Screening of Pear Rootstocks for Armillaria Resistance

By Carolina E. Tweedy
University of California, Davis

Funding for Screening of Pear Rootstocks for Armillaria Resistance was made possible by the U.S. Department of Agriculture's (USDA) Agricultural Marketing Service through grant AM180100XXXXG003. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA.

OVERVIEW

In California and throughout the Pacific Northwest, orchards of pear and other horticultural crops have been established on land cleared of native forests [1]. *Armillaria* is a native soil-borne fungus with a broad range of hardwood hosts along the West Coast [1] [2]. In a variety of woody plants, Armillaria infection causes root system decay, gradual health decline, and ultimately death [3] [4] [5]. The most common species of *Armillaria* in California are *Armillaria mellea* and *Armillaria gallica* [2] [6]. This study focused on *A. mellea* because it is the most lethal and widely distributed species [2].

Image 1: Possible Armillaria infected pear tree in a Lakeport pear orchard. Scarce and minimal leaf development.

PROBLEM

Reports of *A. mellea* infections in pear orchards started to increase in the 1990s [1]. Image 1 shows a tree exhibiting sign and symptoms of possible Armillaria infection. The lack of leaf coverage can result in lower fruit production and ultimately lower yield. Pears in the past were considered "immune or highly resistant" to Armillaria infection and as a result were highly recommended as replants for other tree crops killed by *A. mellea* in California [1] [7]. There is no method to eradicate the pathogen from infected trees nor therapeutic methods that counteract tree death of highly susceptible species. Image 2 showcases the white *Armillaria* fungus penetrating the bark tissue of an infected tree stump. *Armillaria* is a very slow growing fungus and is able to persist in the soil within residual infected root or bark tissue for over 10 years [3].

Image 2: Armillaria infected bark tissue. Sample collected from crown of dead pear tree stump that most likely died from Armillaria infection. The white specks within the bark tissue are the Armillaria fungus.

WHAT TO DO

Currently, there is no pear rootstock known to be resistant to Armillaria. This limits options for replanting trees in infected orchards. Due to the broad host range of *Armillaria*, other tree crops face a similar challenge. In order to identify sources of resistance to be utilized in rootstock breeding programs, a rapid and reliable screening technique for pears is necessary. Studies in other susceptible crops, namely grape, walnut, and *Prunus*, have shown that greenhouse experiments with *Armillaria* are largely unsuccessful, whereas *in vitro* experiments (under sterile lab conditions, Image 3) are more repeatable [4] [8] [9] [10] [11]. In greenhouse experiments, symptoms can take multiple years to be expressed and often a high proportion of plants 'escape' infection [8]. Baumgartner et al. (2010) [10] developed an Armillaria *in vitro* screening technique for grape, and subsequently used a similar approach to identify sources of resistance in walnut and almond rootstocks [3] [4]. For *in vitro* evaluations, plants are micropropagated, rooted *in vitro*, and inoculated with *A. mellea* on tissue culture (agar) medium which supports both the roots of the plant and the pathogen, rather than using soil-based media. This study was performed to optimize a rapid and reliable *in vitro* phenotyping protocol in pears. There were

Image 3: Week 0 (left) is the day of Armillaria inoculation and Week 6 (right) show plantlets six weeks after Armillaria inoculation. Plantlet number 5 has a purple strip of tape to indicate it was a control (was not exposed to the Armillaria inoculum). Black leaves are visible in Week 6, progression of disease development.

85 different clonal genotypes and 185 different seedling genotypes tested. Clonal rootstocks are genetically identical to each other, whereas each individual seedling is genetically unique. The use of seedlings in this study increased the diversity of rootstocks observed. Each clonal rootstock was propagated to at least 20 replicates, while the seedling numbers varied based on germination success. Each plantlet was placed into an individual test tube and inoculated with a homogenized liquid suspension of cultured *A. mellea*, as seen in Image 3. Plantlets were visually inspected on a weekly basis and scored for necrosis on a scale of 0 to 100% for a total of 6 weeks. Necrosis, the blackening of the leaves and stem, was deemed a symptom of infection.

