Christmas Tree Promotion Board

Research Final Report

CTPB Project Number: 22-02-CAES

Project Title: Soil chemistry and biological manipulation to prevent losses from Phytophthora root rot

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Progress Report

Technical report

Disease-suppressive soils prevent root infections of susceptible plants, even when inoculated with a pathogen. Soils suppressive to phytophthora root rot (PRR) in Australia have been intensively studied, but the components associated with suppressive soils have not been fully tested in Christmas tree plantings. These experiments tested the hypothesis that suppressive soils may require complementary conditions: (1) Bacteria and fungi suppressive of PRR are common in soil. To be effective their activity needs to be stimulated by simultaneously adding organic matter and nitrogen to soil. (2) High soil calcium levels, which can be accomplished by adding gypsum, can interfere with phytophthora zoospores. (3) Populations of microorganisms (notably *Trichoderma* spp. fungi) beneficial for suppressing phytophthora can benefit from the addition of elemental sulfur.

In these studies supported by the RCTB, I directly tested each factor and their combinations in two experiments, one using microplots to easily replicate all combinations, and the other with a field trial, to compare native soil condition (the untreated check), the full combination of factors, and systemic acquired resistance elicitors. In this second experiment, root dips of commercially available microorganisms suppressive of phytophthora root rot were compared. Both experiments used Fraser fir seedling transplants from a nursery (Asack and Son, Barton, VT) that irrigates with well water and does not use systemic fungicides. These nursery conditions are required to grow plant material susceptible to PRR but not previously exposed to disease.

Microplot experiment

Methods

Microplot Experiment, Part 1 (2022 - 2023).

Microplots were established at the Valley Laboratory, Windsor, CT, using soil from Humming Grove Farm, from a portion of the farm with nearly total losses in 2021 due to flooded soils and phytophthora root rot. A 2 × 2 × 2 × 2 factorial design required 42 L of soil mixture to fill six replicate microplot tubes for each of the 16 treatment combinations. Factors included (1) presence/absence of organic matter, consisting of 2-year old hardwood chips, mixed in a 1:3 ratio with field soil; (2) presence/absence of amendment with elemental sulfur, using 22 mL of a Sulfur 6F product (containing 15.8 g of elemental sulfur, equivalent to an application of 500 lb per acre) sprayed onto the 42 L of soil; (3) presence/absence of agricultural gypsum, applied at a rate of 63 g per microplot; (4) inoculation/no inoculation of Trichoderma, using 272 mg of Rootshield Plus per 42 L of soil medium. All experimental factors were mixed with soil, using a concrete mixer on May 11, 2022, at the time of planting into the microplots. All microplots were watered using a drip system, after passing the water through a carbon filter to neutralize chlorine from the municipal water supply. All plots were inoculated with rice grains on which *Phytophthora cactorum* had been cultured, and then flood irrigated to provide conditions suitable for infection.

Microplot Experiment, Part 2 (2023 – 2024). Due to poor experimental conditions for inducing disease in the previous year, the Microplot Experiment was repeated in 2023 with some revisions. Fraser fir trees were obtained from the biocontrol inoculation of roots field trial (see below). The best 96 trees were chosen to set up this experiment, based on size, root system, and color, in a 2 x 2 x 3 factorial experiment with eight replicates. Microplots were established by planting trees on May 16, 2023. Half of the trees were planted into cylinders containing a 1:1 mixture of aged wood chips and native soil, which were blended in a cement mixer. The other half were planted into native soil. A second factor was the amount of fertilizer provided. We recognized the poor growth and yellowing could have been due in the previous experiment to nitrogen deficiency. Half the trees were provided one teaspoon of 17-6-12 controlled release fertilizer (Harrell's, 90-day release), and the other half were provided three teaspoons of the same fertilizer. The last factor was calcium application. One-third did not have any calcium added, one-third had a surface application of gypsum (63 g per tree, applied June 6, 2023), and the last third of trees received a liquid calcium drench applied as a custom-produced solution of calcium aspartate (500 mL of 50 mM Ca^{+2} applied on September 13, 2023).

