Distribution and Anti-herbivoral Role of Extra Extrafloral Nectaries and Leaflet Pair Number on Inga sierrae (Fabaceae: Mimosaceae)

Andraya Gough

Department of Biology, Occidental College

Abstract

The purpose of this project is to investigate the distribution and anti-herbivore impact of extra-extrafloral nectaries (EEFNs) and leaflet pair number on *Inga sierrae* (Fabacaeae: Mimosaceae) in Monteverde, Costa Rica. I recorded the number of EEFNs and leaflet pairs per leaf for forty leaves collected from sixty trees from three different locations. EEFNs, though not commonly found on *I. sierrae*, did significantly reduce the percent herbivory on leaves (1-way ANOVA, $P < 0.0001$ and Exponential Regression Analysis; $R^2 = 0.9413$, $P < 0.0001$). The most commonly encountered leaflet pair number was three. However, when the number of leaflet pairs decreased, percent herbivory increased (Exponential Regression Analysis, $R^2 = 0.9905$, $P < 0.0001$ and 1-way ANOVA, $P < 0.0001$). Heavy herbivory pressure could be causing a species change toward increased production of EFNs.

Resumen

El objecto de este proyecto es investigar la distribución y el impacto de anti-herbivore de los nectararios extraflorales (EEFNs) y números de hojuela de parejas en los árboles de *Inga sierrae* en Monteverde en Costa Rica. Yo documenté el número de EEFNs y hojuela de pareja por hoja para cuarenta hojas que yo recojí desde seisenta árboles de tres localidades diferentes. EEFNs, aunque no son comunes a encontrar en *I. sierrae*, reducen significamente el porcentaje de la herbividad de las hojas. Más frequentemente el número de las hojuelas de parejas por cada hoja fue tres, aunque, cuando el número disminuio, el percentaje de herbividad aumentó y el opuesto ocurrió también. Herbividad pesado pueden ser causando un cambio de especies para aumentado producción de EFNs.

Introduction

Herbivory can be physiologically costly to plants both in the short-term and long-term. Herbivores, and in particular folivores, decrease the fitness of plants by destroying their photosynthetic surfaces (Raven et al. 1991). Because plants are stationary, they must develop physical, chemical or biotic defenses in response to herbivore damage (Koptur 1991). Biotic defenses, including both dynamic and complex mutualisms, have not been studied as extensively.

Inga spp. are tall, leguminous, sub-canopy trees commonly found in disturbed areas such as pastures and along forest edges only on the Pacific side of Costa Rica (Bello et al. 2000). The leaves are alternate, parapinnate, and compound with 2-4 opposite leaflet pairs, approximately 12 x 18 cm (Bello et al. 2000). *Inga sierrae* has a winged rachis and when mature, stiff and thick convex, leathery leaves with rough pubescence (Bello et al. 2000). Between the leaflet pairs on the rachis are conspicuous extrafloral nectaries (Coley & Barone 1996).

Nectary glands found outside the flower, called extrafloral nectaries (EFNs). These EFNs are often found near reproductive parts of the plants, or are active on young vulnerable leaves and solicit biotic defenses. Third level trophic organisms will often come to these nectaries and, in exchange for the sugars and amino acids they receive from the plant, will remove herbivores (Koptur 1991). Ants and wasps (Hymenoptera) have been documented visiting several species of trees including *Inga spp*. (Koptur 1991). Herbivore damage is extensive on *Inga spp*. and as with most plants, usually happens on younger, more tender leaves. Folivores on *Inga sp*. include: katydids and grasshoppers (Othoptera), lepidopteran larvae, beetles (Coleoptera), monkeys (Cebidae in Costa Rica), sloths (Brachypodidae), leaf-cutting ants and bees (Hymenoptera) (Koptur 1983). These nectaries are active on young tender leaves but stop producing nectar when the leaves are mature and toughened (Koptur 1991). Suzanne Koptur's study (1985) of elevational gradients and *Inga* spp. nectaries indicate increased concentrations of tannins in higher elevation plants. These tannins retard the development of caterpillars, making them vulnerable to predators. She also reported the presence of parasitic wasps (Hymenoptera) and flies (Diptera) on *Inga sp*. (Koptur 2000). Parasitoids complete their life cycle within or on the body of a host, ultimately killing it. Parasitoids provide protection for trees by ovipositing on the larvae that prey on the leaves.

I. sierrae trees in Monteverde, Costa Rica have been found to have extra-extrafloral nectaries (EEFNs) (Masters, Pers. Comm.). These are nectaries usually found on the base of a leaflet pair on the pulvinus, near the expected nectary glands. I investigated the frequency of EEFNs nectaries on *I. sierrae* and their effectiveness in reducing herbivory. Additionally, since the number of leaflet pairs vary, and with it the number of EFNs, I suspect that there will be lower herbivory on leaves with more leaflet pairs. I hypothesize that leaves with greater number of EFNs and leaflet pairs will experience less herbivory. I infer that the tree is producing additional EFNs in response to a decrease in biotic defenses in an attempt to attract the few third trophic level species that may be present in the area.

Materials and Methods

STUDY SITE- This study was conducted in a tropical lower montane cloud forest in Monteverde, Costa Rica on the property of the Estación Biológica de Monteverde at approximately 1525 – 1555m (Nadkarni & Wheelwright 2000; Sterlmach 2001). Samples were taken from three sites, no larger than one hectare. Twenty trees were found in a pasture or pasture edge in small stands of two or three trees. The second site for twenty trees was the edge of secondary forest. Most of these trees were moderately distant from conspecifics. Twenty trees were on the edge of a secondary growth forest. The last twenty were on the property of Alan and Karen Masters planted on fallow pasture from seeds collected in nearby areas. The distance between nearest neighbor for these trees was usually not more than 1m.

FREQUENCY DISTRIBUTION – I looked at the distribution of the EEFNs and leaflet pair number with a sample size of sixty trees. I documented forty random leaves per tree and counted the number of EEFNs as well as noting the number of leaflet pairs on each leaf. This made a total sample size of 2, 400 leaves. The leaves were collected by hand and with pole clippers for higher branches and examined with a hand lens. Due to the frequent unavailability of the pole clippers, most of the leaves were collected from branches that were within 2m of the forest floor. Each leaf was labeled for organizational purposes. From this information, I was able to create a frequency distribution of the EEFNs and leaflet pair numbers. I then looked at the distribution of EEFNs and leaflet pair number for trees that were planted and those found in natural populations.

