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POLLINATION ECOLOGY OF *AGAVE MACROACANTHA* (AGAVACEAE) IN A MEXICAN TROPICAL DESERT. I. FLORAL BIOLOGY AND POLLINATION MECHANISMS[1]

SANTIAGO ARIZAGA,[2] EXEQUIEL EZCURRA,[3] EDWARD PETERS,[2]
FERNANDO RAMÍREZ DE ARELLANO,[2] AND ERNESTO VEGA[2]

[2]Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 27–3 (Xangari), 58190–Morelia, Michoacán, Mexico; and
[3]Biodiversity Research Center of the Californias, San Diego Natural History Museum, P.O. Box 1390, San Diego, California 92112 USA

In a study of sexual reproduction in long-lived semelparous plants, we observed *Agave macroacantha* in the tropical desert of Tehuacán-Cuicatlán, Mexico, describing duration of flowering, flower phenology, and nectar production patterns. We also performed two manipulative experiments evaluating (a) the seed production efficiency of different crossing systems (selfing, cross-pollination, apomixis, and control), and (b) the effect of different pollinators (diurnal exposure to pollinators, nocturnal exposure, exclusion, and control) on the seeds produced. Flowering occurred from early May to late July and had a mean duration of 29 days in the individual rosettes. The flowers were protandrous; anthesis occurred in the afternoon of the third day after floral opening, and the pistils matured in the afternoon of the fifth day. The stigmas remained receptive from dusk to the following morning. Pollination was mostly allogamous. Nectar was produced principally during the night, from the first stages of floral aperture until the stigmas wilted and flowering ceased. The flowers were visited during the day by hymenoptera, butterflies, and hummingbirds and during the night by bats and moths. Only the nocturnal visitors, however, were successful pollinators. *Agave macroacantha* is extremely dependent on nocturnal pollinators for its reproductive success.

Key words: *Agave macroacantha*; Agavaceae; nectar; nectar-feeding bats; pollination biology; rosette plants; tropical deserts.

Species within the genus *Agave* are monocarpic, i.e., the individual rosettes have only one reproductive event, which leads to their death (Gómez-Pompa, 1963; Gentry, 1982). When flowering starts, agaves develop a large terminal inflorescence or flowering stalk (known botanically as a ''scape,'' and called *quiote* in Mexico), as a result of the rapid elongation of the apical meristem after years of vegetative growth of the basal rosette. The flowers produce abundant nectar with which they attract their natural pollinators, which are hummingbirds, bats, or insects, according to the different *Agave* species (Gentry, 1972, 1982; Schaffer and Schaffer, 1977; Freeman and Reid, 1985; Martínez del Río and Eguiarte, 1987; Eguiarte and Búrquez, 1987; Slauson, 1994).

The floral biology of agaves is of interest from the point of view of ecological theory, as the rosettes are both long lived and semelparous, and it has received substantial attention during the last decades (e.g., Schaffer and Schaffer, 1977; Howell and Hart, 1980; Howell and Roth, 1981; Eguiate, 1983; Freeman and Reid, 1985; Eguiate and Búrquez, 1987; Slauson, 1994). One of the main demographic risks in the life history of long-lived semelparous plants is the threat of reproductive failure induced by random environmental events, which can reduce the fitness of the individual rosette to zero. In a previous paper (Arizaga and Ezcurra, 1995) we showed how bulbils (i.e., small rosettes forming vegetatively on the meristems of the scape) are produced when pollinators fail to arrive, and thus help to recover the individual ramet from a demographic collapse by vegetatively propagating the genet. The aim of this paper is to describe the pollination mechanisms and to characterize the crossing systems of *Agave macroacantha* Zucc. in a tropical desert in southern Mexico.

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MATERIALS AND METHODS

Study area—Our field observations for the studies of floral biology and pollination mechanisms were done between May and September 1994 at the field laboratory of UNAM's Institute of Ecology, located in Zapotitlán Salinas (18°20′ N, 97°28′ W), 30 km south of the city of Tehuacán. Mean annual precipitation in the study site is ~400 mm (García, 1982) and the dominant vegetation type is a dry xerophytic scrub (Rzedowski, 1978) dominated by *Neobuxbaumia tetetzo*, a giant columnar cactus (Zavala-Hurtado, 1982). The rainy season occurs in summer, starting in late May and ending in late September.

The site has a patchy population of *Agave macroacantha* (Agavaceae), a species that is endemic to the Tehuacán-Cuicatlán Valley (Gentry, 1982). This species belongs to the subgenus *Agave* (Gentry, 1982), a group characterized by open paniculate inflorescences, with flowers in large umbellate clusters on long lateral peduncles. (The other subgenus, *Littaea*, presents narrow spicate inflorescences with flowers arranged in clusters along the elongated scape). *Agave macroacantha* produces flowering stalks in April and May, and by September–November the capsules are ripe and start to open. Although rarely consumed by

humans, the early flowering stalks suffer intense damage by foraging goats (Arizaga and Ezcurra, 1995).

