Almond Board of California

Project Report-2002

Project Title: **Effect of pollen S-allele combinations (one or both cross-compatible) on seed-set success**

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Location: Department of Pomology, University of California at Davis

Objective:

1. Determine whether pollinizers that have one pollen cross-incompatibility factor (S-alleles) in common with the seed parent lead to reduced seed-set compared to pollinizers where both pollen cross-incompatibility factors differ from the seed parent.
2. Determine whether otherwise fully cross-compatible pollen cross-incompatibility factors (S-alleles) differ in their effectiveness for achieving honeybee transfer, fertilization and ultimately, seed set in field pollinations.

Summary

Seed set under field conditions is affected by a large number of environmental and biological conditions resulting in a large variation in the seed set success even under the same apparent environments and with the same crossing parents. The effect of pollen S-allele combinations within this larger variability appears negligible. The use of hive inserts did result in the movement of inserted pollen within the targeted hive and among individually examined bees. The movement of tagged pollen was not observed to move from hive to hive however, though poor conditions during pollination may be responsible. The emasculation of flowers prior to controlled pollinations to avoid contamination by self-pollen consistently resulted lower seed sets. The lower sets appear to be the consequence of trauma to the emasculated flower. The continuing study of actual S-allele transfer to resultant seed (i.e. paternity testing) should, however, offer a clearer picture of any advantage of individual pollen genotypes for fertilization success. Over 2000 seedlings from controlled crosses developed for this analysis have now been planted and molecular markers capable of distinguishing between the different test-genotypes have been identified. This paternity analysis should be completed by Fall, 2003.

Because cross-pollination during the limited flowering season has been shown to be the most important determinant of final crop yield, a critical decision facing growers is the appropriate source of the pollinizer pollen. This is a crucial question both in the selection of pollinizer varieties as well as the use of pollen inserts to encourage cross-pollination. Two frequent grower and Farm Adviser questions concerning specific sources of pollen have been: [1] Does a variety such as Mission (S1S5) which has no pollen cross-incompatibility group (CIG) factors (S-alleles) in common with Nonpareil (S7S8) provided a better cross-pollination pollen source than a variety such as Carmel (S5S8) which has one pollen CIG factor different and one factor in common? And [2] Are some pollen CIG factors (S-alleles) better at achieving seed set than others under otherwise cross-compatible field conditions? Although isozyme and PCR based pollen CIG (S-allele) markers have recently become available, these markers could not unequivocally determine pollen paternity following seed-set since these isozymes and PCR markers are shared by a great number of California almond varieties (due to the highly inbred nature of California almonds). We have now developed PCR and SSR based markers which can unequivocally identify the specific pollen donor contributing to seed-set.

1. Assessment of possible seed set disadvantage when donor pollen has one CIG factor in common with seed parent.

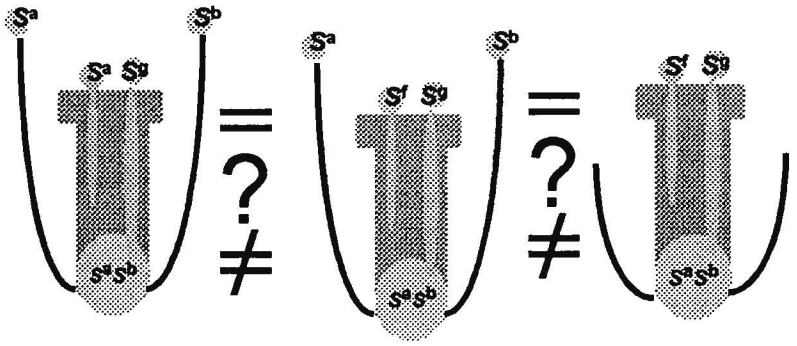


Fig. 1 a b c

Controlled hand pollinations were made using pollen from a variety [SaSg] having one cross- incompatibility factor in common with seed parent [SaSb] and, separately, using pollen from a variety [SfSg] were both cross incompatibility factors differ from those of the seed parent. This test is represented graphically in Figure 1, where [a] represents the pistil of the flower upon which pollen containing one inevitably incompatible S-factor in common (Sa) and a distinct S-factor (Sg), and, [b] represents a flower where both applied pollen are from different S-factors. (S-factors shown attached to the base of the flower by a

curved line represents the self pollen of that flower's anthers]. The question being addressed by this test is whether similar seed sets will be recovered through these two scenarios or more specifically, whether improved seed set will result when both pollen S-factors are different. Figure 1 (c) represents an emasculated flower where the self-pollen containing anthers have been removed prior to applying the test pollen (as discussed later). At least 8,000 controlled pollinations were evaluated. Initially, pollinated flowers were not emasculated in order to remain

