Almond Board of California
Annual Report - 2002-03

Project Title: **Almond Variety Development**

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Objectives:
Develop improved pollinizers for Nonpareil, and ultimately, replacement varieties for Nonpareil and its pollenizers that possess self-fertility, improved disease and insect resistance and a range of bloom times and maturities. Goals for 2002-03 include:

A. Breed new selections through large-scale field crosses and seedling evaluations, characterize their cross-compatibility groupings using molecular as well as field tests. Continue to develop resistant cultivars for important almond diseases and Noninfectious Bud-failure.
B. Develop rapid selection techniques for self-compatibility, disease resistance (particularly Anthracnose and Alternaria), and NOW resistance.
C. Establish field trials of new self-compatible selections, particularly UCD36-52, a high quality Marcona type almond with high crack-out and good shell seal. Continue to monitor performance of 'Winters' low BF *Carmel* sources, and the *Nickels* hybrid rootstock presently in regional trials.

The almond variety improvement program is presently transitioning from the breeding of traditional pollinizers for *Nonpareil* to the development of the next generation pollinizers/replacements for *Nonpareil* which possess self-compatibility for high cropping consistency, and pest and disease resistance to reduce grower inputs. The key to the deployments of these new traits include their identification and transfer to California almond breeding lines, and their eventual incorporation into high yielding, high-quality almond varieties adapted to California conditions. Genes conferring high levels of self-compatibility as well as resistance for a number of important almond pests and diseases have been transferred from related species germplasm previously developed by Dr. Kester. The efficient incorporation of these genes with the wide range of genes required for commercial success in almond has necessitated a large number of controlled crosses to import the genes into an appropriate genetic background and large population sizes to select the rare individuals possessing the full complement of genes required for commercial success. Over 10,000 seedlings from controlled crosses between a number of selected parents were recovered in 2002, surpassing our goal of 6000 seedlings and advancing the breeding program ahead of our previously established timetable. Reductions in a University support for field facilities has led to the needed for more efficient field evaluations including the development of rapid selection techniques for targeted traits. This report highlights current progress in transferring resistance/self-compatibility genes to advanced breeding lines, as well as the development of rapid screening procedures for self-compatibility. An increasing number of advanced selections have been propagated for regional tests in 2003-04.

A. Breed new selections, and test breeding lines for resistance to key pests and diseases.

The release of the 'Winters' variety as a productive, pollinizer for the early Nonpareil bloom, while addressing the initial objective of the breeding program, highlights the importance of the subsequent objectives of yield consistency with reduced grower inputs. The statewide distribution of 'Winters' plantings will be determined, in part, by its susceptibility to Anthracnose and to a lesser degree *Alternaria. Anthracnose* was not even considered a significant almond disease 12 years ago when my breeding program began. The loss of traditional agro-chemical solutions combined with new disease threats from Anthracnose, Almond Leaf Scorch, Silver Leaf, and possibly plum pox virus make timely genetic (resistance) solutions critical. Similarly, while a more consistent cross-pollination of *Nonpareil* by *'Winters'* as well as UC sources of *Carmel* with low Noninfectious Bud-failure (BF) may help stabilize *Nonpareil* production, what will cross-pollinize the early blooming 'Winters' variety to capitalize on its high yield potential? Ultimately, self-fruitfulness (self-compatibility) in the early pollinizers (and subsequently in the major production varieties) is required. Finally, market forces are driving a decline in farm prices for all but the high quality Nonpareil and Carmel type nuts. Concurrent with the selection and testing of advanced lines leading to the release of Winters, and low BF Carmel varieties and the Nickels rootstock, the breeding program has emphasized the incorporation of new germplasm having improved resistance, self-compatibility and improved kernel size and quality. Within the past year, we have begun to recover advanced breeding lines sufficient in these traits to use as parents for the second breeding objective. However, even with high quality and well-matched parents, the probability of a seedling progeny inheriting the best genes for the multitude of desired traits including self-compatibility, high nut quality and yield potential, and disease/pest resistance is low. To increase the likelihood of breeding such an elite individual, large populations of seedlings from controlled crosses are being generated. In addition, since the best crossing combinations can only be verified by evaluating their progeny, a number of different crossing combinations have been employed.

Crossing goals for 2002 were 6,000 seedlings from approximately 20 different crossing combinations. Over 10,000 seed from controlled crosses were ultimately generated in 2002 and planted to greenhouses in Winter, 2003. The most promising 4,000 seelings will be transplanted to field plots this coming May, 2003.Thus, we are slightly ahead of our long-term breeding program time table (Fig. 1) as discussed with the Almond Board last August, 2002. Under this timetable the breeding program will soon enter a transition phase where emphasis on generating

elite seedlings will gradually shift to the generation of fewer crosses with greater attention on the regional evaluation of the increasing number of advanced breeding selections. Approximately 70% of controlled crosses in 2002 were directed towards pest/disease resistance. Disease and pest resistance are being assessed through evaluation of natural infections in regional plantings of advanced selections and parental lines, and through inclusion in the *Disease Evaluation Block* at UC/Davis being managed by Dr. Jim Adaskaveg. The bulk of field activities for 2002, however, involved the evaluation of approximately 20,000 seedling trees developed from controlled crosses between parents with promising levels of disease resistance and or self compatibility. Goals include (1) the collection of tree and nut data to further asses the value of various parental crossing combinations, and (2) the rouging-out or elimination of approximately 8,000 seedling trees to allow a more detailed quality assessment of remaining crossing progeny in subsequent years.

