

November 2005

Extra extrafloral nectaries and anti-herbivory protection in *Inga sierrae* (Fabaceae : Mimosaceae)

Camryn Pennington

Follow this and additional works at: https://digitalcommons.usf.edu/tropical_ecology

Recommended Citation

Pennington, Camryn, "Extra extrafloral nectaries and anti-herbivory protection in *Inga sierrae* (Fabaceae : Mimosaceae)" (2005). *Tropical Ecology Collection (Monteverde Institute)*. 316.
https://digitalcommons.usf.edu/tropical_ecology/316

This Text is brought to you for free and open access by the Monteverde Institute at Digital Commons @ University of South Florida. It has been accepted for inclusion in Tropical Ecology Collection (Monteverde Institute) by an authorized administrator of Digital Commons @ University of South Florida. For more information, please contact scholarcommons@usf.edu.

Extra extrafloral nectaries and anti-herbivory protection in *Inga sierrae* (Fabaceae: Mimosaceae)

Camryn Pennington

Department of Biology, University of Puget Sound

ABSTRACT

Extrafloral nectaries (EFNs) in plants are known to attract insects such as ants and wasps that drink nectar and in turn protect the plant from herbivores (Bentley 1977). In this study, ten year old *Inga sierrae* (Fabaceae: Mimosaceae) trees planted on a fallow pasture at 1535 m in Monteverde, Costa Rica have been observed to have extra EFNs on their leaflets in addition to the normal three located on the rachis between the leaflet pairs. The leaves of 20 trees were sampled to determine the local distribution of EFN number. Percent herbivory was determined for leaves with a varying number of EFNs by dividing damaged leaf surface area by total leaf surface area. In addition, insects were captured on leaves for eight days to find out if more protective insects visit leaves with more EFNs. The number of simulated insects eggs (made of animal lard) removed from leaves over 24 hours was used as a proxy for the level of predatory wasp activity on leaves with varying numbers of EFNs. Thirteen of 20 sampled trees had some leaves with extra EFNs. The number of extra EFNs ranged from one to five, and a linear regression revealed a significant trend between a higher numbers of extrafloral nectaries and lower herbivory on a per leaf basis ($r^2 = 0.0236$, $p = 0.0461$, $n = 169$). No trends between the number of EFNs and either the number of protective insects captured on leaves or the number of simulated herbivore eggs removed by predatory wasps were observed, however more parasitoid than predatory wasps were captured. These results show parasitoid wasps are the primary *I. sierrae* EFN visitors and suggest higher numbers of EFNs confer higher fitness to leaves. Trees with extra EFNs should produce more offspring and, hence, extra EFNs will become more prevalent in the *I. sierrae* population.

RESUMEN

Los nectarios extraflorales (EFN) de plantas atraen a insectos como hormigas y avispa que beben néctar y a cambio protegen las plantas de herbívoros (Bentley 1977). En este estudio, se observó que árboles de *Inga sierrae* (Fabaceae: Mimosaceae) que tienen diez años de edad y que están creciendo en pastos en barbecho a 1535 m en Monteverde, Costa Rica, tienen EFN adicionales en sus hojuelas en exceso de los tres regulares que se localizan en el raquis entre los pares de hojuelas. Las hojas de 20 árboles fueron examinados y los EFN fueron contados para determinar su distribución local. El porcentaje de herbivoría fue determinado para las hojas con números diferentes de EFN; el área dañada de la hoja fue dividida por el área total de la hoja. Además, se capturaron insectos en las hojas por ocho días para ver si las hojas con más EFN atraen a más insectos defensores. Se utilizó el número de huevos falsos de insectos (Hechos de grasa de animales) que fueron removidos como una representación del nivel de protección de las avispa depredadoras. Trece de los 20 árboles examinados tenían entre uno y cinco EFN adicionales. Una regresión lineal entre el porcentaje de herbivoría y el número de EFN mostró una relación significativa ($r^2 = 0.0236$, $p = 0.0461$, $n = 169$). No se encontraron relaciones significativas entre el número de EFN y el número de insectos capturados o el número de huevos falsos de insectos removidos, pero más avispa parasitoides que avispa depredadoras fueron capturadas. Los resultados muestran que las avispa parasitoides visitan los EFN más a menudo que otros insectos defensores y que más EFN aumentan la adaptabilidad de *I. sierrae*. Los árboles con EFN adicionales deben producir más descendientes y, por lo tanto, el número de *I. sierrae* con EFN adicionales debería aumentar en el futuro.

