
Almond Variety Development

Project No.: 07-HORT1-Gradziel/Crisosto

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Interpretive Summary:

In 2007 over 12,000 seed were recovered from 22 separate crossing combinations. Approximately 94% of controlled crosses were directed towards improved yield consistency and pest/disease resistance. Approximately 42,000 seedling trees developed from these and previous controlled crosses between parents with promising levels of kernel quality, disease resistance and or self compatibility were evaluated. Promising selections have been placed in regional evaluation plots in the Sacramento and San Joaquin Valleys. Advanced selections in previously established long-term regional trials continue to perform well, including the previously released Winters variety, the FPS#1 low Bud-failure source for Carmel, the recently released Sweetheart variety and the promising high-yield selection UCD2-19E. Twelve additional self-compatible selections are being propagated for regional test plantings in 2009. Effective molecular markers are being developed for self-incompatibility / self-compatibility and key developmental processes involved in nut maturation (including hull and shell split and associated susceptibility to kernel diseases and insect pests). Molecular based markers, developed as part of an Industry-University Discovery Grant, may allow the more accurate selection of desired traits as well as a clearer understanding of the key genetic and developmental mechanisms controlling those traits. Large breeding populations segregating for important agronomic traits have been developed to advance ongoing trait mapping studies.

Objectives:

Develop (1) improved pollinizers for *Nonpareil*, and ultimately, (2) varieties that possess self-fertility and improved market value and disease/insect resistance.

1. Release *Sweetheart* as a premium quality resistant variety. Expand field trials of new UCD selections and monitor performance of advanced selections now in regional/grower testing.
2. Continue to develop rapid selection/breeding techniques for Noninfectious Bud-failure, self-compatibility, disease resistance, and pest (especially NOW), resistance.
3. Generate the next generation of almonds from controlled crosses and screen progeny trees for self-compatibility, tree productivity, kernel quality and resistance to key pests/diseases.

Materials and Methods:

1. Over 40 advanced selections combining desired traits such as self-compatibility with good commercial quality have been transferred for regional evaluation in grower plots. Field trials are currently underway for three groups of UCD almond selections: a) recent releases including the 'Sweetheart' and 'Winters' almonds and the low Bud-failure Carmel source (FPS#1); b) advanced selections such as UCD2-19E being considered for release, and c) UCD experimental being evaluated for regional adaptability, disease/pest resistance and self-compatibility yield. Twelve additional self-compatible selections are being propagated for regional test plantings in 2009. Twelve, 18 and 20 year old test plantings as well as the FPS foundation sources of the low Bud-failure Carmel source (FPS#1), are being monitored in Sacramento and San Joaquin Valley sites including high summer temperature Kern County sites. In addition, 2nd and 3rd generation nursery propagations from this source are being monitored in Sacramento and San Joaquin valley grower plots.
2. Since genes ultimately determine specific plant characteristics, the best selection marker for a difficult to distinguish trait such as self-compatibility or disease resistance, is the gene itself. The recent explosion in knowledge in the area of molecular genetics has made the identification and utilization of such molecular markers feasible. Molecular markers developed with Dr. Dandekar are proving accurate and efficient in identifying California self- and cross-incompatibility groups. Continued improvements in 2007-08 include additional California almond S-alleles identification and candidate markers for hull/kernel development in almond. Resulting data is being combined with lineage data to classify additional varieties to cross-incompatibility groups and to better characterize relationships between cultivars. This knowledge is necessary for growers to plan orchards with the required cross-compatibility among cultivars and to facilitate parent selection for future crosses.
3. In 2007 over 12,000 seed were recovered from 22 separate crossing combinations. Approximately 94% of controlled crosses were directed towards improved yield consistency and pest/disease resistance with aflatoxin (*Aspergillus* spp.), *Alternaria* leaf spot, hull rot and flower (*Monilinia* spp.), blight being principle disease targets and navel orangeworm being the primary insect pest targeted. Disease and pest

resistance in initial seedling trees are being assessed through evaluation following natural and controlled infections in seedling blocks including Dr. Adaskaveg's almond disease trials at Davis, CA.

The bulk of field activities in 2007-2008 remains the evaluation of the approximately 42,000 seedling trees developed from controlled crosses between parents with promising levels of kernel quality, disease resistance and or self compatibility. Tree and nut data are being collected/analyzed to determine the value of various parental crossing combinations, to rouge-out or eliminate approximately 30% of initial seedlings followed by ~80 % of 3rd & 4th year trees (to reduce field costs while allowing a more detailed quality assessment of remaining crossing progeny in subsequent years), and to select promising genotypes for regional testing in anticipation of future variety releases.

Results and Discussion:

The release of the *Sweetheart* and *Winters* almond varieties and low Bud-Failure *Carmel* sources to the California nursery industry addresses the initial breeding objective of dependable, productive pollinizers for *Nonpareil*. Our next objective is a series of almond varieties having production quality and yields comparable to *Nonpareil* but with self-compatibility to improve insect pollination and yield consistency, and reduce orchard management costs. Additional priorities include good shell-seal to control worm, ant, and fungal (*Aspergillus*) entry, with high crack-out percentages, freedom from Non-infectious Bud-failure and improved disease/pest resistance.

UCD 36-52 has been released as the cultivar 'Sweetheart'. This new cultivar combines very high kernel quality (high oleic acid which confers good processing quality and phytonutrient value and lower susceptibility to rancidity in storage), good productivity, partial self-fertility, and improved resistance to navel orangeworm and associated aflatoxin contamination. Because of its unique 'Marcona'-like heart shape, Sweetheart provides an alternative/replacement variety for the premium quality, niche-market, Spanish variety 'Marcona' which is currently being test-planted in California.

The low Bud-Failure *Carmel* source FPS#1 continues to show very low incidence with commercially insignificant yield losses in 12, 18 and 20 year old test plantings in Sacramento and San Joaquin Valley sites including high summer temperature Kern County sites. Nursery propagations from this source, (which along with the original Carmel seedling tree source), remain the principle foundation stock for this variety. In 2008, individual nursery mother block trees (which were propagated directly or indirectly from the FPS#1 source and so are 1 to 2 generations more advanced than FPS#1), showed varying levels of Bud-failure in the critical 1st 5 years of growth when used as bud-sources for these trees. Nurseries are currently rouging out those mother trees showing BF-expression in propagated trees during their 1st 5 years of growth, shifting future propagation to the fewer remaining Carmel trees showing good field performance. The future of this variety will depend on the nursery industry's ability to identify an adequate number of FPS#1 sourced mother trees to meet the demand for new Carmel plantings. While some nurseries remain optimistic that relatively clean mother block trees can be maintained, other nurseries find this to be more challenging with increased time. Very severe BF incidence in some young 2008 Carmel orchards have also been trace to

the use as propagation stock, of Carmel sources which were either untested or previously tested positive for BF expression in vegetative progeny.

UCD 2-19E is a very productive but potentially alternate bearing pollinizer for the Nonpareil late-bloom. New plantings of this selection have recently been established in Sacramento Valley and San Joaquin Valley test plots to determine whether consistent high yields are possible with higher levels of irrigation/fertilizer inputs. A performance summary for recently completed Regional Variety Trials was included in the 2007 report. An updated description of UCD2-19E is provided in Appendix A.

Weather conditions in the spring of 2007 were very favorable for field crosses and over 12,000 seed were recovered from 22 separate crossing combinations. Parents for these crosses were selected primarily for self-fruitfulness, disease and pest resistance, and high kernel quality and yield. Weather conditions in the spring of 2008 were also favorable, though low chilling during the winter delayed bloom by approximately 8 days. Warm daytime temperatures during bloom accelerated bloom development, reducing the total bloom season and opportunities for cross-pollination. By enclosing both entire trees and scaffold branches of seed donor parents in insect proof screening prior to pollination and then using inserted honeybee and bumblebees (along with bouquets of desired donor pollen), we were able to achieve a large number of successful crosses and anticipate the recovery of over 10,000 additional seed from controlled crosses in the summer of 2008 (barring any unforeseen weather/disease problems).

As in previous years, the bulk of field activities in both 2007 and 2008 involved the evaluation of new and remaining seedling trees (approximately 32,000 in the field and 10,000 in the greenhouse). In addition, a large number of advanced selections are now entering production in regional testing. The goal is to identify the most promising individuals from segregating seedling populations, though our evaluations also target the characterization of the segregating population in general so as to ascertain the merits of the specific parents used, both individually and in specific combinations. Similarly, for advanced selections planted in regional grower plots, the goal is to evaluate not only commercial potential but also the specific advantages/disadvantages of these breeding selections for use as future parents. For example, breeding lines were selected for the most recent regional trials based not only by perceived commercial potential, but also potential disease resistance (particularly to flower blight, hull rot and *Alternaria* leaf spot), and to evaluate the consequences of different tree architectures and bearing habits in diverse California growing regions. (In one test we are evaluating whether a more profuse lateral branching provides an advantage to varieties planted in the Sacramento Valley where climatic conditions typically result in a less dense canopy development than that seen in the southern San Joaquin Valley). Regional trials have also proven very important in justifying the otherwise costly process involved in the patenting, clean-stock development, and release of improved almond varieties. [Because of the increasing costs of the patent process, the University now requires extensive documentation of breeding selection value before the patenting process is initiated].

Thirty advanced UCD experimental selections, demonstrating promising levels of self-compatibility, pest/disease resistance, kernel quality and/or tree productivity, have been planted in medium-scale (10-100 tree), grower test plots in Kern and Colusa Counties

and are now coming into bearing. Results from the first harvests have identified many individuals with continuing promise but have also identified individuals with possible deficiencies (disease susceptibility, undesirable tree structure, low yields, etc.). Tree and kernel characteristics of the most promising seedling selections are summarized in Appendix A. In addition, 12 promising breeding lines were selected in 2007/2008 and are currently being propagated for grower evaluation block planting in 2009 (as described in Appendix A).

The development of molecular markers for almonds continues to make progress in our collaborations with Drs. Crisosto and Dandekar. A large number of new genetic markers have been added to the almond genetic map using approaches described in greater detail in 2007 report. The majority of these markers (the S-allele or self/cross-incompatibility markers are an exception), do not tag commercially valuable genes directly, but can be used to detect the presence of these genes if they are located close together on the DNA strand. The development of new genetic markers continues because with more markers the almond DNA strand becomes more highly saturated with markers and so the likelihood increases that a marker will be located close enough to a commercially important gene so that it can be detected. Detection is achieved by making controlled crosses between parents selected for desired commercial qualities and then evaluating the segregation of those traits in the segregating progeny population. By also evaluating the segregation of the marker genes in these populations, it is possible to identify a marker gene which always segregates with the trait of interest due to its being physically located nearby in the DNA strand. The development, characterization, and maintenance of these large segregating populations from controlled crosses creates a challenge for the breeding program since one of the keys to its cost efficiency is the rapid and early rouging out of inferior individuals from large segregating populations. Thus, while genetic maps will improve the efficiency of such rouging in the future (for example, self-compatibility could be selected using molecular markers at the seedling stage and so eliminate the need to grow out the entire population over several years to the fruiting stage before self-compatibility can be detected). This technology requires the maintenance of previously unselected populations in order to discriminate the positive and negative forms of the different genes/traits. This "field population maintenance" burden is further exasperated because in order to be considered for the outside funding often necessary for molecular work, mature breeding populations segregating for the targeted trait need to be pre-established (a difficult task when it typically takes five to six years to establish mature populations and when the final decision for outside (molecular) funding is very inconsistent owing to federal funding agency hesitancy to fund specialty crops such as almonds). Consequently, we have had to establish and maintain large, unselected populations as a prerequisite for applying for supplemental state and federal molecular research based funding. As an example, Appendix B summarizes breeding populations segregating for seed/seedcoat antioxidants (important both for phytonutrient value and disease resistance), which we are currently maintaining in the hopes of eventually securing federal matching funds for detailed mapping of the controlling genes. Specific knowledge of individual genes in this pathway will allow us to select for those contributing positive attributes (improved phytonutrient value and aflatoxin resistance), from negative attributes (increased postharvest tissue browning and kernel quality deterioration).

