it has increased to $\sim 360 \mu\text{l l}^{-1}$ and is expected to double this century. The increase in CO₂ concentration stimulates growth and productivity of terrestrial ecosystems (Curtis and Wang 1998; DeLucia et al. 1999; Hamilton et al. 2002) but may also alter the amount of productivity lost to herbivores. Net primary productivity removed by foliage-chewing insects can be significant. Estimates typically range from about 2 to 15% in forests and 4–24% in old fields and grasslands (Cebrian 1999; Cyr and Pace 1993), but during outbreaks these percentages can exceed 70%. Changes in leaf structure and chemistry caused by increasing atmospheric CO₂ are very likely to alter the quantity of photosynthetic tissue removed by insect herbivores in the future (Bezemer and Jones 1998; Whittaker 1999).

Plant species show such variability in response to CO₂ that there is currently no general theory that can predict the specific changes in leaf tissue composition for any particular species (Hamilton et al. 2001; Lincoln 1993; Norby et al. 1999). However, it is often the case that increased CO₂ causes a decline in nutritional quality

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(Whittaker 1999). This decline in nutritional quality may result from reduced nitrogen concentration (Cotrufo et al. 1998), increased starch (Lincoln 1993), increased C/N (Lincoln et al. 1993), reduced water content (Hättenschwiler and Schafellner 1999; Lawler et al. 1997), increased toughness (Lawler et al. 1997), or increased concentration of various defense compounds (Bezemer and Jones 1998).

Plant nutritional quality is a main factor limiting the feeding rate of folivorous arthropods (Cebrian 1999), and it might be expected that changes in leaf quality would translate into differences in insect feeding rate and the total amount of leaf tissue consumed. However, the responses of insect herbivores to changes in leaf nutritional quality are varied and complex. As nutritional quality decreases, the first response of insects can be to choose more nutritious food sources. Because plant species differ in their responsiveness to elevated CO₂, generalist herbivores may simply choose neighboring individuals of a different species that do not respond negatively to CO₂ enrichment. Alternatively, herbivores may choose different genotypes of the same species (Goverde et al. 1999; Hwang and Lindroth 1997; Osier et al. 2000) or leaves at different crown positions or ages (Williams et al. 1997, 1998). Specialist herbivores have many of the same options available except they can choose from only a narrow range of suitable host plants. After choosing appropriate food sources, insects from some feeding guilds, particularly leaf miners and chewers, have the ability to increase feeding to offset a reduction in nutritional quality, but species in other guilds do not appear to have this ability (Bezemer and Jones 1998; Whittaker 1999). Changes in insect physiology are also possible. For example, increased nitrogen use efficiency has been observed in insects feeding on leaves grown in a CO₂-enriched environment (Hättenschwiler and Schafellner 1999; Williams et al. 1998).

Because of this complexity of potential responses, community-level responses to elevated CO₂, such as total losses to herbivory, cannot readily be predicted from experiments involving a single species, or even a few species (Bezemer et al. 1999; Diaz et al. 1998; Whittaker 1999). Moreover, multi-trophic interactions, such as increased parasitism (Stiling et al. 1999) and the potentially different responses of generalist herbivores when offered multiple food choices (Bernays et al. 1994; Bezemer et al. 1999; Peters et al. 2000) greatly complicate community response to elevated CO₂.

In this study, we took advantage of a free air concentration enrichment (FACE) installation in a forest ecosystem to study community-level responses, allowing for all of the complexity of intact natural systems (Hendrey et al. 1999). In particular, we asked four questions: (1) Does elevated CO₂ alter the amounts of leaf damage from folivorous arthropods in a forest understory? (2) Does elevated CO₂ affect the amounts of leaf damage on different tree species differently? (3) Are amounts of leaf damage caused by insects from different feeding guilds affected differently? (4) Do amounts of leaf damage correlate with changes in leaf tissue chemistry?

