California Cling Peach Advisory Board
2021 Annual Report

Project Title:	Development of New Cling Peach Varieties
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Summary

Skyrocketing labor cost, stifling regulations, increasing crop stress from changing climates, and market shifts largely defined 2021. While these conditions well-summarize industry realities from recent grower discussions, they have similarly come to define the UCD Breeding Program and its sustainability. As discussed in the 2020 annual report, UCD labor costs have doubled since 2019 and continue to rise. In addition, the program is increasingly responsible for additional field costs such as herbicide sprays which were previously covered by Hatch funds. Mechanization, such as mechanical hedging, has been adopted to decrease labor costs but regulatory restrictions have limited our access to providers. (Our previously contracted mechanical-hedger declined UCD work after the University demanded access to his books to ensure regulatory compliance. An alternative contractor could not be used because one of his equipment yards used security cameras made by a Chinese company that is blacklisted by the US government.). Both the Plant Sciences Field and Greenhouse managers were replaced (again) in 2021 resulting in facilities and management breakdown and loss of plants. The inconsistent management also facilitated additional plant stress exasperated by the mild winter and continuing drought. Similar stresses at the University administrative level, also exasperated by COVID, seem to be shifting University priorities from education and extension to improving profits. As a consequence, Hatch funds, originally intended for applied research and extension, are increasingly being used for other university-related purposes and so not available to buffer current hardships.

Opportunities also exist within these changes, and these are being pursued to maintain, and in some cases improve, breeding program efficacy. Regulatory and labor cost increases have largely removed the option of outside contract-labor, requiring our use of higher-cost but better-trained Plant Sciences career employees. This includes skilled mechanics and fabricators that we have used to repair and update older equipment, including a Darwin flower-thinner and field transplanters. The improved quality and year-to-year consistency of these career-level field crews also allow a higher level of training for such specialized practices as controlled-pollinations of peach flowers and large-scale tree planting/transplanting, thus removing the lower and inconsistent performance associated with using contract-labor and even seasonal student employees. Similarly, University focus on improving profits has put greater emphasis on

securing the more lucrative government grants where universities typically take 40-60% of the grant as overhead. Recently improved government funding for genetic/genomic projects for specialty crops has increased interest within UCD and other universities to develop successful proposals. Because any successful proposal will require established and well-characterized orchards (versus planting new orchards and waiting 4 years until the 1st crop), this funding shift has actually enhanced our ability to successfully join interdisciplinary and often multi-state proposals where we provide the plant material and genetic information, and they do the more tedious and costly genomic analysis (of our breeding lines).

As documented in previous annual reports, all industry funding is directly applied to the breeding of improved processing peach cultivars. The major determinants of breeding success are a) the availability of novel and heritable genetics possessing the desired traits, and b) a breeding strategy allowing the recombination of these genetic solutions into a regionally adapted background. Previous annual reports have documented the availability of novel and heritable genetic solutions to emerging challenges in labor (for example, the stay-ripe trait for more efficient once-over hand or mechanized harvest), agrochemicals (genetic resistance to fruit-brown-rock, etc.) and climate-change (concentrated bloom and crop-set following low chill winters, etc.). Consequently, this report will summarize our efforts to adapt previously successful breeding strategy to the new realities of the 2020s.

Breeding progress.

Controlled field-crosses. Despite starting field irrigation in late February, 2021, trees suffered from some level of drought stress from bloom through harvest. In addition to changes in Plant Sciences field management, irrigation wells at both the Davis and Winters locations were struggling to meet overall irrigation demands resulting in decreased irrigation scheduling. With the exception of a few isolated locations, no visible wilting was observed though the stress was apparent by lower overall orchard performances. The earliest indication of the stress was lower fruit set after controlled pollinations. Over 40,000 controlled pollinations were made resulting in only about 6000 recovered seed rather than the 8000+ expected under normal conditions. Despite the setbacks, over 1400 breeding progeny were planted in 2021 with approximately 2000 additional seedlings currently growing in the greenhouse. While surpassing our more modest target of 3000 breeding progeny, only about half of the progeny was from crosses targeting the Extra-Early season with the other half being primarily in the Early season with a much lower number in the Extra-Late

Year	Target	Planted
2015	3000	3211
2016	5000	3700
2017	8000	3027
2018	9000	3234
2019	6000	4859
2020	4000	2462
2021	3000	1400
2022	**10,000**	

Figure 1. Yearly breeding targets and actual number of trees ultimately transplanted to the field.

season. This was probably due to a higher fruit survival on later-maturing seed-parent trees owing to their greater avoidance of the marginal drought stress.

Seed harvest and stratification. Typically, about 70% of stratified seed survived to the 1st true leaf stage with the remainder failing to germinate, succumbing to primarily fungal infections or being rogued-out as having insufficient vigor. Survival in 2021 was below 60% (decidedly lower in certain crosses). Adding to the higher disease losses, was the regulatory-induced loss of our traditional fungicide which we apply as a slurry to the stratifying/germinating seed. By the end of summer, 2021, we had identified suitable though not yet ideal replacement fungicides (primarily Zerotol) along with more precise temperature controls during stratification and early germination to give greater advantage to plant growth over disease development. Two of our 3 stratification cold rooms broke down in 2021, possibly encouraged by our frequent readjustments while testing maximum/minimum optimal temperatures.

This experience has led to a new protocol for stratification/germination which has promise to give satisfactory levels of seedling recovery in the future. Key components of the protocol include greater use of seed and media sterilization prior to stratification, greater use of a naturally fungicidal sphagnum-moss stratification/germination media rather than the petri dish/fungicide slurry media used previously, and greater use of controlled temperatures, particularly during seed germination (radical emergence to 1st true leaf) stage. For this more precise temperature control, one of the stratification cold rooms has been completely rebuilt with the 2nd currently being rebuilt to optimize germination and early seedling growth temperature regimes, both using University provided funding.

