

## ECOLOGICAL CAUSES AND CONSEQUENCES OF VARIATION IN DEFENSIVE CHEMISTRY OF A NEOTROPICAL SHRUB

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**Abstract.** Prominent models of herbivore regulation focus on predators, low plant biomass, or poor resource quality as factors that limit herbivore populations. We examined predictions of these tritrophic models for herbivores on the understory shrub, *Piper cenocladum*, which is defended by mutualistic ants (*Pheidole bicornis*) and three amide secondary metabolites. To examine sources of variation in *P. cenocladum* amide content and to compare the effects of amides vs. ants on herbivores, we used three linked experiments in the field and in shadehouses. We manipulated light, nutrient availability, and presence of symbionts for experimental plant fragments and shrubs and then quantified leaf amide concentration. We also examined relationships between amide content and damage by the three most common groups of folivores on *P. cenocladum*: generalist orthopterans, specialist coleopterans, and specialist lepidopterans. For all experiments, enhanced resources and absence of symbionts caused higher levels of amides. These increased chemical defenses had strong effects on generalist herbivores in this system, while the ant predators were more effective at depressing herbivory by specialists. The negative effects of amides on specialist lepidopterans were small, suggesting that these herbivores are adapted to chemical defenses in their host plant. It is possible that our results are part of a more general trend where top-down effects are stronger against specialist herbivores, while chemical defenses are more effective against generalists. We concluded that different models of herbivore regulation were supported by components of the *P. cenocladum* arthropod community, depending on resource availability and on the portion of the web examined.

**Key words:** amides; chemical defense; green desert; herbivory; Piper; rain forest; resource availability; trophic cascades.

### INTRODUCTION

Identifying the ecological forces that regulate populations of herbivores in different communities is one of the most important goals of basic and applied ecology. A variety of “top-down” and “bottom-up” trophic interaction models have been developed to understand herbivore regulation, including top-down trophic cascade models in which predators regulate their prey (reviewed by Pace et al. 1999, Persson 1999, Polis 1999, Schmitz et al. 2000, Halaj and Wise 2001), thermodynamics models of bottom-up trophic cascades in which herbivores are regulated by plant biomass (e.g., Slobodkin 1960), and the “green desert” model of bottom-up forces in which herbivores are regulated through plant secondary compounds or nutritional quality (e.g., Murdoch 1966, White 1978, Abe and Higashi 1991). Many studies examining community organization have focused on comparing the first two models (e.g., Hunter and Price 1992, Moran and Scheidler 2002, Dyer and Letourneau 2003, Schmitz 2003),

but it is equally interesting to investigate predictions of top-down cascades vs. green desert regulation of herbivores (Moen et al. 1993).

Studies of antiherbivore defenses of plants have proposed a variety of relationships between resource availability, plant growth rates, plant vigor, and plant defense that are relevant to models of herbivore regulation (e.g., Bryant et al. 1983, White 1984, Coley et al. 1985, Larsson et al. 1986, Nichols-Orians 1991, Price 1991, Herms and Mattson 1992, Shure and Wilson 1993, reviewed by Koricheva et al. 1998). Resource availability hypotheses could either confound or enhance traditional thermodynamic hypotheses of bottom-up control. For example, under low nitrogen conditions, levels of carbon-based defenses will increase for a given plant species, and herbivores will decline both because of increased plant defense and lower plant biomass. But under low light conditions, where herbivore populations might be expected to decline because of reduced plant tissue, they could increase because of lower levels of carbon-based defenses (Bryant et al. 1983). Variation in light availability might also affect nitrogen-based defenses (Bryant et al. 1983), which would alter effects of enhanced plant biomass

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on upper trophic levels. Similarly, despite predictions by bottom-up models, folivore densities have not increased in response to nitrogen additions in large-scale field experiments (Kyto et al. 1996, Letourneau 1997), perhaps because of increases in nitrogen-based defenses. The studies that have examined associations between resource availability, plant biomass, plant chemistry, and herbivory have yielded inconsistent results (Waterman et al. 1984, Larsson et al. 1986, Bryant et al. 1987, Briggs 1990, Dudd and Shure 1994, Koricheva et al. 1998, Hol et al. 2003). Even meta-analyses synthesizing results from resource availability studies have yielded opposing conclusions (Koricheva et al. 1998, Dyer and Coley 2001). One solution to this lack of consistent synthesis is to put resource availability hypotheses into a larger ecological or evolutionary context (Hamilton et al. 2001, Nitao et al. 2002). We chose a novel approach to examining the effects of resource availability by simultaneously studying how resources directly affect growth and plant defense and how they indirectly affect upper trophic levels. This approach combines two bottom-up trophic models (thermodynamic and green desert) with the prediction that an increase in plant resources will cause increases in plant growth and defense.

