Effect of Slow Release Fertilizers on Formation of Mycorrhizae and Growth of Container Grown Pine Seedlings[1]

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Abstract

Slow-release fertilizer formulations at various NPK analyses, release rates, and application rates were evaluated for their effects on seedling growth and mycorrhizal development of container-grown seedlings of *Pinus echinata* (Mill.) inoculated with *Pisolithus tinctorius* (Pers.) Coker and Couch. Fertilizer release rate and application rate affected formation of mycorrhizae. At 9.0 kg/m^3 (15.0 lb/yd^3), greater numbers of seedlings formed mycorrhizae and a greater portion of the root system was mycorrhizal with the 8 to 9 month release fertilizer, regardless of formulation, than with the 1 to 2 or 3 to 4 month release fertilizers. Best seedling growth and mycorrhizae formation was obtained with the 21N-3.OP-11.6K (21-7-14) 8 to 9 month release fertilizer at the 4.5 kg/m^3 (7.5 lb/yd^3) application rate. Because fertilizer release is temperature dependent, deleterious effects of over fertilization may be greater with higher greenhouse temperatures. The low benefits of fertilization rates higher than 4.5 kg/m^3 (7.5 lb/yd^3) do not warrant the risk of interference with formation of mycorrhizae.

Index words: fertilizer, slow-release fertilizer, *Pinus echinata*, plant nutrition, ectomycorrhizae, mycorrhizae, *Pisolithus tinctorius*

Introduction

Formation of mycorrhizae on container-grown tree seedlings is affected by the fertilizer regime used in seedling production. High concentrations of fertilizer salts in the container medium suppress formation of mycorrhizae (5,6,8), and may have a toxic effect on vegetative mycelial inoculum in artificial inoculation of tree seedlings with specific mycorrhizal fungi (1,6). Recent studies with polymer-coated, slow-release fertilizers have demonstrated that slow-release fertilizers are superior to water-soluble fertilizers supplied in irrigation water and permit both good seedling growth and formation of mycorrhizae (3,4,5,6).

Earlier trials with slow-release fertilizers indicated that application rates of 2.3 to 4.5 kg/m^3 (3.8 to 7.5 lb/yd^3) container medium of an 18N-2.6P-9.9K (18-6-12) formulation with an 8 to 9 month release rate may provide an optimum fertility level for container-grown southern pine species inoculated with *Pisolithus tinctorius* (Pers.) Coker and Couch (1,6). However, the fertility level maintained in the plant-growth medium is a function of both the application rate and release rate, and it may be necessary to adjust the application rate if a shorter release rate or different formulation is used. The research reported herein examines the effects of different fertilizer formulations, release rates, and application rates on seedling growth and formation of mycorrhizae on container-grown, shortleaf pine seedlings (*Pinus echinata* Mill.) inoculated with the mycorrhizal fungus, *Pisolithus tinctorius*.

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Materials and Methods

Shortleaf pine seeds obtained from the Kentucky state nursery at Gilbertsville, were surface sterilized in 30% H_2O_2, stratified (11), and sown in flats containing a peat:perlite (1:1 by vol) medium. After one month, seedlings were transplanted into 65 ml (2.2 oz) Leach "Pine Cell" tube containers (Ray Leach "Conetainer" Nursery, 1787 North Pine Street, Canby, Oregon) containing the peat-perlite medium, slow-release fertilizer, and 0.109 kg/m^3 (0.182 lb/yd^3) fritted trace elements. The seedlings were produced in racks of 200 at a density of 100 seedlings/ft^2. Seedlings produced with different fertilizer treatments were arranged in a randomized block design, with 30 seedlings per block, and each block replicated four times. To avoid border effects, the seedlings around the perimeter of each rack were not included in the experiments. The seedlings were grown in a greenhouse under an 18 hr photoperiod [one 60 watt incandescent bulb/0.47 m^2 (5.1 ft^2) at 1.2 m (4 ft) height] for four months, after which height and basal stem diameter (caliper) were measured. The seedlings were then removed from their containers and evaluated for the presence of mycorrhizae and the percentage of short roots that had formed mycorrhizae on infected seedlings (5).

