

POLLINATION BIOLOGY OF TWO CHIROPTEROPHILOUS AGAVES IN ARIZONA¹

LIZ A. SLAUSON

Desert Botanical Garden, 1201 N. Galvin Parkway, Phoenix, Arizona 85008 USA

I studied the pollination biology of two closely related species of agave, *Agave palmeri* and *A. chrysantha* (Agavaceae), which exhibit several chiropterophilous (bat-pollinated) traits. Floral studies, floral visitor observations, and pollination studies were conducted over four summers at six different sites to examine floral traits and determine the relative importance of diurnal vs. nocturnal pollinators. *Agave chrysantha* appears to have developed minor shifts in several floral characters that enhance diurnal pollination. Although floral shifts towards diurnal pollination were fewer in *A. palmeri*, stigmas were diurnally receptive and copious floral rewards were available in the morning, indicating that some adaptations exist to allow for multiple pollinators. Differences in fruit and seed set between naturally day- and night-pollinated umbels for both species were either not significant or significantly higher in day-pollinated plants. Bats were not important pollinators of *A. chrysantha*, and the mutualistic relationship between *A. palmeri* and the lesser long-nosed bat was found to be asymmetric. “Bat-adapted” floral traits appear to be flexible enough to respond to the climatic and pollinator unpredictability experienced by agaves at the northern edge of their distribution. This variability may be a more important factor affecting evolution of floral characters than a particular pollinator.

Key words: *Agave chrysantha*; *Agave palmeri*; century plant; fruit set; *Leptonycteris curasoae*; lesser long-nosed bat; pollination; seed set.

Agaves, or century plants, are perennial, rosette-shaped leaf succulents native to the southwestern United States, Mexico, Central America, and the Canary Islands. Many paniculate agaves exhibit floral characteristics suggestive of chiropterophily, or bat pollination (Howell, 1972; Faegri and van der Pijl, 1979; Gentry, 1982; Sutherland, 1987; Kuban, 1989). These characteristics include flowers with large floral tubes that are presented in clusters on tall candelabra-shaped inflorescences, copious quantities of nocturnally produced nectar and pollen, pale yellow to yellow flowers, often with reddish tinged tepals, and a floral scent similar to fermenting or rotting fruit. Members of the Group Ditepalae of the genus *Agave* (Agavaceae) (sensu Gentry, 1982) possess many of these “bat flower” traits and are largely distributed within the migratory range of the nectarivorous lesser long-nosed bat, *Leptonycteris curasoae yerbabuena* Martinez and Villa (Fleming, Nunez, and Sternberg, 1993). Phenological data suggest that several species of columnar cacti and Ditepalae agaves provide a “nectar corridor” for nec-

tarivorous, migratory bats from spring as they migrate north, through the fall when they return to southern roosts (Gentry, 1982; Fleming, Nunez, and Sternberg, 1993). While columnar cacti form the bulk of available food for the lesser long-nosed bat during spring and early summer, the Ditepalae agaves bloom significantly later in the year (mean peak flowering period is August) than other paniculate agaves and columnar cacti, and can provide a potentially important food source during southern migration when other Crassulacean acid metabolism (CAM) plants are no longer available (Fleming, Nunez, and Sternberg, 1993).

Agave palmeri Engelm. (Agavaceae), one of the northernmost distributed members of the Ditepalae, is native to savanna grassland and oak woodland communities of northern Mexico and southern portions of Arizona and New Mexico. Portions of the range of *A. palmeri* are sympatric with that of the lesser long-nosed bat (Fig. 1). Several studies (Howell, 1974, 1979; Howell and Hodgkin, 1976) have shown the lesser long-nosed bat, currently listed as an endangered species in the United States (Schull, 1988), has a mutualistic association with *A. palmeri*. Howell and Roth (1981) found high seed set in *A. palmeri* populations where bats were present, low seed set where bats were not present, and declines in fruit and seed set in herbarium specimens over a 30-yr period (but see Cockrum and Petryszyn, 1991). They suggested that *A. palmeri* was “strongly dependent” upon the lesser long-nosed bat for pollination, although sphinx moths, carpenter bees, and other solitary nectar-feeding bats were indicated as potential, occasional pollinators (Howell, 1979; Howell and Roth, 1981). Howell and Roth (1981) concluded that reported declines in lesser long-nosed bat populations (Hayward and Cockrum, 1971; Howell and Roth, 1981) could severely limit sexual reproduction of *A. palmeri* and other paniculate agaves. On the other hand, low fruit set has been shown to be com-

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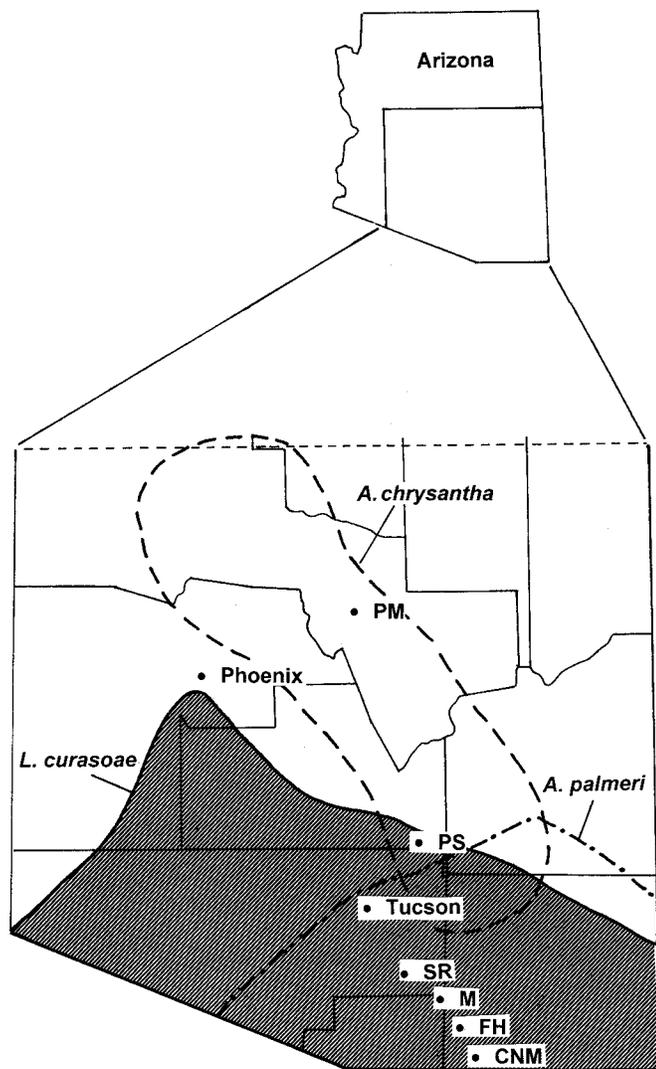


Fig. 1. Distribution of *A. chrysantha*, *A. palmeri*, and *L. curasoae* in Arizona and locations of *Agave* study sites. Figure Abbreviations: CNM = Coronado National Monument site, FH = Fort Huachuca site, M = Mustang site, PM = Parker Mesa site, PS = Peppersauce site, SR = Santa Rita site.

mon in outcrossing, largely self-incompatible hermaphroditic plants such as agaves (Sutherland and Delph, 1984). Sutherland (1982, 1987) found that fruit set in paniculate agaves was primarily resource limited with fruit set averaging 20% and that "excess" or aborted flowers played an important role in pollen donation and male fitness.

Little is known about the pollination ecology of *A. chrysantha* Peebles (Agavaceae), another member of the Ditepalae closely related to *A. palmeri* (Gentry, 1982) with an adjacent and partially overlapping range to the north (Fig. 1). *Agave chrysantha* occupies a variety of habitats, including desertscrub, chaparral, juniper woodland, and the fringes of pinyon pine-oak woodland communities of central and south-central Arizona. It also displays characteristics of bat pollination, such as nocturnal pollen dehiscence and nectar production, and a floral odor suggestive of ripening fruit. However, it has vivid yellow

to yellow-orange flowers more typical of insect- or bird-pollinated flowers, and a distribution that is largely north of the range of nectar and pollen-feeding bats (Baker and Cockrum, 1966) (Fig. 1). Schaffer and Schaffer (1977) noted that a population of *A. palmeri* on the north side of the Santa Catalina Mountains (probably *A. chrysantha*) with bright yellow flowers appeared to depend on large bees for pollination.

