DOE/CE/15270-T15

DOE/CE/15270--T15

DE91 000032

QUARTERLY REPORT

TITLE

Explorations of Mechanisms Regulating Ectomycorrhizal
Colonization of Boron-fertilized Pine

Instrument No. DE-FG01-86CE15270

PERIOD COVERED:
10/1/90-12/31/90

SUBMITTED TO:
Department of Energy

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MASTER

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A. Hypothesis: Boron and IAA act in concert in the root system to increase the flux of sucrose from the foliage to the roots. Such action enhances mycorrhizal colonization of the root system.

Continued data analysis and interpretation have been delayed; but will resume as time permits. Foliar cytokinin and mineral nutrient analyses will be performed following identification of satisfactory analytical procedures.

B. Hypothesis: Phenolic compounds are produced by the host plant following fungal invasion which inhibit mycorrhizal development. Boron fertilization of the host plant restricts inhibition by either directly complexing with phenols or through a disruption of the synthesis of phenols by acting on the enzyme activities regulating phenol production.

Task 1: To determine the effects of hydroxylation of phenolic compounds on the growth of Pisolithus tinctorius, a known ectomycorrhizal fungus, grown in pure culture.

Task 3: To evaluate the effectiveness of phenolase in metabolizing the most and least toxic phenolic compounds studied.

As previously described, complexation between boric acid and phenolic compounds may result in promotion of ectomycorrhizal colonization. It has been proposed that complexation may occur at aromatic ortho-hydroxyl groups of phenolic compounds. Based upon the structure of caffeic acid as well as in vitro fungal growth experiments in our laboratory, we have chosen caffeic acid as a model phenolic compound for complex identification trials.

In addition to spectrophotometric and thin layer chromatographic techniques which have been described previously, fluorometric methods were used for observation of the effect of boric acid on caffeic acid. Results suggested lack of complexation between boric acid and the aromatic ortho-hydroxyl groups of caffeic acid. However, complex formation between boric

acid and caffeic acid may occur at a location other than the ortho-hydroxyl groups on the aromatic ring.

High performance liquid chromatographic (HPLC) techniques have been initiated for further identification of the potential boric acid - caffeic acid complex. Methanolic caffeic acid and boric acid - caffeic acid (1:1) solutions were isocratically eluted using a uBondapak C18 column, a methanol - ethyl acetate -acetic acid (5:4:1) solvent system and a 2.0 ml/min flow rate. Ultra-violet detection at 254 nm indicated that retention times of caffeic acid and boric acid - caffeic acid methanolic solutions did not differ. However, similar retention times of cis- and trans- caffeic acid and boric acid suggest that chromatographic conditions should be modified in an effort to improve resolution of these peaks. Improved resolution of these peaks may allow potential small differences between caffeic acid and boric acid - caffeic acid retention times to be observed. Of additional interest is a reduction in the peak area of trans-caffeic acid when boric acid was added to the eluted solution. Additional HPLC trials will be conducted for further understanding of the relationship between boric acid and caffeic acid.

Task 2: To determine the effect of phenolic glycosides on the growth of Pisolithus tinctorius, grown in pure culture.

Not yet started. Initiation of this task is dependent upon further results obtained in Tasks 1 and 3.

Task 4: To determine the types and quantities of phenolic compounds produced by pine seedlings inoculated with Pisolithus tinctorius and receiving boron fertilization.

As previously described, a greenhouse experiment has been installed for evaluation of the effect of boric acid on the growth, ectomycorrhizal colonization, root morphology and physiology of shortleaf pine seedlings. Seedling subsamples were harvested following 12- and 16- week cultural periods. Specifically, these harvests received 9 and 17 semiweekly boric acid fertilization treatments (20 ml of 25 mg/L boric acid).

Following both 12- and 16- week cultural periods, the IAA oxidase activity of inoculated shortleaf pine seedling root tissue was significantly less than that of non-inoculated seedling root tissue (Graph 1). Fertilization with boric acid did not significantly affect IAA oxidase activity.

The polyphenol oxidase (PPO) activity of inoculated shortleaf pine root tissue was significantly greater following 12- and 16- week cultural periods when compared to that of non-inoculated shortleaf pine root tissue (Graph 2). Furthermore, fertilization with boric acid significantly increased the PPO activity of non-inoculated shortleaf pine seedling root tissue. Fertilization with boric acid did not significantly affect the PPO activity of inoculated shortleaf pine root tissue following 12- and 16- week cultural periods. However, mean root PPO activity of inoculated shortleaf pine seedlings fertilized with boric acid was consistently greater than that of inoculated shortleaf pine seedlings receiving no boric acid fertilization treatment.

