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Anti-Herbivore Defense Tradeoffs: A Comparison of *Passiflora capsularis*, *Passiflora helleri*, and *Passiflora sexflora* (Passifloraceae).

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ABSTRACT

Plants in the Tropics must evolve strategies for combating high levels of herbivory with few available nutrients. This results in possible tradeoffs between chemical and structural defenses. Three species of tropical passion vines, *Passiflora capsularis*, *Passiflora helleri*, and *Passiflora sexflora* (Passifloraceae) were examined for possible tradeoffs in cyanide, leaf toughness, glands and pubescence as anti-herbivore defenses. One young and one old leaf were collected from ten individuals of each species and analyzed for cyanide concentration and leaf toughness. In addition, observations were made regarding habitat, pubescence and presence or absence of blade glands. From the results it is clear that each species analyzed has adapted different anti-herbivore defense strategies. *Passiflora helleri* (mean = 119.88 $\mu\text{g/g}$ leaf, Std Err. \pm 39.54) demonstrated markedly higher concentrations of cyanide production as well as blade gland than *P. capsularis* (mean = 24.04 $\mu\text{g/g}$ leaf, Std Err. \pm 18.15) and *P. sexflora* (mean = 0.05 $\mu\text{g/g}$ leaf, Std Err. \pm 0.006), which did not have blade glands ($N = 59$, $t = 2.01$, $P = 0.05$). *Passiflora sexflora* showed observably more pubescence than either of the other two species. And *P. capsularis* exhibited an observable tradeoff in the levels of cyanide produced in young versus old leaves ($N = 8$, $F = 5.14$, $P = 0.04$, $DF = 1$). All species were found in the understory along well cleared trails. These data suggest that the anti-herbivore defense strategies employed by *Passiflora* spp. are diverse and represent evolutionary tradeoffs between different defenses under similar selective pressures.

RESUMEN

Las plantas en los trópicos deben evolucionar estrategias para combatir los altos niveles de herbivoría con pocos recursos disponibles. Esto puede resultar en compensación diferencial entre defensas químicas y estructurales. Se examinaron tres especies de bejucos, *Passiflora capsularis*, *Passiflora helleri*, y *Passiflora sexflora*, para evaluar la posibilidad de compensación entre cianuro, dureza de hojas, glándulas y pubescencia como defensas anti-herbivoría. De los resultados es claro que cada especie analizada se ha adaptado de forma diferente con respecto a las defensas anti-herbívoras. *P. helleri* demostró altas concentraciones de cianuro, al igual que glándulas ausentes en las otras 2 especies. *P. sexflora* demostró más pubescencia que las otras 2 especies. En *P. capsularis* además se determinó que las hojas más jóvenes contienen mayores concentraciones de cianuro. Los datos sugieren que las defensas anti-herbívoras utilizadas por *Passiflora* son divergentes y representan diferentes caminos evolutivos bajo las mismas presiones.

INTRODUCTION

In Tropical life zones herbivory rates are higher than in Temperate life zones (Coley and Aide 1991), species richness is higher resulting in more specialized herbivores (Marquis and Braker 1994), and the soils of tropical forests are generally nutrient deficient (Baillie 1996, Harms *et al.* 2004, Powers 2004). Thus, plants in Tropical Rain and Moist Forests must be more efficient at allocating the limited available resources to growth,

reproduction, and defense. Because herbivory is higher, defense becomes an important element in the plant's energy budget.

Young leaves are softer and have a higher nutritional value than older leaves; therefore, herbivory is greater on young leaves than on old leaves (Coley and Aide 1991, Kursar and Coley 2003). As a result, plants have developed many mechanisms for minimizing herbivory on young leaves such as rapid expansion, delayed greening, extrafloral nectaries, tougher tissue, pubescence, and chemical defenses in the form of secondary metabolites (Coley 1983, Coley and Barone 1996, Kursar and Coley 2003). Leaf toughness has been shown to be the most efficient defense against herbivory. Once a leaf has toughened, by accumulating lignins and cellulose, the leaves are no longer attacked. Therefore, plants utilize one or a combination of many of these defense mechanisms to minimize herbivory of young leaves, before they are tough enough to eliminate herbivore risk (Kursar and Coley 2003).