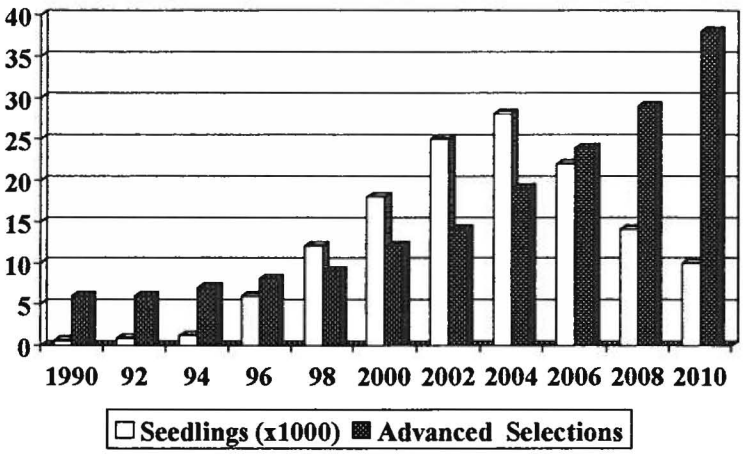


Figure 1. Almond breeding program timetable showing the number of seedlings in field production (x 1000) and also advanced breeding selections generated over the breeding period.

B. Develop rapid selection techniques for self-compatibility, and resistance.

Procedures for the rapid evaluation of resistance of targeted diseases is being evaluated based on the inoculation techniques developed by Dr. Jim Adaskaveg and others. Anthracnose is also routinely evaluated using natural field disease ratings. The level of resistance is evaluated as either the extent of consequent gumming/wounding on the hulls and/or the time to symptom development. Similar inoculation and evaluation procedures are being pursued for Alternaria. Aspergillus resistance is being evaluated as the rate and extend of mold development on wounded mature kernels and, alternately as the level of toxin production following inoculation under optimal conditions (in collaboration with Dr. Noreen Mahoney, USDA/ARS research center, Albany, CA). Almonds to be tested include advanced breeding lines demonstrating good

kernel quality and size, self-compatibility, and/or promising levels of disease resistance. Resistance to Navel Orangeworm has been evaluated in terms of the rate and extend of insect development following the placement of NOW eggs on hulls and nuts under controlled laboratory conditions. Most emphasis in 2001-02 was directed towards understanding the nature of almond hull fracture occurring at and following hull split. Various components associated with NOW resistance include hull, shell and kernel structure and biochemical composition. Our present understanding indicates that the most durable resistance to NOW and ant damage (as well as *Aspergillus* and *Salmonella* contamination) results from the development of an impenetrable shell structure. Approaches and progress in this program were derailed in the 2001 report. Breeding populations for testing the heritability of these resistances are now beginning to come into bearing and will allow a more precise estimate of genetic control and so opportunities for genetic manipulation. Current breeding populations show improving levels of self compatibility and improved horticultural quality (though not necessarily in the same tree). The challenge has been the initial incorporation of new germplasm containing the effective sources on disease/ pest resistance and improved production efficiency, and concurrently, the rapid screening of these increasingly large populations in order to identify rare individuals possessing the highest level of self-compatibility and commercial quality. New germplasm has been incorporated through introgression of selected traits from a genetically improved almond species breeding stock initiated by Dr. Kester (described below). Rapid screening for self-compatibility is being pursued using the PCR techniques developed with Dr. Dandekar and colleagues which are specific to individual self-compatibility alleles. The reliability of PCR based molecular markers for self compatibility have now been tested through field crossing studies.

B(1). Develop genetic sources of disease/ pest resistance and improved production efficiency.

A major limitation to the genetic improvement of almond has been the limited genetic variability typically available to the breeder. The ability to utilize germplasm from related species could greatly expanded genetic options available for crop improvement, however the perceived need for multiple backcrosses for successful gene introgression combined with the longer seed-to-seed cycle for almond have limited efforts for interspecific gene transfer. The historical use of wild species and their hybrids as rootstocks for peach (*Prunus persica*) and cultivated almond

(*Prunus dulcis*) has provided a starting point for further gene introgression. Wild *Prunus* species used directly as a rootstock for dryland almond include *P. spartioides* in Iran, *P. bucharica* and *P. fenzliana* in Russia, *P. webbii* in Turkey, and *P. fenzliana*, *P. bucharica*, *P. kuramica*, *P. argentea* and *P dehiscens* and *P. kotschyi* in these and nearby regions. Crosses within this subgenus, Amygdalus, have also been readily achieved under controlled conditions. Interspecific crosses (mainly *P. persica* x *P. dulcis* but also *P. webbii* x *P. dulcis*) have been used for almond rootstock breeding in France, USA, Spain and Yugoslavia. In addition, a high level of spontaneous interpecific hybridization appears to take place in the wild between species with overlapping ranges.