INTRODUCTION

Tropical forests, compared to temperate forests, experience higher levels of herbivory and as a result, plants in the Tropics have a higher level of protection than plants in the temperate zone (Coley and Barone 1996). Plants may store secondary metabolites in their tissues that are unpalatable, have low leaf nutrition (so little is offered to herbivores), or delay the production of expensive chlorophyll in young leaves, known as delayed greening (Coley and Barone 1996).

Third trophic level plant defenses, or the recruitment of herbivore predators by plants, also occur more commonly in the Tropics. A widespread Tropical example is the pioneer tree *Cecropia sp.*, which provides hollow stems as shelter and glycogen-rich Mullerian bodies as food for ants of the genus *Azteca* (Longino 1989). In return, the ants attack any herbivore that comes in contact with the tree (Longino 1989). Swollen-thorn *Acacia* trees in the neotropics similarly have *Pseudomyrmex* EFN-visiting resident ants (Janzen 1966).

Extrafloral nectaries (EFNs) are often the agents used to attract third trophic level protective insects. Protective insects drink the sugar, water and amino acid containing nectar secreted by EFNs (Pemberton 1992). The success of EFNs as an herbivore defense is apparent in their longevity, prevalence and effectiveness. EFNs have been noted in a fossil of the tree *Populus crassa* dating to the Oligocene period 35 million years ago and at least 93 plant families have EFN-bearing members (Pemberton 1992). Previous work with the EFN bearing plant *Catalpa speciosa* (Bignoniaceae) has shown that recruitment of anti-herbivory protective insects directly increases the fitness and reproductive capability of the plant (Stephenson 1982). When the ants that visit EFNs were excluded from branches of *C. speciosa* with a sticky, the branches experienced higher herbivory and lower fruit set than other branches on the same tree that were left untouched (Stephenson 1982).

Plants of the genus *Inga* (Fabaceae: Mimosaceae) produce extra-floral nectaries (EFNs) on the rachis of their leaves. EFNs are believed to elicit anti-herbivory protection on new leaves by attracting nectar eating ants or wasps that will deter insect herbivores (Bentley 1977). Indeed, folivorous insects are the main agents of herbivory in the Tropics (Coley and Barone 1996). Previous studies by Koptur have addressed the function of *Inga* EFNs in Costa Rica. *Inga punctata* and *Inga densiflora* EFNs have been shown to secrete nectar day and night, continuously attracting ants that remove herbivorous insects such as caterpillar larvae, beetles and katydids (Koptur 1984). Experiments with saplings of these two species show that more leaf damage is incurred when ants are excluded from leaves, leaving them more vulnerable to herbivores (Koptur 1984). Koptur's (1985) study of *Inga* EFN function across an elevational gradient (600 – 1650 m) in Costa Rica showed that nectar was secreted at all elevations, but more herbivore leaf damage is inflicted at higher elevations. Correspondingly, ants were the dominant *Inga* EFN visitor at lower elevations, but were replaced by parasitoid wasps at higher elevations (Koptur 1985).

Inga sierrae planted on a fallow pasture in Monteverde, Costa Rica have been observed to produce extra EFNs on their leaves (Gough 2003). In addition to the normal three EFNs located between the three leaflet pairs on the rachis, these individuals can have up to nine EFNs. Extra EFNs may be located adjacent to the normal EFNs on the

rachis or on any of the leaflet petiolules. Previous work with these *I. sierrae* in spring 2003 show leaves with a higher number of EFNs experience lower levels of herbivory damage. The average percent herbivory for leaves with three EFNs was 27.2%, while the average percent herbivory for leaves with at least one extra EFN was 10.6% (Gough 2003).

