Title: Cataloging the Host Response of Blueberry Cultivars to Phytophthora Root Rot Disease

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Public Abstract

Phytophthora root rot is a major disease in commercial blueberry production in the Southeast US where in-ground planting is the prevalent cultivation practice. Phytophthora root rot is caused by infection with soil-borne oomycete pathogen *Phytophthora cinnamomi*. Blueberry bushes infected with *P. cinnamomi* exhibit root necrosis, discoloration of leaf tissue, yield loss, and plant death. Although fungicide applications and raised bed planting are helpful practices for mitigating the disease, there is no cure for the infected plants and waterlogging from severe weather or overhead freeze protection promotes root rot disease progression. Developing blueberry cultivar resistant to Phytophthora root rot provides long-term sustainable management of this devastating disease. In this study, a reliable greenhouse inoculation method was established that can be used to screen blueberry breeding materials for host resistance. Younger plants were more prone to infection and rapid disease progression than older plants. A diverse range of host responses were observed from screening eight blueberry cultivars commonly grown in the Southeast region. Among them, southern highbush blueberry cultivar 'Suziblue' demonstrated most tolerance to *P. cinnamomi* infection.

Introduction

Cultivated blueberry (*Vaccinium* sect. *Cyanococcus*) consists of northern highbush, southern highbush, lowbush, and rabbiteye blueberries. Geographic distribution of these cultivated species depends on their chilling requirement and cold hardiness. The United States is the world leading blueberry producer and produced 322,595 tons in 2023 (producereport.com/article/us-blueberry-production-exceeds-320000-tons). Expansion of blueberry production is expected due to the increasing consumer demands. However, blueberry production is challenged by various biotic and abiotic stresses. Among these, Phytophthora root rot was recently ranked as the third most important disease by the US blueberry industry (Gallardo et al., 2018). Phytophthora root rot disease is caused by the soilborne oomycete pathogen *P. cinnamomi* (Smith, 2008). Previous studies have reported differential host responses to Phytophthora root rot among blueberry cultivars (Oliver et al., 2021; Smith, 2012; Yeo et al., 2016); however, there have been limited efforts to develop southern highbush cultivars with resistance or tolerance to this disease. Disease tolerant cultivars would be an ideal choice for low-lying lands where waterlogging is frequent, whereas cultivars that are more susceptible to Phytophthora root rot may require more frequent fungicide applications when grown in root rot-prone areas. Nonetheless, there is currently little to no information comparing the response of southern highbush cultivars commonly grown in the southeastern region to Phytophthora root rot disease. As such, to enable

growers to make informed decisions regarding cultivar choices and fungicide application, there is a critical need to determine the host response of commonly grown southern highbush blueberry cultivars to *P. cinnamomi* infection. Therefore, the following specific objectives have been addressed in this research report.

Objective 1. Optimize a greenhouse inoculation assay for Phytophthora root rot resistance screening.

Objective 2. Assess southern highbush cultivars commonly grown in the southeastern region for resistance or tolerance to Phytophthora root rot disease.

Materials and Methods

Two separate experiments were performed on 1.5-year-old and 6-month-old plants of southern highbush cultivars 'Emerald' and 'Rebel' to establish a greenhouse inoculation method. Experiments were carried out using two inoculation methods, grind and millet inoculation. Grind inoculum was prepared from fully-grown mycelia and millet inoculum was prepared by

Figure 1. Scale of visual ratings of disease symptoms following *Phytophthora* inoculation.

inoculating cooked millet grain with *P. cinnamomi*. Disease progression was reported using a scoring system where plants were assessed for foliar symptoms and wilting. Once the first symptom appeared, days-to-first-symptom development among two cultivars was recorded. Later, plants were observed weekly for disease progression and rated using a scale of 0 to10, 0 being for a healthy vigorously growing plant and 10 being plant death. Examples of these root rot symptoms ratings are illustrated in Figure 1. Presence of the disease pathogen was confirmed by growing surface-sterilized roots of recently collapsed seedlings on PARPH-V8 agar media. The presence of *P. cinnamomi* in the root culture indicated inoculation.

