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PLANT ANIMAL INTERACTION

P. D. Coley · M. Massa · C. E. Lovelock · K. Winter

Effects of elevated CO_2 on foliar chemistry of saplings of nine species of tropical tree

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Abstract This study examined the effects of elevated CO_2 on secondary metabolites for saplings of tropical trees. In the first experiment, nine species of trees were grown in the ground in open-top chambers in central Panama at ambient and elevated CO_2 (about twice ambient). On average, leaf phenolic contents were 48% higher under elevated CO_2. Biomass accumulation was not affected by CO_2, but starch, total non-structural carbohydrates and C/N ratios all increased. In a second experiment with *Ficus*, an early successional species, and *Virola*, a late successional species, treatments were enriched for both CO_2 and nutrients. For both species, nutrient fertilization increased plant growth and decreased leaf carbohydrates, C/N ratios and phenolic contents, as predicted by the carbon/nutrient balance hypothesis. Changes in leaf C/N levels were correlated with changes in phenolic contents for *Virola* (r=0.95, P<0.05), but not for *Ficus*. Thus, elevated CO_2, particularly under conditions of low soil fertility, significantly increased phenolic content as well as the C/N ratio of leaves. The magnitude of the changes is sufficient to negatively affect herbivore growth, survival and fecundity, which should have impacts on plant/herbivore interactions.

Keywords Phenolic compounds · Elevated CO_2 · Tropical trees · Fertilization · Panama

P.D. Coley (✉) · M. Massa
Biology Department, University of Utah, Salt Lake City, UT 84112, USA
e-mail: coley@biology.utah.edu
Fax: +1-801-5814668

C.E. Lovelock
Smithsonian Environmental Research Center, Edgewater, MD 21037, USA

P.D. Coley · K. Winter
Smithsonian Tropical Research Institute, Balboa, Republic of Panama

Introduction

Atmospheric concentrations of CO_2 are expected to double within 50–100 years (IPCC 1998) and it is clear that elevated CO_2 will have profound impacts on plant growth and allocation. Plant responses will also have cascading effects on herbivores, perhaps greatly altering plant/herbivore interactions and community structure (Coley 1998; Peñuelas and Estiarte 1998; Coviella and Trumble 2000). Thus, understanding how elevated CO_2 changes plant nutritional quality and defense is an essential first step in predicting how herbivores will respond to this component of climate change.

Two key leaf traits that affect their suitability as food for herbivores are leaf protein and secondary metabolites (Scriber and Slansky 1981; Rosenthal and Berenbaum 1992; Stamp and Casey 1993). The majority of experiments using elevated CO_2 have found a 15–30% reduction in leaf nitrogen per unit leaf dry mass and an increase in the carbon/nitrogen (C/N) ratio (Reekie and Bazazz 1989; Körner and Arnone 1992; Arnone and Körner 1995; Poorter et al. 1997; Bezemer and Jones 1998; Coley 1998; Curtis and Wang 1998; but see Díaz et al. 1998). As insect herbivores are typically limited by dietary protein, their growth and fecundity is significantly lower on leaves grown under conditions of enriched CO_2 (Lincoln et al. 1993; Watt et al. 1995; Lindroth 1996a, b; Kinney et al. 1997; Lindroth et al. 1997; Coley 1998; McDonald et al. 1999; Agrell et al. 2000).

Fewer studies have examined changes in defensive chemistry, and almost all of these are for temperate species. Carbon-based defenses, such as simple phenols and tannins, frequently increase in response to elevated CO_2 (see reviews by Lindroth 1996a, b; Coley 1998; Koricheva et al. 1998; Peñuelas and Estiarte 1998). This is consistent with predictions of the carbon/nutrient balance (CNB) hypothesis, which suggests that carbon in excess of growth demands will be allocated to defenses (Bryant et al. 1983). Thus, high light or CO_2 should lead to an accumulation of carbohydrate reserves relative to nitrogen and thus to an increase in carbon-based defenses.

