Interspecific Breeding Germplasm for Rootstock Research and Development

Project No.: **17-HORT10-Gradziel**

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Objectives:

A. Separate out general hybrid-vigor effects from specific major gene control of desired rootstock traits to allow more predictable progress by public and private breeding programs targeting rootstock improvement.
B. Compile a more comprehensive knowledge of breeding value and deficiencies for this genetically diverse germplasm.
C. Improve methods to generate and clonally propagated large interspecies-hybrid populations to capture targeted traits within a commercially viable background. Concurrently, developed methods to generate large segregating progeny populations from species and hybrids in order to sort out major gene effects of *Objective A*.
D. Generate new and diverse species-hybrids with promising rootstock potential for testing and selection. Also, develop and test methods for generating binary or chimeric rootstocks, that is, rootstocks combining 2 or more segments from different species.

Interpretive Summary:

Changes in orchard land and water quality and subsequent management and cultural practices have led to the need for a new generation of rootstocks with improved disease and environmental stress resistance. Responding to this need, a number of public and private efforts have been initiated to develop and test new rootstocks for California tree

Item	Genetic background
Atlas	Almond, Peach, P. davidiana, Plu8m
Bright Hybrid	Almond, Peach, P. davidiana
Cadaman	Peach x P. davidiana
Citation	Almond, Plum
Compass	P. besseyi x P. americana
Controller 5	P. salicina x Peach
Cornerstone	Peach x Almond
Empyrean#1	Peach x P. davidiana
Flordaguard	Peach x P. davidiana
Hansen 536	Almond, Peach, P. davidiana
Hiawatha	P. besseyi x P. salicina
Ishtara	P. cerasifera, P. saliciana, Peach
Krymsk#86	Peach x P. cerasifera
Marianna 2624	P.munsoniana x P. cerasifera x P. hortulana
Nemaguard	Peach x P. davidiana
Nemared	Peach x P. davidiana
Nickels	Almond, Peach, P. davidiana
Paramount	Peach x Almond
Viking	Almond, P. blireiana, P. cerasifera, P. Mume

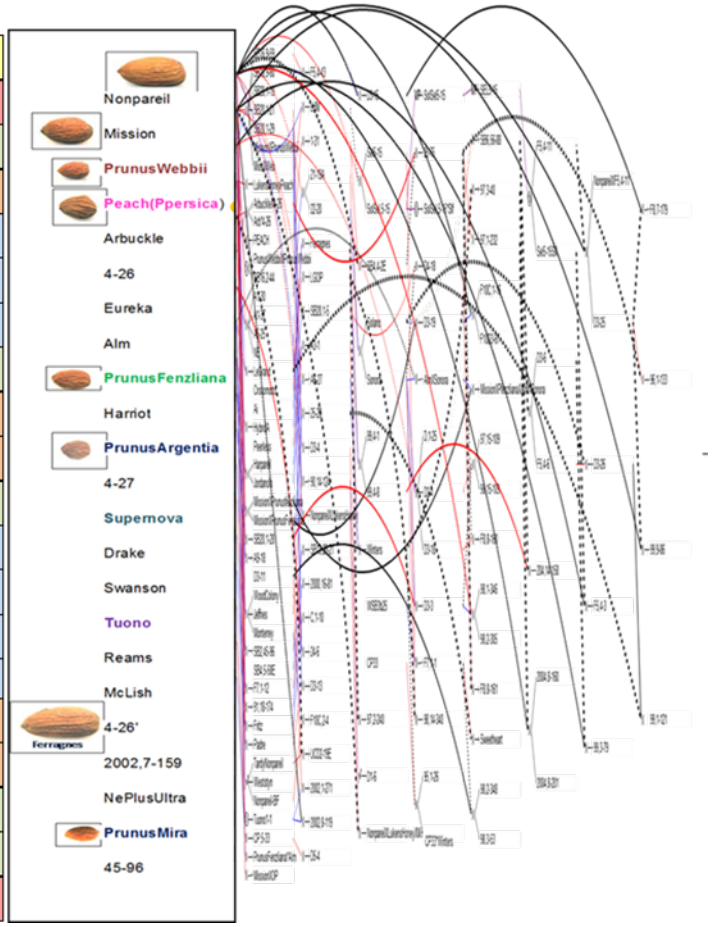

Figure 1. A list of currently important Prunus rootstocks resulting from interspecific hybridization (left). Examples of a diverse range of species parents and breeding crosses from early generations of the UCD almond/peach breeding and germplasm improvement programs (right); {solid lines denote seed parent while dotted lines denote pollen parent}.

crops. Germplasm derived from interspecies hybrids is often pursued to attain the greatest range of vigor and desirable horticultural traits (**Figure 1**) [Citation 1]. However, the development of such exotic germplasm is often difficult and time-consuming and, as has been recently shown with the UCB1 pistachio hybrid rootstock, the genetic, genomic and cultural interactions can be complex and unpredictable. As part of our long-term UCD almond and peach variety development programs, breeding lines have been and continue to be developed combining almond, peach and plum as well as with an extensive diversity within related Prunus species which have been demonstrated to have value for rootstock breeding (**Figure 1 and 2**) [Citations1,3,5].

Early selections within this germplasm have demonstrated traits that appear desirable for rootstocks, including possible drought, nutrient, insect and disease tolerance, as well as modified tree size/structure. This germplasm has being made available to interested public and private rootstock development programs as clonal as well as segregating seedling populations to facilitate and accelerate comprehensive testing. Over 2,000 genetically diverse genotypes derived from this exotic UCD germplasm which includes peach (*P. persica),* almond *(P. dulcis), P. mira, P. davidiana, P. scoparia, P. tangutica, P. webbii, P argentea, P. orthosepala,* and *P. bucharica* (see **Figure 2**) have been transferred for 2017/18 evaluation in several public and private programs for resistance to drought, salinity, boron toxicity, as well as diseases and pests (**Table 1**). The development of effective molecular markers for traits such as nematode and disease resistance should allow improved future selection and so breeding efficiency.

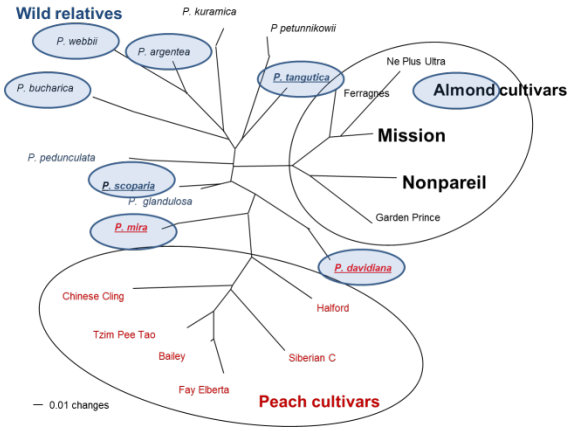

Figure 2. Diagram showing genetic relationships among diverse species currently included in the UCD breeding programs. (Length of connecting lines indicates increasing genetic diversity.)