Floral characters, phenology, and distribution patterns suggest that bats have been an important influence in *Agave* evolution. However, chiropterophilous agave species that occupy habitats at the edges or outside the range of nectarivorous bats can be successfully pollinated by other animals. Sutherland (1987) noted that *A. mckelveyana* Gentry has nocturnal anther dehiscence and nectar production, but is primarily pollinated by diurnal insects. *Agave havardiana* Trel. in southern Texas was pollinated by the greater long-nosed bat (*L. nivalis* Saussure) at higher elevations, but white-winged doves (*Zenaida asiatica* L.) and orioles (*Icterus parisorum* Bonaparte) were the most significant pollinators in desert habitats (Kuban, 1989). A variety of animals other than bats are known to visit flowers of *A. palmeri* and *A. chrysantha* (Schaffer and Schaffer, 1977; Howell, 1979; Howell and Roth, 1981), but the characteristics of these relationships are not well understood. Additionally, the nature of the mutualistic relationship between the lesser long-nosed bat and *A. palmeri* appears to be unresolved based on current data. This study investigates the floral traits of *A. palmeri* and previously unstudied *A. chrysantha* and the importance of various pollinators on fruit and seed set. Floral traits, pollinator observations, and pollinator exclusion experiments were conducted over four summers in four populations of *A. palmeri* and two populations of *A. chrysantha* to address the following questions: (1) How do the floral traits of *A. palmeri* and *A. chrysantha* affect pollination? (2) Does the timing of pollen presentation and nectar production suggest adaptation strictly for nocturnal visitors? (3) Who are the diurnal and nocturnal floral visitors of *A. palmeri* and *A. chrysantha* and what are their relative contributions to fruit and seed set? (4) Does *A. palmeri* depend primarily on the lesser long-nosed bat for pollination? (5) Is pollinator limitation an important factor in sexual reproduction of *A. palmeri* and *A. chrysantha*? (6) What animals pollinate *A. chrysantha* and what are the reproductive implications of chiropterophilous traits in a plant that is largely distributed outside of the range of nectarivorous bats?

METHODS

Study sites—Research was conducted at six study sites in central and southern Arizona (Fig. 1). The study of geographic variation in plant-pollinator relationships can be informative (Thompson, 1994), therefore 2–4 populations of each species were studied along a north-south gradient. *Agave chrysantha* study sites were located at the northern edge of its distribution in the Sierra Ancha Mountains (Parker Mesa site: 33°45' N, 110°58' W; 1400 m elevation), and near the southern edge of its range in the Santa Catalina Mountains (Peppersauce site: 31°33' N, 110°43' W; 1432 m elevation). Study sites for *A. palmeri* were located throughout the northern portion of its distribution in Arizona in the foothills of the Santa Rita Mountains (Santa Rita site: 31°47' N, 110°42' W; 1520 m elevation), the foothills of the Mustang Mountains

(Mustang site: 31°43' N, 110°30' W; 1500 m elevation), Coronado National Monument (Coronado site: 31°21' N, 110°13' W; 1508 m elevation), and Fort Huachuca Military Reservation (Fort Huachuca site: 31°30' N, 110°18' W; 1507 m elevation). The Coronado site was located ~4 km from a transient lesser long-nosed bat roost occupied in July–October (V. Dalton, University of Arizona, personal communication). Research was conducted on *A. chrysantha* at the Parker Mesa site during 14–20 July 1993 and at the Peppersauce site during 5–11 July 1994. *Agave palmeri* studies were conducted at the Santa Rita site 3–9 August 1993, the Mustang site 1–7 August 1994, the Coronado site 14–20 August 1995, and the Fort Huachuca site 20–26 July 1998.

Floral trait studies—To characterize the phenology of each species, the flowering seasons of 13 *A. chrysantha* and 14 *A. palmeri* populations were recorded in addition to the above study sites. Populations were visited 1–3 times per year from June to September, and it was noted whether plants were flowering or not. Flowering dates of the above populations were also obtained from herbarium specimens located at Arizona State University (ASU) and the Desert Botanical Garden (DES).

Flower traits were recorded during flowering at five sites. Twenty flowers on 1–3 plants per site were numbered prior to dehiscence. The length of exerted style and condition of filaments, tepals and stigma were recorded once daily until stigmas wilted.

Anther dehiscence was studied at all sites but the Fort Huachuca site. Twenty numbered predehiscent flowers were observed starting shortly before dusk and examined hourly to determine time of anther dehiscence.

To determine whether nectar was produced diurnally, 24-h nectar accumulation studies were carried out at Parker Mesa (*A. chrysantha*) and Santa Rita (*A. palmeri*) sites. Nectar was measured every 2 h in 20 numbered flowers on 1–2 replicates (separate plants). Nocturnal nectar accumulation was studied at Peppersauce (*A. chrysantha*) and Mustang sites (*A. palmeri*) where nectar was measured every 3 h from 1800 to 0600 in 20 numbered flowers on three replicates (separate plants). For both nectar accumulation studies, the same flowers were sampled each day during the dehiscent, postdehiscent, early pistillate, and pistillate floral stages. Sampled flowers were located on umbels in the middle section of the inflorescence and exclosed from pollinators with a 1.5-mm nylon mesh netting beginning at 1800 (Howell, 1979). Nectar was removed with a tuberculin syringe and blunt needle and was not replaced after removal. Nectar accumulation was also measured at the Fort Huachuca site, but a different nectar collection method was employed. Nectar was measured in ten predehiscent, dehiscent, postdehiscent, and all pistillate stage flowers on 29 replicates (separate plants). The same flowers were sampled throughout each floral stage. Flowers were bagged at dusk, and nectar was allowed to accumulate until dawn, and then measured.

Standing nectar crop was measured at the Peppersauce (*A. chrysantha*) and Mustang (*A. palmeri*) sites at dusk and dawn on 20 numbered predehiscent, dehiscent, postdehiscent, and pistillate stage flowers on 1–2 replicates (separate plants). Nectar sugar concentration (percentage sucrose equivalents on a mass/mass basis) of standing nectar crop flowers was measured at dawn on dehiscent and early pistillate stage flowers in *A. chrysantha* and dehiscent, postdehiscent, and early pistillate stage flowers in *A. palmeri* with a hand-held refractometer.

Nectar statistical analyses were conducted using SYSTAT 6.0 for Windows (SPSS, 1996). Nectar sugar percentage data were arcsine transformed prior to analysis (Zar, 1984), but data are reported as percentages in this paper.

Floral visitor observations—To determine pollinator visitation rates and patterns, observations of pollinators were conducted over 3–4 d at all study sites, except the Santa Rita site where stormy weather limited observations to a single day. Flower visits were observed closely to determine whether or not stigma contact was made. Visitors were clas-

sified as pollinators if they appeared to transfer pollen to exerted (postdehiscent to pistillate) stigmas. Bat visits were generally <1 s, and it was difficult to determine by visual observation whether stigma contact was made. I assumed that bat visits that caused movement of the umbel (a lateral branch of the inflorescence with flowers borne in umbellate clusters) were a result of stigma contact with the bat's body. Night vision goggles (M973, Litton Co., Tempe, Arizona, USA) were used for nocturnal studies. Visitation rates of insects (primarily bees and moths) were determined by counting the number of insect visits to flowers on an umbel for 10 min every 2 h during the day (~0500–1900) and three times during the night (~2100, 2400, and 0400). Insects were observed from a ladder ~1.5 m from the study umbel. Birds and bats were observed 10–20 m from the inflorescence. Visitation rates of birds were measured by counting the number of visits to open flowers on an inflorescence for 1 h shortly after dawn, during mid-day, and 1 h prior to dusk. Bat visitation rates were determined by observing a clumped group of inflorescences for ~1 h after dusk, near midnight, and before dawn at the Santa Rita and Mustang sites. One inflorescence was observed from 2000 to 0430 on 16–17 August and 17–18 August at the Coronado site. Predominant flower stage(s) (predehiscent, dehiscent, postdehiscent, and pistillate) of each observed umbel was recorded. Identity of visitor, visitation behavior, and general environmental conditions were also noted. Bats were identified visually with night vision goggles using tail membrane characteristics; the lesser long-nosed bat has a very reduced tail membrane, which distinguishes it from the sympatric Mexican long-tongued bat (*Choeronycteris mexicana* Tschudi) (Hoffmeister, 1986). Insects were collected while they visited flowers and stored for later identification.

