

May 2002

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Function and size optimization in *Mucuna urens* vexillum

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Abstract

This study examines the role of the vexillum as an aural nectar guide in the species *Mucuna urens*. Field manipulations show that the vexillum of *M. urens* enhances visitation and pollen removal ($X^2 = 52.8$, $p < .001$). Subsequent observational studies conducted on bat visited hummingbird feeders support this finding as well ($X^2 = 10.67$, $p < .005$). Data also show an increase in vexillum size correlated with number of bats visits ($X^2 = 32.06$, $p < .001$). Two explanations are offered for why *M. urens* and other bat pollinated flowers in the genus have not developed as large a vexillum as possible. Investment return optimization is one possible explanation. Niche partitioning of potential pollinators is another.

Resumen

Este estudio examina el papel del "vexillum" como una guía auricular de néctar en la especie *Mucuna urens*. Las manipulaciones del campo muestran que el vexillum de *M. urens* aumenta las visitas y la remoción de polen ($X^2 = 52.8$, $P < .001$). Observaciones en los comederos de colibríes que fueron visitados por los murciélagos apoyaron estas observaciones ($X^2 = 10.67$, $P < .005$). Los datos mostraron que un aumento en el tamaño de vexillum tuvo correlación con el número de visitas de los murciélagos ($X^2 = 32.06$, $P < .001$). Dos alternativas se ofrecen para explicar porque *M. urens* y otras flores polinizadas por murciélagos en mismo género no han desarrollado un vexillum tan grande como es posible. La optimización del regreso de la inversión y la división de nicho de los polinizadores potenciales son las posibles explicaciones.

Introduction

One of the most pressing challenges plants face is finding an efficient method of reproducing sexually. In areas like the Tropics, where a large number of plant species are rare and live in a highly heterogeneous environment, a plant's ability to encounter pollen from conspecifics may be especially difficult. In response to this challenge, plants have developed specialized relationships with insects, mammals, and birds to move pollen (Endress 1994). Bawa (1990) estimates that nearly all flowering plants in tropical lowland rain forest are pollinated by animals. With the large number of plants and potential pollinators in the Tropics, evolving very specific plant-pollinator interactions greatly increases the likelihood that pollen from conspecifics will reach a given individual (Feinsinger 1983). This has led to specialized features to attract specific pollinators, commonly referred to as pollination syndromes (van der Pijl 1960).

Pollination syndromes serve to attract those pollinators that will provide the most effective pollination service (Feinsinger 1983). Pollination syndromes can be generalized by

pollinator. Bat pollinated plants, for example, are typically large and often a dull or white in color. They characteristically have a strong odor that somewhat resembles fermented fruit and produce large quantities of nectar (Janzen 1975). Bat pollinated flowers also usually flower at night and contain some sort of receptacle to hold pollen as a reward for the bat. Finally, many bat pollinated plants devise some way of reducing clutter around the inflorescences, such as hanging the flowers free of the leaves (Altringham 1996).

In addition to the generalized pollination syndromes, some flowers also have nectar guides to direct their pollinators to the flower. Many types of insect pollinated flowers are known to use UV light patterns as visual nectar guides (Brehm and Krell 1975; Penny JH 1983; van der Pijl 1960). The insects that pollinate these flowers are able to see UV light, and are essentially guided towards the flowers by the visual cues provided by the flower. One of the more interesting and rare examples of a nectar guide, however, is that of an aural guide.

The bat pollinated liana *Mucuna holtonii* (Papilionoideae) was shown by von Helverson O and von Helverson D (1999) to contain an aural nectar guide that helps bats find the small green flowers using echolocation. The study was done at the La Selva field station in Costa Rica. Flowers of *M. holtonii* contain a structure called a vexillum (also known as a standard) that has a concave structure and sits on top of the flower (fig. 1). This structure was hypothesized to reflect the echolocation calls of bats, which helps the bat locate the flower. Removing the vexillum resulted in much lower rates of pollination, and tests using microphones supported the hypothesis that *M. holtonii* does have a structure that acts as a nectar guide by reflecting the calls of bats (von Helverson O and von Helverson D 1999).

