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# Almond Variety Development

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**Project No.:** 13-HORT1-Gradziel

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**Objectives:**

Develop (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 2013-14 include:

1. Release UCD2-19E as a late flowering and very productive Nonpareil-type cultivar. Identify candidates for new Regional Variety Trials (RVT).
2. Generate 14,000 new seedling progeny from crosses between elite parents. Prioritize traits in partnership with growers, handlers and processors. Evaluate and reduce by 40% the ~32,000 progeny trees currently in breeding trials through development/implementation of low-input/high-throughput breeding efficiency strategies.
3. Identify effective predictors of yield potential (annual and cumulative) to assess opportunities/limitations of traditional and new biotech approaches including molecular marker-assisted-selection (MAS).

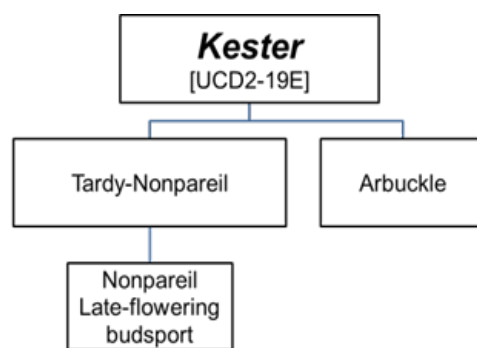
**Interpretive Summary**

Advanced selection UCD2-19E has been released to the industry as the variety *Kester* providing California growers with a late flowering and very productive Nonpareil-type cultivar. Ten advanced UCD breeding selections representing a diverse array of genetic backgrounds while expressing high kernel quality and productivity as well as promising new traits, including self-compatibility and disease/pest/environmental stress resistance, have now been planted as part of the new Regional Variety Trials (RVT). Over 12,000 seed from crosses between advanced breeding selections and established California cultivars were recovered in 2013. Following greenhouse screening, approximately 6000 seeds have been field planted with an additional 2000 anticipated to be planted by mid-2014. Following evaluations of the over 32,000 breeding progeny trees currently in production, approximately 40% of the inferior seedlings have been removed to allow greater

resources and breeding attention to the remaining and more promising individuals. To improve breeding program evaluation efficiency for breeding progeny, improvements in our ability to characterize and codify genetic differences in fruit/kernel productivity and quality are being pursued. Targets for improvement include enhanced cropping consistency through the incorporation of genes conferring self-compatibility and disease/pest/stress resistance, improved kernel size and productivity, and improved spur production consistency including long-term orchard productivity. These goals are being pursued through field research to better understand the individual components involved in productivity and crop consistency, and for the development of molecular markers to more effectively identify and tag crucial traits for breeding improvement through marker-assisted-selection (MAS) and marker assisted breeding (MAB).

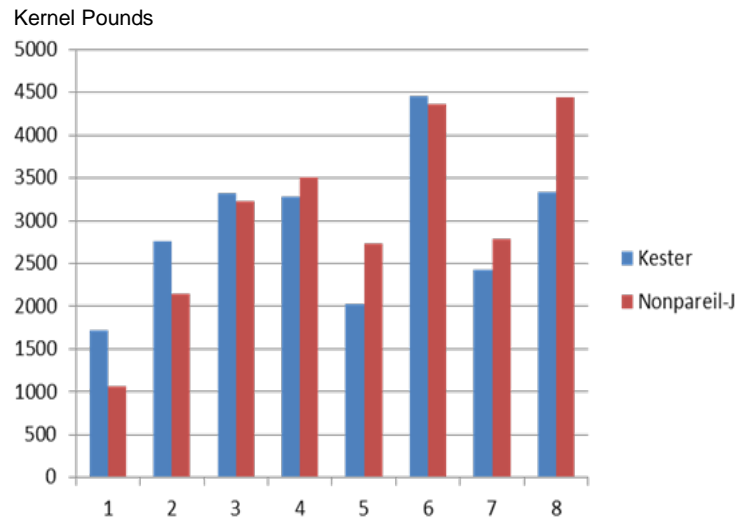
Release UCD2-19E as *Kester*, a late flowering and very productive Nonpareil-type cultivar.

Patenting and release of advanced selection UCD2-19E as the variety *Kester* was completed by June 2014, in time for nurseries to begin propagation for 2015 field plantings. Over 15 years of field testing, including multiple grower locations in the Sacramento and upper and lower San Joaquin valleys has shown *Kester* to be a very productive late flowering Nonpareil-type cultivar. A detailed description of this new release, including field performance data from the 1990s and 2000s were provided in the 2012 annual report (Project 12-HORT1-Gradziel, Almond Variety Development which can be found at [Almonds.com/ResearchDatabase](http://Almonds.com/ResearchDatabase)). The major advantages of *Kester* include: a) cross-compatibility with Nonpareil, b) a flowering time after Nonpareil and so covering Nonpareil late bloom and c) a high and consistent productivity of Nonpareil type kernels from a more compact tree. The smaller tree size allows more light to fall on adjacent Nonpareil trees promoting greater yields on this commercially dominant cultivar. The later bloom offers the additional climate change-buffering advantages of reduce risk from frost damage as well as more consistent cross-pollination of the often straggling later-Nonpareil bloom – increasingly observed in seasons were winter chill is reduced [either



**Figure 1.** Parentage of the *Kester*

through lower cumulative chill units or through unusually warmer mid-winter (typically in early January) temperatures] which appear to degrade the chilling process. The kernel similarities between *Kester* and Nonpareil, as well as the *Kester* harvest time which immediately follows Nonpareil, may allow mixed harvest of these cultivars the growers wishing to improved orchard management efficiency. While these advantages were already recognized to late 1990s, the extended time to patenting is consistent with UCD breeding priority for releasing proven cultivars for grower/industry use. The long time period is required for thorough, regional grower testing to document cultivar advantages over current material and to guard against releasing cultivars with defects such as alternate bearing, noninfectious bud failure or high susceptibility to local diseases/pests which would result in serious grower losses if serious defects were only recognized well into orchard establishment. In addition to comprehensive performance testing, a second major goal of the UCD public breeding program is the incorporation improved germplasm for cultivar breeding by both public and private institutions. The release of *Kester* represents the first of three steps towards this final goal. While the majority of almond cultivars currently under California cultivation are progeny of crosses between Nonpareil in the standard cultivar Mission, the *Kester* variety represents incorporation of unique, though still traditional almond germplasm, as shown in its pedigree (**Figure 1**). *Arbuckle* was a productive cultivar grown in the early to mid-1900s which demonstrated improved disease resistance (though a distinct vulnerability to *Monilinia* flower blight, partially because of its early bloom). Tardy-Nonpareil is a late blooming budspout of Nonpareil which flowers approximately 10 days after Nonpareil but which has been associated with lower productivity. Inheritance studies in the 1990s suggested that the lower productivity and late flowering were probably genetically linked (i.e., the traits were controlled by separate genes but that the genes were on the same chromosome and close enough to each other that they were almost always inherited together). Molecular studies during the last 10 years have generally confirmed this hypothesis. The variety *Kester* is the consequence of several thousand crosses to Tardy-Nonpareil which were required to break the linkage between commercially desired traits such as later bloom, while excluding unwanted



**Figure 2.** Yield of *Kester* relative to adjacent planted Nonpareil for the first eight years of McFarlane RVT production.

