Update on Cactus Pear Breeding and New Products at D*Arrigo Bros.

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INTRODUCTION

D*Arrigo Bros. Co. of California, a grower, packer, and shipper of fresh fruits and vegetables, currently farms about 6000 ha of crops in California and Arizona. We grow, pack, and ship more than 30 different fresh fruits and vegetables, including the well-known vegetables---lettuce, broccoli, and cauliflower---as well as lesser-known Italian specialty items, such as fennel, broccoli rabe (an Italian mustard green), and, of course, cactus pears.

CACTUS PEAR PRODUCTS

Although cactus pears are a small part of our business, they are an important item because of the long history of cactus pears at D*Arrigo Bros. and because they are considered an important and profitable Italian specialty item. We currently grow about 120 ha of cactus (*Opuntia ficus- indica*) in the Salinas valley of California. We grow only the red spineless Italian-type cactus pears, which are harvested from August through March. All of our fruit is mechanically despined, cleaned, and lightly waxed. Our premium fruit is individually tissue wrapped and packed into our Andy Boy label in 8.2 kg boxes with 50, 60, or 70 cactus pears per box.

At the PACD meeting in 1996 I reported on the development of a value-added cactus pear product, the cactus-pear puree (Bunch, 1996). We have made a few changes in this program since last year. First, we have decided to focus on a single, simple, pure product which is the frozen cactus-pear puree and stay out of the retail drink mixes and ready-to-use products. As a grower, packer, and shipper we prefer to concentrate on what we do best and let our customer do the creative product development using our puree as an ingredient. The second change, and at considerable expense, we have stopped using other juice companies' co-pack facilities and have moved into our own new puree plant. This new plant allows us to have complete control over the process and assure the highest product quality and safety. The new plant can process fruit introduced as previously frozen pulp or as fresh fruits. The puree is processed and pasteurized under strict quality-control standards. Food safety is extremely important to D*Arrigo Bros., hence, one of the unique and most impressive areas of the new plant is the ultraclean fill room with restricted access, filtered air, and extremely high cleanliness standards. This room, combined with the process controls in the rest of the plant, allows us to produce a nearly aseptic product while retaining excellent quality. Our customers now have the ability to produce higher quality and safer products from our puree. Our primary package is a 55-gallon drum containing about 400 lb. of puree. The average price is $1.25/lb. and the puree can be packaged in alternative containers at additional cost. One of our customers, Perfect Puree (a puree distributor) sells the frozen puree in smaller containers. The puree can be used in any number of beverages and food dishes, as we have seen over the last few years, thanks to inventive chefs, such a Jay McCarthy.

BREEDING RESEARCH

A primary goal of any new breeding program is to learn the biology of the plant and how to efficiently produce generations of seed-produced plants in order to select improved varieties. Worldwide experience dealing with the breeding of Opuntia sp. is very limited and, until recently, literature on the subject has been nearly nonexistent. Here, we describe some of our experiences as we develop breeding methods suited to the specific environment and genotypes that make up our program. Since 1994, we have been working on developing methods to make controlled pollinations and grow the seedlings from those pollinations.

Crossing Methods

We have learned crossing techniques from many different experts from around the world and are now modifying these methods to fit our program. Each year we have attempted controlled pollinations in the cactus and each year we have varied our methods in an attempt to maximize our crossing success rate. In Bunch (1996), we reported that in 1994 we attempted 39 crosses and obtained no seed. In 1995, we attempted 120 crosses and obtained seed in 14 of them, considerably lower than expected, but others have reported that a 10% to 15% success rate is not uncommon (Pimienta, B. E., 1995 pers. comm.). Over the last three years, we have varied emasculation methods, bag types, pollen collection methods, and kept detailed records of time from emasculation to pollination. In 1995, all of the flowers of the female parent were emasculated at a similar stage of development and nearly all of our successful crosses were from pollinations made 3-5 days after emasculation. In 1996, we expanded the crossing trials to include more pollen sources and to measure pollen storage times as well as time from emasculation to pollination. The number of days from pollen collection until use of the pollen for crossing was assessed to determine if a specific age of pollen (days from collection) was most effective and to determine the length of time we could expect pollen to stay viable under ambient storage conditions.

