Title: Investigating the effects of gypsum soil amendments on Phytophthora root rot in southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrids).

Progress Report

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Research Proposal

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Objectives:

1. Determine the effects of gypsum soil amendments on soil moisture, pH, and nutrient composition.
2. Determine the effects of gypsum soil amendments on blueberry growth and plant nutrient composition.
3. Determine the effects of gypsum soil amendments on Phytophthora root rot infection and pathogen abundance.

Justification and Description

Phytophthora root rot can cause significant damage to the root system of plants and dramatically reduce vigor. In blueberry, Phytophthora root rot is caused by *Phytophthora cinnamomi*, a soilborne oomycete pathogen. Chlamydospores are the prevalent overwintering source of inoculum, and these persist in soil, bark, and on infected plant roots (Milholland and Oudemans, 2017). Though rabbiteye blueberries (*V. virgatum*) can be infected, southern highbush (SHB) blueberry cultivars are particularly susceptible to root rot and Phytophthora is frequently detected in SHB blueberry plantings in the southeastern U.S. Wet conditions promote the growth and spread of *P. cinnamomi*. Zoospores are produced by sporangia, which are mobile under wet conditions. It is zoospores that infect actively growing roots where they cause damage and reduce nutrient and water uptake by the plant, eventually killing the infected plant. The ability for water to freely drain from the soil is essential for controlling the pathogen. If the site has poor drainage, blueberry should not be planted unless drainage issues can be rectified during establishment. Commonly, growers will plant blueberry in contiguous rows across high and low spots in a field. Water collects in the low spots where plant vigor is reduced and production is lost. Growers attempt to manage production losses through the use of fungicides like phenylamides (mefenoxam) and phosphonates.

Prior evidence indicates that high Ca^{2+} levels in the soil can disrupt the infection process of *Phytophthora* sp. by inhibiting the motility of zoospores. Accordingly, gypsum ($CaSO_4$) soil amendments can decrease the incidence of Phytopthora root rot (Messenger-Routh et al. 1996; Messenger et al. 2000a). Gypsum soil amendments can improve soil drainage in some cases, and compared to other sources of soluble Ca^{2+} such as lime, gypsum provides more soluble Ca^{2+} without increasing soil pH (Messenger et al. 2000a; Shainberg et al. 1989). Since blueberry thrives in an acidic soil medium of pH 4.5 – 5.5, this is of significant importance. Work with *P. cinnamomi* in both avocado and northern highbush blueberry (NHB; *V. corymbosum*) has shown that gypsum use reduced sporangial production by the pathogen and increased plant biomass (Messenger et al. 2000a,b; Yeo et al. 2017). Recent work with NHB in Oregon indicated that irrigation method (widely spaced driplines) can impact the effectiveness of the use of gypsum in this manner (Yeo et al. 2017). In the southeastern U.S., SHB are grown in conditions that differ significantly from NHB in the Pacific Northwest. Not only are soil types and weather conditions different, but growing practices in the southeastern U.S. often include the widespread use of pine bark mulch and frequent use of single dripline irrigation. To determine if gypsum soil amendments are capable of providing southeastern U.S. grown SHB blueberries with the same benefits against *P. cinnamomi* as those observed in NHB plants in the Pacific Northwest, we propose to initially examine the effectiveness of gypsum for Phytophthora root rot suppression in both an established blueberry planting and in greenhouse experiments.

Experimental Plan and Methods

Field Experiment Establishment

Field experimentation has been conducted at the University of Georgia Alapaha Blueberry Research Farm in Alapaha, Georgia and at a commercial blueberry farm in Ware County, Georgia. Both sites have a high water table and remain flooded for long periods of time after heavy rains. Phytophthora root rot has been previously noted as a persistent problem in both sites.

The field experiment at Alapaha has been established in a randomized complete block design with three blocks per treatment. Two SHB cultivars (Emerald and Farthing) have been planted contiguously into each row. Each treatment plot has 6 plants in a treatment with two guard plants between treatments. Plant spacing is 3 ft (in-row) by 12 ft (between-rows) in beds 4 ft wide by 1.5 ft in height with pine bark incorporated into the soil at 10 tons/A with 3 inches pine bark mulch. This field experiment consists of four treatments (gypsum+fungicide, gypsum+no fungicide, no gypsum+no fungicide, no gypsum+fungicide). Gypsum was incorporated into the soil of their respective treatments at planting at 2240 kg/ha (1 ton/A). After planting and prior to treatment with fungicide, the availability of Phytophthora inoculum within the plots was determined through isolation of the pathogen. Once leaves were fully expanded, foliar applications of phosphonate fungicide (K-phite) was applied at 2 qts/A to the fungicide treatment blocks on a monthly schedule four times during the year (June through September). Otherwise, standard management practices and pest spray programs were applied to all blocks throughout the year.