PERCENT HERBIVORY – The next phase of the project involved recording the percent herbivory on the leaves and comparing this to the number of EEFNs on the leaves as well as leaflet pair number. I collected mature, toughened leaves that were fully expanded but had not been exposed to non-herbivory related damage. The leaves were collected from both saplings and adults, although none of the trees were smaller than 2m due to lack of sampling material, nor were any larger than 20m. To measure herbivory, I used a grid on transparent paper. Each convex leaf was carefully flattened (making sure not to tear any of the edges) and the grid placed over it. I extrapolated the missing parts of the leaf from the projected shape or from the paired leaflet across, which is nearly a mirror image. Herbivory was calculated for each leaflet as the number of grid boxes missing divided by the total number of grid boxes that the leaf would have been. I then added all the missing parts for each leaflet and divided by the total leaflet area for each leaf. In my inventory of herbivory, I included gall formation, leaf rollers and large discolorations on the surface of the leaf that reduced photosynthetic area. I then looked at the percent herbivory of EEFNs and leaflet pair number for trees that were planted and those found in natural populations.

DATA ANALYSIS – I created frequency distributions to determine the population wide occurrence of EEFNs and leaflet pair numbers. Utilizing an unpaired t-test, I was able to statistically determine if a difference existed between the percent herbivory between trees with EEFNs and those without. I also used a 1-way ANOVA and regression analyses to see if there was a difference between trees with varying numbers of EEFNs numbers of EEFNs and percent herbivory. I employed the same tests for leaflet pair numbers. A Chi Square test was used to determine whether these frequencies deviated from what was expected. I also used regression analyses to see if there was a difference between number of galls per leaf and number of EEFNs per leaf. I then divided the sample size between trees in natural populations and trees planted and created frequency distributions for leaves from trees that had been planted and those that occur naturally. I applied an unpaired t-test and 1-way ANOVA to determine if there was a difference in number of EEFNs, leaflet pair number, and percent herbivory between the natural populations and those planted.

Results

FREQUENCY DISTRIBUTION – There was no statistical difference between trees with the typical number of EFNs (1-way ANOVA, $p > 0.14$). Excluding leaflet pair number, about 75% of the trees had at least one EEFN. However, of all the leaves sampled, approximately 85% of the leaves did not; there is a significant difference between the frequency of leaves with EEFNs and those without (x^2 = 1221.22, df = 1, cv = 3.84) (Figure 1). The frequency of leaves with EEFNs decreased as number of EEFNs increased, except for 6 EEFNs which were nearly as common as finding two EEFNs (Figure 2). There was no significant difference between leaves with two and six EEFNs (x^2 = 0.68, df = 1, cv = 3.84) (Figure 2). There is a significant difference between leaves with 1-4 EEFNs and 5-9 EEFNs (x^2= 82.04, df = 1, cv = 3.84). On any given leaf, there is an 87% chance of finding three leaflet pairs (Figure 3). There was a 7.0% chance of finding leaves with two leaflet pairs, and a 5.7% chance of finding leaves with four leaflet pairs (Figure 3). There was no significant difference between the frequency of leaves with two leaflet pairs and four leaflet pairs (x^2 = 2.96, df = 1, cv = 3.84) (Figure 3). Only 0.125% of the leaves had leaves with a single leaflet pair, which was significantly different from the frequency of leaves with two and four leaflet pairs (x^2= 148.80, df = 2, cv = 5.99) (Figure 3).

Though EEFNs were infrequently encountered in the total population, the frequency distribution of number of EEFNs per leaf significantly differed between leaves found on planted trees and those found in natural populations (1-way ANOVA, P = 0.028) (Figure 4). The frequencies of EEFNs per leaf were more evenly distributed on planted trees than those found in natural populations. There was also a significant difference between the distribution of leaflet pair numbers per leaf found on planted trees versus the ones found on natural population trees (1-way ANOVA, P = 0.0322) (Figure 5). The frequency of two leaflet pairs per leaf is lower on planted population trees, while the frequency of four leaflet pairs is higher in planted populations (2 x 2 Contingency Table, x^2 = 217.20, df = 1, cv = 3.84). The most commonly encountered leaflet pair per leaf was three between both populations.

PERCENT HERBIVORY – There was significantly less herbivory on trees that had EEFNs. (T-test, $P < 0.0001$). The mean number of EEFNs, the mean number of EFNs, and the percent herbivory on population of trees found on the Master's property was significantly different from those found in natural populations (t-test, $p < 0.0001$, $P < 0.0001$, $P < 0.0001$) (Appendix A, Table 1). There was less percent herbivory per tree that had EEFNs versus those that did not (Exponential Regression Analysis, R^2 = 0.80; $P < 0.0001$) (Figure 6). For natural populations, there is no significant decrease in percent herbivory as average EEFN number per tree increases (Simple Regression Analysis, R^2 = 0.02, $P > 0.05$) (Figure 7). There is a significant decrease in percent herbivory as average number of EEFNs per tree increases for planted trees (Exponential Regression Analysis, R^2 = 0.85, $P < 0.0001$) (Figure 7). The most dramatic decrease, greater than 15%, in the percent herbivory per leaf is from zero EEFNs to one EEFN (Figure 8a). The average percent herbivory for leaves with no EEFNs was 27.2%, while the average percent herbivory for leaves with at least one EEFN was 10.6%. The percent herbivory significantly decreased as the number of EEFNs increased (1-way ANOVA, $P < 0.0001$ and Exponential Regression Analysis; $R^2 = 0.94$, $P < 0.0001$) (Figure 8b). The percent herbivory significantly decreased for both planted and natural populations as number of EEFNs increased (Polynomial Regression Analysis, $R^2 = 0.89$, $P < 0.0001$, Polynomial Regression Analysis, $R^2 = 0.89$, $P < 0.0001$) (Figure 9). The percent herbivory decreased as number of leaflet pairs increased (Exponential Regression Analysis, $R^2 = 0.99$, $P < 0.0001$ and 1-way ANOVA, P = 2.62E-35) (Figure 10). The percent herbivory also significantly decreased as number of leaflet pairs increased for both the natural and the planted population (Polynomial Regression Analysis, $R^2 = 0.91$; $P < 0.0001$ and $R^2 = 0.9998$; $P < 0.0001$).

