Integrated Conventional and Genomic Approaches to Almond Rootstock Development

Project No.: **17-HORT16-Aradhya/Ledbetter**

Project Leaders:

Malli Aradhya
USDA-ARS, UC Davis
Davis, CA 95616
530.752.9392
aradhya@ucdavis.edu

Craig A Ledbetter
USDA-ARS
Parlier, CA, 93648
559.596.2817
craig.ledetter@ars.usda.gov

Daniel Kluepfel
USDA-ARS, UC Davis
Davis, CA 95616
530-752-1137
dakluepfel@ucdavis.edu

Gregory Browne
USDA-ARS, UC Davis
Davis CA 95616
530-754-9351
gtbrowne@ucdavis.edu

Andreas Westphal
UC Riverside, KAC
Riverside, CA 93648
559-646-6555
andreas.westphal@ucr.edu

Project Cooperators and Personnel:

John Preece, USDA-ARS, UC Davis
Carolyn DeBuse, USDA-ARS, UC Davis
Georgia Drakakaki, UC Davis Plant Services
Tom Gradziel, UC Davis Plant Services
Dianne Velasco, Doctoral Candidate, UC Davis Plant Sciences
Ali McClean, USDA-ARS, UC Davis
Emily Johnson, Grad Student, UC Davis Plant Sciences
Holly Forbes, Grad Student, Plant Pathology, UC Davis
Franklin Lewis, Grad Student, Plant Sciences, UC Davis
Chuck Fleck, Sierra Gold Nurseries

Objectives:

1. Produce genetically diverse interspecific hybrids involving *Prunus* spp. that are donors of disease resistance.
2. Disease testing of commercial and experimental rootstocks to produce high-quality disease phenotype data.
3. Intensify GBS based high-density genotyping of rootstock breeding populations and perform association analysis to develop efficient marker assisted selection strategies.
4. Develop and use effective marker assisted selection strategies for rapid development of improved rootstocks.

Interpretive Summary:

Improved rootstocks resistant/tolerant to soil borne pathogens and abiotic factors such as drought are critical for long-term sustainability and profitability of California almond industry. Our ongoing rootstock improvement project is addressing many of these issues through breeding for environmentally benign host plant mediated durable resistance to highly genetically variable soil borne pests and pathogens. We are tapping into novel resistance sources occurring in the wild *Prunus* species through interspecific hybridization and disease testing of resulting hybrids.

While durable/polygenic/field resistance is highly desirable, it is difficult, resource intensive and time consuming to exploit. Field resistance is especially important in long-lived tree crops that endure long-term biotic and abiotic stresses in the rhizosphere. We are producing genetically diverse array of hybrids involving both cultivated and wild peach and almond, and other known sources of resistance in wild plum species that are available in the USDA Germplasm Repository. In addition, we are also cognizant of the need to consider graft compatibility and propagability of any promising rootstock selected for improved levels of resistance to soil borne diseases. During 2017 spring, we successfully produced 12 diverse interspecific hybrids and embryo rescued/cultured to micropropagate clonal copies of each of these hybrids for replicated disease screening (**Table 1a**). Additionally, 142 hybrids from six different hybrid combinations have been germinated to produce seedling progenies, which in turn are being propagated through cuttings to produce copies of plants for replicated disease testing (**Table 1b**). During 2018 Spring, we have produced 110 new hybrids from 13 hybrid combinations (**Table 2**). These hybrids are currently in embryo rescue/culture stage and subsequent micropropagation will produce clones of each for replicated disease evaluation (**Figure 1**). We have the following highlights for 2016-17:

1. Identified ten 2016 rootstock hybrids with high levels of tolerance to *Agrobacterium tumefaciens* infection/crown gall formation. Four of these 10 hybrids shown combined tolerance to both crown and root infections (**Figure 2**).
2. A two-year field evaluation of 2015 *Prunus* hybrid identified several hybrids showing high levels of resistance/tolerance to root-knot and root-lesion nematodes (**Figure 3a and b**).

3. Eight promising hybrids (197-190, 197-199, 197-206, 197-214, 198-18, P2-9, P4-10, and P4-25 are being evaluated for tolerance to ring nematode.

