BED DENSITY AND <u>PISOLITHUS</u> ECTOMYCORRHIZAE AFFECT MORPHOLOGY OF LOBLOLLY PINE SEEDLINGS

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Abstract.--Loblolly pine seedlings were grown at 25, 30, 35 and 40 seedlings/ft ^{2} with either Pisolithus tinctorius (Pt) or naturally occurring (NI) ectomycorrhizae at the Taylor State Nursery, Trenton, SC, in 1987. Uniform spacing of seedlings was maintained in each bed density. Average root collar diameter (RCD) and weight decreased as bed density increased, but the averages were within acceptable size limits at all bed densities. Fifty-four percent of the NI seedlings grown at 40/ft ^{2} had <3.6 mm (9/64 in.) RCD while only 23 percent were this small at 25 seedlings/ft2. Pt ectomycorrhizae decreased the percentage of seedlings with <3.6 mm RCD at all densities. Less than half of the NI seedlings had >30 percent ectomycorrhizal development and more than half of the Pt seedlings had >45 percent development at all bed densities.

Using an average RCD of >3.5 mm for the desired seedling population, about 38 seedlings/ft ^{2} with natural ectomycorrhizae and 42 seedlings/ft ^{2} with Pt ectomycorrhizae were produced. However, when culls (<3.0 mm RCD) were removed, 30 and 32 plantable seedlings/ft2, respectively, were produced. At 30 seedlings/ft ^{2}, average RCD was 3.7 and 3.9 mm for NI and Pt seedlings with cull percentages of 10 and 7.5, respectively. Larger seedlings with RCD >4.0 mm of both mycorrhizal treatments were produced at the 25 seedlings/ft ^{2} density with an average of <3 percent culls.

Additional keywords: Seedling quality, carrying capacity, Geotech.

Seedbed density strongly affects morphology of southern pine seedlings and certain morphological traits are known to affect field performance of seedlings (Caulfield and others 1987). Density controls competition between seedlings for above- and below-ground growing space and it affects the ability of seedlings to acquire sunlight, water, and essential nutrients. As bed density increases, top and root weights of seedlings usually decrease with an accompanying increase in seedling culls (Shoulders 1961, Harms and Langdon 1977, Mexal 1981, Brissette and Carlson 1987).

Loblolly pine seedlings with abundant Pisolithus tinctorius (Pt) ecto-mycorrhizae (Pt index >50) frequently grow significantly larger per unit area of soil than routine-run seedlings in nurseries (Marx and others 1976, 1984, 1986). Seedlings with abundant Pt ectomycorrhizae formed early in the season apparently are able to utilize sunlight and to obtain water and nutrients more effectively than seedlings having only naturally occurring ectomycorrhizae formed later in the season. These results suggest that more quality (i.e. good morphological traits) seedlings may be produced at

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higher bed densities with Pt ectomycorrhizae than with only natural ectomycorrhizae using the same nursery cultural practices. The test described here was designed to determine the effects of ectomycorrhizae and increasing bed density on seedling morphology of loblolly pine (*Pinus taeda* L.).

MATERIALS AND METHODS

The experiment was installed at the Taylor State Nursery in Trenton, SC, in April 1987. After routine spring fumigation, soil fertilization, and bed shaping, eight 15-ft long (4.56 m) plots, each separated by a 5-ft long (1.52 m) buffer strip, were laid out in each of five nursery beds (blocks). Four plots/bed were randomly chosen and machine-inoculated with vegetative inoculum of Pt produced using research procedures (Marx and others 1984). Inoculum rate *was* 1.5 liters/40 ft^2 (3.7 m^2) of soil surface. The soil in the four remaining plots/bed (controls) was mechanically disrupted with the inoculating machine to standardize soil disturbance. Seeds of improved coastal loblolly pine were stratified, soaked in triadimefon (Bayleton), and sowed in plots with a precision-vacuum seeder to obtain seedbed densities approximately 10 percent greater than 25, 30, 35, and 40 seed-lings/ft^2 (approximately 270, 320, 375, and 430 seedings/m2). Each seed drill contained two rows spaced 1 inch (2.5 cm) apart. The seeds were placed in an alternating pattern between rows in each drill. Four bed densities with or without Pt inoculation produced a total of eight treatments, which were replicated five times. In early June, seedlings in each plot were thinned by hand to the desired bed density, leaving as uniform a spacing between seedlings as possible. Routine cultural practices, including fertilizer and pesticide application, and root and top pruning, were followed during the growing season with one exception. Ferbam rather than triadimefon was sprayed to control fusiform rust disease so as not to depress Pt ectomycorrhizal development (Marx and others 1986).