Members of the family Passifloraceae in the genus *Passiflora* have a long-standing plant-herbivore relationship with nymphalid butterflies in the genus *Heliconius* (Heliconiinae) (DeVries 1987, Feuillet 2004, Gilbert 1991). There are over 500 species of *Passiflora*, which can occur as small trees or shrubs but are typically vines or lianas, which are commonly referred to as passion vines (Feuillet 2004). This mostly tropical genus can be found in the forest understory or among secondary vegetation (Feuillet 2004, Mabberley 1993). *Heliconius* can be found in much of the Americas from the southern United States through South America and the West Indies (DeVries 1987). Although *Heliconius* adults primarily feed on the pollen of flowers in the genera *Psiguria* (DeVries 1987, Gilbert 1991) and *Gurania* (Gilbert 1991) in the family Cucurbitaceae, they lay their eggs on the leaves and tendrils of vines in the family Passifloraceae, mostly in the genus *Passiflora* (DeVries 1987, Gilbert 1991). When the larvae hatch they use their host plant as a food source until they have become large enough to pupate. This intense herbivory can remove considerable quantities of biomass from the host *Passiflora* plant leading to a reduced fitness for that individual (Gilbert 1991). This intense pressure is believed to be responsible for the evolution of a host of possible anti-herbivore defenses seen in *Passiflora* (Gilbert 1991).

Passiflora and closely-related genera have cyanogenic glycosides, a secondary metabolite (Feuillet 2004, Gilbert 1991). The cyanogenic glycosides are effective at deterring general herbivores (Kursar and Coley 2003), however, they are not useful in preventing herbivory by heliconiine butterfly larvae. In fact, heliconiine butterflies and their larvae possess the same cyanogenic glycoside system as *Passiflora* and some species may sequester cyanide from the leaves, as well as benefiting nutritionally (Brown *et al.* 1991, Engler *et al.* 2000) The ability of heliconiines to overcome cyanide defenses has resulted in a coevolutionary arms race between the Passifloraceae and Heliconiinae (Feuillet 2004, Gilbert 1991, Gilbert 1971). In addition to cyanogenic glycosides, *Passiflora spp.* have evolved variable numbers of other defenses such as extrafloral nectaries, variable leaf shapes, egg mimics (Feuillet 2004, Gilbert 1991), and pubescence on the leaves (Gilbert 1971). Extrafloral nectaries have been shown to reduce herbivory in *P. incarnata* by attracting territorial ants that harvest the nectaries and remove insect eggs and larvae

from leaves. Variable leaf shape is believed to thwart heliconiine females' ability to identify *Passiflora* as suitable for oviposition (Feuillet 2004, Gilbert 1991). Likewise, because *Heliconius spp.* that lay their eggs on passion vines typically have cannibalistic young they will only oviposit one egg on a leaf or tendril at a time (DeVries 1987). It has been suggested that some species of *Passiflora* have adapted small yellow structures, leaf glands, that mimic *Heliconius* eggs in order to deter oviposition and the subsequent herbivory by larval heliconiine (Feuillet 2004, Gilbert 1991, Williams and Gilbert 1981). Finally, the presence of pubescence on leaves makes it difficult for the larvae to move across the leaf (Coley 1983) and when the pubescence are hook shaped they can even result in the death of the larva as seen in *P. adenopoda* (Gilbert 1971).

Tropical species of *Passiflora* must deal with increased herbivory in nutrient poor environments. Therefore, it may not be energetically possible to develop an anti-herbivory strategy that employs all of the defense mechanisms that have been described for this genus. Instead, plants may have trade-offs where investment in one defense precludes others (Coley 1983, Coley and Barone 1996, Gilbert 1991, Kursar and Coley 2003 Marquis 1994). Additionally, given that young leaves experience higher levels of herbivory, it would seem evolutionarily advantageous to invest more energy in the protection of young leaves than old leaves. The purpose of this study is to examine possible tradeoffs made in resources allocated to antiherbivore defense in three tropical passion vine species, *Passiflora capsularis*, *Passiflora helleri*, and *Passiflora sexflora*.

METHODS

Study Site

Leaf samples of *P. capsularis*, *P. helleri*, and *P. sexflora* were collected from a Montane Tropical Moist Forest in Monteverde, Costa Rica (elevation between 1450-1750 m) around the Estación Biológica Monteverde. All leaf samples were collected from plants that were found along well maintained walking trails.

Leaf Collection and Identification

Samples were collected for three days on April 30, May 1, and May 3, 2008. One young and one old leaf were collected from ten different vines of each species sampled. Leaves were only collected from vines when the apex of the vine could be located. When possible, vines were followed to their end point in order to ensure that the same individual was not sampled more than once. The age of the leaf was determined relative to the tip of the vine. The third leaf from the apex was collected as the young leaf sample. To determine the old leaf sample, leaves were counted down from the position of the young leaf and the farthest down or the tenth leaf was collected, whichever came first (Fig. 1). The nearest old leaf collected was the seventh leaf from the young leaf. The majority of the old leaves collected were in the tenth position from the young leaf sample with the exception of *P. sexflora*, in which the old leaf sample was more consistently the eighth leaf. Leaf samples from the same species were all collected and tested the same

day. William Haber and Willow Zuchowski, local botanists, were consulted for the identification of the three species used in this study.

