Selection of Apple Rootstock Breeding Families for *Phytophthora* crown rot resistance

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Abstract

Crown and root rot of apple rootstocks associated with *Phytophthora* species is an important disease causing major losses in apple production areas. Crown and root rots are often associated with major abiotic stresses like prolonged water submergence and poorly drained or compacted soils. *Phytophthora* species are also implicated in the replant disease complex. The Geneva® apple rootstock breeding program has been active in the selection for crown rot resistance within its germplasm. In 2009 we conducted a replicated experiment featuring 16 full-sib families representing crosses between elite rootstocks and wild Malus species to validate the reliability of the selection method used. The method established in the 1970s, required the inoculation of young seedlings, two weeks after emergence, with a mixture of several *Phytophthora cactorum* strains collected throughout the U.S.A. and subsequent root submergence with cool water for 76 hours. The experiments were set up with four replicates of 40 full-sib seedlings per flood basin. Full sib family percent survival and flood images were collected three weeks after inoculation. The inoculation was successful displaying differences between both control treatments (flooded non-inoculated [~83% survival] and non-flooded/non-inoculated [~87% survival]), and the mean survival of all inoculated treatments (~26% survival). There were, for the most part, no significant differences between full-sib family survival means, probably caused by localized variation within inoculation bins and between bins where one full-sib family would display. Only one family ('G.41' x '*Malus sieversii* pool 4') displayed higher than normal survival (~47% survival). This may indicate an improved source of resistance to crown rot within the pollen pool of that family. However, the variance within inoculated family replications may indicate potential escapes of up to 15% of the total survivors. More research is needed to improve the reproducibility of this important selection parameter within Geneva® apple rootstocks.

Keywords: disease resistance, crown rot, rootstock breeding, replant disease.

INTRODUCTION

Phytophthora crown and root rot are among the most important diseases affecting the crown and roots of apple trees worldwide. Several *Phytophthora* spp. have been implicated in the crown and root rot disease, but two, *P. cactorum* (Lebert and Chon) and *P. cambivora* (Petri), have been consistently associated with the disease (Baines 1939; Sewell and Wilson 1973). The severity of the disease is exacerbated when apple trees are planted on wet and low land or in heavy soils (Jeffers and Aldwinckle 1988; Utkhede and Smith 1993;). *Phytophthora* spp. have also been implicated as a component in apple replant disease complex and other fungal, oomycete, and bacterial components (Mazzola 1998). While several fumigation methods have shown success depending on soil type and severity of the disease, genetic tolerance or resistance is by far the best method to overcome this disease (Utkhede et al., 2001). In general, all the advanced selections and releases from the Geneva® breeding program are considered resistant or tolerant to crown and root rots given their survival in the initial inoculation tests and successive field experiments (Robinson et al. 2003).

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Among wild apple species, *Malus sieversii* accessions have shown promise in resisting/surviving to replant disease (Utkhede, 1985; Browne and Mircetich, 1993; Browne et al., 1995; Isutsa and Merwin, 2000; Fazio et al., 2009). This study aimed to understand if the addition of wild *Malus* germplasm pools could increase the overall resistance/survival response to crown and root rot in the Geneva breeding program (Robinson et al., 2003).

MATERIALS AND METHODS

In 2009 we conducted a replicated experiment featuring 17 full-sib families representing crosses between commercial and elite rootstocks (considered to be tolerant to crown and root rot) and wild Malus species (with unknown tolerance to crown and root rot) to validate the reliability of the selection method that we had been using. The full-sib families were 'B.9' x 'G.41', 'B.9' x 'Siev.Pool7', 'CG.5757' x 'B.9', 'G.16' x 'G.65', 'G.16' x 'G.202', 'G.41' x 'G.11', 'G.41' x 'M.26', 'G.41' x 'M.ioensis7', 'G.41' x 'M.ioensis8', 'G.41' x 'Siev.Pool4', 'G.41' x 'Siev.Pool5', 'G.41' x 'Siev.Pool7', 'G.41' x 'Siev.Pool8', 'G.202' x 'Siev.Pool5', 'G.202' x 'Siev.Pool8', 'G.935' x 'G.41', 'M.26' x 'Siev.Pool5' where the notations 'Siev.Pool#' and '*M.ioensis*#' represent a particular pollen pool or accession of *Malus sieversii* or *Malus ioensis* obtained from the *Malus* germplasm collection in Geneva, NY.

