

DECREASED LEAF-MINER ABUNDANCE IN ELEVATED CO₂: REDUCED LEAF QUALITY AND INCREASED PARASITOID ATTACK

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Abstract. Most studies on the effects of elevated CO₂ have focused on the effects on plant growth and ecosystem processes. Fewer studies have examined the effects of elevated CO₂ on herbivory, and of these, most have examined feeding rates in laboratory conditions. Our study takes advantage of an open-top CO₂ fertilization study in a Florida scrub-oak community to examine the effects of elevated CO₂ on herbivore densities, herbivore feeding rates, and levels of attack of herbivores by natural enemies.

Higher atmospheric CO₂ concentration reduced plant foliar nitrogen concentrations, decreased abundance of leaf-mining insect herbivores, increased per capita leaf consumption by leafminers, and increased leafminer mortality. As suggested by other authors, reduced foliar quality contributed to the increase in herbivore mortality, but only partly. The major factor increasing mortality was higher attack rate by parasitoids. Thus increasing CO₂ concentrations may reduce the survivorship of insect herbivores directly, by reducing plant quality, but also indirectly, by changing herbivore feeding and eliciting greater top-down pressure from natural enemies.

Key words: carbon:nitrogen ratios; field experiment; herbivore-enemy interactions; increased leaf consumption; increased parasitism; plant-herbivore interactions; plant nitrogen; CO₂; response to elevation in.

INTRODUCTION

Atmospheric CO₂ concentration is increasing at the rate of 1.5 $\mu\text{L}\cdot\text{L}^{-1}\cdot\text{yr}^{-1}$ and is expected to double by the end of the next century relative to present levels (700 $\mu\text{L}/\text{L}$ vs. 360 $\mu\text{L}/\text{L}$) (Wigley and Raper 1992, Keeling et al. 1995, Houghton et al. 1996). Most work to date on elevated CO₂ has focused on the effects of CO₂ on vegetation (e.g., Potvin and Vasseur 1997, Wilsey et al. 1997). The effects of elevated CO₂ on vegetation are both direct, including an increase in plant photosynthetic rates, reduced foliar nitrogen, and increased C/N ratios (Ceulemans and Mousseau 1994, Wilsey 1996, Hughes and Bazzaz 1997), and indirect, via herbivory. For example, under elevated CO₂, herbivores grow more slowly, take longer to develop, and consume more plant material (Watt et al. 1995, Hughes and Bazzaz 1997). However, most work on herbivory has focused on larval feeding trials in laboratory or greenhouse situations. This approach may be inadequate for predicting how herbivory will be affected in natural communities where plants do not always receive plentiful nutrient supplies (Hassell et al. 1993, Lincoln et al. 1993, Arnone et al. 1995, Lawton 1995), where herbivores can choose where to feed, and where

natural enemies may have major impacts on herbivore abundances. This study provides what we believe to be the first data on the effects of global change (elevated CO₂) on trophic webs in natural ecosystems. Specifically we test (1) whether herbivore population densities are changed by elevated CO₂, (2) whether herbivory increases or decreases under elevated CO₂, and (3) whether attack rates of herbivores by natural enemies are affected by elevated CO₂. The study takes advantage of experimental plots set up by some of us (Drake, Hungate, Dijkstra, Hinkle and Knott) in 1996 to investigate the effects of rising atmospheric CO₂ concentration on carbon accumulation and nutrient cycling in terrestrial ecosystems.

METHODS

This study was conducted on a 2-ha section of a native scrub-oak community at Kennedy Space Center, Florida. This system was chosen to represent a nutrient-poor, woody ecosystem, with mature canopy, seasonal litter fall, high rhizosphere production and nutrient cycling. The vegetation is dominated by two sclerophyllous oaks: myrtle oak, *Quercus myrtifolia*, and sand live oak, *Q. geminata*, which together account for 93% of the plant biomass. This is a fire-influenced community that is exposed to a natural fire return cycle of 8–12 yr (Schmalzer and Hinkle 1996). Prior to our

Manuscript received 3 November 1997; revised 16 March 1998; accepted 13 April 1998.

