Effects of Metalaxyl on Growth and Ectomycorrhizae of Fraser Fir Seedlings

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ABSTRACT

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Effects of metalaxyl on growth and ectomycorrhizae of Fraser fir seedlings were examined at two nurseries and in a natural Fraser fir stand. Dry weights of roots and shoots of seedlings at the Linville River Nursery were found to have a significant ($P = 0.01$) linear and quadratic response to metalaxyl. Lateral root length, number of ectomycorrhizal tips per centimeter of lateral root, and percentage of short roots ectomycorrhizal were significantly greater among metalaxyl-treated than among untreated seedlings at the Linville River Nursery. The number of sporocarps of *Laccaria proxima*, a putative ectomycorrhizal fungus of Fraser fir, increased with increasing dosage of metalaxyl. Dry weights were significantly greater ($P = 0.01$) for treated seedlings in their first year of growth at Wolf Mt. Nursery than for untreated seedlings on one sampling date but not on other dates. Dry weights of seedlings in their second year of growth at the Wolf Mt. Nursery or in a natural stand of Fraser fir were not affected by applications of a single rate of metalaxyl. Ectomycorrhizae were found earlier in the growing season on metalaxyl-treated than on untreated seedlings at the Wolf Mt. Nursery. Average organic matter content of soils was considerably higher in the natural stand (12.3%) and at the Wolf Mt. Nursery (4.3%) than at the Linville River Nursery (0.5%), which may account for the difference in responses to metalaxyl.

Application of chemicals for management of insects, pathogens, and weeds has become routine in the intensive cultivation of Fraser fir (*Abies fraseri* (Pursh) Poir.) by Christmas tree growers in North Carolina (2,3). Metalaxyl, a systematic fungicide of the acylalanine class (Ridomil, Subdue) has been shown effective for control of root rot of Fraser fir incited by *Phytophthora cinnamomi* Rands (4). Metalaxyl is used in spring and autumn as a broadcast spray at state-owned as well as at numerous commercial nurseries in western North Carolina. We observed a marked increase in shoot height and branching and improved needle color of 5-yr-old fir transplants after a spring application of metalaxyl 15G in 1980. These initial field observations and reports by various authors (12,14,16) of the effects of fungicides on ectomycorrhizal development prompted us to investigate the secondary effects metalaxyl may have on root and shoot weight and abundance of ectomycorrhizae of Fraser fir.

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MATERIALS AND METHODS

Linville River Nursery. Ten blocks (1.2 × 4.9 m) were established on 1 May 1980 at a state nursery in Crossnore, NC, in a 1.2-ha bed with 90 rows (1.2 × 55 m) of fir transplants in their fifth growing season. Three contiguous 1-m^2 plots, each containing about 50 transplants, were delineated within each block. One plot was treated with metalaxyl 15G at a rate of 1.12 kg a.i./ha, the second plot with 2.81 kg a.i./ha, and the third plot was not treated. Treatments were assigned randomly within each block and separated by a 0.6-m buffer. To determine dry weights of roots and shoots, 10 transplants were removed from each of the 30 plots immediately before and 4 mo after treatment.

In 1981, a second trial was initiated using a 2E formulation of metalaxyl to treat seedlings during their third growing season in a 0.7-ha bed (40 rows 1.2 × 75 m, 110 seedlings per square meter) within the nursery. Five rates (0.00, 0.14, 0.28, 0.56, and 1.12 kg a.i./ha) were applied by a tractor-mounted hydraulic pump sprayer in a volume of 467 L/ha at 2.76×10^5 Pa to seedlings on two dates (29 April and 4 September). Treatments were assigned randomly among the 35 rows of seedlings chosen for the experiment. Twenty-five seedlings were removed randomly from each of the 35 rows on six sampling dates in 1981 and used to determine dry weights of roots and shoots.