Early analyses indicate no significant effect of boric acid on the numbers of primary, secondary and tertiary lateral roots of

inoculated and non-inoculated shortleaf pine seedlings (Graphs 3-5). However, trends between numbers of secondary and tertiary lateral roots suggests that root morphology may be affected by both ectomycorrhizal inoculation and boric acid fertilization. Further data analyses will be performed in an effort to understand trends in root morphology.

Potential effects of boric acid on root morphology may be associated with new root production and rate of elongation. Table 1 indicates that, following a 12-week cultural period, fertilization with boric acid significantly increased the length of elongating lateral roots of non-inoculated shortleaf pine seedlings. However, the quantity of these roots on non-inoculated root systems was unaffected by boric acid fertilization treatment. Although not significant, a similar relationship was associated with inoculated seedlings in response to boric acid fertilization treatment. This trend was not present following a 16-week cultural period. However, as was apparent following the 12-week cultural period, ectomycorrhizal inoculation significantly increased elongating lateral root length following the 16-week cultural period.

Enzyme activity and root morphology data suggest that boric acid had an effect on shortleaf pine seedlings early during the cultural period. However, this effect was either absent or not apparent following a 16-week cultural period. Ectomycorrhizal colonization reflects this trend. Two parameters of ectomycorrhizal colonization have been presented in Table 2. The

% of primary lateral roots colonized is descriptive of the spread of the fungal symbiont throughout the root system; whereas, the number of infection points per infected primary lateral root describes the intensity of ectomycorrhizal colonization.

Following a 12-week cultural period, the spread of Pisolithus tinctorius throughout the root system was significantly increased due to boric acid fertilization treatment. However, following a 16-week cultural period, no significant effect of boric acid on ectomycorrhizal colonization was apparent.

Moreover, in accordance with only small responses of inoculated and non-inoculated shortleaf pine root systems to boric acid fertilization treatments, no significant effect of boric acid on shoot growth was detected (Table 3). However, shoot growth of 12- and 16- week old inoculated shortleaf pine seedlings was significantly greater than that of non-inoculated seedlings.

Lack of consistency between 12 and 16 week responses of shortleaf pine seedling root IAA oxidase and PPO activity and root morphology may be attributed to a number of factors. Perhaps the effect of boric acid on ectomycorrhizal colonization occurs during seedling growth prior to 12 weeks. Moreover, variability associated with rapid growth between weeks 12 and 16 of the seedling production period may mask early as well as later effects of boric acid fertilization treatments. Further statistical analyses will include analysis of covariance in an effort to control for variability within treatments.

Lack of a boric acid response following a 16-week cultural period may also be associated with extreme greenhouse temperatures which seedlings were exposed to in August, between weeks 12 and 16 of the cultural period. Specifically, greenhouse temperatures may have been too high for efficient photosynthetic activity. Without adequate photosynthesis and carbohydrate translocation, the fungal symbiont may have been exposed to less than optimal nutritional conditions.

The third and final subsample of seedlings in this experiment will be harvested following a 24-week cultural period and will have received 33 boric acid fertilization treatments. Seedling growth, ectomycorrhizal colonization, root morphology and physiology will be assessed. Comparison of these parameters following 12-, 16- and 24- week cultural periods will be made in an effort to understand the effect of boric acid on ectomycorrhizal colonization of shortleaf pine by Pisolithus tinctorius.

Graph 1. IAA Oxidase Activity of 12 and 16-week
Old Shortleaf Pine Seedlings.

* Inoculated or not inoculated with *Pisolithus tinctorius* and fertilized with 0 or 25 mg/L boric acid.

** Within cultural periods, differing lower case letters indicate significance at $P = 0.05$.

Graph 2. PPO Activity of 12 and 16-week Old Shortleaf Pine Seedlings .

* Inoculated or not inoculated with *Pisolithus tinctorius* and fertilized with 0 or 25 mg/L boric acid.
 ** Within cultural periods, differing lower and upper case letters indicate significance at $P = 0.05$ and $P = 0.10$, respectively.

