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The effects of elevated CO_2 atmospheres on the nutritional quality of Eucalyptus foliage and its interaction with soil nutrient and light availability

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Abstract Seedlings of *Eucalyptus tereticornis* (Smith) were grown under two levels of availability each of CO_2 (352 and 793 μmol mol^{-1}), soil nutrients (1/24 and 1/4 Hoagland's solution) and light (full and 30% sunlight). Low soil nutrient availability or high light increased the C:N ratio of leaves, leading to lower leaf nitrogen concentrations, higher leaf specific weights and higher levels of both total phenolics and condensed tannins. These results were consistent with other studies of the effect of environmental resource availability on foliage composition. Similar results were observed when the C:N ratio of leaves was increased under elevated CO_2. The changes in leaf chemistry induced by the treatments affected the performance of 4th-instar larvae of *Chrysophtharta flaveola* (Chapuis) fed on the leaves. Increased C:N ratios of leaves reduced digestive efficiencies and pupal body sizes and increased mortality. Below a threshold nitrogen concentration of approximately 1% dry mass, severe reductions in the performance of larvae were recorded. Such changes may have significant consequences for herbivores of *Eucalyptus*, particularly in view of projected increases in atmospheric CO_2.

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Introduction

The concentration of nutrients and plant secondary metabolites (PSMs) in plants is very variable. This is partly due to genetic differences within and between species, but there is an increasing realisation that the concentration of PSMs in plants is a function of environmental conditions (Waterman and Mole 1989). The availability of resources within a plant's environment is considered to be a major determinant of the allocation of resources by plants to PSMs and of variation in nutrient concentrations in leaves. Several studies have shown that the balance between the availabilities of carbon and soil nutrients is a primary determinant of the phenotypic expression of PSMs (Bryant et al. 1983; Coley et al. 1985; Bazzaz et al. 1987).

Coley et al. (1985) predicted that plants growing under conditions of high relative carbon availability (e.g. high light or low soil nutrient availability) will contain more carbon-based allelochemicals (terpenes, phenolics), and have lower nutrient concentrations, than plants growing under low relative carbon availability. Several studies have provided evidence that this does in fact occur (e.g. Mihaliak and Lincoln 1985; Waterman and Mole 1989; Herms and Mattson 1992).

Relative carbon availability may also be increased through elevated atmospheric CO_2, such as may occur if predictions of a doubling of present day CO_2 concentrations within the next 50–75 years (Taylor and Lloyd 1992) are accurate. Using the carbon:nutrient balance framework, we can predict that increases in carbon relative to nitrogen should increase the concentration of storage and carbon-based PSMs. Although several studies have shown that elevated CO_2 leads to a reduction in foliar nutrients in many plant species (e.g. review by Luo et al. 1994), few have shown increased levels of allocation to PSMs in these conditions. This may be because most studies have concentrated on herbaceous species which allocate relatively few resources to PSMs (e.g. Fajer et al. 1992). In contrast, two recent studies of trees have demonstrated a significant rise in the allocation of resources to carbon-based PSMs under enhanced CO_2 (Lindroth et al. 1993; Roth and Lindroth 1994). This suggests that we need to focus more of our attention on tree species if we are to establish rules governing the partitioning of resources to PSMs under enhanced CO_2.

Previous studies suggest that the changes in the balance between the concentrations of PSMs and nutrients produced by variation in the relative availabilities of carbon and soil nutrients will have a number of consequences for insect herbivores (Lincoln et al. 1993). These include reduced digestibility, growth rates and final body size and increased consumption rates, development times and mortality (Fajer et al. 1989; Johnson and Lincoln 1991; Lincoln and Couvet 1989; Lindroth et al. 1993).

We report here on experiments carried out with an evergreen tree species, *Eucalyptus tereticornis* (Smith), to determine the effects of manipulation of the availability of CO_2, soil nutrients and light on those leaf qualities considered to be of importance to herbivores. We also present data from experiments in which leaves from the experimental trees were fed to final instar larvae of the chrysomelid beetle *Chrysophtharta flaveola* (Chapuis), a common eucalypt herbivore. The data presented show that the experimental manipulations produced changes in leaf chemistry consistent with reduced quality under increased relative availability of carbon and that these changes had effects on herbivores.

Methods

Growth conditions of plants

Forty seedlings of *E. tereticornis* were grown in 25-cm-diameter pots in a medium of 1:1 sand and vermiculite. Sets of five individual plants were assigned to all combinations of two levels each of CO_2, soil nutrient and light availability. Plants were grown in a controlled environment glasshouse at James Cook University of North Queensland, Australia. Descriptions of environmental variables are means±SE. The plants were assigned to either of two separate compartments of the glasshouse: one kept at ambient CO_2 levels (352±10 μmol mol^{-1}) and the other at elevated CO_2 (793±29 μmol mol^{-1}). In each compartment equal numbers of plants were then assigned to each of two nutrient treatments: low availability (1/24 Hoagland's solution) and high availability (1/4 Hoagland's solution). To keep nutrient concentrations constant, the plants were irrigated with nutrient solution to saturation four times daily at three-hourly intervals beginning at 0800 hours. Two light treatments were also applied: plants were grown either in full sunlight or in 30% sunlight with shade applied by placing a shadecloth shelter over one bench in each side of the glasshouse. While CO_2 and light treatments are pseudo-replicated, other variables likely to affect plant growth (temperature, humidity, light period) were similar across all treatments (see below) and thus they have been considered to be replicated for the purposes of statistical analysis.

Glasshouse conditions were as follows: maximum photon flux density above the full sunlight treatment was 2100 μmol m^{-2} s^{-1}; average light period was 11 h; average day/night temperatures were 29.2/25.3° C for the ambient CO_2 compartment and 29.1/25.1° C for the elevated CO_2 compartment. The average water vapour concentrations during the photoperiod were 11.8±0.5 mmol mol^{-1} for the low compartment and 11.5±0.7 mmol mol^{-1} for the high. Conditions are described fully in Woodrow (1993).