Seed germination and greenhouse screening. Most seedling failure occurred during early stages of germination/growth. In many cases of failure, early incidents of fungal diseases were apparent at the start of germination (radical emergence) though a large number also failed prior to achieving the 1st fully developed true-leaf (as previously described). Early seedling failure was primarily a problem for those germinating seedlings which were transplanted and moved to the greenhouse after mid May, 2021 and was characterized by suppressed growth and ultimately fungal disease development (primarily Rhizopus) usually starting in the cotyledons and then spreading to the seedling shoot. Typically, we also use greenhouse screenings to rogue out 10-20% of the weaker plantlets. Because of greenhouse management changes, most plantings after the middle of May were in a larger multiple-user greenhouse where most of our seedling loss occurred and where it was difficult to do good sanitation. Experience with almond seedlings indicates that early germination to 1st true-leaf development is suppressed if it occurs at temperatures above 65° F. Early experiments suggest that this may also be happening with peach seed germination though the temperature threshold may be closer to 80° F, (though, because of the breakdown of the cold room being used, this experiment is still ongoing).

To solve these problems, we have relocated to a smaller greenhouse which is dedicated entirely to our project and so can be sanitized and temperatures managed more effectively. We are currently stratifying test seedlings to verify the high temperature effects on germination suppression and to better characterize the temperatures levels to be avoided. Concurrently, we delayed stratification/germination of our final seedling batches until fall, 2021 so that germination/early seedling growth will occur during the winter months when greenhouse temperatures are much cooler and can be better managed. Another option to facilitate rapid seedling growth and plantlets survival is to field transplant germinating seed just after radical emergence (as described below).

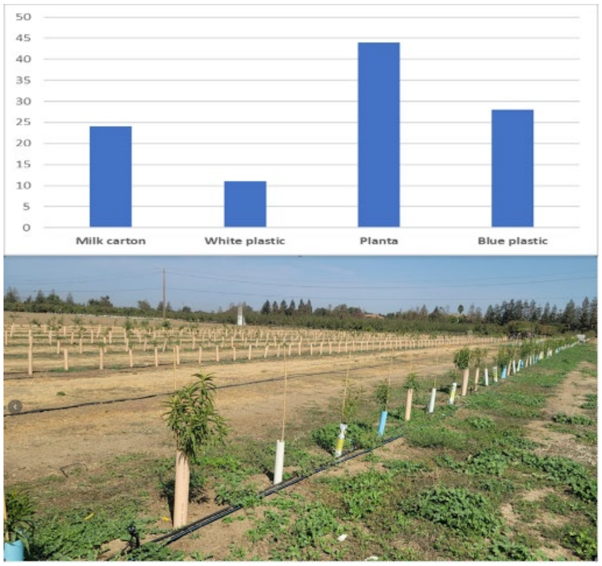


Figure 2. Effect of type of tree protector on 1st season's growth. (Graph is in inches).

Field planting. Direct field planting of breeding seed results in variable and inconsistent stratification and so differences in seed germination and survival. This has been overcome by bulk stratifying seed in sphagnum moss and then field transplant when the root radicals emerge. Seedlings at this early stage, however, a very vulnerable to rodent (primarily ground squirrel) and bird (primarily crow) damage. To some extent this can be avoided by planting in the fall when they tend to feed more on seed rather than seedlings though the high temperatures into late fall and/or early frosts can cause widespread damage. Our primary solution has been to field plant in the mid-spring to early summer and protect individual seedlings with plastic tree protectors. So far this has effectively protected against both ground squirrel and birds, though gophers can still be a problem. As shown in figure 2, the choice of tree protector can be important, we are currently using a rigid, amber-colored plastic tree protectors (Planta) that costs over a dollar wholesale but which we have acquired at a considerable discount by buying through Sierra Gold nursery. The micro-environments within these plastic tube protectors have higher humidity as well as higher temperatures, often exceeding 100° F. The higher humidity appears conducive to early seedling growth but also to disease, particularly mildew (which we control with Abamectin) and earwigs (which we control with insecticidal bait). Interestingly, germination/early growth seems to be enhanced by the higher temperatures, contrary to our greenhouse experience. It may be that it's not the high daytime temperatures that is actually suppressing growth but rather the lack of cool temperatures at night which would be present in the field but absent in a greenhouse. [Consequently, were including variable nighttime temperatures in our ongoing seedling germination/early growth temperature optimization studies].

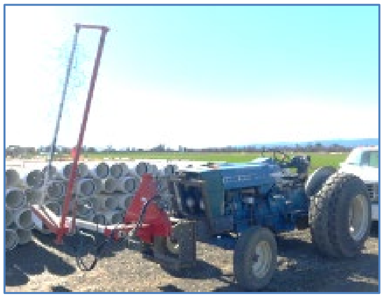


Figure 3. Modified UCD Darwin flower thinner. (Note shortened spin bar).

Field maintenance: thinning and pruning. As summarized in previous reports we use a Darwin flower thinner for all orchard thinning, typically deliberately over-thinning our seedling populations to minimize fruit-to-fruit competition and so allow assessment of the full genetic potential for fruit size and soluble solids. This was done as a collaborative testing with UCD Ag-engineering, who provided a modified (experimental) tractor which had a history of breakdown and repair difficulties (largely owing to its experimental nature and so lack of shop manual). In addition, the electronic controls of the Darwin thinner frequently shut down requiring a system reboot typically about every other tree row of thinning. With the 2020/21 Plant Sciences field labor reorganization and consequent access to required expertise, we transferred the Darwin thinner to a dedicated Plant Sciences tractor and installed direct hydraulic rotor-speed control, eliminating the problematic electronic controls. The system worked well in spring 2021 orchard thinning except when the front tractor tire collapsed into a squirrel burrow damaging the angle adjustment bracket. I called-in the damage to the shop mechanic and he picked up the damage thinner within an hour and had it returned by that evening with a nicely fabricated replacement bracket. Impressive and much appreciated.

Figure 4. USDA cutting-bar type tree hedger.

As also summarized in previous reports, all of our breeding orchards are mechanically hedged and after the difficulties of 2021 in contracting outside mechanical hedgers, we have been contemplating buying a rig, possibly supported by Plant Sciences funding as well as support from the walnut variety improvement program which also requires annual hedging. In addition, in 2020, I was able to secure federal funds for a hedging study at the USDA/ARS Germplasm Repository, also located in Winters, California. Partly as a consequence of favorable study results, the Germplasm Repository has acquired a cutting-bar based mechanical hedger (figure 4). I currently have an informal agreement with the Repository to have access to their mechanical-hedger, if needed, in exchange for their use of the Darwin flower-thinner to reduce their labor costs for peach tree thinning.

Figure 5. Pruning-wall achieved with annual hedging of Compact#3, a 2/3 size processing peach selection in regional testing.

