California Cling Peach Advisory Board
2015 Annual Report

Project Titles:	Development of New Cling Peach Varieties
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Objectives:

a) Generate 3,000 new seedling progeny trees targeting replacements for the *Dixon-Andross* & *Halford-Corona* maturity periods as well as pre-*Loadel* maturity, the capacity for once-over harvest, fruit tolerant of mechanical harvest & transport, reduced requirements for pruning /thinning, and resistance to fruit brown-rot, mechanical bruising and inconsistent winter chilling.
b) Identify the most promising breeding lines as well as the most promising individuals within selected lines for grower testing and further crossing. Use these genotypes and their siblings for the new RosBreed analysis (i.e. provide appropriate tissue for molecular analysis and collect detailed tree and fruit data).
c) Utilize high through-put field selection methods including molecular marker based pedigree analysis, targeting high fruit quality and productivity to reduce 2012-15 field plantings by rouging inferior lines.

Introduction:

The needs for processing peach varieties differ from fresh market fruit since the lower returns for processed fruit require greater production efficiency and crop consistency over an expected orchard life of 20 years or more. Achieving these needs requires the identification and incorporation of new germplasm for increasing productivity and processing quality while reducing grower inputs, as well as long-term regional testing to identify deficiencies prior to large-scale commercial plantings. Industry priorities for new varieties have included replacement of problem varieties particularly in the *Dixon-Andross* and *Halford* seasons, harvest season extension, and novel varieties which allow reduced grower inputs, including resistance to fruit brown rot. Because the traditional germplasm available to the early breeding program did not possess the required genetic material to meet these goals, a major effort of the subsequent breeding process has been the introduction and thorough incorporation of promising genetic material from both peach and its related species, while concurrently completing a thorough regional testing of more traditional selections in the early breeding pipeline. The UCD variety development program has since become an international leader in the identification, testing, and incorporation of new germplasm into commercial varieties as part of our efforts to meet California's rapidly changing needs. We have also been able to leverage the resulting large and

diverse breeding populations to successfully obtain outside funding (USDA, SCRI, etc.) for the more costly and technically demanding analysis required to develop molecular markers for important traits. (Molecular markers for a trait such as fruit brown rot resistance offer improved breeding efficiency since one could select the resistance genes directly rather than the more difficult and costly indirect field screenings, as detailed in earlier reports). While many tree crop breeding programs have solicited industry funding for molecular marker research, our policy has been to apply all industry funds to develop large-scale, field-based variety development programs and to then leverage these established and genetically diverse breeding populations to solicit outside funding for the more demanding molecular studies. In the recent 2009-12 SCRI multi-state RosBreed (www.rosbreed.org) project for developing molecular markers (i.e genotyping) to improve fruit crop breeding efficiency, the largest proportion of peach selections analyzed were from our processing peach program (350 selections) since we had the required large population sizes possessing extensive genetic diversity already established. The subsequent 4-year RosBreed-2 molecular-marker development project is now underway in which the number of UCD peach selections to be molecularly genotyped has been increased to over 1,000 individuals. We have identified the most promising targets for molecular analysis within established populations from 2010 and 2011 plantings since 2013-15 field data show these populations to be segregating for crucial commercial traits including disease resistance, fruit quality and harvest efficiency (once-over-harvest, etc.). However, continued evaluation/maintenance of 2010 and 2011 plantings (originally scheduled for full removal in 2015) will require reductions in newer field plantings in order to stay within breeding budget. The more comprehensive assessment of the large and diverse breeding populations in the 2010 and 2011 plantings, however, also provide an opportunity to investigate potential genetic options for emerging production needs such as greater water use efficiency, reduced winter chill requirements, capacity for mechanical harvesting, and resistance to potentially forthcoming pests and diseases.

Progress Summary- 2015

Over 8,000 seed from controlled hybridizations and subsequent cycles of self-pollinations were generated in 2015, with over 3,000 subsequent seedlings planted to Davis and Winters field evaluation plots (Table 1). As in 2014, conditions for controlled-crosses were difficult because low winter chill caused a more extended bloom time for many peach selections resulting in fewer flowers available controlled hybridization at any particular day. Resulting fruit set was also lower than normal, possibly due to the warmer weather at bloom. To compensate for these conditions, over 20,000 controlled hybridizations were made targeting replacements for the *Dixon-Andross* and *Halford-Corona* maturity periods as well as pre-*Loadel* maturity. Long-term climate predictions suggesting a continuing and perhaps accelerating loss of winter chill/fog conditions have led to a renewed emphasis on identifying selections tolerant to winter chill vagaries, as well as the development of hybridization methods allowing higher fruit-sets under these more challenging environments.

Good fruit quality was achieved for both seedling and advanced selection evaluation blocks despite the very high number of seedlings (~30,000) currently in full production. Key to the consistent recovery of good fruit quality was the implementation of previously developed field practices such as properly applied mechanical tree hedging and flower thinning to facilitate uniform cropping with low labor inputs.

All trees in 2010, 2011 and 2012 seedling blocks were evaluated in the summer of 2015 with approximately 300 selections advanced to processing evaluation at the UCD Mondavi Pilot Plant, and with approximately 400 selections similarly advanced to brown rot disease and fruit quality testing at the labs of Rick Bostock and Carlos Crisosto, respectively. Evaluations focused on identifying promising individuals for the next round of regional testing as well as individuals for the next round of controlled hybridizations.

Using both phenotype-based as well as genotype-based predictors of tree performance, approximately 30% of the seedlings and the 2012 and 2013 seedling evaluation blocks were eliminated in 2015 and the space used for new plantings. Similarly, approximately 50% of the 2015 seedlings have been rogued out prior to field planting using similar selection criteria.

In addition to standard tree productivity and fruit quality characteristics, attention is being given to more unique traits such as improved resilience to warming climates, capacity for once-over, including mechanical harvest, and improved phytonutrient quality of processed product. A consequence of previous UCD breeding efforts to incorporate new and sometimes exotic germplasm possessing desired traits into California adapted breeding lines with good fruit and tree quality, is the availability of an exceptional level genetic diversity in current evaluation blocks, particularly the large 2010 and 2011 plantings. A goal of current selection cycles is the maintenance of as large a genetic diversity as possible within breeding material to maintain the flexibility to address emerging and often unanticipated production/processing problems. At the same time we are working to identify the most promising individuals meeting current production/processing requirements for advancement to regional testing. This is possible because the highly mechanized nature of our current breeding plots allows most trees in the plot to be maintained throughout the selection phase rather than being continually weeded out as they are found inferior. This maintains the population structure needed to identify potentially new traits over multi-year evaluations of this diverse germplasm.

Table 1. Targeted versus actual field plantings by the UCD processing peach breeding program.

Year	Target	Field planting
2008	8,000	9,061
2009	8,000	12,038
2010	6,000	11,637
2011	5,000	8,211
2012	5,000	5,141
2013	5,000	2,073
2014	9,000	3,846
2015	3,000	3,211

The Variety Development or breeding program is thus seen as the engine to generate the genetic diversity to meet current and emerging industry challenges, to consolidate desired genes into trees highly adapted to California production conditions, and to select the best candidates for advancing to regional testing. Subsequently, the objectives of the Regional Testing Program are to thoroughly evaluate these advanced selections in regional UCD and grower plots, including processing evaluations of promising selections and established standards, and to fulfill all phytosanitary, legal, regulatory, and documentation requirements necessary for the release of new varieties to the California industry.

The following sections present recent progress in this characterization, capture and candidate selection, as part of the ongoing breeding effort. Much of this research has been or is in the process of being published in leading peer-reviewed journals, which are reference within the text to allow access to more detailed information concerning materials, methods and results.

Progress Report- 2015

Genetic Diversity.

A fundamental need for continued breeding progress is an accurate characterization of the level of genetic diversity currently available in our advanced breeding generations (i.e. currently showing good tree and fruit quality and adaptability to California growing conditions). Molecular genetic analysis, using data from RosBreed-1 and separate UCD molecular studies provides the most accurate characterization of genetic variability (citations 9,14,15). Germplasm potential for variety improvement has also been analyzed as part of a joint UCD-Spanish evaluation of peach trait diversity (2). A summary for current UCD breeding lineages is presented below based on early results.