RESULTS

This study used the area under the disease progress curve (AUDPC) to quantify disease development over the six-week treatment for each individual plantlet (Figure 1) [12] [13]. The smaller the AUDPC the more resistant the genotype. Two elite *P. communis* rootstocks, OH×F 87 and OH×F 97, stood out as highly resistant based on low overall AUDPC scores, as seen in Figure 2. OH×F 87 and OH×F 97 are two clonal genotypes already in commercial production and also show great resistance to fire blight [14]. The OH×F varieties also have great micropropagation qualities which would facilitate their use in additional *in vitro* studies. It may also be beneficial to field-test the OH×F 87 and OH×F 97 genotypes. OH×F 87 and OH×F 97 are the strongest recommendations for possible replants from this study.

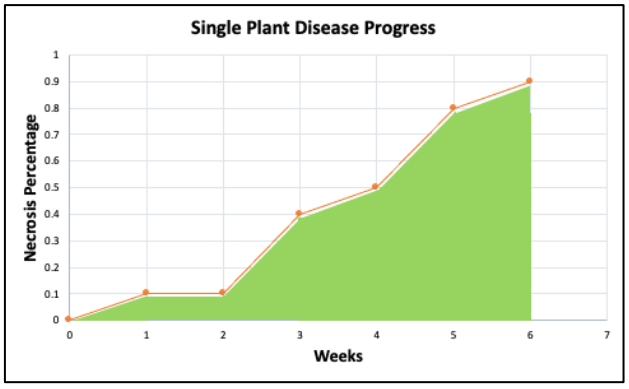

Figure 1: Line graph of a single plant's disease development over the course of 6 weeks. Area under the curve was calculated to give an AUDPC value for each plant. This value was used to determine which genotypes preformed the best.

The long-term goal of breeding pear rootstocks resistant to Armillaria is one step closer to fruition with the development of this optimized *in vitro* protocol for rapid and reliable Armillaria screening.

