

# GROWTH AND CHEMICAL DEFENSE IN RELATION TO RESOURCE AVAILABILITY: TRADEOFFS OR COMMON RESPONSES TO ENVIRONMENTAL STRESS?

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## ABSTRACT

One aspect of plant defense is the production of constitutive secondary compounds that confer toxicity on herbivores and pathogens. The purpose of this study was to compare patterns of plant tissue toxicity across gradients of irradiance and nutrient content. We measured the potential toxicity ( $1/LC_{50}$ ) of extracts of six species of herbaceous Asteraceae grown under controlled conditions of temperature (25°C), humidity (80%), photoperiod (16 h/day), in a range of concentrations of a modified Hoagland hydroponic solution (full-strength, 1/5 dilute, 1/10 dilute, and 1/50 dilute) and under two different light intensities (250 and 125  $\mu\text{mol}/\text{m}^2/\text{s}$ ). The plants grew from seed for 42 days post-germination, and randomly chosen plants were harvested each 7 days. We did a general measure of potential phytochemical toxicity using an alcohol extraction of secondary compounds followed by brine shrimp (*Artemia* sp.) bioassay. Contrary to the carbon/nutrient balance hypothesis, tissue toxicity generally increased with decreasing irradiance and nutrient levels, so that plants whose growth was most restricted had tissues that were most toxic, although there were species-specific differences in this trend.

*Key Words:* Asteraceae, nutrient availability, secondary metabolites, plant defense, RGR.

## RESUMO

### **Crescimento e defesa química em relação à disponibilidade de recursos: compromisso ou resposta comum a estresse ambiental?**

Um dos aspectos da defesa das plantas é a produção de compostos secundários constitutivos que são tóxicos para herbívoros e patógenos. O objetivo deste estudo foi comparar padrões de toxidez nas folhas em diferentes gradientes de nutrientes e intensidade luminosa. Foi medida a potencial toxidez ( $1/CL_{50}$ ) em extratos de seis espécies herbáceas de Asteraceae, desenvolvidas sob condições controladas de temperatura (25°C), umidade (80%) e fotoperíodo (16 h/dias), com diferentes concentrações de solução hidropônica de Hoagland modificada (completa, 1/5, 1/10 e 1/50 diluídas) e sob três intensidades de luminosidade (250 e 125  $\mu\text{mol}/\text{m}^2/\text{s}$ ). As plantas germinadas foram acompanhadas por 42 dias e, então, 15 indivíduos de cada espécie foram coletados ao acaso a cada 7 dias. Foi realizada uma medida geral da toxidez fitoquímica potencial usando extração alcoólica de compostos secundários das plantas estudadas, seguida do teste biológico com *Artemia* sp. Contrariando a hipótese de carbono/nutriente, a toxidez do tecido geralmente aumenta com a redução dos níveis de nutrientes e luminosidade. Isto é, plantas que tiveram crescimento restringido apresentaram-se mais tóxicas, embora as espécies tivessem tendências diferentes.

*Palavras-chaves:* Asteraceae, disponibilidade de nutrientes, compostos secundários, defesa vegetal, TCR.

## INTRODUCTION

Studies of the resource availability hypothesis have tended to contrast the defense capacities of the plant species growing in two different resource states (Bryant & Kuropat, 1980; Coley, 1983; Newberry & de Foresta, 1985). However, in most natural communities, individuals within a population of plants may often experience many different levels of resource availability. These differences have been shown to generate variation in defensive chemistry (Bryant *et al.*, 1987a, b) which, even on a small spatial scale, may influence host selection and subsequent success of insect herbivores (Zangerl & Berenbaum, 1993). Therefore, it is important to understand how a range of resource availabilities influences phenotypic variation in plant allocation to defensive chemistry. Few studies have examined how a range (i.e., more than two levels) of a resource affects allocation to defensive chemistry and growth-related characteristics (Mihaliak & Lincoln, 1985; Waring *et al.*, 1985). How might resource availability constrain secondary metabolism and, thereby, plant defensive responses?

The objective of this study was to investigate if there is any correlation between relative growth rate (RGR) and potential toxicity due to secondary compounds under different combinations of light intensity and nutrient availability for 6 species of Asteraceae.