In previous studies, detailing a four-trophic-level, rain forest understory community (Letourneau and Dyer 1998a, b, Dyer and Letourneau 1999a, b, 2003), we compared the relative contributions of top-down forces and thermodynamically driven bottom-up forces in structuring a diverse animal community. We demonstrated strong top-down effects, with herbivores regulated by their predators, but we found no detectable indirect effects of incremental changes in the amount of plant resources on herbivores. Here, we compare the suppression of herbivores by chemical defenses vs. predators, and we assess the effects of plant resource availability on upper trophic levels via chemical defense. The four-trophic-level system consists of an ant-plant, *Piper cenocladum* C. DC. (Piperaceae), which increases production of amide defensive compounds when ants are not present (Dodson et al. 2000, Dyer et al. 2001); representative specialist and generalist herbivores of the plant; an ant mutualist, *Pheidole bicornis* Forel, that feeds on plant-derived food bodies and kills herbivores; and a specialist clerid beetle, *Tarsoabaenus letourneauae* Barr, that kills *Ph. bicornis*, but maintains plant production of food bodies. The defensive amides in *P. cenocladum* are: piplartine, 4'-desmethylpiplartine, and cenocladamide; standard natural products methods have not uncovered other defensive compounds in this species (Dodson et al. 2000). Opalescent food bodies, rich in lipids and proteins, are produced on the adaxial side of sheathing leaf bases (hollow petioles) when occupied by *Ph. bicornis* (i.e., when amide levels are low) or *T. letourneauae* symbionts. The fact that amide concentrations are significantly lower in ant protected vs. empty plants facilitates direct

comparisons of top-down (ant predation) vs. bottom-up (chemical defense) effects on specific herbivores via manipulation of ants. More extensive natural history of this system, relevant to this paper, is described in Dyer and Letourneau (1999b) and Letourneau (1998).

To assess the potential strength of bottom-up effects of nutrients via amides on the arthropod community associated with *P. cenocladum*, we performed a series of experiments examining the effects of resources on amide concentrations and the preference and performance of generalist and specialist insects on amide-manipulated diets. These experiments were designed to address the following questions: (1) Does resource availability affect concentrations of amide defenses in *P. cenocladum*? (2) Do *P. cenocladum* amides affect relative amounts of generalist and specialist herbivory? (3) Do amides reduce the fitness of specialist herbivores? (4) How do these effects compare to the effects of predation by *Ph. bicornis*? Full or partial answers to these questions provide a means to assess the strength of the green desert trophic model and aid in creating a full, multitiered picture of the ecological forces that structure this complex terrestrial system.

#### METHODS

##### *Experiments: resource availability, amide content, and herbivory patterns*

To examine sources of variation in *P. cenocladum* amide content, we used three linked experiments that manipulated known and suspected sources of variation in secondary metabolites. Experiments were conducted on both full-size shrubs and small fragments because they are both very common in the forest understory, they have different levels of amides (Dodson et al. 2000, Dyer et al. 2001), and they respond differently to various experimental manipulations (Letourneau and Dyer 1998b, Dyer and Letourneau 1999a). Experiments were also conducted in shadehouses to control for unknown sources of variation in amide concentration, such as induced defenses from pathogens. All experiments were conducted in the forest at La Selva Biological Station (Heredia Province, Costa Rica, 10°25' N 84°05' W; for more information on this station see McDade et al. 1994). The first was a 22-month field experiment conducted from November 1994 to August 1996; this experiment is described in full in Dyer and Letourneau (1999b) and is referred to in this manuscript as the "field fragment experiment." We have already reported effects of manipulations on plants and arthropods, but the data on leaf chemistry of plants from these experiments are new and are reported here. Briefly, we established *P. cenocladum* cuttings in the forest understory in a factorial design of three treatments: fertilizer vs. no fertilizer, high light vs. low light, and ant presence vs. ant exclusion. At the end of the experiment, all plants were harvested and dissected to count food bodies and ants (including all adult castes,