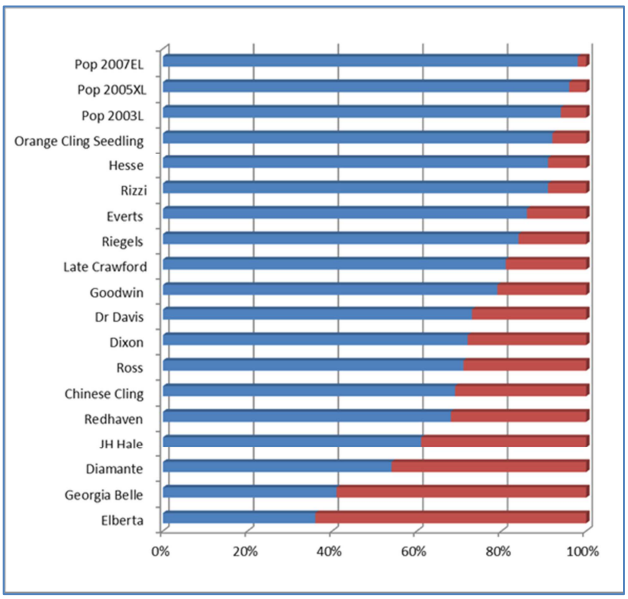

Figure 1. Genetic variability of the more traditional (peach by peach) peach parents/lineages utilized in the early breeding program. (Blue bars show the percent homozygosity (i.e. uniformity) for genetic loci over the range of the peach genome.

Figure 1 shows the percent homozygosity across the genome for a sampling of more traditional parents and breeding lineages (i.e. origins utilized in the early part of the UCD breeding program). Included for reference is *Chinese Cling* which appears to be the principal genetic source from which to most clingstone processing peaches were developed in the US, as well as *Redhaven, JH Hale Georgia Belle* and *Elberta* which were peach introductions which were major contributors to the establishment of both the clingstone and freestone industries in North America. Homozygosity exists at trait loci when both possible alleles are identical. The trait then becomes fixed with further selfing or with crossing to similarly homozygous parents. This restricted genetic variability inherently limits the amount and kind of trait or phenotypic variability that can be achieved in the progeny. While this inbreeding or loss of genetic potential was apparent in the reduced variability and reduced productivity observed in the early breeding progeny (see earlier annual reports), the recent availability of precise molecular markers has allowed an accurate characterization of the percent homozygosity in different varieties and even within their individual chromosomes. The varieties *Hesse* and *Rizzi*, which were released in the 1990s, represent good examples of this inbreeding (Fig. 1). Since homozygosity would be further increased in their progeny (Pop-2003, 2005 and 2007 in Figure 1), they were often uniformly lackluster. Again, this was because the very high levels of homozygosity did not allow much genetic and so trait recombination to occur. Progeny would generally be inferior to the parents as well, since key traits such as fruit size and productivity are invigorated by higher levels of genetic variability.

In response to this observed inbreeding depression in early UCD breeding lines, an extensive new and diverse germplasm was introduced into the breeding program during the late 1990s and early 2000s using promising peach selections from other processing peach growing areas including Europe, Mexico, Brazil and South Africa, as well as from closely related peach species including *Prunus mira, P davidiana, P scoparia, P. argentea, P tangutica,* and cultivated almond, *Prunus dulcis*. By repeated backcrossing to selected peach parents, useful traits including disease resistance, improved productivity, mechanical and once-over harvest ability, and improved tolerance to low-chill winters have been recovered in advanced breeding lineages (i.e. possessing good adaptation to California climates and cultural practices). A recent characterization of the level of genetic diversity within all major US public peach breeding programs (Clemson, Arkansas, Texas A&M, and UCD) shows that despite our more limited focus on processing clingstone types, the level of genetic diversity developed at UCD is 2 to 3 times greater than the combined diversity of all other (primarily freestone) breeding programs (Figure 2). In these studies (see citations 9,14,15),

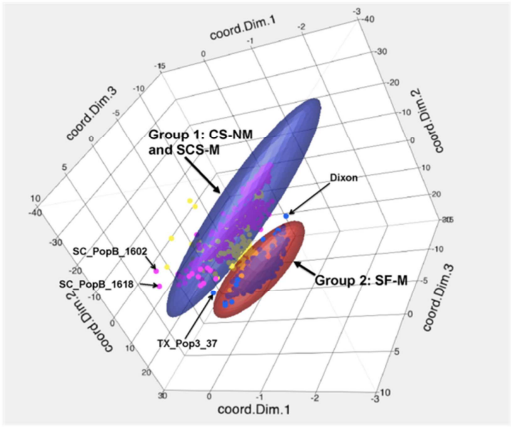

Figure 2. Genetic Structure and extent of genetic diversity of breeding pedigrees analyzed in RosBreed 1 from major public breeding programs at Clemson, Arkansas, Texas A&M, (Group 2-red oval) and UCD (Group 1-blue oval).

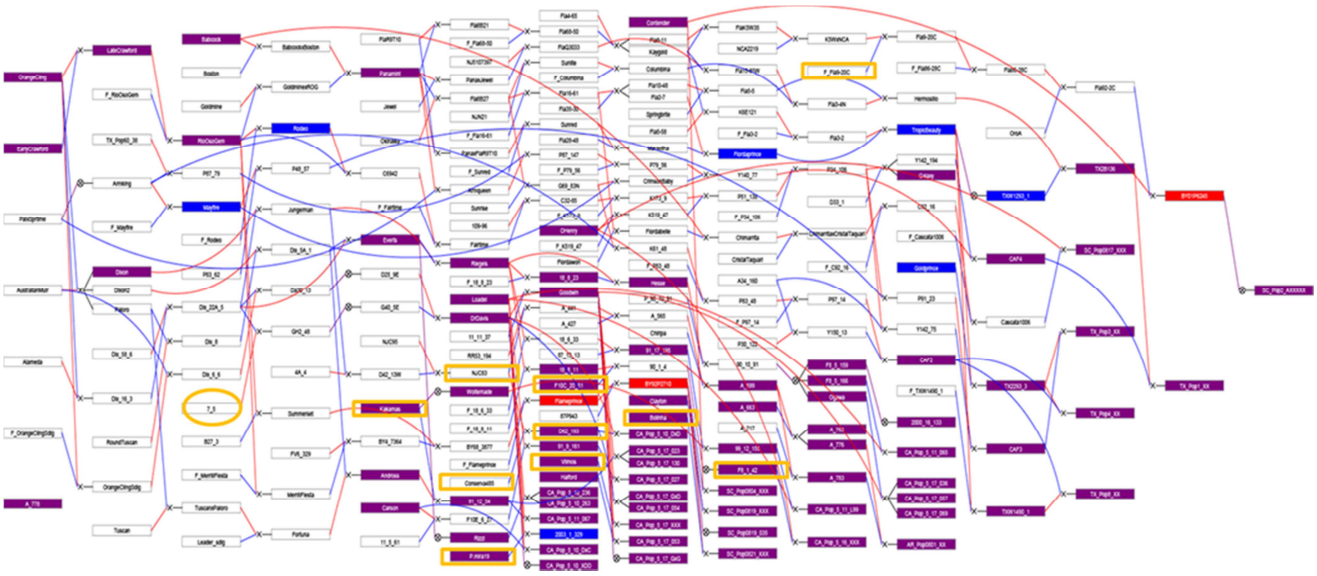

Figure 3. Pedigree depicting the offspring from the '*Orange Cling'* peach as utilized by major US fresh market and processing breeding programs. In purple, genotypes with phenotypic and genotypic information available; in blue, genotypes with genotypic information only; in red, genotypes with genotypic information only. Gold highlighted boxes identify infusions with germplasm from Brazil and South Africa as well as almond and the wild peach *P mira*, which are unique to the UCD breeding lines.

diversity refers to not just genetic variability at different loci, but variability originating from genetically and so phenotypically diverse sources. [This extended diversity, and the large and advanced breeding populations that we have developed from it, have been the main reason for our success procuring outside funds for molecular studies]. Figure 3 shows an example of this backcrossing or introgression process where core heirloom germplasm (*Orange Cling*) has been widely utilized by all major public breeding programs (Clemson, Arkansas, Texas, and UCD) but has been extensively augmented with outside germplasm only at UCD. [Interestingly, the gold highlighted oval identifies a novel plant accession identified in records only as P.I.7-5 which was introduced by LD Davis into the UCD germplasm, eventually resulting in the release of the very successful varieties *Dr. Davis* and *Ross*]. Because of its exotic origin, germplasm introgression from related species can have particularly large affects on increasing genetic diversity. In the genetic analysis summarized in Table 2, inclusion of almond derived breeding line F8, 1-42 (highlighted in bottom right quadrant of Figure 3) increases the traditional peach cultivar diversity (represented by the variety *Lovell*) by approximately 4-fold, despite this accession having very good peach fruit and tree characteristics possessing only about 10% remnant almond genome. (In contrast, *Dr.Davis*, even as a carrier for the introduced diversity from PI 7-5 increases the diversity approximately 1.7 fold).