Selections from 7 species within the *Prunus* subgenus Amygdalus were studied including: *Prunus argentia, P. dulcis, P. bucharica, P. persica* and *P. fenzliana* of the section Euamygdalus; *P. scoparia* which is placed in the section Spartiodes; and *P. webbii* of the section Lycioides. Trees, including parent species, interspecies hybrids, and, when available, backcross and self progeny, were grown under standard fertilizer and water regimes in experimental orchards in Davis, and Winters, CA. Tree, leaf, nut and kernel morphologies of parent species and interspecific offspring were recorded. Targeted traits for breeding program transfer included hybrid vigor from interspecific crosses, self-compatibility (from the self-compatible wild species *P. persica, P. mira,* and *P. webbii* to the normally self-incompatible almond), and disease resistance. Novel variants recovered following interspecific gene introgression include high oleic acid levels in almond progeny following introgression of germplasm from peach, and the modification of peach mesocarp development following introgression of genes from almond.

Over 800 hybrid and backcross lines have now been evaluated. A wide range in morphological and developmental variability has been observed, a portion of which is shown in Fig. 1as a comparison of parental and hybrid leaf and nut morphologies. While parent species typically have unique morphologies for tree and nut structure (Fig. 2), the morphologies of interspecies hybrids often resemble those of related species, supporting the proposals that some named species are the result of natural interspecific hybridization. Selected examples from within the wide variation observed are presented below to demonstrate the phenotypes achievable, and are not necessarily representative of the phenotypes most commonly observed.

B1.1 Evaluation of hybrid vigor in interspecies hybrids

The almond cultivars 'Nonpareil', 'Ne Plus Ultra', and 'Mission' were budded to hardwood cuttings of an interspecific hybrid between almond and the peach rootstock 'Nemaguard' and to standard 'Nemaguard' rootstock seedlings. Scion vigor at sixth-leaf stage of growth was evaluated by measuring the trunk cross-sectional area at 30 cm above soil level.

The almond x peach hybrid rootstock consistently resulted in a more vigorous scion