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experiments vegetation was burned before January 1996 and plants were then allowed to regrow.

From March–April 1996 sixteen 3.6 m diameter plots were enclosed with a clear polyester film open-top chamber of a design similar to that used by Drake (1992) in earlier elevated CO₂ experiments in Maryland, and also used in a small-scale pilot study at Kennedy Space Center (Viegas et al. 1995). The open-top chambers were roofless cylinders measuring 3.6 m in diameter and 3.4 m in height. They were constructed of a polyester film overlaid on an octagonal framework of PVC pipe with a removable access door and frustum to reduce dilution of air within the chamber by outside wind. Beginning in May 1996, all regrowth was cut to ground level and the vegetation in eight of the chambers was exposed to almost twice ambient CO₂ (ambient + 350 μL/L ≈ 700 μL/L) while the other eight chambers were exposed to ambient levels of CO₂ (about 350 μL/L). The CO₂ was continuously supplied, 24 h a day. Monitoring and control of CO₂ injection into each chamber were done by an infrared gas analyzer in conjunction with manually adjusted needle valves. Throughout the experiment, CO₂ was sampled from the chambers and, if CO₂ concentration was below ambient + 350 μL/L, pure CO₂ was injected into the airstream. In ambient CO₂ chambers, the flow of air was identical to that of the high CO₂ chambers but was not supplemented with CO₂. CO₂ concentration was measured continuously throughout the experiment and was stable at 350 ± 20 μL/L depending on wind pattern. In addition, another eight unchambered plots were established to determine the effects, if any, of the chambers.

This study focuses on leafmining insects. Leafminers consume foliage while simultaneously dwelling inside it. Only larvae feed within leafmines and the adults are free living, emerging from pupae, often within the mine, to mate and lay eggs. Most species remain within the natal leafmine. The number of generations per year varies. Leafmining has evolved independently many times and is found in the Coleoptera, Diptera, Lepidoptera, and Hymenoptera (Auerbach et al. 1995). All the species in this study are leafmining moths with multiple generations per year. Leafminers were studied for four main reasons: (1) the most common insect herbivores we encountered in our study plots were leafminers, (2) we could measure area of leaf consumed by leafminers, (3) the leafmine leaves a permanent record of each miner's fate, and (4) leafminers cannot escape their mines and cadavers inside mines indicate host-plant induced death (Auerbach et al. 1995).

Within each chambered plot, all leafminers on 100 leaves were counted every month on each of two different branches of *Q. myrtifolia* and *Q. geminata*. Beginning at the tip of a branch, and moving toward the main trunk, every leaf was censused until 100 had been counted. Different branches were selected each month for 9 mo, for a total of 1800 leaves censused per plot. This is a common method of assessing leafminer den-

sities on trees (Bultman and Faeth 1987, Connor and Beck 1993). Censuses were taken between July 1996 and March 1997, a period that corresponded to leaf flushing after the burn until leaf drop, at which time a new cohort of leaves was formed on the trees. Similar censuses were made on unchambered plots in 1997. Densities of mines were compared between chambered ambient CO₂ and chambered elevated CO₂ plots using *t* tests on the total numbers of mines found between July 1996 and March 1997 in each plot. The effect of the chambers was determined by using a one-way ANOVA on total densities of miners in 1997 from unchambered, chambered ambient, and chambered elevated CO₂ plots.