Emasculation

Selection of flower buds at the optimum stage for emasculation based on external appearance is difficult (Wang et al., 1996). Cactus pear flowers naturally shed pollen prior to opening (Nerd and Mizrahi, 1995) in addition the anthers will dehisce even earlier if disturbed, for example, during the emasculation procedure. If pollen is shed when emasculating a flower, the chance of self-pollination is increased. The best guide to determine if a flower bud is ready for emasculation is the appearance of the anthers after removing the corolla. If the anthers shed pollen while removing the corolla, the bud is too old, if the anthers are not well formed and pressed too closely to the stigma then the flower is too young and there is a high risk of damaging the stigma while removing the anthers. Therefore, emasculation was performed on the oldest possible flower buds in which the anthers did not dehisce during the procedure, assuring that all buds were emasculated as close to the same stage as possible. The emasculation procedure used in 1996 involved removal of the corolla by cutting around the flower just below the attachment point of the corolla and then cutting away anthers in close proximity to the stigma and those easily removed without damaging the style. White first-aid tape was used to cover the cut surface of the receptacle and remaining anthers, leaving just the stigma and a portion of the style protruding through the tape to be pollinated. The flowers were then covered with a 2 lb. white paper bag secured with plastic horticultural tie tape. All emasculations were performed on flowers of `DAB R' (D*Arrigo Bros. commercial spineless red variety).

Pollen Collection

Pollen was collected from 115 different plants representing 97 different clones. The majority of the clones were seedlings from a program with the late Dr. Facundo Barrientos. Other pollen parents included six Texas accessions, the D*Arrigo Bros. commercial variety, a spineless yellow, and a spineless white clone. Flowers for pollen collection were bagged early in the morning prior to opening and allowed to open under the bag before pollen was collected, usually the same day as bagging. Pollen was then brushed into a small plastic cup and covered for transfer to the emasculated flowers or stored for later use. Pollen was stored in the plastic cups at ambient room and/or field conditions.

Pollination

Pollination was performed by brushing the pollen onto the stigma of an emasculated flower with a #3 camel-hair paintbrush. The crossed flower was then re-covered with the 2-lb. paper bag from the emasculation procedure. After observing many emasculated flowers, none were found with any evidence of pollen movement from below the first-aid tape to the stigma to invoke self pollination. In 1996, the first emasculation was made May 20 and the last pollination was made June 10. The time from emasculation to pollination varied from 0 to 6 days and the time from pollen collection until pollen use varied from 0 to 16 days.

Crosses Between Red-Fruited Female Parent (DAR R) and Spineless Yellow-Fruited Variety (DAB Y) and Spiny White-Fruited Variety (BR)

Two small experiments were established, with consistent clones used as male and female parents, to eliminate the effect of genotype on crossing success. This limited the effects measured to the days from emasculation to pollination and the age of pollen used in the crosses. The DAB Y experiment utilized `DAB R' as the female parent and pollen was collected from `DAB Y' (a yellow-fruited spineless variety) as the male parent. The BR experiment utilized `DAB R' as the female parent and pollen was collected from BR (a white-fruited, heavily-spined variety obtained from Dr. Facundo Barrientos in 1992) as the male parent. In conjunction with these experiments, seven flowers of `DAB R' were emasculated on June 1, 1996 and bagged as unpollinated controls.

For the DAB Y experiment (Table 1), 32 flowers from `DAB R' were emasculated on June 1, 1996. Pollen was collected from `DAB Y' beginning on May 27, 1996, then every few days after that until the end of the experiment. The ``relative day pollen was collected'' column in Table 1 lists the day of pollen collection relative to the emasculation date (June 1) with multiple days (ex: 0/- 1) referring to both the day pollen was collected and the day the flower was bagged. On Day 0 (June 1, the day the emasculations were made) we used pollen collected on Day 0, Day 0/-1, and Day -3 to pollinate one each of the emasculated flowers. Then at subsequent 2-day intervals more of the emasculated flowers were pollinated with several different ages of pollen. The result was a series of crosses pollinated at various days after emasculation with pollen collected various days prior to pollination. A successful cross is defined here as a fruit with more than one seed. A single seed in a fruit is suspect and may be apomictic rather than due to pollination.

