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Herbivory and alkaloid concentration in Cecropia obtusifolia (Cecropiaceae) trees with and without ants

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ABSTRACT

Herbivore damage greatly impacts a plant's fitness, and because of this, plants have evolved various defenses. *Cecropia obtusifolia* has a facultative mutualism with *Azteca* ants. Selection could favor plants without ants to compensate in some way, like increasing concentrations of secondary compounds. In this study, percent herbivory and alkaloid concentration were measured in *C. obtusifolia* trees with and without ants in Monteverde, Costa Rica. Herbivory levels were low in both occupied **(**mean ± sd **=** 2.702 ± 2.139**)** and unoccupied (3.029 ± 3.662) trees, and no significant difference was found in the concentration of alkaloids (mean ± sd with ants = 0.57 ± 0.34, without ants = 0.63 ± 0.45). These results could be a consequence of the dry season.

RESUMEN

El exito reproductivo de las plantas se ve altamente afectado por la herbivoría debido a esto, las plantas han evolucionado varias defensas. *Cecropia obtusifolia* tiene un mutualismo facultativo con hormigas del género *Azteca*. La selección puede favorecer plantas sin hormigas para compensar de alguna manera, como aumentando la concentración de compuestos secundarios. En este estudio, el porcentaje de herbivoría y concentración de alcaloides se midio para plantas de *C. obtusifolia* con y sin hormigas en Monteverde, Costa Rica. Los niveles de herbivoría fueron bajos tanto en plantas ocupadas **(**promedio ± ed **=** 2.702 ± 2.139**)** como desocupadas (3.029 ± 3.662), y no existen diferencias en la concentración de alcaloides (promedio ± ed con hormigas = 0.57 ± 0.34, sin hormigas = 0.63 ± 0.45). Estos resultados son una consecuencia de la época seca.

INTRODUCTION

Plants employ a variety of defense mechanisms, both physical and chemical, to protect them from herbivore attack. Some defenses include trichomes (Traw and Feeny, 2008), spines (Gowda and Palo, 2003), leaf toughness (Loney et al., 2006), alkaloids (Elger et al., 2009), cyanogenic glycosides (Goodger and Woodrow, 2002), and phenolics (Elger et al., 2009). These mechanisms are believed to increase plant fitness in the presence of herbivores, but also incur costs. Because of this, they are often found in high levels in seedlings and juveniles, which can experience a large reduction in fitness with herbivore damage (Gowda and Palo, 2003).

Another way plants may defend themselves is by evolving and maintaining specialized ant mutualisms. For example, in mainland Costa Rica, three of four *Cecropia* species have evolved mutualisms with *Azteca* ants that nest within their hollow trunks. The ants are therefore provided with housing and also a proteinaceous food source from the Müllerian bodies produced by the leaves. By housing *Azteca* ants, *Cecropia* trees are aggressively defended: the ants attack herbivores that try to feed on leaves (Longino, 1989). In a study by Schupp (1986), unoccupied *Cecropia* had 5.4 times as much leaf damage as occupied saplings during the dry season. Vasconcelos and Casimiro (1997) had similar findings: leaf-cutting ants attacked 71-100 percent of trees unoccupied by *A. alfari* colonies, while only 15-45 percent of occupied trees were attacked. Both studies concluded that ants play a role in decreasing herbivory, thereby increasing plant fitness.

Cecropia trees unoccupied by ants may compensate by increasing their chemical defenses. In highly mutualistic and obligate associations, such as that between *Acacia* and *Pseudomyrmex* ants, plants with defending ants have no obvious chemical defenses (Janzen, 1966). *Cecropia polyphlebia*, also does not house ants and it has been found to not only have low Müllerian body production (Janzen, 1973), but also slightly higher leaf alkaloid concentrations as compared to *C. obtusifolia* species with ant mutualisms (Gottheiner, 1998). Alkaloids are secondary constituents that are believed to provide herbivore defense due to their toxic effects on many animals (Hartmann, 1991). High alkaloid levels are known to reduce nutrient utilization, feed efficiency, animal productivity, and in some cases cause death in grazing animals and leaf feeding insects (Achakzai et. al, 2009). Selection should favor *Cecropia* plants without ants to produce greater concentrations of chemicals toxic to herbivores (Smiley, 1986).

In this study, I focused on a single *Cecropia* species, *C. obtusifolia*, which has a facultative mutualism with *Azteca* ants (Schupp, 1986). Because of this, some individuals house ants, while others do not. In this paper, I compare percent herbivory and alkaloid concentrations in individuals of *C. obtusifolia* with and without ant mutualisms. I expected plants without ants to have the capacity to compensate by having higher chemical defenses in their leaf tissue.