Pollination experiments—To evaluate the relative importance of diurnal and nocturnal pollinators, the following pollinator exclusion experiments were performed on both *Agave* species. Experimental plants were centrally located within a population, if possible. Test umbels were chosen from the middle section of inflorescences, and 15 plants each were randomly assigned to one of the following treatments: (1) control umbels available to both diurnal and nocturnal visitors, (2) umbels available only to diurnal visitors (umbels bagged at sunset and unbagged at sunrise), (3) umbels available only to nocturnal visitors (umbels bagged at sunrise and unbagged at sunset), and (4) umbels bagged, but receptive stigmas were liberally hand-pollinated daily (from 0700 to 1100) with fresh pollen collected from different individuals within the population (Peppersauce and Mustang sites only) until they wilted. Umbels were bagged prior to stigma receptivity (generally after anther dehiscence), and bagging continued until all styles were wilted (4–5 d). Umbels of experimental and control plants were collected in October and November when fruits were partially dry but undehiscent, number of mature fruits and aborted flower scars on each umbel were counted, and percentage fruit set was determined [number of fruits/(number of fruits + aborted flower scars)]. Mean seed set per fruit [number of black, fertilized seeds/(number of black, fertilized seeds + number of white, unfertilized ovules)] was determined for the Peppersauce, Mustang, and Coronado sites. Fruit and seed set data had non-normal distributions and heterogeneous variances and were analyzed with distribution-free multiple-comparison procedures (Edgington, 1995) using the MRPP procedure (multiresponse permutation procedure) of BLOSSOM Statistical Software (Slauson, Cade, and Richards, 1994). Probability values were adjusted using the Bonferroni procedure (Edgington, 1995).

To determine whether differences existed in diurnal and nocturnal pistil receptivity of *A. palmeri*, anthers were removed from 30 predehiscent flowers from each of 13 plants at the Fort Huachuca site, and flowers were exclosed with nylon mesh netting. Once flowers became receptive (evening of day 4), five flowers per plant were hand-pollinated at dusk (1900–2000) and rebagged. Five additional flowers were hand-pollinated on each plant the next morning (day 5, 0600–0800). This procedure was repeated on the evening of day 5/morning of day 6 and the evening of day 6/morning of day 7. Donor pollen was gathered

from several plants that were not in the experimental group. Fruits were collected in mid-October 1998, and fruit and seed set data were analyzed by one-way repeated-measures ANOVA using SYSTAT 6.0 for Windows (SPSS, 1996). Fruit and seed set percentage data for receptivity experiments were arcsine transformed prior to analysis (Zar, 1984), but are reported in this paper as percentages.

To determine whether *A. chrysantha* and *A. palmeri* were self-compatible, anthers from 15 predehiscent flowers were removed from ten plants each of both species at the Peppersauce and Mustang sites. Flowers were covered with nylon mesh netting, hand-pollinated on the evenings of days 4–6, and rebagged. Donor pollen was gathered from freshly dehiscent flowers from the same or an adjacent umbel. Fruits were gathered in October and November 1994, and mean fruit set was determined for each species.

The total number of fruits and aborted fruits was counted on control and experimental inflorescences at all study sites to determine mean fruit set of inflorescences for each population.

RESULTS

Floral trait studies—Although the flowering periods of the two taxa overlap partially, *A. chrysantha* had an earlier flowering period. At lower elevations (700–975 m) *A. chrysantha* began flowering in late May–early June. Peak flowering at mid-elevations (975–1650 m) where study sites were located occurred through June and July, and flowering continued at higher elevations (1700–2130 m) into early August. *Agave palmeri* began flowering in late June–early July at lower to mid-elevations (1000–1500 m) with peak flowering occurring from late July to August (1220–1828 m) and extending through September–early October.

Both agave species were protandrous, gradually changing from a staminate to pistillate state over a 5–6 d period. On day 1 of flowering (predehiscent stage), the tepals opened and the filaments and anthers were exerted above distal tips of tepals. Flowers remained in this condition until the evening of day 2 or early morning of day 3 (dehiscent stage) when anthers dehisced and pollen was presented. Stigmas were tightly closed at dehiscence, although styles had begun to elongate. *Agave chrysantha* styles were exerted 0–15 mm beyond tepals on the morning of day 3 (postdehiscent stage), whereas styles in *A. palmeri* were exerted 15–35 mm. On the morning of day 4 (early pistillate stage), the tripartite stigmas were usually closed, but were generally moist and open by evening. Thus, flowers became receptive ~48 h after anther dehiscence. Filaments began to wilt on day 4 with styles exerted 16–27 mm in *A. chrysantha* and 25–40 mm in *A. palmeri*. By the morning of day 5 (pistillate stage), stigmas were open and sticky, and filaments were completely wilted by the end of the day. Styles were exerted 21–30 mm and 25–48 mm in *A. chrysantha* and *A. palmeri*, respectively. Tepals were generally wilted by the morning of day 6 (late pistillate stage), and stigmas varied from being widely parted and moist to dry and slightly wilted. Styles of both taxa were thoroughly wilted by day 7. Floral scent differed between taxa. *Agave chrysantha* flowers had a mild odor, often coconut-like, while flowers of *A. palmeri* had a much stronger odor, which resembled fermenting or rotting fruit.

Time of pollen dehiscence differed significantly between taxa (Pearson chi-square, $\chi^2 = 1374$; $df = 3$, $P < 0.001$). Ninety-five percent ($N = 360$) of *A. palmeri* an-

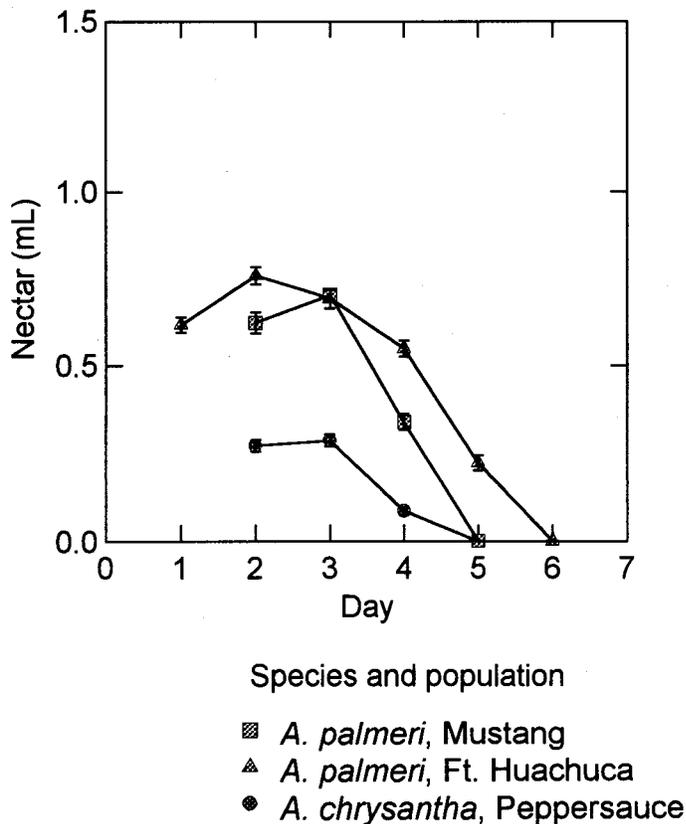


Fig. 2. Mean nocturnal nectar accumulation of *A. chrysantha* (Peppersauce site) and *A. palmeri* (Mustang and Fort Huachuca sites). Sampling methods differed between *A. palmeri* sites (see Methods). $N = 60$ flowers for Peppersauce and Mustang sites, and 290 flowers for Fort Huachuca site. Vertical lines = 1 SE.

thers dehisced shortly after sunset between 2000 and 2200 of day 2, while 96% ($N = 340$) of *A. chrysantha* anthers dehisced later, between 2400 of day 2 and 0200 of day 3. Occasionally, all six anthers in a flower did not dehisce on the same night, and the remaining undehiscent anthers opened the following night. No matter what day anthers dehisced, all *A. palmeri* anthers dehisced between 2000 and 2400 and *A. chrysantha* anthers dehisced between 2400 and 0300.