Part of the difficulty in attracting outside (typically federal or state), funds for tree crop research results from the long generation time and large plant size (and consequently relatively small possible population size), when compared to vegetable or cereal crops. Tree crops are also at a handicap relative to vegetable and cereal crops in that we have made much less progress in identifying the multitude of factors contributing to crop quality and yield (primarily due to our disadvantage of long generation time and small potential population size). Molecular marker strategies provide an opportunity to leapfrog past these obstacles. For example, within the next decade it will be possible to sequence the entire almond genome and, based on current work with other crops, be able to predict the putative role (and so breeding value), of different gene sequences from different parents. To use an analogy from the card game of poker (except in this game each hand [or trait] is determined by tens to hundreds of different cards [genetic factors]), we will be able to know the identity of the different cards [genetic factors] from the beginning and so can pick and choose (either by traditional breeding or genetic engineering), the best combination of genetic factors to optimize the desired end result. Because the full utilization of this molecular technology is still a decade or so away, however, and improved varieties are needed now, we have adopted an approach of essentially spiking the deck of cards with 'genetic aces' to improve our short-term opportunities to achieve breeding goals, as well as the longer-term goal of having the future 'genetic deck of cards' with the most commercially valuable genes for subsequent characterization and exploitation. We're pursuing this goal through the increased introduction and incorporation of novel, commercially valuable genes from outside the traditional California almond germplasm. The objective is to identify and incorporate major genes which can be more readily identified and so transferred into California-adapted varieties and which by themselves can contribute sizable improvements in commercial quality. [This approach, which is called exotic gene introgression, has potential disadvantages, since the very exoticness of the gene can bring with it some undesirable attribute. However, a unique advantage of tree crops over both vegetable and cereal crops is that there is a much greater plasticity in the final tree and nut characteristics acceptable for commercial use]. An example of this type of gene is the self-compatibility gene which, because it is not available in cultivated almond, has to be transferred or introgressed from a related species. Although a single, readily transferred gene, self-compatibility radically changes the pollination requirements for commercial almonds and so offers dramatic improvements for the almond crop set where insect pollinizers are insufficient for normal cross-pollination. A negative consequence of the incorporation of the self-compatibility gene has recently been documented by European almond breeding programs where significant losses in tree productivity were observed in breeding progeny. Decreased productivity was shown a consequence of inbreeding within advanced breeding lines since all self-compatible lines were ultimately derived from the European self-compatible land race 'Tuono'. Tuono is a very old variety/land race which has recently been shown to have originated from the natural transfer to cultivated almonds of a self-compatibility gene from wild *Prunus webbii* trees growing alongside cultivated almond fields in southern Italy. In the California almond breeding program, self-compatibility has been transferred from a multitude of different sources including *Prunus webbii*, *Prunus persica* (peach), *Prunus mira*, as well as induced mutations (Supernova), and we routinely reshuffle or recombine this germplasm to avoid inbreeding depression and to optimize

final genetic composition (see Table 3). Although starting out with very exotic and so commercially unadapted germplasm (in addition to the species listed above, we have also transferred genes from *Prunus argentea*, *Prunus fenzliana*, and *Prunus scoparia* as shown in Table 3, Fig. 6 and Appendix B). We have now completed 6 or more cycles of back crossing of this material into a more commercially accepted California almond background resulting in a large number of breeding lines which in appearance are indistinguishable from commercial varieties (see Appendix A), yet which contain novel and often major genes for self-compatibility, disease and insect resistance, and improved tree and nut quality. To compare the value of genes from related species versus cultivated almonds, we are involved in a series of evaluations as reported in recent (2007-08) publications. In an evaluation of genetic diversity within related species as well as Californian, European and central Asian (as the center of early domestication), cultivars, we have shown that the genetic variation found in related species dwarfs that from within even geographically diverse cultivars. This is graphically shown in Fig. 1 where the genetic diversity is shown as a three dimensional graph of individual cultivar/species data. The dense concentration of points at the center represent all European, Asian and Californian cultivars and shows a relative genetic uniformity despite their diverse geographic origins. This uniformity is due in part to a common use of high-quality cultivars as parents by geographically diverse breeding programs. For example, the self-compatible land race Tuono has been used as the primary source of self compatibility by breeding programs in Spain, France, Italy, Portugal, Morocco, Israel, Greece, Turkey, Iran and Australia. The genetic uniqueness of the related species as well as the general genetic uniformity of geographically diverse cultivars is more thoroughly charted out in Fig. 2 (see publication #1 for greater detail), where, similar to an actual tree (tilt the chart to the left so that the main 'trunk' lines are vertical), the more closely related germplasm is clustered together on small attached branches while the less closely related germplasm is found on isolated branches (such as all the related species), or on its own individual scaffold (as is the case for the species *Prunus tenella*). Interestingly, among the geographically diverse cultivars only seven relatively old Iranian cultivars (uppermost in Fig. 2 or left branches on tilted tree), maintain their uniqueness. The remaining cultivars are thoroughly intermixed suggesting significant genetic relatedness. These findings agree with our earlier published findings (see publication #5, 8, 10), and further validate the use of related species as promising sources of new genetic material for cultivar breeding. In a related study of the same material, we were similarly unable to discriminate these diverse cultivars based on geographic origin when agronomic characteristics rather than genetic markers were analyzed (Fig. 3). The relative uniformity observed in cultivated material

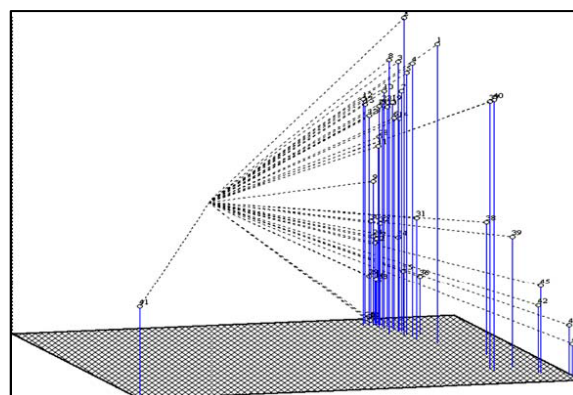


Fig. 1. Principle coordinate map for the first, second and third principle coordinate estimated for 781 AFLP markers using the genetic similarities matrix for 45 cultivated genotypes and related species.

from different geographic origins also underscores a previous finding (publication #9), that while reshuffling genes from within this cultivated germplasm will result in progeny that are genetically (and in appearance), distinct from the parents, the level of performance (productivity, disease resistance, quality, etc.), will typically not significantly exceed that of the stronger parent. To put it differently, to improve the traditional performance of a new variety, one often needs to bring in new genetic factors with pronounced individual effect. California varieties, because most are derived from crosses between the two founding varieties Nonpareil and Mission, offer a unique opportunity to study this genetic constraint. Since the large numbers of varieties resulting from Nonpareil x Mission crosses represent commercially acceptable segregating progeny of this cross, they also offer a useful opportunity to begin to assess the genetic factors controlling important commercial traits.

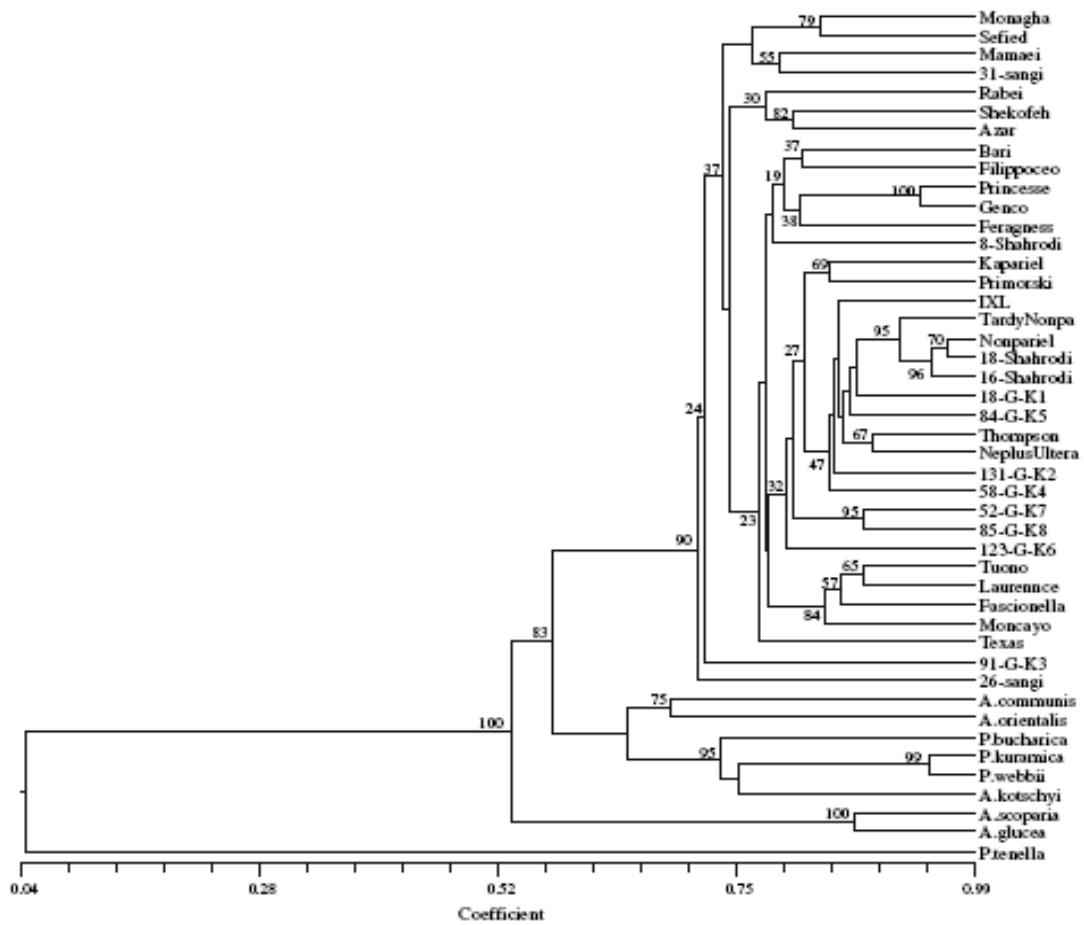


Fig. 2. Dendrogram obtained with the similarity Jacard coefficient pair group method with arithmetical average clustering algorithm from 781 AFLP markers for 45 almond accessions and related species. The value on the dendrogram gives the stability of nodes estimated with a bootstrap procedure (no number indicates support of less than 10%).