Hedge with harvest. Mechanical hedging has been essential in evaluating breeding selection performance where over-thinning maximizes individual fruit size and sugar content, thus showing its true genetic potential. Mechanical-hedging is also an integral component of some of the experimental processing peach orchard configurations, particularly those developed to facilitate mechanical-harvest (figures 5, 6 and 7). Both mechanical harvest as well as harvest platforms facilitating more efficient hand-harvest, perform best on uniform fruiting-walls such as those obtained with pillar apple varieties on semidwarfing rootstocks. Because

Figure 6. Artificial fruiting-walls in processing peach achieved through intensive pruning (left) compared to natural growth habits achieved without pruning using the pillar-gene from *Prunus mira* (right). Comparison of normal growth habits of traditional versus pillar following top-hedging which has promoted extensive watersprouts development in traditional trees with only limited development in pillar trees (center).

traditional processing peach varieties have an upright-spreading to spreading architecture, the only way to achieve something resembling a fruiting-wall is with aggressive pruning, often utilizing mechanical hedging, though this inherently works against normal tree growth and development, particularly the tendency to produce aggressive watersprouts with severe pruning (figure 6). Compact selections bred by this program develop shorter internode lengths resulting in 2/3 to ½ tree size reduction. Regional testing of Compact #2 (Early-season), Compact#3 (Late-season) and Compact#4 (Extra-Late season) demonstrate that these selections also suppress watersprout growth (figure 5) while also possessing the stay-ripe trait which allows fruit to maintain processing integrity for a week or more following tree-ripe stage (as discussed in Regional Testing annual report). More recently we have been breeding for a pillar-type tree architecture utilizing genes from the wild peach *Prunus mira*, and to a lesser extent the pillar-gene from freestone peach which produces a tree architecture more amenable to the development of a fruiting- wall (figures 6 and 7). A more ambitious aspect of this ongoing project is to develop varieties where hedging can be incorporated to facilitate harvest. This "hedge with harvest" strategy would utilize the more natural tendency to form a fruiting-wall and suppress watersprouts growth of our Compact or Pillar selections [or genetically combined compact-pillar types currently being developed] while using a less aggressive hedger such as those use in vineyards for hedging and/or leaf removal. Once the basic fruiting wall was established,

Figure 7. Pillar-type peach tree where fruit-bearing wood was mechanically hedged during 2021 harvest (left) and subsequent extensive new shoot development by the end of the 2021 growing season (right and inset).

most subsequent hedging would primarily be on the more succulent fruit hangers developed during the remaining growing season following harvest. To test whether sufficient time would be available to establish sufficient fruit wood for the next season, we summer-hedged (early August) advanced UCD selections including standard, compact and pillar types. Results (figure 7) suggest that many Extra-Early and Early selections have sufficient time to replenish sufficient bearing-wood to provide for next season's crop. Interestingly, some of the compact and pillar types ripening in the Late-season also appear able to replenish fruitwood, though a more complete analysis awaits bloom and crop development in 2022.

To further facilitate this type of interdisciplinary experimentation, high density Compact#3 and Agromillora propagated Ross trees have been planted to a mechanization-test block at Davis, though the somewhat dwarfing plum rootstock used with the Agromillora trees as resulted in slow tree growth and development. We have also collected dormant budwood from promising pillar-tree to include pillar types in this test-block, but such selections are still 1 to 2 breeding generations away from having good commercial processing quality).

Field evaluations.

As discussed earlier, orchard performance suffered at both the Davis and Winters (Wolfskill) orchards, though tended to be more severe at Davis. Both areas had very limited rainfall during the previous winter though we were able to get irrigation on at Wolfskill earlier as we perform most of our controlled crosses there. Younger trees were more affected than older ones which would also affect Davis more strongly since most of our breeding progeny are located there. Because of our difficulties in contracting an orchard hedger, trees were also hedged very late, just before bloom. As would be expected, the major issue was smaller fruit size, but we also saw an increase in split-pits and associated fragmentation as well as red discoloration of the pit. In many progenies we also observed a more rapid softening of the fruit flesh after tree-ripe stage, even in selections which had not shown similar problems in previous years. This problem was most pronounced in freestone progeny where we were attempting to transfer the stay-ripe trait to increase/maintain fruit firmness. While frustrating and probably preventable with better orchard management, these observations present the type of situation we should anticipate in future production with the warmer summers and drier winters predicted from climate changes. A similar example was seen with the Goodwin variety which was developed as a possible replacement to Andross. While Goodwin initially had similar levels of split-pits as Andross, its other advantages including higher potential yields and higher processing case-yields encouraged its release. However, summer temperatures in the Central Valley were significantly hotter following release then in the 15 years of previous regional testing and this was sufficient to push Goodwin over a threshold for pit-splitting, even though with a very high level of grower management (as presented in earlier annual reports). Recognizing this vulnerability to future climate change, the UCD Variety Development Program has become more conservative in its identification of promising selections, now requiring at least 5 years of good field and processing data before advancement to the next level (regional trials, use as a breeding parent, etc.).

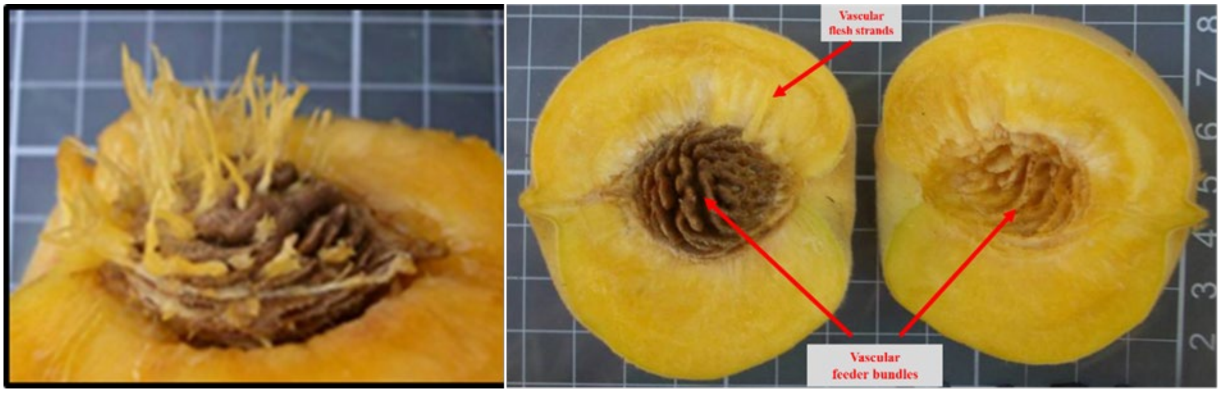


Figure 8. In clingstone peach, the fruit flesh is composed of tissue strands connected to (clinging to) the feeder bundles located in the pit channels or grooves (left). In freestone peaches the rays become physically detached (freed) from feeder bundles (right) so that needed nutrients can no longer be supplied through this pathway leading inevitably (that is, even with genetic reprogramming as with the stay-ripe trait) to tissue breakdown.

