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Field Survival of Loblolly and Slash Pine Seedlings Grown in Trays and Ray Leach Containers

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Loblolly and slash pine seedlings grown in Ray Leach seedling containers had 85 percent field survival, as compared to 64 percent for seedlings transplanted (with all growth media removed from roots) from 20-tree trays filled with a mixture of soil, sand, and peat moss.

The USDA Forest Service Resistance Screening Center, Asheville, N.C., was established in 1973 to test selected pine seedlings for fusiform rust resistance under controlled greenhouse conditions (1). In a standard test, six trays of 20 seedlings from each seedlot are inoculated. These trays contain a growing medium of soil, sand, and peat moss. Because of the labor required to carry out this process, variations in soil fertility, and poor seedling survival following outplanting, an evaluation of the Ray Leach container was undertaken. This article describes the results for the field survival part of the evaluation.

Methods

Considering the 20-tree design at the Resistance Screening Center; the need to handle each tree for fusiform analysis; and the need to fertilize, germinate, transplant, water, and perform other cultural procedures, the super cell Ray Leach tubes were selected. This selection was based only on examination of the containers. A study was then designed to make a side-by-side comparison of the trays currently being used at the Resistance Screening Center and Ray Leach super cell containers.

Currently used trays. The trays are $13^1/_2$ by $5^1/_2$ by 4 inches deep. They were filled to within 1 inch of the top with a 3:2:1 mixture of sterilized soil, sand, and peat. The slash and loblolly pine seeds used in the evaluation were treated with 20-percent hydrogen peroxide for 10 minutes and rinsed in tapwater. The seeds were then placed in germination trays of vermiculite and watered for 15 minutes three times a day. After the seeds germinated but before the seedcoats were shed, 20 germinated seeds were transplanted into each tray. A specially designed planting dowell, with 20 evenly spaced holes, was used to ensure uniform seedling spacing. The trays were fertilized with Miracle-Gro at the rate recommended on the label before transplanting and at 8, 16, and 24 weeks.

Ray Leach super cell containers. The tubes were filled with a 5:4:1 mixture of peat moss, vermiculite, and perlite to within $1^1/_2$ inches of the top. This growing medium was watered, and one seed was placed directly into the top of each tube. The medium was watered daily until the seed germinated and then as needed. The tubes received one-half of the concentration of Miracle-Gro recommended on the label 1 week after germination and a full concentration each month thereafter.

All trays and tubes were maintained in the greenhouse where temperatures varied from 50° (night) to 90° (day) F. The 740 slash and 740 loblolly pine seedlings were inoculated with the fusiform rust spores and grown for 6 months (slash pine) and 9 months (loblolly pine). The complete evaluation was replicated three times, 1 month apart. The seedlings were evaluated for fusiform rust infection to see if the container performed as well or better than the trays at the Resistance Screening Center. The healthy survivors were placed in a shadehouse for 30 days before outplanting.

The trays and containers with healthy seedlings (12 to 18 inches tall) were transported to the Savannah River Plant at Aiken S.C., in July, August, and September 1979 and outplanted. The tray trees had the soil mix removed from the roots on the site and were planted with a planting bar. The tube trees were removed from the tubes and planted using the same bar. The locations were randomized by family and type of container on the sandy loam soil to minimize the effects of site differences. The survival percentage was determined in November 1980 and again in November 1981.

Results

The average survival for slash and loblolly pine in containers (table 1) was about 85 percent, while the survival for tray-grown trees was 64 percent (table 1). In July and September, the tray trees survived as well as container-grown trees; but in the August planting, there was a substantial difference.

Conclusions

Overall, seedlings grown at the Resistance Screening Center in Ray Leach tubes survived outplanting better than the tray-grown trees, particularly when outplanted under hot, dry conditions.

It would seem safe to conclude that the tubes provide increased, or at least equal, survival of trees outplanted from the Resistance Screening Center.

Table 1—*Field survival of loblolly and slash pine seedlings grown at the Resistance Screening Center in trays and containers*

	Survival of slash pine		Survival of loblolly pine	
Planting date	Trays	Containers	Trays	Containers
		%		
July	79 (3.7)[1]	80 (3.7)	67 (4.3)	79 (3.7)
August	47 (4.6)	88 (3.0)	59 (4.5)	81 (3.6)
September	59 (4.5)	66 (4.3)	91 (2.6)	95 (2.0)
Average	64 (2.5)	86 (1.8)	64 (2.5)	85 (1.9)

[1] Standard error is in parentheses next to each percentage.

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Sizing Slash Pine Seeds as a Nursery Procedure

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Three slash pine seedlots were each graded by diameter and weight into 10 seed classes. These classes were evaluated in laboratory tests, nursery seedbeds, and 5-year-old plantations. Lightweight seeds had lower laboratory germination percentages than heavier seeds, while small seeds had lower survival rates in the nursery and produced shorter seedlings than larger seeds. Differences in seedling heights at 5 years were significant only between plantations.

Sizing seeds was a common nursery practice until it was suggested that it may eliminate some genotypes (9, 16). Actually, no genotypes are lost unless one or more seed sizes are discarded. In fact, sizing may preserve slower germinating genotypes (18). Further, Righter (13) showed that there was no relationship between seed weight and inherent vigor. He therefore suggested that selection for genetic improvement through seed sizing was of no benefit within a progeny seedlot.

The real benefits of seed sizing are more uniform germination in the nursery and more uniform seedling density in the seedbed. Both of these qualities are very important in the forest nursery to increase the seedling-to-seed ratio. There are two basic means of grading seeds (screens and gravity), but few comparisons have been made and very little research is available to guide nursery personnel.

The objective of this study was to investigate the effect of sizing seed orchard and wild-collected slash pine seeds by diameter and by density on laboratory tests and on nursery and field performance.

Materials and Methods

Two seed orchard (Pensacola and Georgia) and one wild collection of slash pine seeds from St. Regis seed stores were commercially separated into four physical sizes by screens. Each of the three smaller sizes was then separated on a gravity separator into three density grades. This provided a total of 10 classes per lot (fig. 1).

A sample was drawn from each of the 10 classes in each lot and sent to the National Tree Seed Laboratory for examination. The seed laboratory evaluated the percentage of filled seeds by radiography and the average number of seeds per pound and then tested four 100-seed samples from each lot for germination. The seeds were germinated at 22° C with 8 hours of fluorescent light daily. Counts were made weekly for 4 weeks.

Another sample of each size class was sown in the St. Regis Nursery at Lee, Fla., in April 1973. Each treatment was sown on a 3-foot length of nurserybed. Treatments were randomly assigned and replicated three times. Nursery germination was last measured on May 30 and appeared to be virtually complete. The number of seedlings per square foot and their height were measured on July 11 and November 20. At lifting time (January), 10 seedlings were randomly selected and lifted from the

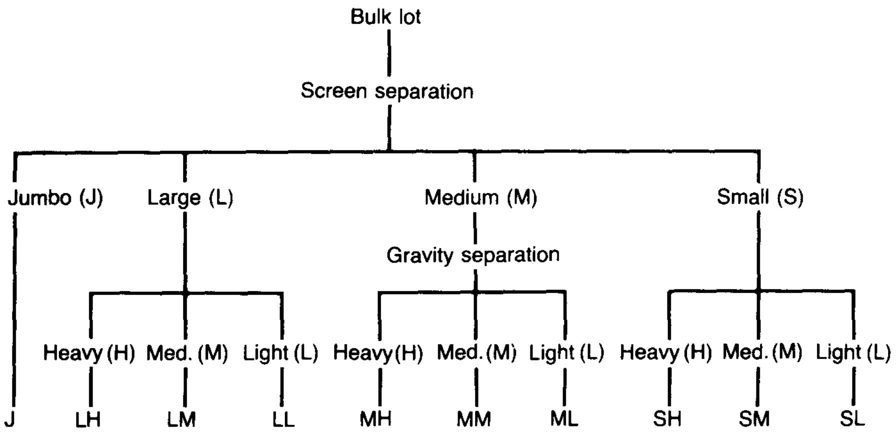

Figure 1—*Diagram of seed classes derived from each seedlot.*

center of each plot. These seedlings were transported in polyethylene bags to the National Tree Seed Laboratory for evaluation. The remaining seedlings were outplanted in 10-tree row plots replicated four times. The seedlings were machine-planted on a 7- by 12-foot spacing in Madison County, Fla.