Overall, temperate species do seem to increase phenolic compounds under enriched CO_2 (Lindroth 1996a, b; Coley 1988; Koricheva et al. 1998; Peñuelas and Estiarte 1998), although responses vary among species and environmental conditions (e.g. Fajer et al 1992; Kinney and Lindroth 1997). However, enriched CO_2 does not appear to increase terpene concentration, raising questions regarding the universality of the CNB hypothesis (Koricheva et al. 1998; Hamilton et al. 2001). In the only study from the tropics, two rainforest species from Australia gave inconsistent patterns for the effect of enriched CO_2 on leaf phenolics (Kanowski 2001).

Many of the above experiments were conducted with potted plants in greenhouses. More recent work in open-top chambers or FACE rings suggests that plants exhibit different allocation patterns in response to elevated CO_2 when they are planted in the ground (Arnone and Körner 1995; O'Neil and Norby 1996; Lovelock et al. 1998). These advances in experimental work may allow more realistic predictions of plant and animal responses to increases in atmospheric CO_2.

In this paper we examine the responses of nine species of neotropical rainforest trees growing in open-top chambers under conditions of ambient and enriched CO_2. Additional experiments on two species focus on the interaction between nutrient fertilization and CO_2 enrichment. Data on photosynthesis are reported elsewhere (Lovelock et al. 1998; Winter et al. 2000, 2001). Here we present data on plant growth, phenolic compounds and C/N contents, as these have important implications for insect herbivores.

Materials and methods

Site description

The experiments were conducted near the Smithsonian Tropical Research Institute's canopy crane site in Parque Natural Metropolitano in central Panama (8°58′ N, 79°34′ W). Annual mean temperature is 27°C and annual precipitation averages 1,740 mm with a dry season lasting from late December until early May.

Elevated CO_2 experiment

In this experiment, we quantified the effect of elevated CO_2 on phenolic content for nine native species of trees grown in six octagonal chambers on vacant grasslands adjacent to the forest at Parque Metropolitano. Each open-top chamber was 2 m across and 2.5 m tall and was constructed with an aluminum frame covered with clear plastic film. Saplings were planted directly in the natural soil, which eliminated pot effects. The soil at the site was poor, having a pH of 5.9, 2.25% organic matter, 0.144% total nitrogen, 0.013% total phosphorus, 1.42% total carbon and a C:N ratio of approximately 10. The CO_2 concentrations within the three ambient chambers were slightly above 400 ppm at dawn and decreased to 350 ppm during the course of the day. The elevated CO_2 chambers were about 300–400 ppm above ambient. CO_2 was bubbled through a saturated solution of potassium permanganate to remove ethylene impurities before it was delivered to the chambers (see Lovelock et al. 1998 for additional details).

Temperature within the chambers varied between 24° and 33°C, exceeding air temperatures outside the chambers by a maximum of 2°C. Photon flux densities (PFD) reached up to 2,000 µmol m^{-2} s^{-1} and were similar inside and outside the chambers. By the end of the experiment, PFD at ground level in the chambers was approximately 10% of the PFD incident at the top of the chamber. Relative humidity declined during the day from almost 100% to approximately 65% and was similar inside and outside the chambers.

Nine native species of trees (three late-successional, three mid-successional, three early-successional) were grown in each chamber. Shade-tolerant late-successional species consisted of *Calophyllum longifolium* Willd. (Gutteriferae), *Tetragastris panamensis* (Engler) O. Kuntze (Burseraceae) and *Virola surinamensis* (Rol.) Warb. (Myristicaceae). Mid-successional species consisted of *Anacardium excelsum* (Bertero & Balb.) Skeels (Anacardiaceae), *Antirrhoea trichantha* (Griseb.) Hemsl. (Rubiaceae) and *Cordia alliodora* (R. & P.) Cham. (Boraginaceae). Light-demanding early-successional species consisted of *Cercropia longipes* Bertol. (Moraceae), *Ficus insipida* Willd. (Moraceae) and *Luehea seemannii* Tr. & Planch. (Tiliaceae).