Composition of foliage

All fully-expanded leaves (minus petioles) less than 6 weeks old were used for analysis of leaf chemistry. Leaves were removed from plants after 1700 hours to ensure similar water and photosynthate status. A subsample of fresh leaves was frozen for later extraction of steam volatile terpenes and the remainder freeze dried to minimise loss of terpenes (W.J. Foley, unpublished work) and to maximise the extraction of condensed tannins (Cork and Krockenberger 1991). Once dried, leaves were ground to pass through a 1-mm sieve.

The concentration of total carbon and nitrogen was determined with a Perkin-Elmer Series 2400 CHN analyser. Total non-structural carbohydrates (TNC) were extracted with 0.2 M sulphuric acid (Smith et al. 1964) and the resulting monosaccharides were determined colorimetrically using dinitrosalicylic acid (Englyst and Cummings 1988).

To measure cell-wall carbohydrates and lignin (dietary fibre), samples of ground leaf were extracted in neutral detergent solution (Van Soest et al. 1991), omitting sodium sulphite. Subsamples of the residue were then hydrolysed in two ways: (1) 12 M sulphuric acid for 1 h at 25° C, then 1 M sulphuric acid for 2 h in a boiling water bath (conditions were found optimal for liberating monosaccharides from cellulose); (2) hydrolysis in the 1 M sulphuric acid only, to liberate non-cellulosic monosaccharides (Englyst and Cummings 1988; Hoebler et al. 1989). The monosaccharides were determined as described above. Lignin was determined as the washed and dried residue remaining after acid hydrolysis.

Total phenolics and condensed tannins were extracted in 50% acetone and then assayed by the Folin-Ciocalteu method (total phenolics) and the HCl-butanol method (condensed tannins). Results are expressed as milligram quebracho equivalents per dry weight (Cork and Krockenberger 1991).

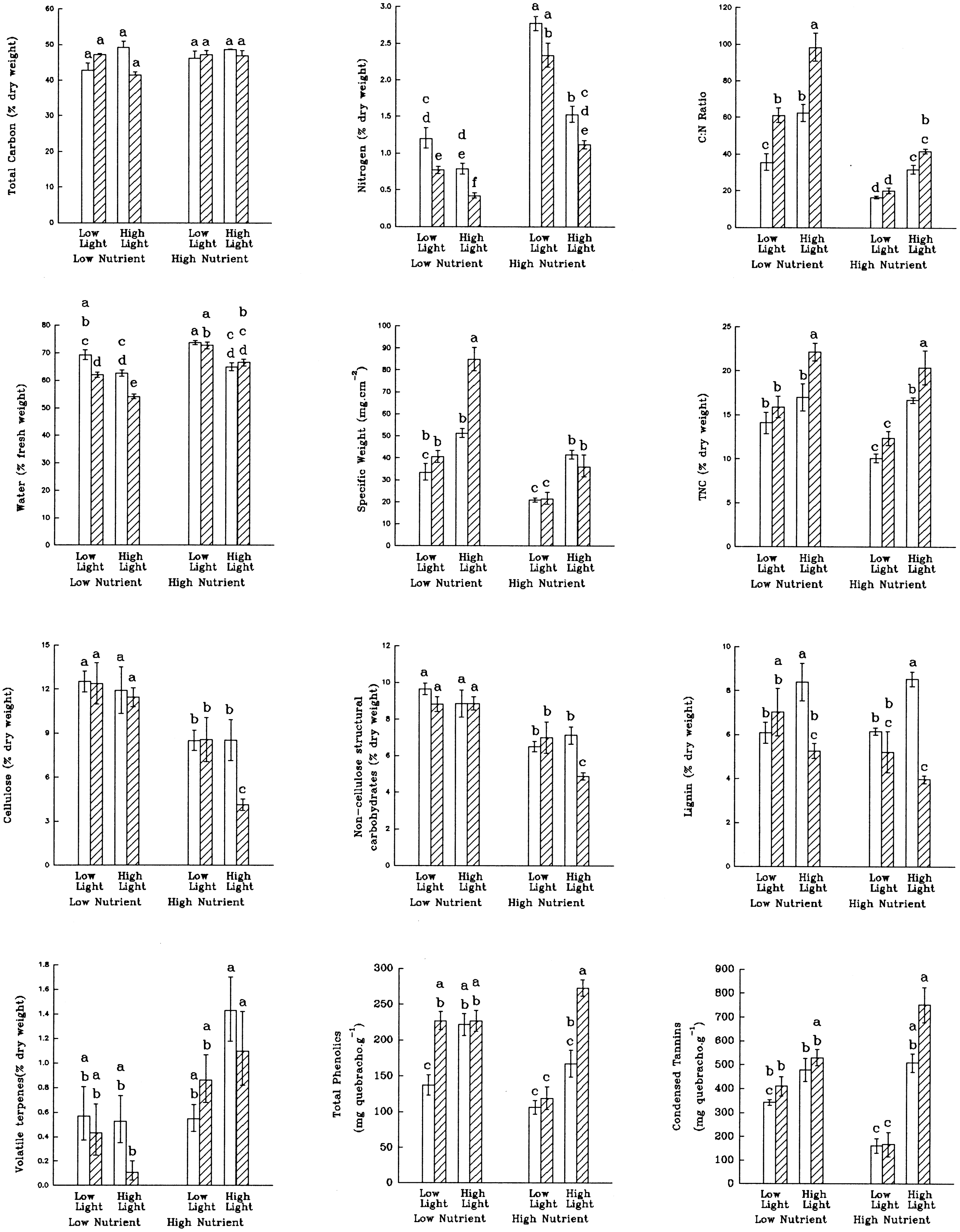
Feeding experiments with *Chrysophtharta flaveola*

Two replicate feeding experiments were carried out for each plant in each treatment combination (a total of 80 replicates). In each replicate, four newly moulted 4th-instar larvae of *C. flaveola* were placed in 9-cm plastic petri dishes with a moistened filter paper. Leaf material from one of the treatment trees was then placed in the petri dish which was kept at 28.0±0.5° C with a 12 h light/dark cycle. Only the youngest fully-expanded leaves were used, and these were replaced every two days, or earlier as required. Experiments were continued until all larvae had either died or pupated. Pupae were then sexed according to the method of Reid and Ohmart (1989) and dried for 24 h at 50° C. Initial dry weights of larvae were determined using conversions from the moisture content of a subsample of 25 other larvae, allowing calculation of total dry weight gained for each replicate.