Due to the low density of the species, in March 1994, 16 budding rosettes were transplanted to the experimental site from other areas located within a radius of 5 km that showed a similar vegetation. We used in total 32 flowering individuals (16 transplanted and 16 native to the site), which were fenced to protect them from large herbivores. When experimental treatments were applied to individual plants, the transplanted individuals were randomized among treatments. The individuals used in the experiments were also used for observational procedures that did not require experimental manipulation.

Floral biology—*Duration of flowering*—On a random subset of nine flowering individuals we monitored the time spent in each flowering stage, both at the level of the flowering ''branches'' (i.e., umbellate clusters at the end of the long lateral peduncles) and of the whole inflorescence. For the branches, we registered the duration of the different flowering phases in the first eight lateral umbellate clusters. In each one, we considered that flowering started when the suture lines of at least one floral bud split and concluded when the style of the last flower wilted. Flowers were classified into three categories: (a) developing, from floral aperture to pollen release (anthesis), (b) staminate, from pollen release to stamen decay, which coincides with dehiscence of the stigma, and (c) pistilate, from stigmal lobes spreading to style wilting. Total flowering duration in a single branch was computed from the aperture of the first flower to the wilting of the stigma of the last flower. Individual branches were observed twice each day, at 0700 and 1800. In each observation, we registered the developmental stage of each flower. With these data, we could calculate for each branch the mean time a flower spent in each flowering stage. Once flowering in the first eight branches concluded we continued monitoring the plants until flowering in the last lateral branch concluded. Observations were made daily at noon. In each observation we registered for the whole individual the presence of active flowers, the cumulative number of flowering branches produced, and the cumulative number of fruit capsules formed.

Flower phenology—To quantify in more detail the phenology of individual flowers, we selected ten flowers from the lowest six branches of each of ten randomly selected plants, obtaining a total of 100 flowers. The phenological stages were determined by observations in previous seasons, defining ten categories (Table 1). We systematically selected the first ten flowers to open from the lowest six branches and followed the transitions of each flower from 16 May to 8 June 1994, registering daily the phenological phase of each flower every 3 h from 0700 to 1900. With these results we calculated the mean duration of each phenological state.

Crossing systems—On ten randomly selected flowering rosettes we performed four treatments. (1) Selfing. In order to avoid uncontrolled pollination, the stigmas of the flowers were covered before anthesis with a thin polyethylene tube closed at one end (we defined anthesis as anther dehiscence). Once the stigmas were receptive (as shown by the presence of exudates) they were fertilized with pollen from flowers of the same individual (pollen of the same flower cannot be used, as the flowers are protandrous). The pollen was deposited in the stigmas by rubbing open anthers against them. In most cases, the anthers were collected immediately before pollination. In rainy periods, however, we used dry anthers collected for such contingencies <24 h before. (2) Cross-pollination. Flowers in this treatment were protected with plastic tubes as with the previous group and were fertilized with pollen from anthers of other individuals. Not all the flowers in a scape were cross-pollinated by the same individual, as we were restricted by the availability of dehiscent anthers. Pollen was obtained from the nearest individuals when available, or otherwise from anthers previously collected and stored in vials. (3) Apomixis. To investigate the potential formation of fruits and seeds through asexual mechanisms, we left a third group of flowers with their stigmas covered by the plastic tubes throughout the flowering period. (4) Control. A fourth group of flowers was marked, but their stigmas were not covered. Pollination in these flowers was left to the natural agents in the field. The plastic tubes allowed flowers of the four treatments to coexist in a flowering branch. Each treatment employed between ten and 30 flowers per individual scape. The number of flowers in each treatment was limited by the availability of pollen and by the effort involved in the procedure. Thus, the experiment was not balanced; the treatments consisted of 124, 197, 204, and 274 flowers for the selfing, cross-pollination, apomixis, and control groups, respectively. The experiment was carried on in early June, and by late July the number of fruits developed under the different treatments was counted. In mid-September we collected all the fruits, eliminating those that were dehiscent and had already shed seeds, and we quantified in each fruit the number of fertile and sterile seeds, which are deep black and light gray in color, respectively.

TABLE 1. Description and mean duration of the phenological stages of flower development in *Agave macroacantha* (see also Fig. 1). The first number of each phenological stage defines the development phase of the protandrous flowers: (1) immature flowers, (2) flowers in male phase, (3) flowers in female phase, and (4) fruit development.

