Rev. Chilena Ent.
2000, *26*: 63 - 68

POLLINATION AND FRUIT PRODUCTION OF TWO SPECIES OF *ALSTROEMERIA* (AMARYLLIDACEAE) IN PURE AND MIXED PLANT PATCHES[1]

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ABSTRACT

When a pollinator visits flowers of two or more species during a single foraging bout it may loose the pollen grains of one species while visiting another. However, some pollinators will visit the same plant species in a mixed plant patch, therefore increasing the probability of fruit and seed set. In the present study visit rates by insect pollinators to plants of *Alstroemeria ligtu* L. and *A. angustifolia* Herb. growing together or in mixed patches were evaluated. Overall fruit and seed production and mortality was determined in pure and mixed patches. Insect visits to both species of *Alstroemeria* were significantly fewer in mixed patches. When both species grew together individuals of *A. angustifolia* were more visited than individuals of *A. ligtu.* Seed and fruit production were approximately the same in mixed and pure patches, but the number of non-developed seeds was significantly higher for *A. angustifolia* when it grew in pure patches.
Key words: *Alstroemeria ligtu*, *Alstroemeria angustifolia*, pollination, mixed patches.

RESUMEN

Cuando un polinizador visita flores de dos o más especies durante una visita, puede perder granos de polen de la primera especie cuando está visitando una segunda. Por otro lado, cuando los polinizadores visitan la misma especie de planta en un parche mixto, la probabilidad de generar frutos y semillas aumenta. En el presente estudio, se evaluó la tasa de visita por polinizadores a plantas de *Alstroemeria ligtu* L. y *Alstroemeria angustifolia* Herb. que crecían en parches puros o mixtos, y también la producción de frutos y de semillas, y la mortalidad de estas últimas. Cuando ambas especies crecían juntas, los individuos de *A. angustifolia* fueron más visitados que los de *A. ligtu*. La producción de frutos y semillas fue aproximadamente igual en parches puros y mixtos, pero el número de semillas que no se desarrollaron fue significativamente mayor en *A. angustifolia* cuando crecía en parches puros.
Palabras claves: *Alstroemeria ligtu*, *Alstroemeria angustifolia*, polinización, parches mixtos.

INTRODUCTION

A pollinator that visits flowers of two or more plant species during a single foraging bout may lose some pollen grains of one species while visiting another (Murcia & Feinsinger, 1996). This "interspecific pollen transfer" may reduce the reproductive success of the plants involved (e.g. Waser, 1978; Feinsinger, 1987; Feinsinger *et al.*, 1991). Two aspects of pollinator behavior which may affect pollen dispersal: are directionality of movement and the ability of some insects to discriminate between different flower colors or flower forms in a mixed population (Proctor *et al.*, 1996).

Pollinating animals sometimes restrict their visits to flowers of a single species or morph within a species, even when rewarding alternative flowers must be bypassed in the process (Waser, 1986). This

[1] This work was produced during the International Field Course on Insect-Plant Interactions held at Reserva Nacional Río Clarillo and Laboratorio de Química Ecológica, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, from November 28 to December 14, 1999. The results presented are based on 6 days of field work.

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(Recibido: 3 de diciembre de 1999. Aceptado: 5 de junio de 2000)

tendency has been referred to as *flower constancy* (Heinrich, 1976, 1979), and there is good evidence for constancy based solely on color (Waser, 1986). However constancy can also be determined by other factors, such as travel costs, flower density, morphology and the energetic rewards of the flowers (Brown & Clegg, 1984).

Coexisting flower species often differ in morphology, odor, color, flowering time, nectar and pollen rewards. These patterns have been interpreted as adaptations to reduce competition for pollinators by promoting specific plant-pollinator associations (Heinrich, 1976; Waser, 1978; Brown & Kodric-Brown, 1979). However there are some exceptions to this general pattern, where plants of similar characteristics grow and flower together (e.g. Grant & Grant 1968), presumably leading to increased pollinator competition and reduced pollinator effectiveness.

This paper examines the pollinators visit rate and behavior, as well as fruit and seed production and mortality in two species of *Alstroemeria* coexisting together and separately in patches within the Central Chilean Matorral.