The nine native species were planted within a 3×3 matrix, and then surrounded with a border of a tenth species exotic to Panama, *Swietenia macrophylla* (Meliaceae). We used *Swietenia* as a border in order to minimize edge effects on experimental plant species. *Swietenia* was not included in the analysis. Among the three pairs of chambers there was a different arrangement of the three successional groups. Late-successional species were dispersed among other faster growing species in order to generalize the results for any arrangement of species. Beginning in February 1996, species were transplanted into the chambers and watered for the remainder of the dry season. All plants had at least 50 cm^2 leaf area at the beginning of the experiment and were harvested after 6 months. Harvested plant material was dried to a constant weight at 60°C. Dried leaf material was ground in a Wiley Mill and stored at –20°C until further analysis.

Carbon and nitrogen fertilization experiment

The second experiment addressed the effects of both elevated CO_2 and high nutrient availability on phenolic content for *Ficus insipida*, an early successional species and *Virola surinamensis*, a late-successional species. The plants used in this experiment were grown in the same six chambers described above plus two more identical chambers. The experimental design was split into two different time periods in order to compare fertilized versus unfertilized chambers. In the first series of this experiment, the upper 30 cm of soil was replaced with uniform soil from another site to increase comparability between chambers and experiments. In the second series of experiments the top 30 cm of soil was also replaced with uniform soil and Osmocote-plus fertilizer (0.56 kg per m^2, N-P-K 16–8–12 and Mg, Fe, Mn, Cu, Mo and B; Scotts-Sierra, Maryland, Ohio) was evenly distributed on the soil surface and covered with leaf litter. Eight weeks later an additional 0.56 kg per m^2 of fertilizer was added to each chamber. Eighteen seedlings of each species, up to 20 cm tall, were planted in each chamber. The first series of this experiment (unfertilized chambers) began on 10 December 1996 and lasted 30 weeks. The second series (fertilized chambers) began on 25 August 1997 and lasted 16 weeks. Due to logistical constraints, the fertilization and non-fertilization experiments were not run simultaneously, possibly confounding season with treatment. However, measures of environmental variables taken within the chambers were extremely similar in both experiments. Temperatures ranged from nighttime values of 24°C to daytime maxima of 33°C and relative humidity varied from 65% to 98% (see Winter et al. 2000, 2001 for additional details). As there were more cloudy days during the second experiment, total solar radiation averaged 13.2% less than during the first (29.5 vs 25.6 mol m^{-2} day^{-1}). Thus plants in the second experiment experienced conditions of lower C/N primarily due to greatly enhanced nutrient fertilization as well as slightly less light than in the unfertilized experiment. Plants were watered if necessary. Plants were also classified as being in the center or edge of the chamber, as

light levels were higher in edge positions. Plants were harvested and dried at 60°C.

Foliar chemistry analysis

Leaf tissue was analyzed for total phenolic content using the Folin-Denis method (Mole and Waterman, 1987; Torti et al. 1995). All 54 plants were analyzed in the first experiment. In the second experiment, a random subset of plants was sampled, with a minimum of two replicates per position (center and edge) in each chamber, for a total of 66 *Ficus* and 70 *Virola* plants. A 20 mg sample was taken from the total ground leaf sample for each individual plant. Each sample was extracted in 10 ml of 80% methanol then placed in an ice bath and homogenized with a polytron for 30 s. Samples were centrifuged, the pellet was discarded, and the supernatant was lyophilized and stored at –20°C until further assay. For analysis, samples were resolubilized in 80% methanol and measured at three different concentrations. Tannic acid was used as a standard.

Because species differ in the reactivity of their phenolic defense compounds, values obtained with a tannic acid standard do not necessarily reflect the true investment in phenolic defenses (Wisdom et al. 1987; Appel et al. 2001). Thus, it may be misleading to compare absolute values among species. However, we were interested in how the CO_2 and fertilization treatments affected phenolic content within a species, a relative comparison for which it is legitimate to use a single standard.

Tissue concentrations of starch and total non-structural carbohydrates (TNC) were determined on mature leaves. Leaves were harvested at dusk, immediately placed in liquid nitrogen and freeze-dried for storage. Results and details of the extraction methods for starch and soluble sugars are described in previous publications (Winter et al. 2000, 2001). We also present the C/N ratio, which is the ratio of the total carbon and leaf nitrogen as measured in a CHN Element Analyzer (Heraeus, Hanau, Germany).