Results of 24-h nectar accumulation experiments showed that nectar production was nocturnal in both species with no nectar produced from ~0600 to 2000. Flowers in nocturnal nectar accumulation studies produced the most nectar on days 1–3 (predehiscent, dehiscent, and postdehiscent stages), with nectar production declining thereafter (Fig. 2, Table 1). Daily nocturnal nectar accumulation was greater in *A. palmeri* (repeated-measures nested ANOVA, $F_{1,4} = 21.051$, $P = 0.010$) (Fig. 2), but the pattern among days was somewhat dissimilar between species ($F_{3,12} = 5.834$, $P = 0.011$). Thus, nectar accumulation among days was analyzed separately for each species. Nectar production significantly decreased between days 3 and 4 in *A. chrysantha* (postdehiscent/early pistillate) (Tukey's multiple-comparisons procedure with Bonferroni adjustment, $P < 0.05$) and between days 3–4 (postdehiscent/early pistillate) and days 4–5 (early pistillate/pistillate) in *A. palmeri* (Tukey's multiple-compar-

TABLE 1. Standing nectar crop and nocturnal nectar accumulation of *Agave chrysantha* (Peppersauce) and *A. palmeri* (Mustang). Data are mean values, SE (in parentheses) represents nectar variation among flowers. Standing crop and nocturnal nectar values with different lowercase superscripts differ significantly within days for each species (*t* test with Bonferroni adjustment, $P < 0.005$ for all pairwise comparisons). Standing crop values with different uppercase superscripts differ significantly between adjacent days within species (repeated-measures ANOVA, $P < 0.001$). Nocturnal nectar values with different uppercase superscripts differ significantly between days within species (Tukey's multiple-comparisons procedure with Bonferroni adjustment, $P < 0.05$).

Floral stage	Standing crop (mL)		Nocturnal nectar accumulation (mL)
	Dusk	Dawn	Dawn
<i>A. chrysantha</i> (Peppersauce)			
<i>(N = 20)</i>			
Day 2 (dehiscent)	0.104 (0.010)	0.190 (0.016) ^{a,A}	0.275 (0.016) ^{b,A}
Day 3 (postdehiscent)	0.003 (0.001)	0.180 (0.015) ^{a,A}	0.288 (0.017) ^{b,A}
Day 4 (early pistillate)	0.000 (0.000)	0.007 (0.005) ^{a,B}	0.087 (0.011) ^{a,B}
Day 5 (pistillate)	0.000	0.000 ^{a,B}	0.001 (0.000) ^{a,B}
<i>A. palmeri</i> (Mustang)			
<i>(N = 20)</i>			
Day 2 (dehiscent)	0.141 (0.018)	0.332 (0.027) ^{a,A}	0.623 (0.030) ^{b,A}
Day 3 (postdehiscent)	0.61 (0.007)	0.419 (0.027) ^{a,B}	0.702 (0.014) ^{b,A}
Day 4 (early pistillate)	0.083 (0.008)	0.354 (0.039) ^{a,C}	0.341 (0.023) ^{a,B}
Day 5 (pistillate)	0.008 (0.002)	0.008 (0.003) ^{a,D}	0.002 (0.001) ^{a,C}

isons procedure with Bonferroni adjustment, $P < 0.05$) (Table 1).

Nectar accumulation for successive 3-h periods of day 2 (dehiscent) flowers showed that the shape of nectar production curves within species were similar for each

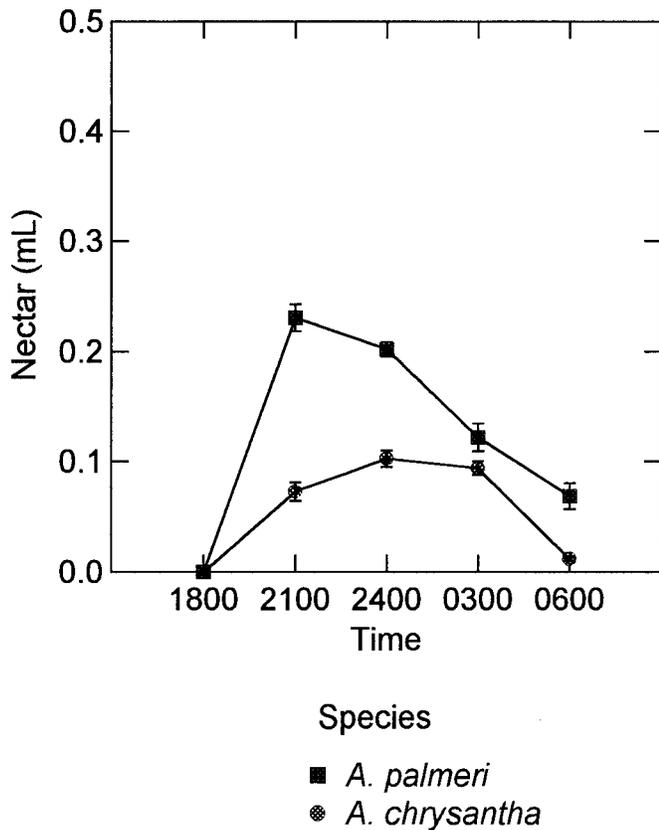


Fig. 3. Mean nocturnal nectar production of day 2 (dehiscent) flowers during successive 3-h periods for *A. chrysantha* (Peppersauce site) and *A. palmeri* (Mustang site). $N = 60$ flowers for each site, and vertical lines = 1 SE.

day, except for day 5 flowers (pistillate), which produced a small amount of nectar from dusk until 2100 with negligible production afterwards (Fig. 3). Nectar production of *A. palmeri* exceeded that of *A. chrysantha* (repeated-measures nested ANOVA, $F_{1,4} = 6.016$, $P = 0.070$). Nectar accumulation through time was similar between species ($F_{3,12} = 1.463$, $P = 0.274$), with highest production occurring from 1800 to 2100 and 2100 to 2400, and decreasing thereafter. For both species, nectar production significantly decreased linearly during the night ($P < 0.001$). Although not significant, some differences in nectar production between species were observed. Nectar production in *A. chrysantha* began at dusk, increased slightly from 2100 to 2400, remained fairly constant from 2400 to 0300, and then declined to near zero by 0600. For *A. palmeri*, nectar production increased from 1800 to 2100, decreased slightly from 2100 to 2400, and then decreased steadily until dawn.

Standing nectar crops were significantly lower than nocturnal nectar accumulations for days 2–4 (dehiscent, postdehiscent, and pistillate flowers) in *A. chrysantha* and days 2–3 (dehiscent and postdehiscent) in *A. palmeri* (Table 1) (*t* test with Bonferroni adjustment, *A. chrysantha*: dehiscent, $t = 2.976$, $df = 77$, $P = 0.004$; postdehiscent, $t = 3.600$, $df = 76$, $P = 0.001$, pistillate, $t = 3.983$, $P = 0.001$; *A. palmeri*: dehiscent, $t = 5.451$, $df = 78$, $P = 0.000$; postdehiscent, $t = 0.283$, $df = 78$, $P = 0.000$). It appears that a substantial amount of nectar was consumed during the night, presumably by moths, as bats were not observed at these sites (see next subsection). Approximately 20–37% of nectar was consumed at night during days 2–4 in *A. chrysantha*, and a similar pattern (41–47%) was present for days 2–3 in *A. palmeri*. However, a majority of nectar produced during the night was available at dawn for diurnal visitors that harvested most, but not all, of the nectar. By dusk, diurnal visitors had harvested 59–100% of nectar in *A. chrysantha* and 83–98% in *A. palmeri* (e.g., standing crop value at dawn on day 2 vs. standing crop at dusk on day 3).

Mean nectar sugar concentrations of dawn samples of

TABLE 2. Dawn standing crop nectar sugar concentration (sucrose percentage) of *Agave chrysantha* (Peppersauce) and *A. palmeri* (Mustang). Data are mean values, $N = 20$ flowers, and SEs (in parentheses) represent variation among flowers. Nectar sugar concentrations with different lowercase superscripts differ significantly between days within a species (repeated-measures ANOVA, $P < 0.001$). Nectar sugar concentrations with different uppercase superscripts differ significantly between species within a particular day (t test with Bonferroni adjustment, $P < 0.01$).

Floral stage	Dawn standing crop (% sucrose)	
	<i>A. chrysantha</i>	<i>A. palmeri</i>
Day 2 (dehiscent)	17.5 (0.395) ^{a,A}	13.4 (0.215) ^{a,B}
Day 3 (postdehiscent)	—	17.3 (0.478) ^b
Day 4 (early pistillate)	15.5 (0.395) ^{b,C}	13.8 (0.326) ^{a,D}

day 2 (dehiscent) and day 4 (pistillate) flowers were significantly higher in *A. chrysantha* than *A. palmeri* (t test with Bonferroni adjustment: dehiscent, $t = 9.229$, $df = 36$, $P < 0.01$; pistillate, $t = 3.030$, $df = 38$, $P < 0.01$) (Table 2). Nectar sugar concentrations significantly decreased between dehiscent (day 2) and pistillate (day 4) flowers of *A. chrysantha* (repeated-measures ANOVA, $F_{1,18} = 29.862$, $P < 0.001$). For *A. palmeri*, postdehiscent nectar sugar concentrations were significantly higher than dehiscent or pistillate concentrations (repeated-measures ANOVA, $F_{2,17} = 42.639$, $P < 0.001$).

