California Cling Peach Advisory Board

2008 Annual Report

Project Title: **Development of New Cling Peach Varieties**

Project Leaders: Tom Gradziel & Carlos Crisosto

Cooperating Personnel: M.A. Thorpe, E. Ogundiwin, R. Bostock & J. Adeskaveg

Location: Dept. of Plant Sciences, University of California at Davis

Summary:

Ideal conditions in 2008 resulted in a second consecutive year of record crossing success in the Processing Peach Variety Development Program. Priority breeding traits included improved fruit quality (particularly fruit integrity to withstand the physical and biochemical abuses of mechanical harvesting, long-distance transport, and processing), disease resistance (particularly fruit brown rot) and tree architectures facilitating reduced labor inputs during pruning, thinning and harvest operations. Over 16,000 seed were recovered from controlled pollinations with approx. 10,000 seedlings to be field transplanted in 2009 (following greenhouse screenings). While the early breeding program balanced the development of improved *Dixon/Andross* & *Halford/Corona* period varieties with the identification/incorporation of new germplasm required by ongoing losses in labor and agrochemicals, recent progress in integrating these desired resistance and fruit/tree improvement genes into the breeding program have allowed a greater emphasis on final variety development. A series of distinct genetic sources of for each targeted trait has been evaluated and selected. The current challenge is to recombine these genes into such a way as to optimize the final performance while also incorporating the large range of other traits (vigor, yield, uniformity, size, quality, etc) required for commercial variety success. Heritability and molecular studies to allow the efficient transfer these traits from more their exotic to cultivated backgrounds are underway. Major and so readily manipulated genes contributing to fruit integrity and some disease resistances have been identified. Genetic control other desired traits may be more subtle is so difficult to manipulate using traditional breeding methods. Molecular markers are currently being developed for these traits to allow their accurate manipulation/recombination even when full expression is masked by confounding genetic/environmental backgrounds.

Objectives:

1. Accelerate breeding efforts towards lower production costs including once-over harvest and fruit resistance to mechanical damage with improved postharvest flesh texture and firmness without increased pitting problems such a split-pits, fragments and red-staining of pit cavities.

2. Continue breeding for Ultra-Early, Extra-Early, Early and Extra-Late maturity periods, as well as resistance to brown rot, mildew and aphids. Develop and test strategies for identifying the most promising seeding candidates within the first 2 years of field production

3. Generate 8,000 new seedling trees through the controlled recombination among advanced 2nd and 3rd generation breeding selections and locally adapted genotypes.

Controlled Crosses and Field Selections.

Cooler February temperatures followed by warm March weather with low precipitation resulted in a concentrated peach bloom in 2008. While a more concentrated bloom will reduce the overall flowering and so crossing season, it also allows greater numbers of flowers to be handled per cross using our controlled hybridization techniques. (Because peach is self-fruitful, flowers to be cross-pollinated need to be emasculated at the flower-bud stage prior to flower opening and anther dehiscence. With a concentrated bloom, more flowers are at the desired stage for emasculation allowing greater number of total flowers to be crossed on any given crossing combination/day). Fortunately, our cross-hybridization crews were already geared-up from the just completed 2008 almond pollination season, allowing the breeding program to take full advantage of the favorable peach crossing opportunities. Resulting seed set from controlled crosses was almost doubled our targeted goal of 8,000 seed. Given the significant and ongoing university cutbacks in support of field programs, the high 2008 controlled-cross seed-sets might not seem entirely welcome. However, as the breeding program is entering its final 9 years using traditional hybridization/large seedling progeny evaluation approaches, the opportunity to inject large numbers of seedlings from crosses between high-quality parents was pursued. Fewer pollen parents were utilized in 2008 as compared to 2007, with an emphasis on pollen parents contributing to one or more of four major areas: disease resistance, improved fruit quality (particularly harvest and postharvest fruit integrity), improved tree architectures to facilitate mechanization and once-over harvest, and the incorporation of new traits such as season extension, fruit flavor and phytonutrient content (Figure 1). Seed generated in 2008 was comparable to the record number of seed (20,000) generated in 2007, despite the fewer number of pollen parents, due to an increased seed-set in most crossing combinations. The higher than usual seed-sets initially caused some concern that the crossed flowers were being emasculated too late in development, thus allowing higher levels of self-pollination. Resultant seedling progeny populations were subsequently evaluated using seedling markers developed earlier in this program to discriminate between progeny resulting from selfed-pollination and out-crossing. Marker segregation ratios confirm that the majority of the progeny were derived from the desired crosses, indicating the higher sets were the result of the near ideal weather conditions. Over 16,000 seed from controlled crosses were germinated in the fall of 2008 and grown in greenhouses. Screenings is taking place in the greenhouse for both shoot architecture and disease resistance using previously developed marker-assisted selection. Eight to 12,000

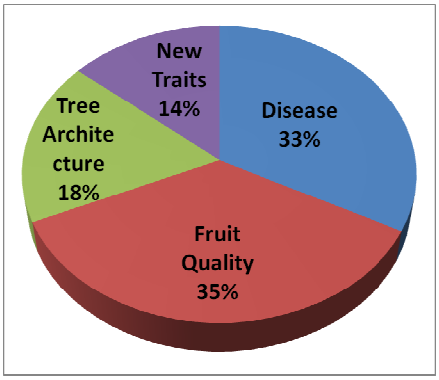

Figure 1. Source of germplasm used as parents for 2008 controlled crosses.

seedlings resulting from selection will be field transplanted in April, 2009. To reduce field costs, trees will be planted in high-density double-rows. Approximately 75% of trees from earlier planted blocks will be rouged- out by June 2009 based on tree and/or fruit performance. Important sources of initial germplasm for priority traits will be propagated and maintained at USDA Tree Fruit Repository at Davis California. Advanced selections showing high-quality for targeted traits as well as good adaptation to Central Valley conditions will be maintained at the UCD breeding program for continued evaluation and for further use in controlled crosses. As will be shown, most of these advanced breeding lines have already integrated desirable genes for several of the high-priority breeding traits, including those for improved fruit integrity, tree architecture, and disease resistance.