pupae, larvae, and eggs) in the petioles and to quantify levels of herbivory by specialist chrysomelid beetles (*Physimera* spp.), specialist geometrids (*Eois apyraria* and *Eois* sp.), and generalist orthopterans (Tetigoniidae, Acrididae, *Microtylopteryx hebaridi*; and Eumastacidae, *Homeomastax robertsi*). Herbivory by these different herbivores is very distinct; a plastic overlay with a 1-cm<sup>2</sup> grid was used to quantify percentage herbivory. Dry mass was determined for all leaves, petioles, stems, and roots and leaves were saved for chemical analyses (described in the *Methods: Quantification*).

For the second experiment, referred to as the “field shrub experiment,” we examined leaves from a field experiment that was conducted on existing shrubs in the forest at La Selva. In July 2000, 30 naturally occurring stands of *P. cenocladum* shrubs were randomly selected using Arc/Info (Geographic Information System) at La Selva. We designated these stands as occurring on rich soils if they were on inceptisols (immature soils with poorly developed lower horizons) or on poor soils for ultisols (highly weathered, low stock of basic cations), using soil maps generated for La Selva (Sollins et al. 1994). Relative availability of light to each understory plot was estimated for each of three *P. cenocladum* shrubs in each plot, by measuring the amount of canopy cover at four cardinal directions with a spherical densiometer. An average percent canopy cover was derived, and designated the high light treatment if the cover value was less than the average cover for all plots, and as low light if it was more than the mean value for percent canopy cover (96.4%). The high light category is associated with significantly decreased herbivory, increased leaf size, and increased diversity of the detrital community living within the plant (Dyer and Letourneau 1999b, 2003). All plants initially contained ants. These ants were removed and beetles were added (1 beetle/plant) to half of the plants, while ants were not removed from the other half (following the methods of Dyer and Letourneau 1999b). Leaves were harvested for chemical analyses after 16 months.

A third experiment, the “shadehouse fragment experiment,” was conducted using fragments grown in a controlled environment that excluded herbivores and other parasites. Sixty plant fragments were cut from plants in 60 discrete patches growing in ultisols at La Selva; these fragments were grown in pots containing ultisols in a 20% ambient light shadehouse. The fragments included only the top two leaves with unoccupied (by ants or beetles) petioles and the third node, which was placed under the soil. The petioles were unoccupied because the chambers only become hollow after a new leaf emerges from it. The cuttings were left for one month to establish, and then they were randomly assigned to fertilizer, light, and symbiont treatments in a factorial design. There were three levels of the symbiont treatment: controls, one early instar beetle larva added to the newest hollow petiole, and ant col-

onies (queen, brood, workers) added to all hollow petioles. When adult beetles left a plant, another larva was placed in the newest hollow petiole. For the light treatment, half of the plants were placed under shade cloth frames to reduce the ambient light to ~2%. The two levels of fertilizer treatment were 0 and 5 g of Once slow release fertilizer (NPK 13:13:13 with trace levels of Ca, Mg, S, B, Cu, Fe, Mn, Mo, and Zn); these levels were chosen to be the low vs. high nutrient treatments based on a previous study (Dyer and Letourneau 1999b). After nine months (the same duration as previous shadehouse experiments), the number of new leaves, leaf area, and stem height were recorded, and plants were harvested and dried at room temperature for chemical analyses.

#### *Quantification of amides*

A modification of the procedure described in Dodson et al. (2000) was used to quantify the three amides. For all experiments, leaves were air dried at room temperature, ground to a fine powder, and 1-g aliquots from each plant were extracted overnight, twice, at room temperature with 95% ethanol. The crude residue of the extract was resuspended in 3:1 water/ethanol and exhaustively extracted with chloroform in a separatory funnel. Combined chloroform extracts were dried in vacuo and the residue redissolved in 10 mL of methylene chloride. One mL aliquots of samples were transferred to autosampler vials. Each sample was quantitatively analyzed by GC/FID using the method of internal standardization. Piperine was used as the internal standard and was added to the samples at the 80 µg/mL level. Five point calibrations (50, 100, 200, 300, and 500 µg/mL;  $r^2$  values for calibration were 0.99 or better) were prepared with synthetic piplartine, 4'-desmethylpiplartine, and cenocladamide (Dodson et al. 2000, Dyer et al. 2001).