Potential herbivores found on the plant included many of the insects found in previous studies. The following insects, both adult and larval, were found on or near the leaves of *I. sierrae*: Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, and Orthoptera. Several arachnids were also seen, but their impact on herbivory is unknown. There were no mammals seen preying on the leaves. Two species of ants that vigorously defended the leaves were seen visiting the EFNs. Gall number decreased as number of EEFN increased (Simple Regression Analysis, $R^2 = 0.9905$, $P < 0.0001$) (Figure 12).

Discussion

There was no statistical difference in herbivore damage between trees that had normal numbers of EFNs. This means that the *I. sierrae* trees are subject to equivalent herbivore pressures, and that other factors such as location or size were not an important factor in this study. This means that the differences in percent herbivory stem from other factors such as increased EFN production.

Though over 75% of the trees had EEFNs, over 85% of leaves surveyed did not have any EEFNs at all. The production of EEFNs is probably costly to the tree. It would be in the tree's best interest to produce the fewest number of EEFNs while receiving the maximum benefit of herbivory reduction.

The most commonly found leaflet pair number per leaf was three. The production of four leaflet pairs may be an adaptation to increase the number of EFNs. The production of two leaflet pairs may have to do with a lack of resources to allocate to that particular leaf.

There was a significant difference between the percent herbivory on trees with EEFNs and those that did not. Having EEFNs on the tree seems to have increased fitness for the entire tree. Even though all leaves did not have EEFNs, they may have been enough to attract the needed amount of biotic defenses. Increasing the number of EEFNs may increase the number of visits by any nectar seeking insect, making the overall activity at the site higher, discouraging smaller or less aggressive insects. An increased number of EEFNs may have increased herbivore defense by ants. In accordance with the findings of Haff (1995), there were at least two species of ants, possibly three, visiting the EEFNs (Koptur 1991). The number of parasitoid visitors which prey on the larvae of ovipositing herbivores could also have increased attracted to the additional EFNs, reducing the percent herbivory found on *I. sierrae* leaves.

The increase in leaflet pair number per leaf correlating with a decrease in herbivory supports these ideas. As leaflet pair number increases so do the number of EFNs. Between each leaflet pair is an EFN, so if there are four leaflet pairs, there are 4 EFNs. The increase in leaflet pairs could be a strategy not only to increase photosynthetic capacity, but also to increase the number of EFNs. Production of two leaflet pairs, less than the 3 leaflet pair norm, may be due to the lack of resources to allocate to a particular leaf. It is interesting to note that there was no significant difference between the frequency of leaves with two leaflet pairs and those with four leaflet pairs. This is in spite of the fact that, in this study, two leaflet pairs sustained much more damage than four leaflet pairs which tended to decrease percent herbivory to the leaf. One possible explanation is that it may be that, though the percent of herbivory is changing, the quantity of herbivory is remaining constant as leaflet pair is increasing.

Gall number per leaf decreases as EEFN frequency per leaf increases. If the increased EFN production also increases visitation by and abundance of mutualistic organisms, this may increase defense and decrease desirability of the leaf when a female galling insect tries to oviposit.

The trees planted on the Masters' property have, on average, more EEFNs, more leaflet pairs per leaf, and reduced percent herbivory. This could be due to the fact that the seeds planted on this property are from a few, unusual individuals. The increased production of EFNs is most likely genetic as is indicated by the localized population of genetically similar individuals. Additionally, if the production of EEFNs was a physiological response to high predation, then the frequency of EEFNs would be more common in the population. However, the reduction in herbivory may not necessarily be because of the increase in EEFNs. Insects tend to be attracted to the food and waste of people. The proximity of these trees to their house may increase the number of insects present, especially insects that would be biotic defenses to the *I. sierrae*. Moreover, since most of the plants in their yard have been placed intentionally, competition may be reduced allowing *I. sierrae* to allocate more resources to leaf toughness, leaflet pair and EEFNs. It was noted that large saplings from the secondary forest edge that had EEFNs may be offspring of the planted trees.

The average number of EEFNs per tree may not significantly reduce the percent herbivory on natural population of trees because the frequency is to low to have an impact on the entire tree. As the frequency of average number of EEFNs increased on planted trees the average percent herbivory decreased. The high densities of EEFNs may be attracting enough visitors to confer fitness for the entire tree.

With increasing elevation there is often a decrease in the species richness of insects (Koptur 1994). The cause of increased herbivory may be that as elevation increases, there is a disproportionate number of mutualistic organisms that decrease. In order to compensate for the increased herbivore pressure, the trees may invest more in costly chemical protection and EEFN production (Koptur 1994). Since this study was conducted at a high elevation, it would be interesting to find out whether the *I. sierrae* on the Masters' property have higher level of phenols in addition to the EEFNs and increased leaflet pair number.

The direct mutualism between ants and EFNs and the indirect mutualism between parasitoids, may have led to an increase in production of EEFNs in the *I. sierrae* trees in the Monteverde Cloud Forest Preserve. It could be that we are witnessing a change in species morphology and/or it could be that the introduction and propagation of these particular alleles has increased the abundance of *I. sierrae* trees with EEFNs.

Improvements on this project include data from the wet season when insect abundances are higher. For example, I noticed Chrysomelid beetles preying on the young leaves of the planted trees that were present only after I had finished collecting data. Future research would include investing the relationship between fitness and percent herbivory. Another interesting study would be the cost-benefit analysis of the production of EEFNs and leaflet pair number. Also, it is imperative to create an inventory of the species visiting the nectaries, ovipositing on the leaves, parasitizing the galls, and causing the herbivory damage on the *I. sierrae* trees.

Acknowledgements

I would like to thank my advisor Alan Masters for his guidance through the course of this project and his family for the use of their *Inga sierrae* trees, and Mauricio Garcia, Rick Smith and Andrew Rodstrom were invaluable resources. Additionally, I give thanks to those apart of La Estación Biológica Monteverde for the use of their *I. sierrae* trees, and CIEE for all their support. And of course, thanks to the *I. sierrae* for all the leaves they donated. A special thanks goes to my roommates: Ruth, Crystal, and Chrissi, for nursing me through this paper.

Literature Cited

Bello, E., W.A. Haber and W. Zuchowski. 2000. An Introduction to Cloud Forest Trees. Monteverde Costa Rica Mountain Gem Publications. Monteverde, Costa Rica pp. 62.