Areas of mines of three different leafmining species (*Stigmella*, *Cameraria*, and *Stilbosis*) were measured by tracing 20 mined leaves from each plot onto acetate sheets and scanning with a CID-201 leaf area meter (CID, Incorporated, Vancouver, Washington, USA). This procedure was performed once, between December 1996 and January 1997. Plot averages were used for statistical determination of treatment effects using a *t* test. Fates of each censused mine were assigned to different mortalities based on the condition of the mine (Stiling et al. 1991). Successfully developed leafminers leave a crescent- or circular-shaped exit hole at the end of the mine when the larvae cut their way out of the mine and drop to the forest floor to pupate. Parasitized mines exhibit small shotgun-like holes on the upper surface from which the parasites emerge. Common parasites include the wasps *Zagrammosoma multilineatum*, *Chrysonotomyia* sp., *Anchrysocharoides* sp., *Closterocercus trincinctus*, *Elasmus* sp., *Chrysocharis* sp., and *Chelonus cosmopteridis* (Stiling et al. 1991, Mopper and Simberloff 1995). Predated mines are ripped open by ants, bugs, spiders, or lizards, and larvae killed by host plant defenses or nutritional deficiencies can be seen dead in the mine when it is backlit by the sun. Treatment differences between percentage dead in mine, percentage preyed on, and percentage parasitized were compared using *t* tests on untransformed percentages, which were normally distributed.

Leaf samples were taken from the same position of the branches of censused trees in each plot during July 1996, freeze dried, and analyzed for carbon and nitrogen using a Perkin-Elmer CHN elemental analyzer.

RESULTS

There was no exclusion of leafminers by the chambers. All species that were found on nonchambered plots were found in chambered plots. Furthermore, densities of all species were not reduced by the chambers, indicating recruitment was not affected. In fact there was a nonsignificant trend toward increased leafminer abundance inside chambers (e.g., total mines on myrtle oak in 1997, unchambered $\bar{X} = 29.0$, $SD = 14.37$, chambered CO₂ $\bar{X} = 30.0$, $SD = 12.09$, chambered ambient $\bar{X} = 43$, $SD = 15.07$; one-way ANOVA $F_{2,21}$, $\bar{X} = 2.524$,

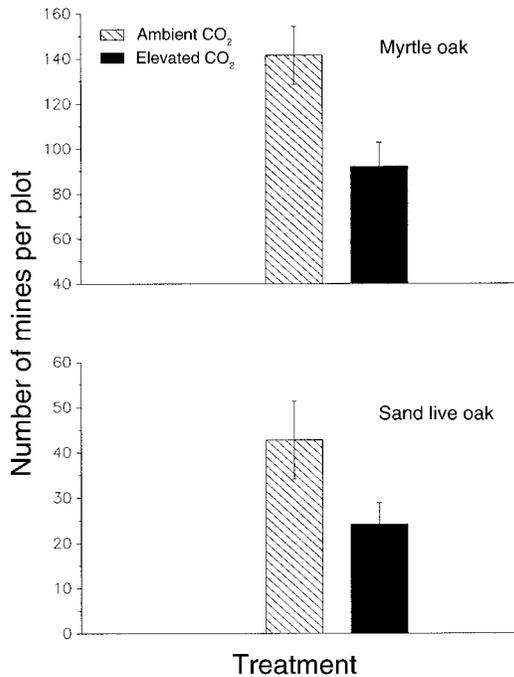


FIG. 1. Average numbers of leaf miners per plot (sum of monthly counts, July 1996–March 1997) ± 1 SE, in ambient CO₂ and elevated CO₂ treatments on myrtle oak and sand live oak at Kennedy Space Center.

$P = 0.104$). This may be due to the “Krebs effect”; that is, emigration of insects from the chambers may be prevented by the chamber walls (Strong and Stiling 1983).

The densities of leafminers were reduced inside the elevated CO₂ chambers as compared to the ambient CO₂ chambers. There was a significant reduction in the numbers of leafminers per plot (i.e., per 1800 leaves) in the CO₂ treatments on myrtle oak ($t_{14} = 2.961$, $P = 0.011$) and a marginally significant reduction on sand live oak ($t_{14} = 1.891$, $P = 0.086$) (Fig. 1). The trend of elevated insect densities in ambient CO₂ chambers compared to elevated CO₂ chambers held for all six common species of leafminer (Table 1).