I. sierrae trees bearing extra EFNs provide a unique opportunity to study the overall effectiveness of EFNs attracting protective insects and deterring herbivory. Individuals such as these have not been observed elsewhere. It was the intent of this study to look for changes in the distribution and effectiveness of the study *I. sierrae* EFNs since spring 2003. Looking at the distribution now that the trees are two and a half years older will help elucidate if extra EFNs are age related. New *I. sierrae* leaves flush in the fall and may show different levels of herbivory when compared to the fully expanded leaves in the spring. It was predicted that leaves with higher numbers of EFNs would experience less herbivory. Also, experiments were conducted to determine if more protective insects are recruited to leaves with a higher number of EFNs and if leaves with more EFNs have a demonstrated higher level of protection. More parasitoid wasps were expected to visit leaves with higher numbers of EFNs (six to seven) than those with fewer (three to four) (Koptur 1985). Predatory wasps have been seen flying in the area of the trees, so an experiment was designed to test the amount of predatory wasp protection elicited by leaves with varying numbers of EFNs. It was predicted that more simulated herbivore eggs would be removed from leaves with higher numbers of EFNs than those with fewer.

MATERIALS AND METHODS

Study site

This study was conducted on fallow pastureland at 1535 m elevation in Monteverde, Costa Rica October- November, 2005. *I. sierrae* trees were planted in a row ten years ago on the edge of the pasture along a road. Seeds were taken from a few adults, meaning many of them were siblings. Three to four seeds were planted per plastic bag placed in the ground, resulting in 1-4 trees growing in close proximity, often with intertwined trunks and branches. Tree groups were separated by approximately 2 m.

EFN distribution

To determine the distribution of EFN number found on the *I. sierrae* trees, the number of EFNs on 60 leaves was counted on 20 trees for a total of 1,200 leaves. Trees were assigned a letter (A-T) to keep samples separate. Leaves selected were the first young leaves encountered that had a complete set of leaflets (Figure 1b). Young leaves, easily identifiable by a smaller size, tenderness and a lighter green color, have EFNs that are easy to see. Leaves with missing leaflets were excluded because extra EFNs can be located adjacent to the normal EFNs on the rachis or on the petiolule of the leaflet, so the absence of a leaflet may give an inaccurate count of EFNs for the leaf. If accessible, EFNs were counted on leaves on the tree. Pole-clippers were used to collect other leaves.

Percent herbivory

To determine if the number of EFNs found on a leaf affects the amount of herbivory

damage per leaf, percent herbivory was tabulated for leaves with varying numbers of EFNs. Herbivore defenses of *I. sierrae* leaves are age related. When the leaf is young, it is also tender and at its most vulnerable state (Figure 1a). Hence, young leaves employ EFNs to defend themselves against herbivores. *I. sierrae* EFNs stop secreting nectar once the leaf has expanded to their full size (Bentley 1977) (Figure 1c). Once leaves are fully expanded, they toughen, negating the need for EFNs. Leaves were collected from a variety of trees just after their EFNs dried up because any herbivory damage inflicted after this point would not be related to EFNs.