Fruit quality (integrity).

To be successful, a processing peach cultivar requires desirable attributes for a broad range of fruit quality traits, including fruit firmness, acidity, soluble- solids, color, pitting-quality and size and shape uniformity. With the movement of the California industry towards one-pick harvesting, both to reduce labor costs and to facilitate mechanization, the maintenance of good fruit integrity both during and after fruit full-ripe stage has become an increasingly important requirement and will be the focus of this section. Because the time between first and last fruit-ripening on the same tree may be as much as 5 to 10 days, it would be desirable to have a 'long-keeping' capacity of first ripening fruit so that they could be harvested with the final ripening fruit. Ideally, fruit should have good firmness, as well as good biochemical and structural integrity during pre-and post-harvest periods. Fruit should tolerate the rough treatment from (mechanical) harvest, transport and processing without significant fruit loss from bruising, softening, physical damage, or pit-problems (such as red-pit).
Currently the breeding program utilizes four principal sources of germplasm targeting fruit integrity: a) Eastern US/European, b) Mutations within California material, c) South African, and d) Related species (including almonds).

Eastern US/European. Advanced selections within this group are best represented by UCD Ultra-Early#1 (Figure 2). The parent germplasm was breeding lines developed by Dr. Fred Hough at Rutgers University during the mid-1900s using parents from the eastern US and western and eastern Europe. In addition to the required post-ripening keeping ability of 10 days or more, Ultra-Early#1 has a very early harvest season of approximately 10 days before Loadel, making it an important parent for the Ultra-Early, Extra-Early, and Early harvest periods. Fruit firmness, size and tree yield are exceptional for its early ripening time. Fruit flesh is yellow-gold to orange-gold,

Figure 2. Ultra-Early#1

allowing good color grades even when fruit skin is somewhat green (see Figure 3) while the Ultra-Early harvest season diminishes the risk of mixing with lighter, yellow-colored fruit. Fruit is large and has good appearance, and has a well-defined pit cavity. Because of its size and rapid ripening, fruit shape may sometimes be asymmetrical, making this cultivar best suited for sliced and diced product typical for early peach production. Ultra-Early#1 also demonstrates improved resistance to fruit brown rot and has become an important parent in the breeding program for this trait, particularly for Extra-Early-season cultivars.

Figure 3. Ultra-Early#1 raw fruit sample.

Mutations within California adapted material. Mutations selected in our early (1990s) breeding efforts have become important sources for both fruit integrity as well as tree architecture, as represented by the advanced selection Compact #2 (Figure 4). Compact #2 combines 5-7 day post-ripening fruit long-keeping ability with a compact tree structure (as discussed in the next section). While both modified tree and modified fruit types were derived from mutations in the same breeding line (UCD 18, 8-11) they appear genetically distinct and inherited separately. While the genetics of the fruit long-keeping ability is still not completely understood for this mutation, it appears to be controlled by one to two genes with major effect, and so readily transferred to progeny. Advanced breeding lines have combined the traits for good fruit integrity with compact tree architecture, good flesh color and size and freedom from red-pitting in overripe fruit. Improved resistance to both fruit brown rot and flesh bruising have also been incorporated into this and related breeding lines.

Figure 4. Compact #2

South African. The South African 'Kakamas' variety, while having an unfortunate spelling for Spanish-speaking areas such as the Central Valley, does possess good processing ability with high yield potentials, a yellow-orange flesh color and improved fruit brown rot resistance. Consequently, this variety has become the basis for much of the South African processing peach industry. Poor flesh firmness has, however, been one of its shortcomings, as fruit can quickly soften at ripening. However, certain genetic recombinations with California material have resulted in breeding lines showing very good fruit integrity in the otherwise difficult to achieve Late-ripening season. Advanced UCD selection Late#4 combines a keeping-ability of 10 days or more with good fruit size, shape, orange-gold color, low flesh-bruising and good yield potential. Fruit brown rot resistance is moderate which may be sufficient for this harvest season and which also makes it a useful parent for crossing with other breeding lines possessing major resistance genes (see brown rot section).

Figure 5. Late#4

The genetics controlling fruit integrity in this material is more complex, and thus more difficult to transfer to progeny. Efforts to concentrate different fruit-integrity genes in advanced breeding lines are currently underway using molecular markers described below.

