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J. Amer. Soc. Hort. Sci. 107(6):1104–1110. 1982.

Slow-release Fertilizers Optimize Mycorrhizal Development in Container-grown Pine Seedlings Inoculated with *Pisolithus tinctorius*[1]

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Additional index words. *Pinus rigida, Pinus Virginia*, pitch pine, Virginia pine, ectomycorrhizae, plant nutrition

Abstract. **Seedlings of pitch pine (*Pinus rigida* mill.) and Virginia pine (*P. virginiana* mill.) were grown with and without inoculum of the ectomycorrhizal fungus *Pisolithus tinctorius* [(Pers.) Coker & Couch] in a sphagnum peatmoss-perlite medium supplemented with various rates of the slow-release fertilizer (18N–2.5P–10K Osmocote or single rates of 14N–6P–11.6K Osmocote and 19N–3P–8.3K Sierrablen plus ON–19.8P–OK superphosphate) or a soluble 20N–8.6P–16.4K fertilizer treatment. Mycorrhizal development was evaluated after 5 months of growth and then after a 3-month cold storage period. Seedlings heavily mycorrhizal with *P. tinctorius* and of acceptable planting size were produced with 2.3 to 4.5 kg 18N–2.5P–10K Osmocote/m^3 medium. Higher fertilizer rates reduced or eliminated mycorrhizal development and reduced plant growth. Seedlings grown with soluble fertilizer were comparable in size to those produced with slow-release fertilizers, but mycorrhizal development was eliminated. The 3 slow-release fertilizer formulations produced seedlings of comparable size and mycorrhizal development. Superphosphate with or without slow-release or soluble fertilizer did not influence seedling growth or mycorrhizal development. Mycorrhizae continued to develop while plants were in cold storage. The ITW One-Way tube produced seedlings equal in size to those produced in the Leach Pine Cell, but mycorrhizal development appeared to be more sensitive to high fertilizer rates with the ITW tube. Mycorrhizal development did not affect seedling size.**

During the past decade, mycorrhizal fungi have been found to be important in the efficient production of a number of woody plants, and in most instances, some mycorrhizal fungal isolates have been found superior to others. Seedlings infected with mycorrhizal fungi which were selected for ecological adaptation to adverse conditions have been found to survive and grow better on adverse sites than nonmycorrhizal plants or plants mycorrhizal with fungi naturally present in nursery soil. The exploitation of mycorrhizal fungi to improve plant growth has just begun (8).

Common nursery production practices are not suitable for the production of seedlings infected with specific mycorrhizal fungi (8, 15). One cultural factor which requires modification for production of mycorrhizal seedlings is fertilization. Fertility influences both development of mycorrhizae and the effects fungi have on their hosts. The conventional method of fertilizing container- and field-grown nursery seedlings with large amounts of soluble fertilizer inhibits mycorrhizal development (3, 11, 13). Consequently, current production methods of producing mycorrhizal seedlings often involves a reduction in soil fertility which also may be at the sacrifice of target sizes for plantable nursery stock (12).

Slow-release fertilizers incorporated into the medium before seeding may permit both adequate plant growth and mycorrhizal

[1]Received for publication Jan. 6, 1982. The assistance of Janet Finley, Margaret Gregory, Mark Leopold, and Robert Perrone is gratefully acknowledged. Supported by Grants G5105004, G5115003, and G5115212, U.S. Department of Interior, Office of Surface Mining, and by grants from the Horticultural Research Institute and Sierra Chemical Company.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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development in the production of containerized mycorrhizal tree seedlings (6, 7, 9). Slow-release fertilizers at or below the manufacturer's recommended rate were used successfully to produce containerized mycorrhizal conifer and deciduous seedlings at either an adequate or accelerated growth rate, depending on the plant and fungal species (7, 9), but optimal rates or formulations of slow-release fertilizers required for the production of mycorrhizal seedlings of acceptable size were not determined. Incorporation of slow-release or other granular fertilizers into the container media is not a widely accepted practice among growers producing container-grown seedlings. The conventional method of applying water-soluble fertilizers of various formulations when needed is often preferred (2). The major disadvantages to fertilizer incorporation into the container medium before seeding are the inability to control seedling growth and hardening off of seedlings before the onset of low temperatures, the inability to match fertilizer release rates with needs of seedlings grown over an extended period of time, and the alteration of media pH (14).

