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Müllerian body production by Cecropia obtusifolia (Cecropiaceae) with and without Azteca ants

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ABSTRACT

Cecropia obtusifolia (Cecropiaceae) has a facultative mutualism with *Azteca* ants to minimize herbivory. In this study, Müllerian Bodies (MBs), glycoprotein bodies the plant produces to feed its mutualist ants, were systematically removed, from *C. obtusifolia*. A second study tested at what rate the tree would decrease its production of MB if the ants were restricted from removing them. It as found that removal of the MBs did result in an increase in MB production for two of the five trees in the treatment group. The *C. obtusifolia* where the ants were restricted from removing the MB, in all but one tree, showed a rapid decrease in MB production in the first few days. The results were also looked at in terms percent canopy cover and herbivory. It was found that plants without ants suffered significantly higher herbivore damage (Mann Whitney U-test, df=1, $\chi^2 = 5.51$, $p<.05$). A trend between an increase in percent canopy cover and a decrease in MB production was noted but was not statistically significant. It seems that MB production is a costly process for *C. obtusifolia* and worth the trade off when they receive the protection from the *Azteca* ants but when ants are not present they will quickly divert that energy elsewhere

RESUMEN

Cecropia obtusifolia (Cecropiaceae) tiene un mutualismo facultative con las hormigas del género *Azteca* para reducir herbivoría. En este estudio, cuerpos de Muller (CM), glicoproteínas que la planta produce para alimentar la hormiga mutualista, fueron sistemáticamente removidos de *C. obtusifolia*. Un Segundo estudio probo a que tasa la planta decrece en la producción de CM si se restringe el acceso a los mismos por parte de las hormigas. Encontré que la remoción de CM resulta en un aumento en la producción de los mismos en dos de cinco árboles utilizados en este tratamiento. En las plantas en las que se restringió el acceso a las hormigas a los CM, en todos excepto uno, se desmostró un rápido decline en la producción de CM a los pocos días. Se analizó también el porcentaje de cobertura de dosel y la herbivoría. Encontré que las plantas sin hormigas sufren mayor daño por herbivoría (Mann Whitney U-test, df=1, $\chi 2 = 5.51$, $p<.05$). Existe una tendencia a una menor producción de CM al aumentar la cobertura de dosel, pero no es estadísticamente significativa. Parece que la producción de CM es un proceso costos para *C. obtusifolia* y vale la pena producirlo cuando tienen la protección por parte de las hormigas, pero cuando no están estas presentes en mejor utilizar la energía en otros procesos.

INTRODUCTION

Plants employ an array of defenses to protect themselves against herbivore attacks, and these defenses can be both chemical and physical. An example is the facultative symbiotic relationship of many ant-plants, in which ants provide an herbivore defense in exchange for receiving housing and food from the plant, though neither organism completely relies on the other for survival (Folgarait & Davidson 1995, Karban *et al*. 1999). Ants are an excellent herbivore defense for plants for several reasons: (1) they are aggressive and have well-developed defense mechanisms such as biting and stinging, (2) they are willing to sacrifice themselves in defense of the colony, and (3) they can release chemical cues telling others of a disturbance (Hölldobler & Wilson 1990).

The genus *Cecropia* (Cecropiaceae), a neotropical pioneer tree, has around 70-80 different species, of which 80% are myrmecophytic and inhabited by symbiotic ants

(Davidson & Fisher 1991). The symbiotic ants include four subfamilies: Dolichoderinae, Formiciae, Myrmicinae and Ponerinae (Folgarait *et al*. 1994). In Costa Rica there are only five species of *Azteca* (Dolichoderinae) ants that are mutualistic with *Cecropia* three of which: *C.peltata*, *C. obtusifolia*, and *C. insignis*, occur in the Monteverde region, where I conducted my study.

Myrmecophytic *Cecropia* trees produce Müllerian bodies (MB), which are small ovoid structures, located on and produced by special petiolar pads called trichilia (Rickson 1976, Davidson & Fisher 1991). These MBs are full of glycogen and small amounts of protein, and are a main food source for *Azteca*. Along with food Cecropia also provides housing for the ants; the hollow internodes in the tree form domatia which the ants inhabit (Longino 1991). In this facultative relationship with *Cecropia Azteca* ants, in turn, contribute by helping protect the plant from herbivores (Janzen 1969, Schupp 1986). This relationship clearly benefits the *Cecropia* in at least two ways. The sapling growth rate when occupied by ants is significantly greater than that of unoccupied trees (Schupp 1986). Additionally, there is a positive correlation between the number of ants inhabiting the tree and the tree's resistance against herbivores (Rocha & Bergallo 1992).