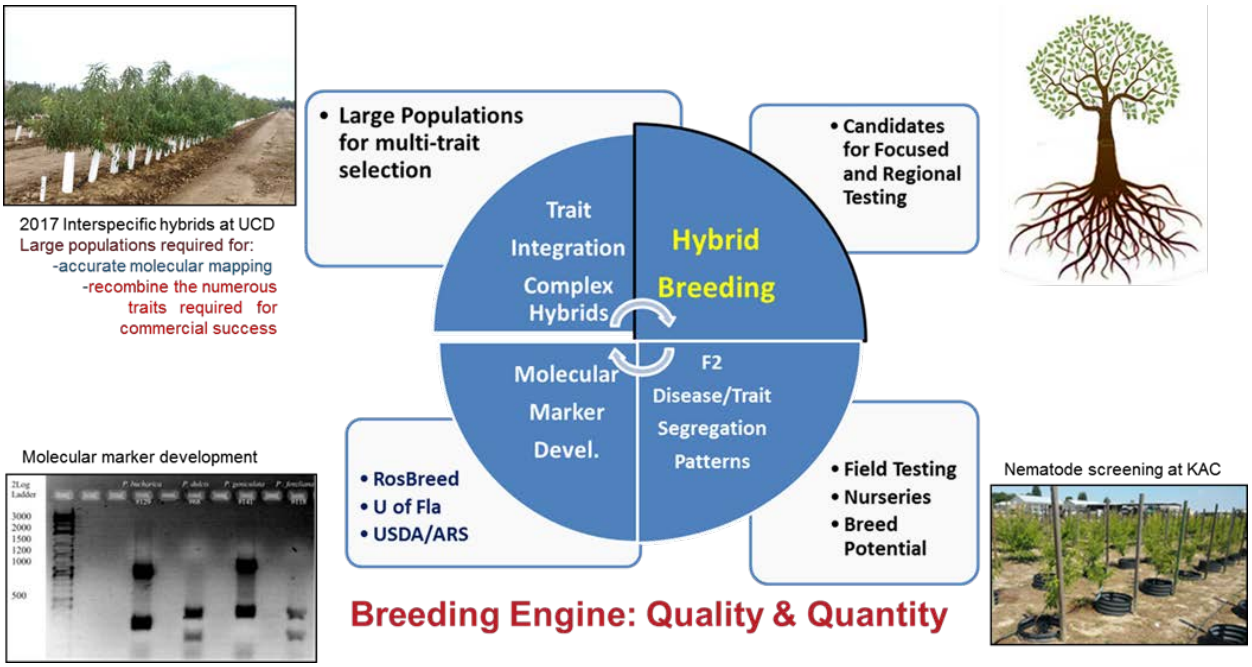

Figure 3. UCD Prunus Breeding cycle. Interspecies hybrid rootstock candidates from Hybrid Breeding are evaluated simultaneously for specific disease resistance and overall field performance, as well as trait inheritance patterns in progeny and subsequently, molecular marker development. The driving engine **of** the breeding program remains the ability to produce/propagate large numbers of diverse interspecies hybrids.

The number and diversity of rootstock characteristics needed for commercial success, require the development of breeding populations well beyond those manageable through traditional molecular marker assisted breeding alone [1,2] (see **Figure 7).** Consequently, an aggressive breeding strategy has been developed which allows efficient concurrent assessment of both hybrid rootstock candidates as well as progeny populations derived from these hybrids, for subsequent inheritance and molecular marker studies. The most promising species, as well as individual genotypes within species are then selected (based on disease resistance, molecular marker assessment as well as overall field performance) for the next round of hybridization and testing (**Figure 3**). As of March, 2018, over 300 additional species-hybrids and over 1,400 segregating F2 (selfed progeny of hybrids) seed were recovered from controlled pollinations.

Current methods for generating chimeric rootstocks have been summarized and published [19], including their potential appication for applied breeding.

An updated draft compiliolation of current knowledge/experience of breeding value and deficiencies for Prunus intra- and interspecific germplasm is included in the Appendix. Updates for individual collaborations listed in (**Table 1**) are provided in the following text.

Materials and Methods:

Workplans and methods are generally the same for different years but with differing levels of detail.

Year 1 has completed the 1st stage of cooperator test-plantings including plot mapping, and the collection of initial information on species hybrid growth-vigor, potential disease resistance and plant architecture. Hybrid, F2 and/or BC2 seed has been generated for concurrent in-house and cooperator studies. Segregating F2 (selfed-seed of hybrid) and BC (seed from crosses back to parent selection of breeding line) populations from targeted interspecies hybrids have been generated from controlled crosses for heritability, including molecular-marker studies.

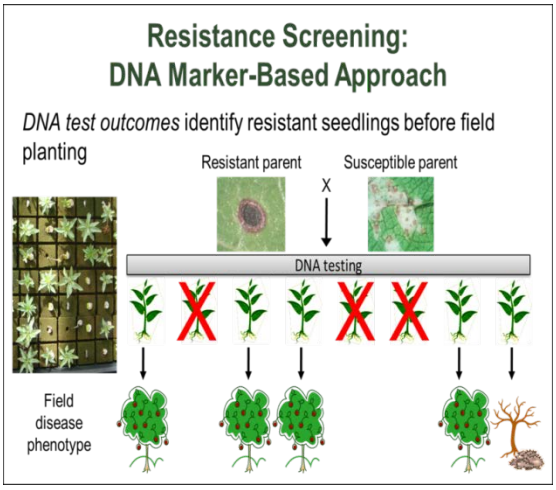

Figure 4. For Breeding populations segregating for a gene with a major resistance, hundreds to thousands of established molecular markers can be evaluated to find a marker that co-segregates with resistance because it is located close enough to the actual (but unknown) disease gene.

Year 2 and 3 will involve the collection of data from different cooperators and the compilation of results for different species donors. New interspecies hybrid as well as F2/BC populations will also be developed based on cooperator feedback. Crossing goals for 2018 target the generation of over 500 additional inter-species hybrids between almond, peach, plum as well as related species. Preliminary molecular data available from RosBreed [8] and other UF, Clemson University and USDA cooperators may allow initial characterization of interspecies potential. If year 2&3 results continue to look promising, future funding will be solicited primarily from the Almond Board of California.