The primary objective of this study was to determine the effect of selected fertilizer types, formulations, and rates on growth and mycorrhizal development of pitch and Virginia pine seedlings in 2 types of containers.

Materials and Methods

General procedures. Seed of pitch and Virginia pine were obtained from commercial seed collectors, screened for viability, and stratified (16). Isolate M3 (6) of *Pisolithus tinctorius* was reisolated from a sporocarp produced on a mycorrhizal plant within 1 year before use in these experiments. Vegetative mycorrhizal inoculum was produced in 3.8-liter jars by the procedures of Marx and Bryan (10). After 3 months, the inoculum was leached with tap water, excess water was removed by squeezing through cheesecloth, and the inoculum was stored at 3°C for 24 hrs before use.

The plant growth medium, 1 sphagnum peatmoss: 1 perlite (by volume) containing 0.11 kg/m^3 of fritted trace elements, was steam-pasteurized. Mycorrhizal inoculum was incorporated uniformly at the rate of 1 inoculum: 8 medium. Vermiculite was substituted for the inoculum in noninoculted control treatments.

Fertilizers evaluated were as follows: 18N–2.6P–10K Osmocote (18–6–12 Osmocote, 8–9 month release rate), 14N–6P–11.6K Osmocote (14–14–14 Osmocote, 3–4 month release rate) or 19N–3P–8.3K Sierrablen plus iron, (19–7–10 Sierrablen plus Iron, 3–4 month release rate). These slow-release fertilizers are manufactured by Sierra Chemical Company, Milpitas, Calif. The water-soluble fertilizer used was a 20N–8.6P–16.6K formulation (Peters 20–20–20, manufactured by W. R. Grace & Company, Allentown, Pa.). Superphosphate (ON–19.8P–OK) was also evaluated. The fertilizers were incorporated into the medium except for soluble fertilizer which was applied at each watering at the rate of 200 ppm N.

Containers were 65-ml Leach 'Pine cells' (Ray Leach Cone-Tainer Nursery, 1787 N. Pine St., Canby, Ore.) and 65-ml ITW 'One Way' containers (Illinois Tool Works, Inc., Itasca, Ill.).

To minimize the risk of uninoculated control seedlings becoming mycorrhizal, seedlings of each treatment were grouped as 5 rows of 10 tubes in a single block within container racks. Each rack contained 200 tubes. To minimize environmental variables, racks were removed randomly on the bench weekly.

Equal numbers of seed were sown in each container. After emergence, seedlings were thinned to 1 per container and grown in a greenhouse for 5 months under an extended 18-hr photoperiod (one 60 W incandescent bulb/0.47m^2) and prevailing greenhouse temperatures of 20° to 29°C (day) and 18° to 20° (night). When needed, plants were watered with sufficient tap water (or soluble fertilizer solution for that treatment) for copious drainage from the tubes as a measure to prevent salts accumulation.

After 5 months, 5 seedlings were selected randomly from each treatment, removed from their containers, and evaluated for mycorrhizal development by the procedures of Maronek et al. (9). The ''percentage of plants mycorrhizal'' is the proportion of plants having mycorrhizae, with individual plants being rated either positive or negative; whereas, ''percentage of root systems mycorrhizal'' is a mean of scores for individual plants rated positive above, with each score being a visual estimate of the proportion of short roots which are mycorrhizal. Root systems then were gently washed and oven-dry weights were obtained for shoot and root. The remaining seedlings from all experiments were subjected to low temperature (3 to 5°C) storage and an 8-hr photoperiod (one 60 W incandescent bulb/0.40m^2) for 3 months. During storage, seedlings were watered as needed with tap water.