More than 1500 meters above the *M. holtonii* study site at La Selva there grows a different species in the same genus, *Mucuna urens* (McDade and Hartshorn 1994, Diller GW, 2000). *M. urens*, like *M. holtonii*, is known to be bat pollinated and also has a vexillum structure like that of *M. holtonii*. The purpose of this study is to examine the role of the vexillum in *M. urens*, and to test different sizes of vexillum for size optimization.

Materials and Methods

Site Description:

All studies were conducted in the lower montane cloud forest in Monteverde, Costa Rica. The entire study took place from mid April to mid May 2002. The first part of the study was conducted at the Estación Biológica Monteverde. All feeder experiments were conducted at the hummingbird gallery, 50 meters from the entrance to the Monteverde reserve. Both sites are at nearly the same elevation, approximately 1550 meters above sea level (Masters K, pers. comm.). Mist nets set up between hummingbird feeders for use in two concurrent studies frequently caught three species of nectivorous bats, *Glossophaga commisserissi*, *Anoura geoffroyi*, and *Hylonycteris underwoodi*, and one species of frugivorous bat, *Artibeus toltecus* (Majewski JJ 2002 ; Miller RM 2002). All of these bats are in the family Phyllostomatidae. Of these, the likely pollinators of *M. urens* are *G. commisserissi* and *H. underwoodi*. *A. geoffroyi* is most likely too large for the relatively small *M. urens* flower and *A. toltecus* is a fruit eating bat (LaVal, Pers. Comm.; Diller GW, 2000).

Study organism: *Mucuna urens*:

M. urens is a relatively common liana around the Monteverde region and is most often seen high in the forest canopy or along roadsides where bats have easy access to the flowers (von Helverson and von Helverson, 1999). Individuals tend to be fairly far away from each other, so bats are the ideal vectors for *M. urens*, since bats are known to travel up to 16 km in one night to visit a plant (Janzen 1975). Inflorescences of *M. urens* take the form of a long pendant raceme (Endress 1994). Up to 20 flowers may be found on a single inflorescence. When a flower on an inflorescence matures, the vexillum opens, also exposing the keel and wings. The pollination strategy of *M. urens* is explosive. Visitation by a bat triggers the keel to open, which projects the pollen onto the rear of the bat and exposes the sexual organs of the flower (Endress 1994). Thus, visitation by bats is easy to note because of the change in appearance of the flowers.

(a) Testing functionality of *M. urens* vexillum:

The first part of the experiment is necessary to verify that *M. urens* vexillum serves the same function as the vexillum in *M. holtonii* as a nectar guide for bats. The methodology of the original La Selva study was copied as closely as possible. Since *M. urens* flowers open sequentially through the course of a night, the number of open flowers available for visitation was optimized by covering all mature inflorescences with a large mesh bag between noon and 6:00 pm (von Helverson O and von Helverson D 1999). The bags were then removed between 8:30 and 9:00 pm. One half of the flowers had the vexillum cut off and the other half were left as control. Sampling was continued for five nights and a total of 215 flowers were tallied.

(b) Testing visitation and vexillum at feeders:

In order to determine whether using detached *M. urens* vexillum on feeders would solicit more visits by bats; one hummingbird feeder with four holes was assembled. Attached directly behind two of the holes on opposite sides of the feeder were actual *M. urens* vexilla. The other two holes were left unaltered as a control. The feeder was then observed for up to three hours a day between 6:00 and 9:00 pm for four days. A red filter was put on a small 40 watt light, so that just enough red lights were produced to see the feeder. For every bat that came to the feeder, the specific hole visited was written down. Every fifteen minutes to half hour, the feeder was rotated a quarter turn to minimize the possibility of placement affecting the data.

(c) Testing optimal vexillum size at feeders with actual vexilla:

This test was done to test the effect of using paper vexilla of varied size along with an actual vexillum. Ten actual vexilla were measured and both the height and width were found to be 2.5 ± 1 cm. The average of 2.5 was used for subsequent vexilla created using bond paper as a basis for medium paper vexilla. Two paper vexilla were created, one 5 cm in diameter and height, which is twice the size of an actual *M. urens*, and one a little more than half the size of the actual, about 1.5 cm in height and diameter. Fake vexilla made of notebook paper were shaped into the general form of an actual vexilla and attached to the same four hole feeder as the first feeder experiment. A real vexilla from a

plant was also attached and one hole was left open as a control. Data were collected using the same observational methods used in the first part of the feeder experiment.

