
Almond Variety Development

Project No.: 11-HORT1-Gradziel

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Objectives:

Key goals of this project are: (1) improved pollenizers for Nonpareil, and ultimately; (2) varieties that possess self-fertility and improved market value and resistance to disease, insects and environmental stress. Specific objectives for 2011-12 include:

- A. Streamline the variety development cycle through more efficient controlled hybridizations and screening of progeny trees for self-compatibility, tree productivity, kernel quality and resistance to key pests, diseases and environmental stresses, including water use efficiency.
- B. Prioritize required traits for future commercial success in partnership with growers, handlers and processors, and increase selection efficiency for productivity and resistance to disease/pest/environmental stresses.
- C. Expand data collection from regional trials to more fully evaluate commercial potential of advanced breeding lines and to identify the most promising genes/selections for inclusion in new Regional Variety Trials (RVT).

Interpretive Summary:

The California almond industry is in a historic period of transformation driven by increasing Central Valley acreage along with increasing environmental and market requirements, reductions in natural resources, including natural pollinators, and changing climates. While almond represents a diverse and highly adaptable species, commercial production in California is dependent almost entirely on the variety Nonpareil and a relatively few pollenizers, most of which have Nonpareil and Mission as direct parents. A long-term emphasis of the UC Davis (UCD) almond breeding program has been the identification and incorporation of new and diverse germplasms. Genetic solutions to emerging production challenges are now becoming available from this new germplasm. These include regionally-adapted selections expressing high levels of self-compatibility as well as increased insect, disease and environmental stress resistance. These improved breeding lines also offer opportunities to expand market demand by optimizing phytonutrients in new varieties while minimizing potential health and marketing risks including aflatoxins, salmonella and allergens. The recent release of the Sweetheart variety as a premium quality, heart-shaped Marcona-type almond possessing partial self-

compatibility, very high levels of the heart-healthy phytonutrient oleic-acid, as well as improved resistance to navel orangeworm and aflatoxin contamination, demonstrates the potential of this new germplasm. Additional advanced selections combining self-compatibility, California-market types and a range of resistances have come into production in small-scale regional trials in the Sacramento and San Joaquin Valleys. For commercial success, however, varieties will need to combine very high quality and productivity with adaptability to the changing (and yet to be fully determined) needs of tomorrow's orchards and markets. Continued breeding progress depends upon efficient characterization and introduction of genes conferring the required commercial traits, the efficient recombination of these often novel genetic solutions with established genes for local productivity and marketability, and the generation of the large numbers of progeny from controlled crosses to ensure recovery of the rare individuals possessing the best genetic combination of genes for productivity, marketability and sustainability. The almond breeding program is thus pursuing coordinated progress in the three required areas: new germplasm procurement, genetic improvement, and ultimately, cultivar development.

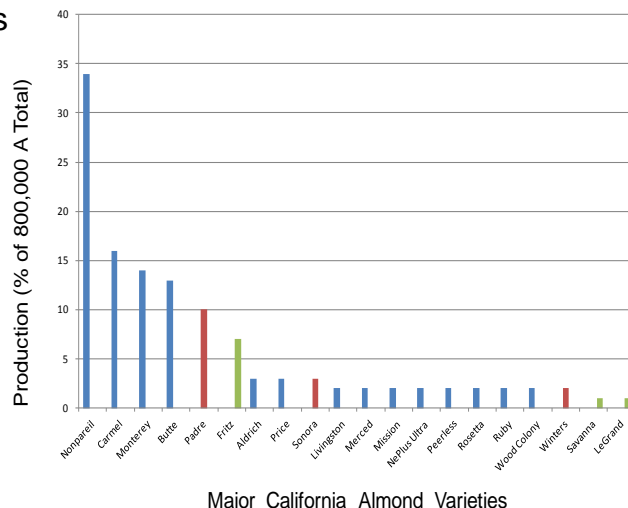


Figure 1. California almond varieties of current commercial importance and different derivations (blue-Nonpareil and the progeny of crosses of Nonpareil by Mission, red-UCD varieties, green-private breeder varieties).

During this current project period, approximately 6,000 progeny trees from the over 10,000 seedlings generated from 2010 hybridizations were transplanted in the spring of 2011 following greenhouse screenings for vigor and disease resistance. Weather conditions for controlled field-crosses in the spring of 2011 were poor, with rain during and after bloom, and frosts during early fruit development which dramatically reduced seed-set success. From over 28,000 controlled pollinations, less than 4,000 viable seed were recovered. An additional 3,000 seed were obtained from open-pollinated isolation blocks (where the desired seed parent is inter-planted with the desired pollen parents with paternity to be determined later by molecular methods). Most isolation blocks were located in the more moderate climates of the San Joaquin Valley where weather conditions allowed higher seed sets. Seedlings from 2011 controlled crosses are currently in the final stages of greenhouse evaluation prior to field transplanting at the Wolfskill Experimental Orchard in Winters, California. Approximately 1,000 of the isolation-block generated seed was direct seeded in Davis, California test plots in 2011 in a test of high-volume/low-labor alternatives to traditional hand transplanting. Initial seedling take was moderate to poor owing to soil compaction and related irrigation problems and remaining isolation-block seedlings are being greenhouse germinated/grown to be used to replace missing Davis field trees. The rainy and freezing conditions of spring 2011 also resulted in generally low seed sets in the majority of the 263 breeding selections tested for self-compatibility, though the same conditions allowed very good opportunities to field-screening for disease resistances. Weather conditions in the spring of 2012 were much more favorable and over 30,000 controlled crosses were made with good seed sets apparent as of early summer, 2012. Over 400 of the most promising advanced selections were tested for self-

compatibility under controlled (bagged branches) conditions. While final seed set will be determined in fall of 2012, initial early summer, 2012 sets have identified over 50 individual genotypes with promising levels of self-compatibility and kernel quality. Over 16,000 bearing trees from diverse genetic sources (summarized in **Figure 4**) were evaluated in 2010-11 for productivity, fruit quality and disease resistance. The most promising of these selections will be evaluated under controlled conditions for self-compatibility in the spring of 2013.

Materials and Methods:

Previous Almond Variety Development reports have presented thorough descriptions of UCD breeding strategies, specific methodologies, advanced breeding lines and individual selections. This report presents an overview of the 3 core breeding projects (new germplasm procurement, genetic improvement, and cultivar development) with an emphasis on recent progress and final integration).