Midstudy assessments in early August showed that Pt ectomycorrhizal development on seedlings in one test bed was erratic compared to others. Pt index for the four inoculated plots in this bed averaged only 12 compared to 78 for the remaining 32 test plots. This bed had been sprayed inadvertently over its entire length with Geotech bed binder during operational application of this material to adjacent beds. A similar suppression in Pt ectomycorrhizal development was also observed at the same time on seedlings in a Pt spore study installed on the opposite end of this bed. However circumstantial, this observation suggests that Geotech may decrease survival of both vegetative and spore inoculum of Pt in nursery soil, resulting in erratic and suppressed Pt ectomycorrhizal development. The impacted test bed was dropped from the bed density experiment leaving four replicate blocks for evaluation.

In early December 1987, seedling beds were undercut to 8 in. (20 cm) and vertically cut between rows. Three 1 x 4 ft. (0.3 x 1.2 m) subplots of seedlings were randomly removed by hand from each test plot and combined to represent plot seedlings. All seedlings were counted and 50 per plot, regardless of size, were randomly chosen as sample seedlings. These were measured for height, root-collar diameter (RCD), and shoot and root fresh weights. Ectomycorrhizal development was visually assessed at 5X magnification. Fusiform rust cankers were not observed on any sample seedling.

Analyses of variance and Duncan's new multiple range test were used to determine differences between treatments. For each seedling measurement, seedlings were sorted into five different size classes to determine frequency distribution of the seedling population. Regression equations were also fitted to seedling measurements obtained from each plot and predicted values were plotted.

Remaining plot seedlings were graded and culls (RCD <3 mm or 1/8 in.) were discarded. Randomly selected seedlings of plantable size from each of the eight treatments were outplanted on a good-quality reforestation site at the Savannah River Forest Station, Aiken, SC, to determine the effects of nursery treatments on their field performance. Results from this field study are not available at this time.

RESULTS AND DISCUSSION

Average RCD and weights of the seedlings decreased significantly as bed densities increased (Table 1). Top/root ratios were not strongly affected by bed density but the significantly lower ratios were in the Pt treatment. Even at the highest densities, however, averages of each measurement were within acceptable limits for plantable-size seedlings (Wakeley 1954). Generally, the decrease in size with increasing bed density was less for seedlings with Pt ectomycorrhizae. As with seedling size, development of Pt ectomycorrhizae decreased with increasing bed density. Hatchell (1985) observed a similar relationship in longleaf pine (P. *palustris* Mill.) seedlings.

Percentage distributions of the seedling population in size classes for morphological traits furnish further insight into the effects of bed density and ectomycorrhizae on seedling response. For brevity, only distributions for RCD (Figure 1) and total ectomycorrhizal development (Figure 2) are shown. Other morphological traits, such as top and root fresh weights, followed the same distribution patterns. While 54 percent of the control seedlings grown at 40/ft^2 had <3.6 mm (approx. 9/64 in.) RCD only 23 percent were this small when grown at 25/ft^2. The two other bed densities fell between these ranges. Pt ectomycorrhizae decreased the percentage of seedlings with <3.6 mm RCD at all densities (Figure 1). Nearly half of the Pt seedlings grown at 40/ft^2 had <3.6 mm RCD while only 15 percent were this small at 25/ft^2. It should be pointed out that in Figure 1 there is only 1/16 inch difference between the smallest size class of 3.0 mm (1/8 in.) and the largest size class of 4.6 mm (3/16 in.).