## MATERIALS AND METHODS

### *Plant material*

The family Asteraceae make up one of the largest and most successful flowering plant families, consisting of 12-17 tribes, approximately 1,100 genera of over 20,000 species, and having a cosmopolitan distribution (Gleason & Cronquist, 1991). We used six herbaceous species from 4 different tribes (*Achillea millefolium*, *Arctium minus*, *Chrysanthemum leucanthemum*, *Cichorium intybus*, *Matricaria matricarioides*, and *Rudbeckia hirta*). Taxonomy follows Gleason & Cronquist (1991). These six species had high, intermediate, and low values of RGR and of potential phytochemical toxicity ( $LC_{50}$ ) based on a previous experiment (Almeida-Cortez *et al.*, 1999). Of the 6 species, there was 1 annual (*Matricaria matricarioides*), 1 biennial (*Arctium minus*), and 4 perennial growth forms (Marie-Victorin, 1964).

Seeds were collected from wild populations across southwestern Quebec (Canada), stored in paper bags in a refrigerator at 4°C prior to germination, and were germinated on wet filter paper. Within 2-3 days of germination, seedlings were transplanted individually into separate small blocks of rock wool ( $2 \times 2 \times 4 \text{ cm}^3$ ) that served as a support medium. Rock wool is a sterile and inert mineral fiber, without phytotoxic substances, and commonly used in hydroponic culture. To minimize algal growth and reduce evaporation, aluminum foil was placed around each seedling on the upper surface of the rock wool.

### *Hydroponic system*

The aerated standing nutrient system consisted of 12 polyethylene containers ( $36 \times 36 \times 30 \text{ cm}^3$ ). Each container was divided into 144 compartments ( $2.5 \times 2.5 \times 21.5 \text{ cm}^3$ ) using polyethylene sheets; there were therefore approximately 10 cm of undivided space at the bottom of each container, thus allowing free circulation of the hydroponic solution between compartments. The four corner compartments of each container were used to introduce aeration tubes and to monitor the temperature, pH, and nitrate concentration. Therefore, each container originally held 140 plants; this number was reduced as harvests proceeded. Each compartment contained a block of rock wool ( $2 \times 2 \times 4 \text{ cm}^3$ ) which functioned as a support medium, and roots hung freely in the solution. Aquarium pumps were used to aerate and circulate the solution inside of each container. Each container was filled with 30 L of modified Hoagland solution (Hoagland & Arnon, 1950). The full-strength (1/1) solution has a nitrogen concentration of 3 mM Ca ( $\text{NO}_3$ )<sub>2</sub>·4H<sub>2</sub>O, 2 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 5 mM KH<sub>2</sub>PO<sub>4</sub>, 2 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 9.07 mM MnSO<sub>4</sub>, 0.765 mM ZnSO<sub>4</sub>·7H<sub>2</sub>O, 46.4 mM H<sub>3</sub>BO<sub>3</sub>, 0.09 mM Na<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O, 0.01 mM CuSO<sub>4</sub>, plus 36 mM FeSO<sub>4</sub>·7H<sub>2</sub>O as iron-EDTA. The conductance was measured and the solution was topped off daily with the same solution or distilled water as required to compensate for water loss due to evaporation and transpiration. The nutrient solution in each container was completely renewed every week; the pH of a freshly prepared solution was 6.1. The pH and the nitrate concentration were monitored daily with a NO<sub>3</sub> selective electrode for readjustment.

### *Experimental design*

The experiment was conducted under controlled conditions in a Conviron (PGW36) growth chamber. Plants were supplied with a photosynthetic

photon flux density (PPFD) of 250 and 125  $\mu\text{mol}/\text{m}^2/\text{s}$ , provided by a combination of fluorescent tubes and incandescent bulbs, for 16 hours a day. This provided a daily integrated photon flux of 14.4 and 7.2 moles/ $\text{m}^2$  respectively. The temperature was maintained at 25°C by day and 20°C by night and the relative humidity was 80%. Plants from each light intensity treatment were grown separately but always in the same growth chamber.

