Almond Variety Development

Project No.: 18HORT1-Gradziel

Project Leader: Tom Gradziel Department of Plant Sciences UC Davis One Shields Ave. Davis, CA 95616 530.752.1575 tmgradziel@ucdavis.edu

Project Cooperators and Personnel:

- B. Lampinen, S. Marchand, J. Fresnedo, C. Crisosto,
- S. Metcalf, and M. Gilbert, UC Davis
- J. Adaskaveg, UC Riverside
- D. Doll, UCCE Merced County
- P. Gordon, UCCE Madera County
- J. Connell, Emeritus, UCCE Butte County
- F. Niederholzer, UCCE Colusa/Yolo/Sutter Counties
- R. Duncan, UCCE Stanislaus County
- L. Kinney Milliron Butte County

A. Summary

The goal of the Variety Development Program is to breed genetic solutions for current and emerging industry needs. These include self-fruitfulness, improved kernel quality and water use efficiency, improved disease and pest resistance, and adaptation to a changing climate. Objectives are organized into three time-frames: Genetic options available for immediate deployment, Next-generation varieties combining and consolidating proven elite genetics/genomics, and, Identification & maintenance of genetic options allowing continued production & profitability for anticipated as well as for unanticipated changes in future climatic/market/regulatory environments. UCD advanced breeding selections currently in Regional Variety Trials continue to show good performance for desirable traits such as self-fruitfulness, productivity and quality that were previously identified in smaller, multi-year, regional grower plots. The introduction of new genetic traits as part of the multi-decade project for transferring selffruitfulness to almond from peach and its wild relatives has also made available desirable new genetic options for improving production efficiency, including improved disease and pest resistance and orchard performance. Second-generation selections currently coming out of the UCD breeding pipeline combine desirable traits from different genetic backgrounds in order to improve the level of expression of traits such as self-compatibility while also improving consistency of performance under a wider range of environments and climates. Using food-safety as a case- study on the availability within current UCD breeding germplasm of traits required to address future industry needs, we have demonstrated that promising germplasm currently exists but risks being lost if not identified/maintained prior to being selected-against as part our current breeding focus to rapidly address more immediate production challenges.

Β. **Objectives**

The goal of the UCD Variety Development Program is to breed genetic solutions for current and emerging industry needs. These include self-fruitfulness, improved quality and water use efficiency, improved disease and pest resistance and adaptation to a changing climate. Objectives are organized into three time-frames:

- 1. Genetic options for immediate deployment.
- 2. Next-generation varieties combining/consolidating available elite and proven genetics/genomics.
- 3. Genetic options allowing continued production/profitability for anticipated as well as unanticipated changes in future climatic/market/regulatory environments.

С. Annual Results and Discussion

1. Genetic options for immediate deployment. Yield data is presented in Appendix-1 for the the 12 recent UCD releases and/or advanced selections currently in their 4th year of commercial production at Regional Variety Trials (RVT) in Butte, Stanislaus and Madera Counties. All selections had previously been evaluated for 6 to 8 years at smaller, regional grower trials to rogue-out genotypes showing any important deficiencies (and thus, from a commercial-confidence perspective, are similar to many of the recent privately released varieties). Continued RVT assessment provides

accurate and long-term regional performance appraisals for growers and processors and is required to identify the best genotypes for different production regions, market needs and climates by the time of their formal release. However, all items are concurrently also available for grower plantings under test- agreement as large, commercial-scale plantings. RVT data is crucial for not only assessing current performance but also identifying important production trends. For example, the bar-graph in Fig. 1 shows the correlation (and so predictability) of early-year yields with final Table 1 10-year cumulative yields for 20 selections over 10 years of evaluation at an earlier Kern County RVT. Only the 4th-year harvest data provided significant predictability of long-term productivity (Pearson correlation coefficient = 0.63). The reason that early yields are poorly correlated with overall performance was that different selections achieve productivity through different strategies. For example, the line graphs in Fig. 1 show yearly yields (x 10,000 lbs) for Mission (+) producing primarily on spurs versus Nonpareil

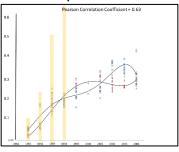


Figure 1

Variety	Lbs./Acre	\$\$/lb.	Classification	\$ Value
Kester / Hansen	2630	\$2.49	Carmel	6547
UCD18-20	2121	\$2.39	Monterey	5070
UCD8-160	1992	\$2.40	Wood Colony	4780
UCD7-159	1780	\$2.40	CA	4272
Kester	1618	\$2.49	Carmel	4029
UCD8-201	1660	\$2.40	CA	3983
UCD1-232	1646	\$2.40	CA	3950
UCD1-271	1630	\$2.40	CA	3913
Sweetheart	1554	\$2.40	CA	3730
Nonpareil	1377	\$2.65	Nonpareil	3650
Aldrich	1480	\$2.40	CA	3551
UCD3-40	1341	\$2.49	Carmel	3339
Winters	1341	\$2.49	Carmel	3338
UCD1-16	1295	\$2.54	Sonora	3289
UCD8-27	1062	\$2.40	CA	2549

(x) producing on a combination of terminal shoots and spurs. RVT yield data for 2019 (Appendix-1) show that Nonpareil is beginning its anticipated rise in the yield rankings at all sites. UCD selections are also prominent in high 4th-year yield positions at all sites. A strong UCD emphasis on nut quality is demonstrated in the desirable shell and kernel characteristics of samples collected for the ABC 2019 Crackout event (Appendix-2). Promising guality-based performance against Nonpareil and Aldrich standards becomes

more apparent following 'Duncan-testing' (i.e., showing predicted grower-returns by multiplying yields by anticipated market value, as in Table 1 for the 2019 Stanislaus RVT). Individual selection performance differed at the different RVT sites (Appendix-1) which was anticipated because the wide difference in RVT environments would favor different tree and growth characteristics. An example of this can be seen in the different capture levels of photosynthetically active radiation (PAR) in different selections at different sites. Because PAR is considered highly correlated with photosynthetic rate and so plant productivity, it is traditionally a good estimator of potential yield (see earlier annual RVT reports by Lampinen). While high yield per unit PAR is generally observed for more traditional varieties, sizable differences were observed particularly for the UCD self-fruitful selections. Because the traditional varieties have a very narrow genetic base and so a very limited variability in traits affecting production, a uniform yield/PAR ratio would be expected. Greater variability observed in new selections suggest that in addition to introducing the novel trait of self-compatibility/self-fruitfulness, we have also been able to introduce novel traits affecting productivity and thus may be able to overcome traditional germplasm-based production limits.