Materials and Methods:

Objective 1. Production of interspecific hybrid rootstocks

Production and evaluation of genetically diverse hybrids are the major focus of a rootstock breeding program. The more diverse and greater the number of hybrids, better the chance of getting some desirable genetic recombinants with combined durable resistance. Embryo rescue/culture and micropropagation are the critical and limiting steps in the rootstock breeding/evaluation. We are using these techniques to obtain hybrids otherwise not possible and then multiply these valuable hybrids to provide clones for the various disease screening procedures. Hybridization in *Prunus* is laborious and time consuming since a single pollination produces a single fruit/seed and thousands of pollinations are required to produce genetically diverse hybrids. Fruit development is carefully monitored to identify the right maturity stage for embryo extraction prior to abortion.

Objective 2. Disease evaluation of rootstock hybrids (Aradhya)

a) Evaluation of resistance/tolerance to Agrobacterium mediated crown gall (Daniel Kluepfel) Rootstock plants grown in 10-inch pots are bare-rooted, washed, and inoculated by stabbing the crown region with a scalpel blade dipped in a suspension of four virulent *A. tumefaciens* biovar1 strains representing the genetic diversity of the pathogen across the almond growing regions of California. The plants are lightly root pruned to create wounds and sprayed with the inoculum and immediately replanted into pots and placed under mist until established. The disease expression is rated during the following summer. All trials will include the standard rootstocks of Nemaguard, Hansen 536, and Marianna 2624 or Krymsk 86.

b) Evaluation of resistance/tolerance to Phytophthora crown and root rots (Greg Browne) The rootstocks are established in 500-ml pots and 20 plants receive the substrate permeated with *Phytophthora* sp. and 10 receiving sterile substrate as a control. Starting one week after inoculation, all the plants will be subjected to biweekly 48-hr episodes of flooding and weekly disease severity ratings (0 to 5 scales) to quantify aboveground disease progress. One to two months after inoculation, final data will be collected, including: plant top fresh weight, percentage of root and root crown lengths rotted, and root fresh weight. The data will be subjected to analysis of variance. Resistance of each rootstock will be assessed according to disease severity and fresh weight reductions induced by the pathogens, as compared to the control. All trials will include the standard rootstocks of Nemaguard, Hansen 536, and Marianna 2624 or Krymsk 86.

c) Evaluation of resistance/tolerance to root-knot, root-lesion, and ring nematodes (Andreas Westphal)

Host status of *Prunus* hybrids to root-knot nematode (*Meloidogyne incognita*), root lesion nematode (*Pratylenchus vulnus*), and ring nematode (*Mesocriconema xenoplax*) are assessed under field conditions. Plants supplied by the breeder in field plots are inoculated with known numbers of root-knot nematode *M. incognita*, ring nematode (*M. xenoplax*), or lesion nematode (*P. vulnus*). While root-knot and root-lesion nematodes probably can be co-inoculated, ring nematode may need to be tested separately. Final population densities of the nematodes that develop on the genotypes will be determined and compared to standards of susceptible or known resistant plants. The nematode reproductive potential will be used to classify the hybrids. Preliminary evaluations are conducted after one vegetation period but more reliable evaluations in the second year are needed to confirm the information. All trials will include the standard rootstocks of Nemaguard, Hansen 536, and Marianna 2624 or Krymsk 86.

Objectives 3 and 4. Develop and identify SNPs linked to genes mediating disease resistance (Aradhya)

We are getting ready to perform low depth genome sequencing of about 200 experimental and commercial rootstocks for which we have disease evaluation data. Sequencing libraries are under preparation and will be sequenced during fall, 2018. Low depth sequencing is used an alternative to the GBS approach, which has become proprietary. Association analyses will be performed with all experimental and commercial rootstocks for which we have disease evaluation data.

Results and Discussion:

a) Production of rootstock hybrids and testing rootability of *donor Prunus species*

A critical step in the rootstock breeding program is to generate and evaluate large numbers of genetically diverse interspecific *Prunus* hybrids with wild *Prunus* species that are potential donors of resistance to soil borne diseases within the peach-almond-plum genetic backgrounds. During 2017 spring, we successfully produced a dozen diverse interspecific hybrids with plum (*P. ceresifera* and *P. salicina*), peach (*P. persica, P. kensuensis, P. davidiana*) and almond (*P. dulcis*) and the hybrids are being embryo rescued/cultured and micropropagate to produce clonal copies of each of these hybrids for disease resistance testing (**Table 1a**). Another set of 142 hybrids from six different hybrid combinations have been germinated to produce seedling progenies, which are in turn being propagated through cuttings to produce copies for replicated disease testing (**Table 1b**). These embryos rescued, and clonally propagated seedling hybrids will be evaluated during 2018-19 fall and spring seasons. During 2018 spring, we have produced 110 new hybrids using *P. cerasifera, P. dulcis, P. persica* and wild peach, almond, and plums (**Table 2**). These hybrids are in the embryo rescue/culture stage and subsequent micropropagation to produce plants for replicated disease evaluation (**Figure 1**).