Statistical analyses. Since little growth occurred with the nonfertilized treatments in experiment I, they were not included in the analysis of growth data to prevent bias in the variance used for comparisons among the other treatments. The data were unbalanced because of unequal numbers of observations on various treatments. Consequently, data were analyzed by least squares using the Statistical Analysis System (SAS) General Linear Models (GLM) procedure (5). Since preliminary analyses of data over the 2 species in the ITW 'One Way' containers and over the 2 containers using the pitch pine species showed numerous cases of interaction with species and with container type, final interpretation was based on separate analyses of data from each container type and species combination. Least squares estimates (i.e, ''least squares means'') of individual treatment means, fertilizer level means, and fungus level means were obtained, and *t*-tests of significance of differences were used to compare means in cases where F-tests indicated such comparisons were appropriate. Analyses performed also included sets of contrasts among fertilizer levels and their interactions with levels of inoculum (with and without) in the analyses. Fertilizer-level contrasts also were made within levels of inoculum to aid interpretation in cases where a significant interaction of fertilizer contrast by inoculum was found. Contrasts used were linear, quadratic, cubic and quartic effects of the 18N–2.6P–10K Osmocote fertilizer levels expressed on a logarithmic scale, and also among 19N–3P–8.3K Sierrablen, 14N–6P–11.6K Osmocote, and 18N–2.6P–10K at the 4.5 kg/m^3 level.

Growth data from experiment II were analyzed in 2 parts. One part consisted of data from the nonfertilized control and phosphate-only treatments because there exhibited little growth compared to the other treatments. Sums of squares owing to phosphate, inoculum, and phosphate × inoculum interaction were partitioned in this analysis of variance. The second part consisted of data from a 2 × 2 ×2 factorial arrangement of inoculum levels (inoculated and noninoculted), super phosphate levels (none and 2.4 kg/m^3 superphospate) and fertilizer types (18N–6P–10K Osmocote and 20N–8.6P–16.6K water-soluble). In this analysis, sums of squares owing to all main effects and 2- and 3-factor interactions were partitioned. Since interactions with fungus levels were found in a number of cases, the sums of squares owing to superphosphate, fertilizer, and superphosphate by fertilizer within each inoculum level were also computed and tested for significance. One-, 2- and 3-factor least squares means were also obtained in these analyses.

Because 8-month percentage mycorrhizal data was not separated by experiment, all percentage mycorrhizal data from the 2 experiments were analyzed in 1 analysis. In experiments I and II where fertilizer treatments were identical (4.5 kg/m^3 of 18N–2.6P–10K Osmocote and nonfertilized treatments with each species container combination), the mean 5-month mycorrhizal percentages in most instances were within a few percentage points of each other. Fertilizer treatments which consistently showed no infection were assumed to have a parametric mean of zero and were deleted from the analysis in order to prevent downward bias in the estimated variance used for making other treatment comparisons. For "percentage of root systems mycorrhizal," least squares means were obtained and pairwise *t*-tests of treatment differences were made if F-tests indicated such comparisons were appropriate. Since the data for "percentage of plants mycorrhizal" were binomial data, an analysis was done by the SAS procedure FUNCAT (5). This procedure is a linear models procedure for the analysis of linear, logarithmic, or exponential functions (or combinations of these) of categorical data. We used a linear response function, which caused the procedure to do a weighted analysis of variance of the observed proportions of mycorrhizal plants. Sums of squares obtained by this procedure are directly interpretable as chi-square statistics for testing significance of their respective sources of variation. Chi-square statistics were also obtained for specific pairwise comparisons of treatments within a date (5 months or 8 months) or between the 2 dates within a treatment. Since FUNCAT cannot work with zero frequencies, a small constant (0.01) was added to all cell frequencies before analysis (while most cases of zero frequencies of infection were not included in the analysis, there were cases of zero frequency of noninfected plants). Owing to small cell frequencies for the 5-month data, tests of comparisons of 2 treatments at 5 months should be considered as rather roughly approximate. However, tests within the 8-month data or between dates for a particular treatment can be considered quite reliable. Specific treatment comparisons, other than pairwise comparisons which were appropriate and interesting, were not made in these analyses.