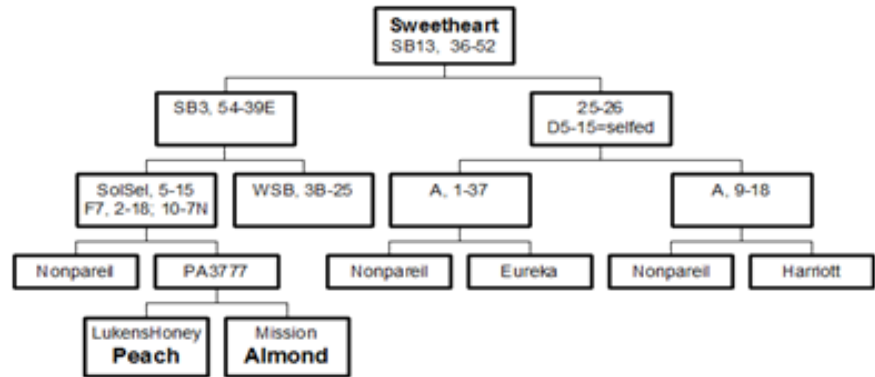
	Scab Rating	Alternaria rating	Hull Rot Strikes		
Nonpareil-DR	0.0 a	Nonpareil-J	0 a	Marcona	2.83 a
Nonpareil-7	0.0 a	Nonpareil-DR	0 a	Kahl	3.33 a
Nonpareil-J	0.0 a	Nonpareil-7	0 a	Chips	9.50 a b
Nonpareil-5	0.0 a	Nonpareil-Newell	0 a	Winters	16.83 a b
Nonpareil-6	0.0 a	Nonpareil-Nico	0 a	Nonpareil-Nico	21.00 a b c
Nonpareil-Newell	0.0 a	Nonpareil 3-8-2-70	0 a	Nonpareil-6	21.50 a b c
Kochi	0.0 a	Nonpareil-5	0 a	Sweetheart	22.00 a b c
Nonpareil 3-8-2-70	0.0 a	Nonpareil-6	0 a	Nonpareil 3-8-2-70	22.67 a b c
2-19E	0.0 a	Kochi	0 a	Nonpareil-5	23.17 a b c
Sweetheart	0.0 a	2-19E	0 a	Nonpareil-7	24.17 a b c
Nonpareil-Nico	0.0 a	Sweetheart	1 a	Nonpareil-J	28.17 a b c
Chips	0.0 a	Chips	1 b	Nonpareil-DR	30.33 a b c
Kahl	0.2 a b	Kahl	1 b	Nonpareil-Newell	33.33 b c
Marcona	0.3 b	Marcona	1 b	Kochi	51.00 c d
Winters	1.2 c	Winters	2 c	2-19E	68.67 d

**Figure 3.** Disease scores for 2013 season at McFarland RVT. 2-19E is *Kester*.

traits such as low productivity. As such, it represents not only an improved cultivar, but also a potential parent for further breeding progress, which combines the previously difficult combination of late flowering with high productivity of Nonpareil type kernels. Success of this process demonstrates the value of traditional breeding methods to effectively

manipulate/recombined even difficult and poorly understood

trait combinations. An inherent limitation in using only traditional germplasm is what essentially is just reshuffling the same traditional potential as well as vulnerabilities. **Figure 2** shows the yield per acre of *Kester* compared to adjacently planted Nonpareil (J or Jones clone) over the past eight years in the McFarland Regional Variety Trial. While individual year yields, as well as average yields, have been comparable, production was significantly lower in 2010 and 2013 (year 5 and 8) primarily due to greater losses from hull rot (**Figure 3**). Both flower blight and hull rot can be caused by *Monilinia* spp., suggesting the greater susceptibility to hull rot may be a legacy of the *Arbuckle* parent, but the pathogen subspecies are different (*fructicola* versus *laxa*) and *Kester* shows relatively high resistance to flower blight, (being comparable to Nonpareil). The greater susceptibility to hull rot appears to be a result of *Kester*'s more compact tree size, resulting in a more humid and so more disease vulnerable spur micro-environment.



**Figure 4.** Pedigree of the Sweetheart almond.

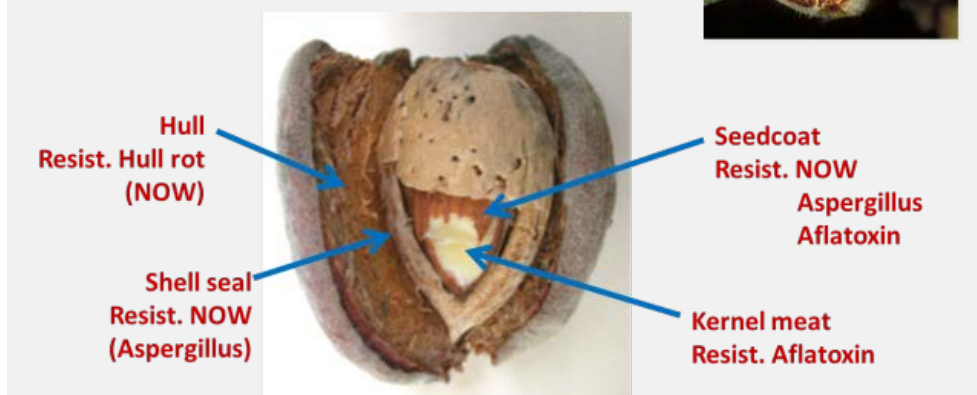
To overcome such limitations inherent in traditional germplasm, the UCD almond breeding program has established and advanced a parallel project for new trait introduction and introgression (i.e., advancing novel traits into a commercially adapted background). Targeted traits include self-compatibility, improve disease and pest resistance, and improved tolerance to climate change. While relatively simple traits such as self-compatibility can be transferred relatively quickly (i.e., decade), the complexity of most traits (such as consistent self-pollination to complement self-compatibility, or disease or pest resistance) requires longer periods of genetic recombination (as detailed in previous annual reports). In addition, changing cultural practices driven by losses in agrochemicals and water quantity/quality represent a constantly moving target. Given our lack of knowledge concerning the genetic control for many traditional and most introgressed traits, as well as the uncertainty as well as regional variability in predicting future yield determinants (water, disease, pests, climate-change?), A pragmatic solution is seen as the incorporation of a range of new genetic options in various combinations and subsequently to test performance in high risk grower-sites to identify the most promising materials. An early product of this longer effort is the recently released cultivar *Sweetheart*. As shown in **Figure 4**, the pedigree for *Sweetheart* is much more complex than *Kester* or traditional California cultivars as it involves a greater range of germplasm including the interspecific transfer of traits from the related species peach. Peach was initially included primarily as a source of self-compatibility and while *Sweetheart* shows degrees of self-compatibility, the level and year-to-year consistency are not sufficient for to be truly classified as self-compatible. (This finding also demonstrates that even 'simple' traits often prove to be not so simple when transferred through wide crosses). As with most early to mid-introgression breeding lines, *Sweetheart* kernels were intermediate between peach and almond and so generally undesirable (**Figure 5**). Kernels of *Sweetheart*, however, were very similar to the premium quality

Spanish variety Marcona, though with twice the crack-out ratio of Marcona-making them particularly desirable (**Table 1**).