b) Rootability of donor species

A second-year greenhouse evaluation of rootability of *Prunus* species that are potential donors of resistance to soil borne diseases was undertaken during April-July 2018. Twenty genotypes representing ten wild peach, almond, and plum species were included in the study with four concentration of IBA rooting hormone (0, 500, 1000, 2000, and 4000 ppm and a water control) using a factorial Randomized Complete Block Design with five replications. The preliminary results indicate that wild peaches and plums rooted better than wild almond species (**Table 3**). Treatment with 4000 ppm IBA gave the best result, however 1000 and 2000 ppm treatments gave comparable results.

c) Disease testing of hybrids

Thirty-nine rootstock hybrids from 2016 were screened for crown gall (CG), Phytophthora (CG), root-knot and root-lesion nematodes (RKN and RLN). In the CG evaluation, nine hybrids showed high levels of resistance to root inoculation whereas five among them showed resistance to combined crown and root inoculations (**Figure 2**). The two-year evaluation of 2015 hybrids identified several promising hybrids showing high levels of tolerance to RKN and RLN nematodes (**Figures 3a and b**). The promising ones are currently being tested for ring nematode tolerance. In addition to 2016 hybrids, a backlog of 36 hybrids from 2015 were evaluated for PHY in two experiments. On average, control treatments showed roughly half the amount of crown and root infections (**Figure 4**). However, the results were inconclusive as there was insufficient levels of infection to challenge rootstocks in both experiments. These experiments will be repeated in a field setting during 2018-19 for a realistic evaluation.

d) Single nucleotide polymorphism genotyping and association analyses

A second round of single nucleotide polymorphism discovery and genotyping using a low depth genome sequencing of ~200 experimental and commercial rootstocks is in progress. Association analysis will be performed on the SNP genotype and disease phenotype data to identify the linkage between SNPs and disease resistance.
A previous association analysis using the mixed linear modeling (MLM) results indicated several SNPs across the genome with significant association ($p<0.05$) with CG with R^2 values ranging from 0.09 to 0.11, which is considered significant for traits such as disease resistance with complex inheritance patterns with generally low heritability. It appears that the genetic or quantitative loci (QTLs) modulating CG resistance occurs in four different linkage groups. The marker with highly significant association is found on chromosome 8 based on the peach reference genome used in this study for SNP discovery.

Research Effort Recent Publications:

Browne, G.T. 2017. Resistance to Phytophthora species among rootstocks for cultivated *Prunus* species. HortScience 52: 1471-1476.

Table 1a. *Prunus* hybrids from 2017 spring hybridization – embryo rescued and micropropagated.

Pistil Parent	Pollen Parent	Hybrid ID	No. of hybrids	No. of Plants
P. cerasifera	*P. persica*	CP	6	364
P. dulcis	*P. salicina*	DS	3	155
P. dulcis	*P. angustifolia*	DA	1	27
P. dulcis	*P. kansuensis*	DN	1	234
P. dulcis	*P. davidiana*	DV	1	120

Table 1b. *Prunus* hybrids from 2017 spring hybridization – seed propagated.

Pistil Parent	Pollen Parent	Hybrid ID	No. of hybrids
P. dulcis	*P. davidiana*	DV	72
P. dulcis	*P. kansuensis*	DS	14
P. dulcis	*P. persica (Nemared)*	DP	1
P. dulcis	*P. mira*	DM	7
P. persica	*P. bucharica*	PU	30
P. persica	*P. davidiana*	PV	18
Total			142

Table 2. *Prunus* hybrids produced in 2018 spring (in embryo culture)

Pistil Parent	Pollen Parent	Hybrid ID	No. of hybrids
P. ceresifera	*P. fenzliana*	CF	9
P. ceresifera	*P. kuramica*	CK	20
P. ceresifera	*P. argentea*	CA	21
P. ceresifera	*P. arabica*	CB	14
P. ceresifera	*P. bucharica*	CB	22
P. ceresifera	*P. davidiana*	CD	2
P. dulcis	*P. mira*	DM	1
P. dulcis	*P. angustifolia*	DA	1
P. dulcis	*P. nemaguard*	DN	1
P. dulcis	*P. persica (FG)*	DP	1
P. persica	*P. tomentosa*	PT	2
P. persica	*P. tangutica*	PG	2
P. persica	*P. arabica*	PA	14
Total			110

Table 3. Rootability assessment (% rooting) of some of the *Prunus* species used in rootstock breeding.

