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FUNGUS SHOWN TO BE AN EFFECTIVE BIOLOGICAL CONTROL OF GRAY MOLD ON CONTAINER-GROWN CONIFERS

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Category: Disease management
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INTRODUCTION

Gray mold, caused by *Botrytis cinerea* Pers.:Fr., is a destructive disease of container-grown conifers. It develops on the lower needles and progresses as the seedlings age and the foliage becomes more dense. The disease can cause weakened seedlings and mortality. If left unchecked, fungicides are the mainstay of gray mold control. However, this dependency on chemical fungicides has some serious disadvantages. One is the ability of the fungus to develop a resistance to fungicides; an event documented in a number of instances (Staub 1991). In addition to this, the routine use of fungicides in enclosed environments can result in the high exposure of greenhouse workers to chemicals. A reduced dependency on fungicides is possible through the control of environmental factors that predispose seedlings to the disease (Zhang et al. 1994a).

Biological control agents might also be used as part of an integrated pest management program. Biological organisms have been used successfully to control selected diseases of other greenhouse or field crops (Sutton and Peng 1993). In work carried out at the University of Guelph and the Canadian Forest Service in Sault Ste. Marie, greenhouse and controlled environment experiments identified agents that were highly effective in reducing the incidence of gray mold. The use of these agents and their effectiveness is discussed in this technical note.

MATERIALS AND METHODS

Black spruce (*Picea mariana* [Mill.] B.S.P.) seeds were sown in a 3:1 mixture of peat and vermiculite in paperpot containers. Seedlings were grown under greenhouse conditions at 20–30°C with a 16-hour photoperiod and fertilized once a week with 20-20-20 (N-P-K) fertilizer. Seedlings were inoculated with *B. cinerea* 14 weeks after planting to ensure infection; no attempt was made to prevent infection by natural populations of the fungus. Biocontrol agents, water plus surfactant, and recommended fungicides were applied at 16, 18, 20, 22, and 24 weeks after planting. In the fungicide program in the first-year test, chlorothalonil (Daconil 2787®, 2 mL product /L) was applied twice and iprodione (Rovral 50® WP 0.01 g product/L) was applied three times. In both years, treatments were initiated after the seedling canopies had become dense and a few of the lower needles had turned yellow or brown and showed signs of gray mold. Biocontrol agents used in the greenhouse study were one isolate each of *Gliocladium roseum* Link:Bainer, *Fusarium* sp., *Myrothecium verrucaria* (Alb & Schw.) Ditm. Ex Stendel., and *Penicillium* sp. These isolates were previously found to effectively suppress *B. cinerea* in black spruce seedlings in a controlled study (Zhang et al. 1994b); a number of other fungi and bacteria were tested but found to be ineffective. After treatment, seedlings were covered with clear vinyl sheeting to maintain high humidity for 14 hours after the first application of *B. cinerea* and also after the treatments were applied. Thirty seedlings were sampled arbitrarily from each treatment 28 weeks after planting. The seedlings were surface sterilized and kept in high humidity at 20°C for 7–8 days, after which sporulation incidence of *B. cinerea* on the seedlings was estimated.

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To measure the concentration and frequency of application, inoculum of *G. roseum* was applied at various concentrations beginning 14 weeks after planting (coinciding with closure of the seedling canopy), and then at 2-week intervals. Inoculation with *B. cinerea* occurred 2 weeks after the initial inoculation with *G. roseum*. Seedlings were treated as previously described, but subsamples of 30 seedlings each were removed at 2-week intervals and the level of gray mold infection was measured. Seedling mortality was assessed 22 weeks after planting.

RESULTS

Biological Control

The incidence of gray mold did not differ between control seedlings that were artificially inoculated with *Botrytis* and those inoculated with water only. This indicates that levels of naturally occurring *Botrytis* were high. The incidence of gray mold in the inoculated and uninoculated checks was high (83–97 percent) in the first year, and moderate (34–36 percent) in the second year (Fig. 1). In the first year, three applications of *G. roseum*, *M. verrucaria*, and *Fusarium* spp. significantly suppressed the incidence of gray mold in the black spruce seedlings by 69, 60, and 64 percent, respectively, as compared to the controls (Fig. 1). Each of these biocontrol treatments was as effective as the recommended fungicides. Applications of *Penicillium* spp. were ineffective. In the second year, applications of *G. roseum* significantly suppressed the incidence of *B. cinerea* by 50–55 percent relative to that in the controls (Fig. 1). However, the other biological control agents were ineffective.