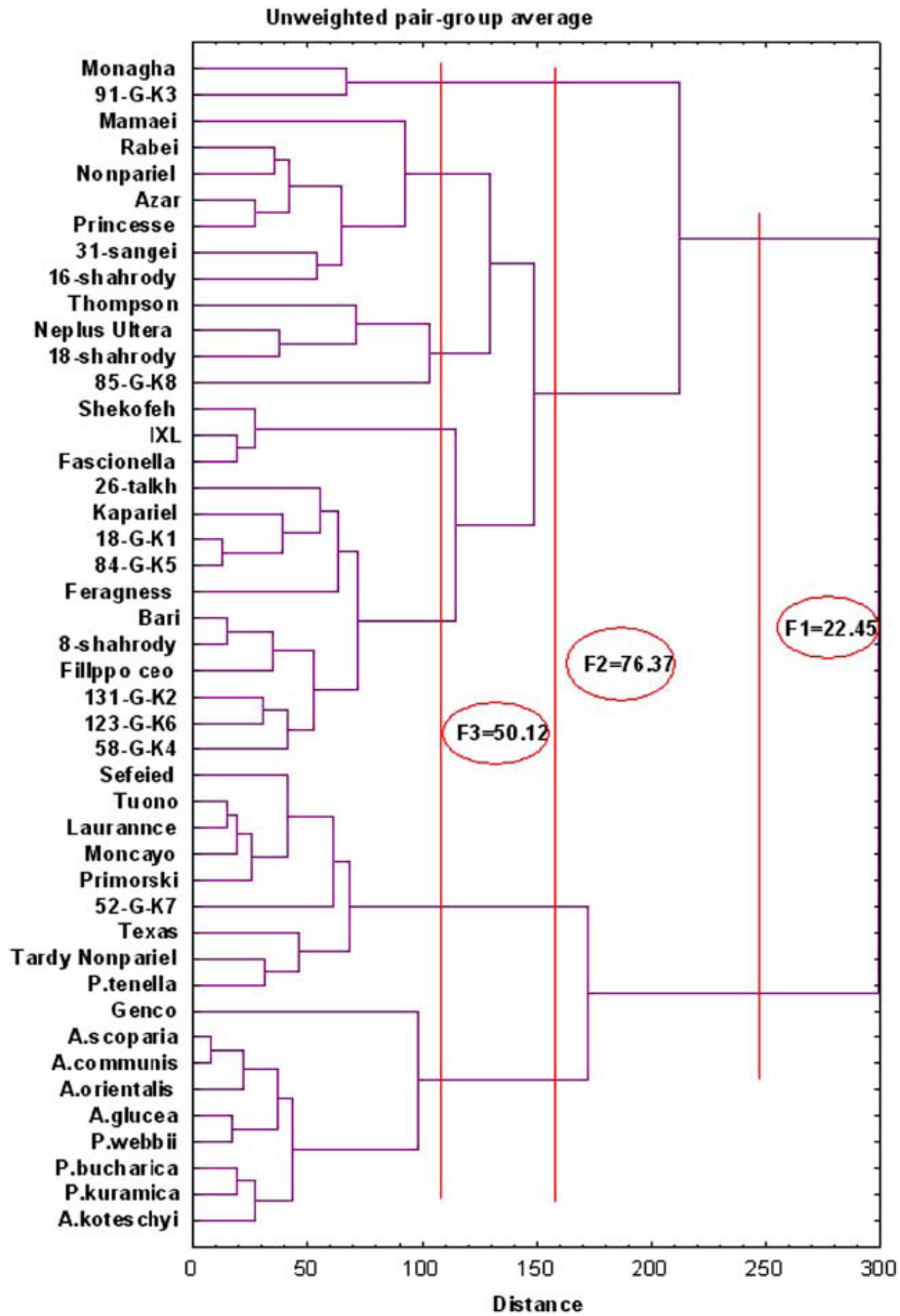


Fig. 3. Dendrogram obtained with the dissimilarity matrix of the agronomic characteristics and un-weighted pair group method with arithmetic average clustering algorithm for 45 almond accessions and related species. The value on the cut-off point on dendrogram gives the optimal clustering estimated with a MANOVA procedure.

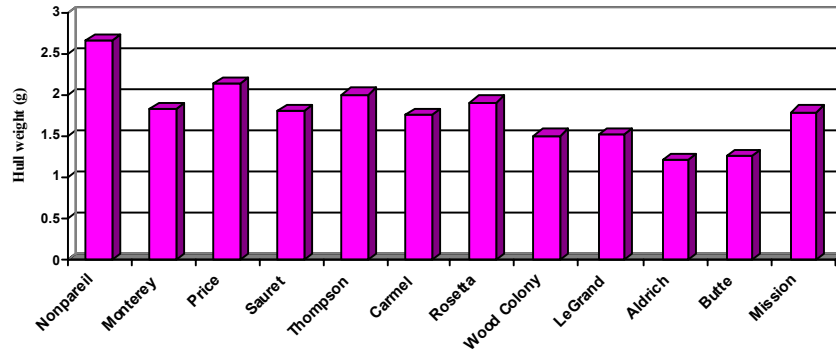


Fig. 4. Average hull weight of Nonpareil, Mission and cultivars resulting from a Nonpareil x Mission cross.

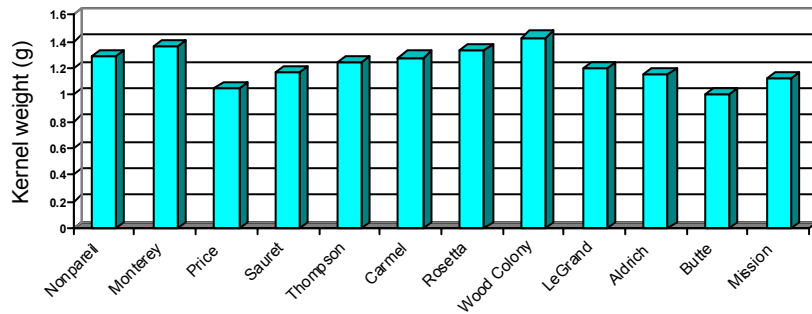


Fig. 5. Average kernel weight of Nonpareil, Mission and cultivars resulting from a Nonpareil x Mission cross.

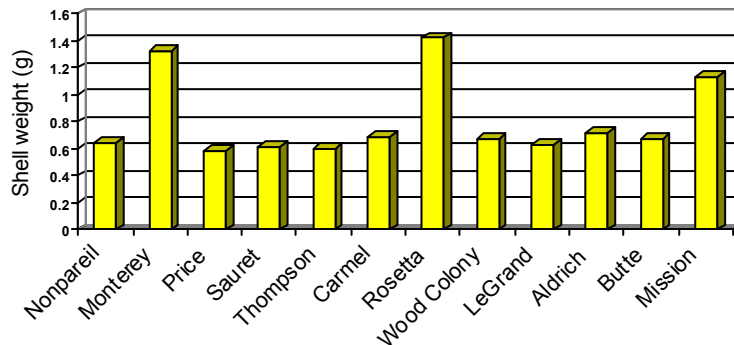


Fig. 6. Average shell weight of Nonpareil, Mission and cultivars resulting from a Nonpareil x Mission cross.

In this analysis, 10 characteristics of commercial importance: hull weight, kernel weight, shell weight, lignin composition, kernel lipid content and composition, shelling proportion and yield are compared for Nonpareil, Mission, and 10 of their progeny varieties. Data evaluated is average performance over eight years from fully mature commercially producing orchards.

In Fig. 4, average hull weight of progeny varieties tends to be intermediate that of the parents with some particularly high-yielding varieties such as Butte showing reduced hull weight, presumably resulting from the from the greater crop loads in this high-yield cultivar. The relatively uniform distribution of progeny between parental means is indicative of a trait controlled by many genes, each with relatively small effect. The genetic potential for hull weight of any individual progeny would be determined by the total number of such additive genetic factors acquired from both parents. Because of the averaging affect, it would be difficult to breed varieties with hull weight much higher than the levels observed in the higher Nonpareil parent.

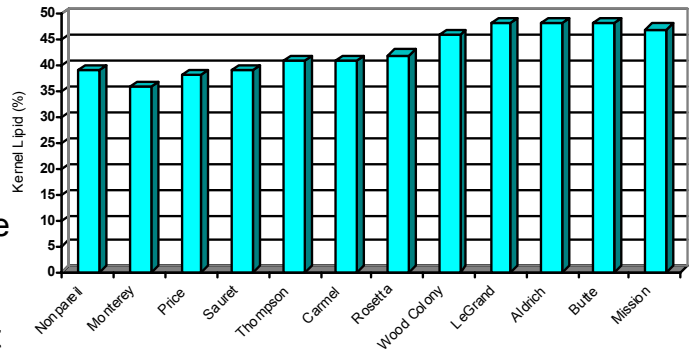


Fig. 7. Average kernel lipid content of Nonpareil, Mission and cultivars resulting from a Nonpareil x Mission cross.

A somewhat similar distribution is observed in Fig. 5 where kernel weight is plotted.

Again, most progeny are similar to the average between the two parents, though several progeny, most notably Monterey and Wood Colony are measurably larger than the larger Nonpareil parent. Again, the additive effect of many genes with relatively many small individual contributions can explain the results

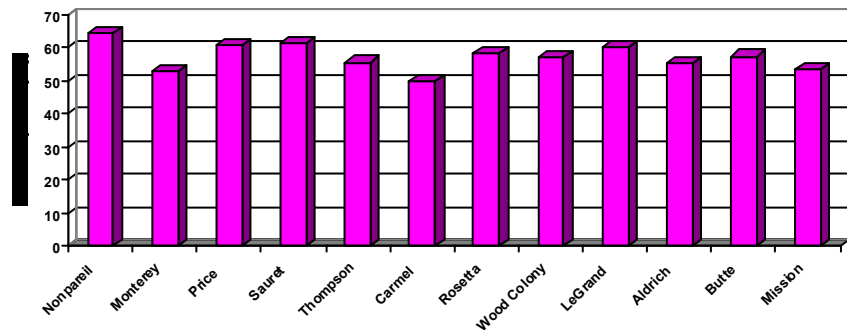


Fig. 8. Average oleic acid content of Nonpareil, Mission and cultivars resulting from a Nonpareil x Mission cross.

assuming that the larger kernel varieties have inherited a large number of genetic factors contributing positive to kernel weight from both parents. The skewing of the population towards larger kernels would be anticipated since the commercially desirable large kernels would be selected in these populations and small kernel individuals rouged out. Despite the commercial desirability of large kernel size, even the largest kernels observed in these progeny varieties is not considerably larger than either parent, as would be expected in a trait controlled by additive genetic factors since there is a parental determined limit to the number of kernel increasing factors available. Several European cultivars such as Almendro de la pie have kernel sizes easily exceeding 3 g which if

crossed with California material would result in significant increases in kernel mass in the progeny. However these larger kernels are also associated with losses in kernel quality, particularly an increase in kernel creasing and shape distortions.