Figure 6. Extra-Late#4

Related species. Interspecies crosses, particularly between peach *(Prunus persica)* and almond *(Prunus dulcis),* were initially developed as part of a hybrid rootstock breeding program, and as a core component of an almond breeding program to develop self-fruitful varieties. Recognizing the relative ease of genetic transfer between almond and peach and the presence in almond of disease resistances which could be useful in California peach varieties, in the early 1990s we initiated a program to transfer almond and related germplasm (including the wild almonds *Prunus webbili, Prunus argentia, Prunus scoparia, Prunus tangutica,* and the wild peaches *Prunus mira* and *Prunus davidiana)* to cultivated peach. While this germplasm has proven to be important sources of resistance to disease (including fruit brown rot, fruit sour, rot mildew, and leaf curl), insects (aphid and peach twig borer) and drought stress, perhaps its most surprising contribution was to improved fruit integrity. While a wide range of fruit flesh modifications have been achieved, the most significant have been our ability to develop preliminary breeding lines possessing both non-melting flesh with freestone characteristics (a previously unattainable holy grail of peach breeding as it combines the best characteristics of processing with freestone peach, -as summarized in earlier reports) and Extra-Late selections showing exceptional fruit long-keeping ability. The Extra-Late series Extra-Late#4, Extra-Late#5, Extra-Late#6, and Extra-Late#7 which were recently released for grower testing represent this 'long-keeper' germplasm (Figures 6 & 7). This Extra-Late series are all sister lines developed from the crossing of almond-derived breeding lines to the processing peach variety Reigles, then selecting and selfing the most promising progeny (Figure 10). Although these different Extra-Late selections differ somewhat in agronomic characteristics such as size and disease resistance, they're notable for their exceptional uniformity (given the recent lineage from almond) and exceptional long-keeping ability. Fruit will consistently hold on the tree for 4 to 6 weeks after normal maturity, with acceptable processing quality fruit harvested as late as late October in Davis (see 2008 regional testing report). Fruit also possess good sizing ability with minimal thinning, good structure and shape (Figure 6), a high orange-gold flesh color which allows good fruit grading even with some green at the fruit epidermis (Figure 7), and good resistance to fruit brown rot. While these selections have shown good horticultural promise without apparent deficiencies in over 10 years of field testing, the transfer of

Figure 7. Extra-Late #6 raw fruit.

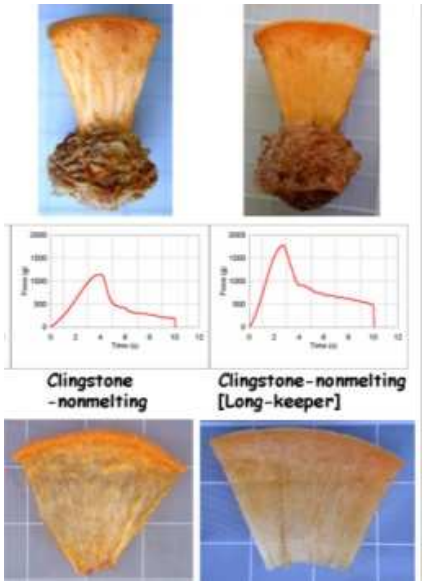

Figure 8. Fruit structural differences between Extra-Late #6 and Corona.

their exceptional long-keeping ability to progeny through controlled crosses has proven more difficult and appears to be controlled by both genetic and genomic interactions (see 2007 annual report).

The uniqueness of the different fruit integrity sources is indicated by both their distinct genetic origins as well as observable differences in flesh characteristics. For example, both the fruit flesh structure and firmness gradient have been shown to differ between the Extra-Late 'long-keeper' selections and standard California processing peaches (Figure 8) as discussed in the 2007 report. Even when present, however, structural differences are difficult to score particularly in young trees and more accurate

Table 1. Summary of allelic combinations for endoPG observed in peach and nectarine. Because some allelic combinations were expected to give the same banding pattern as others, only 43 different banding patterns were possible. Alleles listed in bold were those with the highest observed frequency. Phenotypes: FMF = freestone melting flesh; CMF = clingstone melting flesh; CNMF = clingstone non-melting flesh; sc = semi-clingstone; sf = semi-freestone; parentheses indicate that only some varieties with this allelic combination have the "semi-" phenotype. Frequencies were the proportion of varieties with each allelic combination across the species (n = 109) or within FMF + CMF varieties (n = 80) and CNMF varieties (n = 29). Example cultivars in parentheses are seedlings of a mapping population derived from 'Dr. Davis' and 'Georgia Belle', where the trees listed were the result of outcrossing to unknown pollen parents.

Allelic combination	**Phenotype**	**Frequency in species**	**Frequency in phenotype**	**Example cultivar**
F$_{205}$F$_{205}$/F$_{205}$f1$_{201}$/F$_{205}$f1$_{null}$ (sc)	FMF	10%	14%	'Georgia Belle'
F$_{205}$F$_{207}$ sc	FMF	1%	1% '	Crimson Baby'
F$_{205}$F$_{231}$/F$_{231}$f1$_{201}$ (sc)	FMF	7%	10%	'Redhaven'
F$_{205}$f$_{211}$ (sc)	FMF	8%	11%	'Fantasia'
F$_{205}$f$_{213}$	FMF	1%	1%	(Tree 309)
F$_{205}$f$_{229}$ (sc)	FMF	3%	4%	'Suncrest'
F$_{231}$F$_{231}$/F$_{231}$f1$_{null}$ (sc)	FMF	6%	9%	'Loring'
F$_{231}$f$_{209}$	FMF	1%	1%	'Babcock'
F$_{231}$f$_{211}$ (sc)	FMF	2%	3%	'Earliglo'
F$_{231}$f$_{229}$	FMF	5%	6%	'Elberta'
f$_{209}$**f$_{211}$**	CMF	2%	3%	'Spring Bright'
f$_{211}$f$_{211}$/f$_{211}$f1$_{null}$	CMF	12%	16%	'Mayglo'
f$_{211}$f$_{227}$	CMF	1%	1%	'August Glo'
f$_{211}$f$_{229}$ (sf)	CMF	6%	8%	'Fairlane'
f$_{213}$f$_{213}$/f$_{213}$f1$_{null}$	CMF	2%	3%	(Tree 308)
f$_{213}$**f1$_{201}$**	CMF	1%	1%	(Tree 379)
f$_{229}$f$_{229}$/f$_{229}$f1$_{null}$ (sf)	CMF	6%	8%	'Chinese Cling'
f$_{229}$f1$_{201}$	CMF	1%	1%	(Tree 114)
f1$_{201}$f1$_{201}$/f1$_{201}$f1$_{null}$	CNMF	20%	76%	'Andross'
f1$_{null}$f1$_{null}$	CNMF	6%	24%	'Fla9-20C'