In addition to a kernel appearance similar to Marcona (**Figure 5**), *Sweetheart* also possesses a very high oleic acid content (~70%) conferring a premium roasting and eating quality comparable to Marcona. In addition, *Sweetheart* shows high level of resistance to hull rot, navel orangeworm, as well as aflatoxin contamination (**Figure 5**). Thus, *Sweetheart* represents both the undesirable aspects of new

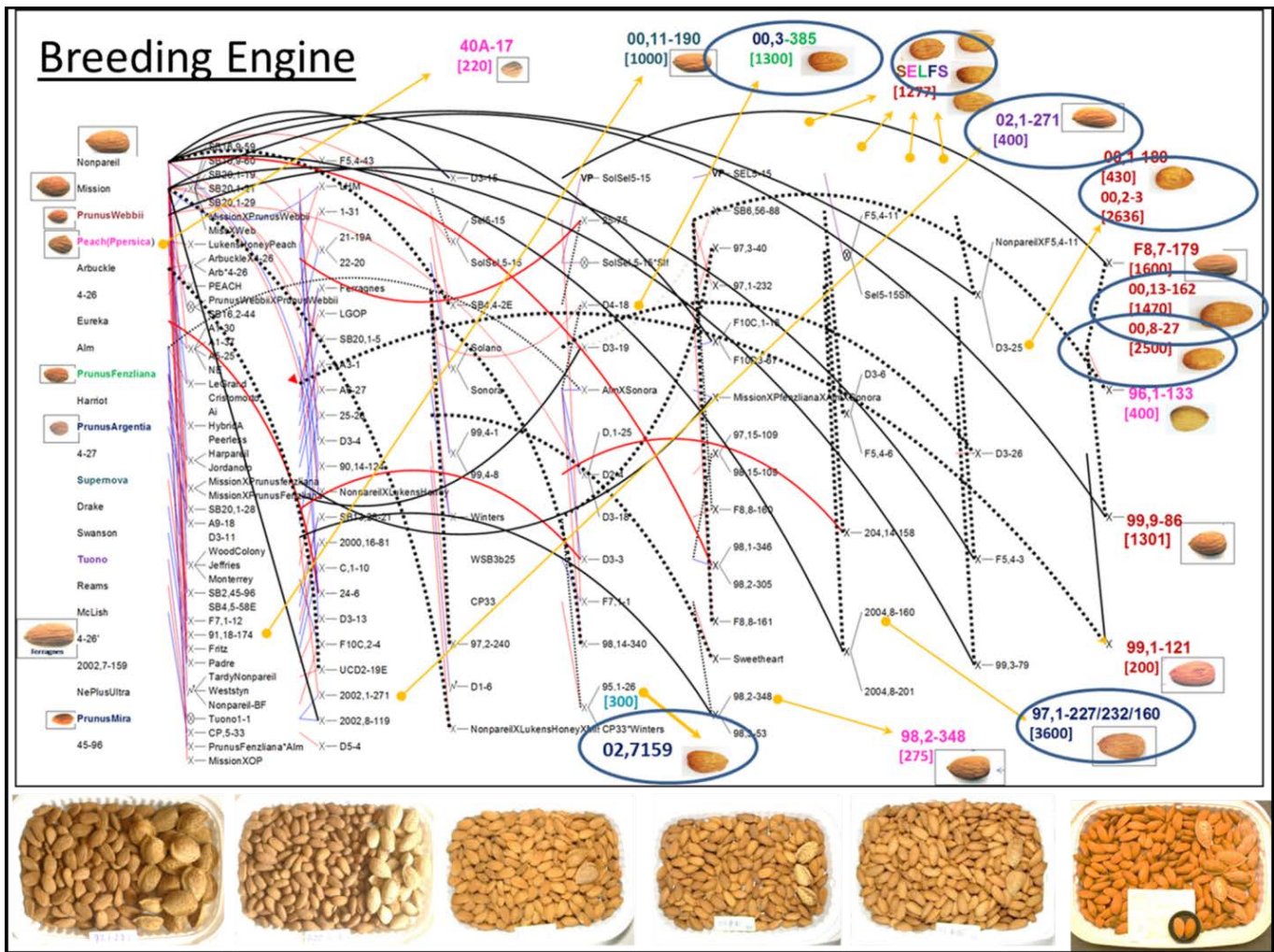
gene introgression (particularly the intermediate kernel characteristics between wild and commercial types) as well as the anticipated and often unanticipated desirable traits. Advanced UCD breeding pedigrees representing a broad diversity of genetic origins have been advanced to the level where kernel quality is indistinguishable from traditional California almonds (**Figure 6**). Many of these have also undergone limited regional grower testing to begin to rogue out important deficiencies (poor tree structure, noninfectious bud failure, etc.) and so represent the most promising candidates for the next generation RVT planted last spring, 2014. While kernel productivity and quality were important considerations for choosing the limited number of advanced UCD selections to be included in the new RVT, priority was also given to maintaining the maximum genetic (germplasm) diversity within the pooled selections, including the most promising materials for disease/pest/climate change resistance. The goal was to not only advance the most productive material, but also to advance material where productivity appeared based on tolerance to a wide range of biotic and abiotic stresses. The most promising selections would consequently have value both immediately as potential commercial cultivars and more broadly as novel yet adapted germplasm for continued public/private breeding. A more detailed description of UCD selections which have been included in the new RVT are presented in the following section.

Performance	Nonpareil	Sweetheart	Marcona	Heritable
Lipid (%)	38.8 a	43.4 b	42.6 b	No
Oleic Acid (%)	66.8 a	73.0 b	72.2 b	No
Self-compatibility (%)	3.1 a	28.7 b	3.8 a	Yes
Aflatoxin	83.7 a	18.1 b	-	No
NOW (%)	79.5 c	4.1 b	0 a	No
Hull Rot (%)	97.3 c	23.1 a	82.4 b	Partial



**Figure 5.** Integrated disease/pest resistance of the *Sweetheart* almond, many derived from its interspecies origin.





**Figure 6.** Summary of the breeding strategies for introducing new genes including self-compatibility and disease/pest/stress resistance, showing advanced selections at the bottom (circled items have been advanced to regional variety trial testing).

**Table 1.** Performance summary for breeding selections advancing to RVT trials (averages from three seasons).

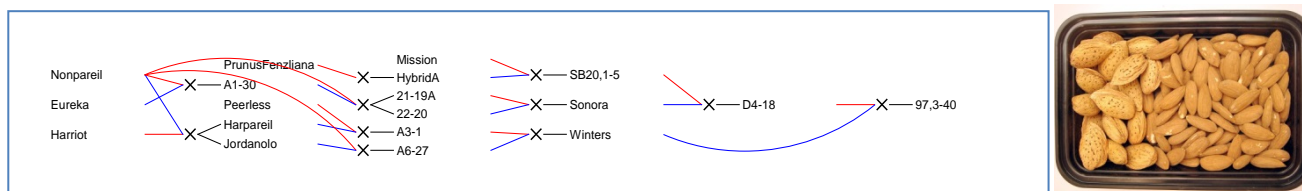
Selection	Bloom	Harvest	Origin	Kernel (g)	Shell-out (%)	Doubles (%)	Twins (%)	Crease (%)	Shrive (%)	Length (mm)	Width (mm)	Thick (mm)	Self-set (%)	Self-compat.
	relative to Nonpareil	relative to Nonpareil												
UCD3-40	-5	11	<i>P.fenzliana</i>	1.8	0.45	1	3	17	0	32	15	9.3	7	no
Sweetheart	-2	18	<i>P. persica</i>	1	0.51	0	1	7	0	20	13	8.7	12	part.
UCD-Marcona	-2	28	heirloom	1.5	0.24	0	0	11	6	26	17	8.3	6	no
UCD18-20	1	20	<i>P. persica</i>	1.2	0.6	0	0	11	0	29	13	7.6	11	part.
UCD1-16	3	12	<i>P.fenzliana</i>	1.2	0.68	3	2	6	1	26	11	8.5	7	no
UCD8-160	4	15	<i>P. mira</i>	1.9	0.65	0	2	20	0	30	15	8.5	96	yes
UCD8-27	4	12	<i>P. webbii</i>	1.1	0.51	10	2	2	6	24	12	8.4	92	yes
UCD1-271	5	14	Tuono	1.2	0.65	2	0	1	1	26	11	8.1	74	yes
UCD7-159	5	16	<i>P. webbii</i>	1.3	0.61	1	1	5	0	24	12	7.3	66	yes
UCD97,1-232	5	13	<i>P. mira</i>	1.3	0.66	0	1	0	0	24	12	7.3	61	yes
Kester	6	10	TardyNonp.	1	0.62	1	1	2	1	21	11	8.1	6	no
UCD8-201	7	18	<i>P. mira</i>	1.2	0.73	1	1	6	5	26	14	8.2	81	yes