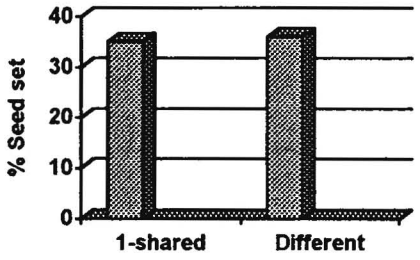


Fig. 2. Shared or different self-incompat. alleles

true to actual field conditions (thus some self-pollen was deposited on the stigma with the donor pollen). To determine whether the addition of self-pollen influences final seed set, similar crosses were made to emasculated flowers (to eliminate self-pollination when applying the donor-pollen).

Seed set data for either single years (Figure 2 and Table 2) or multiple years (Table 1) show no clear advantage of having both cross-incompatibility factors different. Examination of compiled data from these two types of crosses over the past five years demonstrates the presence of a large variability within each type of cross as well as between years (Table 1). For example, a comparison of 1997 data for Sonora as seed parent crossed with pollen which had no incompatibility alleles in common showed a range in set from 0.08 to 0.54 (Table 1). Other Sonora crosses to D3-25, where one of the pollen alleles is common to Sonora gave sets of 0.32 and 0.14. Similar crosses in 1998 gave sets of 0.16, 0.22 and 0.31 for crosses to a pollen parent which had no alleles in common. Paired crosses of Sonora to D3-6, which

had a shared allele with Sonora resulted in some of the highest sets of 0.41 and 0.45 as well as the lowest set of 0.01. The same trend is repeated for other varieties and other years. The crosses in 2000 of Mission as the seed parent to pollen sources possessing no incompatibility alleles in common resulted in relatively low sets overall but containing individual sets as high as 0.38 and as low as 0.02. The crosses of Mission to LeGrand with its shared S-5 allele resulted in somewhat better sets on average, though with a maximum set of 0.24.

This wide variability in seed set following controlled pollinations is typical for breeding programs of this type. These varying sets demonstrate the wide range of outside factors affecting final seed set, including weather conditions at bloom, pollen viability, flower age and fecundity, pollen dosage, and conditions following bloom. This range in natural variability is also demonstrated in Table 2 were selected pollinations have been replicated and also made in the reciprocal (seed parent becomes the pollen parent). Final sets where both pollen S-alleles are distinct from those of the seed parent are comparable to crosses were only a single as allele is different. A wide range in variability is again evident, particularly were more replications were performed. Even crosses between parents where both S-alleles were identical resulted in sets as high as 18 percent in the cross of Butte to Monterey (Table 2) although most such crosses result in sets of less than 10 percent (as do self pollinations).

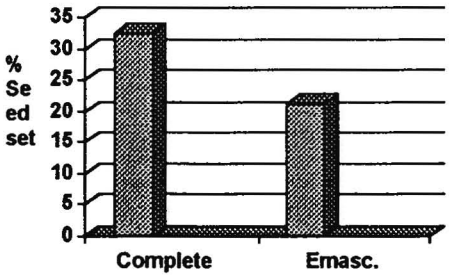


Fig. 3. Flowers

The self-incompatibility in almond thus involves a leaky system rather than rigid barriers described in other plan systems. Earlier work by my program has shown that this leakiness may actually enhance seed set in almond. This is because the (ultimately incompatible) self-pollen tubes are still capable of growth through the length of the style. Larger numbers of pollen tubes growing through the style, even when