2. Next-generation varieties combining/consolidating available elite and proven genetics/genomics. UCD selections currently in RVT evaluation include breeding selections where self-fruitfulness has been introgressed or brought in from several different related species, including Prunus webbii, P. mira, and peach. As discussed in the previous section, in addition to self-fruitfulness we also appear to have been successful in bringing in novel traits that may allow us to overcome current production barriers. The next generation of varieties are the result of interbreeding parents from different sources to consolidate and enhance performance for targeted traits. For example, the mechanisms for self-compatibility (a requirement for self-fruitfulness) are different in P webbii vs. peach sources. Combining both traits in new 2nd generation selections could increase its total level of expression and should increase consistency of performance under a wider the range of environments and climates. (Because each gene/trait will have an environmental optimum, having 2 distinct genes/traits will expand environmental conditions were optimal performance occurs). A total of 25 selections are currently being propagated that combine desired traits such as self-fruitfulness, good nut and kernel quality (Appendix-2), improved disease/pest resistance, and greater genetic/genomic diversity (i.e., derived from multiple germplasm sources; see 2018 annual report). Based on performance this coming spring, 2020 as well as performance data since 2016, 12 of these genetically diverse genotypes will be selected for propagation for inclusion in the interim assessment evaluation plot to be planted in the fall/winter of 2020. The challenge is to capture as much germplasm diversity as possible while at the same time focusing selection towards those traits most needed for California production. As discussed in the next section, a greater challenge is to anticipate changes in the production, climatic, market, and regulatory environments over the next 30 to 50 years so that possible genetic solutions can be identified, retained and incorporated into third-generation breeding lines. [As an example, self-fruitfulness has become a grower priority only in the last several years. Fortunately, because it has been a UCD breeding priority since the mid-1990s, we now have a number of diverse and well adapted self-fruitful selections available to the California industry].

3. Genetic options allowing continued industry profitability. Self-fruitfulness has become highly desirable because of the rapid increase in California almond production acreage with a concurrent decrease in dependable pollinator availability (owing to colony-collapse and other honeybee afflictions). Neither of these conditions were widely anticipated in the 1990s when we began the long process of introgressing the novel trait of self-fruitfulness to almond from peach and its wild relatives. Relative to the past 20 years, the next 20 years will almost certainly involve significant changes in production, processing and marketing. The extraordinary and unparalleled intraspecific-breeding germplasm developed at UCD over the last 30 years offer similarly unparalleled opportunities to develop genetic/genomic solutions to these problems if anticipated and targeted before the necessary germplasm is lost through the equally desirable focused breeding towards more immediate goals. Examples of such opportunities are presented in the following study-results that we presented at the 2019 National Institute of Food and Agriculture sponsored workshop on breeding for future food safety. In this study we examined potential genetic solutions to nut allergenicity, aflatoxin contamination, and soilborne contaminants within the existing UCD breeding germplasm (includes relevant research citations).

Almond allergenicity (immunoreactivity). An extensive variability for all nut traits evaluated, including size, shape, soluble protein content and R-ELISA immunoreactivity was documented in this diverse UCD germplasm (Appendix-4 and Appendix-5). Kernel mass, a critical commercial trait, ranges from 0.11g to 2.08g. All commercial varieties were approximately 1 g or greater, which has been shown to be an important threshold for optimizing orchard yield (Gradziel and Lampinen, 2013). R-ELISA immunoreactivity values ranged from 0.26 to 2.18 times the level found in the Nonpareil standard, while soluble protein, an important trait in both processing and nutritional quality, ranges from 12.4 to 26.5 (g/100g). The lower immunoreactivity scores were more strongly associated with interspecific hybridizations lineages having peach or the wild almond species P. argentea or P. webbii, while the higher scores were associated with hybridizations with P. fenzliana, which is generally considered to be one of the species from which cultivated almond was derived (Gradziel, 2011). No correlation was observed between almond seed size and either total soluble protein or amandin content. R-ELISA did show a general increase with increases in soluble protein content when only commercial varieties were analyzed. This positive association between amandin and immunoreactivity is consistent with previous reports analyzing a broader range of commercial varieties that identified amandin, also known as almond major protein (AMP), prunin, 11S globulin, and Pru du 6, as the major storage protein in commercial almond seed (Sathe et al., 2001). This relation does not hold up, however, within the species, interspecies hybrids and introgressed germplasm. Of the 15 selections showing R-ELISA values of approximately one-half or less of the Nonpareil standard, four are found in those commercially desirable selections having an average kernel mass of approximately 1g or greater. All commercial varieties show R-ELISA values approaching or exceeding that of the Nonpareil standard with the exception of the Italian variety Tuono. Tuono is unique among Mediterranean and California varieties in that it is self-compatible. Recent molecular analysis has demonstrated the source of this self-compatibility was a natural introgression from P. webbii which is native in the regions of southern Italy were Tuono originated (Gradziel and Martínez-Gómez, 2013).

Similarly, the soluble protein content of 17.14 for Tuono is unusually low for a commercial cultivar, being well below the 20g/100g level

Table 2	Nonpareil	Sweetheart	Mission	Sonora
Total oil (% dry weight)	38.8 (0.3)	47.3 (1.2)	43.4 (1.2)	43.8 (2.3)
Oleic acid (%)	66.8 (0.8)	73.0 (1.3)	71.9 (2.3)	69.3 (2.3)
Aflatoxin (ug g-1 dry wt.)	0.17 (0.02)	0.04 (0.003)	0.20 (0.04)	0.25 (0.05)
Hull rot (%)	97.3 (8.8)	23.1 (6.9)	64.5 (6.7)	83.7 (6.1)
NOW (%)	79.5 (5.3)	4.1 (0.8)	39.8 (4.7)	64.1 (6.3)