METHODS

SITE DESCRIPTION.—The study was conducted during the dry season in Monteverde, Costa Rica, between April and May 2010. *Cecropia* trees were found in San Luis (1,200 m), Bajo del Tigre (1,300 m), and Santa Elena (1,400 m), all of which are Premontane Moist Forests according to the characteristics of the Holdridge life zones. The mean annual rainfall is 2000-4000 mm, the mean annual temperature is 17-24 °C, and the dry season is 4-5 months in duration (Haber, 2000). I chose *C. obtusifolia* that were 2-8 m tall. To be certain they were *C. obtusifolia*, I chose only trees that had fruits near 50 cm in length, and had rough leaf surfaces with ten or more lobes. Once individuals were found, the tree trunks and/or branches were vigorously shaken to determine if ants were present. The third youngest leaves were clipped from trees with ants and trees without ants. Young plant parts generally contain greater levels of alkaloids as compared to old parts (Achakzai et al., 2009). Also, the third youngest leaves have lived adequate time to experience herbivore damage. And because young leaves are approximately half as tough, less fibrous, and significantly more nutritious than mature leaves (Coley, 1983), herbivores may target them. Other than the absence of ants, there were no apparent differences between these groups of plants.

HERBIVORY.—Herbivory was calculated for each leaf by placing the leaf under a transparent, plastic grid. First, the total number of squares occupied by leaf tissue was counted. Then, the total number of squares missing leaf tissue was counted. These squares had fifty-percent or more leaf tissue removed. Percent herbivory was equal to the total number of squares missing divided by the total number of squares times 100.

ALKALOID CONCENTRATION.—Collected leaves were dried for at least 12 hours, or until they were completely dry. A mortar and pestal was then used to break the leaves into small pieces and 4.0 g samples were weighed out. The samples were saturated with methanol, and left soaking for 12-15 hours. In the morning, the methanol was filtered out, and then the beakers containing methanol were heated until all methanol evaporated. Next, 5 mL of 2N H_2SO_4 was added to the beaker. The solution was then transferred to a test tube and washed 3 times with equal volumes of chloroform. The chloroform layers were discarded each time. NH_4OH basified the solution to a pH of 10. The alkaloids were again washed 3 times with equal volumes of chloroform. This time, the chloroform layers were preserved in test tubes. The test tubes were placed in a hot water bath to evaporate the chloroform. Now isolated alkaloids were mixed with 4.0 mL of 0.1N H_2SO_4 and 0.4 mL of Dragendorff's reagent. A spectrophotometer at wavelength 530 nm measured transmittance (Scalley, 1993).

RESULTS

Eighteen leaves from trees with ants and twenty-one leaves from trees without ants were collected and measured.

HERBIVORY.—The percent herbivory of trees with ants (mean ± sd = 2.702 ± 2.139) and the percent herbivory of trees without ants (3.029 ± 3.662) were not significantly different (t test, df = 37, t = 0.334, P = 0.7405, see Figure 1). Plants with ants had 0.9% to 8.9% leaf tissue removed, and those without had 0.2% to 12.5% removed.

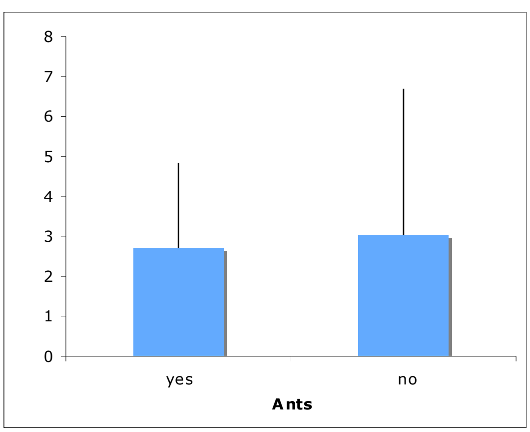

Figure 1. Mean percent herbivory (± SD) of *C. obtusifolia* trees with (n=18, 2.702 ± 2.139) and without (n=21, 3.029 ± 3.662) ants. Percent herbivory is not significantly different between the two groups (t test, df=37, t=0.334, P=0.7405).

ALKALOID CONCENTRATION.—Alkaloid concentrations of leaves were not significantly different between plants with ants (n=5) and plants without ants (n=7) (Mann Whitney u-test, df = 1, chi squared = 0.0595, P = 0.8072, see Figure 2). No relation was found between alkaloid concentration and percent herbivory (Spearman rank, z = 0.53, P = 0.59, see Figure 3).

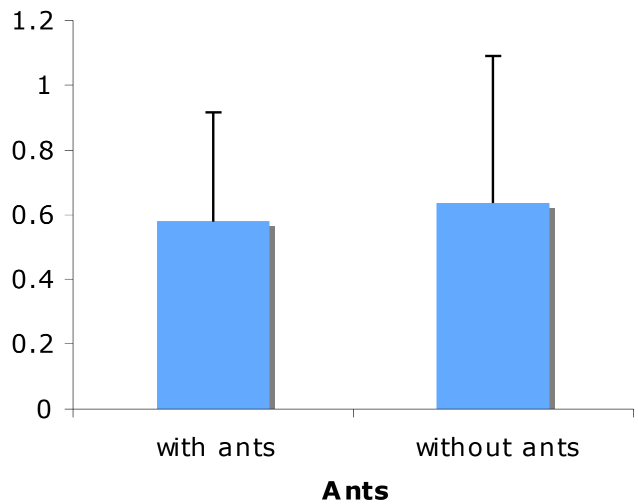

Figure 2. Mean absorbance (± SD) of *C. obtusifolia* trees with (n=5, 0.57 ± 0.34) and without (n=7, 0.63 ± 0.45) ants. Transmittance was measured with a spectrophotometer and the values were converted to absorbance with the equation absorbance = -log(transmittance/100). No difference was found between the two plant groups (Mann Whitney U-test, df=1, chi squared = 0.0595, P = 0.8072).