Part of the reason for the differences in herbivore abundance between treatments may be linked to nitrogen levels and C/N ratios. For both myrtle and sand live oak, foliar nitrogen on elevated CO₂ plots was depressed below levels on ambient CO₂ plots (Fig. 2) though only for myrtle oak was the trend significant ($t_{13} = 2.335$, $P = 0.036$). Reduced N lowers the nutritional value of the plants to consumers (Scriber and Slansky 1981, Bryant et al. 1983, Herms and Mattson 1992). C/N ratios were also higher on elevated CO₂ plots compared to others, but again only for myrtle oak was this significant ($t_{13} = 2.720$, $P = 0.018$). According to the C/N balance hypothesis, nutrient deficient plants accumulate carbon based secondary metabolites, some of which reduce plant quality for herbivores (Bryant

TABLE 1. Leaf miner abundance on myrtle oak in ambient and elevated CO₂ plots. Data are sums of monthly counts, July 1996–March 1997 ± 1 standard error.

Leaf miner species	Treatment	
	Ambient CO ₂	Elevated CO ₂
<i>Stilbosis</i>	6.25 \pm 1.11	1.87 \pm 1.19
<i>Cameraria</i>	16.6 \pm 6.84	5.4 \pm 1.74
<i>Tisheria</i>	9.7 \pm 2.87	5.6 \pm 2.01
<i>Buccalatrix</i>	4.9 \pm 1.34	3.1 \pm 1.34
<i>Stigmella</i>	79.7 \pm 10.78	54.1 \pm 8.84
<i>Acerocercops</i>	28.2 \pm 4.62	20.1 \pm 2.82

et al. 1983, Herms and Mattson 1992, Ruohomaki et al. 1996).

Treatments also affected herbivore feeding. All three species of leafminers that we measured on myrtle oak had bigger mines on elevated CO₂ plots than on ambient CO₂ plots (Fig. 3) (*Stigmella*, $t_{14} = 3.086$, $P < 0.01$; *Stilbosis*, $t_{14} = 3.755$, $P < 0.001$; *Cameraria* $t_{14} = 2.544$, $P < 0.05$). This supports other studies that have shown increased consumption of plant tissue by Lepidoptera larvae in elevated CO₂ environments (Fajer et al. 1989, Lincoln and Couvet 1989, Lindroth et al. 1993, Kinney et al. 1997), including leafminers on *Rumex* where a 20% increase in mine area was apparent (Salt et al. 1995).

Finally, we found a significant treatment effect on leafminer mortality. A higher percentage of leafminers died in their mines in elevated CO₂ chambers than in ambient CO₂ chambers ($t_{14} = 4.349$, $P = 0.001$) (Fig.

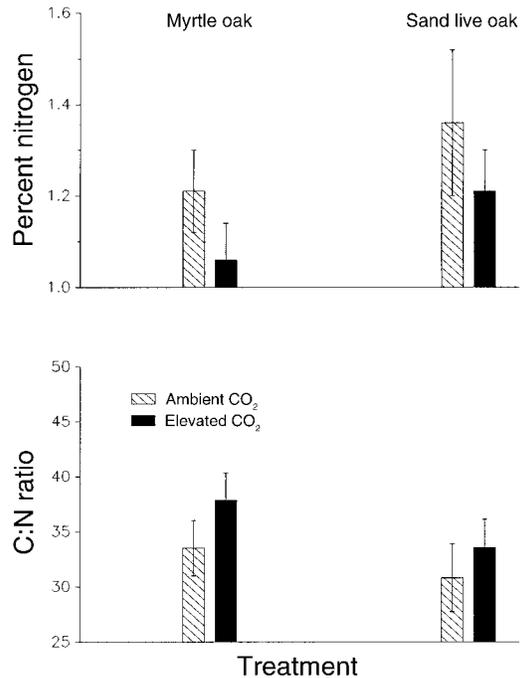


FIG. 2. Average leaf nitrogen and carbon : nitrogen ratios, ± 1 SE in ambient CO₂ and elevated CO₂ treatments on myrtle oak and sand live oak.