Increasing bed densities also had an effect on total ectomycorrhizal development (Figure 2). Not only did inoculation with Pt increase the overall average incidence of ectomycorrhizae (Table 1), it sharply decreased the percentage of seedlings with few ectomycorrhizae (Figure 2). Generally, less than half the control seedlings had >30 percent ectomycorrhizal development, regardless of bed density (Figure 2), even though their average development was about 37 percent (Table 1). About half of the seedlings with Pt ectomycorrhizae had >45 percent total ectomycorrhizal development at all bed densities (Figure 2). This represents an increase in total ectomycorrhizal development of 25 percent.

Table 1.--Average measurements of nongraded loblolly pine seedlings with Pisolithus tinctorius (Pt) or natural ectomycorrhizae from four bed densities)

Treatment		Height	Root-collar diameter	Fresh weights (g)			T/R	% Ectomycorrhizae with		Pt
Fungus	Seedlings/ft[2]	(cm)	(mm)	Tops	Roots	Total		Pt	All fungi	index
Pisolithus tinctorius	25(27)[2]	26.3a[3]	4.1a	9.2a	3.2a	12.4a	3.06b	36a	50a	66a[4]
	30(30)	26.1a	3.9b	8.3b	2.6b	10.9b	3.26ab	29ab	46ab	62ab
	35(36)	25.8a	3.8bc	7.9b	2.5bc	10.4b	3.29ab	29ab	45ab	64a
	40(40)	25.1a	3.5c	6.9c	2.3c	9.2c	3.12b	24b	42abc	56b
Natural	25(26)	25.8a	3.9b	8.6ab	2.7b	11.3ab	3.27ab	0	35c	0
	30(31)	26.0a	3.8bc	7.8b	2.5bc	10.3bc	3.34a	0	38c	0
	35(35)	25.4a	3.6c	6.9c	2.3c	9.2c	3.25ab	0	39c	0
	40(38)	25.5a	3.5c	6.9c	2.2c	9.1c	3.26ab	0	35c	0

[1] Numbers in a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at P = 0.05.

[2] Numbers in parenthesis are actual average bed densities determined at time of seedling lifting. Each test density was significantly different from the others.

[3] Seedling tops pruned to 20 cm on August 11, 1987.

[4] Pt index = **A x** (B/C) where A = percent of assessed seedlings with Pt ectomycorrhizae, B = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for seedlings without Pt), and C = percent of feeder roots with ectomycorrhizae formed by Pt and other fungi (total development).

Table 2 shows the percentage of seedlings in the smallest size class for selected morphological traits. For all traits, the percentage of seedlings in this class increased with increasing bed density. This increase, however, was consistently less in the Pt ectomycorrhizal treatments. Using RCD as the selection criterion, percentages for both ectomycorrhizal treatments with RCD <3.0 mm nearly doubled to over 22 percent between 35 and 40 seedlings/ft2.

Table 2.--Percent of loblolly pine seedlings with minimum morphological characteristics grown with and without Pisolithus ectomycorrhizae at four bed densities

Treatment: Fungus	Treatment: Seedlings/ft^2	Height ≤20 cm	Root collar diameter ≤3.0 mm	Root weight ≤1.5 g	Top weight ≤5.0 g	Total ectomycorrhizae ≤30 %
	25	5	4	3	4	7
Pisolithus	30	5	7	8	8	16
tinctorius	35	6	12	10	10	24
	40	6	22	16	22	28
	25	5	7	7	9	46
Natural	30	5	9	13	10	41
	35	6	16	22	18	44
	40	6	24	22	22	50

In part because seedling tops were pruned to 20 cm in August, seedling height at the end of the growing season *was* not affected by bed density and was not a good indicator of seedling response. Decreasing RCDs due to increasing bed densities without a corresponding decrease in seedling height have been observed by Brissette and Carlson (1987) with shortleaf pine (P. echinata Mill.) seedlings.