Table 2. Genome conservation matrix among the three genotypes and the peach genome genetic sequence showing a 4-fold increase in genetic diversity though incorporating almond derived breeding line F8,1-42.

	'Lovell'	**'Georgia Belle'**	**'Dr. Davis'**	**'F8,1-42'**
'Lovell'	0	0.0264	0.0167	0.0430
'Georgia Belle'	-	0	0.0268	0.0429
'Dr. Davis'	-	-	0	0.0405
'F8,1-42'	-	-	-	0

Despite their exotic and often wild origins, continued introgression through multiple backcrosses have developed advanced breeding selections within these diverse lineages with good fruit and tree qualities as documented in the following sections as well as the images in Figures 7 and 8.

Phenotypic or Trait Diversity.

Results from a recently published joint Spanish-UCD study examining the extent of trait diversity within all Spanish breeding germplasm (including heirloom material and ancient land races) as well as traditional California clingstone varieties (including *Andross, Carolyn, Carson, Dixon, Everts, Halford, Klampt, Loadel, Lovell, Starn* and *Wiser*) are presented in Table 3. Data were collected for 4 consecutive years from trees entering full production in an experimental orchard in southern Spain having a production climate comparable to the central Valley of California (see citation 2). While the germplasm evaluated in this study was selected to represent the extent of diversity available to breeding programs, similar data developed from the RosBreed-1 project demonstrates that current expanded UCD germplasm exceeds even this diversity for key horticultural traits, (including fruit weight, soluble solids, firmness, titratable acidity, and a*- flesh color). [Traditional California varieties typically performed within the upper 25% performance for the traits evaluated in Spain, with *Andross, Klampt* and *Wiser*

Table 3. Minimum, maximum and mean values for the traits evaluated, and ANOVA analysis of the effect of 94 Spanish and UCD peach accessions for the average of all years of study

					Source of variation[1]		
Trait	Units/Description	Minimum	Maximum	Mean ± SE	Cultivar (C)	Year (Y)	Y x C
Bloom beginning	Julian days	72	83	78 ± 0.19	ns	ns	ns
Full Bloom	Julian days	79	87	82 ± 0.15	ns	ns	ns
Harvest date	Julian days	185	275	224 ± 2.5	ns	ns	ns
TCSA	cm^2	44	280	92 ± 3.9	ns	ns	ns
Yield	Kg/tree	1.0	46.5	13.4 ± 1.9	ns	ns	ns
Yield efficiency	Kg/cm^2	0.11	1.31	0.30 ± 0.02	ns	ns	ns
Fruit weight (FW)	Grams	64	315	178 ± 2.8	ns	ns	ns
Soluble Solids Content (SSC)	ºBrix	12	18	15 ± 0.13	***	ns	ns
Flesh firmness (FF)	Newtons	9	61	38 ± 0.9	ns	ns	ns
Titratable acidity (TA)	g malic acid/100 g FW	0.4	0.9	0.6 ± 0.01	***	ns	ns
Ripening index (RI)	SSC/TA	15	67	25 ± 0.43	***	ns	ns
L*	Lightness	10.6	76.8	61.9 ± 9.0	ns	ns	ns
a*	Greenness/redness	-1.18	60.8	22.4 ± 5.2	ns	ns	ns
b*	Blueness/yellowness	8.9	69.1	52.0 ± 11.5	ns	ns	ns
C*	Chroma	25.3	80.6	58.9 ± 9.1	ns	ns	ns
h*	Lightness's angle	16.9	91.4	62.7 ± 14.0	ns	ns	ns
Sucrose	g/kg FW	35	98	75 ± 0.9	ns	ns	ns
Glucose	g/kg FW	4	15	10 ± 0.19	*	ns	ns
Fructose	g/kg FW	2	14	11 ± 0.18	***	ns	ns
Sorbitol	g/kg FW	2	35	13 ± 0.76	***	ns	ns
Total sugars (TS)	g/kg FW	63	136	110 ± 1.35	ns	ns	ns
Vitamin C	mg AsA/100 g FW	3	28	13 ± 0.41	ns	ns	ns
Total phenolics	mg GAE/100 g FW	18	62	44 ± 0.65	ns	ns	ns
Flavonoids	mg CE/100 g FW	3	63	24 ± 1.49	ns	ns	ns
Anthocyanins	mg C3GE/kg FW	0.7	12	2.5 ± 0.21	ns	ns	ns
Relative Antioxidant Capacity (RAC)	mg TE/g FW	186	1184	840 ± 19.0	*	ns	ns

AsA ascorbic acid, *GAE* gallic acid equivalents, *CE* catechin equivalents, *C3GE* cyanidin-3-glucoside equivalents, *TE* trolox equivalents
[1]Data were evaluated by two-way variance (ANOVA); ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns, not significant

Table 4. Variability in total phenols and polyphenol oxidase (PPO) as well as tissue browning potential (DL* or change in L*flesh lightness with time) for selected California processing peach varieties and advanced selections.

Cultivar	DL*	Total phenols	PPO
		(mg/l)	(unit/µl)
Late#4	44.2 ± 4.6	21.1±0.7	15.0±1.9
Kader	43.9 ± 0.9	3.0±0.2	22.6±0.9
Late Ross	43.1 ± 0.8	8.7±0.7	13.2±0.7
Ross	40.4 ± 0.5	8.0±0.3	22.9±1.4
97-7-79	31.9 ± 0.9	13.6±0.4	11.3±0.7
99-09-231	25.3 ± 4.1	11.5±0.2	18.4±1.6
Dr.Davis	23.8 ±2.8	7.0±0.2	48.5±2.2
Halford	19.3 ±2.7	11.6±0.6	30.5±4.4
92-11-57	15.5 ±2.5	10.7±1.1	25.8±2.3
Evert	10.9 ±0.1	14.1±3.0	30.2±0.4
Riegels	8.9± 1.2	9.3±1.9	22.8±9.7
Lilleland	8.8±4.3	5.6±0.9	7.0±1.6
Andross	2.6±0.9	9.5±0.5	44.4±3.7

performing in the upper 15% for key traits such as fruit size]. Similarly, recent collaborative work with Dr. Chukwan Techakanon in Diane Barrett's Food Science Laboratory at UCD has also documented extensive variability for both total phenols and polyphenol oxidase (PPO) within advanced UCD germplasm (Table 4). High levels of total phenols are desirable from a nutritional aspect as they are a potent antioxidant. In our breeding material, they are also associated with improved resistance to fruit brown rot (see previous annual reports). Traits desirable for one objective, such as nutrition or disease resistance, may prove undesirable for other aspects of fruit quality. In this case the higher phenol content is associated with higher levels of fruit flesh-browning with time (DL* or change in the tissue lightness color value) and previous work has shown it to be associated with higher susceptibility to fruit bruising at harvest. [Upon mechanical injury and/or exposure to air the colorless phenols will bind together to form quinone chemical-chains which confer the brown color to bruising but whose resinous qualities