Species	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	Mean
P. argentea	0	0	0	0	0	0
P. bucharica	0	0	0	2.5	2.5	1
P. cerasifera	2.5	10	22.5	32.5	22.5	18
P. dulcis	0	0	0	0	0	0
P. davidiana	5	10	5	15	32.5	13.5
P. kansuensis	7.5	25	30	60	50	34.5
P. mira	10	40	57.5	47.5	60	43
P. persica	0	5	12.5	30	20	13.5
P. salicina	0	2.5	0	12.5	10	13.3
P. tangutica	0	0	2.5	0	2.5	1
Lovell	0	5	25	35	0	13

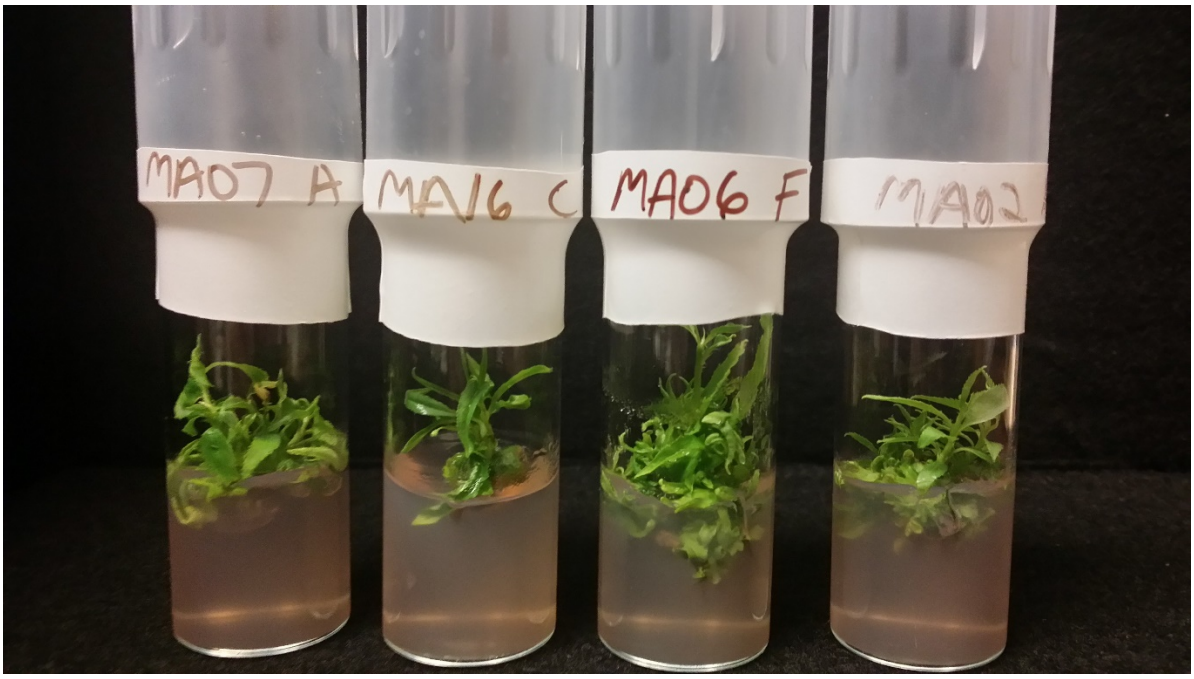

Figure 1. Embryo rescued *Prunus* hybrids.