Results

In general, the optimum rate of 18N–2.5P–10K Osmocote slow-release fertilizer for growth of both pitch and Virginia pine seedlings in both containers was 4.5 kg/m^3, the manufacturer's recommended rate (Table 1). Stem heights and diameters at this rate generally exceeded 13 cm and 1.7 mm, respectively. Higher rates of fertilizers often were deleterious to 1 or more growth response variables. In some cases, seedlings grown with 2.3 kg fertilizer/m^3 were nearly as large as those grown with 4.5 kg/m^3. A few cases of significant cubic or quartic effects were found. Such results indicate that the results do not statistically fit a parabolic response curve. Most of these cases apparently were the consequence of the marked superiority of the 4.5 kg fertilizer/m^3 rate (sufficiently so that a satisfactorily fitting curve must have a point of inflection on each side of a prominent peak in the neighborhood of 4.5 kg/m^3). In 2 cases, most notably stem diameter of uninoculated pitch pine grown in the Leach pine cell, an increase in growth from rate 9.0 to rate 18.0 kg/m^3 contributed to significant high order effects. Growth of seedlings with 2 other slow-release fertilizer formulations, 14N–6P–11.6K Osmocote and 19N–3P–8.3K Sierrablen at 4.5 kg/m^3, was similar to that with the same rate of 18N–2.5P–10K Osmocote. Inoculation with *Pisolithus tinctorius* had no consistent effect on growth, except in the complete absence of fertilizer, in which case stunting of stem height usually occurred.

Development of mycorrhizae on inoculated seedlings was excellent over the range of 0 to 2.3 kg 18N–2.5P–10K Osmocote/m^3 medium with both pine species grown in either container. Mycorrhizae developed quite well also with 4.5 kg/m^3, although

Table 1. The effect of various slow-release fertilizer rates and formulations on *Pisolithus tinctorius* ectomycorrhizal formation and growth of pitch and Virginia pine seedlings grown in Leach 'Pine Cell' or Illinois Tool Works (ITW) 'One-Way' containers after 5 months. The 8-month mycorrhizal rating was completed after 3 months of low-temperature storage at 5°C ± 2°C.[z]

Fertilizer treatment and (kg/,m^3) rate	Height (cm)		Stem diam (mm)		Shoot dry wt (g)		Plants mycorrhizal (%)		Root systems mycorrhizal (%)	
	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	5 mo.	8 mo.	5 mo.	8 mo.
Pitch pine, Leach 'Pine Cell' container										
18N–2.5P–10K Osmocote										
I) None	3.5 a	3.1 a*	0.60 a	0.46 a	0.10 a	0.07 a	100 b	80 c**	68 bc	80 c
II) 1.1	11.6 b	11.1 b	1.54 c	0.84 b*	0.51 bc	0.15 b	100 b	92 cd*	62 bc	91 de*
III) 2.3	12.0 b	12.3 bc	2.10 cd	1.98 cd	0.68 cd	0.47 bcd	100 b	100 e	92 c	100 de
IV) 4.5	14.2 cd	14.9 d	1.74 cd	2.32 d	0.50 bc	0.82 d	80 b	97 de	52 b	95 de**
V) 9.0	13.4 c	13.3 cd	0.90 b	2.08 cd**	0.26 b	0.72 cd*	80 b	52 b	48 b	42 b
VI) 18.0	12.9 bc	12.8 c	2.00 cd	NA	0.80 cd	NA	0 a	3 a	0 a	3 a
14N–6P–11.6K Osmocote										
VII) 4.5	15.4d	14.4d	2.40 d	1.46 bc**	1.02 d	0.39 bc**	80 b	100 e	44 b	96de**
19N–3P–8.3 K Sierrablen										
VIII) 4.5	15.3d	13.9 cd*	2.04 cd	1.64 c	0.66 bcd	0.46 bcd	100 b	87 cd*	50 b	83 cd*
Treatment comparisons[y]										
II–VI Linear	**	**	NS(*)[y]	(**)	NS(NS)	(**)				
II–VI Quadratic	**	**	NS(**)	(**)	NS(NS)	(NS)				
II–VI Cubic	NS	NS	**(NS)	(NS)	*(NS)	(NS)				
II–VI Quartic	*	**	NS	NA	NS	NA				
Among 4.5 rates	NS	NS	NS	*	*	NS				