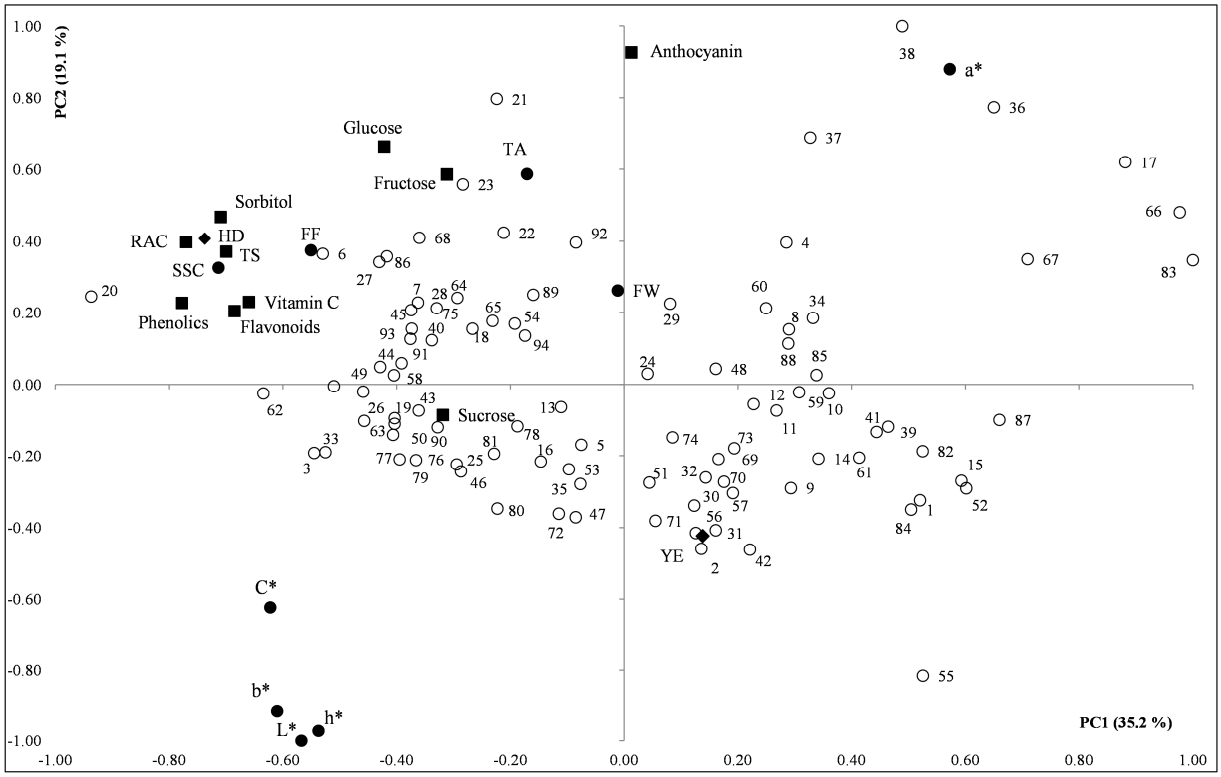

Fig. 4. Principal components analysis axes of the agronomic, basic fruit quality traits, sugars and phytochemical compounds evaluated on 94 peach accessions. Symbols for the different quality traits are: (♦) agronomical traits, (●) basic fruit quality traits, (▲) sugars and (■) phytochemical compounds. Numbers refer to individual peach accessions. [citation 2].

also contribute-to wound-healing and so disease resistance.] The large data set developed in the Spain-UCD peach diversity study allows a more comprehensive analysis of individual traits relationship to final quality using principal component analysis (Figure 4). The concentration of most desirable traits in the upper left quadrant of the analysis suggest that breeding for higher

quality in one trait positively influences desirable levels of other traits. This is particularly true when working with more exotic germplasm since aspects of fruit quality (size, sweetness, texture, etc.) tend to increase together and not be in conflict when introgressed to a greater peach genetic background. The exceptions identified in Figure 4 relate primarily to color (black dots) since these are relative scales of perceived color rather than ratings based on increasing merit. Anthocyanin is also in this group since, as with the color scale for (a*), increasing levels confer a redder color to the flesh which is generally horticulturally undesirable.

Trait Recovery

Fruit quality.

Field evaluations in 2015 targeted general tree and fruit quality in the 2010 in 2011 seedling blocks with an emphasis on fruit brown rot resistance, fruit size and quality, and the long-keeper trait (i.e. ability to maintain fruit quality on the trees for one to 2 weeks after initial ripening) as part of our RosBreed-2 initiative. Over 600 genetically diverse selections were evaluated for fruit

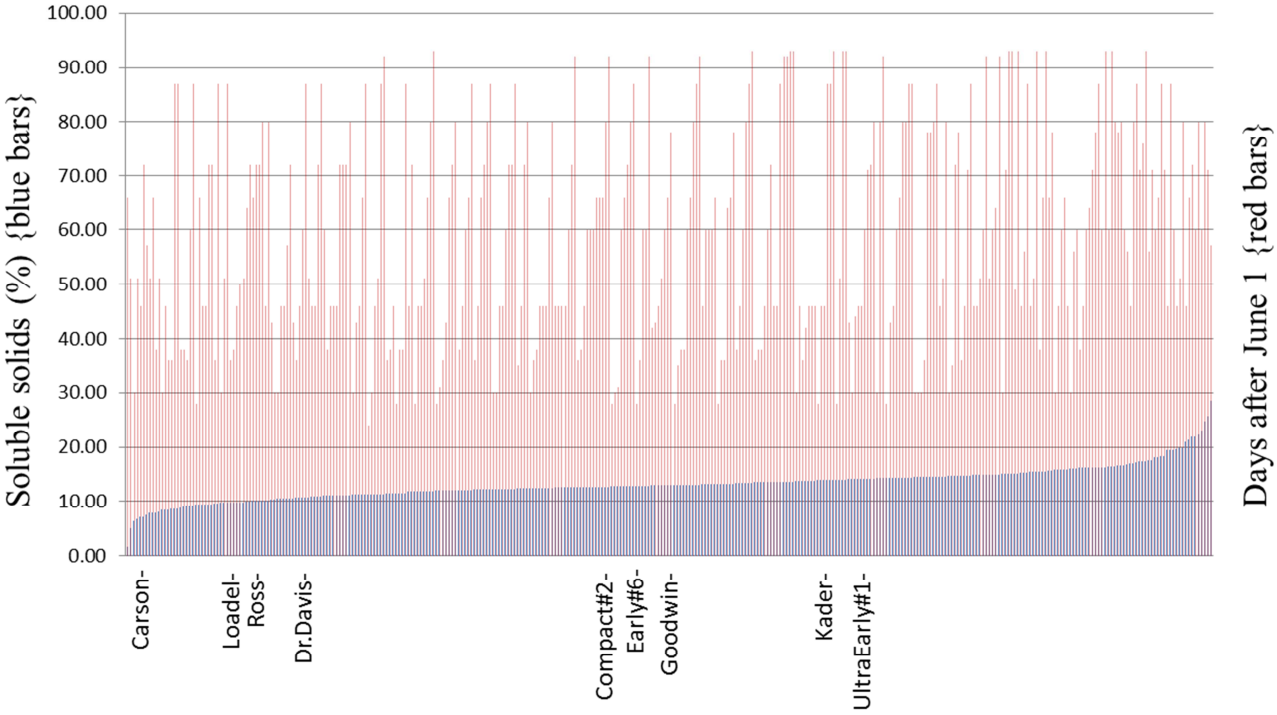

Figure 5. Distribution for fruit soluble solids (% -blue bars) and maturity time (given in red bars as days after June 1) for 371 progeny from RosBreed-2 populations. Data for standard cultivars and selected advance selections are plotted for reference.

processing quality and/or brown rot resistance. Genetic diversity was maintained by including breeding lines derived from Brazilian, Mexican, European and South African germplasm and the related species *Prunus dulcis* (almond), *Prunus scoparia* and *Prunus argentea* (wild almond species), and *Prunus mira* and *Prunus davidiana* (wild peach species). All tested selections were

from advanced breeding lines showing good fruit and tree characteristics. Figure 5 shows the distribution for fruit soluble solids (blue bars) and maturity time (given in red bars as days after June 1). While the soluble solids tested ranged from less than less than 5 to almost 30%, the great majority of the fruit were within the range of acceptable for processing, demonstrating the high horticultural quality of selections tested. As expected, early-maturing fruit tend to have lower soluble solids then later fruit with the notable exception of *UltraEarly#1* which, despite its ripe date of approximately 12 days before *Loadel* had distinctly high soluble solids at approximately 14%. [*UltraEarly#1*, which is derived from Brazilian (Conserva parent in figure 3) and possibly Eastern European germplasm, is also unique in that it confers exceptional fruit size, color and firmness for such an early maturing peach].

Fruit brown rot resistance

A much greater range of response was observed for fruit brown rot resistance as determined using the artificial inoculation and lab incubation method of Rick Bostock, Plant Pathology, UCD. [While RosBreed-2 will fully genotype all 1000 UCD selections free of charge, only limited funds remain for phenotyping, including disease screenings. Because of the importance and technical difficulties with disease evaluations, all RosBreed-2 funding has been targeted for brown rot disease screening at the Bostock lab (and even then at a Departmental subsidized rate).] The greater range in response was expected, since this material was not strongly preselected for resistance while it had been preselected for general good fruit and tree quality. Results document promising sources of potential resistance to this disease, though previous experience has shown that up to half of the resistant material will fail to show year-to-year stability in subsequent disease screenings and so prove undesirable. The observed range of resistance-susceptibility is also desirable as greater differences facilitate the accurate identification of genes associated with traits (either because they are actively contributing to the resistance or located adjacent to genes contributing to resistance (i.e. linked)). UCD varieties *Goodwin* and *Kader* showed high levels of resistance in this first round of testing, demonstrating the consequence of extensive screening for this trait in advanced UCD selections. *UltraEarly#1* also shows high levels of resistance and has been an important parent for resistance in our breeding program particularly when targeting the development of early ripening resistant cultivars. [The high disease resistance of *UltraEarly#1* combined with its previously demonstrated exceptional fruit quality has encouraged its test-planting in organic orchards as a source for organic processed peaches.] Co-plotting resistance and maturity time highlights a dearth of good resistance in late maturing items. UCD advanced selections ExtraLate#4 thru #7 have shown good resistance in previous studies but matured too late (mid-September) to be included in this disease screening. While some of their progeny were include in the study, the inheritance of resistance is not well demonstrated suggesting a need for other sources.

