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SEED PRODUCTION AND POLLEN TUBE GROWTH FOLLOWING CROSS- AND SELF-POLLINATIONS IN *SPHAERALCEA LAXA* WOOT. & STANDL.

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ABSTRACT.—Little is known about reproductive biology in *Sphaeralcea*, a remarkably variable genus of mostly xerophytic and weedy perennial herbs. Understanding mating systems in *Sphaeralcea* may help explain the basis for the ecological success and complex variability exhibited in the genus. Our objective was to describe seed production and pollen tube growth following controlled self- and cross-pollination in *Sphaeralcea laxa* Woot. & Standl. from southern Arizona. Seed production after self-pollination was only about 12% of that present after cross-pollination. Significant reductions in seed production were also observed in crosses among siblings from a small, presumably colonial population. No consistent differences were observed in the number of pollen tubes in pistils between cross- or self-pollinations. *S. laxa* appears to be predominately cross-pollinated with no evidence of typical self-incompatibility mechanisms.

The genus *Sphaeralcea* (Malvaceae) contains over 50 taxa of mostly short-lived perennial herbs or sub-shrubs, commonly called “globemallows,” which are native to arid regions of North and South America (Kearney, 1935; La Duke, 1986). In the southwestern United States, species of *Sphaeralcea* are excellent colonizers of disturbed sites, and are common along roadsides and in vacant lots in urban areas (Shreve and Wiggins, 1964; La Duke, 1985). Their drought tolerance, and profuse flowering and seed production, have made some *Sphaeralcea* species attractive for use in revegetation of disturbed desert sites (Pendery and Rumbaugh, 1990).

Sphaeralcea species are extremely variable and complex hybrid forms are commonly observed even between morphologically distinct taxa. Taxonomic analyses have been complicated by this variability and the presence of diploid and polyploid individuals within taxa (La Duke, 1986). Relatively little is known about the genetic basis for this variability or of mating systems in *Sphaeralcea*. *Sphaeralcea* flowers are hermaphroditic (Kearney, 1935), but based on the propensity for wide hybridization (La Duke, 1986), it could be assumed that cross-pollination is more common than self-pollination. The possibility for self-fertilization cannot be ruled out, however, since anthesis frequently begins before the corolla opens, and stigmas typically exert through the stamen

after anthesis (Kearney, 1935). Moreover, the apparent success of many globemallows in establishing colonial populations in disturbed sites in the absence of vegetative reproduction (S. E. Smith, unpubl. obser.) suggests that seed production may be possible following self-pollination or cross-pollination among closely related individuals (Brown and Burdon, 1987). Understanding the mating systems of *Sphaeralcea* may help to explain both its ecological success and the basis for the complex patterns of variability observed within the genus.

Many mechanisms have evolved to prevent self-fertilization and elevate outcrossing in plants with hermaphroditic flowers (de Nettancourt, 1977). These mechanisms may act both prior to and following fertilization. Prefertilization mechanisms are typified by a variety of self-incompatibility systems, where self-fertilization is prevented by inhibiting the growth of male gametophytes in genetically identical pistils (Charlesworth, 1985). Alternatively, inbreeding depression in the fitness of self progenies is one of the most common mechanisms acting after fertilization to maintain outcrossing (Olmstead, 1989). In species where outcrossing appears to predominate, the basic mechanisms controlling mating behavior can be elucidated by studying seed production and pollen tube growth following self pollinations and cross pollinations among

plants differing in genetic relationship. Furthermore, comparing seed production in individuals from relatively small populations, which may contain only close relatives, with that from larger, more variable populations permits a better understanding of the relationships between mating system and colonization success.