Materials and methods

Site

The Duke Forest FACE experiment (FACTS-1 research site) is located near Chapel Hill, North Carolina (35°58'N 79°05'W). The forest is dominated by loblolly pine (1,733 stems ha⁻¹; 92% of total woody biomass), with sweetgum (*Liquidambar styraciflua* L., 620 stems ha⁻¹) and yellow poplar (*Liriodendron tulipifera* L., 68 stems ha⁻¹) as sub-dominants (DeLucia et al. 1999). Forty-eight species of woody plants (trees, shrubs and vines) have established naturally in the understory of this forest (J. Pippen and W. Cook, unpublished data). The soil is a clay-rich Alfisol with low nitrogen and phosphorus availability (Schlesinger and Lichter 2001). This section of the Duke forest was farmed a century ago, and the current plantation was established in 1983 after a regenerating forest was clear-cut in 1979.

The FACE system increases atmospheric CO₂ concentration in 30-m diameter experimental plots within this continuous pine forest (Hendrey et al. 1999). Each FACE plot consists of a circular plenum that delivers air to an array of 32 vertical pipes. The pipes extend from the forest floor through the 14-m tall forest canopy and contain adjustable ports at 50-cm intervals. These ports are tuned to control atmospheric CO₂ concentration through the entire forest volume. Fumigation with CO₂ in three "elevated" plots began in August 1996. Three "ambient" control plots receive the same volume of air without additional CO₂. CO₂ levels in the understory (1 m height) were measured at 366 μl l⁻¹ for ambient plots and 616 μl l⁻¹ for elevated plots (DeLucia and Thomas 2000). Each plot is ~100 m from its nearest neighbor. To control for topographic variation (~5 m) between plots and potential gradients in site fertility, the three control and three fumigated plots are arranged in three blocks.

Damage estimates

We estimated the amount of leaf tissue damage four times, in early June, mid-July, and early September 1999, and again in mid-July 2000. In 1999 we chose sweetgum (*Liquidambar styraciflua* L.), red maple (*Acer rubrum* L.), and winged elm (*Ulmus alata* Michx.). In 2000 we again used these three species and added redbud (*Cercis canadensis* L.) to the study. On each sampling date we haphazardly chose three (in 1999) or four (in 2000) understory individuals of each species in each plot in the range from 1 m to 3 m in height. We visually stratified the branches of each individual into bottom, middle, and top. Within each group, we used a random number generator to choose one branch, and on each branch we randomly chose three leaves. Thus, we chose 1,458 leaves in 1999 and 1,296 leaves in 2000. Leaves were removed and photographed with a high-resolution digital camera (Nikon Coolpix 950). The number of saplings chosen was constrained by the naturally occurring numbers and distribution of individuals within the FACE plots, and the number of leaves removed per tree was constrained by the need to avoid damage to small trees from too much leaf area removal. Disks of known areas were taken from the leaves; one set of disks was immediately frozen in liquid nitrogen for later analysis and another set was dried at 70°C to constant mass and weighed.

The digital image of each leaf was converted to binary black and white and its area was measured using the ScionImage software (Scion, Frederick, Maryland, USA). Leaf damage was quantified into one of four damage classes (missing tissue, galls, skeletonized/mined, discolored). We measured the area present in each leaf directly and the total leaf area (before folivory) by reconstructing the missing area. Depending on the specific pattern and extent of leaf area removed, the pre-damaged leaf area was estimated from the images by filling in holes, redrawing margins, doubling area of remaining lobes, or, occasionally, allometric regressions based on mid-vein length.

Insect censuses

To qualitatively assess the insect community at the site, we sweep netted each tree species during each sampling period. Sweep netting was done on a minimum of 30 individuals of each species outside of the rings to avoid damaging plants or affecting herbivory levels within the rings.