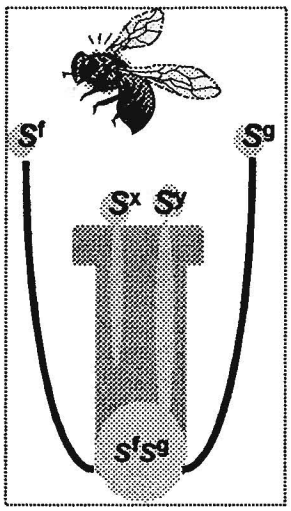
many may be self-incompatible pollen, results in a synergistic effect of improving pollen growth and fertilization for compatible pollen. (For example, one to a few compatible pollen tubes would be much less successful in traversing the style on their own than when accompanied by the large number of self-pollen typically applied during hand for honeybee pollinations).

Under our field conditions, these outside factors that lead to a high variability in final seed-set success effectively mask any differences, if they exist, of S-allele composition. In addition, both hand and honeybee mediated cross-pollination will result in some self-pollen from the pollinated flower also being applied to the stigma, further diluting the cross pollen. The magnitude of this dilution, particularly for honeybee cross-pollinations, probably minimizes any differences in cross-compatible pollen dosage for most field cross-pollinations.

The elimination by the artificial emasculation (removal of all flower anthers) of this dilution by self-pollen prior to cross-pollination did not result in improved seed sets (Figure 4 and Table 3) but rather significantly reduced sets. These reductions, however, are probably due to subtle damage to the pistil at emasculation, resulting in later abortion of a larger number of flowers.

1. **Determine whether otherwise fully cross-compatible pollen CIG factors differ in their fertilization effectiveness following honeybee transfer of pollen.** Two field tests have been undertaken; one involving caged trees and the other involving honeybee cross-pollination of an isolated single variety Mission block using a pollen insert to one of 2 hives.

Caged trees. Two 13-year-old almond trees at full production and having entirely different CIG factors were enclosed with mesh screening to exclude outside pollinators. A honeybee hive was placed in this enclosure at flowering to provide pollinators. Over 1,000 seed were harvested from each tree in September 2002. At least 200 of the resultant seed will be germinated during Winter 2003 and are being prepared for analysis using PCR, and if necessary SSR markers developed at UCD to determine



pollen source (paternity) and identify whether any CIG factor showed significantly higher rates of seed set as compared to the others. The expected outcome is that all CIG factors (x and y in Fig. 4) will be equally represented in resultant seed. Significantly higher proportions of any one factor would indicate a field advantage for that factor and so differences in CIG efficacy for setting seed.

Figure 4

Use of pollen inserts in an isolated Mission block.

Two honeybee hives were placed at bloom in an isolated solid Mission planting (of approximately 100 trees) located at UCD. Fresh pollen having entirely different CIG factors (SxSy) than Mission (designated SaSb in Fig. 3) was prepared and placed in a hive-insert at one of the to hives. Pollen sized fluorescent microspores were added to the pollen in order to trace later dispersal in both the hive containing the inserts as well as the adjacent hive without an insert. Fluorescent microspores use can be identified by illuminating hive frames with ultra-violent light causing the microspores to fluoresce with a distinct blue-violet color. Later examinations under ultra-violent light demonstrated that while fluorescent markers were observed throughout the donor hive, no fluorescent markers and presumably no pollen was exchanged hive-to-hive. Field conditions during and following pollinations in 2002, however, led to poor bee flights and subsequent poor seed set. This experiment is thus being repeated in 2003. At the 2003 harvest, at least 200 Mission nuts will be harvested, germinated, and the pollen donor (paternity) determined using PCR and/or SSR markers. As with the previous experiment, the assumption is equal seed set success by the different CIG (x and y) pollen. Significantly greater rates of

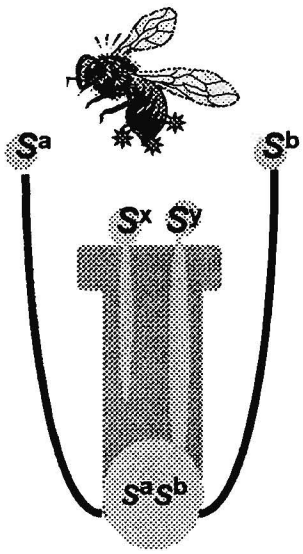


Figure 5

success of one CIG factor relative to the other would indicate different pollination efficiencies of different though otherwise compatible CIG factors or (less likely) different selection/preferences by honeybee pollinators. [The fluorescent microbead study will allow a preliminary estimate of insert pollen movement through the main hive and through spatially separated hives as well as the degree of insert pollen grooming (removal) by worker bees within the hive].