The goal of this study was to describe the breeding system in *Sphaeralcea laxa* Woot. & Standl., which is native to plains and foothill areas from central Arizona to Texas and south into Sonora, Mexico, occurring most frequently on highly calcareous soils (Shreve and Wiggins, 1964). Using greenhouse-grown plants from diverse *S. laxa* populations from southern Arizona, our objectives were to 1) quantify seed production following controlled self- and cross-pollination among genetically related and unrelated plants from relatively small and large populations; and 2) examine pollen tube growth following self- and cross-pollinations to determine whether self-incompatibility reactions are present before fertilization.

MATERIALS AND METHODS—All plants used in this study were diploid ($2n = 10$) and were derived from seed of open-pollinated flowers. Classification followed Kearney (1935) and chromosome counts were made using the procedure described by Smith et al. (1984). Three populations used in seed production studies (nos. 1, 4 and 26) were located in moderately disturbed urban lots in Tucson, Arizona that were at least 1 km apart. Population 1 was located along Allen Road approximately 300 m E of Campbell Avenue. Population 5 was situated along Calle Chaparita, 80 m S of Broadway Boulevard. Population 26 was located SE of the intersection of Irving Avenue and Timrod Avenue. A fourth population (no. 6) was in an undisturbed Upper Sonoran Desert Shrub community, 50 m E of Sandario Road, 11.4 km S of Arizona State Highway 86. This site is approximately 32 km SW of central Tucson. Voucher specimens from each population were deposited at the University of Arizona Herbarium. Individuals in each population were contained within less than 2500 m² and no other *Sphaeralceas* were present within 500 m of the margin of the population. All living plants were counted in each population at the time of seed harvest. The number of plants differed among populations (Pop. 6, $n = 8$; Pop. 1, $n = 30$; Pop. 4, $n = 52$; Pop. 26, $n > 100$). Plants used in studies of pollen tube growth were derived from Populations 1, 6, and 26 and additional populations isolated by at least 1 km from other sampled sites.

Seed was collected separately from randomly selected plants in all populations in October through December 1989. Seeds were scarified with sandpaper and sown

in the greenhouse in March 1990. All plants used in seed production/pollen tube studies were grown in 1-l containers with 24-h fluorescent lighting. Mean daily minimum temperatures in the greenhouse during the pollination period was $16 \pm 2^\circ\text{C}$ and the mean daily maximum was $31 \pm 2^\circ\text{C}$.

Seed production was studied using a 12-plant diallel cross (including reciprocal crosses) performed in the greenhouse. Plants from three half-sib families (progenies from randomly selected plants in the field) from Populations 1, 4, and 6 were included as were two plants from Population 26. Each of the 144 pollinations (including self pollinations) in the diallel was made once. All pollinations were made by hand without emasculation. Flowers were considered receptive when styles were fully exerted from the staminal column. Pollen was applied to the stigmas by using detached, entire staminal columns of the pollen parent. A total of five flowers were also self pollinated on each of 78 plants from the four populations (Pop. 1: 33; Pop. 4: 30; Pop. 6: 15). For self pollinations, pollen was applied to five flowers per plant as in crosses using staminal columns from flowers on the same plant. For all pollinations, fruit development was monitored at least twice each day and fruit abortion was recorded. Mature fruits were harvested before dehiscence. In these fruits, the number of carpels and mature and immature/aborted (shriveled) seeds were recorded.

Fluorescence microscopy was used to examine the growth of pollen tubes following self- and cross-pollination (Martin, 1959). Two flowers pollinated on the same day were analyzed from both self- and cross-pollinations on six plants from Populations 1, 4, and 6. For all crosses, pollen was taken from a single plant from Population 26. Plant growth and pollination conditions were as in seed production studies. Flowers were fixed, 48 h after pollination, in 80% ethanol, glacial acetic acid, and formalin (8:1:1 ratio by volume) at 4°C . After 24 h, flowers were placed in 8M NaOH for 18 h and then held in water at 4°C . Before microscopic observation, flowers were transferred to a 0.1 M K_3PO_4 , 0.1% (w/v) aniline blue solution for one h. Stained pistils were removed from each flower and five individual styles observed under a fluorescence microscope (356 nm). For each style, two observers recorded the number of pollen grains on the stigma, pollen tubes penetrating the stigma surface, and pollen tubes at the base of the stigma. Observers did not know the pollination history of the pistil.

