By combining the results of blight resistance breeding and the application of hypovirulence as a biocontrol, populations of resistant hybrid trees could be deployed together with a less pathogenic strain of Cryphonectria parasitica (Muir). Barr used a small stem assay to screen seedlings in thirteen half-sibling backcross F2 families with an attenuated strain of C. parasitica containing the Cryphonectria parasitica hypovirus-1 (CHV-1) Euro7 virus. The experiment was set up as a randomized complete block design in 2-gallon containers. Measurements of canker length and morphology will be gathered at four, eight, 12, and 16-weeks post-inoculation. A secondary experiment was conducted to further understand the in vivo effects of hypovirulence. Trees from five of the thirteen half-sibling families were inoculated with isolates of virulent (virus-free) and hypovirulent (virus-containing) strains of the fungus. Virulence of the virus-free (V) and virus-containing (H) strains was measured using the Granny Smith apple assay. The possibility of in vivo canker conversion from V to H will be tested later (after eight weeks) by inoculating half of the V-infected trees with the H strain. Measurements of canker length and morphology will be gathered weekly for another eight weeks post-inoculation. Only preliminary results are presented here as the SSA portion of this project is expected to continue through November 2022. All surviving trees will be planted in an experimental orchard in Middle Tennessee to create a potentially long-lasting population of disease resistant trees together with an effective, long-term biocontrol for chestnut blight.

**Experiments**

**Small Stem Assay using a Hypovirulent Strain:** We plan to continue measuring the canker growth of hypovirulent C. parasitica on the progeny of C. mollissima, C. dentata and selected backcross hybrids expected to have varied levels of blight resistance.

**Small Stem Conversion from V to H:** Measuring the Canker Growth of converted C. parasitica from virulent to hypovirulent on the progeny of hybrids with intermediate blight resistance.

**Small Stem Conversion from V to H:** Measuring the Canker Growth of converted C. parasitica from virulent to hypovirulent on the progeny of hybrids with intermediate blight resistance.

**Granny Smith Apple Assay:** Measuring the lesion growth of hypovirulent and virulent C. parasitica on Granny Smith apples.

**Preliminary Results**

**Small Stem Assay using a Hypovirulent Strain**

- Inoculation and Data Collection: 365 open-pollinated progeny of selected backcross hybrid trees with intermediate levels of blight resistance were planted in four completely randomized blocks. Only 321 trees were inoculated with a hypovirulent Weekly strain of C. parasitica. Infected with the CHV-1 Euro7 hypovirus. At four, eight, twelve, and sixteen weeks, the cankers will be measured for canker length and morphology (Cipollini et al. 2021).

**Small Stem Conversion from V to H**

- Inoculation and Data Collection: A total of 109 trees were inoculated in the secondary experiment. For each family, eight out of 24 trees were inoculated with hypovirulent C. parasitica and sixteen out of 24 were inoculated with virulent C. parasitica. After four weeks, the cankers were measured weekly using a caliper for canker length and morphology. After eight weeks post-inoculation, eight of the sixteen trees originally inoculated with the virulent fungus were re-inoculated with the hypovirulent fungus to convert the canker in vivo. The cankers will be measured weekly for canker length and morphology for the next four weeks.

**Granney Smith Apple Assay**

- Ten Granny Smith apples were inoculated with a control plug of PDA, virulent C. parasitica, and hypovirulent C. parasitica (Kuhlman, 1983). Measurements were recorded with a caliper at five, ten, and fifteen days. Each lesion was recorded for three different diameters from which the mean area was calculated. Photos were also taken for each lesion on each measurement date and the surface area was determined using ImageJ.

**Granny Smith Apple Assay**

- Ten Granny Smith apples were inoculated with a control plug of PDA, virulent C. parasitica, and hypovirulent C. parasitica (Kuhlman, 1983). Measurements were recorded with a caliper at five, ten, and fifteen days. Each lesion was recorded for three different diameters from which the mean area was calculated. Photos were also taken for each lesion on each measurement date and the surface area was determined using ImageJ.

**Conclusion**

**Small Stem Assay using a Hypovirulent Strain**

- The data collection and analysis of the main experiment is still ongoing. I plan to record two more canker measurements at 12- and 16-weeks post-inoculation. Only two measurements have been recorded for the main experiment so far. Because analysis of already gathered data is still underway, no conclusion can be drawn yet.

- The results of the Small Stem Assay will help to develop screening methods for progeny testing. Surviving trees will be grown in an orchard setting with the prospect of establishing a population of hypovirulent C. parasitica along with blight resistant trees.

**Small Stem Conversion from V to H**

- I have already completed the first set of measurements prior to re-inoculation, the re-inoculation of half the V-infected trees, and two out of four post-inoculation measurements. I am currently analyzing the data already obtained but I cannot yet draw any conclusions.

**Granney Smith Apple Assay**

- The results of the Granny Smith Apple assay were statistically insignificant, which does not support my prediction that the virulent lesion would grow faster than the hypovirulent lesion. The two strains—virulent and hypovirulent—of the isogenic fungus may have similar levels of pathogenicity, resulting in no distinction in terms of growth rates. An excised stem assay may provide more distinctive growth differences in comparison to the Granny Smith Apple assay.

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