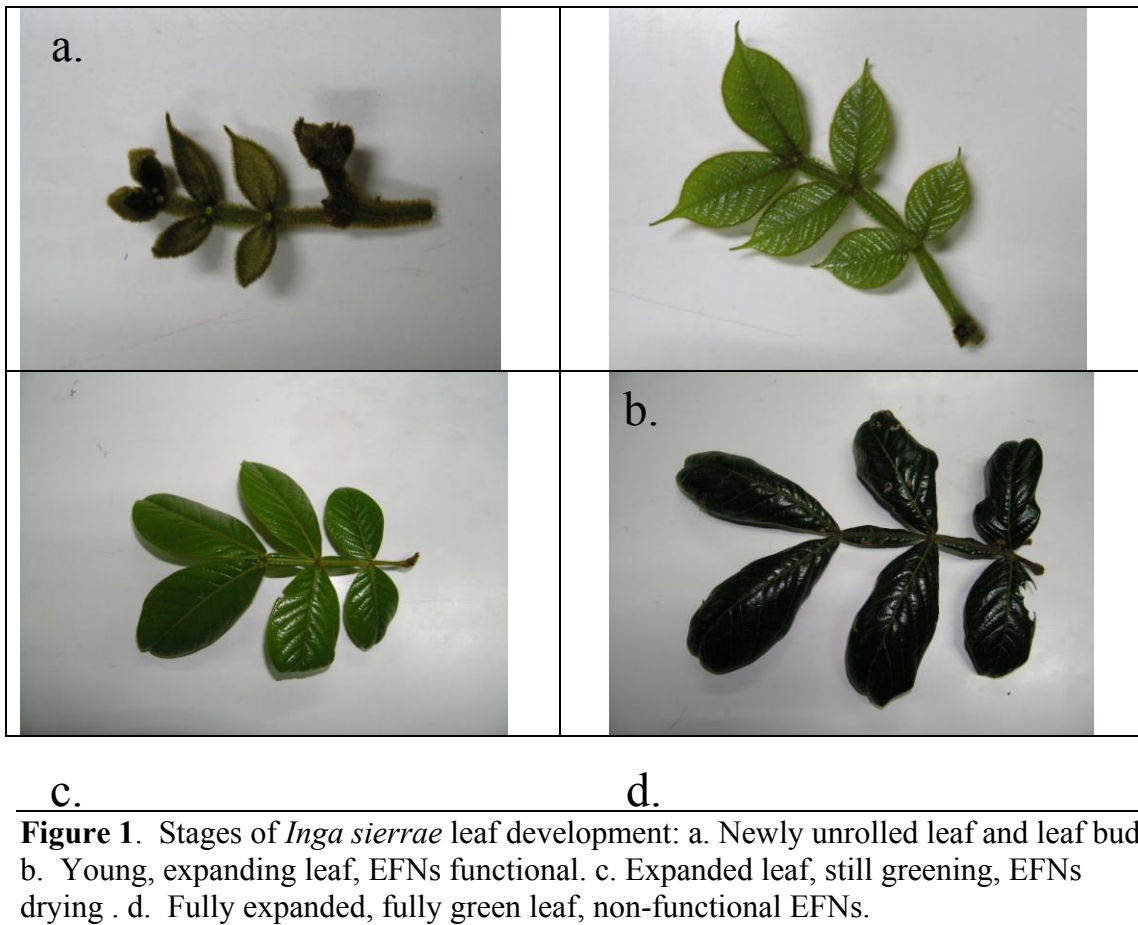




Figure 2. *Inga sierrae* leaf bearing extra extrafloral nectaries (EFNs) at the base of its leaflets. Study trees on a fallow pasture (1535 m) near La Estación Biológica de Monteverde, Costa Rica have two to nine EFNs, compared to normal leaves that have three EFNs.

Percent herbivory was calculated as the amount of leaf surface damage divided by the total surface area of the leaf. A one cm by one cm grid was placed over each leaf and any square filled at least halfway by leaf surface was counted. Missing leaflet parts were estimated relatively easily by comparing opposite leaflets, which are typically the same size and shape. Herbivory damage included missing tissue and discoloration due to scraping on the leaf surface, both of which result in reduced photosynthetic ability.

Protective insect quantification

To determine if extra EFNs recruit more protective insects to leaves, insects were collected on a per leaf basis. Tanglefoot Pest Barrier (The Tanglefoot Company, Grand Rapids, Michigan), a highly viscous liquid, was put on the leaf rachis close to the EFNs on 50 leaves, ten each with three, four, five, six or seven EFNs. Leaves were examined for captured insects every morning for eight days. Insects were brought to the laboratory, examined under a dissecting microscope and identified based on morphological characteristics. Insects were categorized as ants, parasitoid wasps, predatory wasps, or “other,” which included insects such as flies and mosquitoes that do not participate in the third trophic level interaction.