(d) Testing optimal vexillum size with paper vexilla:

This observational study was virtually identical to (c) with two important exceptions. The first was that the *M. urens* vexillum was replaced by a paper one of the same dimensions, so that the feeder had three paper vexilla, one small, one medium (*M. urens* sized), and one large. Dimensions for the small and large remained the same. As in all other studies, one hole was left as a control. The second difference was the use of more resilient note card paper instead of the more flimsy notebook paper vexillum. The time of observation for this and the next study was moved to 8:00 to 11:00 pm

(e) Testing optimal vexillum size with waterproof vexillum:

This study was identical to (d) except for the use of contact paper around the vexillum to minimize damage by rain.

All results were analyzed with a chi-square test (X^2) to determine significance.

Results

(a) Functionality of vexillum

Results of the five days of data were shown to be significant ($X^2 = 52.8$, $p < .001$). A total of 46% of the control flowers were visited while only 3% of the modified flowers were visited. When compared to the original study by von Helverson (1999), the sample size is considerably smaller, 524 compared to 215, but the overall pattern of pollination is consistent (Figure 2).

(b) Viability at feeders

Observational results with *M. urens* vexillum and control yielded significant results ($X^2 = 10.67$, $p < .005$). A total number of 225 bats were observed. Out of total visits, 61% were to the holes with vexillum attached (Figure 3). A total of two hours were spent at the feeders for three nights. Distinguishing type of bat visiting the feeder was impossible. Many bats seemed a little hesitant to feed from the feeder. Often a bat would fly to about half a meter from the feeder, and turn away. It's hard to say, however, if this is a function of the light, the vexillum, or if this is part of their normal behavior.

(c) Artificial vs. real vexillum

Results from this study once again are significant ($X^2 = 12.93$, $p < .005$). Sample size was 98 bats. The numbers for the large and small artificial vexillum are nearly identical, though; in general, all holes with vexillum show a greater number of visits than the control (Figure 4; small- 26% of visits, Mucuna- 39%, Large- 21%, Control- 14%). Light rains severely affected the paper vexillum, which were destroyed and remade several

times through the course of the night.

(d) **Optimal size with vexilla of paper**

These results turned out not to be significant ($X^2 = 4.59$, critical value 7.85). A total of 162 bats were observed. These data, however, are fairly suspect due to problems with rain and the large vexillum. In this study in particular, the rain affected the paper nectar guide by saturating it with water and causing it to fall forward, blocking the hole. This was not always visible immediately, and since it rained constantly through entire sampling time, data from the large portion of the study was probably severely affected. When the large vexillum is not included into the final calculations, a significant difference is found between the medium vexillum vs. the small plus the control ($X^2 = 8.01$, $p < .025$, $cv = 5.99$). The small and control had nearly identical numbers of bat visits. The graph (Figure 5) also looks very similar to the graph in figure 6, with the exception of a sizable dip in the number visiting the hole with the large vexillum. One final part was added to the study to combat the effects of the rain on previous feeder studies.

(e) **Optimal size with waterproof vexillum**

These results ended up being significant ($X^2 = 32.06$, $p < .001$, $cv = 7.81$). The sample size was 325 bats. This graph shows a clear numerical trend towards larger vexillum, with the biggest jump being between the medium and small vexillum. There is also a clear, though smaller, jump between the large and medium vexillum (Figure 6). Note that in (c), (d), and (e), the control is always one of the least popular visited.

Discussion

From the collective data presented in my report, there is little doubt in my mind that the vexillum in *M. urens* serves as an aural nectar guide, as was shown for *M. holtonii* (von Helverson and von Helverson, 1999). Though the visitation figures are different between the two studies, the same pattern is clear. There are simply too many variables to accurately predict why only 48% of my control flowers were visited while those at La Selva had a nearly 90% visitation rate. Accounting for variations in plant species, time of year, number of flowers, varied topography, bat population structure, rain, a full moon, and the fact that the plant in Monteverde is more widespread than in La Selva makes concluding anything about the fact that only half of the unmodified flowers were pollinated nearly impossible (LaVal, pers. comm.). In general, however, the results of both studies were very clear. In addition, these results were further supported by the data from the later studies at the hummingbird feeders.