**Candidates for new Regional Variety Trials (RVT).**

A summary of the basic characteristics of the UCD selections included in the new RVT are presented in **Table 1**. In addition to more traditional California almond germplasm, the selections capture a broader range of inter-species genetic diversity, including genes introgressed from peach (*P. persica*), *P. fenzliana*, *P. mira* and *P. webbii*. Most selections show high levels of self-compatibility. Most also show relatively large kernel size (Nonpareil kernels average approximately 1 g). As described in earlier annual reports, overcoming the small kernel size and quality characteristic of related species has been one of the greatest obstacles to the utilization of this important germplasm. (See also the discussions in the following sections for advances in developing molecular markers for larger fruit and kernel size). The large kernel size has advantages for marketing and, particularly for continued cultivar improvement (since a much larger proportion of breeding progeny would now inherit commercially-acceptable larger kernel sizes from continued backcrossing of this expanded germplasm to more traditional California-adapted material). Disease screening in advanced introgression lines occurs initially in unsprayed evaluation blocks at WEO and Jim Adaskaveg’s almond disease evaluation block in Davis California, with the more promising materials going to evaluation in grower blocks in high disease pressure areas of the Sacramento (Nickels) in southern San Joaquin (McFarland RVT plot) valleys.

**Summary of basic characteristics of UCD selections included in the new RVT trials.**

**UCD3-40**



UCD3-40 combines a large, high-quality kernel with good tree form and productivity. Regional testing in the Sacramento and lower San Joaquin valleys has also demonstrated good disease and pest resistance. The pedigree includes a complex parentage with a sizable contribution from *P. fenzliana*, which is often considered the species from which cultivated almond was derived. Parentage also includes Nonpareil and Jordanolo which have a history of noninfectious bud failure. Extensive and long-term testing of this selection has thus far been free from any indication of noninfectious bud failure risk. Bloom has consistently been just before Nonpareil, even in low chill years. The large, attractive kernels may also facilitate the development of new premium quality (Sonora, etc.) markets.

### Sweetheart and UCD Marcona

UCD selections targeting the ultra-premium (i.e., Spanish Marcona) roasted almond market. The recently released *Sweetheart* cultivar has performed well alongside standard Marcona at the McFarland RVT as well as long-term testing at the Nickels soils lab. Kernel size, shape and quality



have been comparable to Marcona, with mature tree yields surpassing Marcona. (In years of particularly high *Sweetheart* per tree production, individual kernel size may be reduced to below 1 g which would frustrate its inclusion in the international Marcona market. Crackout for *Sweetheart*, however, is over twice that of Marcona. [Detailed performance and quality data have been provided in earlier annual reports]. *Sweetheart* has also shown better disease resistance and production consistency than adjacently planted Marcona. Traditional sources of Marcona used in these propagations, however, were often virus infected which would inherently reduce yields. Standard Marconas did show very high yields in the early growing seasons and consistently produced medium to large high-quality kernels. Prunus Necrotic Ringspot Virus (PNRV) symptoms first became apparent in the McFarland RVT at about fifth leaf, becoming more extensive with season until tree yield was clearly affected. UCD Marcona is a UCD clonal selection which is genetically identical to the standard Marcona which has been selected to be virus free. Multiyear field (leaf symptoms on the tree and symptoms on buds propagated onto Shiro indicator plants) and molecular testing (ELISA and molecular markers) have documented a freedom from PNRV and other commercially important Prunus viruses and foundation stock now available to nurseries through FPS.

### UCD18-20

The seed parent of this selection is F10D5-11, a USDA item from the early Professor Kester/Dr. Jones UCD/USDA breeding collaboration which appeared to have high levels of self-compatibility. The pollen parent is the UC cultivar *Winters* which, while genetically self-incompatible, demonstrates relatively high background levels of self-compatibility (which unfortunately has not been consistent from year to year).



This selection has similarly shown moderate levels of self-compatibility in some years, but is more erratic in others and so is considered self-incompatible. Long-term trials at WEO in the Davis pathology block have demonstrated good productivity as well as good general disease and pest resistance.

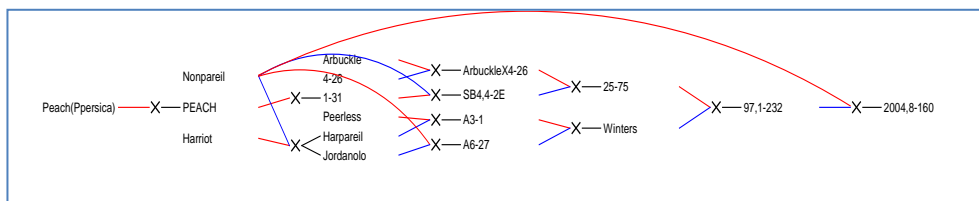


### UCD1-16

A second fenzliana lineage [Nonpareil X D3-19 {(Mission X *P. fenzliana*) X Solano}]. The selection is considered self-incompatible (*P. fenzliana* has not been a useful breeding source for self-compatibility). Both shell and kernel quality have been very good in long-term WEO and Nickels Soils Lab testing. Selections have also showed good general disease resistance in grower regional trials. Kernel pellicles also show a very desirable blonde-yellow color, comparable to Sonora. Trees are medium in size and productive.

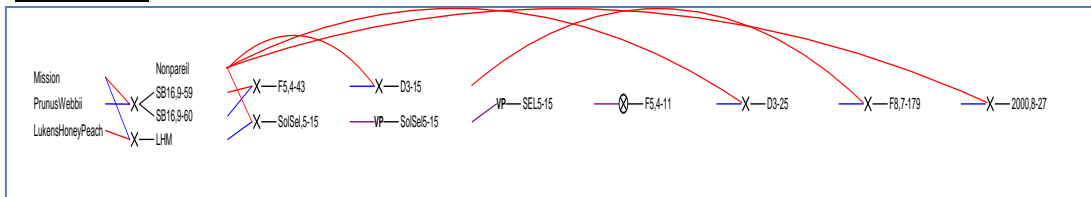


### UCD8-160



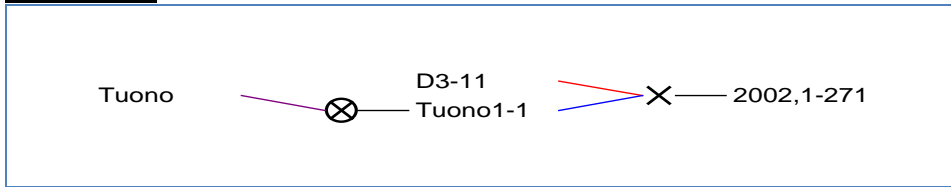
UCD8-160 has become one of the most promising of the new UCD breeding parents because of its combination of good kernel size and quality and consistently high self-compatibility. It is a progeny of UCD2-232 which is also included in the regional trials. Trees, while more compact than Nonpareil, are very productive and with production primarily on spurs. Multiyear testing at Nickels, WEO, and McFarland has demonstrated good general disease resistance despite the more compact structure of the trees.

