

The effect of *Armillaria* root disease on conifer nutrients

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SUMMARY

Greenhouse grown lodgepole pine and white spruce trees were inoculated with *Armillaria ostoyae* and *Armillaria sinapina* to determine the effect of *Armillaria* root disease on tree nutrients. Twelve months after the inoculation trees were examined for evidence of the disease and then analyzed for nutrient content. Potassium (K) concentrations were significantly greater in the stems and needles of diseased trees than in healthy trees for both tree species. There was no significant difference in K concentration in the roots of the diseased versus the healthy trees. None of the other nutrients, such as nitrogen (N), phosphorus (P), sulfur (S), calcium (Ca) and magnesium (Mg) showed consistent concentration differences between the diseased and healthy trees. The increased K in the aboveground parts of the trees could indicate a physiological response of the tree to the disease. Exudate samples from cultures of the *A. ostoyae* and *A. sinapina* isolates used in the inoculation studies were analyzed for elemental composition. Significant quantities of K (690 mg kg⁻¹) were found in the isolates exudate. Thus, the higher K in the tree tissue was more probably related to the ability of the fungi to accumulate K.

INTRODUCTION

Armillaria root disease (ARD) is one of the most important tree diseases in the world today. The realization that there are many different species of *Armillaria* capable of inciting ARD has improved our understanding of the disease and its epidemiology. The infection process has been studied to some extent; however, our understanding of pathogenesis is far from complete.

There are two competing hypotheses on how (ARD) causes trees to die (Morrison *et al.*, 1991). The first hypothesis is that the fungus disrupts the vascular tissues in the roots and the second is that the fungus produces a toxin that affects host metabolism. According to Morrison *et al.* (1991), the vascular tissue disruption hypothesis has been largely accepted because of the nature of the symptoms, particularly foliar symptoms.

If vascular tissues were affected by ARD one would expect a reduction in the flow of water to the crown of the tree and with this a reduction in the mineral nutrients in the tree. Our objective in this study was to determine if there were any difference in the mineral nutrient concentrations in young lodgepole pine (*Pinus contorta* ex. Loud. var. *latifolia* Engelm.) and white spruce (*Picea glauca* (Moench) Voss) trees inoculated with *Armillaria ostoyae* (Romagnesi) Herink or *Armillaria sinapina* Bérubé & Dessureault and non-inoculated healthy trees.

MATERIALS AND METHODS

Two *Armillaria* isolates were used in the experiments: *A. ostoyae*, isolate NOF-1076, and *A. sinapina*, isolate NOF-894. The species identity was determined by haploid pairing tests (Anderson and Ullrich, 1979). Inoculum was prepared from live branch segments of *Populus tremuloides* (Michx.) as described by Mallett and Hiratsuka (1988).

Lodgepole pine and white spruce seeds were seeded in 3 L plastic pots containing limed peat moss (pH 5). After each seedling had emerged, an inverted 2 × 25 cm test tube was placed parallel next to the seedling's root. Trees were grown in a greenhouse compartment with artificial light (high pressure sodium vapor lamps, 400 W, with an intensity of 363 $\mu\text{mol m}^{-2}\text{s}^{-1}$) with a photo period of 18 hours and daytime and nighttime temperatures of 25°C and 20°C, respectively. The trees were watered twice weekly and fertilized with 20-20-20 fertilizer.

Two hundred and ten lodgepole pine and 210 white spruce were randomly assigned to the treatments in a randomized complete block. There were five replications of each treatment combination. Six months after seeding the test tube was replaced with a piece of inoculum in each pot. The heights and diameters (at soil line) of all trees were measured when the inoculum was placed in the pots. The trees were grown for one year before the experiment was terminated. Trees that died during the experiment were examined for characteristic signs of *Armillaria* and heights and diameters were measured. Height and diameter measurements were taken for all the remaining trees before they were unpotted and the root systems carefully examined for evidence of *Armillaria* infection at the end of the experiment. A tree was considered attacked if there was a resinous lesion with an attached rhizomorph or a white

mycelial fan beneath the surface of the bark. Roots, shoots, and foliage were separated and placed in paper bags before drying in ovens at 60°C.

Total nitrogen (N), phosphorus (P), sulfur (S), calcium (Ca), magnesium (Mg), and potassium (K), were determined on the needles, stems, and roots of all trees. Total analysis of the plant material was determined on oven-dried samples (60° C for 24 h) ground in a Wiley mill and passed through a 0.25 mm sieve. The samples were digested with $\text{HNO}_3\text{-H}_2\text{O}_2\text{-HCl}$ in a microwave oven (Kalra *et al.*, 1989) and analysed for P, S, Ca, Mg, and K. Total N was determined using a modified Kjeldahl digestion technique. Samples were digested in an aluminum block digester using a H_2SO_4 and K_2SO_4 - CuSO_4 catalyst mixture. Total N was determined by distillation with a Tecator Kjeltac 1030 automated system (Kalra and Maynard, 1991). Fungal exudate was obtained from isolates NOF-1076 and NOF-894 as described in Mallett and Colotelo (1984). The exudate was digested with $\text{HNO}_3\text{-H}_2\text{O}_2\text{-HCl}$ in a microwave oven and analysed for P, S, Ca, Mg, K, Mn, Na, and Fe.