Phenological stage	Description	Mean duration (hours ± SE)
1	Beginning of dehiscence of the corolla.	9: 48 (±1: 44)
2.1	Tepals become separated, stamens start to elongate.	19: 53 (±4: 36)
2.2	Anthers appear above the tepals.	19: 50 (±6: 47)
2.3	Stamen filaments appear showing a marked bent below the anthers.	8: 58 (±0: 49)
2.4	Filaments elongate completely, style appears above the tepals.	19: 06 (±0: 49)
2.5	Anthers become dehiscent and release pollen.	10: 54 (±0: 59)
2.6	Anthers lose functionality, style elongates above the stamens.	19: 03 (±0: 50)
3.1	Style reaches maximum elongation, stigmal lobe spreading begins.	26: 42 (±0: 56)
3.2	Stigma fully open with maximum exudates.	10: 05 (±1: 36)
3.3	Style begins to wilt, stigma dries up.	4: 40 (±1: 16)
4	If flower was fertilized, fruiting begins.	—
Total	Flowering time, from stage 1 to stage 3.3.	147: 00 (±8: 51)

Pollination mechanisms—*Nectar production*—In mid-July, we isolated six randomly selected flowering plants inside a gauze mesh in a shade house, protecting them both from the uncontrolled arrival of pollinators and from excessive evaporation of nectar as a result of exposure to direct solar radiation. In each plant we selected six flowers, all at the stage in which the stamens were starting to elongate (phenological phase 2.3; Fig. 1). Every 6 h (0600, 1200, 1800, and 0000) we collected the nectar available inside the flowers with a 1-mL syringe. The total daily production was calculated by accumulating the four values. The experiment was continued for several days, until the wilting of the stigmas indicated the end of flower development. During each collection we registered both the phenological phase of the flower and the volume of nectar.

Pollinators—In parallel with the observations of floral biology, we also made periodical observations of the fauna associated with nine randomly selected flowering rosettes. Observations were made every 3 h, from 0700 to 2200. In each observation we counted all pollen-col-

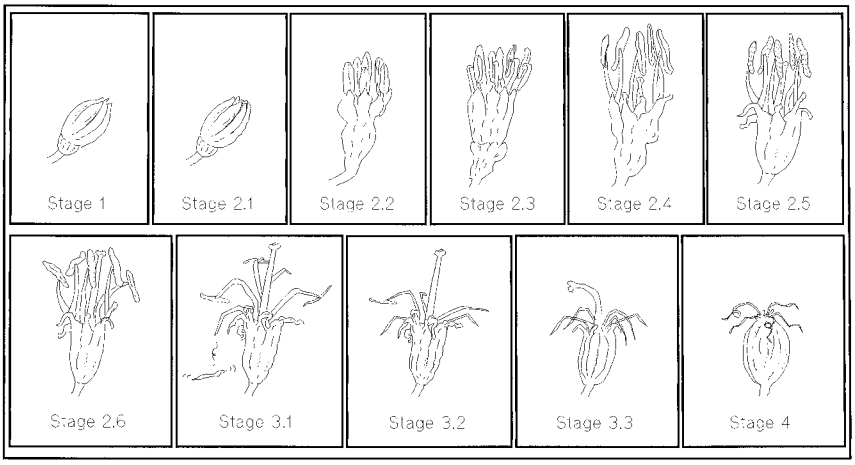

Fig. 1. Phenological phases of flower development in *Agave macroacantha* (see also Table 1).

lecting or nectar-feeding animals, found either directly on the inflorescence or flying less than 1 m away from the scape. The observations were continued until flower development ended in the rosette. The daily sampling effort was, on average, ~22 min per plant (4–5 min/observation). In total, the sampling effort added up to ~13 h/scape. Because of the difficulties involved in directly observing after-dark visitors, only one nocturnal observation was done at 2200. All other observations were diurnal.

Efficiency of pollinators—The following four treatments were applied within each of nine randomly selected reproductive rosettes (each treatment was applied to a single flowering branch within each rosette). (1) Diurnal pollinators. A branch was covered with green gauze mesh during each night, from 1900 to 0700, until flowering ended. (2) Nocturnal pollinators. A second branch was covered with green gauze mesh during each day, from 0700 to 1900, until flowering ended. (3) Exclusion of pollinators. A third branch was covered with green gauze mesh during the whole flowering period. (4) Control. A fourth branch was marked, but not covered.

In all plants, the treatments were applied in four of the first six branches. In order to avoid the mesh in the covered branches interfering with the arrival of animals to the uncovered branches, we consistently located treatment (4) in the upper branch, and treatment (3) in the lower branch. The other two treatments were randomized among the remaining branches. In all four branches, in each of the nine plants, we counted the number of flowers produced. In late July we counted the number of capsules produced, and in September we counted the number of fertile and infertile seeds in each capsule.