The increased vulnerability to softening for freestone peach selections being bred for extended maintenance of fruit-firmness beyond the tree-ripe stage (stay-ripe trait), prodded us to test a hypothesis on why the freestone trait is so vulnerable. The successful incorporation of the stay-ripe trait (allowing processing clingstone peach fruit to maintain firmness and processing quality for up to several weeks after tree-ripe) demonstrates that normal fruit deterioration after ripening is not an inevitable consequence of aging, but rather is part of the genetic programming that in nature would encourage desirable flesh softening and seed dispersal. By incorporating new genetics from outside the relatively narrow processing peach germplasm, we have been able to reprogram tree-ripe peaches to maintain firmness and processing quality for up to several weeks in certain Extra-Late selections, that previously had been only a matter of days. Achieving similar success in freestone peaches was much less successful, however, and we hypothesized that while the fruit flesh might be successfully reprogrammed away from the previous programs for rapid tissue breakdown, the fruit flesh would continue to require water and nutrients from the tree to maintain tissue integrity. Because the majority of peach flesh is composed of vascular strands radiating from the pit feeder bundles (located in the pit grooves or channels, see figure 8), the breakage of these vascular connections with fruit ripening, which would result in the free (from)stone trait (highly desirable in the fresh market), would essentially lead to starvation of the associated flesh tissue. We tested this hypothesis in 2021 by injecting non-toxic, water-soluble dyes into fruiting branches for both

	Clingstone	Freestone
Underripe	++	++
Overripe	++	- -

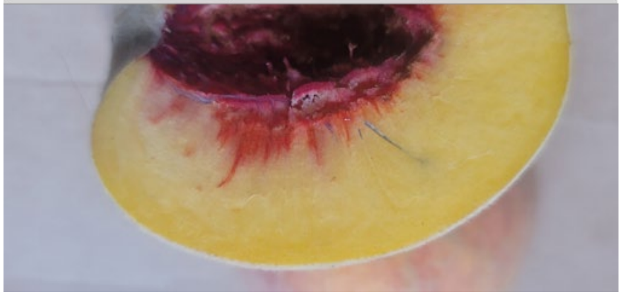


Figure 9. Freestone peach half showing movement of stem injected biological stains (red- *acid fuchsin* and blue-*aniline* blue) from vascular bundles in the pit channels radially out to the developing fruit flesh. Biological stains (and presumably fruit nutrients) continue to move through these channels after fruit ripening in clingstone but not freestone-type peaches (top chart).

freestone and clingstone progeny in some of our stay-ripe crosses. The dye readily moved from the stem to the pit feeder bundles to the fleshy rays radiating out from the feeder bundles to fruit flesh in all clingstone types as well as those freestone fruit tested prior to fruit ripening (figure 8). Once the freestone trait was expressed by the abscission of the pit to flesh connections, the red and blue dyes utilized would accumulate in the pit feeder bundles but not transfer to the flesh. While this seems rather obvious in hindsight, it does demonstrate that the range of what we can accomplish with both traditional genetics and even the more advanced biotechnologies will ultimately be limited by basic plant development patterns. Results also suggest opportunities for more accurate selection of stay-ripe trait in processing, clingstone types. To achieve the stay-ripe trait after normal fruit maturity, the fruit must be able receive required nutrients including water, sugars and essential chemicals such as calcium. Understanding which nutrient is most limiting may further breeding progress in this area. For example, if calcium is needed to maintain cell-to-cell integrity we should be able to develop a more accurate assay (such as selective tissue stains for calcium or a molecular marker for the controlling calcium gene) and so eliminate confusions caused by background environments such as warm temperatures or desiccating winds.

Molecular screening.

During this last year we have also been able to establish a state-of-the-art genomics lab managed by Dr. Gina Sideli using both SCRI peach and almond rootstock breeding grants and funds from the Almond Board of California. We anticipate using this facility to further track major genes for resistance and crop quality. An example is seen in Appendix C where we have identified effective markers for fruit brown rot resistance (as detailed and earlier reports). In addition, I recently compiled a list of known traits and putative molecular markers of value for peach breeding (Appendix A). We do not plan, however, to do molecular screening on seedling progeny. (For example, screen all progeny for the fruit brown rot resistance marker, eliminating all plantlets that lack it). This is because our focus is on genes with major and dominant to co-dominant inheritance. For example, in Appendix C, the dominant, major-affect nature of the resistance gene ensures that all progeny of a cross will inherit this trait as long as at least one of the parents is homozygous for it. Consequently, it is much easier and cost-effective to genotype the limited number of parents utilized rather than the many thousand progeny. In addition, the seedlings rogued out by bulk molecular screening may contain that rare and complex genetic complement to represent the commercially viable replacement for Andross (as an example) and so would be valuable to the industry even with limited vulnerability to brown rot disease. [Unless a trait is essential, such as nematode resistance for peach rootstocks in the San Joaquin Valley, bulk molecular screening/rogueing of progeny may actually undermine breeding progress since it inherently reduces breeding progeny size and so reduces the probability of finding those very rare individuals combining the multitude of traits (yield, disease resistance, insect resistance, tree structure, bearing habit, fruit size, color, soluble solids, etc.) required for commercial success.

Crossing plan: 2022.

