Cross-Hybridization and Seed Germination in *Opuntia* Species

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ABSTRACT

The objective of this study was to examine aspects of floral biology of several *Opuntia* clones as a prerequisite to initiation of a comprehensive breeding program. The first phase examined anthesis as a function of flower development and pollen viability (*in vitro*). The second phase examined techniques necessary to ensure reliable hybridizations, including fruit set in (1) natural pollination, (2) flowers bagged before emasculation, (3) flowers bagged after emasculation, (4) hand pollination of bagged, emasculated flowers with pollen from same plant, (5) hand pollination of bagged, emasculated flowers with pollen from a different accession. No fruit set was obtained following emasculation without pollination, while up to 50% fruit set was obtained with some hybridizations. This suggested that selfing can be avoided and that reliable

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hybridizations can be made. A seed germination trial compared 25°C, 30°C, and 35°C soil temperatures with sand, a commercial cactus soil, and coarse vermiculite. With the commercial cactus soil seeds with and without a gibberellic acid soak were compared. Optimum germination without gibberellic acid occurred at 35°C in vermiculite. The germination of gibberellic-acid-treated seeds was greater than non-gibberellic-acid-treated seeds.

INTRODUCTION

Opuntia is becoming increasingly important as a crop for exotic fruit, vegetable, and forage production in Mexico, USA, Chile, Argentina, Israel, Italy, and South Africa (Pimienta-Barrios and Munoz-Urias, 1994; Flores-Valdez, 1994; Felker, 1994). Fruit varieties of *Opuntia* are restricted to subtropical areas, which rarely encounter freezing temperatures (Russell and Felker, 1987). By contrast, *Opuntia* has about 170 species (Gibson and Nobel, 1986) that are distributed from below sea level in the deserts of California to elevations of over 4700 m in the mountains of Peru and from tropical regions of Mexico where temperatures are always above 5°C, to regions in Canada that experience –40°C each winter (Nobel 1988, 1994; Keeley and Keeley, 1989). This tremendous ecological diversity hints at remarkable prospects for improving cold tolerance of *Opuntia* fruit varieties through breeding.

Unfortunately, there is little quantitative data in the literature on which to base a traditional plant-breeding program. The *Opuntia* genetic structure has been modified via natural hybridization in geographically isolated areas and have various ploidy levels (Gibson and Nobel, 1986). This has been facilitated by the presence of self-compatibility in Sicilian cultivars and *Opuntia rastrera* (Damigella, 1958; Mundujano et al., 1996). Apomixis also occurs in some species, including *O. aurantiaca* Lindl., *O. dillenii* Haw, *O. glaucophyla*, *O. leucantha* Link, *O. rafinesqui*, *O. tortispina* Engelm (Mondragon-Jacobo and Pimienta-Barrios, 1994).

MATERIALS AND METHODS

Opuntia species were collected from Texas, Mexico, and Chile and planted at Kingsville in south Texas. Six-year-old plants from cuttings were used in this study, including a clone native to Texas, i.e., *O. lindheimeri*, a naturalized possible hybrid of *O. lindheimeri* (1233); the very cold-hardy *O. ellisiana* (1464); the Mexico fruit clones, *O. streptacantha* (1280 and 1281), *O. hyptiacantha* (1287), *O. ficus-indica* (1294), *O. megacantha* (1383); and the Chilean fruit clones, *O. ficus-indica* (1319), *O. ficus-indica* (1320), *O. ficus-indica* (1321).

Floral development as a function of anthesis was measured in two contrasting clones. *O. ficus-indica* (1320) from Chile was used as a model for fruit clones, while the naturalized, cold-hardy, spineless clone 1233 with small (30 g) low-sugar fruits was used as a model for sources of cold-hardy germplasm. The age of flower buds was estimated by observing the development of marked buds at beginning bloom and 1 day, 2–3 days, or 4–6 days before bloom. Four to seven flower buds were collected at each age, and the diameter and length of flower bud, the largest diameter of perianth, perianth diameter at the mouth of receptacle, perianth height over and inside the receptacle, and pistil length were measured (Figure 1). Anther dehiscence and pollen spread in the flower were observed with a stereo microscope. Pollen spread was examined in 30 anthers and was deemed to have occurred when pollen was spread from 1 of the 30 anthers.