Toughness

The toughness of the leaves was determined using a penetrometer, which measures the toughness of the leaf. Leaf samples were placed between two metal plates that were 10 x 7.5 cm. Each plate had a hole drilled through the center. The hole in the bottom plate had a diameter of 3 mm and the hole in the top plate had a diameter of 2 mm. The bottom plate had two metal posts, one in each opposite corner that matched up with two holes in the top plate, this worked to keep the plates and sample from moving during the test. A plastic plate, diameter of 7.4 cm, with a metal rod, length 1.5 cm and diameter 1.1 mm, in the center was set on top of the sample through the hole in the top plate (Fig. 2). In order to ensure that a proper measurement was taken, the leaf sample was placed on the bottom plate so that no venation was centered above the hole. A container of known mass was placed on the plastic plate and water was added to the container until the metal rod punctured the leaf. The amount of water was measured in milliliters and converted to grams. The mass of the water was added to the mass of the container and this was used as the measure of toughness in grams. When the mass of the plastic plate was sufficient for puncturing the sample a toughness value of zero was recorded. This test was conducted on three different locations of each leaf sample (Fig. 3) and the average was calculated and used as the final toughness value.

Habitat Preference and Anti-herbivore Defense Inventory

Habitat type was noted during collection, whole leaf weight was obtained, and pubescence and gland observations were recorded prior to the toughness test. Pubescence was determined to be high, medium, or none in relation to the pubescence on the other species sampled as demonstrated in Figure 4.

Cyanide Analysis

The sodium picrate test was used to determine the concentration of cyanide in each leaf sample (Seigler, 1991). Sodium picrate paper was prepared by dipping 9 x 50 mm chromatography paper into a prepared aqueous solution of 0.5% (w/v) picric acid and 5% (w/v) sodium bicarbonate and allowed to dry fully. Each leaf sample was weighed out to the approximate weight of the lightest sample from that species to the nearest thousandth of a gram and placed in a 21 mm glass vial. The leaf sample was macerated in the vial and three drops of toluene was added as a solvent. The sodium picrate paper was then suspended over the sample by attaching it to the rubber stopper and the vial was placed in the window seal for ten minutes at room temperature. The presence of cyanide is indicated by a change in color from yellow to red-orange. The sodium picrate paper was then placed in 3 ml of de-ionized water for 30 sec and then removed. The solution was then placed into a cuvet and read in an MRC UV-200-RS Ultraviolet and Visible spectrophotometer at 540 nm, the percent transmittance was recorded. The blank was prepared by following the same procedure without a leaf sample. A standard curve was

developed by plotting the percent transmittance against known values of cyanide concentration in a serial dilution. A stock solution of aqueous potassium cyanide (KCN) was made by dissolving 1 g of KCN in 1 ml of de-ionized water. Each subsequent solution was made by diluting 0.1 ml of the solution with 0.9 ml of de-ionized water so that there were five solutions that decreased in cyanide (CN) concentration by a factor of ten from 1 g CN ml⁻¹ to 0.0001 g CN ml⁻¹. A sodium picrate test was performed on 0.1 ml of each CN solution as described above. The concentrations of CN were divided by 0.1 in order to account for the volume tested and then the unit of measure was converted to micrograms. The percent transmittance was converted to absorbance for both the standard curve and sample. The sample absorbance was then converted to concentration of CN using the standard curve and divided by the sample weight to obtain the amount of CN per leaf reported in units µg / g.

RESULTS

As predicted, the younger leaves of all three species were not as tough as the older leaves sampled (Fig. 5, N = 59, F = 21.60, P < 0.0001, DF = 1). *P. capsularis* old leaves, with a mean +/- SE of 64.3g +/- 2.76, were more than twice as tough as its young leaves, with a mean of 23.73g +/- 6.26 (N = 10 for old and N = 9 for young). Likewise, old leaves for *P. helleri* had a mean of 78.5g +/- 13.07, while young leaves were less than half as tough, with a mean of 31.48 +/- 12.88 (N = 10 for both). *P. sexflora*, on the other hand, had young and old leaves that were much closer to one another, in terms of toughness: old leaves were near the toughness of the other species, at 73.87g +/- 13.07, but young leaves were far tougher than the other *Passiflora* spp., having a mean toughness of 53.83g +/- 6.22, which is nearly the toughness of old *P. capsularis* leaves (see figure 5). In general, there was no difference observed in the mean toughness between each species with young and old leaves combined (Fig. 5, N = 59, F = 2.25, P = 0.12, DF = 2). The LSMeans differences student's t test suggested that the mean toughness of all *P. helleri* leaves combined was statistically equivalent to the toughness of both *P. capsularis* and *P. sexflora* leaves. By contrast, *P. capsularis* and *P. sexflora* had statistically different mean leaf toughnesses, in that *P. helleri* generally had tougher leaves (Fig. 5, N = 59, t = 2.01, P = 0.05).