New Germplasm

Almond production in California is heavily dependent on a single cultivar, Nonpareil, which represents about 40% of current commercial acreage as well as being a parent for most of the remaining commercial California pollinizer varieties (**Figure 1**). (Most of these varieties also have Mission as the second parent, further limiting genetic variability and increasing crop vulnerability). Of the California almond varieties currently in commercial production, only Padre, Fritz, Sonora and Winters originate from other than a Mission by Nonpareil cross and of these only Padre does not have Nonpareil prominently in its lineage. The resulting low genetic variability and high genetic inbreeding increases crop vulnerability to Nonpareil-type afflictions - such as Noninfectious Bud Failure and susceptibility to diseases - such as Alternaria leafspot as well as plum rootstock incompatibilities. The low genetic diversity also inherently makes current production more vulnerable to ongoing changes in climate, market expectations and regulatory demands. High inputs of good quality water, agrochemicals, along with the availability of good orchard soils and progressive horticultural practices, have contributed to an ongoing increase in both total almond production as well as average yield per acre (**Figure 2**). Year-to-year fluctuations in per acre yields, however, remain comparable to those observed in the 1990s and higher than those observed in the 1980s and earlier and partly represent failures of cross-pollination and seed set during the short and often stormy spring flowering

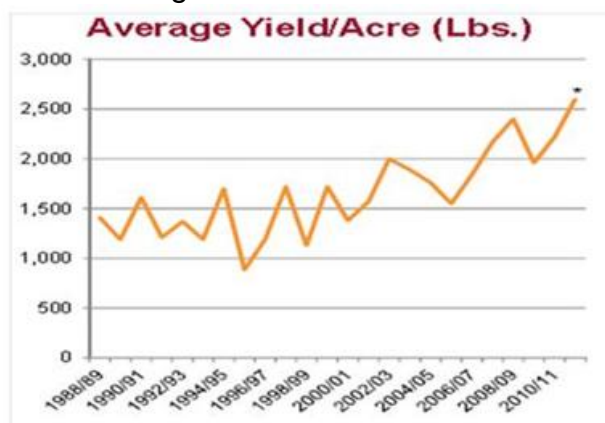


Figure 3. California per acre yield fluctuations over the last 25 years.

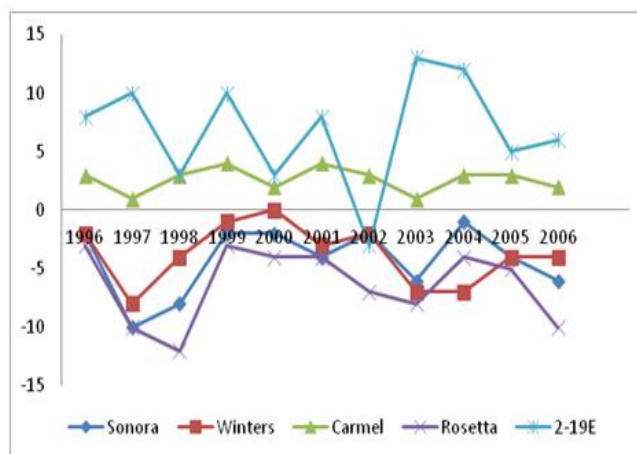


Figure 2. Bloom data for important Nonpareil pollinizers from the 1996-2006 Kern Co. RVT. Bloom initiation date relative to Nonpareil given for all except 2-19E which is end of bloom date).

season. Effective pollenizers for the crucial early Nonpareil bloom have been identified (i.e., the pollenizer consistently blooms a few days ahead of Nonpareil to allow honeybee pollinators to efficiently move cross-pollen to the relatively low-numbered but high-fecundity early Nonpareil flowers). The consistency of specific pollenizer-Nonpareil bloom overlap appears to be becoming more erratic, particularly in the lower San Joaquin Valley with its ongoing tendency for warmer winters and so more variable winter-chilling conditions (**Figure 3**). Since all current Nonpareil-pollenizers are closely related to Nonpareil, they share its general vulnerabilities. A long-term objective of the UCD almond variety development program has been to develop improved Nonpareil pollenizers through the transfer

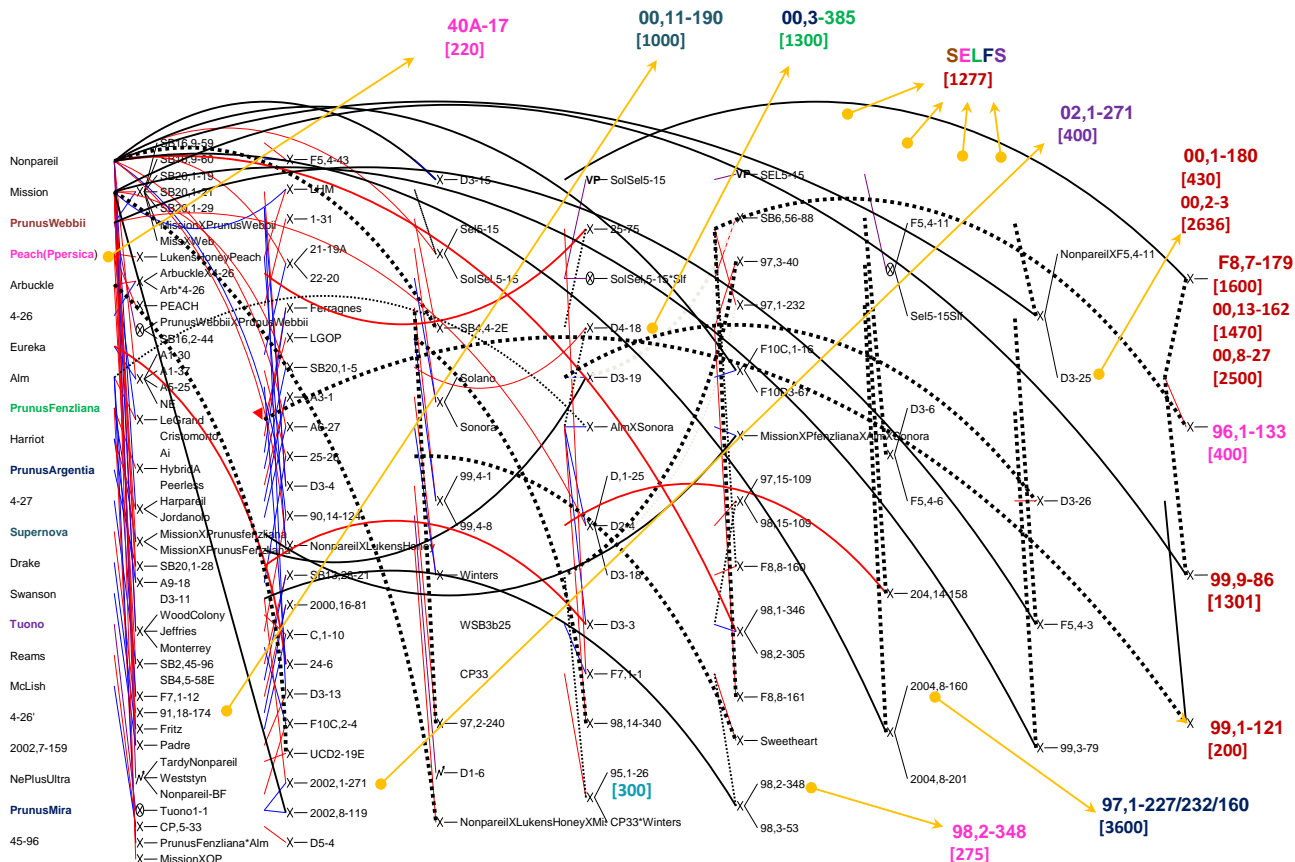


Figure 4. Crossing diagram showing major breeding lineages and their often exotic species origin. {Numbers in brackets denote the total number of progeny trees from different advanced lineages evaluated in 2011-12 while color-codes refer to species origin; solid lines denote seed parent; dotted lines denote pollen parent}.