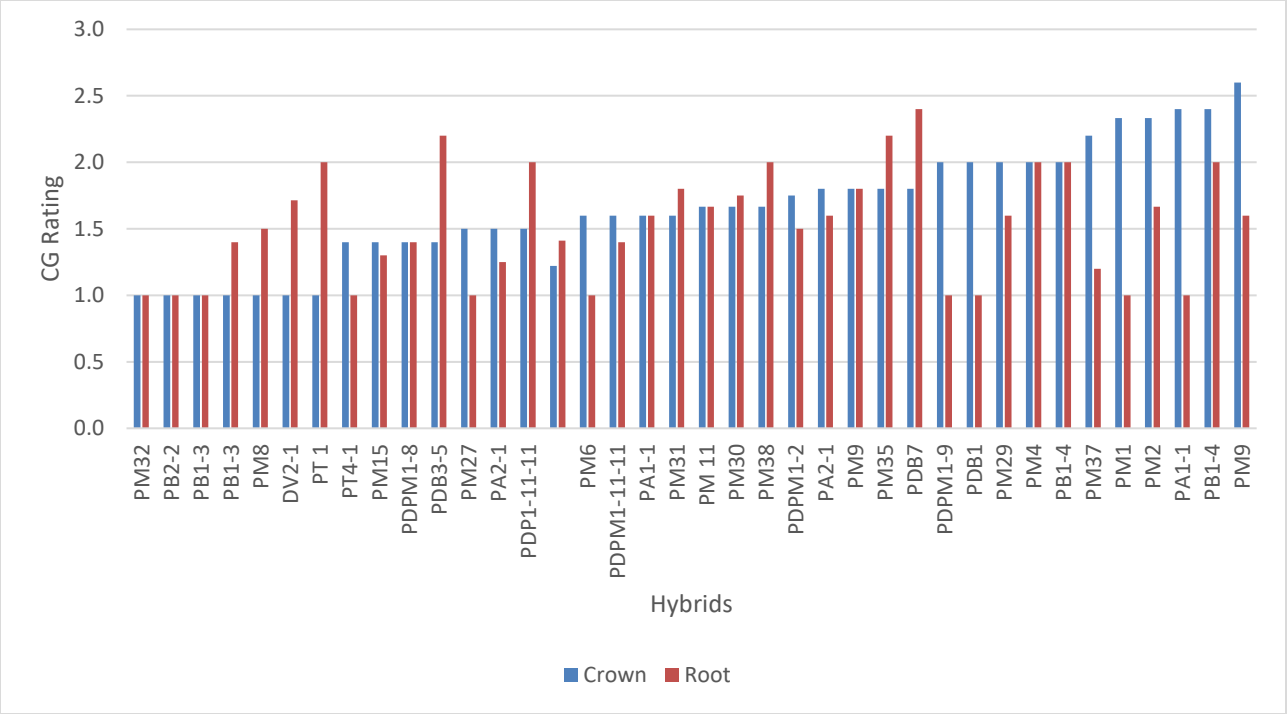

Figure 2. Results of crown gall evaluation of 2016 *Prunus* hybrids. (Y axis: CG rating 1 = No disease symptoms).

Figure 3a. Evaluation of 2015 hybrids for root-knot nematode resistance/tolerance (Box plot showing descriptive statistics on rootstock response to nematode).