Continued on next page

Table 1. Continued

Fertilizer treatment and (kg/,m³) rate	Height (cm)		Stem diam (mm)		Shoot dry wt (g)		Plants mycorrhizal (%)		Root systems mycorrhizal (%)	
	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	5 mo.	8 mo.	5 mo.	8 mo.
Pitch pine, ITW 'One-Way' container										
18N–2.5P–10 K Osmocote										
I) 0.0	2.6 a	2.1 a	1.00 a	0.68 a	0.07 a	0.07 a	100 c	79 bc**	60 c	77 bcd
II) 1.1	10.9 b	11.6 b	1.58 b	1.80 bc	0.36 b	0.50 bc	80 c	78 bc	74 c	66 bc
III) 2.3	13.4 c	13.2 c	1.62 bc	1.90 bc	0.65 bc	0.64 bc	100 c	90 cd	64 c	85 d
IV) 4.5	14.3 cd	14.6 d	1.96 bcd	1.96 c	0.94 c	0.80 c	20 b	64 b*	10 b	60 b**
V) 9.0	13.6 cd	12.0 bc*	1.68 bc	1.82 bc	0.62 bc	0.70 bc	0 a	0 a	0 a	0 a
VI) 18.0	10.6 b	13.2 cd**	2.14 cd	2.24 c	0.70 bc	1.00 c	0 a	0 a	0 a	0 a
14N–6P–11.6K Osmocote										
VII) 4.5	14.8 d	14.0 cd	2.44 d	1.32 b**	0.88 c	0.31 b**	80 b	100 d	52 bc	89 d*
19N–3P–8.3K Sierrablen										
VIII) 4.5	14.0 cd	13.9 cd	1.98 bcd	1.76 bc	0.87 c	0.49 bc	100 b	95 d	88 c	92 d
Treatment comparisons[y]										
II–VI Linear	NS	NS	*	NS	NS	*				
II–VI Quadratic	**	*	NS	NS	NS	NS				
II–VI Cubic	NS	*	NS	NS	NS	NS				
II–VI Quartic	NS	**	NS	NS	NS	NS				
Among 4.5 rates	NS	NS	NS	NS	NS	NS				
Virginia pine, ITW 'One-Way'										
18N–2.5P–10K Osmocote										
I) 0.0	3.2 a	2.2 a**	NA	1.00 a	NA	0.08 a	100 c	77 b**	32 b	72 b**
II) 1.1	12.4 c	10.6 b**	1.44	1.88 bc	0.45 a	0.38 b	100 c	74 b**	72 b	70 b
III) 2.3	11.7 c	13.1 c*	1.40 a	2.00 c	0.36 a	0.64 b	80 bc	76 b	44 b	67 b
IV) 4.5	14.0 d	13.4 c	2.02 a	1.32 b*	0.72 a	0.36 b	40 ab	87 bc*	30 b	74 bc*
V) 9.0	12.0 c	11.0 b	1.62 a	1.80 bc	0.54 a	0.59 b	0 a	0 a	0 a	0 a
VI) 18.0	9.2 b	10.9 b*	1.65 a	1.70 bc	0.48 a	0.56 b	0 a	0 a	0 a	0 a
14N–6P–11.6K Osmocote										
VII) 4.5	14.6d	13.5c	1.76 a	1.80 bc	0.72 a	0.61 b	80 bc	77 b	44 b	70 b
19N–3P–8.3K Sierrablen										
VIII) 4.5	12.2 c	12.9 c	1.54 a	1.76 bc	0.40 a	0.61 b	80 bc	95 c	48 b	95 c*
Treatment comparison										
II–VI Linear	**	**	NS	NS	NS	NS				
II–VI Quadratic	**	**	NS	NS	NS	NS				
II–VI Cubic	**	NS	NS	NS	NS	NS				
II–VI Quartic	**	NS	NS	NS	NS	NS				
Among 4.5 rates	**	NS	NS	NS	NS	NS				

[z]Means separation within a column for each species and container are by chi-square tests for percentage of plants mycorrhizal and pairwise *t*-tests for other variables 5% level. Height, stem diameter, and 8-month mycorrhizal data are means of 30 tests while all other data are means of 5 trees. Osmocote 18N–2.5P–10K has a 8–9 month release rate while all others are 3–4 month release rates. Significant differences between inoculated and noninoculated seedlings or between sampling times were determined by chi-square or pairwise *t* tests at 5% (*) or 10% (**) level.

[y]Tests of comparisons among treatments (by F-test in the analysis of variance). Because of missing data in treatment VI, data included in parentheses indicates test of comparisons using treatment II–V only.