of novel genes for self-compatibility as well as the concurrent transfer of similarly novel genes for improved resistance to abiotic (climate change, noninfectious bud-failure, lower quality soils, reduced water quantity/quality, etc.) as well as biotic (diseases and pests) threats. A graphical summary of current breeding progress has been compiled in **Figure 4**, which updates previous

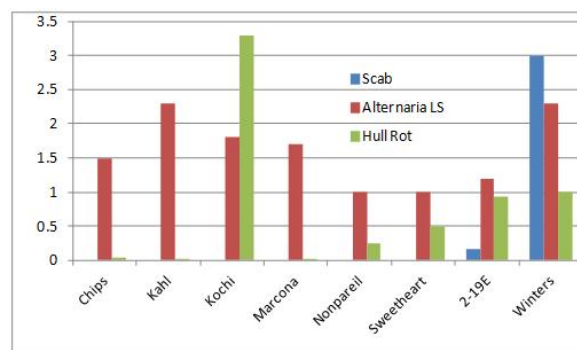


Figure 5. Disease ratings from 2011 Kern RVT . (1-low, 5-high disease).

figures showing the highly diverse genetic origins (including gene transfer from the related species *Prunus persica* (peach), *P. webbii*, *P. mira*, *P. argentea*, *P. fenziiana* and the European self-compatible varieties Tuono and Supernova). Numbers in brackets in **Figure 4** denote the total number of progeny trees from advanced lineages evaluated in 2011-12. All advanced lineages show promising levels of self-compatibility and/or disease/pest resistance along with improved kernel quality and adaptability the Central Valley production conditions. Examples of improved overall disease resistance is presented in **Figure 5**, where Sweetheart and advanced UCD selection 2-19E continued to show good resistance to Alternaria and scab (though moderate levels of hull rot were observed in 2-19E, which are partly a consequence of its very high yields (see **Table 2**)).

Although numerous opportunities for improved disease resistance are present within this exotic new germplasm (for example, see **Figure 6**), successful transfer to advanced California breeding lines presents significant challenges as the genetics are often complex and poorly understood and the resistances typically represent a less immediate priority when compared to self-compatibility, kernel quality and tree productivity. Consequently, in our more focused efforts to rapidly transfer self-compatibility and improved productivity, promising opportunities for disease and pest resistance, as well as improved nutritional and food-safety value, are given lower priority and so pursued less aggressively, particularly in times of budget cutbacks. To avoid the possible loss of these potentially useful exotic traits which have often already been incorporated into improved California-adapted breeding lines, the most promising

Variety	OP (Avg.)
Marianna	-21.7 a
Lovell	-24.2 a,b,c,d
Winters	-24.5 a,b,c,d,e
Sonora	-25 a,b,c,d,e
Padre	-25 a,b,c,d,e
Carmel	-25.1 b,c,d,e
Nemaguard	-25.3 b,c,d,e
Titan	-25.6 c,d,e
NE Plus Ultra	-25.6 c,d,e
Fritz	-26 c,d,e
Mission	-26.3 c,d,e
Butte	-26.3 c,d,e
Hansen 536	-26.9 d,e
Nonpareil	-27.2 d,e
Price	-27.9 e
Probability	0.02

Table 1. Differences in leaf osmotic potential among selected almond varieties and peach rootstocks (Ed Russel M.S., Thesis)



Figure 6. Segregating resistance (left) and susceptibility (right) for flower/leaf blight in a 2011 breeding line.

germplasm is being transferred to USDA National Prunus Germplasm Repository, where it is freely available to both public and private breeders. [Over 20 such advanced breeding genotypes were transferred to USDA Germplasm Repository in 2011-12].

A similar challenge is encountered when pursuing resistance to potential biotic and abiotic changes in future California production systems. In the early stages of interspecific gene transfer (for example, transferring self-compatibility from peach to almond) an extensive genetic variability is available, which often includes resistances to abiotic/biotic stresses which go well beyond current commercial germplasm. An example is shown in **Figure 7** where all summer irrigation was shut off for 100 genotypes from a series of complex almond species crosses to evaluate drought tolerance. While the resultant drought conditions dramatically decreased crop productivity for most genotypes, a few individuals such as A00, 7-180 (180 in **figure 7**) showed surprisingly high productivity. (As these were seed propagated breeding progeny, the source of resistance could be morphological and/or physiological differences within roots and/or shoots). The high levels of stress resistance in some of these intermediate-stage exotic breeding lines indicate that multiple mechanisms are probably active. While individual mechanisms for this type of stress tolerance remain poorly understood, the availability of breeding lines segregating for tolerance (or disease/pest resistance) provides a powerful opportunity for dissecting out the principal genetic control and associated biological mechanism (as has been successfully done in our peach breeding work to develop improved processing quality and fruit brown rot resistance in peach; see references 4, 10, 14, 15, and 16). Towards this almond stress resistance objective, over 20 intermediate-level exotic almond breeding lines have been planted in San Joaquin (Delano) and Sacramento (Arbuckle) Valley test plots. Several aspects of stress resistance/tree productivity are currently being evaluated with collaborating researchers and some early progress is taking place in characterizing stress tolerance differences among different breeding material through quantitative measures like leaf osmotic potential (see **Table 1**).

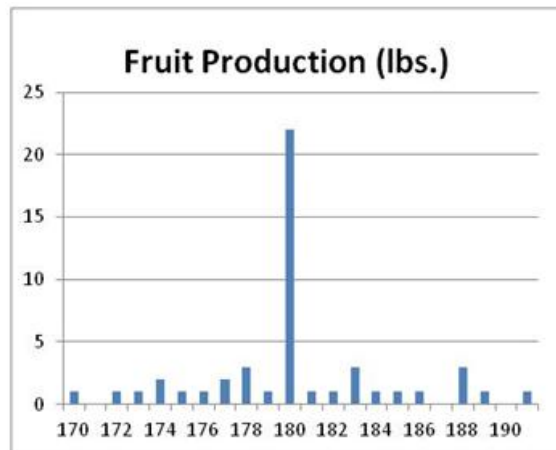


Figure 7. Drought tolerance as demonstrated by total tree fruit production in population of progeny from complex interspecific crosses.