Chemical analyses

Sugars present in the foliage of each species were identified by gas chromatography/mass spectrometry. For quantification, duplicate 12 mg samples of dried leaf tissue were each extracted with 2 ml of a 75% methanol/water solution containing 0.15 g/l mannitol as an internal standard. Solutions were agitated for 1 h, centrifuged, and subsamples (400 μ l) of this solution were vacuum evaporated. When completely dry, the residues were dissolved in 200 μ l of Tri-Sil Z silylation reagent (Pierce, Rockford, Ill., USA) and incubated at 70°C for 10 min. A 1 μ l aliquot of each sample was automatically injected into a gas chromatograph (Hewlett Packard 5890A, triple ramp temperature program: start 160°C, increase at 1°C min⁻¹ to 180°C; 1 min pause then increase at 20°C min⁻¹ to 220°C; then increase at 2°C min⁻¹ to 240°C) equipped with a 30 m capillary column (SE-30, Alltech, Deerfield Ill.) and an FID detector. The identity of sugars was confirmed by gas chromatography—mass spectroscopy and reference standards (Sigma, St. Louis) were used to calculate concentrations. Concentrations of all simple sugars (glucose, fructose, scyllo-inositol, myo-inositol, sucrose, one unidentified) were summed for total sugar concentration.

Starch concentrations were determined by enzymatic assay using amyloglucosidase followed by glucose hexokinase (Starch assay kit SA-20, Sigma, St. Louis, Mo., USA). The principle of the assay is that starch is converted to glucose; the glucose is phosphorylated and subsequently oxidized. The last reaction is quantified by the amount of NAD that is reduced. Duplicate 12 mg samples of dried leaf tissue were each extracted twice with 1.5 ml of 80% hot ethanol for 45 min. The supernatant was discarded, the pellet re-suspended by topping off up to 1.6 ml with distilled water. The pH was adjusted to pH 5–7, and the solution autoclaved for 2 h at 121°C. After autoclaving, the sample volume was restored to 1.6 ml. The sample solution (500 μ l) was mixed with an equal amount of starch assay reagent and incubated at 60°C for 15 min. This solution (140 μ l) was added to 1 ml of glucose assay reagent in a UV absorbance cuvette, stirred, incubated for 15 min and the absorbance read at 340 nm (Ultraspec 2, Biochrom, Cambridge).

Total leaf phenolics were quantified on fresh-frozen tissue as catechin equivalents using the Folin-Ciocalteu method (Booker et al. 1996; Singleton and Rossi 1965). Leaf tissue was ground in liquid nitrogen and extracted five times with 70% acetone, and extracts combined. Triplicate runs were performed on each sample. Aliquots (500 μ l) were diluted 1:10 with 70% acetone and 475 μ l of Folin-Ciocalteu (Sigma, St. Louis, Mo., USA) reagent was added. Each solution was stirred, and after 3 min 475 μ l of Na₂CO₃ was added to each tube. Tubes were sealed and left for 1 h. Afterwards, the solutions were centrifuged and absorbance read at 724 nm. The Folin-Ciocalteu assay is most appropriately used to compare within a species only.

Concentrations of leaf carbon and nitrogen were measured on dried tissue by micro-Dumas combustion (NA1500, Carlo Erba Instrumentazione, Milan, Italy).

Statistical analysis

Statistical comparisons of the effects of CO₂ on levels of herbivory and on leaf chemistry were performed with analysis of variance (ANOVA) (Proc Mixed version 8.2; SAS, Cary, N.C., USA). The FACE plots were originally established in three blocks of two plots each. Herbivory data for 1999 were modeled with plots as a random effect, treatment, species and month as fixed effects, with month as a

repeated measure in a mixed model analysis. All fixed effect interactions were included in the model. Interactions with the blocks were included as necessary to ensure the proper error structure. Percent damage was log transformed to meet the assumptions of normality. Separate analyses were performed for the 2000 data and each of the leaf chemistry variables. All of the leaf chemistry variables were also log transformed to meet the assumptions of normality. Each leaf chemistry factor was included in the analysis of percent damage to check whether any of the factors were significant covariates. Because of the small sample size inherent in FACE studies ($n=3$ in this case), the level of statistical significance was taken as $P=0.1$.