For the BR experiment (Table 2), 43 flowers from `DAB R' were emasculated on May 25, 1996. Pollen was collected from `BR* (a white-fruited, heavily spined variety obtained from Dr. Facundo Barrientos in 1992) beginning on May 24, 1996, then every few days after that until the end of the experiment. Again, this crossing pattern resulted in a series of crosses pollinated at various days after emasculation with pollen collected various days prior to pollination.

Crossing Results

No seeds were set in the seven flowers emasculated on June 1 but never pollinated. This result lends additional evidence to the integrity of our emasculation and pollen-control methods.

Experiment With DAB Y as Male Parent and DAR R as Female Parent

In the DAB Y experiment (Table 1), on Day 0 (the day the flowers were emasculated) three crosses were attempted, but none was successful. Four successful crosses were obtained out of six attempts when pollinated at two days after emasculation for a 67% success rate. At four days after emasculation, five out of 14 crosses were successful for a 36% success rate and at six days after emasculation, two of nine crosses set seed for a 22% success rate (see values at the bottom of Table 1). Crosses pollinated on Day 4 gave the highest average number of seeds per fruit at 141. These results indicate that with our current emasculation procedure and under our field conditions, most flowers are being emasculated two to four days before the flowers would naturally open and be receptive. This period is similar to that reported by Mondragon and Bordelon (1996). On examination of pollen storage time in the DAB Y experiment, results show pollen collected the same day the cross was made (0 days from pollen collection to pollen use) resulted in two successful crosses out of six for a 33% success rate. For pollen used two days after it was collected, we obtained seed from four of eight crosses for a 50% success rate. Crosses made with pollen stored up to nine days after collection were at least partially successful (see the right side of Table 1). Two crosses with 11-day-old pollen, two with 14-day-old pollen, and one with 16-day-old pollen were attempted; however, these gave no seed set and were not included in the Table 1 values.

Experiment With BR as Male Parent and DAR R as Female Parent

In the BR experiment (Table 2), crosses pollinated at the time of emasculation (Day 0) gave no seed set (zero successful of seven attempted). Of the 10 crosses pollinated on Day 2 none set seed. For crosses pollinated on Day 4, we obtained seeds with three of nine crosses. Pollination of flowers six days after emasculation gave the best results with six successful out of ten attempts. This apparent shift from an optimum pollination time of 2 to 4 days after emasculation in the DAB Y experiment to 4 to 6 days in the BR experiment can be explained by differing weather patterns during the two experiments (Figure 1). The emasculations for the BR experiment were made May 25 at the beginning of a cool foggy period while the emasculations for the DAB Y experiment were made June 1 at the beginning of a very warm and sunny period. In Salinas during the Spring and Summer it is not uncommon to have alternating periods of sunny warm weather and foggy cold weather. The warm temperatures appear to speed up the development of the stigma and the cold weather appears to slow the development of the stigma and thus decrease or increase the time between emasculation and receptivity. This strong influence of weather on optimum days from emasculation to pollination needs to be considered when attempting crosses. Examination of pollen storage times in the BR experiment (Table 2) revealed that pollen collected on the day of the cross (0 days from pollen collection to pollen use) produced three successful crosses from eleven attempts and pollen used two days after it was collected gave seed set in four of seven crosses while four-day old pollen produced only one of six successful crosses. Six-day old pollen produced one fruit from two crosses but with only three seeds.