There is an interesting extra factor in all of the hummingbird feeder studies that may have had an effect on the total number of visitors to each hole, but probably not on the final numbers. The fact that *A. geoffroyi* is too large to use *M. urens* would presumably mean that *A. geoffroyi* could ignore any reflectance from *M. urens* vexilla (LaVal pers. comm.). This could have several effects. In my opinion, the most likely is that the bats would simply ignore the vexillum and go to the holes indiscriminately. There is also the possibility, however, that they would actually avoid the vexillum and go towards the control hole. When all the data are looked at and weighed, however, it seems more likely that *A. geoffroyi* is probably feeding indiscriminately. If this is the case, then *A. geoffroyi* only impacts the data

when total number of bats are considered, not individual holes. This should not impact my data. There is a possibility, discussed in detail later in this paper, that the larger vexillum did attract the larger *A. geoffroyi* to the feeders more effectively than other studies did.

At the hummingbird feeders, the initial data comparing modified holes to unmodified holes yielded the expected trend. The fact that just the vexillum and no other part of the flower was used to attract a significant amount of bats further supports the findings of the first part of the study, as well as the La Selva study. The first test with the artificial paper vexilla showed nothing more than paper could be used to simulate the function of the nectar guide. Nothing more can be concluded from this study due to the fact that rain badly warped the large vexillum, rendering it useless for regular intervals. Still, the data clearly showed that the holes with vexilla were preferred over the control. This is consistent with all other studies. The fact that paper was shown to effectively serve as a vexillum indicates that the vexillum on *M. urens* serves mainly as a reflective structure and probably does not contain any undetected chemical or visual cues to the bat.

The final two studies, probably the two most dependable data sets, show a clear trend toward favoring larger vexillum size. The first of the two suffered once again from the effects of rain on the large paper vexillum. The medium, *M. urens* sized vexillum performed well, however. Especially when the data from the large vexilla is thrown out, it is clear that paper is an acceptable substitute for actual *M. urens* vexillum, given the same result as the previous experiment.

The final study clearly shows the trend favoring largeness in vexilla (Graph E). This is a fascinating trend which begs the question why *M. urens* has not evolved to have larger flowers and take advantage of the increased numbers of visits. One possible explanation for this is that the plant is maximizing the output from the vexillum while still minimizing the investment needed in the flower. This is supported by the fact that there was virtually no difference between the control and small vexillum, while a significant difference was found between the medium and small. This indicates that, for a relatively small amount of biomass, visitation increases by 56%. When more investment is put into the vexillum, however, the advantage given to the plant, while numerically greater only grants a 22% increase. This suggests that the *M. urens* sized flower is optimized to get the most out of the least possible material.

It is interesting to note that other plants in the genus *Mucuna* also have vexillum approximately the same size (Table 1); (Stevens et al. 2001; Woodson et al. 1980). This suggests that selection everywhere has converged upon 2-3 cm as an optimum size of vexillum. Evolutionarily, the sub-family Papilionoidea is widespread throughout the world, and is pollinated by just about every type of pollinator known. The vast majority of Papilionoideae are insect pollinated and the vexillum works as a visual flag for the insects (Judd et al. 1999). It has been hypothesized that bat pollinated flowers in general originated from insect pollination and evolved from there to bat specialization (Stebbins 1970). It is not too much of a stretch to imagine the visual flag being modified through evolutionary time to reflect bat echoes. The fact that a large amount of the bat pollinated species have vexillum of nearly the same size reveals a remarkable amount of convergence. Selection must strongly favor the current flower size of *Mucuna* in bat pollinated species. The genus *Mucuna* has undergone remarkable adaptive radiation. Insects, bats, birds, and possums all visit *Mucuna*, but data about differences in vexilla size is not readily available (Endress 1994). However, in the species *Mucuna rostrata*, which probably is visited by hummingbirds, the vexillum has grown to nearly 5 cm long. This further suggests that a 2-3 cm vexillum is a specific adaptation to bat pollinated flowers. Other species in the genus *Mucuna* that are not visited

by bats probably have highly varied *Mucuna* vexillum sizes.

A second explanation takes into account the larger nectar bat, *Anoura geoffroyi* that was found at the site where sampling was done. It was assumed that since *A. geoffroyi* would not normally visit *M. urens*, it was unlikely that the bats would respond to vexilla. It is possible, however, that the larger bat might respond to the larger vexillum. There may very well be nectar guides in larger flowers that *A. geoffroyi* pollinate. If so, they would act similarly to the vexillum in *M. urens*, but on a larger scale. Putting on an artificially large vexillum onto a feeder may reflect the echolocation of the bat in a way that closely resembles a species with a larger flower, one that *A. geoffroyi* could visit. Much more information is needed, though, test this hypothesis.