#### *Feeding experiments*

To experimentally test the effects of elevated *P. cenocladum* amide concentrations on the herbivore species that causes the most amount of damage to this plant (Dyer and Letourneau 1999b), we conducted rearing experiments with a geometrid, *Eois apyraria*, at La Selva Biological Station. Immature geometrids in this genus are very difficult to distinguish, especially in early instars, so it is likely that there were individuals of another unidentified species of *Eois* that were included in this experiment, and these caterpillars are hereafter referred to as *Eois*. A total of 130 larvae in second through fourth instar, were collected from *P. cenocladum* shrubs at La Selva and each larva was randomly assigned a shadehouse leaf diet (from the shadehouse fragment experiment). The youngest, fully expanded leaves on the shadehouse plants were selected for feeding larvae, and each subsequent leaf was chosen from the top of the plant down. Leaf disks were cut to fit the lid of a 9-cm petri dish, and the larvae

TABLE 1. Total amide content (piplartine + 4'-desmethylpiplartine + cenoctadamide) in *Piper cenocladum* shrubs and fragments, expressed as percentage dry mass of the leaf tissue (1 SE in parentheses).

Location	Resources				F	df	P
	Soil nutrients		Light level				
	High	Low	High	Low			
Field shrubs	0.44 (0.03)	0.43 (0.02)	0.48 (0.02)	0.40 (0.02)	3.47	1, 74	0.066
Shadehouse	2.0 (0.16)	1.3 (0.15)	1.5 (0.15)	1.7 (0.18)	8.4	2, 46	0.0058
Field fragments	1.8 (0.18)	1.6 (0.28)	1.8 (0.25)	1.7 (0.23)	4.7	1, 24	0.044

Notes: Ellipses indicate not measured. The *F* statistic reported for each source of variation is Wilks' lambda from MANOVAs for each experiment, with percentage dry mass of the three amides as dependent variables. Levels of the resource and symbiont manipulations for each experiment are described in the *Methods: Experiments*.

were placed on the underside of the leaf disks in the lids of the petri dishes so that the larvae could hang from the underside of the leaf disk, which is how they feed in the field. Cutting the plant does not affect amide concentrations (L. A. Dyer, unpublished data). The initial mass and length of the larva and the initial fresh mass of the leaf disk were measured. All larvae were reared under ambient conditions (~25°C). Each day until pupation, the length and the fresh masses of the larvae, leaf disks, and frass were measured. When the leaf disk became too dry (~4–5 d after initial rearing), a new leaf disk was cut from either the same leaf (if the size of the leaf permitted more than one leaf disk) or from a new leaf taken from the same shadehouse plant. The fresh mass of the new leaf disk was recorded and the old leaf disk was discarded. The potential outcomes of the experiment were pupation or mortality, and if a larva reached pupation, the pupal mass was recorded. Some mortality (11% of all caterpillars) was caused by unidentified braconid wasps, but these results were included in the analyses since diets with high levels of amides can enhance parasitoid success after oviposition (Dyer et al. 2003b). Parasitoids had no access to the caterpillars in petri dishes, so caterpillars were probably parasitized in the field, before they were collected. New leaves from the shadehouse plants were harvested and analyzed for amides approximately one month before the feeding experiments. Standard gravimetric methods (Waldbauer 1968) were used to examine the effect of amides on the feeding efficiency of *Eois* larvae.

#### Statistics

For all resource availability experiments, multivariate analysis of variance (MANOVA) was used to examine the effects of light, fertilizer, and symbiont (ants or beetles vs. no symbiont) on concentrations of cenoctadamide, 4'-desmethylpiplartine, and piplartine. Other studies have documented the effects of these independent variables on biomass in the field and shadehouse (Dyer and Letourneau 1999a, b). Total amide concentration was not used as a dependent variable because the amide concentrations are not multicollinear, and each amide can respond differently to manipulations (e.g., Dyer et al. 2001). However, since

MANOVAs were not followed by profile analyses, mean amide concentrations are simply reported as total amides. For the two field experiments, where we uncovered complex interactions between light and fertilizer, we used the same statistical approach followed in similar experiments where we found that plant biomass and food body production responded favorably to balanced resource availability (Dyer and Letourneau 2003) and created a resource variable with two levels: balanced (high light, high nutrients and low light, low nutrients) vs. unbalanced (high light, low nutrients and low light, high nutrients).