[NS,*,**]Nonsignificant (NS) or significant at 5% (*) or 10% (**) level.

[NA]Not available.

with pitch pine in the ITW tube, mycorrhizal development was reduced. Mycorrhizae failed to develop on either pitch or Virginia pine seedlings grown in the ITW tube with 9 or 18 kg fertilizer/m³, whereas, in the Leach tube, mycorrhizae developed on about half the plants with 9 kg fertilizer/m³. At the fertilizer manufacturer's recommended rate of 4.5 kg/m³, the other 2 formulations evaluated, 14N–6P–11.6K Osmocote and 19N–3P–8.3K Sierrablen, were equal to or superior to 18N–2.5P–10K Osmocote.

In experiment II, seedlings produced by slow-release and soluble fertilizers were comparable in growth variables (Table 2). In contrast, mycorrhizal development was excellent with slow-

Table 2. The effect of slow-release and water-soluble fertilizers applied alone or in combination with superphospate on *Pisolithus tinctorius* ectomycorrhizal formation and growth of pitch and/or Virginia Pine seedlings grown in Leach 'Pine cells' or Illinois Tool Works (ITW) 'One-Way' containers after 5 months. The 8-month mycorrhizal rating was completed after 3 months of low-temperature storage at 5°C ± 2°C.[z]

Treatment number and fertilizer[y]	Height (cm)		Stem diam (mm)		Shoot dry wt (g)		Plants mycorrhizal (%)		Root systems mycorrhizal (%)	
	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	5 mo.	8 mo.	5 mo.	8 mo.
Pitch pine Leach 'Pine Cell' container										
I None	3.5 a	3.1 a*	0.60 b	0.46 a	0.10 a	0.07 a	100 b	80 b	68 b	80 b
II ON–19.8P–OK Superphosphate	4.1 b	3.3 a**	0.50 a	1.16 b**	0.14 a	0.15 b	NA	NA	NA	NA
III 18N–2.6P–10K Osmocote	18.4 d	16.0 b**	2.36 cd	2.20 c	1.04 c	0.96 c	80 b	97 c	52 b	95 c**
IV Osmocote & Superphosphate	16.7 c	15.9 b	2.77 d	2.26 c	1.26 c	0.94 c	100 b	96 c	56 b	91 bc
V 20N–8.6P–16.4 Soluble	15.9 c	18.5 c**	1.93 c	2.45 c	0.64 b	0.95 c	0 a	0 a	0 a	0 a
VI Soluble & Superphosphate	16.9 cd	17.6 c	2.00 c	2.36 c	0.62 b	0.92 c	0 a	0 a	0 a	0 a
Treatment comparisons[x]										
I vs. II	**	NS	*	**	NS	*				
III, V vs. IV, VI (Phosphorous, P)	NS	NS	NS	NS	NS	NS				
III, IV vs. V, VI (Fertilizer, F)	*	**	**	NS	**	NS				
III, VI vs. IV, V (P × F)	*	NS	NS	NS	NS	NS				
Pitch Pine 'ITW Tube' container										
I None	2.6 a	2.1 a*	1.00 b	0.68 a*	0.07 a	0.07 a	100 c	79 b**	60 c	77 b
II) ON–19.8P–OK Superphosphate	4.4 b	2.9 b**	0.42 a	0.60 a	0.07 a	0.07 a	NA	NA	NA	NS
III) 18N–2.6P–10K Osmocote	17.6 d	18.0 c	2.22 cd	2.10 b	0.86 b	0.94 b	20 b	64 b*	10 b	60 b*
IV) Osmocote & Superphosphate	16.0 c	18.5 c**	2.50 d	2.72 c	0.70 b	0.97 b	80 c	73 b	41 bc	61 b
V) 20N–8.6P–16.4K Soluble	17.4 d	18.5 c	2.14 cd	2.18 bc	0.70 b	0.70 b	0 a	3 a	0 a	0 a
VI) Soluble & Superphosphate	18.7 d	18.0 c	1.86 c	1.94 b	0.64 b	0.76 b	0 a	0 a	0 a	0 a
Treatment comparisons[x]										
I vs. II	**	**	**	NS	NS					
III, V, vs. IV, VI (Phosphorous, P)	NS	NS	NS	NS	NS					
III, IV vs. V, VI (Fertilizer, F)	*	NS	NS	NS	NS					
III, VI vs. IV, V (P × F)	**	NS	NS	*	NS					
Virginia pine 'ITW Tube' container										
I) None	3.2 a	2.2 a**	NA	1.00 a	NA	0.08 a	100 c	77 c**	32 bc	72 c**
II) ON–19.8P–OK Superphosphate	3.4 a	2.9 b**	NA	1.00 a	NA	0.13 b	50 b	100 d**	24 b	91 c**
III) 18N–2.6P–10K Osmocote	17.1 b	15.9 c	2.46 a	2.00 b	0.98 a	0.70 c	40 b	87 c*	30 bc	74 c*
IV) Osmocote & Superphosphate	16.3 b	17.0 c	1.90 a	2.40 b	0.76 a	0.76 c	90 c	41 b**	61 bc	38 b
V) 20N–8.6P–16.4 Soluble	15.8 b	15.6 c	2.04 a	1.72 b	0.72 a	0.61 c	0 a	0 a	0 a	0 a
VI) Soluble & Superphosphate	16.7 b	16.3 c	1.92 a	1.92 b	0.64 a	0.76 c	0 a	0 a	0 a	0 a