In summary, results from the first year of analysis demonstrate extensive variability in successful seed set. Variability from outside factors, however, dwarf any real differences between pollen sources. Paternity testing of resultant seed, which will take place in the summer of 2003, should provide clearer insights concerning differences in pollen quality affecting final seed set success.

Table 1. Effect of having 1 or no S-alleles in common on final seed set in controlled crosses where the S-genotype has been determined.

Alleles in common	Year	Seed Parent	1stt allele	2nd allele	Pollen Parent	1stt allele	2nd allele	Total pollinations	Set	Set %
0	1997	D3-25	y	8	LeGrand	5	7	202	97	0.48
0	1997	D3-25	y	8	UCD25-75	p	8f	197	50	0.25
0	1997	Padre	1	h18	D3-25	y	8	76	14	0.18
0	1997	Padre	1	h18	D3-25	y	8	53	11	0.21
0	1997	Padre	1	h18	D3-25	y	8	158	42	0.27
0	1997	Padre	1	h18	LeGrand	5	7	165	35	0.21
0	1997	Sonora	13	8	D10-3	5f	7f	57	31	0.54
0	1997	Sonora	13	8	LeGrand	5	7	120	39	0.33
0	1997	Sonora	13	8	LeGrand	5	7	50	3	0.06
0	1997	Sonora	13	8	LeGrand	5	7	46	7	0.15
0	1997	Sonora	13	8	UCD25-75	p	8f	108	9	0.08
0	1997	UCD25-75	p	8f	D3-25	y	8	160	20	0.13
	1997	UCD25-75	x	8f	D3-25	y	8	111	43	0.39
1	1997	Sonora	13	8	D3-25	y	8	110	35	0.32
1	1997	Sonora	13	8	D3-25	y	8	58	8	0.14
0	1998	Butte	1	8	TRUSITO	r	s	56	9	0.16
0	1998	Butte	1	8	TRUSITO	r	s	97	29	0.30
0	1998	D1+2-10	13	8	F7,1-11	i	m	41	11	0.27
0	1998	D3-25	y	8	F7,1-11	i	m	120	11	0.09
0	1998	D3-25	y	8	UCD25-75	p	8f	107	3	0.03
0	1998	D3-25	y	8	UCD25-75	p	8f	160	17	0.11
0	1998	Mission	1	5	TRUSITO	r	s	156	41	0.26
0	1998	Mission	1	5	TRUSITO	r	s	84	27	0.32
0	1998	Padre	1	h18	TRUSITO	r	s	97	29	0.30
0	1998	Price	1	7	TRUSITO	r	s	49	26	0.53
0	1998	Sonora	13	8	F7,1-11	i	m	39	12	0.31
0	1998	Sonora	13	8	F7,1-11	i	m	55	12	0.22
0	1998	Sonora	13	8	F7,1-11	i	m	189	30	0.16
0	1998	UCD25-75	n	8f	D3-6	z	8	127	22	0.17
	1998	UCD25-75	x	8f	D3-6	z	8	49	2	0.04
0	1998	UCD25-75	x	8f	D3-6	z	8	72	8	0.11