All Crosses

Combined analysis of all crosses made in 1996 (including DAB Y and BR experiments) revealed that seed set was obtained in 73 out of 266 attempted crosses for a 27% success rate (Table 3). For 49 of the attempted crosses, pollination of the emasculated flower was made on two different days using the same pollen. This procedure was used to increase the chances of the stigma being receptive during pollination. The headings across the top of Table 3 show the days from emasculation to pollination for both the first and second pollination. No seed set was obtained from the 28 emasculated flowers left unpollinated (``None" on Table 3). The crosses made on the same day as emasculation, Day 0, were not very effective but slightly better success rates were obtained when the same flower was repollinated at Day 4 or 5. Similarly crosses made one day after emasculation were not effective but a little better when repollinated on Day 3 or 5. On Day 2 after emasculation greater success was achieved with 13 out of 52 for a 25% success rate; however, success rates of 67, 50, and 46% were obtained when the flowers were pollinated on Day 2 and repollinated on Day 3, 4 or 5 respectively. A single pollination on Day 3, 4, 5, or 6 after emasculation was also moderately successful. A fairly wide range of successful intervals between emasculation and pollination exist primarily because of variation in weather as discussed above. Under our conditions, crossing success rates of over 50% can be expected when pollinating at two days and again at three to five days after emasculation, depending on the weather and how fast the stigma develops. It appears that pollen collected fresh or stored in ambient room and field conditions for up to 6 days will be viable and effect successful pollination. In addition, one pollen sample appears at least partially viable after 9 days of storage.

After seeing these results, our 1997 crossing program was modified so each cross was pollinated two times with the first pollination at Day 2 after emasculation and then based on the observed condition of the stigma repollination on Day 3, 4, or 5 after emasculation. Nearly all of the pollen was collected and air dried for use 2 - 5 days after collection.

Seed Germination

In 1996, we reported (Bunch, 1996) several different methods to germinate the seeds as well as several different seed treatments.We have found that two factors consistently increase germination (data not shown):

- Dry storage of the seeds for several months before planting
- Germination at elevated temperatures

Both factors have also been reported by others to increase seed germination of various *Opuntia species* (Mondragon and Pimienta, 1995; Deno, 1996).

During 1996, we attempted to germinate seeds from 25 crossed (Figure 2) and 66 self-pollinated fruits (27 shown in Figure 3) from the 1995 crossing season. The seeds were harvested in September 1995 and planted November 19, 1996, nearly 14 months after harvest. Several of the crosses resulted in only a few seeds. Seeds were sown into a commercial cactus soil and kept at near 100% humidity by covering with plastic dome covers. The soil temperature was kept at 25°C to 29°C. We obtained 67% germination for crossed seeds and 63% germination for self-pollinated seeds. Overall 75% of those seeds that germinated did so within the first four weeks (data not shown). Multiple seedlings (``Multiples'' in Figures 2 and 3) indicate polyembryony and were produced in 18% of the seeds that germinated, similar to figures reported for several *Opuntia* species (Mondragon and Bordelon, 1996; Mondragon and Pimienta, 1995). These seedlings present a problem for plant breeders because they are most likely apomictic, arising from nucellar tissue (female parent cells) rather from fertilization by the pollen parent. In general, these multiple (the second or third) seedlings are later to emerge, smaller, and weaker than the primary seedling, but not always. We presume the primary seedling to be the sexually produced embryo and the second and/or third seedling to be apomictic as reported by Mondragon and Bordelon (1996). Due to the high percentage of polyembryonic seeds, some of the seedlings that we expect are sexually derived are possibly instead apomictic; however, this can only be proven through genotypic testing, such as a RAPD analysis.

With much of this basic biological understanding now within our grasp, we are well on our way to producing and evaluating the genetic variation necessary to develop improved cactus varieties.

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Table 1. DAB Y Experiment: Number of Successful Crosses and Attempted Crosses Using `DAB Y* Pollen at Various Days After Collection and `DAB R* (female parent) Flowers at Various Days After Emasculation

