Integrated Conventional and Genomic Approaches to Almond Rootstock Development

Dianne Velasco[1,2], Emily Johnson[1], Malli Aradhya[2], Craig Ledbetter[3], Daniel Kluepfel[4], Greg Browne[4], Andreas Westphal[5]

[1]UC Davis, Plant Sciences, Davis, CA; [2]USDA-ARS, National Clonal Germplasm Repository, Davis, CA; [3]USDA-ARS, Crop Diseases, Pests and Genetics Research , Parlier, CA; [4]USDA-ARS, Crops Pathology and Genetics Research, Davis, CA; [5]UC Riverside, Kearney Agricultural Center, Parlier, CA

Introduction

Rootstocks resistant to soil borne diseases are crucial for sustainable production of almonds in California. In this project, we are focusing on developing rootstocks with host-plant mediated field resistance. Cultivated and wild relatives of peach, almond, and plums are important donors of durable resistance to soil borne diseases (*Agrobacterium* induced crown gall (CG), *Phytophthora* (PHY) root and crown rots, root-knot (RK) and root-lesion (RL) nematodes (NEM), and drought tolerance. We have assembled a novel genepool of wild relatives of peach, almond, and plum (**Fig. 1**) for evaluation of resistance to soil borne diseases, rootability (**Fig. 2**), and infusion into the rootstock breeding program. Extensive hybridization to produce genetically diverse hybrids, embryo rescue of interspecific hybrids, and clonal propagation for comprehensive disease evaluation are the key to identifying resistant or tolerant rootstock hybrids (**Table 1, Fig. 3**). Graft compatibility, propagability (**Fig. 2**), and drought tolerance are important measures for successful rootstock breeding program. Peach and almond genetic backgrounds offer graft compatibility, rootability (**Fig. 2**), and drought tolerance. During the past five years 68 different cross combinations in 141 different interspecific hybrids involving wild species of peach, almond and plum were produced embryo rescued, and clonally propagated into ~10,800 clonal plants for disease evaluation. During 2016-17, a set of 40 hybrids produced in 2013 were subjected to a second round of CG evaluation and the same set was screened for the first time for response to RK and RL nematodes at the Kearney Ag. Center. A second round of PHY screening is under way.