METHODS

Alstroemeria ligtu L. and *A. angustifolia* Herb. are frequently seen growing in the Río Clarillo National Reserve (33°51' S, 70°29' W, 45 km southeast of Santiago, Chile) either forming almost pure monocultures or together in mixed patches. Flowering shoots range from 20 to 60 cm in height, each producing a terminal umbelliform inflorescence of 3 – 15 flowers (Hoffmann, 1998). Both species present androdiocy, having male and hermaphrodite flowers in the same inflorescences. The two species can be recognized mainly by flower color, *A. ligtu* being reddish and *A. angustifolia* pink.

Twenty-four flowering patches of approximately 16 m^2 each were chosen along equatorial facing slopes at an altitude of between 850 and 900 m. These sites were divided into three sets of eight patches each. Each set was categorized as either a pure patch of *A. ligtu* (red patches), a pure patch of *A. angustifolia* (pink patches), or as mixed patches with both species growing together. There were no apparent differences among patches in light conditions (flowering patches were under full light); however, in some sites patch size had to be adjusted prior to the experiment, so that all sites would have approximately the same size.

Four different floral traits in both species of *Alstroemeria* were measured in order to assess flower morphology: 1) total length (measured as the length from the base of the stigma to the tip of the flower), 2) stigma length, 3) male flower anther length and 4) hermaphrodite flower anther length. These measurements were then compared between species with a t-test for two independent samples.

The foraging hours of flower visitors for both species of *Alstroemeria* were determined with one-day observations in red and pink patches. The total number of visits per insect species was quantified every hour from 9:00 a.m. to 6:30 p.m. The visits were then plotted in relation to time of day and the activity of the flower visitors was obtained. To assess the way in which the flower resource was used by different insect species in red and pink patches, a Kolmogorov-Smirnov test for two samples was performed. This was repeated for all main visitors.

The insects found foraging at *Alstroemeria* flowers were collected and identified. However, in all the analyses only the most frequent species of visitors were considered, since others were observed only seldom during the study.

The number of visits and the number of flowers visited per 20-minute periods were observed in pure and mixed patches. During this period the time spent per visitor at each flower and the total time spent at the patch were also registered. To evaluate differences among these variables between pure and mixed patches, Mann-Whitney tests were performed. To see if the mean number of visits differed significantly in each type of patch, a Kruskall-Wallis test was used, and multiple comparisons between treatments to see which groups were different. In mixed patches, the number of pink and red flowers visited by an insect during a foraging bout was observed, and also the movements between species were recorded.

The number of inflorescences that had at least one fruit was counted and this number was divided by the total number of inflorescences in the patch. In mixed patches each species was assessed separately, considering the total number of inflorescences per species. Differences in a species between patches were tested with a Mann-Whitney test.

TABLE 1
FLOWER BIOMETRY (MEASUREMENTS IN CENTIMETERS).

	Mean anther length in male flowers	Mean anther length in hermaphrodite flowers	Mean stigma length	Mean total length
A. ligtu	7.08	3.00	36.5♣	41.4♣
A. angustifolia	6.73	3.13	23.7♣	27.2♣

♣ Statistical difference, $P< 0,05$.

To evaluate the proportion of fruits per inflorescence in each patch, the total number of fruits in each plant of *Alstroemeria* were counted and divided by the total number of flowers and flower buds in each inflorescence. This procedure was repeated for all inflorescences in a patch for all 24 patches. Differences between patches were tested with Mann-Whitney tests.

The mortality of fruits per patch was estimated by dividing the number of aborted fruits over the total number of fruits produced in a patch. Mann-Whitney tests were performed to detect differences between patches.

To determine if seed mortality was different within a species in different patches, the proportion of aborted and non-fertilized seeds per fruit per patch was calculated. Both of these parameters were categorized as non-developed seeds, and the values obtained were divided by the total number of developed seeds per fruit.