Plant growth

At the end of the experiments, above- and below-ground plant material was harvested and weighed. Roots were carefully excavated using a high-pressure water hose. Tissue was dried to constant weight at 60°C and total biomass determined. For the comparison of nine species growing in ambient and enriched CO_2, the amount of biomass accumulated during the 6-month experiment was used as a measure of growth. For the fertilization experiments, plants were harvested when *Ficus*, the tallest plants, were about 1.5 m tall. In the unfertilized treatments where growth was slower, plants reached this height and were harvested after 30 days, whereas in the fertilized treatments, plants grew more quickly and reached 1.5 m in only 16 weeks. Thus, the only meaningful growth comparisons are within a fertilization treatment.

Statistical analysis

In order to assess the change in phenolic content between species in ambient and elevated CO_2 environments, data for the nine native species were analyzed by analysis of variance (ANOVA, SAS 1997), with chamber nested within CO_2 treatment. For each ANOVA we used a type III SS. In the second experiment, plant responses to CO_2 and fertilization were analyzed by ANOVA, with chamber nested within CO_2 treatment. Because there was a significant species × fertilization interaction, we also analyzed the two species separately.

Results

Effects of elevated CO_2 on nine species

There was a significant effect of CO_2 concentration on leaf phenolic content (Tables 1, 2). In eight of the nine species, there was a tendency for tannin concentrations to increase under elevated CO_2 levels (Fig. 1). *Cecropia longipes*, an early-successional species, was the only species that responded to elevated CO_2 with a decrease in phenolic concentrations (n.s.). On average, tannin concentration (percent dry weight of leaves) increased by 47.6% (Table 1). The C/N ratio and carbon allocation to non-structural carbohydrates also increased under conditions of elevated CO_2 (Table 1).

Effects of CO_2 and nutrient fertilization on two species

There was a significant effect of nitrogen fertilization on phenol production for both *Virola* and *Ficus* (Table 3), with concentrations being lower in fertilized plants regardless of the CO_2 levels. There was no significant

Table 1 Responses of phenolic compounds and non-structural carbohydrates to elevated CO_2 for nine species of rainforest plants. Species are classified as early, mid and late successional. The percent dry weight concentration of phenolic compounds in mature leaves is given for plants grown in ambient and elevated CO_2 (mean and standard error). Total non-structural carbohydrates (*TNC*) and C/N values are from Lovelock et al. (1998). Changes in phenols, TNC and C/N are calculated as the percent increase in response to elevated CO_2

Species	Status	Phenols ambient CO_2 (% dry weight)	Phenols elevated CO_2 (% dry weight)	Percent change in phenols	Percent change in TNC	Percent change in C/N
Cecropia longipes	Early	6.51 (1.1)	4.75 (1.5)	–27.0	1.3	14.5
Ficus insipida	Early	0.64 (0.1)	0.86 (0.1)	34.4	20.0	25.5
Luehea seemannii	Early	4.53 (0.8)	6.87 (0.6)	51.7	25.3	29.9
Anacardium excelsum	Mid	12.73 (0.3)	13.56 (1.5)	6.5	43.4	41.9
Antirrhoea trichantha	Mid	5.20 (1.0)	7.51 (0.2)	44.4	20.4	29.7
Cordia alliodora	Mid	2.47 (0.6)	5.42 (1.6)	119.4	44.2	41.1
Calophyllum longifolium	Late	3.72 (1.9)	7.48 (1.4)	101.1	25.3	36.4
Tetragastris panamensis	Late	9.76 (3.7)	14.66 (2.0)	50.2	27.9	32.2
Virola surinamensis	Late	4.82 (0.9)	7.14 (1.4)	48.1	23.3	40.1
Average overall	–	–	–	47.6	25.7	25.4

Table 2 ANOVA results for phenolic responses of nine species to elevated CO_2 Species was nested within successional status and chamber was nested within the different CO_2 levels

Source of variation	*df*	*F* value	*P* value
CO_2	1	11.80	0.0014
Species	8	18.65	0.0001
Chamber (CO_2)	4	3.17	0.0237
CO_2×Species	8	1.28	0.2797