Each leaf was split along the midrib and one half was freeze-dried to constant mass so that the dry matter content could be determined. The other half was offered to the larvae. Any leaf remaining after 24 h was freeze-dried to constant mass.

The intake of dry matter was calculated by subtracting total dry mass remaining from that offered. Frass output was determined by subtracting the initial dry weight of the filter paper (dried at 50° C for 24 h) from the final dry weight of the filter paper plus frass. Nitrogen concentrations in larvae and of the leaves offered were determined colorimetrically following Kjeldahl digestion.

Fig. 1 Leaf characteristics of *Eucalyptus tereticornis* seedlings grown under different combinations of light, nutrients and ambient CO_2. *Unshaded bars* represent low CO_2 (350 μmol mol^{-1}), *shaded bars* represent high CO_2 (800 μmol mol^{-1}). Nutrient treatments were 1/24 strength Hoagland's solution (low) and 1/4 strength Hoagland's solution (high). Light levels were 30% of sunlight (low) and full sunlight (high). *Vertical bars* represent ±1 SE from the mean. Bars labelled with *different letters* are significantly different (Tukey's test, α=0.01)


Total Carbon (% dry weight)
0
10
20
30
40
50
60
Nitrogen (% dry weight)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
C:N Ratio
0
20
40
60
80
100
120
Water (% fresh weight)
0
10
20
30
40
50
60
70
80
Specific Weight (mg.cm⁻²)
0
10
20
30
40
50
60
70
80
90
100
TNC (% dry weight)
0
5
10
15
20
25
Cellulose (% dry weight)
0
3
6
9
12
15
Non-cellulose structural carbohydrates (% dry weight)
0
2
4
6
8
10
12
Lignin (% dry weight)
0
2
4
6
8
10
Volatile terpenes(% dry weight)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Total Phenolics (mg quebracho.g⁻¹)
0
50
100
150
200
250
300
Condensed Tannins (mg quebracho.g⁻¹)
0
100
200
300
400
500
600
700
800
900
Low Light
High Light
Low Light
High Light
Low Nutrient
High Nutrient

Insect performance

Suitability of the diets was first assessed by calculation of standard indices of digestibility (Waldbauer 1968). Performance was also assessed by comparing mortality rates and final body sizes of both male and female pupae. Data for total consumption are presented rather than relative consumption rates (Waldbauer 1968), due to the difficulty in accounting for the effects of differential mortality and time of death or pupation of individuals.

Statistical analysis

Treatment effects on all variables were analysed by three-way orthogonal analysis of variance. Investigation of the relationships between the C:N ratio and other leaf variables was carried out using linear regression. Transformations were used where necessary and means and standard errors calculated by back-transforming the statistics for the transformed data. Data for dry matter and nitrogen intake in each replicate of the feeding experiments were pooled for all larvae such that a single data point was recorded for each replicate. Proportion of female pupae in each replicate was initially used as a covariate, to allow for possible differences between sexes in digestive efficiencies. It was found to have no effect and was therefore omitted from the final analysis. Final body weights of pupae were recorded individually, which allowed for separate analysis of the effects of the treatments on body weights using unbalanced ANOVAs. Due to the large number of tests used we have used a conservative α level of 0.01.

Results

Composition of foliage

Leaf nutrients

There was little effect of CO_2, light or nutrient availability on total carbon concentration in the plant with the exception of the significant, but small, interaction between CO_2 level and light availability (*P*=0.0007) (Fig. 1). The effects on leaf nitrogen were much stronger, with means for treatment combinations ranging from as low as 0.42% to 2.77% (Fig. 1). Leaf nitrogen was reduced by increased atmospheric CO_2 or light or decreased nutrient availability (*P*<0.0001 for all). Due to the small effects on total carbon and the strong nitrogen effects, those on the C:N ratio were similar, but opposite to, those of nitrogen (Fig. 1). The effect of CO_2 availability appeared to be relatively greater under low nutrient conditions (*P*=0.0119) (Fig. 1).

Water content varied significantly between treatments, being generally lower under high light or CO_2 or low nutrients (*P*<0.0001 for all). Specific weights showed much stronger responses with increases due to increased light or decreased nutrients (*P*<0.0001 for both), with a CO_2 effect under low nutrient, high light conditions, causing the CO_2 by nutrient interaction (*P*=0.0029). Total non-structural carbohydrates (TNC) were also higher when carbon availability was relatively higher (CO_2: *P*=0.0005, nutrients: *P*=0.0070, light: *P*<0.0001) (Fig. 1). Both cellulose and non-cellulose cell-wall constituents were significantly higher at low nutrient availabilities (*P*<0.0001 for both) (Fig. 1). Ligning concentrations were lower at high CO_2 (*P*=0.0002) concentrations but only when high light was available (*P*=0.0002) (Fig. 1).

Leaf PSMs

The changes in the concentration of the total phenolic compounds were consistent with the predictions of carbon-nutrient balance theories. The concentration of total phenolics increased when relative carbon availability was increased by any of the main effects (CO_2, light: *P*<0.00001, nutrients: *P*=0.0010) (Fig. 1). A significant interaction occurred between nutrients and light (*P*=0.0029) as the nutrient effect was stronger under low light. The three-way interaction (*P*<0.0001) was due to the CO_2 effect being significant only under high light, high nutrient or low light, low nutrient conditions (Fig. 1). Condensed tannins responded similarly though there was no main effect of nutrients (CO_2: *P*=0.0063, light: *P*<0.0001). Increased nutrients increased the difference between the light treatments (*P*<0.0001).

The response of volatile terpenes was more variable; the only significant main effect being an increase with increased nutrient availability (*P*=0.0003) and also an interaction which indicated that high incident light also increased concentrations of volatile terpenes, but only under high nutrient availability (*P*=0.0139) (Fig. 1).