Interestingly, I did not observe an overall difference in the mean cyanide concentration between the young and old leaves of the three species (Fig. 6, N = 59, F = 0.77, P = 0.39, DF = 1). *Passiflora Capsularis* is the exception to this trend, the young leaves, with a mean +/- SE of 47.75 µg / g leaf +/- 35.59, has 40 times the cyanide concentration as the old leaves, with a mean +/- SE of 0.33 µg / g leaf +/- 0.09 (N = 10 for both). Two outliers were removed from the analysis of *P. capsularis* as they resulted in a very large standard error (Fig 7 and 8, [CN] = 351.97 with Toughness = 0 and [CN] = 113.37 with Toughness = 6). When these data were removed from the analysis the difference in mean cyanide concentration between young and old leaves of *P. capsularis* is statistically significant (N = 8, F = 5.14, P = 0.04, DF = 1, data not shown). *Passiflora helleri* young leaves, with a mean +/- SE of 134.64 µg / g leaf +/- 53.01 µg / g leaf, had slightly higher cyanide concentrations than the old leaves, with a mean +/- SE of 106.61 µg / g leaf +/- 63.71 (N = 9 for young leaves and N = 10 for old leaves). *Passiflora sexflora* produced

very little cyanide and the young leaves, with a mean \pm SE of $0.05 \mu\text{g} / \text{g leaf} \pm 0.007$, had equal concentrations of cyanide as the old leaves, with a mean \pm SE of $0.05 \mu\text{g} / \text{g leaf} \pm 0.009$ ($N = 10$ for both). Due to the equal concentrations of cyanide in the young and old leaves of *P. sexflora*, the data point for the old leaf is being covered by the data point for the young leaf in figure 6. At the species level, *P. helleri* produced greater concentrations of cyanide in both young and old leaves than did *P. capsularis* and *P. sexflora*, which showed statistically equivalent concentrations of cyanide (Fig. 6, $N = 59$, $t = 2.01$, $P = 0.05$).

A trend of decreasing cyanide concentrations with increasing leaf toughness was observed for all three species of *Passiflora* (Fig. 9). Due to the large scale used on the y-axis in the linear regression, *P. sexflora* does not appear to follow this trend, however, when the scale is lowered the cyanide concentrations do show this trend despite the fact that nearly all of the data points for this species are near to zero. The linear regression also clearly shows that *P. helleri* produces larger quantities of cyanide. As with the relationship between age and cyanide concentrations, *P. capsularis* was the only species to show statistical significance in this relationship ($N = 20$, $P = 0.02$, $R^2 = 0.26$, $y = 95.02 - 1.61*x$). No statistically significant relationship was observed for *P. helleri* ($N = 19$, $P = 0.13$, $R^2 = 0.13$, $y = 200.28 - 1.43*x$) and *P. sexflora* ($N = 20$, $P = 0.35$, $R^2 = 0.05$, $y = 0.06 - 0.00017*x$), though all trends were in this direction.

Additional observations

All species of *Passiflora* that were sampled were located along well cleared trails in understory habitat and often times occurred within a few feet of another species of passion vine sampled (Table 1). Therefore, habitat type did not differ for the three species examined here. In terms of pubescence, *Passiflora sexflora* appeared to have substantially more pubescence than either of the two other species, whereas *P. capsularis* had relatively more pubescence than *P. helleri*, which appeared to have little to no pubescence (Fig. 4, Table 1). Finally, of the three species, *P. helleri* was the only one to possess observable glands on the leaf blades (Table 1).

DISCUSSION

Tradeoffs in anti-herbivore defenses assume a limited availability of resources that restricts the number and types of defenses any one plant species can employ to protect themselves (Coley and Aide 1991). Tropical species should show clear tradeoffs, as nutrients are generally limiting and herbivore pressure is high. Given the large number of described anti-herbivore defense mechanisms utilized by *Passiflora spp.* against their primary heliconiine herbivores, it is unlikely to be energetically feasible for one species to employ all available mechanisms. Therefore, many species in this genus will combine different mechanisms in order to evolve a subset of anti-herbivore defenses. Because the most effective protection a plant can have against herbivores is tougher, less nutrient rich tissue, such as that found in older leaves (Coley and Aide 1991, Kursar and Coley 2003), all plants should evolve to toughen old leaves, but may have to employ other strategies until leaves can acquire toughness.