The mycorrhizal fungus used to inoculate the seedlings was the M3 isolate of *Pisolithus tinctorius* (2) produced as vegetative mycelium in 2 l (0.5 gal) glass jars according to the procedures of Marx and Bryan (7). After 12 weeks' growth, the inoculum was washed, squeezed in cheesecloth to remove excess water, spread on a laboratory bench, and air-dried for 24 hours. Inoculum was applied to the seedling containers as a single band of inoculum (1), 1 cm (0.4 in) beneath the surface of the container medium at a ratio of 1:5.5 (by vol) inoculum:plant growth medium.

The slow-release fertilizers evaluated were Osmocote (Sierra Chemical Co., Milpitas, California) polymer-coated fertilizer formulations, 21N-2.9P-11.6K (21-7-14), 15N-6.3P-12.4K (15-15-15), and 15N-2.0P-20K (15-5-24). Each formulation was coated to provide release rates of 1 to 2, 3 to

4, and 8 to 9 months. At the end of one experiment, the foliar nutrient contents of N, P, and K were determined using a micro Kjeldahl procedure for nitrogen and phosphorus, and atomic absorption spectrophotometry for potassium. The amount of tissue available for foliar analysis was too small to permit replication and, therefore, statistical analysis.

The release rates of the three 8 to 9 month release fertilizer formulations were determined by placing 5-g samples of the fertilizers in vials containing 15 ml distilled water and incubating at room temperature (approx. 22°C, 72°F). At 5-day intervals, the water was decanted and the salt content measured as conductance (mohms), using a solubridge conductance meter. Decanted solutions were replaced with fresh distilled water. Total fertilizer release over time was calculated cumulatively.

Results and Discussion

Formation of mycorrhizae was significantly affected by all three variables, release rate, rate of fertilizer application, and fertilizer NPK ratio (Table 1). Regardless of formulation, the percentage of seedlings forming mycorrhizae was usually greater when the fertilizers were applied at the 4.5 kg/m^3 (7.5 lb/yd^3) rate compared to the 9.0 kg/m^3 (15.0 lb/yd^3) application rate, and when the fertilizers had extended release rates of 8 to 9 months rather than the shorter 1 to 2 and 3 to 4 month release rates. On seedlings which became mycorrhizal, formation of mycorrhizae was sometimes affected by fertilizer release rate [the 21N-2.9P-11.6K (21-7-14) fertilizer at the higher application rate and the 15N-6.3P-12.4K (15-15-15) fertilizer at both application rates] and application rate (all fertilizers at the 1 to 2 month release rate). Although there was little effect of release rate at the 4.5 kg/m^3 (7.5 lb/yd^3) application rate, the detrimental effect on formation of mycorrhizae by fast releasing 1 to 2 and 3 to 4 month release fertilizers became readily apparent when the seedlings were fertilized at the 9.0 kg/m^3 (15.0 lb/yd^3) rate.

Uniform seedlings of acceptable size for outplanting were produced with all of the fertilizer treatments, except for seedlings grown with the 15N-6.3P-12.4K (15-15-15), 8 to 9 month and 15N-2.0P-20K (15-5-24), 1 to 2 month fertilizers (Table 1). The high nitrogen fertilizer, 21N-2.9P-11.6K (21-7-14), usually produced seedlings with the greatest height. Increasing the application rate of this fertilizer from 4.5 to 9.0 kg/m^3 (7.5 to 15.0 lb/yd^3) did not increase seedling height. Smallest size seedlings were produced with 15N-6.3P-12.4K (15-15-15), 8 to 9 month release fertilizer. Stem diameter was related to height.

The foliar content of N, P, and K in seedlings produced with different fertilizer formulations generally reflected the relative amounts of N, P, and K in the formulations (Table 2). For any formulation at a particular application rate, the highest nutrient content values were usually obtained with 3 to 4 and 8 to 9 month release fertilizers. The data indicated that the 1 to 2 month release fertilizer was largely expended before the end of the 4-month seedling growth period. Although there were substantial differences in foliar nutrient content values, seedling height and foliar N content were usually related up to ca. 2.1% N.