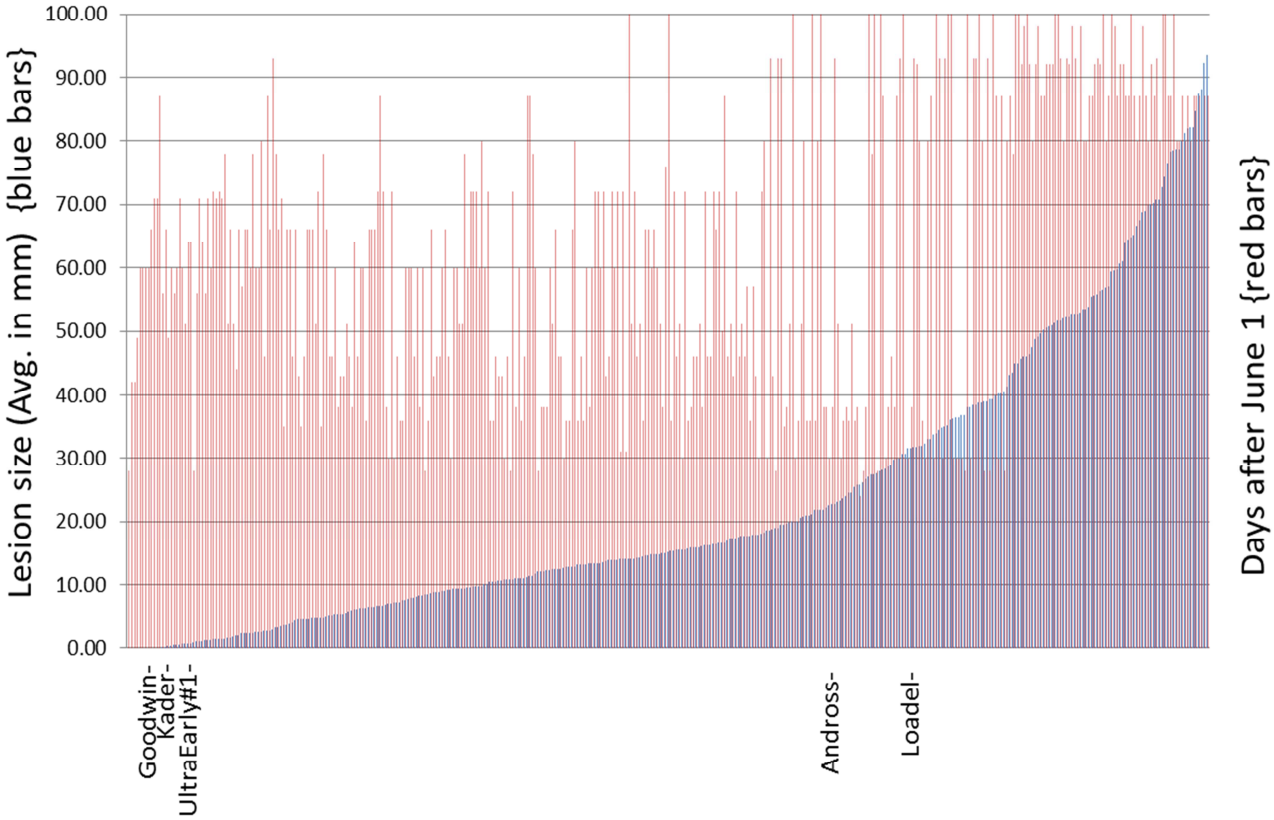

Figure 6. Distribution for fruit brown rot lesion size (mm -blue bars) and fruit maturity time (given in red bars as days after June 1) for 384 progeny from RosBreed-2 populations.. Data for standard cultivars and selected advance selections are plotted for reference.

The patterns or distribution of resistance also differ depending upon resistance source as summarized in Figure 7. The Traditional population shows a typical response for population with no strong selection for brown rot resistance. Individuals show varying levels of diseases susceptibility but the observed resistance is often the results of variables such as skin pubescence density, extent of the waxy cuticle, previous wounds to the skin, etc. that are more influenced by environment than basic genetic composition. The remaining sources all possess progeny showing high levels of resistance but generally low frequencies. If a major gene for resistance was involved, 25% or more of the population should show its presence as resistance. Because this pattern is not observed in these populations, the evidence points to multiple resistance genes with relatively small effects contributed by each. Multiple genes are particularly difficult to select because a relatively small effect is often confounded by random environmental effects. If the number of genes is relatively small (2-3), however, they can be identified and effectively

selected using appropriate molecular markers. This is one of the main goals of the RosBreed-2 project and builds on previous successful marker development at UCD for this disease as summarized in previous annual reports and recent citations (1, 9, 14). Our previous experience

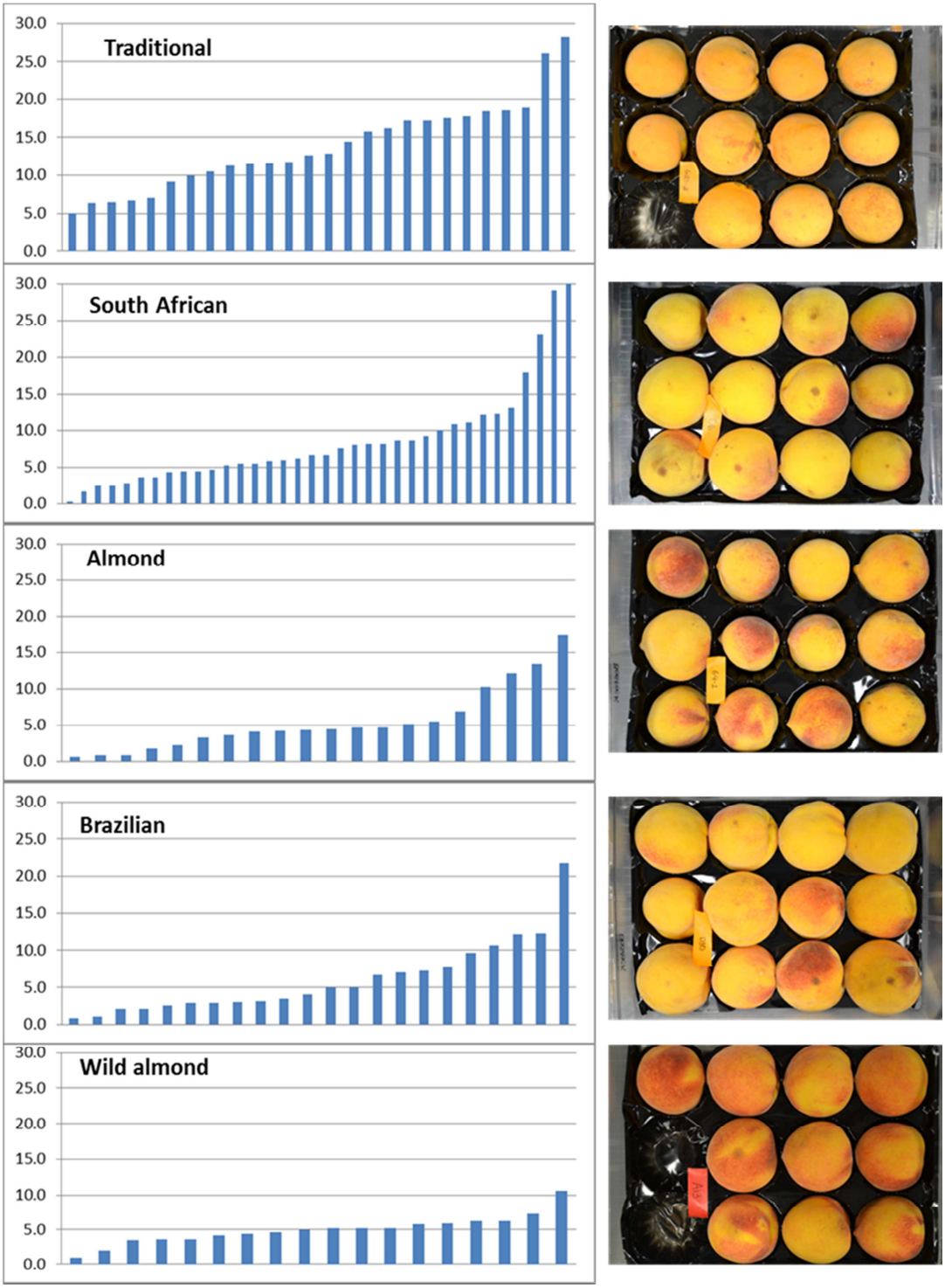

Figure 7. Differences in disease resistance distribution for different resistance sources. Each bar represents one individual of that population with the bar height representing the average lesion size after controlled inoculation. Fruit image at right of each plot shows a selected individual from that population at 2 days after inoculation.

when breeding for this disease resistance has been that resistance from different distinct sources typically has distinct mechanisms and controlling genes which allows the development of more complex resistance by using multiple resistance sources/mechanisms. This also confers more durable resistance over different environments and years. The absence of even a few individuals showing strong susceptibility (i.e. average lesion size is greater than 10 mm) in the Wild almond disease source suggest a similar stability across different environments and genetic backgrounds. The wild almond source (*Prunus scoparia*) is a species native to extremely arid climates and is often noted for its high susceptibility to foliar diseases and in particular Monilinia flower blight.

