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Almond Board of California

Annual Report - 1997

Project Title: **Almond Variety Development**

Project Leader: Tom Gradziel

Cooperating Personnel: W. Micke, M.A. Thorpe and C. Walters

Location: Department of Pomology, University of California at Davis

Objectives:

Develop replacement varieties for 'Nonpareil' and its pollenizers which possess self-fertility, improved disease and insect resistance, and a range of bloom times and maturities.

1. Develop crossing strategies that can consistently generate large progeny populations from crosses between selected parents particularly in weather conditions which reduce bud viability, flower set, and early fruit development.

2. Evaluate advanced selections and rootstocks in regional variety trials. Identify the most promising lines for grower testing. Find genotypes and crossing combinations with high levels of self-fertility, high quality and yield, and having later flowering period. Continue studies on the underlying control and inheritance of these traits.

3. Continue to test genetic strategies for developing self-fertility and improved production consistency, protection from Bud-failure, Navel orangeworm and aflatoxin contamination, and other disease and insect problems.

4. Develop rapid yet accurate evaluation guidelines for characterizing nut and tree quality, and yield potential, to allow rapid eliminate inferior seedlings from breeding populations and to identify the best parent combinations for overall breeding goals.

Summary:

Approximately 25,000 new crosses were made in 1997 to further improve nut and tree quality, and approximately 5,000 seedlings from 1996 crosses have now been planted in field evaluation plots for selection of the rare seedlings having high quality for all necessary traits. Advanced breeding selections planted in 1993 continue to show promise in the 1997 Regional Almond Variety Trial (RVT) performance. U.C. Davis Selection 13-1, with a bloom period similar to Carmel and so possibly covering the critical early bloom of Nonpareil, outproduced Carmel and Nonpareil at two of the three RVT with an average kernel/tree production of 25.9 lbs. vs 23.4 lbs. and 22 lbs. for Carmel and Nonpareil, respectively. Selection 13-1 shells out at approximately

56% kernel compared to Carmel at approximately 59%. Tree and kernel quality were also very good with crop loss from insect, disease, doubling and other kernel problems being low. Other promising U.C. Davis selections include 2-43W, 2-19E, 1-87, and 1-102W, all of which performed well at the Butte and Kern County RVT. High proportions of double kernels (> 10%) were observed for 2-43W and 2-19E with lower yields on the more sandy San Joaquin County RVT observed for 2-19E, 1-87 and 1-102W. The particularly good performance for 13-1 make it a promising candidate for additional grower trials in the different almond production areas. Approximately 5 to 7 additional years of testing for possible regional production problems are necessary before final decision on naming and release of these items. Selections have also been made for navel orangeworm and disease resistance, and improved kernel oil quality and storage stability.

Self-compatibility has been shown to be readily transferred to almond from its wild relatives. While agronomic quality suffered in hybrids and first generation backcrosses, quality approached commercial levels by the second backcross. In addition to self compatibility, other novel tree and nut traits were transferred including self-pollinating flower type, novel bearing habits, disease and insect resistance, and improved kernel fatty-acid composition. The level of self-compatibility and, where present, self-pollination varied considerably in different genetic and environmental backgrounds . Successful development of a commercially acceptable self-fertile cultivar will require an improved understanding of the genetic and environmental determinants of field selfing and outcrossing, as well as progress in overcoming the small size, hard-shell characteristic, and poor kernel quality associated with many of the genetic sources of self-fertility.

The breeding program has now made a transition from responding to immediate needs of the industry (low Bud-Failure sources), to current needs (more consistent pollinizers for Nonpareil - particularly during the early bloom stage), to addressing the future need for genetic options to anticipated reductions in pesticides, pollinators, and increased production costs. Early crosses made in this breeding program (1990, 1991, & 1992) had been directed towards the testing of parents and progeny for Bud-failure potential for Carmel and other selections. Information provided by the evaluation of progeny from these test-crosses have helped to identify low Bud-failure potential lines that are presently being adopted by nurseries. UCD almonds developed to meet current industry needs, include *13-1,* the early blooming pollinizer for Nonpareil and late flowering, productive varieties *2-43W* and *2-19E* . Advanced selections from these trials are now in Regional Variety Trials. Recent crosses (1993 to 1997) have concentrated on bringing needed genetic improvements previously not accessible to California almond improvement, into a high quality nut adapted to Central Valley conditions. These needs include self-compatibility to decrease the present crop vulnerability to cross-pollination problems at flowering, disease and insect resistance to reduce grower costs and processor losses, and improved nut quality. Progeny now coming into bearing are derived from a much larger genetic base, including wild almond species and European and Asian breeding lines. These breeding lines should offer exceptional opportunities for developing new genetic solutions to almond production problems. The incorporation of this more exotic germplasm to expand breeding options also results in a greater risk of bringing in undesirable traits as well (as in the more peach-like growth habit of the self-fruitful selection 25-75 now in Regional Trials). Careful and multi-year/multi-location trials of new selections will thus be required before their release for widespread planting.

ALMOND VARIETY DEVELOPMENT

1. Develop crossing strategies that can consistently generate large progeny populations from crosses between selected parents particularly in weather conditions which reduce bud viability, flower set, and early fruit development.

The objective is to generate both a large quantity and high quality of almond seedlings. The large quantity or large progeny population size is necessary due to the overall improbability of obtaining an individual seedling possessing the large number of desirable tree and nut traits necessary for a new variety's success. The large size of progeny populations from controlled crosses also allows better understanding of specific parental (genetic) combinations [i.e. parent quality] which work best for targeted goals (for example, pollen self-compatibility, flower self-pollination and good nut size and tree yield), thus leading to improved program efficiency. Early crosses made in this breeding program (1990, 1991, & 1992) had been directed towards the testing of parents and progeny for Bud-failure potential. Almonds tested included established varieties (including the *Carmel* clones presently being adopted by nurseries), and advanced selections and promising breeding lines. Information provided by the evaluation of progeny from these test-crosses have helped to identify low Bud-failure potential lines that are now being used (as in Carmel source-clones). Also tested are the several selections presently under consideration for release as new varieties (13-1, 2-19E & 2-43W), and/or as parents for new crosses. Recent crosses (1993 to 1997) have concentrated on bringing needed genetic improvements previously not accessible to California almonds, into a high quality nut adapted to Central Valley conditions. These needs include self-compatibility to decrease the present crop vulnerability to cross-pollination problems at flowering, disease and insect resistance to reduce grower costs and processor losses, and improved nut quality.