Relationships between C:N ratio and other leaf variables

Relationships between leaf C:N and other variables are summarised in Fig. 2. Significant relationships were demonstrated between the C:N ratio and most other leaf characteristics, though the strength of relationships varied. In accordance with resource availability theories, specific weights, total non-structural carbohydrates, total phenolics and condensed tannins all increased with increases in the C:N ratio, while water content decreased. However, in contradiction to predictions, the concentration of volatile terpenes decreased with increasing C:N, although the relationship was weak, and no significant relationship was found between C:N and any of the fractions of the plant cell wall.

Consequences of treatment – induced leaf chemistry changes for beetle larvae

Consumption of foliage by beetle larvae was greater under high light, high nutrient conditions than under all other conditions (*P*=0.0085) (Fig. 3). Increasing the concentration of nutrients applied to the trees had the greatest effect on all indices of digestive efficiencies except for approximate digestibility (AD). AD was high and positive in all cases, while efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were slightly negative on the low

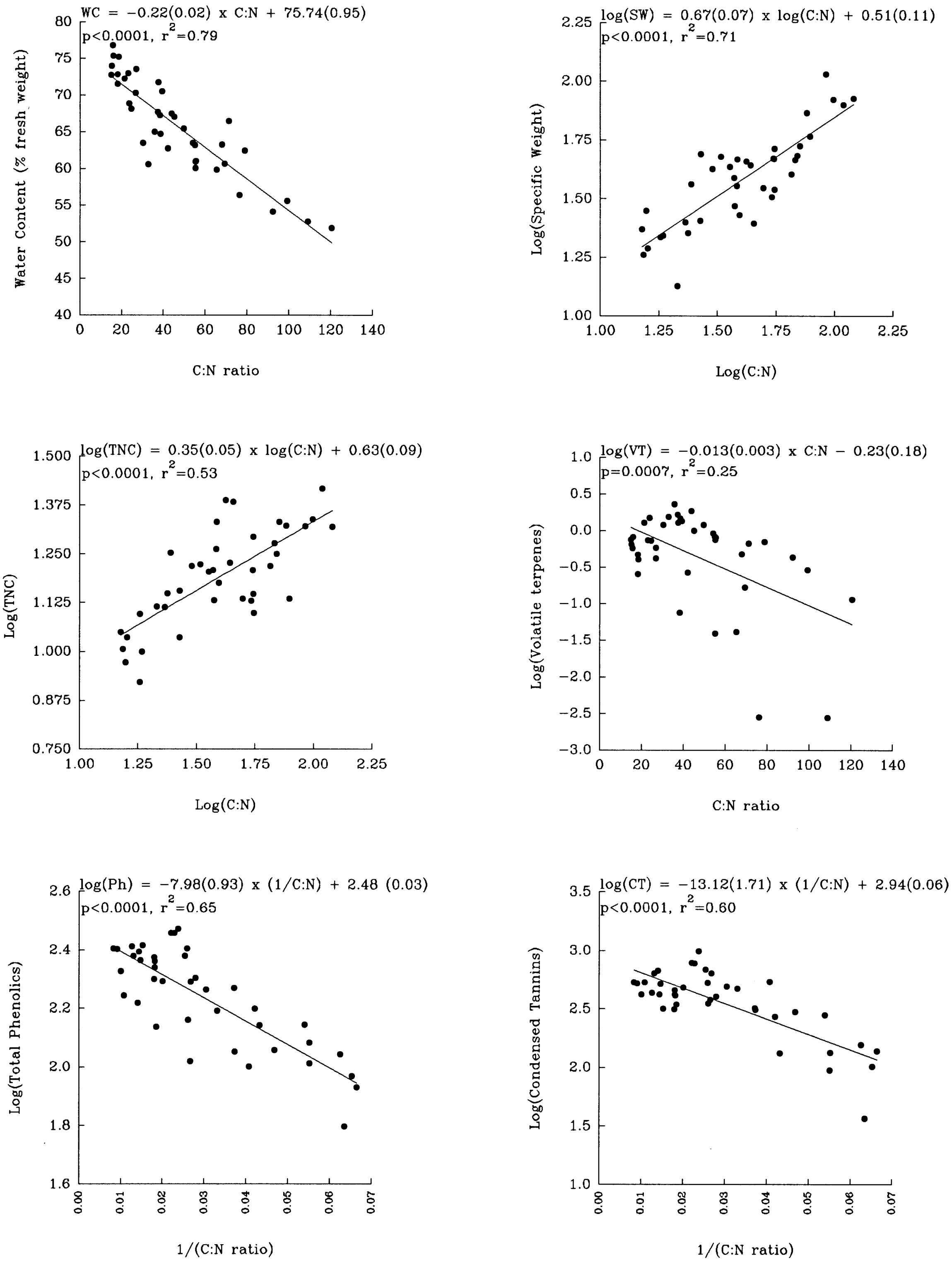
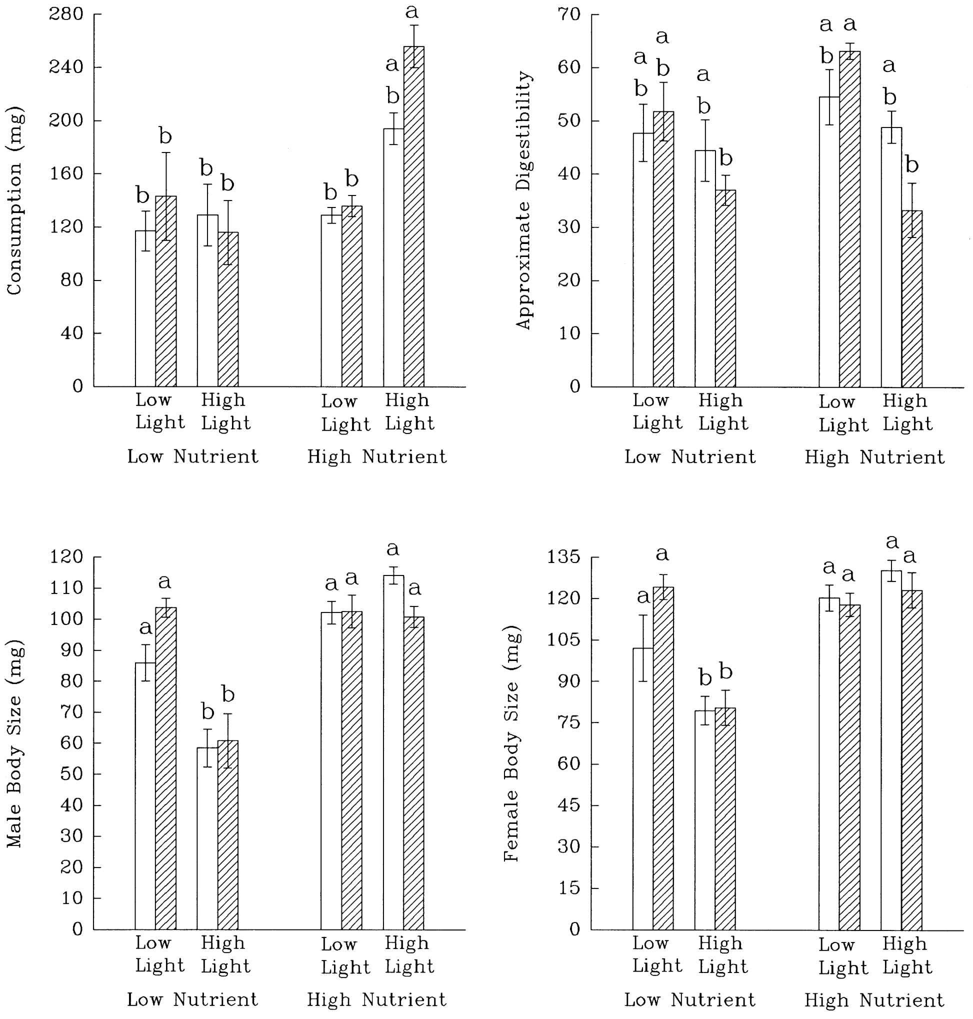