Coley, P.D. and Barone, J.A. 1996. "Herbivory and Plant defenses in Tropical Forests". Ann. Rev. Ecol. Syst. 27: 305-335.

Haff, T. 1995. "Effects of Elevation, Ants and Parasitoid Wasps on Herbivory in the Guaba Tree *Inga punctata* (Fabaceae: Mimosoideae)". In UCEAP Tropical Biology. Fall, pp. 29-41.

Koptur, S. 2000. "Interactions among *Inga*, herbivores and ants and insect visitors to foliar nectaries". In Nadkarni, N.M. and N.T. Wheelwright. (Eds.) Monteverde: Ecology and Conservation of a Tropical Cloud Forest. Oxford University Press. New York, New York. pp. 277-278.

-----, S. 1994. "Floral and extrafloral nectars of Costa Rican *Inga* trees: a comparison of their constituents and composition". Biotropica 26: 276.

-----, S. 1991. "Extrafloral nectaries of herbs and trees: modeling the interaction with ants and parasitoids". In Huxley, C.R. and D.F. Cutler (Eds.) Ant – Plant Interactions. Oxford University Press. Walton Street, Oxford.

-----, S. 1983. “ Inga (Guaba, Guajiniquil, Caite, Paterno)”. In D.H. Janzen (Ed.) Costa Rican Natural History. University of Chicago Press. Chicago, Illinois. pp. 259-261.

Masters, A. 2003. Personal Communication: Unpublished data regarding EEFNs in *Inga sierrae* and herbivory damage.

Nadkarni, N.M. and Wheelwright N.T. (Ed.) 2000. Monteverde: Ecology and Conservation of a Tropical Cloud Forest. Oxford University Press. New York, New York. pp. 9.

Raven, P.H., R.F. Evert and A.E. Eichhorn. 1991. Biology of Plants. W.H. Freeman and Company and Worth Publishers. New York, New York. pp. 779-783.

Sterlmach, D.K. 2001. Levels of Herbivory on Inga sierrae Along an Elevational Gradient. In Tropical Ecology and Conservation, Unpublished: Council for International Educational Exchange, Monteverde, Costa Rica, pp. 103-107.

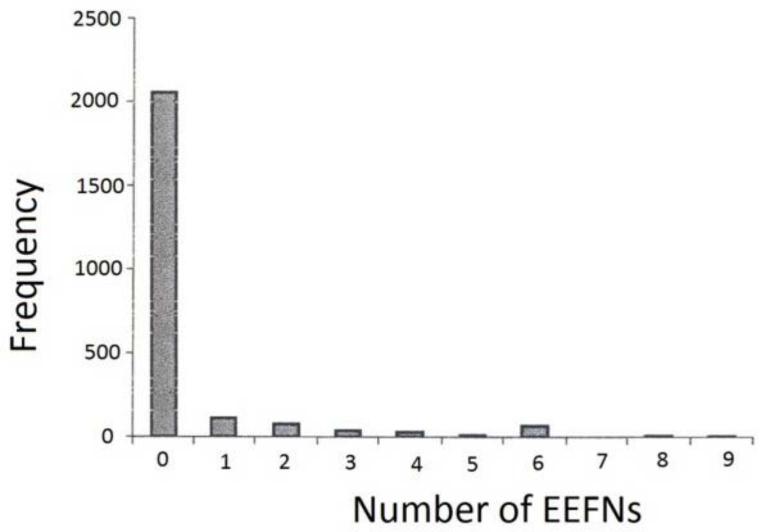


Figure 1. The frequency of *I. sierrae* leaves that have extra extrafloral nectaries (EEFNs) compared to leaves that do not have any EEFNs. Over 85% of the 2, 400 leaves sampled did not have any EEFNs at all. There is a significant difference between the frequency of leaves with EEFNs and those without (x^2 = 1221.22, df = 1, cv = 3.84).

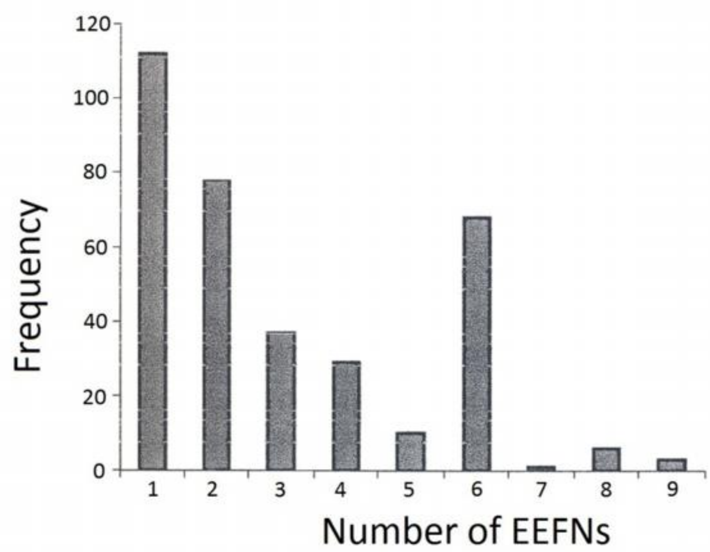


Figure 2. The frequency *I. sierrae* leaves that have extra extraflora nectaries (EEFNs). Frequency decreases as number of EEFNs increases expect for leaves with 6 EEFNs. There is no significant difference between leaves with two and six EEFNs. (x^2= 0.6849, df = 1, cv = 3.84). There is a significant difference between leaves with 1-4 EEFNs and 5-9 EEFNs (x^2 = 82.04, df = 1, cv = 3.84).