Pollen Germinability (in vitro)

Five flowers (at anthesis) were sampled from each clone. Fresh pollen was spread on a 1% agar film on a glass slide containing 13% sucrose, 100 ppm H_3BO_4, 100 ppm $Ca(NO_3)_2 4H_2O$, 100 ppm $MgSO_4$, and 100 ppm KNO_3 as previously described (Weiss et al., 1994). The slide was closed in a petri dish with 100% humidity at 25°C for 24 hours, then 13% sucrose water solution was used to dilute the agar surface, after which pollen germinability was examined under a stereo microscope. Grains were scored as germinated when the tube length exceeded the diameter of the grain itself.

Breeding System and Pollination

Brown paper bags were used to cover the flower buds to avoid cross pollination. Two degrees of emasculation were examined. A very tedious full-emasculation treatment removed all the anthers and the perianth over the receptacle, and washed the stigma with water. A less tedious partial emasculation removed only the perianth over receptacle and anthers over stigma, and washed the stigma with water. To prevent pollen from the anthers below the stigma from pollinating the stigma, masking tape was used to separate stigma from anthers. Flower buds were emasculated or partially emasculated at more than four days before bloom for breeding experiments. Emasculated and partially emasculated flower buds were hand-pollinated with fresh pollen one day after emasculation and every 2 days until the stigma wilted.

The following pollination/emasculation treatments were examined:

- Control - tagged flower bud
- Flower bud bagged to exclude floral visitors
- Fully emasculated and bagged
- Partially emasculated and bagged without pollination
- Fully emasculated, self-pollinated and bagged
- Partially emasculated, self-pollinated and bagged
- Fully emasculated, cross-pollinated with a different clone and bagged
- Partially emasculated, cross-pollinated with a different clone and bagged

Paper bags were removed two to three weeks after pollination. Because ants visited emasculated flower buds, Sevin insecticide was used in partially emasculated non-pollinated and cross-pollinated and bagged in clone 1280.

Due to past difficulty in germination of *Opuntia* seeds by our group and others (Dr. Ueckert), we examined various treatments to enhance seed germination. Seeds of *Opuntia ficus-indica* accession number 1294 were used for this trial. The experimental unit consisted of a 10 cm by 10 cm by 10 cm plastic pot that contained 25 seeds. Four pots were used for each treatment combination. Temperatures of 25°C, 30°C, and 35°C were examined. The 25°C treatment was a room-temperature treatment. Stanfield heating pads were used to achieve the 30°C and 35°C temperatures. The thermostat for each heating pad was placed inside one of the pots. The pots

were placed under a fluorescent-light bench in the laboratory. Three soil media [coarse vermiculite, washed river sand, and a commercial cactus-nursery mix ("Schultz-instant" cactus and succulent soil)] were examined at each temperature. At each temperature four pots of the commercial cactus soil mix were examined with *Opuntia* seeds that had been soaked for one hour in a 35 mg/l solution of gibberellic acid (GA3).

RESULTS

Over the 4 to 6 day period prior to anthesis there was no relationship between the time to bloom and the length or diameter of flower bud (Table 1). The diameter and height of perianth, and pistil length, increased greatly before bloom. The fruit clone 1320 had longer flower buds than the nonfruit, cold-hardy clone 1233, and had a smaller perianth over the receptacle and deeper perianth part inside the receptacle. The anthers dehisced about 2 to 3 days before bloom in both clones. Some anthers were over the stigma. There was some pollen spread on the stigma at 2 to 3 days before bloom in clone 1233, suggesting the possibility of self-pollination. Much pollen occurred on the stigma 1 day before bloom in both clones 1233 and 1320 (Table 1). Pollen grains were easily washed away from the stigma with water at 2 to 3 days before bloom, but hard to clear totally from the stigma at 1 day before bloom. Although the pollen grains were washed away, it is not known whether the pollen tube penetrated the stigma; that would have permitted self-pollination. To avoid pollen spread to the stigma and self-pollination, flower buds should be emasculated at about 4 days before bloom, and washed with water. Masking tape was used to separate the stigma from anthers in partial emasculation. To ensure there was no problem with pollen viability, pollen germinability was tested *in vitro*, and found to be more than 70% in all clones (Table 2).