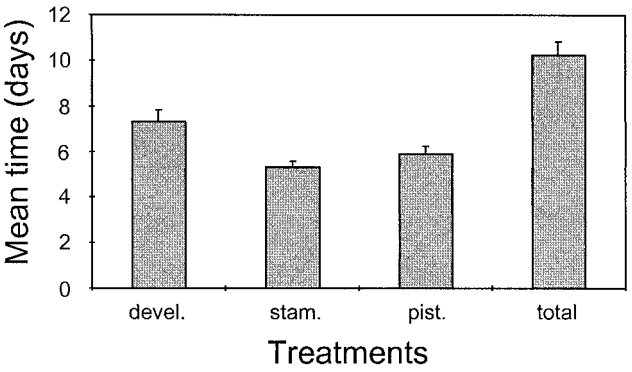

Fig. 2. Duration of flowering for the first eight branches in nine scapes: The columns indicate the mean duration of umbellate clusters with developing (devel.), staminate (stam.), and pistilate (pist.) flowers, and mean total flowering duration (total). The total flowering duration times are not necessarily equal to the sum of the first three columns, as there is some overlap between the phenological phases. Vertical lines indicate standard errors.

Statistical analyses—The relationship between frequency variables (i.e., discrete counts such as numbers of flowers, fruits, seeds, or pollinators) as dependent variables and their statistical predictors (including both continuous variables and factors, or categorical variables) was analyzed by means of Poisson regression, i.e., log-linear regression with continuous predictors and a χ^2 measure of fit, in which the discrepancy between the observed and the predicted data is not evaluated by means of least squares but rather by means of the log-likelihood statistic $[\Sigma_{i=1}^{n} y_i \log(y_i/\hat{y}_i)]$; (see Crawley, 1993; Everitt, 1994; and Krause and Molson, 1997)]. Proportions (i.e., relative frequencies such as proportion of flowers yielding fruits or proportion of seeds that are fertile) were analyzed by means of logistic models (Crawley, 1993). When the residual errors in these models showed overdispersion (i.e., the variance of the residuals was significantly higher that that predicted by the Poisson or the binomial distributions), the frequency data were rescaled to correct for biases in the statistical tests of hypotheses (Crawley, 1993). For the cases in which the dependent variable was continuous (e.g., nectar production), we used ANOVAs to analyze their association with statistical predictors, with the *F* value as test of hypothesis. The residuals of all the ANOVAs were tested for independence, normality, and homoscedasticity. In all cases, the analyses were made with the GLIM (Generalized Linear Interactive Modeling) statistical package, version 3.77 (McCullagh and Nelder, 1983; NAG, 1986). Both for ANOVAs and for log-linear models, when the response variables were obtained from repeated measurements, we analyzed the data following the procedure suggested by Von Ende (1993) to first test the pooled effects of the main factor between subjects independently of time and then to test for the effect of time within subjects and the corresponding interactions. In order to make the results of this study comparable with other similar studies, all our measurements of time were transformed to local mean time, that is, our time measurements were referred to the 97°28′ W meridian, and are hence ~30 min less than Mexican Central winter time, which is referred to the 90° W meridian.

RESULTS

Floral biology—*Duration of flowering*—Within a branch, the phase of developing flowers lasted longer than the pistillate or the staminate stage (Fig. 2). Branch-

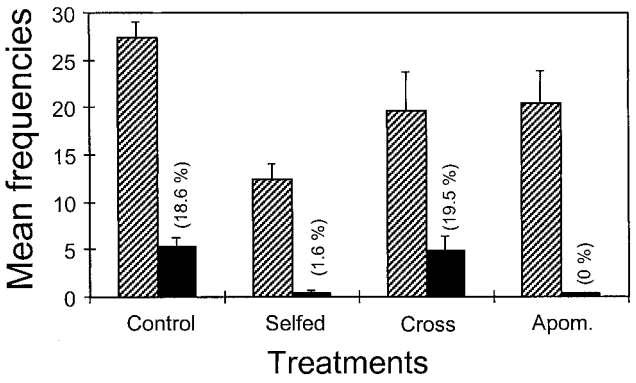

Fig. 3. Production of flowers (hatched bars), and capsules (black bars) in *Agave macracantha* for the different pollination treatments. The numbers in parenthesis indicate the percentage of flowers that yielded capsules (fruit set).

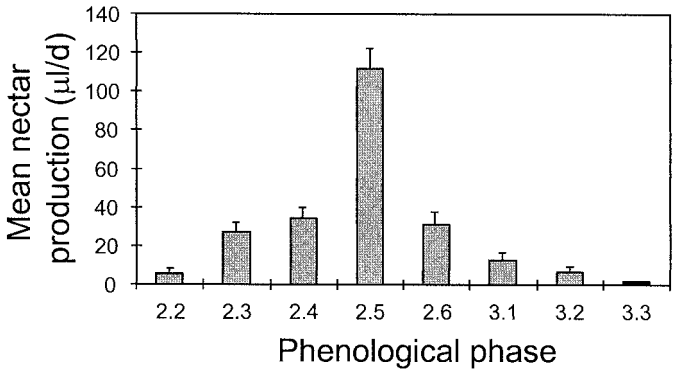

Fig. 4. Mean daily nectar production for each phenological phase. Symbols for phenological phases as in Fig. 1.