Continued on next page

Table 2. Continued

Treatment number and fertilizer[y]	Heights (cm)		Stem diam (mm)		Shoot dry wt (g)		Plants mycorrhizal (%)		Root systems mycorrhizal (%)	
	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	5 mo.	8 mo.	5 mo.	8 mo.
Treatment comparisons[x]										
I vs. II	NS	**	NA	NS	NA	*				
III, V vs. IV, VI (Phosphorous, P)	NS	NS	NS	NS	NS	NS				
III, IV, vs. V, VI (Fertilizer, F)	NS	NS	NS	NS	NS	NS				
III, VI vs. IV, V (P × F)	NS	NS	NS	NS	NS	NS				

[z]Mean separation within a column for each species and container combination are by chi-square tests for percentage of plants mycorrhizal and pairwise *t*-tests for other variables, 5% level. Height, stem diameter, and 8-month mycorrhizal data are means of 30 trees. All other data are means of 5 or 10 trees.

[y]Fertilizer rates were: superphosphate, 2.4 kg/m^3; 18N–2.6P–10K Osmocote, 4.5 kg/m^3; and 20N–8.6P–16.4K water-soluble applied daily at 1 gm/liter. Significant differences between inoculated and noninoculated seedlings or between sampling dates were determined by chi-square or *t*-tests; *, 5% and **, 1% level.

[x]Tests of comparisons, among treatments (by F test in the analysis of variance).

[NS,*, **]Nonsignificant (NS), or significant at 5% (*) or 10% (**) level.

[NA]Not available.

release fertilizer but was eliminated by soluble fertilizer. Superphosphate had little effect on either growth or development of mycorrhizae except in the absence of other fertilizer; in this case, a small growth stimulation usually was recorded.

Noninoculated treatments were completely nonmycorrhizal. Contamination with either *Pisolithus tinctorius* or air-disseminated spores of other mycorrhizal fungi was not observed.

Mycorrhizae continued to develop during the 3 months in cold storage (Tables 1 and 2). In some instances, there appeared to be reductions in the percentage of plants which were mycorrhizal, some of them significant statistically. Because only 5 plants were rated at 5 months, the 8-month data consisting of 30 observations probably are more reliable, and we doubt that these apparent reductions are real. We have more confidence that differences between 5- and 8-month means of percentages of root systems which were mycorrhizal are real because these are based on a quantitative rather than a qualitative rating. In many treatments, the percentages of roots systems which were mycorrhizal increased during cold storage. The effect was pronounced particularly at rates of slow-release fertilizer, which were beginning to inhibit development of mycorrhizae prior to storage (Table 1).