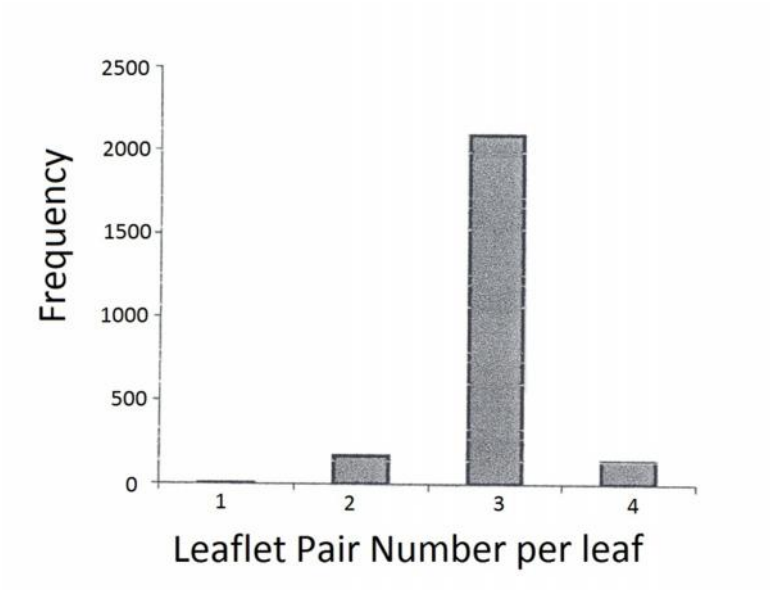


Figure 3. Frequency of leaflet pair number on leaves of *I. sierrae*. The median number of leaflets per leaf is three. One leaflet per leaf was rare, only three leaves out of 2,400 sampled were found. There was a significant difference between the one pair of leaflets and two and four leaflet pairs (x^2 = 148.8, df = 2, cv = 5.99). However, there was no significant difference between the frequency of leaves with two and four leaflets (x^2 = 2.96, df = 1, cv = 3.84).

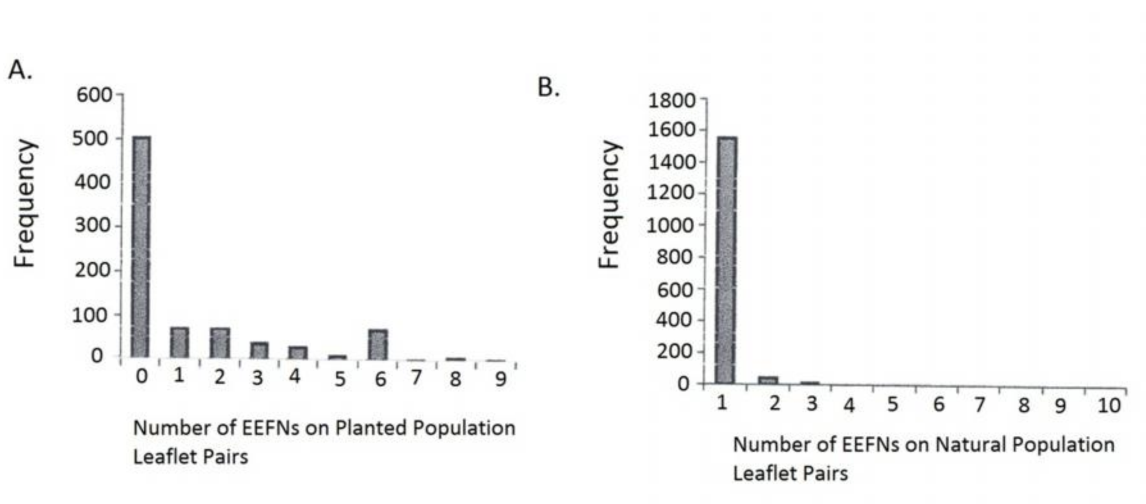


Figure 4. The frequency of *I. sierrae* leaves that have extra extrafloral nectaries (EEFNs) compared to leaves that do not have any EEFNs in a. planted and b. planted populations. There is a significant difference between the frequency of leaves with EEFNs and those without for natural and planted populations (x^2 = 2229. 89, df = 1, cv = 3.84; x^2= 10, 710.26, df = 1, cv = 3.84).

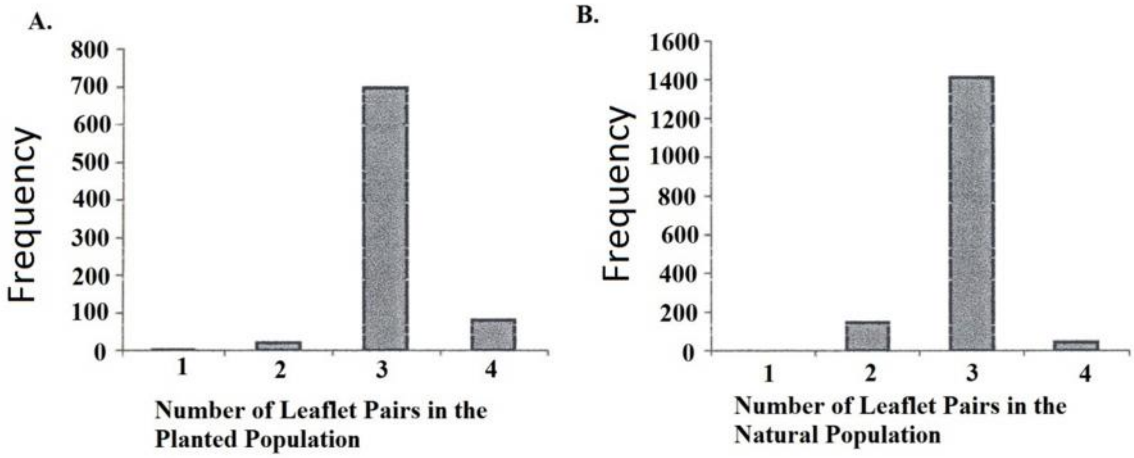


Figure 5. Frequency of leaflet pair number on leaves of *I. sierrae* for a. planted and b. natural populations. The median number of leaflets per leaf is three. The frequency of two leaflet pairs per leaf is lower on planted population trees, while the frequency of four leaflet pairs is higher in planted populations (2 x 2 Contingency Table, x^2 = 217.2, df = 1, cv = 3.84).