### UCD8-27



This selection represents a complex pedigree combining traits from both peach and the wild almond, *Prunus webbii*. High levels of self-compatibility have also been recovered as have good tree architecture and uniform crop distribution, primarily on spur bearing wood. [Earlier research has shown that the control of self-compatibility from *P. webbii* is in the pistil of control from peach is in the pollen. By combining or pyramiding not only different genes but different mechanisms for self-compatibility, we are attempting to improve both maximum performance and year-two-year consistency.] The tree also exhibits improved levels of foliar disease resistance when compared to 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 paper shells give good crack out but have poorer seals (60%) though the worm infestation has not been a problem to date. Kernels show good-quality though double kernels (~10%) may be a concern.

## UCD1-271



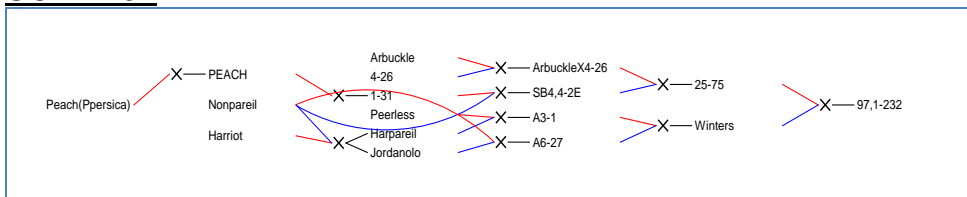
UCD1-271 utilizes the heirloom Italian cultivars Tuono as the source of self-compatibility. This source has been heavily utilized by the earlier USDA Fresno almond breeding program as well as most Spanish and Italian almond breeding programs. While the Italian Tuono almond is a very hard shelled, irregularly shaped kernel cultivar, its commercial almond background allows self-compatibility to be readily transferred to advanced breeding lines. Our experience has been that this breeding source also contributes poorer kernel quality, in particular significant kernel creasing in progeny. Most of the undesirable traits have been bred out in this selection while retaining good kernel quality and high self-compatibility. Current efforts are targeting the increasing year-to-year production/self-compatibility consistency through combining with other breeding sources.

## UCD7-159

This selection resulted from the cross of Nonpareil by 95,1-26 (USDA selection CP33 crossed with *Winters*) based on previous USDA data which indicated that 95,1-26 had a novel source of self-compatibility. Recent test crossing at UCD has shown only moderate levels of self-compatibility in the 95,1-26 parent. Relatively high levels of self-compatibility have been identified in UCD7-159 suggesting that the 95,1-26 may still be a useful and unique source of self-compatibility but that expression is masked in the parent by its particular genetic background. Both tree and kernel show promising quality with good yields and low disease, look trees have only been evaluated at WEO and Nickels plots.



## UCD1-232



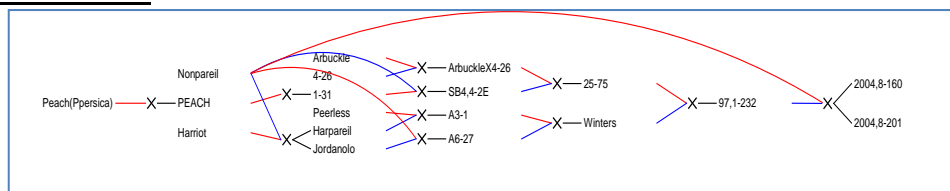
UCD1-232 has been one of our most effective parents for the transfer of self-compatibility as well as good disease resistance. It combines desirable traits from peach as well as a range of heirloom California cultivars. Kernel qualities are not as good as the other advanced RVT selections yet within the range of commercially important current California cultivars. Long-term testing has shown consistent levels of productivity, self-compatibility and disease resistance in this selection as well as in many breeding progeny using this selection as a parent. [In addition to assessing commercial value of these accessions, the new multisite RVTs will allow more detailed evaluations of susceptibility to different diseases in an array of environments. This full or data will allow better assessment of these items both as potential cultivar releases as well as parents for future crosses].

## Kester

This selection was one of the highest producing varieties when tested as UCD2-19E at the Kern RVT plot with an accumulated (1996-2005) yield of 26,112 pounds per acre following an exceptionally high crop of 4890 pounds per acre in 2003. UCD2-19E had also shown evidence of alternate bearing. In the recent McFarland RVT, we have been successful in maximizing year-to-year production by monitoring current season crop yield and providing increases in both irrigation water and fertilizer nutrients as needed. Under these conditions, UCD2-19E has yields and kernel quality comparable to Nonpareil (see data and discussion in earlier section). Low *Alternaria* blight disease levels have also been observed in all plots. Hull rot incidence is generally comparable to Nonpareil but yields were significantly lower than Nonpareil 2010 in 2013 due to hull rot flare-ups in high disease and high crop years. The more compact tree size relative to Nonpareil while increasing adjacent Nonpareil tree yield potential; also appear to increase the potential for hull rot when airflow and so treat relative humidity are impacted.



## UCD8-201



UCD8-201 is a sister line to UCD8-160, described previously as one of our most promising sources of self-compatibility and kernel quality. Although kernels of this selection do not show the uniform high quality of UCD8-160 (kernels are medium to large and somewhat flat), the tree is particularly productive with a desirable upright spreading structure. Nuts are well-sealed. Branches show high density of spur production and have shown no significant disease despite the consistently high crops.

## Generate new seedling progeny while reducing trees currently in breeding trials through low-input/high-throughput breeding strategies.

Over 12,000 breeding seed were recovered 2013-14 through a combination of hand-crosses to bagged (to prevent insect cross-pollination) branches and scaffolds, caging the entire trees of desired seed parents and using honeybees and bumblebees to transfer of pollen from desired male parents, and growing desired seed parents (typically advanced breeding selections) in isolation blocks with desired male parents and allowing natural honeybee cross-pollination to make the crosses. In caged tree and isolation block crosses, multiple pollen sources are commonly used, since cost-efficient and effective molecular markers have been developed to identify paternity in resultant seedlings. [Paternity testing would typically only be performed on advanced selections showing particular merit]. Over 6000 seedling progeny have been field transplanted thus far in 2014 following standard cold room stratification/greenhouse seedling establishment procedures (as described in previous reports) and following initial greenhouse selections for tree vigor and growth form. Because planting space in remaining 2014 UCD WEO evaluation plots is limited to only an additional ~3000 progeny seedling, and because we have a large number of seedlings from current crosses currently growing in greenhouses/shade houses for 2015 plantings, the decision was made to subject remaining 2014 seedlings more intensive disease screening. While still ongoing, it appears the more intensive screening will result in higher seedling loss and it is

currently anticipated that only about 1,200 seedlings will be field transplanted during the remainder of the summer. Progeny selection plots planted in 2005 through 2007 are scheduled to be removed this fall following final evaluation. Approximately 40% of progeny trees in remaining bearing selection plots have now been removed through a combination of physical cutting and Roundup application. An additional 10% of remaining bearing trees are expected to be rogued-out following fall evaluations. While the rapid rouging and so cycling of breeding populations is crucial to remain in budget, it also increases the risk that promising genotypes will be lost. This is because it typically takes four years for almond seedlings to come into production and an additional four years to accurately assess self-compatibility and initial disease resistance. The consequence is that by the end of 2013, competent assessment of seedling progeny extends only to progeny planted in 2005 (05 block) despite pressures for the early removal of these older blocks. Sequential, annual selections with propagations of the most promising materials have allowed a balancing act between these two priorities. A large numbers of propagation is also expensive and prone to plant loss during early greenhouse growth and later field transplantation.