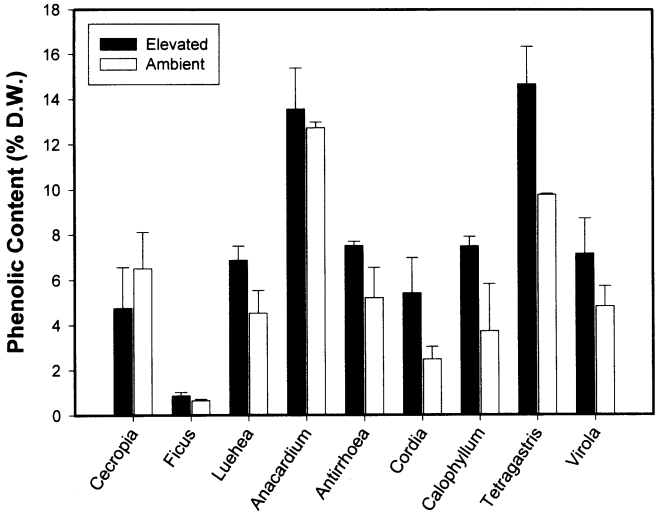

Fig. 1 Phenolic content (% dry weight) of mature leaves for nine species of tropical tree grown in open-top chambers under conditions of ambient and elevated CO_2. *Error bars* indicate SE

main effect of CO_2 concentration. However, because there was a significant species × fertilization effect (P<0.001) and a marginally significant species × CO_2 effect (P<0.1), we analyzed the species separately in order to determine how they were responding to particular treatments.

Virola surinamensis: *interactions between nitrogen and carbon enrichment*

For *Virola*, there was a strong fertilization effect on phenolic content (Table 4). Unfertilized plants had approximately 1.5 times the phenolic concentrations of fertilized plants (Fig. 2). In both fertilization treatments, plants grown at elevated CO_2 had non-significant, but slightly higher phenolic concentrations.

Phenolic concentrations were also analyzed for plants growing on the edges of the chamber where they received more light as compared to plants growing at the center, although light levels were well below saturation in both cases (between 1% and 8% of light levels above the canopy; Winter et al. 2000, 2001). There was no significant effect of location for *Virola* nor was there a significant fertilization × location effect (Table 4). Although phenolic concentrations in *Virola* responded strongly to

Table 3 Results of ANOVA analysis for the effects of CO_2 and nutrient fertilization on foliar phenolic content. The sources of variation are CO_2 levels (ambient or elevated), fertilization (fertilized or unfertilized) and species (*Ficus insipida* or *Virola surinamensis*). Chamber was nested within the different CO_2 levels

Source of variation	*df*	*F* value	*P* value
Species	1	683.13	0.0001
Fertilization	1	36.61	0.0001
CO_2	1	2.16	0.1442
Chamber (CO_2)	6	1.45	0.2016
Species×Fertilization	1	34.65	0.0001
Species×CO_2	1	2.80	0.0965
Fertilization×CO_2	1	0.86	0.3552
Species×Fertilization×CO_2	1	0.90	0.3435

Table 4 Results of ANOVA analysis for phenol production by *Virola surinamensis* in the fertilization experiment with CO_2 (ambient or elevated) and nutrients (fertilized or unfertilized). Location within the chamber (center or edge) was also included. Chamber was nested within the different CO_2 levels

Source of variation	*df*	*F* value	*P* value
Fertilization	1	39.45	0.0001
CO_2	1	2.59	0.1132
Location	1	0.43	0.5167
Chamber (CO_2)	6	1.43	0.2207
Fertilization×CO_2	1	0.92	0.3425
CO_2×Location	1	2.09	0.1543
Fertilization×Location	1	0.19	0.6677
Fertilization×CO_2×Location	1	0.37	0.5438

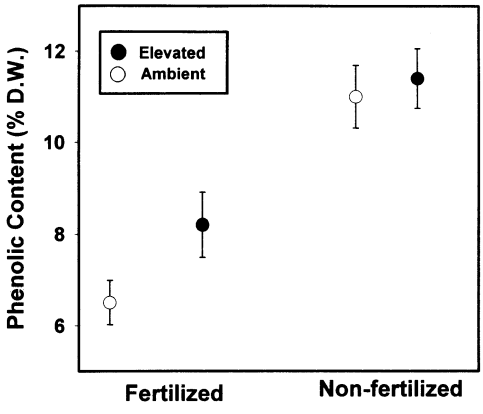

Fig. 2 Phenolic content (% dry weight) of mature leaves for *Virola surinamensis* grown under conditions of high and low nutrients and ambient and elevated CO_2 (n=70 plants). *Error bars* indicate SE

fertilization and marginally to elevated CO_2 (Fig. 2), light had no effect.