Simulated herbivore egg removal

Simulated herbivorous insect eggs were placed on leaves to establish if a higher number of EFNs cause a higher amount of predatory wasp protection for the leaf. Animal lard, a lipid and protein source, was mixed with unscented gray clay as a thickener to make simulated eggs. Twenty one to two mm diameter balls of this mixture were placed on the middle leaflets of one leaf per EFN category (three, four, five, six or seven), then reexamined 24 hours later to record how many of the simulated herbivore eggs had been

removed.

RESULTS

Nineteen of the 20 sampled *I. sierrae* trees had at least one leaf with an extra EFN (Figure 3). The average number of extra EFNs was 0.784 ± 0.036 . More importantly, only 13 of the 20 trees had a substantial number of leaves with extra EFNs (Figure 3). Leaf EFN number data points outside of the 25th to 75th quantiles were not considered accurate representations of the entire tree, and thus seven trees did not have a substantial number of extra EFNs. Unexpectedly, three trees had a few leaves with only two leaflet pairs and subsequently only two EFNs, demonstrating that in addition to extra EFNs, some *I. sierrae* trees at this site may be missing EFNs. Tree E had the largest range of EFN number, with two to nine EFNs, although most trees with variable EFN number had a maximum of seven EFNs (Figure 3). Both the range of EFN number and the average number of extra EFNs was smaller than that found in the spring 2003 study. Gough (2003) found an average number of 1.62 ± 0.0076 extra EFNs and anywhere from 0-12 EFNs per leaf. Despite a smaller range of EFNs per leaf and a lower average number of extra EFNs compared to spring 2003, these results indicate extra EFNs are not limited to juvenile trees.

The majority of herbivore damage observed can be characterized as small holes or missing tissue on leaf margins. A linear regression of percent herbivory versus leaf EFN number showed a weak negative trend, as leaves with more EFNs had lower herbivore damage (Figure 4a). However, the trend was not significant ($r^2 = 0.0196$, $p = 0.0667$, $n = 172$), due to three outliers above 10% herbivory (11.57, 19.14, 44.72%). When outliers were excluded, the second linear regression showed a significant trend ($r^2 = 0.0236$, $p = 0.0461$, $n = 169$) (Figure 4b). In this case, leaves with a high number of EFNs were damaged by herbivores less than those leaves with fewer (Figure 4b).

Forty-two insects were caught with Tanglefoot on leaves over eight days. No trends were observed when comparing insects to the number of EFNs on leaves. As seen in Table 1, no more parasitoid or predatory wasps were caught on leaves with more EFNs than those with fewer. No ants were captured over the eight days, but it is important to note that more parasitoid wasps (26) were caught than predatory wasps (16).

Some simulated herbivore eggs were removed over the 24-hour period for trials 1 and 2. However, no trend was observed between number of simulated insect eggs removed from leaves and the number of EFNs on the leaf (Table 2). The leaf with five EFNs had the most eggs removed in both trials, with ten eggs remaining after 24 hours in both trials. During the third trial no eggs were removed from any of the leaves, probably due to the constant rainfall that day, which limits insect movement.