In terms of toughness and cyanide, the three *Passiflora* spp examined are very different and seem to exhibit very different tradeoffs. *Passiflora capsularis* produces more cyanide when the leaves are untoughened and young, but have no cyanide in older, tougher leaves. Thus, as the leaf toughens, *P. capsularis* stops its investment in cyanide defenses. *Passiflora helleri* did not show a trade off in the production of cyanide with leaf age or toughness. In part, this is because even old, tough leaves retain high cyanide concentrations in *P. helleri*; cyanide concentrations of *P. helleri* old leaves are equal to its young leaves and surpass even the young leaves of the other species. In *P. sexflora*, leaves are never very tough and yet their leaves contain little or no cyanide.

Passiflora helleri may maintain high cyanide production in old leaves because they lack pubescence. Pubescence has been shown to be an effective anti-herbivore defense mechanism (Coley 1983, Gilbert 1971). However, small chemical compounds, such as cyanide, are less energetically costly than a structural defense, such as pubescence (Coley and Aide 1991). Therefore, it may be more efficient for *P. helleri* to invest in the consistent production of an inexpensive defense rather than invest more energy at one time to a structural defense. Another possible explanation may be that the heliconiine butterflies that specialize on *P. helleri* lay their eggs on mature leaves as well as young leaves and therefore the older leaves require added protection. This is one possible scenario in the selection of the anti-herbivore defense mechanisms employed by *P. helleri*.

Passiflora sexflora, having neither cyanide nor tough leaves, appears to utilize yet another type of anti-herbivory defense strategy. This species seems to invest more in pubescence. *Passiflora sexflora* had considerably more pubescence than either of the other two species sampled for this study. Gilbert, 1971, suggests that hooked pubescence is the ultimate deterrent against herbivory by *Passiflora* specialists heliconiine. Hooked pubescences have been shown to rip the soft flesh of new larvae causing them to lose hemolymph and eventually die (Gilbert 1971). The use of pubescence may be so effective at deterring herbivory that *P. sexflora* has no reason to toughen its leaves or invest in cyanide defenses.

Perhaps the abundance of pubescence observed on *P. sexflora* is indicative of an advantage over *P. capsularis* and *P. helleri*. It could be that *P. sexflora* is moving toward one of the most effective defense strategies against heliconiine larvae, hooked pubescence, and is farther along in the *Passiflora* - *Heliconius* coevolutionary arms race than are *P. capsularis* and *P. helleri*. If this is the case, then perhaps *P. capsularis* is farther along in the coevolutionary arms race than *P. helleri* and the cyanide/toughness tradeoff observed in the former is not due to resource allocation but rather, is due to the eventual phasing out of a defense mechanism that is no longer relevant.

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Figure 1. Leaf collection points, *P. helleri* vine, arrows indicate the approximate positions of leaves collected from the sampled *Passiflora*, young (yellow) and old (orange).



Figure 2. Penetrometer technique used to determine the toughness of each *Passiflora* leaf sampled.



Figure 3. Locations of toughness test shown on *P. helleri* leaf, arrows indicate the three positions of the leaf toughness test, first – yellow, second – orange, and the third – purple. The average toughness was calculated and reported as the sample toughness.



Figure 4. Relative pubescence of (a) *Passiflora capsulari*, (b) *P. helleri*, and (c) *P. sexflora*.