Floral visitor observations—Floral visitors included a diverse range of animals: honey bees, bumble bees, car-

penter bees, hummingbirds, orioles, sphinx moths, butterflies, wasps, moths, bats, and a variety of small solitary bees. Diurnal visitor frequency was greater than nocturnal visitation at all sites (Table 3). Although visitors varied in composition and numbers between sites, introduced honey bees (*Apis mellifera* L.) had the highest overall visitation rates, except for the Coronado site where bumble bees (*Bombus pennsylvanicus sonorus* Say) were more numerous. Small moths (Family Noctuidae) and white-lined sphinx moths (Sphingidae: *Hyles lineata* Fabricius) were the predominant nocturnal insect visitors; their visitation rates were lower than those of diurnal insects. Hummingbirds visited at four sites, but varied in territorial behavior. The broad-tailed hummingbird (*Selasphorus platycercus* Swainson) was a significant visitor (1.70 flower visits \cdot min⁻¹ \cdot umbel⁻¹) at the Coronado site where each hummingbird appeared to actively defend a territory containing 2–3 inflorescences and exhibited frequent foraging bouts. At other sites this species and black-chinned hummingbirds (*Archilochus alexandri* Bourcier & Mulsant) were not territorial. Bats were not observed at either *A. chrysantha* site (over 15 total nighttime hours of periodic observations during early to mid-July) and at only one of the *A. palmeri* sites, which was observed latest in the season (Coronado, 14–20 August). Nocturnal vertebrate (bat) visitation rates were lower than diurnal vertebrate (bird) visitation rates at all sites, except for the Santa Rita site.

Peak visitation occurred at dawn, but a smaller burst of activity also took place prior to dusk. Both honey bees

TABLE 3. Diurnal and nocturnal floral visitors of *Agave chrysantha* and *A. palmeri* at five study sites.

Visitors	Flowers visits \cdot min ⁻¹ \cdot umbel ⁻¹ (minutes observed)				
	<i>A. chrysantha</i>		<i>A. palmeri</i>		
	Parker Mesa 14–20 Jul 93	Peppersauce 5–11 Jul 94	Santa Rita 3–9 Aug 93	Mustang 1–7 Aug 94	Coronado 14–20 Aug 95
Diurnal visitors					
Insects	0.63 (480)	1.56 (690)	3.32 (90)	3.01 (300)	4.39 (140)
Honey bee	0.34	1.38	2.40	1.42	0.93
<i>Apis mellifera</i>					
Bumble bee	0.26	0.04	0.92	0.25	3.35
(<i>Bombus sonorus</i>)					
Carpenter bee	0.02	0.06	0.00	0.52	0.01
(<i>Xylocopa californica</i>)					
Unidentified bee	0.00	0.00	0.00	0.50	0.10
Wasp	0.00	0.08	0.00	0.32	0.00
(<i>Polistes</i> sp.)					
Pipevine swallowtail	0.01	0.00	0.00	0.00	0.00
(<i>Battus philenor</i>)					
Housefly	0.00	0.01	0.00	0.00	0.00
Birds	0.15 (360)	0.03 (480)	0.00 (90)	0.12 (540)	1.70 (840)
Broad-tailed hummingbird	0.13	0.00	0.00	0.00	1.70
(<i>Selasphorus platycercus</i>)					
Black-chinned hummingbird	0.00	0.03	0.00	0.12	0.00
(<i>Archilochus alexandri</i>)					
Scott's oriole	0.02	0.00	0.00	0.00	0.00
(<i>Icterus parisorum</i>)					
Nocturnal Visitors					
Insects	0.20 (60)	0.25 (100)	0.10 (60)	0.28 (90)	0.24 (140)
Moths (Noctuidae)	0.16	0.25	0.05	0.18	0.00
White-lined sphinx moth	0.03	0.00	0.05	0.10	0.24
(<i>Hyles lineata</i>)					
Lesser long-nosed bat	0.00 (120)	0.00 (120)	0.00 (120)	0.00 (570)	0.03 (1020)
(<i>Leptonycteris curasoae</i>)					

TABLE 4. Effect of four pollination treatments on fruit and seed set in *Agave chrysantha* and *A. palmeri*. Within a site, mean values of fruit or seed set with the same superscript do not differ significantly ($P > 0.05$). At each site N = number of plants assigned to each treatment (one umbel treated per plant).

Characteristic	Treatment	<i>A. chrysantha</i>		<i>A. palmeri</i>		
		Parker Mesa ($N = 10$)	Peppersauce ($N = 15$)	Santa Rita ($N = 13-14$)	Mustang ($N = 15$)	Coronado ($N = 13-15$)
Mean % fruit set per umbel \pm SE	night-pollinated	14.9 \pm 5.7 ^a	2.2 \pm 0.89 ^a	10.3 \pm 4.3 ^a	6.5 \pm 2.5 ^a	6.9 \pm 1.9 ^a
	day-pollinated	22.2 \pm 4.8 ^a	15.7 \pm 3.7 ^b	14.3 \pm 5.1 ^a	8.8 \pm 3.4 ^a	10.4 \pm 2.9 ^a
	control	23.8 \pm 4.7 ^a	18.8 \pm 4.2 ^b	16.6 \pm 3.7 ^a	17.9 \pm 3.7 ^a	22.8 \pm 3.9 ^b
	hand-outcrossed during day		51.0 \pm 9.4 ^c		54.3 \pm 5.8 ^b	
Mean % viable seeds per fruit \pm SE (N of plants, N of fruits)	night-pollinated		19.5 \pm 2.7 ^a (7, 48)		23.2 \pm 3.8 ^{a,b} (5, 45)	26.3 \pm 1.8 ^a (11, 62)
	day-pollinated		13.7 \pm 2.3 ^a (12, 284)		15.6 \pm 2.7 ^a (12, 186)	33.4 \pm 4.2 ^a (11, 84)
	control		16.9 \pm 2.8 ^a (13, 333)		25.9 \pm 3.8 ^{a,b} (14, 268)	33.5 \pm 4.7 ^a (12, 239)
	hand-pollinated during day		36.1 \pm 4.2 ^b (10, 313)		37.8 \pm 3.7 ^b (14, 303)	

and bumble bees foraged most actively in the early morning, first gathering pollen and later collecting nectar. As observed by Schaffer et al. (1979), carpenter bee (*Xylocopa californica arizonensis* Cresson) activity tended to peak later in the morning and continue through the afternoon. Peak visitation (2.4 visits/min) for hummingbirds at the Coronado site was during mid-day, but was generally during mid-morning and prior to dusk at other sites. Noctuid moths and sphinx moths were most active shortly after dusk, although sphinx moths were active near dawn as well. Moths differed in their foraging behavior. Sphinx moths hovered above flowers and probed for nectar, only occasionally landing on the umbel, while noctuid moths landed and crawled upon umbels.

Visitation rate varied as a function of nectar production. Umbels with the highest visitation rates were predominately predehiscent/dehiscent (3.5–4.7 flower visits/min) and dehiscent/postdehiscent (3.5–3.9 flower visits/min), while rates were lowest in umbels containing predominately pistillate stage flowers (0.13–2.3 flower visits/min).

The proportion of stigma contacts in *A. chrysantha* (Parker Mesa and Peppersauce sites) was highest for pipevine swallowtail butterflies (*Battus philenor* L.) (0.25, $N = 20$ total visits scored), followed by carpenter bees (0.15, $N = 13$), houseflies (0.06, $N = 16$), bumble bees (0.04, $N = 156$), wasps (*Polistes* sp.) (0.01, $N = 58$), and honey bees (0.003, $N = 1115$). For *A. palmeri* (Santa Rita, Mustang, and Coronado sites), stigma contacts were most frequent for lesser long-nosed bats (1.00, $N = 32$), white-lined sphinx moths (0.18, $N = 49$), carpenter bees (0.07, $N = 158$), small unidentified bees (0.02, $N = 111$), bumble bees (0.01, $N = 628$), honey bees (0.002, $N = 774$), and broad-tailed hummingbirds (0.007, $N = 1431$). Of the total observed visits, diurnal insects only contacted stigmas ~1% of the time in both *Agave* species. Of the three diurnal insects (carpenter bee, bumble bee, and honey bee) that contacted stigmas of both *A. chrysantha* and *A. palmeri*, bumble bees had significantly higher stigma contact rates in *A. palmeri* (Fisher exact test, $P = 0.017$). Stigma contact rates for nocturnal insect visitors were 0% for *A. chrysantha* and 6% for *A. palmeri*, while 0 and 0.6% of bird visitations resulted in stigma contact in *A.*

chrysantha and *A. palmeri*, respectively. All of the observed bat visits to *A. palmeri* resulted in stigma contact.