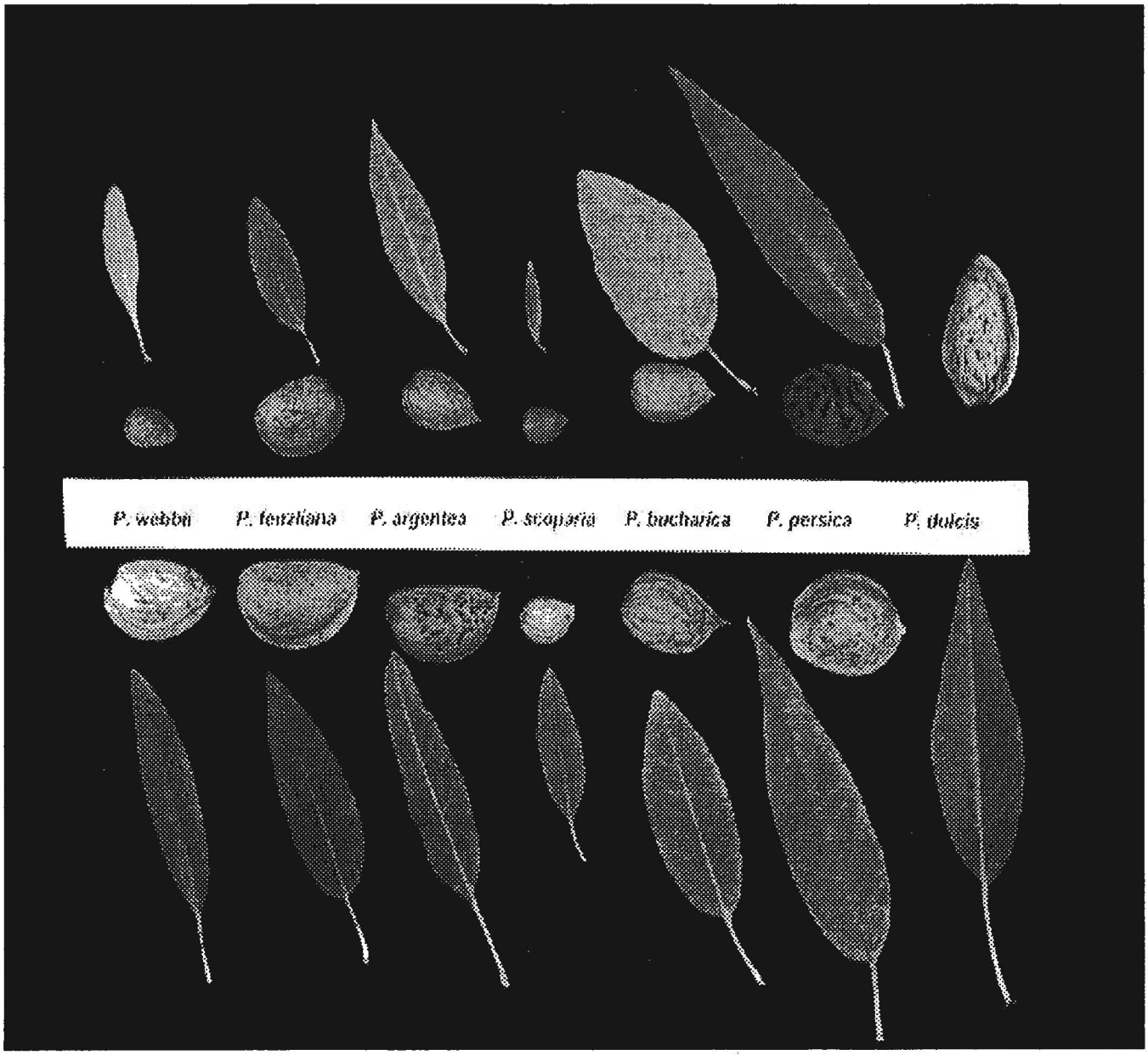


Figure 2. Leaf and nut morphologies representative of parent species (top) and hybrids with cultivated almond (bottom). Typical cultivated almond (*P. dulcis*) nut and leaf shown at right.

development for the three almond cultivars tested (Fig. 2). Such increased vigor has been previously documented and is particularly useful on poor quality soils and in replant situations where the replanted tree must compete with older and so better established adjacent trees. Increased scion vigor typically results in a larger tree than would occur on the peach rootstock, with the potential for larger yields. In some almond x peach hybrids greater tolerance to soil-born pest and diseases has been observed though this may be the result of a more vigorous replacement of damaged tissue rather than actual resistance.

B1.2 Transfer of self-compatibility in backcross progeny from interspecific hybrids

Genotypes evaluated included selected backcross (BC_1) progeny from interspecific hybrids between almond and *P. mira, P. persica* and *P. webbii.* Self-compatibility was tested by enclosing individual branches with insect-proof, mesh bags. At anthesis, all open flowers on bagged branches were self-pollinated. Viability of self-pollen was verified through in-vitro germination as described previously. Each self-pollination was repeated at least twice on alternate days to allow for a more complete pollination of late opening flowers. Fruit set was recorded at least 12 weeks following pollination to allow for late drop of abortive nuts.

Self-compatibility was readily transferred to almond from related self-compatible species. In addition, backcross and F_2 progeny usually segregate in the Mendellian ratio expected for a single dominant gene. While average seed set following controlled self-pollination vary considerably within self-compatible backcross lines, selections showing self-seed sets comparable to the parent species (between 30 and 40 percent) are readily recovered (Table 1 and Fig. 3). Self-seed set under field conditions was also strongly influenced by differences in flower morphologies, including the position of the anthers relative to the pistil, and the attractiveness of of the flower to insect pollinators.

B1.4 Evaluation of oleic acid content in backcrosses from interspecific hybrids

Total oleic acid content was determined for 2 almond selections derived from backcrosses of almond x *P. persica* and 3 cultivars important to California production ('Nonpareil', 'Mission' and 'Ne Plus Ultra'). Three separate replicates of each genotype were tested. High levels of the

monounsaturated fatty-acid oleic acid are desirable in almond kernels as it confers improved flavor and lower levels of rancidity. Selection for improved eating quality in the cultivated almond has resulted in the selection for high levels of oleic relative to linoleic and other kernel fatty acids. It was thus unexpected to find the highest oleic acid concentrations in backcross progeny from almond x peach hybrids rather than any of the 3 almond genotypes tested. While other peach derived backcross lines also possess some of the lowest oleic acid concentrations, this finding demonstrates the occurrence of unanticipated, novel variants in introgression progeny which may have important value for future breeding.

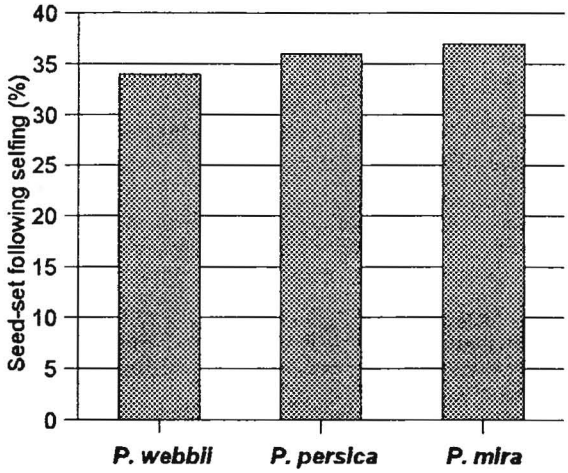


Figure 3. Average seed-set following self-pollinations of interspecies BC_1 to almond.