Fig. 2 Relationships between the C:N ratio of leaves and other leaf characteristics for *E. tereticornis* seedlings. The *solid lines* represent the regression equation for the two variables, which is given above each graph

nutrient treatment when averaged over CO_2 and light treatments (Table 1). The light treatment significantly affected AD and ECI and both decreased when larvae were fed leaves from plants grown under high light. CO_2 alone did not have any statistically significant effects on any of the digestive efficiency measures but interacted slightly with light as increased CO_2 increased AD under

Fig. 3 Measures of performance for larvae of *Chrysophtharta flaveola* fed leaves from the experimental *E. tereticornis.* See Fig. 1 for explanation of treatments



low light but decreased it under high light (P=0.0337) (Fig. 3).

Mortality was marginally increased only for those larvae fed plants grown with low concentrations of nutrients. This low nutrient treatment also resulted in substantially reduced body size for both males and females, whereas male body size was sightly reduced when larvae were fed leaves from plants grown under high light intensity (Table 1). The nutrient and light treatments also interacted significantly, as body weights increased when larvae were fed plants grown under low levels of light but only when this was combined with the low nutrient treatment (male: P=0.0007, female: P=0.0034) (Fig. 3).

We also investigated the relationships between the leaf variables measured and all measures of larval performance (Fig. 4). No relationship was found between consumption and any of the variables, whereas only a very weak negative linear relationship was found between AD and the log of the C:N ratio. The other three digestive indices (ECI, ECD, and nitrogen use efficiency NUE) all showed slight decreases with decreased leaf N until a threshold, at approximately 1%, at which they decreased sharply. Weak linear relationships were found between body size and the C:N ratio for both male and female pupae, with a tendency for body size to decrease with increasing leaf C:N.

Discussion

Our results show that the chemical composition of *E. tereticornis* seedlings varies according to the provision of resources in their environment, and that this has direct consequences for the herbivores feeding on their leaves. Manipulations of resource availability that increased the relative availability of carbon caused increases in the C:N ratio of leaves and these led to increases in concentrations of carbonbased compounds. These included increases in total non-structural carbohydrates, PSMs and specific weight. These manipulations also resulted in decreases in the nutritional quality (water and nitrogen content) of leaves. Significantly, few studies apart from this one have shown that carbon-based PSMs increased under elevated CO_2 (Lincoln et al. 1993; Lindroth et al. 1993).

Specific weights showed nearly two-fold differences between nutrient and light treatments. CO_2 generally had less effect on specific weight but its effects were strongest under high light and low nutrients. Specific weight is strongly correlated with penetrometer measurements of leaf toughness in *E. tereticornis* (I. R. Lawler, unpublished data) and so the increase in specific weight at low nutrient concentrations may have large impacts on insects, particularly early instar larvae, as feeding may be significantly limited by leaf toughness (Ohmart et al. 1987).

C:N ratio as a predictor

The C:N ratio was a good predictor of several factors considered to be of importance to herbivores. The responses of phenolics and condensed tannins were in accordance with theoretical predictions (Bryant et al. 1983). In general, increases in the C:N ratio caused increases in phenolics. Light had the strongest effect, particularly on condensed tannins.

Significant relationships were also identified between C:N and all other variables, although the relationship with the concentration of volatile terpenes was weakly negative. This is strong evidence of a direct causal link between the C:N ratio and the allocation of carbon to potential defense compounds. It has been suggested that secondary compounds accumulate via "overflow metabolism", where carbohydrates in excess of those required for primary metabolic functions are diverted into secondary compound production (Waterman and Mole 1989). The C:N ratio may be a useful indicator of the amount of this overflow, as the nitrogen content should be related to enzyme concentrations, and therefore the metabolic activity, of the leaf. The different effects on volatile terpenes, as compared to phenolic compounds (discussed in the next section) however, suggest that other, as yet unidentified, factors are also important.

Why do all PSMs not respond similarly?