<u>1.1. 1996-97 Controlled Crosses.</u> Over 5,000 seedlings from 1996 crosses have now been planted for selection of the rare seedlings having high quality for all necessary traits. Over 8,000 seed from 260 different crossing combinations between advanced selections and California adapted lines were produced in 1997. Roughly 6,000 seed from 1997 crosses have now been planted in greenhouses for the initial roughing of inferior seedlings prior to field transplanting later this spring. The large number and size of 1997 crosses combined with very good crossing and growing conditions has allowed us to more accurately characterize important limiters of crossing success. Pollen cross-incompatibility, poor pollen or seed parent quality (virus, disease, age, etc.) and inclement weather at pollination have previously been identified as limiters to successful nut sets. Although 1997 conditions allowed these problems to be minimized, large variations in final set were seen at harvest. Analysis of the resulting data has shown inherent differences in the seed parent cultivar or selection to set fruit. An example is shown in Fig. 1. Varieties such as *Ferragnes* (French origin) and *Sonora* consistently show high sets despite differences in pollen sources (and different trees and tree locations for crosses to these varieties). Consistently lower sets are seen in the breeding lines used in this example. Final yields may often still be higher in

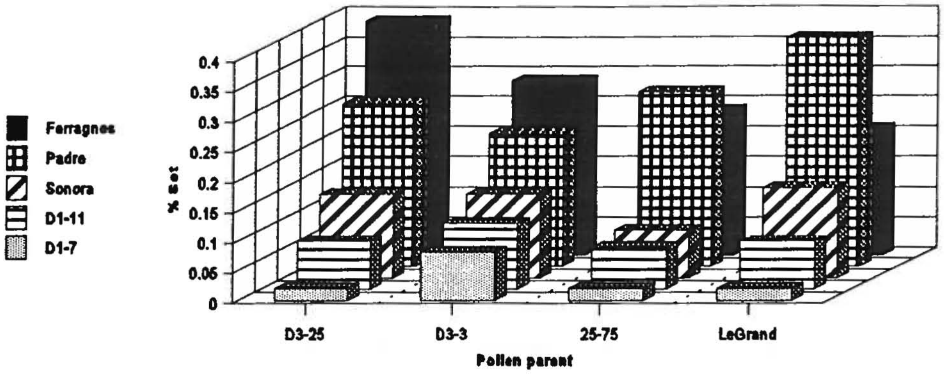

Figure 1. Crossing results (% sets) of differing seed parents with identical pollens showing strong influence of seed parent on fecundity.

breeding lines since many ultimately produce more flowers. Both flower quality and quantity thus need to be better understood. *Ferragnes*, for example, already has substantial fruit development at flowering. Pistils as large as 5 mm are common at first petal opening and anther dehiscence, (compared to 1-2mm for California almonds). The larger and more pubescent pistils also appear to disperse (volatilize) the flower nectar more effectively making them more attractive to pollinators. This variety appears to commit limited nutrient reserves to flowers early in order to improve individual flower set but also limiting the total number of flowers that can be formed. Breeding lines demonstrating both high set rates as well as high flower number have been identified leading to the possibility of dependable and high production, but also the possibility of alternate bearing if final crop size is so large as to starve out the next season's flower initiation.

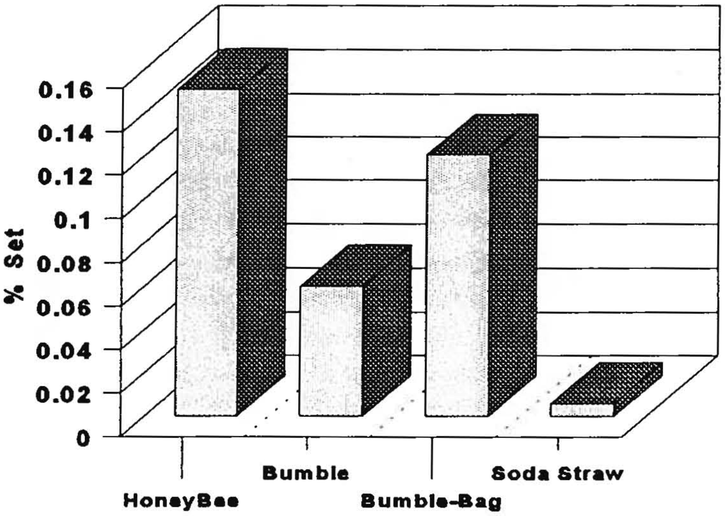

Figure 2. Efficiency of selected insect pollinators for making controlled crosses on caged trees.

1.2 Pollinator efficiency. Three different almond pollinators; honeybees, bumblebees (from BeesWest), and soda straw bees (from Dr. R. Thorpe) were tested for their efficiency in transferring pollen between cut branches of a pollen donor variety and a large tree scaffold enclosed in a mesh cage to contain the bees and exclude outside pollinators. Results, summarized in Fig. 2, show the best efficiency is seen with honeybees even when placed in relatively small (6x6x20 ft.) cages. The advantage of Soda Straw bees lies in their being able to be handled in very small numbers and in the methods developed by Dr. Thorpe to readily overwinter these bees yet induce their emergence at the time of almond bloom. Soda straw bees, while successful in emerging from overwintering tubes at the required time showed only moderate to low activity with resulting very low sets. Bumble-bee advantages include their ease of management and transport in the hive cartons developed by BeesWest, their more solitary working nature and habit of flying in colder and wetter conditions, and lower agitation (relative to honeybees) when confined. Final sets from 3 separate cages were disappointing, being lower than hand pollinations (which average approx. 10%). However, when individual bumblebees were selected for their pollen foraging behavior, dusted with a donor pollen and introduced into small mesh-bag containers (approx. 8 cu. Ft.), relatively good sets were achieved (Bumble-Bag). This last approach could be very useful for making large numbers of different but low volume crosses in years where weather conditions make hand pollinations difficult. The relatively low sets with non-honeybee pollinators undoubtedly also reflect a lack of experience and so understanding of these other bees. Initial honeybee trials also resulted in poor sets, but improved efficiencies have been developed over the years through special management of honeybee colonies. Once honeybee conditions are optimized, however, they are a very effective pollinators due to their dependable supply, their large numbers/hive and the high activity of individual worker bees.

2. Evaluate advanced selections and rootstocks in regional variety trials. Identify the most promising lines for grower testing. Find genotypes and crossing combinations with high levels of self-fertility, high quality and yield, and having later flowering period. Continue studies on the underlying control and inheritance of these traits.