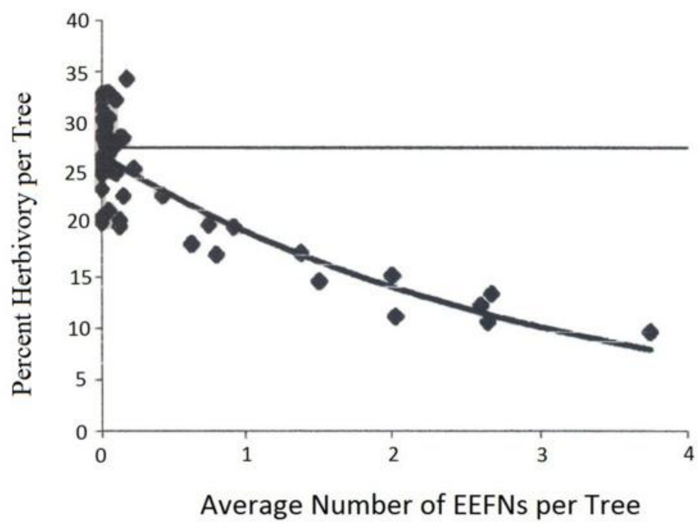


Figure 6. Percent herbivory on *I. sierrae* trees with increasing extra extrafloral nectaries (EEFNs). As the average number of EEFNs per tree increases the percent herbivory decreases (Exponential Regression Analysis, R^2 = 0.7981, P < 0.0001). The horizontal line indicates the average percentage of herbivory (26.53) on trees without EEFNs. The standard deviation from that average is 3.77.

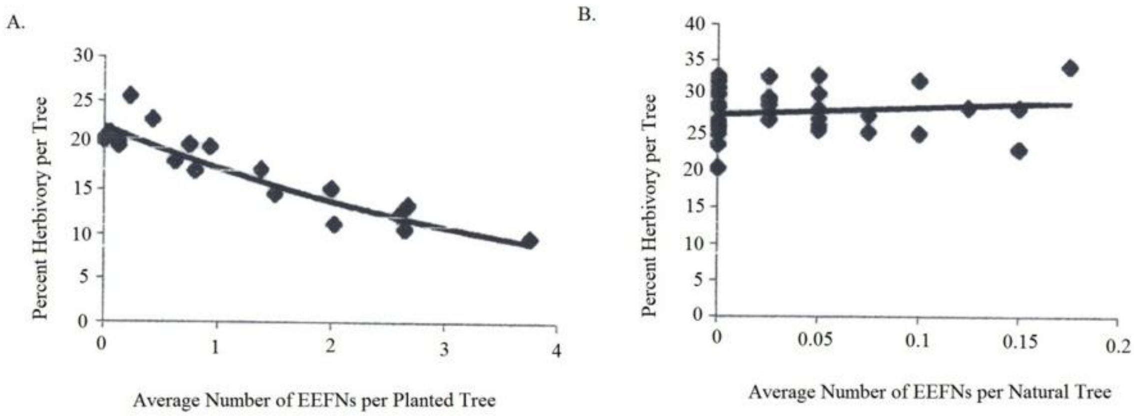
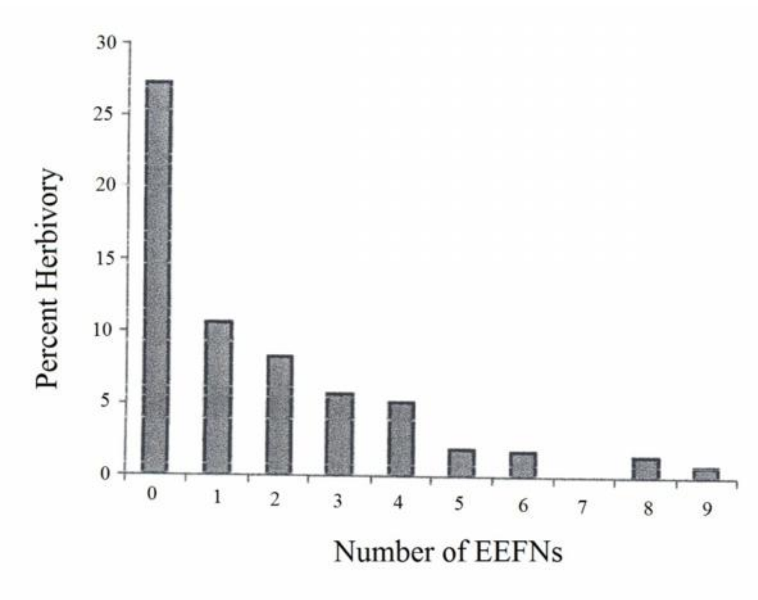


Figure 7. Percent herbivory on *I. sierrae* trees with increasing extra extrafloral nectaries (EEFNs) for a. planted population trees and b. natural population trees. For natural populations, there is no significant decrease in percent herbivory as average EEFN number per tree increases (Simple Regression Analysis, $R^2 = 0.0239$, $P > 0.05$). For planted populations, there is a significant decrease in percent herbivory as average number of EEFN per tree increases (Exponential Regression Analysis, $R^2 = 0.8518$, $P < 0.0001$).



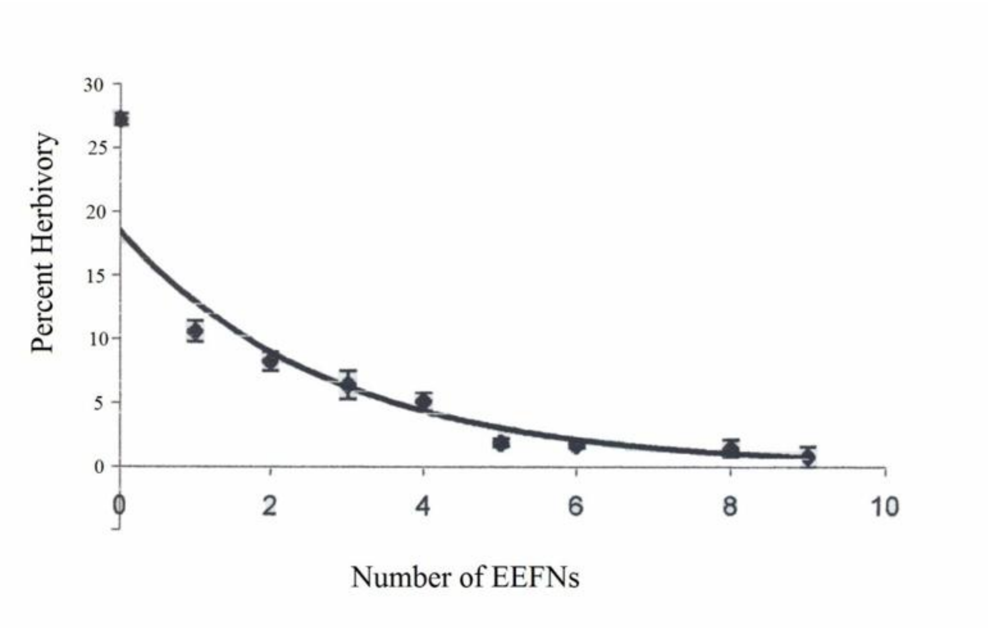


Figure 8. a. Percent herbivory for all leaves on *I. sierrae* with respect to number of extra extrafloral nectaries (EEFNs) per leaf. b. Percent herbivory decreases as number of EEFN increases with standard error (Appendix A, Table 2) (Exponential Regression Analysis, R^2 = 0.9413, $P < 0.0001$).