RESULTS

The biometry of *A. ligtu* and *A. angustifolia* flowers is shown in Table 1. No differences were found in the mean length of the two types of anthers between both species, suggesting that the pollen offer - in terms of quantity - is approximately the same. However, stigma length ($t = 7.26$, d.f.= 28, $P< 0.009$) and total length ($t= 13.86$, d.f.= 48, $P< 0.009$) were greater in *A. ligtu* than in *A. angustifolia*.

Seven morphospecies of flower visitors were identified which were present in both *Alstroemeria* species. They were (in order of abundance): white bumblebee, black bumblebee, black Nemestrinidae, striped Nemestrinidae, yellow Nemestrinidae, Scarabidae, and bee fly.

The activity peaks of the flower visitors in *A. ligtu* and *A. angustifolia* are illustrated in figures 1A and 1B, respectively. Among the species of flower visitors, only the white bumblebee (D_{obs} 0.46 > $Da_{0.05}$ 0.24) and the black bumblebee (D_{obs} 0.51 > $Da_{0.05}$ 0.32) differed in the resource exploitation behavior between *A. ligtu* and *A. angustifolia*. All other species used the resource similarly in pink and red patches.

Insect visits to both species of *Alstroemeria* were higher in pure patches than in mixed patches. Flowers of *A. ligtu* were more visited in pure patches (Me= 8.5, range= 21, n= 8) than in mixed patches (Me= 1.0, range= 2, n= 8) ($U= 12.5$, $P= 0.03$). The same pattern was observed for *A. angustifolia* ($U= 10.0$, $P= 0.02$), the number of visits in pure patches (Me= 7.5, range= 19, n= 8) being higher than in mixed ones (Me= 2.5, range= 6, n= 8). Additionally, in mixed patches, flowers of *A. angustifolia* (Me= 2.5, range= 6 n= 8) were more visited than those of *A. ligtu* (Me= 1.0, range= 2, n= 8) ($U= 13.0$, $P= 0.04$). No evidence was found for differences in the time spent per visitor at each flower ($U= 26.5$, $P= 0.56$), nor in the total number of flowers visited in the patch ($U= 26.5$, $P= 0.5$). Finally, the average number of visits in red, pink and mixed patches were different from each other ($H= 9.46$, d.f.= 2, $P< 0.009$); however, only the number of visits between pink and mixed patches were statistically different ($|R_1-R_2| = 11.16 > Z\alpha_{0,05}$ 9.72), the number of visits in mixed patches being lower than in pink patches.

All insects visiting the mixed patches were constant with flower type during each foraging bout. If a pollinator chose flowers of *A. ligtu*, it would only forage the flowers of that species during the visit; the same happened in case it chose flowers of *A. angustifolia*. Only on one occasion during the study a pollinator was observed to change from visiting pink flowers to visiting red ones.

The average proportion of inflorescences of *A. ligtu* ($U= 29.0$, $P=0.75$) and *A. angustifolia* ($U=28.0$, $P= 0.88$) with fruit per patch did not differ

