Effects of Elevation on Pollinaria Removal in *Asclepias curassavica* (Asclepiadaceae)

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RESUMEN

En este proyecto se estudio la remoción de polen en tres grupos de plantas *Asclepias curassavica* (Asclepiadaceae) a diferentes elevaciones (815m, 1175m, 1440m). Además se tomo: número de floraciones, número de flores por floración, número de polen removido por flor, número de frutos, presencia de herbívoros, y presencia de pulgones. Los datos se colectaron por nueve días durante Octubre y Noviembre del 2000. Los resultados indican una diferencia significativa entre todas las elevaciones con respecto a número de polen removido por flor (ANOVA P-value < 0.0001; Fisher's PLSD alta y intermedia, intermedia y baja, alta y baja, todos P-values < 0.0001). La remoción de polen fue mas baja en la elevación baja, intermedia en la elevación intermedia y más alta en la elevación alta. Los resultados se pueden explicar por una presencia mayor de polinizadores en la elevación alta, específicamente la Monarca (Danaus plexippus). La elevación alta tuvo el mayor número de frutas por planta y más número de plantas con presencia de herbívoros. La elevación intermedia tuvo el mayor número de plantas con presencia de pulgones. El número de flores por planta fue mayor en la elevación intermedia y menor en la elevación alta. De cualquier modo, la elevación alta tuvo el mayor número de las flores por floración. La elevación es un factor importante que afecta la remoción de polen en las flores de A. curassavica.

ABSTRACT

Pollinaria removal was studied in three populations of *Asclepias curassavica* located on an elevational gradient in and around Monteverde, Costa Rica (815m, 1175m, 1440m). The following plant characteristics were also studied: number of inflorescences, number of flowers per inflorescence, number of pollinaria removed per flower, number of pods. Pollinaria removal in plants with the presence of herbivory was compared to pollinaria removal in plants without herbivory at all three elevations. Pollinaria removal and the presence of aphids were compared the same way as herbivory. Data were collected over a nine day period during the misty season (October – November). Results showed a significant difference in pollinaria removal between all three elevations (ANOVA P- value < 0.0001). Pollinaria removal was lowest at the low elevation site, intermediate at the middle elevation site and highest at the high elevation site. These results may be explained by an increase in pollinators, specifically the Monarch butterfly (*Danaus plexippus*), as elevation increases, or the density of the plants per site. The highest numbers of pods per plant were observed at the high elevation site, which may indicate those plants also have the highest fitness. Significant differences in number of pods per plant were seen between high and low, and low and middle elevations (ANOVA P- value < 0.0001; Fisher's PLSD both P-values < 0.0001). Neither the presence of herbivory nor presence of aphids had a significant effect on pollinaria removal. The number of flowers per inflorescence was highest at the high elevation site and lowest at the low elevation significantly affects pollinaria removal in *Asclepias curassavica*.

INTRODUCTION

Asclepias curassavica, commonly referred to as milkweed, is an herbaceous species found along roadsides and in clearings throughout the tropics. It flowers and fruits throughout the year (Croat 1978). Along with other members of the Asclepiadaceae family, *A. curassavica* contains alkaloids and cardenolides, heart poisons that are toxic to most vertebrate herbivores (Taiz & Zeiger 1991). Due to this characteristic, *A. curassavica* has developed a interesting relationship with a number herbivorous insects, a classic example of which involves the Monarch butterfly (*Danaus plexippus*). Monarchs lay a single egg under each of several leaves of *A. curassavica*. The larvae then feed on the leaves and accumulate the cardenolides in their bodies. Both the caterpillars and the adults are toxic to predators (Opler & Krizek 1984). If a predator such as a bird should eat a Monarch, severe gastric distress and vomiting will result (Raven et al. 1986). *Asclepias curassavica* benefits from this relationship because the adult Monarch butterflies serve as important pollinators.

The success of flowering plants is largely dependent on their relationship with pollinators. Cross-pollination provides plants with an evolutionary advantage by increasing variability within a species. Thus, plants can develop adaptations or modifications enabling them to maintain fertility and possibly increase their fitness (Heywood 1993). Pollination success can be inferred by measuring the amount of pollen removed in flowers. *Asclepias curassavica* is well suited for this type of study because its flowers contain pollen grains packed into structures called pollinaria. The pollinaria are visible to the naked eye and thus easily observed and counted (Wilson & Melampy 1983). Pollinaria consist of two pollinia containing the compacted pollen, and connected via a wishbone-shaped translator. The two arms of the translator, each referred to as a retinaculum, are joined by a structure called the corpusculum (Proctor et al. 1996). The corpusculum is a visible dark spot, on either side of which are cavities containing the pollinia (Wilson & Melampy 1983). Each flower of *A. curassavica* contains five pollinaria, located in five separate stigmatic chambers. Each chamber occurs between a cucullum (hood) of the corona and the adjacent anther (Zomlefer 1994). The gynostegium, encompassed by the stigmatic chambers, consists of five stamens surrounding an enlarged stigmatic head. Nectar is produced by secretory cells located in the lining of the stigmatic chambers (Somlefer 1994). The nectar accumulates in pouches formed by the cuculli of the corona (Proctor et al. 1996).