When total shell weight is evaluated (Fig. 6) the progeny segregate into two groups: high shell weight types similar to the Mission parent and low shell weight types comparable to the Nonpareil parent. This type of dramatically punctuated response differences in

progeny populations is indicative of control by major genes, and major genes have been identified as being involved in the control of this trait. The most important of these genes is the hard-shell (D-) locus which when present confers very hard and highly lignified, thick shell as compared to Nonpareil's soft paper

shell. While thick and hard shells are desirable because of their protection against insect damage and associated

aflatoxin contamination, they're undesirable because they result in low kernel/nut crack-out ratios and because the thick shells can often damage the kernel meat during processing. Novel and major genes controlling shell composition have also been transferred from related species (described later in this section), allowing significant increases in shell seal integrity while at the same time reducing total shell mass and so increasing kernel/nut crack out ratios.

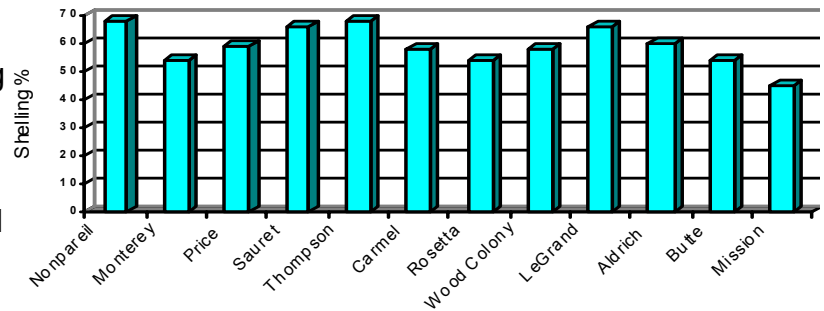


Fig. 9. Average shelling percentage of Nonpareil, Mission and cultivars resulting from a Nonpareil x Mission cross.

Both total kernel lipid (Fig. 7) and oleic acid composition of kernel lipid (Fig. 8) show the intermediate distribution between parent levels expected when a number of genetic factors, each having a relatively small contribution to final trait performance. If control is additive, it would be difficult to dramatically increase either total lipid or lipid composition using crosses within this germplasm since only a relatively small incremental gain would be expected. This has been our experience in early efforts at breeding for these traits. When genes from outside this germplasm were incorporated, however, opportunities for dramatic increases were observed. Total kernel lipid contents as high as 52% have been observed in some peach (*Prunus persica*), derived lines. Even higher increases in oleic acid proportion had been recovered in advanced lines resulting from introgression of genes from peach and its relative *Prunus mira*. For example, the recently released Sweetheart variety has oleic acid proportions as high as 77%, an important factor contributing to its Marcona-like buttery roasting quality as well as resistance to rancidity in storage. Similarly, the advanced selection F1-1 now in regional grower trials (described in Appendix A), combines a desirable high oleic acid proportion of 74% with high levels of self-compatibility as well as high levels of resistance to flower blight and bacterial blast.



Fig.10. *Marcona* almond shell showing fractures at the inner and outer endocarp layers associated with the site of abortive ovule vascular strand.



Fig.11. Cross-section of advanced selection from BC2 to *Nonpareil* showing good shell-seal with the high crack-out of 77%.

Shelling percentage is a trait of great commercial importance since high shelling percentage (kernel mass/kernel plus shell mass), is associated with higher kernel yields while lower shelling percentage is associated with thicker shells (see Fig. 10) and so greater potential resistance to insect damage. Progeny cultivars segregate fairly uniformly between the *Nonpareil* parent with its very thin paper shell and the *Mission* parent with its thicker shell and so lower shelling percentage (Fig. 9). While the *Mission* shell is considerably thicker than a *Nonpareil* shell, both are considered soft shells since they lack the dominant (D-) gene which confers a very high lignification resulting in a thick and very durable shell as exemplified by the variety *Peerless* and the European variety *Marcona* (see Fig. 10). Again, our experience has shown that extensive crossing within the traditional California leading germplasm results in shelling percentages that are typically intermediate between the parents. Shelling percentages as high as 75- 77% (Fig. 11), however, have been recovered in good commercial quality, advanced almond breeding lines derived from the wild species *Prunus webbii* (see advanced selection 97,2-240 in Appendix A). The high shelling percentage results from more pronounced lignification of the inner shell wall and an absence of lignification in the typical outer wall (Fig. 10 and 11). Previous research has shown that perturbations in the lignification of the outer wall are a primary cause of the subsequent shell fracturing and loss of shell seal integrity (and so vulnerability to navel orangeworm). The absence of cell lignification in the outer wall of these advanced selections appears to avoid this vulnerability to shell fracturing, resulting in high proportion of resultant nuts having well-sealed shells. As discussed earlier, shell hardness is controlled by the D/d-locus where D- shells are hard and dd are soft. In addition to the final degree of lignification, these genotypes also differ

in their development time. D- genotypes become lignified at the beginning of Stage II while in dd genotypes (California types), lignification does not occur until after completion of Stage II, leaving these genotypes vulnerable to earlier insect feeding on the developing nuts. Within this traditional germplasm, the timing of shell hardening has been positively correlated to the final shelling percentage. Consequently, the P. webbii-type endocarp, because it appears to primarily suppress outer endocarp layer development, may not only facilitate the breeding of high-sealed high crack out almond cultivars, but when paired with the high lignin D- allele may promote earlier shell lignification and so greater resistance to insects such as Lygus sp. which feed on the early developing fruit. Despite the high crack-out ratios, shell seal integrity can range from moderate to high depending on individual genotype. High shell seal

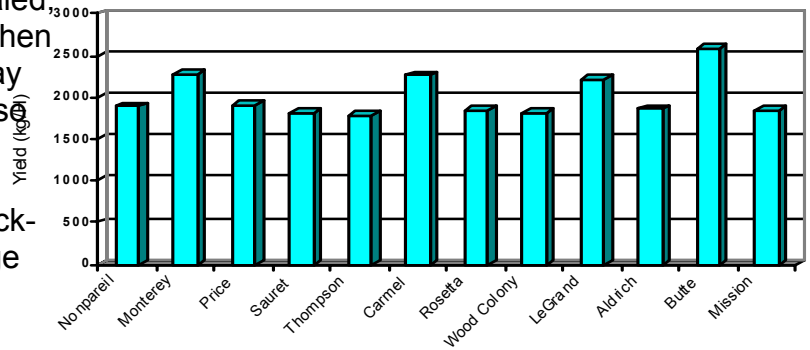


Fig.12. Average yield of Nonpareil, Mission and cultivars resulting from a Nonpareil x Mission cross.

integrity results from both the high lignin density found in fully developed shells as well as a modified endocarp development pattern which appears to avoid internal structural stresses which can result in shell fractures in cultivated almond types.

In comparing performance of progeny to parents for the most important commercial trait—yield, the constraints of parent performance on progeny potential appear to fall apart (Fig. 12). There are two major reasons for this. First, these progeny have been rigorously selected for high yields of good-quality kernels. In our unselected breeding populations we typically find the progeny sort out mainly between parental values, though often, significantly higher and significantly lower values are also observed. The high yields typically result from several factors which are commercially undesirable such as very small kernel size, or excessive flowering and fruit set on young trees, which is undesirable as it diverts energy from needed vegetative structural growth. A second major reason for the greater independence of progeny performance to parent performance when it comes to yield is that virtually every functional almond gene contributes the final yield potential. The myriad of interactions of these numerous genes can result in progeny performance which is significantly superior to the parents. For example, the variety Butte can produce very high yields as a consequent of it inheriting a multitude of favorable factors from each parent, such as small but acceptable kernel size, early flowering and fruiting on terminal shoots, aggressive development of spurs throughout its lifetime, and the maintenance of vegetative vigor needed for fruit wood renewal well into maturity. Most key factors which determine final yield potential in almond remain poorly resolved. Consequently, one of our major challenges to improve almond yield consistency (and fully utilize molecular markers towards this goal), is to identify the most important development/physiological factors contributing to yield. Advances in both genetic and field assessment may facilitate this process. For example, Bruce Lampinen is developing procedures for the rapid evaluation of light interception efficiency which his previous data has shown to be a primary determinant in final yield

potential. (The recently released Sweetheart variety was the most efficient in these preliminary studies supporting our earlier field-production based estimates of yield potential). Ideally, a yield enhancing trait would have high heritability (i.e. readily transferred to progeny), and offer sizable increases in yield potential. An example of this type of trait may be the 'dard'-type of short shoot development in the Winter's variety (Fig. 13). These long spurs (or very short shoots), have been shown to be readily transferred to progeny and promote aggressive and uniform fruit wood renewal throughout the tree, and also promote efficient use of tree branching architecture to avoid excessive shading of interior wood. This would be of particular advantage in northern regions such as the Sacramento Valley whose environment does not encourage the rapid canopy growth/development as found in the southern San Joaquin. [In support of this conjecture, Winters has been the most productive of all tested varieties in the long term Butte County Regional Variety Trials].

The gene controlling cross-incompatibility/self-compatibility in almond is a classic example of single, highly heritable gene having the potential for dramatic improvements in yield and yield stability. PCR-based molecular markers developed in the lab of Dr. Dandekar have allowed unprecedented opportunities for the genetic manipulation/breeding for this trait, yet still have important limitations which are not widely recognized. Our current progress in identifying the S-genotypes of important commercial varieties and breeding lines is summarized in Table 2 (which represents an expansion of the 2007 reported results). Varieties in bold represent previously characterized cultivars. Varieties in red are recently added or updated results, while selections in blue identify contradictory results from different evaluation methodologies. The methodology used also confers different levels of confidence in the results. For example, most cross incompatibility groupings of varieties in bold were determined by actual field crossing studies which were replicated over several years and among several orchards. Based on the actual seed set data, we are comfortable that these represent actual performance under California conditions [reference numbers 1 and 2 in citations]. Non-bolded varieties in black were determined primarily using the PCR-based molecular approaches [as summarized in citation #4]. Items in red or blue were characterized primarily by examining the two-dimensional gel separation of isolated



Fig.13. Very productive 'dard' type short lateral shoot development on 'Winters' almond.

S-proteins [as summarized in citation #3], where blue labeled varieties represent conflicts between different evaluations. Most conflict varieties such as Norman, Northland, and Reams, represent minor cultivars and confusion concerning true S-identities may not be commercially important. Others such as Butte in cross-incompatibility group VI would have important commercial consequences if mislabeled. From repeated molecular evaluations, the consensus for Butte is that it belongs in group VI with the genotype S1S8. This grouping is also supported by our limited field cross data, though important deviations were observed.