There was remarkable variation among clones in fruit set of the unemasculated, open-pollinated flowers and in the bagged flowers (Table 3). Clone 1233, a thornless, cold-hardy clone, which we believe may be a hybrid between the native *O. lindheimeri* and an unknown parent, set no fruit under open-pollinated or bagged conditions in 10 replications. It only set one fruit out of 10 pollinations when crossed with the Texas native *O. lindheimeri*. This "apparent sterility" under open-pollinated conditions suggests this clone might be a sterile hybrid. Also *O. lindheimeri* 1464 had very low fruit set under open-pollinated bagged or unbagged treatments. In contrast, the highly fruitful clones *O. ficus-indica* 1294 and 1320 and *O. streptacantha* 1281 had 100% fruit set in both bagged and unbagged treatments. There was no significant difference between the control and bagged treatments for any of the clones.

In the highly self-fertile fruit clones 1294, 1320, and 1281, emasculation totally eliminated fruit set. Partial emasculation in clone 1280 with the Sevin (to control pollinating ants) but without pollination also had no fruit set. In all cases the fruit weight produced from emasculation with either artificial self-pollination or artificial cross-pollination was lower than the control fruit weight (Table 3).

There were clear effects of temperature, germination media, and hormone treatment in the germination of *Opuntia ficus-indica* accession 1294 (Table 4). Clearly, the 35°C germination temperature was superior to both the 25°C and 30°C treatments. The coarse vermiculite medium had significantly greater germination than the commercial cactus soil mix. Coarse river sand had practically little or no germination at any temperature. The seeds that were soaked in gibberellic acid had much faster germination than the equivalent treatment without gibberellic acid. Unfortunately, the gibberellic acid treatment was not used in the vermiculite medium.

DISCUSSION

This study provides key information about artificial pollination and seed germination necessary to implement serious breeding programs for *Opuntia*. Different *Opuntia* clones vary widely in self-compatibility and crossability, however, we clearly demonstrated that many hybrids can be made. Full emasculation (as defined earlier) at 4 days before anthesis, followed by bagging to exclude insects, was necessary to ensure that artificial hybridizations were reliable. Germination in coarse vermiculite was more successful than other media, and gibberellic-acid-stimulated germination.

The size of perianth (diameter and height) could be used for estimating the bloom time of flower buds, but not the size of flower bud. The native Texas clone (*O. lindheimeri*), and 1281, 1294, and 1320 were self-compatible, as previously reported in other species (Damigella, 1957; Mundujano et al., 1996). It was not possible to determine whether clones 1233 and 1464 were self-compatible. Due to self-compatibility, emasculation is required to avoid self-pollination. As the flowers became large, they became easier to emasculate, due to greater space around the anthers and pistil before bloom. *Opuntia* flowers should be emasculated about 4 days before bloom to avoid self-pollination because all clones tested had high pollen germinability (*in vitro*). However, if the pollen germinated at the stigma only after bloom (*in vivo*), emasculation could be done later, since the pollen grains were easily washed from the stigma with water 2 to 3 days before bloom.