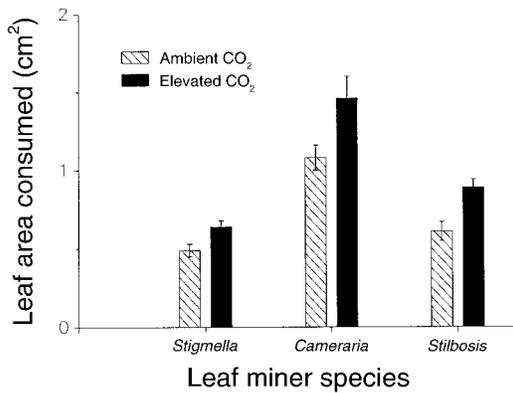


FIG. 3. Average leaf area consumed per leaf mine, ± 1 SE, for three species of leafminer on myrtle oak.

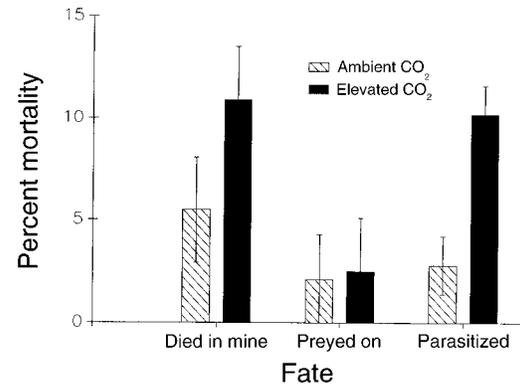


FIG. 4. Fates of leaf miners on myrtle oak ± 1 SD in ambient and elevated CO₂ treatments.

4) suggesting changes within the leaf detrimental to the leafminers. There was also significantly greater mortality from parasitoids in elevated CO₂ chambers ($t_{14} = 10.495$, $P < 0.001$). Death due to predation was unchanged ($t_{14} = 0.429$, $P = 0.676$).

DISCUSSION

Our results show three main things. First, leafminer population densities per 1800 leaves are depressed under elevated CO₂. However, because leaf production increases under elevated CO₂ (P. Dijkstra, *unpublished data*), the exact reduction in insect densities per plot is not yet known. Second, per capita herbivory by leafminers increases under elevated CO₂. Third, death rates of leafminers are elevated under elevated CO₂ which helps explain the lower abundances there. The same reduction of density inside the elevated CO₂ chambers was true of other insects we counted such as leaf-tiers on myrtle oak (average count of 8.5 ± 4.5 SD in ambient CO₂ chambers, 4.5 ± 2.3 SD in elevated CO₂) and woolly aphids on sand live oak (487.75 ± 408.4 SD in ambient CO₂ vs. 377.5 ± 350.4 SD in elevated CO₂). The latter is an interesting result because there have been relatively few studies on the performance of sucking insects under elevated CO₂, and the few studies that do exist show no effect of elevated CO₂ on sap-suckers (Butler et al. 1986, Tripp et al. 1992, Salt et al. 1996).

There are at least two reasons for the elevated death rates of leafminers under elevated CO₂. First, there is a direct effect of reduced leaf quality, lowered N, and elevated C/N, on leafminer death. Second, there is an indirect trophic cascade of elevated atmospheric CO₂ involving the plants, their herbivores, and the herbivores' natural enemies. It is not easy to separate out these effects. For example, it is possible that leafminers showed elevated levels of parasitism because (1) their mines are bigger and more obvious to searching parasitoids (Mopper et al. 1984) (2) possibly because delayed growth may prolong exposure to enemies (Lindroth et al. 1993), or (3) the larvae are in a weakened state and are more susceptible to parasites. However,

we do know that reduced plant quality caused a doubling of leafminer mortality, whereas death due to parasitism increased four-fold in elevated CO₂, showing that indirect effects through trophic interactions can outweigh the direct effects of altered resource quality. However, both effects operate in the same direction, so the reduction in overall herbivore densities in elevated CO₂ is especially pronounced. These results highlight the importance of trophic interactions in natural communities, and suggest that laboratory and greenhouse experiments that exclude top trophic levels may underestimate the detrimental effects of elevated CO₂ on insect herbivores.

ACKNOWLEDGMENTS

This research was supported by NSF grants DEB 9633717 to Peter Stiling and DOE 94-005 to Bert Drake.

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