Days from pollen collection to pollen use	Day pollen was collected		Days from emasculation to pollination and date of pollination				Total success, attempt	% success	Average seeds/ fruit
	relative	date	0 6/1/96	2 6/3/96	4 6/5/96	6 6/7/96			
			(Successful, attempted crosses)						
0	Day 0	6/1/96	0,1						
0	Day 0/-1	6/1/96	0,1						
0	Day 2	6/3/96		0,1					
0	Day 4	6/5/96			2,3		2,6	33 %	158
2	Day 0	6/1/96		1,1					
2	Day 0/-1	6/1/96		2,2					
2	Day 2	6/3/96			1,3				
2	Day 4	6/5/96				0,2	4,8	50 %	65
3	Day -3	5/29/96	0,1				0,1	0 %	
4	Day 0	6/1/96			1,2				
4	Day 0/-1	6/1/96			0,2				
4	Day 2	6/3/96				0,2	1,6	17 %	197
5	Day -3	5/29/96		1,2			1,2	50 %	2
6	Day 0	6/1/96				1,2			
6	Day 0/-1	6/1/96				0,2	1,4	25 %	18
7	Day -3	5/29/96			1,2		1,2	50 %	21
9	Day -3	5/29/96				1,1			
9	Day -5	5/27/96			0,2		1,3	33 %	15
Total successful, attempeted			0,3	4,6	5,14	2,9	11,32	34 %	75
% success			0 %	67 %	36 %	22 %	34 %		
Averaged seeds7 fruit				23	141	17	75		

Table 2. BR Experiment: Number of Successful Crosses and Attempted Crosses Using `BR* Pollen at Various Days After Collection and `DAB R* (female parent) Flowers at Various Days After Emasculation

Days from pollen collection to pollen use	Day pollen was collected		Days from emasculation to pollination and date of pollination				Total success, attempt	% success	Average seeds/ fruit
	relative	date	0 5/25/96	2 5/27/96	4 5/29/96	6 5/31/96			
			(Successful, attempted crosses)						
0	Day 0	5/25/96	0,3						
0	Day 2	5/27/96		0,2					
0	Day 2/0	5/27/96		0,2					
0	Day 4	5/29/96			1,2				
0	Day 6	5/31/96				2,2	3,11	27%	136
1	Day -1	5/24/96	0,2						
1	Day -1/-3	5/24/96	0,2				0,4	0%	
2	Day 0	5/25/96		0,2					
2	Day 2	5/27/96			0,2				
2	Day2/0	5/27/96			2,2				
2	Day 4	5/29/96				2,2	4,7	57%	133
3	Day -1	5/24/96		0,2					
3	Day -1/-3	5/24/96		0,2			0,4	0%	
4	Day 0	5/25/96			0,1				
4	Day 2	5/27/96				0,2			
4	Day 2/0	5/27/96				1,2	1,6	17 %	108
5	Day -1	5/24/96			0,2		0,2	0 %	
6	Day 0	5/25/96				1,2	1,2	50 %	3
Total successful, attempeted			0,7	0,10	3,9	6,10	9,43	21 %	117
% success			0 %	0 %	33 %	60 %	21 %		
Averaged seeds7 fruit					83	133	117		

Table 3. All 1996 Crosses: Number of Successful Crosses and Attempted Crosses Using Pollen from 115 Different Plants (representing 97 different clones) at Various Days after Collection and `DAB R* (female parent) Flowers at Various Days after Emasculation.
Crosses from the DAB Y and BR experiments are included in this table.

Days from pollen collection to pollen use		Days from Emasculation to Pollination																			Total	
	First[1,2]:	None[3]	0	0	0	0	1	1	1	2	2	2	2	2	3	3	3	4	5	6	Success,	%
First[1]	Second[2]	Second[1,2]:		3	4	5		3	5		3	4	5	7		5	7				Attempt	
		(Number of successful, attempted crosses)																				
None[3]		0,28																			0,28	0%
0			0,8				0,3			1,14					3,10			3,10	1,4	2,2	10,51	20%
0	2											3,6									3,6	50%
0	3			0,5									0,3								0,8	0%
0	4				1,4																1,4	25%
0	5					2,3								0,1							2,4	50%
1			0,4				0,3								0,6						0,13	0%
1	3							1,4													1,4	25%
1	5								0,1								1,1				1,2	50%
2			0,2							4,11					5,10			10,17	1,12	2,4	22,56	39%
2	3										4,6										4,6	67%
2	5												6,10								6,10	60%
3			0,1				0,1			0,4					0,8						0,14	0%
3	5							0,1								1,3					1,4	25%
3	7								1,1												1,1	100
4			0,6							6,17								3,11	0,3	1,6	10,43	23%
5							0,2			1,2					1,5			0,2	2,4		4,15	27%
6			1,1							1,3								0,2		2,6	4,12	33%
7															1,2			1,2	0,2		2,6	33%
9										0,1					0,1			0,2		1,1	1,5	20%
10															0,2						0,2	0%
Total Success, Attempt		0,28	1,22	0,5	1,4	2,3	0,9	1,5	1,2	13,52	4,6	3,6	6,13	0,1	10,44	1,3	1,1	17,46	4,25	8,19	73,266	27%
% Success		0%	5%	0%	25%	67%	0%	20%	50%	25%	67%	50%	46%	0%	23%	33%	100	37%	16%	42%	27%	

