# Incorporation of soil amendments for managing Phytophthora root rot of Fraser fir in North Carolina

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## Background and Objectives

Valued at over $85 million, North Carolina ranks in second in the United States for Christmas tree production. Fraser fir (*Abies fraseri*), native to high elevations of the Southern Appalachian Mountains, is the predominant species of cultivated Christmas trees in NC. Commercial Fraser fir production generally occurs at much lower elevations in soil with poorer drainage and lower pH. While often more fertile, such soils tend to me more conducive for infection by the Phytophthora root rot pathogens, *Phytophthora* spp. Escalating risk for infection is the highly susceptible nature of Fraser fir to PRR. Surveys evaluating the incidence of 58 commercial field sites revealed that 57% of Frasier fir plantings in Western NC had trees symptomatic of PRR. Within all plantings, PRR incidence ranged from 0 to 75%, with approximately 10% loss of trees annually.

Despite integration of host resistance and cultural practices, utilization of fungicides targeting *Phytophthora* spp. remains the most reliable method for *Phytophthora* spp. in crops that are harvested annually. Unfortunately for Fraser fir, frequent calendar-based applications of fungicides such as mefanoxam (e.g. Subdue Maxx) or fosetyl-Al fungicides (e.g. Aliette) have been either economically prohibitive due to the long production cycle of Fraser fir (7 to 10 years), have been difficult to apply due to the hilly terrain in which trees are grown, or have demonstrated poor efficacy in wet years. Furthermore, given the slopes in which Fraser fir trees are planted in western NC, maintaining fungicides within the root zone long enough to interact with and kill the pathogen is challenging.

In other cropping systems the addition of soil amendments for PRR management has resulted in a reduction in plant mortality and could be a valuable tool for integrated management of PRR in Fraser fir. Infection of avocado seedlings grown in *P. cinnamomi* infested soil was reduced by over 70% following the incorporation of gypsum compared to the untreated program. Similarly, in PRR infested soils of red raspberry plantings, soil amendments with gypsum had a faster growth rate, greater yield, and reduced PRR symptoms by 83% compared to an untreated program. Amendment of soil with elemental sulfur represents another method for suppression of PRR in Fraser fir plantings. In addition to having a direct fungicidal effect, the incorporation of sulfur lowers soil pH and limits the survivability of zoospores-the motile spores of *Phytophthora* spp.

This proposal to the Christmas Tree Promotion Board aims to evaluate soil amendments, alone or integrated programs, for the suppression of PRR of Fraser fir Christmas trees. Besides generating research on the potential of these programs for PRR management, the effect of these programs on tree health parameters, including growth, mid-day stem water potential, transpiration rate, and stomatal conducted will be evaluated for trees in soils both infested and non-infested with *Phytophthora* spp. In addition, as demonstrated previously in avocado and Fraser fir, wood-based mulches have reduced PRR incidence due to their ability to lyse hyphal cell walls of *P. cinnamomi* and reduce zoospore survival and mobility. While alone wood-based mulches may not be sufficient due loss in cellulase activity over time, we hypothesize that integration of wood-based mulches with soil amendements and/or minimal phosphorous acid fungicide applications can improve the management of Fraser fir PRR in North Carolina.

**Objective 1. Evaluate standalone programs of varying rates of gypsum and sulfur for the suppression of Phytophthora root rot in container grown Fraser fir seedlings with soil infested with or absent of *Phytophthora cinnamomi*.**

***Seedling production.*** The effect of gypsum and sulfur programs on soil pH, tree growth, and Phytophthora root rot was evaluated for Fraser fir seedlings. In September 2021, 624 Fraser fir seedlings were acquired from Stuewe and Sons Inc. (Tangent, OR) and planted into 5 inch pots (2.38 inches deep) and placed in Anderson bands (36 25 sq. inch containers per flat). Metro Mix 852 (Sun Gro Horticulture), containing 20% perlite and an 80% 50/50 mixture of nursery bark and peat moss was utilized as the growing medium. Seedlings were maintained in a glasshouse at the NCSU Mountain Horticultural Crops Research and Extension Center in Mills River North Carolina at a constant temperature of 75F, with minimal of 16 hours light, 8 hours or less of darkness.