The seedlings transported to the seed laboratory were weighed upon arrival, and the tops were cut off at the root collar. The tops were measured for length and both tops and roots were weighed and ovendried at 105° C for 24 hours. The stem diameter was measured at the root collar with a caliper. Finally, the dry weight of the top and roots was recorded.

The entire experiment was repeated a second year, and the seedlings were outplanted in Escambia County, Fla. The field design was modified slightly by using block plots of 50 seedlings at an 8- by 10-foot spacing replicated three times. Analysis of variance was conducted on all data. Duncan's multiple range test was used to determine differences between treatment means.

Results and Discussion

Laboratory tests. Both screens and gravity separations gave significantly different seed sizes (table 1). Seeds per pound varied continuously from the largest diameter class, at 9,630 seeds per pound, up to the lightest seeds in the smallest diameter class, at 18,870 seeds per pound.

Germination percentage was significantly less for the lightest seeds in each diameter class. Within the light-seed category, the germination percentage decreased as the diameter class increased (fig. 2). This result was attributed to the number of partially filled seeds (as seen in the radiograph).

Larger seeds reportedly have more stored energy. Also, seed vigor is related to seed density (10). Therefore, larger seeds are expected to germinate first and get a faster start than smaller seeds (2, 3, 5, 7). However, large seeds have been surpassed by medium-sized seeds (4, 8); and in some cases, no relationship was found between seed size and germination (1, 6, 12, 19). These differences may have several explanations: (1) Not recording germination often enough to fully identify the rate of germination, (2) the reduction in variability with improved seed stock, and (3) variations within a given seedlot, with storage.

We noted, in this study, that 7-day intervals were too long to examine germination rate accurately. In the first year of the study, 63 to 68 percent of the seeds germinated in 7 days. In the second year, germination was only 15 to 18 percent in 7 days, but nearly 100 percent in 14 days. Also, the greater variability of wild-collected seeds produced slower germination. Apparently, each seedlot must be analyzed on its own merits to obtain accurate germination rates.

Table 1—*Average measurements made on slash pine seed grades in a laboratory evaluation*

Grade	Seeds per pound	Germination of filled seeds	Days to 90% of total germination
		%	
Screens			
Jumbo	9,373a[1]	87.5a	12.5a
Large	10,278b	84.8b	12.2a
Medium	13,182c	87.8a	13.0a
Small	17,737d	89.4a	13.1a
Gravity			
Heavy	13,022a	91.8a	12.7a
Medium	13,513b	89.7a	12.6a
Light	14,662c	79.6b	12.9a

[1] Means followed by the same letter are not significantly different at $p = 0.01$. The multiple range tests were used within each method of sizing and not in their combination.

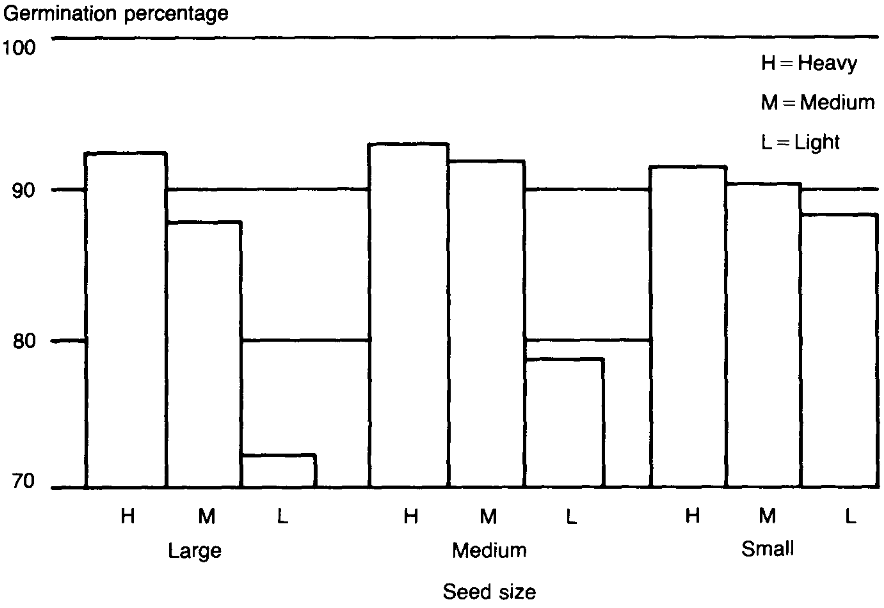

Figure 2—*Germination of filled seed within grading classes.*

Nursery data. The differences between laboratory and field germination were inversely related to seed diameter (table 2). Small seeds survived poorly in the field. Much of their loss may be attributed to slower germination and possibly weaker seeds. No relationship was noted with seed weight. Sowing rates could therefore be adjusted to give desired seedling densities among lots with varying seed sizes.

Height differences between seedlings from various seed sizes became more distinct with time (table 3). Differences in seed density did not affect seedling height, while differences in seed diameter produced three significantly different seedling heights. Overall, seedlings developed an array of heights, uniformly decreasing from a high of 12.5 inches with jumbo seeds to 9.6 inches with small, light seeds.

Seedling data. Initial seed diameter significantly affected all seedling measurements, while seed weight had no significant effect (table 4). Seedling height was correlated to seed size ($r^2 = 0.84$): the jumbo seeds produced seedlings 19 percent taller than those developed from small seeds, while the large seeds produced seedlings with a larger root collar diameter and heavier seedling weight.

Table 2—*Comparison of laboratory germination and nursery survival*

Treatment	Laboratory prediction	Actual field count	Difference
	%	%	%
Screens			
Jumbo	86	80	6
Large	82	70	12
Medium	85	70	15
Small	88	64	24
Gravity			
Heavy	90	74	16
Medium	87	70	17
Light	75	60	15
Source			
Georgia seed orchard	92	81	11
Florida seed orchard	86	78	8
Wild collection	73	70	3

Table 3—*Seedling height in the nursery*

Grade	Month May	 July	 November
		In	
Screens			
Jumbo	2.63a[1]	6.30a	12.47a
Large	2.56a	5.94b	11.64b
Med.	2.29b	5.58c	10.91c
Small	2.10c	5.12d	10.13c
Gravity			
Heavy	2.34a	5.64a	11.13a
Med.	2.33a	5.60a	10.91a
Light	2.26a	5.40b	10.64a

[1] Means with the same letter, within the grade and month, are not significantly different at $p = 0.01$.

Stem diameters and mean seedling weights were correlated with seed size for wild-collected seeds ($r^2 = 0.64$), but no relationship existed for orchard seedlots. The reason for this is not clear, but a possibility is that sizing of wild collections may result in a sorting of genetic parents, especially if the seeds were collected from relatively few parent trees.

The generally larger size of improved seeds is because of increased storage material resulting from cultural treatments in seed orchards. Seedlings derived from large seeds produced 25 percent more dry matter than those from small seeds, and they also produced 10 percent greater stem diameters.

Plantation data. Significant differences occurred in all measurements between locations. The seedlings in the second planting (Escambia County) were shorter (table 5), were smaller in diameter, had better survival, and exhibited less rust infection (table 6). However, there were no significant differences among the seed classes in the combined data related to either seedling height or rust infection rates.