desired for some forms of processing. Several advanced introgression breeding selections combine the desirable characteristics of sweet kernels with high mass and high soluble protein content with low immunoreactivity. These include selections #86, UCD,8-27 (Almond × (P. webbii × P. persica))BC3 [i.e., three consecutive backcrosses to almond], and selection #98, UCD,2-240 (Nonpareil × P. webbii)BC3. Both of these intraspecific breeding selections are currently being considered as improved almond selections based on their desirable kernel characteristics and high crop productivity. Aflatoxin. Sweetheart is a UCD released commercial cultivar originating as a Mission almond by peach introgression line (Mission × P. persica)BC3 in an effort to transfer self-fruitfulness from peach (Gradziel et al., 2001). While not expressing sufficiently high levels of self-fruitfulness to be commercially distinct. Sweetheart possesses an exceptionally high oil content as well as quality as demonstrated by its very high oleic acid content (Table 2) placing it in a premium roasting-quality category with the Spanish variety Marcona (Gradziel et al., 2013). Sweetheart is also exceptional in that, since its release in 2007, very few positive findings for aflatoxin contamination have been reported in commercial shipments. Early analysis by Gradziel et al. (2000) had shown significantly lower levels of aflatoxin production following inoculation under controlled laboratory conditions. More recent studies have shown that this variety also has higher resistance to hull-rot as well as NOW infestation (Table 2). Improved performance in a number of unrelated traits is not unusual in interspecific introgressions because of the inherently higher genetic and so trait variability compared with the highly inbred and so trait limited nature of most Californian varieties (Gradziel et al. 2001). In Sweetheart, however, these traits appear to be complementary in reducing the overall risk of aflatoxin contamination. Under field conditions, Aspegillus flavus infection usually occurs following kernel damage by NOW, where infestation acts to inoculate the normally shell-protected kernel and subsequent feeding creates a suitable environment for Aspegillus flavus growth and aflatoxin development (Hamby et al., 2011). Kernel infestation/infection can occur in the field from the time of fruit maturity (where the hull splits exposing the almond nut), to field harvest and again during storage prior to hulling and shelling. Because of the size of the 1 billion kg. (kernel meat) crop, fruit are often infield air-dried and held in bulk storage for several months or more. When properly dried, nuts are relatively resistant to new NOW infestation because the 1st-instar larvae are very small and particularly vulnerable to desiccation or starvation before it can access the kernel meat (Hamby et al., 2011). The occurrence of hull-rot during storage, however, acts to both macerated and hydrate hull tissue making it much more vulnerable to NOW infestation. Under these conditions, the multiple barriers found in the Sweetheart almond, including increased resistance to NOW as well as hull-rot development and the reduced tendency for aflatoxin production combined with a highlysealed shell have resulted in a high level of field resistance to this economically important insect-disease complex.

- 5 -

Soil-born contaminants. A major problem with soil contaminants such as salmonella, E. coli and pesticide residues is the difficulty in defining safe concentrations and so even trace level detection can lead to crop rejection. Avoiding contamination remains the most



effective strategy for ensuring food safety. Like peach, the almond kernel Figure 2 is enclosed in a lignified endocarp or shell (Fig. 2), which, if highly sealed, confers protection from infestation by NOW and other insect pests. Unfortunately, an important post-harvest role of the shell is to facilitate the uptake of moisture for seed hydration/germination. Danyluk et al. (2008) have demonstrated that this moisture uptake pathway also provides a ready conduit for the entrance of bacteria and contaminated water. A strategy currently being pursued by the California almond industry is the use of catch-frame harvesting as currently practiced for pistachio in California and some orchards in Spain because it avoids off-ground nut harvest with its high risk of soil contamination. In current practice, California almonds are shakeharvested to the orchard floor and allowed to dry in the Central Valley's warm, dry environment to kernel moisture levels of 7% or less to suppressed post-harvest disease. Dried fruit (hulls plus nuts) are then collected and bulk-stored until hull removal (hulling) and shelling in specialized industrial facilities. While off-site drying is feasible with the relatively limited production of California pistachio and Spanish almond, it present huge technical challenges for the 4 billion kg almond crop (2 billion kg in hulls, 1 billion kg in shells and 1 billion kg in kernel-meat). Infield hulling at harvest would reduce the postharvest handling tonnage by half and allow the vegetative hulls to be reincorporated into orchard soils in a more sustainable manner. Unlike Spanish almonds where the thick, highly lignified shells typically constitute about two thirds of the nut mass (Fig. 2); California almonds have relatively thin, 'paper' shells that dramatically improve harvest index and shelling efficiency. The fragile nature of traditional California almond shells would result in unacceptable levels of nut and kernel damage with the mechanically intensive in-field hulling, while the highly lignified Spanish-type shells would dramatically reduce harvest efficiency and would require extensive retooling of industrial shelling equipment. Certain wild almond species such as P. argentea, P. bucharica and P. webbii (#99, #105, & 107 in Appendix-4 and 5) possess a thin, highly lignified shell that confers high structural strength while allowing a high kernel-to-nut 'crack-out' ratio. This trait has proven highly heritable in certain P. webbii introgression lines allowing the development of California-adapted almonds possessing thin yet highly lignified P. webbii-type shells. An example can be seen in the previously discussed low-aflatoxin selection UCD,2-240 (#98 in Appendix-4 and Appendix-5, and Fig. 2). Combining good kernel size and quality with a durable, highly-sealed shell having a kernel to nut crack out ratios of 70%, UCD,2-240 is currently undergoing field testing as a candidate for almond catch-frame harvest.

D. Outreach Activities

15-Jan	PAG Conference	Almond phytomediomics	(~30 participants)
5-Jun	NIFA workshop	Breeding for food safety	(~50 participants)
21-Jan	Washington Post Interview	Climate change	(~30 participants)
8-Apr	Nursery/grower visit	Issues in new nursery varieties	(~16 participants)
14-May	Nursery Visits	Almond Bud-Failure	(~14 participants)
21-May	Farm Advisor Tour	Self-Fruitfulness in Almond	(~35 participants)
24-Jul	ABC Almond breed.workshop	UCD almond breeding	(~ 30 participants)
30-Sep	Almond Short Course	Flower develop. and pollination	(~800 participants)
1-Oct	Western Nut Grower	Article on Kester variety	(~4000 readers)
13-Nov	ABC Crackout	UCD samples	(~ 40 participants)
10-Dec	ABC Annual Conference	poster presentation	(~40 participants)
16-Dec	UCD Plant Breeders Conf.	Unique genomics for clone breed	(~90 participants)
3-Feb	UCD seminar	Breed. for Food Safety and Almond	(~30 participants)
5-Feb	American Society of Agronomy	Almond breeding challenges	(~80 anticipated)
7-Feb	ABC Rootstock workshop	UCD almond breeding	(~30 anticipated)