For example, while Butte appears fully cross incompatible with Plateau (as would be expected), Butte, when used as the pollen parent, will set higher than expected seed with Monterey though the reciprocal is incompatible. Similarly, within Group IV, Rosetta will set higher than expected when crossed to Price as the seed parent (again the reciprocal is incompatible), while Wood Colony pollen shows lower sets at Price than would be expected from their placement in different (and so theoretically fully cross-compatible), groups. Part of the difficulty is that the incompatibility system in almonds does not represent a complete crossing barrier. Self pollinating even Nonpareil or Mission can result in up to 10% of the selfed flowers setting selfed seed (depending on season). We are currently initiating research to better characterize the true variability within these genetically leaky systems, both to allow better translation of molecular data for field applications and also to maximize self-fruitfulness in future varieties by combining the major gene for self-compatibility with a optimized genetic background that has a natural tendency towards (leaky) self pollinations. The rare, but potentially important, inconsistencies between molecular-based diagnostics and true incompatibility groupings is further demonstrated in Table 1 where the identities of different California S-alleles were tested against the varietal standards (see citation #4). Although the majority of tests returned expected results, several (indicated by an 'x'), did not amplify the genes as expected, while in one test (shaded) amplification occurred were it was not expected based on current genotyping.

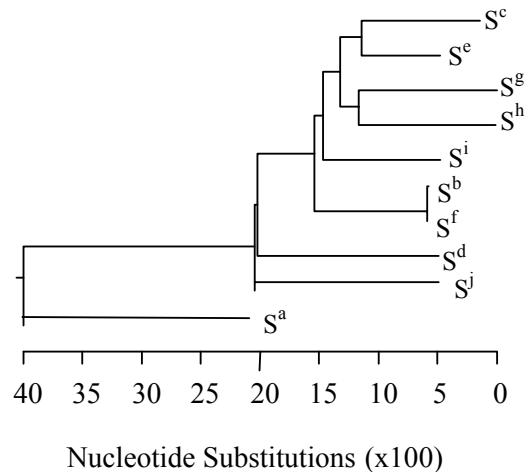


Fig.14. Phylogenetic tree of S-RNase alleles. A phylogenetic tree was constructed using the Clustal W amino acid alignment based on the number of nucleotide substitutions.

Part of the difficulty in accurately characterizing the different S-alleles in California almond, results in the low total number of alleles involved and their often being closely related (Fig. 14). Consequently, it is difficult to distinguish S_b from S_f using molecular approaches, since molecularly they're very similar and appear to produce proteins that are very similar as well. More perplexing, however, is the finding that many of our self-compatibility alleles remain difficult to distinguish from the major cross-incompatibility alleles. The self-compatibility allele S_d (derived from *Prunus mira* and *Prunus webbii*) appears very similar to the California cross-incompatibility allele S1 using PCR-based molecular diagnostic techniques, while a second major self-compatibility allele S_b appears similar to California cross-incompatibility allele S8. [The self-compatible 'b' and 'd' designations used here and in Table 3 are unrelated to the letter superscripts used in Table 2]. While more precise molecular diagnostics are currently being developed, the true identity of the self-compatibility alleles can only be accurately determined by using both molecular and lineage data (Table 3). Table 3 also summarizes the major breeding parents used on current and recent crossing efforts.

Compiled data in Table 3 represent the current status of our efforts at incorporating new, useful germplasm into high commercial quality selections. It documents our ongoing efforts to maximize genetic variability in advance breeding lines through the maintenance and incorporation of self-compatibility and disease/pest resistance from the widest possible diversity of sources. The extent of genetic diversity is shown by the diverse origins of these breeding selections, their complex and integrated parentage, and the diversity of S-alleles recovered. These breeding selections have also been placed in regional grower plantings to field test self-compatibility and disease/pest resistance under actual commercial conditions. Nut and kernel samples from these trials are presented in the Appendix-A to demonstrate that, despite their diverse and often exotic origins, these advanced selections possess high levels of tree and nut commercial quality with many indistinguishable from traditional California cultivars. As documented in this report, many of these advanced selections, while being well-adapted to California growing conditions also demonstrate superior performance in key areas (self-fruitfulness, kernel oil quality, disease resistance, shell structure, etc.). Because a large number of genetic traits need to be optimized for commercial success, most of these selections will still be found deficient in the test of time and need further breeding/genetic reshuffling to produce advanced selections/new cultivars with optimal genetic composition for all key traits.

	S ^a	S ^b	S ^c	S ^d	S ^e	S ^g	S ^h	S ⁱ	S ^j
Nonpareil (S ^c S ^d)			32.51	27.71					
Mission (S ^a S ^b)	39.21	36.1							
Peerless (S ^b S ^e)		X	33.91		33.33				
Sonora (S ^g S ^d)				31.74		31.7			
Padre (S ^b S ^h)		36.92					26.6		
UCD25-75 (S ⁱ S ^d)				X				29.82	
Arbuckle (S ^a S ⁱ)	X							31.33	
Winters (S ^b S ^j)		37.9							34.57
Harriot (S ^e S ^j)					X				36.99
Rosetta (S ^b S ^c)		X	35.61						

Table 1. Real-Time PCR mRNA expression analysis. Each variety was tested with TaqMan primers specific for alleles Sa-Sj. The alleles listed after the variety name is the expected allele expression pattern. The numbers correspond to the CT value, which is directly related to the amount of PCR product. The x designates expected amplifications that did not occur. The shaded box in the Peerless row indicates an unexpected miss-amplification.

Table 2. Cross-incompatibility chart of California almond varieties and S-allele genotype. Bolded varieties indicate previously genotyped varieties. The varieties genotyped in 2006-07 are not bolded. Red indicates recent additions/updates. Blue indicates inconsistent designations.

CIG	Almond Cultivars and Breeding Lines	European Genotype
I	Nonpareil, Tardy Nonpareil , Grace, West Steyn, UCD F8:7-180, Galaxy, IXL , Long IXL, Riedenhore, Golden State	S ⁷ S ⁸
II	Mission , All in One (S7S8)	S ⁵ S ¹
III	Thompson, Sauret #2, Mono, Wood Colony , Durango, Le Grand, Wassum, Granada, Mono, Harvey, Robson	S ⁵ S ⁷
IV	Merced, Ne Plus Ultra, Rosetta, Price cluster , Aldrich, Pearl, Jeanette, Sano, Ripon (S5S17)	S ¹ S ⁷
V	Carmel, Sauret # 1, Livingston, Tioga	S ⁵ S ⁸
VI	Monterey , Dottie Won, Plateau, Avalon, Folsom, Blue Gum, Butte (S1S6?)	S ¹ S ⁸
	Ferragnes	S1S3
	Languedoc	S1S5
VIII	Fritz, Ruby, Peerless (S6S16/S1)	S ¹ S ⁶
	Ripon, Norman (S5S8)	S1S7
	Le Grand, Northland (S6S8), Grace (S6S8)	S1S8
X	Winters	S ¹ S ¹⁴
	UCD 36-52 (Sweetheart)	S ¹ S ^{14x}
IX	Padre	S ¹ S ¹⁸
VII	Arbuckle	S ⁵ S ⁷
XVIII	Carrion	S ⁵ S ¹⁴
XI	Tokyo	S ⁶ S ⁷
	Drake, Smith XL	S6S8
XVII	Harriet	S ⁶ S ¹⁴
	Jeffries	S7AS8
XII	Milo	S ⁷ S ¹³
XIII	Jordanolo, Harpareil ,	S ⁷ S ¹⁴
	Pearl ,	S7S16/S1
	Milow (S7S19)	S7S19
XIV	Kochi	S ⁸ S ⁶
	Yosemite	S8S10
XV	Solano, Sonora, Vesta, Kapareil, Eureka, Wawona	S ⁸ S ^{13/19}
	Jubilee, Reams (S1S3)	S8S15
XVI	Bigelow, Kutsch, Rivers Nonpareil	S8S16/S1
	Titan	S8S14?
	UCD 25-75	S⁸S[?]
	UCD D3-25, UCD F8:7-179,	
	UCD F8:8-160, UCD F8:7-161,	
XIX	Jeffries, UCD 3-6, Johlyn	S ⁸
XX	Tuono, Supernova ,	S ^f
	Vesta (S8S13)	S10Sx
	Marcona	S11S12
	UCD 54P455 (peach)	S [?]

Table 3. Cross-incompatibility chart of California almond breeding lines and S-allele genotype. Color identifies different putative self-incompatibility alleles and their sources where known. ORIGIN code: A-Prunus argentia; E-European source; F-P. fenzliana; H-heritage almond germplasm; M-P. mira; P-P. persica (peach); S-Supernova mutation; T-Tuono.

Selection	ORIGIN	Parentage	S-alleles
91-18-174	S	Supernova OP	Sc Ss ?
96A,1-133	P	F7,2-9 (Sel5-15 slf) op	Sb? S14?
97,1-232	M	25-75 [Arb * 4-26][SB4, 4-2E] * WINTERS	S1 Sd?
97,3-40	F	D4-18 (Mis * [P.fenzliana * Alm])** 13-1	S14
98,15-109	WA	D2-4 SB20,1-19 (Miss*Web)*Sonora) * D3-3 SB20,1-28 (Miss*Arg)*Sonora	Sd S14
99,1-121	WP	D4-26 (F5,4-6[{{W*W}}*{SolSel, 5-15*24-6}])*LG	Sb S8
99,3-189	M	F56-22 * 25-75	Sd?
D3-25	WP	NP * F5,4-11 [W*W][SEL5-15SLF])	Sb S8
95,1-26	H	D5-4 (USDA CP33) * 13-1	S1 S14
F7,1-1	P	(Sel5-15[NP*LukensHoneyXMis]*WSB3b25)	Sb S8
F8,7-179	WP	D3-15 (NP*F5,4-43[W*W][SEL5-15SLF])) * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	Sb S8
F8,7-180	WP	D3-15 (NP*F5,4-43[W*W][SEL5-15SLF])) * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	S7 S8
F8,8-160	FM	D4-18 (Mis * [P.fenzliana * Alm])* Sonora * 25-75	S6 Sd
F8,8-4	WP	D3-15 (NP*F5,4-43[W*W][SEL5-15SLF])) * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	Sb S8
F8-8-161	FM	D4-18 (Mis * [P.fenzliana * Alm])* Sonora * 25-75	Sd S13
2000,11-190	S	F7,1-1 * 91,18-174 (Sopernova OP)	Sb? Ss ?
2000,1-180	WP	D3-15 (NP*F5,4-43[W*W][SEL5-15SLF])) * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	Sb S8
2000,13-162	WP	F7,1-1 * F8,7-179	Sb?
2000,16-81	WP	F7,1-12 * 91,18-174	Ss ?
2000,2-3	WP	D3-15 * D3-25	Sb?
99,4-97	WP	USDA CP33 * D3-25	Sb?
99,9-86	WP	Mission * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	Sb?
2000,3-385	FM	D4-18 (Mis * [P.fenzliana * Alm])* Sonora * 25-75	Sd?
99,4-8	P	Ferragnes * LGOP	Sb?
2000,8-27	WP	NP * F8,7-179	Sb S7
2002,1-271	T	F10 D, 3-11 * Tuono1-1	S1 S8
2002,8-119	T	Mission * Tuono1-1	
2004,14-158	FP	99,4-8 (Ferragnes * LGOP) * 97,3-40 (D3-18 (Mis * [P.fenzliana * Alm])** 13-1)	Sb?
2004,14-31	W	99,4-1 (Ferragnes * LGOP) * 97,2-240 (Ferragnes * D3-6)	Sb?
Ferragnes	E		S1 S3
LEGRAND	P		Sb S8
LGOP	P	LeGrand OP	Sb S8?
Sweetheart	P	F7,1-1 (Sel5-15=[NP*LukensHoneyXMis]*WSB3b25) * USDA 25-26{SF?Harriot?}	S1 S14
UCD25-75	M	[Arb * 4-26][SB4, 4-2E]	SiSd
WINTERS	H		S1 S14
Tuono	T		S1 Sf
97,14-340	P	Sonora * LGOP	Sb S8

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5. Appendix A.