Figure 1. Experimental setup for the 2009 full-sib seedling families resistance test arranged in randomized replicated groups prior to inoculation with *Phytophthora spp.*

The inoculation method was established in the 1970s (Aldwinckle et al., 1972; Aldwinckle et al., 1974) and briefly described hereafter: "Difeo" lima bean agar is prepared using half the recommended amount and then adding extra agar to bring the concentration back to 1.5%. The sterile melted "LBA" is poured into sterile 20 mm deep Petri plates. The plates are seeded with an agar plug of *Phytophthora cactorum* (combination of 13 different isolates). Plates are incubated at room temperature. When the colonies are about 3 cm in diameter, the plates are flooded with sterile distilled water to a depth of about 0.5 cm and incubated at room temperature covered with clean cheesecloth to allow spores to suspend in solution. This process should coincide with the time the seeds to be screened are sown. The seedlings are inoculated when they are about 2.5-4 cm high and have at least two true leaves. A cork is put in the hole of each tray. Then the soil is flooded with water sufficiently to produce some puddles. However, the water level is not significantly higher than the soil level. The final zoospore release is performed by exposing the flooded plates to -17 °C temperatures for 20 min and then room temperature for 30-45 min. A microscope and hemocytometer are used to observe zoospores and adjust the inoculum to 100,000 spores mL^{-1}. One hundred milliliters of inoculum are poured evenly into each tray. The trays are left flooded for 76 h.

The experiments were set up with four replicates of 40 full-sib seed per cross and 4 crosses per flood basin for a total of 160 seed for each cross. Crosses were assigned to flood basins randomly. To test the effectiveness of the inoculation treatment an additional four replicates (40 seeds) for four full sib families were given the no flood/no inoculation treatment and the flood/no inoculation treatment. Full sib family percent survival (sprouted seedlings/survivors) and images were collected three weeks after inoculation. Mixed models analyses were used to obtain individual full sib population means (treatment and full sib population as fixed effects and flood bins as random effects). Statistical analyses and graphs were obtained with SAS JMP15-Pro software (Cary, NC 27513-2414, USA).

RESULTS AND DISCUSSION

The inoculation was effective in causing the death of seedlings belonging to all full-sib families compared to the non-inoculated flooded and the non-inoculated treatments. Parental sources of full-sib families had a significant effect on seedling survival (Figure 2). The control flooded non-inoculated treatment displayed ~83% survival, the control non-flooded/non-inoculated displayed ~87% survival, whereas the mean survival of all flooded inoculated treatments was ~26%. For the most part, no significant differences between full-sib family survival mean. 'G.41' x '*Malus sieversii* pool 4' displayed higher than normal survival (~47%), whereas 'G.41' x '*Malus sieversii* pool 8' had the lowest survival (Figure 3).

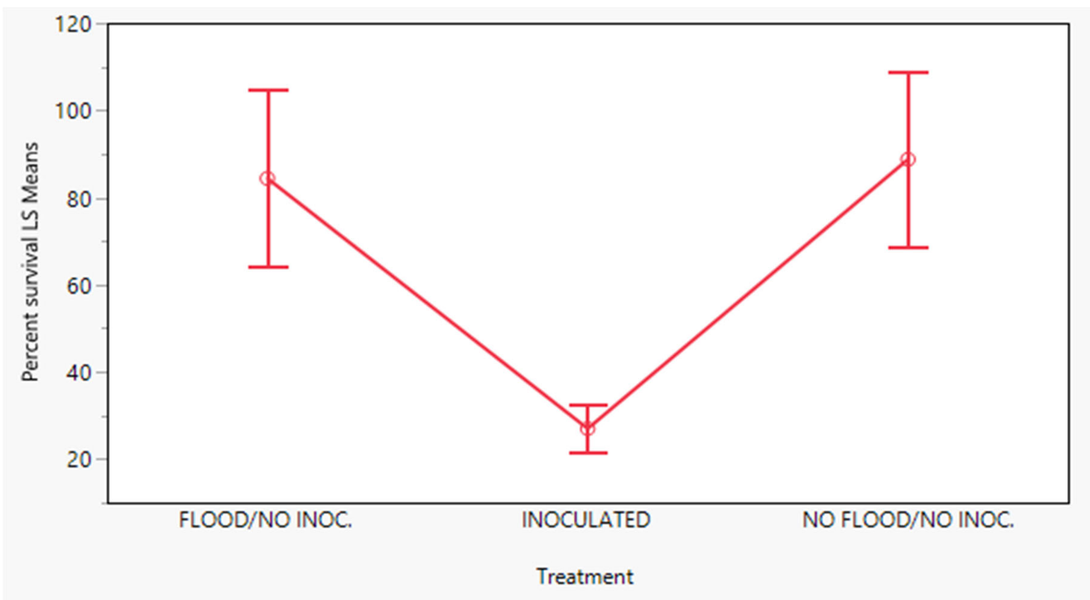

Figure 2. LS means and error bars showing the effectiveness of the inoculation treatment compared to the non-inoculated controls.