The experimental design consisted of all possible combinations of two levels of light intensity (250 and 125  $\mu\text{mol}/\text{m}^2/\text{s}$ ) and four levels of nutrient concentration (1/1, 1/5, 1/10, and 1/50 dilution of full-strength modified Hoagland solution), giving 8 environmental combinations in all for each of the 6 species. Fifteen plants per species per environmental combination were randomly chosen for each harvest period, yielding a total of 120 plants per species per harvest. Harvest dates were at 21, 28, 35, and 42 days after transplanting into the hydroponic system.

At each harvest, plants were separated into leaves, stem, bud flowers or flowers, and roots. Roots were separated at the base of each plant at ground level and washed free of rock wool with tap water. All plant parts were blotted dry with paper towels and fresh weights were measured. Leaf blades and flowers were placed in a plant press, and roots and stems were placed in paper bags. Enough plants were randomly chosen at each harvest/species/treatment combination to provide approximately 1 g fresh weight of tissue (between 1 and 5 plants); these plants were immediately placed in 100% ethanol and used for the toxicity measurement. The remaining plants were allowed to dry at 80°C in a forced air drying oven to a constant dry weight for a minimum period of 48 hours and were used to estimate RGR.

The relative growth rate (RGR,  $\text{g g}^{-1} \text{day}^{-1}$ ) of each species was estimated as the slope of the linear regression of the natural logarithm of seedling dry mass on time. Units are grams of new biomass produced per gram of pre-existing biomass per day ( $\text{g g}^{-1} \text{day}^{-1}$ ). Thus, RGR was a mean taken over the 21-42 day growth period.

#### *Bioassay of plant chemical potential toxicity*

To measure this potential toxicity, we use the brine shrimp bioassay (Arnason *et al.*, 1991). This bioassay is widely used as an initial screening technique in pharmacology and is known to be sensitive to a large number of secondary compounds present in seeds of 41 species of Euphorbiaceae (Meyer *et*

*al.*, 1982), alkaloids (Lopez *et al.*, 1997), and sesquiterpenes (He *et al.*, 1997).

Details of the method are given in Arnason *et al.* (1991) and Almeida-Cortez *et al.* (1999); only the logic of the test is presented here. Ethanol was used because its intermediate polarity allows most biologically active secondary compounds to be extracted, including phenolics, alkaloids, acetylenes, terpenes, and other less common secondary compounds (Liskens & Jackson, 1992). After a general extraction of the fresh tissues in 100% ethanol followed by evaporation, the extractant is diluted again in ethanol at a ratio of 1 ml per gram fresh weight of plant tissue. Four-day-old brine shrimp nauplii were used in the bioassay. Approximately 40 nauplii in 4.9 ml of brine solution were placed in a test tube. A further 100  $\mu\text{l}$  addition was added based on serial logarithmic dilutions (0:100; 1:99, 10:90, 100:0  $\mu\text{l}$ :  $\mu\text{l}$  of solution: ethanol).

#### *Statistical analysis*

The concentration required for 50% mortality in the brine shrimp assay after 24 hours ( $\text{LC}_{50}$ ;  $\mu\text{g}/\text{ml}$ ) was calculated using probit regression (SAS, Inc. 1990). These values were then transformed to their inverse ( $1/\text{LC}_{50}$ ) so that larger values indicate a greater toxicity and, therefore, that a lower concentration of tissue extract is needed to produce 50% mortality within 24 h. Controls were included with each analysis and results were accepted only when mortality in the controls was less than 5%.

Treatment effects were tested using ANOVA and type III sums of squares. In the case of overall growth rate, the error term was based on the replicate mean square within each light/nutrient/species treatment combination. Because tissues of more than one plant per light/nutrient/harvest/species treatment combination had to be pooled in order to obtain 1 g of fresh weight, we did not have replication at this level. Instead, we used the "light X nutrient X harvest X species" interaction term as the error term. Statistical significance was evaluated at the  $p < 0.05$ .

## RESULTS

#### *General observations*

Daily measures of solution nitrate concentration showed that target levels were always maintained within narrow limits. The nitrate concentration in the solution ranged from 7.4 to 9.3; 1.5 to 1.7; 0.7 to 0.8; and 0.16 to 0.15 millimoles for 1/1, 1/5, 1/10,

and 1/50 dilution of the full-strength modified Hoagland solution, respectively. Toxicity (as measured by the brine shrimp bioassay) was measured weekly in the hydroponic solutions from each container. Measurable toxicity of the samples was never different from the controls, indicating that no detectable secondary compounds were diluted into the hydroponic solution.