For grind inoculation, *P. cinnamomi* mycelia was ground to make a dilute slurry which was used to inoculate the plants. For this, V8 Agar media plates were inoculated with agar plugs from a fresh *P. cinnamomi* culture. After 7-10 days (enough time to allow for the *Phytophthora* to fully colonize the plate), the inoculation slurry was prepared according to a previously published method (Yeo et al., 2016) with minor modifications. The mycelial sheet from 3 fully colonized V8 Petri plates were excised and blended with 1 liter of distilled water with a food processor for about a minute. This slurry was then diluted with additional distilled water to reach a total volume of 2 liters. Following this, 200 ml of the diluted slurry was applied to the soil around the roots in each pot.

For millet inoculation, inoculum was prepared by using cooked millet from a method previously described (Drenth & Sendall, 2001). Briefly, 50 g of millet was first rinsed with distilled water and then soaked with 100 ml of distilled water in glass flask. The millet soaked in water further incubated for 5 hours in a water bath at 65ºC. Excess water was then removed, and the millet was autoclaved at 121ºC for 25 minutes. Each flask of millet was inoculated with 15 agar plugs of *P. cinnamomi* culture grown on V8 media. The millet was fully colonized following incubation for 15 to 21 days in dark, with flasks being shaken daily to avoid the formation of big clumps. To inoculate individual pots, a portion of this inoculated millet (10 g) was spread on the soil around root zone and covered with additional soil.

Following inoculation using either method, plants were grown in 1-gallon pots with the bottom half of the pots immersed in water for 30 days until symptoms developed. At the conclusion of each experiment (100 days), Susceptibility of blueberry cultivars to phytophthora inoculation was evaluated in the greenhouse study using the grinding method. Eight blueberry cultivars included in this study were northern highbush cultivar ‘Legacy’, southern highbush cultivars ‘Farthing’, Suziblue’, Patrecia’, Rebel’, and ‘Optimus’, and rabbiteye cultivars ‘Brightwell’ and ‘Titan’. These plants were one year old at the time of inoculation. An experiment following the completely randomized design (CRD) with three replications was implemented. Fresh and dry weights of both shoots and roots were estimated to determine the susceptibility to phytophthora root rot. The plants and seedlings from both experiments were washed and divided into shoots and roots, fresh weight of both shoots and roots were collected separately. Further oven dried at 70ºC for 24 to 48 hours to determine dry biomass. Relative tissue weight was calculated by dividing the tissue weight of inoculated plants by the average tissue weight of mock inoculated plants (Yao et al., 2016). ANOVA and Tukey’s test were performed for data analysis using SAS Enterprise (Version 8.3).

Results and Discussion

Blueberry plants and seedlings inoculated with *P. cinnamomi* displayed a range of leaf symptoms which increased over time with both the inoculation methods. Most of the plants started to exhibit arial symptoms after one month of inoculation. No significant difference was found between the millet and grind inoculation methods, however, with millet inoculum, fungal growth was occasionally observed on the surface of the soil, likely on the organic matter in the millet substrate. No fungal growth was observed in the pots inoculated by the grind method. Therefore, the grind method was concluded to be the preferred inoculation method and was used for subsequent cultivar screening (in Objective 2). In terms of tissue weight, no significant difference in cultivar susceptibility to *P. Cinnamomi* was identified between 'Rebel' and 'Emerald' in these experiments. However, significant differences were found between older and younger plants in relative tissue weight of both shoots and roots (Figure 2). The younger plant inoculated with *P. Cinnamomi* retained less than 20% of the tissue weight of the mock-inoculated (uninoculated) controls, whereas the older plants inoculated with *P. Cinnamomi* retained 60% to 80% of the tissue weight of the controls. Although significant differences in relative biomass were found between both the young and old plant sets, no significant differences were found in the visual ratings of the disease progression expressed as weekly area under disease progression curve. This indicates that it was not possible to differentiate between the impacts of infection on the young and old plants based on visual assessments of canopy symptoms alone. Due to the rapid decline in plant vigor, the experiment with the younger plants was able to be terminated 9 weeks upon inoculation. In contrast, the experiment with 1.5-year-old plants ended 15 weeks after inoculation. Taken together, these results suggest that using younger plants (rather than older plants) for inoculation studies would be more efficient in terms of showing more pronounced differences in disease effects on the host in a more timely manner.