E. Materials and methods

- **Genetic material.** A diverse germplasm, including heirloom varieties, and related Prunus species and inter-species hybrids and introgression lines has been developed at the UCD almond breeding program as detailed in 2017 and 2018 annual reports
- **Hybridizations, introgression in general breeding methods**. Breeding strategies, including standard and modified intra-and interspecific hybridization methods as well as marker assisted breeding are routinely employed as detailed in 2017 and 2018 annual reports.
- **Production and harvest quality analysis**. Methods for characterizing orchard yield and production traits have been presented in detail in previous RVT annual report by Lampinen et al.
- Seed soluble protein and immunoreactivity. Whole seeds were ground to pass through a 20-mesh sieve and soluble proteins were extracted in borate saline buffer). Flours were defatted and subjected to previously reported amandin cryoprecipitation methods (Su et al. 2015, 2017; Liu et al. 2017). Soluble protein was determined by Bradford and Lowry methods. Solubilized proteins were analyzed using electrophoresis and immunoassays employing mAbs 4C10 to assess conformational epitope immunoreactivity as described in Su et al. (2015).
- **Aflatoxin.** Whole seeds were ground to a fine powder as described above. A mixture of 5% almond kernel powder and 1.5% agar in 40 mL water was autoclaved and 10 mL sterile solution poured into 60-mm petri dishes. Each petri dish was inoculated with 200 spores of A. flavus and incubated at 30 °C for 7 d as described by Gradziel et al. (2000).
- **Oil content and composition**. Total fat content and fatty-acid methyl esters (FAMEs) were determined according to the procedure of Garces and Mancha (1993). The FAMEs were identified based on retention times of known standards (Sigma, St. Louis). The presence of 17:0 as an internal standard allowed the calculation of the

total lipids based on the area of the standard. Data were recorded on a dry-weight (DW) basis and analyzed as previously described by Abdallah et al. (1998).

- Navel Orangeworm (NOW) infestation. Fruits were collected from UCD research plots at Winters, CA and inspected visually to ensure no previous infestation by navel Orangeworm (NOW). A total of 24 nuts of each selection were tested as exposed kernels (shells broken to expose kernels). Samples were placed in individual plastic containers with 15 NOW eggs added and incubated at 25° C for 90 days. Proportion of samples containing mature NOW moths at the end after 90 days were recorded.
- **Hull-rot.** Disease assessment was as described by Fresnedo-Ramírez et al. (2017). Fruit from each selection were harvested from UCD research plots at Winters, CA and surface sterilized, rinsed in deionized water, and dried. A total of 24 unblemished hulls for each selection were inoculated with a 10 μL droplet containing conidia of Monilinia fructicola. (mixed field isolates) at a concentration of 2.5×104 spores per mL from 7 to 10-day-old cultures. Disease severity for each selection was calculated as the proportion of fruit with lesions greater than 3 mm. at 3 days after inoculation and incubation of the hulls in the humidified containers at room temperature.

NIFA Study References

- Abdallah, A., Ahumada, M.H., and Gradziel, T.M. (1998). Oil content and fatty acid composition of almond kernels from different genotypes and California production regions. Journal of the American Society for Horticultural Science. 123, 1029-1033.
- Danyluk, M.D., Brandl A., and Harris L.J. (2008). Migration of salmonella enteritidis phage Type 30 through almond hulls and shells. Journal of Food Protection. 71, 397–401.
- Fresnedo-Ramírez, J., Famula, T.R., and Gradzie,I T.M. (2017). Application of a Bayesian ordinal animal model for the estimation of breeding values for the resistance to Monilinia fruticola (G.Winter) Honey in progenies of peach [Prunus persica (L.) Batsch). Breeding Science Preview doi:10.1270/jsbbs.16027.
- Garces, R., and Mancha M. (1993). One-step lipid Extraction and fatty acid methyl esters preparation from fresh plant tissues. Analytical Biochemistry. 211, 139-143.
- Gradziel, TM. (2017). History of cultivation. In: Socias I Company and T. Gradziel {Editors} Almonds: Botany, Production and Uses. Pp. 43-69. CABI Press, Boston 494 pgs.
- Gradziel, T.M. (2011). Almond origin and domestication. In J. Janick (ed.) Horticultural Reviews. 38, 23-82.
- Gradziel, T.M., Curtis, R., and Socias i Company, R. (2017) Production and growing regions. In: Socias I Company and T. Gradziel {Editors}) Almonds: Botany, Production and Uses. Pp. 70-86. CABI Press, Boston 494 pgs.
- Gradziel, T.M. and Lampinen B.D. (2013). Defining the limits of almond productivity to facilitate marker assisted selection and orchard management. Acta Hort. 912, 33-39.
- Gradziel, T., Lampinen, B., Niederholzer, F., and Viveros, M. (2013). 'Sweetheart' almond: A fully cross-compatible pollenizer for the early Nonpareil bloom that exhibits very high Marcona-type kernel quality. HortScience. 48, 1320–1322.
- Gradziel, T., Mahoney, N. and Abdallah, A. (2000). Aflatoxin production among almond genotypes is unrelated to either kernel oil composition or Aspergillus flavus growth rate. HortScience. 35, 937-939.