Alleles in common	Year	Seed Parent	1stt allele	2nd allele	Pollen Parent	1stt allele	2nd allele	Total pollinations	Set	Set %
0	1998	UCD25-75	x	8f	TRUSITO	r	s	262	16	0.06
0	1998	UCD25-75	x	8f	TRUSITO(EMAS)	r	s	116	10	0.09
1	1998	D3-25	y	8	D3-6	z	8	135	13	0.10
1	1998	Sonora	13	8	D3-6	z	8	76	31	0.41
1	1998	Sonora	13	8	D3-6	z	8	90	1	0.01
1	1998	Sonora	13	8	D3-6	z	8	55	25	0.45
1	1998	Sonora	13	8	D3-6	z	8	111	14	0.13
1	1998	Sonora	13	8	D3-6	z	8	139	38	0.27
0	1999	D3-25	y	8	F8,7-179	q	8f	82	30	0.37
0	1999	D3-25	y	8	F8,7-179	q	8f	125	9	0.07
0	1999	D3-25	y	8	F8,7-179	q	8f	225	25	0.11
0	1999	D3-25	y	8	F8,7-179	q	8f	128	20	0.16
0	1999	D3-25	y	8	F8,8-160	e6	8f	120	50	0.42
0	1999	D3-25	y	8	F8,8-160	e6	8f	100	22	0.22
0	1999	D3-6	z	8	F8,7-179	q	8f	107	7	0.07
0	1999	D3-6	z	8	F8,8-160	e6	8f	191	13	0.07
0	1999	F7,56-89	i	m	D3-25	y	8	50	14	0.28
0	1999	F7,56-89	i	m	D3-25	y	8	100	9	0.09
0	1999	F7,56-89	i	m	D3-25	y	8	90	19	0.21
0	1999	F7,56-89	i	m	D3-25	y	8	60	3	0.05
0	1999	F7,56-89	i	m	D3-25	y	8	46	1	0.02
0	1999	F7,56-89	i	m	D3-25	y	8	55	15	0.27
0	1999	F7,56-89	i	m	D3-25	y	8	70	13	0.19
0	1999	F7,56-89	i	m	D3-6	z	8	75	11	0.15
0	1999	F7,56-89	i	m	D3-6	z	8	102	23	0.23
0	1999	F7,56-89	i	m	D3-6	z	8	77	39	0.51
0	1999	F7,56-89	i	m	D3-6	z	8	75	26	0.35
0	1999	F7,56-89	i	m	D3-6	z	8	106	13	0.12
0	1999	F7,56-89	i	m	F8,7-179	q	8f	23	10	0.43
0	1999	F7,56-89	i	m	F8,7-179	q	8f	62	6	0.10
0	1999	F7,56-89	i	m	F8,7-179	q	8f	92	13	0.14
0	1999	F7,56-89	i	m	F8,7-179	q	8f	13	3	0.23
0	1999	F7,56-89	i	m	F8,7-179	q	8f	40	12	0.30
Alleles in common	Year	Seed Parent	1stt allele	2nd allele	Pollen Parent	1stt allele	2nd allele	Total pollinations	Set	Set %

Alleles in common	Year	Seed Parent	1stt allele	2nd allele	Pollen Parent	1stt allele	2nd allele	Total pollinations	Set	Set %
0	1999	F7,56-89	i	m	F8,7-180	q	8	131	11	0.08
0	1999	F7,56-89	i	m	F8,7-180	q	8	131	11	0.08
0	1999	F7,56-89	i	m	F8,7-180	q	8	42	7	0.17
0	1999	F7,56-89	i	m	F8,8-160	e6	8f	65	13	0.20
0	1999	F7,56-89	i	m	F8,8-160	e6	8f	65	7	0.11
0	1999	F7,56-89	i	m	F8,8-160	e6	8f	71	29	0.41
0	1999	F7,56-89	i	m	F8,8-160	e6	8f	78	22	0.28
0	1999	Ferragnes	1	3	F7,1-11	i	m	180	28	0.16
0	1999	Ferragnes	1	3	F7,1-11	i	m	216	35	0.16
0	1999	Ferragnes	1	3	F7,1-11	i	m	145	19	0.13
0	1999	Ferragnes	1	3	F8,7-179	q	8f	151	60	0.40
0	1999	Ferragnes	1	3	F8,7-179	q	8f	188	13	0.07
0	1999	Ferragnes	1	3	F8,7-179	q	8f	83	17	0.20
0	1999	Ferragnes	1	3	F8,7-179	q	8f	193	45	0.23
0	1999	Ferragnes	1	3	F8,7-179	q	8f	155	25	0.16
0	1999	Ferragnes	1	3	F8,7-179	q	8f	246	80	0.33
0	1999	Ferragnes	1	3	F8,7-179	q	8f	266	60	0.23
0	1999	Ferragnes	1	3	F8,7-180	q	8	119	13	0.11
0	1999	Ferragnes	1	3	F8,7-180	q	8	98	11	0.11
0	1999	Ferragnes	1	3	F8,7-180	q	8	189	21	0.11
0	1999	Ferragnes	1	3	F8,7-180	q	8	214	80	0.37
0	1999	Ferragnes	1	3	F8,7-180	q	8	208	70	0.34
0	1999	Sonora	13	8	F8,7-179	q	8f	118	68	0.58
0	1999	Sonora	13	8	F8,7-179	q	8f	147	56	0.38
1	1999	D3-25	y	8	F8,7-180	q	8	40	2	0.05
1	1999	D3-25	y	8	F8,7-180	q	8	130	34	0.26
0	2000	D3-25	y	8	F8,8-160	e6	8f	139	18	0.13
0	2000	D3-25	y	8	LeGrand	5	7	126	1	0.01
0	2000	D3-25	y	8	LeGrand	5	7	273	4	0.01
0	2000	D3-25	y	8	LeGrand	5	7	193	6	0.03
0	2000	D3-6	z	8	UCD25-75	x	8f	57	14	0.25
0	2000	D3-6	z	8	UCD25-75	x	8f	33	8	0.24
Alleles in common	Year	Seed Parent	1stt allele	2nd allele	Pollen Parent	1stt allele	2nd allele	Total pollinations	Set	Set %