Figure 3A shows the regression analysis predicting average RCD at increasing bed densities. The slopes for Pt and natural ectomycorrhizae are significantly different from zero and the lines are significantly different from each other. Assuming a desired average RCD of >3.5 mm for the entire seedling population, about 38 seedlings/ft^2 could be produced with natural ectomycorrhizae and 42 seedlings/ft^2 could be produced with Pt ectomycorrhizae. This relationship, however, must take into consideration the percent of cull seedlings associated with these densities.

Figure 3B and Table 3 show predicted cull percentages (RCD <3.0 mm or 1/8 in.) over a range of bed densities. The natural ectomycorrhizal treatment maintained at 38 seedlings/ft^2 would produce over 20 percent culls or about 8 cull seedlings/ft^2 producing an average of about 30 plantable seedlings/ft^2. The Pt ectomycorrhizal treatment maintained at 42 seedlings/ft^2 would produce about 23 percent culls or 10 cull seedlings/ft2 producing 32 plantable seedlings/ft^2. However, if the Pt seedlings were grown at 38 seedlings/ft^2, the seedling population would have an overall larger RCD of 3.7 mm (Figure 3A) with 18 percent culls and would produce just as many plantable seedlings/ft^2, i.e. between 31 and 32, as produced at 42 seedlings/ft^2. At 38 seedlings/ft^2, Pt ectomycorrhizae would produce about one more plantable seedling/ft^2 than natural ectomycorrhizae and the overall seedling population would be larger.

Table 3.--Predicted relationships between initial seedling bed densities, percent culls (<3.0 mm RCD), and number of plantable seedlings of loblolly pine with Pisolithus tinctorius or natural ectomycorrhizae

Mycorrhizal condition	Initial seedling density/ft2	% of culls	No. plantable seedlings/ft2
Pisolithus tinctorius	26	2.1	25.5
	30	7.3	27.8
	34	12.5	29.8
	38	17.7	31.3
	42	22.9	32.4
Natural	26	4.9	24.7
	30	10.1	27.0
	34	15.2	28.8
	38	20.4	30.2
	42	25.5	31.3

Assuming that RCD is a valid criterion of seedling quality, results of this study suggest a maximum effective seedbed density of about 30/ft^2 for loblolly pine. Above this density, regardless of ectomycorrhizal condition, RCD decreases quite rapidly with a corresponding sharp increase in seedling culls. Also, at the higher seedbed density, more seed to produce proportionately fewer plantable seedlings, is inefficient use of valuable seed. At 30 seedlings/ft^2, average RCD is 3.7 mm and 3.9 mm for natural and Pt ectomycorrhizal seedlings with cull percentages of 10 and 7.5, respectively (Figure 3). Substantially larger seedlings (average RCD >4.0 mm) with minimal cull percentages (<3 percent) were produced at a seedbed density of 25/ft^2 (Figure 3) which is the most frequently used density for loblolly pine in southern nurseries.

At a density between 25 and 35 seedlings/ft^2, inoculation with Pt resulted in about one additional plantable seedling/ft^2 (Figure 3). Although seemingly insignificant, this one seedling translates to about 30,000 more plantable seedlings/acre of nursery bed. Inoculation also ti improves seedling weights, RCD, and total ectomycorrhizal development which should translate into improved field performance for all the seedlings.

Seedling response to high densities indicate that the carrying capacity of the soil and its associated environmental factors cannot be exceeded without a significant increase in the number of seedlings with below standard morphological traits. This does not mean that this carrying capacity cannot possibly be increased by changing irrigation, pesticide, fertility, or root and top pruning practices. Even with precision sowing of seed and uniform spacing of seedlings, morphological traits of seedlings will vary over a range of sizes. Much of this will be caused by genetic variability within seed lots collected from multiple mother trees in the seed orchard. Variability would be even greater with wild seed.