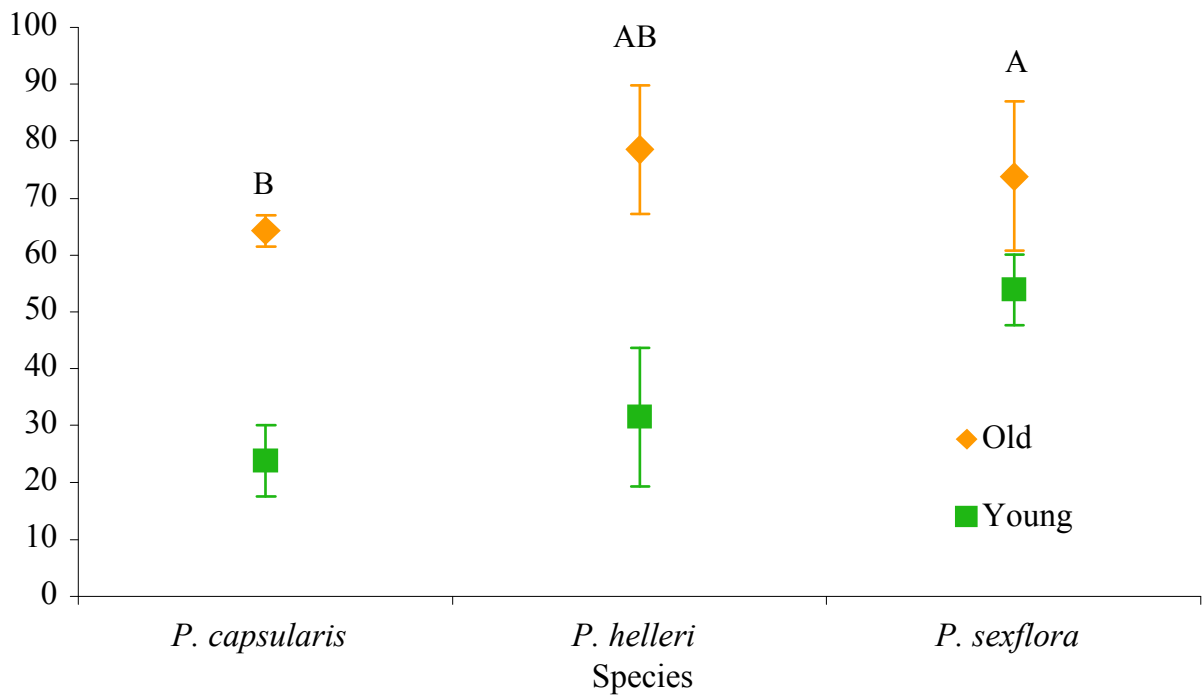


Figure 5. The difference between the means of toughness as a relationship of species ($N = 59$, $F = 2.25$, $P = 0.12$, $DF = 2$), age ($N = 59$, $F = 21.60$, $P < 0.0001$, $DF = 1$), and the interaction between species and age ($N = 59$, $F = 1.11$, $P = 0.34$, $DF = 2$) for *Passiflora capsularis* (young: $N = 10$, mean = 23.73 ± 6.26 Std. Err., old: $N = 10$, mean = 64.3 ± 2.76 Std. Err.), *P. helleri* (young: $N = 9$, mean = 31.48 ± 12.88 Std. Err., old: $N = 10$, mean = 78.5 ± 11.30 Std. Err.), and *P. sexflora* (young: $N = 10$, mean = 53.83 ± 6.22 Std. Err., old: $N = 10$, mean = 73.87 ± 13.07 Std. Err.). Letters represent significantly different means between species ($N = 59$, $t = 2.01$, $P = 0.05$).