**Table 2.** Performance summary of a sample of breeding selections promoted to advance testing.

Item	Sealed #/50	Hull g/50	Inshell g/50	Kernel g/50	Crack-out	Double (%)	Twin (%)	NOW (%)	Blank (%)	Broken (%)	Crease (%)	Gum (%)	Sl. Shr (%)	Sev. Shr (%)	Rupture (%)	Seedparent	Pollen parent	Origin
05,3-103	50	64.1	216.6	58.2	0.27	7	-	-	4	7	3	-	47	-	2	Ferragnes	A97,2-240	Frgs
05,3-55	49	80.5	175.5	63	0.36	15	-	-	1	6	4	-	42	1	-	Ferragnes	A97,2-240	Frgs
05,3-69	33	100.6	109.4	63.3	0.58	11	-	1	1	-	10	-	8	2	-	Ferragnes	A97,2-240	Frgs
05,3-99	50	101.8	256.1	86.1	0.34	-	-	-	3	8	3	-	33	3	-	Ferragnes	A97,2-240	Frgs
05,6-234	45	86.5	128.1	56.9	0.44	7	-	-	4	4	7	-	45	-	-	Winters	<b>A97,1-232</b>	M
07,2-292	46	82.1	139.9	60	0.43	6	1	2	1	1	11	-	42	8	1	Winters	A97,1-227	M
05,5-17	46	88.5	126	60.5	0.48	2	-	-	1	4	5	4	41	6	-	Winters	<b>A97,1-232</b>	M
05,5-11	24	74.2	121.4	63.5	0.52	1	1	-	1	5	4	-	49	-	-	Winters	<b>A97,1-232</b>	M
05,5-169	46	94.7	132.5	64.4	0.49	1	4	-	-	2	12	6	32	12	-	Winters	<b>A97,1-232</b>	M
04,17-210	27	90.1	136	69	0.51	-	1	3	-	4	-	-	49	-	-	NP	97A,1-232	M
06,4-27	50	127.8	287.5	65.6	0.23	-	1	-	2	14	7	-	24	2	-	NP	A96,1-133	P
06,3-330	48	123.2	179.8	73.5	0.41	19	1	-	-	2	9	-	30	5	-	NP	A96,1-133	P
06,5-160	47	99.5	136.9	76.2	0.56	5	2	2	2	-	16	-	30	8	-	Tardy NP	A96,1-133	P
06,3-238	37	195.2	155.3	79.3	0.51	2	4	2	1	-	9	6	32	11	-5	NP	A96,1-133	P
06,4-134	27	170.2	128.5	80.3	0.62	2	1	4	2	1	30	1	38	9	-	NP	A96,1-133	P
06,3-297	47	154.4	315.9	83.3	0.26	16	2	-	1	9	2	-	38	14	-	NP	A96,1-133	P
06,3-105	37	183.7	202.6	86.4	0.43	35	-	6	3	3	11	-	35	15	2	NP	A96,1-133	P
06,3-82	44	198.9	138.8	89.4	0.64	-	-	5	1	3	1	25	13	34	-	NP	A96,1-133	P
06,4-188	40	123.3	278.7	91.6	0.33	18	-	2	-	6	6	-	43	7	-	NP	A96,1-133	P
06,3-319	26	152	176.3	94.6	0.54	2	1	3	-	6	11	-	28	4	-2	NP	A96,1-133	P
06,3-121	48	189.1	278.9	100.4	0.36	3	3	-	-	8	23	4	37	13	-	NP	A96,1-133	P
05,8-317	40	88	132.4	55.7	0.42	5	-	-	-	6	3	-	24	-	-	Winters	F8,7-179	WP
05,9-100	44	79.6	127.8	58.4	0.46	6	1	-	-	-	-	-	49	1	2	Winters	F8,7-179	WP
05,8-402	34	74.4	114.8	60.5	0.53	6	-	2	1	-	2	-	47	2	-	Winters	F8,7-179	WP
05,9-281	42	73.4	100.9	64.3	0.64	5	1	-	2	4	4	3	32	6	-	Winters	F8,7-179	WP
05,9-249	29	87.2	163.7	68.9	0.42	31	-	-	-	6	1	-	48	2	-	Winters	F8,7-179	WP
06,2-60	5	79.6	134.8	75.6	0.56	1	1	1	-	-	7	-	48	2	-	NP	A00,1-180	WP
06,2-24	N/A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NP	A00,1-180	WP



Potential strategies to alleviate this dilemma have been discussed in previous reports and are still in initial testing.

A summary of the more promising seedling progeny is presented in **Tables 2** and **3** and in the following descriptions. Because of the 8 to 10 year initial evaluation period, most are progeny from early to mid-generation parents. (For example, while **Table 1** summarizes current breeding material with the highest potential for commercial success, the requirement for initial seedling growth and multiyear trait testing dictate that these progenies will typically be about 10 years old.

**Table 3.** Sampling of promising breeding selections from 2013 evaluations.

Selection	Seed Parent	Pollen Parent	Origin	Pedigree	
2005,10-270	Winters	F8,7-179	WP	D3-15 (NP*F5,4-43[W*W][SEL5-15SLF])) * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	
2005,2-108	Nonpareil	A98,2-348	P	D3-11 * F7,1-1 (Sel5-15[NP*LukensHoneyXMis]* WSB3b25)(Sel5-15[NP*LukensHoneyXMis]*	
2005,5-17	Winters	A97,1-232	M	25-75 [Arb * 4-26]*[SB4, 4-2E] * WINTERS	
2005,9-340	Winters	F8,7-179	WP	D3-15 (NP*F5,4-43[W*W][SEL5-15SLF])) * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	
2006,2-24	Nonpareil	A00,1-180 +A97,3-40	WP	D3-15 (NP*F5,4-43[W*W][SEL5-15SLF])) * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	
2006,2-60	Nonpareil	A00,1-180 +A97,3-40	WP	D3-15 (NP*F5,4-43[W*W][SEL5-15SLF])) * D3-25(NP*F5,4-11[W*W][SEL5-15SLF])	
2006,3-19	Winters	A00,8-27	WP	NP *F8,7-179	
2006,4-134	Nonpareil	A96,1-133	P	F7,2-9 (Sel5-15 slf) F2	
2006,5-134	Tardy NP	A96,1-133	P	F7,2-9 (Sel5-15 slf) F2	
2006,5-40	Nonpareil	A96,1-133	P	F7,2-9 (Sel5-15 slf) F2	
2006,5-46	Nonpareil	A96,1-133	P	F7,2-9 (Sel5-15 slf) F2	
2007,2-127	Winters	A97,1-227	M	25-75 [Arb * 4-26]*[SB4, 4-2E] * WINTERS	
2008,3-125	Nonpareil	A00,8-27	WP	NP *F8,7-179	

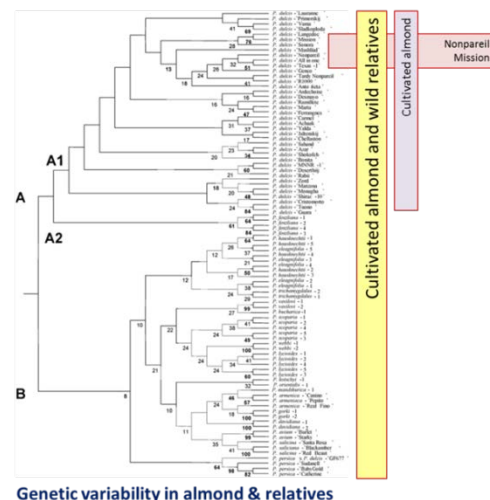
Similarly, while these elite materials are being extensively used in new breeding crosses, it will be approximately 10 years before we can identify the most promising of these progeny. However, while this long cycling time can be frustrating in any one lineage, the constant year-to-year advancement and improvement in multiple and diverse progeny lineages results in constantly improving selection quality and concurrently, constantly improving breeding parent quality which appears to be sufficient to maintain breeding program momentum.