<u>2.1 Regional Variety Trials of Advanced UCD Almond Selections.</u> Advanced breeding selections planted in 1993 continue to show promise in the 1997 Regional Almond Variety Trial (RVT) performance. U.C. Davis Selection 13-1, with a bloom period covering the critical early bloom of Nonpareil (Fig. 3), outproduced Carmel and Nonpareil at two of the three RVT with an average kernel/tree production of 25.9 lbs. vs 23.4 lbs. and 22 lbs. for Carmel and Nonpareil, respectively (Fig. 4,5 & 6 -next page). While Carmel bloomed later than normal in the low-chill 1997 season, resulting in poorer coverage of the early Nonpareil bloom, 13-1 showed a strong and protracted bloom. Selection 13-1 shells out at approximately 56% kernel compared to Carmel at approximately 59%. Tree and kernel quality were also very good with crop loss from insect, disease, doubling and other kernel problems being low. The particularly good performance for 13-1 make

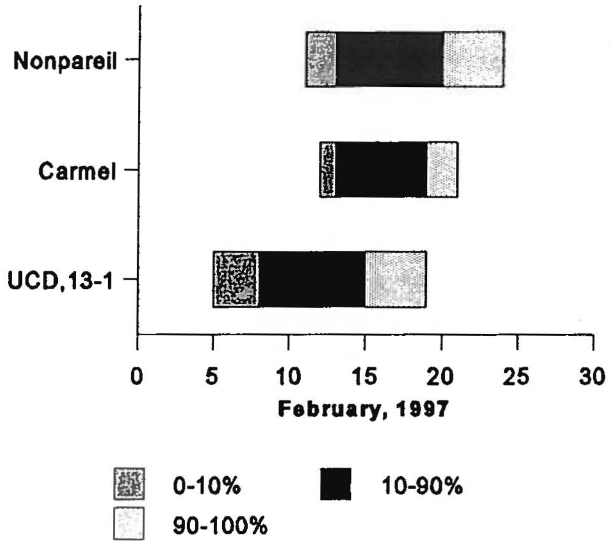

Figure 3. Bloom overlap of UCD,13-1 with Nonpareil.

it a promising candidate for additional grower trials in important production regions. UCD,13-1 is a mid-season variety, maturing just ahead of Carmel in most trials. The self-compatible and self-fruitful selection 25-75 showed relatively poor performance at all sites though this was expected due to its very late bloom and poor tree type. The purpose for planting this selection at the RVTs was to assess year-to-year production consistency of a self-fruitful type flower. Other promising U.C. Davis selections include the late blooming selections 2-43W, 2-19E, 1-87, and 1-102W, all of which performed well at the Butte and Kern County RVT. High proportions of double kernels (> 10%) were observed for 2-43W and 2-19E with lower yields on the more sandy San Joaquin County RVT observed for 2-19E, 1-87 and 1-102W. The lower yield on these later maturing varieties (including 13-1) were due in part to harvest problems at this site for late varieties. Approximately 100-200 trees of 13-1 will be propagated in 1998 for grower trials (provided 1998 performance continues to be promising). Approximately 5 to 7 additional years of testing for possible regional production problems are necessary before final decision on naming and release of these items.

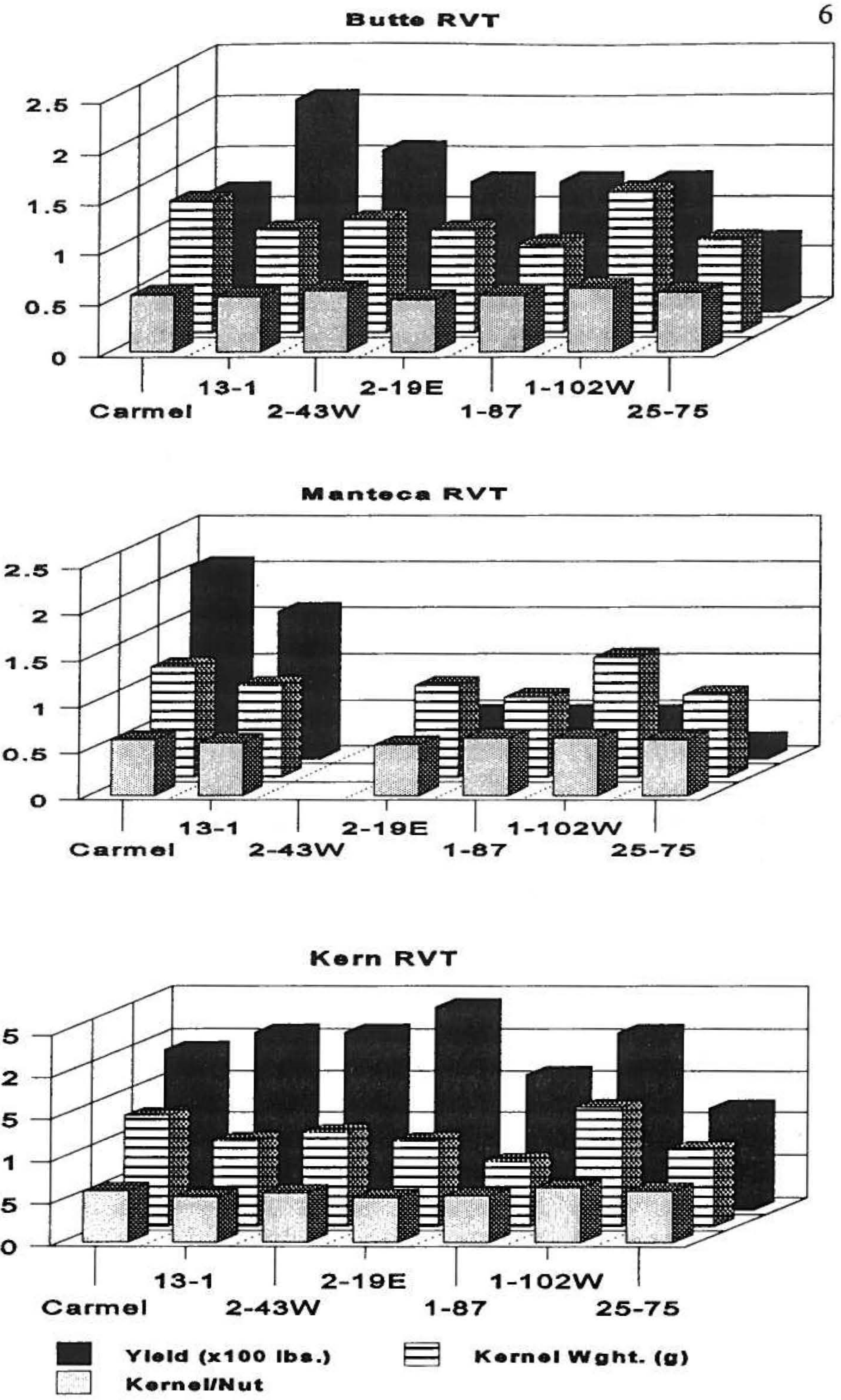

Figure 4,, 5, 6. Performance of advanced breeding selections at Regional Variety Trials (RVT)

2.2 Rootstocks.
Regional almond rootstock trials are now established at several almond production sites. While it will be several seasons before any informed evaluation can begin, 1997 results support a greater nematode as well as pre-plant storage disease resistant for the peach x almond hybrid rootstock 1-82 when compared to the Hansen hybrid.