es with flowers in the staminate stage showed the shortest duration. Highly significant differences were found between individuals in the duration of the developing stage of the flowering branches ($F = 14.77$; df = 7, 47; $P < 0.00001$), the staminate stage ($F = 13.29$; df = 7, 47; $P < 0.00001$), and the pistillate stage ($F = 9.88$; df = 7, 47; $P < 0.00001$). In contrast, no significant differences were observed between branches in the mean duration of these three phases. A similar trend was found when analyzing the total duration of flowering in the branches; no significant differences were found between branches, but highly significant differences were found between individual scapes ($F = 17.11$; df = 7, 47; $P < 0.00001$). On average, the total duration of the flowering process in lateral branches (from the development of the first flowers in the branch to the last wilting of pistils) was 10.3 d (SE = 0.58).

Flowering in individual scapes started in early May and ended in late June. In each scape, the mean flowering duration was 29.3 d (SE = 2.3; $N = 9$). The number of flowering branches in the individual scapes varied between eight and 18 ($X = 12.00$; SE = 1.05). No significant relationships were found between the number of flowering branches and the total duration of flowering in the scape ($\chi^2 = 0.81$; df = 1; $P = 0.37$) or between the number of capsules and the duration of flowering ($\chi^2 = 1.68$; df = 1; $P = 0.19$).

Flower phenology—The ten phenological states of the flowers of *A. macroacantha* showed different periods of duration (Fig. 1, Table 1). Anthesis lasted, on average, 10.9 h, usually occurring in the afternoon. Pollen was available the next day and could still be obtained from wilting anthers 2 d after anthesis. Spreading of stigma lobes occurred the morning after anthesis. In the following afternoon, the release of exudates marked the initiation of stigma receptivity. The flowers lasted, on average, 26.7 h in the pistilate stage. The total mean time elapsed between the initiation and the end of flowering was 147 h (~6 d).

Crossing systems—A large number of the flowers selected aborted during the experiment (Fig. 3). A fraction of these abortions may be attributable to experimental manipulation, but most of them occurred well after manipulation had taken place and are more likely a consequence of internal mechanisms of the plant and/or the treatments themselves.

Highly significant differences were observed between treatments in the proportion of flowers giving capsules ($\chi^2 = 107.3$; df = 3; $P < 0.00001$; Fig. 3). Apomictic formation of fruits or seeds did not occur in *A. macroacantha*. A significant ($P < 0.00001$) depression in fruit formation was observed in the self-pollinated treatment when compared to the control group (of the nine flowering branches submitted to self-pollination, only one yielded fruits). No significant differences in fruit set were observed between the cross-pollinated treatment and the control plants ($\chi^2 = 0.24$; df = 1; $P = 0.62$). The cross-pollinated plants produced a mean of 222 fertile seeds per capsule (SE = 14.2), representing 72.3% of the total amount of seeds. The control plants produced a mean of 155 fertile seeds per capsule (SE = 13.3), representing 61.1% of the total amount of seeds. These differences, however, were not statistically significant ($\chi^2 = 2.39$; df = 1; $P = 0.12$). In summary, *A. macroacantha* seems to be almost exclusively an outbreeder, with a strong degree of self-incompatibility and a marked dependence on pollinators for its successful reproduction.

Pollination mechanisms—*Nectar production*—The flowers of *A. macroacantha* have three nectaries in the lower part of the tepals, localized between the locules of the ovary. Nectar production lasted slightly more than 4 d. It started just before anthesis and continued until the end of flowering. The maximum daily production of nectar coincided with the anthesis (Fig. 4). Significant variations in the production of nectar were observed between the different phenological stages ($F = 99.0$; df = 7, 256; $P < 0.00001$).

Nectar production also varied during the day (Fig. 5). During the diurnal hours (from 0600 to 1800) the production of nectar was significantly ($P < 0.0001$) lower than in the nocturnal samples (from 1800 to 0600). Significant differences in nectar production were observed between the hours of collection nested within the individual plants ($F = 24.9$; df = 6,398; $P < 0.0001$), and also in the interaction term between the phenological stages and the hours of collection nested within plants ($F = 16.9$; df = 30,398; $P < 0.0001$). The trend in all plants