A particularly interesting resistance source/distribution is shown in Figure 8. Although only 10 progeny have been tested, all but one show very low average lesion size characteristic of a major resistance gene. Fruit tested are from an F2 population from the cross between the Mexican white melting clingstone peach *Pallas* and an almond resistance source. Fruit are clingstone and characteristically medium in size with moderately soft yellow flesh and high soluble solids ranging from 13 to 21%. Resistant fruit also appeared to have very short skin pubescence, almost appearing as nectarine. Fungal resistance is so complete in the selections that the fruit will usually dry-down prune-like without any noticeable brown rot lesions. A similar resistant selection, (2009,41-42) also shows an association with a very short pubescence (Figure 9) and has firm fruit which also holds well on the tree despite being freestone. We plan to make additional crosses with this material in 2016 in attempts to improve fruit size and firmness.

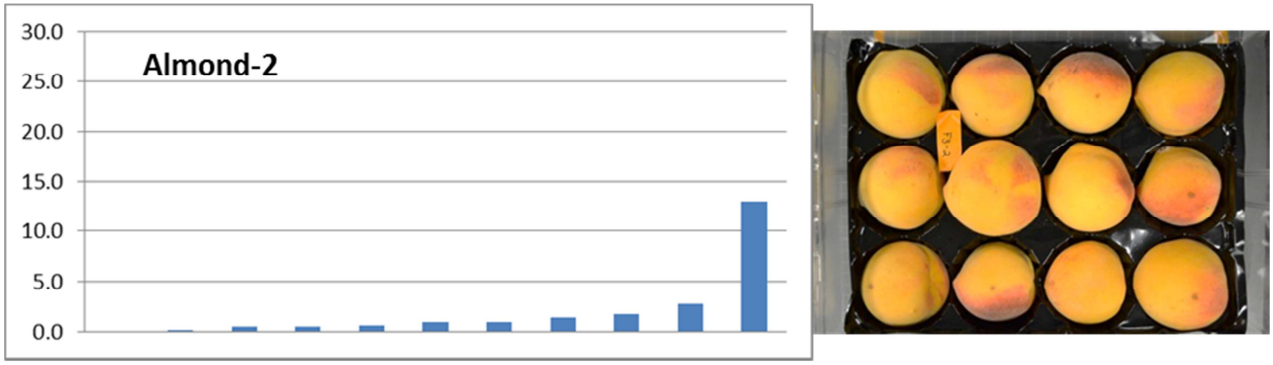

Figure 8. Differences in disease resistance distribution for a *Pallas* by almond resistance source. Each bar represents one individual of that population with the bar height representing the average lesion size after inoculation. Fruit image of selected individual from that population shown at right of each graft at approximately 2 days after inoculation.

Figure 9. Resistant selection, 2009, 41-42 which also shows an association of resistance with a very short skin pubescence but has firm yellow fleshed fruit which also holds well on the tree despite being freestone.

Results from Jim Adaskaveg' s 2015 peach blossom blight resistance study (Figure 10) also show a range in resistance depending upon germplasm source. Interestingly, sources which confer good fruit resistance, such as Bolinha, do not appear to confer good flower blight resistance. (This is particularly remarkable since in the Pelotas region Brazil were Bolinha was developed, rains during bloom a common). Other fruit resistance sources are also inconsistent, with some appearing to confer flower blight resistance while others do not.

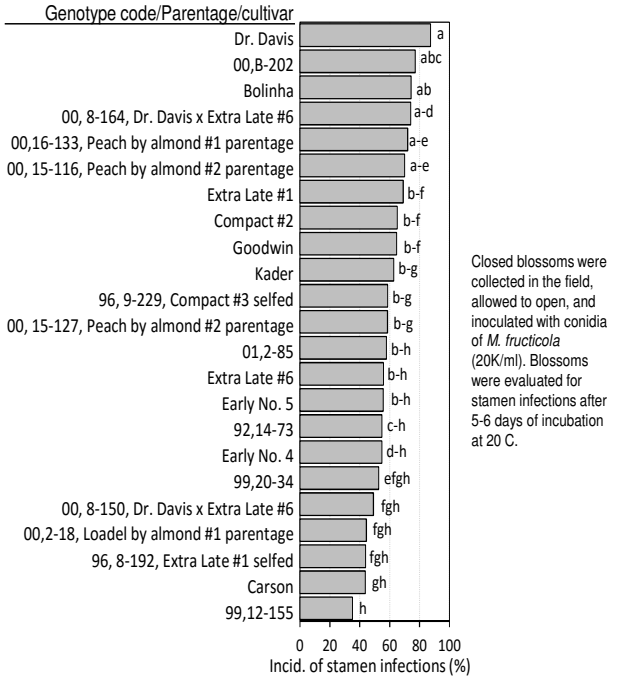

Figure 10. Results from Jim Adaskaveg' s 2015 peach blossom blight resistance study, showing a range in resistance depending upon germplasm source. Interestingly, sources which confer good fruit resistance, such as Bolinha, do not appear to confer good flower blight resistance.

Fruit size and integrity.

Fruit size and fruit firmness remained among the most critical traits required for successful commercialization of new cultivars. Fruit size has traditionally been considered a quantitative trait (similar to yield). Thus, it was thought to be controlled by a large number of genes each with relatively minor effect so that average progeny size would be intermediate to the size of the 2 parents and only a low proportion of the progeny would approach the larger parent size. For fruit firmness, the melting/non-melting trait was shown to be qualitative, i.e. controlled by a single gene which would determine a defined quality (i.e. - freestone and clingstone respectively). Because non-melting flesh is required for canning, this gene has largely been fixed in our breeding program. Even fruit fixed for the non-melting gene will vary in harvest firmness, which has again been shown to be a quantitative trait controlled by many smaller-effect genes. Recent work in our program has changed this view significantly, however. Genes with relatively large impact on fruit size had been identified as part of our RosBreed-1 and earlier UCD research. While the gene effect is large enough to make a significant impact on fruit size alone, their effect

is not easily detected within the complex background of other genetic and environmental effects so that the use of molecular markers greatly facilitates their selection. A more detailed presentation of this finding was presented in the 2014 annual report and only an update will be presented here. The most recent data, summarized in Table 5 shows patterns for molecular markers within the previously identified qFSz.5 candidate fruit size locus, along with two-year averages for breeding efficiencies for the related traits fruit size and fruit mass. Recent analysis confirm that the consistent alignment of this unique set of markers (and so associated genes) results in improvement in fruit size and fruit mass beyond what would be expected from purely quantitative inheritance (9, 13, 14). As discussed in the 2014 annual report, key components of this elite genetic combination appeared to of been lost early in the introduction of peach to North America (and probably Europe). It was only with the incorporation of more exotic breeding material in our program that these powerful combinations were recovered and identified. Using more complex quantitative analysis, advanced selections containing these elite combinations have been already achieved, as documented by the incorporation in both the UCD released *Goodwin* processing peach variety, and the selection of the Ultra-Early#1 advanced selection (whose exceptional size and firmness has been previously highlighted in this report). Interestingly, the PI 7-5 introduction effectively utilized by LD Davis to develop the superior attributes of both the *Dr.Davis* and *Ross* varieties also appears to contribute previously lost components. The more precise characterization of the controlling genes and associated markers involved will similarly allow a more precise and efficient selection for this important trait, thus allowing it to be handled in both a qualitative and quantitative manner. Even without detailed marker information, results have proven useful for the breeding program in the specific insights they have allowed. For example, is now recognized that *Dr. Davis* is a more effective parent than *Ross* for contributing both fruit size and fruit mass to breeding progeny because its genetic component set has been found to be more complete.