[1,2] "First" and "Second" refer to the first and second pollination, respectively, for crosses involving two pollination dates.
[3] "None" means that the emasculated flowers were not pollinated.

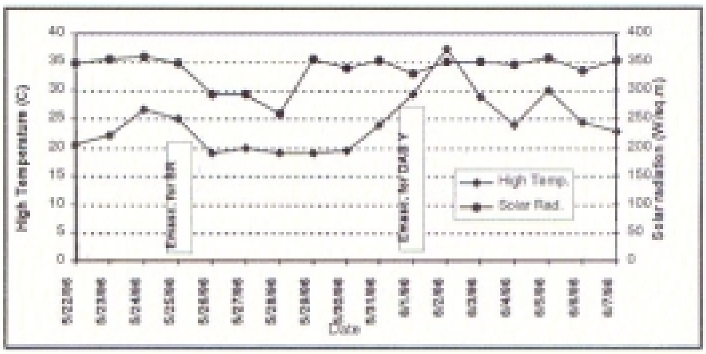

Figure 1. Daily High Temperature and Average Solar Radiation Records
During DAB Y BR Crossing Experiments

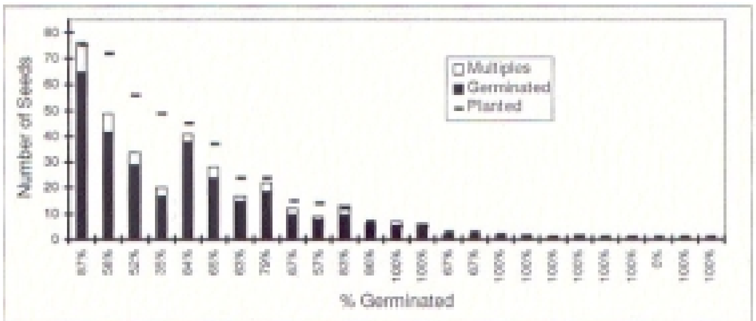

Figure 2. Germination Results During 1996 of Cactus Seed Lots from Crosses Made in 1995
(-) Number of Seeds Planted, (■)Number of Seeds Germinated, and (□) Number of Germinated Seeds with More than One Emerged Seedling

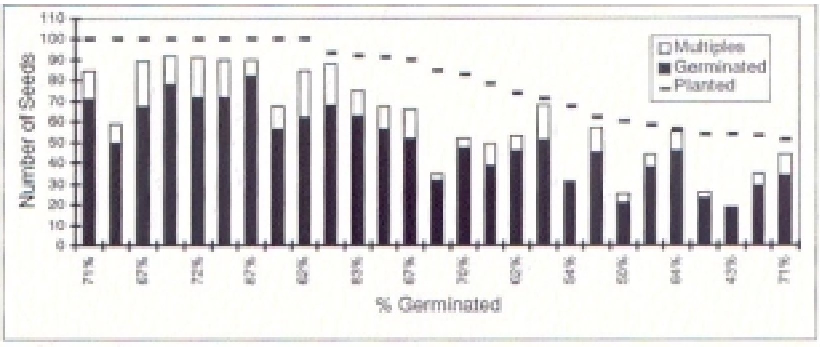

Figure 3. Germination Results, During 1996, of Cactus Seed Lots
from Flowers Self-Pollinated in 1995
(-) Number of Seeds Planted, (■)Number of Seeds Germinated, and (□) Number of Germinated Seeds with More than One Emerged Seedling