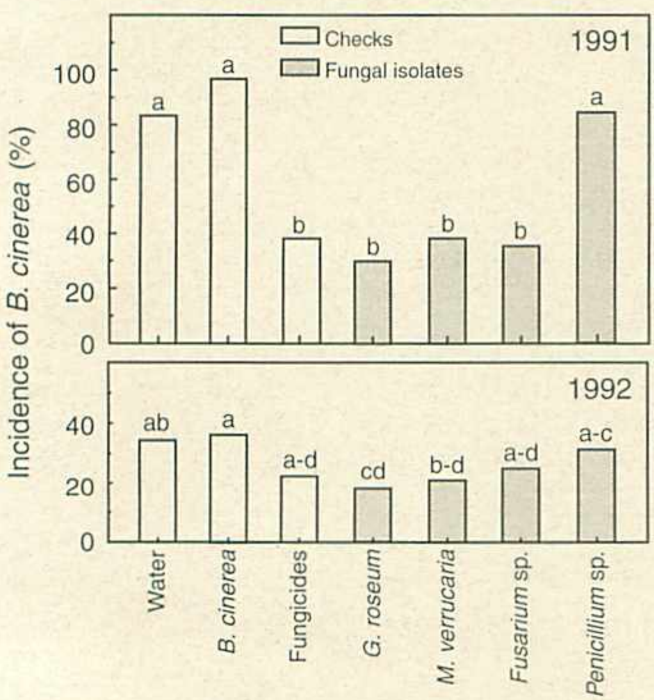


Figure 1. The incidence of Botrytis cinerea *on greenhouse-grown black spruce seedlings after treatment with fungicide or fungal isolates. Data bars assigned the same letter are not significantly different.*

Gliocladium roseum was chosen for further testing as it markedly suppressed *B. cinerea* on black spruce seedlings in the greenhouse in both years of evaluation. This fungus suppressed gray mold as, or more, effectively than did the fungicide program normally recommended for managing gray mold in the container production of conifers. The isolate of *G. roseum* was effective under conditions that favored high infection by the pathogen in the first year, and a moderate infection incidence in the second year.

Concentration and Time of Application of Inoculum

The ability of *G. roseum* to control gray mold was dependent primarily on the concentration of the control agent. Concentrations of 10^6 and 10^8 mL per L were found to be effective. These concentrations reduced both the amount of sporulation (Fig. 2) and the mortality (Fig. 3) caused by severe infections

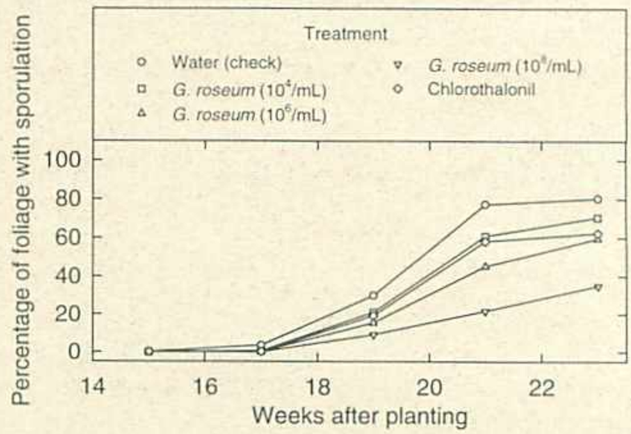


Figure 2. The effect of various concentrations of Gliocladium roseum, *each applied four times, and of a standard fungicide treatment on the level of gray mold sporulation on containerized black spruce seedlings in a greenhouse.*

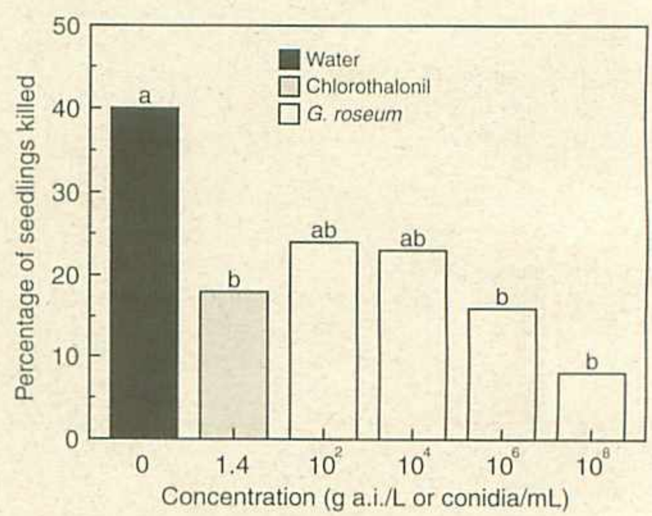


Figure 3. Effects of conidial concentrations of Gliocladium roseum *each applied four times, and of fungicide treatment on the percentage of black spruce seedlings killed by gray mold, at 22 weeks after planting. Data bars with the same letter are not significantly different.*

of gray mold. The higher concentrations of fungal spores provided control equal to that of fungicide at 22 weeks after planting. Concentrations of less than 10⁶ mL per L failed to provide effective control as compared to fungicides.