- 8 -

- Gradziel, T.M. and Martínez-Gómez, P. (2013). Almond Breeding. Plant Breeding Reviews 37. 207-258.
- Gradziel T.M., Martínez-Gómez P., Dicenta, F. and Kester, D.E. (2001). The utilization of related almond species for almond variety improvement. J. Amer. Pomol. Soc. 55, 100-108.
- Hamby, K., Gao, L.W., Lampinen, B., Gradziel, T. and Zalom, F. (2011). Hull split date and Shell seal in relation to navel Orangeworm (Lepidoptera: Pyralidae) infestation of almonds J. Econ. Entom. 104-965-969.
- Liu, C, Chhabra, G. S., Zhao, J., Zaffran, V. D., Gupta, S, Roux, K. H., Gradziel, T. M., Sathe, S. K. (2017). Comparison of laboratory developed and commercial monoclonal antibody-based sandwich enzyme-linked immunosorbent assays for almond (Prunus dulcis) detection and quantification. J Food Sci. 10, 2504-2515. doi: 10.1111/1750-3841.13829. Epub
- Sathe, S. K., Teuber, S. S., Gradziel, T. M., and Roux, K. H. (2001). Electrophoretic and immunological analyses of almond (Prunus dulcis L.) genotypes and interspecies hybrids. J. Agric. Food Chem., 49, 2043-2052.
- Socias i Company, R., Kodad, O., Alonso, J. M., and Gradziel, T. M. (2008). Almond quality: A breeding perspective. In J. Janick (ed.) Horticultural Reviews. 34, 197-238.
- Su, M., Liu, C., Roux, K. H., Gradziel, T. M., Sathe, S. K. (2017). Effects of processing and storage on almond (Prunus dulcis L.) amandin immunoreactivity. Food Res Int. 100, 87-95. doi: 10.1016/j.foodres.2017.06.061. It's
- Su, M. N., Venkatachalam, M., Gradziel, T. M., Liu, C. Q., Zhang, Y., Roux, K. H., and Sathe, S. K. (2015). Application of mouse monoclonal antibody (mAb) 4C10-based enzyme-linked immunosorbent assay (ELISA) for amandin detection in almond (Prunus dulcis L.) genotypes and hybrids. LWT - Food Science and Technology. 60, 535-543.
- Zeinalabedini, M., Khayam-Nekoui, M., Grigorian, V. Gradziel, T. M., and Martinez- Gomez, P. (2010). The origin and dissemination of the cultivated almond as determined by nuclear and chloroplast SSR marker analysis. Scientia Horticulturae 125, 593-601.

F. Publications that emerged from this work

- Gradziel T, B. Lampinen and J.E. Preece. (2019). Propagation from Basal Epicormic Meristems Remediates an Aging-Related Disorder in Almond Clones. Horticulturae 2019, 5(2), 28; https://doi.org/10.3390/horticulturae5020028
- Gradziel, Thomas M. and Jonathan Fresnedo-Ramírez. (2019). Noninfectious Budfailure As a Model for Studying Age Related Genetic Disorders in Long-Lived Perennial Plants. Journal of the American Pomological Society 73(4): 240-253 2019
- Liu, Ting-Hang, Mohammad A. Yaghmour, Miin-Huey Lee, Thomas M. Gradziel, Johan Leveau, and Richard M. Bostock. 2019. A roGFP2-based bacterial bioreporter for redox sensing of plant surfaces. Phytopathology September 4, 2019. https://doi.org/10.1094/PHYTO-07-19-0237-R
- Gradziel, T. M., and. B. Lampinen. 2019. 'Kester' Almond: A Pollenizer for the Late 'Nonpareil' Bloom with High Yield and Kernel Quality. HORTSCIENCE 54(n):1–2. 2019. Https://doi.org/10.21273/HORTSCI14398-19
- Gradziel, T. M. 2019. 'Kester' Almond: A Pollenizer for the late 'Nonpareil' bloom as a possible Padre replacement. Western Nut Grower, Fall, 2019.

Appendix 1. Cumulative yield data (left), 2019 yield, (center left) midday PAR interception (center right) and yield per unit PAR intercepted (right) are presented below. Note that Wood Colony at the Madera site is one year younger. Common letters indicate differences are not significantly different at the 5% level of significance.