Genetic Background:

Traits are controlled by genes in which the required information is coded by specific sequences of DNA. Rootstock breeding, like variety breeding, essentially involves selecting for desirable genes while selecting against undesirable genes. Some traits, such as rootknot nematode resistance, are controlled by single genes with major affects, while others such as vigor are controlled by a large number of genes, each having only small individual affects. While we cannot yet identify most of the important genes by their DNA sequence, by statistically comparing large populations segregating for targeted traits, we can use the association of known molecular markers with the trait of interest as markers or indicators for the presence of that trait (**Figure 4**). This is because the marker is located on the DNA close enough to the trait of interest that, on average, they are inherited together. Because we know the DNA sequence of the marker, we now have a powerful tool to select for that trait even at the seedling stage. Molecular markers tend to be filler-DNA so that the DNA sequence does not have to be precise and so often mutates or changes over evolutionary time. Because of this, markers developed for one species, such as peach, may not be useful for even closely related species, such as almond, because of the large amount of time separating their evolution from a common ancestor [2]. Normally this is not a problem because most crop breeding involves only the single ancestral species.

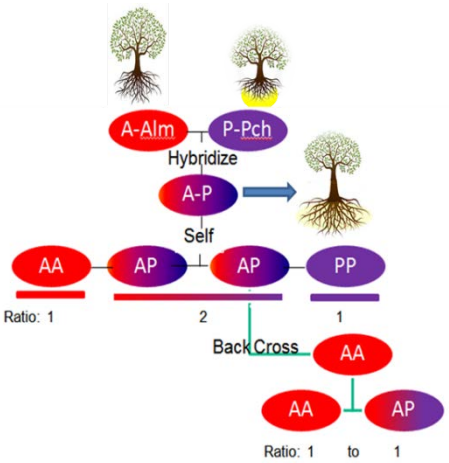

Figure 5. Segregation of root knot nematode resistance in Almond by Nemaguard peach lineages. *Self* generates F2 progeny; *BackCross* generates BC progeny, eaxch with distinct segregation ratios].

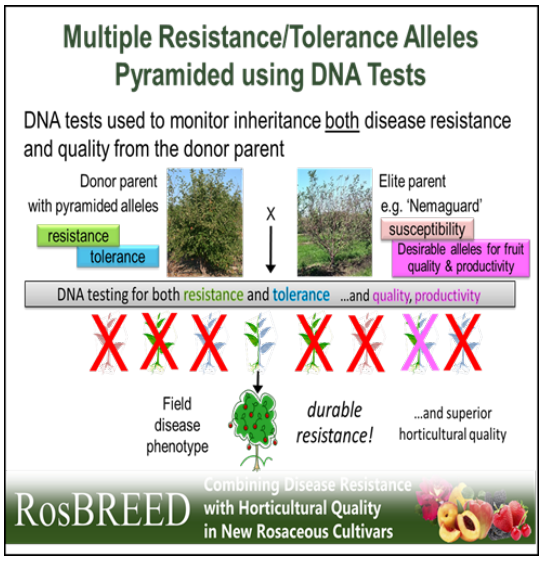

Figure 6. By simultaneously selecting for multiple molecular markers targeting separate resistance/quality genes, seedlings can be recovered in which the desired genes have been consolidated or pyramided.

In rootstock breeding, however, diverse interspecies hybrids are common because they tend to be exceptionally vigorous and so inherently tolerant of wider environmental differences [1,2]. Because this growth vigor will often mask and so delay disease expression, is very difficult to determine whether the hybrid shows disease tolerance because of the possession of useful resistance genes or whether the vigorous growth can initially compensate for the lost diseased tissue and so delay final disease expression. To overcome this difficulty, we are developing both a series of inter-species hybrids as well as segregating seedling progeny populations that allow the identification of major resistance genes through their inheritance patterns (**Figure 5**). For example, in (**Figure 5**), the inherent vigor of almond by peach hybrids will confer tolerance to nematode damage, thus requiring many additional years before resistance or susceptibility can be truely determined. By generating segregating progeny populations, the hybrid vigor is removed while specific inheritance patterns denote the segregation of major resistance genes (1:2:1 for F2 populations, and 1:1 segregation for backcross (BC) backcross populations). Species hybrids identified by progeny inheritance patterns as having desired major genes can then be advanced to further field-testing as well as the generation of more advanced species hybrids. Similarly, individual F2 or BC progeny possessing the desired trait as well as good general rootstock potential can be selected for additional targeted inter-species crosses.

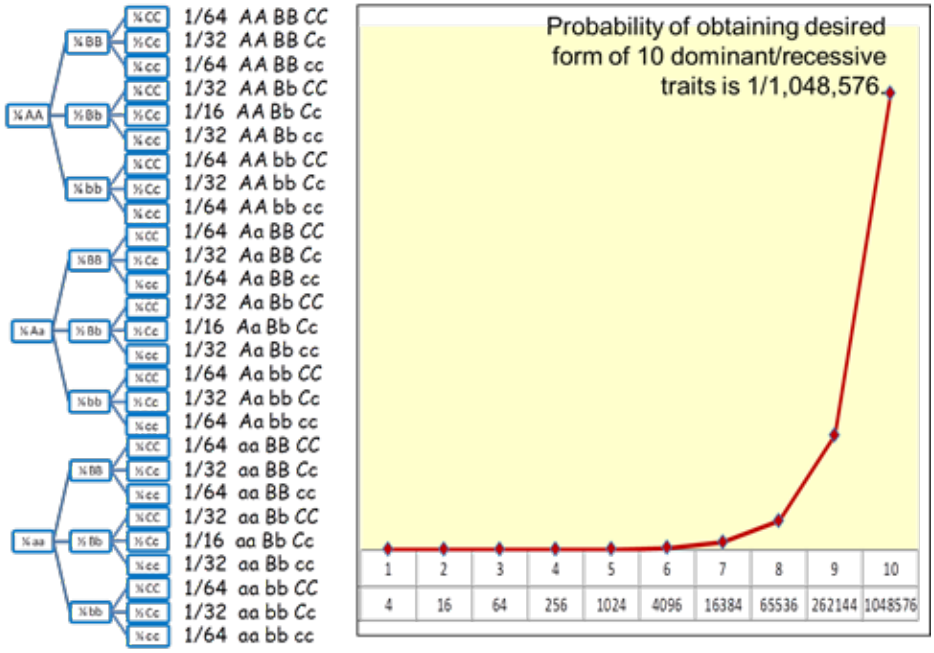

Figure 7. Segregation ratios following selfing for 3 separate genes (left). Minimum population sizes required to recover at least one of each possible genotype for increasing number of genes (right).