Results

In 1999 and 2000, the amount of leaf area damaged by arthropod herbivores depended on the species of tree (Tables 1, 2, Fig. 1). In 1999, on a basis of percent (Table 1a) or absolute (Table 1b) leaf area removed, there was a significant interaction between CO₂ and herbivory such that elm showed lower herbivory in elevated CO₂ plots, whereas maple and sweetgum did not (Fig. 1). In 2000, our results did not achieve statistical significance because of loss of power without the repeated measures design, but the magnitude of the result was consistent between the two years of the study. For example, in July 1999 herbivory on elm was 67% lower in elevated CO₂ plots ($P=0.04$), and in July 2000 it was 55% lower ($P=0.09$).

Community-level averages of percent herbivory ranged from 10% to 46% lower in elevated CO₂ plots (Fig. 2) although this difference was only statistically significant in July 1999 ($P=0.02$). Again, our results were not statistically significant in 2000 but the magnitude of the

Table 1 ANOVA table for percent and absolute area leaf tissue damaged in 1999 in a deciduous forest understory. Data were log transformed prior to statistical analysis

Effect	Num <i>df</i>	Den <i>df</i>	<i>F</i>	Pr> <i>F</i>
Percent leaf tissue				
CO ₂	1	2	2.66	0.2445
Species	2	8	6.66	0.0198
CO ₂ ×Species	2	8	4.58	0.0473
Month	2	94.3	10.82	<0.0001
CO ₂ ×Month	2	94.3	1.77	0.1754
Species×Month	4	94.3	6.70	<0.0001
CO ₂ ×Species×Month	4	94.3	0.99	0.4179
Absolute leaf tissue				
CO ₂	1	2	2.88	0.2318
Species	2	8	13.62	0.0027
CO ₂ ×Species	2	8	2.98	0.1076
Month	2	94.3	21.70	<0.0001
CO ₂ ×Month	2	94.3	1.33	0.2699
Species×Month	4	94.3	5.87	0.0003
CO ₂ ×Species×Month	4	94.3	0.70	0.5922

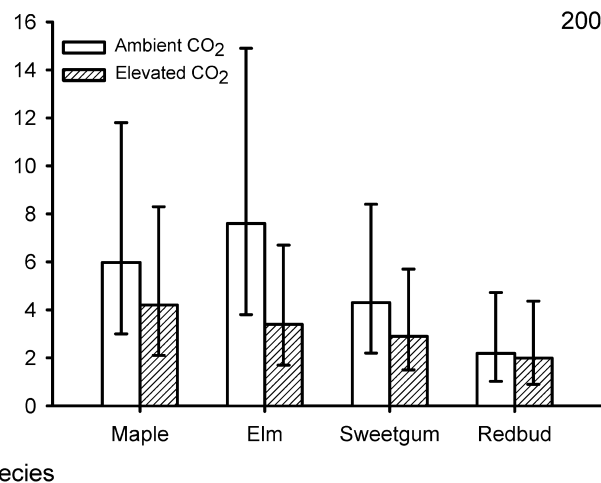
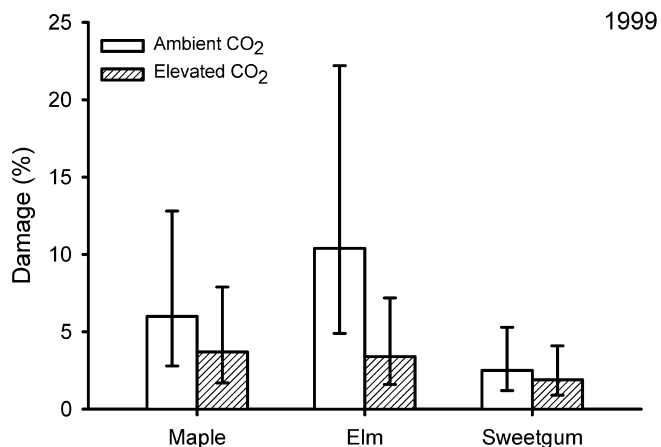