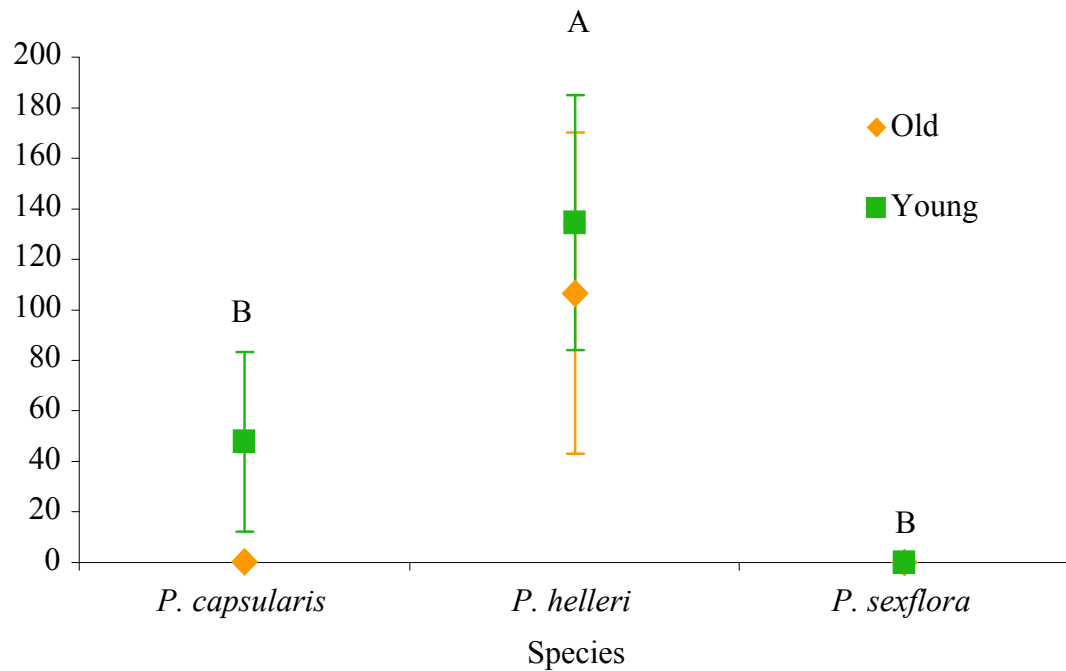


Figure 6. The difference between the means of cyanide concentration ($\mu\text{g} / \text{g}$ leaf) for three species of *Passiflora* from Monteverde, Costa Rica ($N = 59$, $F = 6.46$, $P = 0.003$, $DF = 2$), age ($N = 59$, $F = 0.77$, $P = 0.39$, $DF = 1$), and the interaction between species and age ($N = 59$, $F = 0.23$, $P = 0.79$, $DF = 2$) for *Passiflora capsularis* (young: $N = 10$, mean = 47.75 ± 35.59 Std. Err., old: $N = 10$, mean = 0.33 ± 0.09 Std. Err.), *P. helleri* (young: $N = 9$, mean = 134.64 ± 53.01 Std. Err., old: $N = 10$, mean = 106.61 ± 63.71 Std. Err.), and *P. sexflora* (young: $N = 10$, mean = 0.05 ± 0.007 Std. Err., old: $N = 10$, mean = 0.05 ± 0.009 Std. Err.). Young leaf samples shown as a green square and old leaf samples shown as a gold diamond. Letters represent significantly different means between species ($N = 59$, $t = 2.01$, $P = 0.05$).

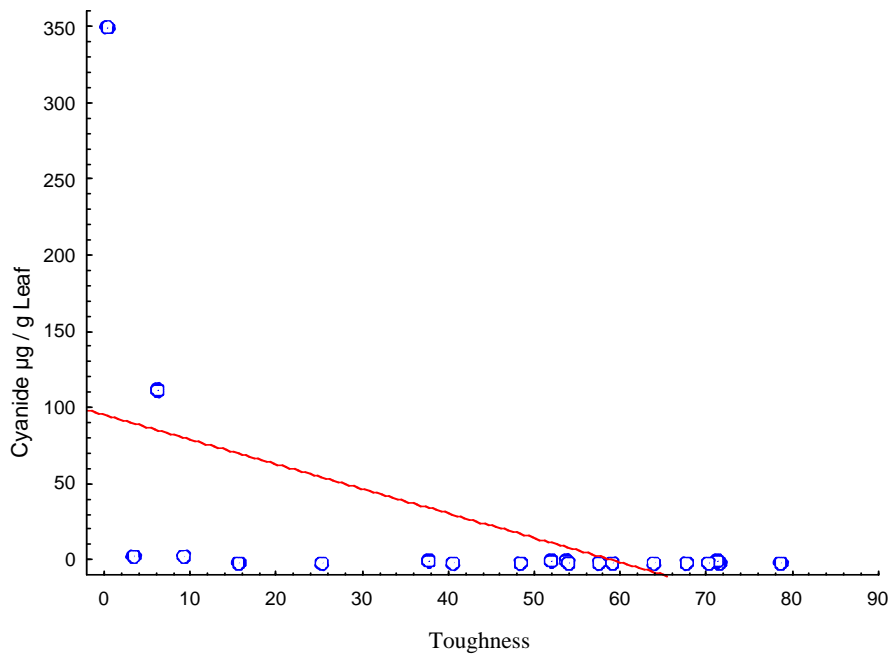


Figure 7. Cyanide concentrations ($\mu\text{g} / \text{g}$ leaf) of *Passiflora capsularis* against leaf toughness with outliers included ($[\text{CN}] = 351.97$ with Toughness = 0 and $[\text{CN}] = 113.37$ with Toughness = 6, $N = 20$, $P = 0.02$, $R^2 = 0.26$, $y = 95.02 - 1.61 \cdot x$).