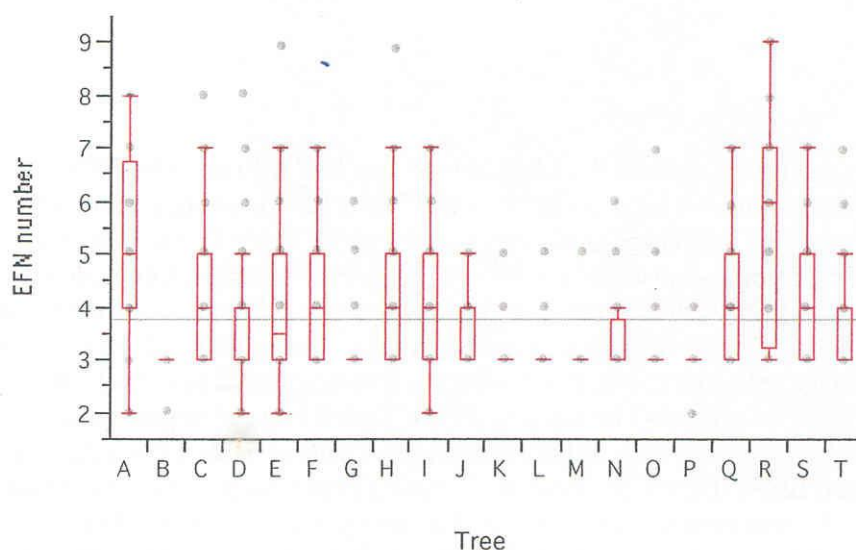


Figure 3. One-way analysis of extrafloral nectary number by tree. Boxes indicate data ranges from the 25th to 75th quantiles, the line across the middle of the box denotes the median EFN number per tree and whiskers illustrate the acceptable ranges of EFN number per tree. Data points outside of the whiskers are outliers and thus not a good representation of the whole tree. The gray bar indicates the mean number of EFNs for the entire community. Trees B, G, K, L, M, O, and P had leaves with the normal 3 EFNs. Trees A, C, D, E, F, H, I, J, N, Q, R, S and T had some leaves with extra EFNs. Tree E had the widest range of leaf EFN number with two to nine EFNs, although seven was the most common maximum. Monteverde, Costa Rica, 1420 m, October- November, 2005, n = 1200.

Table 1. Number of parasitoid wasps, predatory wasps and other non-protective insects captured on 10 *I. sierrae* leaves with 3, 4, 5, 6 and 7 EFNs (for a total of 50 leaves). Wasps did not seem to be attracted to leaves with higher numbers of EFNs. The “other” category includes flies, mosquitoes and very small beetles, which play no significant role in the herbivory/EFN relationship. It is important to note that no ants were captured, probably due to the high elevation of the study site.

Leaf EFN number	Parasitoid wasps	Predatory wasps	Other
3	4	3	3
4	4	0	3
5	6	1	4
6	4	1	5
7	2	1	1

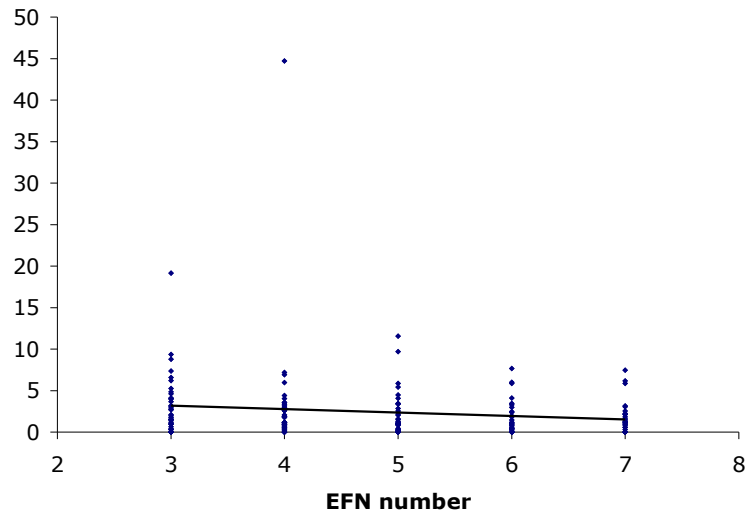
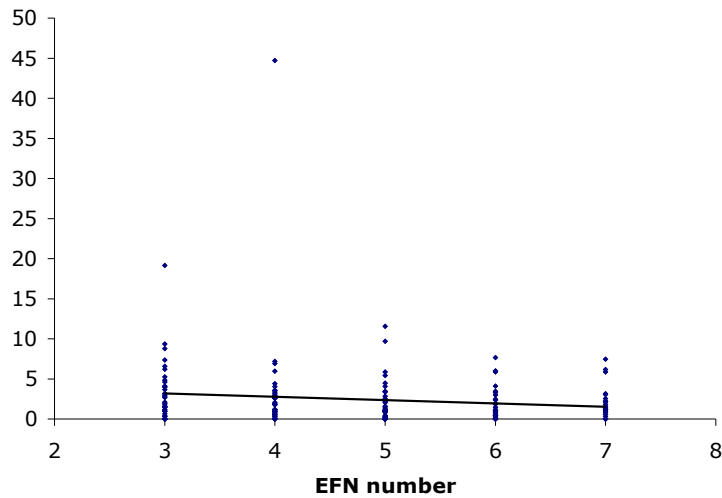