Fig. 1 Average percent leaf area damaged under ambient and elevated CO₂ by tree species for 1999 and 2000. In both years the amount of herbivory depended on tree species, and in 1999 there

was a significant treatment by species interaction ($P < 0.1$) with elm showing significant reductions in herbivory under elevated CO₂ ($P = 0.02$). Error bars are back-transformed 95% confidence intervals

Table 2 ANOVA table for percent and absolute area leaf tissue damaged in 2000 in a deciduous forest understory. Data were log transformed prior to statistical analysis

Effect	Num <i>Df</i>	Den <i>df</i>	<i>F</i>	<i>Pr > F</i>
Percent leaf tissue				
CO ₂	1	2	2.61	0.2475
Species	2	8	3.36	0.0587
CO ₂ × Species	2	8	0.44	0.7276
Absolute area leaf tissue				
CO ₂	1	2	2.78	0.2371
Species	2	8	5.88	0.0120
CO ₂ × Species	2	8	0.57	0.6492

result was consistent between the 2 years of the study, with 46% decline in July 1999 and 43% July 2000.

Breaking down total foliar damage into classes that had approximate correspondence with insect feeding guild, we found that most of the damage was missing tissue, presumably from leaf chewers (66%; Fig. 3). The only instance where this was not the case was for sweetgum in July 2000 where there was a large amount of discolored tissue possibly the result of ozone damage (The region frequently experiences elevated levels of tropospheric ozone; D. Stephan and T. Creswell, personal communications).

Our insect trapping methods did not capture enough individuals of any species to accurately estimate population numbers, but we were able to determine the range of species responsible for herbivory (Table 3). We found representatives from a number of different feeding guilds, but chewers predominated in the insect community. The preponderance of chewers was consistent with our estimates that missing tissue was the predominant form of leaf damage.

We found no evidence for an effect of elevated CO₂ on leaf chemical composition. In July, when the effects on

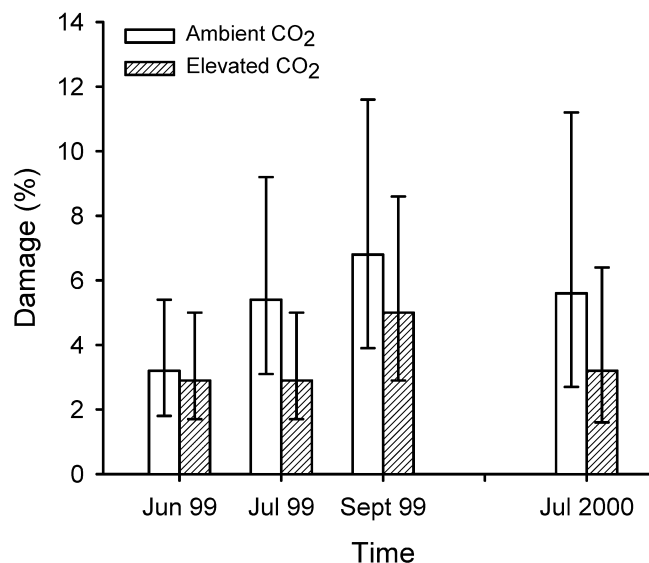


Fig. 2 Average percent leaf area damaged under ambient and elevated CO₂ in the deciduous understory at the Duke FACE site. Damage was calculated as an average across three (1999) or four (2000) tree species. Month-by-month comparisons were significant for July 1999 ($P = 0.02$). Error bars are back-transformed 95% confidence intervals

elevated CO₂ on herbivory were greatest, we found no significant differences for leaf nitrogen ($F = 1.8$, $P = 0.3$), carbon ($F = 2.27$, $P = 0.3$), C/N ratio ($F = 3.42$, $P = 0.2$), sugars ($F = 0.4$, $P = 0.6$), starch ($F = 1.85$, $P = 0.3$), or total phenolics ($F = 0.17$, $P = 0.3$) (Table 4). Leaf chemical composition was very similar between 1999 and 2000 (Table 4), and none of the compounds were significant covariates with damage.