The brightly colored flowers (reddish – orange corolla and yellow gynostegium) attract a variety of pollinators. The main pollinators of *A. curassavica* are butterflies; however, beetles, bees, wasps and flies also frequent the flowers (Wilson & Melampy 1983). When an insect visits a flower, its legs slide down the slippery stigma apex to the space between the anthers. The translator is snagged by the spurs or hairs on the insect's legs, and the entire pollinaria is dislodged when the insect departs. When the insect visits another flower and its ladened leg slips into a stigmatic chamber, the pollinaria can be deposited. However, successful cross-pollination will only occur if the more convex side of the pollinaria is properly inserted into the chamber (Zomlefer 1994). A wind dispersed pod develops following pollination, and multiple pods can, but not always, develop on a single plant (Wilson & Melampy 1983).

Many studies have been done on pollinaria removal in *A. curassavica* flowers. A previous study in Monteverde found a higher rate of pollinaria removal in plants from lower elevations as compared to those at higher elevations (Wilson & Melampy 1983). In addition to elevation, the presence of herbivory and aphids may also affect pollinaria removal. The purpose of this study is to determine the effect of elevation on pollinaria removal in *A. curassavica*. In addition, the effects of elevation on other factors relating to pollinaria removal are also discussed.

METHODS

This study was conducted over a nine-day period, from October 25 to November 2, 2000. The three study sites in and around Monteverde, Costa Rica, were selected on an elevational gradient. The low elevation site (815m) was located in San Luis Abajo, the middle elevation site (1175m) was located in San Luis Arriba, and the high elevation site (1440m) was located in the community of Monteverde. At each site 40 *Asclepias curassavica* plants were tagged and the following characteristics recorded: number of inflorescences, number of flowers per inflorescence, number of pollinaria removed per flower, and number of pods. Also, the presence or absence of herbivory and aphids were recorded for each plant. Over the length of the study, initial budding and death of flowers were also recorded. Because the flowers on each inflorescence do not bloom simultaneously, data were collected for every flower independently. In order to monitor every flower over the length of the study, each inflorescence was drawn and each flower assigned a letter. The plant tags were oriented so that inflorescences were viewed from the same perspective each time the site was visited, to reduce the probability of confusing flowers. The study sites were visited on a rotational basis over the nine day period, so that each site was visited four times. The average length of time between visitations for a given site was two days. Over the nine day study, inflorescences often died, in which case a second would almost always bloom from the same plant. When this happened, data for the second inflorescence were also recorded.

All data were analyzed using ANOVA and Fisher’s PLSD post-hoc tests. The following factors were each compared to elevation: pollinaria removal per flower, number of pods, number of flowers per inflorescence, and number of flowers per plant. For each elevation, the number of pollinaria removed from plants on which aphids were present was compared to the number of pollinaria removed from plants without aphids. Finally, the number of pollinaria removed from plants on which herbivory was present was compared to the number of pollinaria removed from plants without herbivory for each elevation.

RESULTS

Data were recorded for 40 plants from each site and a total of 1087 flowers (low N= 395, 73 inflorescences; middle N = 356, 78 inflorescences; high N = 336, 57 inflorescences). There was a significant difference between all elevations regarding pollinaria removal per flower (ANOVA P-value < 0.0001; Fig, 1). Results showed a significant difference between high and middle, high and low, and middle and low elevations (Fisher's PLSD all P-values < 0.0001).

There was a significant difference between the number of pods per plant and elevation (ANOVA P-value < 0.0001; Fig. 2). The low elevation was significantly different from both the middle and high elevations (Fisher's PLSD both P-values < 0.0001), and there was not a significant difference between the high and middle elevations (Fisher's PLSD P- value = 0.90).

Twenty-seven of the 40 plants in the high elevation showed the presence of some herbivory. Herbivory was present on 11 plants in the middle elevation and only one plant in the low elevation. There was not a significant difference regarding pollinaria removal between plants with herbivory and plants without herbivory (ANOVA P-value = 0.2232; Fisher's PLSD P-value = 0.6951; Fig. 3).