B1.5 Assessment of novel variants and disease resistance

Mesocarp structure and development in peach-type progeny selected after backcrosses to peach of almond x peach hybrids, were evaluated at 3 to 5 d intervals from fruit maturity to 20 d following full-maturity. Fruit flesh firmness was measured using a Magness-Taylor frimness tester with an 8 mm flat tip and freestone to clingstone fruit type was recorded. In cultivated peach varieties as well as the known examples of wild peach germplasm, peach texture segregates to either a soft, melting-type flesh with an associated freedom of stone adhesion to the flesh (i.e. freestone) or a firm, non-melting-type flesh which has always been associated with a strong adhesion of flesh fibers to the stone (i.e. clingstone). In cultivated almond, the mesocarp cleanly separate from the stone at fruit maturity resulting in a freestone type. The almond mesocarp or hull never becomes fleshy and so appears phenotypically as a non melting flesh. In backcrosses to peach of almond x peach hybrids, the full range of variability for both flesh texture and stone adhesion were observed from melting clingstones to the rarer non-melting freestone types. Additional novel variants (i.e. not observed in either parent) are seen in other aspects of mesocarp development following ripening.

Table 1. Average seed set following self-pollinations of selected interspecies crosses to almond. (Sample standard deviations given in parenthesis).

Source	Introgression	Selection	Percent Seed Set	
P. persica	F_2	10C,20-51	20.8	(1.8)
P. persica	BC_1	10C,12-28	32.3	(2.1)
P. persica	BC_1S_1	6,56-89	34.4	(2.4)
P. persica	BC_2	13,28-21	43.9	(2.1)
P. persica	BC_2	3,54-39E	36.1	(3.4)
P. mira	BC_2	13,25-75	37.2	(3.5)
P. argentia	BC_1	7920-45	11.5	(1.4)
P. fenzliana	BC_1	7906-13	9.0	(1.3)
P. fenzliana	BC_1	7906-22	9.3	(1.6)
P. webbii	BC_1	7927-54	9.6	(0.9)
$LSD_{(0.05)}$			5.3	

Table 2. Range of tree characteristics in BC1 selections from interspecific almond crosses. ('Ross' peach provided as reference; S-small, M- medium, L-large).

Source	Selections Examined	Tree Size	Crop Size	Bloom Time	Kernel Size(g)	Promising traits
P. argentia	5	S-M	M-L	Early	0.55-0.92	Pest resistance Tree Architecture
P. bucharica	2	L	S-M	Late	0.53-0.73	Late flowering Early ripening
P. fenzliana	9	M-L	L	Mid	0.67-0.94	Drought resistance Late flowering
P. scoparia	2	S-M	S-M	Mid	0.53-0.61	Drought resistance Insect resistance
P. webbii	14	S-L	S-L	Mid	0.51-0.87	Insect resistance Bearing habit
P. persica	12	S-L	L	Late	0.67-1.86	Pest resistance Architecture Bearing habit
P. persica	'Ross'	M	M	Late	0.68	

Several advanced species crosses also demonstrated high levels of resistance to important diseases including anthracnose, Monilinia blight and hull rot, Alternaria and Aspergillus (Table 2). Insect resistance was also identified for NOW, PTB, ants and Lygus damage. Additional traits of potential for almond breeding include modified bearing/fruiting habit, drought tolerance, and early production. Individuals possessing the desired traits plus the best horticultural quality are being selected for use as parents in further crosses to California almond .

C (2). Develop rapid screening procedures for self-compatibility.

Most commercial California plantings of the normally self-incompatible almond are of the older varieties 'Mission' (S1S5) and 'Nonpareil' (S7S8) and more recent varieties which have largely resulted from cross-pollination between 'Mission' and 'Nonpareil' . Six pollen-pistil cross-incompatibility groups, corresponding to the to parental S-genotypes and the four expected recombinant genotypes (S1S7, S1S8, S5S7, S5S8) have been identified through field crossing trials, with the subsequent identification of specific S-allele proteins (SRNases) and determination of their N-terminal amino acid sequences. Associated mRNA for each S-allele was determined through cDNA analysis, including DNA sequencing . Results have allowed the development of an S-allele specific PCR analysis which can clearly distinguish almond cultivars belonging to any the six major cross-incompatibility groups (see earlier reports by Dandekar and us). These DNA markers have proven useful for the determination of cross-incompatibility grouping for new varieties and breeding lines. In addition, knowledge of parental S-genotypes allows the breeding of progeny with known S-genotypes including a newly identified self-compatibility (Ssc) genotype. The further objective of this research is the development of strategies for ensuring that all progeny in a breeding program for self-fruitfulness possess the self-compatibility allele. These populations would then serve as the basis for longer-term studies on the genetic control of self-fruitfulness, particularly the role of modifier genes on Ssc expression as well as the genetic modification of the capacity for self-pollination to (autogamy).