3. Continue to test genetic strategies for developing self-fertility and improved production consistency, protection from Bud-failure, Navel orangeworm and aflatoxin contamination, and other disease and insect problems.

3.1 Self-fertility. All commercially important California almond cultivars are self-incompatible. The resulting self-sterility has necessitated the interplanting of cross-compatible pollinizer cultivars and the bringing of large numbers of honeybee pollinators into the orchard during flowering to transfer compatible pollen between trees. Often, two to three pollinizer cultivars having overlapping bloom periods relative to the main cultivar, are interplanted in the same orchard to maximize cross-pollination with the main cultivar. Despite these efforts, seed-set at bloom remains the most important determinant of final crop yield. While poor weather at bloom results in some flower loss to disease, its suppression of honeybee cross-pollination flights is believed to be the most important limitation. Thus, the development of self-fruitful cultivars may both stabilize production and reduce field management complications resulting from the differing needs of separate pollinizer cultivars.

Self-compatibility is present in the California cultivar LeGrand', though the level is probably too low for commercial sets from selfing alone. Several self-compatible European cultivars have also been reported though most have not yet been tested in the U.S. Many of these new self-compatible selections have been derived from the Italian cultivar 'Tuono' whose very hard shell and growth habit is not adapted to California conditions. The source of self-compatibility in 'LeGrand' and 'Tuono' is believed to be peach (*P. persica)* and *P. webbii* respectively. Hybrids have been obtained between California almond cultivars, including 'Nonpareil' and 'Mission', and the related species *Prunus persica, P. mira, P. webbii, P. argentia,* and *P. fenzliana.* Promising selections were backcrossed for one to two generations with recurrent selection for desired traits. (See previous annual reports). Trees were selected for vigor and desired tree and nut shape before testing for self-fruitfulness. Self-compatibility and self-pollinating ability were tested by bagging individual branches of trees with insect-proof, mesh bags. Separate bags on each tree were either hand crossed with self-pollen, hand crossed with cross-compatible pollen, or left untouched to evaluate natural selfing. Viability of donor pollen was verified through in-vitro germination and/or vital staining tests. Fruit set was recorded 8 weeks following pollination to allow for late drop of abortive nuts. Fruit set following self-pollination was compared with sets from outcrossing to assess level of self-compatibility. In general, self-sets greater than 20% were considered self-compatible, and sets of less than 10% considered self-incompatible. Similarly, sets in unpollinated bags of greater than 20% or which approximated sets from controlled out-crosses of adjacent limbs which were allowed to out-cross normally, were considered self-pollinating. Crossing tests have been performed multiple times for each selection. Results are summarized below by both genetic (species) source, and selected advanced breeding line performance.

P. persica. Peach parents used include 'Lukens Honey', 'J.H. Hale', 'Fay Elberta', the very early flowering and nematode resistant breeding line '40A-17, and the brachytic dwarf breeding line '54P455'. Hybrids were very vigorous with large trees, and leaf and fruit morphology intermediate to parents. Backcrossing to almond resulted in moderately vigorous and productive trees, often with single, pink, narrow-petaled and showy flowers. Other phenotypes recovered include double flowered, very late flowering, and pistil sterile types. Fruiting habit ranged from predominantly spurs to terminal shoot bearing habits, with the specific almond backcross parent showing strong influence on final predominance of spur production. F_2 populations segregating for the brachytic dwarf gene appeared to show some incomplete dominance for this trait rather than the complete dominance observed in peach. Up to 30% of the progeny showed degrees of pistil sterility though remaining individuals demonstrated appreciable self-compatibility. Approximately half of the self-compatible trees also exhibited some capacity for self-fertilization as well.

P. mira. Parents were very peach-like, though with small, poor-quality fruit and small, somewhat smooth, and flattened seed. Kernels tended to be bitter. Hybrids and backcrosses were very similar to peach populations though with smoother pits. Both parent and progeny trees tend to be smaller, often with a weepy or willowy growth habit.

P. webbii. The P. webbii parent was a small, thorny bush with small leaves. Bloom is very late with dense flowering occurring on many spurs and short lateral branches. Nuts are hard shelled and small with bitter kernels. Hybrids are upright, varying in size, with flowering on long flowering shoots and many lateral shoots. Flowers are almond-like; appearing pink in buds then becoming white. Anther length varies from short to long. Backcrosses to almond often retained the characteristic bearing habit on many short, lateral branches.

P. fenzliana. Parents were bushy and small. Nuts were small, flat and hard shelled with the shell surface showing sparse, shallow holes. Kernels were bitter. Hybrid nuts are intermediate to parents. Trees tend to be upright and vigorous with nut production on both spurs and terminal shoots.

P. argentea. Parents were medium to large trees with the characteristic silvery, pubescent leaves. Nuts are small with very hard shells having shallow pits to slight exterior grooves. Hybrids tend to show the complete range of shell markings but most having roughly equal pits and grooves. Kernel shapes range from long narrow to short plump. Trees are often large, almond-like, ranging in shape from spreading to upright and of good quality though fruit quality is often poor. May progeny are late blooming and late leafing, often with varying levels of leaf pubescence conferring the silvery appearance of the leaves.

3.2 Selected Advanced Breeding Lines. Tree and nut characteristics of 10 self-compatible selections are presented in Table 1 with 'Nonpareil included for reference. All demonstrate sets exceeding 20% following controlled self-pollination during flowering seasons favorable to pollination. Natural self-pollination in selection SB13,25-75 (UCD,25-75) achieves sets of better than 20% without the need for hand pollination. Honeybee foraging behavior, however, appeared erratic for this cultivar. Examination of floral nectaries revealed that the nectar often coalesced into droplets at the hypanthium wall rather than being dispersed by hypanthium trichomes, which were absent, or pistil trichomes, due to a small pistil size at anthesis.