Advanced UCD Self-compatible Almond Selections in Regional Grower Evaluations

These breeding selections represent a very wide genetic variability due to their interspecific origins. In addition to self-compatibility, novel genetic options for disease and insect resistance have been incorporated in this material. By establishing evaluation plots for these selections in different areas of the Sacramento and San Joaquin valleys, we hope to more thoroughly evaluate the value for further resistance breeding, as well as their potential and deficiencies as possible cultivar releases.

UCD2-19E - Lineage: Tardy Nonpareil X Arbutle. This selection is one of the highest producing varieties at the Kern RVT plot with an accumulated (1996-2005) yield of 26,112 pounds per acre following an exceptionally high crop of 4,890 pounds per acre in 2003. UCD2-19E shows a strong alternate bearing habit where years of high crop yield are followed by low crops. It is believed that on years of very high crop, insufficient nutrients are available to the overloaded fruiting spurs to initiate the number of flowers needed to maintain the crop, and in some cases to maintain the very viability of the spur into the next season. An alternate bearing habit is undesirable for California production, and usually breeding selections showing this behavior would be discarded. However, many Kern County growers have been successful in maximizing year-to-year production in other strongly alternate bearing varieties such as Price, by closely monitoring current season crop yield and providing increases in both irrigation water and fertilizer nutrients as needed. To evaluate this opportunity to capitalize on its very high crop in potential and because of its good kernel quality and late bloom, and resistance to flower blight. Additional grower plantings have been made in Kern County in 2004. Virus-free, nursery foundation plant material has also been provided to California nurseries to facilitate greater grower experimentation with the selection.



2000,2-3 - Lineage: D3-15 (Nonpareil X F5,4-43{P.webbii X P.webbii}{SEL5-15Selfed})) X D3-25 [(Nonpareil X F5,4-11{P.webbii X P.webbii}{SEL5-15Selfed})]. A relatively recent selection, 2000,2-3 represents an advancement of the previously described D3-25 selection by incorporating improved tree structure disease resistance and productivity. Self-compatibility and a Nonpareil-type kernel were derived from the D3-25 parent. The D3-15 parent contributed a more upright-spreading tree structure, a more uniform, spur based productivity, and a more durable and well-sealed shell. Tree structure is upright to upright-spreading with a very high productivity resulting from a uniform and high nut distribution. The original tree also shows evidence of improved foliar disease resistance. The tree is semi-upright with radial branching. Anticipated size will be 10% narrower than Nonpareil but similar height. Expected bloom is approximately 6 d after Nonpareil with harvest approx. 21 d after Nonpareil. Kernel quality is good; however this selection may produce some double-kernels. Average kernel length/width/thickness is 2.4/1.2/0.9 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crack out is 0.55. Shell-seal is moderate with approximately 70% of the nuts showing complete seals. This selection resulted from a complex series of crosses involving *Prunus persica* (peach) and *Prunus webbii* in its lineage.



2000,8-27 - Lineage: Nonpareil X F8,7-179. As with selection 2000,2-3, (above), this selection represents the next breeding generation derived from selection F8,7-179 (described below). The backcross to Nonpareil has resulted in an improved Nonpareil-type kernel quality and improved shell seal. High levels of self-compatibility have also been recovered as have good tree architecture and uniform crop distribution, primarily on spur bearing wood. The tree also exhibits improved levels of foliar disease resistance when compared to both parents. Kernel uniformity is very high with low levels of doubled or damaged kernels. The tree is upright-spreading and approx. 20% smaller than Nonpareil. The bearing-habit is similar in terms of the ratio of spur to shoot flower buds. The selection blooms approximately 4 d after Nonpareil and harvest approx. 18 d after Nonpareil. Average kernel length/width/thickness is 2.2/1.2/0.9 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crack out is 0.64. The paper shells give good crack out but have poor seals (60%) though the worm infestation has not been a problem to date. Kernels show good-quality though double kernels may be a problem. This selection resulted from a complex series of crosses involving *Prunus persica* (peach) and *Prunus webbii* in its lineage.



F8,7-179 - Lineage: D3-15 (Nonpareil X F5,4-43 {P.webbii X P.webbii}{SEL5-15Selfed})) X D3-25 [(Nonpareil X F5,4-11{P.webbii X P.webbii}{SEL5-15Selfed})]. Combining multiple and distinct sources of self-compatibility (from both peach and P. webbii), this selection has shown consistently good levels of self compatibility even in seasons where spring storms have suppressed flower development. Improved levels of both foliar and blossom diseases have also been observed though moderate susceptibility to hull rot has also been observed. Kernels show good Carmel-like quality and are of uniform size and shape with some doubles. The shells are paper, though only 50% sealed. Regional trials at Winters, California have shown the tree to be productive and a regular bearer. Early productivity in regional trials has been moderate. Regional trials are being watched closely for disease susceptibility and bearing wood renewal. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).



F7,1-1 - Lineage: (Sel5-15{Nonpareil X LukensHoney X Mission} X WSB3b25). Breeding selection Sel5-15, has proven to be one of our most effective sources for both self-compatibility and improved disease resistance and has been derived from peach and more exotic almond germplasm. F7,1-1 combines a high levels of self-compatibility with high tree productivity and good disease resistance. The Butte-type kernel is a medium to small in size, and uniform in its good appearance and freedom from defects. It has a paper shell with 74% seal. This selection has been a consistent and dependable performer in terms of both self-compatibility, foliar disease resistance and tree productivity both in the initial evaluation in regional evaluation plots. The small kernel size is its most apparent handicap, though its consistent productivity and disease resistance may lead to commercial profitability. Tree has an upright to upright -spreading tree which can be similar to 10% larger than Nonpareil. Bloom occurs 7 to 10 d after Nonpareil. Flower densities and levels of self compatibility are high, resulting in a high yield potential. Harvest occurs approximately 28 days after Nonpareil. Nuts are small and teardrop-shaped which appear desirable to the confectionery industry. Average kernel length/width/thickness is 1.8/1.1/0.8 cm. Ave. kernel weight is 0.8 g; kernel/kernel + shell crack out is 0.68 F7,1-1 has been one of the most resistant selections in Dr. Adaskaveg's UCD almond disease evaluation block with demonstrated resistance to bacterial blast and Monilinia flower blight.



F8,8-160 - Lineage: D4-18 [(Mission X {P.fenzliana X Alm}) X Sonora] x 25-75.

This and the following selection have incorporated genes from the wild almond species *P. fenzliana* into a cultivated almond background. F8,8-160 was selected for its consistent level of self-compatibility and its high-quality Carmel-type kernel. Shells are paper, and poorly (70%) sealed. Trees have shown good productivity both at the Winter's, California and southern San Joaquin evaluation plots. Kernels are uniformly elliptical and relatively thick resulting in good kernel weights and so improved yield potential. In regional test plantings, trees are upright-spreading with moderate to good crop distribution primarily on spur bearing wood. Pollen is fully cross compatible with Nonpareil. This selection is upright-spreading resulting in a tree size similar to Plateau or Carmel. Bloom occurs approximately 7 d after Nonpareil and can be profuse. Harvest occurs approximately 6 weeks after Nonpareil. Average kernel length/width/thickness is 2.2/1.2/0.9 cm. Ave. kernel weight is 1.0 g; kernel/kernel + shell crack out is 0.57.



F8,8-161 - Lineage: D4-18 [(Mission X {P.fenzliana X Alm}) X Sonora] x 2575.

This and the previous selection have incorporated genes from the wild almond species *P. fenzliana* into a cultivated almond background. Like F8,8-160, F8,8-161 was selected for its consistent level of self-compatibility and its high-quality Carmel-type kernel. Shells are comparable to, to slightly thicker than Carmel, having good (98%) seals. Trees have shown good productivity both at the Winters, California and southern San Joaquin evaluation plots. Trees are upright-spreading with good crop distribution, primarily on spur bearing wood. Doubled nuts (two nuts developing on a unique T-shape spur) are often observed and may contribute to the higher yield potential this selection. Pollen is fully cross compatible with Nonpareil and most major commercial almond varieties. This selection is a sister line to F8,1-160 and the tree is upright and similar in size and vigor to Fritz. Bloom occurs approximately 10 d after Nonpareil and is also profuse. Harvest occurs approximately 28 d after Nonpareil. Shells are medium, thick and are moderately well sealed. Kernels are medium in size and of good quality. Average kernel length/width/thickness is 2.3/1.2/0.8 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crack out is 0.63. Doubles may be a problem and Monilinia flower blight and Alternaria leafspot lesions have been observed in early San Joaquin valley plantings.



F8,7-180 - Lineage: D3-15 (Nonpareil X F5,4-43{P.webbii X P.webbii}{SEL5-15Selfed})) X D3-25 [(Nonpareil X F5,4-11{P.webbii X P.webbii}{SEL5-15Selfed})]. Although a sister line to F8,7-179, the selection is self-incompatible, having inherited none of the self-compatibility factors from the parents. Kernels are

of good quality being similar to Nonpareil though larger. Shells are thin, paper consistency and only moderately well sealed. This selection has shown only moderate productivity at Winter's, California evaluation sites but has shown stronger productivity at southern San Joaquin test sites. Trees at the Winter's, California evaluation site show problems in bearing wood renewal leading, eventually to lower yields. Trees flower just after Nonpareil and pollen is fully cross compatible with Nonpareil and Carmel. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).