Clones 1233 and 1464 had low fruit set, thus were not suitable for use as the female in hybridization experiments. The native *O. lindheimeri* had no fruit following full emasculation and either self- or cross-pollination. Perhaps less damaging partial emasculation treatments could be developed. Alternatively, *O. lindheimeri* could be used as the male in hybridizations. As there was no fruit set after emasculation without pollination, the possibility of apomictic seed production must be ruled out. This is in contrast to other reports for *Opuntia* species (Mondragon-Jacobo and Pimienta-Barrios, 1994). The seeds from partial emasculation without pollination were perhaps caused by visiting ants, because there was no fruit set in 1280 after the application of Sevin in partial emasculation. The fact that lower fruit set, fewer seeds, and smaller fruits occurred in emasculated self-pollinated and cross-pollinated clones 1281, 1294, and 1320 than in the open-pollinated control suggests that physical damage occurred to the pistil or the ovary during emasculation. This physical damage may have been the cause for decreasing fruit development, because partial emasculation resulted in higher fruit set than full emasculation. Sevin should be used in partial emasculation to protect the flower buds from floral visitors to avoid self-pollination. The hybridization that occurred between species supports the proposed importance of natural hybridization in the evolution of *Opuntia* population (Gibson and Nobel, 1986).

The native *O. lindheimeri*, and clones 1233 and 1464, although cold hardy, albeit with small fruit, cannot be recommended for use as the female in hybridization because they had lower fruit set than clone 1281, 1294, and 1320. This is unfortunate because they had a large perianth before bloom that made emasculation easy. In *Opuntia* breeding, clones such as 1281, 1294, and 1320 should be used as female parents because of higher fruit set. All clones of *Opuntia* could be used as male in hybridization since they had over 70% pollen germinability. Emasculation or partial emasculation with Sevin protection should be conducted before bloom since *Opuntia* is self-compatible.

As production of numerous hybrids without the ability to germinate seeds would be futile, it is most reassuring that treatments have been identified to enhance the germination of *Opuntia* seed. While temperature, media type, and gibberellic acid treatment all had profound effects on seed germination, none of these variables has been optimized for seed germination. Perhaps other as yet unidentified combinations of media type, hormone treatment, and environmental parameters will stimulate further seed germination.

With the identification of successful techniques for emasculation, pollination, and seed germination of resulting hybrids, all the requisite methods are in hand to begin serious breeding programs for *Opuntia.*

REFERENCES

Damigella, P., 1958. I1 fico d'india e le cultivar della Sicilia orientale. Tecnica Agricola. 10:474–502.

Felker, P., 1994. Forage and fodder production and utilization. *In*: Barbera et al. (ed.). "Agro-ecology, cultivation and uses of cactus pear. FAO International Technical Cooperation Network on Cactus Pear. pp. 144–154.

Flores-Valdez, C. A., 1994. "Nopalitos" production, processing and marketing. *In*: Barbera et al. (ed.). "Agro-ecology, cultivation and uses of cactus pear. FAO International Technical Cooperation Network on Cactus Pear. pp. 92–99.

Gibson, C. G. and P. S. Nobel, 1986. The cactus primer. Harvard Univ. Press, Cambridge. pp. 286.

Keeley, J. E. and S. C. Keeley, 1989. Crassulacean acid metabolism (CAM) in high elevation tropical cactus. Plant Cell Environ. 12:331–336.

Mondragon-Jacobo, C. and E. Pimienta-Barrios 1994. Propagation. *In*: Barbera et al. (ed.). "Agro-ecology, cultivation and uses of cactus pear. FAO International Technical Cooperation Network on Cactus Pear. pp.64–70.

Mundujano, M. D. C., C. Montana, and L. E. Eguiarte, 1996. Reproductive ecology and inbreeding depression in *Opuntia rastrera* (Cactaceae) in the Chihuahuan desert: Why are sexually derived recruitments so rare. Amer. J. Bot. 83(1):63-70.

Nobel, P. S., 1988. Environmental biology of agaves and cacti. Cambridge Univ. Press, New York. pp. 270.

Pimienta-Barrios, E., and A. Munoz-Urias, 1994. Domestication of *Opuntia* and cultivated varieties. *In*: Barbera et al. (ed.). "Agro-ecology, cultivation and uses of cactus pear. FAO International Technical Cooperation Network on Cactus Pear. pp. 58–63.

Russell, C. E. and P. Felker, 1987. The prickly pear (*Opuntia spp.* Cactaceae): a source of human and animal food in semiarid regions. Econ. Bot. 41:433–445.