Aphids were present on 12 plants at the middle elevation, two plants at the high elevation, and four plants at the low elevation. There was not a significant difference in pollinaria removal between plants with aphids and plants without aphids (ANOVA P-value = 0.1966; Fisher's PLSD P = 0.7405; Fig. 4).

Elevation did have a significant affect on the number of flowers per plant (ANOVA P-value = 0.0041; Fig. 5). The number of flowers was highest in the middle elevation, followed by the low elevation, and then the high elevation. There was not a significant difference between numbers of flowers per plant in the high and low elevations (Fisher's PLSD P-value = 0.79). However, there were significant differences between both high and middle elevations, and middle and low elevations (Fisher's PLSD P-value = 0.0027, P-value = .0060).

Elevation also had a significant effect on the number of flowers per inflorescence (ANOVA P-value = 0.0084, Fig. 6). Again, there was no significant difference between high and middle elevations (Fisher's PLSD P-value = 0.74). Significant differences in number of flowers per inflorescence were seen between high and low, and middle and low elevations (Fisher's PLSD P-value = 0.0066, P-value = 0.0093).

DISCUSSION

The results of this study show that elevation has an effect on pollinaria removal in Asclepias curassavica (Fig. 1). A possible explanation for these results may be the locations of the plants in each site. Plants in the low elevation were located in a pasture. Plants in this site also occurred at the lowest density, meaning the most area had to be covered to obtain 40 plants. Plants in the middle elevation were located by a gravel road and occurred at an intermediate density compared with the other sites. Plants in the high elevation were located in a small dense patch next to a lake. The high density of plants in an area, also known as clumping, has been suggested to affect pollinaria removal (Hayon 1996). The flowers of plants found close together could be expected to receive a high number of visitations by pollinators because many flowers would be available in a small area, and the pollinators would not have far to go to reach the next flower. The flowers of plants that are less clumped could be expected to have a lower rate of visitation by pollinators because flowers would be more spread out. Clumping of plants in this study did increase from low to middle to high elevation, and although not test was done, increasing density of plants may be related to increasing pollinaria removal. A previous study (Hayon 1996) found there to be no significant difference in pollinaria removal in *A. curassavica* between single plants and plants occurring in clumped patches; however, these results may not accurately reflect trends on a larger scale due to the small sample size used in the study (N=45). Clumping affects on pollinaria removal is something to be researched in further studies.

The biology of the Monarch, a major pollinator of A. curassavica, may also help explain the results of this study. Monarchs are common in all open areas on both slopes of Costa Rica, and are especially abundant at and above 1500m. Also, unlike their migratory North American relatives, Central American Monarchs are thought to remain sedentary (De Vries 1987). The high elevation site, at 1440m, was the closest site to the preferred elevation of Monarchs. Therefore, there may have been a greater abundance of these pollinators at that site. Monarchs also live in groups, and the high density of the plants at the high elevation would provide the pollinators with many easily accessible plants. Since Monarch populations are also sedentary, flowers of A. curassavica would have a continual supply of pollinators. A previous study in Monteverde, Costa Rica also found that butterfly abundance increased with elevation (Araujo 1999). Therefore, the results of the present study, which report an increase in pollinaria removal with elevation, may be due to an increase in butterfly abundance.

Although the presence of aphids varied with elevation, it did not significantly affect pollinaria removal in *A. curassavica*. Only in the middle elevation did plants without aphids have a higher number of pollinaria removals. In both the high and low elevations, plants with aphids had more pollinaria removed than those without aphids (Fig. 4). Therefore, it cannot be concluded that presence of aphids reduces pollinaria removal in the flowers.

The location of the sites may explain the differences in presence of herbivory between sites. Herbivory was present on the most plants at the high elevation. The high density at which these flowers occurred may account for these results. Because these plants were located in a dense patch, herbivores, just like pollinators, would have access to many plants in a small area at one time. The lowest occurrence of plant herbivory occurred in the middle elevation. These plants were located along a gravel road and occurred among many other weedy species so that *A. curassavica* was not the most common or most densely occurring plant. Because herbivory was dispersed over many different, but closely occurring species, the frequency of herbivory per plant would be reduced.

The low number of pods in the low elevation may be a reflection of the low pollinaria removal in that site. Low pollinaria removal would result in low rates of pollination, thus producing few pods. However, the high and middle elevations showed almost the same number of pods, so factors other than elevation, perhaps random ones, are affecting pod development. A low number of pods may result in reduced fitness for the plant because it will have reduced dispersal ability. The more seeds a plant can disperse the greater chance it will have for successful establishment in other, perhaps more desirable areas. A plant having several pods can produce more offspring than a plant with one or no pods.