Unfertilized plants accumulated less biomass than fertilized plants, even though the unfertilized experiment ran for almost twice as long (Table 5). In addition to lower growth, non-fertilized plants accumulated more carbon in the leaves (Table 5). Increases in starch accumulation were positively associated with phenolic con-

Table 5 Effects of nutrient and CO_2 fertilization on growth and phenolic and carbohydrate concentrations in mature leaves of *Virola surinamensis*. Growth was measured as the total above-and below-ground biomass accumulated per plant during the experiment. Starch and total non-structural carbohydrates (*TNC*) were measured on leaves collected at dusk. Values for growth and carbohydrates are from Winter et al. (2000, 2001). Values are means (SE)

Treatments	Phenols (% dry weight)	Biomass (g)	Starch (mg g^{-1})	TNC (mg g^{-1})	C/N
Fertilized, ambient	6.5 (0.48)	5.1 (0.35)	6.6 (0.6)	67 (6.3)	19.3 (0.2)
Fertilized, elevated	8.2 (0.71)	6.5 (0.28)	14.2 (3.3)	58 (4.2)	21.3 (0.6)
Non-fertilized, ambient	11.0 (0.69)	4.5 (0.34)	33.0 (1.6)	145 (4.3)	39.1 (1.1)
Non-fertilized, elevated	11.4 (0.66)	4.3 (0.43)	82.6 (8.3)	174 (10.1)	48.7 (0.9)

Table 6 Results of ANOVA analysis for phenol production by *Ficus insipida* in the fertilization experiment with CO_2 (ambient or elevated) and nutrients (fertilized or unfertilized). Location within the chamber (center or edge) was also included. Chamber was nested within the different CO_2 levels

Source of variation	*df*	*F* value	*P* value
Fertilization	1	6.10	0.0168
CO_2	1	0.76	0.3876
Location	1	23.08	0.0001
Chamber (CO_2)	6	2.01	0.0814
Fertilization×CO_2	1	2.40	0.1272
CO_2×Location	1	0.00	0.9928
Fertilization×Location	1	31.74	0.0001
Fertilization×CO_2×Location	1	0.06	0.8040

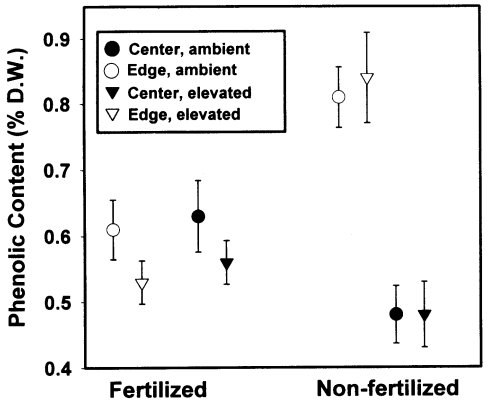

Fig. 3 Phenolic content (% dry weight) of mature leaves for *Ficus insipida* grown under conditions of high and low nutrients, and ambient and elevated CO_2. In addition, plants were divided depending on location within the chamber, as edge plants received higher light and had less root competition (n=66 plants). *Error bars* indicate SE

centrations. Phenolic content was most highly correlated with the C/N ratio (r=0.95, P<0.05). Fertilization had a large effect on C/N ratios, while CO_2 did not.

Ficus insipida*: interactions between nitrogen and carbon enrichment*

Ficus had significantly lower phenolic concentrations in fertilized as compared to unfertilized chambers (Table 6). There was also a significant interaction between fertilization and location of plants within the chamber suggesting that the phenolic concentration of fertilized and non-fertilized plants responded differently to light (Table 6). For example, all fertilized plants had similar phenolic concentrations regardless of their location (Fig. 3). In contrast, unfertilized plants responded to the increased light availability at edge locations by greatly increasing phenolic concentrations (Fig. 3). In ambient and elevated CO_2 chambers, *Ficus* plants located on the edge had 69% and 66% higher phenolic levels, respectively (Fig. 3). There was no effect of elevated CO_2 on phenolic concentrations (Fig. 3). Thus, for *Ficus*, light and nutrients caused significant effects on carbon allocation to phenolics, but elevated CO_2 did not.

