SECONDARY METABOLITES IN PLANTS WITH KNOWN MEDICINAL USES IN SUN AND SHADE HABITATS

CHRISTINA H. MAY, SARAH L. EMEL, AND KATHRYN M. SULLAN

Research advisors: Faculty and graduate students. Faculty editor: Matthew P. Ayres

Abstract: Herbivory pressure in the tropics has selected for high secondary metabolites. We investigated the effect of habitat (sun versus shade) on the production of secondary metabolites within species of plants. We hypothesized that plants with less access to light have higher cost of leaf replacement, resulting in a higher investment in chemical defenses. Alternatively, plants growing in sun habitats may shunt excess carbon (from high photosynthesis relative to nutrient acquisition) into chemical defenses. We tested these alternatives by comparing the secondary metabolites in leaves of the same four species between sun and shade habitats. Using a double blind smell test, pH measurements, and paper chromatography analyses, we detected significant differences in secondary metabolites between species. However, no difference was detected between light habitats for each species. Our results are consistent with relatively tight genetic regulation of secondary metabolites within these species.

Key Words: secondary metabolites, plant defense theory, phenotypic plasticity

INTRODUCTION

High levels of herbivory in the tropics have selected for increased levels of secondary metabolites, which can act as chemical defenses. (Coley et al. 2003). In Cerro de la Muerte, Costa Rica, local people use many different plants to remedy common ailments. The biologically active compounds within these plants, which confer their medicinal value, are generally thought to have evolved because of their function in plant defense systems.

Plant defense theory predicts that because replacing lost leaves entails a greater cost in low-resource environments, mature leaves of shade tolerant species should have higher levels of chemical defenses than mature leaves of species found in high-light conditions (Coley et al. 2003). Based on this theory, we hypothesized that varying light availability would produce the same pattern within a single species, but through phenotypic changes rather than genotypic changes. We therefore predicted that there would be higher levels of secondary compounds in individual plants growing in shade habitats for a given species.

Alternatively, high light conditions can lead to increased plant defense. If low nutrient (e.g. nitrogen or phosphorous) availability limits plant growth, surplus carbon fixed through photosynthesis in high light conditions may be shunted into the production of secondary metabolites (Bryant et al. 1983). Under this model, individual plants growing in sun habitats would be predicted to have higher levels of secondary metabolites than those growing in shade habitats.

Finally, it is possible that plant species may lack adaptive phenotypic plasticity in regulating secondary metabolite production. In this case, we would see no difference in the levels of

secondary metabolites across light habitats within a given species. To investigate the difference in the levels of secondary metabolites between sun and shade habitats, we selected four species of plants with known medicinal properties, distributed in both shade and sun habitats (Kehas 2003).

METHODS

On 27 January 2006 we sampled shaded understory habitat northeast of open pasture north of the Cuerici Biological Station in Cerro de la Muerte, Costa Rica. With the help of Sr. Carlos Solano we identified and collected samples in both open (sun) and understory (shade) environments. We selected one plant species each from the families *Urticaceae* (10 plants in sun, 10 plants in shade), *Winteraceae* (5, 5), *Verbenaceae* (6, 3), and *Clusiaceae* (1, 5). We took 1-4 mature leaves from each plant, sealed them in airtight bags, and kept them shaded until processing.

We conducted a double-blind smell test of each sample and rated scent intensity (1-3; 1 = weak, 3 = strong). We ran paper chromatography (PC) tests (modeled after thin layer chromatography) on 3 randomly selected replicates of each species in each habitat, using Whatman 4 filtration paper to create a PC plate. We placed the selected leaf over the bottom 1 cm segment of the filtration paper and rolled a quarter across the leaf to transfer a chemical extract onto the paper. We modified 5 oz transparent plastic cups, filled with 2 cc of 96% alcohol to support the PC plate and run the chromatography test, covering the samples during each run to prevent evaporation. The first orange band represented the presence of secondary metabolites. The second two bands, green and yellow, were identified as chlorophyll and disregarded. If the distance migrated by the orange band was consistent throughout all samples for a given species, we concluded that that band represented the same secondary metabolite. All orange bands were visually rated by intensity (0-3; 0 = not present, 3 = strong).

We then macerated a 6.25 cm^2 leaf sample of 3 randomly selected replicates of each species in each habitat, mixed it with 3 cc of water using a mortar and pestle, and measured the pH using Fisher Scientific short range alkacid no. 2 strips.

We ran three two-way ANOVAs to test how smell intensity, pH, and PC band intensity varied according to species, habitat, and the interaction between species and habitat (JMP 5.0.1).