Ten seedlings were collected from each row on 8 December 1981 to determine lateral root length and abundance of ectomycorrhizae. Abundance was expressed as numbers of ectomycorrhizal tips per centimeter of lateral root and the percentage of short roots ectomycorrhizal. For this, five lateral roots from each of 10 seedlings per row were selected randomly and measured with a stereoscopic microscope at ×4–20 (7). In addition, several ectomycorrhizae from each row were selected randomly to assess Hartig nets and mantles.

Abundance of *Laccaria proxima* (Boud.) Pat., a putative ectomycorrhizal fungus occurring in the nursery beds, was determined by counting the sporocarps per 50-m portion (randomly selected) of each of the 35 nursery bed rows. Attempts were made to isolate *L. proxima* from ectomycorrhizae that appeared to be macroscopically connected to sporocarps in the beds and from fresh sporocarp tissue (13). Mercuric chloride (15–60 sec) was used as the surface disinfestant before rinsing and placing the ectomycorrhizae onto modified Melin-Norkrans medium (9).

Wolf Mt. Nursery. A second study was established on 29 May 1980 in a private Fraser fir nursery located on Wolf Mt., Jackson County, NC. Two nursery bed rows (1.2 × 30 m) were divided into two blocks with four plots (each plot 1.2 × 1.4 m, separated by a 0.5 m buffer) in each block. One-year-old greenhouse-grown seedlings were transplanted into each of two plots per block. Each transplant was examined to ensure that only macroscopically nonmycorrhizal seedlings were used. The other two plots per block were seeded with Fraser fir seed. One plot in each block of each age class (1-yr-old transplants and seeded fir) was treated (by hand sprayer) with metalaxyl 2E at a rate of 1.12 kg a.i./ha. Metalaxyl was applied on 2 July and 3 September 1980 and 29 April 1981. Plant material (seeds and seedlings) and treatments were randomly assigned within a block, resulting in a randomized complete block design with four replicates.

Three collections were made in 1981 (13 April, 8 July, and 29 October) to determine dry weights of roots and shoots. Percentage of seedlings ectomycorrhizal and abundance of ectomycorrhizae were recorded from five collections in 1980 (2 and 24 July, 13 August, 3 September, and 29 October). Abundance of ectomycorrhizae was expressed in terms of an ectomycorrhizal index on the following scale: 1 = 0–25, 2 = 26–50, 3 = 51–75, and 4 = 76–100% of feeder roots ectomycorrhizal (10). Fifteen seedlings were chosen randomly from each plot at each sampling date to count ectomycorrhizae and an additional 15 seedlings per plot were collected to determine dry weights of roots and shoots.

Richland Balsam natural sand. A third study was conducted at Richland Balsam, Jackson County, NC, in a natural stand of Fraser fir. On 28 May 1980, 1-yr-old greenhouse-grown seedlings were transplanted into four blocks, with each block divided into two plots (each plot 1.2 × 1.4 m separated by a 0.5-m buffer). All transplanted seedlings were determined by macroscopic inspection to be nonmycorrhizal. Seedlings in one plot per block were hand-sprayed with metalaxyl 2E at a rate of 1.12 kg a.i./ha on 28 May and 2 September 1980 and 29 April 1981. The other plots were sprayed with the same volume of water as the metalaxyl-treated plots. Sampling dates, numbers, and procedures were the same as in the Wolf Mt. Nursery. Eight soil samples were collected from each of the three locations in July 1981 and submitted to the North Carolina Department of Agriculture, Agronomic Division, for a standard analysis.

RESULTS

Linville River Nursery. Before application of metalaxyl 15G on 1 May 1980, there were no significant differences in dry weights of roots or shoots among transplants in their fifth growing season (Table 1). Four months after a single application of metalaxyl at 1.12 kg a.i./ha or 2.81 kg a.i./ha, root and shoot dry weights of treated seedlings were greater ($P = 0.01$) than those of the untreated seedlings (Table 1).