In contrast to *Virola*, phenolic concentrations in *Ficus* were not correlated with starch, TNC or C/N ratios. Carbohydrate concentrations were most affected by fertilization and CO_2 concentrations, while phenolic content was

Table 7 Effects of nutrient and CO_2 fertilization on growth and phenolic and carbohydrate concentrations in mature leaves of *Ficus insipida*. Location of plants within the chambers (edge vs center) affects light levels and root competition. Growth was measured as the total above-and below-ground biomass accumulated per plant during the experiment. Starch and total non-structural carbohydrates (*TNC*) were measured on leaves collected at dusk. Values for growth and carbohydrates are from Winter et al. (2000, 2001). Values are means (SE)

Treatments	Phenols (% dry weight)	Biomass (g)	Starch (mg g^{-1})	TNC (mg g^{-1})	C/N
Fertilized, ambient, edge	0.61 (0.05)	200 (2.5)	31 (4.4)	149 (11.2)	9.5 (0.1)
Fertilized, elevated, edge	0.53 (0.03)	307 (9.0)	83 (6.7)	195 (12.6)	10.0 (0.1)
Fertilized, ambient, center	0.63 (0.05)	111 (1.3)	40 (2.1)	169 (12.8)	9.0 (0.1)
Fertilized, elevated, center	0.56 (0.03)	168 (6.8)	68 (5.7)	175 (13.4)	9.3 (0.2)
Non-fertilized, ambient, edge	0.81 (0.05)	159 (6.0)	68 (3.9)	219 (6.4)	16.1 (0.5)
Non-fertilized, elevated, edge	0.84 (0.07)	193 (10.5)	187 (4.1)	299 (2.4)	23.8 (0.5)
Non-fertilized, ambient, center	0.48 (0.04)	77 (6.0)	73 (8.6)	188 (10.2)	18.7 (1.3)
Non-fertilized, elevated, center	0.48 (0.05)	90 (1.8)	194 (6.7)	294 (8.2)	28.9 (0.3)

more affected by light (edge vs center locations, Table 7). Growth responded strongly to both fertilization and light, and less so to CO_2 (Table 7).

Discussion

Phenolic content increases in response to elevated CO_2

This is the first study to examine the effects of elevated CO_2 on secondary metabolites for tropical trees grown in their natural environment and in soil rather than pots. In temperate trees, leaf phenolic contents tend to increase by 20–60% under conditions of enriched CO_2, although there is substantial variation among species (Koricheva et al. 1998; Peñuelas and Estiarte 1998; McDonald et al. 1999; Agrell et al. 2000; Hartley et al. 2000). We found similar results for the nine tropical tree species in our study. The average increase in phenolics was 48%, with one species showing a decline, and the most responsive species showing a two-fold increase (Table 1). In the only other tropical study, potted seedlings of one species showed no response to CO_2, and the other species increased phenolics in one soil type but not the other (Kanowski 2001). Thus, both temperate and tropical trees show large interspecific variation in the extent of their response to CO_2, although the overwhelming pattern is for an increase in phenolics by approximately 50%.

Phenolic content is correlated with starch accumulation

A possible explanation for the increase in phenolics when trees are grown under conditions of elevated CO_2 is the CNB hypothesis (Bryant et al. 1983). This hypothesis says that if plants increase photosynthesis and carbon gain under enriched CO_2, the "excess" carbon will be allocated to carbon-based defenses. Although this prediction is not borne out with all carbon-based defenses, particularly terpenes, it does seem to apply to products of the shikimate pathway such as phenolics (Reichart et al. 1991; Koricheva et al. 1998; Peñueles and Estiarte 1998). In our study with nine species, CO_2 treatments did not enhance growth, suggesting that plants were limited by other resources, presumably nutrients. Thus, an increase in CO_2 would be expected to lead to an accumulation of carbon in the leaf and an increase in the C/N ratio, as it did in all nine species (Table 1). A decrease in leaf nitrogen and an increase in the C/N ratio have been seen in other studies of tropical trees (Arnone and Körner 1995; Kanowski 2001; but see Arnone et al. 1995).