These results do not agree with reports by Sluder (17) and Robinson and Van Buijtenen (14), which showed seed size had a significant effect on tree height and volume at age 15 in loblolly pine. In Sluder's study, only one bulk lot of unimproved seeds was used, and planting was done at only one location. If the bulk lot was composed of seeds of relatively few parent trees, separating the lot into size classes could result in genetic differences among the size classes. This is because seeds from a given parent tree are fairly uniform in size, and are closely related (at least as half-sibs, with many full-sibs likely). Therefore, the comparison of seed size classes could really be a comparison of different families, and substantial family variation in slash pine is well known. Planting at only one location would enhance this ge-

Table 4—*Data summary (nursery seedlings)*

Measurement	Laboratory evaluations Seedling height	 Root collar diameter	 Top:root ratio	 Root dry weight	 Top dry weight	 Weight of 10 seedlings
	---------- *In* ----------			------------------ *Oz* ------------------		
Screens						
Jumbo	9.76a[1]	0.206b	3.95a	0.039ab	0.153a	6.33a
Large	9.68a	.211a	3.95a	.040a	1.157a	6.52a
Medium	8.86b	.203b	3.99a	.035bc	.140b	5.79b
Small	8.23c	.191c	3.92a	.032c	.126c	5.26c
Gravity						
Heavy	9.09a	.201a	3.88a	.036a	.139a	5.76a
Medium	8.94a	.199a	4.11a	.034a	.140a	5.77a
Light	8.78a	.204a	3.92a	.037a	.145a	6.04a
Source						
Georgia seed orchard	9.21a	.203a	4.16a	.034a	.141ab	5.90ab
Florida seed orchard	8.78b	.198b	3.91a	.034a	.134b	5.56b
Wild collection	8.78b	.204a	3.99a	.037a	.148a	6.11a

[1] Within screens, gravity, and source, data having the same letters are not significantly different at $p = 0.01$.

Table 5—*Mean seedling height in the plantation at 5 years from seeds of different diameters, weights, and sources*

	Seed diameter				Seed weight			Seed source		
Location	Jumbo	Large	Medium	Small	Heavy	Medium	Light	Florida seed orchard	Georgia seed orchard	Wild
						Ft				
Madison Co.	15.4b[1]	15.4b	16.4a	16.8a	16.6a	16.0a	16.1a	16.3b	16.8a	15.5c
Escambia Co.	14.6a	15.1a	14.7a	14.5a	14.8a	14.7a	14.8a	15.1a	14.6a	14.6a
Combined data	15.0a	15.2a	15.6a	15.6a	15.7a	15.4a	15.4a	15.7a	15.7a	15.6a

[1] Means with the same letter, within each location, are not significantly different at $p = 0.01$.

Table 6—*Percentage of rust infection in plantation at 5 years from seeds of differnent diameters, weights, and sources*

	Seed diameter				Seed weight			Seed source		
Location	Jumbo	Large	Medium	Small	Heavy	Medium	Light	Florida seed orchard	Georgia seed orchard	Wild
						%				
Madison Co.	38.7a[1]	33.3a	36.9a	37.1a	38.6a	33.4a	35.3a	41.0a	34.7b	31.6c
Escambia Co.	6.7a	11.0a	9.2a	10.0a	11.2a	10.0a	9.0a	11.1a	11.9a	7.2a
Combined data	22.7a	22.2a	23.0a	23.6a	24.9a	21.7a	22.2a	26.0a	23.3ab	19.4b

[1] Means with the same letters, within each location, are not significantly different at $p = 0.01$.

netic effect if there was a significant family by location interaction. Sluder recognized these limitations in the loblolly pine study. Since our slash pine study involved three bulk lots and two planting locations, these confounding factors are not likely to have an effect.

In the study of east Texas loblolly by Robinson and Van Buijtenen, the separation by seed weight may have really been a separation of natural shortleaf loblolly pine hybrids from loblolly pine. Since natural interspecific hybridization is not common in slash pine, it is not likely that seed weight differences were caused by hybridization.

These findings of our study support Langdon's results (11) that seedlings from a given seed size had only a slight influence on field survival; and those of Shoulders (15), which showed that height differences developed in the nursery from sized seeds were soon eliminated in plantations.

Conclusion

Seed sizing is a useful tool in the forest nursery. These data show that large seeds will produce large seedlings and that, by separating the seeds into classes, a more uniform seedbed density may be obtained. The uniformity in density is derived through more uniform germination of the sized seeds. After outplanting, however, the range of variation is expected to be large and the distribution approximately normal, since seed weight is not correlated with inherent vigor (13).

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Bayleton Applied to Bareroot Nursery Stock Reduces Fusiform Rust in First Year After Outplanting

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Bayleton applied to bareroot nursery stock at 600 milligrams per liter as a top dip, root dip, or clay slurry prevented fusiform rust infections in loblolly pine seedlings artificially inoculated 3 months later. Clay slurries containing 600, 800, 1,000, and 1,500 milligrams of Bayleton per liter prevented natural infections during the first infection season after outplanting, but top and root dips prevented natural infections only at Bayleton concentrations of 1,500 milligrams per liter.

Fusiform rust caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* is the most important disease in forest nurseries and young plantations in the Southeastern United States. The systemic fungicide Bayleton (triadimefon; 1-(4-chlorophenoxy)-3, 3-dimethyl -2-(1 H-1,2, 4-triazol-1-yl)-2-butanone) has 24-C registration for use as a foliar spray in forest tree nurseries for control of fusiform rust. The fungicide also controls the disease when applied as a seed treatment or soil drench (6, 7). Because most damaging fusiform rust infections occur during the first 5 years after planting (2), a treatment of nursery stock that provides some control in the young plantation would be most valuable.

This paper reports results of applying Bayleton to 1-0 loblolly pine seedlings for control of fusiform rust after outplanting.

Methods

Loblolly pine seedlings were lifted from a Georgia nursery and groups of 100 seedlings in Wakeley's grade 2 were exposed to various Bayleton treatments (table 1). Five concentrations were applied in water as root and top dips and five concentrations were applied in clay slurry as a root dip. Roots of control seedlings were either dipped in a Bayleton-free clay slurry or received no treatment. Aqueous suspensions of Bayleton were formulated to contain 600, 800, 1,000, and 1,500 milligrams of active ingredient and 2.5 milliliters of the adjuvant Agridex per liter. The clay slurry used as a root dip was formulated to contain Bayleton and 45.35 percent kaolinitic clay (less than 2-

Table 1—*Efficacy of Bayleton*[1] *for control of fusiform rust in 1-0 loblolly pine nursery stock*[2]

Treatment	Bayleton concentration	Seedlings galled: Greenhouse—artificial inoculations	Seedlings galled: Nursery—natural infections
	Mg/l	%	%
Check	0	10.9a[3]	4.0a
Check:clay slurry	0	4.8a	6.3a
Top dip	600	.0b	4.0a
	800	.0b	4.2a
	1,000	.0b	2.1b
	1,500	.0b	.0c
Root dip	600	.0b	2.0b
	800	.0b	4.2a
	1,000	.0b	2.0b
	1,500	.0b	.0c
Clay slurry	600	.0b	.0c
	800	.0b	.0c
	1,000	.0b	.0c
	1,500	.0b	.0c

[1] Bayleton was formulated to contain 2.5 milliliters of the adjuvant Agri-dex per liter. The clay slurry contained 45.35 percent kaolinitic clay.

[2] Seedlings were artificially inoculated 3 months after treatment or exposed to first-year natural-field inoculum.