Genetic improvement

In order to improve the performance of new cultivars, the specific genes controlling those traits need to be transferred and consolidated. As detailed in the 2010 annual report, the efficiency of this genetic transfer depends upon

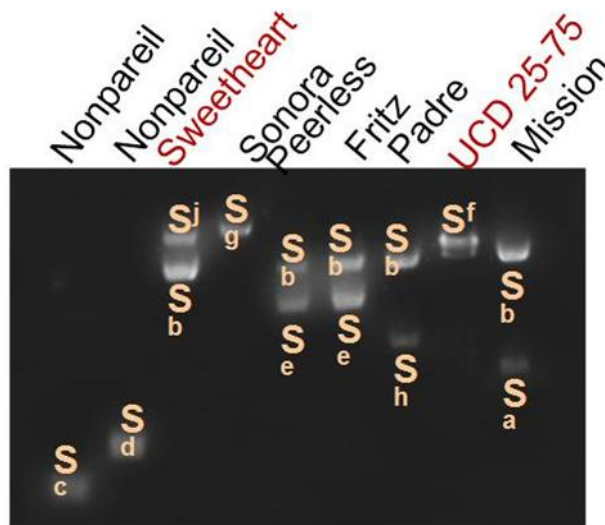


Figure 8. Gel separation of major almond self/cross incompatibility alleles as well as the self-compatibility (Sf) allele from peach in almond advanced selection UCD25-75

the nature of genetic control for the trait of interest. In almond, there are only a few commercial traits which are controlled by one to a few major genes (which makes them relatively easy to manipulate). (As documented in the 2010 annual report, however, effective manipulation of even such discrete major gene traits becomes increasingly difficult with an increase in the number of traits targeted by breeding). Examples of relatively easily manipulated major genes include bitterness in almond kernels as well as pollen self-compatibility. While all commercial California almonds have sweet kernels, nearly all carry a gene form (or allele) for bitterness which is dominated in the kernel by the allele for sweetness. [We have found the exceptions to be the cultivars Butte and Winters which possess two alleles for sweetness and none for bitterness]. While having a single allele for bitterness has only a very small affect on kernel sweetness, it does confer a degree of bitterness (as well as increased cyanide content) in the leaves which makes them less desirable to leaf foragers and so are sometimes commercially preferable. Current research indicates that other, smaller-affect genes are also involved in conferring both kernel bitterness (which is undesirable), as well as its associated amaretto or 'cherry-Coke' flavor (which can be desirable for certain markets). We are currently initiating a large molecular analysis of kernel flavor to try to more clearly characterize the nature genetic control and so the opportunities for its manipulation.

Research also continues with collaborators in Japan, Spain and Iran, on characterizing the genetic control of self/cross-incompatibility as well as self-compatibility. Early molecular data indicate that self-incompatibility is the ancestral form and still predominates in wild populations as it enforces outbreeding and so the greater genetic diversity required for plants to adapt to changing

environments. Our data indicate that self-compatibility is a relatively recent development, which appears to have occurred independently within related species such as *P webbii* and the closely related peach species. In addition, research with Prof. Tao's group in Kyoto



Figure 9. Flower structure encouraging outcrossing at left (showing style and stigma extending well beyond the pollen bearing anther-sacs and so not readily self pollinated without honeybee visits) versus flower structures encouraging self-pollination (right) showing a flower type where the stigma-style grow (bend) back into the anther area.

Japan, has shown that the mutations for self-compatibility have occurred in different organs in peach species (mutations in the pollen, see **Figure 8**) compared with almond species (where the mutation has occurred in the flower pistil tissue). Consequently we are moving to combine or 'pyramid' the pollen as well as the pistil self-compatibility genes to maximize both field expression and environmental stability of self-compatibility in new UCD cultivars. [For example, in **Figure 4**, *P webbii* sources are identified by a rust color while peach sources identified with a pink-red color. Advanced selections evaluated in 2011-12 combining both forms are designated with a rust-red color (with a total of almost 8000 individual genotypes (trees)).

Ongoing work with Jerry Dangl at USDA germplasm repository has also verified that some putative self-incompatible genotypes can show higher levels of self-compatibility under certain environments and with certain genetic backgrounds. Both recent UCD almond variety releases (Winters and Sweetheart) while genetically SjSb and so self-incompatible (**Figure 8**) possess major and modifier genetic factors to allow relatively high levels of self-pollination in certain environments. For this reason, Winters has been used in recent crossing cycles to combine this more complex genetic background with major genes for self-compatibility from both the peach (pollen mutation) group [cultivated peach (*P. persica*) and the related species *P. mira*] as well as the almond (pistil mutation) group [primarily *P. webbii* in the European self-compatible cultivars Tuono and Supernova (see **figure 4**)].

High levels of cultivar self-fruitfulness will require both high levels of self-compatibility (i.e. self-pollen shows high success at self-fertilization when self-pollination occurs) as well as flower structures/development which promotes high levels of self-pollination (i.e. pollen transfer from anther sacs to adjacent flower stigma). Unlike self-compatibility, which is under relatively simple genetic control, self-pollination is a very complex traits controlled by many independent genetic factors and strongly influenced by environment. For example, in **Figure 9** flower structures encouraging outcrossing (left image showing style and stigma extending well beyond the pollen bearing anther-sacs and so not readily self-pollinated without honeybee visits) versus flower structures encouraging self-pollination (right side (under certain environments) where the stigma-style grow (bend) back into the anther area. Although some advanced breeding UCD selections (such as UCD 25-75 in **Figure 8**) contain both self-compatibility and consistently high levels of self-pollination (i.e. high levels of selfing within enclosures which exclude honeybees and other pollinators), the genetic control is complex and so difficult to manipulate with predictability.

Figure 10 summarizes the breeding progress for another novel interspecies-origin trait with potential value for commercial California production. The trait confers a highly-lignified but very thin and well-sealed shell. While we are continuing to determine the nature of genetic control, the trait dominates in progeny containing at



Figure 10. Transfer of the thin, hard shell trait from *P. webbii* by Mission cross (top), sample progeny from 1st backcross to California almond (center) and 2n backcross with selection for specific market types (bottom).

least one parent having the trait, indicating control by a few dominant genes, thus allowing ready genetic manipulation. The trait has commercial application in the development of thin but hard shelled and well sealed almonds as possible use in the in-shell Peerless market. (The distinctive shell sculpturing as seen in the bottom left of **figure 10** may improve value for this market). A more widely applicable use may be the development of thin-shelled, highly-sealed commercial cultivars combining high navel orangeworm and ant resistance with high kernel crack-out ratios. (At the bottom right of **figure 10** is a 2011 field selection showing good shell-seal with a crack out ratio of greater than 70%). Breeding work is also continuing to combine his novel thin-shelled, high-seal trait with the very hard shelled trait of European cultivars such as Ferragnes and Marcona. In addition to a very highly lignified shell, these cultivars also initiate shell lignifications weeks earlier than California thin-shelled cultivars and so develop earlier resistance to damage from probing insects such as Leaffooted bugs and may also reduce hull-rot strikes since earlier lignifications has been reported to protect some European varieties from pathogen transfer from the infected hulls to attached shoots. Progeny from these crosses will be coming into nut production (and so be amenable to selection) within the next 1-2 years.

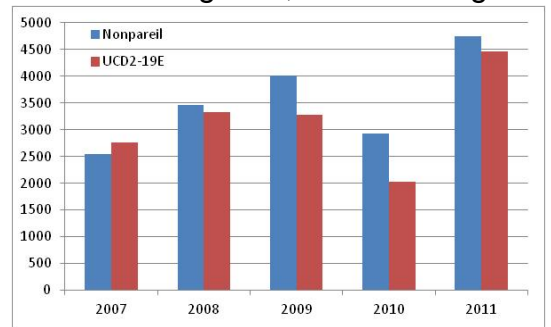


Figure 11. Yield of UCD2-19E and adjacent Nonpareil for 1st 5 years of Kern RVT.