The CNB hypothesis also predicts that excess carbon should be allocated to phenolics, and this was true for eight of the nine species. Across all species, there was a positive but marginally significant correlation between the percent increase in TNC and the percent increase in phenolics (r=0.53, P=0.09). In other words, species that responded to enhanced CO_2 with a greater accumulation of carbohydrates also exhibited a greater increase in phenolics (Table 1).

The CNB hypothesis also suggests that nutrient fertilization will affect the C/N ratio of the plant, and hence the allocation of resources to carbon-based defenses (Bryant et al. 1983). With fertilization, carbon can be shunted into growth so that other carbon pools, such as carbohydrates and phenolics, should decrease. In the experiments with *Virola*, fertilization increased growth and reduced leaf carbohydrates (Table 5), as predicted by the CNB hypothesis. There was a marginally significant positive correlation between TNC and phenolic contents (r=0.88, P=0.08) and an even stronger one for the C/N ratio and phenols (r=0.95, P=0.05). Fertilization had a much larger effect on phenolics than did CO_2 or light (Fig. 3). This is not surprising as fertilization also had a much larger effect on TNC, starch and the C/N ratio (Tables 4, 5). Thus, in this late successional species, the levels of carbohydrates in the leaf were strongly linked to the concentrations of phenolic compounds.

For *Ficus*, the allocation of resources to growth and phenolics also followed predictions of the CNB hypothesis (Bryant et al. 1983). At high light and low nutrients phenols accumulated, while under fertilization, growth increased and phenols dropped (Fig. 3, Table 7). However, the phenolic changes were not correlated with changes in the C/N ratios of plants in different treatments. Thus, it appears that storage carbohydrates and phenolics are not linked and are responding to different carbon sources. For example, the C/N ratio was more affected by CO_2 and phenols were more affected by light (location) (Table 7).

Impacts of leaf quality on herbivores

Although species differed in their responsiveness to treatments, elevated CO_2 had significant effects on both phenolic and nitrogen contents of leaves. An average increase of 48% phenolic content is likely to have substantial negative effects on herbivore performance. In general, studies have shown that increased phenolic content lengthens the developmental time, increases mortality and reduces adult size and fecundity for insect herbivores (Fajer et al. 1989; Lincoln et al. 1993; Lindroth et al. 1993, 1995; Roth and Lindroth 1995; Kinney et al. 1997; Coley and Kursar 2001). In addition, increases in phenolics were accompanied by increases in C/N ratios (Table 1), a common, but not universal response for trees (Lincoln et al. 1993; Arnone et al 1995; Watt et al. 1995; Lindroth 1996b). This dilution of the protein content of the leaf can also limit herbivore growth and fitness (Scriber and Slansky 1981). Finally, a nutritionally poor diet can exacerbate the negative effects of secondary metabolites (Slansky 1992, 1993). Thus the combined increases in phenolics and C/N ratios of leaves under elevated CO_2 may have substantially negative impacts on herbivores.

Under conditions of elevated CO_2, herbivore mortality could be increased because of direct effects of high

phenolic content and low protein, as discussed above. In addition, longer developmental times allow greater opportunities for predation and parasitism, further reducing herbivore survival (Price et al. 1980; Benrey and Denno 1997; Stiling et al. 1999). Thus, one might expect densities of herbivores and rates of herbivory to drop. However, a survey of 56 studies showed that herbivores tend to eat between 20 and 90% more on high phenol diets, presumably in an attempt to compensate for poor diet quality (Coley 1998; Coley and Kursar, 2001). It is therefore difficult to predict the outcome of elevated CO_2 for rates of herbivory. What is clear is that across a broad range of temperate and tropical tree species, elevated CO_2 decreases leaf nutritional value and increases phenolic compounds. These two traits strongly shape herbivore fitness and may therefore have a major impact on plant/herbivore interactions.

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