The foliar, stem and root nutrient and the growth data were analysed by a multivariate analysis of variance (ANOVA) using the general linear model (GLM) procedure of the Statistical Analysis System (SAS) Institute, Inc. (1990). Least square means (Lsmeans) were used for comparisons where significant effects were detected by the multivariate ANOVAs.

RESULTS

No differences were seen in the nutrient concentration of trees infected by *A. ostoyae* or *A. sinapina*; consequently, data from trees infected by these two different species were combined for analysis. There was no difference in the shoot and root dry weights, heights or diameters of the healthy trees compared to the infected trees.

The concentrations of N, P, and S among healthy, infected and dead pine followed a similar pattern (Table 1). Concentrations of N, P, and S in stems from dead pine were higher than for healthy trees. In contrast, concentrations in dead roots of pine were lower than roots from healthy trees. No differences in N, P, and S needle concentrations among health classes of pine were observed.

Differences in N, P, and S concentrations among healthy, infected and dead spruce were limited to the roots for P and S only (Table 1). The P concentration of roots from healthy and infected spruce were higher than from roots of dead trees. The S concentrations of the roots varied among soil moisture content and health class of trees.

Table 1. Total Nitrogen (N), Phosphorus (P), and Sulfur (S) concentrations (g kg^{-1}) in the needles, stems, and roots of 16-month old lodgepole pine and white spruce by health class (healthy, infected or dead). Values are means \pm standard error.

	Lodgepole pine			White spruce		
	healthy	infected	dead	healthy	infected	dead
Needles						
N	$12.75 \pm 0.35\text{a}^*$	$12.44 \pm 0.84\text{a}$	$10.59 \pm 1.13\text{a}$	$21.36 \pm 0.79\text{a}$	$21.35 \pm 1.55\text{a}$	$15.29 \pm 3.16\text{a}$
P	$1.66 \pm 0.05\text{a}$	$1.94 \pm 0.12\text{a}$	$1.66 \pm 0.17\text{a}$	$3.15 \pm 0.08\text{a}$	$3.16 \pm 0.15\text{a}$	$3.66 \pm 0.31\text{a}$
S	$1.56 \pm 0.12\text{a}$	$1.38 \pm 0.05\text{a}$	$1.22 \pm 0.16\text{a}$	$1.78 \pm 0.05\text{a}$	$1.71 \pm 0.10\text{a}$	$1.38 \pm 0.21\text{a}$
Stems						
N	$7.22 \pm 0.32\text{b}$	$8.88 \pm 0.77\text{a}$	$10.99 \pm 1.04\text{a}$	$16.76 \pm 1.04\text{a}$	$17.00 \pm 2.07\text{a}$	$16.14 \pm 4.13\text{a}$
P	$1.31 \pm 0.06\text{b}$	$1.54 \pm 0.14\text{ab}$	$1.82 \pm 0.19\text{a}$	$2.31 \pm 0.04\text{a}$	$2.36 \pm 0.08\text{a}$	$2.39 \pm 0.17\text{a}$
S	$0.76 \pm 0.04\text{b}$	$0.90 \pm 0.09\text{ab}$	$1.13 \pm 0.12\text{a}$	$0.92 \pm 0.02\text{a}$	$0.95 \pm 0.04\text{a}$	$1.10 \pm 0.09\text{a}$
Roots						
N	$11.33 \pm 0.47\text{a}$	$10.76 \pm 1.13\text{a}$	$8.17 \pm 1.53\text{a}$	$17.95 \pm 0.64\text{a}$	$18.51 \pm 1.28\text{a}$	$13.84 \pm 2.54\text{a}$
P	$2.86 \pm 0.10\text{a}$	$2.39 \pm 0.23\text{ab}$	$1.72 \pm 0.31\text{b}$	$3.12 \pm 0.07\text{a}$	$3.05 \pm 0.14\text{a}$	$2.09 \pm 0.30\text{b}$
S	$2.28 \pm 0.08\text{a}$	$1.94 \pm 0.20\text{a}$	$1.16 \pm 0.27\text{b}$	$1.64 \pm 0.05\text{a}$	$1.46 \pm 0.09\text{a}$	$1.52 \pm 0.18\text{a}$

*Nutrient means for a given tree species and tree part followed by the same letter are not significantly different at $P \geq 0.05$.