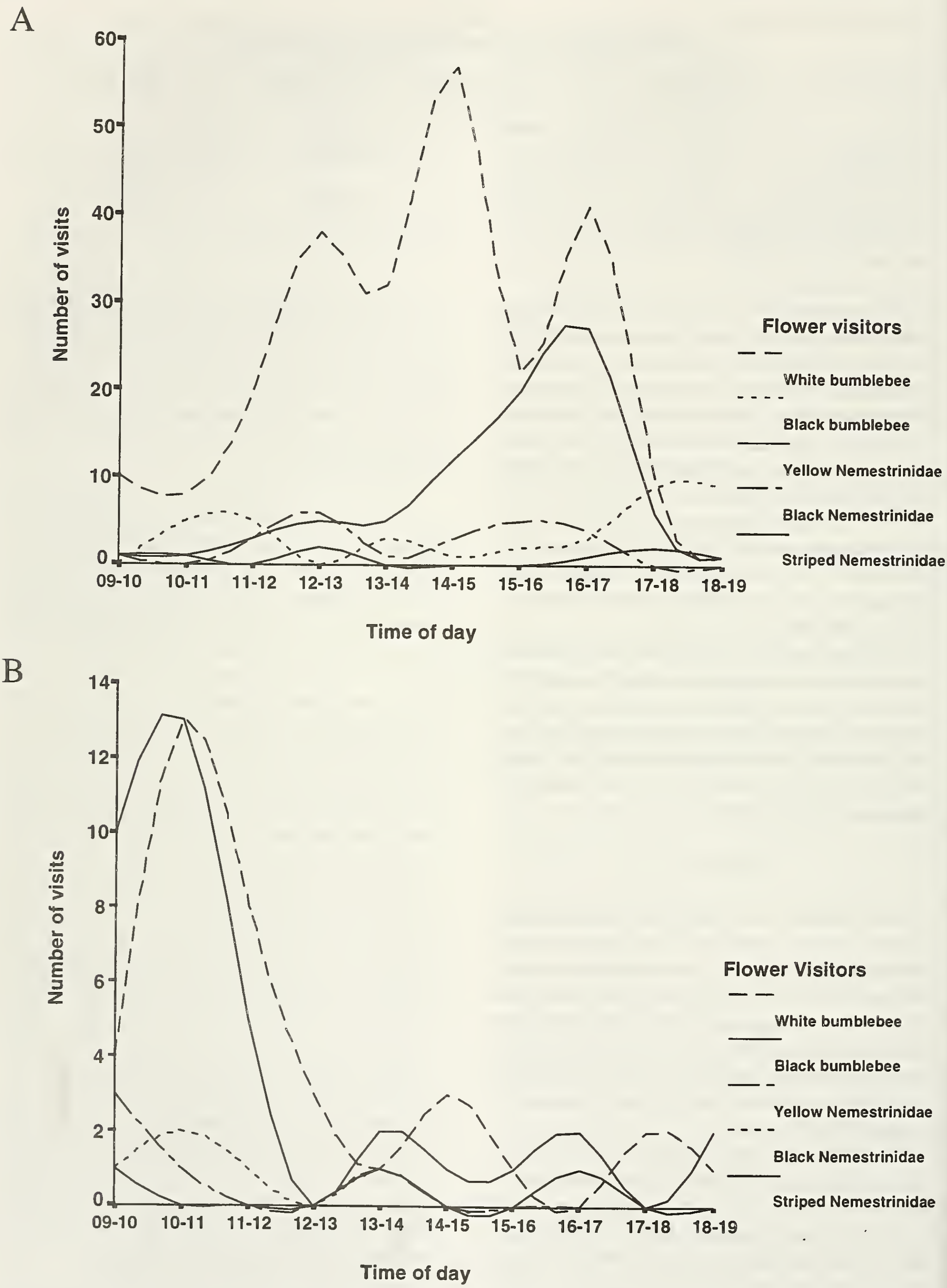

Figure 1. Activity peaks of the main flowers visitors of *Alstroemeria ligtu* (A) and *Alstroemeria angustifolia* (B). Numbers are based on one-day observations.

with patch type. Both species of *Alstroemeria* in pure and mixed patches presented approximately the same proportion of fruits per inflorescence. There were no evidences of a patch effect either on *A. ligtu* (U=27, *P*=0.59) or on *A. angustifolia* (U=22.5, *P*=0.32). The average proportion of aborted fruits did not differ in *A. ligtu* (U=58, *P*=0.33) or *A. angustifolia* (U=2.48, *P*=0.09) between mixed and red patches. The average proportion of non-developed seeds per fruit of *A. ligtu* in each patch did not differ with patch type (U=24.0, *P*=0.37). However, there was a patch effect on seed of *A. angustifolia* (U=27, *P*= 0.03), indicating that there were, on average, less non-developed seeds per fruit in mixed patches (Me= 0.33, range= 0.32, n= 8) than in pure ones (Me= 0.45, range= 0.18, n= 8).

DISCUSSION

In this study, pollination and fruit production were examined in two species of *Alstroemeria* when they grew in pure or mixed patches. The results suggest that the mean number of visits is related to the patch type - flowers had a greater chance of being visited when they grew in pure patches, than when they grew in mixed patches. Also, when both species are found together, individuals of *A. angustifolia* are more visited than those of *A. ligtu*. Seed mortality also seemed related to patch type, at least for *A. angustifolia* since there was evidence that more developed seeds were present in mixed patches. However, no differences were found between pure and mixed patches in pollinator behavior, or fruit and seed production.