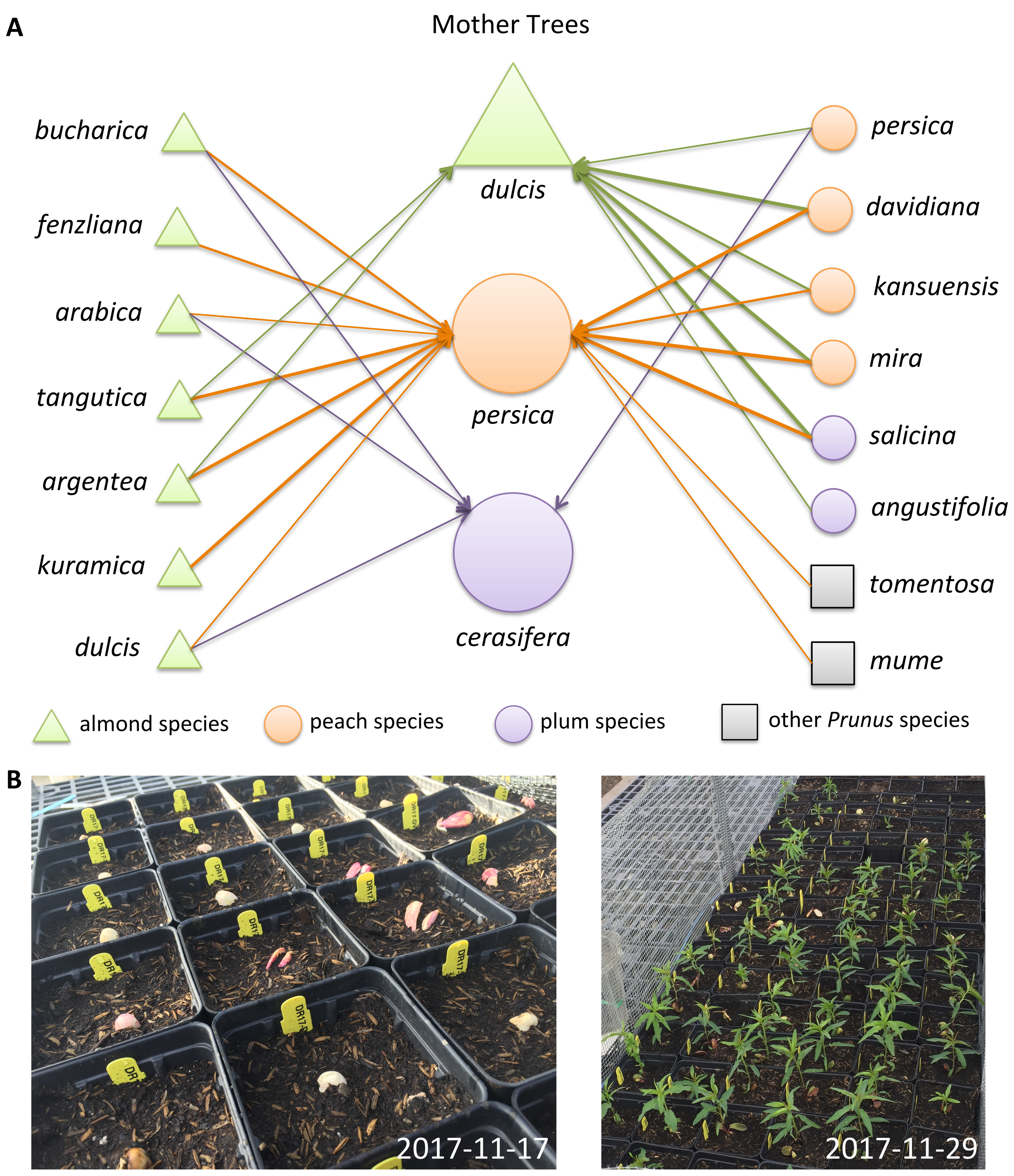

Fig. 1. Interspecific *Prunus* Hybrid Production. A) Crossing scheme for interspecific hybrid production highlighting the diversity of species utilized. **B)** Germination of hybrid seed produced in 2017. Summer-harvested fruit produced from Winter 2017 crosses was and stratified in Fall 2017. We sowed several of the crosses with *P. dulcis* mother trees in mid-November. These crosses require less chilling time than seeds from *P. persica* mother trees, which we will sow when their radicles are ~7-10 cm in length.

Acknowledgements

california almonds
Almond Board of California
Sierra Gold Nurseries

Table 1. Rootstock hybrids showing promising levels of resistance to soil borne diseases.

Hybrid ID	Parentage	CG	PHY	RK_NEM	RL_NEM
P-2-1	Nemared x *P. argentea*				X
P-2-2	Nemared x *P. argentea*			X	
P-2-4	Nemared x *P. argentea*			X	
P-2-9	Nemared x *P. argentea*	X		X	
P-4-1	Nemared x *P. argentea*		X		
P-4-10	Nemared x *P. argentea*			X	X
P-4-25	Nemared x *P. argentea*	X	X	X	X
L-1-2	*P. cerasifera* (OP)	X	X	X	
197-190	*P. persica* x *P. dulcis*		NT		X
197-198	*P. persica* x *P. davidiana*	X	NT	X	
197-199	*P. persica* x *P. davidiana*		NT	X	
197-204	*P. persica* x *P. kansuensis*	X	NT	X	
197-209	*P. persica* x *P. kuramica*		NT		X
197-214	*P. persica* x *P. bucharica*		NT		X
198-10	*P. argentea* (OP; likely *P. dulcis*)		NT		X
198-18	Nemaguard x *P. kansuensis*		NT	X	
197-112	*P. persica* x *P. tangutica*		NT		(X)
197-113	*P. persica* x *P. tangutica*	X	NT		(X)
197-133	*P. persica* x *P. tangutica*	X	NT		
197-137	*P. persica* x *P. tangutica*		NT		(X)
197-162	*P. persica* x *P. tangutica*		NT		(X)
197-214	*P. persica* x *P. bucharica*	X	NT		
197-217	*P. persica* x *P. kuramica*	X	NT		