The Ca concentrations of the needles of healthy trees were higher than in the needles of dead trees for pine ($4.68 \pm 0.13 \text{ g kg}^{-1}$ for healthy and $3.54 \pm 0.41 \text{ g kg}^{-1}$ for dead; $P = 0.01$) and white spruce ($11.61 \pm 0.26 \text{ g kg}^{-1}$ for healthy and $8.84 \pm 1.06 \text{ g kg}^{-1}$ for dead; $P = 0.01$). Needles of infected trees had Ca concentrations intermediate between those of healthy and dead trees. There was no consistent pattern for Mg concentrations among healthy, infected and dead trees for any tree parts or either tree species (data not shown).

The K concentration in needles from dead trees of lodgepole pine ($6.26 \pm 1.80 \text{ g kg}^{-1}$, $P = 0.004$) and white spruce ($8.75 \pm 2.43 \text{ g kg}^{-1}$, $P = 0.0001$) were higher than in the needles of healthy trees (4.80 ± 1.33 and $5.04 \pm 1.74 \text{ g kg}^{-1}$ for pine and spruce, respectively). Needles of infected trees had K concentrations (5.82 ± 0.84 and $5.98 \pm 2.12 \text{ g kg}^{-1}$ for pine and spruce respectively) midway between those in healthy and dead trees. Total K concentrations in the stems followed a similar pattern to the K concentrations in the needles. Total K was higher in

the stems of dead lodgepole pine ($6.49 \pm 1.86 \text{ g kg}^{-1}$, $P = 0.0001$) and white spruce ($7.60 \pm 1.26 \text{ g kg}^{-1}$, $P = 0.0001$) compared to the stems of healthy trees ($4.25 \pm 1.32 \text{ g kg}^{-1}$ and $4.76 \pm 1.31 \text{ g kg}^{-1}$ for lodgepole pine and white spruce, respectively). In contrast, K concentration of roots from dead trees were lower than from healthy trees for both tree species ($P = 0.0001$ for pine and $P = 0.0001$ for spruce).

Armillaria ostoyae and *A. sinapina* exudate analysis is shown in Table 2. The exudate of both species had similar elemental concentrations.

Table 2. Total Phosphorus (P), and Sulfur (S), Magnesium (Mg), Sodium (Na), Potassium (K) Calcium (Ca), Manganese (Mn), and Iron (Fe) concentrations (mg kg^{-1}) in the rhizomorph exudate of *Armillaria ostoyae* and *Armillaria sinapina*.

Element	<i>A. ostoyae</i>	<i>A. sinapina</i>
P	47.96	42.32
S	181.7	217.8
Mg	41.48	29.62
Na	594.7	580.1
K	697	662
Ca	35.43	25.29
Mn	0.081	0.075
Fe	0.261	0.195

DISCUSSION

The infection of tree seedlings by *A. ostoyae* and *A. sinapina* had a significant effect on the K concentration in the needles, stems, and roots. This may be important in understanding the pathogenesis of the disease. Potassium was the only element that consistently had concentration differences among the health class of trees with the various tree parts. The aboveground (needles and stems) parts of the dead and infected trees had higher K concentrations than in healthy trees. Lower concentrations of K may have been expected in dead or damaged plant tissue because K is one of the most readily leached elements from plant tissue (Tukey 1970).

The higher K concentration in the needles and stems could indicate a physiological response of the tree to the disease. Rykowski (1981) also found K concentrations in the needles from infected trees increased 10-50% compared to the K concentrations in needles from healthy

trees. It is unclear if the changes observed in the chemical composition of the trees were related to the reaction of the trees to infection by *Armillaria* or whether changes can be attributed to the fungus. Basidiomycete fungi (Vogt *et al.*, 1983, Cromack *et al.*, 1975) and *Armillaria* sp. (Mallet and Colotelo, 1984) have been shown to accumulate large quantities of K within rhizomorphs. Both fungal isolates used in this study had rhizomorph exudate with large quantities of K. Rhizomorphs of *Armillariella mellea* on decaying wood in forests of western Washington had K concentrations of 3280 and 6728 mg K kg⁻¹ (Vogt and Edmonds 1980). Increased K in sapwood of Norway spruce attacked by *Fomes annosus* has also been observed (Johansson and Theander, 1974). Rennerfelt and Tamm (1962) suggested high K levels in infected tree root tissue was due to the ability of the fungi to accumulate K; however, Rykowski (1981) attributed higher K in needles infected with *Armillaria* to defensive mechanism of the tree due to the role of K in the activation of enzymatic systems.

CONCLUSION

The lack of consistent differences among health classes suggests that the infection of the trees by *Armillaria* had minimal affect on the nutrient balance of the tree with the exception of K. The high concentrations of K may disrupt stomatal cell control as well as other plant cell membrane functions. Further studies are needed to determine if the high K concentration in rhizomorph exudate is responsible for the elevated levels of K in the plant and whether these concentrations of K can cause similar foliar symptoms.

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