#### *Variation in the growth parameters*

Differences in the mean of relative growth rates (RGR), root: shoot ratios, specific leaf area (SLA), and chemical characteristics (% nitrogen in leaves and mean of measurable toxicity in the brine shrimp test) among treatments are summarized in Table 1.

The slowest growing species was *Arctium minus* (RGR = 0.031 g g<sup>-1</sup> day<sup>-1</sup> grown under light intensity 250 μmol/m<sup>2</sup>/s PAR and 1/50 dilution of the full-strength modified Hoagland solution), and the fastest growing species was *Rudbeckia hirta* (RGR = 0.250 g g<sup>-1</sup> day<sup>-1</sup> grown under light intensity 250 μmol/m<sup>2</sup>/s PAR and full-strength modified Hoagland solution). The same two species show similar values for the SLA *Arctium minus*, 156.576 (250 μmol/m<sup>2</sup>/s PAR and 1/50 dilution of the full-strength modified Hoagland solution) and *Rudbeckia hirta*, 580.611 cm<sup>2</sup> g<sup>-1</sup> (125 μmol/m<sup>2</sup>/s PAR and full-strength modified Hoagland solution). The means of the root:shoot ratios varied 10.6-fold between 0.123 to 1.298 g/g for *Matricaria matricarioides* (125 μmol/m<sup>2</sup>/s PAR and 1/5 dilution of the full-strength modified Hoagland solution) and *Arctium minus* (250 μmol/m<sup>2</sup>/s PAR and 1/50 dilution of the full-strength modified Hoagland solution). The means of the leaf nitrogen content varied 2.7-fold between 1.762% for *Arctium minus* (250 μmol/m<sup>2</sup>/s PAR and 1/50 dilution of the full-strength modified Hoagland solution) and 4.734% for *Matricaria matricarioides* (250 μmol/m<sup>2</sup>/s PAR and full-strength modified Hoagland solution).

The measurable toxicity in the brine shrimp test (1/LC<sub>50</sub>: μg/ml) means varied 44.6 fold between 0.01 for *Arctium minus* (250 μmol/m<sup>2</sup>/s PAR and 1/10 dilution of the full-strength modified Hoagland solution) and *Matricaria matricarioides* (125 μmol/m<sup>2</sup>/s PAR and 1/50 dilution of the full-strength modified Hoagland solution) to 0.446 μg/ml for *Chrysanthemum leucanthemum* (125 μmol/m<sup>2</sup>/s PAR and 1/50 dilution of the full-strength modified Hoagland solution), respectively.

#### *Effects of experimental manipulations on growth*

**Relative Growth Rate (RGR):** ANOVA showed significant differences between species (p = 0.0001), light (p = 0.0001), and nutrients (p = 0.02), and no significant interactions. The ANOVA on dry weights showed the same effects but (consistent with the first analysis above) Tukey's Studentized range showed a decrease only in the lowest (1/50 dilution of the full-strength solution) nutrient level. The lowest light level produced a decreased dry weight of the plants.

The analysis of covariance with dry weight at the end of each harvest period as the covariate shows that, after standardizing to common size, there were no significant differences in mean RGR between species (p = 0.07), between the two light levels 250 and 125 mmol/m<sup>2</sup>/s PAR (p = 0.62), or between the four nutrient levels (p = 0.09), but with a hint of an interaction between light levels and nutrient levels (p = 0.05).

**Specific Leaf Area (SLA):** The SLA values differed between the 6 species (p < 0.0001), and between the two light levels (p < 0.0001), and between the four nutrient levels (p = 0.0002) but there were no significant interactions among the treatments (Table 1). The mean values of SLA were 235 and 400 g/cm<sup>2</sup> for 250 and 125 mmol/m<sup>2</sup>/s PAR, respectively.

**Root:Shoot Ratios:** Root:shoot ratios differed between the 6 species (p < 0.0001), between the two light levels (p < 0.0001), and between the four nutrient levels (p < 0.0001). The only interactions were between light and nutrients (p < 0.0001). The means values of the root:shoot ratios were 0.615 and 0.329 g/g for 250 and 125 mmol/m<sup>2</sup>/s PAR, respectively (Table 1).