A second major change in breeding approach involves the standard view of processing fruit firmness. Traditionally, fruit having about 10 pounds firmness and maturity were desirable, with fruit much softer showing damage during transport and processing, while very hard fruit would reduce pitting efficiency and possibly final caning quality. While the importance of good fruit firmness at ripening remains unchallenged, the changing industry need for the capacity for once-over and even mechanical harvest has put a priority on harvest uniformity. Because flowering and initial fruit-set times can significantly vary, and because resulting fruit in different parts of the tree will develop and ripen at different rates, the breeding of varieties with a single uniform ripe-date would be particularly difficult. However, we have had good success in breeding for fruit which can hold on the tree for 1 to 2 weeks or more without loss of fruit quality. [Essentially this represents a paradigm shift in how we view after-ripening fruit. Instead of a chaotic deterioration of fruit integrity and quality, the softening and deterioration of fruit quality post-ripening is now perceived as following an internal programming which is amenable to genetic reprogramming in such a way that on-tree fruit quality can be maintained well after initial ripening stage.] The change in breeding approach thus emphasizes fruit firmness at one to

Table 5. Genotypes for the molecular markers within the interval of *q*FSz.5 and two-year average genomic breeding values (GBVs) for fruit diameter (FD) and fruit weight (FW) for individuals and their parents represented in the pedigree studied here and from the four breeding programs. AR - University of Arkansas, CA - University of California at Davis, SC - Clemson University and TX - Texas A&M University.

Name	Parent 1	Parent 2	ss_551012 2,085,084	ss_553912 2,580,963	ss_556209 3,212,441	ss_556975 3,427,608	ss_556982 3,427,826	ss_559057 3,731,230	FD[¶] 2-Year	FW[§] 2-Year
Goodwin	*DrDavis*	*11_11_37*	*AC*	*AG*	*AA*	*AA*	*AA*	*CC*	0.17	0.08
Vilmos	F8_72_33	F_F10C_12_28	*AC*	*AA*	*AG*	*AC*	*AG*	*AA*	-0.28	-0.13
CA_Pop_5_17_109	Goodwin	Vilmos	AC	AG	AG	AC	AG	AC	-0.21	-0.18
CA_Pop_5_17_047	Goodwin	Vilmos	AC	AG	AG	AC	AG	AC	-0.21	-0.17
CA_Pop_5_17_088	Goodwin	Vilmos	AC	AG	AG	AC	AG	AC	-0.21	-0.18
CA_Pop_5_17_093	Goodwin	Vilmos	AC	AG	AG	AC	AG	AC	-0.21	-0.18
CA_Pop_5_17_081	Goodwin	Vilmos	AC	AG	AG	AC	AG	AC	-0.18	-0.17
TX2B136	*Hermosillo*	*TXW1293_1*	*CC*	*AA*	*AG*	*AC*	*AG*	*CC*	-0.09	0.01
CAF4	*Y140_77*	*Y142_194*	*CC*	*AA*	*AA*	*AA*	*AA*	*CC*	0.00	0.03
TX_Pop1_15	TX2B136	CAF4	CC	AA	AG	AC	AG	CC	-0.16	-0.04
TX_Pop1_37	TX2B136	CAF4	CC	AA	AG	AC	AG	CC	-0.12	-0.04
TX_Pop1_19	TX2B136	CAF4	CC	AA	AG	AC	AG	CC	-0.15	-0.03
TX_Pop1_11	TX2B136	CAF4	CC	AA	AA	AC	AG	CC	-0.15	-0.02
TX_Pop1_35	TX2B136	CAF4	CC	AA	AA	AC	AG	CC	-0.15	-0.01
OHenry	MerrillBon	F_OHenry	*CC*	*AA*	*AA*	*AA*	*AA*	*CC*	0.08	-0.01
Cascata1006	*C92_16*	*F_Cascata1006*	--	--	--	--	--	--	-0.05	-0.01
SC_Pop0817_036	OHenry	Cascata1006	AC	AA	AG	AC	AG	AC	-0.07	-0.03
SC_Pop0817_052	OHenry	Cascata1006	AC	AA	AA	AC	AG	CC	-0.07	-0.01
SC_Pop0817_015	OHenry	Cascata1006	AC	AG	AG	AC	AG	CC	-0.06	0.01
SC_Pop0817_095	OHenry	Cascata1006	CC	AG	AA	AC	AG	CC	-0.06	0.01
SC_Pop0817_056	OHenry	Cascata1006	CC	AG	AA	AC	AG	CC	-0.06	0.02
A_776	*A_699*	*A_663*	*AC*	*AA*	*AG*	*AC*	*AG*	*AC*	0.05	-0.02
A_783	*A_699*	*A_717*	*AC*	*AA*	*AA*	*AA*	*AA*	*CC*	0.05	0.01
AR_Pop0801_09	A_776	A_783	AC	AA	AG	AC	AG	AC	0.04	-0.08
AR_Pop0801_10	A_776	A_783	AC	AA	AG	AC	AG	AC	0.05	-0.08
AR_Pop0801_12	A_776	A_783	AC	AA	AG	AC	AG	AC	0.05	-0.07
AR_Pop0801_01	A_776	A_783	AC	AA	AG	AA	AA	CC	0.04	-0.01
AR_Pop0801_14	A_776	A_783	AC	AA	AG	AA	AA	CC	0.04	0.01
BY92P2710	*Flameprince*	*87P943*	--	--	--	--	--	--	0.02	0.07
Bolinha	-	-	*CC*	*AA*	*AA*	*AA*	*AA*	*AA*	0.02	0.04
SC_Pop0821_001	BY92P2710	Bolinha	CC	AA	AA	AA	AA	AC	-0.04	0.05
SC_Pop0821_005	BY92P2710	Bolinha	CC	AA	AA	AA	AA	AC	-0.03	0.04
SC_Pop0821_010	BY92P2710	Bolinha	CC	AA	AA	AA	AA	AC	-0.03	0.04
SC_Pop0821_013	BY92P2710	Bolinha	CC	AA	AA	AA	AA	AC	-0.01	0.04
SC_Pop0821_017	BY92P2710	Bolinha	CC	AA	AA	AA	AA	AC	-0.01	0.04
DrDavis	*D25_9E*	*G40_5E*	*AC*	*AG*	*AG*	*AA*	*AA*	*CC*	0.11	0.01
D62_193	*NJC83*	*Conserva485*	*CC*	*AG*	*AA*	*AA*	*AA*	*CC*	0.22	0.16
CA_Pop_5_10_245	DrDavis	D62_193	AC	AG	AG	AA	AA	CC	0.19	0.08
CA_Pop_5_10_247	DrDavis	D62_193	AC	AG	AG	AA	AA	CC	0.19	0.10
CA_Pop_5_10_253	DrDavis	D62_193	AC	AG	AA	AA	AA	CC	0.19	0.14
CA_Pop_5_10_244	DrDavis	D62_193	CC	AG	AA	AA	AA	CC	0.19	0.16
CA_Pop_5_10_139	DrDavis	D62_193	CC	AG	AA	AA	AA	CC	0.21	0.17

[¶] Green shading cells represent individuals with small size fruits, yellow shading cells represent individuals with medium size fruits and red shading cells represent individuals with large size fruits.

[§] Green shading cells represent individuals with light weight fruits, yellow shading cells represent individuals with medium weight fruits and red shading cells represent individuals with heavy weight fruits.

2 weeks after initial tree ripening rather than at tree ripening. Where we would previously evaluate fruit quality at tree ripening stage, we now just flag trees whose fruit looks promising for all selection criteria (fruit size, shape, firmness, color etc.; tree vigor, productivity, disease resistance etc.) at ripening for further assessment at 1, 2 and even 3 weeks after the 1st fruit ripening stage. Only selections having this 'long-keeper' attribute will be advanced for cannery evaluation. Consequently, while their fruit firmness at harvest 2 weeks after initial tree ripe stage may only be 6 to 7 pounds, this firmness number has a very different meaning than the traditional harvest firmness. Examples of such a 'long-keeper' selections are presented in Figure 8 with samples of *Andross* and *Ross* harvested at tree ripe stage included for comparison. [More details on these samples, including processing quality, are presented in the Regional Testing Annual Report.] In many ways, this trait behaves similarly to the fruit size situation previously described. Within traditional breeding lines it is poorly expressed and under complex inheritance. However, the introduction of exotic germplasm has allowed the introgression/assembling a relatively small number of genes (to allow their controlled and efficient recombination) with a sufficiently high level of expression to allow pragmatic change in tree performance. As with our experience for breeding for fruit size, progress is very difficult though achievable and would be greatly facilitated if good molecular markers were identified. While the genetic control of fruit size was a major objective successfully realized in RosBreed-1, the genetic control of the 'long-keeper' trait (along with brown rot resistance) is a major UCD objective of the RosBreed-2 multiyear project. Research in 2015 has shown that we've successfully developed and sampled diverse populations segregating for both this trait and fruit brown rot resistance. Genotyping of this material is now underway with RosBreed collaborators followed by preliminary testing for correlation between specific genes and specific traits. Since different environmental conditions will also strongly affect trait expression, multiple years of data collection will be required to separate out the proportion of expression resulting from genetic control from that influenced by environment. If successful, the selection for once-over, including mechanical harvest ability could significantly change grower management options in farming efficiencies. The demonstration that the required trait variability is present and captured within current segregating UCD breeding populations along with the proven performance of molecular-based genetic improvement strategies for fruit size in this germplasm, appear to improve the probability that this type of selection could also be successful for the 'long-keeper' and similar traits in the near future.