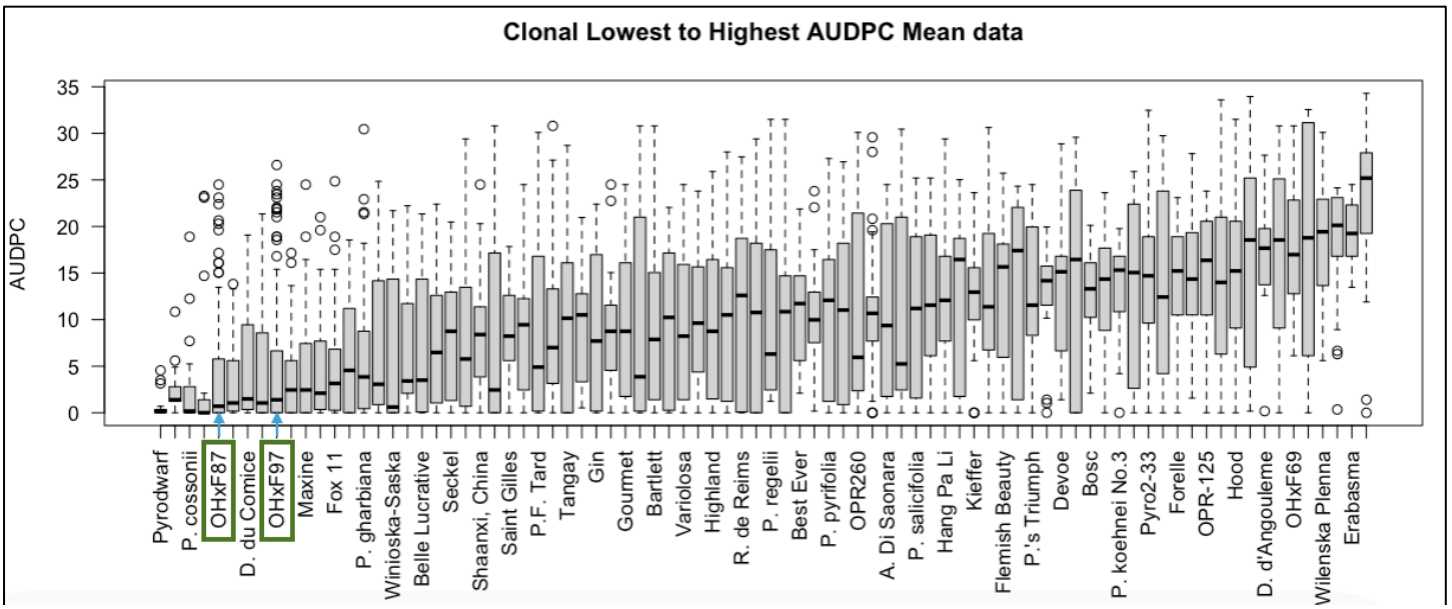

Figure 2: Boxplot of average AUDPC scores for all 85 clonal genotypes inoculated. The green boxes show the genotypes OH×F 87 and OH×F 97. Ordered from lowest AUDPC mean scores (more resistant) to highest AUDPC mean scores (more susceptible).

References

* All images and graphs were taken and produced by Carolina Tweedy*

1. Rizzo, D. M., E. C. Whiting, and R. B. Elkins. "Spatial distribution of Armillaria mellea in pear orchards." *Plant Disease* 82.11 (1998): 1226-1231.
2. Baumgartner, Kendra, and David M. Rizzo. "Distribution of Armillaria species in California." *Mycologia* 93.5 (2001): 821-830.
3. Baumgartner, Kendra, et al. "Evaluating paradox walnut rootstocks for resistance to armillaria root disease." *HortScience* 48.1 (2013): 68-72.
4. Baumgartner, Kendra, et al. "Screening almond rootstocks for sources of resistance to Armillaria root disease." *HortScience*53.1 (2018): 4-8.
5. Baumgartner, Kendra, Martin PA Coetzee, and Dirk Hoffmeister. "Secrets of the subterranean pathosystem of Armillaria." *Molecular Plant Pathology* 12.6 (2011): 515-534.
6. Wargo, Philip M., and Charles G. Shaw III. "Armillaria root rot: the puzzle is being solved." *Plant Disease* 69.10 (1985): 826-832.
7. Raabe, R. D. "Resistance or susceptibility of certain plants to Armillaria root rot." *Leaflet-University of California, Cooperative Extension Service (USA)* (1979).
8. Beckman, T. G., and P. L. Pusey. "Field testing peach rootstocks for resistance to Armillaria root rot." *HortScience*36.1 (2001): 101-103.
9. Baumgartner, Kendra, and David M. Rizzo. "Spread of Armillaria root disease in a California vineyard." *American Journal of Enology and Viticulture* 53.3 (2002): 197-203.
10. Baumgartner, Kendra, Ravi Bhat, and Phillip Fujiyoshi. "A rapid infection assay for Armillaria and real-time PCR quantitation of the fungal biomass in planta." *Fungal biology*114.1 (2010): 107-119.
11. Prodorutti, Daniele, et al. "Susceptibility to Armillaria mellea root rot in grapevine rootstocks commonly grafted onto Teroldego Rotaliano." *Phytopathologia mediterranea* 48.2 (2009): 285-290.
12. Jeger, M. J., and S. L. H. Viljanen-Rollinson. "The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars." *Theoretical and Applied Genetics* 102.1 (2001): 32-40.
13. PARASCHIVU, Mirela, Otilia COTUNA, and M. Paraschivu. "The use of the area under the disease progress curve (AUDPC) to assess the epidemics of Septoria tritici in winter wheat." *Research Journal of Agricultural Science* 45.1 (2013).
14. Elkins, Rachel B., and Mitcham, Elizabeth J.. Pear Production and Handling Manual. United States, University of California, Agriculture and Natural Resources, Communication Services, 2007.