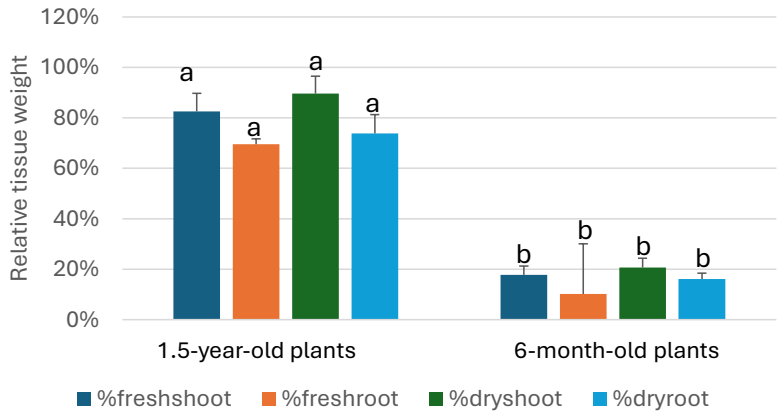

Figure 2. Significantly lower relative tissue weight was found in 6-month-old plants compared to 1.5-year-old plants ($P<0.05$).

Blueberry cultivars were inoculated with *P. Cinnamomi* (Figure 3). Differential responses to *Phytophthora* infection were identified amongst the cultivars (Figure 4). Southern highbush blueberry 'Suziblue' appeared to have the highest level of tolerance to *Phytophthora* infection. Compared to the mock-inoculated 'Suziblue' plants, the infected plants maintained 80% of shoot and root weight upon disease challenge. On the other hand, 'Patrecia' was the most susceptible line, as the inoculated plants had less than 20% of root and shoot biomass present in the mock-inoculated plants. The other cultivars demonstrated intermediate level of tolerance to the inoculations with root growth (but not necessarily shoot growth) being significantly reduced by *Phytophthora* inoculation.

Figure 3. A southern highbush blueberry cultivar inoculated with *Phytophthora* (left) and mock inoculated (right).

Most of the cultivars included in this study were introduced in the Southeast US growing region over 10 years ago. In-ground planting is the common practice of cultivation within this region. Dependent upon the natural distribution of *Phytophthora* populations, disease pressure varies in the commercial blueberry fields. Given this, and based on the results from this study, it is advisable that blueberry growers in the Southeast region attentively manage Phytophthora root disease. Apart from 'Suziblue', which demonstrated the most tolerance to *Phytophthora* infection in this study, the growth of all the other cultivars was significantly impacted by *Phytophthora* infection.

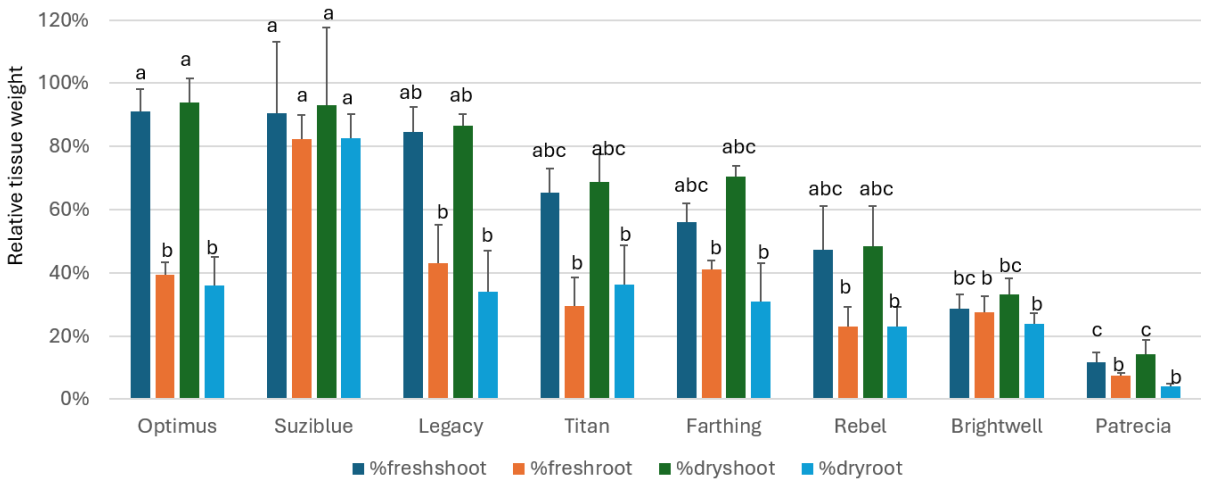

Figure 4. Differential disease responses among blueberry cultivars inoculated with *Phytophthora cinnamomi*. Statistically difference was marked by different letters above the bars ($P<0.05$).

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