Alleles in common	Year	Seed Parent	1stt allele	2nd allele	Pollen Parent	1stt allele	2nd allele	Total pollinations	Set	Set %
0	2000	Ferragnes	1	3	D3-25	y	8	189	38	0.20
0	2000	Ferragnes	1	3	D3-25	y	8	68	23	0.34
0	2000	Ferragnes	1	3	D3-6	z	8	98	38	0.39
0	2000	Ferragnes	1	3	D3-6	z	8	105	60	0.57
0	2000	Ferragnes	1	3	F8,7-179	q	8f	82	28	0.34
0	2000	Ferragnes	1	3	F8,7-179	q	8f	109	37	0.34
0	2000	Ferragnes	1	3	F8,7-179	q	8f	95	34	0.36
0	2000	Ferragnes	1	3	F8,7-180	q	8	71	37	0.52
0	2000	Ferragnes	1	3	F8,7-180	q	8	60	35	0.58
0	2000	Ferragnes	1	3	LeGrand	5	7	92	26	0.28
0	2000	Ferragnes	1	3	LeGrand	5	7	139	58	0.42
0	2000	Mission	1	5	D3-25	y	8	29	2	0.07
0	2000	Mission	1	5	D3-25	y	8	60	1	0.02
0	2000	Mission	1	5	D3-25	y	8	76	11	0.14
0	2000	Mission	1	5	D3-25	y	8	67	5	0.07
0	2000	Mission	1	5	D3-25	y	8	46	4	0.09
0	2000	Mission	1	5	D3-25	y	8	16	6	0.38
0	2000	Mission	1	5	D3-25	y	8	111	2	0.02
0	2000	Mission	1	5	D3-6	z	8	24	4	0.17
0	2000	Mission	1	5	D3-6	z	8	75	4	0.05
0	2000	Mission	1	5	D3-6	z	8	52	2	0.04
0	2000	Mission	1	5	D3-6	z	8	170	18	0.11
0	2000	Mission	1	5	F8,7-179	q	8f	48	13	0.27
0	2000	Mission	1	5	F8,7-179	q	8f	52	2	0.04
0	2000	Mission	1	5	F8,7-180	q	8	68	21	0.31
0	2000	Mission	1	5	F8,7-180	q	8	67	5	0.07
1	2000	D3-25	y	8	F8,7-180	q	8	113	32	0.28
1	2000	D3-25	y	8	F8,7-180	q	8	109	7	0.06
1	2000	D3-25	y	8	F8,7-180	q	8	202	15	0.07
1	2000	Mission	1	5	LeGrand	5	7	53	7	0.13
1	2000	Mission	1	5	LeGrand	5	7	50	12	0.24
1	2000	Mission	1	5	LeGrand	5	7	80	2	0.03
Alleles in common	Year	Seed Parent	1stt allele	2nd allele	Pollen Parent	1stt allele	2nd allele	Total pollinations	Set	Set %