[3] In each column, means followed by a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

micron particle size). This formulation is thicker than that operationally used (36.0 percent) in packing seedlings at nurseries and, consequently, caused more material to adhere to roots (1). Seedling tops or roots were immersed for 30 minutes in each aqueous Bayleton suspension.

Ten seedlings of each treatment were transplanted to each of 10 replicate flats (33 by 13 by 11 cm) containing a sandy loam, sand, and vermiculite soil mixture in a 2:1:1 ratio by volume. Five replicates of each treatment were placed on a greenhouse bench and five were placed on a bed in the Georgia Forestry Commission's Davisboro Nursery on April 15, 1981. Miracle-Gro, a commercially available liquid fertilizer, was applied every other month (5). Seedlings grown in the greenhouse were inoculated 3 months after treatment with the fusiform rust fungus (300,000 basidiospores/ml) (3). Aeciospores collected from loblolly pine galls in Clarke County, Ga. (source 2-74), were used to produce the basidiospore inoculum on northern red oak seedlings. Seedlings in the nursery were in place fort year; thus they were exposed for a full infection season of approximately 3 months (2, 4). One year after the inoculations were made, the percentages of seedlings infected (galled) were determined. Nursery and greenhouse studies were arranged in randomized complete block designs and variances in each study were analyzed independently.

Results and Discussion

Among seedlings grown in the greenhouse, all Bayleton treatments fully controlled fusiform rust (table 1). The low incidence of rust infections in check seedlings (10 percent and 4.8 percent) after artificial inoculation with 300,000 basidiospores per milliliter (a very high inoculum density) reflects the small amount of newly formed tissue on seedlings at the time of inoculation. However, the amount of infection obtained on control seedlings was sufficient to test the effectiveness of the treatments.

Seedlings in the nursery produced considerable amounts of new shoot tissue during the 3month exposure to the rust fungus. On these seedlings, Bayleton concentrations below 1,500 milligrams per liter were effective in only the clay-slurry treatments (table 1). The clay-slurry root dip was fully effective at all concentrations used. Mixing a fungicide with clay and dipping pine seedling roots in the mixture before planting is easily adaptable to nursery operations; a similar clay slurry is applied at some nurseries to improve field survival of seedlings after storage (1). The clay apparently provides a reservoir of Bayleton for systemic translocation after planting and during the time when seedlings are likely to become infected. The lowest concentration of Bayleton applied in this study (600 mg/l in a clay slurry root dip appears promising for reducing fusiform rust in loblolly and slash pine regeneration programs. No phytotoxicity was noted in any treatment concentration tested indicating that the 600-milligram- per-liter rate of application in a clay slurry is well below phytotoxic rates. Because none of the treatments reduced survival or inhibited growth of seedlings in the greenhouse or nursery, it is un likely that they were a serious detriment to mycorrhizal or root development. Plans are underway to test the clay-slurry treatments on operationally planted seedlings to determine effects on field survival, growth, mycorrhizae, and rust control.

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Sample Size for Evaluating Treatment Effects on Red Pine Seedlings

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Basal diameter and total height measurements of 990 3-0 red pine seedlings provided the variance data to compute sample sizes for various probabilities and allowable errors. A precision level ± 5 percent of the mean would require approximately 20 to 100 seedlings, depending upon the probability level chosen.

Many studies involving the effects of various treatments on growth of tree seedlings rely on measurements of stem diameter and seedling height as variables (2, 4). A common question that occurs in the planning of such studies centers around the appropriate sampling size or the number of seedlings to include in each study replicate. The number of seedlings depends upon the variability associated with diameter and height, the degree of precision desired, and the appropriate probability level.

The objectives of this study were to determine the variation associated with diameter and height of red pine seedlings and to compute the sample sizes required for varying probabilities and levels of precision.

Methods

In April 1983, 990 3-0 red pine seedlings were obtained from the Griffith State Nursery in Wisconsin Rapids, Wis. On each seedling, basal stem diameter (mm) and total height (cm) were measured. Means, standard deviations, and coefficients of variation were computed, and the iterative procedure of Avery and Burkhart (11) was used to determine sample sizes. The following formula was used:

$$n = \left[\frac{(t)\ (cv)^2}{A}\right]$$

n = sample size
t = Student's t
cv = coefficient of variation (%)
A = allowable error expressed as a percentage of the mean

Results and Discussion

Sample statistics are presented in table 1, and calculated sample sizes for diameter and height for various probabilities and allowable errors are presented in tables 2 and 3.

Table 1—*Sample statistics based on measurement of 990 3-0 red pine seedlings*

Statistic	Basal diameter	Total height
	Mm	*Cm*
Mean	4.36	26.3
Standard deviation	.88	4.3
Range	2.18-8.82	14.0-43.5
Coefficient of variation (%)	20.2	16.2

Table 2—*Number of seedlings to sample for various probabilities and allowable errors for basal diameter*

	Allowable error as a percentage of the mean				
Probability	1%	5%	10%	15%	20%
80	699	28	8	4	2
90	1,102	46	13	6	4
95	1,564	65	18	9	6
99	2,702	112	31	16	9

Table 3—*Number of seedlings to sample for various probabilities and allowable errors for total height*

	Allowable error as a percentage of the mean				
Probability	1%	5%	10%	15%	20%
80	433	19	5	2	1
90	714	30	9	5	2
95	1,013	43	13	7	3
99	1,750	75	21	11	4

Coefficients of variation for diameter and height were fairly close, 20.2 percent for diameter and 16.2 percent for height. Thus, for those studies where both seedling diameter and height are to be measured, approximately the same number of seedlings would provide the desired precision for both variables.

For both diameter and height, an allowable error of ±1 percent of the mean results in an inordinately high sample size, regardless of the probability level chosen. Most investigators would probably prefer to operate in the +/- 5-percent range, where the sample size varies from about 20 seedlings to slightly over 100, depending upon the probability level chosen.

Sample sizes for other probability levels can be computed using the data in table 1 and the sample size formula.

Conclusion

If appropriate phenotypic variation data is available, numbers of seedlings that should be used in various studies need not be based on guesswork or perceived convenience. As shown by this study, there is a great deal of variation in sample size based upon the desired level of precision alone. According to Freese (3), "the aim in planning a survey should be to take enough observations to obtain the desired precision-no more, no less." The information presented here should aid those workers faced with setting up studies that involve the measurement of seedling diameter and height.

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Second-Season Top and Root Development of Potted, 1-0, Bareroot *Paulownia Tomentosa* Seedlings[1]

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Paulownia tomentosa *seedlings were grown in a nurserybed at a density of 64 seedlings per square meter for one growing season. Dormant, 1-0, bareroot seedlings were lifted the following spring and grown in a greenhouse in large pots filled with sand. Three seedlings were removed every 7 days for a period of 49 days to measure top and root growth. Shoot bud break and new roots developed 7 days after planting. Shoot and root growth dramatically increased 21 days after planting with a reduction in original taproot dry weight.*

Paulownia tomentosa (Thunb.) Steud. has been of recent interest because of newspaper and magazine articles *(4, 5, 6, 7, 8)* and recent scientific literature *(1, 3, 9)* on the growth, site adaptability, and wood value of this species. The University of Maryland's Paulownia Research Center is currently investigating numerous aspects of the site adaptability and silviculture of paulownia *(2)* . Unfortunately, American literature providing even the simplest botanical information on paulownia is scarce.

Paulownia plantations must either *be* planted with containerized seedlings, bareroot seedlings , or large root portions for adequate survival and spacing control. Utilizing large root portions is not practical in large plantations because of inaccessibility of root sources and the possibilities of decay and dessication of wounded root portions when planted. Containerized and bareroot seedlings have been successfully grown *(1)* and planted with good to excellent survival *(3).*

This study was conducted to investigate the second-season top and root development of 1-0, bareroot *Paulownia tomentosa* seedlings. This information should familiarize foresters and tree farmers with the initial field growth and establishment of planted paulownia seedlings.