The poor growth and low foliar nutrient content of seedlings fertilized with the 15N-6.3P-12.4K (15-15-15), 8 to 9 month release fertilizer suggested that there may have been a problem with the polymer coating of this fertilizer. Although this fertilizer had a rapid initial release of fertilizer, the total amount of salts released over time was less than that released from the other two formulations (Fig. 1). The initial rapid release of fertilizer from both the 21N-2.9P-11.6K (21-7-14) and 15N-6.3P-12.4K (15-15-15) formulations may have been due to rapid salt release from damaged fertilizer prills and/or a certain portion of irregularly coated prills.

Because the best combination of seedling growth, foliar nutrient content, and development of mycorrhizae was obtained with the 21N-2.9P-11.6K (21-7-14), 8 to 9 month fertilizer, this fertilizer was selected for further evaluation

Table 1. Effect of fertilizer formulation, release rate, and application rate on growth and mycorrhizae formation of 4-month-old container-grown *Pinus echinata* seedlings inoculated with *Pisolithus tinctorius*.

Fertilizer	Application rate kg/m^3 (lb/yd^3)	Release rate (months)	Height (cm)	Stem diam. (mm)	% Plants mycorrhizal	% Root systems mycorrhizal
21N-2.9P-11.6K (21-7-14)	4.5 (7.5)	1–2	14.9 a[z]	2.3 ab	46 bc	52 b
		3–4	14.2 ab	2.2 b	69 ab	59 b
		8–9	14.2 ab	2.1 b	91 a	58 b
	9.0 (15.0)	1–2	15.5 a	2.3 ab	24 c	21 c
		3–4	14.7 ab	2.5 a	36 c	42 bc
		8–9	14.0 b	2.4 a	64 b	59 b
15N-6.3P-12.4K (15-15-15)	4.5 (7.5)	1–2	13.2 b	2.2 b	55 b	55 b
		3–4	13.8 b	2.1 bc	39 c	63 b
		8–9	8.6 e	1.4 d	93 a	80 a
	9.0 (15.0)	1–2	13.6 b	2.2 b	31 c	37 c
		3–4	14.6 ab	2.3 ab	23 d	57 b
		8–9	10.2 d	1.4 d	83 a	84 a
15N-2.0P-20K (15-5-24)	4.5 (7.5)	1–2	10.1 d	1.7 d	77 ab	71 ab
		3–4	13.5 b	2.1 bc	78 ab	68 b
		8–9	13.2 b	2.0 c	90 a	69 b
	9.0 (15.0)	1–2	11.3 c	1.9 c	38 c	46 bc
		3–4	15.0 a	2.3 ab	38 c	52 b
		8–9	14.1 ab	2.2 b	72 ab	77 ab

[z]Mean separation with columns followed by the same letter or letters are not significantly different at the 5% level according to Duncan's multiple range test.

Table 2. **Effect of fertilizer formulation and application rate on foliar nutrient contents of nitrogen, phosphorus, and potassium in 4-month-old container-grown seedling *Pinus echinata* inoculated with *Pisolithus tinctorius*.**

Fertilizer	Application rate kg/m³ (lb/yd³)	Release rate (months)	Nitrogen %	Phosphorus %	Potassium %
21N-2.9P-11.6K (21-7-14)	4.5 (7.5)	1–2	2.10[z]	.26	1.01
		3–4	2.45	.31	1.13
		8–9	2.40	.28	1.11
	9.0 (15.0)	1–2	2.30	.29	1.01
		3–4	2.80	.32	1.09
		8–9	2.70	.32	1.15
15N-6.3P-12.4K (15-15-15)	4.5 (7.5)	1–2	2.05	.28	1.07
		3–4	2.30	.32	1.06
		8–9	1.50	.23	0.94
	9.0 (15.0)	1–2	2.05	.35	1.19
		3–4	2.50	.37	1.09
		8–9	1.95	.27	0.97
15N-2.0P-20K (15-5-24)	4.5 (7.5)	1–2	1.45	.19	0.94
		3–4	1.95	.25	1.14
		8–9	2.20	.27	1.16
	9.0 (15.0)	1–2	1.70	.21	0.97
		3–4	2.20	.27	1.24
		8–9	2.45	.30	1.31

[z]Values are for a subsample of foliage from all seedlings produced in each treatment.