While this facultative relationship increases the plants fitness when herbivores are present in the environment, it is costly to the plant in terms of energy and nutrient expenditure. If herbivores are absent, producing MBs would be taxing and wasteful, and actually decreases the plant's fitness (Agrawal 1998).

This cost-benefit trade off leads to the idea that without herbivore pressure or ant occupation the *Cecropia* would limit production of MB (Putz & Holbrook 1988, Scalley 1993, Karban *et al*. 1999). Studies have shown that when ants are not present, the *Cecropia* reduces production of MB but when ants return, the *Cecropia* will increase production of MBs (Folgarait *et al*. 1994, Karban 1999) but this has not been attempted outside of a greenhouse. These are indications of both the costliness of producing MBs and the ability of *Cecropia* to 'detect' if the MBs are being removed (Janzen 1973, Folgarait *et al.* 1994).

My study examined the facultative mutualism of *C obtusifolia,* and *Azteca* (Schupp 1986) ants in relation to canopy cover and herbivore damages, in the Monteverde region of Costa Rica The two main questions the study was designed to answer were:

(1) At what rate is MBs production increased by *C. obtusifolia,* when MBs are manually removed, simulating ant inhabitation, compared to *C. obtusifolia* in which MBs are not removed;

(2) If ants are restricted from removing MBs, how quickly does *C. obtusifolia* reduce MB production?

MATERIALS AND METHODS

SITE DESCRIPTION.— The study was conducted during the wet season in Monteverde, Costa Rica, between October and November 2010. *Cecropia* trees were encountered in Bajo del Tigre (1,300m), Santa Elena (1,400m), Cerro Plano (1450m), and the biology station (1550m).

FIGURE 1. Newly produced Müllerian bodies on the trichila of *C. obtusifolia* from a plant in Monteverde, Costa Rica.

FIGURE 2. Vaseline and mosquito netting to prevent ant removal of Müllerian bodies

(1500m). Each is Premontane Moist Forest according to Holdridge. Mean annual temperature is 17-24 °C and the mean annual rainfall is 2000-4000 mm (Haber 2000). I chose *C. obtusifolia* that were 1.5-2.5m tall. Once the trees were found, the trunks were shaken to determine if ants were present. Seven trees with ants were found and labeled A2-A8. Ten trees with-out ants were found and labeled B1-B10.

MB REMOVAL EXPERIMENT.— Ten *C. obtusifolia* without ant inhabitants were located, divided into a control (B3,5,6,7,9) and treatment (B1,2,4,8,10) group of five each. Vaseline was placed around the bottom of the trunk of each tree to prevent new ants or any other insect from reaching trichilia. In the treatment group, the presence of ants was simulated by manually removing MBs daily. In the control group, MBs were allowed to accumulate on plants, this treatment simulating the absence of ants. Because MB production by *C. obtusifolia* normally peaks just after dusk (Davidson & Fisher 1991) I counted the number of MB every morning on the three youngest leaves of each plant. The three youngest leaves were chosen because I observed only the three youngest trichilia tended to produce MB. I record the number of new MBs-- which were detectable by their brighter white color (Figure 1). The production of MB was monitored for 16 days, from November 3 to November 18, 2010.

ANT REMOVAL EXPERIMENT.— On the *C. obtusifolia* that had ants (n=7) I used a combination of Vaseline and mosquito netting (Figure 2) to prevent ants from removing MBs on the three youngest leaves by covering the trichilia. I returned every morning and recorded the new MBs that were produced for 13 days between Nov. 5 and 18, 2010. The new MBs were again identified by their brighter white color.

CANOPY COVER.— Percent canopy cover was measured for each plant by using a Densitometer. In order to gage the amount of direct sunlight each tree was receiving in relation to each other.

HERBIVORY.— Herbivory was calculated for each plant by placing each leaf under a transparent grid with $1cm^2$ squares. Younger leaves were chosen because they tend to have higher herbivory (Garnsey 1999), normally the third of forth leaf down on the plant. The leaf that was chosen seemed to be a good representation of the overall herbivore damage to the plant. First the total area within the perimeter of the leaf was calculated. Then the total number of squares missing leaf tissue was counted. Percent herbivory was equal to the total number of squares missing leaf tissue divided by the total number of squares within the perimeter of the leaf multiplied by 100.