Successful apple rootstock breeding requires the introduction of novel alleles to diversify the gene pool and increase the broad-spectrum resistance to diseases (Robinson et al., 2003; Aldwinckle et al., 2004; Fazio et al., 2015). Early screenings and experiments have resulted in the combination of crown and root rot resistance with fire blight resistance in the Geneva® breeding program (Aldwinckle et al., 1972; Cummins et al., 1972). Breeding rootstocks is a continuous process aimed at improving field performance where crosses and selection of superior genotypes recur every few years. These experiments were conducted to assess resistance to crown and root rot of recently developed gene pools within the Geneva breeding program. All crosses featured a crown rot resistant elite parent ('G.41', 'G.935', 'G.202', 'CG.5757', and 'G.16') and pollen pools from various *Malus* wild species accessions. Crosses between elite parents all displayed lower survival rates (20-35%) than most crosses with wild germplasm pools.

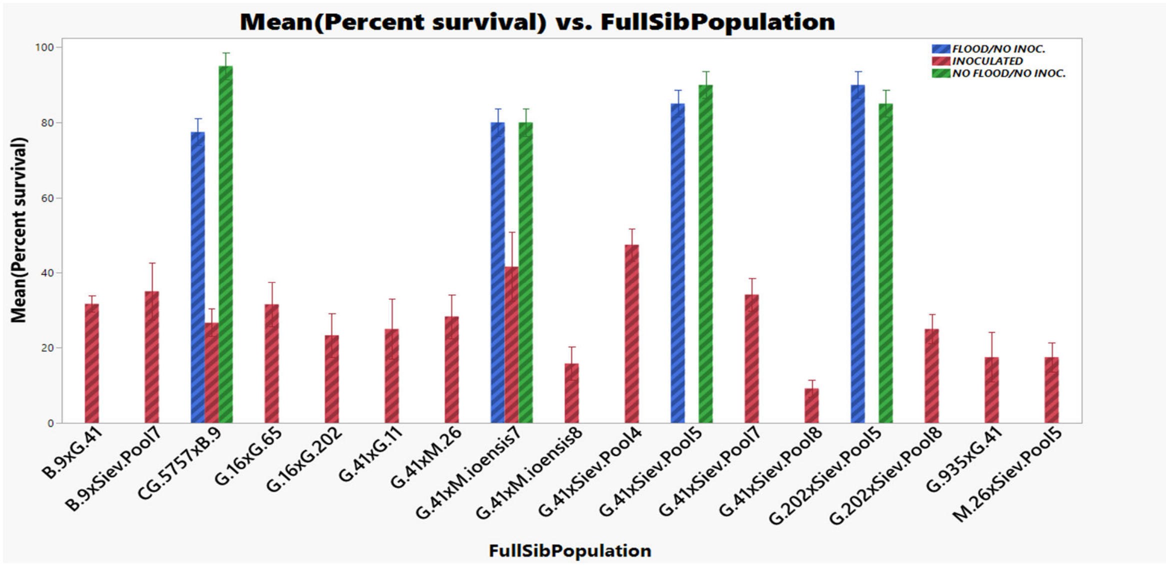

Figure 3. Mean percent survival by treatment and sib population. Some treatments could not be replicated in all sib populations because of the lack in the number of seedlings available.

The *M. sieversii* pollen pools were chosen because of early indications of resistance to other components of the replant disease complex. Interestingly, not all *M. sieversii* pollen pools performed the same, where pool eight displayed survival under 20% and pool 4 displayed survival close to 50%. Interestingly, '*M. sieversii* pool 7' crossed with 'B.9' (sensitive to crown rot) and 'G.41' (resistant to crown rot) displayed similar survival levels, suggesting a stronger influence by that '*M. sieversii*' pollen pool. Similarly, '*M. ioensis* pool 8' was less than 20%, whereas pool 7 was higher than 40%. A significant difference was detected between full sibs 'G.202' and 'G.41' when crossed with the same '*M. sieversii* pool 8' where the number of survivors in the 'G.202' cross was greater than the cross with 'G.41'. This difference may indicate additional tolerance/resistance genes inherited by 'G.202' from parents 'Robusta 5' and 'M.27', however at this point without additional inheritance tests it is not possible to ascertain what parent is the source of this additional resistance. The population obtained from the cross between 'G.935' and 'G.41' had a lower survival score compared to the 'B.9' by 'G.41' cross which is somewhat unexpected given the tolerance to components of replant disease (Mazzola, 1998) displayed by 'G.935' when compared to 'B.9' (Zhu et al., 2017; Zhu et al., 2020) and perhaps highlights the difference between Phytophthora species and Pythium species in the way they are antagonized by the host plant.

CONCLUSIONS

Broadening gene pools in a breeding program allows the discovery new sources of tolerance to biotic and abiotic stresses as well as preparing for new threats (Volk et al., 2015). In this work we have demonstrated the value of including some Malus wild species in broadening the apple rootstock germplasm pool to increase tolerance/resistance to crown and root rots. While this is an important trait in the breeding program, it is only one of the many traits that make apple rootstocks productive and desirable by industry, therefore additional research resources need to be dedicated to the material featured in this work to identify superior rootstocks and/or potential parents for the next generation of apple rootstocks.

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