Both self-compatibility and (when present) self-pollination varied from year-to-year indicating a strong environmental effect. Similarly, the level of self-compatibility varies within sibling groups from the same cross. Since previous work has shown self-compatibility to be controlled by a single dominant gene, all self-compatible progeny have probably inherited this gene but the level of its expression is strongly affected by the genetic background. To be commercially viable, self-sets need to be closer to 30-40% and stable from year-to-year. Thus strong selection needs to be applied not only to the presence of self-compatibility but for high levels and stability of self-compatibility. Factors controlling the level and stability of self-compatibility are presently poorly understood. The diverse genetic origins of these interspecific selections may allow important insights into the influence of genetic background for this trait.

Tree and relative crop size of self-compatible selections tended towards the larger ratings due partly to the residual hybrid vigor in these backcrosses, as well as to possibly more productive tree architectures as in the proliferation of short lateral branches in *P. webbii* backcrosses. Bloom time ranges from early to very late. While all parent species were hard shelled with low meat-to-nut crack-out ratios, backcross progeny were fairly easily selected for relatively high crack-out ratios and softer and often less-well-sealed shells (Table 1). Double kernels, which often occur in higher frequencies in some interspecific crosses were fairly low with the exception of F8S,59-1 which had almost 20% doubles. Kernel size, though, consistently smaller than the 'Nonpareil' standard was in the range of other commercially acceptable cultivars.

The finding of appreciable capacity for self-pollination in only one of these advanced self-compatible selections supports the continued need for insect pollinators for most future self-compatible cultivars. Previous reports have also indicated that self-pollinating cultivars produce higher sets when cross pollen was available. Self-compatible cultivars, however, could be an important first step in improving almond production by both allowing production on the more easily managed single cultivar orchards as well as by allowing improved efficiency of the honeybee pollinator. Honeybee cross-pollination efficiency is limited by the strong preference by the foraging bee to work primarily on a single cultivar at a time. Cross-pollination only occurs on the relatively rare cross-flights to a different cultivar, or by some mixing of pollen in the hive so that a small proportion of the foragers pollen is compatible pollen from the hive. By comparison, every bee visit in a self-compatible orchard is potentially an effective pollination since the foraging bee only has to move pollen a few millimeters within the flower to achieve a compatible cross. Since final crop yield is closely tied to honey bee pollination efficacy during the short flowering period, even relatively small improvement in pollination efficiencies could lead to significant crop improvements, particularly when poor weather at flowering or poor colony strength resulting from disease or mite infestation reduce effective bee flights.

3.3 Ability of self-compatibility to buffer against poor pollination conditions at flowering.

Yields from 26 trees of the self-compatible almond cultivar 'LeGrand' and the self-incompatible cv. 'Livingston' (which otherwise has very similar bloom periods, production characteristics, and kernel size) were analyzed over 13 years of production of the Manteca RVT. Rows of the cross-compatible and similar bloom period cultivars 'Mission', 'Padre', and 'Thompson' were planted as pollinizers between these test rows. Cultivar yields for each year were converted to equivalent yields per acre and compared to determine whether relatively greater sets occur on the self-compatible cultivar during years when poor weather at flowering limits bee pollinator flights, since, if self-compatibility acts to buffer against reduced honeybee efficacy by improving pollination efficiency, the effect should be most apparent in years of low crop set resulting from poor crossing conditions at bloom. However, this 13 year comparison of yields of the self-compatible cultivar 'LeGrand' with the self-incompatible and so cross-pollen requiring cultivar 'Livingston' shows no evidence of any significant yield buffering (Fig. 7) even though most of the low crop years resulted from inclement weather at bloom. While reports from Spain and Italy support the ability of self-compatible cultivars to set at commercial crop levels, the typical yields in these countries are well below the 3000 lbs/A yields common in this orchard. Similarly, while 'LeGrand' possesses the self-compatibility gene, sets following hand self-pollination have resulted in lower than commercial crops levels. Nevertheless, for these field trials, the 'LeGrand' would have the same level of cross pollen available as 'Livingston' in addition to any benefit of self-compatibility to pollination efficiency. Thus, the expected improvements in pollination efficiency with self-compatible cultivar orchards remains unsubstantiated. Results from 1997 crossing studies, however, demonstrate sizable differences in both self-and cross-fertilization success which appears to be largely seed parent genotypes dependent. (See section 1.1).

Table 1 - Tree and nut characteristics of self-compatible selections resulting from interspecific introgressions. ('Nonpareil' and the self-compatible 'LeGrand' provided as references; S-small, M-medium, L-large).

Species Selection	Source	Tree Size	Crop Size	Bloom Time	Percent Sealed	Percent Double	Kernel-to-Nut Ratio	Kernel mass (g)
7920-45	*P. argentia*	S	L	Early	100	4	53	0.92
7906-13	*P. fenzliana*	L	L	Mid	62	0	49	0.94
SB13,25-75	*P. mira*	M	L	Late	25	5	42	0.86
F8S,59-1	*P. persica*	S	L	Late	25	17	31	0.72
SB6,56-89	*P. persica*	L	L	Mid	10	2	45	0.79
SB13,45-8	*P. persica*	L	L	Mid	11	4	46	0.84
SB3,54-39	*P. persica*	M	L	Mid	35	4	50	0.85
8024-12	*P. webbii*	M	L	Early	80	8	61	0.71
8011-22	*P. webbii & P. persica*	M	M	Mid	80	0	59	0.87
LeGrand	*P. persica*	L	L	Late	4	0	56	1.03
Nonpareil		L	L	Mid	8	1	63	1.19

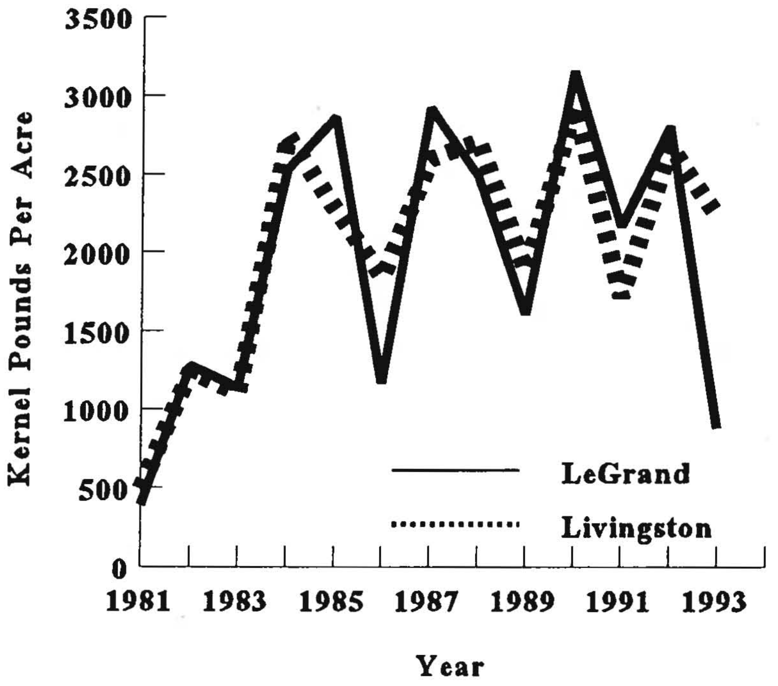

Figure 7. Production pattern for self-compatible cv. LeGrand relative to self-incompatible cv. Livingston, showing absence of year-to-year yield buffering by self-compatibility.