Alleles in cc ion	Year	Seed Parent	1stt allele	2nd allele	Pollen Parent	1stt allele	2nd allele	Total pollinations	Set	Set %
1	2000	Mission	1	5	LeGrand	5	7	45	6	0.13
1	2000	Mission	1	5	LeGrand	5	7	83	8	0.10
1	2000	Mission	1	5	LeGrand	5	7	93	12	0.13
1	2000	Mission	1	5	LeGrand	5	7	49	4	0.08
0	2002	NePlusUltra	1	7	A96,1-133	5	8	327	75	0.23
0	2002	NePlusUltra	1	7	F8,7-179	q	8f	331	130	0.39
0	2002	NePlusUltra	1	7	F8,7-180	q	8	376	102	0.27
0	2002	NePlusUltra	1	7	F8,8-160	e6	8f	380	101	0.27
1	2002	Mission	1	5	Padre	1	h18	235	25	0.11
1	2002	Mission	1	5	Padre	1	h18	174	10	0.06
1	2002	Nonpareil	7	8	A96,1-133	5	8	706	68	0.10
1	2002	Nonpareil	7	8	F8,7-180	q	8	682	19	0.03
1	2002	Nonpareil	7	8	F8,8-160	e6	8f	658	159	0.24
1	2002	Nonpareil	7	8	F8,8-161	g13	8f	521	170	0.33

Table 2. Effect of having 2, 1, or no S-alleles in common on final seed set in controlled crosses where the S-genotype has been determined.

Seed	1st	2n	Pollen	1st	2nd	Set-1	Set-2	Set-3	Set-4	Set-5	Average	StdDev	Recip-	Recip-	Recip-	Recip-	Recip-	Averag	StdDev
Aldrich	1	7	Rosetta	1	7	1.5	2.1	4.8			2.80	1.44	0.6	2.3	0.7			1.20	0.78
NePlusUltra	1	7	Rosetta	1	7	0.6	12				6.30	5.70	11.5	2.3	0.9			4.90	4.70
Butte	1	8	Monterey	1	8	18	3.2	16	16	7	12.04	5.84	4.9	2.3	0.7	7.4	8.3	4.72	2.90
Price	1	7	Rosetta	1	7	13.5	40	38			30.50	12.05	1.1	2	0.7	1.3		1.28	0.47
Price	1	7	WoodColony	5	7	67	56	12.4	15	37	37.48	21.67	23	11	0.7	6	6	9.34	7.57
Padre	1	18	Monterey	1	8	54.1	42				48.05	6.05	53	43.2	19.2			38.47	14.20
Fritz	1	6	WoodColony	5	7	53	49				51.00	2.00	37.2	46.7	9.3			31.07	15.87
Fritz	1	6	Aldrich	1	7	36	59				47.50	11.50	13.2	33.7	4.3			17.07	12.31
Fritz	1	6	Rosetta	1	7	32.5	37				34.75	2.25	18	37.8	4.3	0.8		15.23	14.53

Table 3. Effect of having 1 or no S-alleles in common on final seed set in controlled crosses where the S-genotype has been determined and where flowers were emasculated before controlled crosses to eliminate contamination by own pollen

Seed	1st	2n	Pollen	1st	2nd	Treatment	Set-1	Set-2	Set-3	Set-4	Set-5	Average	StdDev
F8,7-179	z	x	A96,1-133	w	8	Normal	0.49	0.27	0.55	0.80		0.53	0.19
F8,7-179	z	x	A96,1-133	w	8	Emasculated	0.08	0.14				0.11	0.03
F8-8-161	13	y	F8,7-180	7	x	Normal	0.71	0.97				0.84	0.13
F8-8-161	13	y	F8,7-180	7	x	Emasculated	0.47	0.51	0.40	0.32	0.48	0.44	0.07