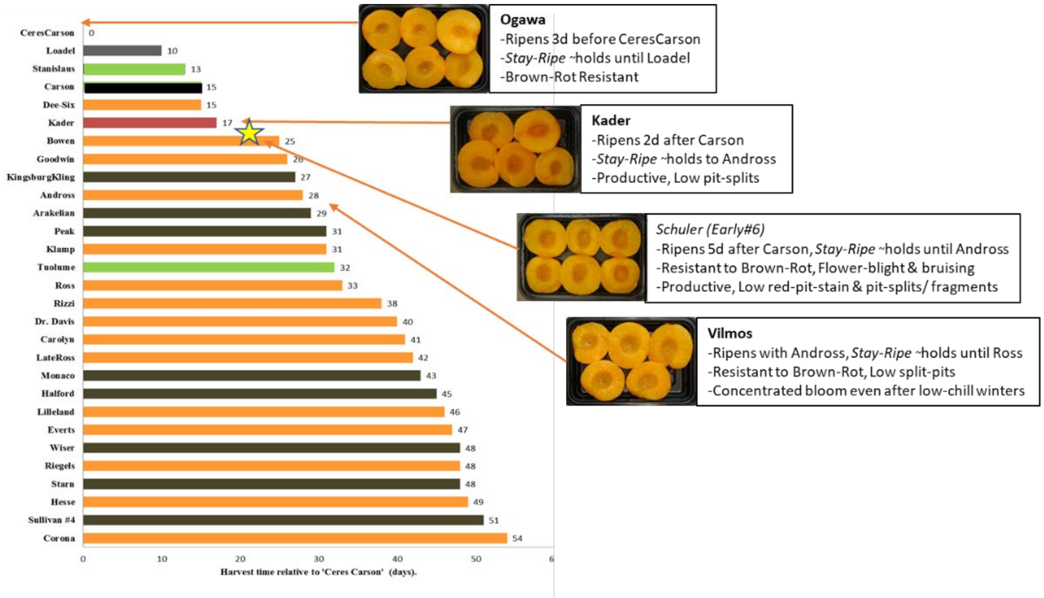
As discussed earlier, a major goal for 2022 is to achieve the very large population sizes required to incorporate the range of new traits for productivity, quality and resistance into Central Valley adapted varieties with desired harvest times. As presented in the Regional Testing Report, recent variety releases as well as breeding selections advanced to regional testing demonstrate significant improvements for these attributes, but often from different genetic sources (Appendix B). Recombining diverse genetic sources for desirable traits, such as the stay-ripe trait, should improve trait performance as well as its year-to-year stability and provide a greater range of harvest times. Breeding orchards are now in much better shape than 2021 and good chilling at Wolfskill indicates a more concentrated bloom is likely. This combined with the identification of a list of proven parents with both genetic and harvest-date diversity (Appendix D), a readily available, pre-trained workforce and our recent improvements in seedling recovery to field plantings, encourages a particularly ambitious breeding effort in 2022. This would also allow adequate time for fruiting progeny evaluations prior to my approaching retirement.

Recent or Relevant Publications

1. Gradziel, T. and S. Marchand. 2019. 'Kader' Peach: a Processing Clingstone Peach with Improved Harvest Quality and Disease Resistance, Ripening in the 'Dixon' Maturity Season. HORTSCIENCE 54(4):754–757. 2019. https://doi.org/10.21273/HORTSCI13708-18
2. Gradziel, T., and S. Marchand. 2019. 'Vilmos' Peach: A Processing Clingstone Peach Expressing a Novel 'Stay-Ripe' Trait With Improved Harvest Quality, Ripening In The 'Andross' Maturity Season. HORTSCIENCE 54: 2078-2080. 2019. https://doi.org/10.21273/HORTSCI14291-19
3. Gradziel, T. M. (2022) Exotic genes for solving emerging peach production challenges. Scientia Horticulturae Volume 295, https://doi.org/10.1016/j.scienta.2021.84801
4. Martinez Garcia, P.J.; Dan E. Parfitt; Richard M. Bostock; Jonathan Fresnedo- Ramirez; Alejandra Vazquez-Lobo; Ebenezer Ogundiwin; Thomas M. Gradziel; Carlos H. Crisosto.. Application of Genomic and Quantitative (2013) Genetic Tools to Identify Candidate Resistance Genes for Brown Rot Resistance in Peach. PLoS ONE 8(11): e78634.
5. Gradziel, T.M. 2020. Redomesticating Almond to Meet Emerging Food Safety Needs. Frontiers in Plant Science, Volume 11, 12 June 2020. 89/fpls.2020.00778. https://doi.org/10.33
6. Johnson, E.P., Preece, J.E., Aradhya, M., Gradziel, T. 2019. Rooting response of Prunus wild relative semi-hardwood cuttings to indole-3-butyric acid potassium salt (KIBA). Scientia Horticulturae, Volume 263, 15 https://doi.org/10.1016/j.scienta.2019.109144.
7. Felipe Pérez de los Cobos, Pedro J Martínez-García , Agustí Romero , Xavier Miarnau, Iban Eduardo, Werner Howad, Mourad Mnejja, Federico Dicenta, Rafel Socias i Company, Maria J Rubio-Cabetas, Thomas M Gradziel, et al. (2021). Pedigree analysis of 220 almond genotypes reveals two world mainstream breeding lines based on only three different cultivars. Hortic. Res. (2021) 8:11. https://doi.org/10.1038/s41438-020-00444-4.
8. Moral, J. , M. Teresa Garcia-Lopez1, A.a Gordon, A. Ortega-Beltran, R. Puckett1, K. Tomari, T. M. Gradziel, and T. J. Michailides. 2021., Resistance of almond cultivars grown in California to Aspergillus flavus and A. parasiticus. Plant Disease. manuscript ID is PDIS-05-21-0892.
9. D'Amico-Willman, K.M.; Anderson, E.S.; Gradziel, T.M.; Fresnedo-Ramírez, J. Relative Telomere Length and Telomerase Reverse Transcriptase (TERT) Expression Are Associated with Age in Almond (Prunus dulcis [Mill.] D.A.Webb). Plants 2021, 10, 189. https://doi.org/10.3390/plants10020189
10. Gradziel, T.M.; Shackel, K.A. Propagation of an Epigenetic Age-Related Disorder in Almond Is Governed by Vegetative Bud Ontogeny Rather Than Chimera-Type Cell Lineage. Horticulturae 2021, 7, 190. https://doi.org/10.3390/horticulturae7070190
11. Vahdati, K.; Sarikhani, S.; Arab, M.M.; Leslie, C.A.; Dandekar, A.M.; Aletà, N.; Bielsa, B.; Gradziel, T.M.; (et al.). Advances in Rootstock Breeding of Nut Trees: Objectives and Strategies. Plants 2021, 10, 2234. https://doi.org/10.3390/plants10112234
12. D'Amico-Willman, K.M.; Anderson, E.S.; Gradziel, T.M.; Fresnedo-Ramírez, J. et al. Identification of putative markers of noninfectious bud failure in almond (Prunus dulcis [Mill] D.A. Webb) through genome wide DNA methylation profiling and gene expression analysis in an almond □ peach hybrid population. Frontiers Front. Plant Sci. 13:804145. doi: 10.3389/fpls.2022.804145.
13. Angela S. Prudencio, Raquel Sánchez-Pérez, TM Gradziel, Pedro J. Martínez-García, Federico. (2020) Genomic Designing for New Climate-Resilient Almond Varieties . In: Chittaranjan Kole (Ed.) Genomic Designing of Climate-Smart Fruit Crops pp. 1-21. . ISHS Jhlpd68505c015976.
14. Gradziel TM. (2022) utilizing wild species for rootstock development. In: C. Kole, (ed.) Genomic Designing for Biotic Stress Resistant Fruit Crops. Springer DOI : 10.1007/978-3-030-91802-6