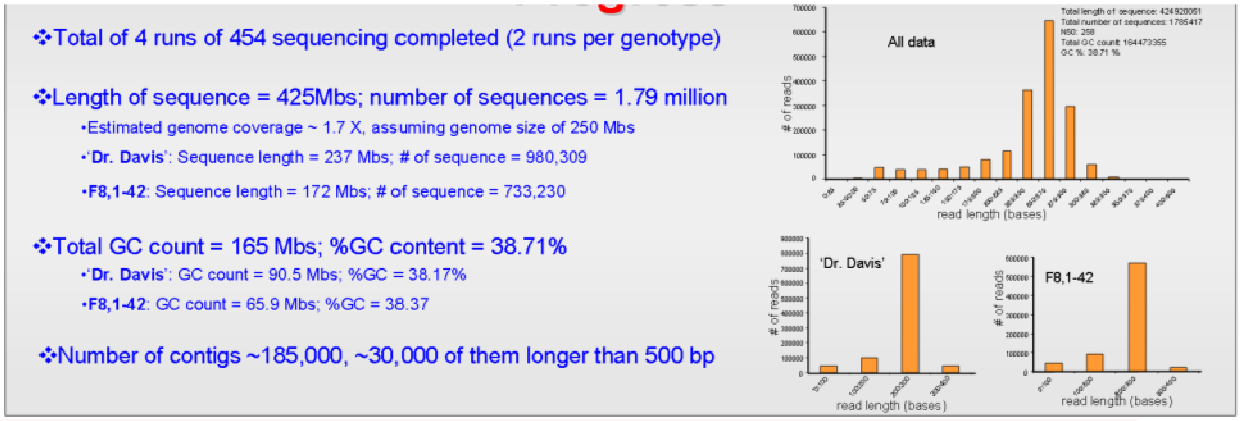

Figure 9. Progress in developing molecular markers via whole genome sequencing for peach flesh integrity in progeny derived from initial almond (F8,1 42) x peach crosses.

markers for fruit structure/ integrity traits are required for the optimal recombination of the different controlling genes in advanced breeding progeny. Not surprising, many of the same genes also appear to play significant roles in improving fruit brown rot resistance, so that fruit structure/integrity markers would also be useful in optimizing resistance in future varieties. Using candidate gene approaches, we have demonstrated the importance of the endopolygalacturonase (endoPG) allelic series as a major determinant of fruit flesh integrity in peach (Table 1) and are now developing molecular markers to be used in its marker assisted selection. While these molecular markers have helpful to identify some of the key determinants of flesh integrity in our advanced sources, many of the determinants appear to be distinct from endoPG (which was somewhat expected given their unique origins). We are currently pursuing a more comprehensive sequencing approach to identify these novel integrity/resistance genes and develop molecular-markers to allow their rapid selection in breeding operations (see Figure 9).

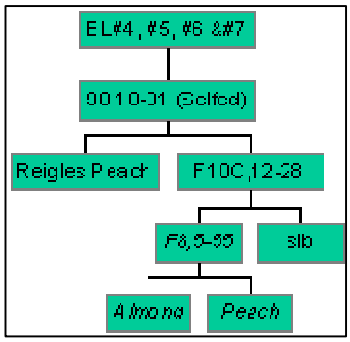

Figure 10. Breeding lineage for Extra-Late Long-Keeper selections showing recent almond parentage.

In addition to the ability to discriminate among specific flesh integrity genes, this more detailed genome sequence data should also shed light on the more complex and poorly understood genome interactions which may play important roles in interspecies-derived breeding lines/varieties. For example, a horticultural uniformity of the Extra-Late advanced selections may result from chromosomal rearrangements in the backcross generation which essentially fixes the desirable characteristics. Evidence for certain types of rearrangements such as inversions, crossovers and translocations might be identified using the more comprehensive sequencing approach.

Figure 11. *Prunus scoparia* shoots, fruits and nuts (inset, length ~6mm).

Figure 12. *Prunus scoparia* crossed to Mission almond.

Figure 13. Progeny from cross to Andross peach

Figure 14. Stone from the cross to Andross peach.

Figure 15. Stone from a further backcross to Carson peach.

Figure 16. Raw fruit samples of advanced processing peach breeding lines develop from crosses to wild almond.

Figure 17. Processed fruit samples of advanced processing peach breeding line 2005,20-117 develop from crosses to wild almond.