For the field fragment study, sequential multiple regressions were used to examine the effects of the three amides on percentage herbivory by specialist chrysomelids, specialist geometrids, and generalist orthopterans. Based on other studies on the relative toxicity of these amides, 4'-desmethylpiplartine was entered into the model first, followed by piplartine and cenoctadamide. Because relatively few plants are attacked by orthopterans, only plants that had orthopteran damage were included in the orthopteran analysis. For the specialist geometrids, only plants without ants were used, since lepidopteran damage on ant plants is almost never found (Letourneau 1983). Path analysis was used to test the fit of the data to specific causal pathways.

For the *Eois* performance experiments, we used a regression of relative consumption rate on relative growth rate (Waldbauer 1968) to obtain linear estimates of efficiency of incorporation. The amide concentrations for the diets fed to the larvae were added as independent variables (one for each type of amide), to determine if they cause an improved fit in the regression model, thus affecting feeding efficiency (modified from Blau et al. 1978). Amides were added in the same order as described above for the multiple regressions. We used the incremental *F* ratio (Tabachnick and Fidell 1996) to test for the significance of the improved model fit. The analysis of covariance method described by Raubenheimer and Simpson (1992) is not appropriate for our hypothesis tests, since our model is more complex (with several continuous variables) than the models they modified. For caterpillars that survived to pupation, we used multiple regression to examine the effects of amide concentrations on pupal mass. We also

TABLE 1. Extended.

Symbionts			Symbiont × Resources					
Ants	Beetles	None	F	df	P	F	df	P
0.48 (0.02)	0.39 (0.03)	...	1.28	1, 74	0.0086	3.37	1, 74	0.071
1.4 (0.17)	1.5 (0.20)	2.0 (0.22)	3.3	2, 46	0.045	3.1	2, 46	0.057
1.4 (0.22)	...	1.9 (0.22)	5.9	1, 24	0.027	2.8	1, 24	0.12

used logistic regression to test for the effects of total amide concentration on survivorship, with mortality of each caterpillar as a dichotomous response variable.

RESULTS

Amide concentrations varied significantly due to manipulations of resources and presence/absence of symbionts in all experiments (Table 1). For the fragment experiments, removal of symbionts and enhancement of resources caused significantly higher amide concentrations (Table 1). For field fragments and field shrubs, levels of amides were higher in balanced vs. unbalanced resource conditions, but this difference was only significant for the field fragments (Table 1). In the shadehouse, where fertilizers were added and differences in light availability were more consistent, there was no significant effect of light manipulations ( $F_{1,46} = 1.7, P = 0.20$ ), but there was a large and significant effect of fertilizers (Table 1). The optimal conditions for production of amides were fertilized fragments at low light conditions without symbionts (e.g.,  $2.8 \pm 0.31\%$  dry mass in the shadehouse) and the conditions associated with the lowest levels of amides were poor soils, with beetles, and high light (e.g.,  $0.34 \pm 0.05\%$  dry mass in the field shrubs). A path analysis comparing sources of variation in plant food bodies, biomass, and chemistry revealed that both resources and symbionts had significant effects on all plant attributes (the model correlation matrix was not significantly different from the observed matrix,  $\chi^2 = 0.009, df = 1, P = 0.98$ ). The effect sizes were all medium to large (0.28–0.8) for all pathways except the symbiont to plant biomass path (Fig. 1).