F8,8-4 - Lineage: D3-15 (Nonpareil X F5,4-43{P.webbii X P.webbii}{SEL5-15Selfed})) X D3-25(Nonpareil X F5,4-11{P.webbii X P.webbii}{SEL5-15Selfed}). This complex interspecies cross, combines genes from P. mira (a wild peach), P. webbii (a wild almond) and P. dulcis (cultivated almond). Kernels are of good quality, ovate in shape, and with good thickness resulting in good individual kernel mass. Shells have a medium thickness and are generally well sealed but can show moderate (~60%) seal with heavy crops. Doubled-nuts have been observed in this selection as have a relatively high number of shriveled nuts. Nuts are borne primarily in spurs but also at current shoot terminals, particularly in younger trees. Kernel quality problems, particularly double kernels, need to be watched with this selection. The tree is upright and approx. 10-20% smaller than Nonpareil. Flowering occurs 1-2 d after Nonpareil. Flowering has been profuse (high flower density), making it a good pollinizer for the later Nonpareil bloom. Harvest occurs approx. 30 d after Nonpareil. Average kernel length/width/thickness is 2.1/1.2/1.0 cm. Ave. kernel weight is 1.1 g; kernel/kernel + shell crack out is 0.55. Kernels have good size, shape and texture. (Sample Nonpareil and Carmel kernels shown inside grey circle).



LG-OP - Lineage: LeGrand-Open-Pollinated. Kernels have a good quality, Padre-type shape though are somewhat larger. Shells are soft, moderate in thickness with good seals. The tree is more compact, like Carmel, but allowing good productivity because of a shorter internode distance between leaves and spurs. Most production in the mature trees is on spurs which are well distributed throughout the canopy. The level of self-compatibility appears consistent from year to year, unlike the LeGrand parent. Trees have shown good productivity both at the



Winter's, California and southern San Joaquin evaluation plots. As these trees mature, particular attention will be given to the evaluation of the stability of self-compatibility, the level of stick-tights, in the incidence of foliar diseases and potential for Bud-failure. A few double kernels have also been observed.

C,1-10 - Lineage: Wood Colony X Fritz.

The result of a cross between two traditional, commercial varieties, this selection combines a Nonpareil-type kernel and paper shell with a later flowering and later maturing tree. In early regional trials, tree productivity has been unexceptional. The selection also seems more susceptible to foliar diseases than other advanced selections. Its similarity to Nonpareil, however, would make this a very useful variety if it performs well in regional trials.

In addition to productivity and disease susceptibility, an overly spreading tree architecture may also be problematic with the selection.



C,1-16 - Lineage: F10D,3-67 = Nonpareil X D3-19 {(Mission X P.fenzliana) X Solano}.

This selection is derived from a cross to P. fenzliana with the goal of transferring improved disease resistance and cropping architecture. Tree is productive in both Sacramento and San Joaquin Valley test plots and consistently yields uniform and good-quality Price-like kernels. Shells are paper and relatively well sealed. Tree is upright to spreading and it appears to have improved levels of disease resistance. Mature tree productivity and architecture continue to look promising in regional trials. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).



C,2-4 - Nonpareil-BF X Monterrey. [Tested as selection F10D, 5-39 in some locations].

Similar to selection C1-10 (above), this selection resulted from an early cross between a high bud-failure Nonpareil selection and a variety Monterey (to evaluate latent bud failure potential in the variety Monterey). Kernels show good-quality, and are somewhat between Carmel and Sonora in shape and size. Shells are paper in texture and moderately well sealed. Propagated trees have shown good productivity in regional testing although some non-bearing blind wood has also been observed in the southern San Joaquin Valley. Experience has shown that crosses to high bud-failure



sources have approximately 50% probability of inheriting the predisposition to noninfectious bud-failure. In addition to bud-failure potential, regionally planted trees will be closely monitored for tree bearing habit, particularly fruiting wood regeneration. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).

D,1-25 - Lineage: (Mission X P.webbii) X Sonora. This selection represents a unique class of kernels being relatively long, wide and flat and thus well tailored for the sugar coating or panning market. In addition, kernels possess a unique and desirable amaretto flavor. The combination of kernel shape and kernel flavor quality has made it of interest to specialty markets processors. The tree is medium in size and spreading. While the selection appears highly productive, there is some evidence of bearing decline in older trees associated with bark cracking and in some cases bark deterioration (an occasional consequence from wide crosses). The selection is being released for regional testing for growers and processors interested in this particular niche market. In addition to attention to those kernel characteristics required for this market, other characteristics to be watched in regional trials include consistency of productivity and a renewal of bearing wood. It is not self-compatible and has not been noted as having promising disease resistance. It produces a more spreading tree approx. 30% smaller than Nonpareil. The tree blooms approximately 8 d after Nonpareil and harvests approx. 18 d after Nonpareil. The kernels are symmetrical, medium in size and shallower than a typical Nonpareil shape making it desirable for the panning (Jordan-type sugar coated) almond. As mentioned, the kernel has a slight but pleasant amaretto flavor, but this can vary year-to-year. Average kernel length/width/thickness is 2.3/1.2/0.8 cm. Ave. kernel weight is 0.9 g; kernel/kernel + shell crack out is 0.044. Shells are attractive and well-sealed, but we still see some NOW damage (I suspect the amaretto flavor (benzaldehyde) is an attractant to NOW at these low levels). Prunus webbii and Sonora are in its lineage.



D,1-6 - Lineage: 90,14-124= (Jeffries X Nonpareil). This selection represents a forced self pollination of Nonpareil as part of a project to dissect the genetic components of this important variety. D,1-6 was selected for its very good Nonpareil-type kernel and thin paper shell conferring a high crack out with an improved barrier to insect pests. Shell seal is good at 96%. Trees appear productive in regional trials with no evidence of reduced vigor or increased susceptibility to disease as is often associated with selfed or inbred genotypes. The selection also shows partial self-compatibility which appears associated with the unilateral breakdown of the self-incompatibility



mechanism in the Jeffries bud-sport of Nonpareil. Some double kernels and twin kernels have been observed at low proportions. In addition to the general signs of inbreeding depression, other deficiencies to be watched for in this selection are an increased susceptibility to stem canker which is frequently seen in inbred lines of Nonpareil. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).

D3-25 - Lineage: Nonpareil X F5,4-11 {P.webbii X P.webbii}{SEL5-15Selfed}). This interspecies cross, combines genes from P. mira (a wild peach), P. webbii (a wild almond) and P. dulcis (cultivated almond). The selection combines Nonpareil-type kernel quality with good upright tree architecture and good levels of self-compatibility. Both tree productivity and bearing wood renewal have declined with age in the initial tree



though propagated trees in regional trials have shown good tree growth and scaffold architecture, but a tendency for weepy growth at branch terminals. This selection is also associated with greater susceptibility to flower blight. Initial crops in regional trials have been moderate. Shell seal is poor at 8% sealed. Cropping potential and levels of disease resistance are being watched closely in the selection as regional trial trees mature.

D3-26 - Lineage: F5,4-6 {P.webbii X P.webbii} X Solano. [Labeled as F10D, 3 - 216 in some plots]. Tree is large with dense foliage, upright-spreading, and productive. Nuts are ovate, similar to Peerless, and like Peerless have a semi-hard shell resulting in good resistance to insect damage but moderate crack out ratios. Some evidence for alternate bearing has been observed in the parent trees, but trees in initial regional trials continued to show good growth and productivity. In addition to alternate bearing, the selection is being watched for consistency of bearing wood regeneration and susceptibility to peach twig borer damage and Noninfectious Bud-failure.



97,1-227 - Lineage: 25-75 [{Arbuckle X 4-26} X {SB4, 4-2E}] X Winters. The seed parent 25-75 is highly self-fertile (derived from the peach species Prunus mira), and productive but suffers from poor tree structure. The Winter's parent contributes a more upright-spreading tree structure as well as an improved kernel size and quality. Kernels are large and uniform with a desirable Nonpareil-type elliptical shape. Shells have a medium thickness and are well sealed with good resistance to NOW and damage. Self-compatibility in this election is rated as good, meaning that 50% or more of viable flowers will set seed when selfed under artificial (mesh bag limbs, limited



controlled pollinations, etc.), conditions. The tree is upright to spreading and productive. Blooms approx. 5 d after Nonpareil and harvest approx. 21 d after Nonpareil. Average kernel length/width/thickness is 2.6/1.5/0.8 cm. Ave. kernel weight is 1.5 g; kernel/kernel + shell crack out is 0.42. Shells are easily cracked yet have good seals.

97,1-232 - Lineage: 25-75 [{Arbuckle X 4-26} X {SB4, 4-2E}] X Winters. A sister line to 97, 1-227, resulting in good levels of self-compatibility and tree productivity. Nuts are large and ovate, being similar to Monterrey. Kernel shells are thinner than 97, 1-227 resulting in greater crack out but greater susceptibility to insect damage. The tree is productive and more spreading than the 97, 1-227. Flower self-compatibility is rated as good, being slightly better than its sister line when compared over several years. Potential deficiencies include a possible predisposition to shriveled kernels and kernel gumming and a tree architecture that may develop to be overly spreading. The tree is approx. 20% smaller than Nonpareil. The tree blooms approximately 5 d after Nonpareil and harvests approx. 21 d after Nonpareil. Average kernel length/width/thickness is 2.3/1.4/0.8 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crack out is 0.40.



97,2-240 - Lineage: D3-4 {(Mission X P.webbii) X (Mission X P.webbii)} X

Ferragnes. The seed parent is a backcross from the cross between Mission and P. webbii, a wild bushy almond species with small but well sealed nuts. The French variety *Ferragnes* was used as the pollen parent to increase kernel size and quality and contribute a more upright architecture to the tree. Kernels are Carmel-type in size and appearance with few doubles. [For reference, a Nonpareil kernel (left) and Carmel kernel (right) are placed within a



black disk within the sample tray]. The kernel shell has been reduced to a very thin but very durable inner shell resulting in crack outs exceeding 70%, combined with very high shell seals and so low vulnerability to insect damage and aflatoxin contamination. Trees are medium in height and productive, though the spreading architecture results in a higher incidence of early limb splitting and possible tree loss if early orchard trees are not managed properly. The possible excessive spreading nature of this tree remains its most serious potential deficiency. Flowers are self-incompatible but are cross-compatible with all major California cultivars. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).

97,3-40 - Lineage: D4-18 [(Mission X {P.fenzliana X Alm})] X Winters. The seed parent is a backcross of Mission and the wild almond species *P. fenzliana*. *P. fenzliana* was selected to bring in early flowering, good shell seal, a more upright tree productivity, and disease resistance. Winters was selected as the pollen parent to contribute better kernel quality and to maximize tree productivity through its

tendency to produce consistently productive lateral branching. This selection exhibits very large Sonora-type kernels in a Peerless-type well-sealed shell. Twin kernels (2 embryos within the same seed-coat), have been observed but do not appear to be a problem. Flowering time is very early, occurring with Sonora or before. Despite its early flowering, the selection has been very productive in our southern San Joaquin test site. Flowers are self-incompatible but compatible with all commercial California almonds because of its unique S-allele genotype. The large, high-quality kernel combined with its attractive, durable shell make this selection a possible replacement for Peerless. Potential deficiencies include its very early flowering and an uncertain production consistency.