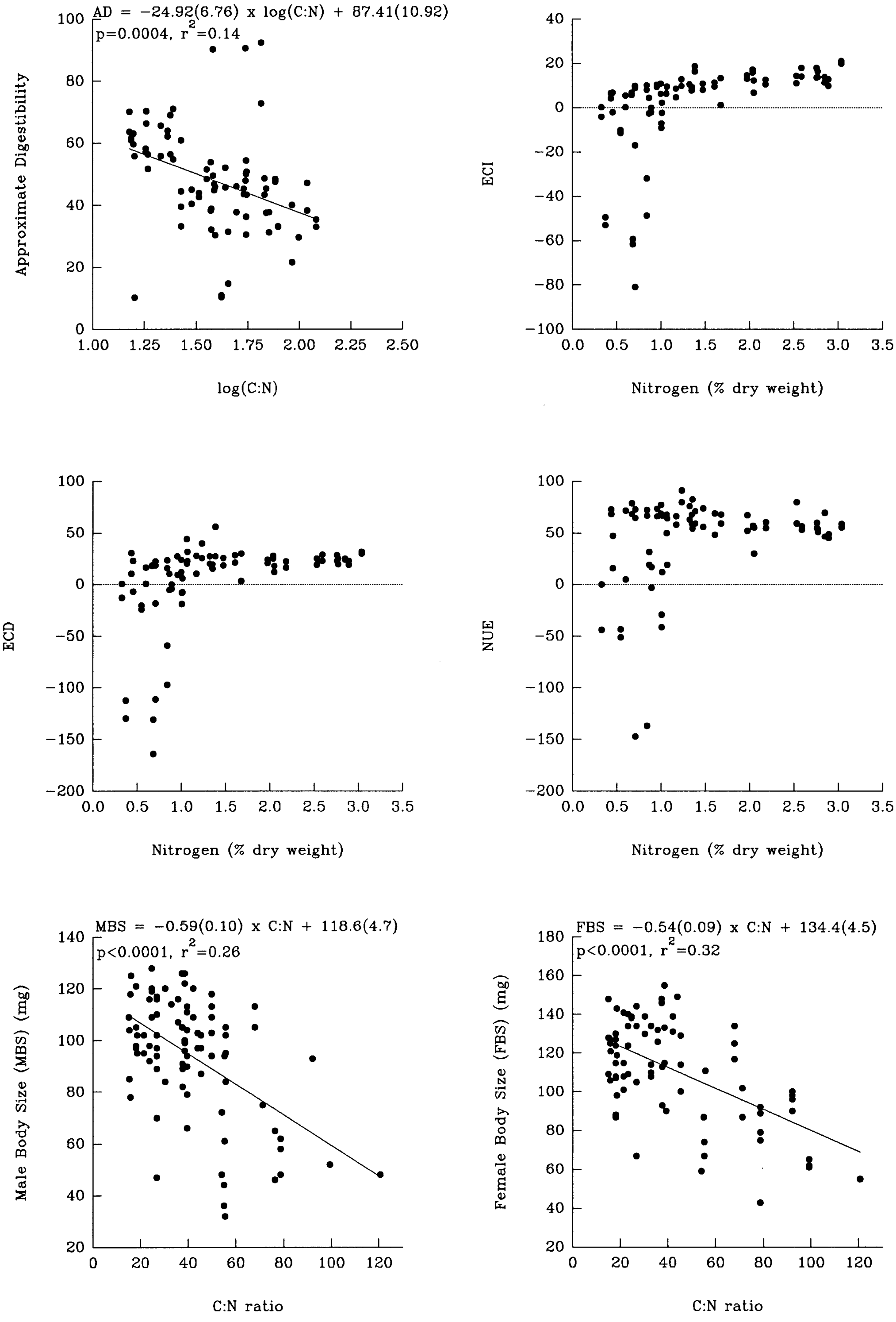
The concentration of volatile terpenes did not behave as predicted by the carbon-nutrient balance hypothesis. Increasing light intensity increased terpene concentration, though this only occurred at high soil nutrient availability. Counter to the hypothesis, terpene concentrations were increased by increased soil nutrient availability. Other studies have reported variable responses (Waterman and Mole 1989) including decreases in terpenes in increased nutrients (Fajer et al. 1992). Similarly, previous studies have failed to observe any effects of increased CO_2 on the concentration of total (Lincoln and Couvet 1989; Johnson and Lincoln 1990) or individual (Fajer et al. 1992) terpenoids. This has led to the suggestion that although increasing CO_2 increases the C:N ratio, it does not stimulate the enzymes necesary to increase allelochemical production (Johnson and Lincoln 1991; Fajer et al. 1992).

The increases in phenolics under high CO_2 (Lindroth et al. 1993; this study) suggest that the responses of plants depend on the type of PSMs present. The differ-

Table 1 Summary of means of measures of beetle performance for main treatments averaged over both levels of each of the other treatments, with significance levels of differences. (n.b. values in parentheses are standard errors. Where data have been back-transformed, positive and negative errors are provided respectively.) See Fig. 1 for explanation of treatments. (*AD* approximate digestibility, *ECI* efficiency of conversion of ingested food, *ECD* efficiency of conversion of digested food, *NUE* nitrogen use efficiency)

Treatment		Amount eaten (mg)	AD	ECI	ECD	NUE	Male body size	Female body size	Mortality
CO_2	Low	142.0 (8.8)	48.917 (2.452)	9.027 (1.513, 1.382)	17.131 (3,352, 3.152)	56.855 (5.013, 4.371)	91.840 (3.7373)	109.95 (4.2907)	0.4375 (0.0496)
	High	162.6 (13.9)	46.326 (2.741)	4.611 (2.268, 2.041)	7.799 (5.220, 4.816)	39.93 (9.300, 7.798)	97.209 (2.9282)	111.88 (3.7896)	0.4875 (0.0551)
P		0.2626	0.5244	0.1222	0.1894	0.1664	0.5673	0.8303	0.5861
Nutrients	Low	125.9 (11.9)	45.253 (2.560)	−0.739 (2.771, 2.501)	−0.243 (6.582, 6.018)	32.947 (12.980, 10.472)	81.095 (3.9786)	92.129 (4.6924)	0.5437 (0.0606)
	High	178.7 (9.8)	49.989 (2.602)	12.347 (0.728, 0.689)	23.183 (1.063, 1.039)	60.930 (2.013, 1.889)	105.22 (1.9518)	122.58 (2.4012)	0.3812 (0.0390)
P		0.0062	0.2482	0.0001	0.0021	0.0274	0.0001	0.0001	0.0833
Light	Low	131.0	54.315	9.920 (1.789, 1.600)	16.024 (4.315, 3.995)	51.235 (6.879, 5.843)	96.750 (2.7823)	116.56 (3.1803)	0.4938 (0.0486)
	High	173.6 (12.9)	40.927 (2.320)	3.403 (1.716, 1.589)	9.074 (4.237, 3.962)	47.245 (7.149, 6.112)	92.143 (3.8705)	105,69 (4.5062)	0.4313 (0.0559)
P		0.0242	0.0022	0.0259	0.3251	0.7405	0.0035	0.1023	0.4966