Discussion

This study demonstrates that Southern pine seedlings specifically infected with a mycorrhizal fungus and of acceptable size for planting may be produced with a slow-release fertilizer. While generally accepted minimum stem height and diameter standards have not been established for container-produced seedlings as they have for bareroot seedlings (1), minimum specifications of 12.8-cm height and 1.55-mm root collar diameter have been used for container-produced loblolly seedlings (4). Extensively mycorrhizal seedlings which exceed these limits usually were produced with fertilizer rates of 2.3 or 4.5 kg 18N–2.5P–10K Osmocote/m^3 medium. Inoculated seedlings of a comparable size produced with soluble fertilizer were nonmycorrhizal. Controlling the amount of soluble fertilizer to which the root system is exposed at any one time is apparently critical in obtaining good infection and production of mycorrhizae.

It is apparent, however, that excessive rates of slow-release fertilizer are deleterious to both seedling growth and and mycorrhizal development. Heavily infected seedlings may be produced with little or no fertilizer, but fertilizer is, of course, necessary to produce plants suitable for planting. The rate of 18N–2.5P–10K Osmocote for optimum growth and mycorrhizal development appears to be 2.3 or between 2.3 and 4.5 kg/m^3 medium.

The continued development of mycorrhizae during cold storage on seedlings grown at high rates of slow-release fertilizer suggests that these rates were inhibiting mycorrhizal development but not infection. In contrast, the soluble fertilizer apparently inhibited infection.

While it is evident that high rates of even slow-release fertilizer will inhibit mycorrhizal development, it is not clear which component, if only 1, of the fertilizer is inhibitory. Phosphorus is often implicated in deleterious effects of mycorrhizal development, but the lack of effect of superphosphate in these experiments suggests that a component other than phosphate is responsible for the inhibition in this system.

The ITW One-Way tube was developed to meet a need for an economical disposable container permitting seedling growth comparable to reusable containers, such as the Leach pine tube, a standard in the container seedling industry. Seedlings produced in the Leach Pine tubes and ITW One-Way tubes were comparable in size. Mycorrhizal development appeared more sensitive to high fertilization rates in the ITW tube than in the Leach tube, but we feel that with slight reductions in fertilization rates, heavily mycorrhizal pine seedlings of acceptable size may be produced in the ITW tube.

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J. Amer. Soc. Hort. Sci. 107(6):1110–1113. 1982.

Influence of Cultivar, Extraction and Storage Temperature, and Time on Quality of Muscadine Grape Juice[1]

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Additional index words. *Vitus rotundifolia*, color

Abstract. **A processing study was conducted on 2 cultivars, 3 extraction temperatures, 3 storage temperatures and 3 storage times to assess differences in quality of juice of muscadine grape (*Vitis rotundifolia* Michx.) The 2 cultivars responded differently to all other variables in color changes. 'Carlos', a bronze-skinned grape was higher in acidity and lower in pH and total phenols than 'Noble', a black-skinned cultivar. Higher extraction temperatures leached out more acids, total phenols, and color. Color and overall quality changes were rapid at 32°C storage, making the juice unacceptable at 7 months storage. Greater changes in quality occurred when juice was extracted at 60° than at 24° or 80°.**

Muscadine grape products have not attained the prominence at the marketplace as such products as juice and jelly of 'Concord' grapes. There have been several active breeding and development programs in the Southern states during the past 40 years from which a number of both black-skinned and bronze-skinned cultivars have been released. The commercial area of muscadine grapes in Arkansas is quite small, being less than 40 ha. However, more interest has been shown by growers in the past 5 years in expanding the acreage of production in Southern and Eastern Arkansas. New uses must be explored for muscadine grape products before production can be increased.

A number of studies have been conducted on processing characteristics of muscadine grape juice (2, 3, 4, 5, 11). Flora (4) demonstrated differences in cultivar suitability for juice manufacture and that color of juices made from black-skinned cultivars deteriorated rapidly when stored at room temperature. Hot-pressing of muscadine grapes increased the color extracted, but flavor scores were not as acceptable when compared to cold-pressed juice.

Earlier studies on 'Concord' have shown that more color was extracted when grapes were heated to 80° to 90°C and cooled to 60° for depectinization than in grapes heated only to 60° (8). Polyphenoloxidase (PPO) degraded 50% of grape color during depectinization at 60° during 1 hour unless the enzymes were inactivated initially (1, 7).

The production of a high-quality muscadine juice for markets in the Southern United States that are familiar with the product would greatly increase the demand for production of grapes. The

[1]Received for publication April 19, 1982. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

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