***Phytophthora spp. inoculum preparation.*** Isolates of *Phytophthora cinnamomi* were obtained from the programs of Meadows et al. (Waynesville, NC) and Benton et al. (Raleigh, NC). Isolates were originally collected between 2006-2011 from the roots and crowns of symptomatic Fraser fir trees in Avery County, NC. Prior to inoculum preparation for the seedling study, four isolates were cultured onto PARPH medium (selective and differential for *Phytopthora* spp.) and the ITS region for each isolate was sequenced to confirm the genus and species for each isolate. Although originally identified as *Phytophthora cinnamomi,* advances in sequencing resolution revealed that two of the isolates were *P. cinnamomi*, and two of the isolates were actually *Phytophthora cryptogea*. Since this species has previously been confirmed to be highly aggressive on Fraser fir and these isolates were originally selected from Fraser fir in NC, preliminary pathogenicity assays were conducted by inoculating Fraser fir seedlings with each isolate and repeating the experiment. Only one isolate (*P. cryptogea* 002) colonized the seedling roots for both experiments and was therefore selected for further greenhouse experiments.

Preparation of inoculum was attempted and ultimately achieved using two different methods of inoculation: vermiculite amended with V8 juice and rice grains amended with V8 juice. The rice grain method yielded the purest cultures and was thus selected for inoculum preparation for the inoculation of seedlings being growth in the greenhouse.

***Media amendment and acidification of potting mix.*** After 8 months of growth in the greenhouse, seedlings were topdressed with varying rates of granular gypsum (Southern Ag) or pelleted elemental sulfur (Southern Ag) (Table 1). The media amendment trial was a 7x2 factorial, completely randomized design with six replications of 25 seedlings included per treatment replicate. For each topdressing treatment, seedlings were either inoculated or not inoculated with *P. cryptogea* (Table 1).

**Table 1.**

| Treatment # | Soil Amendment | Rate | Mean pH (day 7) | Inoculated (Y/N) |
|---|---|---|---|---|
| 1 | Untreated (no amendement) | N/A | 6.3 ± 0.1 | N |
| 2 | Untreated (no amendement) | N/A | 6.2 ± 0.1 | Y |
| 3 | Gypsum | 500 lb/A | 6.3 ± 0.0 | N |
| 4 | Gypsum | 1500 lb/A | 6.3 ± 0.1 | N |
| 5 | Gypsum | 4000 lb/A | 6.4 ± 0.1 | N |
| 6 | Sulfur | 250 lb/A | 5.7 ± 0.1 | N |
| 7 | Sulfur | 1000 lb/A | 6.1 ± 0.0 | N |
| 8 | Sulfur | 3000 lb/A | 6.4 ± 0.0 | N |
| 9 | Gypsum | 500 lb/A | 6.2 ± 0.1 | Y |
| 10 | Gypsum | 1500 lb/A | 6.0 ± 0.0 | Y |
| 11 | Gypsum | 4000 lb/A | 6.0 ± 0.0 | Y |
| 12 | Sulfur | 250 lb/A | 6.0 ± 0.0 | Y |
| 13 | Sulfur | 1000 lb/A | 6.3 ± 0.0 | Y |
| 14 | Sulfur | 3000 lb/A | 6.1 ± 0.0 | Y |

Prior to soil amendment, an initial pH of the potting media was determined using a pour through method. Briefly. Approximately one hour after all containers were thoroughly watered, 50 ml of leachate from each of ten randomly selected containers per treatment was collected and pH was determined. The average pH across all treatments was determined to be 5.93 +/- 0.03. Seven days following top dressing, a pour-through was once again conducted for three randomly selected trees/containers per treatment. The average pH is noted in the table above. One week following top-dressing, no significant changes were observed within any of the treatments, nor were there any significant differences were observed across any of the different treatments. Pour-through assays were continued on 3 to 4 day intervals for an additional five weeks. Unfortunately no significant changes in pH were observed ($P$ = 0.05).