Trait/Resistance	Organism	Pathogen	Inoculation	Resistance/tolerance	Inheritance	Gene name	LG	Reference
P. cerasifera								
Root-knot nematode	Nematode	*Meloidogyne* spp.	Controlled	Resistance	Single dominant gene	*Ma*	7	Claverie et al. (2004a)
Oak root fungus	Fungi	Armillaria mellea	Controlled	Resistance	Quantitative	Not-mapped		Adelberg et al. (2021).
P. davidiana								
Powdery mildew	Fungi	*P. pannosa*	Natural	Tolerance	Quantitative	QTLs	1, 2, 4, 6, 8	Foulongne et al. (2003)
Sharka	Virus	*Plum pox virus*	Controlled	Tolerance	Quantitative	QTLs	1, 2, 4, 5, 6,7	Rubio et al. (2013)
Green peach aphid	Insect	*Myzus persicae*	Natural	Tolerance	Quantitative	QTLs	1, 2, 3, 4, 5,6, 8	Sauge et al. (2012)
Root-knot nematode	Nematode	Meloidogyne spp.	Natural	Tolerance	Major gene		Not-mapped	Reighard and Loreti (2011)
Brown rot		Fungi	Monilinia spp.	Controlled	Tolerance Quantitative		Not-mapped	Pascal et al. (1998)
P. dulcis								
Brown rot	Fungi	*Monilinia* spp.	Controlled	Tolerance	Quantitative	QTLs	1, 4	Martinez-Garcia et al. (2013a)
Powdery mildew	Fungi	*P. pannosa* var. *persicae*	Natural	Resistance	Single dominant gene	*Vr3*	2	Donoso et al. (2016)
Peach gummosis	Fungi	*Botryosphaeria dothidea*	Natural	Resistance	Single dominant gene	*Botd8*	6 or 8	Mancero-Castillo et al. (2018)
Root-knot nematode	Nematode	*Meloidogyne* spp.	Controlled	Resistance	Single dominant gene	*RMja*	7	Van Ghelder et al. (2010)
Root-knot nematode	Nematode	*Meloidogyne* spp.	Controlled	Resistance	Single dominant gene	*RMia*	2	Saucet et al. (2016)
Sharka	Virus	Plum pox virus	Controlled	Tolerance	Quantitative	Not-mapped		Martinez-Gomez et al. (2004)
Sharka	Virus	Plum pox virus	Controlled	Tolerance	Quantitative	Not-mapped		Cirilli et al. (2016)
Fruit firmness					Quantitative	Not-mapped		Peace et al. (2005))
P. insititia								
Crown gall	Bacteria	Agrobacterium spp.	Controlled	Resistance	Not analyzed			Bliss et al. (1999)
silver leaf	Fungi	Stereum purpureum	Controlled	Resistance	Not analyzed			Bliss et al. (1999)
P. kansuensis								
Root-knot nematode	Nematode	*Meloidogyne* spp.	Controlled	Tolerance	Major gene	*PkMi*	2	Cao et al. (2011)
Root-knot nematode	Nematode	*Meloidogyne* spp.	Controlled	Resistance	Single gene	*Mf*	2	Maquilan et al. (2018b)
Root-knot nematode	Nematode	*Meloidogyne* spp.	Controlled	Resistance	Single gene	Not-mapped		Maquilan et al. (2018a)
P. mira								
Root-knot nematode	Nematode	Meloidogyne spp.	Natural	Resistance	Single gene	Not-mapped		Cao et al. (2011)
Powdery mildew	Fungi	P. pannosa var. persicae	Natural	Resistance	Single dominant gene	Not-mapped		Layne and Bassi (2008)
Peach mosaic	Virus	Peach mosaic virus	Natural	Tolerance				Pine (1976)
Pillar tree architecture					*Quantitative*	*Not-mapped*		*Gradziel (unpublished))*
P. persica								
Brown rot	Fungi	*Monilinia* spp.	Controlled	Tolerance	Quantitative	QTLs	2, 3, 4	Pacheco et al. (2014)
Powdery mildew	Fungi	*P. pannosa* var. *persicae*	Natural	Resistance	Single dominant gene	*Vr2*	8	Pascal et al. (2017)
Xanthomonas	Bacteria	*X. arboricola pv. Pruni*	Controlled	Tolerance	Quantitative	QTLs	1, 4, 5, 6	Yang et al. (20
Sharka	Virus	*Plum pox virus*	Controlled	Tolerance	Quantitative	QTLs	2, 3	Cirilli et al. (2017)
Green peach aphid	Insect	*Myzus persicae*	Natural	Resistance.	Single dominant gene	*Rm2*	1	Lambert and Pascal (2011)
Green peach aphid	Insect	*Myzus persicae*	Natural	Resistance	Single dominant gene	*Rm1*	1	Pascal et al. (2017)
Root-knot nematode	Nematode	*Meloidogyne* spp.	Controlled	Resistance	Single dominant gene	*RMiaNem*	2	Claverie et al. (2004b)
P. salicina								
Root-knot nematode	Nematode	*Meloidogyne* spp.	Controlled	Resistance	Single dominant gene	*Rjap*	7	Claverie et al. (2004a)
P. petunnikowii								
Crown gall	Bacteria	Agrobacterium spp.	Natural	resistance	Not analyzed Not-mapped			Evreinov (1952)

Appendix B. Recent UCD releases targeting the Extra-Early and Early maturity seasons (orange bars identify previous UCD releases).