The small size of the majority of insect visitors allowed them to “rob” nectar by entering flowers above the tepals, avoiding the exerted, receptive stigmas. Honey bees were large exploiters of floral rewards, and probably performed little pollination due to their small size and foraging habits. Honey bees tend to gather pollen from a single inflorescence (McGregor et al., 1959; Alcorn, McGregor, and Olin, 1961), suggesting that if contact with receptive stigmas occurred, fertilization was unlikely as these species are largely self-incompatible (see *Pollination studies* section; Howell and Roth, 1981). Stigma contact generally occurred when insects landed awkwardly on umbels and touched stigmas, or while foraging on freshly dehiscent anthers with erect filaments, they crawled over adjacent receptive stigmas. Native bumble bees and carpenter bees were fairly successful as pollinators as their intermediate size and awkward landing abilities often made it difficult for them to avoid exerted stigmas. Although larger than bees, hummingbirds and sphinx moths could generally hover and avoid stigmas unless foraging in the middle of moderate to large-sized umbels. Hummingbirds, despite their larger size, were more adept at avoiding stigmas than sphinx moths and were only slightly more effective than honeybees as pollinators. Although bat visitations rates were low, their large size and foraging behavior (dropping their head into or very near the umbel to lap nectar) resulted in regular contact with exerted stigmas.

Pollination studies—No significant differences were observed in mean fruit set between naturally day- and night-pollinated experimental umbels, except for *A. chrysantha* at the Peppersauce site where diurnally pollinated umbels had significantly higher fruit set than those which were nocturnally pollinated (Table 4). Although differences were nonsignificant at other sites, day-pollinated treatments had higher mean values than night-pollinated treatments at each site, including the *A. palmeri* population (Coronado site) where bats were seen. Day-pollinated umbels were generally pollinated as well as flowers open to both day and night visitors; control fruit set was

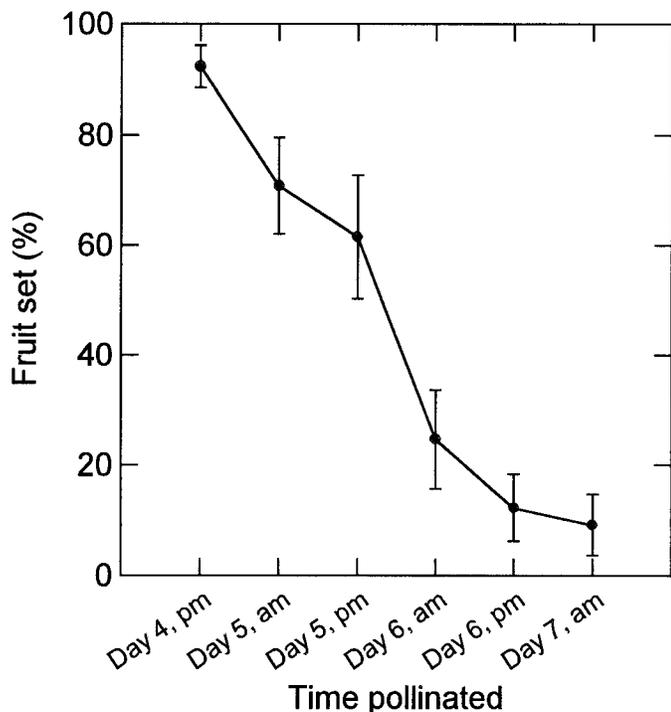


Fig. 4. Fruit set of *A. palmeri* as a function of nocturnal vs. diurnal pollination (Ft. Huachuca site). Vertical lines = 1 SE.

only significantly higher than day-pollinated fruit set at the *A. palmeri* Coronado site. Among populations of the two species, control fruit set was quite similar (16.6–23.8%).

Hand outcrosses at two sites (Peppersauce and Mustang) revealed that umbels of both species were pollen limited during the study periods, with hand-outcrossed fruit set being ~2.5 times as productive as controls. High variability in fruit set was observed within all treatments at all sites (Table 4).

No significant differences were noted between seed set of naturally night-, day-, or control-pollinated plants at the Peppersauce site (*A. chrysantha*) or the two *A. palmeri* sites (Mustang and Coronado) where seeds were counted (Table 4). Several fruits had <1% seed set. Hand-outcrossed umbels had significantly higher seed set than all naturally pollinated treatments of *A. chrysantha* and *A. palmeri*.

Fruit set in receptivity experiments of *A. palmeri* (Fort Huachuca site) was greatest in flowers that were hand-pollinated at the onset of receptivity (evening of day 4), and receptivity steadily decreased over the remaining floral stages (Fig. 4). Fruit set was significantly higher in flowers pollinated on the evening of day 4 than those pollinated the next morning (day 5) (one-way repeated-measures ANOVA, $F_{1,12} = 6.720$, $P < 0.024$). Flowers pollinated on the evening of day 5 also had significantly higher fruit set vs. those pollinated on the morning of day 6 (one-way repeated-measures ANOVA, $F_{1,12} = 8.094$, $P < 0.015$). However, there were no significant differences in fruit set between flowers pollinated in the morning or evening of day 5 or the morning or evening of day 6. Similar to fruit set results, seed set was signif-

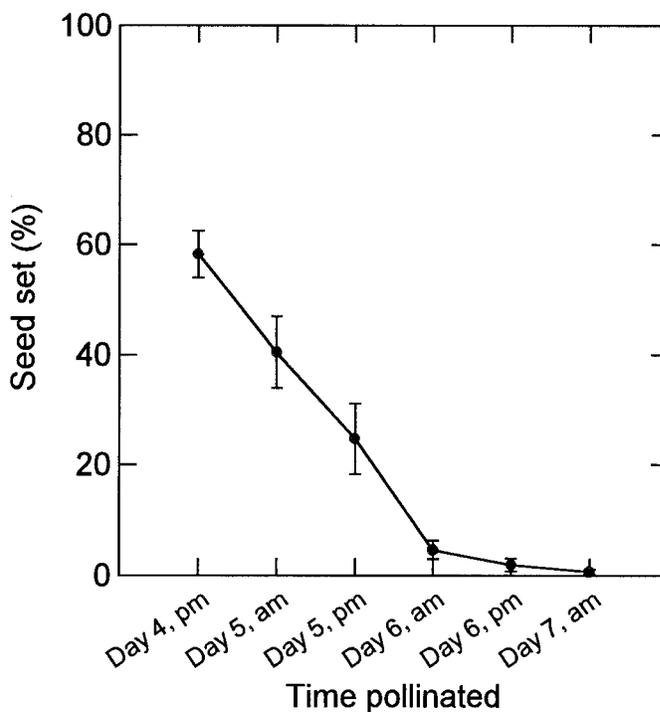


Fig. 5. Seed set of *A. palmeri* as a function of nocturnal vs. diurnal pollination (Ft. Huachuca site). Vertical lines = 1 SE.

icantly higher in flowers pollinated on the evening of day 4 than flowers pollinated the next morning on day 5 (one-way repeated-measures ANOVA, $F_{1,12} = 12.995$, $P < 0.004$) and in flowers pollinated on the evening of day 5 vs. those pollinated on the morning of day 6 (one-way repeated-measures ANOVA, $F_{1,12} = 12.957$, $P < 0.004$) (Fig. 5). Seed set was significantly different between flowers pollinated in the morning and evening of day 5 (day 5 a.m./day 5 p.m., one-way repeated-measures ANOVA, $F_{1,12} = 4.541$, $P < 0.050$).

Mean fruit set of self-pollinated flowers of *A. chrysantha* was $1.2 \pm 2.0\%$ and $4.8 \pm 1.8\%$ for *A. palmeri*. Mean fruit set for entire inflorescences for each population (experimental and control inflorescences pooled) are presented in Table 5. Fruit set was similar between populations, ranging from 17 to 25%, but was quite variable within populations.