Weiss, J., Nerd A. and Y. Mizrahi, 1994. Flowering behavior and pollination requirements in climbing cacti with fruit crop potential. HortScience 29(12):1487–1492.

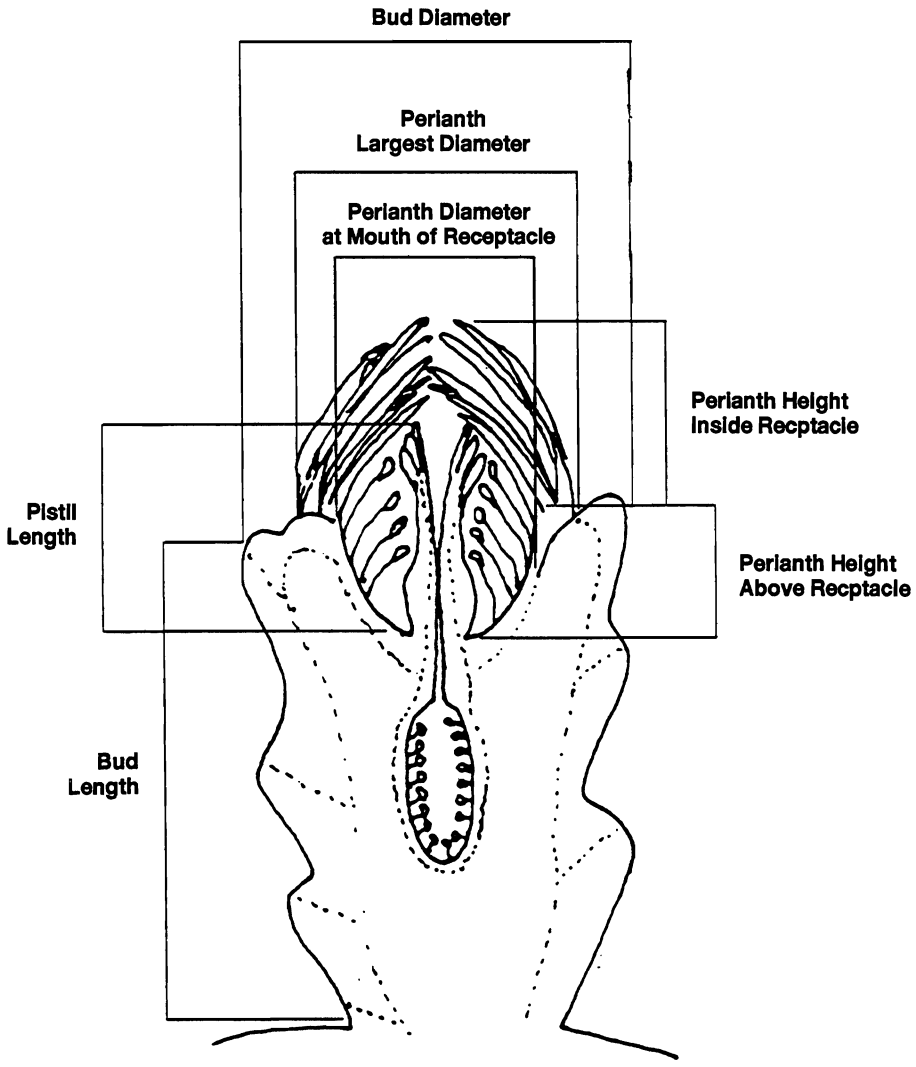

Figure 1. Longitudinal Section of *Opuntia* Flower Bud

Table 1. Flower Bud Structure, Anther Dehiscence, and Pollen Spread Before Bloom

***O. spp.* (1233) (Texas)**				
	0	**1**	**2--3**	**4--6**
Bud length	3.9 ±0.83	4.0 ±0.48	4.4 ±0.38	4.2 ±0.56
Bud diameter	3.2 ±0.50	2.8 ±0.26	2.9 ±0.31	3.0 ±0.30
Perianth largest diameter	3.4 ±0.26	2.8 ±0.21	2.8 ±0.37	2.4 ±0.15
Perianth diameter at the mouth of receptacle	1.7±0.23	1.5 ±0.28	1.5 ±0.11	1.3 ±0.09
Perianth height above the receptacle	4.2 ±0.22	3.2 ±0.31	3.1 ±0.63	2.4 ±0.23
Perianth height inside the receptacle	0.73 ±0.13	0.55 ±0.13	0.55 ±0.10	0.53 ±0.08
Pistil length	2.9 ±0.12	2.6 ±0.34	2.3 ±0.35	1.7 ±0.12
Anther dehiscence	100%	100%	100%	0
Pollen spread	100%	100%	< 5%	0
***O. ficus-indica* (1320) (Chile)**	**Days Before Bloom**			
	0	**1**	**2--3**	**4--6**
Bud length	4.9 ±0.48	5.5 ±0.15	5.4 ±0.18	4.7 ±0.33
Bud diameter	3.0 ±0.21	2.7 ±0.24	2.8 ±0.42	2.6 ±0.27
Perianth largest diameter	2.9 ±0.31	2.4 ±0.17	2.0 ±0.20	1.8 ±0.19
Perianth diameter at the mouth of receptacle	1.9 ±0.24	1.8 ±0.10	1.5 ±0.08	1.3 ±0.14
Perianth height above the receptacle	2.6 ±0.10	1.8 ±0.10	1.2 ±0.15	1.0 ±0.13
Perianth height inside the receptacle	1.2 ±0.08	1.3 ±0.21	1.0 ±0.16	1.0 ±0.14
Pistil length	2.4 ±0.10	2.4 ±0.13	1.8 ±0.11	1.5 ±0.12
Anther dehiscence	100%	100%	100%	0
Pollen spread	100%	100%	0	0

Values are means (n=4 to7) ± standard deviation (cm), or percentage.

Table 2. Pollen Germinability (*in vitro*) of *Opuntia* Species

Clones	Germinability (%)
Native	80 ±5.3
1233	76 ±6.3
1280	83 ±6.2
1281	80 ±2.4
1287	85 ±6.9
1294	79 ±3.7
1319	71 ±3.8
1320	82 ±7.9
1321	86 ±4.3
1383	72 ±9.6
1464	77 ±5.3

Pollen germinability was measured at anthesis.
Values are means (n=5) ± standard deviation.