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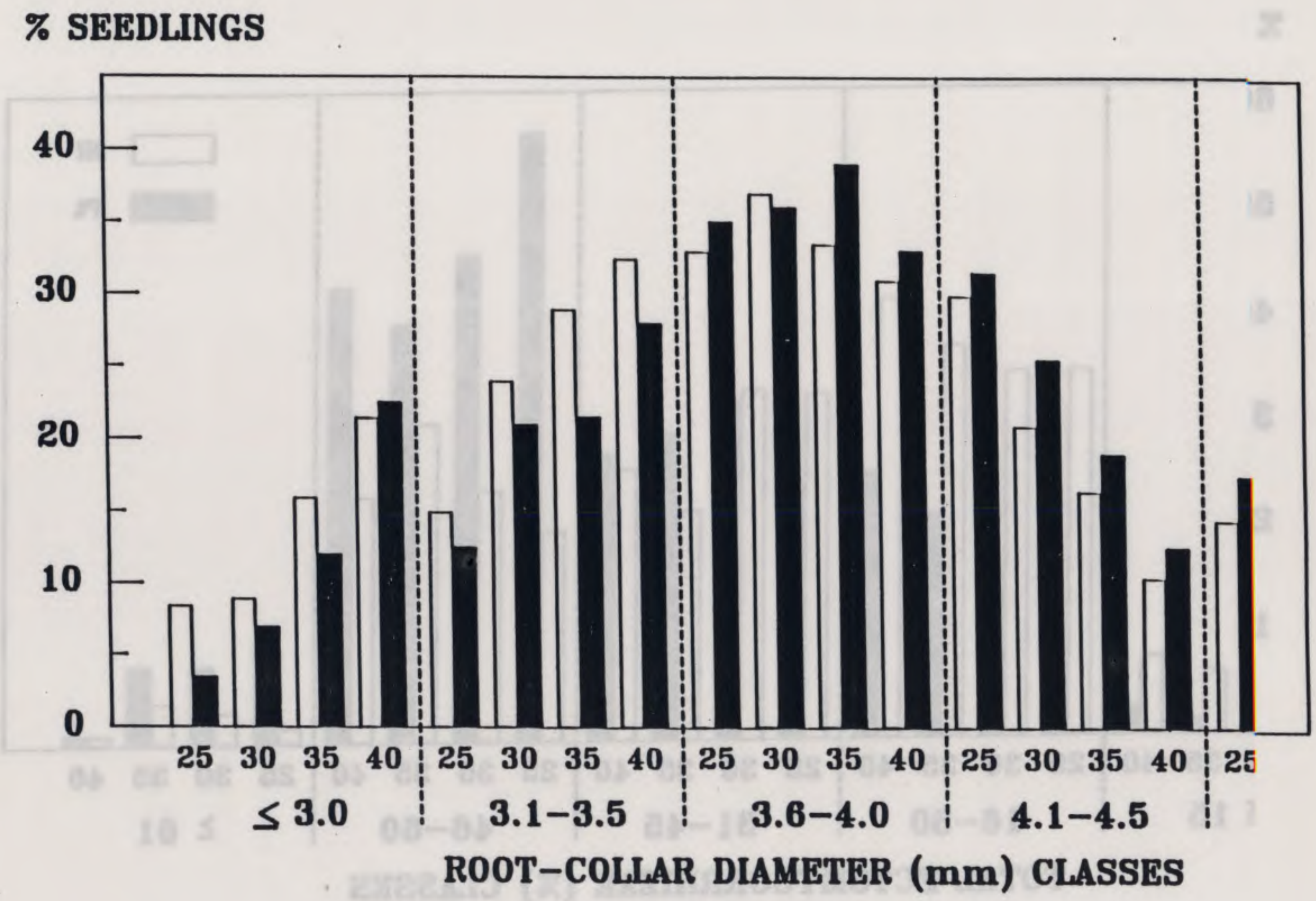

Figure I. Percent distribution of loblolly pine seedlings by size classes of root-collar diameter from bed densities of 25, 30, 35 and 40 seedlings/ft^2 with <u>Pisolithus tinctorius</u> (Pt) or naturally occurring (NI) ectomycorrhizae.