ANT RATIO.— In order to gage the ant population of each tree, I shook each tree vigorously, counted to ten and then recorded that number of ants along a 10cm portion of the trunk between the first and second leaves.

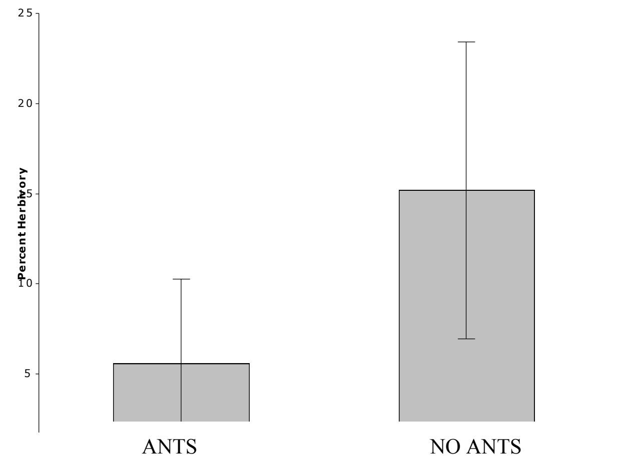

FIGURE 3. Mean percent herbivory (±SD) of *C. obtusifolia* trees with (n=7, 5.57± 4.69) and without (n=10, 15.2± 8.23) ants in Monteverde, Costa Rica. Percent herbivory was significantly different between the two groups (Mann Whitney U-test, df=1, χ^2 = 5.51, p<.05) [on November 18, 2010]

RESULTS

MB REMOVAL EXPERIMENT.— In the treatment group, over the 16-day study period in which MBs were removed from each plant daily, the average MB production per day did not show an overall change. For trees B1 and B8 there was actually a decrease (Table 1). A linear regression analyses for MB production by day was run for each tree in both treatment and control groups. All the trees in the control group had positive slopes and the equation of the line was significant (p<.05). Two *C. obtusifolia* in the treatment group demonstrated a strong positive correlation between MB production and day. There was a two fold and five fold increase in the slope over the largest slope in the control group, respectively, for trees B2 and B10. Tree B2 (Figure. 4) and B10 demonstrated that MB production per day was greater for those trees with daily removal than those in the control, though this trend only help true for two of the five trees. It should be noted that the weather changed and the last five days of the

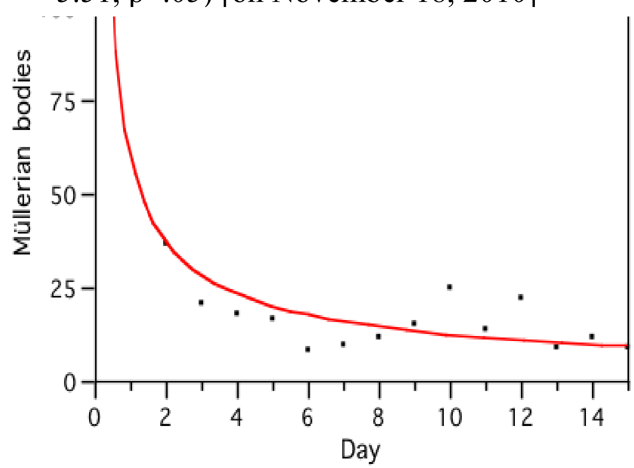

FIGURE 4. Relationship between the number of Müllerian Bodies found on *C. obtusifolia* plant A2 per day this trend is constant for six of the seven sampled (Nov. 3-18, 2010). A regression fit log to log was run. Lny= 4.07 – 0.67ln(x). One way ANOVA, F= 19.8, R^2=0.60, n=16, p<0.05. Data taken in Monteverde, Costa Rica.

experiment were considerably warmer and sunnier.

	Equation	R^2	n	F	p
B3 c	**Y= -4.48 + 1.75x**	**0.40**	**16**	**9.45**	**.0083**
B5 c	**Y= -4 + 2.02x**	**0.73**	**16**	**38.75**	**<.0001**
B6 c	**Y= -1.33 + 0.85x**	**0.72**	**16**	**36.68**	**<.0001**
B7 c	**Y= 1.68 + .6x**	**0.34**	**16**	**7.36**	**.016**

TABLE 1: Results of linear regression analyses for Müllerian bodies (MB) production in Monteverde, Costa Rica over 16 days for *Cecropia obtusifolia* without ants. B1-10 each represents an individual *C. obtusifolia*, followed by a 'c' or 't' for control or treatment group, respectively. Trees with a significant relationship between time and MB production are in bold ($p<.05$).