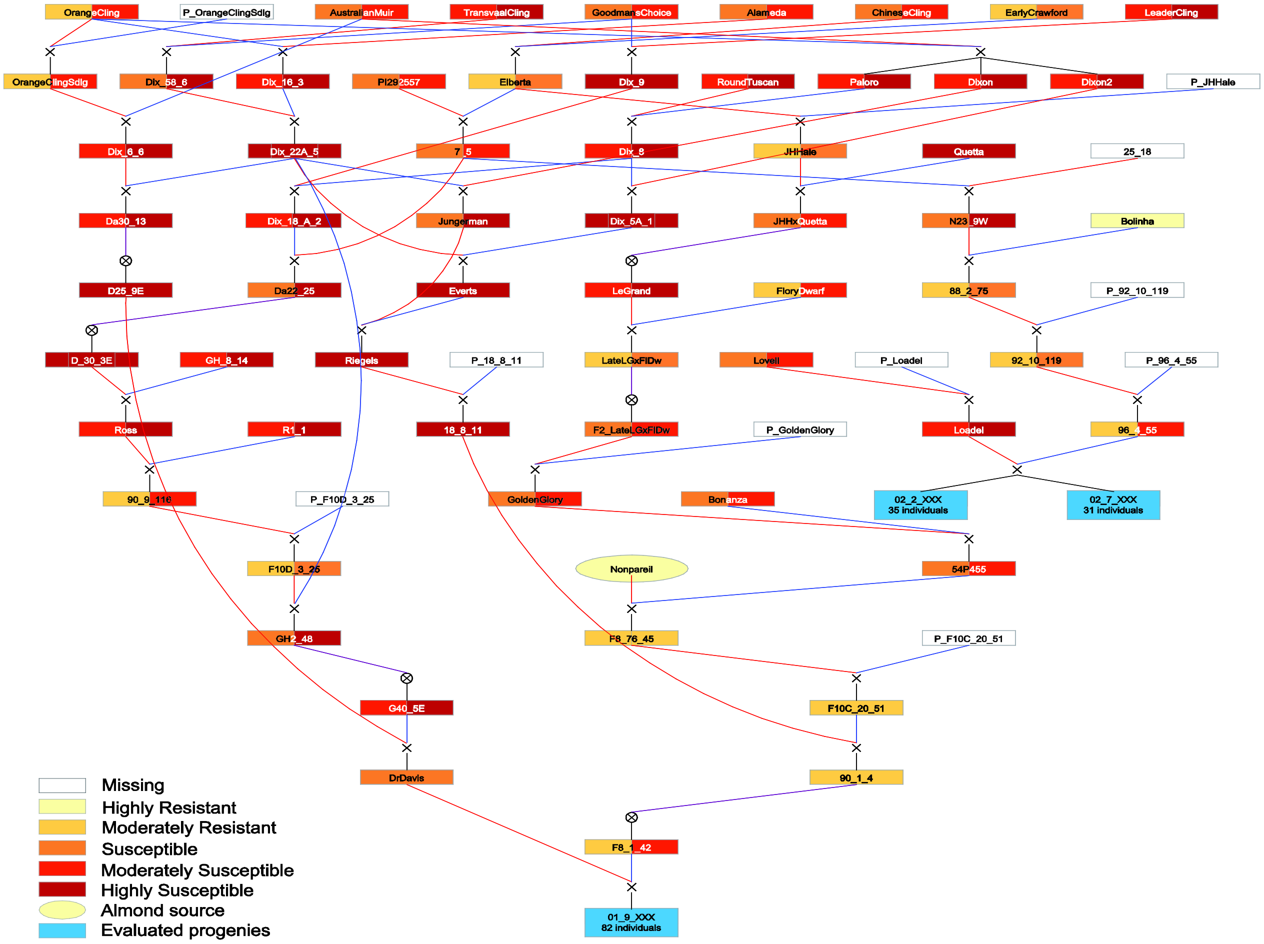
Ogawa
-Ripens 3d before CeresCarson
-*Stay-Ripe* ~holds until Loadel
-Brown-Rot Resistant

Kader
-Ripens 2d after Carson
-*Stay-Ripe* ~holds to Andross
-Productive, Low pit-splits

Schuler (Early#6)
-Ripens 5d after Carson, *Stay-Ripe* ~holds until Andross
-Resistant to Brown-Rot, Flower-blight & bruising
-Productive, Low red-pit-stain & pit-splits/ fragments

Vilmos
-Ripens with Andross, *Stay-Ripe* ~holds until Ross
-Resistant to Brown-Rot, Low split-pits
-Concentrated bloom even after low-chill winters

Appendix C. Use of molecular markers to precisely track trait inheritance through breeding parents.



Appendix D. Description of advanced UCD selections currently being considered as breeding parents for 2022 crosses. [In addition, all selections considered as candidates for regional testing as described in the Regional Testing annual report are also considered as possible parents for 2022 breeding crosses].

Extra-Early season

Carson harvest time.

11,23-110. Ripening with to just before Carson, this selection combines good fruit size, color and firmness a desirable harvest time. The early and rapid sizing combined with the fruit firmness result in an increased incidence in splitpits, otherwise this selection would under consideration for regional testing. Fruit skin is generally free from any red blush in both pit cavity and flesh remained free from any red imprinting even under high spring/summer temperatures, the trait it appears to have acquired from South African breeding germplasm utilized by our program.

16,10-388. An advanced fourth-generation selection combining traits from different species including almond and wild peach. Fruit are large and firm and free from red-staining of the pit. Some split-pits were observed in over thinned trees 2020 though not in previous years. Fruit show very good firmness, with the 2020 canning example (shown) averaging 7 pounds at 12 days after full ripe. Fruit stay firm for up to two weeks after tree ripe but can develop water-soaked, browning of flesh after that. The fruit skin is totally free of any red pigmentation which contributes to the greater resistance of red-staining of the pit.

Dixon harvest time.