DISCUSSION

Floral traits and pollinators—Species diversity in *Agave* is highest in the mesic habitats of central Mexico (Gomez Pompa, 1963) where the evolution of inflorescence and floral characters in paniculate agaves may have been significantly influenced by bat pollination (Howell, 1972; Gentry, 1982). However, many species have successfully radiated into more arid environments of northern Mexico and the southwestern United States, which are beyond or near the edges of the range of nectarivorous bats. *Agave palmeri* and *A. chrysantha* are two such species, representing the northernmost members of the Group Ditepalae, which is centered in the Sierra Madre Occidental. Kuban (1989) has argued that floral adaptations that arose as a result of bat pollination pre-adapted

TABLE 5. Fruit set (entire inflorescence) of *Agave chrysantha* and *A. palmeri* by population. Data are mean values, numbers in parentheses represent SE. Total potential fruits = number of fruits + number of aborted fruits. *N* = number of plants.

Population	Fruits/plant	Total potential fruits	Fruit set (%)	Range (%)
<i>A. chrysantha</i>				
Parker Mesa (<i>N</i> = 21)	406 (40.6)	1720 (155.3)	25.7 (2.7)	9.5–55.2
Peppersauce (<i>N</i> = 60)	332 (30.0)	1537 (93.8)	22.3 (1.4)	0.0–57.0
<i>A. palmeri</i>				
Santa Rita (<i>N</i> = 29)	281 (40.8)	1602 (92.8)	16.9 (1.9)	3.5–42.7
Mustang (<i>N</i> = 57)	336 (29.3)	1569 (91.9)	21.4 (1.5)	3.0–56.9
Coronado (<i>N</i> = 41)	430 (48.1)	2241 (166.2)	19.5 (1.8)	0.0–54.2

many agaves to a multiple vertebrate pollinator syndrome, and that these floral characters have been so effective that only minor changes in the agave “bat pollination syndrome” have occurred. For example, keeping flowers open during the day may be the key adaptation for using diurnal pollinators, as in some columnar cacti (Fleming, Tuttle, and Horner, 1996). Kuban (1989) noted that *A. havardiana*, although retaining many chiropterophilous traits, had differences in floral characters (such as nectar sugar concentrations and pistil receptivity) between populations that suggested adaptation to different levels of bat and bird visitation. *Agave chrysantha* has also retained several typical chiropterophilous features such as nocturnal anther dehiscence, nocturnal nectar production, and peak stigma receptivity on the first night of receptivity. Although *A. chrysantha* produces less nectar per night than *A. palmeri*, volumes are still great enough to be attractive to bats. However, results of this study suggest *A. chrysantha* has evolved minor shifts in floral characteristics (such as pollen dehiscence closer to dawn, production of nectar later at night, a sweeter floral scent, and bright yellow flower color) that may promote a multiple or generalist pollinator syndrome. The high fruit and seed set of hand-outcrossed (pollinated from 0700 to 1100, Peppersauce site) and naturally day-pollinated treatments (Parker Mesa and Peppersauce sites) suggest that pistil receptivity is also relatively high diurnally. These floral changes may have evolved to attract more diurnal visitors as *A. chrysantha* expanded its range northward where bats were no longer reliable visitors.

Although the floral characteristics of *A. palmeri* have not shifted towards diurnal pollinator adaptations to the degree shown by *A. chrysantha*, several traits indicate that some adaptations exist to allow for multiple pollinators. Pollen dehiscence and nectar production occurred nocturnally in *A. palmeri*, but large amounts of pollen and nectar were available in the morning to attract diurnal pollinators (Table 1). This study found nectar accumulation to be 2–2.5 times greater at all sites than that reported by Howell (1979) in the Chiracahua Mountains, but similar to values reported by Schaffer and Schaffer (1977) in the Huachuca Mountains. Hand-pollination (conducted from 0700 to 1100 on days 4–6, Mustang site; Table 4) and daytime receptivity experiments (conducted from 0600 to 0800 on days 5–7, Fort Huachuca

site; Figs. 4–5) demonstrated that significant diurnal fruit and seed production took place and that stigmas remained receptive throughout a 3-d period. These results are similar to the diurnal receptivity reported by Kuban (1989) in *A. havardiana* and Fleming, Tuttle, and Horner (1996) in saguaro cacti. Diurnal stigma receptivity was found to be higher in this study than in previous reports. Seed set the morning of day 5 was 40% and remained above 20% until the evening of day 6 (Fig 5), while Howell and Roth (1981) found diurnal seed set was ~20–30% between 0600 and 1800 on day 5. Although pistil receptivity decreased over time (Figs. 4–5), no significant differences in fruit or seed set were observed between naturally day- and night-pollinated treatments (Table 4). Thus, despite the existence of several floral features that indicate bat pollination in *A. palmeri*, these characters are general enough to allow visitation by a large variety of animals that can affect pollination, including many diurnal animals.

Gregory (1963, 1964) and Waser (1978) have noted that plants with characteristics of a particular pollination syndrome may often depend on other animals for the majority of pollination. While both *A. chrysantha* and *A. palmeri* exhibit characteristics of chiropterophily, fruit and seed set results showed diurnal animals were important pollinators in both *Agave* species. At one of the *A. chrysantha* study sites (Peppersauce), diurnal pollinators were the primary pollinators. Although a large number of diurnal animals visiting *Agave* flowers were “thieves,” pollination was achieved when animals accidentally made contact with receptive stigmas while foraging. Insect pollination (particularly by bumble bees and carpenter bees) occurred primarily by the “mess and soil” procedure, an imprecise method of pollination whereby insects become covered with pollen and eventually contact a stigma by “messing about” in simple flowers (Faegri and van der Pijl, 1979). Although the relative effectiveness of this rather primitive method was observed to be low, the high density and visitation rates of diurnal insects (Table 3) contributed substantially to pollination. Prolonged viability of agave pollen may also improve the pollination success of diurnal insects. Although pollen viability was not tested in these field experiments, Kuban (1989) reported that pollen may remain viable for 3 d after dehiscence. Pollen deposited on unreceptive stigmas by diurnal in-

sects during the flurry of postdehiscence visitation may later contribute to pollination when stigmas become receptive.

Diurnal pollination appeared to be largely due to insects as hummingbirds were seldom observed touching stigmas and other bird visitors were rare (Table 3). Kuban (1989) found many bird visitors to *A. havardiana* besides hummingbirds, while Martinez del Rio and Eguiarte (1987) found several passerines commonly visiting at one *A. salmiana* Otto ex Salm site, and only one species at another site. It is possible that birds were wary of observers within 10 m of plants, but approach and avoidance behavior was only rarely observed in this study.

Nocturnal pollinators (moths or sphinx moths) were important pollinators in three of the four sites where bats were not observed. Night pollinators contributed little to fruit set (2.2%) in *A. chrysantha* at the Peppersauce site, and this rate approximated self-pollination rates of fruit set (1.5%) reported by Sutherland (1982) and results of selfing experiments (1.2%) conducted at the site. Interestingly, sphinx moths were not observed at the Peppersauce site, while they were observed at the Parker Mesa site where nocturnal fruit set was 14.9%. Results of this study suggest that sphinx moths, along with large native bees (bumble bees and carpenter bees), are more important as pollinators of both *A. chrysantha* and *A. palmeri* than previously thought.

The nectarivorous Mexican long-tongued bat (*Choronycteris mexicana*) is present from roughly May–September in southeastern Arizona where its range overlaps with *A. palmeri* and southern populations of *A. chrysantha*. Although it is known to use *A. palmeri* as a food source (Howell and Roth, 1981) and may potentially use *A. chrysantha* as well, it was not observed at any of the study sites. Little is known about this rather solitary bat that roosts in very small groups, but it is a potentially important pollinator of agaves in localized areas.

Importance of the lesser long-nosed bat as a pollinator—Bats were not observed at any of the *A. chrysantha* study sites and were probably not present in southeastern Arizona during study periods. Lesser long-nosed bats do not normally arrive in southeastern Arizona until late July and were not known to be present in the region during early July when enclosure experiments were conducted. In fact, the arrival period of the lesser long-nosed bat to southern Arizona is well past the peak flowering period of *A. chrysantha* throughout most of its range, and many populations have completed flowering by the time of the bats' arrival. Because lesser long-nosed bats may forage 24–32 km from roosts when feeding on columnar cacti (Dalton, Dalton, and Schmidt, 1994; Sahley, Horner, and Fleming, 1993), some southern populations such as the Peppersauce site (16–32 km away from the closest known roosts) are presumably within the bats' foraging range. However, the majority of *A. chrysantha* populations are located well beyond this distance. The Parker Mesa site is located over 160 air km north of the closest known roost (Cockrum and Petryszyn, 1991), making even sporadic bat visitation unlikely. Results of pollination experiments, combined with present knowledge about the distribution and phenology of *A. chrysantha* and the migratory patterns of the lesser long-nosed

bat, suggest that bats are not currently important pollinators of *A. chrysantha*.