Graph 3. Number of Primary Lateral Roots of 12 and 16-week Old Shortleaf Pine *

* Inoculated or not inoculated with *Pisolithus tinctorius* and fertilized with 0 or 25 mg/L boric acid.

Graph 4. Number of Secondary Lateral Roots of 12 and 16-week Old Shortleaf Pine .

• Inoculated or not inoculated with *Pisolithus tinctorius* and fertilized with 0 or 25 mg/L boric acid.

Graph 5. Number of Tertiary Lateral Roots of 12 and 16-week Old Shortleaf Pine *

* Inoculated or not inoculated with *Pisolithus tinctorius* and fertilized with 0 or 25 mg/L boric acid.

Table 1. New root growth of 12 and 16-week old shortleaf pine seedlings inoculated or not inoculated with Pisolithus tinctorius and fertilized with 0 or 25 mg/L boric acid.

Variable	Treatment			
	-Pt -B*	-Pt +B	+Pt -B	+Pt +B
-----12 Weeks-----				
Number of New Roots	14.0 a**	14.6 a	16.1 a	18.9 a
Length of New Roots (cm)	12.6 c	18.2 b	23.5 ab	30.4 a
Dry Weight of New Roots (mg)	2.9 b	3.3 b	6.5 a	7.6 a
-----16 Weeks-----				
Number of New Roots	5.9 b	9.8 b	22.4 a	23.6 a
Length of New Roots (cm)	10.8 c	17.2 bc	34.7 a	34.4 ab
Dry Weight of New Roots (mg)	7.8 b	10.3 b	19.1 a	17.6 a

* +Pt: inoculation with Pisolithus tinctorius; +B: semiweekly fertilization with 25 mg/L boric acid.

** Means within rows followed by the same lower case letter are not significantly different at $P = 0.05$ using the LSD test.

Table 2. Ectomycorrhizal colonization of 12 and 16-week old shortleaf pine seedlings inoculated or not inoculated with Pisolithus tinctorius and fertilized with 0 or 25 mg/L boric acid.

Variable	Treatment			
	-Pt	-B*	-Pt +B	+Pt -B +Pt +B
-----12 Weeks-----				
% Primary Lateral Roots Colonized	1.6 ± 20.4	0.6 ± 2.3	77.3 ± 7.3	94.7 ± 2.5
Mean Number of Infection Points Per Infected Lateral	3.4 ± 4.5	0.6 ± 1.3	14.1 ± 2.6	14.2 ± 1.6
-----16 Weeks-----				
% Primary Lateral Roots Colonized	0	0	80.0 ± 6.1	78.8 ± 8.0
Mean Number of Infection Points Per Infected Lateral	0	0	19.3 ± 4.3	14.9 ± 6.5

* +Pt: inoculation with Pisolithus tinctorius; +B: semiweekly fertilization with 25 mg/L boric acid.

Table 3. Shoot growth of 12 and 16-week old shortleaf pine seedlings inoculated or not inoculated with Pisolithus tinctorius and fertilized with 0 or 25 mg/L boric acid.

Variable	Treatment			
	-Pt -B*	-Pt +B	+Pt -B	+Pt +B
-----12 Weeks-----				
Shoot Length (cm)	3.8 b**	3.7 b	5.0 a	5.0 a
Root Collar Diameter (cm)	0.161 b	0.156 b	0.188 a	0.184 a
Shoot Dry Weight (g)	0.19 b	0.21 b	0.35 a	0.36 a
Number of Branches	3.7 b	3.7 b	5.0 a	5.0 a
Branch Dry Weight (g)	0.021 b	0.026 b	0.126 a	0.119 a
-----16 Weeks-----				
Shoot Height (cm)	4.8 b	4.9 b	6.1 a	5.9 a
Root Collar Diameter (cm)	0.193 b	0.196 b	0.264 a	0.255 a
Shoot Dry Weight (g)	1.29 b	1.25 b	3.52 a	3.32 a
Number of Branches	4.9 b	5.3 a	5.6 a	5.6 a
Branch Dry Weight (g)	0.28 b	0.30 b	1.61 a	1.50 a

* +Pt: inoculation with Pisolithus tinctorius; +B: semiweekly fertilization with 25 mg/L boric acid.

** Means within rows followed by the same lower case letter are not significantly different at P = 0.05 using the LSD test.

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