Since an objective of this study was to evaluate the effect of pH on seedling growth and health and topdressing soil in our greenhouse medium was not effective in reducing pH, sulfuric acid was incorporated into the medium of the treatments topdressed with elemental sulfur to achieve potting mix pHs of 5.5, 4.8, and 3.9 for the 250, 1000, and 2000 lb/A of elemental sulfur treatments, respectively. For each sulfur treatment, seedlings were drenched every 5 to 6 days to maintain the expected pH (± 0.2).

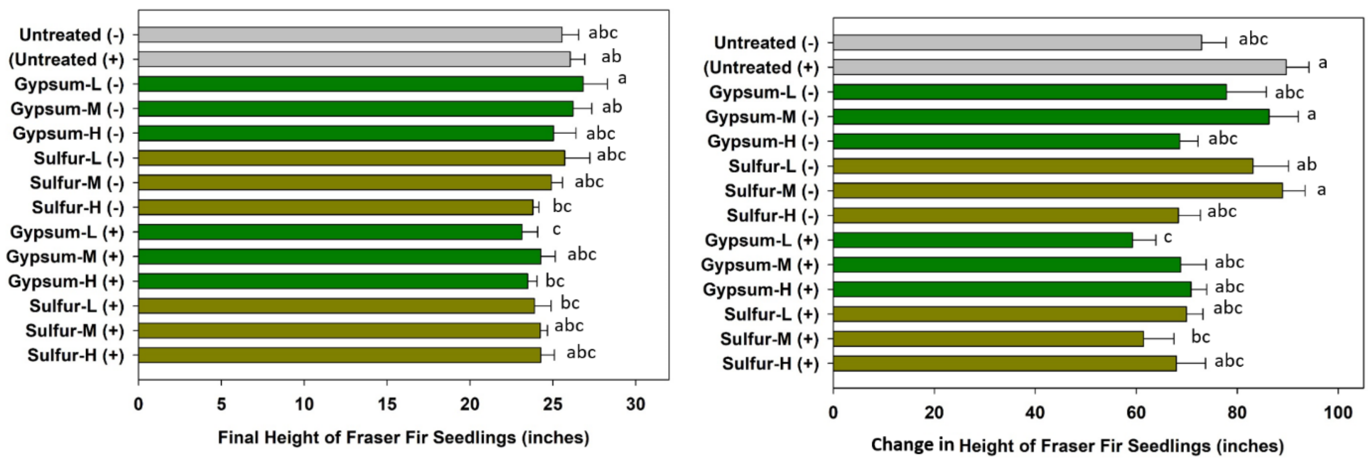
***Inoculation of seedlings with P. cryptogea***. Following maintenance of media pH for three weeks, six rice grains inoculated with *P. cryptogea*, were added equidistant from each other at a depth of 1" and a distance of 1" around the base of the seedling. All seedlings were watered daily and sulfur treatments drenched weekly for a total of 12 weeks. After 12 weeks, the incidence and severity of visible PRR treatments was evaluated. From each of five plants per treatment replicate, roots were harvested, surface sterilized (10% bleach for 90 s followed by two washes with sterile distilled water for 30 seconds) and sections of root tissue were plated on PARPH medium to detect the presence or absence of *P. cryptogea.* Measurements of seedling height (base of soil to top of seedling) were taken to just prior to acidification and at the end of the trial to determine treatment effects on plant growth.

**Figure 1.**

***Objective 1 Observations, Results, Conclusions***

Twelve weeks after inoculation, seedling height across all treatments ranged from 23.1 to 26.8 inches with lowest rate of gypsum (500 lb/A) + inoculated with *P. cryptogea* (trt 9) and the lowest rate of gypsum without *P. cinnamomi* inoculation (trt 3, 250 lb/A) representing the shortest and tallest seedling growths, respectively. Although we hypothesized that we would see differences in seedling growth rate due to i) inoculation of a pathogenic isolate of *P. cryptogea* and ii) differing rates of gypsum and sulfur, no trends in final seedling height or change in seedling height from the beginning of the experiment to the final measurements. No difference in plant height were observed across any of the sulfur treatments regardless of inoculation treatment nor between the inoculated and non-inoculated control. Treatments inoculated with *P. cryptogea*

**Figure 2.**




did not differ significantly from those that were not inoculated. While this could be suggestive that the treatments were effective in reducing the severity or consequences of PRR, unfortunately the untreated inoculated treatment (trt 2) did not differ in seedling height or growth rate from the untreated non-inoculated treatment. This is more suggestive that colonization of the isolate was not successful or was not severe. Furthermore, any seedling that died from PRR or an abiotic condition was not included in seedling height measurements, which could have skewed results. Regardless, our results demonstrated that high rates of gypsum, high rates of elemental sulfur, and low pH (minimum 3.8) did not have an impact on seedling growth over an ~ 4 month period.