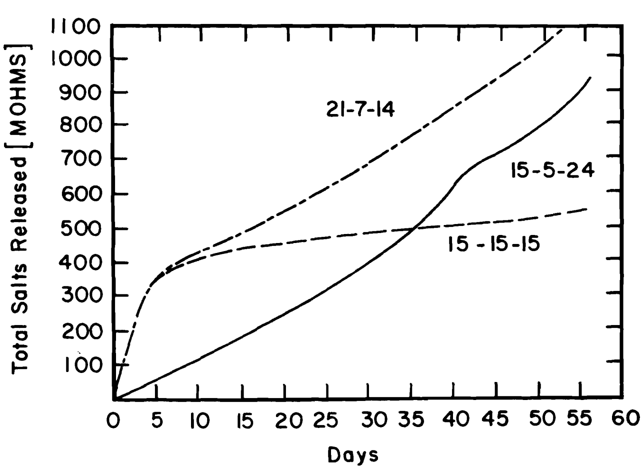

Fig. 1. **Cumulative release of salts from three slow release, 8 to 9 month fertilizers. Measurements were taken at 5-day intervals.**

of application rate. Seedling height and stem diameter increased with increasing fertilizer application from 0–4.5 kg/m³ (0–7.5 lb/yd³) (Fig. 2). No further increase in height was obtained at the 9.0 kg/m³ (15.0 lb/yd³) rate, although there was an increase in stem diameter. Mycorrhizal infection and formation of mycorrhizae were reduced by fertilization at the 9.0 kg/m³ (15.0 lb/yd³) rate. At the 4.5 kg/m³ (7.5 lb/yd³) rate, 90% of the seedlings became infected, with 88% of the short roots on infected plants mycorrhizal. At the 9.0 kg/m³ (15.0 lb/yd³) rate, only 64% of the seedlings became infected, and formation of mycorrhizae was reduced to 59%.

Fertilizer release rates and application rates significantly affected mycorrhizae formation on shortleaf pine seedlings inoculated with *Pisolithus tinctorius*. Rapid fertilizer release or excessive application of fertilizer resulted primarily in a decreased percentage of seedlings forming mycorrhizae. These data are in agreement with earlier studies (1,6), which similarly suggested that high fertilizer salt concentrations are deleterious to efficacy of vegetative mycelial inoculum. The use of 1 to 2 and 3 to 4 month release fertilizers at the 9.0 kg/m³ (15.0 lb/yd³) application rate was especially detrimental to formation of mycorrhizae. With 8 to 9 month release fertilizers, the primary effect of high fertilizer application at the 9.0 kg/m³ (15.0 lb/yd³) rate was a reduction in the percentage of seedlings forming mycorrhizae. After mycorrhizal infection was established, the effect of high fertility on subsequent development of mycorrhizal short roots was less consequential. In the first experiment (Table 1), there was no significant reduction in root formation of mycorrhizae at the higher fertilizer rate. However, in the second experiment (Fig. 2), with 21N-2.9P-11.6K (21-7-14), 8 to 9 month release fertilizer, there was a significant reduction as fertilizer application was increased from 4.5 to 9.0 kg/m³ (7.5 to 15.0 lb/yd³). The difference between these two experiments may be due to higher temperatures in the greenhouse during summer months. Fertilizer release from polymer-coated prills is temperature-dependent, with faster release at higher temperatures. It may be possible to leach excess salts due to accelerated fertilizer release by increased application of irrigation water during watering. The reduction in percent mycorrhizal short roots may be inconsequential (9), but the reduction in percent seedlings becoming mycorrhizal is not. Fertilization should be limited to the 4.5kg/m³ (7.5 lb/yd³) rate, although small sacrifices in seedling size may sometimes occur (Table 1).