Table 3. Effect of Breeding Methods on Fruit Set, Fruit Weight, and Seed Number

Breeding system \ Female: Native (*O. lindheimeri*)	**Number**	**Fruit set**	**Fruit weight (g)**	**Seeds/Fruit**
Control	10	5	48.0 ±8.5	42 ±22
Bagged	10	5	43.1 ±10.8	87.1 ±82.1
Emasculation and bagged without pollination	10	0	/	/
Emasculation, self-pollination and bagged	10	0	/	/
Emasculation, cross-pollination with 1294 and bagged	10	0	/	/
Breeding system \ Female: 1233 (*O.* spp., cold hardy and spineless)	**Number**	**Fruit set**	**Fruit weight (g)**	**Seeds/Fruit**
Control	10	0	/	/
Bagged	10	0	/	/
Emasculation and bagged without pollination	10	0	/	/
Emasculation, self-pollination and bagged	10	0	/	/
Emasculation, cross-pollination with Native and bagged	10	1	26.6	69
Breeding system \ Female: 1294 (*O. ficus-indica*, Mexico fruit clone)	**Number**	**Fruit set**	**Fruit weight (g)**	**Seeds/Fruit**
Control	10	10	61.0 ±15.0	153 ±31
Bagged	10	10	51.7 ±14.8	106 ±52
Emasculation and bagged without pollination	10	0	/	/
Emasculation, self-pollination and bagged	10	2	70.0 ±24.7	161 ±81
Emasculation, cross-pollination with 1321 and bagged	10	4	34.8 ±26.3	6 ±4
Breeding system \ Female: 1281 (*O. streptacantha*, Mexico fruit clone)	**Number**	**Fruit set**	**Fruit weight (g)**	**Seeds/Fruit**
Control	10	10	89.2 ±20.0	160 ±36
Bagged	10	10	51.6 ±15.5	83 ±41
Emasculation and bagged without pollination	10	0	/	/
Partial emasculation and bagged without pollination	10	1	59.8	35
Emasculation, self-pollination and bagged	10	3	24.7 ±1.5	5 ±3
Partial emasculation, self-pollination and bagged	10	6	33.6 ±28	27 ±41
Emasculation, cross-pollination with 1287 and bagged	10	7	37.3 ±7.5	49 ±53
Emasculation, cross-pollination with 1294 and bagged	10	4	47.3 ±9.3	71 ±34
Partial emasculation, cross-pollination with 1287 and bagged	6	5	42.6 ±15.1	17 ±14
Partial emasculation, cross-pollination with 1383 and bagged	6	6	44.5 ±21.0	20 ±20