Identify predictors of yield.

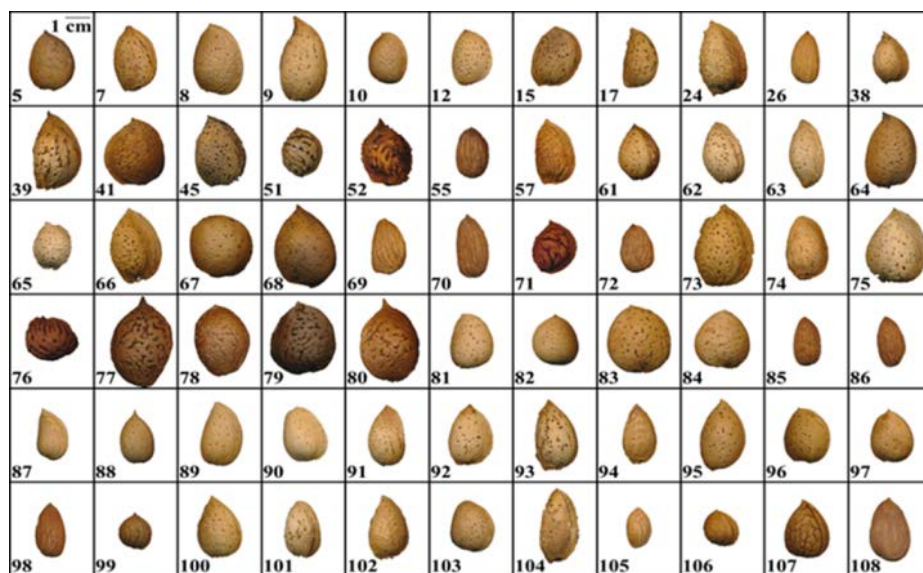
Basic yield determinants include the average size of the fruit/kernel and the number of fruit per tree (or more accurately, per unit orchard area). Final commercial yields will be determined by the average productivity over the lifespan of the orchard, highlighting the importance of consistently high yields rather than an occasional season of exceptionally high yield. In considering productivity over the lifespan to the orchard, a key variable is the expected orchard life.

Current orchard- life expectancies of approximately 20 years are not a consequence of an inherent limitation in almond tree productivity (trees in the Sacramento Valley have remained productive for over 100 years) but typically result when the number of trees lost in the orchard surpasses a threshold of economic returns. High tree loss in modern almond orchards is typically the consequence of disease and environmental damage to trees under maximum stress from the typically high water and nutrient applications to maximize yearly yields. Thus, it may be possible to increase final orchard yields through more judicious application of water and nutrients as this would reduce premature tree loss and extend commercial orchard life. Improved genetic

resistance to disease/pest/environmental stress would be augmented by lower inherent stress from lower input systems. Lower grower expenses for agrochemicals and water would also facilitate economic viability of such a system. Currently, most California almond cultivars are derived from a very narrow germplasm, typically Nonpareil by Mission crosses (**Figure 7**) with inherently limited environmental adaptability. Studies have shown a much greater potential for dealing with environmental stresses (heat, drought, low chill, etc.) in related germplasm, including both in related species but



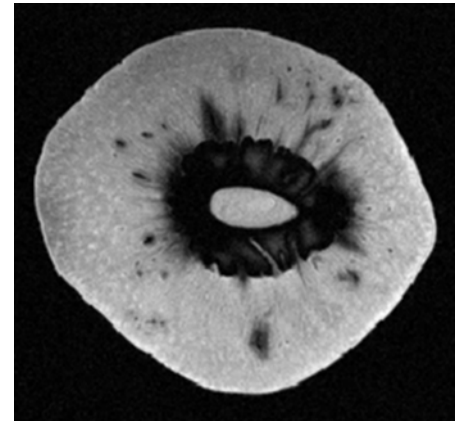
**Figure 7.** Characterization of genetic variability within almond and its close relatives. Red box estimates the amount of variability currently utilized within California varieties. Violet box estimates the amount of variability accessible within heirloom cultivars and land races. Yellow box estimates of genetic variability available within closely related species.



**Figure 8.** Representative endocarp shapes and sizes from germplasm plotted in **Figure 7**.

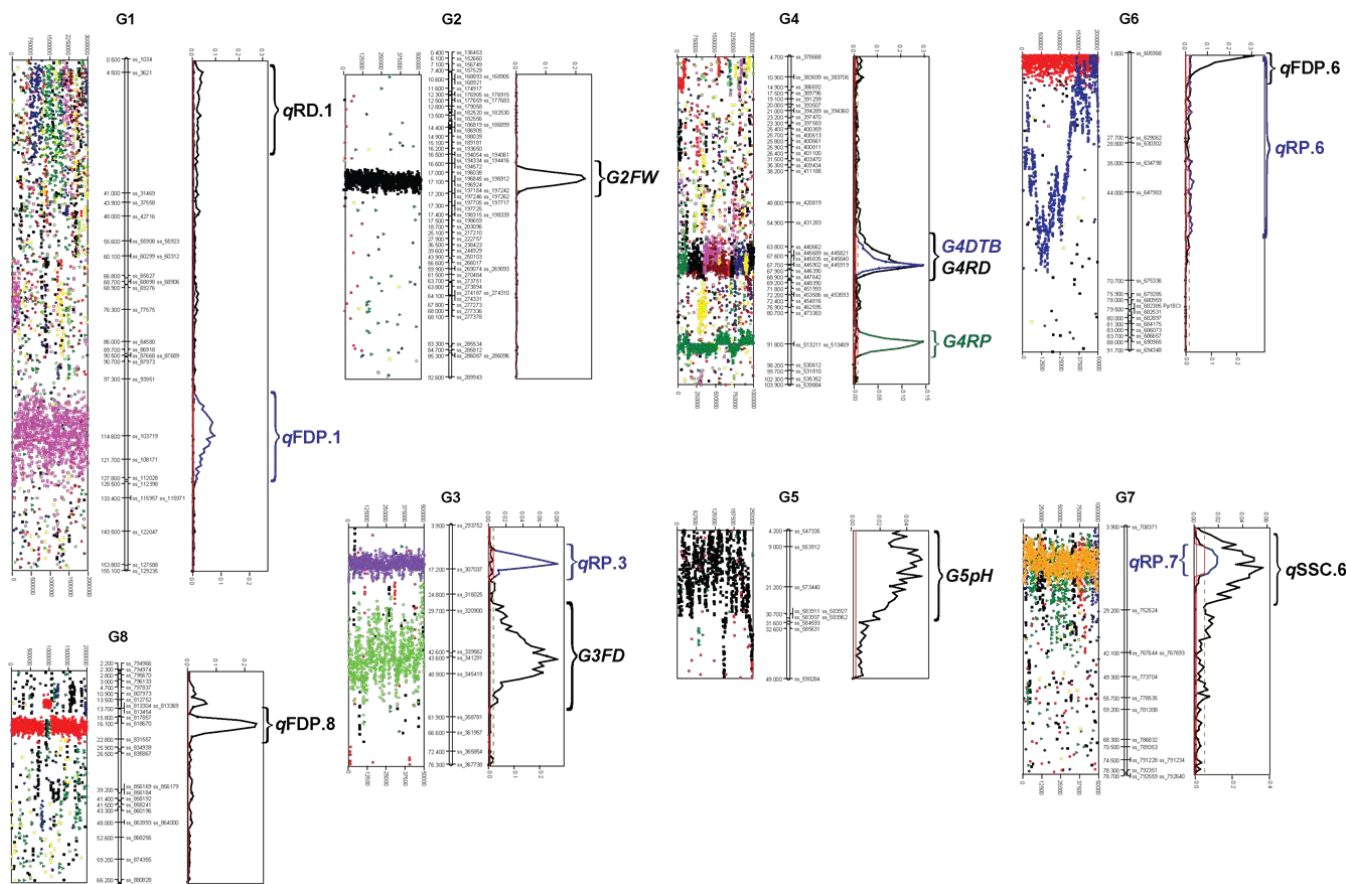
also commercial cultivars developed in areas of central Asia and the Mediterranean were such stresses are commonly encountered (See Reference, Gradziel T.M. & Martinez-Gomez, P. 2013). Current advanced selections, including those in the new RVT represent a much expanded germplasm and consequently greater opportunities for environmental resilience. We are currently working with Matthew Gilbert, UCD Plant Sciences, to better characterize the potential and limitations of this material.