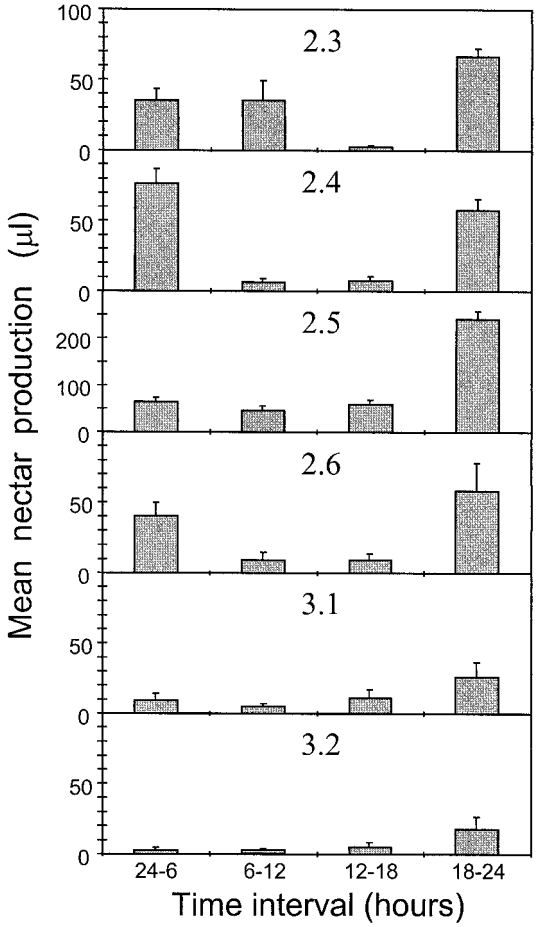

Fig. 5. Daily patterns of nectar production for different phenological phases: (2.3) developing flowers, (2.4) flowers with maximum elongation of stamens, (2.5) flowers in anthesis, (2.6) flowers with developing style and wilting stamens, (3.1) flowers with dehiscence of the stigma, (3.2) flowers with maximum production of stigmal exudates. The vertical lines indicate 1 SE.

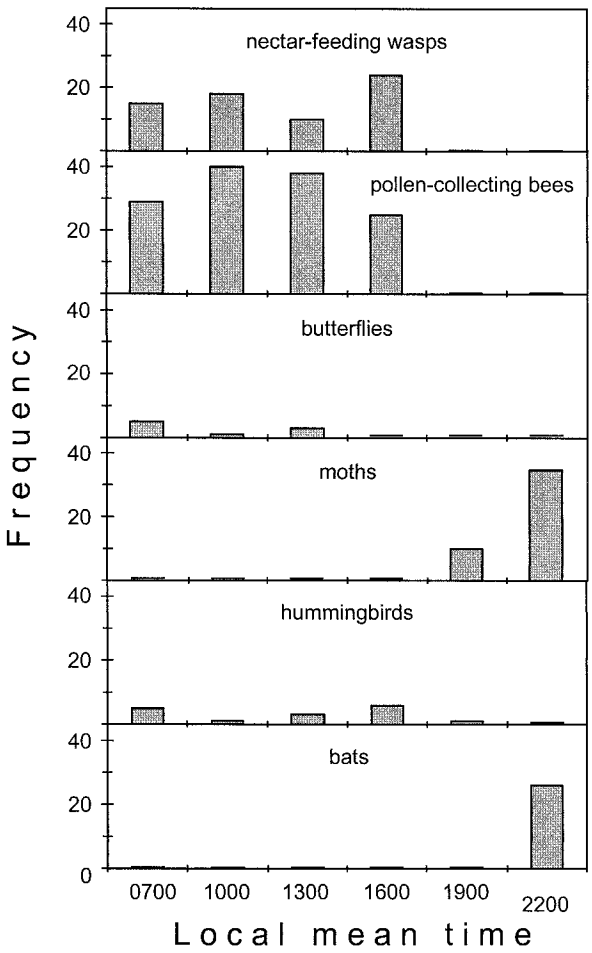

Fig. 6. Frequencies (no. of individuals) of the main potential pollinators that visit the flowers of *A. macroacantha*, classified into six functional groups: nectar-feeding Hymenoptera (five species); pollen-collecting Hymenoptera (four species); butterflies (one species); moths (around six species); hummingbirds (two species); and bats (two species). Time measurements are referred to the local mean time.

was similar, and the interaction term was largely due to the differences between early (1800 to 0000) nocturnal production compared to late (0000 to 0600) nocturnal production that are observed in some phenological stages (Fig. 5).

In short, nectar secretion was higher during the night than in the diurnal hours, it was higher in staminate flowers than in pistillate flowers, and it was extremely high in staminate flowers during the early night. Finally, the total production of nectar also varied significantly between individual plants ($F = 10.5$; df = 5, 30; $P < 0.0001$), but was independent of the size of the scape.

Pollinators—A diverse fauna was observed visiting the inflorescences of *A. macroacantha*, of which the insects were the most diverse taxon. A number of nonpollinating species were found, including a spider (Aracnidae: Salticidae), a tenebrionid beetle (Coleoptera: Tenebrionidae), a fly (Diptera: Muscidae), two species of fruit flies (Diptera: Drosophilidae), a lacewing (Neuroptera: Crysopidae), and a parasitoid wasp (Hymenoptera: Ichneumonidae). The dipteran species were observed visiting the flower stigmas when the production of exudates was abundant. The spider and the beetle were observed during the night.