When molecular markers are available for several separate traits, the opportunity exists of identifying plants with a high probability of having each desired traits even at the seedling stage (**Figures 4 & 6**) [4,6,7]. However, because the traits are usually inherited independently, the minimum probability of obtaining multiple desired traits is the product of the probability of each individual trait. For example, to obtain the desired genotypes AABBCC in (**Figure 7**), the minimum population size would have to be at least 64 and the number increases logarithmically for each additional gene/trait added

quickly reaching over a million trees required for just 10 genes. Because commercially successful rootstocks will require more than just rootknot and/or ring nematode resistance (for example, graft compatibility, lack of high susceptibility to other diseases or pests, the ability to propagate in high numbers and remain true-to-type, desirable root and scion architectures, etc.), molecular marker assisted breeding has limited value and needs to be combined with quantitative breeding methods requiring large breeding populations.

Consequently, the UCD Prunus Breeding cycle (**Figure 3**) incorporates four complementary components: hybridization, progeny inheritance assessment, molecular marker development, and the development of breeding methods for generating large numbers of diverse inter-species hybrids, including successful recovery of viable plantlets and subsequent successful clonal propagation for replicated testing.

Results and Discussion:
Breeding. Approximately 80 clones (~50 species hybrids and 30 species parents) and over 2,000 seed/seedlings from segregating progeny populations were advanced to cooperator evaluation in 2017. Subsequently, over 300 additional species-hybrids and over 1,400 segregating F2 seed were recovered from controlled pollinations made in spring 2017. Goals for 2018 include an additional 500 species hybrids and approximately 3,000 segregating seedling progeny from select parents.

Propagation. Over 50 species hybrids were propagated for replicated trials. Using methods optimized for individual species combinations, propagation 'takes' using harwood cuttings usually exceeded 60%. (**Figure 8**). {Lower propagation successes were frequently attributed to poor initial plant quality}. Similar or improved takes are being recovered by current propagation trials using softwood cuttings. [Propagation taqes of >50% are considered satisfactory for this stage of replicated testing since typically only 5 to 20 plants are required for each replicated test].

ID	Origin	Prop.	Rooted	Take (%)
NSW6-50	Almond by P.argentea	20	12	60.00
NSW6-18	Almond by P.argentea	20	14	70.00
FSC2-29	Almond by P.bucherica	20	7	35.00
STU2-29	Almond by P.bucherica	20	7	35.00
NSW6-24	Almond by P.scoparia	20	5	25.00
06,1-107	Almond by P.tangutica	20	15	75.00
08,7-310	Almond by P.webbii	20	2	10.00
PG13-6	Almond interspecies	20	2	10.00
PG13-3	Almond interspecies	20	15	75.00
07,13-250	Peach by Almond by P.scoparia	20	3	15.00
05,20-139	Peach by Almond by P.scoparia	20	8	40.00
2008,18-63	Almond by P.mira	20	2	10.00
NSW6-16	Almond by P.mira	40	28	70.00
08,6-210	Almond by P.mira	50	36	72.00
05,20-192	Peach by Almond	30	19	63.33
2009,19-32	Peach by Almond complex	20	4	20.00
2009,19-85	Peach by Almond complex	20	4	20.00
2009,20-89	Peach by Almond complex	20	6	30.00
2009,17-336	Peach by Almond complex	20	7	35.00
2009,33-175	Peach by Almond complex	20	7	35.00
2009,19-150	Peach by Almond complex	20	10	50.00
2009,21-168	Peach by Almond complex	20	11	55.00
2009,18-87	Peach by Almond complex	20	12	60.00
07,12-209	Peach by Almond complex	40	25	62.50
2009,22-1	Peach by Almond complex	20	13	65.00
2008,53-47	Peach by Almond complex	20	13	65.00
2008,61-38	Peach by Almond complex	30	21	70.00
2009,28-152	Peach by Almond complex	30	21	70.00
2009,32-214	Peach by Almond complex	40	29	72.50
2009,19-112	Peach by Almond complex	40	29	72.50
08,58-18	Peach by Almond complex	30	22	73.33
2009,23-28	Peach by Almond complex	20	15	75.00
2009,23-109	Peach by Almond complex	20	16	80.00
2009,29-15	Peach by Almond complex	20	16	80.00
2008,25-101	Peach by Almond complex	20	16	80.00
2009,19-18	Peach by Almond complex	20	16	80.00
10,10-420	Peach by Almond complex	30	24	80.00
2009,29-107	Peach by Almond complex	40	33	82.50
2009,19-252	Peach by Almond complex	20	17	85.00
2008,25-113	Peach by Almond complex	50	37	74.00
05,17-186	Peach by P.davidiana	40	32	80.00
2008,44-28	Peach by P.mira	40	12	30.00
STU2-32	Almond by P.orthosepala	40	22	55.00
NSW7-32	Peach by Plum	20	2	10.00
NSW7-34	Plum interspecies	40	14	35.00
PG3-29	Plum interspecies	20	14	70.00
PG14-2	Plum interspecies	30	19	63.33

Figure 8. Propagation success in 2017 for selected species hybrids using hardwood cuttings.

Progress for individual projects is summarized in (**Table 1**) with details, including goals for 2018 provided in the following updates.

Table 1. Status of current cooperator and in-house resistance evaluation projects.