**Toxicity:** The only significant factor in the ANOVA was between the species means (p = 0.004).

**Leaf nitrogen content:** Mean leaf nitrogen values were significantly different between species means (p < 0.0001 and p = 0.0004 for the two groups), between the means of the light levels (p = 0.02), and between nutrients (p < 0.0001). There were no interactions between the variables.

#### *Comparisons between measured variables*

**Phytochemical parameters:** There was a weak positive significant correlation between leaf nitrogen and measurable toxicity in the brine shrimp test (r<sub>s</sub> = 0.195, p = 0.01).

TABLE 1

Means of growth, dry matter partitioning, and chemical characteristics in six species of Asteraceae grown under 250 and 125  $\mu\text{mol}/\text{m}^2/\text{s}$  PAR with four different nutrient supply (full-strength, 1/5, 1/10 and 1/50 dilution of strength Hoagland solution). Means by species (a); by light treatment (b); by nutrient treatment (c). Means followed by the same superscript letters (vertical columns) are not significantly different (HSD, 0.05 level).

## a) Means by species

Species	Mean				
	RGR (g/g/day)	Root/Shoot (g/g)	SLA (g/cm <sup>2</sup> )	Nitrogen (% dw)	Toxicity ( $\mu\text{g}/\text{ml}$ )
<i>Achillea millefolium</i>	0.136 <sup>b</sup>	0.421 <sup>b</sup>	272.982 <sup>dc</sup>	3.15 <sup>bc</sup>	0.072 <sup>ab</sup>
<i>Arctium minus</i>	0.108 <sup>d</sup>	0.637 <sup>a</sup>	228.019 <sup>d</sup>	3.14 <sup>bc</sup>	0.017 <sup>b</sup>
<i>C. leucanthemum</i>	0.126 <sup>c</sup>	0.396 <sup>b</sup>	319.337 <sup>bc</sup>	3.68 <sup>ab</sup>	0.143 <sup>a</sup>
<i>Cichorium intybus</i>	0.127 <sup>c</sup>	0.606 <sup>a</sup>	368.321 <sup>ab</sup>	3.44 <sup>ab</sup>	0.032 <sup>b</sup>
<i>M. matricarioides</i>	0.110 <sup>d</sup>	0.387 <sup>b</sup>	324.681 <sup>bc</sup>	3.84 <sup>a</sup>	0.019 <sup>b</sup>
<i>Rubdeckia hirta</i>	0.152 <sup>a</sup>	0.385 <sup>b</sup>	391.584 <sup>a</sup>	2.66 <sup>c</sup>	0.027 <sup>b</sup>

Pooled samples, n = 32.

## b) Means by light treatment

Level of light ( $\mu\text{mol}/\text{m}^2/\text{s}$ PAR)	Mean				
	RGR (g/g/day)	Root/Shoot (g/g)	SLA (g/cm <sup>2</sup> )	Nitrogen ( $\mu\text{g}/\text{ml}$ )	Toxicity (% dw)
250	0.111 <sup>b</sup>	0.615 <sup>a</sup>	234.969 <sup>b</sup>	3.19 <sup>b</sup>	0.029 <sup>b</sup>
125	0.142 <sup>a</sup>	0.329 <sup>b</sup>	400.005 <sup>a</sup>	3.56 <sup>a</sup>	0.076 <sup>a</sup>

Pooled samples, n = 96.

## c) Means by nutrient treatment

Level of nutrients	Mean				
	RGR (g/g/day)	Root/Shoot (g/g)	SLA (g/cm <sup>2</sup> )	Nitrogen (% dw)	Toxicity ( $\mu\text{g}/\text{ml}$ )
full-strength	0.139 <sup>a</sup>	0.254 <sup>c</sup>	338.885 <sup>a</sup>	3.95 <sup>a</sup>	0.047 <sup>a</sup>
1/5	0.135 <sup>a</sup>	0.326 <sup>c</sup>	337.559 <sup>a</sup>	3.76 <sup>a</sup>	0.055 <sup>a</sup>
1/10	0.116 <sup>b</sup>	0.489 <sup>b</sup>	315.49 <sup>ab</sup>	3.08 <sup>b</sup>	0.042 <sup>a</sup>
1/50	0.116 <sup>b</sup>	0.820 <sup>a</sup>	278.025 <sup>b</sup>	2.61 <sup>b</sup>	0.068 <sup>a</sup>

Pooled samples, n = 48.