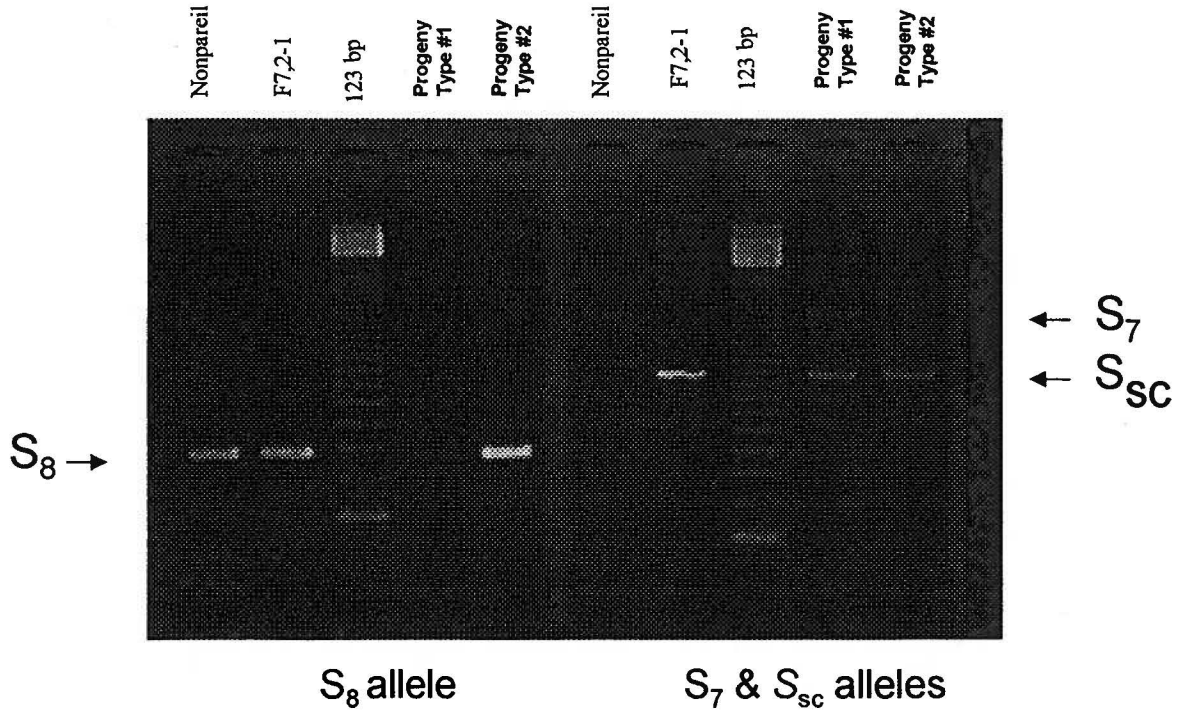
C2.1 PCR probe development for S-alleles

PCR probe development was as described by Dandekar (2000-01 Report) except that primers AS1-II (forward, 5'-TTTCAATTTGTGCAACAATGG-3') and AmyC5R (reverse, 5'-

CAAAATACCACTTCATGTAACAAC-3') were employed for the F7 self-compatible (Ssc) allele amplification. Total RNA was isolated from freeze-dried styles that were at the balloon stage of flower development. c DNA encoding S^a RNase was amplified from total stylar RNA of Mission and (S_aS_b) by 3'RACE with the gene specific primary AS-1 and an adapter primer as described previously. The 5' coding region, which was not contained in the 3'RACE clone of was amplified by the 5' RACE using the primer Pru-C5 in the adapter primer BSRACE -2. PCR fragments amplified by RACE were cloned and sequenced. Total DNA was isolated from young almond leaves of genotypes tested using a modified CTAB method. PCR analysis was conducted using approximately 20 ng of DNA, 2.5 ul of 10x PCR buffer, 0.12mM of dNTP mix, 0.125 uM of each primer and 0.2ul of *Taq* polymerase in a 25 ul reaction mixture. Cycling parameters were 5-min initial denaturation at 94 C, 30 cycles of 1-min denaturation at 94 C, 1 min of annealing at 53C and 2 min of extension at 72 C, and a 5 min final extension. PCR products were cloned using TOPO TA cloning kit and sequences determined by Mac DNAsis.

S-allele-specific PCR analysis was effective in identifying all S-genotypes in progeny

Fig. 1. PCR analysis of different genotypes expected from the cross Nonpareil (S_7S_8) x F7.2-1 ($S_{SC}S_8$).



populations resulting from both 1) selfing and 2) controlled crosses using a self-compatible parent (Fig. 4). The size of the PCR fragments amplified with the different primer pairs were approximately 1.9 kb for S_7 and 1.1 kb for S_{sc}. All progeny of the F7,5-7 selfed population possessed the Ssc allele, while all but two progeny resulting from the cross 'Nonpareil' x F7,2-1 possessed the S_{sc} allele with roughly equal proportions of the S_{sc} and S_8 allele contributed by pollen, (final data analysis not yet complete). Both of the two seedling trees not possessing the S_{sc} allele had the apparent S-phenotype of S_7S_7 (S_7-), as would result from low levels of selfing (relatively common in California almond). An accidental out-crosse to foreign pollen with an unidentified S-allele is also possible.