Analysis of variance of the weight data of 1981 demonstrated significance of all first-order interactions. Thus, data were sorted and analyzed by date using regression analysis which, according to Chew (5), is the most appropriate technique for a quantitative factor. This showed no significant differences among the plots for root and shoot dry weight before fungicide application on 29 April. Thereafter, a significant linear and quadratic relation between root or shoot dry weight and metalaxyl dosage accounted for more than 86% of the variability among the dry-weight responses (Figs. 1 and 2). Examination of residual plots and F-values of analyses of variance showed the data to fit a quadratic model.

A significant linear and quadratic response was found between lateral root length and fungicide dosage in December 1981 (Fig. 3). The ectomycorrhizal variables examined (number of ectomycorrhizal tips per centimeter of lateral root and percentage of short roots ectomycorrhizal) were also related by a quadratic model to fungicide dosage (Figs. 4 and 5).

Sporocarps of *L. proxima* were found to be associated with ectomycorrhizal roots and the fungus was isolated from the ectomycorrhizae. The number of sporocarps increased (significant linear and quadratic response) as the rate of metalaxyl increased (Fig. 6).

Wolf Mt. Nursery. Dry weight and ectomycorrhizal data for each sampling date were analyzed separately because there was a significant date-by-treatment interaction. Metalaxyl-treated seedlings had significantly greater ($P = 0.01$, F-test) dry weights than untreated seedlings in their first year of growth on the last sampling date (29 October 1981). Metalaxyl-treated and untreated seedlings had mean root weights of 0.15 and 0.07 g and shoot weights of 0.35 and 0.15 g, respectively. There were no significant

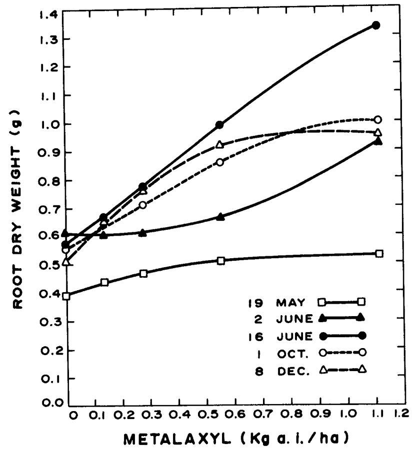

Fig. 1. Regression curves for root weight of Fraser fir seedlings in their third growing season in relation to dose of metalaxyl in 1981 at the Linville River Nursery, Crossnore, NC. Coefficients of determination for the curves ranged from 0.86 to 0.95.

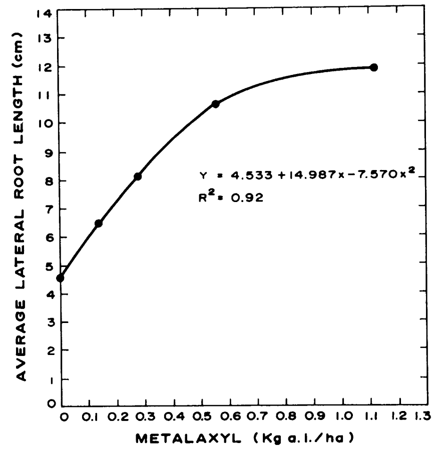

Fig. 3. Average lateral root length of Fraser fir seedlings in their third growing season in relation to dosage of metalaxyl at the Linville River Nursery, Crossnore, NC.

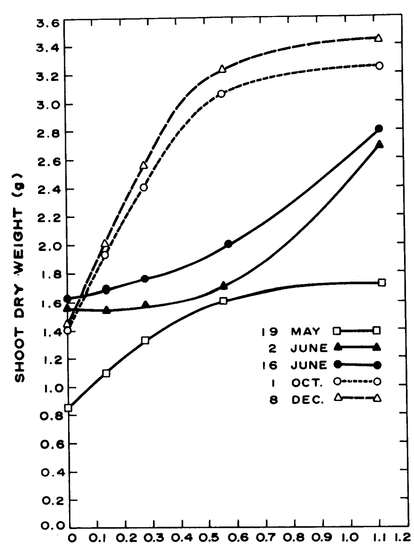

Fig. 2. Regression curves for shoot weight of Fraser fir seedlings in their third growing season in relation to dosage of metalaxyl during 1981 at the Linville River Nursery, Crossnore, NC. Coefficients of determination for the curves ranged from 0.86 to 0.99.