Trait	Cooperator	Material under evaluation	*Speciesevaluated*	Status
Heat Tolerance	M. Gilbert	15 clones	***a, f, m, p, w***	Under analysis
Botryophaeria resistance	J. Chaparro (U. Fla)	40 cl., 100 sdlings	***a, b, f, m, pd, p, plsp, t, tr, w***	Field plots established with preliminary results
Root lesion Ring, and Root-knot nematode	A. Westphal	25 clones	***a, dv, m, p, t, w***	Field plots established for 7 cl. with 19 clones propagated.
Phytophthora	Greg Browne	3 clones	***pl***	Plants established
Crown gall	D. Kluepfel	~200 seedlings	***p, t,***	>100 sdlings in field, ~100 sdlings greenhouse,
Salinity tolerance	P. Brown	12 clones	***d, a, , f, m, p, t, w***	Greenhouse testing
Botryophaeria, Oxyporus and other wood rot diseases	Rizzo/Johnson	15 clones	***d, a, , f, m, p, t, w***	10 clones under test with 10 to 20 additional clones to be added
Effect on scion architecture	Fowler/Wonderful	7 clones	***a, dv,***	Field plots in commercial production
Nonpareil Compat. & Replant decline	Burchell Nursery	50 clones	***a, b, dv, m, p, plsp, s, t, w***	Field testing
Replant decline	Sierra Gold Nursery	20 clones & ~1000 seed	***a, dv, m, p, s, t, w***	Field testing
Dryland culture	A. Langford	Almond seedlings	***d***	Field testing
Armillaria	In-house	~200 seedlings	***d, p***	Seed being prepared for planting
Asphyxia	In-house	~100 seed	***d, p***	Seed being prepared for planting
Verticillium & Phytophthora	In-house	6 cl. & ~240 sdlings	***d, p***	Seed being prepared for planting
Architecture & disease	In-house	90 cl., ~40, 000 sdlings	***a, b, dv, m, p, s, t, w***	Field testing
High density plantings.	G. Thorp, Australia	20 cl., ~400 seedlings	***d, , f, m, p, w***	12 clones propagated, >1000 crosses (hybrids and F2's)
Tissue culture, plant-regeneration, transformation	Abhaya Dandekar	~200 developing seed; 6 clones	***d, p, dv***	Ease of in-vitro regeneration underway
Almond {P.dulcis} (d), Peach {P.persica} (p), P.argentea (ar), P.fenzliana (f), P.mira (m), P.webbii (w),				
P.bucharica (b), P.pedunculata (pd), Plum spp. (pl), P.tangutica (t), P.triloba (tr), P.davidiana (dv), P.scoparia (s)				

1. <u>Heat Tolerance</u>. Cooperator: Mathew Gilbert Material under evaluation: 15 clones. Species evaluated: P. *argentea, P. fenzliana, P. mira, P. persica*, *P. webbii.* Status: Germplasm differences based on a preliminary screening of critical leaf temperature completed and presented in annual report [5]. Recent models have

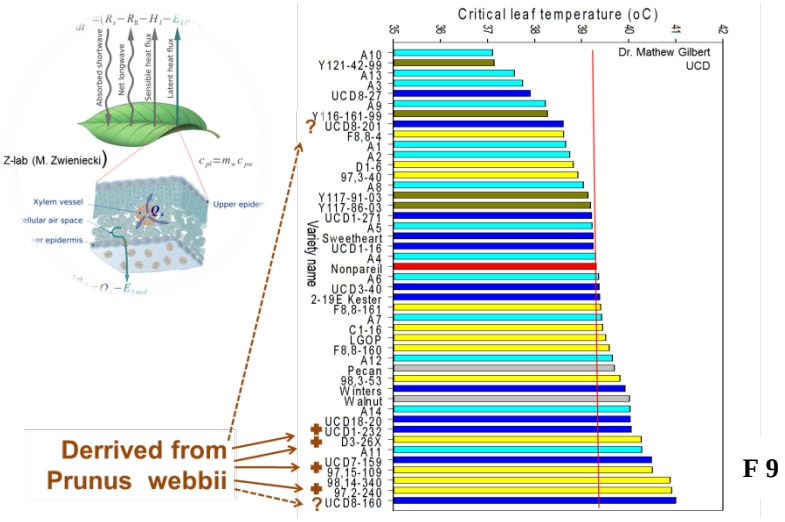

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Figure 9. Differences in critical leaf temperatures for USDA ('A' and 'Y' prefix) and UCD (all others) selections currently in regional variety trials, UCD selections show a range in ratings including all 5 of the high-end scores, demonstrating the value of utilizing diverse germplasm in the cultivar breeding program.

suggested that vascular structure in addition to leaf architecture may be a component contributing to heat tolerance. Whether such vascular structural differences among species has important rootstock implications has yet to be determined though vascular structure has been shown by Ted Dejong to affect both rootsctock-to-scion vigor and final scion size. Notably, most of the UCD clones showing exceptional levels of heat tolerance are almond introgression lines derived from Prunus webbii. Future goals: identify improved predictors of rootstock-conferred heat/drought tolerance for future studies. Evaluate own-rooted accessions of almond-P webbii introgression lines, including accessions UCD04, 8-160 and UCD04, 8-210 for dryland production assessment [see project 10].

2. Botryophaeria resistance. Cooperator: Jose Chaparro (UF). Material under evaluation: 40 clones, 200 seedlings. Species evaluated include P*. argentea*, *P. bucharica, P. fenzliana, P. mira, P. pedunculata, P. persica, Plum spp., P. tangutica, P. triloba, P. webbii*. Status: disease plots established and the first round of disease and molecular marker evaluations completed. A major molecular marker for botryophaeria resistance in several Prunus dulcis (almond) accessions has been identified by UF cooperators with a summary of results in preparation for submission for publication in 2018/19. Future goals: an additional 60 almond by P davidiana and almond by P mira hybrids were generated in 2017 at UCD for UF testing beginning in 2018. In addition, based on present results, 80 additional hybrids as well as a complex intra-species cross (Jeffries by Nonpareil) will be made in 2018 with a target of 100 segregating seedling progeny. [Botryophaeria resistance is a major breeding goal for rootstock development in Florida as it has been found to be a previously underrecognized but important problem (**Figure 18**) and the gene may also be associated with resistance to other rootstock, scion and/or interstock diseases].

3. Root lesion, Ring, and Root knot nematode. Cooperator: Andreas Westphal and Burchell nursery. Material under evaluation: 40 clones. Species evaluated: *P. argentea, P. davidiana, P. mira, P. persica, P. tangutica, P. webbii.* Status: Field plots established. Within the propagated group is a tandemly-grafted rootstock composed of a Nemaguard upper rootstock cleft-grafted to a Hansen basal-rootstock as a test-of-concept of binary-rootstocks engineered to rapidly combine desirable traits from different rootstocks or to selectively target soil strata differences in pathogen, nutrient, drought, etc. conditions. Selection showing promise in 2017 for both root knot and ring

Figure 10. Almond by P orthosepala (left) in ring nematode infested evaluation block

nematode include UCD 05, 17-186 ((P persica x P davidiana) x P persica) and STU 2-32 [4]. (P dulcis x P. x orthosepala). Future goals: continue multiyear evaluation of resistance. Add 10 clones currently being propagated for 2018 planting. Generate F2 of UCD05, 17-186 as well as an 100 additional ((P persica x P davidiana) x P persica) hybrids.