**Growth parameters versus defense parameters:** The correlation was positive and significant for measurable toxicity in the brine shrimp test and RGR ( $r_s = 0.453$ ,  $p = 0.01$ ), as was the correlation between measurable toxicity in the brine shrimp test and SLA ( $r_s = 0.215$ ,  $p = 0.004$ ) but the correlation was negative and significant for measurable toxicity in the brine shrimp test and root:shoot ratio ( $r_s = -0.381$ ,  $p = 0.002$ ). There was a weak positive significant correlation between the mean of leaf nitrogen content and the mean of RGR ( $r_s = 0.176$ ,  $p = 0.02$ ). Finally, leaf nitrogen content and SLA ( $r_s = 0.387$ ,  $p = 0.0001$ ) showed a positive significant correlation but there was a negative strong

significant correlation between leaf nitrogen content and root:shoot ratio ( $r_s = -0.6$ ,  $p = 0.0001$ ).

Correlations may be due to common responses to changing environments or to “genetic” linkages between variables in a constant environment. In order to distinguish between these two possibilities, we fit generalized linear models using the GLM procedure of SAS (SAS Institute Inc., 1990) relating measurable toxicity in the brine shrimp test, in which the experimental treatments and species were included as covariates in order to control for their effects. These results were then compared to models in which these effects were not controlled.

### *Correlations without controlling for the different environments*

**Relative growth rate:** There were significant linear relationships between relative growth rate (RGR) and SLA ( $p < 0.0001$ ), between RGR and root:shoot ratio ( $p < 0.0001$ ), and between RGR and leaf nitrogen ( $p = 0.01$ ). There were no significant linear relationships between RGR and total phenolic ( $p = 0.09$ ) or between RGR and measurable toxicity in the brine shrimp test ( $p = 0.2$ ).

**Toxicity:** There were no linear relationships between measurable toxicity in the brine shrimp test and either RGR ( $p = 0.2$ ), or SLA ( $p = 0.3$ ), or root:shoot ratio ( $p = 0.08$ ), or leaf nitrogen content ( $p = 0.1$ ).

## DISCUSSION

This study investigated how a range of resource availabilities influences the growth and chemical parameters of six species of Asteraceae by measuring fast-growing versus slow-growing plants and toxic versus non-toxic plants.

As a previous study demonstrated (Almeida-Cortez *et al.*, 1999; Almeida & Shipley, 2002), studies of the resource availability hypotheses have tended to contrast the defense capacities of the plant species growing in two different resource states (Bryant & Kuropat, 1980; Coley, 1983; Newberry & de Foresta, 1985), but there exists no unified interpretation of the results even though nutrient supply rates may be used to vary relative growth rates of young plants (see Ingestad 1982 for a review). According to Ågren (1985), the nutrient supply rate in the environment, notably nitrogen, controls the nutrient uptake rate by the plant, which exerts a strict control over growth. Differences in resource availability have been shown to generate variation in defensive chemistry within a single species (Bryant *et al.*, 1987b). In recent years, much attention has been focused on the mechanisms by which the environment may alter the plant's production of chemical defenses, thereby altering susceptibility to herbivores (Mattson, 1980; Bryant *et al.*, 1983; Tuomi *et al.*, 1984). Carbon/nutrient balance is viewed as a key to understanding why plant susceptibility changes under different growing conditions. We might expect that carbon-based constitutive defensive chemicals (e.g., phenols, terpenes, acetylenes) should be scarce in plants subjected to reduced carbon uptake or very high

respiration, where a low carbon/nutrient ratio would result. On the other hand, plants provided with adequate light, but subjected to suboptimal nutrient availability, should exhibit a high carbon/nutrient ratio and resistance to herbivory (Bryant *et al.*, 1983).

