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# Identification of Almond Rootstocks with Resistance to *Armillaria* Root Disease

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**Project No.:** 12-PATH7-Baumgartner

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**Objectives:**

- 1) Identify *Armillaria*-resistant rootstocks for almond, by first screening a set of commercially-available *Prunus* rootstocks
- 2) Determine the relationship between the results of our infection assays in the lab and field observations

**Interpretive Summary:**

*Armillaria* root disease affects all almond production regions of California. The causal fungus, *Armillaria mellea*, colonizes and kills the roots, and then decomposes the root wood as its source of nutrition. Such destruction to the roots significantly reduces crop yield and growth, inhibits nutrient and water uptake from the soil, and eventually kills infected trees. Soil fumigants like methyl bromide are only effective at preventing *Armillaria* root disease to the extent that they reach and penetrate residual roots. Methyl bromide is no longer available for use in almond due to its phase out as part of the Montreal Protocol and other soil fumigant options are under increasing regulatory pressure. We propose to identify resistant rootstocks as an effective, long-term solution. We screened the following rootstocks: Empyrean 1 (*aka* Barrier 1), Lovell, Nemaguard, Brights 5, Hansen 536, Krymsk 1 (*aka* VVA 1), Krymsk 86 (*aka* Kuban 86), and Marianna 2624. More resistant than the resistant control (Marianna 2624) was Krymsk 86. More susceptible than the susceptible control (Nemaguard) were Brights 5 and Hansen 536. Krymsk 1 was similar to Marianna 2624. Lovell and Empyrean 1 were similar to Nemaguard.

**Materials and Methods:**

To screen *Prunus* rootstocks for *Armillaria* resistance in the lab, we use an infection assay that has been used to screen rootstocks of grape (Baumgartner et al. 2010) and walnut

(Baumgartner et al. 2013). In the greenhouse, *Armillaria* inoculations are notoriously difficult to establish, and what happens is that the experimental results are often too variable to identify resistant rootstocks. Our approach was to propagate plants in tissue-culture medium, which supports both the plant and the pathogen. With this infection assay, we have overcome the major barriers of the greenhouse approach, namely eliminating ‘escapes’ and bringing about consistent, repeatable levels of mortality. Experiments are replicable within a one-year period. On the other hand, we did not know initially how this assay would reflect field observations. Furthermore, I need to stress that resistance of the same rootstocks in the field might be different, possibly under the influence of natural conditions that may affect root susceptibility (e.g., root system architecture, beneficial soil microbes). This is just a screen.

Our inoculations included two *Armillaria* species. In almond production areas of California, the species that attacks planted hosts is *Armillaria mellea*, and so we included two aggressive strains originally isolated from dead and dying plants in *Prunus* orchards. The species that attacks *Prunus* (namely peach) in the southeastern US is *A. tabescens*, and we included one such strain originally isolated from symptomatic peach. Plants were inoculated with a homogenized 6-day culture of the three strains of *Armillaria*. On a weekly basis, dead plants were tallied. The majority of plants died within 2 months of inoculation, which was also the point at which the non-inoculated controls plants began to run out of nutrients in the rooting medium. As such, our analyses focused on comparing rootstock mortality after 2 months (**Table 1**, see below). This experiment was replicated three times.

## Results and Discussion:

Our resistant and susceptible controls were Marianna 2624 and Nemaguard, respectively. Their relative resistance was based on past field observations, and so we were not sure how they would perform in our laboratory assay. Fortunately, the results were consistent with field observations. Mortality after 2 months was 63% for Marianna 2624, compared to 85% for Nemaguard (**Table 1**).

**Table 1.** Relative resistance of six almond rootstocks, compared to resistant control Marianna 2624 and susceptible control Nemaguard. Each mean is the average of three replicate experiments, averaged across three *Armillaria* isolates.

Rootstock	Mortality after 2 months (% dead plants)
Krymsk 86	27.3a
Krymsk 1	35.8ab
Marianna 2624	63.1bc
Lovell	71.8cd
Empyrean 1	77.8cd
Nemaguard	84.5d
Bright 5	87.2d
Hansen 536	89.1d

We identified Krymsk 86 as the most resistant of the six rootstocks and Hansen 536 as the most susceptible. Therefore, in future screening experiments, we should use Krymsk 86 and Hansen 536 as resistant and susceptible controls.

Across all rootstocks, the strains of the pathogen behaved similarly. Both strains of *A. mellea* were equally virulent. The strain of *A. tabescens* was in all rootstocks less virulent.

#### **Research Effort Recent Publications:**

A manuscript will be submitted to HortScience.

#### **References Cited:**

Baumgartner K, Bhat R, Fujiyoshi P, 2010. A rapid infection assay for *Armillaria* and real-time PCR quantitation of the fungal biomass *in planta*. *Fungal Biology* 114: 107-119.

Baumgartner K, Fujiyoshi PT, Browne GT, Leslie C, Kluepfel DA, 2013. Evaluating Paradox walnut rootstocks for resistance to *Armillaria* root disease. *HortScience* 48: 68-72.