4. *Phytophthora*. Cooperator: Greg Browne. Material under evaluation: 3 clones. Species evaluated: Plum interspecies. Status: Plants established. Future plans: four additional P. dulcis accessions identified as having potential resistance based on long-term survival in Phytophthora infested soils are being clonally propagated for control testing. Concurrently, these items are also being used as parents in 2018 inter-and intra--species hybridizations.

5. Crown gall. Cooperator: Dan Kluepfel and M. Aradhya. Material under evaluation: ~200 seedlings, ~400 seed. Species evaluated: *P. persica x P. tangutica* (F_2, F_3). Status: The Kluepfel research lab had previously identified potential crown gall resistance in the interspecies hybrid *P. persica x P. tangutica* [4]. To test for progeny segregation patterns indicating a control by major genes (i.e. heritable in any future

Figure 11. {Left} Tree segregating for bacterial canker susceptibility in an F2 of peach by P tangutica.

Figure 12. [Right] Lethal crown gall on a P tangutica accession in the USDA germplasm collection.

controlled hybridizations), we have generated F_2 and F_3 peach by *P. tangutica* progeny populations from the USDA *P. tangutica* source used. Over 200 seed have been field- planted for test inoculations, with an additional 400 seed held in reserve to allow inoculations/testing under laboratory conditions. While controlled inoculations have not yet occurred, field plantings have shown evidence for a high heritability for susceptibility to bacterial canker in F2 progeny (**Figure 11**)

while a sibling of one of the *P. tangutica* USDA parents was found to had been killed by crown gall (**Figure 12**) though whether the infection was on own-rooted P. tangutica or a different rootstock species remains unknown. Future goals: provide hybrid clones and F2 seed/seedlings for 2018 controlled inoculations.

6. Salinity tolerance: Cooperator: Patrick Brown et al. Material under evaluation: 6 clones. Species evaluated: Almond, *P. argentea, P. fenzliana, P. mira, P. persica, P. tangutica, P. webbii*. Status: 12 different interspecies clones were initially targeted for evaluation. However, we were unable to supply the 8-10 trees per clone required for 2017 trials. Future goals: propagate 8-12 trees of an additional 6-8 clones to be included in 2018-19 evaluations.

7. Oxyporus, Botryophaeria, and other wood rot diseases. Cooperator: Rizzo/Johnson. Material under evaluation: 16 clones (9 from UCD breeding and 7 standard rootstocks). Species evaluated: Almond, P. argentea, P. fenzliana, P. mira, P. persica, P. tangutica, P. webbii. Status: preliminary studies have identified potential resistance and several species sources. Future goals: provide additional accessions (species hybrids and species parents) for 2018/19 evaluations as requested.

8. Effect on scion architecture. Cooperator: Fowler Nursery/Paramount. Material under evaluation: 7 clones; in-house-5 clones. Species evaluated: *P. argentea, P. davidiana, P. dulcis, P. persica, P. scoparia.* Status: Field plots established and in commercial production with multi-year evaluations completed. Future goals: evaluate relative production performance as well as orchard longevity for different species sources. (see also project 14).

9. Replant decline. Cooperators: Burchell Nursery, Sierra Gold Nursery. Material under evaluation: 50 clones. Species evaluated: *P. argentea, P. bucharica, P. davidiana, P. mira, P. persica, Plum spp., P. scoparia, P. tangutica, P. webbii*. Status: Field plots established (see ring nematode evaluations for project 3). Future goals: continue multiyear field evaluations combined with 2018 nematode intensity ratings by Andreas Westphal.

10. Dryland culture. Cooperator: Andrew Langford and in-house. Material under evaluation: Almond spp., seedlings & seed. Status: Potted almond seedlings have been planted are currently being prepared for field propagations in dryland Capay orchards. In addition, we continue to evaluate own-rooted accessions of almond-P webbii introgression lines including accessions UCD04, 8-160 and UCD04, 8-210, identified in project 1 as having potential heat tolerance, under dryland production conditions [see **Figure 13** and project 1]. We also are monitoring a 60-year-old almond-rooted dryland Nonpareil/Mission orchard for growth habits and production consistency.

Figure 13. Own-rooted accessions of almond-P webbii I introgression lines including accessions UCD04, 8-160, UCD04, 8-210 and siblings (identified in project 1 as having potential heat tolerance), under dryland production conditions (trees in 2018 fowwowing 5 years without supplementary water; inset: selfed crop of drland UCD04, 8-160 in 2017)

11. Armillaria. In-house. Material under evaluation: ~200 seedlings. Species evaluated: almond and peach spp., Seedlings & seed. Status: seed was collected from parent trees from almond by peach introgression lines showing continued good growth in known *Oak root fungus* hotspots (**Figure 14**). Open-pollinated seed was collected and will be tested in plots previously shown to have high *Armillaria* damage. New plantings are scheduled for fall 2018. Heritability will be estimated from progeny inheritance patterns. At a very basic level, if no evidence of any heritable resistance/tolerance is evident in the progeny, the value of that parent for continued breeding will have been greatly diminished. Over 300 plum F2 seed have been generated for future inheritance and molecular studies. Future goals: Replant UCD Oak-root fungus plot with next

generation of accessions for screening. Generate 300 almond by resistant/tolerant plum and plum interspecies hybrids in 2018.

Figure 15. Five- year survival of almond introgression lines derived from peach by *P. webbii* in a test plot highly infested with oak root fungus

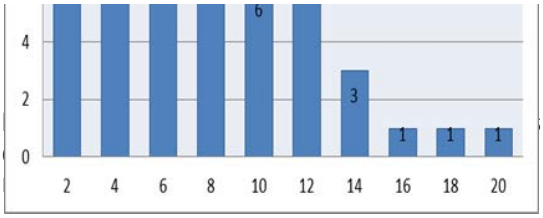
12. Asphyxia. In-house. Material under evaluation: ~100 seed. Species evaluated: Almond and Peach spp. seedlings & seed. Status: Seed was collected from almond species lines showing continued good growth in areas having highly saturated soils. Open-pollinated seed was collected and is being prepared for testing

under greenhouse conditions were soil saturation can be more accurately maintained. Almond by almond and almond by peach crosses were made in 2017/18 for resistance and heritability trials. Heritability of tolerance/resistance will be estimated from progeny inheritance patterns as described above. Future goals: generate an additional 20 almond by peach and almond by plum hybrids from parents showing promise of resistance.