Note: nematode responses after one year are of tentative nature and require confirmation). Abbreviations: CG = Crown gall; PHY = Phytophthora; RK_NEM = Root knot nematode; RL_NEM = Root lesion nematode; X = promise; (X) = somewhat reduced nematode numbers compared to highly susceptible genotypes; NT = Not tested; OP = open pollinated

Research Highlights

- Produced 68 interspecific crosses, each with variable numbers of hybrids.
- ~10,800 clonal hybrid plants were produced for disease testing during 2015-16.
- Evaluated 61 hybrids for resistance to crown gall for two years, root-knot and root-lesion nematodes (**Fig. 3**) for one year, and 21 hybrids from three cross combinations were tested for *Phytophthora* root and crown rots.
- Tentatively identified a number of hybrids showing promising levels of tolerance to soil borne diseases (**Table 1**)
- We generated over 300 hybrid seeds during winter 2016. Twenty-two were embryo rescued and clonally propagated to produce ~5100 plants for disease evaluation. The remaining were germinated at the NCGR and are being clonally propagated.
- In winter 2017 we generated over 200 hybrid seeds, of which twenty-seven are undergoing embryo rescue and propagation at Sierra Gold Nursery. The remaining are being germinated (**Fig. 1**) at the NCGR with clonal propagation to begin in spring 2018.

Future Plans

- Continue generating diverse interspecific hybrids, incorporating hybrids with promising resistance (**Table 1**) into the parental pool, enlarging the hybrid diversity for improved genetic gains.
- Expand high density genotyping of rootstock breeding populations and combine with high quality disease phenotypes of commercial and experimental rootstocks for analyses to identify markers associated with disease/pest response.
- Validate markers associated with disease/pest response, develop marker assisted selection strategies, and utilize for rapid development of improved rootstocks.