The number of flowers per plant and number of flowers per inflorescence (Fig. 5 & Fig. 6) did vary between elevations, but did not show the same trend as pollinaria removal per flower. These results can be explained by random variations. However, previous studies have suggested that inflorescence size (number of flowers per inflorescence) affects the number of pollinaria removed per flower (Wolfe 1987).

The results of the present study are not consistent with those of a previous study, that found pollinaria removal per flower decreased with increasing altitude (Wilson & Melampy 1983). The previous study, conducted in Monteverde during the start of the dry season (January – February), reports an average of 1.72 (N=352) pollinaria removed per flower in San Luis (900m), as compared with results from the present study, which found an average of 0.54 (N=395) pollinaria removed per flower. A difference in the time of year in which the studies were conducted may help explain the contrasting results. Wilson & Melampy suggest that climate differences between study sites may have been a major factor affecting pollinaria removal. During the transition and dry seasons, the San Luis Valley experiences little wind-driven precipitation and minimal cloud immersion (Clark et al. 2000). In the previous study, the middle and high elevations were subjected to more mist and lower temperatures than the low elevation, which was considerably warmer and drier (Wilson & Melampy 1983). The drier climate of the low elevation may have provided more favorable conditions for pollinators and less favorable conditions for potential herbivores. The present study was conducted during the misty season (October – November), and dramatic climate differences between sites were not noted.

Further studies are needed to help determine whether differences between this study and the previous one are merely a result of seasonal differences or if other factors are involved.

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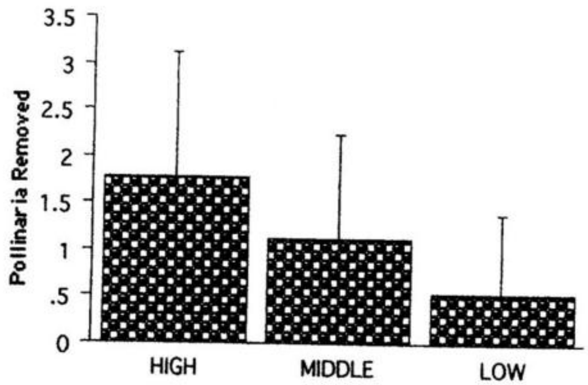

FIGURE 1. The number of pollinaria removed per flower of *Asclepias curassavica* at three elevations in Costa Rica (1440 m, 1175 m, 815 m). The bars represent standard deviations.

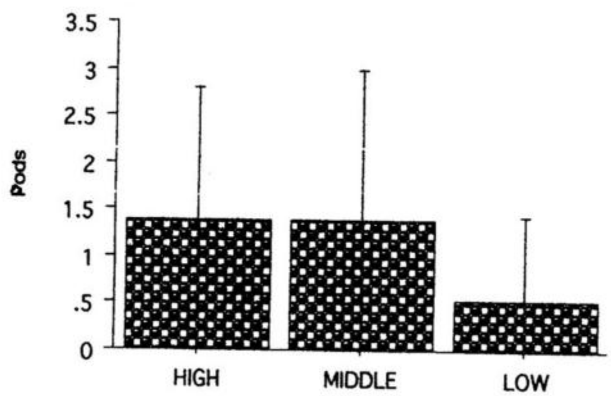

FIGURE 2. The number of pods per plant of *Asclepias curassavica*.at three elevations in Costa Rica (1440 m, 1175 m, 815 m). The bars represent standard deviations.

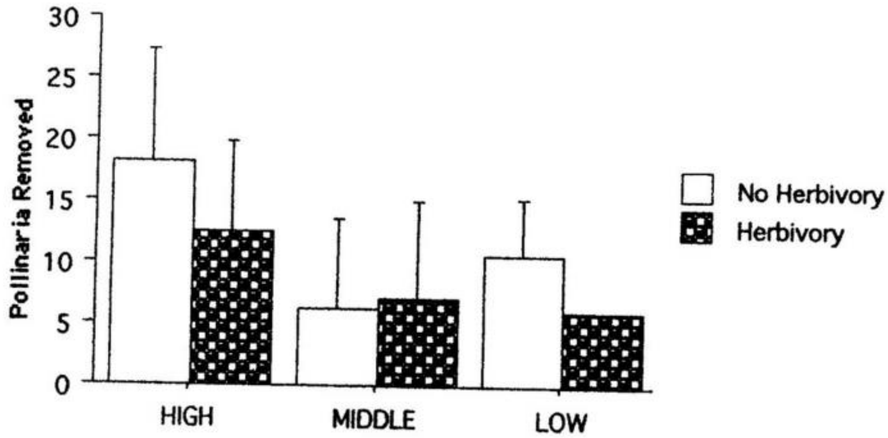

FIGURE 3. The number of pollinaria removed per plant of *Asclepias curassavica* for those with and without herbivory at three elevations in Costa Rica (1440 m, 1175 m, 815 m). The bars represent standard deviations.