In production of container-grown southern pine species within a 3 to 4 month period, the 3 to 4 and 8 to 9 month fertilizers may be preferred to 1 to 2 month release fertilizer. With the exception of 21N-2.9P-11.6K (21-7-14), 1 to 2 month release fertilizer, applied at 9.0 kg/m³ (15.0 lb/yd³), foliar nutrient content values and seedling growth were reduced in comparison to 3 to 4 and 8 to 9 month release fertilizers. Tinus (10) reported average foliar N, P, and K values for narrowleaf evergreens of 2.18, 0.33, and 1.05%, respectively. In this study, reduced seedling growth was

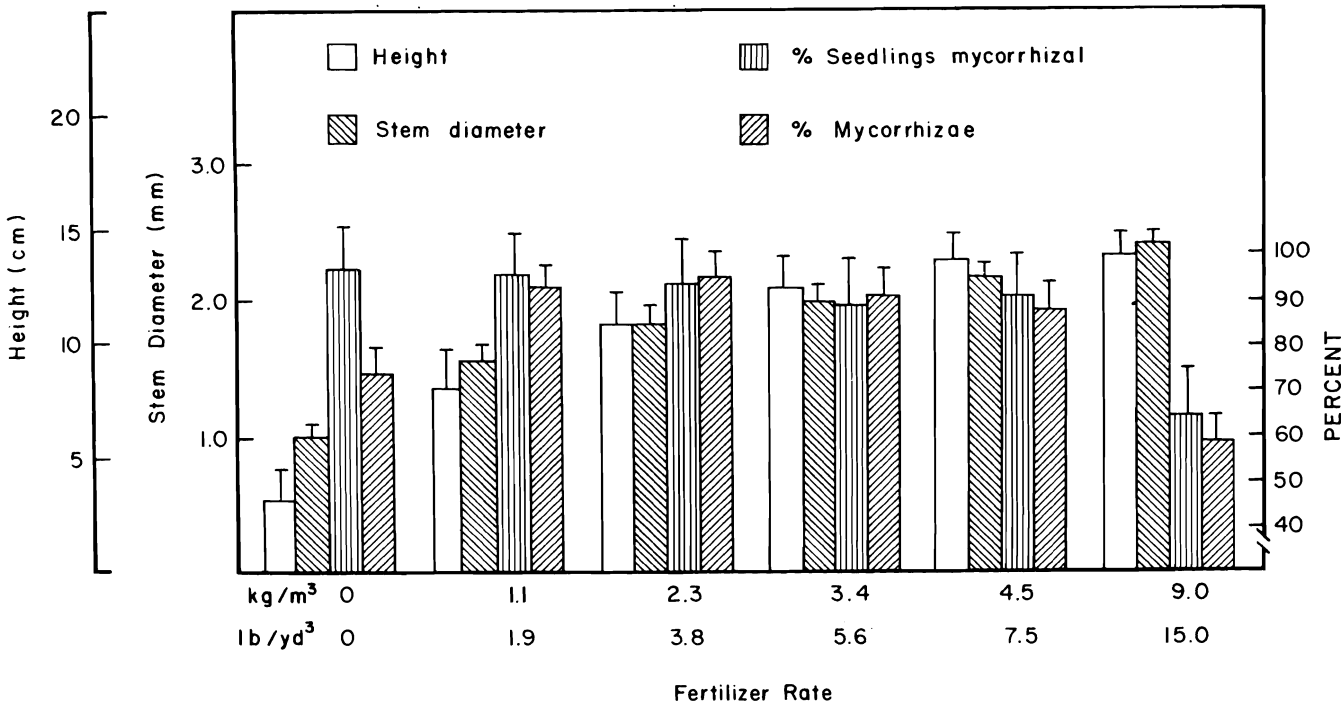

Fig. 2. Effect of rate of fertilization with 21N-2.9P-11.6K (21-7-14), 8 to 9 month release fertilizer on height, stem diameter, percent seedlings with mycorrhizae, and percent of mycorrhizal short roots of seedlings of *Pinus echinata* inoculated with *Pisolithus tinctorius*. Bracket is standard error.

associated with foliar N values below 2.1%. Foliar P content of seedlings produced with all of the formulations examined in this study were slightly below the 0.33% value reported by Tinus (10). This may be a result of species variation or the use of high P fertilization regimes in the studies from which the average values were derived.

The slow-release fertilizer formulations used in this research were prepared for research purposes and may not be representative of those sold commercially. However, the problems experienced with the 8 to 9 month formulation of the 15N-6.3P-12.4K (15-15-15) fertilizer (Fig. 1) suggest that commercial growers producing specifically-infected mycorrhizal seedlings should test fertilizer batches before use.