ANT REMOVAL EXPERIMENT.— During the 13 day sampling of *C. obtusifolia,* where ants were restricted from removing the MB, all but one tree showed an overall decrease in MB production (Table 2). Five of the seven trees had a significant relationship ($p<.05$) between MB production and day, when both were log transformed. Tree A5 was close to being significant with p=.08. Figure 3 demonstrates the rapid decline in MB production over the first few days for tree A2, this trend is similarly represented for trees A4, A5, A6 and A7, thought not as pronounced. In all the trees that followed this trend within around 3 days they all dropped and hit a baseline MB production of usually around 15 or so MB.

CANOPY COVER.—Canopy cover had a trend but no significant impact on MB production for plants without ants (n=5) and no MBs removed manually (F=0.68, R^2=0.18 p=.46 see Figure 6). In the treatment group the tree that had the most MB produced with 1,811, had the lowest percent canopy cover 7.5% and the tree that had the least with only 20 MB, had the highest percent canopy cover (76%).

HERBIVORY.—The percent herbivory of trees with ants (mean ± Sd= 5.57± 4.69) and without ants (mean± sd = 15.2± 8.23) were significantly different (Mann Whitney U-test, df=1, χ^2 = 5.51, $p<.05$ see Figure 3). Plants with ants had from 1% to 13% leaf tissue removed, and those without had from 2% to 28% removed. Of the ant-plants sampled, all appeared to have substantial populations of ant inhabitants.

TABLE 2: Regression analyses for Müllerian body (MB) production in Monteverde, Costa Rica over 13 days for *Cecropia obtusifolia* whose ants were restricted from removing them. MB production and day were log transformed. A2-8 each represents one of the 7 individual *C. obtusifolia* sampled. Trees with a significant relationship between time and MB production are in bold (p<.05).

	Equation	R^2	n	F	p
A2	**Ln(y)=4.07-.67ln(x)**	**0.60**	**15**	**19.8**	**.0006**
A3	**Ln(y)= -2.64 + 1.7ln(x)**	**0.53**	**13**	**4.58**	**.01**
A4	**Ln(y)= 5.14 – 2.34ln(x)**	**0.91**	**13**	**60.03**	**.0002**
A5	Ln(y)= 2.81 – 1.29ln(x)	0.85	13	11.61	.080
A6	**Ln(y)= 3.85 – 0.57ln(x)**	**0.56**	**13**	**13.99**	**.0033**
A7	**Ln(y)= 2.68 – 1.03ln(x)**	**0.73**	**13**	**16.60**	**.0065**
A8	Ln(y)= .46 – 0.31ln(x)	0.09	13	.86	.38

DISCUSSION

Overall the treatment group did not show an increase in MB production as expected. Two of them even stopped producing MBs all together. Folgarait *et al.* (1994) did a similar study in a green house where they controlled for certain abiotic factors. In two of their three experiments they found a significant increase in MB production for the plants in which they systematically removed the MBs. Trees B2 (Figure 5) and B10 (Table 1) in my treatment group did demonstrate that what Folgarait *et al.* (1994) found in their greenhouse experiment could be replicated outside of a greenhouse. In nature there are many more variables to consider. Canopy cover could possibly contribute to why some of these plants did not produce many MBs. The tree in my treatment group with the greatest canopy cover produced the least MBs overall, and the tree that produced the most MBs also received the most direct sunlight. It would seem that percent canopy cover plays into MB production. While there was more herbivore damage to the trees without ants a study by Agrawal & Rutter (1998) showed that in the case of *C. obtusifolia* it did not appear that damage to the plant to reduce the MB production significantly.

The control group had an overall increase in MB production. I can only think to attribute this to the fact that the weather in the last few days of the experiment was considerably sunnier and warmer.

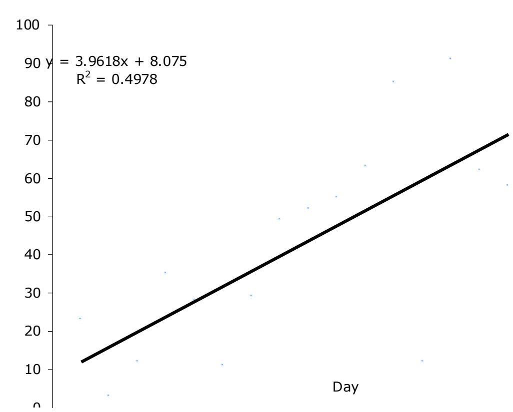

FIGURE 5. Relationship between the total number of Müllerian bodies (MB) produced per *C. obtusifolia* tree per day. Tree B2 was sampled in Monteverde, Costa Rica from Nov.3-18, 2010 (y= 8.08 + 3.96x, R^2= 0.50, p<.05).