Genomic rearrangements are notoriously difficult to manipulate in standard breeding approaches, however, they can be readily fixed in crops by vegetative propagation. The challenge would be to re-create the (possibly rare) genomic recombinations conferring the desired plant traits. We are currently working to re-synthesize a large number of interspecies backcrosses (gene introgressions) in order to test this strategy. (If successful, this would represent a novel strategy for breeding tree crops in general, and may have important implications on the opportunities for epigenetic manipulation for future crop improvement). The power of this approach to rapidly incorporate exotic genes into commercial cultivars can be demonstrated by recent crosses (introgressions) made between processing peach and the very exotic almond species *Prunus scoparia*. *Prunus scoparia* is wild almond native to very high altitude deserts in central Iran. It is a bush with very small leaves which abscise in early summer as a drought tolerance mechanism. Fruit are composed of 6mm-long nuts surrounded by a thin leathery hull (Figure 11). The initial cross with *Mission* almond resulted in very upright trees producing nuts approximately 1 cm long with distinctly scribed shells (Fig. 12). Further crosses (introgression) of this material to the processing peach variety *Andross* produced distinctly upright trees forming slightly fleshy almond-type fruit with hard somewhat peach-type shells (Fig. 13). Shells are distinctly scribed in this material (Fig. 14), which is significant because these channels house the primary vascular bundles which supply the secondary outwardly-radiating vascular strands which make up the bulk of the fruit flesh. The position of the bundles exterior to the stony endocarp rather than within the endocarp as in almond, allows the development of a peach-type flesh when other required peach genes are present. Further backcrossing this advanced material to the processing peach variety *Carson* results in fruit having a highly channeled peach-type seed (Figure 15) and associated characteristically peach type fruit (Fig. 16). As demonstrated in Fig. 16, progeny from the final backcross develop good commercial quality fruit with good fruit size and symmetry and exceptionally high yields for the very early maturity season (approximately 10 days before Loadel). Both soluble solids and flavor have been rated highly in the processed product (Figure

17) and high levels of brown-rot resistance are indicated from field observations but not yet verified under controlled lab conditions.

Figure 18. Tree architecture for the Extra-Late Long-Keeper series.

Tree architecture. Ongoing research by Drs. Ted DeJong and Bruce Lampinen at UCD have shown that tree yields are directly dependent on their ability to capture sunlight energy in leaves adjacent to the developing fruit. Associated physiological and modeling studies continue to increase our understanding of the best tree architectures for optimal light capture efficiency. This research is usually based on traditional open-vase or perpendicular-V plantings which require sizable management inputs in training, pruning and thinning. For example the Extra-Late Long-Keeper series develops a standard processing peach tree architecture (Figure 18), which, while facilitating traditional orchard management, limits its value as a mechanically-harvestable peach since the traditional architectures are difficult to integrate with mechanization. Before the 2008 season, 95% of our variety development efforts targeted traditional perpendicular-V/open-vase tree types with only minor modifications (for example advanced selection Late#2 combines standard tree structure with a greater ability to fruit on older wood). A significant emphasis towards tree architectures more amenable to mechanical orchard management (training, pruning, thinning, harvest) began in the 2008 season and has progressed to the point that approximately 40% of 2009 crosses will target mechanical-amenable tree architectures to accelerate these options in the UCD cultivar development pipeline. The ideal mechanical-amenable tree architecture would be a uniform fruiting-wall requiring low cultural (prune, thin, harvest) inputs and be genetically controlled by major genes which would allow a rapid transfer to the range of ripening periods required by the processing peach industry.

Figure 20. Limited compact-shoot growth habit following pruning the terminal bud any processing peach advanced selection.

Figure 19. Compact #3 tree architecture following minimal training/pruning.

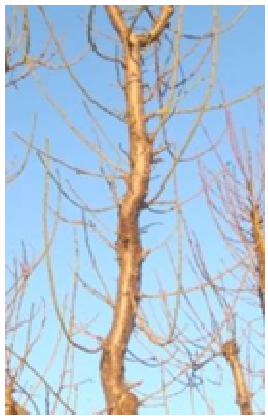

Figure 21. Limited growth are dard-type secondary shoot production and compact tree scaffolds.

Recent efforts in our breeding program to develop a compact (1/2 to 2/3 size) processing peach tree architecture shows some recent success. Advanced selections Compact#1, Compact #2 & Compact #3 are now in regional trials (see 2008 Regional Testing Report). [In addition to directly testing the feasibility of these novel tree architectures to facilitate the mechanization of processing peach in California, these compact tree plantings provide valuable feedback on the opportunities and limitations of similar tree-size controlling rootstocks being concurrently tested by Dr. DeJong]. The compact (50-75% of normal, see Figure 19) tree size is determined a single incompletely dominant major gene which allows a rapid incorporation into breeding lines with a range of fruit maturity times and which can be selected at the seedling stage, eliminating the need for costly field evaluations. As detailed in the 2007 Annual Report, the shorter tree size is determined by shorter internode lengths; thus the number of nodes and so the number of potential flowers and fruit remains similar to standard trees. In early field trials, this compact tree architecture resulted in only limited shoot extension following severe pruning (Figures 19 & 20). Establishment of a uniform peach fruiting-wall amenable to mechanization is often frustrated by the vigorous development of secondary shoots (including waterspouts) following pruning of the central scaffolds. To reduce/eliminate the mechanization difficulties associated with the often erratic secondary shoot development in peach, we are experimenting with options to limit fruit production to the primary scaffold. One strategy would be the development of short 'dard-type' shoots (or extended spurs) on the primary scaffold as shown in a breeding selection in Figure 21. This type of short dard-type shoot is similar to the secondary shoot architecture observed in apple cultivars optimize for mechanical harvest. (With minimal pruning, spur-fruit production can also be achieved in peach similar to that observed in apple (as seen in Figure 22)). Other genetic opportunities for similar dard-type secondary shoot development have recently been identified in processing peach germplasm derived from wild almond (Figure 23). In these genotypes (of incompletely determined genetic control), the shoot angle is more horizontal to the primary scaffold, further facilitating mechanization as well as reducing the induction of the more vertical and troublesome waterspouts.

Figure 22, Spur-based fruit production in UCD processing peach Compact breeding lines.

Figure 23. Limited growth of dard-type secondary shoot production derived from almond.

Figure 23, Multiple spindle peach seedling tree architecture derived from P. mira.

A second genetic option to develop more horizontal dard-type shoots in future processing peach varieties may involve the incorporation of the weepy-trait from ornamental peaches. Vertical secondary shoots development in weepy-genotypes is strongly suppressed, allowing a more uniform and open secondary shoot architecture.