Naturally occurring levels of amides in the field fragments had significant negative effects on percentage damage by orthopterans ( $r^2 = 0.74, P = 0.019$ ). In contrast, amides in the field fragments had little to no effect on specialist lepidopterans and coleopterans. Regressions of percentage *Eois* and *Physimera* herbivory on total amides were not significant (*Eois*,  $r^2 = 0.051, P = 0.77$ ; *Physimera*,  $r^2 = 0.062, P = 0.71$ ). For *Eois*, the feeding efficiency was negatively affected by amide content, demonstrated by the fact that the regression of relative consumption on relative growth ( $r^2 = 0.19, P < 0.0001$ ) was improved when individual amide concentrations in the leaf diets were sequentially added to the regression model ( $F_{1,128} = 5.16, P < 0.03$ ), with 4'-desmethylpiplartine having the strongest negative effect. When 4'-desmethylpiplartine was added to the regression,  $r^2 = 0.25$  with a standardized parameter estimate of  $-2.6$  ( $t = -2.5, df = 1, P = 0.01$ ); when other amides were subsequently added,  $r^2 = 0.25$ , with insignificant parameter estimates. There were no effects of amides on pupal masses ( $r^2 = 0.0005, P = 0.83$ ) or survivorship ( $\chi^2 = 0.14, df = 1, P = 0.71$ ) of the caterpillars.

Hypotheses depicted in Fig. 2 were tested for orthopterans and lepidopterans using path analysis when appropriate. None of the bottom-up models via food bodies or plant biomass were tested, because for field fragments there were no significant effects of resources on food bodies and no significant effects of biomass on herbivores (these analyses are reported in Dyer and Letourneau 1999b). For the generalist orthopterans, the model that was the best fit was the green desert model (Fig. 2,  $\chi^2 = 0.009, df = 1, P = 0.98$ ), with resources causing increases in amide content, which subsequently caused decreases in orthopteran herbivory. For the specialist caterpillars, the model that was the best fit was the top-down model, with ants negatively affecting lepidopteran herbivory and herbivory negatively affecting plant biomass (Fig. 2,  $\chi^2 = 0.009, df = 1, P = 0.98$ ). The same model fit for *Physimera* ( $\chi^2 = 0.23, df = 1, P = 0.63$ ), with ants negatively affecting these herbivorous beetles (path coefficient =  $-0.15$ ), but there was no significant effect of *Physimera* damage on plant biomass. The bottom-up model via amides was not tested for the specialist lepidopterans and coleopterans because there were no significant effects of amides on these herbivores (see previous paragraph).

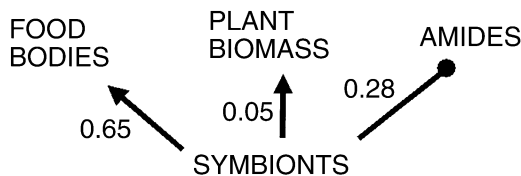


FIG. 1. Results of path analyses from shadehouse experiments with *Piper cenocladum* fragments. Direct positive effects on plants are indicated by an arrow; the bullet-headed line from symbionts to amide content indicates a direct negative effect. The numbers to the right of the lines are path coefficients from a single path analysis; all effects depicted here were significant ( $P < 0.05$ ).

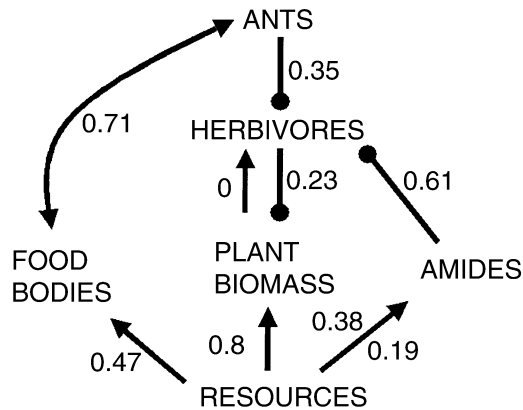


FIG. 2. Results of path analyses from field and shadehouse experiments with *Piper cenocladum* fragments. Potential direct effects are indicated by a solid line between two trophic levels. A negative effect of one trophic level on the other is drawn with a bullet-head; a positive effect is drawn with an arrowhead. The effect is on the trophic level next to the arrow- or bullet-head. Numbers to the left of lines are path coefficients from the shadehouse path analysis, except the 0 for the biomass to herbivore pathway, which reflects the lack of effects uncovered by the field experiment. Numbers to the right of lines are path coefficients from the field experiment, except for the ant–food body value, which is a correlation coefficient calculated from field data and is included to demonstrate the possibility of a pathway leading up to the ants and back down to the herbivores. “Resources” refer to the fertilizer manipulation for the shadehouse experiment, and to balanced (high light, high soil nutrients; low light, low soil nutrients) or unbalanced (high light, low soil nutrients; low light, high soil nutrients) plant resources for the field experiment.