Two guilds of potential pollinators with contrasting behavior were observed. Diurnal pollinators included nine species of wasps and bees (Hymenoptera), a butterfly (Lepidoptera: Papilionidae), and a hummingbird (Aves: Trochilidae). Hymenoptera did most of the diurnal visits. Nocturnal pollinators included two species of nectarivorous bats (Chiroptera: Phyllostomidae) and at least five species of moths (Lepidoptera: two or more species of Noctuidae, one Sphingidae, and two or more Microlepidoptera; less than 10% of the visits by moths were done by the sphingid species). Diurnal visitors start their visits early in the morning, and tend to decrease their activity during the day. Nocturnal visitors appear at dusk (Fig. 6) and keep a sustained activity until dawn. Significant differences were observed between taxa ($\chi^2 = 194.6$; df = 5; $P < 0.0001$), indicating that some taxa (Hymenoptera, moths, and bats) are more common than others (butterflies and hummingbirds) in their visits to the flowers.

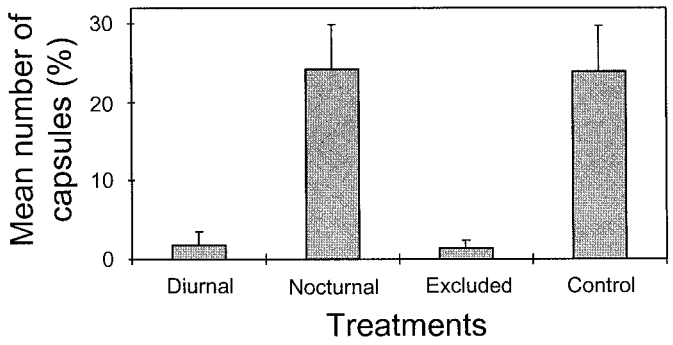

Fig. 7. Mean proportion of capsules formed (fruit set) from flowers subject to the four pollination treatments.

Also, a very significant variation was found between hours of the day nested within taxa ($\chi^2 = 407.3$; df = 30; $P < 0.0001$), underscoring the fact that the different groups of pollinators have marked and obvious hourly preferences in their visit behavior.

Efficiency of pollinators—The branches open to nocturnal pollinators did not differ significantly from the control treatment in the proportion of fruits produced (Fig. 7). In both treatments ~25% of the flowers yielded fertile capsules. Both treatments, however, differed very significantly ($\chi^2 = 42.61$; df = 3; $P < 0.00001$) from the diurnal pollination treatment and from the branches excluded to pollinators. In these two last treatments only ~2% of the flowers yielded capsules, and they did not differ between themselves. That is, plants open to diurnal visitors yielded the same amount of fruits as those open to wind-pollination.

In the fruiting branches open to nocturnal pollinators, 62.2% of the seeds in the capsules were fertile, and each capsule showed a mean of 150.22 fertile seeds ($N = 32$, SE = 2.75). In the control branches, 57.7% of the seeds in the capsules were fertile, and each capsule showed a mean of 140.46 fertile seeds ($N = 24$, SE = 3.47). The differences in the proportion of fertile seeds per capsule were not statistically significant ($\chi^2 = 0.64$; df = 1; $P = 0.42$).

DISCUSSION

The development of the flowering stalk in *A. macroacantha* takes ~10 wk, while the flowering process within the elongated scape takes ~1 mo and occurs towards the end of the dry season. Flowering in a season in which forage is scarce stimulates the consumption of the agave scapes by domestic and wild animals, and the ensuing damage to the flowering stalk triggers in turn the formation of vegetative bulbils in the flowering stalk (Arizaga and Ezcurra, 1995). The plants that escape from predation, however, produce an abundant seed rain that may add up to some 2800 seeds per individual rosette (24 capsules per scape × 117 seeds per capsule). This apparently large seed set, however, is ~4.2% of the mean total number of ovules in a scape (170 floral buds per scape × 393 ovules per floral bud, totaling some 66 870 ovules). Our data show that the arrival of nocturnal pollinators to these outbreeding plants may be a crucial factor in the final reproductive success of the rosette. The results presented here complement those of our previous work (Arizaga and Ezcurra, 1995), in which we showed that the exclusion of nocturnal visitors in plants of this species led to reproductive failure and induced vegetative production of bulbils in the flowering stalk.

Protandria in the hermaphroditic flowers of *A. macroacantha* may reduce self-pollination in a single flower, but it is unlikely that it may do so at the level of the whole inflorescence as there is temporal heterogeneity in the production of flowers within the scape. Thus, flowers in the same inflorescence may show dehiscent anthers, while others at the same time may present receptive stigmas. The main mechanism insuring outbreeding in this species seems to be the high level of self-incompatibility that the reproductive system presents. This means that the transport of pollen from one flowering stalk to the other is of great importance. The activity of nocturnal pollinators was the single factor that appeared to be of the uttermost importance in the reproductive success of the plants. A pattern similar to that of *A. macroacantha* was described for *A. palmeri, Manfreda barchystachya,* and for *Yucca elata,* three species that are preferentially allogamous but may present a low level of self-breeding (Howell and Roth, 1981; Eguiarte and Búrquez 1988; Craig et al., 1993).