Objectives

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

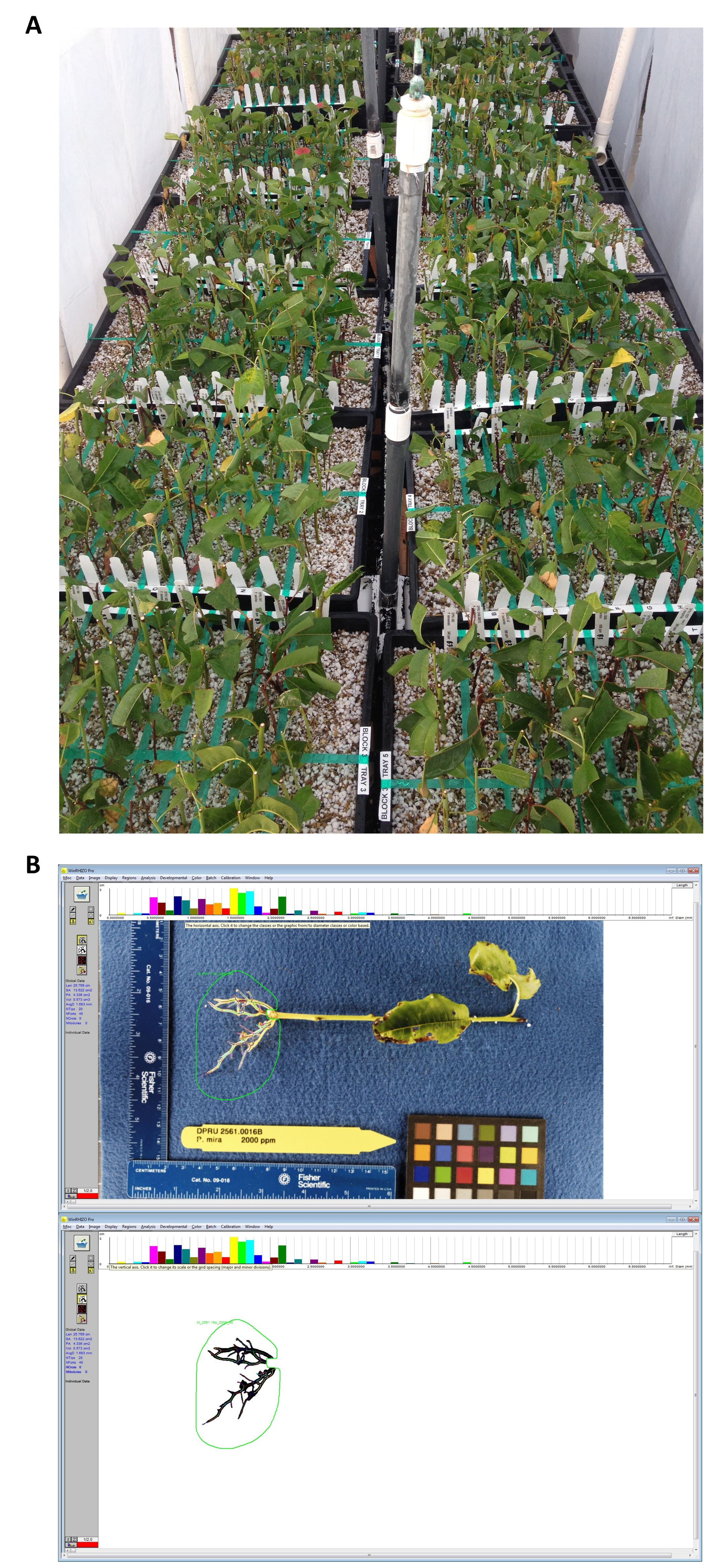

Fig. 2. Evaluation of *Prunus* spp. for Rooting Ability. A) Randomized Complete Block Design with five replications each with nine species and fice KIBA treatments at 0, 500, 1000, 2000, and 4000 mb/L. **B)** Quantification of root generation analyzed from digital photographs using WinRHIZO Pro software. The top image shows the original picture with the root area selected and the bottom image demonstrates detection and analysis of the roots by the software.

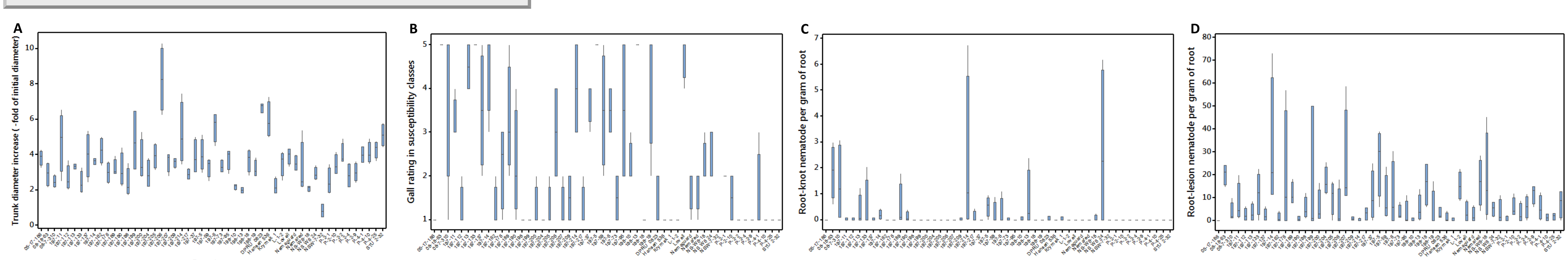

Fig. 3. Nematode Screening Evaluations. Box plots of data collected during the first *Prunus* nematode evaluation at Kearney Agricultural Center in Fall 2016. **A)** Trunk diameter increase of rootstocks at the first evaluation from planting. **B)** Root galling induced by root-knot nematodes among rootstock genotypes (ratings: 1 = no visible gall, 2 = 1-4 galls, 3 = 5-10 galls, 4 = 10-20 galls, and 5 > 21 galls). **C)** root-knot nematode populations densities in roots co-infested with root-knot and root-lesion nematodes. **D)** Root-lesion nematode population densities in roots co-infested with root-lesion and root-knot nematodes.