These approaches towards a uniform, economically manageable fruiting walls attempt to suppress and so control the high vigor of peach (relative to apple or almond). An alternative and highly experimental strategy which would exploit rather than suppress the peach tree vigor as demonstrated by some of the multiple spindle processing peach types developed from interspecific crosses to *Prunus mira* (Figure 24). While both primary and secondary shoot growth is very upright in these genotypes, the secondary growth is structurally more pliable, apparently due to lower lignification of shoots. Fruit development on these more pliable secondary shoots will cause the shoots to drop to a more horizontal position allowing better sunlight penetration to interior leaves. The flexibility of both primary and secondary shoot would also allow sufficient pliability within the fruiting column (or wall in high-density plantings) for thinning, pruning and harvesting by mechanized equipment, (the inherent flexibility of the system would tolerate extensive physical disturbances without appreciable branch damage while the same flexibility would open up initial scaffold to allow improved light penetration and so cropping in the tree interior. Breeding selections incorporating the upright growth habit (controlled by as few as one recessive gene) have been developed for field testing in 2009.

Figure 24.Shoots of mildew-susceptible and resistant segregating progeny in a 2008 disease screening block.

Disease Resistance

Fruit brown rot resistance is a principal goal of the processing peach breeding program, particularly for early-season and late-season varieties where rain is more likely to occur. Ongoing projects are also pursuing resistance for other diseases, typically by identifying and incorporating major resistance genes. Resistance opportunities are currently being pursued for mildew, leaf-curl, green peach aphid and post-harvest peach sour rot. Progeny segregating in 2008 for high levels of field-resistance to mildew are shown in Figure 25. Fruit quality is moderate to good, comparable to that shown previously in Figure 16. Mildew resistant progeny were derived from an earlier interspecific peach-almond cross with the immediate parent for this segregating population appearing similar to the fruit shown in Figure 13. Segregation ratios of susceptible to resistant progeny indicate that the resistance may be controlled by a single recessive gene. If confirmed with further genetic studies , resistance could be readily incorporated into

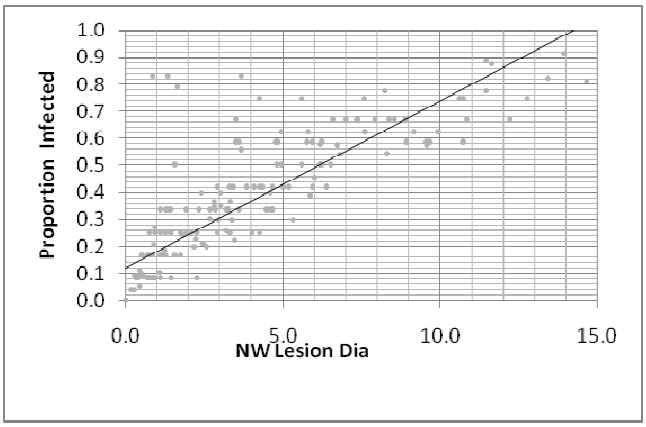

Figure 26. Positive correlation observed between lesion diameter and proportion of inoculated fruit infected after three days of culture.

advanced breeding lines. Interestingly, mildew resistance in this population appears to be independent of leave gland type which has been associated with known mildew resistances derived from peach germplasm.

Fruit brown-rot resistance. The fruit brown rot resistance breeding program has developed multiple, independent resistance lines demonstrating moderate to good levels of resistance and fruit quality. Major resistance sources include a) the Brazilian cultivar *Bolinha*, b) the very early ripening germplasm developed in Dr. Fred Hough's Rutgers University peach breeding program, and c) peach germplasm derived from almond and its wild relatives. Beginning in 2007 and continuing in 2008, a large-scale collaboration with Drs. Dan Parfitt & Rick Bostock, UCD Plant Pathology, has pursued a detailed genetic/molecular analysis of selected breeding populations (derived from *Bolinha* and almond, respectively) to identify effective molecular markers for individual resistance components (see 2007 Annual Report). Analysis of inoculated fruit samples from 2008 (Figure 26) demonstrate a good correlation between average lesion diameter and proportion of fruit infected following inoculation of non-wounded fruit (under previously described laboratory test conditions -see Bostock 2008 Annual Report). This good correlation supports the breeding program's use of lesion diameter as a major indicator of resistance. The relationship between lesion diameters for 2007 wounded fruit with the lesion diameter of the same inoculated genotype in 2008 was much poorer for progeny derived from the *Bolinha* resistant source (Figure 27) with only slightly better agreement for progeny develop from almond-resistance sources (Figure 28). This year-to-year inconsistency in resistance performance continues to be a significant impediment to breeding program progress and appears to be the result of the multiple resistance mechanisms involved and their differing response to differing environments. While the controlled lab inoculations minimize much of the environment induced variability in the early disease development, significant differences in fruit characteristics within the same genotype

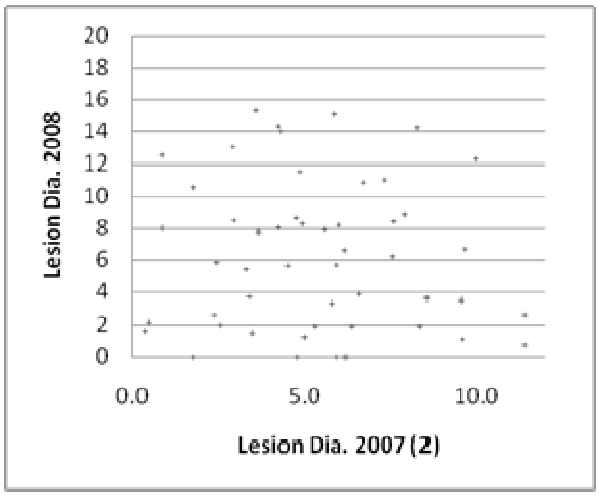

Figure 27. Lesion diameter and 2007 compared with lesion diameter in 2008 for the same Bolinha derived genotype after controlled laboratory inoculations.