Materials and Methods

Twenty-one dormant, 1-0, bareroot paulownia seedlings were removed from a nurserybed in March 1983 and stored in moist sphagnum moss in a paper bag at 3ºC for 3 weeks. Seedlings were selected to be of a uniform root diameter (approximately 1 centimeter) and cut to 18-centimeter lengths. Taproots were 17 centimeters and the stem 1 centimeter long. All 21 seedlings were cut to have equivalent fresh weights and were mixed and then separated into seven groups of three seedlings each. Each group of three seedlings was planted at even spacings in a 20-liter plastic pot filled with washed river sand. These pots were placed on a greenhouse bench. Seedling groups received one-half liter of water every day. The photoperiod

Table 1—*Second-season[1] shoot and root growth of potted, 1-0, dormant* Paulownia tomentosa *seedlings over a 49-day period*

Days of growth	Original taproot dry weight	New root dry weight	New primary lateral roots	Shoot buds and shoots	Top dry weight	Total seedling dry weight	New root-to-shoot ratio
	Gm	*Gm*			*Gm*	*Gm*	
7	1.60	—[1]	3.3	6.0	—	1.60	—
14	1.61	—	3.7	5.3	0.10	1.72	—
21	1.17	0.22	12.3	6.3	.53	1.92	0.42
28	1.28	.40	16.3	5.3	.57	2.25	.70
35	1.52	.46	17.3	4.7	.99	2.97	.46
42	1.75	.40	16.0	5.7	.90	3.15	.44
49	1.89	.40	16.0	6.0	1.41	3.70	.28

[1] Mean of three seedlings per entry.
[2] — = not measurable.

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[1] This study was supported in part by McIntire-Stennis Cooperative Forestry research funds. Scientific Article No. A-3530, Contribution No. 6604, of the Maryland Agriculture Experiment Station.

was not enhanced artificially and greenhouse temperatures were maintained continuously at 24° C.

Three seedlings from a single pot were removed for analyses every 7 days. Shoot buds and new primary lateral roots that developed were counted. Shoot lengths and their locations (distance from the root collars) and locations of new primary lateral roots were tabulated. Paulownia tops (shoots plus leaves), new roots, and original taproots were dried at 65° C for 48 hours and weighed. Photographs were taken before all measurements.

Results and Discussion

Shoot buds and new primary lateral roots developed after 7 days, but these had no measureable dry weight. Numbers of shoot buds and new primary lateral roots of seedlings after 14 days were similar to those after 7 days and also had no measurable dry weight (table 1 and fig.1). After 21 days of growth, top and root growth had accelerated to measurable weights and shoot growth continued geometrically throughout the remainder of the 49-day period. Root dry weight and number of new primary laterals did not increase between the 28th and 49th day, resulting in a decline in new root-to-shoot dry-weight ratios during that period. Original taproot dry weight was reduced sometime between the 14th and 21st days of growth (table 1 and fig. 1) in conjunction with top and new root growth.

Taproot growth increased linearly between 21 and 49 days of growth.

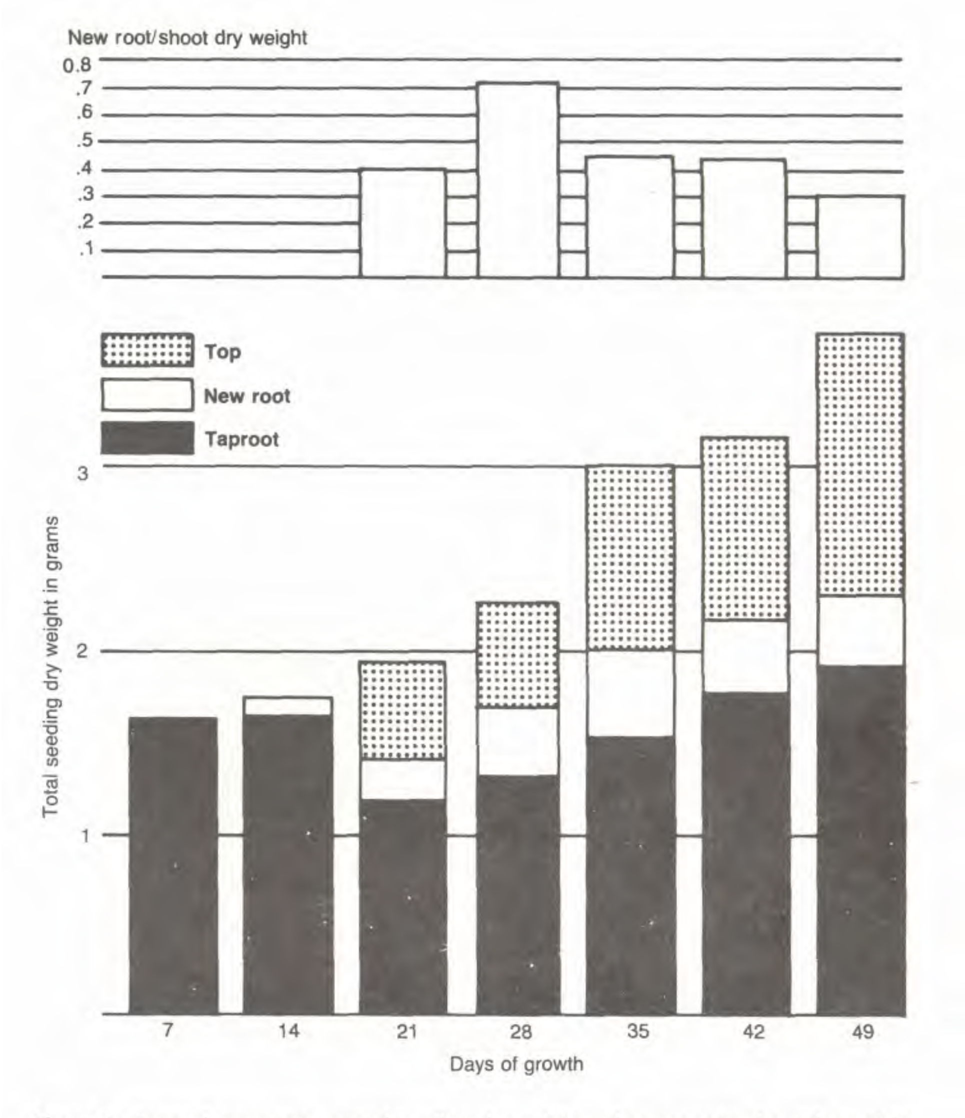

Figure 1—*Second-season mean total seedling dry weight and new root-to-shoot dry-weight ratio of potted, 1-0, dormant* Paulownia tomentosa *seedlings over a 49-day period. The mean is of three seedlings per entry. The total seedling dry weight is separated into three components.*

Figures 2 through 8 show the new growth from 1-0, bareroot seedlings over a 49-day period.

Figure 4 shows the dramatic increase in top and root development of seedlings after 21 days of growth. Most new shoots developed within a 3-centimeter zone below the root collar, but some appeared up to 6 centimeters below the root collar. Figure 5 shows an increase in secondary root development. New roots developed from many locations on the taproot including the callused zone at the cut end of the taproot.