Fig. 4 Relationships between measures of performance of larvae of *Chrysophtharta flaveola* and the quality of *E. terticornis* leaves that they were fed, expressed as the C:N ratio or nitrogen concentration. For those graphs in which linear relationships are shown (approximate digestibility and body size), the *solid line* represents the regression equation which is given above the graph. For the graph of nitrogen use efficiency (NUE) six values between –200 and –710 have been omitted to increase resolution



ence in responses between volatile terpenes and phenols may be due to their differing biosynthetic pathways. Terpenes are products of the mevalonic acid pathway while phenolics are derived from the shikimic acid pathway, although some of the carbon in condensed tannins is also derived from acetate (Waterman and Mole 1989). Secondary metabolism is regulated according to the availability of the precursors (Waterman and Mole 1989) thus perhaps those for phenolics are produced relatively more with high C:N than are those for volatile terpenes.

Alternatively, the production of volatile terpenes may be under stronger genetic control (e.g. Lincoln and Couvet 1989). Muzika (1993) suggests that direct competition for carbon between phenolics and amino acids makes a response to increased carbon more likely for phenolics than for terpenoids. Muzika (1993) found increases in individual phenolics in response to nutrient addition, even though the concentration of total phenolics decreased. Thus there is evidence that excess carbon is used in the shikimic acid pathway but it is not yet clear what controls its allocation to individual phenolics.

Another factor that may be important is the fact that increases in phenolics have been reported only in tree species, while other studies have used herbaceous species (e.g. *Plantago:* Fajer et al. 1992) or woody shrubs (e.g. *Artemisia:* Johnson and Lincoln 1990, 1991). Allocation of carbon may differ between these growth habits because of different investment in storage and structural components. Clearly, future studies of carbon partitioning in response to elevated CO_2 have to take account of different growth habits.

Consequences of treatment effects on leaf chemistry for an insect herbivore

The results of our experiments with *C. flaveola* fed leaves from *E. tereticornis* grown under varying availabilities of CO_2, soil nutrients and light show that the changes induced in leaf chemistry have important consequences for herbivores. Consumption changed with changes in the availability of light and soil nutrients. However, the observed effect of nutrients was not consistent with previous studies (e.g. Lincoln and Couvet 1989; Johnson and Lincoln 1990, 1991); consumption of plants grown with high nutrient concentrations actually increased.

This increase was due to the combined effects of the nutrient and light treatments, because plants grown under the low nutrient regime and with high levels of light, appeared too tough for the larvae to eat. The larvae attempted to eat these leaves but were unsuccessful, as evidenced by their chewing around the whole perimeter of the leaf, but never eating a substantial amount at any point, in contrast to their usual tendency to chew a semicircular patch out of the edge of the leaf (I. R. Lawler, personal observations). This suggests that compensatory consumption could not occur where it might have been expected to be greatest. These results are consistent with the suggestion of Ohmart et al. (1987) that leaf toughness may be of vital importance for insect herbivores. These effects are likely to be even more important for early instar larvae.

Digestibility of the diets was related to either the C:N ratio or N alone. Approximate digestibility had a significant, though weak, relationship with C:N. Approximate digestibility was reduced by increasing C:N, although this was not related to increases in the concentration of cell wall carbohydrates or lignin. The results for ECI, ECD and NUE are consistent with other studies that have found a threshold nitrogen concentration, at approximately 1% dry weight, below which digestive efficiency of larvae is greatly reduced, leading to high mortality and reduced body sizes (Fox and Macauley 1977; Ohmart et al. 1985a). The main effects of each treatment produced differences of up to 50%, and in combination were strong enough to reduce leaf N to below the 1% threshold observed.

It is important to use absolute measures of performance, such as pupal body size and mortality, as well as indices, to assess the effects of variable plant chemistry on insects. This because population dynamics are affected by absolutes rather than by relative estimates, such as consumption and digestive efficiencies (Ohmart et al. 1985b). Fecundity is directly related to body size in species closely related to *C. flaveola* (Ohmart et al. 1985a, b). On this basis, the negative relationships between C:N and body size for both males and females suggest that the resources available to *E. tereticornis* plants can directly affect the population dynamics of their herbivores. Similarly, our results are indicative of an increase in mortality for larvae feeding on leaves from the plants grown under low nutrient regimes and this may be a significant modulator of insect herbivore populations.

Our data illustrate the complexity of predicting impacts of elevated atmospheric CO_2 on animals in forests. The chemical composition of *E. tereticornis* changed sufficiently under elevated CO_2 so that it could be expected to affect insect herbivores (Lincoln et al. 1993; Lindroth et al. 1993). However, elevated CO_2 had different effects on leaf consumption and digestibility, depending on light and nutrient treatments, and had no statistically significant effect on male or female body size. At high nutrient concentrations and high light availability, increased CO_2 reduced leaf digestibility but this was balanced by compensatory feeding (Fig. 3). However, at low concentrations of nutrients and/or low light, it appeared that leaf consumption was already so limited that elevated CO_2 could not reduce it further. The effect of CO_2, then, may be important only in conditions in which leaf quality is marginal, such as may occur with intermediate soil nutrient availability and high light.