Discussion

We found that elevated CO₂ led to a trend toward reduced herbivory in a deciduous understory in a situation that

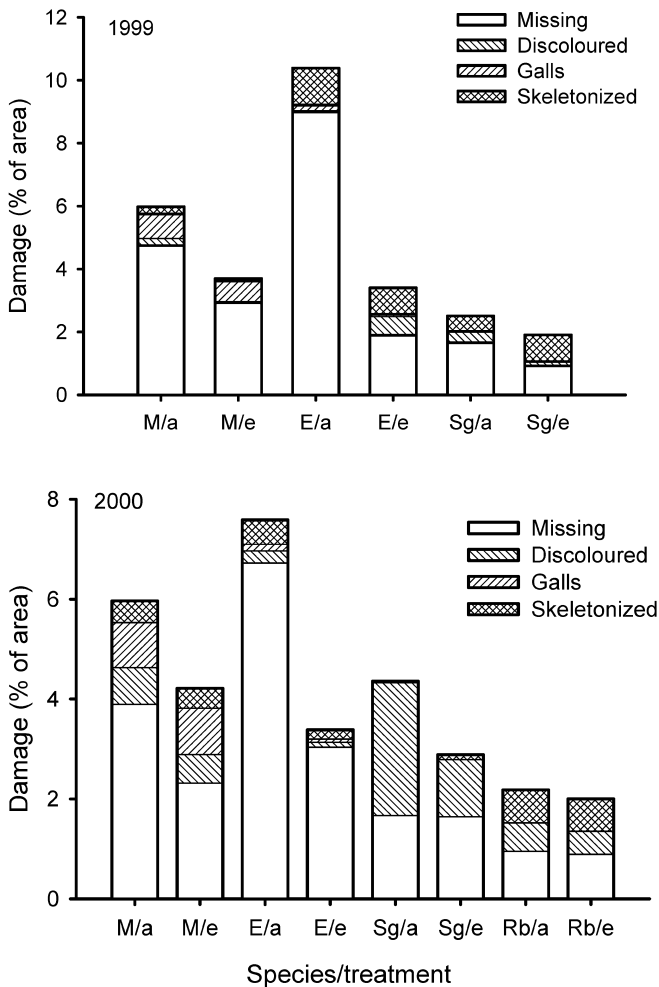


Fig. 3 Average percent leaf area damaged under ambient and elevated CO₂ by tree species for 1999 and 2000 broken down by damage type. (*M* maple, *E* elm, *Sg* sweetgum, *Rb* redbud; treatment designations are *a* ambient CO₂, *e* elevated CO₂)

included the full complement of naturally occurring plant and insect species. The overall levels of herbivory we measured are typical for forested ecosystems (Cebrian 1999). In 1999, elevated CO₂ reduced overall herbivory by more than 40% with elm showing greater reduction than either red maple or sweetgum. We observed the same pattern and magnitude of reduction in 2000 although our sampling scheme in that year lacked the power to statistically verify this result. In general, missing tissue (removed by leaf chewers) represented the primary type of tissue damage, and appeared to be the damage type most affected by elevated CO₂. If, under elevated CO₂, trees grew larger leaves or produced more leaves while insect consumption remained constant, this would produce an apparent reduction of herbivory when expressed on a basis of percent leaf tissue removed (Hughes and Bazzaz 1997). This was not the case in our study because we found reductions in herbivory whether expressed on an absolute or a percent basis. Our results are surprising because data from many growth chamber feeding trials strongly suggest that leaf chewers in particular will increase their con-

sumption of foliage grown in elevated CO₂ to offset a loss of nutritional quality (Bezemer and Jones 1998; Cannon 1998; Coviella and Trumble 1999; Hunter 2001; Lincoln et al. 1993; Whittaker 1999). However, in agreement with our results, another relatively large-scale study found a reduction in the number of leaves damaged by insect herbivores under elevated CO₂ (Stiling et al. 2002, 2003).