3.4 Breeding for low Bud-Failure.

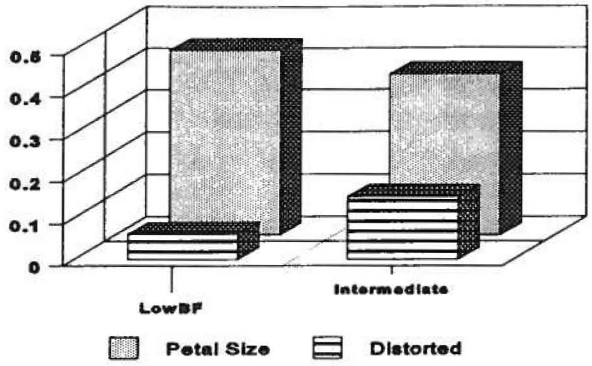

Figure 8. Deviations in petal size on same flower (Petal Size) and proportion of distorted petals (Distorted) as markers to identify low BF potential Nonpareil clones from similarly asymptomatic clones which will show BF symptoms in the next generation of propagation (Intermediate).

A genetic study of Bud-Failure (BF) in Carmel and Nonpareil almond has just been completed as part of an separate Almond Board project with Drs. K. Shackel, D. Kester and W. Micke. That project emphasized the identification of clonal sources with low potential for developing BF, and the final report has been submitted. Work continues in this program to screen-out breeding lines and potential variety releases with medium to high potential for developing bud-failure if planted widely. Selection 13-1 has now been shown to have low a probability of developing BF through the use of previously developed test-crossings followed by several year of field observations of test cross progeny. Similar tests continue for remaining advanced selections. In addition, more rapid tests are being developed based on molecular markers and/or developmental patterns. The development of BF within a shoot suggests that it first begins as a small and for the most part invisible sector within the shoot. Consequently, we have attempted to compare individual petals of a flower since the whorl of petals should fairly reliably sample most possible sectors within a shoot growing point and a petal developing from a bud-failure sector might have reduced viability and so size. Very precise analysis using digital measurements and image analysis in 1997 has been able to distinguish between asymptomatic clonal sources of Nonpareil which have relatively high BF (i.e. will show BF in later propagations) and those which will not (Fig. 8). In addition, it was observed that BF prone clonal sources had a sizable increase in the number of petal distorted flowers (flowers where one of the petals developed abberrantly) (Fig. 8). [Initial results in 1998 (a low BF year relative to 1997) also show subtle but reproducible differences in petal size though distorted flowers occur a similar rates for both sources.] While this approach may prove too tedious for large scale field testing, it may represent the first 'real time' test for BF potential and as such would be useful in developing and testing molecular marker based assays.

3.5 Breeding for Disease and Insect Resistance. A multi-year project has been developed to reduce the risk of aflatoxin development on almond through support from the Almond Board and a USDA grant. Resistance to aflatoxin contamination is being pursued through 3 interconnected strategies: (1) resistance to the aflatoxin producing fungal pathogen (*Aspergillus spp.),* (2) resistance to nut damage from worms which allow initial *Aspergillus* infection, and (3) breeding almond varieties which are less supportive to aflatoxin development once infection has occurred. While progress in each of these approaches will be summarized in 1998, a breakthrough of sorts was achieved in 1997 when breeding lines were identified which failed to support extensive toxin production even after controlled inoculations with high toxin producing strains (Fig. 9). [This finding was the result of a collaborative effort between our program and USDA, WRRC (Albany, CA) plant pathologist Dr. N. Mahoney].

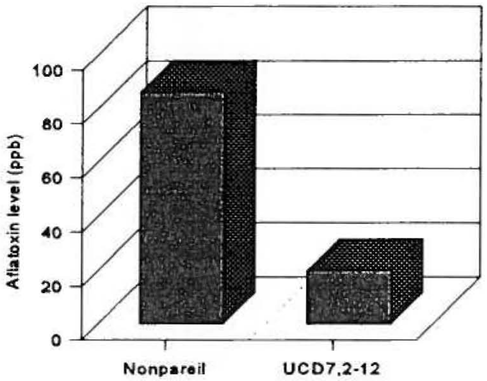

Figure 9. Reduced ability to produce aflatoxin following controlled inoculation of self-compatible advanced breeding line.

3.6 Breeding for Improved Kernel Oil Quality and Storage Stability. [Preliminary results as well as more detailed methods for this multi-year study were reported in the 1996 Annual Report. The following section summarizes findings of this now completed study]. Chemical analysis of kernel oil content showed large variation among samples of varieties and breeding lines harvested in 1996 & 1997 seasons, ranging from 36 to 53% of the kernel dry-weight (Table 2). The oil content of 'Price' was consistently low, while that for 'Padre' was consistently high, with the remaining cultivars being intermediate. Oil content from 1995 harvested kernels was generally slightly higher than those from 1996. Contrary to initial expectations, the oil content of selections possessing peach germplasm (having PA prefix in Table 2) was generally higher and more stable between seasons. Most of these selections resulted from the use of peach as a pollen parent followed by 1-2 backcrosses, or in the case of 'PA-F7,5-7" one backcross from *Prunus mira* (a wild peach species) to almond. Peach and almond are fully inter-fertile, thus peach genes should constitute approximately 12-25% of the selection's genome. Since kernel oil composition appears to be under polygenic control, peach genes are probably active during some aspect of fatty acid metabolism. The absence of any observable detrimental effect of introgressed genes on kernel oil quality reinforces the value of this more exotic germplasm to almond cultivar improvement.