The measurement of the floral phenology and the nectar-production pattern in *A. macroacantha* presented the following characteristics. (1) The reproductive parts mature asynchronously inside the flower. (2) Anthesis occurs at dusk and lasts for 2 d, although the summer rains may wash a high proportion of the available pollen the next afternoon. (3) The stigmas become receptive in the late afternoon of the second day after anthesis, and they remain so for ~13 h. (4) The total daily secretion of nectar is correlated with the production of pollen, and the daily maximum of secreted nectar occurs at night. (5) The flowers are visited during the day by hummingbirds and wasps, which take nectar from the flower, and by bees, which collect pollen. (6) During the night, the inflorescences are visited by bats and moths, which feed on nectar and transport the pollen that incidentally sticks to their bodies. As in many other plants, nectar is the basic reward for nocturnal pollinators (Real and Rathcke, 1991).

It is not completely clear to us at this point why diurnal pollination fails so much in *A. macroacantha,* and why, in contrast, nocturnal pollination is the main mechanism for a successful fruit set. The most likely hypothesis lies in the small size of the wasps and bees that make most of the diurnal pollinators. These small insects are capable of reaching the inner perianth of the *Agave* flowers without really being in contact with the long exerted stigma and often without even touching the stamens, which are also exerted away from the petals. In *A. palmeri,* Howell and Roth (1981) have shown that the behavior of nectar and pollen foraging in bees and hummingbirds minimizes their contact with the sexual parts of the flower. In *Manfreda brachystachya* and *Pseudobombax ellipticum,* the hymenoptera and birds remove pollen and nectar, hampering fertilization by nocturnal pollinators (Equiarte and Búrquez, 1987, 1988; Eguiarte and Martínez del Río, 1988). It is also likely that the nectar-search behavior of the diurnal pollinators is wider and that they visit other inflorescences than those of the agaves. Additionally, it

is also possible that the microclimate during the day is adverse for pollination, drying-up the stigma and hindering the growth of the pollen tube. Finally, the summer rains in this part of Mexico fall almost invariably in the mid-afternoon. These short but intense showers may wash out the pollen accumulated in the stigmas. Whatever the true cause, the high secretion of nectar at night suggests that the plants are adapted to nocturnal visitors. This floral syndrome in *A. macroacantha*, favoring nocturnal cross-pollination, is common in other Agavaceae (Eguiarte and Búrquez, 1987; Craig et al., 1993), mainly in paniculate species of the subgenus *Agave* in which the pollinators are either moths or nectarivorous bats (Howell, 1974, 1979; Howell and Hodgkin, 1976; Schaffer and Schaffer, 1977; Howell and Roth, 1981).

In another member of the Agavaceae, *Manfreda brachystachya*, it has been found that the stealing of nectar by diurnal visitors has a negative effect on the visits of the nocturnal pollinators and decreases reproductive success (Eguiarte and Búrquez, 1987). In *A. macroacantha*, however, we found that the mean number of seeds produced in the plants that were open to nocturnal pollinators but excluded from diurnal visitors was not significantly higher than the mean number of seeds produced by the control plants, which were open to both diurnal and nocturnal visitors. Thus, the negative effect of diurnal nectar-robbers was not observed in our species. Additionally, pollination by the nocturnal visitors was lower than that achieved by cross-pollinating the plants by hand. While there was a mean of 150 fertile seeds per capsule in the former, in the artificially pollinated plants there was a mean of 222 fertile seeds per capsule. In short, the proportion of fertile seeds seems to be limited by the capacity of nocturnal pollinators to bring pollen from other plants. Diurnal pollinators, in contrast, seem to have no effect in the formation of fertile seeds in *A. macroacantha*, as flowers exposed to diurnal visitors did not differ in their fruit set from flowers excluded from all visitors. Finally, reproductive success can also be limited by direct damage to the inflorescence, usually by herbivores consuming the flowers or the whole scape and by seed predators.

We may conclude then that the diurnal visitors play no functional role in the reproductive success of *Agave macroacantha* and act mainly as nectar and pollen robbers. In agreement with our results that underscore the importance of nocturnal visitors (bats and moths) in *A. macroacantha*, several authors (e.g., Howell, 1974, 1979; Schaffer and Schaffer, 1977; Howell and Roth, 1981; Fleming, Núñez, and Sternberg, 1993) have suggested that paniculate agaves (i.e., species with branched scapes belonging to the subgenus *Agave*, like our study species) are pollinated chiefly by bats, while spiculate agaves (i.e., species with unbranched scapes and with flowers forming directly on the main shoot, belonging to the subgenus *Littaea*) show pollination syndromes that suggest a predominance of entomophilic mechanisms.

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