Plants growing under nitrogen-limiting conditions generally have a slower growth rate than those growing under nitrogen-rich conditions (Almeida-Cortez *et al.*, 1999; Almeida & Shipley, 2002). Carbon supply does not limit plant growth under low nitrate conditions and subsequently, increased quantities of carbon-based defenses should be selected as nitrate availability decreases (Bryant *et al.*, 1983; Coley *et al.*, 1985; Mihaliak & Lincoln, 1985).

A negative correlation between two traits can be caused in two general ways. One possibility is that there is no genetic link between the two traits, but each responds in an opposite way to some common environmental change. The other possibility is that the negative correlation is generated by the physiology or morphology of the plant even when the environment is constant. This second possibility is a "genetic" correlation and provides an operational definition of a "trade-off". The existence of a trade-off between growth and defense has created some controversy. Even if some studies have found a negative correlation between RGR and attack by herbivores (Coley, 1983; Sheldon, 1987), others (Meijden *et al.*, 1988; McCanny *et al.*, 1990) did not find any correlation, and still others (Denslow *et al.*, 1987, 1990; Briggs & Schultz, 1990) show a positive correlation between the two variables.

### *Is there any trade-off between measured variables?*

**Growth parameters:** The Spearman correlation between the mean of relative growth rate (RGR; i.e., 21-42 days) and the mean of specific leaf area (SLA) was strong and positive ( $r_s = 0.606$ ,  $p = 0.0001$ ). Poorter & Remkes (1990) reported a strong positive correlation between RGR and SLA under constant environmental conditions of high nutrient supply but low light intensity (225  $\mu\text{mol}/\text{m}^2/\text{s}$ ). McKenna (1995) did not find such a correlation when light intensities were doubled. Shipley (1995) provided evidence that maximizing relative growth rate involves maximizing specific leaf area, which in turn involves maximizing leaf area with the least amount of biomass. Reich *et al.* (1992) in their review of the literature found a strong positive relationship between these two variables.

**Phytochemical parameters:** There was a weak positive significant correlation between leaf nitrogen and measurable toxicity in the brine shrimp test ( $r_s = 0.195$ ,  $p = 0.01$ ).

**Growth parameters versus chemical parameters:** There was a positive and significant correlation between the mean of measurable toxicity in the brine shrimp test and the mean of RGR ( $r_s = 0.453$ ,  $p = 0.01$ ), as was the correlation between measurable toxicity in the brine shrimp test and SLA ( $r_s = 0.215$ ,  $p = 0.004$ ). There were no significant linear relationships, however, between measurable toxicity in the brine shrimp test and SLA ( $p = 0.3$ ) before controlling for species and experimental treatments. In Crankshaw & Langenheim (1981), only one (Type III caryophyllene) of the sesquiterpenes studied increased when leaf area increased.

Finally, we would like to emphasize that most of the information on plant/herbivore interactions comes from studies on the effectiveness of specific defenses from the viewpoint of the herbivore rather than the plant. These include surveys with generalists and investigations of more tightly coevolved systems between host and herbivore (Edmunds & Alastad, 1978; Trigo, 2000; Mello & Silva-Filho, 2002).

Another approach has been to document broad-scale associations of plant life history, successional status, habitat preference, or leaf age with either herbivory or plant defense. Since these community-level studies have examined patterns of herbivory and defense separately, their relationships can only be inferred (but see Rhoades, 1977; Milton, 1979; Furlan *et al.*, 1979). The general trend, however, is towards higher concentrations and more effective characteristics as well as lower grazing susceptibility in late successional or woody species, mature leaves (but see Crankshaw & Langenheim, 1981), and plants of nutrient-poor areas (Bryant & Kuropat, 1980; Coley, 1980).

The idea that a plant must accept tradeoffs because it must allocate limited resources among growth, reproduction, and defense has been central to ecological and evolutionary theories, but the existence of a trade-off between growth and defense has generated some controversy. The data and analyses in this study suggest that there is no necessary trade-off between growth rate and the types of toxic chemical defenses that are detected by the bioassay when species are grown under the same environmental conditions. This does not exclude the possibility of tradeoffs between growth and structural defenses or

of chemical defenses that affect palatability or digestibility without inducing any toxic effect. The “trade-off” that has been reported from field experiments may arise because researchers have failed to control for different soil fertilities, and differing soil fertilities affect secondary compounds production and growth in opposite ways.

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