Fruit and seed set results of this study did not support the hypothesis that *A. palmeri* is strongly dependent upon the lesser long-nosed bat for pollination. Although *A. palmeri* is believed to be the primary food source of the lesser long-nosed bat during August–September in southeastern Arizona, the lesser long-nosed bat was not found to be the sole pollinator of *A. palmeri*. In no case did night pollinators contribute to higher fruit set than daytime pollinators or controls, even when bats were nocturnal visitors to flowers. Rather, both diurnal and nocturnal pollinators played an important role in the pollination of *A. palmeri*. Although a trend towards greater diurnal fruit set was observed in both species, high variability in fruit and seed set was present in all populations (Tables 4–5) and may be a factor in the lack of detectable significant differences between some treatments. Factors other than pollinator availability may also influence agave fruit and seed set, such as umbel position within the inflorescence (Sutherland, 1987), spatial arrangement of inflorescences (Howell and Roth, 1981), genetic factors, and resource availability (Sutherland, 1982).

In addition to fruit and seed set results, bat migration and phenological data do not provide strong evidence for an obligate mutualism between *A. palmeri* and the lesser long-nosed bat. Several workers (Cockrum and Petryszyn, 1991; Fleming, Nunez, and Sternberg, 1993) have suggested that seasonal movements of lesser long-nosed bats may be quite variable, particularly in the northern portion of their range. Lesser long-nosed bats arrive at known roosts in southeastern Arizona anytime from late July to mid-August (B. Alberti, Coronado National Monument, personal communication), while *A. palmeri* growing in the vicinity of these roosts may begin flowering as early as late June–early July. Flowering is asynchronous in *A. palmeri*, and one-third–one-half of the total floral crop may have finished flowering before the arrival of bats. Early-flowering plants would be completely or nearly through flowering in a late bat-arrival year. Kuban (1989) also noted unpredictable arrival of the greater long-nosed bat in relation to peak flowering of *A. havardiana*, a chiropterophilous agave that uses multiple pollinators presumably as a result of pollinator variability. Even when bats arrive during peak flowering, their numbers are often low. At the Fort Huachuca site, 87% of plants were flowering on 28 July 1998, but only a few hundred bats were present in nearby roosts (B. Alberti, Coronado National Monument, personal communication). The lack of synchrony in the flowering of *A. palmeri* and the arrival of lesser long-nosed bats does not indicate a tightly coevolved relationship, but suggests that *A. palmeri* employs a more generalist pollinator strategy, at least in northern populations.

Pollinator limitation—Fruit and seed set of hand-pollinated umbels greatly exceeded that of control umbels and indicated some pollinator limitation existed at the time of flowering of experimental umbels. Low numbers of bats, inefficient pollination by diurnal pollinators, and removal of pollen by bees may have all contributed to low pollination rates. Sutherland (1982) also found that hand-pollinated single branches (umbels) of *A. chrysan-*

tha had significantly higher fruit set than single branches that were naturally pollinated, but when all umbels (entire inflorescences) were hand-pollinated, there were no significant differences in fruit set between hand-, hand + natural, and naturally pollinated inflorescences (~20% fruit set). Sutherland concluded that paniculate agaves were primarily resource, rather than pollen, limited.

Although some pollen limitation was observed in outlying plants and some umbels of control plants in this study, percent fruit set of control umbels and entire inflorescences averaged ~20% (Tables 4–5), even at sites where bats were not observed. The “high” fruit set reported by Howell and Roth (1981) in bat-pollinated populations of *A. palmeri* may have been a result of inadequate sample size or the normal variability that can occur among branches, while the “low” results may be more representative of normal fruit set. A few plants had zero fruit set despite hand-pollination, implying that genetic incompatibilities or meiotic problems may have existed. However, results of this study suggest that pollen limitation and genetic factors play a smaller role than resource limitation in the pollination biology of agaves, and in particular, bats were not critical for adequate sexual reproduction in the populations of *A. palmeri* and *A. chrysantha* examined. Nonetheless, on a per visit basis, lesser long-nosed bats appear to be effective pollinators because some stigma contact generally occurs when bats visit. Kuban (1989) noted the greater long-nosed bat had higher pollinator effectiveness vs. other pollinators when visiting *A. havardiana*. As very effective pollinators, even low numbers of bats may enhance fruit and seed set to levels that can be supported by a particular plant’s resource pool.

Mutualisms and agaves—In a review of mutualisms, Bronstein (1994) noted that it is common for dependence within a mutualistic relationship to show some degree of asymmetry. While Valiente-Banuet et al. (1996) and Nassar, Ramirez, and Linares (1997) have shown that the relationship between nectar-feeding bats and columnar cacti is mutualistic and partially symmetrical in tropical zones (bats depend on cacti for food during part of the year while plants depend on bats for pollination), Fleming, Nunez, and Sternberg (1993) and Fleming, Tuttle, and Horner (1996) have illustrated an asymmetrical pattern in temperate zones (bats depend on cacti for food during part of the year, but plants do not require bats for pollination). Year-round residency of nectar feeding bats in tropical areas may have promoted tighter mutualistic relationships between some columnar cacti and bats, while the unpredictability of migrant nectarivorous bats in the Sonoran Desert may have favored the development of floral characters that enhance diurnal pollination (Fleming, Tuttle, and Horner, 1996). This same scenario may be equally as valid for chiropterophilous agaves distributed in temperate regions.

It is noteworthy that several striking similarities in floral and reproductive biology exist between columnar cacti and agaves distributed at the edges of the range of nectarivorous bats. Nectar production curves of organ pipe [*Stenocereus thurberi* (Engelm.) Buxbaum] and cardon [*Pachycereus pringlei* (S. Watson) B. & R.] cacti are like that of *A. palmeri*, with nectar production peaking

between 2000 and 2400 and gradually declining, while nectar production in saguaro [*Carnegiea gigantea* (Engelm.) B. & R.] peaks after 2400 and is more evenly distributed, similar to *A. chrysantha* (see Fleming, Tuttle, and Horner, 1996). Flowers of cardon and organ pipe opened shortly after sunset and closed by 0900, but flowers of saguaro were more similar to agave flowers, opening later at night (2300–2400) and remaining available to diurnal pollinators until 1500–1700 the next day. Like agaves, diurnal visitors contributed to the majority of fruit set in two of the three cacti species: birds were responsible for most fruit set in organ pipe, birds and bees were the principle pollinators in saguaro, while bats accounted for most fruit set in cardon. The relatively low importance of bats as pollinators of columnar cacti in the northern Sonoran Desert appears comparable to Ditepalae agaves that are distributed near the northern edge of the bats’ range.

The variable climatic conditions and low number of agave and cacti species in the southwestern United States and northern Mexico may have contributed to the variable and unpredictable migration behavior in northern populations of lesser long-nosed bats (Valiente-Banuet et al., 1996), and this may also explain why columnar cacti in the southwestern United States are not specialized for a particular type of pollinator (e.g., Alcorn, McGregor, and Olin, 1961; Fleming, Tuttle, and Horner, 1996) as compared to the highly specialized flowers of columnar cacti of southern Mexico (Valiente-Banuet et al., 1996) or Venezuela (Nassar, Ramirez, and Linares, 1997). Climatic variability may also be a factor in lack of pollinator specialization in agaves that are at the northern edge of the distribution of the genus. Community composition and species associations in the southwestern United States and northern Mexico have been in flux throughout recent geological time, and the effectiveness of mutualistic relationships would also seem to be unstable over the long term (Howe, 1984). Waser et al. (1996) noted that pollinator specialization is less likely in plants that experience spatial variation in pollinator fauna and temporal variation in pollinator service, particularly in species with few reproductive episodes. *Agave chrysantha* and *A. palmeri* seem to fit the bill as generalist species; they are monocarpic (after a prolonged vegetative stage, plants flower once and die), generally nonsuckering, and experience pollinator unpredictability and variability.

Both *A. chrysantha* and *A. palmeri* have diffuse interactions with a variety of organisms that may result in their pollination. A generalist pollinator strategy may be more adaptive in monocarpic species that occur in diverse habitats (*A. chrysantha*) or have large geographic ranges (*A. palmeri*), allowing plants to use a variety of pollinators that may vary both temporally and spatially. Reproductive characteristics of agave species at the northern edge of their distribution may be subject to more intense selection as a result of climatic and pollinator variability rather than selection for particular pollinators or dispersal agents. The conservation of “bat-adapted” traits may reflect the variable and relatively unpredictable suite of diurnal and nocturnal animals that inhabit the fluctuating climates at the edges of agave geographic ranges.

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