RESULTS—A total of 25.6% of the plants in populations 1, 4 and 6 produced seed when self-pollinated. Only 7.2% of all self-pollinated flowers set fruit, and a mean ($\pm SE$) of 1.9 ± 0.4 mature seeds were produced per self-pollinated flower. In fruits producing seed, an average of an additional 9.5 ± 0.7 immature/aborted seeds were

recovered following self pollination. This compares to the diallel crosses where 100% of plants produced at least some seed. Of all flowers cross pollinated, 88.2% set fruit and a mean of 14.8 ± 1.2 mature seeds were produced per flower pollinated. A mean of 3.9 ± 0.3 immature/aborted seeds were present in fruits producing seed.

There were no significant differences in seed production either as a pollen or seed parent among the three populations when all half-siblings from populations 1, 4 and 6 were crossed with the same two plants from Population 26 (Table 1). However, in crosses among siblings from the same population, seed production was significantly reduced in plants from the smallest population (no. 6) compared to the two larger populations. Seed production in crosses among siblings from Population 6 was about 15% on average of that observed in crosses with presumably unrelated individuals but was about twice that expected for matings representing the most severe inbreeding (self pollination).

The six plants used in cytological studies of pollen tube development did not produce seeds following self pollination of five flowers but did produce a mean of 18.7 ± 0.9 seeds per flower pollinated in crosses with a single unrelated plant. Cross pollination resulted in significantly more pollen tubes in the stigma (as % of pollen grains on stigma) than self pollination for only one of the six plants (no. 2-1, Table 2). In none of the six plants were there significantly more pollen tubes at the base of the style in pistils following cross pollination than following self pollination. In fact, over all six plants, there were significantly more pollen tubes in the stigma in self-pollinated pistils than in those cross pollinated. Pollen tube number at the base of the style near the most distal ovule did not differ between the types of pollination. There also was no evidence of any differences in the basic morphology of the pollen tubes among self- and cross-pollinations as has been associated with self incompatibility in certain species (Williams et al., 1982). Clearing did not permit consistent observation of pollen tubes near the ovules, however, pollen tubes were occasionally observed around distal ovules in pistils from both self- and cross-pollinated flowers.

DISCUSSION—While not directly addressing the relative frequency of self- or cross-pollination that occurs in nature, data from controlled pollinations suggest that some mechanism(s) limit the

TABLE 1.—Mean number of mature seeds produced per flower pollinated ($\pm SE$) in reciprocal crosses among plants from three half-sib families and two unrelated plants of *Sphaeralcea laxa* in a 12-plant diallel with self pollinations excluded.

Popula- tion number	Original popula- tion size	Number of siblings	Number of crosses within diallel			Seeds per flower pollinated							
			Among siblings	All outcrosses	With common seed parents ¹	As pollen parent		As seed parent		With common pollen parents ¹		All outcrosses	
						Among siblings	All outcrosses	Among siblings	All outcrosses	Among siblings	All outcrosses		
4	52	3	6	54	22.2 \pm 1.7	21.6 \pm 0.7	18.1 \pm 0.9	20.9 \pm 0.7	21.5 \pm 0.3	15.8 \pm 1.7	14.1 \pm 1.7	14.6 \pm 1.6*	
1	30	3	6	54	16.2 \pm 3.5	14.9 \pm 3.1	14.8 \pm 0.8	16.3 \pm 1.8	14.8 \pm 3.0	14.1 \pm 1.7	14.1 \pm 1.7	14.1 \pm 1.7	
6	8	4	12	64	21.0 \pm 1.4*	3.4 \pm 1.8*	12.1 \pm 1.9*	21.0 \pm 1.4*	3.4 \pm 1.8*	14.6 \pm 1.6*	14.6 \pm 1.6*	14.6 \pm 1.6*	

¹ Two unrelated plants from Population 26 (i.e., not half-siblings) crossed to all other plants.