This study shows the sequence of new shoot and root development and growth of 1-0, bareroot paulownia. These seedlings were grown under greenhouse conditions with adequate moisture, light, and atmospheric and soil temperatures in a porous, sandy medium, which allowed rapid development and growth. Field conditions would probably delay development and growth and alter

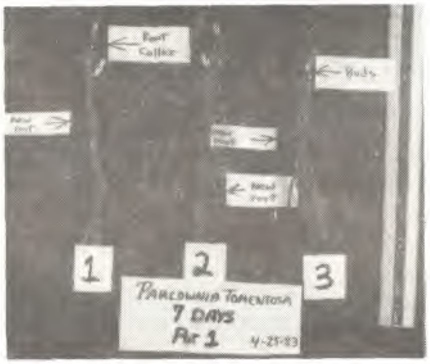

Figure 2—*Seedlings after 7 days of growth. Note early roots and shoot buds.*

Figure 3—*Seedlings after 14 days of growth. Note shoot elongation and shoot formation several centimeters below root collar.*

Figure 4—*Seedlings after 21 days of growth. Note the dramatic increase in root initiation and growth.*

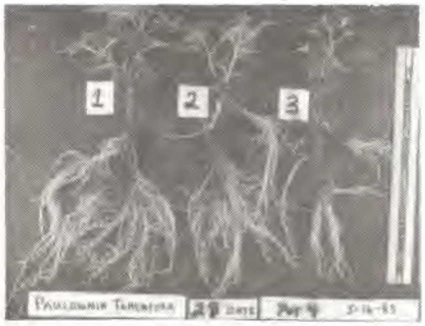

Figure 5—*Seedlings after 28 days of growth. Note the advanced development of a fibrous root system with new secondary lateral roots.*

Figure 6—*Seedlings after 35 days of growth.*

Figure 7—*Seedlings after 42 days of growth.*

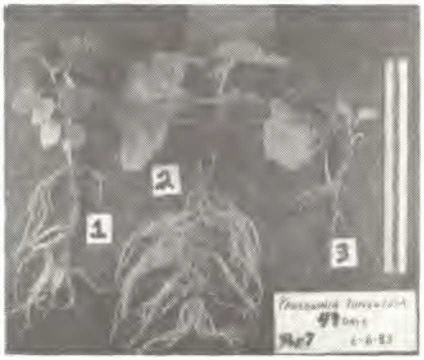

Figure 8—*Seedlings after 49 days of growth.*

new root-to-shoot ratios because of various edaphic and atmospheric variables. Nonetheless, this study may provide the tree planter a better understanding of how paulownias develop and grow after planting.

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Comparison of Quick- and Slow-Release Fertilizers in Young Plantings of Eucalyptus Species[1]

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Eucalyptus saligna and E. grand is seedlings fertilized twice per year over a 2-year period with a soluble fertilizer (12-24-12) were taller and had greater diameters than those given one application per year of this quick-release fertilizer or one application per year of the slow-release fertilizers Osmocote, Nitrogano, or Agriform.

Fertilization is required for maximum biomass production in short-rotation, intensively cultured eucalyptus forests in Hawaii (5). Along the Hamakua Coast of Hawaii Island, rainfall exceeds 5,000 millimeters annually and leaching of nutrients may be a problem in forest establishment and maintenance.

Slow-release fertilizers have been shown to stimulate the growth of trees, especially on nutrient-deficient sites (1, 2, 4, 7). Meskimen (4) demonstrated the beneficial effect of slow-release fertilizer tablets on the establishment and growth of *Eucalyptus camaldulensis* on sandy soils in Florida. Walters (7) found that the slow-release fertilizer Osmocote significantly increased the early growth of Australian toon and Queensland-maple seedlings on the strongly acidic forest soils of Hawaii Island. No previous work has been published concerning the effect of slow-release fertilizers on the early growth of eucalyptus in Hawaii.

It was thought that slow-release fertilizers might be beneficial for eucalyptus growth in high rainfall areas because of decreased leaching of soluble nutrients. Slow-release fertilizers make small concentrations of nutrients available over a long period of time (6), thus decreasing the amount of nutrients susceptible to leaching at one particular time.

This paper reports on two tests that were installed to compare tree growth and application costs associated with three slow-release fertilizers and a soluble fertilizer applied at two dosages in a short-rotation eucalyptus plantation.

Materials and Methods

Eucalyptus saligna Sm. and *E. grandis* hill ex Maiden. seedlings were outplanted in January 1980 at the Kamae site, located 24 kilometers to the northwest of Hilo, Hawaii. The elevation at Kamae is 480 meters and the average annual rainfall exceeds 5,000 millimeters. The test site is located in a poorly drained area that had formerly been in sugarcane production.

The soil belongs to the Akaka silty clay loam series (Thixotropic Isomesic Typic Hydrandept) and slopes range from 10 to 20 percent. Thirty soil samples were sent to Brewer Analytical Laboratory for determination of pH, total N, and sulfuric acid extractable P and ammonium acetate extractable K, Ca, and Mg. Color in the extracted P solution was developed by the phosphomolybdate method (3), and concentration was determined using a Klett-Summerson photoelectric colorimeter. Potassium was analyzed with a Beckman model D-U flame spectrophotometer, while other elements were analyzed with a Perkin-Elmer model 303 atomic absorption spectrophotometer. Results were averaged and compiled in table 1.

The site was disk-harrowed, then sprayed twice with glyphosate at 2.2 kilograms per hectare of active ingredients per application before outplanting. Tubed E. saligna and *E. grandis* seedlings, which averaged 30 centimeters in height, were outplanted at a 0.75- by 3.0-meter spacing. The plot size was 7.5 by 27.0 meters with 90 trees per plot.

The following five fertilizer treatments were placed in holes 15 to 20 centimeters away from the trees and covered with soil. Based on preliminary results from a eucalyptus N-P fertilizer trial at Akaka in Hawaii (5), fertilizer rates were calculated to give 12.6 grams of N per tree per application.

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[1] This research work was funded in part by the U.S. Department of Energy under subcontract 9061.

1. Soluble DC-153 fertilizer[2] (12-24-12) applied at planting and 12 months later (101 grams/tree per application).
2. Soluble DC-153 fertilizer (12-24-12) applied at planting and 6, 12, and 18 months later (101 grams/tree per application).
3. Slow-release Osmocote[3] (14-14-14) applied at planting and Osmocote (13.5-13.5-13.5) applied at 12 months (90 grams/tree per application).
4. Slow-release Agriform[4] tablets (20-10-5) applied at planting and 12 months later (three 21-gram tablets/tree per application).
5. Slow-release Nitrogano[5] (3-4-2.5) applied at planting and 12 months later (420 grams/tree per application).

The two experiments were laid out in a randomized complete block design with five treatments and four replicates of *E. saligna* in one test and five treatments and four replicates of *E. grandis* in a second test. Measurements of height of the inner 40 trees in a plot were taken at 3, 6, 9, 12, 18, and 24 months after outplanting. Diameter at breast height was measured at 18 and 24 months after outplanting. Analysis of variance was carried out to determine the significance of the treatment effect, and means were statistically separated by Duncan's multiple range test.

Results and Discussion

Levels of extractable P, K, Ca, and Mg in the soil were found to be low at this site (table 1). Preliminary results from a eucalyptus N-P fertilizer trial located 4 kilometers to the southeast of the Kamae site on soils belonging to the same soil series showed that the primary limiting nutrient was N and there was no significant growth response to Pat 12 months after outplanting (5). Based on these preliminary results, it was decided to apply soluble and slow-release fertilizers at a constant rate of N per application.

The annual rainfall recorded at the Honohina mauka weather station located 3 kilometers to the north of the Kamae plantings was 5,625 millimeters for 1980 and 3,282 millimeters for 1981. The first fertilizer treatments were applied at the time of outplanting in January 1980. In March 1980, more than 2,000 millimeters of rain fell along the Hamakua Coast during a major rainstorm. This rainstorm presumably increased fertilizer loss by leaching soluble nutrients below the rootzone of the seedlings. Fertilizer loss in the surface runoff was somewhat minimized by the subsurface applications of the fertilizers.