Figure 4. a. Linear regression of percent herbivory versus *I. sierrae* leaf EFN number (N = 172). The best fit line ($y = 4.48 - 0.423x$) illustrates a trend between more EFNs and lower herbivory, but it is insignificant ($r^2 = 0.0196$, $p = 0.0667$, $n = 172$). **b.** Linear regression of percent herbivory versus *I. sierrae* leaf EFN number when outliers above 10% herbivory are excluded. Exclusion is justified because any leaf herbivory above 10% is likely caused by an herbivore too large to be deterred by protective insects recruited by *I. sierrae* EFNs and thus is not important to the system being analyzed in this study. The best fit line ($y = 3.19 - 0.24x$) illustrates the significant relationship between higher EFN number and lower leaf percent herbivory ($r^2 = 0.0236$, $p = 0.0461$, $n = 169$). Monteverde, Costa Rica, 1420 m, October- November, 2005.

Table 2. Number of simulated herbivore eggs out of an original 20 remaining on *I. sierrae* leaves with 3, 4, 5, 6 and 7 EFNs, used as a proxy for the amount of predatory wasp protection. There were no observable trends in eggs removed versus EFN number, suggesting EFN number plays little role in recruiting predatory wasps as anti-herbivore protection at the study site. The absence of simulated egg removal activity in trial 3 can be accounted for by constant rain at the study site during which insects are not active.

Trial	Eggs remaining after 24 hours				
	3 EFNs	4 EFNs	5 EFNs	6 EFNs	7 EFNs
1	19	14	10	18	19
2	15	16	10	16	19
3	20	20	20	20	20

DISCUSSION

The distribution of EFN numbers across the *I. sierrae* trees at the study site shows that the presence of a substantial number of extra EFNs is limited to 13 of the 20 sampled trees, so their existence is not universal (Figure 3). The majority of trees with extra EFNs had a maximum of seven. Thus, the number of extra EFNs a leaf can produce seems to be variable but limited. Both the average and range of EFN number was less than those observed in spring 2003 (range = zero to nine, average = 1.62 ± 0.0076 EFNs) (Gough 2003). The results do show, despite smaller values, that the existence of *I. sierrae* extra EFNs is not age limited; two and a half years later, the trees still bear extra EFNs.

The presence of extra EFNs would logically confer higher fitness to a tree because less herbivore damage means a leaf has higher photosynthetic ability. However, previous work shows the existence of extra EFNs is limited to this particular study site; *I. sierrae* trees in the surrounding area do not have them (Gough 2003). The variable and limited distribution suggests EFNs are genetically controlled. The study trees are experiencing the same weather conditions and are the same age, so the variety must due to varying genotypes.

Because herbivory resulting in more than 10% leaf surface damage was most likely inflicted by a large herbivore that would not be thwarted by the protective insects *I. sierrae* EFNs recruit and thus is beyond this study, data points above 10% were excluded from a second linear regression (Figure 2b). The results show that higher numbers of EFNs correspond to lower percent herbivory on a per leaf basis, confirming that similar to the dry season (Gough 2003), the number of EFNs correlates to herbivory damage in the wet season (Figure 4b). Hence, extra EFNs appear to be an adaptive defense against small insect herbivory.

Further, percent herbivory was typically lower compared to the spring 2003 data. For example, the average percent herbivory for leaves with three EFNs in the spring was 27.2% (Gough 2003), while the maximum for this study was 9.36%. Lower herbivory rates may reflect new leaves flushed in the fall, and thus herbivory damage only covered the period EFNs are functional. The spring study had to use fully expanded leaves that

probably incurred damage after their EFNs stopped secreting nectar and thus the amount of herbivory is inflated.