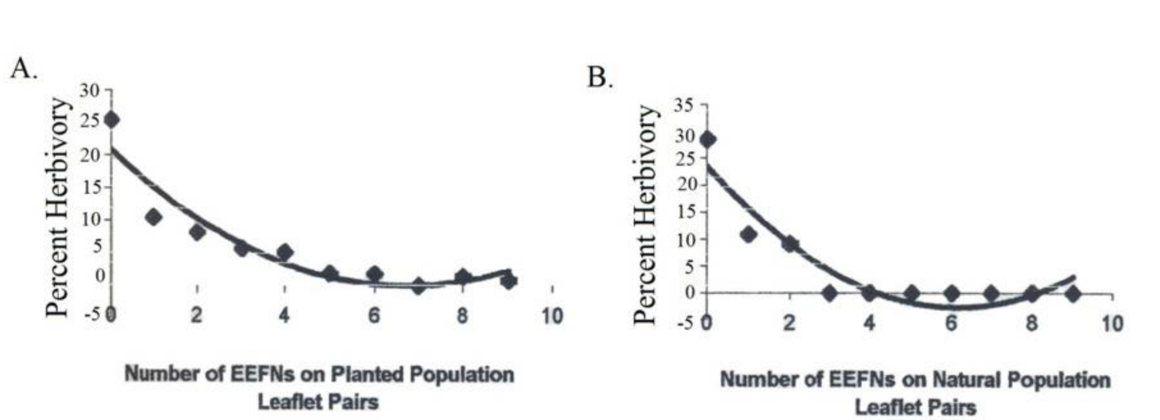


Figure 9 a. Percent herbivory for leaves on *I. sierrae* found in the planted population with respect to number of extra extrafloral nectaries (EEFNs) per leaf. b. Percent herbivory for leaves found in natural populations. Percent herbivory decreases as number of EEFN increases with standard error (Appendix A, Table 5 and 7) (Polynomial Regression Analysis, R^2 = 0.8932, $P < 0.0001$, Polynomial Regression Analysis, R^2 = 0.885, $P < 0.0001$).

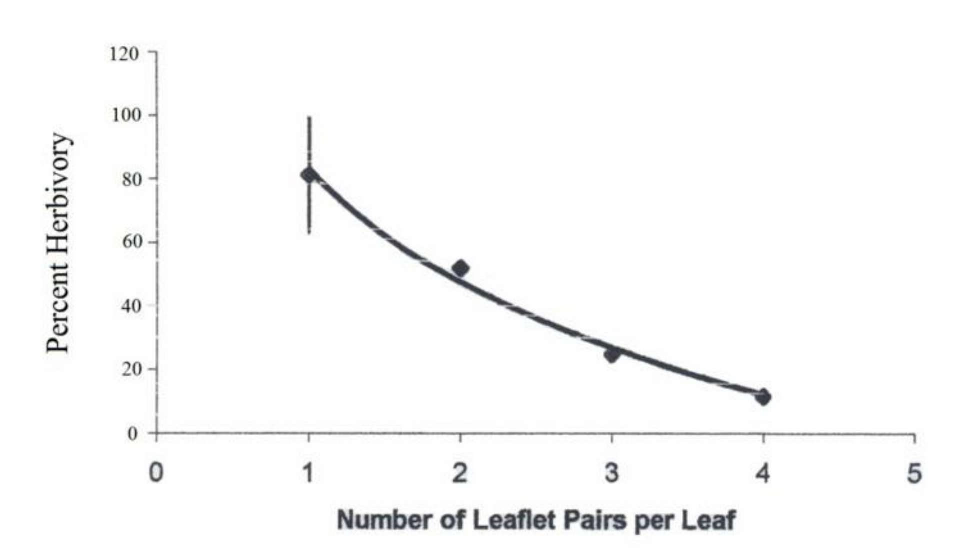


Figure 10. Percent herbivory for all leaves of *I. sierrae* with respect to number of leaflet pairs per leaf with standard error (Appendix A, Table 3). As the number of leaflet pairs increases, the percent herbivory decreases (Logarithmic Regression Analysis, $R^2 = 0.9905$; $P < 0.0001$ and 1-way ANOVA, $P = 2.62E-35$).

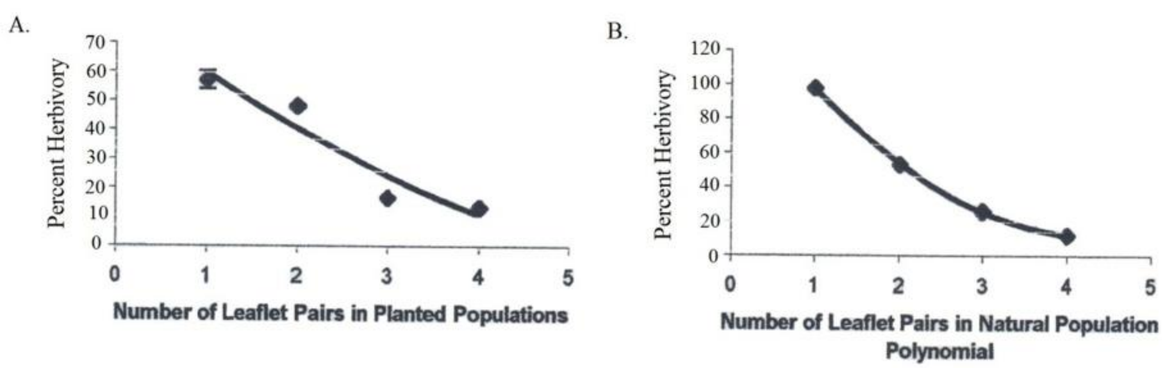


Figure 11. Percent herbivory for a. planted and b. natural population leaves of *I. sierrae* with respect to number of leaflet pairs per leaf with standard error (Appendix A, Table 6 and 8). As the number of leaflet pairs increases, the percent herbivory decreases (Polynomial Regression Analysis, $R^2 = 0.9128$; $P < 0.0001$ and $R^2 = 0.9998$; $P < 0.0001$).