At 3 months after outplanting, there were no significant differences between fertilizer treatments in height of the *E. saligna* or *E. grandis* seedlings (figs. 1 and 2). By 6 months after outplanting, the

Table 1—*Soil pH, total N, and 0.02-N sulfuric acid extractable P and 1-N ammonium acetate extractable K, Ca, and Mg averaged from 30 sites at Kamae, Hawaii*

Depth	pH	N	P	K	Ca	Mg
		P/m				
0 to 15 cm	5.0	6,845	18	52	64	37
45 to 60 cm	5.1	4,701	10	43	40	13

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[2] DC-153 is composed of urea, treble superphosphate, and muriate of potash.

[3] Osmocote is a product of Sierra Chemical Co., Milpitas, Calif. Osmocote 14-14-14 is a 3- to 4-month-release fertilizer, while Osmocote 13.5-13.5-13.5 is a 6- to 7-month-release fertilizer.

[4] Agriform is a product of Sierra Chemical Co. In addition to N, P, and K, it contains 2.6 percent Ca, 1.6 percent S, and 0.35 percent Fe.

[5] Nitrogano is a product of Wilbur-Ellis Co., Los Angeles, Calif. It is derived from activated dried chicken manure and, in addition to N, P, and K, contains 5 percent Ca, 0.75 percent Mg, 0.35 percent Fe, and 50.0 percent organic matter.

eucalyptus trees fertilized with Osmocote were significantly taller than those given all other treatments (figs. 1 and 2), indicating the greater effectiveness of Osmocote fertilizer, particularly after heavy rains.

The eucalyptus trees that received a second application of soluble DC-153 fertilizer at 6 months after outplanting were significantly taller at 9 months than those given all other treatments (figs. 1 and 2). This second application at 6 months appeared to be critical since growth of the fertilized trees sharply increased between 6 and 9 months (figs. 1 and 2). Soluble nutrient loss in these plots was probably accelerated by the rainstorm in March, resulting in soil nutrient deficiencies by 6 months.

Eucalyptus trees receiving soluble DC-153 fertilizer at 6-month intervals generally outperformed all other treatments from 9 through 24 months after outplanting (figs. 1 and 2). At 24 months after outplanting, *E. saligna* trees given the double dosage of DC-153 fertilizer were significantly taller and had significantly greater diameters than those given all other treatments (table 2). At 24 months after outplanting, *E. grandis* trees given the double dosage of DC-153 fertilizer were significantly taller and had significantly greater diameters than those given all other treatments except for Osmocote (table 2).

Among the fertilizer treatments given once per year, eucalyptus trees fertilized with Osmocote were the tallest from 6 through 24 months after outplanting (figs. 1 and 2). At 24 months after outplanting, Osmocote-fertilized *E. saligna* trees were significantly taller than those given a single application per year of the other fertilizers, except for Nitrogano (table 2). In the case of *E. grandis,* the height of Osmocote-fertilized trees was significantly greater than those given Agriform (table 2).

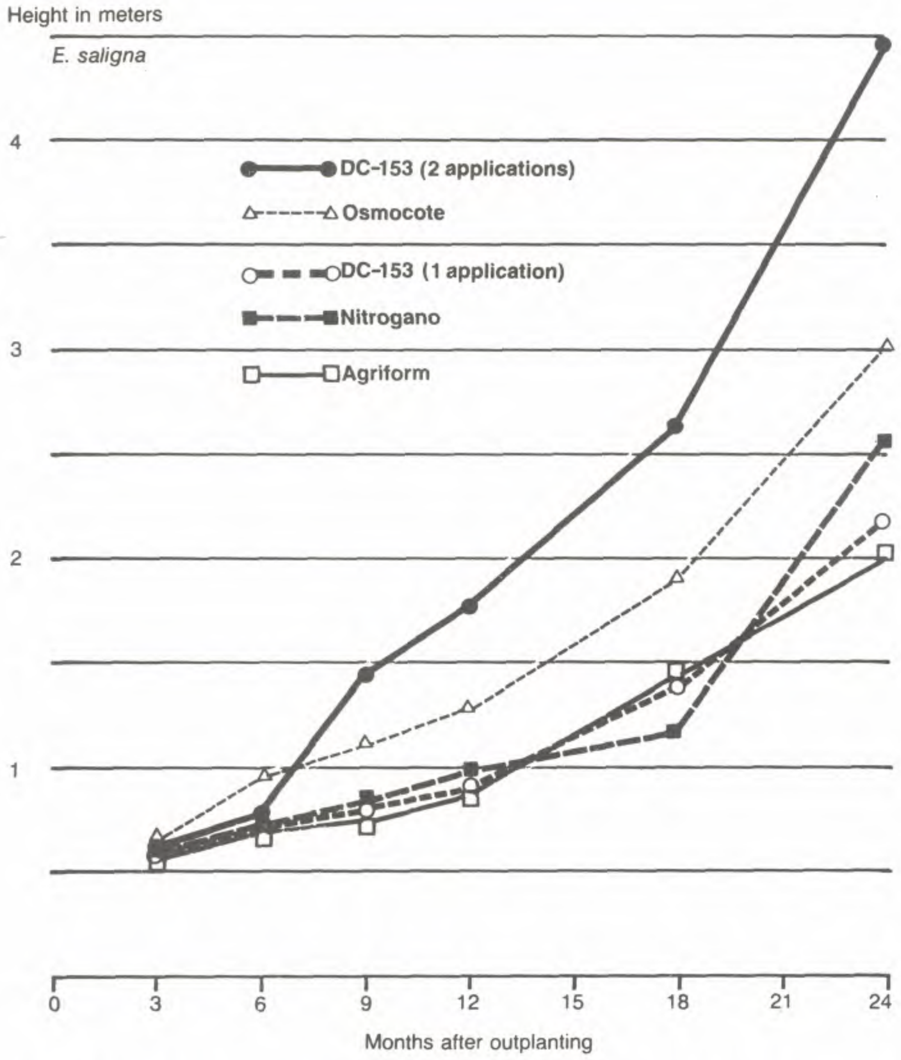

Figure 1—*The effect of various slow- and quick-release fertilizers on height growth of* E. saligna *over a 24-month period.*