The inoculum used consisted of one Petri dish each with cultured *Phytophthora abietivora* (mycelium and sparse oospores), *P. cactorum* (mycelium and abundant oospores), and *P. plurivora* (mycelium with sporangia), added to 500 mL of deionized water and homogenized in a blender for 30 seconds. Three milliliters of mixed inoculum were pipetted into the soil, 2 cm below the soil surface, near the base of each tree on September 14, 2023. Trees were then irrigated continuously with drip irrigation for 24 h to saturate the soil.

Trees were evaluated for color on December 8, 2023, April 17 and June 10, 2024.

Results

Experiments set up in 2022

The Microplot Experiment, Part 1, and the Field Test of Biocontrol experiments were conducted during a drought. Poor infection conditions, despite our best efforts to establish flooded conditions and inoculation, probably interfered with our ability to induce disease.

The microplot experiment did not detect differences among treatment factors or combinations, other than a transient and significantly poorer color among trees associated with the sulfur exposure (*P* = 0.01), which had improved by the time of the second plot evaluation, and similarly transient but significant affect on color associated with a gypsum x *Trichoderma* inoculation interaction (*P* = 0.03), in which the best color was observed with the Trichoderma-only and the gypsum-only treatments.

Table 1. Summary of tree color in response to combinations of the treatments to prevent phytophthora root rot infection. Reported values are main effects means averaged over all treatment combinations. With 6 replicates in the factorial experiment, the reported means are averages of 24 trees. Color 1 and Color 2 are data recorded at two evaluation dates in 2022, using a color scale of 0 = dead, 1 = yellow, 2 = greenish yellow, 3 = yellowish green, 4 = green.

	Color 1			Color 2		
	None	Present	P	None	Present	P
Sulfur	2.04	1.56	0.01	1.69	1.79	n.s.
Gypsum	1.85	1.75	n.s.	1.79	1.68	n.s.
Wood chips	1.81	1.79	n.s.	1.88	1.60	n.s.
Trichoderma	1.81	1.79	n.s.	1.73	1.75	n.s.

The results from the Microplot Experiment, Part 2, were consistent with the expectation that the greater level of N fertility would provide better color (P = 0.0035), and that the presence of wood chips would tie up available nitrogen (P = 0.016). Color ratings (4 = dark green, 3 = green, 2 = yellow green, 1 = dying, 0 = dead) were as follows:

	October 2023		June 2024
Nitrogen level	Compost present	Color rating	Mortality (out of 24)
Low	none	2.66	4
Low	present	2.21	9
High	none	3.49	4
High	present	2.79	8

This anticipated effect on overall color and vigor of the trees did not translate into significant differences in survival related to phytophthora infection and subsequent mortality. Mortality was nearly identical with respect to nitrogen level, when holding the compost effect constant, and nearly, but not quite significant with respect to the presence of incorporated wood chips (P = 0.06, Fisher's Exact test). There was no evidence that higher levels of calcium or the method of applying calcium had any influence of tree survival when challenged by Phytophthora spp. (P > 0.05), with the total number of trees dying in each group (out of 32 trees) being 10, 9, and 6 for the no calcium, gypsum, and calcium aspartate treatments, respectively.

Field test of biological control inoculation of roots

Methods

A field test was set up in the same area of Humming Grove Farm in Broad Brook, CT, from where disease-conducive soil had been removed to set up the microplots. The experiment was set up as a split-plot factorial with six replicates. The main plots consisted of a comparison of Fraser fir with balsam fir, both sourced from Asack and Son in Barton, VT, planted into unamended soil. There were two additional main plot treatments using Fraser fir: transplants were either (1) planted into soil amended with gypsum, sulfur, and woodchips, like the rates used (per unit area) in the microplot experiment, or (2) at the time of applying root dips, products (imidacloprid and potassium phosphite) were added to the root dip to stimulate the trees' systemic acquired resistance traits. The subplot factor was application of commercially available biological control products as a root dip at the time of planting. Products tested were (A) the fungal-parasitic fungus *Trichoderma harzianum* and *T. virens*, available as the product Rootshield Plus, applied at 68.1 g per 2 gallons; (B) a *Bacillus subtilis* product, sold as Cease, applied at 150 mL per 2 gallons; (C) *Bacillus amyloliquifaciens*, sold as Triathlon, applied at the rate of 1 quart per 2 gallons; (D) an actinomycete, *Streptomyces griseoverdis*, sold as MYCOSTOP, applied at 2.43 g of product mixed into two gallons of root dip. Products were applied in the following combinations: A, B, C, D, A+B, A+C, A+D, B+D, and C+D. Together with the untreated check, these constituted 10 root dip treatments that were randomized in position within the main plots. Four transplants were dipped and planted 15 cm apart within a row across the main plot, and different treatment groups were spaced 20 cm apart. Trees were planted on May 12, 2022.