Research on other nectar guides in different bat-pollinated plants is scarce. In order to confidently support this theory, aural nectar guides would have to be found in other, larger plants. Looking at most bat pollinated plants, a common characteristic is that they are cone or trumpet shaped (Janzen 1975). I propose that these flowers are the actual nectar guide, formed by the petals of the flower. The concave shape of the flower serves to reflect back echolocations, just as the vexillum did in *Mucuna*. It is interesting to note that in areas of the old-world tropics where bats do not echolocate; *Mucuna* species that are visited by bats do not possess raised and concave vexillum (von Helverson and von Helverson 1999). It would be worthwhile to compare other bat pollinated flowers from these areas with those in the new world tropics.

In the broader context of plant-pollinator evolution, my results suggest an optimization of vexillum size. With a larger nectar guide, the plant simply can not justify the extra investment required for a small increase in pollinator visits. In addition, *M. urens* has selected for pollination by smaller bats, which gives it a few very efficient pollinators. Making the nectar guide larger may attract other bats, who have their own suite of specific plants that they pollinate. This makes them much less efficient pollinators that do not necessarily visit conspecifics.

Acknowledgements

I would like to thank Alan, Andrew, and Richard for providing so much assistance with this project. I would also like to thank Jay and Rhett for keeping me company up at the gallery. I would also like to thank the owners of the hummingbird gallery for allowing this project. No bats were harmed in the making of this project.

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Table 1. Size differences among varied species of *Mucuna*. The species *rostrata* is the only species to have a vexillum over 3 cm. The pollinator of this species may not be bats, however. It is an orange flower, unusual for a bat flower. It is probably hummingbird pollinated (Stevens et al. 2001; Woodson et al. 1980)

<i>Mucuna</i> spp.	Vexillum Length (cm)
<i>holtonii</i>	1.8-2.2
<i>mutisiana</i>	2.5
<i>rostrata</i>	5
<i>sloanei</i>	2.5-3
<i>urens</i>	2.5