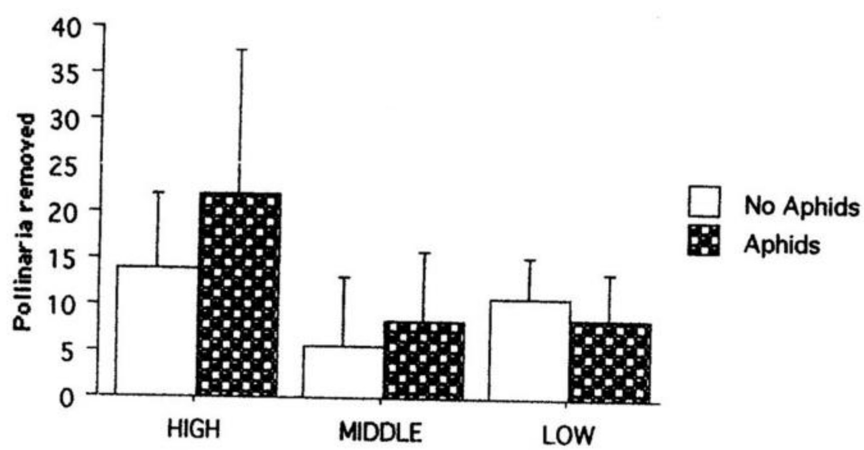

FIGURE 4. The number of pollinaria removed per plant of *Asclepias curassavica* for those with and without aphids at three elevations in Costa Rica (1440 m, 1175 m, 815 m). The bars represent standard deviations.

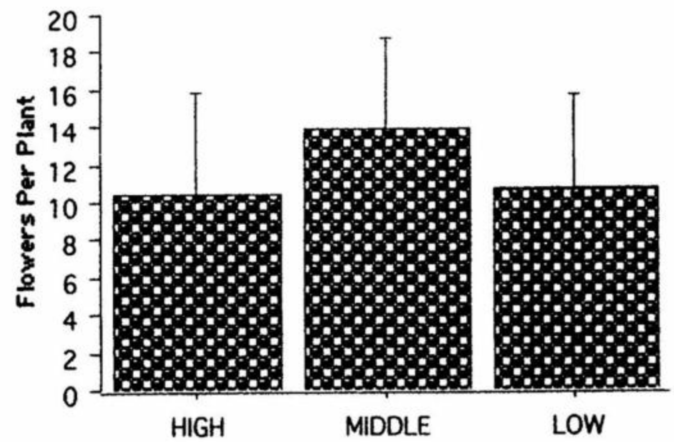

FIGURE 5. The number of flowers per plant of *Asclepias curassavica* at three elevations in Costa Rica (1440 m, 1175 m, 815 m). The bars represent standard deviations.

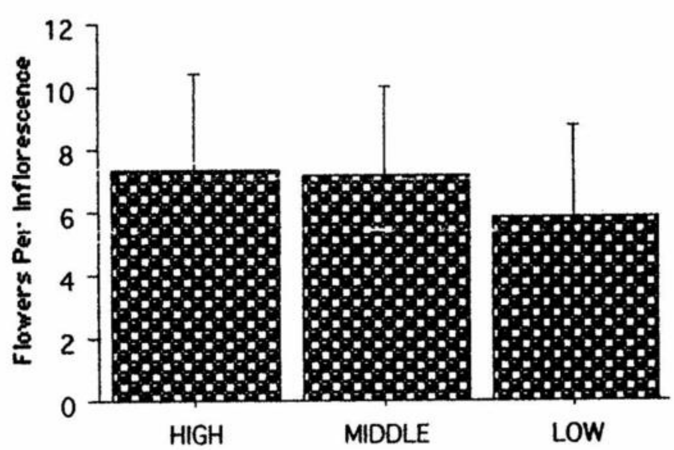

FIGURE 6. The number of flowers per inflorescence of *Asclepias curassavica* at three elevations in and around Monteverde, Costa Rica (1440 m, 1175 m, 815 m). The bars represent standard deviations.