The field experiment in Ware County was conducted in a randomized complete block design with three blocks per treatment. Gypsum was applied to the soil of the respective treatment plots of 5-year old Farthing plants at 2240 kg/ha (1 ton/A). This field experiment consisted of two treatments (gypsum and no gypsum), and standard management practices and pest spray programs were applied to all blocks throughout the year.

Field Experiment Assessments

Soil and leaf tissue sampling and testing was performed on each block at the Ware County site. The Alapaha site was planted late in the spring and will be sampled next spring (2019). Soil parameters assessed at the Ware County site were: soil pH, organic matter, and mineral nutrient composition. Plant parameters assessed at the Ware County site were: leaf nutrient composition and fruit quality.

Leaf samples were rinsed with distilled water and dried to a constant weight at 80°C prior to sending the samples to an analytical laboratory. The samples were analyzed for nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulfur (S), boron (B), zinc (Zn), manganese (Mn), and iron (Fe) (Waters Agricultural Laboratories, Inc., Camilla, GA), where the dried leaves were ground to pass through a 20-mesh screen. The samples were reduced to ash in a muffle furnace, acid digested, and measured by an inductive coupled plasma spectrophotometer (ICP) coupled to a Digiblock 3000 (SCP Science, Baie D'Urfé, Quebec, Canada). Nitrogen was determined through combustion of plant tissue using a LECO FP-428 N analyzer (LECO, ST. Joseph, MI, U.S.).

A composite soil sample was taken in June 2018 from under the drip line of the same plants used in this study, each sample was taken at 0 to 15 cm depth with the surface 2.5 cm removed. Soil nutrients were extracted using a Mehlich I procedure and pH was measured using a 0.01M calcium chloride solution in a 1:1 soil to $CaCl_2$ mixture and reported as an adjusted pH value of + 0.6 units (Kissel and Sonon, 2008).

Field Pathogen Isolations

To confirm the presence of inoculum in the treatment plots, *P. cinnamomi* was isolated from the soil on selective PAR(PH)-V8 media prepared according to the protocol of Jeffers (2006). The identity of obtained isolates was confirmed by PCR and sequencing of the ITS4 and ITS6 region. Primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS6 (5'-GAAGGTGAAGTCGTAACAAGG-3') were used for amplification of a 941 bp product (EPPO 2004). A GoTaq® (Promega®, Fitchburg, WI) PCR kit was used, with reagents as follows: 10 µL 2x GoTaq® Green Master Mix, 2 µL each of forward and reverse primers (10 µM), 1 µL DNA (1-3 ng/µL) and 5 µL dH_2O. PCR conditions were as follows: 94°C (3 min), 34 × [94°C (30 sec), 55°C (30 sec), 72°C (1 min)], and 72°C (10 min). Resulting amplicons were sequenced in both directions by Sanger sequencing at Eurofins Genomics (Louisville, KY). Obtained sequences were compared to known *P. cinnamomi* sequences available on the GenBank database (www.ncbi.nlm.nih.gov) to verify isolate identity.

Greenhouse Experiments

Greenhouse experiments using both cultivars 'Emerald' and 'Farthing' are currently underway. Blueberries were planted in three parts pine bark mulch to one part sand. For the greenhouse experiments, there are four treatments: gypsum+non-inoculated, gypsum+inoculated, no gypsum+inoculated, and no gypsum+non-inoculated. Based upon their assigned treatment regime, pots have been inoculated with *P. cinnamomi* inoculum prepared from an isolate obtained at the field site. Five plants of each cultivar have received each treatment. Inoculations have just recently been performed, and it is expected that three replications of greenhouse experiments will be completed by mid-2019.

Results and Current Progress

Field Experiments

In spring 2018, blueberry plants of cultivars Emerald and Farthing were established at the University of Georgia Blueberry Research Farm in Alapaha, Georgia, and gypsum was incorporated into the soil at planting. The presence of *P. cinnamomi* in the treatment plots was confirmed by isolation, PCR, and sequencing, and confirmed isolates of *P. cinnamomi* obtained from the field were stored for later use in greenhouse experiments. Foliar applications of phosphonate fungicide (K-Phite) were applied monthly from June through September 2018.

Soil and leaf nutrient assessments were carried out at the Ware County site on 1-June. No significant differences among the soil parameters assessed were noted between any of the treatments (Table 1), and only a small difference in Mg was noted in the leaf analysis (Table 2). Fruit harvested from the Ware County site on 1-June also did not indicate substantial differences (Table 3), but differences in fruit weight, % acid, firmness, and diameter were noted (Table 3). At this site, percent root infection with *P. cinnamomi* was assessed twice during the growing season (7-August, and 3-October). Low overall disease incidence was noted, and statistical analysis did not indicate any significant differences between treatments (data not shown).