Table 3. Effect of Breeding Methods on Fruit Set, Fruit Weight, and Seed Number (continued)

Breeding system \ Female: 1320 (*O. ficus-indica*, Chilean fruit clone)	**Number**	**Fruit set**	**Fruit weight (g)**	**Seeds/Fruit**
Control	10	10	52.7 ±11.0	155 ±37
Bagged	10	10	57.8 ±10.2	131 ±27
Emasculation and bagged without pollination	10	0	/	/
Partial emasculation and bagged without pollination	10	1	30.9	3
Emasculation, self-pollination and bagged	10	2	18.4 ±8.0	3 ±2
Partial emasculation, self-pollination and bagged	10	10	38.3 ±8.9	106 ±13
Emasculation, cross-pollination with 1319 and bagged	10	4	17.5 ±1.1	4 ±4
Partial emasculation, cross-pollination with 1319 and bagged	5	4	44.9 ±16.3	85 ±59
Partial emasculation, cross-pollination with 1287 and bagged	5	5	53.0 ±12.7	119 ±29
Breeding system \ Female: 1280 (*O. streptacantha*, Mexico fruit clone)	**Number**	**Fruit set**	**Fruit weight (g)**	**Seeds/Fruit**
Control	8	8	75.0 ±15.5	190 ±39
Partial emasculation and bagged without pollination	8	1	30.3	4
Partial emasculation and bagged without pollination plus Sevin	8	0	/	/
Partial emasculation, cross-pollination with 1320 and bagged	8	4	64.3 ±25.2	112.9 ±40.2
Partial emasculation, cross-pollination with 1320 and bagged plus Sevin	8	3	58.3 ±20.3	81.7 ±63.9
Partial emasculation, cross-pollination with 1320 and bagged	8	5	67.7 ±31.6	100.6 ±16.0
Breeding system \ Female: 1464 (*O. ellisana*, Texas cold hardy)	**Number**	**Fruit set**	**Fruit weight (g)**	**Seeds/Fruit**
Control	10	2	7.4 ±1.0	11 ±11
Bagged	10	0	/	/
Emasculation and bagged without pollination	10	0	/	/
Emasculation, self-pollination and bagged	10	5	7.4 ±1.9	20 ±20
Emasculation, cross-pollination with 1233 and bagged	10	2	11.0 ±1.4	111 ±15
Emasculation, cross-pollination with 1320 and bagged	20	5	7.4 ±1.3	10 ±15
Emasculation, cross-pollination with 1286 and bagged	20	1	8.7	50

Values are means ± standard deviation when samples are =>2.

Table 4. Seed Germination (%) in Two Months

Media	Temperature		
	25°C	30°C	35°C
Vermiculite	11 ±2	27 ±6	32 ±11
Sand	0 ±0	0 ±0	2 ±2
Cactus soil	0 ±0	5 ±3	9 ±4
Cactus soil + GA3	17 ±8	47 ±15	55 ±5

Values are the means ±SE.