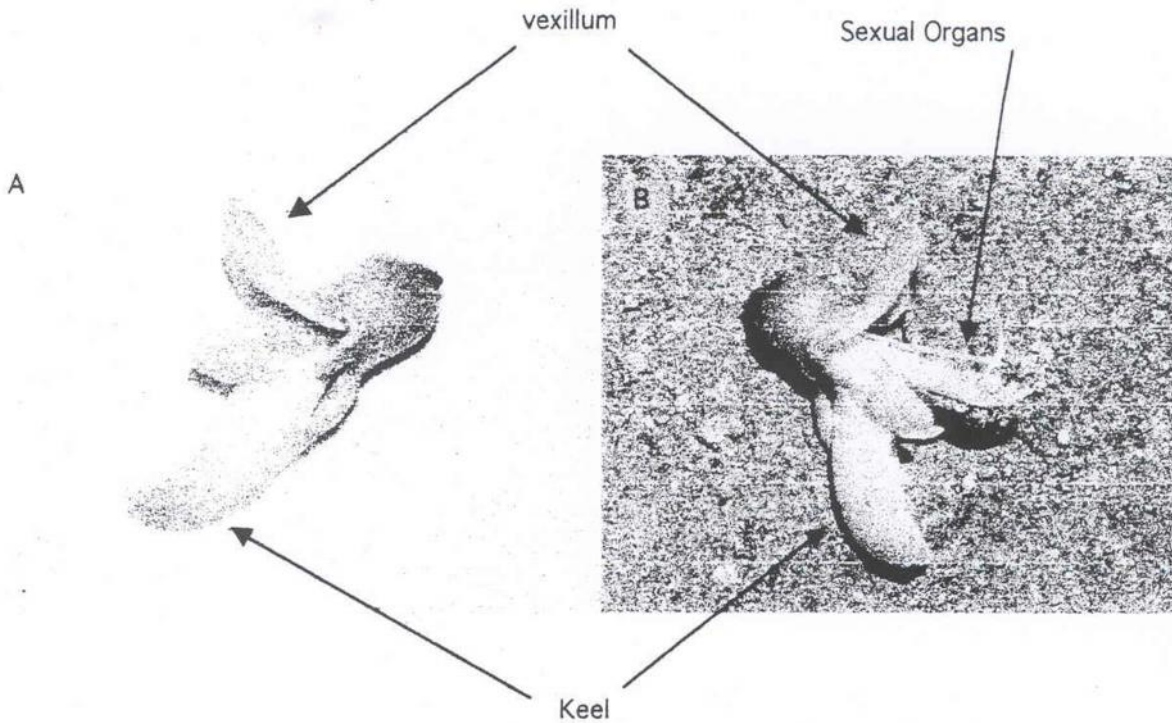


Figure 1. Two *M. urens* flowers. Note the reproductive structures visible in the already visited flowers (B). These structures burst out of the keel and throw pollen onto the bat. This allows easy identification of pollinated flowers

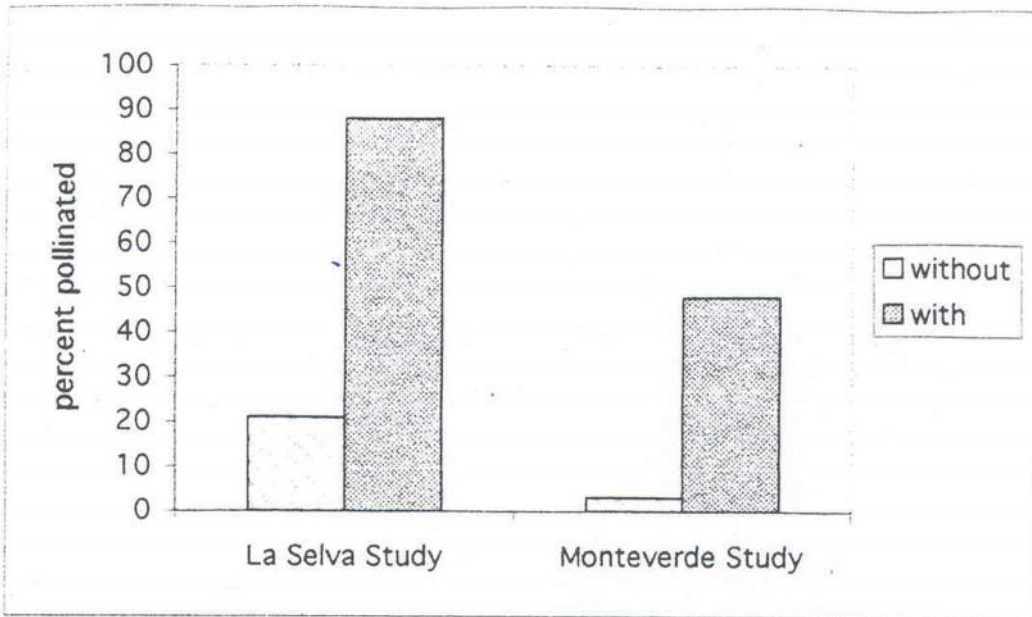


Figure 2. Results from five day study of cutting vexillum and leaving flowers with vexillum. Comparison of my data vs. data gathered at La Selva (von Helverson and von Helverson 1999). The same general pattern can be seen in both. There is statistical significance in the Monteverde study ($X^2 = 52.8, p < .001$)