Almond samples analyzed contained very low (<10%) saturated fatty-acids, high MUFA (mono-unsaturated fatty acid) and low PUFA (polyunsaturated fatty acid). Almond oil consisted primarily of 5 fatty-acids: palmetic (16:0), palmetoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2) (Table 2). Linolenic acid (18:3) was found in proportions of less than 0.02% of total kernel oil in a few samples. Minor differences were found in the proportions of 16:0, 16:1 and 18:0 fatty-acids. Palmetic (16:0), which ranged from 5.0-7.3% of total kernel oil, was the major saturated fatty-acid in all genotypes tested. Palmetoleic acid (16:1) ranged from 0.3-0.7% of total kernel oil. Stearic acid (18:0) was also a minor saturated fatty-acid, ranging from 1.1-2.3%. The proportion of the saturated palmetic and stearic fatty-acids was generally slightly higher in 1995 than 1996.

The major differences in fatty-acid composition among genotypes was found in the proportions of the MUFA (oleic (18:1)) and PUFA (linoleic (18:2)). The proportion of oleic acid in the two seasons was highest, ranging from approximately 62 - 77%. Most genotypes contained a similar proportion of oleic acid, in 1996 as 1995. A few cultivars, including 'Rosetta', 'Price', 'Ne Plus Ultra', 'Padre', and 'Sonora' had lower proportions in 1995.

Most advanced selections from peach germplasm introgression contained high proportions of oleic (18:1) for both seasons tested. The exception was the selection 'PA-F7,5-7' which resulted from a cross to *P. mira*, a wild peach species, followed by a single backcross to almond, showed low oleic and high linoleic acid levels similar to ratios reported in the generative organs of similar hybrids. As first reported in the 1996 Annual Report, the proportion of linoleic acid (18:2) was highly and negatively correlated with oleic levels for both years for all samples (R^2=0.96). This high correlation between the two predominant fatty acids of almond kernels should allow accurate future predictions of total fatty acid composition by analyzing only linoleic acid levels. The general applicability of this finding to almond and its close relatives is supported by the maintenance of this relationship even in interspecific almond x peach hybrids and their backcross progeny.

The pool of oleic acid (18:1) thus appears to be controlled by its conversion to linoleic acid (18:2). [Oleic desaturase has been proposed to control the variation in fatty-acid composition in pistachio]. A higher ratio of oleic to linoleic acid (18:1/18:2) is associated with lower rancidity and so greater oil stability. High ratios were observed in the cultivar 'Le Grand' and breeding selections having peach germplasm. 'Le Grand' is unique among the cultivars tested in that it possesses partial self-compatibility, probably derived from peach . As with kernel oil content, results suggest that introgression of genes from related species such as peach, (where no selection has been applied for high oil quality), do not necessarily result in loss in oil quality in backcross progeny but may offer opportunities for improved quality.

3.7 Variation among production regions. Oil content varied among production regions tested, being consistently lower in the Manteca plot. Yields also varied greatly with region. The southernmost region, Bakersfield, produced the highest yields, followed by the northernmost region, Chico. The central regions of Winters and Manteca had 'Nonpareil' yields only 37% and 17% as large as similar test plantings in Bakersfield. Very similar yield ratios were reported for 'Carmel', 'Sonora' and 'Mission', with regional differences being attributed to differences in weather conditions at bloom. Contrary to expectations, higher yields were associated with higher oil content (as per-cent kernel dry weight) and higher oleic/linoleic acid ratios. However, due to the range of important production factors varying at these sites causal relationships cannot be determined from this data. Differences in oil content were not due to differences in kernel moisture content since moisture content for all genotypes at all locations ranged from only 2-3%.

No significant changes in the proportions of 16:0, 16:1, and 18:0 were observed among production regions. Variation in oleic acid (18:1) generally mirrored differences in total oil content but to a lesser degree. Variations in linoleic acid (18:2) closely followed the inverse relationship previously reported with oleic acid levels. The greater environmental stability of 'Mission' has been previously noted when grown in different European environments.

The performance of these cultivars at Manteca, both in terms of ranking and actual oil composition, is in good agreement with the separate evaluation of genotype performance at this regional variety trial site reported in Table 2. The considerable variation in cultivar performance among regional sampling sites suggests important genotype by environment interactions for both oil content and composition similar to that reported for walnut.

Opportunities thus exist for optimizing oil content, resistance to rancidity during storage, and quality through the proper selection of both genotype and production environment. The introgression of new germplasm from peach and related species does not appear to result in reduced oil quantity or quality and may offer opportunities for further genetic improvement of oil quality. The importance of production environment on final genetic potential, however, supports the need for improved understanding of the environmental factors affecting kernel oil quality, including the possible relationship between oil content and yield.

Table 2. Kernel oil (% DW) and fatty acid composition (% of total oil) for almond genotypes sampled in 1995 and 1996. Data sorted on the proportion of oleic acid (18:1) for 1996 samples.

	Oil (%DW)		C 16:0		C 16:1		C 18:0		C 18:1		C 18:2	
Cultivar	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
LeGrand	51	48	6.1	5.8	0.4	0.4	2.0	1.5	71.9	76.0	19.5	16.3
Rosetta	42	42	6.6	5.4	0.4	0.4	1.3	1.6	67.0	73.4	24.7	19.2
PA-F7,5-9	47	43	5.7	5.9	0.5	0.6	1.6	1.5	76.8	73.0	15.4	19.1
Price	38	38	6.3	5.0	0.4	0.4	1.5	1.5	65.6	72.8	26.3	20.3
PA-F7,1-1	46	44	6.2	5.8	0.5	0.5	1.6	1.4	74.1	72.8	17.6	19.5
Mission	52	47	5.6	5.3	0.3	0.3	2.0	1.8	70.4	71.9	21.7	20.6
Thompson	44	41	6.0	5.6	0.3	0.4	2.0	1.6	72.6	71.5	19.1	20.8
PA-F7,5-11	47	45	5.6	6.3	0.4	0.4	1.6	1.3	75.7	71.3	16.6	20.6
Aldrich	52	48	6.2	5.5	0.3	0.3	1.4	1.4	68.9	71.0	23.1	21.8
Padre	52	51	6.1	5.3	0.3	0.3	2.3	1.6	64.8	70.9	26.5	21.8
Ne Plus Ultra	50	46	6.3	6.1	0.3	0.5	1.9	1.3	65.5	70.7	26.0	21.4
Fritz	49	38	6.5	5.8	0.5	0.4	1.3	1.3	68.0	70.6	23.7	21.8
Wood Colony	44	46	6.2	5.6	0.4	0.4	1.3	1.1	72.0	70.5	20.1	22.4
Sauret #2	43	39	6.5	5.8	0.5	0.5	1.5	1.2	69.8	70.2	21.5	22.3
Sonora	41	44	7.3	5.8	0.5	0.4	1.6	1.6	61.7	69.3	28.9	22.9
Monterey	48	36	6.7	6.3	0.4	0.5	2.2	1.4	68.2	67.5	22.5	24.2
Nonpareil	48	39	6.8	6.4	0.5	0.5	1.6	1.2	68.1	66.8	23.0	25.0
Carmel	49	41	6.4	6.0	0.4	0.5	1.3	1.4	62.4	66.6	29.5	25.5
Butte	53	48	6.6	6.2	0.7	0.5	1.8	1.6	63.9	64.7	27.0	26.9
Peerless	44	39	6.5	5.8	0.4	0.5	1.4	1.3	65.8	64.6	25.8	27.7
PA-F7, 5-7	42	46	6.6	6.7	0.4	0.4	2.3	1.9	64.0	61.8	26.7	29.2
LSD (5%)	5	3	0.7	0.6	0.2	0.1	0.3	0.3	3.0	3.2	2.7	2.8