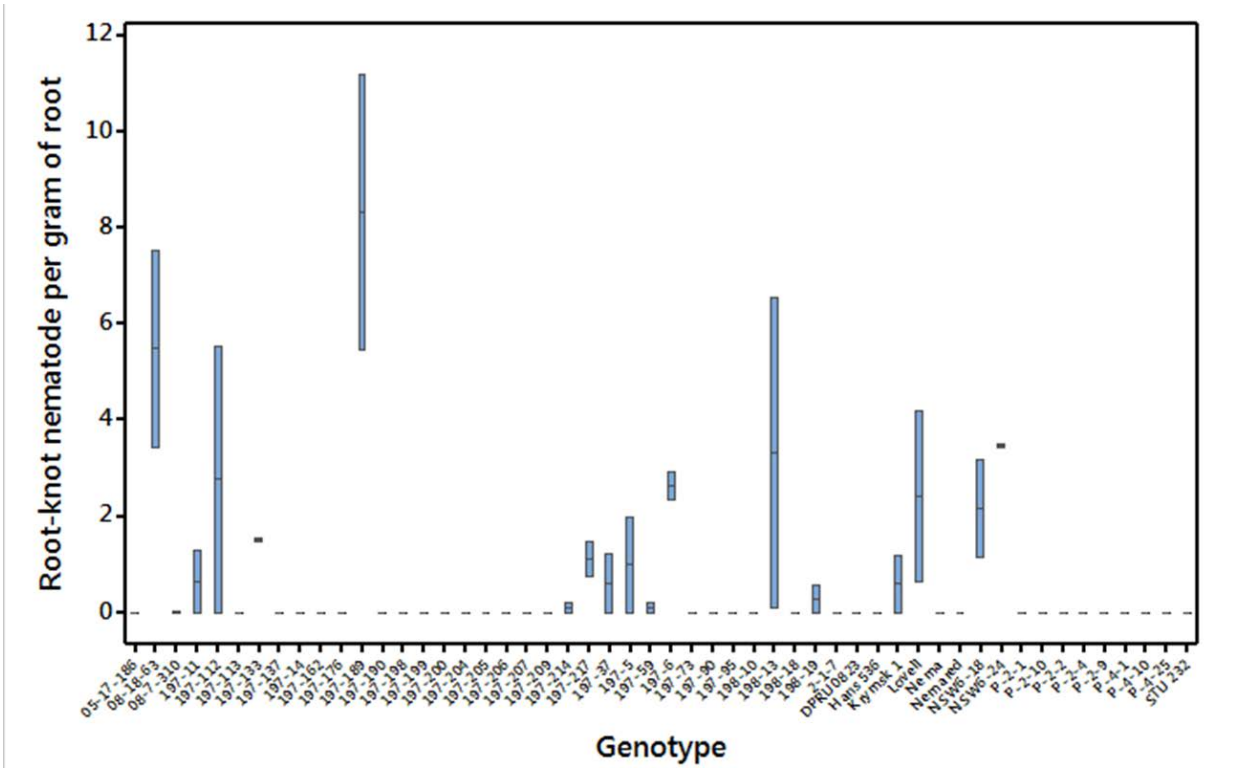

Figure 3b. Evaluation of 2015 hybrids for root-lesion nematode resistance/tolerance.

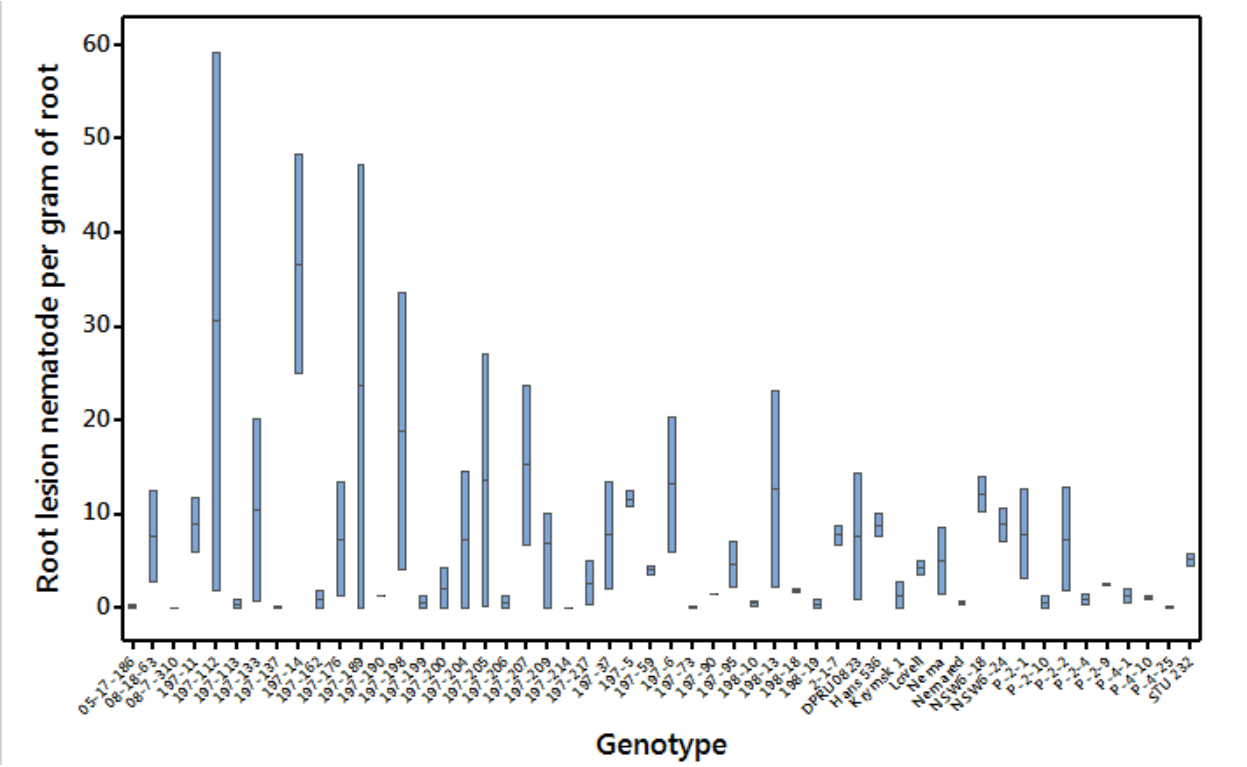

Figure 4. Aboveground disease severity ratings among rootstock standards and experimental hybrids (disease severity scale; 0 to 5 in the order of increasing severity of disease).