Table 1. Soil analysis results from Ware County site taken June 1st, 2018 (n=3).

	P (lb/A)	K (lb/A)	Mg (lb/A)	Ca (lb/A)	Mn (lb/A)	Zn (lb/A)	pH	OM (%)
Untreated	31.25 a	94.47 a	163.53 a	863.7 a	5.46 a	3.52 a	4.17 a	6.69 a
Gypsum	32.73 a	77.25 a	119.63 a	1064.8 a	4.72 a	3.66 a	4.14 a	6.48 a

Different letters represent significant differences between means at $P<0.05$ according to Fisher's least significant difference test.

Table 2. Leaf tissue nutrient analysis results from Ware County site taken June 1st, 2018 (n=3).

	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
Untreated	1.89 a	0.08 a	0.51 a	0.32 b	0.87 a	0.19 a	61.4 a	18.6 a	180.7 a	50.0 a
Gypsum	2.05 a	0.12 a	0.50 a	0.35 a	0.93 a	0.22 a	76.4 a	19.6 a	231.0 a	77.7 a

Different letters represent significant differences between means at $P<0.05$ according to Fisher's least significant difference test.

Table 3. Fruit harvested from Ware County site on June 1st, 2018 (n=100).

	100 fruit count weight (g)	Individual Fruit Weight (g)	Soluble Solids (Brix)	% Acid	pH	Firmness (g/mm)	Diameter (mm)
Untreated	197.6 b	1.98 b	10.6 a	0.47 b	3.85 a	155.7 a	16.6 b
Gypsum	216.2 a	2.16 a	10.7 a	0.54 a	3.66 a	151.8 b	17.2 a

Different letters represent significant differences between means at $P<0.05$ according to Fisher's least significant difference test.

Plant growth from 9-May to 31-October was assessed at the Alapaha site and relatively poor growth was noted during the course of the season (data not shown). Percent root infection with *P. cinnamomi* was assessed at this site three times during the growing season (27-June, 17-September, and 18-October). A relatively low overall disease incidence was observed, and no significant differences in root rot incidence between gypsum treated and untreated plots were noted; however, there was a significant fungicide x cultivar interaction, and fungicide and cultivar both had a significant effect on root rot incidence (Table 4).

Table 4. Root infection at Alapaha site by *P. cinnamomi* for (a) fungicide x cultivar, (b) cultivar, and (c) fungicide.

(a)	Root infection by *P. cinnamomi* (%)
No Fungicide+Farthing	7.0 a
No Fungicide+Emerald	0.4 b
Fungicide+Emerald	0.3 b
Fungicide+Farthing	0.3 b

(b)	Root infection by *P. cinnamomi* (%)
Farthing	2.2 a
Emerald	0.3 b

(c)	Root infection by *P. cinnamomi* (%)
No Fungicide	2.4 a
Fungicide	0.3 b

Different letters represent significant differences between means at $P<0.05$ (LS Means in Proc GLIMMX in SAS)

Due to poor growth of new plants during the initial year of establishment, it was decided that an additional year of growth at the Alapaha field site would be necessary. Gypsum will be reapplied to the treatment plots in spring 2019, and phosphonate fungicide (K-Phite) will be applied monthly four times from June through September 2019. Plant and soil parameters will be evaluated three times in 2019 (at mid-summer, early September, and early October). Root samples will also be collected using a core sampler and evaluated for percent root infection at each of these sample timings. Likewise, the field experiment in Ware County, Georgia will be continued into 2019, with additional gypsum applied across the beds in spring 2019. Plant and soil parameters will be subsequently re-evaluated at this site as well in 2019 (at mid-summer, early September, and early October).

Greenhouse Experiments

Due to an unexpected delay in obtaining and confirming viable *P. cinnamomi* isolates from blueberry, the start of greenhouse experiments was accordingly delayed. Experimental work in the greenhouse commenced in fall 2018 and plants are currently being monitored and evaluated. As a result, greenhouse experiments are expected to be completed in early- to mid-2019.

Potential Impact

Management of Phytophthora root rot currently relies on the continued suppression of this pathogen through the use of fungicides, but disease-suppressive cultural practices are needed. Once complete, the primary practical benefit of this study will be to determine if soil amendment with gypsum can significantly impact Phytophthora root rot in southern highbush blueberry. If an impact is noted from these initial studies, additional field experiments can be performed which may lead to new management recommendations that will be valuable for SHB growers in the southeastern U.S.

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