Cultivar development

The definitive objective the UCD almond variety development program is the development of cultivars with sufficient value to promote their commercial plantings in the Central Valley. While this and earlier annual reports have clearly documented success in genetic improvement (as with the continuous and sustained progress in the transfer of self-compatibility to locally adapted almond breeding lines), cultivar development goes beyond the discrete, incremental (and so predictable) genetic improvement of specific traits as it involves the optimization of all traits important for commercial success. As demonstrated in the 2010 annual report, the large number of such traits precludes a readily calculated and additive genetic improvement approach, but rather requires a holistic integration across all commercial traits. Currently, the variety Nonpareil dominates the industry as it is the result of over 150 years of selection (originally for the initial Nonpareil genotype and subsequently for clonal sources or budsports with even higher production value (see **Table 2**)). In addition, Nonpareil's dominance has made it the preferred tree and kernel type in the industry, so that for a new cultivar to achieve preferred pricing it not only has to match Nonpareil in productivity but also kernel quality. This aspect of clonally propagated crops, including most fruit and nut crops, makes it very difficult to breed new varieties superior or even comparable to the established market variety for all major commercial traits. A summary of currently important California almond varieties (**Figure 1**) both documents the predominance of Nonpareil and also demonstrates that the other cultivars are mainly utilized as

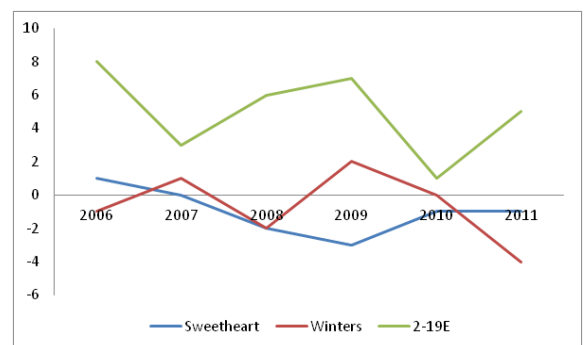


Figure 12. End of bloom date for 2-19E relative to Nonpareil at Kern RVT. (Initial bloom date vs. Nonpareil plotted for remaining early bloom pollenizers).

pollenizers for Nonpareil. Most of these pollenizers are the results of early chance seedlings between Nonpareil and Mission, with only 4 cultivars (Padre, Fritz, Sonora and Winters) the product of breeding programs. Commercial success of a new variety is thus determined not by any one special trait, but rather by optimized performance among the many traits required for commercial viability. The development of new cultivars incorporating new and often exotic traits such as self-compatibility from other species such as peach is particularly difficult as it inherently reshuffles the optimized target cultivar genotype with both desirable and undesirable genes from the exotic donor. Despite this constraint, a large number of advanced UCD selections demonstrating self-compatibility and or improved disease/pest resistance are currently in small-scale regional variety trials as described in the 2010 annual report. [Since the presence or absence of trait deficiencies ultimately determine cultivar success, long-term regional testing under a range of environments and climates is essential for determining true commercial value prior to release to the California industry.] Currently, the most promising UCD breeding selection is the high-value, productive genotype UCD2-19E. Multiyear regional evaluations have shown both productivity and kernel quality to be comparable to Nonpareil (**Table 2** and **Figure 11**). Results are consistent with earlier regional variety and grower trials where UCD2-19E demonstrated superior yields and kernel quality as well as good disease resistance (**Figure 5**), improved shell-seal (though lower crack-out) compared to Nonpareil (**Table 2**) and good bloom overlap with the later Nonpareil bloom (**Figures 3** and **12**). Early trials showed a tendency for UCD2-19E to alternate bear in later years because of its high crop density and smaller tree size (**Figure 13**). While the more recent (2007 to 2011) regional trial data also suggest a tendency to alternate bear (**Table 2**), a plotting of UCD2-19E yields relative to the Nonpareil Newell clonal source (which was typically planted adjacent to it) shows both to be tracking the same pattern, indicating that changes are due to environmental (or irrigation) differences (**Figure 11**). Data on UCD2-19E bloom overlap with Nonpareil (**Figures 3** and **12**) plot the end of bloom date as this would be the important criteria for a pollenizer for the late Nonpareil bloom. A comparison of **Figures 11** and **12** shows the end of bloom date for UCD2-19E closely tracks its productivity for that year. This is primarily because high crops years are predetermined by high flower densities at bloom, and these high flower densities also result in an extended bloom for that particular year. Years with low initial flower densities will suffer not only the inevitable reduction in final crop potential but also run the risk of reduced flower numbers and so reduced bloom-overlap with adjacent Nonpareil trees.



Figure 13. Smaller tree size of UCD2-19E (center) relative to 2 flanking Nonpareil rows which allows more light, and so greater productivity for Nonpareils.

The consistently higher yields of the Nonpareil clones planted adjacent to UCD2-19E (**Figure 11**) is both expected and targeted by the breeding program since the smaller size of these late-bloom pollenizer trees allows more light to be captured by the adjacent high-value Nonpareil trees (**Figure 13**). [The early-pollenizers variety Winters with its smaller tree size also shows similar trends]. This relationship confounds the analysis of differences among clonal sources of

Nonpareil as it may be the result of a location rather than genetic/clone effect. For this reason, Bruce Lampinen has initiated routine light interception measurements for all regional variety and grower trials so that genetic and orchard location affects can be separated. **Figure 14** shows a recent analysis by Bruce Lampinen where the pollenizer yields are plotted against the yields of the pollenizer plus adjacent Nonpareil clones to compensate for location affects. Results show that UCD2-19E is very similar in productivity to Nonpareil even given its smaller size. Finally, kernel characteristics of UCD2-19E are so similar to Nonpareil that the growers are receiving premium Nonpareil pricing.

Based and these and earlier regional variety trial data, UCD2-19E will probably be released within the next two years. It will also be included in the next and more extensive regional variety trials to track its performance and provide early and accurate feedback to growers on any unique characteristics observed. Remaining UCD regional variety trial candidates are currently being selected from the most recent breeding cycle for self-compatibility and improved yield, quality and resistance. Good shell and kernel quality are increasingly being recovered in this next-generation breeding population, with some individuals approaching Nonpareil in their kernel characteristics (**Figure 15**).

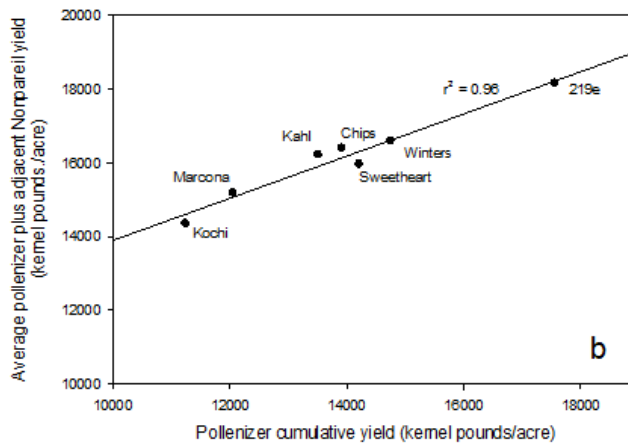


Figure 14. Plot of cumulative yields of Kern RVT selections relative to average pollenizers and adjacent Nonpareil yields.