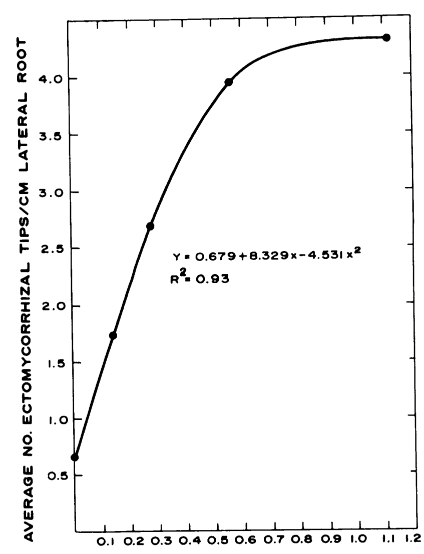

Fig. 4. Average number of ectomycorrhizal tips per centimeter of root of Fraser fir seedlings in their third growing season in relation to dosage of metalaxyl at the Linville River Nursery, Crossnore, NC.

Table 1. Dry weights of Fraser fir seedlings treated with metalaxyl 15G in their fifth growing season at Linville River Nursery, Crossnore, NC, in 1980

Treatment	Pretreatment weights (g)[a] (1 May)		Posttreatment weights (g)[a] (2 September)	
	Root	Shoot	Root	Shoot
Control	45.7	88.3	59.7	102.3
1.12 kg/ha	43.8	91.2	80.8*[b]	141.8*
2.81 kg/ha	40.1	89.4	154.8*	141.8*

[a] Per seedling; mean of 100 seedlings from 10 plots per treatment.
[b] * = Significantly greater than control at $P = 0.01$.

differences in dry weight between metalaxyl-treated and untreated seedlings in their first year of growth on the other sampling dates. Also, significant differences were not found between metalaxyl-treated and untreated seedlings in their second growing season. Ranges of dry weights of roots and shoots of these seedlings on 29 October 1981 were 0.47–3.14 and 1.11–8.62 g, respectively.

One-year-old transplants treated with metalaxyl formed ectomycorrhizae about 1 mo sooner than those not treated (Table 2). No differences in percentage of seedlings ectomycorrhizal or ectomycorrhizal abundance were found between treated and untreated seedlings 8 wk after transplanting (Table 2). In the second series of experiments at the Wolf Mt. Nursery, germinating seedlings treated with metalaxyl initiated formation of ectomycorrhizae during the first year, whereas untreated seedlings did not (Table 2). Ectomycorrhizal development during the winter months was restricted to seedlings previously ectomycorrhizal; seedlings from untreated areas were not ectomycorrhizal by 13 May of the following year. By 8 July 1981, all seedlings sampled were ectomycorrhizal, but those treated with metalaxyl had more abundant ectomycorrhizae (Table 2). As the summer progressed, ectomycorrhizal abundance became similar for metalaxyl-treated and untreated seedlings.

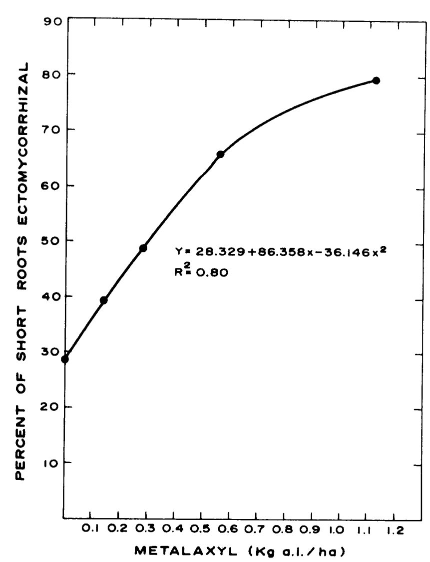

Fig. 5. Average percentage of short roots ectomycorrhizal on Fraser fir seedlings in their third growing season in relation to dosage of metalaxyl at the Linville River Nursery, Crossnore, NC.