4. **Develop rapid yet accurate evaluation guidelines for characterizing nut and tree quality, and yield potential, to allow rapid eliminate inferior seedlings from breeding populations and to identify the best parent combinations for overall breeding goals.**

Almond descriptors and a numeric scoring index have been developed (Table 3) and Regional Variety Trail selections have been evaluated in cooperation with Joe Connell (Farm Advisor, Butte Co.) and Warrren Micke (Table 4). These descriptors are intended to serve as a basis for the development of more comprehensive descriptors both to better characterize present varieties and breeding lines and to better understand the relation between these parameters and field performance. A second phase of this study is to develop useful correlations and so prediction parameters between seedling performance and final grafted tree performance. Such predictors would allow rouging of inferior seedlings earlier in the breeding program. As in 1996, growth parameters of selected seedlings have been characterized for later comparison with mature tree performance.

Recent Publications on Almond Research.

1. Tao, R., H. Yamane, H. Sassa, H. Mori, T.M. Gradziel, A.M. Dandekar and A. Sugiura. 1997. Identification of stylar Rnases associated with gametophytic self-incompatibility in almond (*Prunus dulcis*). Plant Cell Physiol. 38:304-311.

2. Marcotrigiano, M. and T.M. Gradziel. 1997. Genetic mosaics and plant improvement. In J. Janick (ed.) Plant Breeding Reviews 15: 1-84.

3. Bartolozzi, F., M.L. Warburton, S. Arulsekar and T.M. Gradziel. (In-press). Genetic characterization and relatedness among California almond cultivars and breeding lines detected by Randomly Amplified Polymorphic DNA (RAPD) analysis. J Amer Soc Hort Sci

4. Gradziel, T.M. and D.E. Kester. (In-press). Breeding for self-fertility in California almond Cultivars. Acta Hort.

5. Micke, W.C., D.E. Kester, T.M. Gradziel, J.H. Connell, P.S. Verdegaal, M. Viveros, J.T. Yeager and M.A. Thorpe. (In-press). Almond cultivar evaluation using regional trials. Acta Hort.

6. Kester, D.E., K.A. Shackel, T.M. Gradziel, W.C. Micke and M. Viveros. (In-press). Variability in BF-potential and BF-expression among nursery propagules of 'Carmel' almond. Acta Hort.

Table. 3

Scale for Characteristics of Almond Varieties - June 1997

Height

8	7	6	5	4
Very Tall	Tall	Moderately Tall	Medium	Relatively Short

Size

8	7	6	5	4
Very Large	Large	Medium to Large	Medium	Medium to Small

Shape

9	7	6	5	4	3	2
Drooping	Spreading	Somewhat Spreading	Upright to Spreading	Somewhat Upright	Upright	Very Upright

Vigor

7	6	5	4	3
Strong Vigor	Good Vigor	Moderate Vigor	Fair Vigor	Low Vigor

Branching Density (Number of Tertiary Branches)

7	6	5	4	3	2
Large #	Moderately Large #	Moderate #	Relatively Small #	Few	Very Few

Foliage Density

6	5	4	3	2
Moderately Dense	Medium	Somewhat Low	Low	Very Low

Bearing Habit

8	7	6	5
Almost entirely on spurs	Mostly on spurs some on shoots	Usually on spurs. considerable production on shoots	Equally on spurs and shoots

Bearing on Type (size) of Wood

7	6	5	4	3
Mostly on smaller fruiting wood	Often on smaller fruiting wood	On all sizes of wood	Mostly close to larger branches	Close to larger branches

Table 4. Quantification of important growth and production characteristics for varieties and selections at the Almond Regional Variety Trials based on ratings developed in Table 3.

CULTIVAR	HEIGHT	SIZE	SHAPE	VIGOR	BRANCH	FOLIAGE	HABIT	CROP
NONPAREIL	7	7	5	5	3	3	7	7
MISSION	7	5	3	5	5	5	7	4
CHIPS	5	4	5	5	5	6	6	7
JOHLYN	5	5	4	3	3	6	7	7
JENETTE	7	6	4	5	6	5	7	7
KAHL	5	6	4	5	5	3	7	3
SANO	5	6	6	5	5	3	7	7
YOKUT	6	5	4	5	4	2	7	6
SAVANA	6	5	3	5	4	4	8	5
PLATEAU	7	7	5	6	3	3	7	7
2-43W	7	7	4	6	3	5	6	5
MORLEY	7	6	4	6	6	6	6	6
KAPAREIL	8	8	6	7	4	6	5	7
SONORA	5	6	7	5	3	6	7	6
ROSETTA	7	7	4	5	6	3	8	6
13-1	7	8	5	5	3	4	7	6
PRICE	6	6	4	6	5	6	7	5
ALDRICH	8	5	2	5	7	4	8	4
WOODY	5	4	4	4	3	3	8	4
CARMEL	5	5	5	6	5	4	7	6
JIML	6	5	4	6	5	3	7	6
DONNA	5	4	3	5	2	3	8	4
MONTEREY	5	6	6	5	4	3	8	4
LIVINGSTON	6	5	4	5	3	3	7	6
1-87	8	6	4	5	4	3	7	4
PADRE	7	6	3	6	5	6	7	3
2-19E	6	6	7	4	3	6	8	5
1-102W	7	7	5	6	6	3	7	6
RUBY	5	5	3	4	5	5	7	4
25-75	5	5	9	6	4	6	7	7
BUTTE	6	5	4	5	6	4	7	4