We are also pursuing the molecular analysis of advanced almond and peach breeding lines to improve our understanding of the underlying genetic control and ultimately improve our selection efficiencies. Initial results have been provocative.

Figure 16 is a preliminary plot of the occurrence of genetic exchange (translocations) between chromosomes in advanced almond by peach breeding lines relative to the Lovell peach rootstock. Results support a preferential pairing and genetic exchange among specific

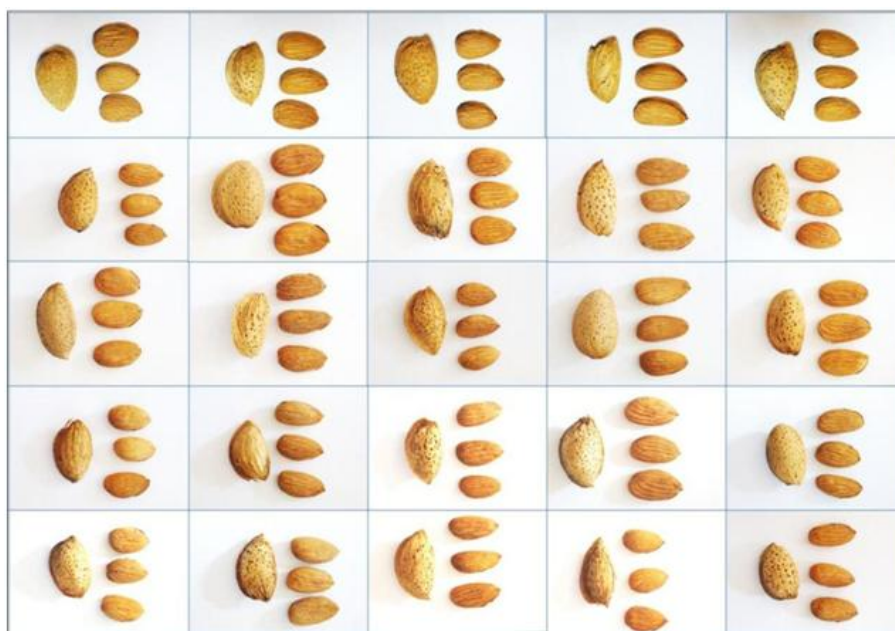


Figure 15. Sampling of different nuts types of some of the advanced UCD selections showing good self-compatibility in 2010-11.

chromosomes, which is consistent with recent human genome research which shows that chromosome orientations within the cell nucleus is not random but predetermined and this preferential chromosome positioning can strongly affect the level expression of trait on separate but proximal chromosomes (**Figure 17**). Consequently, the expression of genes controlling specific traits is controlled not just by the presence or absence of DNA but by a myriad of nuclear factors including chromosome architecture (i.e. chromosome pairing, level of DNA compaction, histone structure, etc.) and chromosome chemistry (i.e. DNA methylation patterns, histone and chromosome chemistry differences, DNA repair mechanisms, etc.). While these factors would be rescrambled each time a genotype goes through seed propagation (as occurs with most agronomic and vegetable crops) their unique nuclear architecture/chemistry is effectively captured in clonally propagated crops. While the mechanisms remain poorly understood and so currently beyond our ability for intelligent manipulation, they would be subject to both natural and human selection. If confirmed, this would further explain the predominance in fruit and nut crop production of elite clones which are often the product of hundreds to thousands of

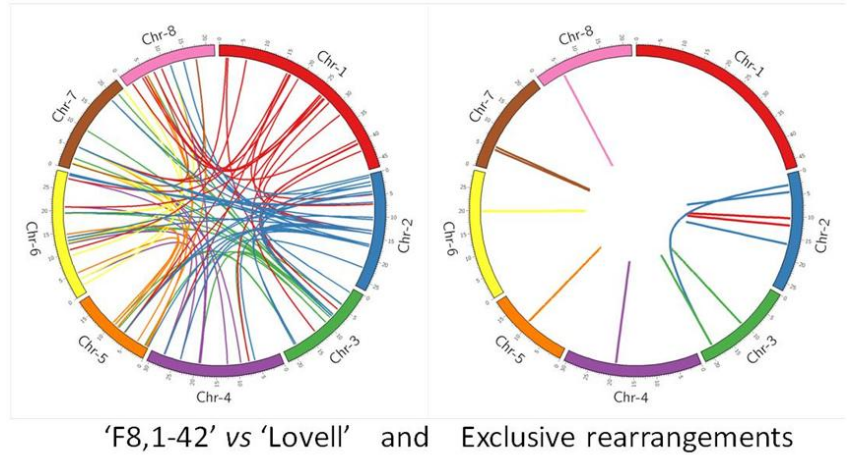


Figure 16. Evidence for preferential genetic exchange (translocations) between chromosomes in an advanced peach by almond breeding line (left) and translocation (curved interconnected lines between chromosomes) and unique insertions/deletions (straight radiating lines) unique to the almond by peach backcross (right). [Identified through the alignment of more than 20 million reads from 'F8,1-42' against the Reference Genome Sequence of Peach 'Lovell' double-haploid].