C2.2 Selective crossing strategies for producing high proportions of self-compatible progeny

Two crossing strategies were employed: 1) the self-compatible selection F7,5-7 was self-pollinated, and, 2) the self-compatible selection F7,2-1was used as pollen parent in a controlled cross to 'Nonpareil' (S7S8). PCR analysis using probes developed as previously described, identified the S-genotype of both F7, 5-7 and F7, 2-1 as (S_8S_{sc}). Thus, stylar recognition and arrest of pollen tubes expressing the S_8-allele in the pistils of the seed parent would selectively eliminate the pollen S_8 allele from progeny while allowing transfer only of the Ssc allele. All cross- and self-pollinations were done using flowers which had been previously enclosed in nylon mesh bags from the pink-tip to after petal-fall stage to prevent unwanted insect pollinations. Thirty-five progeny recovered from the self-pollinations of F7,5-7, and 70 progeny recovered from the cross-pollination of 'Nonpareil' x F7,2-1 were planted. Progeny trees were tested for level of self-compatibility as well as S-allele genotype. Self-compatibility was evaluated by enclosing 100 pre-anthesis flowers in the insect-proof nylon mesh bags followed by self-pollinating on at least three separate occasions between anthesis and initial petal fall. In addition, 100 flowers on adjacent limbs of each progeny trees were allowed to be open-pollinated as flower fecundity checks. S-genotype of progeny was determined using PCR analysis as described above.
Both crossing populations produced high proportions of self-compatible progeny as determined by both the successful seed set (Fig. 5) as well as by the presence of the S_{sc} allele. Two individuals seedling trees resulting from the selfing of F7,5-7 produce no seed despite carrying the S_{sc} allele. Very low seed set on the adjacent controlled branch, however, indicated a low flower-fecundity for these individuals, and a general loss in vigor in most seedlings was attributed to inbreeding depression. In the 'Nonpareil' x F7,2-1 progeny, three individuals failed to set seed; one of

which had the genotype S_7S_{sc} with the remaining 2 having the apparent genotype S_7 S_7 (as described above).

Despite the presence of the S_{sc} allele in a majority of progeny for both populations, the self-seed set performance varied widely (Fig. 5). Progeny from the out-cross showed a roughly normal distribution, though with some skewing toward low seed-set. A similar distribution with an even greater tendency towards low self-seed-set was observed in the selfed-population. No evidence of Mendelian patterns of inheritance patterns were observed in the levels of self-fruitfulness in these populations, suggesting genes modifying the level of expression of S_{sc} have low individual affect requiring quantitative analysis for their further characterization.

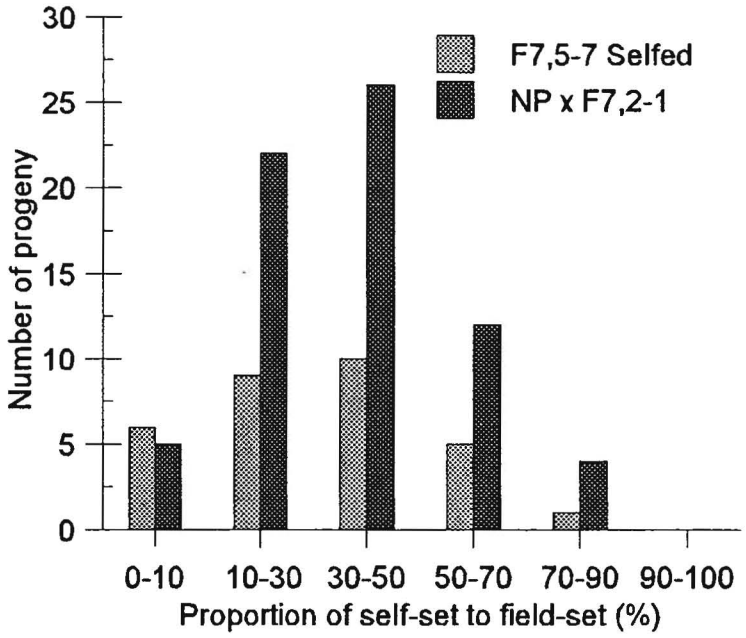


Figure 5. Self-set distribution for progeny from controlled selfs and out-crosses.

2.3. Flower structure facilitating self-pollination.

Flower structure, particularly pistil-stigma position relative to anther position, and pistil and hypanthium pubescence, were characterized for progeny of self-compatible populations. In addition, honeybee-pollinator flower preference was evaluated by observing pollinator behavior in different progeny and by evaluating the presence and quantity of flower nectar using UV fluorescence. A wide variability was observed in a flower structures within the two populations studied. This variability was further confounded by different pistil dynamics for different genotypes. For some genotypes the ratio of stigma to styles length remained fairly constant, while many others were distinguished by a more rapid extension of the pistil and stigma stalk relative to the anther filaments. A common pistil phenotype observed in the selfed population involved the rapid elongation of the upper pistil and stigma-stalk with a simultaneous bending to spiraling of the pistil so that it often would twist back into the dehiscing anthers. Temporal differences in maturity