The incidence of PRR, as determined by symptoms observed above the soil line, ranged from 0.0-4.6% with several treatments having no seedlings symptomatic of PRR (see Fig 3). The high rate of gypsum that was inoculated with *P. cryptogea* (trt 11) had the highest difference of plant death but did not differ significantly from any other treatments, including the high rate gypsum program without inoculation (trt 5). Culturing of roots from dead seedlings onto PARPH did not isolate *P. cryptogea*. While this could simply be due to the fact that seedlings were dead, perhaps the high rate of gypsum also caused death to weak seedlings under greenhouse conditions. Further experiments should be conducted to i) better understand the effect of gypsum on Fraser fir seedling health and ii) to try to accomplish more consistent infection by *Phytophthora* spp.

**Figure 3.**

**Objective 2. Evaluate standalone and integrated programs of sulfur for the suppression of Phytophthora root rot (PRR) on Fraser fir transplants in a minimum of 5 commercial Fraser fir plantings in NC with a history of PRR.**

At a commercial Fraser fir farm in western NC with a history of PRR, plots were split and either i) top-dressed with 1000-3000 lb of pelletized elemental sulfur or were left untreated. Approximately one year following top-dressing, soil samples were taken using a soil corer at a depth 3-6 inches. This depth was chosen as Fraser fir roots are relatively shallow, rarely reaching a depth much greater than 6 inches. The pH in untreated plots average 5.8, whereas plots top-dressed with sulfur ranged from 4.3 to 5.1 with an average of 4.7. Given difficulties of travel due to COVID-19 and only partial funding of this grant, this objective could only be partially fulfilled and while seedlings were replanted into the experimental plots, it was difficult to follow through and monitor progress. To our best knowledge, growers did not see differences in mortality or tree quality between the two treatments. Despite the ending of this project, it is our intention to continue to monitor the progress of transplants within these plots. If differences in mortality do arise by through the life of the transplants, we will report these results to the Christmas Tree Promotion Board and to stakeholders. Since this portion of the proposal could not be fulfilled in its entirety by the end of completion of the grant, we have forfeited approximately 1/3 of our funding, which should be returned to the CTPB.

**Objectives 3 and 4.** Objective 3, which focused on the influence of sulfur and soil pH on the root and soil microbiome was not completed as the fully requested amount of funding would have been needed for us to accomplish this task. Given our interest and the interest by the CTPB, we are currently looking into additional funding sources to accomplish this. If we are able to procure additional funding through another organization we present the results to stakeholders and provide an update to your board. Given our seedling results and Dr. Richard Cowles' research on pH, sulfur, and PRR, we are currently assembling factsheet to post on the Christmas Tree Portal at NCSU.

## Lessons learned and advice for future projects

Although several challenges regarding methods in this project have already been discussed, there are other challenges in regards to methodology that remain. In regards to the greenhouse trial, the utilization of a different potting mix should have been considered. The Metro Mix is a common medium for growing Fraser fir seedlings which is why it was selected. Unfortunately, the mix was not the best option for *Phytophthora* establishment. It drains well and has a relatively high porosity. In addition, the Metro Mix 852 (80% 50/50 nursery bark and peat moss, 20% perlite) has a high level of organic material and thus a high cation exchange capacity. This required frequent (every 6 days) drenches with sulfuric acid to maintain a low pH. Even at this low pH, we were unable to reduce pH below 3.5, which would have been a pH that would have been less conducive for *Phytophthora* growth and survival. In regards to Obj. 2, soil pH also did not get low enough that it would have been extremely toxic to *Phytophothora*. Higher rates of sulfur and considerations of incorporating into the soil rather than topdressing may have produce pH values more favorable to the study.