RESULTS

We detected significant differences in smell, pH, and PC band intensity among species (Figure 1). However, we detected no overall difference between habitats, and no interaction between species and habitat (Table 1). We estimated that our analysis for habitat had a power of 0.82 to detect a difference in band intensity of 0.5, a power of 0.99 for a difference in pH of 0.2, and a power of 0.99 for a difference in smell intensity of 0.3.

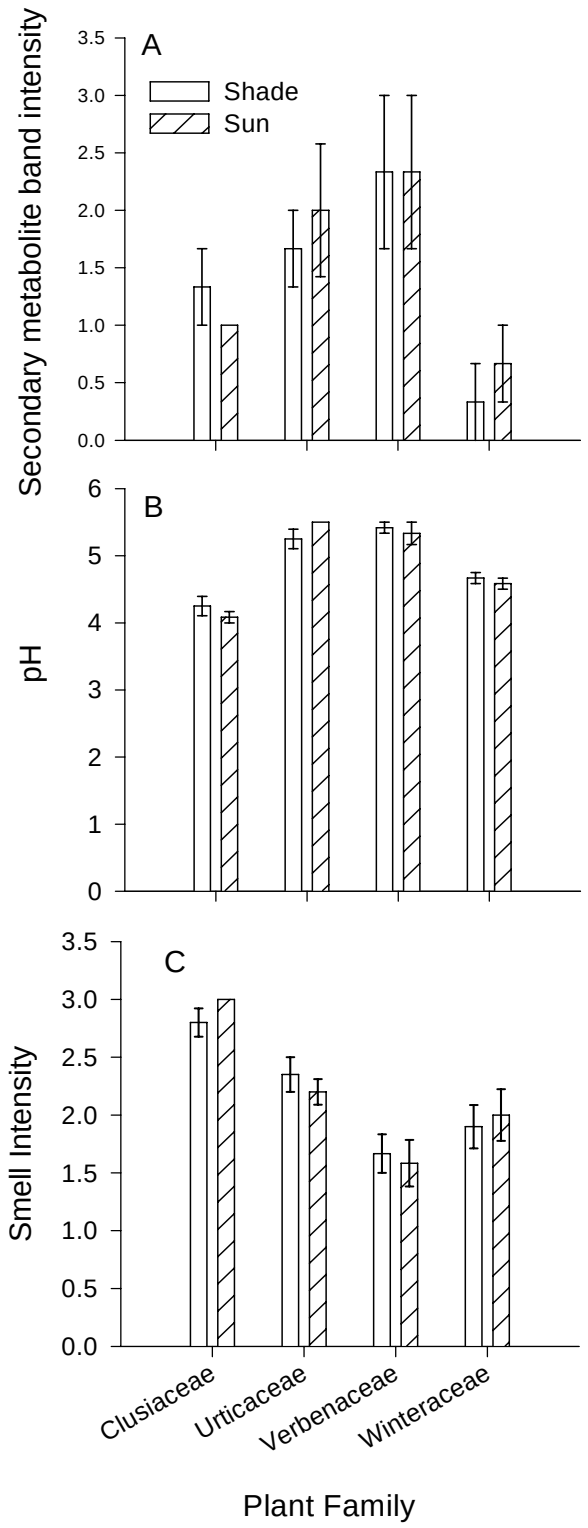

Figure 1. Variation in secondary metabolite concentration based on (A) paper chromatography band intensity, (B) pH, and (C) smell intensity (to the investigators) across four species of plants in both sun and shade habitats. Data were collected near the Cuerici Biological Station in Cerro de la Muerte, Costa Rica.

DISCUSSION

Although different plants showed clear differences in the amount and nature of their secondary metabolites, we found no evidence that light environment (sun vs shade) influenced intraspecific variation in plant chemistry. These results support the hypothesis that plants may lack adaptive phenotypic plasticity in regulating the production of secondary metabolites on the basis of light availability. These results do not match either: (1) the pattern predicted by extension to phenotypic variation of the resource availability hypothesis (Coley et al. 2003), or (2) the pattern of increased production of secondary metabolites in high light conditions, as predicted by the carbon shunting hypothesis (Bryant et al. 1983).

Even though we had power to detect differences in secondary metabolites between species, our methods could have masked variation in individual compounds which may have medicinal uses. Nevertheless, we found no evidence suggesting an individual seeking these species should discriminate between sun and shade habitats to find the most effective medicinal plant within a given species.

TABLE 1. Two-way ANOVA results for effects of pH, band intensity, and smell intensity on species and habitat (sun versus shade). Degrees of freedom for error = 16.

		pH		Band Intensity		Smell Intensity	
	df	F	P	F	P	F	P
Species	3	58.35	<.0001	6.11	0.0057	9.03	0.0001
Habitat	1	0.071	0.79	0.067	0.80	0.011	0.92
Species*Habitat	3	1.40	0.28	0.24	0.86	0.30	0.82

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