#### DISCUSSION

Based on the patterns uncovered in this study, top-down effects are stronger against the most abundant specialist herbivores in the *P. cenocladum* arthropod community, while the green desert is a more appropriate concept for the generalist orthopterans in the community. Other studies with this system have demonstrated the strong top-down effects of ants on caterpillars and the potential for strong bottom-up effects on leafcutting ants, orthopterans, and generalist caterpillars (Dyer and Letourneau 1999b, Dyer et al. 2003b). All of these results fit predictions from a general hypothesis that when pressure from generalist herbivores is more intense (both in space and time), bottom-up regulation is more important, whereas when specialists are more common, predators are better regulators of herbivory. Many authors have presented data that support components of this hypothesis (Whittaker and Feeny 1971, Blau et al. 1978, Bernays and Cornelius 1989, Dyer 1995, van der Meijden 1996, Cornell and Hawkins 2003), but it has not often been tested at a broader level. For example, assuming that dietary specialization by herbivores is more common in the tropics, we would predict that predation has larger effects on tropical herbivores, while plant chemistry has

larger effects on herbivores in temperate systems; this prediction was supported by a recent meta-analysis (Dyer and Coley 2001).

In previous experiments (Dyer and Letourneau 1999b, 2003), we found that predictions from thermodynamic bottom-up models (Slobodkin 1960) are not relevant to external arthropods in this system, despite the fact that experimentally increasing plant resource availability caused significantly higher plant biomass. The relationship uncovered here between resource availability and amide content in leaves helps explain this result (improved resources lead to higher plant biomass that is more toxic to herbivores). These changes in total amide concentrations for fertilized vs. unfertilized plants are biologically significant and are comparable to those found in typical studies of variation in alkaloids due to resource limitation (e.g., Hoft et al. 1996), induced defenses (e.g., Khan and Harborne 1991), or genetic variation (e.g., van Dam and Vrieling 1991). In related feeding studies, we found that increases in total amide concentration of this magnitude (Dyer et al. 2003a, b) caused increased mortality of generalist herbivores or caused leaf-cutting and omnivorous ants to reject food items.

Most studies on bottom-up effects focus on increased productivity in response to resource availability, and indirect effects on upper trophic levels are not commonly uncovered (Hunter and Price 1992, Floyd 1996, Dyer and Letourneau 1999b, Moran and Scheidler 2002). The bottom-up cascade depends on either an increase in plant biomass (Fretwell 1977, Hunter and Price 1992) or an increase in plant quality (greater concentrations of amino acids), but this cascade may not occur if plant quality declines due to increased defense. Another prominent trophic model, proposed by Oksanen et al. (1981) incorporates the bottom-up cascade, and predicts that predators should limit herbivores in areas of high plant productivity due to the indirect positive effects of plant biomass on predators. One recent study (Van Bael 2003) supported the Oksanen model, but many studies in other communities do not, possibly because the effects of resources on plant defense swamp out the potential for increased plant biomass to cascade upwards. Closer examination of the studies that have not uncovered bottom-up cascades could reveal that, like the *Piper* system, indirect positive effects are not possible due to an increase in plant defense. For example, Dawes-Gromadzki (2002) investigated the thermodynamic bottom-up model for arthropods of a chenopod shrubland community and found only weak direct effects of enhanced nutrients on herbivores and no effects on upper trophic levels. Plants in the Chenopodiaceae are rich sources of both carbon-based (e.g., flavonoids, terpenoids, and steroids) and nitrogen-based (alkaloids) defenses (Aljaber et al. 1992, Alsaleh et al. 1997). Thus, the failure to find classic bottom-up effects in the chenopod system could be due to the fact that resource availability increased plant quantity

but decreased quality. Enhanced defenses may prevent increased herbivore and predator densities despite increased plant productivity.