97,4-333 - Lineage: Nonpareil X F7,1-1 [(Sel5-15={Nonpareil X LukensHoney X Mission} X WSB3b25) X 25-26. The seed parent is Nonpareil crossed with the very productive, self-compatible and disease resistant selection F7,1-1. The self-compatibility from the pollen parent is derived from peach but was not transferred to this selection, which is self-incompatible. The kernel possesses good quality and is Carmel-like in appearance but typically not as thick. Nuts have a paper shell which is only moderately sealed. Seedling tree productivity was high though productivity in propagated trees remains mediocre. Medium yields and the relative flatness of the kernels remain potential deficiencies of concern. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).



98,11-77 - Lineage: Nonpareil (F5,3-12) X 90,13-59 (Jeffries X Nonpareil). The variety Nonpareil was essentially selfed in this cross since the pollen parent 90,13-59 resulted from the cross between Jeffries (a Nonpareil bud-sport), to Nonpareil. Seedlings from this cross showed a range of variation for many important commercial qualities, confirming the genetic diversity inherent to this important commercial variety. Many seedlings in this population also showed reduced vigor and increased disease susceptibility, particularly to certain stem cankers, demonstrating the detrimental effect of selfing in almonds since it concentrates otherwise deleterious but recessive genes so that they are expressed to the detriment of the final selfed plant. This selection was chosen for its high-quality Nonpareil-type kernels combined with a good sealed shell and good tree productivity. The semi-



hard shell confers greater insect resistance but results in lower crack out ratios. Besides potentially greater disease susceptibility (because of the selfed nature of this selection), other potential concerns with this selection include possible deterioration in tree architecture and reduced yields as trees age. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).

98,14-340 - Lineage: Sonora X LeGrand-OP. LeGrand-OP is a selection from crossing the partially self-compatible variety LeGrand with Sonora. Flower self-compatibility is rated as good. Kernels are medium large and somewhat resemble the Sonora parent though slightly shorter. Shriveled and creased kernels were apparent in initial harvests but these were from essentially dry land farmed trees with better kernel quality observed in irrigated orchards. Kernel eating quality is very good. Trees are upright-spreading and productive. Early yields of test trees in the southern San Joaquin remain promising though deficiencies and kernel quality remain a concern. Trees tend to be more compact with dense, somewhat willowy foliage.



98,15-109 - Lineage: D2-4 SB20,1-19 (Missions X P.webbii) X Sonora) X D3-3 SB20,1-28 (Missions X P.argentia) X Sonora. The result of a complex interspecies cross involving cultivated almond (*P. dulcis*), and the wild almond species *P. webbii* and *P. argentia*, this selection combines good levels of self-compatibility, disease resistance, and a more spreading tree architecture. Erratic



(cross-over), branching have been observed, however. Kernels are medium quality, with slightly beaked kernels and shells. Some kernel creasing and doubling were apparent in Winter's, California seedling blocks, though this could have been the consequence of the near dry land farming methods used for this and related selections. Kernel quality remains a concern in ongoing evaluations as is the possibility that the tree habit may be overly spreading.

98,2-305 - Lineage: Nonpareil X F7,3-11/D3-3 (SB13,28-21 X P.webbii hybrid). The result of a cross between Nonpareil and F7, 3-11, a self-compatible and aflatoxin resistant selection. Tree is upright and appears to be only moderately productive. Both shell and kernel are of medium quality as they are somewhat flat and elongated. Kernels consistently express the distinct and desirable amaretto flavor which combined with their elongated and relatively flat structure may make this a useful variety for panning or sugar coating. Potential deficiencies being examined in regional



testing include inconsistency of amaretto flavor, kernel shape, and productivity. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).

98,3-53 - Lineage: D3-11 (=F8S,53-60) X F7,1-1 [(Sel5-15{Nonpareil X LukensHoney X Mission} X WSB3b25)]. The seed parent, D3-11, was selected for its very good kernel quality, good disease resistance and high tree productivity. Tree architecture, however, was excessively spreading, and in particular, the terminals and laterals bearing the crop were too weepy or feathery for consistent mechanical harvest. The pollen parent, F7, 1-1, was selected for its high disease resistance, high productivity, and good spur production. Kernels tend to be small and Ruby-like though with occasional blank nuts. The resultant selection has combined the better attributes of both parents with good-quality Sonora-type kernels within an upright and productive tree. Very thin shells confer high crack out ratios but the poor seal (8%) result in increased vulnerability to insect damage.



99,1-121 - Lineage: D3-26 (F5,4-6{{P.webbii X P.webbii} X {SolSel, 5-15 X 24-6}}) X LeGrand. A cross between D3-26 with its good tree, kernel and shelf characteristics (described previously), and the variety LeGrand to incorporate improved bearing habit and self-compatibility. The tree is upright and productive. Flowers show good levels of self compatibility and kernels show consistent high-quality. Kernels and shells are somewhat similar to Monterey in appearance with a slight but distinct beaking. The tree is very upright, and approx. 20% smaller than Nonpareil. Bloom occurs approximately 6 d after Nonpareil, while harvest occurs approx. 28d after Nonpareil. Kernels show good-quality but double-kernels may be a problem. Average kernel length/width/thickness is 2.4/1.4/0.9 cm. Ave. kernel weight is 1.3g; kernel/kernel + shell crack out is 0.30. The shell is similar to the variety Mission, having a very good shell-seal and so low worm damage. Bud-failure has been observed in 2008 on siblings of this cross and so a higher potential for it eventually showing bud-failure need to be considered.



99,4-8 - Lineage: Ferragnes X LeGrand-OP (LGOP). (Sometimes listed in regional trials as 99, 4-2). Combining the upright tree architecture and large kernel size of Ferragnes with the productivity and self-compatibility of LGOP, this selection has proven only partially self-compatible (i.e. between 25 to 50% of all viable flowers artificially selfed pollinated will set seed). Kernels are of good quality, being somewhat similar to Solano. Shells are hard, with a very good seal and insect resistance but moderate crack out ratios. The tree is upright-spreading and very productive. The shell is durable, attractive, and bleachable.



Because of its high productivity, and high-quality kernel and shell, the selection is being considered as replacement for the Peerless variety. (Sample Nonpareil (left) and Carmel (right) kernels shown inside grey circle).

1999,3-189 - Lineage: D5-24 (SB20,1-5 (Mission x Hybrid-A[fenz]) x Sonora)* UCD25-75. Tree is vigorous and upright spreading. Bloom occurs approximately 3 days after Nonpareil with harvest approximately 1 week after Nonpareil. Flowers are fully self-compatible and productive. Kernels are well sealed but showing a tendency to double.



1999,4-97 - Lineage: CP,5-33 * D3-25. Tree is upright and productive. Nuts are uniform and of good quality. Bloom occurs with Nonpareil and harvest is approximately 2 weeks after Nonpareil. Flowers are fully self-compatible with production primarily on spurs. Shells are thin but with moderate to good seal. Appears to have some resistance to flower blight.



1999,9-86 - Lineage: Mission * D3-25 [(Nonpareil X F5,4-11{P.webbii X P.webbii}){{SEL5-15Selfed}}]. Tree is upright-spreading and productive but with some tendency to alternate bear. Bloom occurs approximately with Nonpareil with harvest approximately 3 weeks after Nonpareil. Kernels are of very high quality and uniform with a slightly darker pellicle color. Flowers are self-compatible.



2000,11-190 - Lineage: F1-1 * 91,18-174 (Supernova op). Tree is upright to upright-spreading and productive. Bloom occurs approximately 5 days before Nonpareil with harvest occurring approximately with Nonpareil. Flowers are fully self-compatible. Kernels are uniform with moderate to good quality and moderate to good shell seal. May produce some double kernels and some scabwas evident in 2008.



2000,13-162 - Lineage: F7,1-1 * F7,7-179. Tree is more compact, similar to Carmel, but somewhat more spreading. Blooms approximately 10 days after Nonpareil and harvest approximately 1 week after Nonpareil. Flowers are fully self-compatible. Kernels are elongated, being somewhat similar to Sonora and of good eating quality. Some double kernels are produced with some shot-hole evident in 2008.



2000,16-81 - F7,1-12 * 91,18-174 (Supernova op).
Trees are upright and productive producing well sealed kernels. Kernel size is moderately large occasionally showing shriveling. Bloom is approximately with Nonpareil and harvest is approximately 2 weeks after Nonpareil. Flowers are fully self-compatible and appear more resistant to flower blight. Some shot-hole was observed in 2008.



2000,3-385 - Lineage: D3-18 * UCD25-75.
Tree is a bright-spreading to spreading and very productive though prone to alternate bearing if under fertilized. Bloom is approximately with Nonpareil as is harvest. Flowers are fully self compatible. Kernels are Sonora-like in shape the somewhat smaller. Nuts are well sealed and of high quality.



2002,1-271 - Lineage: D3-11 * Touno1-1. Tree is upright to upright-spreading. Flowers are fully self-compatible. Kernels are large and have a tendency to crease. Bloom occurs approximately 5 days after Nonpareil with harvest occurring approximately 2 weeks after Nonpareil. Flowers appear more resistant to flower blight. Nuts are well sealed with high crack out ratios.



2002,8-119 - Lineage: Mission * Tuono1-1.
Tree is upright to upright-spreading. Bloom occurs approximately 1 week after Nonpareil with harvest approximately with Nonpareil. Flowers are fully self-compatible. Kernels are large and broad similar to Sweetheart and Marcona with well sealed shells but moderate crack out ratios. Some kernel creasing has been observed at levels similar to Marcona. Leaves appear more resistant to leaf-blight in 2008.



2004,14-158 - Lineage: 99,4-8 (Ferragnes * LGOP) * 97, 3-40 (P. webbii * Winters). Tree is upright-spreading to spreading. Bloom occurs approximately with two just before Nonpareil. Harvest is approximately 3 weeks after Nonpareil. Flowers are fully self-compatible. Kernels are large and of uniform, with good quality and with moderately thin but well sealed shells. Branches are very productive leading to some breakage of seedling trees.



Appendix B:

Example of the array of test populations developed and maintained for molecular studies of the genetic control of kernel antioxidant levels in almond. [Antioxidants are important for consumer phytonutrient benefit, resistance to diseases such as aflatoxin, and postharvest storage life]. (Red –parents with high anti-oxidant levels; pink – moderate levels & grey –low antioxidant levels).

Pollen Parent	25-75	95, 1-26	97,3-40	98,14-340	Mission	Mission-X02	Tuono1-1	97,2-240	98,1-346	98,2-348	A96, 1-133	D3-15	D3-25	F7,7-179	F7,8-4	F8,8-160	F8,8-161	LeGrand	98,15-109	99,1-121	F7,7-180	Nonpareil	
Seed Parent																							
99,4-8			40																				
D3-26													180										
F8,7-179											40												
Ferragnes	20							30						40	20	30		30				20	
Mission	40				13	50								80	20							10	
99,4-1								40															
D3-11							18																
D3-13	20											50						25					
D3-18	25																						
F8,8-161																						25	
NPU														20									
Sonora														40		20							
Nonpareil		70		25			100	35	20	80	25			250	15	40	80		30	35		5	
Weststyn													80										