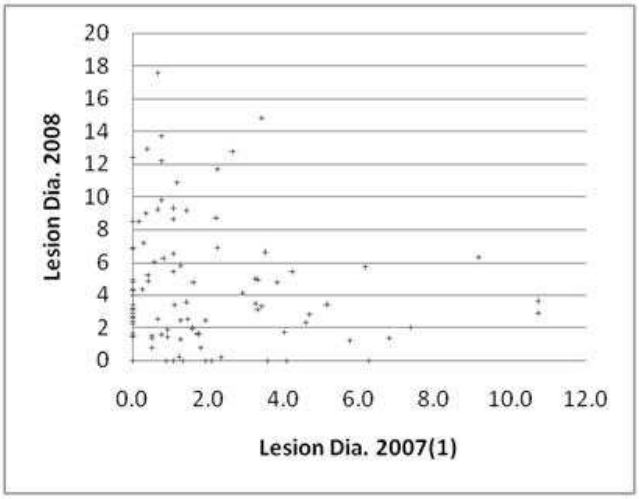

Figure 28. Lesion diameter and 2007 compared with lesion diameter in 2008 for the same almond derived genotype after controlled laboratory inoculations.

are often observed from one season to another (as detailed in the 2006 and 2007 reports). Variability results from physical and biochemical differences in the tested fruit (for example, protective epidermal waxes will vary with different environments depending on temperature, humidity and fruit growth conditions). Because of this extensive year to year variability, appropriate replicated testing is required. Typically, before a genetic selection is utilized as a parent for further crosses , it must show resistance over three consecutive seasons.

Figure 29. Resistance selection E4 derived from almond.

The variability in responses observed for the Bolinha derived progeny (Figure 27) support the previously reported multiple mechanisms with smaller individual contribution to final resistance. An example of the often indirect nature of resistance mechanisms is demonstrated by the relatively good correlation of 0.70 for resistance with flesh color hue as observed in almond-derived population E4 (Figure 29 and 30). More careful evaluation of the color data, however, demonstrates that a more direct correlation exists between resistance level and remnant chlorophyll levels in fruit epidermis (which would also be highly correlated with fruit ripening (Figure 30)). Even higher correlation between remnant chlorophyll level and resistance was found in another almond derived population G-11 (Figure 31), though the presence of remnant chlorophyll (and associated secondary resistance compounds) could only be detected by analyzing the light absorbance spectrum of chlorophyll at its characteristic 670nM wavelength using a spectrophotometer rather than by visual inspection.

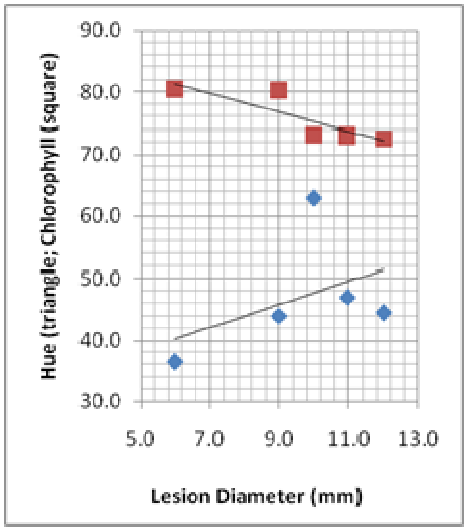

Figure 30. Relation of resistance in selection E4 with both fruit epidermis color (diamonds) and remnant chlorophyll content (red squares).

Better, but only moderate correlations were observed when 2007 results from lesion diameter of non-wounded fruit was compared with wounded fruit of the same almond-resistance derived genotype (Figure 32). This was expected because the breeding program has targeted epidermis-based resistance rather than flesh resistance to avoid deterioration in fruit flesh quality (for example, the higher flesh bruising associated with resistance phenolic compounds cited in earlier reports). Concentrating the resistance mechanisms to the fruit epidermis also has unique advantages for processing peach since the epidermis is removed during processing. Consequently, damage to the epidermis would obviate resistance barriers allowing disease to develop in previously resistant material as probably occurred for the lower-left fruit in Figure 31. This is particularly true in genotypes with poor fruit flesh integrity, either from advancing maturity (overripe) or characteristic of that genotype. For example, when the 2007 data is

Figure 31. Almond derived selection G11 resistance level is correlated with remnant chlorophyll content which , however, remains undetectable visual observation.

analyzed after separating genotypes by fruit firmness (blue circle <5lbs.; red square > 5lbs) a significantly improved correlation was observed for the firmer fruit (Figure 32). Interestingly, when data was separated by fruit-type (blue circle –freestone; red square -clingstone) the relationships were not as strongly affected for either the 2007 (Figure 33) or 2008 (Figure 34) results.