This research demonstrates the relationship between release rate and application rate when slow release fertilizers are used for production of mycorrhizal tree seedlings. Our research would support the use of extended release formulations. In fall production of container-grown seedlings for spring planting, release of the fertilizer is effectively shut off during seedling dormancy by low temperature storage. In addition to optimizing mycorrhizal infection and providing for good seedling growth, long-term release fertilizer may also aid in establishment of seedlings on the outplanting site by continued supply of fertilizer after outplanting.

Significance to the Nursery Industry

It is important to control fertilizer salts when growing ectomycorrhizal seedlings. Formulations with extended release rates (8 to 9 months) should be used. The optimum rate for 18N-2.6P-9.9K (18-6-12) and 21N-2.9P-11.6K (21-7-14) slow-release fertilizers with 8 to 9 month release rates is 4.5 kg/m^3 (7.5 lb/yd^3). Higher rates reduce the mycorrhizal condition without increasing plant size appreciably; lower rates reduce plant size. Slow-release fertilizers should be handled carefully to avoid rupturing the plastic capsules, and slow-release fertilizers should be tested before use. Damaged fertilizer will release too much salt at the beginning of the production cycle, the time that the mycorrhizal fungus is infecting the seedlings; and excess soluble salt will reduce the percentage of plants which becomes infected. Slow-release fertilizers can be tested by leaching a sample in deionized water and testing for salts over a period of days with a solubridge, an inexpensive apparatus present in most commercial greenhouses.

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Fingerprinting Apple Cultivars by Electrophoretic Isozyme Banding Patterns[1]

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Abstract

Anionic electrophoretic isozyme patterns of peroxidase, esterase and acid phosphatase and cationic peroxidase isozyme patterns from shoot bark protein extracts were used to identify clonal apple scion cultivars. Each of the 21 cultivars included in this study developed a unique combination of isozyme patterns which allowed it to be distinguished from the others. Sports within cultivars exhibited identical patterns of enzymes, with the exception of 'Wijcik', a natural compact mutant of 'McIntosh' which could be distinguished from the latter, although it was indistinguishable from the cultivar 'Spartan'. Isozyme patterns remained constant when samples were taken from wood of different ages, at several times of the year and with trees growing in different locations and on different rootstocks.

Index words: cultivar identification, *Malus domestica*, protein analysis

Introduction

Cultivar identification has recently gained considerable attention because of its financial and legal implications (5). Currently, classification of fruit cultivars is based on morphological characteristics (1, 4, 11, 18). These classifications are based chiefly on flowers and fruit and other easily observed, often subjective, characteristics that may vary widely with the environment. Since morphological classifications are based on descriptions of adult specimens with flowers and fruits, they are largely useless for identifying immature or juvenile individuals.

Chemical markers have proven to be extremely useful for the identification of many economically important crops. Isozyme polymorphism (genetic variance) has been researched in several horticultural plants for the purpose of cultivar identification, i.e. strawberry (2), 'Kentucky' blue grass (16), grapes (17), and roses (6), among others. Apples and other clonally propagated temperate fruits are well suited for isozyme analysis because there should be minimal genetic variability within the individuals of a cultivar (13, 15).

In our previous work (7, 8, 9), we demonstrated that: a) isozyme diversity could be used for positive identification of apple rootstock clones, and b) for the enzymes reported in the present study, banding patterns were not altered by sampling time or environment and were constant within individuals of the same clone.

The objective of this study was to test the applicability of techniques previously developed (for the study of rootstock clones) in our laboratory (7, 8, 9) for the identification of apple cultivars used for fruit production (scion cultivars) and their natural mutations (sports), and to determine the effect of age of sampled wood, rootstock, sampling time, and growing location on banding patterns.

Materials and Methods

One-year-old scion shoots were obtained from commercial nurseries in the Yakima and Wenatchee areas of WA, from the Washington State University horticultural farm at Pullman, and the Washington State University Tree Fruit Research Center at Wenatchee, WA. Cultivars and sports used are as listed in Table 1. All those available in Pullman were sequentially sampled 4 times throughout the year (Feb-

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