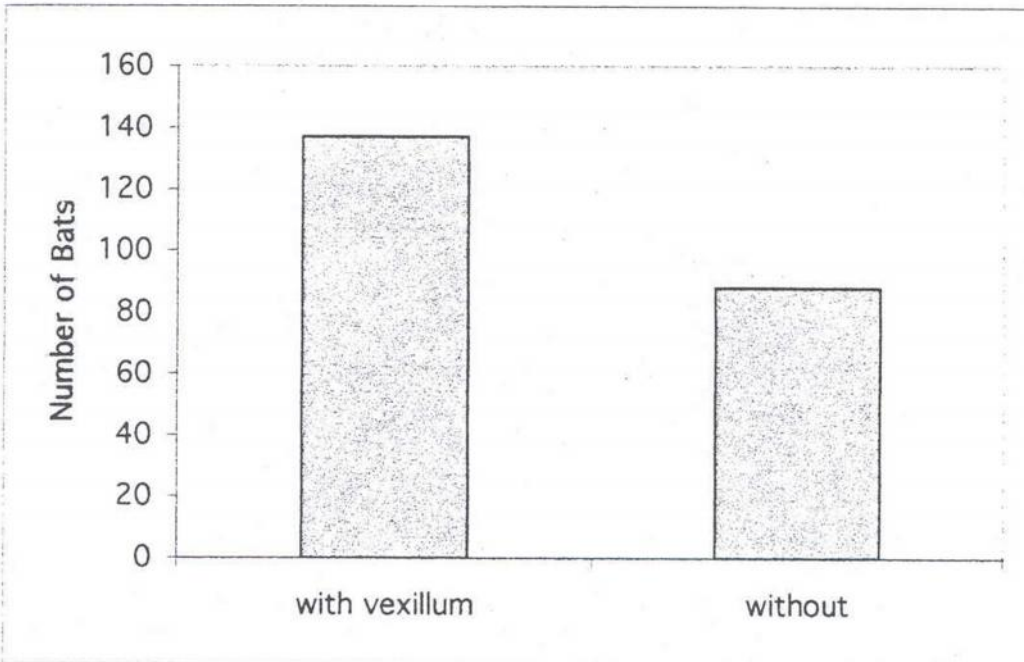


Figure 3. First hummingbird feeder result comparing feeders with and without actual vexillum. This test was done to determine whether or not bats would respond to vexillum without a flower. It clearly shows a difference between the holes with and without vexillum ($X^2 = 10.67, p < .005$).

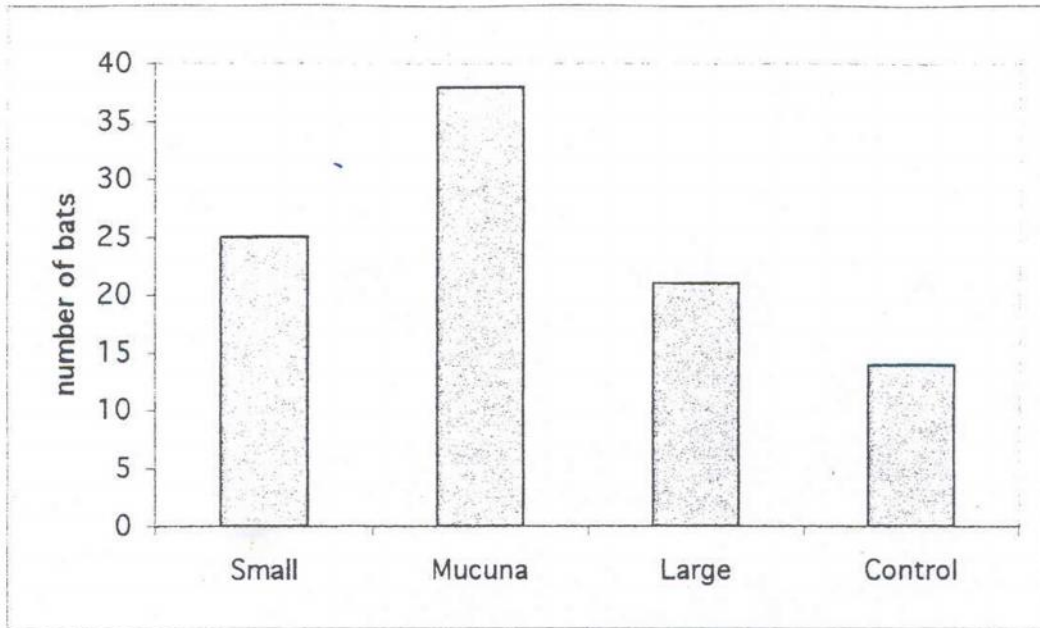


Figure 4. Hummingbird feeder results comparing a real *M.urens* vexillum to large and small paper vexillum. Rain was a damaging factor in the fragile paper vexillum, and low numbers in these categories reflect this. Still, all numbers are larger than control, showing that they still somewhat effective. ($X^2 = 12.93$, $p < .005$)