The foliage of *Eucalyptus* species in many forests is considered to be of marginal quality for herbivores, particularly with respect to nitrogen concentrations. Typical nitrogen concentrations in *Eucalyptus* are rarely above 2% and thus their beetle herbivores may be considered to be feeding on a suboptimal diet (Fox and Macauley 1977). If increased atmospheric CO_2 reduces the nitrogen concentration of these leaves to below the threshold of approximately 1%, the consequences for insect herbivores may be great. The effects are likely to be greater than is indicated by results here, because of stronger effects on early instar larvae, particularly as a result of increased leaf toughness (Ohmart et al. 1987). Further effects may be observed for adults, as reduction in nitrogen contents of leaves may lead to reduced fecundity, egg viability and adult survival (Ohmart et al. 1985b). The increased phenolic compounds observed under elevated CO_2 may also affect other insect herbivores of Eucalyptus, such as psyllids (Ohmart and Edwards 1991).

Conclusion

The treatments used in these experiments clearly affected the nutritional quality of *E. tereticornis* leaves for *C. flaveola*. The magnitude of the differences between nutrient (6-fold) and light (3-fold) treatments was much greater than that of the CO_2 treatment (2-fold) and this appears to have obscured any possible effects of CO_2 on beetle performance. In reality, the nutrient concentrations of soil, and therefore the quality of *Eucalyptus* leaf, is far more marginal than in the treatments used here. Hence, there is a clear need for further studies using a range of nutrient concentrations that more precisely reflect those found in nature, to truly estimate the likely impacts of elevated CO_2 atmospheres on insect herbivores of *Eucalyptus*. These will likely be greater than indicated here and will primarily occur via CO_2-induced effects on leaf nitrogen and specific weight.

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References

Bazzaz FA, Chiarello NR, Coley PD, Pitelka LF (1987) Allocating resources to reproduction and defense. Bioscience 37:58–67

Bryant JP, Chapin FS III, Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357–368

Coley PD, Bryant JP, Chapin FS III (1985) Resource availability and plant antiherbivore defense. Science 230:895–899

Cork SJ, Krockenberger AK (1991) Methods and pitfalls of extracting condensed tannins and other phenolics from plants: insights from investigations on *Eucalyptus* leaves. J Chem Ecol 17:123–124

Englyst HN, Cummings JH (1988) Improved method for measurement of dietary fiber as non-starch polysaccharides in plant foods. J Assoc Off Agric Chem 71:808–814

Fajer ED, Bowers MD, Bazzaz FA (1989) The effects of enriched CO_2 atmospheres on the buckeye butterfly, *Junonia coenia*. Ecology 72:751–754

Fajer ED, Bowers MD, Bazzaz FA (1992) The effects of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in *Plantago:* a test of the carbon/nutrient balance hypothesis. Am Nat 140:707–723

Fox LR, Macauley BJ (1977) Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. Oecologia 29: 145–162

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or to defend. Q Rev Biol 67:283–335

Hoebler C, Barry JL, David A, Delort-Laval J (1989) Rapid acid hydrolysis of plant cell wall polysaccharides and simplified quantitative determination of their neutral monosaccharides by gas-liquid chromatography. J Agric Food Chem 37:360–367

Johnson RH, Lincoln DE (1990) Sagebrush and grasshopper responses to atmospheric carbon dioxide concentration. Oecologia 84:103–110

Johnson RH, Lincoln DE (1991) Sagebrush carbon allocation patterns and grasshopper nutrition: the influence of CO_2 enrichment and soil mineral limitation. Oecologia 87:127–134

Lincoln DE, Couvet D (1989) The effect of carbon supply on allocation to allelochemicals and caterpillar consumption of peppermint. Oecologia 78:112–114

Lincoln DE, Fajer ED, Johnson RH (1993) Plant-insect herbivore interactions in elevated CO_2 environments. Trends Ecol Evol 8:64–68

Lindroth RL, Kinney KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry, and insect performance. Ecology 74:763–777

Luo Y, Field CB, Mooney HA (1994) Predicting responses of photosynthesis and root fraction to elevated CO_2: interactions among carbon, nitrogen and growth. Plant Cell Environ 17: 1195–1204

Mihaliak CA, Lincoln DE (1985) Growth pattern and carbon allocation to volatile leaf terpenes under nitrogen-limiting conditions in *Heterotheca subaxillaris* (Ateraceae). Oecologia 66: 423–426

Muzika RM (1993) Terpenes and phenolics in response to nitrogen fertilisation: a test of the carbon/nutrient balance hypothesis. Chemoecology 4:3–7

Ohmart CP, Edwards PB (1991) Insect herbivory on *Eucalyptus*. Annu Rev Entomol 36:637–657

Ohmart CP, Stewart LG, Thomas JR (1985a) Effect of food quality, particularly nitrogen concentrations, of *Eucalyptus blakelyi* foliage on the growth of *Paropsis atomaria* larvae (Coleoptera: Chrysomelidae). Oecologia 65:543–549

Ohmart CP, Stewart LG, Thomas JR (1985b) Effect of nitrogen concentrations of *Eucalyptus blakelyi* foliage on fecundity of *Paropsis atomaria* (Coleoptera: Chrysomelidae). Oecologia 68:41–44

Ohmart CP, Stewart LG, Thomas JR (1987) Nitrogen, leaf toughness and the population dynamics of *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae) – a hypothesis. J Aust Entomol Soc 26:203–207

Reid CAM, Ohmart CP (1989) Determination of the sex of pupae of *Paropsis atomaria* Olivier, and related Paropsina (Coleoptera: Chrysomelidae). J Aust Entomol Soc 28:29–30

Roth SK, Lindroth RL (1994) Effects of CO_2 mediated changes in paper birch and white pine chemistry on gypsy moth performance. Oecologia 98:133–138

Smith D, Paulsen GM, Raguse CA (1964) Extraction of total available carbohydrates from grass and legume tissue. Plant Physiol 39:960–962

Taylor JA, Lloyd J (1992) Sources and sinks of atmospheric CO_2. Aust J Bot 40:401–418

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583-3597

Waldbauer GP (1968) The consumption and utilisation of food by insects. Adv Insect Physiol 5:229–289

Waterman PG, Mole S (1989) Extrinsic factors influencing production of secondary metabolites in plants. In: Bernays EA (ed) Insect-plant interactions, vol I. CRC, Boca Raton, pp 107–137

Woodrow IE (1993) Control of steady-state photosynthesis in sunflowers growing in enhanced CO_2. Plant Cell Environ 17: 277–286