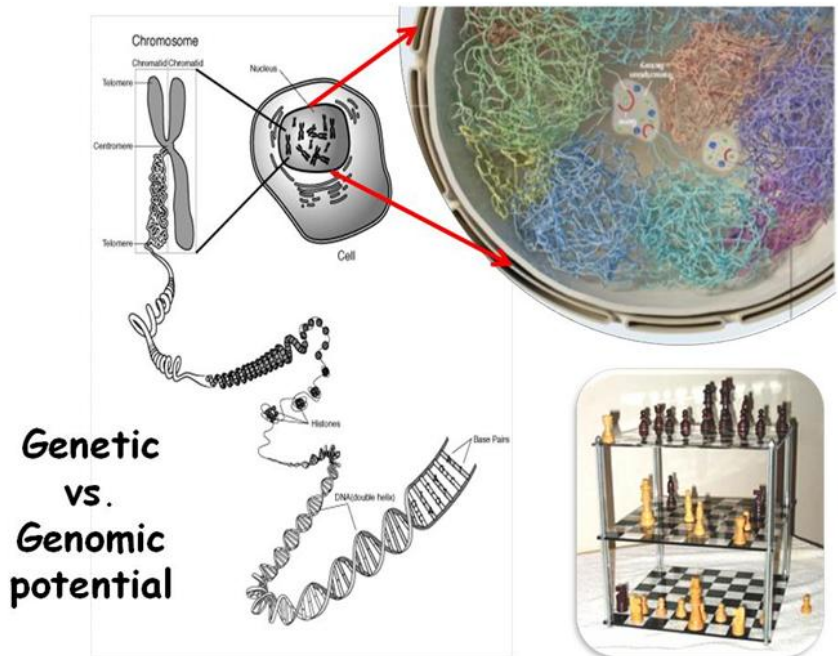
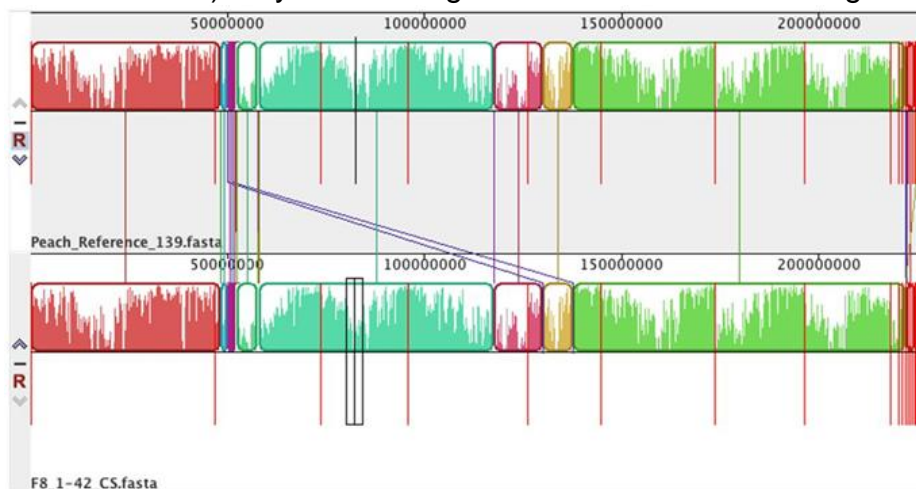


Figure 17. Model of the complex interactions of chromosome architecture and orientations within the cell nucleus. Specific chromosome location is not random but predetermined and this preferential chromosome positioning can strongly affect the level expression of trait on proximal, paired chromosomes,

years of selection (such as the Tuono almond, Thompson seedless grape, Mission fig and Sweet oranges). Consequently, to match the performance of established elite varieties, the breeder has to optimize not only the genetic composition but also the full complexity of the above genomic architecture. [An analogy depicted in **Figure 17** is that while the science of traditional genetics and breeding may be considered a traditional chess game, this holistic genomic selection would be analogous a multi-dimensional chess game (except that we are presently only aware of-and capable of intelligently manipulating the pieces {DNA complement} on a single dimension].

A second (yet to be verified) application of these findings is that inbreeding (such as would occur by interbreeding related individuals) may act to devograte at both the DNA and genomic architecture level. Wide crosses, including interspecies crosses, appear to result in novel chromosome rearrangements which can lead to both invigoration (as in the hybrid vigor shown by peach x almond rootstocks) as well as the expression of novel traits (such as improved



resistance to certain diseases in interspecific hybrids and the early backcrosses). While the underlying mechanisms remain poorly understood, some patterns are emerging. For example, in certain interspecies (almond by peach) crosses, specific groups of genes appear to be preferentially transferred in advanced breeding lines (**Figure 18**).

Figure 18. Possible rearrangements of contigs (set of overlapping DNA segments that together represent a consensus region of DNA) in the assembled sequence (preliminary) of F8,1-42 in comparison with the Peach Genome Reference Sequence of dihaploid Lovell.

The role of this larger genome architecture/chemistry to trait and so genotype performance in clonally propagated crops is preliminary and speculative. However it is consistent with breeding experience which has shown very little success for improving on established commercially elite clones in crops such as citrus, winegrape, prune, fig, apple, pear, plantain/banana, coffee, pineapple, mango and papaya, among others). If confirmed it would indicate that a significant proportion of the field variation that is currently being dismissed as being environmental in origin is actually of genetic/genomic origin and so available for selection. While the mechanisms to effectively achieve such selection remain unknown, the potential gains may be required if the performance of new cultivars is to match that of the current elite clonal standard.

Table 2. Five year performance of UCD selections and Nonpareil propagation sources at the Kern RVT (from B. Lampinen et al., 2011 Annual RVT Report).

2007						
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Kernel pounds per		Cumulative kernel yield (lbs/acre)
				Tree	Acre	
2-19e	13149 a	0.78 e	54.3 d	22.8 a	2756 a	4474 a
Winters	11972 ab	0.83 de	60.2 b	21.8 ab	2634 ab	4173 a
Nonpareil-Newell	10659 bc	0.90 bc	67.3 a	20.9 abc	2536 abc	3626 b
Nonpareil-Nico	9260 cde	0.92 bc	66.0 a	18.8 abcde	2279 abcde	3511 b
Nonpareil-Driver	9793 cd	0.91 bc	65.6 a	19.6 abcd	2370 abcd	3474 b
Nonpareil-3-8-2-70	9340 cde	0.92 bc	66.3 a	18.9 abcde	2291 abcde	3393 b
Nonpareil-5	8905 cdef	0.95 b	67.0 a	18.6 abcde	2251 bcde	3323 bc
Marcona	6938 fg	1.08 a	29.8 f	16.5 defg	1995 defg	3252 bcd
Kahl	9594 cd	0.91 bc	47.6 e	19.3 abcd	2332 abcd	3222 bcd
Nonpareil-J	9137 cde	0.89 bcd	65.5 a	17.8 bcde	2152 bcdef	3218 bcd
Nonpareil-6	8396 def	0.94 b	67.1 a	17.4 def	2103 def	3178 bcd
Nonpareil-7	9517 cd	0.92 bc	67.9 a	19.3 abcd	2332 abcd	3140 bcd
Chips	7681 defg	0.87 cd	54.4 d	14.7 efq	1780 efq	2766 bcd
Kochi	6006 g	1.08 a	59.4 bc	14.3 fg	1729 fg	2694 de
Sweetheart	6767 fg	0.89 bcd	66.6 a	13.1 g	1588 g	2165 e

2008						
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Kernel pounds per		Cumulative kernel yield (lbs/acre)
				Tree	Acre	
2-19e	13472 a	0.93 g	54.3 d	27.5 cd	3321 cd	7795 a
Nonpareil-Nico	13879 a	1.10 cd	66.0 a	33.5 a	4056 a	7567 ab
Nonpareil-Newell	11916 bcd	1.09 de	67.3 a	28.6 cd	3456 cd	7110 bc
Nonpareil-3-8-2-70	12506 bcd	1.17 cd	66.3 a	30.7 b	3714 b	7106 bc
Nonpareil-Driver	12729 abc	1.07 de	65.6 a	29.8 bc	3611 bc	7085 bc
Nonpareil-5	12883 ab	1.08 de	67.0 a	30.5 b	3692 b	7001 bc
Winters	9872 e	1.02	60.2 b	22.1 fg	2670 fg	6843 c
Nonpareil-7	13250 ab	1.06 de	67.9 a	31.1 ab	3763 ab	6802 c
Nonpareil-6	10707 de	1.16 c	67.1 a	27.3 cd	3300 cd	6478 cd
Nonpareil-J	11071 d	1.09 cde	65.5 a	26.6 de	3224 de	6442 cd
Kahl	10720 de	0.96 fg	47.6 e	22.6 fg	2733 fg	5954 de
Chips	11465 cd	0.97 fg	54.4 d	24.4 ef	2956 ef	5722 e
Sweetheart	13149 ab	0.82 g	66.6 a	23.9 ef	2893 ef	5059 f
Marcona	4721 f	1.39 a	29.8 f	14.4 h	1748 h	5001 f
Kochi	5882 f	1.28 b	59.5 bc	16.5 h	2002 h	4996 f