* Seeds per flower significantly different in crosses among half-siblings and outcrosses to common parents or all outcrosses (LSD, $P \leq 0.05$).

TABLE 2—Mean number of stigmatic pollen grains and pollen tubes ($\pm SE$) at two positions in pistils of *Sphaeralcea laxa* following self and cross pollination.

Plant number	Pollination type	Number of pollen grains on stigma	Number of pollen tubes (as % of pollen grains)	
			In stigma	At style base ¹
12-1	Self	54.4 \pm 3.1	34.6 \pm 2.5	29.1 \pm 3.0
	Cross	47.5 \pm 3.5	30.7 \pm 2.6	21.2 \pm 3.0
14-1	Self	25.5 \pm 2.3	40.1 \pm 3.7*	43.7 \pm 3.9*
	Cross	36.1 \pm 3.8	21.4 \pm 1.9*	30.5 \pm 2.5*
2-1	Self	24.9 \pm 2.0	13.5 \pm 2.8*	24.0 \pm 5.6
	Cross	48.8 \pm 4.0	21.0 \pm 2.4*	29.4 \pm 1.9
2-3	Self	29.8 \pm 1.2	38.9 \pm 2.1	39.7 \pm 2.0*
	Cross	30.5 \pm 1.4	41.0 \pm 2.6	55.3 \pm 2.5*
6-3	Self	48.5 \pm 3.2	40.1 \pm 2.8*	35.1 \pm 3.7*
	Cross	53.2 \pm 5.3	22.5 \pm 2.2*	17.2 \pm 3.9*
7-5	Self	33.1 \pm 2.2	49.7 \pm 3.3*	38.9 \pm 2.6*
	Cross	51.0 \pm 4.8	32.6 \pm 2.6*	23.2 \pm 2.4*
Mean	Self	35.4 \pm 1.4	36.0 \pm 1.6*	35.1 \pm 1.6
	Cross	44.1 \pm 1.8	29.3 \pm 1.3*	31.6 \pm 1.7

¹ At junction between ovary and style, immediately distal to most distal ovule within ovary.

* Percentages significantly different between two types of pollination (LSD, $P \leq 0.05$).

production of seed from matings among close relatives in *S. laxa*. Reduced seed production from crosses between siblings suggests that members of Population 6 were comparatively more closely related genetically to each other than were siblings within the other populations. This may indicate that this population was in the early stages of colonization. Nevertheless, seed production was possible between close relatives (either through self- or sib-mating), suggesting that successful colonization is possible by a single individual without cross pollination. Importantly, all plants in these studies were grown in the greenhouse where they were presumably under conditions of limited abiotic stress. Based on results from other species (e.g., Waller, 1980), the possibility exists that higher levels of fertility in matings among genetically related individuals may occur under the generally more xeric conditions which *S. laxa* experiences in nature.

Cytological observations of pollen tubes indicate that reduced self fertility in *S. laxa* was not due to typical self-incompatibility reactions, i.e., inhibition of pollen germination or pollen tube

growth in the style (de Nettancourt, 1977). Therefore, reduced seed production following self-pollination in *S. laxa* is apparently due to either differences in fertilization success between self- and cross-pollination and/or to differences in the development of ovules from the two types of pollination. The comparatively high number of immature/aborted seeds observed following self pollination suggests that the development of seeds resulting from self pollination is frequently inhibited by events occurring post-fertilization. Increased immature/aborted seed with selfing may represent development failure in the most inbred embryos. This may represent one mechanism of maintaining or increasing mean heterozygosity in populations where matings among close relatives predominate (Olmstead, 1989).

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