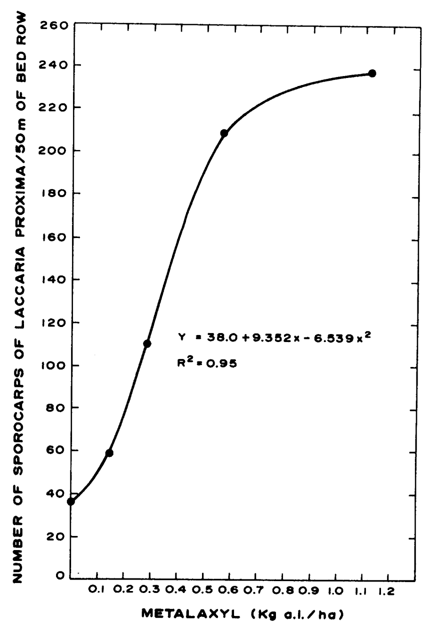

Fig. 6. Average number of sporocarps of *Laccaria proxima* in nursery bed rows with Fraser fir seedlings in their third growing season in relation to dosage of metalaxyl at the Linville River Nursery, Crossnore, NC.

Richland Balsam natural stand. Dry weights of shoots of metalaxyl-treated seedlings in their second growing season were greater ($P = 0.05$) than those of untreated seedlings from the natural stand of Fraser fir on 13 April 1981. There were no differences in weights between treated and untreated seedlings on the other sampling dates. Ranges for dry weights of roots and shoots sampled on 29 October 1981 were 0.13–0.88 and 0.18–1.79 g, respectively.

Metalaxyl-treated seedlings had more ectomycorrhizae ($P = 0.01$) than untreated seedlings collected on 28 November 1980, but percentage of seedlings ectomycorrhizal and ectomycorrhizal abundance were unaffected by metalaxyl on all other sampling dates. Fifty-seven days after transplanting, >10% of the seedlings were ectomycorrhizal and ectomycorrhizal abundance was >25%. By the end of the first year after transplanting, percentage of seedlings ectomycorrhizal and abundance of ectomycorrhizae were both between 50 and 75% for treated and untreated seedlings. Seedlings sampled from any of the plots at the onset of the second growing season (13 April 1981) all had ectomycorrhizae on 75% or more of the feeder roots (Table 2).

Soil analyses. Average percent organic matter contents of soils in the Linville River and Wolf Mt. nurseries and Richland Balsam natural stand were 0.54, 4.26, and 12.27, respectively (F-test significant, $P = 0.01$, least significance difference = 1.53). There were significant differences (F-test, $P = 0.01$, least significant difference = 0.25) among average pH values at the three locations (Linville River, 4.3; Wolf Mt., 5.8; and natural stand, 3.8). The average percent base saturation was 22.38, 79.25, and 15.13 (significant, $P = 0.01$) at the Linville River and Wolf Mt. nurseries and the natural stand, respectively. Calcium and magnesium contents were significantly

Table 2. Percentage of seedlings ectomycorrhizal and abundance of ectomycorrhizae on Fraser fir seedlings treated with metalaxyl of two locations in Jackson County, NC