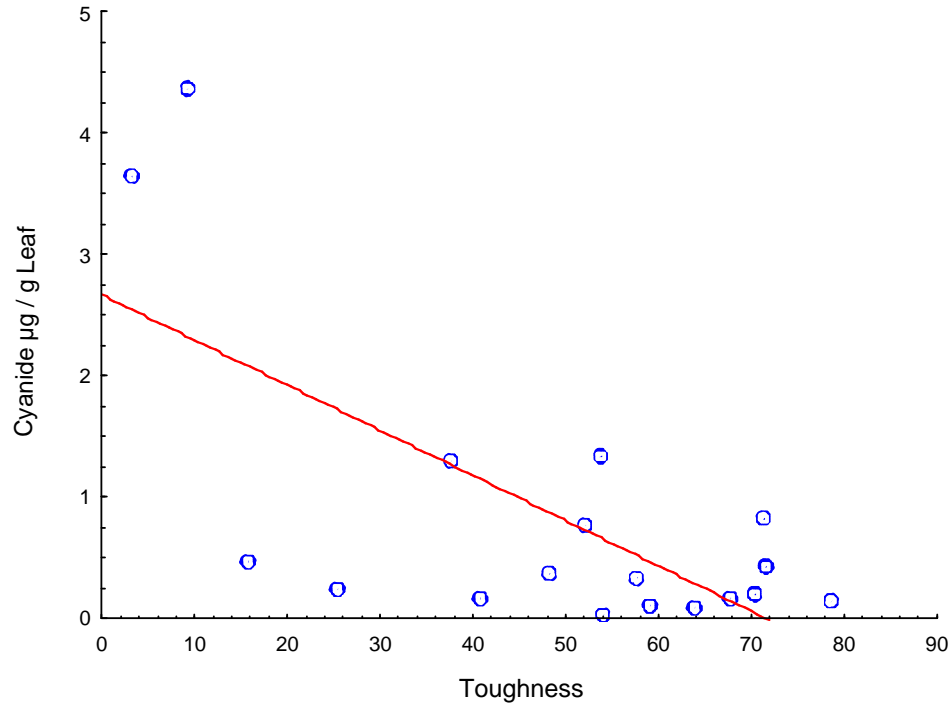


Figure 8. Decreasing cyanide concentrations ($\mu\text{g} / \text{g}$ leaf) with increasing toughness of *Passiflora capsularis* having removed two outlier points, $[\text{CN}] = 351.97$ with Toughness = 0 and $[\text{CN}] = 113.37$ with Toughness = 6 ($N = 18$, $P = 0.0017$, $R^2 = 0.47$, $y = 2.67 - 0.04 \cdot x$).

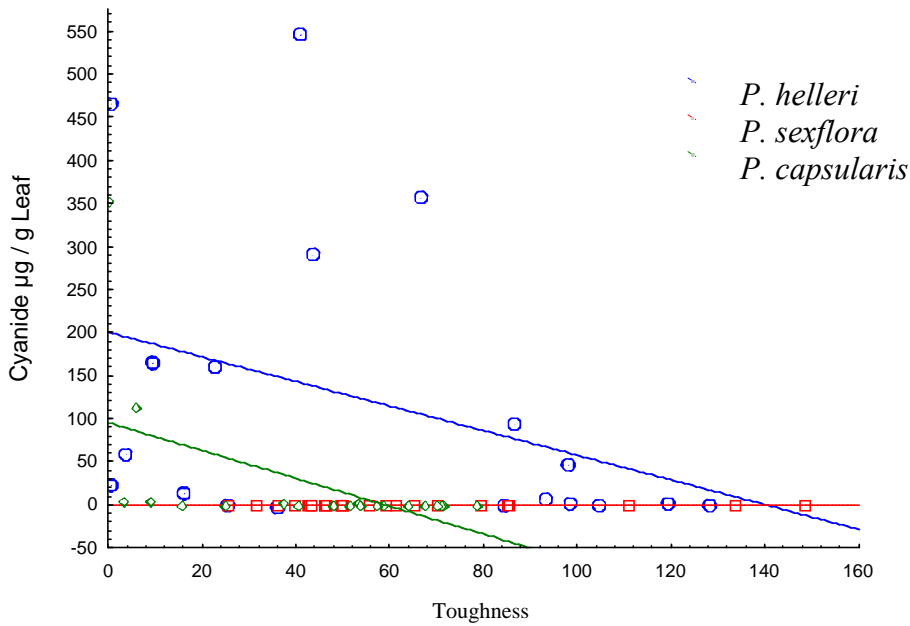


Figure 9. Plots of cyanide concentration ($\mu\text{g/g}$ Leaf) against leaf toughness of *Passiflora capsularis* (open green diamond and green regression line, $N = 20$, $P = 0.02$, $R^2 = 0.26$, $y = 95.02 - 1.61*x$), *P. helleri* (open blue circle and blue regression line, $N = 19$, $P = 0.13$, $R^2 = 0.13$, $y = 200.28 - 1.43*x$), and *P. sexflora* (open red square and red regression line, $N = 20$, $P = 0.35$, $R^2 = 0.05$, $y = 0.06 - 0.00017*x$).

Table 1. Observed habitat type at time of sample collection, the presence of pubescence in relative amounts, and the presence of glands on the blades of *Passiflora capsularis*, *P. helleri*, and *P. sexflora* in Monteverde, Costa Rica Cloud Forest habitat between 1450 and 1600 meters.

| Species | Habitat | Pubescence | Glands on the Blade |
|----------------------|--|------------|---------------------|
| <i>P. capsularis</i> | Understory - along trail | Medium | None |
| <i>P. helleri</i> | Understory - along trail In trees - along trail | None | Yes |
| <i>P. sexflora</i> | Understory - along trail | High | None |