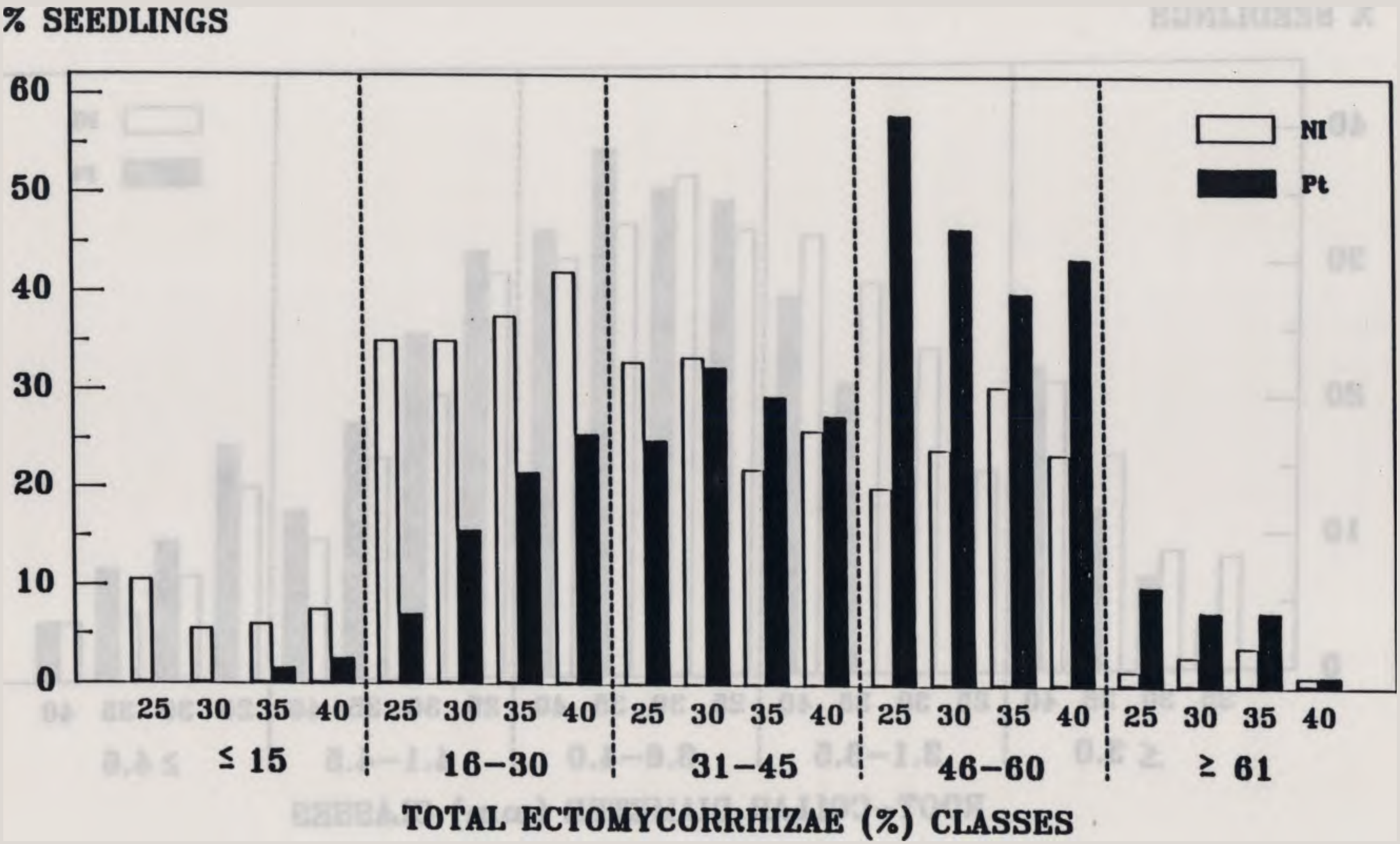

Figure 2. Percent distribution of loblolly pine seedlings by classes of percent total ectomycorrhizal development from bed densities of 25, 30, 35 and 40 seedlings/ft^2 with Pisolithus tinctorius (Pt) or naturally occurring (NI) ectomycorrhizae.