Figure 14. Drought

13. Hybrid architecture. UCD Almond and Peach variety breeding programs material under evaluation: 90 clones & ~70,000 seedlings. Species evaluated: *P. argentea, P. bucharica, P. davidiana, P. mira, P. persica, P. scoparia, P. tangutica, P. webbii*. Status: Over 90 species hybrids or species introgression parents, as well as ~70,000 seedling progeny are currently being evaluated as part of the UCD Almond and Peach breeding programs. Genetic opportunities for rootstock improvement are now being considered concurrently with

Figure 17. Vigor differences in peach by P. mira hybrids after 4 mo. field growth in 2017.



those for scion (variety) improvement in the ongoing evaluation of this germplasm. For example, interspecies progeny breeding blocks scheduled for removal are now retained for an additional season but without supplemental irrigation to evaluate differences in survival. Drought tolerance associated with a strong vertical tap-root was identified in some almond introgression lines derived from peach by P. webbii hybrids (**Figure 15**). Similarly, (**Figure 16)** shows progeny from a peach by P mira hybridization after 4 months of field growth demonstrating variability in the levels of hybrid vigor and plant architecture in these species hybrids. Height/vigor distribution profile of 60 hybrid progeny from a peach by P. mira cross are shown in (**Figure 17)**. Future goals: continue drought and tree/root architecture studies. Identify predictors of tolerance/resistance. Assess Crown gall resistance in this interspecies germplasm.

14. High-density plantings. Work with Bruce Lampinen and Australian collaborators, Grant Thorp at *Plant and Food Research,* and Michelle Wirthensohn, at the *University of Adelaide* has found that the greatly reduced lower-tree productivity is a major limitation in the development of very high-density orchards. Reduced spur-bearing from harvest damage as well as lower-wood shading has been identified as a major contributor to this loss. High-density, compact and lateral-bearing selections are being developed from *Prunus webbii* and *Prunus mira* lineages that contribute to 2nd and 3rd year fruit-bearing wood having higher bearing-densities as well as greater fruitwood. (Much of the production is on short dard-type lateral shoots similar to those seen on the *Winters* variety). Advanced UCD breeding selections showing this and similarly promising traits for very high-density plantings have been propagated and planted to a very high-density evaluation block in the Wolfskill Experimental Orchards. In addition, a large (> 200 seedlings) F2 population segregating for this bearing habit, self-compatibility, kernel quality and tree architecture has been generated in 2017 for studying desirable/undesirable trait associations and the possible development of molecular markers for targeted traits. In 2017, novel pillar-type almond breeding lines derived from P mira introgression was also identified which show promise for productive, high density plantings. In addition, peach by P mira hybrids and BC1 progeny also

Figure 18. Botryophaeria infected rootstock in Florida peach orchard [Left]. [Right] Botryophaeria susceptibility segregating in a UCD P. tangutica interspecies F2 population, demonstrating that both resistance as well as susceptibility can be introduced with new germplasm.

show a pillar trait with evidence for control by a single major gene. Future goals: continue to evaluate the inheritance of the compact, pillar-type growth habits and determined what affect compact, and pillar-trees confer when used as rootstocks.

15. Tissue culture, plant-regeneration, transformation. Cooperator: Abhaya Dandekar. Material under evaluation: ~300 developing seed. Species evaluated: *P. dulcis, P. persica, P. davidiana*. Status: The Dandekar project aims to re-synthesize commercially successful almond by peach hybrids to use as foundation for plantlet-regenerable tissue-culture callus for future genetic engineering of desirable rootstock characteristics. Over 50 seed approximating the initial parentage of the Nickels almond by peach by P. davidiana rootstock have been generated in 2017 and provided to the Dandekar lab for culture and evaluation. In 2018, 200 additional almond by peach/P davidiana / P mira hybrid seed was generated but the developing seed was harvested at very early stages of embryo development (globular to heart stage) to test whether early embryonic tissue is more amenable to the adventitious callus/plantlet regeneration required for successful genetic engineering. Future goals: assess methods for direct meristem transformation using recent modifications of the Biolistic gene gun [see 9, 10].

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Appendix.

Draft summary of Rootstock characteristics based on published data, discussions with collegues and field experience.