Results

For the comparison among main plots, there were highly significant differences ($P < 0.0001$) apparent due to the tree genetics (balsam fir grew more and had better color than the Fraser fir), and the modification of the soil. While the systemic acquired resistance-inducing root dips were statistically equivalent to the unamended soil, the soil amended with wood chips, sulfur, and gypsum greatly suppressed tree growth and color ratings.

The root dip treatments did not produce any pronounced differences with respect to tree color, but there were small, statistically highly significant differences among treatment effects on tree growth ($P < 0.01$). The untreated check had average terminal growth (7.03 cm) and was not significantly worse than the growth with the best performing root dip (7.8 cm leader growth with the D treatment, or MYCOSTOP), nor better than the growth for the worst treatment (6.2 cm growth for the C+D treatment). The C treatment (Triathlon) may have stunted growth, as three of the four treatments rated as having poorer average growth than the untreated check included the Triathlon product. It is possible that the dosage of this product used in inoculating the roots in the root dip process may have been excessive.

Discussion

The incorporation of high-cellulose organic material with these experiments was problematic. We have observed that surface applications of wood chip mulch greatly enhance conditions for Christmas tree growth under normal fertility practices. However, incorporation of wood chips into soil clearly ties up nitrogen and led to nitrogen deficiency symptoms in these experiments. Nitrogenous fertilizers were not part of the 2022 experiments and were not applied. The follow-up experiment in 2023 demonstrated, surprisingly, that even though incorporation of aged wood chip compost into soil did tie up nitrogen, that this had no impact on mortality to trees challenged by phytophthora inoculated into microplots. The role of N fertility should be investigated further relative to susceptibility of firs to phytophthora: on the one hand color and growth should improve with N application, but on the other hand this could also detract from the trees’ ability to defend themselves from infection, due to trade-offs between growth and defense.

Despite recent studies by other workers demonstrating that chelated calcium can be as effective as potassium phosphite treatments for protecting against phytophthora infection, and from earlier work showing that incorporation of gypsum can interfere with phytophthora infection in other crops, my experiments did not support claims that manipulation of calcium prevented tree mortality when challenged by phytophthora. Although surface application of wood chips as a mulch are known to be beneficial for improving the growth and survival of Christmas trees, there was no evidence that incorporation of such material into the soil profile could stimulate antagonistic microorganisms to phytophthora and thus suppress infections and plant mortality. Rather, incorporation of composted wood chips appeared to be detrimental, almost certainly through the mechanism of tying up available N for tree growth.

Overall, this project has been disappointing from the perspective of finding new tools to combat phytophthora losses by creating suppressive soil conditions. That leaves growers with conventional methods (raised beds, improved drainage, and resistant plant species or accessions), as tried and true approaches to mitigate the risk of losses due to root rot.

Summary of Research Report for Public Release by CTPB

Phytophthora organisms that cause root rot of Christmas trees have spores that can persist in the soil for several years. One approach to protect Christmas trees from infection could be to create conditions in which the disease organism either cannot persist, because antagonistic microorganisms destroy it, or to change the soil chemistry to interfere with the infection process, specifically by elevating calcium levels in soil. Tests conducted in Connecticut in 2022 and 2023 did not find effective ways to take advantage of these processes. Subtle improvements in growth were observed through inoculation of roots with a product containing a strain of *Streptomyces*. Tree growth was inhibited and color was poorer when partly decomposed wood chips were incorporated into the soil. These effects might be explained by depletion of available nitrogen from the soil. Nitrogen depletion effects due to incorporation of wood chips were independent of ultimate mortality of trees challenged from exposure to phytophthora. Growers should rely on conventional methods such as raised beds, improved drainage, and resistant plant species or accessions to mitigate the risk of losses due to root rot.