	201 Variety or selection kern	19 yield el lbs/ac	inte Variety or selection	PAR erception (%)	Yield per unit PAR Variety or selection intercepted				
Butte	Nonparel Nonparel UCD3-40 Booth Jenette Capitola UCD18-20 Winters UCD7-159 Durango Supareli Aldrich Folsom Kester Wood Colony Bennett UCD1-16 Y117-91-03 Y117-86-03 UCD8-201 Sterling UCD1-232 Y116-161-99 UCD8-161-99 UCD8-161-99 UCD8-161 Self-fuitful P16.013 UCD1-271	Bask C 2999 a 2701 a b 27613 a b c 25613 a b c 22653 a b c 22683 a b c 22683 a b c 22144 b c d 2016 b c d 20204 b c d 20206 b c d 20206 b c d 1989 b c d 1988 b c d 1846 d d 1846 d d 1848 d d 1849 d d 1803 d d 1803 d d 1803 d e 1804 d e 1748 <th>Valley of Selection Capitola Suparell Nonparell Sweetheant Y117-91-03 Folsom UCD3-40 Kester Booth Winters UCD18-20 UCD1-16 Sterling Bennett UCD8-27 Y117-86-03 Aldrich Kester/Hansen Self-fruitful P13.019 Eddie UCD8-201 UCD7-159 UCD8-201 UCD7-159 UCD8-201 UCD7-159 UCD8-201 UCD7-159 UCD1-232 Jenette Y116-161-99 UCD1-21 self-fruitful P16.013 Wood Colony UCD8-160</th> <th>78.8 a 78.6 a 78.7 a 75.7 a 73.6 a 73.6 a 73.6 a 73.7 a 73.6 a 72.0 a b c 72.0 a b c 72.0 a b c 73.6 a b c a b a b a b c d a b b c b c b c b c b c a b c d b c</th> <th>Variety of selection Jenette UCD3-40 Nonpareli UCD8-160 Wood Colony Booth UCD7-159 Warcona UCD7-159 Winters UCD7-320 Aldrich UCD8-201 UCD1-322 Aldrich UCD1-322 Aldrich UCD1-320 UCD1-16 Capitola Bennett Self-fruitful P13.019 Folsom Kester Y117-86-03 Eddie Sterling Kester/Hansen UCD8-27 Supareli Y117-91-03 Sweetheart self-fruitful P16.013 UCD1-271</th> <th>$\begin{array}{cccccccccccccccccccccccccccccccccccc$</th>	Valley of Selection Capitola Suparell Nonparell Sweetheant Y117-91-03 Folsom UCD3-40 Kester Booth Winters UCD18-20 UCD1-16 Sterling Bennett UCD8-27 Y117-86-03 Aldrich Kester/Hansen Self-fruitful P13.019 Eddie UCD8-201 UCD7-159 UCD8-201 UCD7-159 UCD8-201 UCD7-159 UCD8-201 UCD7-159 UCD1-232 Jenette Y116-161-99 UCD1-21 self-fruitful P16.013 Wood Colony UCD8-160	78.8 a 78.6 a 78.7 a 75.7 a 73.6 a 73.6 a 73.6 a 73.7 a 73.6 a 72.0 a b c 72.0 a b c 72.0 a b c 73.6 a b c a b a b a b c d a b b c b c b c b c b c a b c d b c	Variety of selection Jenette UCD3-40 Nonpareli UCD8-160 Wood Colony Booth UCD7-159 Warcona UCD7-159 Winters UCD7-320 Aldrich UCD8-201 UCD1-322 Aldrich UCD1-322 Aldrich UCD1-320 UCD1-16 Capitola Bennett Self-fruitful P13.019 Folsom Kester Y117-86-03 Eddie Sterling Kester/Hansen UCD8-27 Supareli Y117-91-03 Sweetheart self-fruitful P16.013 UCD1-271	$\begin{array}{cccccccccccccccccccccccccccccccccccc$			
Stanislaus	Kester/Hansen UCD18-20 UCD7-159 UCD7-159 Y117-91-03 Y116-161-99 UCD1-271 Kester Folsom Self-fruitful P13.019 Sweetheart Booth Durango Aldrich Y117-86-03 Sterling Bennett Nonpareil Y121-42-99 UCD3-40 Winters Jenette UCD1-16 Capitola UCD1-27 Eddie self-fruitful P16-013	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Kester/Hansen Sweetheart Supareii Y117-91-03 Booth Eddie Capitola UCD3-40 Self-fruifut P13.019 UCD18-20 Sterfing UCD18-20 Sterfing UCD2-21 Bennett Folsom Durango UCD1-232 Aldrich Jenette UCD1-232 Aldrich Jenette UCD1-16 Nonpareii UCD1-16 Nonpareii UCD1-16 Nonpareii UCD1-16 Nonpareii UCD1-16 Nonpareii UCD1-16 Nonpareii UCD2-19 Y117-86-03 Y116-161-99 UCD8-201 Winters Self-fruifutµP16-013 UCD8-160		Y116-161-99 UCD8-160 Nonpareli Y121-42-99 Y117-91-03 UCD18-20 Kester/Hansen UCD7-159 UCD8-201 Winters UCD1-232 Y117-86-03 Folsom UCD1-232 Y117-86-03 Folsom UCD1-232 Y117-86-03 Folsom UCD1-271 Supareli Aldrich 2-19E Durango Stelf-fruit/ful P13.019 Stelffruit/ful P13.019 Stelfnette Bennett Bennett Bennett Booth Swetheart Eddid UCD3-40 Capitola self-fruit/ful P16-013 UCD8-27	$\begin{array}{cccccccccccccccccccccccccccccccccccc$			
Madera	Winters Capitola Sweetheart UCD-1-16 Y-116-161-99 Folsom Booth Suparell Kester UCD-7-159 Sterling UCD-8-160 Jenette Y-117-91-03 Wood Colony Y-121-42-99 Y-117-86-03 UCD-1-232 UCD-8-27 Eddle Aldrich Self-fr-P13-019 UCD-8-201 Durango Self-fr-P16-013 Bennett UCD-3-40 UCD-1-271	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Folsom Capitola Booth Suparell Sterling Eddie Y-121-42-99 UCD-1-271 Nonparell Aldrich Sweetheart Kester Self-fr-P16-013 UCD-3-40 Durango UCD-8-27 UCD-7-159 Self-fr-P13-019 Bennett Winters UCD-1-322 Y-116-161-99 UCD-18-20 Y-117-91-03 Jenette Wood Colony Y-117-96-03 UCD-8-201 UCD-8-160	91.2 a b s <td>Winters UCD-1-16 Y-116-161-99 UCD-8-160 Sweetheart UCD-18-20 Jenette Capitola UCD-7-159 Wood Colony Y-117-91-03 Kester UCD-8-201 Y-117-86-03 Self-fr-P13-019 Folsom Supareli Booth UCD-1-232 Sterling UCD-8-27 Y-121-42-99 Aldrich Eddie Durango Self-fr-P16-013 Bennett UCD-3-40 UCD-1-271</td> <td></td>	Winters UCD-1-16 Y-116-161-99 UCD-8-160 Sweetheart UCD-18-20 Jenette Capitola UCD-7-159 Wood Colony Y-117-91-03 Kester UCD-8-201 Y-117-86-03 Self-fr-P13-019 Folsom Supareli Booth UCD-1-232 Sterling UCD-8-27 Y-121-42-99 Aldrich Eddie Durango Self-fr-P16-013 Bennett UCD-3-40 UCD-1-271				

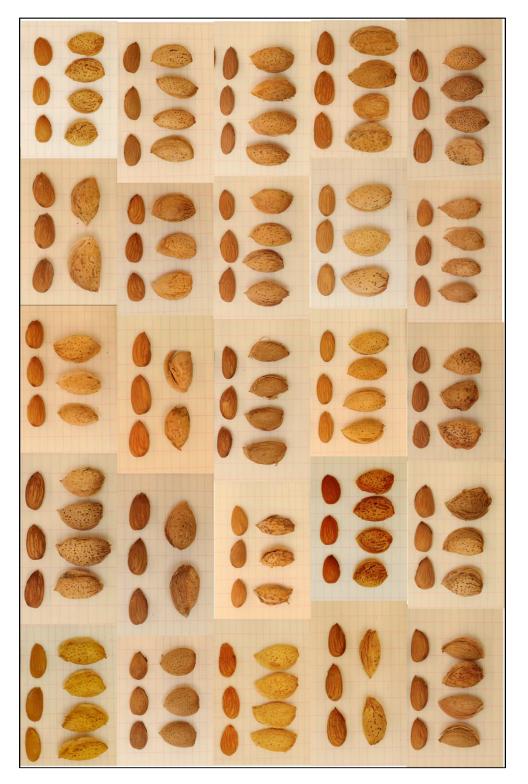
Stanislaus

Madera

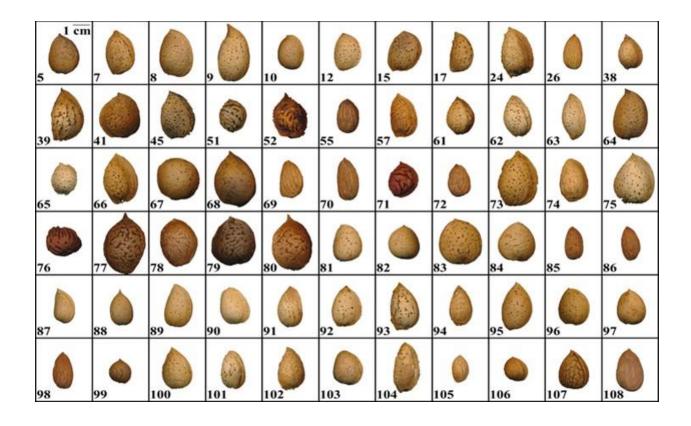
Appendix 2. In-shell nut and and kernel characteristics advanced UCD selections sampled from RVT's for the ABC 2019 crackout event.