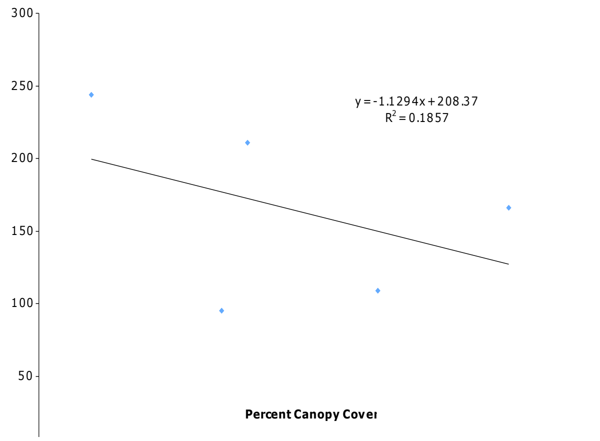

FIGURE 6. Relationship between the total number of Müllerian bodies (MB) produced over a 16 day span (Nov. 3-18, 2010) and the percent canopy cover for the control group of *C. obtusifolia* (n=5), in Monteverde, Costa Rica. (F=0.68, R^2=0.18 p=.46)

This trend did not hold for all the plants in the treatment group, possibly due to other contributing factors such as lower nutrients in the soil or just the small sample size. Folgarait *et al.* (1994) did find that there was no significant difference in their control and treatment groups when the MB removal experiments were done in low nutrient soil.

In the second test where the ants were restricted from removing the MB, there was an overall rapid decrease in MB production. This is an ideal example of how these inducible defenses increase plant fitness in the presence of herbivores (Agrawal 1998) but in the absence of ants the plant will allocate these resources elsewhere, such as to growth, reproduction or other defenses (Karban *et al*. 1997). Generally, within three days the production of MB is reduced to a new, lower baseline level. The tree was able to 'detect' the absence of ants and reduce production very rapidly.

Herbivory damage was significantly higher on unoccupied plants. These results differ from a previous study done in Monteverde last spring, where they found no significant difference (Riha 2010). The discrepancy in these results could be contributed to several factors: (1) that there has been observed about a two fold increase in herbivore damage from the dry season to the wet (Coley 1983), (2) that in *Cecropia,* ant number is positively associated with resistance against herbivores (Rocha & Bergallo 1992). All of the ant-plants sampled had substantial population of ants, which would have aided in protection. And perhaps the ants are more helpful for the trees during the wet season.

Three main factors seem to contribute to MB production: weather, leaf age and rate of MB accumulation. Weather, being the amount of sunlight and energy the plant receives was able to cause a substantial increase in MB production. Leaf age contributes as well, where only the youngest three leaves or so, produce MB. Since the youngest leaves tend to suffer more to herbivory, the plant is possibly trying to draw the ants up towards the younger leaves. And finally the rate of accumulation of MBs plays a major role in MB production, the more MBs left on the Trichilia the less MBs the plant will produce. It appears from this study that *C. obtusifolia* can quickly 'detect' both the removal and accumulation of MBs and adjust accordingly. This adjustment is rapid, with obvious signs seen within about a day or two, an example of an induced defense. The energy saved when the *C. obtusifolia* decrease its MB production is most likely diverted elsewhere, to growth or secondary metabolites for defenses. I would expect the plant to boost its other defense when ants are not present, since when comparing herbivore damage between trees inhabited by ants and those without, the ant mutualism appears quite beneficial.

Abiotic factors such as light exposure also play into the plant's fitness. Further work could be done to fully understand how each plays into the species fitness. A similar larger scale study could help conclude how this variation in MB accumulation likely to impact the mutualism.

While ant-plant mutualisms may appear ideal we must remember that ants and chemical defenses are similar in that their numbers (concentrations) are dependent on various environmental factors (Karban *et al.* 1999). There will always be this constant cost-benefit trade-off between energy put towards defenses versus herbivory damage, where the plant is trying to maximize its fitness. To conclude the costliness of MB production is worth the trade off for the defenses received from the Azteca ants, though as soon as the MB are no longer removed the plant will quickly divert that energy elsewhere.

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