The fact that pure patches were more visited than mixed ones could be explained on the basis that the spatial distribution of resources is an important determinant of searching behavior and searching success (Bell, 1991) and eventually visitation rates. Pure patches of *A. ligtu* and *A. angustifolia* may be considered more "apparent" – and therefore easier to locate - to insects than mixed patches. Plants in monoculture patches in the study area were usually more aggregated than plants in mixed patches - where the presence of an additional species *per se* reduced the aggregation of the first species- so it is reasonable to predict that a group of more aggregated plants (more evident) will have a greater chance of being found, and therefore visited, by an insect.

Within a mixed patch flowers of *A. angustifolia* were more visited than flowers of *A. ligtu*. A possible reason for this is that floral rewards, such as nectar, are more accessible in *A. angustifolia* than in *A. ligtu*. The mean length from the base of the stigma -presumably where the nectaries are– to the tip of the flower in *A. angustifolia* was significantly smaller than in *A. ligtu*. Presumably, a shorter distance to the nectaries will increase the spectrum of pollinators with access to them and consequently the chances a flower has of being visited. Alternatively, both species could be offering differing quantities or quality of the resource, and hence the visits would simply reflect resource availability. Although nectar production was not measured, based on the same mean length of anthers for both species, it may be inferred that the pollen reward should be the same for both species.

During a single foraging bout the pollinators always visited the same plant species. This indicates that, at least during each visit, pollinators presented high flower constancy. This constancy could have been due to fixed preferences for a flower species a pollinator may have, or to the choice by pollinators of flowers partly on the basis of their energetic rewards. It has been suggested that if a pollinator encounters a highly energetic reward within the flowers of a given plant species, it will continue visiting those flowers versus visiting new ones. This can go on until the reward received from the current flower is less than a certain threshold, in which case the pollinator leaves in search of new flowers (Pyke, 1979; Hodges, 1985a, 1985b). However, these hypotheses remain to be tested in the present study system.

Fewer visits to mixed patches could eventually lead to fewer fruits produced per plant in a particular patch or fewer seeds produced per fruit in a plant. Although differences were found in the visitation rates between mixed and pure patches, no differences were observed in the proportion of either of the variables mentioned above. This suggests that a greater occurrence of pollinator visits does not necessarily indicate an increase in pollinator effectiveness. However it is important to stress at this point that the fruit production and seed set observed are the consequences of past events. Since some of the patches in this study were experimentally modified, the visitation rates observed cannot be directly related to the pollination events that took place before it.

The presence of non-developed seeds was higher in pure patches than in mixed patches for *A. angustifolia*. No differences were observed for *A. ligtu*. Again this result stresses the fact that more visits does not imply a "better" seed set, measured as the proportion of developed seeds per fruit. On the contrary, it suggests that patches more visited by insects are also the ones that have fewer developed seeds. It may be speculated that this could be related with the optimal foraging distance. If an insect forages within a patch where plants are close together, pollination effectiveness will be reduced because of the degree of spatial autocorrelation between plants. Plants that are neighboring are likely to be similar than plants that are far away from each other. If there are too many visits within a small patch, the mixing and recombination of genes could cause recessive features to express themselves, some of which could make the seeds non-viable and cause abortion within the fruit. However, this hypothesis needs further study.

ACKNOWLEDGMENTS

Many people contributed to this study. We would like to particularly thank Hermann M. Niemeyer, Christer Björkman, Mattias Jonsson, Richard J. Hopkins, Cecilia Smith and Claudio C. Ramírez, for their comments, help and most of all support. We would also like to say a very big thank you to all our classmates in the International Field Course on Insect-Plant interactions; no doubt their help, support and good mood was essential in finishing this manuscript. We gratefully acknowledge the financial support of MISTRA/IFS for our participation in the course, and the help and advice received from staff at CONAF.

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