Appendix 3. In-shell nut and kernel characteristics UCD selections being advanced and propagated for consideration of inclusion in 2020 Interim Almond Evaluation Trials. [A total of 12 genotypes will be selected for the initial 2020 plantings].



Appendix 4. In-shell nut characteristics for representative of the intra- and interspecific almond breeding germplasm at UCD. (Identifying numbers refer to the first column of Appendix-5).



Almond Variety Development

Appendix 5. Nut and kernel characteristics, including R-ELISA immunoreactivity values, for an intra- and interspecific almond breeding germplasm at UCD. (Values for cultivated varieties given in bold for reference).

No.	Genotype	Origin	Percent Almond	Kernel Length (mm)	Kernel Width (mm)	Kernel Breadth (mm)	Kernel Mass (g)	Nut Length (mm)	Nut Width (mm)	Nut Breadth (mm)	Nut Mass (g)	Soluble protein (g/100g)	R- ELISA
51	40A-17	Peach (P. persica) (bitter seed)	0	13.4	7.2	3.4	0.11	24.3	16.8	12.5	1.81	23.74	0.51
105	A10-4	P. bucharica (bitter seed)	0	14.3	6.6	4.7	0.21	19.1	10.3	7.4	0.58	20.94	0.59
71	P11-58	P. mira (bitter seed)	0	14.5	9.9	4.3	0.29	26.6	17.8	12.8	2.48	23.39	0.53
52	Andross	Peach (P. persica) (bitter seed)	0	17.8	11.4	3.9	0.36	35.3	26.1	19.5	6.21	20.65	0.39
99	A7-23	P. argentea (bitter seed)	0	13.4	9.7	6.0	0.37	19.0	15.3	12.1	1.47	17.28	0.61
76	A13-1	<i>P. persica</i> \times <i>P. davidiana</i> (bitter seed)	0	13.8	11.4	6.1	0.46	21.5	20.7	17.8	3.83	23.41	0.45
87	A7-28	P. webbii (bitter seed)	0	18.4	9.1	6.3	0.49	25.7	14.1	10.2	1.39	21.04	0.88
106	A2-11	P. tangutica (bitter seed)	0	13.4	10.3	8.3	0.49	16.5	15.2	12.4	1.34	25.44	0.70
88	F5,4-42	Almond x P. webbii (F2)	50	18.5	9.5	6.7	0.55	26.8	15.0	10.8	1.96	25.80	0.64
81	F10D,3-7	$(Almond \times (P. webbii \times P. persica))$ (BC1)	75	20.5	10.6	6.7	0.69	26.3	16.6	12.6	1.41	15.35	0.42
38	SB13,54-39E	(Nonpareil $\times P.$ persica) BC3	94	16.9	10.2	8.2	0.70	26.2	15.8	12.3	1.05	21.51	1.96
97	F10D,3-24	Almond x P. webbii (BC1)	75	19.3	13.2	6.1	0.71	25.7	19.5	13.3	2.66	13.39	1.27
17	F8N,7-4	F5,4-10 \times Sonora	62	22.7	10.7	6.2	0.76	32.0	16.1	10.7	1.17	19.52	0.65
94	F10D,2-5	Almond x P. webbii (BC1)	75	20.8	9.8	8.1	0.76	28.7	14.6	11.3	1.23	17.99	0.47
93	F10D,3-2	Almond x P. webbii (BC1)	75	19.7	11.1	7.0	0.77	30.6	17.8	13.6	1.53	17.84	0.66
101	F10D,2-12	Almond x P. fenzliana (F2)	50	20.6	10.8	7.0	0.77	26.5	16.1	11.5	1.41	21.38	1.53
5	F5,4-10	<i>P. webbii</i> \times (Nonpareil \times <i>P. persica</i>)	25	19.7	11.9	7.2	0.78	27.5	18.3	12.8	2.69	22.12	0.53
82	F10D,2-18	Nonpareil almond x P. webbii (BC1)	75	19.0	10.8	8.5	0.80	24.9	17.5	13.1	1.95	22.40	0.76
10	F5,10-9	(Mission \times <i>P. fenzliana</i>) BC1 \times Sonora	88	21.1	12.2	7.0	0.82	27.3	18.8	14.2	3.08	18.11	0.61
107	A7-25	P. webbii (bitter seed)	0	20.4	11.8	7.3	0.82	29.0	18.3	13.7	2.93	19.09	0.51
96	F10D,3-13	Almond x P. webbii (BC1)	75	19.4	12.0	8.0	0.83	25.4	19.1	13.7	1.85	17.07	0.47
7	F5,6-13	(Mission \times <i>P. fenzliana</i>) BC1 \times Sonora	88	22.1	10.8	6.7	0.84	32.0	17.3	10.5	1.66	25.60	0.95
84	F10D,3-23	Padre almond \times P. webbii (BC1)	75	20.4	11.9	7.7	0.84	27.5	19.8	13.4	2.32	14.48	1.49
92	F10D,1-2	Almond x P. webbii (BC1)	75	20.8	12.2	7.2	0.84	30.0	19.8	14.2	1.59	20.40	0.68
57	F5,16-60	(Mission almond $\times P$. argentea) F2	50	23.8	11.1	7.3	0.87	32.9	17.1	11.9	1.56	24.08	0.44
95	F10D,3-26	Almond $\times P$. webbii (BC1)	75	24.1	11.4	7.5	0.93	33.6	20.3	14.4	3.23	21.17	1.06
91	F10D,1-4	Almond x P. webbii (BC1)	75	23.1	11.9	7.6	0.95	30.8	18.1	13.3	1.94	20.50	1.32
62	Chips	Almond variety	100	21.5	12.7	8.2	0.96	28.7	19.5	14.7	2.02	26.46	1.68
15	F8N,6-68	F5,4-10 \times Solano	62	21.6	12.5	7.2	0.96	30.7	19.9	14.4	1.89	23.47	0.88
89	F10D,3-15	Almond \times P. webbii (F2BC1)	75	24.0	12.9	7.2	0.96	33.3	21.0	14.6	4.10	18.58	0.33