2009							
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Kernel pounds per			Cumulative kernel yield (lbs/acre)
				unit PAR int.	Tree	Acre	
Nonpareil-Nico	13773 ab	1.05 bcd	74.7 ab	69.3 bc	32.9 a	3977 a	11417 a
Nonpareil-Newell	14513 a	1.03 bcd	74.8 ab	72.8 abc	33.1 a	4004 a	11145 ab
2-19e	14706 a	0.84 f	65.6 f	71.6 bc	27.1 c	3285 c	11080 ab
Nonpareil-Driver	13856 ab	1.08 ab	75.8 a	76.1 bc	32.9 a	3977 a	11062 ab
Nonpareil-3-8-2-70	13756 ab	1.04 bcd	74.6 ab	71.8 bc	31.4 ab	3798 ab	10905 abc
Nonpareil-5	12070 bcd	1.08 ab	74.2 ab	72.0 bc	28.7 bc	3476 bc	10494 bcd
Nonpareil-7	13051 ab	1.03 bcd	72.6 abc	72.9 bc	29.5 bc	3571 bc	10393 bcd
Nonpareil-6	13505 ab	1.02 bcd	71.2 cd	68.9 bc	30.3 abc	3661 abc	10139 cd
Nonpareil-J	12803 abc	1.04 bcd	71.6 bcd	63.4 bc	29.0 bc	3513 bc	9955 de
Winters	9434 ef	0.96 bcde	61.6 g	63.9 cd	20.0 e	2415 e	9258 ef
Kahl	11035 cde	0.87 ef	59.1 g	85.2 a	21.1 de	2559 de	8513 fg
Chips	9771 ef	0.93 def	58.6 g	55.9 cd	20.0 e	2422 e	8144 gh
Sweetheart	12798 abc	0.85 ef	73.3 abc	69.6 bc	24.0 d	2906 d	7965 gh
Marcona	8977 fg	1.07 abc	32.5 f	77.7 ab	21.2 de	2562 de	7563 h
Kochi	7252 g	1.17 a	68.9 de	52.6 d	18.7 e	2259 e	6955

2010							
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Kernel pounds per			Cumulative kernel yield (lbs/acre)
				unit PAR int.	Tree	Acre	
Nonpareil-Nico	9521.8 abc	1.24 abcdef	72.5 ab	49.7 ab	25.9 ab	3141 ab	14558 a
Nonpareil-Newell	8429.4 cde	1.31 ab	73.6 a	45.2 abc	24.2 ab	2931 ab	14099 ab
Nonpareil-3-8-2-70	8823.4 bcd	1.28 abcd	72.3 ab	47.0 ab	24.8 ab	3011 ab	13915 abc
Nonpareil-Driver	8368.2 cde	1.28 abcd	71.0 ab	46.2 abc	23.5 ab	2849 ab	13910 abc
Nonpareil-5	9410.2 abc	1.24 abcde	72.3 ab	50.8 a	25.8 ab	3130 ab	13579 abc
Nonpareil-7	10611.8 ab	1.16 bcdef	69.8 ab	49.4 ab	27.1 a	3282 a	13510 abc
Nonpareil-6	9498.9 abc	1.21 abcdef	71.8 ab	48.7 ab	25.4 ab	3081 ab	13219 bc
2-19e	6832.8 efg	1.10 bcdef	56.1 e	33.7 e	16.6 cd	2020 cd	13100 bc
Nonpareil-Jones	8314.7 cde	1.23 abcdef	70.9 ab	43.8 abcd	22.6 b	2737 b	12691 c
Winters	8601.3 efg	1.11 bcdef	60.7 cde	38.5 cde	16.0 de	1945 cd	11203 d
Chips	9089.0 abc	1.15 bcdef	65.9 abc	48.4 ab	23.0 b	2789 b	10933 d
Sweetheart	10915.5 a	0.80 g	71.8 ab	42.1 bcd	19.3 ab	2803 ab	10768 de
Kahl	7587.0 cde	1.01 f	56.5 de	43.4 abcd	16.9 c	2048 c	10561 de
Marcona	5072.7 gh	1.28 abc	26.2	36.7 de	14.4 cde	1745 cde	9307 fg
Kochi	3902.2 h	1.40 a	64.4 bcd	23.5 f	12.1 e	1466 e	8421 g

2011							
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Kernel pounds per			Cumulative kernel yield (lbs/acre)
				unit PAR int.	Tree	Acre	
Nonpareil-Nico	18776.9 a	0.99 bcde	68.0 abc	86.7 a	41.0 a	4964.2 a	19522.7 a
Nonpareil-3-8-2-70	17744.2 abc	1.05 bc	70.7 a	87.9 a	41.0 a	4962.3 a	18878.1 ab
Nonpareil-Newell	17790.9 abc	1.00 bcd	70.1 ab	81.0 ab	39.2 a	4744.7 a	18746.5 ab
Nonpareil-Driver	17943.0 ab	0.98 bcde	66.0 abcd	84.3 a	38.7 ab	4682.6 ab	18593.4 abc
Nonpareil-5	15744.6 de	1.03 bc	70.4 ab	78.0 ab	35.9 abc	4341.9 abc	17886.9 bcd
Nonpareil-6	16630.0 bcde	1.04 bc	70.0 ab	81.6 ab	38.1 ab	4618.5 ab	17838.3 bcd
2-19e	18253.3 ab	0.91 bcde	64.8 abcd	73.6 ab	36.8 ab	4459.7 ab	17560.0 bcd
Nonpareil-7	17078.8 abcd	0.83 e	69.2 abc	76.1 ab	31.4 bcd	3804.0 bcd	17235.0 cd
Nonpareil-Jones	16992.6 abcd	0.96 bcde	70.0 ab	81.6 ab	36.0 abc	4359.4 abc	17050.7 d
Winters	15979.0 cde	0.83 e	58.7 ef	76.3 bc	29.3 cde	3553.5 cde	14757.0 e
Chips	11900.6 f	0.94 bcde	60.3 de	51.4 de	24.6 de	2984.7 de	13917.8 e
Sweetheart	14969.2 e	0.86 de	64.1 bcde	52.5 de	28.2 de	3411.8 de	13712.5 e
Kahl	12420.0 f	0.89 cde	53.5 f	59.1 cd	24.4 de	2953.2 de	13514.3 e
Marcona	9633.4 g	1.07 b	30.8 g	51.8 de	22.7 e	2746.0 e	12053.7 f
Kochi	8701.4 g	1.22 a	63.5 cde	43.4 e	23.3 e	2825.2 e	11246.5 f

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