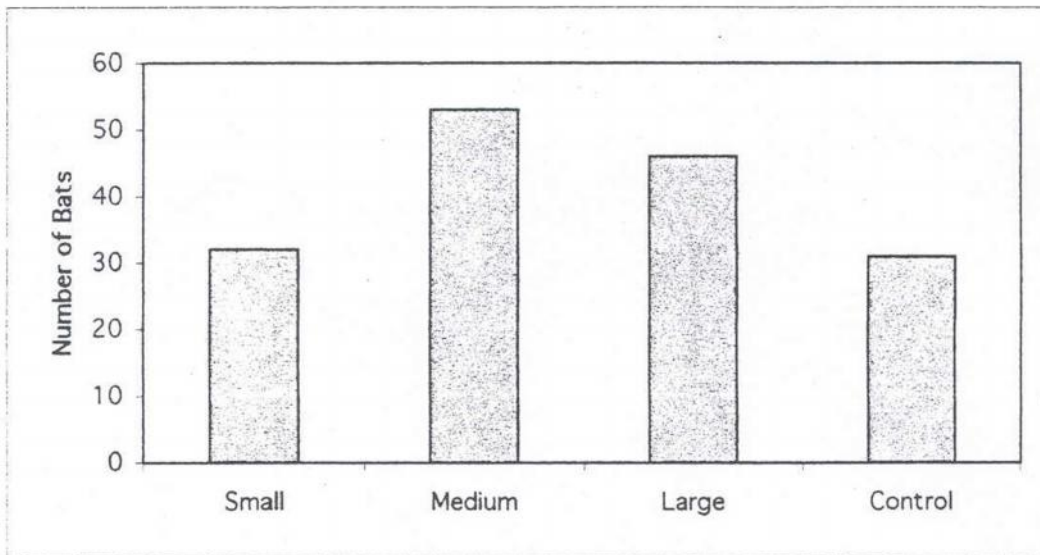


Figure 5. Observational study nearly identical to figure 4. The *M. urens* vexilla was replaced by a paper one of equal size. Stiffer paper was used but rain still negatively impacted the study, especially the large. Results were not significant. ($X^2 = 4.59$, critical value 7.85).

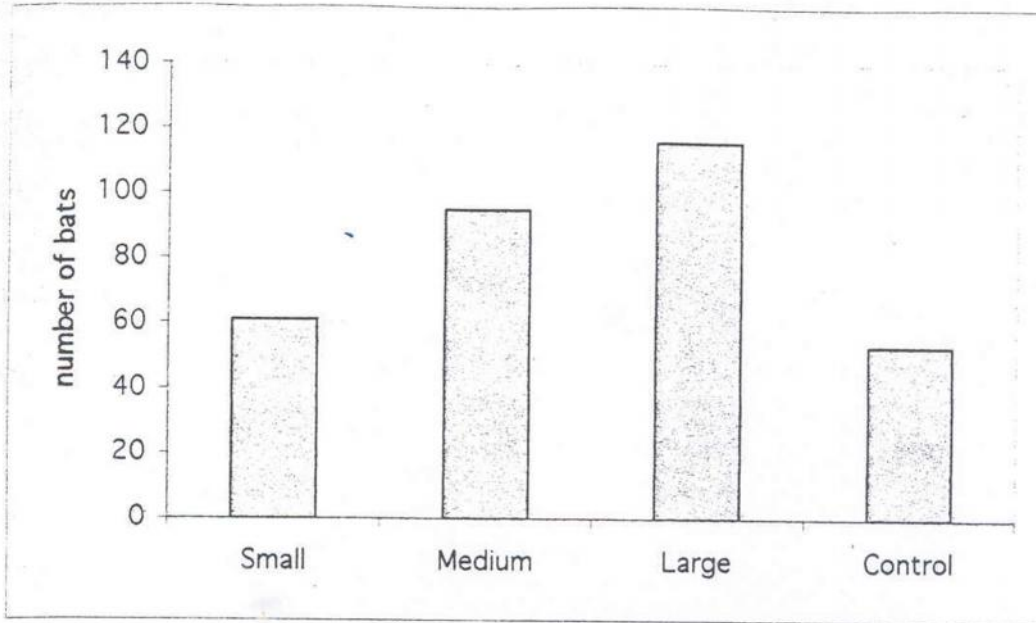


Figure 6. Final observational study. Waterproof vexilla were used to ensure that the previous impact from the rain on the paper vexilla were minimized. There is a clear progression toward larger vexillum. The difference between Medium and Small is significant ($X^2=13.76$, $p<.05$). The difference between medium and large, however, are not ($X^2=2.1$, $p>.05$)