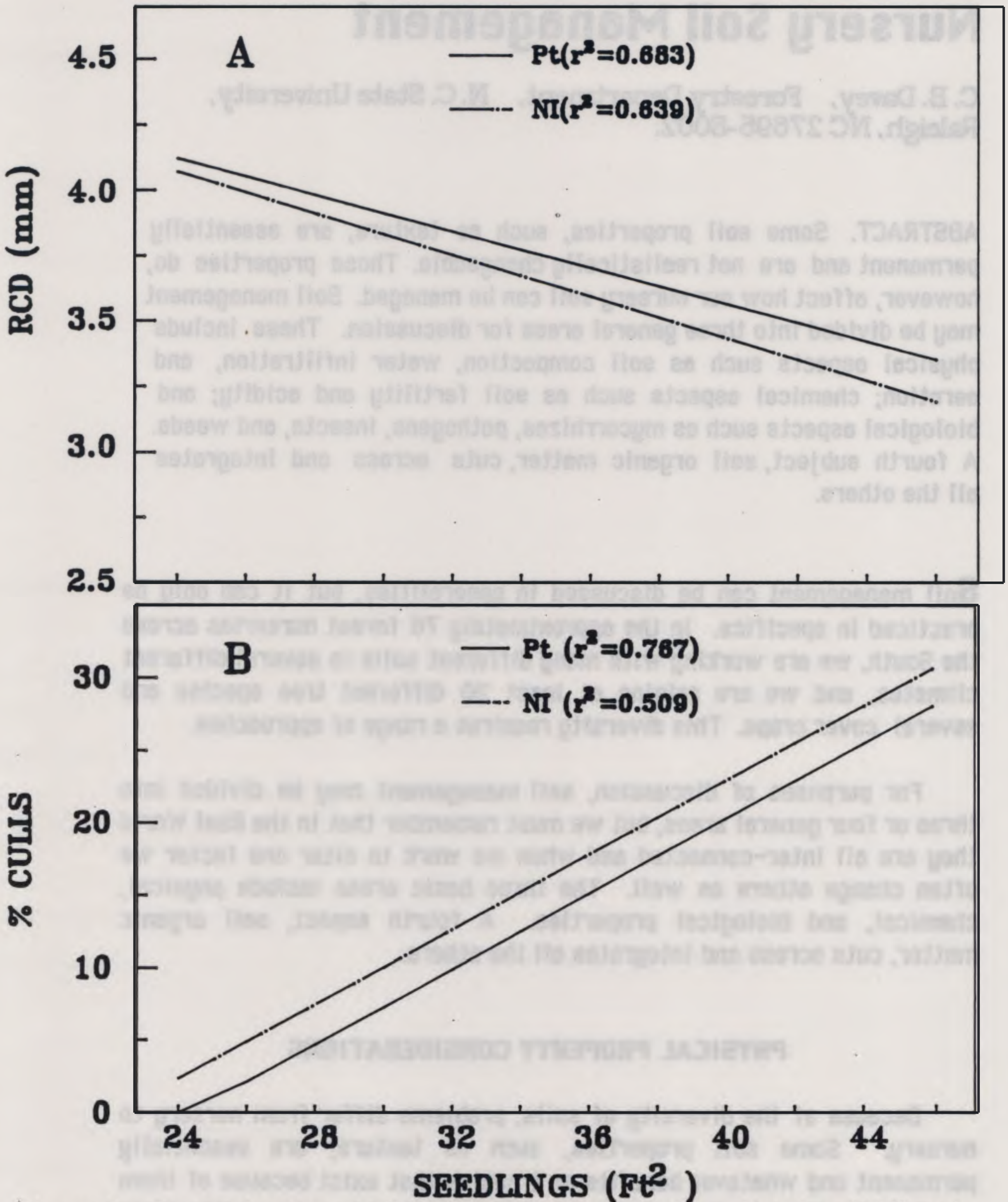

Figure 3. Regression for predicted values of root collar diameter (RCD) and percent cull seedlings (<3 mm RCD) showing relationship of seedlings with Pisolithus tinctorius (Pt) or natural occurring (NI) ectomycorrhizae grown at different bed densities.