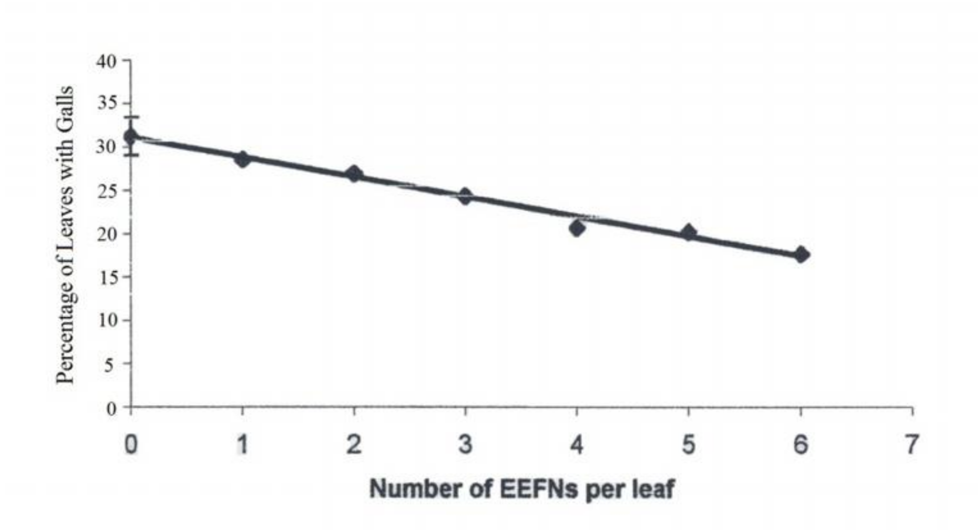


Figure 12. Percentage of leaves with galls on *I. sierrae* with respect to number of extra extrafloral nectaries (EEFNs) per leaf with standard deviation (Appendix A, Table 4). As number of EEFNs increases, the percentage of leaves with galls decreases (Simple Regression Analysis. $R^2 = 0.9865$, $P < 0.0001$).

APPENDIX A.

Table 1. The differences between the average number of EEFNs, EFNs, and percentage of herbivory of the trees in the pasture and secondary forest edge and those planted by the Masters'. An unpaired T-test was used to examine the differences between the EEFNs, leaflet pairs, and percent herbivory on trees that were planted and those found in nature.

	Planted Trees	Trees in Natural Populations	P-value
Average # of EEFNs	**1.62**	**0.04**	**< 0.0001**
Range	0-9	0-2	
Standard Error	0.0076	0.1764	
Average # of Leaflet Pairs	**3.11**	**2.94**	**< 0.0001**
Range	0-4	0-4	
Standard Error	0.355	0.362	
Average % of Herbivory	**17.12**	**27.91**	**< 0.0001**
Range	0-100	0-100	
Standard Error	0.481	0.7278	

Top Population

Table 2. The average, maximum number for range and standard error for number of EEFNs per leaf for the entire sample. The minimum number for range for all categories was zero. All numbers are rounded to the nearest tenth.

	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Average	**2.2**	**10.6**	**8.3**	**6.4**	**5.1**	**1.9**	**1.7**	**0**	**1.4**	**.8**
Range	**100**	**56.1**	**32.3**	**21.2**	**15.4**	**5.6**	**15.6**	**0**	**8.1**	**2.3**
Standard Error	**0.4**	**0.8**	**0.7**	**1.1**	**0.7**	**0.3**	**0.2**	**0**	**0.7**	**0.8**

Table 3. The average, range and standard error for number of leaflet pairs per leaf for the entire sample. All numbers are rounded to the nearest tenth.

	1	**2**	**3**	**4**
Average	**81.2**	**52.7**	**22.8**	**12.4**
Range	**45.7 -100**	**0-100**	**0-99**	**0-38.3**
Standard Error	**17.7**	**1.7**	**0.4**	**0.49**

Table 4. The average, maximum number for range and standard error for number of galls per leaflet pair with respect to EEFNs. The minimum number for range for all categories was zero.

	0	1	2	3	4	5	6	7	8	9
Average	**0.2673**	**0.0133**	**0.0087**	**0.0037**	**0.0025**	**0.0008**	**0.0050**	**0**	**0.0004**	**0**
Range	**45**	**4**	**4**	**2**	**1**	**1**	**2**	**0**	**1**	**0**
Standard Deviation	**2.20**	**0.19**	**0.17**	**0.07**	**0.05**	**0.03**	**0.09**	**0**	**0.02**	**0**

Planted Populations

Table 5. The average, maximum number for range and standard error for number of EEFNs per leaf for planted populations. The minimum number for range for all categories was zero.

	0	1	2	3	4	5	6	7	8	9
Average	**25.4**	**10.5**	**8.2**	**5.7**	**5.1**	**1.9**	**1.8**	**0**	**1.4**	**0.78**
Range	**100**	**56.1**	**23.3**	**21.2**	**15.7**	**5.6**	**15.6**	**0**	**8.1**	**2.34**
Standard Error	**0.008**	**0.004**	**0.034**	**0.064**	**0.067**	**0.157**	**0.012**	**0**	**0.301**	**0..0387**

Table 6. The average, range and standard error for number of leaflet pairs per leaf for planted populations. All numbers are rounded to the nearest tenth.

	1	2	3	4
Average	**57.15**	**46.24**	**17.89**	**0.687**
Range	**47.5 -100**	**14.3 -89**	**0-89.2**	**0-29.4**
Standard Error	**3.098**	**0.247**	**0.005**	**0.030**

Natural Populations

Table 7. The average, maximum number for range and standard error for number of EEFNs per leaf for natural populations. The minimum number for range for all categories was zero.

	0	1	2	3	4	5	6	7	8	9	
Average	**28.45**	**10.87**	**9.06**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Range	**99**	**54.3**	**32.32**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Standard Error	**0.003**	**0.083**	**0.376**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 8. The average, range and standard error for number of leaflet pairs per leaf for natural populations.

	1	2	3	4
Average	**97.8**	**53.22**	**25.84**	**11.91**
Range	**97.8**	**0-100**	**0-99**	**0 – 38.3**
Standard Error	**0**	**0.032**	**0.003**	**0.055**