The responses of amides to enhanced resources were consistent with simple ecological predictions of the carbon-nutrient balance hypothesis (CNB, Bryant et al. 1983) and other resource limitation hypotheses (Lorio 1986, Hamilton et al. 2001). Whether the CNB is useful (Lerdau and Coley 2002) or not (Hamilton et al. 2001, Koricheva 2002, Nitao et al. 2002), resource limitation in general often has predictable effects on nitrogen-based defenses such as alkaloids, amides, and cyanogenic glycosides (meta-analysis reported in Dyer and Coley 2001). As recommended by Nitao et al. (2002), we tested nutrient limitation in a broader context and demonstrated that correlations between resource availability and chemical defense affected the arthropod community associated with *P. cenocladum*. Despite valid criticisms of the interspecific predictions of CNB, it is worthwhile to test one useful and straightforward ecological aspect of the resource limitation hypotheses: enhanced resources can cause higher levels of secondary metabolites for particular plant species. Critics of CNB acknowledge that resource availability likely affects all physiological processes, including secondary metabolites (Nitao et al. 2002). If resource enhancement does lead to increased levels of secondary metabolites, by whatever mechanism or for whatever adaptive reason, then increased resources will result in effects on upper trophic levels that are different from the effects predicted by bottom-up thermodynamic models (Fretwell 1977, Oksanen 1981).

If the amides only have weak effects on specialists, why there are no significant positive effects of increased biomass on specialist herbivores in this system? It is likely that increased plant biomass is positively associated with increased food bodies, possibly resulting in larger ant colonies that are more effective at controlling the specialist herbivores. It is also possible that other enemies of the specialist herbivores (e.g., parasitoids of the caterpillars, Dyer et al. 2003b) may cause higher herbivore mortality when feeding efficiency is compromised by high levels of 4'-desmethylpiplartine. Lower feeding efficiency can render herbivores more vulnerable to natural enemies (Feeny 1976, Clancy and Price 1987, Williams 1999).

Although we did not directly test for induction of chemical defense and we cannot rule out the possibility that these amides can be induced, it is important to point out that the increase in amides associated with ant exclusion occurred both in the absence (shadehouse experiments) and presence (field experiments) of herbivores. A quick comparison of ant-excluded vs. control plants in the field might lead one to conclude that the high levels of herbivory on the ant-excluded plants caused the higher levels of amides in these plants via induction. However, amide production was significantly lower for the field shrubs with beetles compared

to plants with ants, and beetle plants experience the highest levels of herbivory by specialist geometrids, while ant plants are almost never attacked by geometrids (Letourneau and Dyer 1998b, Dyer and Letourneau 1999a). Based on the experiments reported here along with several other experimental and correlational measures of amide concentrations (Dodson et al. 2000, Dyer et al. 2001), allocation of resources to amides in *P. cenocladum* is determined by symbionts and resource availability, regardless of the presence and absence of herbivores (Table 1, compare shade and field fragments). The trade-off between metabolically expensive food bodies (Fischer et al. 2002) vs. amide production is a potential mechanism explaining the effects of symbionts on plant chemistry. Heil et al. (2002) report that such trade-offs between ant and chemical defense are uncommon in myrmecophytes and suggest that in the instances where they occur, the secondary compounds may be harmful to the ants. This alternative hypothesis is definitely plausible for some systems (e.g., Heil et al. 1999), but not for *Ph. bicornis*, since the major food items (food bodies and caterpillar eggs) are unlikely to contain amides.

In conclusion, all three prominent models of herbivore regulation have been supported by components of the *P. cenocladum* arthropod community, depending on the portion of the web examined. These models include: (1) herbivores are limited by natural enemies (Hairston et al. 1960, true for specialists in the *P. cenocladum* web), (2) resource availability limits chemical defense when the defensive benefit is high (Bryant et al. 1983, Hamilton et al. 2001, true for amides in *P. cenocladum*), and (3) herbivores are limited by plant chemical defenses (the green desert hypothesis, Moen et al. 1993, true for generalists in the *P. cenocladum* web). In all cases the relative roles of these top-down and bottom-up forces are strongly dependent on the presence of ant mutualists, which itself varies across habitats and ecological communities (Letourneau and Dyer 1998b). It is possible that our results are part of a more general trend where top-down effects are stronger against specialist herbivores, while the green desert is a more appropriate concept for generalists.

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