We found that herbivory levels on different tree species were affected differently by elevated CO₂. In both years, elm showed much larger reductions in herbivory under elevated CO₂ than either maple or sweetgum (although the interaction term was not significant in 2000). These differences in herbivory could translate into community or ecosystem-level responses by altering competitive relationships between plants (Bentley and Whittaker 1979), causing changes in plant flowering (Awmack and Harrington 2000), or even affect nutrient availability by changing nutrient cycling (David et al. 2001).

Reduced herbivory may be caused by reduced populations of herbivores, reduced feeding by herbivores, or some combination of both. We were unable to determine whether herbivore populations were affected by elevated CO₂. Our sweep net sampling procedure provided qualitative information on the composition of the insect community, but because of the generally low levels of insect herbivores in the forest, we were unable to obtain quantitative population counts. It is possible that elevated CO₂, acting through small (statistically insignificant) changes in leaf chemistry, affected insect populations by reducing insect fecundity or survival (Awmack and Leather 2002; Joutei et al. 2000; Percy et al. 2002), and indeed Stiling et al. (2003) found that elevated CO₂ reduced densities of leaf miners in a scrub oak community. Reduced herbivore numbers are particularly plausible for our experiment because at the beginning of our measurements, the Duke FACE site had been under continuous fumigation with elevated CO₂ for 3 years allowing effects on insect populations to accumulate. Studies on the effects of elevated CO₂ acting across multiple generations of insects are few, but there is evidence that effects such as reduced numbers of eggs, reduced survival, and increased rates of parasitoid attack acting across multiple generations can reduce insect numbers (Brooks and Whittaker 1998, 1999; Stiling et al. 1999; Watt et al. 1995; Williams et al. 1997).

In addition to possible changes in overall insect populations in the treatment plots, is the potential for generalist herbivores to switch host species within a plot since the foliar chemical composition may be affected differently for different tree species (Cotrufo et al. 1998; Kinney et al. 1997; Williams et al. 2000). Even though this is a possibility, the scant literature thus far has failed to provide evidence for host shifting under elevated CO₂ (Diaz et al. 1998; Ledergerber et al. 1997, 1997; Peters et al. 2000). Further characterization of this as a potential mechanism of changes in herbivory will require measurement of many more plant species in the understory.

At the same time that insect populations may have been affected, it is known that alteration in leaf chemical

Table 3 Insects captured in sweep net samples in 1999 and 2000 at the Duke FACE site in the deciduous forest understory