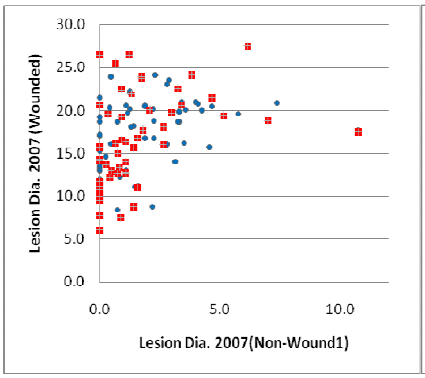

Figure 32. Relation of lesion size in 2007 for nonwounded to wounded (dot-soft; square-hard) fruit

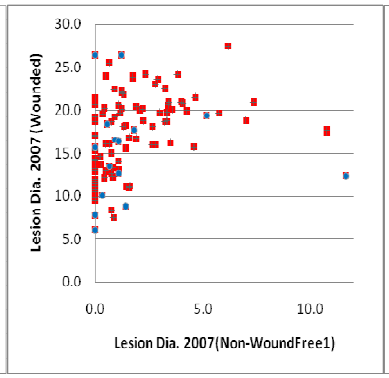

Figure 33. Relation of 2007 lesion size for nonwounded to wounded (dot-freestone; square-clingstone) .

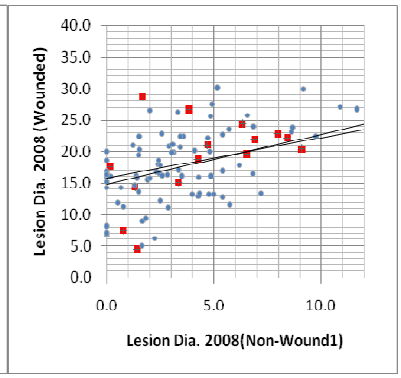

Figure 34. Relation of 2008 lesion size in for nonwounded to wounded (dot-freestone; square-clingstone) fruit.

As demonstrated by these examples, the challenge in evaluating overall resistance quality is the accurate characterization of individual resistance component. Frequently, however, resistance components interact, either masking or exaggerating the effect of other components as seen with the interaction between freestone/clingstone fruit type and flesh firmness. It is because of this biochemical and development uncertainty that we are actively pursuing molecular markers. For example, DNA-based markers for fruit flesh integrity would be distinct from those for freestone/clingstone and so individual resistance/susceptibility effect could easily be identified and manipulated. Preliminary marker-development work continues to look promising with putative QTL markers identified for epidermis-based resistance (Table 2 and Table 3). Linkage mapping also suggests that key resistance genes may be genetically linked (Table 3, Fig. 35) and associated with known SSR markers, both of which should facilitate their future genetic manipulation. Additional markers are expected as the data becomes more completely analyzed. Markers would thus be used directly in selecting the best resistance combinations. Markers for known resistance genes would also allow insight concerning both the mechanism of resistance and the potential positive and negative effects on fruit quality. The emerging understanding of the key genetic components for successful brown rot resistance provides the necessary knowledge base for the eventual deployment of these resistance mechanisms in future cultivars.

Table 2: Putative QTLs for resistance to BR detected by interval mapping analysis of MapQTL®

Trait	Marker Interval	LG	Position (cM)	LOD	% explained
Wound 07	CPPCT003-BPPCT022	G1	28.18	3.74	52.0
Wound 08	ChillPPN04A01-CPPCT003	G1	2.0	3.39	24.0

Table 3: Putative QTLs for resistance to wounded and nonwounded BR inoculations detected by non-parametric Kruskal-Wallis test

Trait	Markers	LG	K*	*P*
Wound 07	BPPCT034	G2	10.14	0.05
Nonwound 07	CPPCT003	G1	6.85	0.01
	ChillPPN09C01	G1	7.10	0.01
	BP2fOLE1122-E	G2	7.57	0.01
Wound 08	CPPCT003	G1	9.46	0.005
	BPPCT034	G2	8.37	0.05
Nonwound 08	CPPCT003	G1	4.86	0.05

K* = the Kruskal-Wallis test statistic, LG = linkage group, *P* = significance level

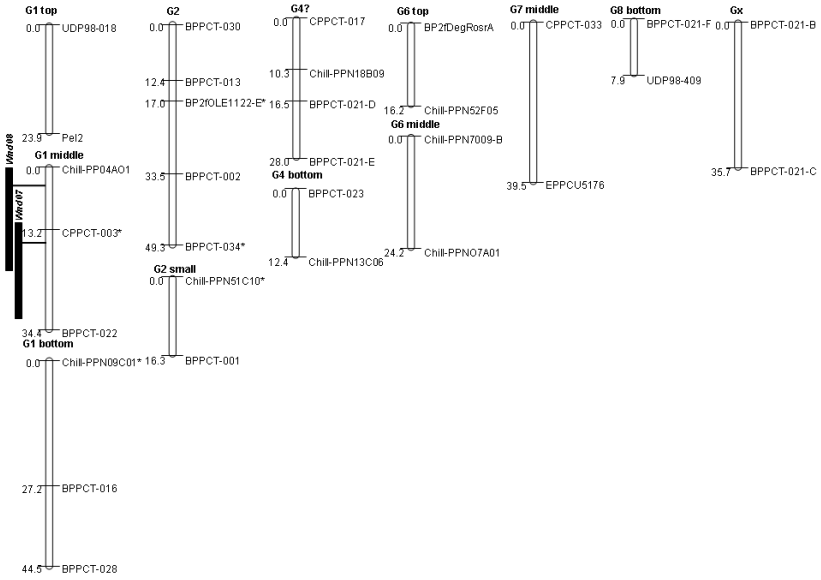

Figure 35. Partial linkage map of Pop-BR1; linkage group numbers and orientation derived from SSR markers in common with the *Prunus* reference T×E map; Group Gx could not be assigned a known number; BR resistance QTL markers detected by non-parametric Kruskal-Wallis test asterisked; black bars represents putative BR resistance QTLs located by interval mapping analysis.