Other areas that we are pursuing to increase productivity include tree size and structure and spur studies with Drs. Lampinen and DeJong. Other areas include disease/pest resistance studies with Drs. Adaskaveg, Bostock, and others), and increasing average kernel size. Previous work (reported in earlier annual reports) has shown that fruit and kernel size is typically controlled by a large number of genes quantitative in their inheritance, and that an average kernel size of just over 1 gram is associated with cultivars such as Nonpareil which give consistently high yields. [This may represent a trade-off between increasing kernel mass and increasing stress which could compromise continued bearing of the individual spur]. In addition, while fruit (hull), endocarp (shell) and kernel mass and three-dimensional structure are generally correlated within California cultivars, this relationship varies considerably when outside germplasm, including Asian and Mediterranean cultivars are examined (see **Figure 8**). Preliminary work by graduate student Shawn Overstreet indicates that endocarp structure may be a major determinant of both kernel and hull development, since the endocarp basically represents the preliminary vascular development required to feed these subsequent developing tissues. As part of his ongoing work, Shawn is utilizing both NMR and CAT scans as well as 3-D printing to better characterize endocarp structure (particularly vascular development) and later fruit and kernel development (see **Figure 9**).



**Figure 9.** Nuclear magnetic resonance (NMR) image of early fruit development showing progression of vascular strands within the endocarp and mesocarp.

Molecular markers are also being pursued for key determinants of crop yield, particularly fruit and kernel size. The RosBREED (USDA Specialty Crop Project) data has been particularly useful in this research since it includes almond as well as a broad range of species (primarily peach but also including introgression lines from *Prunus argentea*, *Prunus scoparia*, *Prunus webbii*, *Prunus mira*, and *Prunus davidiana*). Preliminary results document a quantitative inheritance for these traits but fail to identify potential markers for use in marker assisted breeding (summarized in previous reports). Re-analysis of RosBREED data using a Bayesian statistical approach which allows previous breeding experience to inform and improve marker detection has identified several promising marker sites for fruit size (FD) as well as time to flower (DTB) and maturity date (RD).



**Figure 10.** Identification of putative markers on the different *Prunus* chromosomes (G1-G8) for genes for fruit weight (FW) and fruit diameter (FD) as well as fruit development period (FDP) and ripe date (RP) based on a novel Bayesian analysis strategy.

These are summarized in **Figure 10** for each of the eight *Prunus* chromosomes showing a trace plot on the left and an intensity or probability plot on the right. We are currently comparing the predicted locations of important size controlling genes with breeding results from the different introgression lines. Such markers, if confirmed, could be useful for the early elimination of inferior seedlings through standard marker assisted selection approaches. Although this approach still remains relatively costly compared to field evaluations under our more mechanized management, and would also reduce our ability to identify important disease/pest/stress resistances. More effective use of these markers may be through marker assisted breeding strategies, similar to what we are already utilizing identify self-compatibility genes from related species. In both systems, the markers are used to better characterize potential crossing parents, to identify the specific genetics controlling the trait of interest, and to determined how complementary the paired parents from that crossing combination might be based on previous breeding data.



## Research Effort Recent Publications:

- Gradziel, T., B. Lampinen, F. Niederholzer, M. Viveros. 2013. 'Sweetheart' Almond: a fully cross-compatible pollenizer for the early 'Nonpareil' bloom that exhibits very high 'Marcona'-type kernel quality. *HortScience* 48:1320–1322.
- Martínez-García P., J. Fresnedo-Ramírez, D. Parfitt, T. Gradziel, C. Crisosto. 2013. Effect prediction of identified SNPs linked to fruit quality and chilling injury in peach [*Prunus persica* (L.) Batsch]. *Plant Molecular Biology*: 81:161–174. DOI 10.1007/s11103-012-9989-8.
- Martínez-García, P. J., D. E. Parfitt, E.A. Ogundiwin, J. Fass, H. M. Chan, R. Ahmad, S. Lurie, A. Dandekar, T. M. Gradziel, and C. H. Crisosto. 2013. High Density SNP Mapping and QTL analysis for fruit quality characteristics in peach (*Prunus persica* L.) *Tree Genetics and Genomes*. 9:19-36 DOI 10.1007/s11295-012-0522-7.
- Fresnedo-Ramírez J., P. Martínez-García, D. Parfitt, C. Crisosto, T. Gradziel. 2013. Heterogeneity in the entire genome for three genotypes of Peach [*Prunus persica* (L.) Batsch] as distinguished from sequence analysis of genomic variants. *BMC Genomics*. 2013 14:750. <http://www.biomedcentral.com/1471-2164/14/750>
- Martínez-García P., J. Fresnedo-Ramírez, D. Parfitt, T. Gradziel, C. Crisosto. (2013) Effect prediction of identified SNPs linked to fruit quality and chilling injury in peach [*Prunus persica* (L.) Batsch]. *Plant Molecular Biology*: 81:161–174. DOI 10.1007/s11103-012-9989-8.
- Rahemi, A., Fatahi, R., A. Ebadi, T. Taghavi, D. Hassani, T. Gradziel, K. Folta, J. Chaparro. 2013. Genetic diversity of some wild almonds and related *Prunus* species revealed by SSR and EST-SSR molecular markers. *Plant Systematics and Evolution*, 298: 173-192.
- Martinez Garcia, P.J.; Dan E. Parfitt; Richard M. Bostock; Jonathan Fresnedo-Ramirez; Alejandra Vazquez-Lobo; Ebenezer Ogundiwin; Thomas M. Gradziel; Carlos H. Crisosto. (2014). Application of Genomic and Quantitative Genetic Tools to Identify Candidate Resistance Genes for Brown Rot Resistance in Peach. *PLOS ONE*.
- Gradziel, T. M. & Martínez-Gómez, P. 2013, Almond Breeding. *Plant Breeding Reviews* 37:207-258.
- Font i Forcada, C; T. M. Gradziel; C. Y. Gogorcena; M. A. Moreno. 2014. Phenotypic diversity among local Spanish and foreign peach and nectarine [*Prunus persica* (L.) Batsch] accessions. *Euphytica* 197:261–277. DOI 10.1007/s10681-014-1065-9.
- Hanada, T., A. Watari, T. Kibe, H. Yamane, A. Wünsch, T. M. Gradziel, Y. Sasabe, H. Yaegaki, M. Yamaguchi and R. Tao. 2014. Two Novel Self-compatible S Haplotypes in Peach (*Prunus persica*). *J. Japan. Soc. Hort. Sci.* doi: 10.2503/jjshs1.CH-099.