Although the trend in percent herbivory suggests a higher level of protection with an increasing number of EFNs, protective insects were not captured more often on leaves with an increasing number of EFNs (Table 1). No ants were captured, consistent with work by Koptur that demonstrated at higher elevations, ants do not visit *Inga* EFNs (1985). Based on the insect capture and simulated egg removal experiments, insect activity was clearly low. Heavy rain throughout the study is most likely the cause, as insects are less active during rain.

None-the-less, the results provide evidence for both parasitoid and predatory wasp protection of *I. sierrae*. Parasitoid and predatory wasps were captured close to EFNs (Table 1). Some simulated insect herbivore eggs were removed in two trials (Table 2), despite the fact no correlations with EFN number were found. Furthermore, more parasitoid wasps were captured than predatory wasps, highlighting the possible importance of parasitoid wasps as the dominant local *I. sierrae* protector.

The future of extra EFNs in the Monteverde *I. sierrae* population depends on the costs and benefits incurred by the trees to produce them. Based on current research, it seems they should be selected for because they lessen herbivory damage and subsequently increase a tree's fitness. Extra EFNs should become increasingly prevalent in the population as the genotype coding for them is disproportionately passed on to future generations. However, the energetic cost of producing extra EFNs may outweigh the cost of herbivory. As seen in Figure 2b, the difference in percent herbivory between leaves with three and seven EFNs is less than 0.5%. If the cost of producing four more EFNs is greater than the cost of 0.5% herbivory, extra EFNs will be selected against and become less common with time.

Future studies should use similar insect capture methods when there is little rain. Coupled with visual censuses, these studies should help elucidate if the lower percentage of herbivory on *I. sierrae* leaves with more EFNs is due to a higher density of protective insects being attracted to these leaves, or if protective insects more aggressively protect leaves with extra EFNs by visiting them for longer periods of time. Other studies should determine if the trees bearing leaves with extra EFNs have a correspondingly higher reproductive output.

ACKNOWLEDGMENTS

I would like to thank Alan Masters for his much needed advice and suggestions throughout my project. Thank you Maria and Ollie for putting up with my constant questioning and demands for supplies. Thanks to both Alan and Karen Masters for the use of their property and trees for this study and mi familia tica for a caring home to live in while carrying out this study. Thanks to my classmates for such fun times in this beautiful country and special thanks to Taegan for being my walking buddy.

LITERATURE CITED

Bentley, B. L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. Annual Review of Ecological Systematics 8: 407-427.

- Coley, P. D. and J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology Systematics* 27: 305-335.
- Gough, A. 2003. "Distribution and anti-herbivoral role of extra extrafloral nectaries and leaflet pair number on *Inga sierrae* (Fabaceae: Mimosaceae)." In CIEE Spring 2003 Tropical Ecology and Conservation, pp 45-61.
- Janzen, D. H. 1966. Coevolution of mutualism between ants and *Acacia* in Central America. *Evolution* 20: 249-275.
- Koptur, S. 1984. Experimental evidence for defense of *Inga* (Mimosoideae) saplings by ants. *Ecology* 65: 1787-1793.
- , S. 1985. Alternative defenses against herbivores in *Inga* (Fabaceae: Mimosoideae) over an elevational gradient. *Ecology* 66: 1639-1650.
- . 1994. Floral and extrafloral nectars of Costa Rican *Inga* trees: a comparison of their constituents and composition. *Biotropica* 26: 276-284.
- Longino, J. T. 1989. Geographic community structure and community structure in ant-plant mutualism: *Azteca* and *Cecropia* in Costa Rica. *Biotropica* 21: 126-132.
- Pemberton, R. W. 1992. Fossil Extrafloral nectaries, evidence for the ant-guard antiherbivore defense in an Oligocene *Populus*. *American Journal of Botany* 79: 1242-1246.
- \Stephenson, A. G. 1982. The role of extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology* 63: 663- 669.