Group	Item	Genetic background	Wet Soil	Anchorage	Vigor	Phytophthora	Bacterial Canker	Crown Gall	Root Knot Nematode	RingN	Lesion	Oak Root Fungus	Non-Pareil Compatible?	Suckers?
Pe	11 1 1	(Flordaguard x Alnem) x Y105 208 94												
Pe	11 3 1	(Tskuba No. 4 x Flordaguard) x Y105 208 94												
Pe	11 3 2	(Tskuba No. 4 x Flordaguard) x Y105 208 94												
Pe	11 7 1	Y115 175 97 *x P. davidiana* 'Potanni'												
Pl	12 hybrids of	P. dulcis x P. webbii												
Al	198 13	*P. webbii* x *P. dulcis*												
Al	198 17	*P. tangutica* x *P. dulcis*												
Pe	198 18	Nemared x *P. kensuensis*												
Pe	198 3	*P. argentia* x *P. dulcis*												
Al x Pl	AC 941(Mirobac)	P. dulcis x P. cerasifera												
Pe	AC 9000	P. dulcis x P. persica												
Pe	AC 9502	P. dulcis x P. persica												
Pl	AC 952	P. insititia												
Pl	AC 959	P. insititia x P. domestica												
Pl	AC 960	P. insititia x (dulcis x persica)												
Pe	Adarcias	'Garnem' almond x 'Nemared peach'												
Pe	Adefuel	P. dulcis x P. persica	1		2	1					1	1		
	Adesoto				2									
Al	Alnem 1	bitter almond												
Al	Alnem 201	?												
Al	Alnem 88	bitter almond												
ex hyb	Atlas	Nemaguard * (Jordanolo * Prunus blireiana.	2?	3	3	2	2	2	3	2	2	1	Yes	ne
Pe	Bright Hybrid #2	P. dulcis x Nemaguard												
Pe	Bright Hybrid #3	P. dulcis x Nemaguard												
Pe	Bright Hybrid #6	P. dulcis x Nemaguard												
Pe	Bright Hybrid 106	P. persica x P.dulcis	2						3		2			
Pe	Bright Hybrid"	P. persica x P. davidiana												
Pe	Bright Hybrid 1	P. persica x P.dulcis	1	4	4	1	1	2	3	2		1	Yes	ne
Pe	Bright Hybrid 4	P. persica x P.dulcis												
Pe	Bright Hybrid 5	P. dulcis (Titan) x Nemaguard				1	1	1	1	1	1	1		
Pe	Cadaman	P. persica x P. davidiana	1		2	3	3	1	1	2	1			
Pl	Citation	OP Red Beaut Plum OR Siberian C x (plum x almond)				2	1	1	3	2	2			
unk	Compass	Prunus besseyi x Prunus americana					2		2	2	2	1		
Pe	Contoller 9.5 (HBOK	P. persica (cv Harrow Blood) x (cv Okinawa)							3					
Pl	Controller 5 (=K146 4	P. salicina x P. persica				2	1		2	1	1			
unk	Controller 7 (HBOK 32)					1			3					
Pe	Controller 8 (HBOK 1	P. persica (cv Harrow Blood) x (cv Okinawa)												
unk	Controller 9					2	1		1	1	2		no	
Pe	Cornerstone	P. persica x P.dulcis	1			2	1	2	2	2	2	1		
Pl	Empyrean 101 (Aedes	P. isititia	3	2		2	1		3	1	1	2		
unk	Empyrean 3 (Tetra)	(Prunus domestica)	2	2	1	2	2	3	2		2	1		
Pe	Empyrean#1 (Barrier	P. persica x P. davidiana				1	1	1	3	2	1			
Pl	Empyrean#2 (Penta)	O.P. P. domestica	2	2	2	2	2		3	1	1	2		
Pe	Flordaguard	{('Shau Thai OP' x Prunus davidiana) x (3 OP	1	2			3		3	2	2			
unk	Flordaguard	P. persica x P. davidiana							4	2.4	2.4			
Pe	Flordaguard x Alnem	P. persica x Israeli bitter almond												
Pe	Flordaguard x Israeli I	(P. persica x P. davidiana) x P. dulcis												
Pe	Flordaguard x weepin	P. persica x P. davidiana												
Pe	Floridaguard x almon	(P. persica x davidiana) x P. dulcis	1											
	Guardian	P. persica sdling							3	3	4			
	Guardian	P. persica sdling					2			2	2	1		
Pe	Guardian (SC 17)	Nemaguard							3	2	2			
Pe	GxN 15(Garnem)	P. dulcis x P. persica (Nemared)												
Pe	GxN 22 (Felinem)	P. dulcis x P. persica (Nemared)												
Pe	GxN 9(Monegro)	P. dulcis x P. persica (Nemared)												
Pe	H184	Titan almond x Nemaguard												
Pe	Hansen 536	almond MVM	1	4	4	1	2	2	3	1	2	1	Yes	ne
Comple	Hansen 536 x P. blieriana									RingN				
Comple	Hansen 536 x P. cerasifera					3								
Comple	Hansen 536 x P. domestica					3		3						
Pe	Harrow Blood x Okina	P. persica x P. davidiana												
Pe	Harrow Blood x Okina	P. persica x P. davidiana												
Pl	Havens 2B	P. insititia												
unk	HBOK 15							3	2	2				
unk	HBOK 28													
unk	HBOK1							4	3	3				
unk	HBOK10								3					
	HBOK15								4	3	1			
unk	HBOK17													
Pl	Hiawatha	P. besseyi x P. salicina							2	1	2			
Pe	IS 5/19	P. dulcis x P. persica												
Pe	IS 5/8	P. dulcis x P. persica												
Pe	IS 29 5	P. dulcis x P. persica												

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Pe	Adefuel	P. dulcis x P. persica	1		2	1					1	1		
	Adesoto				2									
Al	Alnem 1	bitter almond												
Al	Alnem 201	?												
Al	Alnem 88	bitter almond												
ex hyb	Atlas	Nemaguard * (Jordanolo * Prunus blireiana.	2?	3	3	2	2	2	3	2	2	1	Yes	ne
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Pe	Bright Hybrid #6	P. dulcis x Nemaguard												
Pe	Bright Hybrid 106	P. persica x P.dulcis	2						3		2			
Pe	Bright Hybrid"	P. persica x P. davidiana												
Pe	Bright Hybrid 1	P. persica x P.dulcis	1	4	4	1	1	2	3	2		1	Yes	ne
Pe	Bright Hybrid 4	P. persica x P.dulcis												
Pe	Bright Hybrid 5	P. dulcis (Titan) x Nemaguard				1	1	1	1	1	1	1		
Pe	Cadaman	P. persica x P. davidiana	1		2	3	3	1	1	2	1			
Pl	Citation	OP Red Beaut Plum OR Siberian C x (plum x almond)				2	1	1	3	2	2			
unk	Compass	Prunus besseyi x Prunus americana					2		2	2	2	1		
Pe	Contoller 9.5 (HBOK	P. persica (cv Harrow Blood) x (cv Okinawa)							3					
Pl	Controller 5 (=K146 4	P. salicina x P. persica				2	1		2	1	1			
unk	Controller 7 (HBOK 32)					1			3					
Pe	Controller 8 (HBOK 1	P. persica (cv Harrow Blood) x (cv Okinawa)												
unk	Controller 9					2	1		1	1	2		no	
Pe	Cornerstone	P. persica x P.dulcis	1			2	1	2	2	2	2	1		
Pl	Empyrean 101 (Aedes	P. isititia	3	2		2	1		3	1	1	2		
unk	Empyrean 3 (Tetra)	(Prunus domestica)	2	2	1	2	2	3	2		2	1		
Pe	Empyrean#1 (Barrier	P. persica x P. davidiana				1	1	1	3	2	1			
Pl	Empyrean#2 (Penta)	O.P. P. domestica	2	2	2	2	2		3	1	1	2		
Pe	Flordaguard	{('Shau Thai OP' x Prunus davidiana) x (3 OP	1	2			3		3	2	2			
unk	Flordaguard	P. persica x P. davidiana							4	2.4	2.4			
Pe	Flordaguard x Alnem	P. persica x Israeli bitter almond												
Pe	Flordaguard x Israeli I	(P. persica x P. davidiana) x P. dulcis												
Pe	Flordaguard x weepin	P. persica x P. davidiana												
Pe	Floridaguard x almon	(P. persica x davidiana) x P. dulcis	1											
	Guardian	P. persica sdling							3	3	4			
	Guardian	P. persica sdling					2			2	2	1		
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unk	HBOK 15							3	2	2				
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unk	HBOK1							4	3	3				
unk	HBOK10								3					
	HBOK15								4	3	1			
unk	HBOK17													
Pl	Hiawatha	P. besseyi x P. salicina							2	1	2			
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