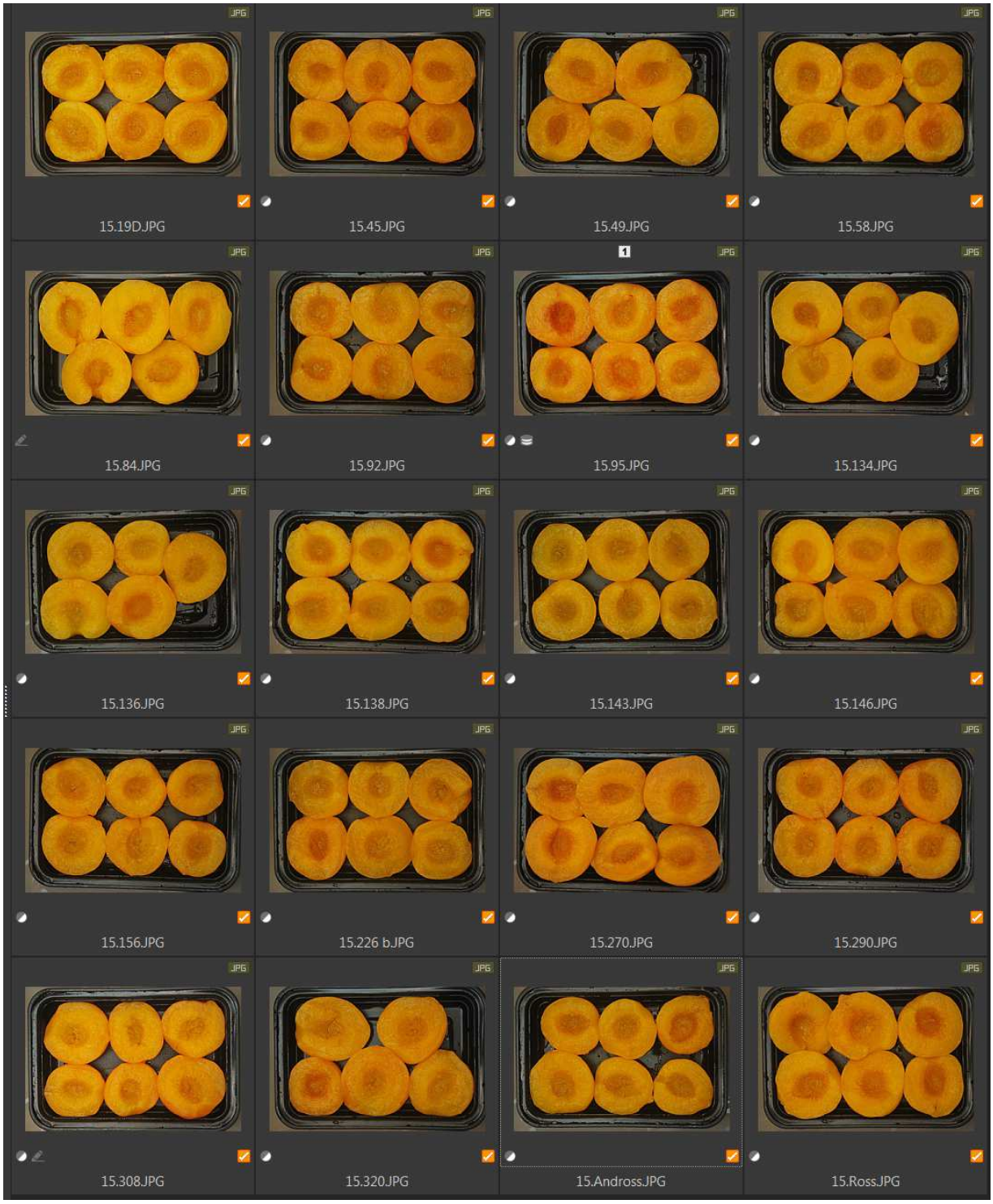

Figure 8. Sample processed fruit from genotypes selected for their long-keeper attributes (i.e. harvested at 1 to 3 weeks after initial tree ripe stage). Andross and Ross fruit harvested at tree ripe are shown at bottom right for comparison.

Recent Relevant Publications

1. 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 8(11): e78634. doi:10.1371/journal.pone.0078634.
2. 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.
3. 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.
4. Overstreet, S.M.; Choi, S.; Park, C.R.; Lee, D.; Gradziel, T. 2015. Acorn Production and Utilization in the Republic of Korea. In: Standiford, R. B. and K. Purcell. 2015. Proceedings of the Seventh California Oak Symposium: Managing Oak Woodlands in a Dynamic World, November 3-6, 2014, Visalia, CA. USDA Forest Service General Technical Report PSW-GTR-XX.
5. Limane, A., S. Noria and T. Gradziel. Root architecture of Atlas pistachio in relation to underlying soil properties under arid conditions. 2014. African Journal of Agricultural Research. DOI: 10.5897/AJAR20, ISSN 1991-637X.
6. Fresnedo-Ramírez J. Martínez-García P. Parfitt D. Crisosto C. Gradziel T. 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.
7. Angel Fernández i Martí, Thomas M. Gradziel, and Rafel Socias i Company. 2014. Methylation of the Sf locus in almond is associated with S-RNase loss of function Plant Mol Biol DOI 10.1007/s11103-014-0258-x.
8. Mengna Su, Mahesh Venkatachalam, Thomas M. Gradziel, Changqi Liu,Ying Zhang, Kenneth H. Roux, Shridhar K. Sathe. 2015. Application of mouse monoclonal antibody (mAb) 4C10-based enzyme-linked immunosorbent assay (ELISA) for amandin detection in almond (Prunus dulcis L.) genotypes and hybridsLWT - Food Science and Technology 60 (2015) 535e543.
9. Fresnedo-Ramírez J, Bink MCAM, van de Weg E, Famula TR, Crisosto CH, Frett TJ, Gasic K, Peace CP, Gradziel TM (2015). QTL mapping of pomological traits in peach and related species breeding germplasm. Molecular Breeding 35:166. Weblink: http://link.springer.com/article/10.1007%2Fs11032-015-0357-7.
10. Minas IS, Font i Forcada C, Dangl GS, Gradziel TM, Dandekar AM, Crisosto CH (2015). Discovery of non-climacteric and suppressed climacteric bud sport mutations originating from a climacteric Japanese plum cultivar (*Prunus salicina* Lindl.). Frontiers in Plant Science 6:316.
11. Yue, C. Gallardo, R.K. Luby, J. Rihn, A. McFerson, J. McCracken, V. Gradziel, T. Gasic, K. Reighard, G. Clark, J. Weebadde, C. Sebolt, A. and A. Iezzoni. 2015. An Investigation of United States Peach Fruit Producers Trait Prioritization—Evidence from Audience Clicker Surveys. HortScience.
12. Rahemy, A., T. Gradziel, et al. (in-press). Allelic diversity of S-RNase in almond and related species. Canadian Journal of Plant Science

13. Fresnedo-Ramírez J., Salgado A., Frett T.J., Clark J., Anderson N., Hartman T., Byrne D., Famula T.R., Crisosto C., Gasic K., Peace C.P., Bink M.C.A.M., Van de Weg E., Gradziel T.M. (in-press). Bayesian QTL mapping for two components of fruit size in Peach [*Prunus persica* (L.) Batsch] in a pedigree including accessions from four breeding programs in the USA. *Tree Genetics and Genomes.*

14. Fresnedo-Ramírez J., Famula T.R., Crisosto C., Parfitt D.E., Gradziel T.M. (in-press). A Bayesian ordinal model for the estimation of breeding values for the resistance to *Monolinia fruticola* (G.Winter) Honey in progenies of peach [*Prunus persica* (L.) Batsch]. *Breeding Research.*

15. Fresnedo-Ramírez J., Martínez-García P., Parfitt D., Crisosto C. Gradziel T.M. Implications of genome heterogeneity in peach [Prunus Persica (L.) Batsch] as distinguished from by sequence analysis of genomic variants. *Abstract and oral presentation at the Plant and Animal Genome Conference, San Diego, California, January 2014.*