of the stigma (evidenced by the presence of exudate) and the anthers (sac dehiscence) was also observed, particularly in the progeny of the selfed population. Thus, the close proximity between stigma and anther sacs required for unassisted autogamy necessitates a spatial-temporal developmental congruence. In a few instances the anther sacs would dehisce before bud break resulting in a sort of cleistogamy, though often sigmas did not yet appear receptive. Since bagged flowers were manually self-pollinated and honeybee pollinators were available to pollinate unbagged checks, variables affecting unassisted autogamy were not evaluated at this time. Appreciable differences were observed in honeybee-pollinator preference as determined by frequency of nectar and pollen forager visits to tagged flower branches. In some instances, honeybee non-preference could be explained by poor pollen or nectar production. Most variables affecting pollinator preference remain unknown, however. For example, the parent of the selfed population, (F7,5-7) while demonstrating high self-fruitfulness (self-compatible with high levels of autogamy), appeared to be relatively unattractive to honeybee pollinators despite having ample pollen and nectar. A closer examination of flower structure, revealed that while most almonds possess appreciable pubescence at the base of the hypanthiun near the nectaries (as with 'Mission') or at the base of the pistil (as in 'Ferragnes'), both the pistil and hypanthium of F7,5-7 were glabrous resulting in the nectar 'beading-up' and so not readily volatilized.

In summary, self-fruitfulness is a complex trait with complex genetic control. PCR analysis of S-genotype greatly facilitates breeding schemes designed to yield populations in which a high proportion of seedling progeny possess the self-compatibility allele. These more uniform populations, in turn, both allow a more precise dissection of the genetic components controlling self-compatibility and offer a more organized approach to addressing the complex genetic and developmental interactions associated with self-fruitfulness and its ultimate incorporation into commercial varieties..

Field tests promising new selections and field trial performance of 'Winters,' low BF *Carmel* sources, and the *Nickels* rootstock.

Approximately 100 trees of the self-compatible selection ' UCD36 - 52 ' which was selected as a substitute to the hard shelled Spanish variety *Marcona* (which is considered the premium almond in the European market) has been planted in a Sacramento Valley grower trial in 2002 alongside Marcona standards. Besides being self-compatible, selection' UCD 36-52 ' has the additional advantage of being a well-

Table 3. The proportion of BF expression (%) for FPMS clonal sources of Carmel.

FPMS#	Source ID	No.	1997	1999	2001	2002
3-56-1-90	D2	38	0	5	2	9%
2	D7	40	2	47	85	89
6	Nursery	NOT TESTED				
7	Nursery	NOT TESTED			BF-field	BF-FPMS
3-56-8-92	D4	10	0	0	10	15
9	D8	10	0	0	40	>40
10	VG 11-6	10	0	0	70	>70
11	D21	14	42	85	92	>92
12	VG 8-8	10	0	0	80	>80
14	VG 8-12	10	40	80	90	~100
15	VG 8-7	6	16	100	100	100
	VG 11-3	10	0	0	0?	15
	VG 8-13	10	0	30	60	>60
	VG 8-14	10	0	10	60	>60
	VG 8-18	10	0	0	40	>40
	VG 8-23	10	0	10	40	>40

sealed NOW resistant almond but with crack-out ratios in excess of 55 percent (vs. approx. 35% for Marcona). Several hundred additional trees are being propagated for test planting in the lower San Joaquin Valley. Approximately 100 2-19E trees as well as 20 trees each of 15 advanced UC selections are also being propagated for 2003-04 plantings in Sacramento and San Joaquin Valley plots. Field trials results of the Winters almond, the Nickels rootstock, low BF Carmel sources, as well as previously planted advanced selections 2-19E, 25-75, 1-87,), located at regional variety trial sites, are presented in the RVT reports for 2002..

The only clonal sources of Carmel that has demonstrated consistently low Bud-failure in the field is the UCD selection FPMS 3-56-1-90. Presently, it is the primary certified propagation source used by most California nurseries. UCD clonal selections FPMS 3-56-1-90 and FPMS 3-56-2-90 were the only Carmel sources that had been progeny tested according to UCD/Industry recommendations. It has taken several years following initial release to provide enough

propagating wood of these sources to meet industry needs. In the interim, other clonal sources originating from commercial nurseries (including FPMS 3-56-6-94 and FPMS 3-56-7-94) had been entered into FPMS through the legally available 10 step program for virus detection. These were CDFA registered and certified, which does not require the UCD BF vegetative propagation test. In addition, some commercial nurseries continued to use their pre- 1994 sources. Various amounts of BF have been observed in these non-UCD sources. In some cases, separate identity of the specific sources was maintained through the digging, sorting and size- grading process and the nursery stock was sold as source identified trees. We were assured in such instances that the BF trees did not come from the two UCD sources. In some cases, separate identity of source was not maintained through the digging and selling process and the origin of BF trees could not be determined. Severe BF in two year-old trees in 2001 and again in 2002 was traced to FPMS 3-56-6-94 and FPMS 3-56-7-94. These nursery originated sources have been eliminated from the FPMS inventory. One source of Aldrich developed BF but this turned out to be another cultivar, which we now believe to be Carmel.