Tests on the number of applications of *G. roseum* required to provide control showed that one application at the time of canopy closure was as effective as multiple applications in reducing sporulation (Fig. 4) and mortality (Fig. 5) due to gray mold. Here, as well, biological control was equal to that of fungicide treatments. Later treatments might not be needed for effective biocontrol, possibly because the organism becomes established and maintains biocontrol activity in the seedlings. The fact that one application of *G. roseum* at canopy closure, and under conditions conducive to gray mold

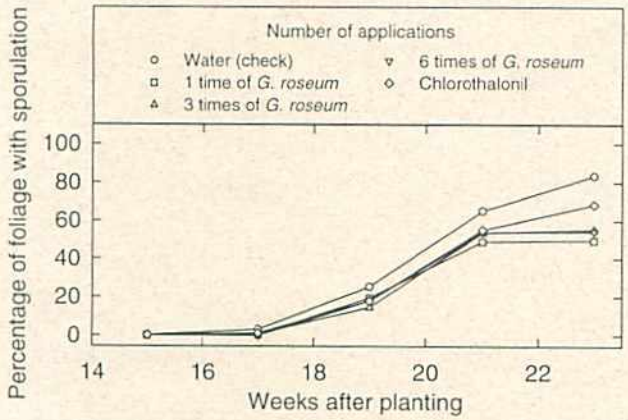
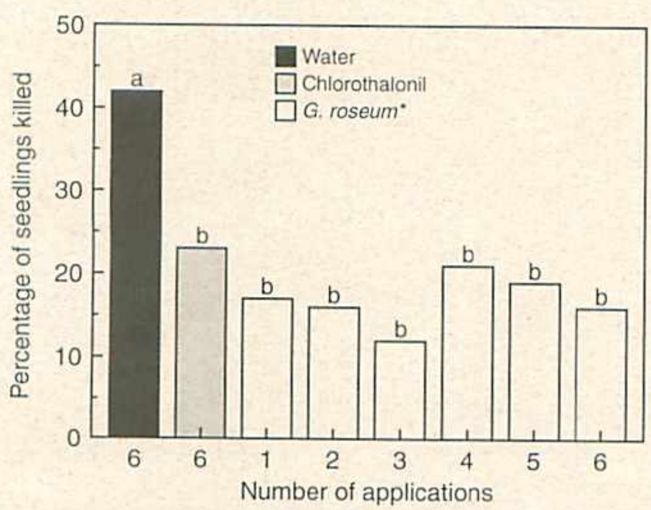


Figure 4. The effect of one to six applications of Gliocladium roseum *(10^6 conidia/ml) and of six fungicide applications on the level of gray mold sporulation on containerized black spruce seedlings in a greenhouse.*



* Spore concentration at 10^6 conidia/ml

Figure 5. Effects of one to six applications of Gliocladium roseum, *and of fungicide treatment on the percentage of black spruce seedlings killed by gray mold, at 22 weeks after planting. Data bars with the same letter are not significantly different.*

production, suppressed the fungus as effectively as did 2–6 applications suggests that persistent and effective control through biological means should be possible. Observations from previous work (Zhang et al. 1996) also suggest that *G. roseum* penetrates the needle surface and competes with *B. cinerea* for substrate, but induced resistance and hyperparasitism are also possible.

RECOMMENDATIONS AND CONCLUSIONS

The fungus *G. roseum* proved to be the most effective biological control agent tested on black spruce. This particular fungus has additional advantages over other potential biocontrol organisms. It seems to have no deleterious effects on seedlings; inoculum production is easy and inexpensive; the inoculum is viable for several months in the cold; and spores are not readily airborne, thereby minimizing allergic reactions. It remains to be clarified whether the application of *G. roseum* at the time of canopy closure is optimal for protection, although other studies (Zhang et al. 1994b, 1996) would suggest it could be advantageous to suppress *B. cinerea* at early stages of epidemics. Canopy closure generally creates a microclimate conducive to the development of gray mold application; after this, treatment with biocontrol agents would likely result in reduced control. It should be emphasized that biological control agents should be used as part of an integrated approach to disease management and not as a "silver bullet" solution. Reduction of plant stress, proper cultural practices, and sanitation will improve the efficiency of any disease control program.

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