100	F10D,3-3	Almond x P. argentea (BC1)	75	23.4	12.4	7.0	0.96	29.6	18.6	13.8	1.88	17.47	0.26
90	F10D,1-22	Almond x P. webbii (F2BC1)	75	21.6	12.7	7.7	0.97	28.9	21.4	15.2	2.45	21.05	1.78
65	Sweetheart	Almond variety (Peach x Almond)BC3	94	19.1	12.5	8.8	0.98	22.5	19.0	14.3	1.54	25.52	1.73
80	2005,20-192	(Nonpareil $\times P.$ persica) BC3	94	20.6	14.6	7.4	0.99	37.1	26.5	19.3	7.31	23.91	0.63
12	F5,20-42	Padre \times F5,4-10	62	21.4	12.1	8.2	1.00	26.8	17.9	14.0	1.87	16.72	0.65
102	F10D,2-14	Almond x P. fenzliana (F2)	50	22.3	11.4	8.4	1.03	30.6	16.5	11.3	4.54	19.21	1.66
61	Mission	Almond variety	100	20.8	12.4	8.9	1.04	27.9	19.8	15.8	2.55	19.17	0.86
9	F5,13-54	(Mission \times <i>P. fenzliana</i>) BC1 \times Sonora	88	23.7	11.9	8.3	1.05	37.2	19.5	16.7	2.94	16.28	0.70
39	8010-22	Nonpareil × F5,4-10	62	24.6	12.5	7.1	1.05	37.6	19.3	14.1	1.90	21.06	2.09
41	F10C,12-28	(Nonpareil $\times P. persica$) F2	50	20.2	13.0	9.0	1.08	35.1	23.9	18.0	4.96	19.32	1.76
45	F10C,20-51	(Nonpareil \times <i>P. persica</i>) F2 (bitter seed)	50	25.1	12.6	7.3	1.10	35.1	21.3	15.0	2.43	23.87	0.56
83	F10D,1-26	Nonpareil × F5,4-10	62	23.1	14.2	6.9	1.11	30.8	24.8	15.8	3.88	17.64	1.61
78	Hansen5	Almond $\times P$. persica	50	23.8	13.9	7.5	1.12	34.5	24.6	18.9	7.44	21.06	0.66
103	F10D,2-3	(Mission \times <i>P. fenzliana</i>) BC1 \times Sonora	88	21.8	13.2	8.9	1.13	27.6	20.1	16.3	3.24	20.71	1.56
55	SB13,25-75	Nonpareil × F5,4-10	62	23.1	12.5	7.8	1.17	30.0	22.3	14.7	2.56	22.18	1.78
85	UCD,2-3	$(Almond \times (P. webbii \times P. persica)) (BC3)$	94	23.9	11.6	9.0	1.17	31.8	22.4	14.6	4.74	19.89	1.93
63	Kahl	Almond variety	100	26.0	12.1	8.0	1.20	34.3	17.0	15.0	2.20	26.29	1.22
86	UCD,8-27	(Almond \times (P. webbii \times P. persica)) (BC3)	94	24.3	12.1	8.6	1.20	30.4	20.9	14.2	3.36	23.92	0.55
66	Winters	Almond variety	100	26.3	11.9	8.1	1.21	36.4	19.3	14.1	2.09	22.37	1.05
75	2004,9-1	Nonpareil \times 97,1-232	91	25.0	13.5	7.5	1.24	34.3	23.8	18.1	3.15	14.54	1.89
74	2004,8-201	Nonpareil × 97,1-232	91	24.1	13.0	8.1	1.26	32.1	21.5	14.0	2.06	15.81	1.67
98	UCD,2-240	(Nonpareil $\times P$. webbii) BC3	94	23.8	12.6	9.5	1.28	30.3	24.3	14.3	5.62	22.22	0.40
72	97,1-232	SB13,25-75 \times Winters (see No. 55)	81	23.6	13.4	8.2	1.29	31.3	20.4	13.5	2.27	20.61	2.06
26	Nonpareil	Almond variety	100	24.7	13.5	7.9	1.31	34.3	17.0	15.0	2.20	23.07	1.02
8	F5,6-1	(Mission \times <i>P. fenzliana</i>) BC2	88	23.0	14.6	7.4	1.33	33.8	23.7	16.8	5.08	25.88	0.92
77	Hansen2	Almond $\times P$. persica Rootstock	50	28.0	15.7	7.3	1.44	44.1	28.5	18.3	9.07	12.35	1.57
64	Ferragnes	Almond variety (France)	100	26.8	14.2	8.3	1.48	36.4	23.1	17.0	4.09	19.37	1.56
24	Sonora	Almond variety	100	27.7	13.1	7.8	1.52	37.0	18.9	12.7	2.25	22.07	0.74
79	Nickels	Almond $\times P$. persica	50	23.9	16.4	8.8	1.53	36.9	28.7	20.9	9.18	13.79	0.75
67	Marcona	Almond variety (Spain)	100	22.0	17.3	8.8	1.55	29.4	25.8	19.6	5.55	22.22	0.88
68	Tuono	Almond variety (Italy)	94	26.4	16.3	8.2	1.58	38.4	27.7	18.3	5.45	17.14	0.52
104	F10D,3-50	Almond $\times P$. fenzliana (BC1)	75	27.3	13.9	8.8	1.59	36.2	19.3	13.3	2.37	15.37	2.18
73	2004,8-160	Nonpareil almond \times 97,1-232 (see No. 72)	91	28.6	14.2	8.6	1.77	38.5	22.5	15.4	2.96	19.84	2.00
108	97,3-40	$(Almond \times (P. webbii \times P. persica)) (BC2)$	88	33.3	15.1	8.7	2.08	39.2	29.6	18.8	9.21	25.31	0.90