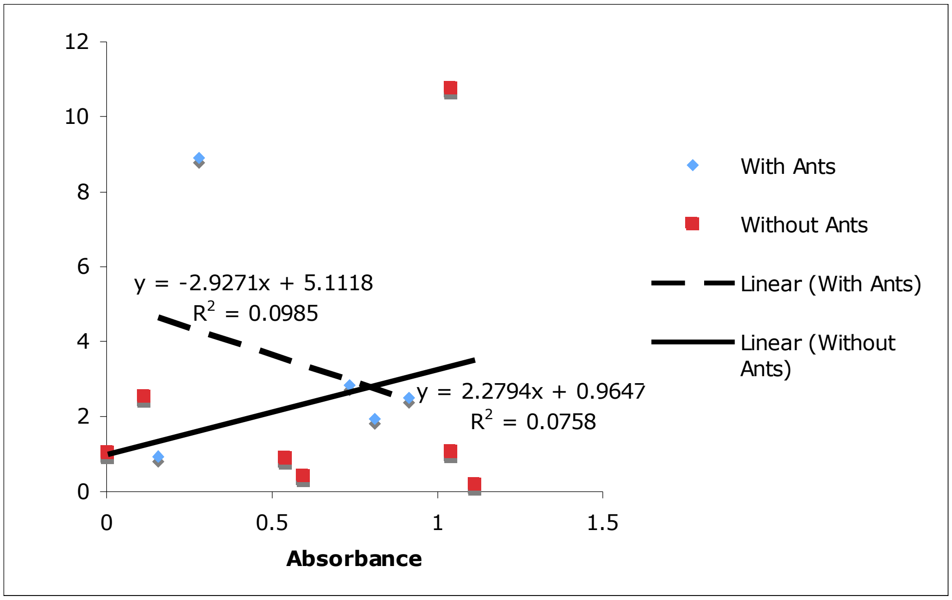

Figure 3. Absorbance versus percent herbivory in *C. obtusifolia* trees with and without ants. No relation was found between alkaloid concentration and percent herbivory (Spearman rank, z = 0.53, P = 0.59).

DISCUSSION

Herbivory percentages were very low for both occupied and unoccupied plants. This study was conducted in April, the end of the dry season, and therefore it is probable that herbivores were less active. Coley (1983) observed that 46% of pioneer species were grazed during the dry season, whereas 89% of species were grazed in the wet season. The decrease in abundance of herbivores during the dry season may explain why neither occupied nor unoccupied plants experienced much herbivory in this study. The results of a study done by Fáveri and Vasconcelos (2004) strongly suggested that when *Cecropia* suffers low incidence of attack, it is able to survive in the absence of defensive ants.

Cecropia trees respond to draught in various ways. For example, host-tree Müllerian body production decreases and internode humidity also decreases to damaging levels during the dry season. Branch exit holes are also smaller and fewer (Longino, 1991), perhaps an adaptation for reducing water loss. This suggests that trees allocate resources differently during the dry season, thus possibly affecting their ability to house ants. *A. alfari* does not forage away from its host-plant, relying entirely on the plant's resources (Vasconcelos and Casimiro, 1997). Plants may therefore not house ants during the dry season when resources are limited. Or, plants may induce ant activity only in the presence of herbivores (Agrawal and Rutter, 1998).

However, the individuals studied showed much variation in the amount of herbivory. *Azteca* species were not identified in this study, and they vary greatly in their behaviors towards herbivores (Longino, 1991). The amount of tissue removed in an individual most likely depends on which species of *Azteca* is occupying the plant. Furthermore, *A. alfari*, the least aggressive of all *Cecropia*-inhabiting *Azteca*, is one of the two most abundant species in Costa Rica. Perhaps occupied individuals in this study were housing *A. alfari* and were only weakly protected, therefore having similar herbivory rates as unoccupied individuals. Frequently chopped roadsides and agricultural land generally inhabit *Cecropia* tress with *A. alfari* (Longino, 1991) and collected leaves came from roadsides in Santa Elena and pasture land of San Luis.

Regarding alkaloids, they may be an induced defense in this species, only being "turned on" by a plant following damage. By controlling this expression, plants are resistant to herbivory when necessary and can allocate more resources to growth and reproduction when they are not under attack (Karban et. al, 1997). The low levels of alkaloids found in this study can be explained by inducible defenses: the lack of insects resulted in low herbivory, and the lack of herbivores resulted in low alkaloid production.

It is also possible that alkaloids are not an inducible defense in this species. Instead, the low concentrations found may be sufficient in protecting the leaves, resulting in the low herbivory found here. A constant alkaloid concentration may mean that ants are unreliable, and plants instead produce alkaloids for protection, and they are effective even at low levels.

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