11,2-84. This selection resulted from a cross between the very firm but small selection 2000,9-79 with Ultra-Early- 1 resulting in improved firmness and size. Fruit color is a desirable golden-yellow and fruit tend to be large with moderate small pit. Fruit have good firmness at tree-ripe and in many years will maintain this firmness for a week or more. Fruit also show a low tendency to bruise and show low fruit brown rot disease in the field. In some years, such as 2019, over-ripe fruit tend to soften in the outer flesh resulting in pit or-cup bruising during processing. Split pits were concern in 2019 and 2021, though the fruit remained relatively free in previous years. 25-68,54

10,8-456. Also representing a second-generation breeding selection derived from a cross with *Dr. Davis* and advanced selection *UltraEarly-1* as grandparents, this Dixon-period selection shows good size for the season. Good field resistance is also observed which has been verified following Bostock lab inoculation and testing. In addition to good size and flesh color, fruit demonstrate good firmness and freedom from pit-staining and fragmentation. Good processing fruit quality is maintained on-tree for over 2 weeks post-ripe though the fruit may soften to below 5 pounds pressure by this time. The seedling tree shows good productivity but continued to show only moderate vigor in 2018 in our very high density selection block so in 2019 it was propagated to a more standard planting density.

Early season

Andross harvest time.

11,11-233. A progeny from a 3rd-generation introgression line, this selection has consistently shown good tree productivity and fruit size, quality and firmness. Fruit quality has been maintained for up to 2 weeks following tree-ripe though the fruit tend to be a bit softer than other advanced selections in this harvest time. Fruit are generally free from red-staining of the pit and associated pit fragmentation, though a light pigmentation can occur on overripe fruit that cooks out with processing but discourages its consideration as a possible variety release. Pits are medium to small in size. The uniform yellow-gold skin and flesh color are also maintained up to 2 weeks following tree-ripe contributing to a good-quality processed product (images) as well as enhance consumer nutrition (i.e. higher levels of antioxidants). Fruit show low levels of fruit brown rot in natural field inoculations but have not yet been tested under laboratory conditions. Fruit also show reduced levels of flesh bruising.

10,18-528. This selection is derived from 00,16-92 as a grandparent. Pubescence or fuzz on this and other selections derived from 00,16-92 appears denser and more compact with better resistance to initial fruit brown rot infection as well as improved lye-peeling. Fruit show moderate size and good firmness but greater susceptibility to flesh bruising. As with other 00,16-92 breeding selections, epidermis and fruit pit are free from any red pigmentation, even in overripe fruit. Breeding line 00,16-92 is from a 2nd and independent almond germplasm source, which appears associated with greater fruit structural integrity and so firmness, particularly for the inner flesh near the pit cavity. It is one of the firmest selections in this maturity season and the flesh firmness holds well to 2 weeks or more after tree ripe. The seed parent, 2000,16-92, also possesses exceptional firmness but had a tendency to soften rapidly at about 10 days after tree ripe. By selfing (seed were derived from self-pollinations of the parent), we appear to have been successful in roguing-out some of the

softening factors while maintaining good fruit quality but have also lost a little in fruit size. Good brown-rot resistance is observed following Bostock lab inoculation (image) as well as in field evaluations in 2018, 2019 and 2020. Fruit is generally free from red skin blush or pit-staining except in very overripe fruit. 15.212

Klampt harvest time.

11,22-233. This selection ripens with Klampt and is derived from the F8,5-166 almond by peach hybrid source. This germplasm has been a good source for brown-rot resistance (see image following Bostock lab-controlled inoculation) and has also been a promising source for mildew resistance. The skin may show a slight blush covering about 40% of the fruit. Fruit flesh remains generally free of any red pit staining and associated pit fragmentation. Fruit maintained good firmness and a bright yellow-gold color 2 weeks or more after tree ripe. The tree is vigorous and productive producing large fruit of uniform size and shape. No leaf curl was observed in 2018 and 2019 field evaluations and a reduced mildew is generally observed in this selection. Although ripening with Klampt, the staying-power of this selection can be seen in the image at right showing fruit harvested on September 6, 2020.

11,9-104. Derived from the almond by peach F8,5-166 germplasm, from a cross between *ExtraLate-1* with *ExtraLate-6*, this selection harvests with *Klampt* despite the very late maturation of its parents. [As previously discussed, this transgressive harvest shift has been a useful strategy to target the Dixon maturity gap]. Fruit are large with uniform size and shape and free from red-blush as well as red pit-staining and associated pit fragmentation. Flesh color is a dark yellow gold that may be too dark for commercial production but acts two complement color when crossed with lighter colored breeding selections such as 17,3-185 described above. Good resistance to fruit brown-rot has been observed in the field and following Bostock lab inoculation (image). Good fruit firmness and quality is maintained up to 2 weeks following tree ripe. Processed fruit firmness averaged 8.4 pounds despite been harvested 10 days after tree ripe stage. Tree is moderately vigorous and productive.

11,6-80. This selection is also derived from almond germplasm but through a different lineage than the previously described selections. A earlier ripening budsport of Carson was used as the seed parent with the intention of targeting this maturity time with good size fruit and productive tree characteristics. Fruit share many of the same characteristics, being of good size and shape. Color is a commercially-desirable golden-yellow. Fruit skin can show about 30% red blush and flesh is free of red-staining and associated pit fragments. Some slight pinking can be observed in peach pits when fruit become overripe and while this cooks out and processing it precludes it probable release as a variety. A relatively small pit also contributes to improved case yields. Fruit flesh maintains good firmness and integrity for up to 2 weeks following tree-ripe, though rapid softening can occur in excessively overripe fruit. Some fruit brown-rot disease has been observed in the field, including 2018 and 2020 but this item has not yet been tested under controlled conditions of the Bostock lab. Tree is moderately vigorous and productive. Tree architecture is upright to upright spreading.

11,9-90. The last selection also has the most exotic lineage. The germplasm is derived from the wild almond species *Prunus argentea*, which shows promising levels of resistance to a number of diseases and environmental stresses but generally produces small poor quality fruits on a plant that is more shrub than tree. The result of a series of backcrosses to cultivated peach, culminating in self-pollinations to sort-out desirable from undesirable genes, this selection continues to show promise as a parent for future crossing and as a possible candidate for regional grower testing. Fruit are medium-sized and uniform with a moderate red blush, depending on year. Flesh is yellow to golden-yellow with some red pit staining in very over-ripe fruit in 2019 (but not 2020) with no serious pit fragmentation. Pit size is medium to small. Fruit have shown good resistance to fruit brown-rot disease in both Bostock lab evaluations (image) and field, including 2018, 2019 and 2020. Fruit show good firmness, which is maintained to 2 weeks after full-ripe. Fruit integrity is maintained even with overcooking during processing as occurred in 2016 (center image). The tree is vigorous, upright spreading and productive.