			Ectomycorrhizal measurements[b]															
			2 July 1980		24 July 1980		13 Aug. 1980		3 Sept. 1980		28 Oct. 1980		13 Apr. 1981		8 July 1981		29 Oct. 1981	
Location	Treatment	Age[a]	%M	MI	%M	MI	%M	MI	%M	MI	%M	MI	%M	MI	%M	MI	%M	MI
Wolf Mt.[c] (nursery)	Control	1 yr old	0	0	0	0	6.5	1	12.5	1	44	1	50	1.5	100	2.3	100	2.3
	Metalaxyl[d]	1 yr old	0	0	6.5*[e]	1*	6.5	1	12.5	1	38	1	45	1	100	2.8	100	3.1
	Control	Planted seeds	0	0	0	0	0	0	0	0	0	0	0	0	100	1.2	100	3.4
	Metalaxyl[d]	Planted seeds	0	0	0	0	0	0	5*	1*	26*	1*	28*	1*	100	3.2*	100	3.4
Richland[c] Balsam (natural stand)	Control	1 yr old	4	1	16	1	27	1.6	55	1.08	55	2.6	100	3.1	100	3.5	100	3.5
	Metalaxyl[d]	1 yr old	10	1	22	1	30	1.7	55	2.1	83*	2.8	100	3.2	100	3.5	100	3.6

[a] Age of seedlings at time of transplanting on 28 May 1980.
[b] %M = Percentage of seedlings ectomycorrhizal. MI = ectomycorrhizal index for percentage of shoot root ectomycorrhizal, where 1 = 0–25, 2 = 26–50, 3 = 51–75, and 4 = 76–100.
[c] Average value of four blocks, 15 seedlings per block sampled.
[d] 1.12 kg a.i./ha.
[e] * = Significantly greater than control at $P = 0.01$.

higher ($P = 0.01$) in soil samples from the Wolf Mt. Nursery than elsewhere, but there were no differences found among the three locations for other soil nutrients (P, K, Mn, Zn, and Cu) tested.

DISCUSSION

Our data suggest that metalaxyl promotes growth of seedlings and abundance of ectomycorrhizae in addition to protecting against Phytophthora root rot of Fraser fir (4). There were no detrimental effects of metalaxyl on seedling growth or ectomycorrhizal formation. Marx and Rowan (12) and Pawuk (14) demonstrated that some fungicides applied to nursery soil or pine bark growing medium increase ectomycorrhizal development in loblolly and longleaf pine seedlings. The increase in occurrence of ectomycorrhizae in this study may be a direct result of the action of metalaxyl in reducing the abundance of many oomycetous pathogens (17), thereby increasing the effectiveness of potential ectomycorrhizal inocula. An indirect effect may be the enhanced root growth that increases the probability of a short root encountering a propagule of a potential ectomycorrhizal fungus.

Other workers have reported an increase in plant or root weight (1,6,8) after application of metalaxyl, but these increases were compared to controls in infested soil. Such increases may result from absence of root rot in treated samples rather than a stimulatory effect of metalaxyl. In this study, there were no macroscopic symptoms of Phytophthora root rot or other root diseases of Fraser fir seedlings collected from untreated rows at the Linville River Nursery. The increase in weights of seedlings at this location appeared to be mediated by the application of metalaxyl; however, a direct effect of metalaxyl on fir growth cannot be separated from the indirect effect of metalaxyl in promoting ectomycorrhizae, which could in turn affect the growth of seedlings. Ectomycorrhizae have been shown to increase nutrient availability (11) and stimulate growth by releasing hormones and regulators (15).

At the Wolf Mt. Nursery, there were significant differences in root and shoot dry weights between metalaxyl-treated and untreated seedlings in their first year of growth on the last sampling date but not on other sampling dates or in other age classes of seedlings. Also, there were no significant responses to metalaxyl among seedlings in the natural area. One component of the soil that may account for this overall lack of response to metalaxyl in Jackson County is the organic matter content. Organic matter content was greater than 10% for plots at Richland Balsam, 4–5% at Wolf Mt., and less than 0.6% at the Linville River Nursery. The effectiveness of metalaxyl is reduced as the organic matter content of soils increases (P. Kennedy, *personal communication*). Additional studies of soil factors that reduce effectiveness of metalaxyl are warranted.

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