Tree	Order	Family	Genus	Species	Feeding guild	
Maple	Lepidoptera	Coleophoridae			case bearer	
	Lepidoptera	Geometridae		3 species	chewer	
	Lepidoptera	Saturniidae	<i>Dryocampa</i>	<i>rubicunda</i>	chewer	
	Lepidoptera	Noctuidae	<i>Acronicta</i>	<i>americana</i>	chewer	
	Orthoptera	Tettigoniidae			chewer	
	Coleoptera	Chrysomelidae	<i>Rhadopterus</i>	<i>picipes</i>	rasper	
	Diptera	Cecidomyiidae	<i>Acericecis</i>	<i>ocellaris</i>	closed gall	
	Sweetgum	Orthoptera	Decticinae	<i>Atlanticus</i>	sp.	chewer
		Lepidoptera	Saturniidae	<i>Actias</i>	<i>luna</i>	chewer
		Lepidoptera	Saturniidae	<i>Citheronia</i>	<i>regalis</i>	chewer
Lepidoptera		Geometridae		2 species	chewer	
Lepidoptera		Lymantriidae	<i>Dasychira</i>	sp.	chewer	
Coleoptera		Scarabaeidae	<i>Macroductylus</i>	sp.	skeletonizer	
Orthoptera		Gryllidae			chewer	
Orthoptera		Tettigoniidae			chewer	
Elm		Coleoptera	Cerambycidae			
	Homoptera				sucker	
	Homoptera				sucker	
	Lepidoptera	Notodontidae	<i>Lochmaeus</i>	<i>manteo</i>	chewer	
	Lepidoptera	Notodontidae	<i>Lochmaeus</i>	<i>bilineata</i>	chewer	
	Lepidoptera	Arctiidae	<i>Halysidota</i>	<i>tessellaris</i>	chewer	
	Orthoptera	Gryllidae	<i>Oecanthus</i>	sp.	chewer	
	Orthoptera	Gryllidae			chewer	
	Homoptera	Cicadellidae			sucker	
	Coleoptera				skeletonizer	
	Acari	Eriophyidae			galler	
	Lepidoptera	Arctiidae	<i>Hyphantria</i>	sp.	tied leaves	
	Acari	Tydeidae			yellowing tissue	
	Red bud	Orthoptera	Gryllidae	<i>Oecanthus</i>		chewer
Orthoptera		Gryllidae				
Lepidoptera		Coleophoridae			case bearer	
Lepidoptera			Gelechiidae	<i>Fascista</i>	<i>cercerisella</i>	
Homoptera					skeletonizer	

characteristics can affect insect feeding (Bezemer and Jones 1998; Hunter 2001; Lincoln et al. 1993). Such changes include altered nutritional quality or changes in the concentrations of defensive compounds. Although changes in leaf tissue chemistry have been commonly

found for plants grown under elevated CO₂ (Cotrufo et al. 1998; Yin 2002), and many of these changes appear to affect insect herbivores (Bezemer and Jones 1998), we found no evidence for significant changes in leaf nitrogen, C/N ratio, sugar, starch or total leaf phenolics in either

Table 4 Leaf chemical composition of deciduous understory trees at the Duke FACE site. Numbers are averages across all species measured in July of 1999 and 2000. There were no significant differences or treatment × species interactions for any of the compounds measured (see text)

Year	Treatment	Total N (% w/w)	Total C (% w/w)	C/N	Sugar (% w/w)	Phenol (g/g leaf)	Starch (% w/w)
1999	Ambient	2.00	44.60	22.64	3.44	0.111	1.01
	Elevated	1.89	44.70	23.79	3.52	0.115	1.41
2000	Ambient	2.10	45.40	22.10	4.99	0.111	0.81
	Elevated	2.07	45.20	22.37	4.89	0.111	0.81

year of our study. This agrees with another study performed at the Duke Forest FACE site that also found no effect of elevated CO₂ on the chemical composition of leaves of understory trees (Finzi and Schlesinger 2002). Perhaps small, statistically non-significant, but biologically important, changes in one or more of these factors did affect herbivory. This explanation is supported by our finding of a significant interaction effect between CO₂ and tree species in 1999.

Predictions based on single species experiments of the effects of elevated CO₂ on herbivory, have not been supported at the community level (Bezemer et al. 1999; Diaz et al. 1998; Hughes and Bazzaz 2001). Apparently the additional options available to herbivores in complex multi-species systems, coupled with tri-trophic interactions (Percy et al. 2002; Stacey and Fellowes 2002; Stiling et al. 1999, 2002) are hindering efforts to generalize simple controlled environment experiments to the field. Despite the large number of studies that predict increased herbivory, particularly from leaf chewers, under elevated CO₂ (Bezemer and Jones 1998; Cannon 1998; Coviella and Trumble 1999; Hunter 2001 Lincoln et al. 1993; Whittaker 1999), our study found a trend toward reduced herbivory 2 years in a row. Although the mechanisms causing reduced herbivory under elevated CO₂ remain unclear, our results agree with the only other large-scale study field experiment that quantified herbivory for a community exposed to elevated CO₂ (Stiling et al. 2002, 2003).

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