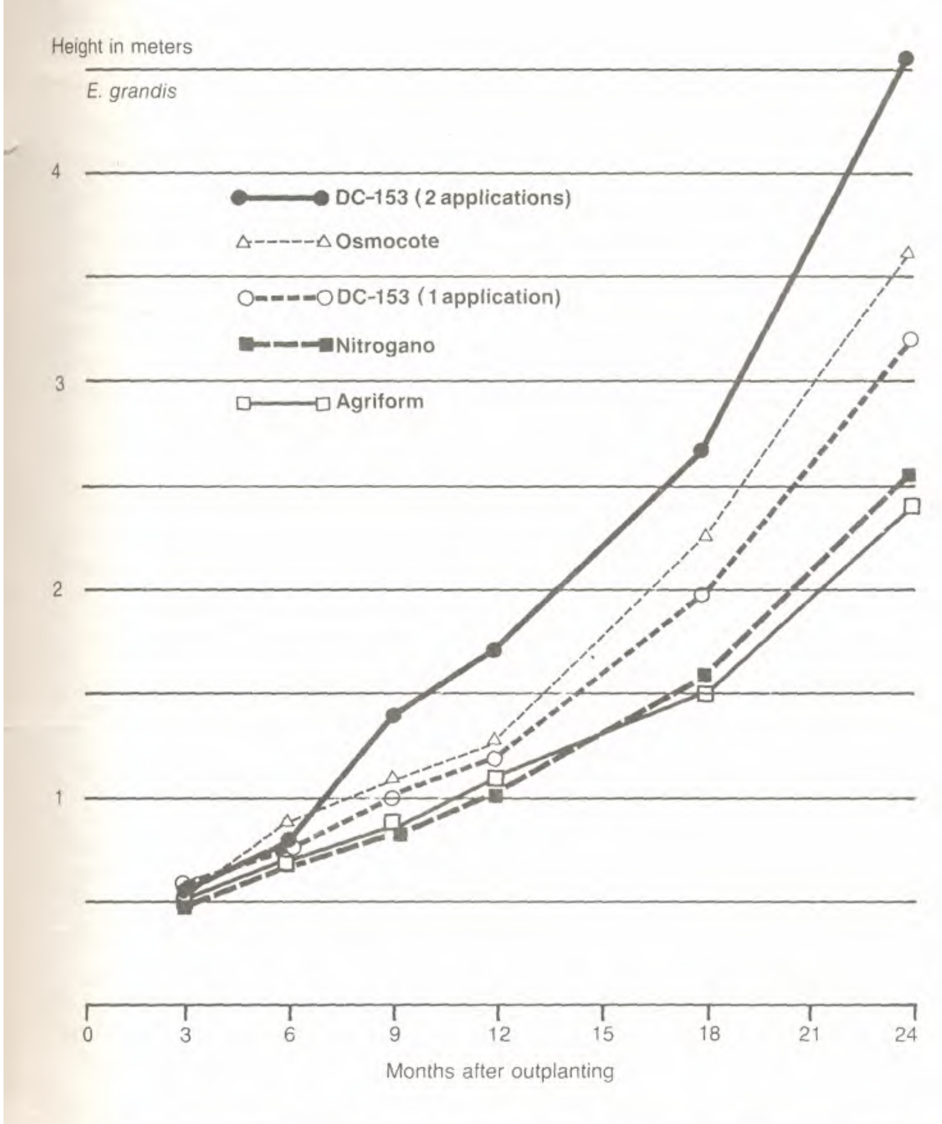

Figure 2—*The effect of various slow- and quick-release fertilizers on height growth of E. grandis over a 24-month period.*

At 24 months after outplanting, *E. saligna* trees fertilized with Osmocote had significantly greater diameters than those given a single application per year of all other fertilizers (table *2).* In the case of *E. grandis,* trees fertilized with Osmocote had significantly greater diameters than those given a single application per year of any other fertilizer, except for DC-153 (table 2). The greater effectiveness of Osmocote, relative to all other fertilizers applied once per year, could be because of its temperature-dependent release of nutrients (6), which would reduce the soluble nutrient concentration available for leaching.

Fertilization costs were calculated to include both materials and labor (table 2). The least expensive fertilizer treatment was the single application per year of soluble DC-153, while the most expensive treatment was Agriform (table 2).

The double application per year of soluble DC-153 fertilizer (12-24-12) resulted in the best growth of *E. saligna* and *E. grandis* compared to all other treatments at 2 years after outplanting (figs. 1 and 2, table 2). The second best growth of eucalyptus trees at 2 years after outplanting was found in Osmocote-fertilized plots (table 2). The cost of a double application per year of soluble DC-153, however, was less than the single application per year of Osmocote fertilizer (table 2).

Conclusions

The double application per year of soluble DC-153 fertilizer (12-24-12) produced the greatest growth of *E. saligna* and *E. grandis* compared to all other treatments at 2 years after outplanting. Slow-release fertilizers, such as Agriform, Nitrogano, and Osmocote, were more expensive than the double

Table 2—*The effect of various slow- and quick-release fertilizers on height and diameter at breast height (d.b.h.) of E. grandis and E. saligna at 24 months after outplanting and the associated costs of the fertilizer treatments*

Fertilizer treatment	*E. grandis*[1]		*E. saligna*		Costs per year per hectare		
	Height	D.b.h.	Height	D.b.h.	Labor[2]	Materials[3]	Total
	M	*Cm*	*M*	*Cm*	*Dollars*		
DC-153 (1 application)	3.2bc	2.3bc	2.2cd	1.3cd	95	160	255
DC-153 (2 applications)	4.6a	3.7a	4.5a	3.7a	190	321	511
Osmocote	3.6ab	2.7ab	3.0b	2.3b	95	494	589
Agriform	2.4c	1.4c	2.0d	1.1d	95	776	871
Nitrogano	2.6bc	1.6c	2.6bc	1.6c	95	427	522

[1] Means in a column followed by the same letter are not significantly different at the 95-percent probability level as determined by Duncan's multiple range test.

[2] Labor costs were calculated at $35 per day of labor with 2.7 days of labor needed to apply fertilizer per hectare.

[3] Material costs were based on bulk order rates. DC-153 cost $0.37 per kilogram. Osmocote 14-14-14 or 13.5-13.5-13.5 cost $1.28 per kilogram. Agriform cost $2.86 per kilogram. Nitrogano cost $0.24 per kilogram.

application per year of soluble DC-153.

The growth of the *E. grandis* given one application per year of soluble DC-153 fertilizer did not significantly differ from that of trees given slow-release fertilizers. In the case of *E. saligna*, only Osmocote-fertilized trees among those given slow-release fertilizers grew significantly more than trees given a single application of DC-153. The cost of the single application per year of DC-153 was less than half that of any slow-release fertilizer.

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Predicting Cold Hardiness of Douglas-Fir Nursery Stock With an Oscilloscope/Square-Wave Apparatus

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The oscilloscope/square-wave apparatus was not useful in predicting the cold hardiness of Douglas-fir nursery stock. No correlations were observed between square-wave trace form and damage or kill in seedlings subjected to freezing tests.

Efforts to evaluate the quality of forest nursery stock have included investigations of dormancy and cold hardiness. Dormancy and cold hardiness often occur in parallel (5). As conifer tree seedlings are exposed to lower temperatures, hardiness increases, a progression that often coincides with the development of the dormant state as reviewed by Cleary and others (2). If dormancy and cold hardiness are correlated, a method that measures the dormant state might also be used to estimate cold hardiness.

Ferguson and others (3) developed an oscilloscope/square-wave apparatus to evaluate dormancy in nursery stock. This article reports the final results of a further study using the same procedures to predict cold hardiness of Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) seedlings. Preliminary results of the current study were reported earlier (4).

Materials and Methods

The Douglas-fir seedlings used were from the Humboldt Nursery at McKinleyville, Calif., and the D. L. Phipps State Forest Nursery at Elkton, Oreg. Seedlings were from two seed sources at the 1,500-foot (457 m) elevation in the Umpqua River drainage of southwest Oregon. Samples were lifted in mid-September, mid-October, and mid-November of 1978.

Seedlings were classified as very active, slightly active, dormant, or dead, according to their response to an electric current delivered through an electrode inserted in the tissue just below terminal bud (fig. 1). Responses were classified visually according to trace patterns observed on an oscilloscope screen (fig. 2). The equipment used for this procedure was a portable oscilloscope (Tektronix Model 212) connected to a portable square-wave generator (Wavetek Model 30) (fig. 3). Attached to this instrument was an electrode consisting of four surgical stainless steel needles in clear plastic.

Figure 1—Douglas-fir seedling with electrode from oscilloscope/square wave apparatus attached.

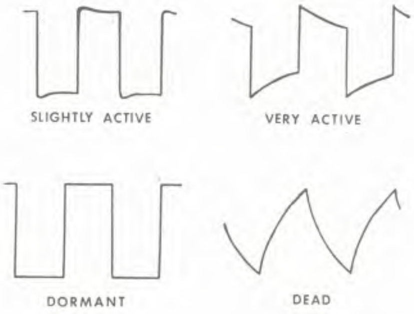

Figure 2—Growth conditions indicated by square-wave trace forms (3).

Trace patterns were observed on 18 groups of 10 seedlings immediately after each lifting at the two nurseries (hereafter called nursery observations). These seedlings were then discarded. Another 18 groups of 10 seedlings were potted after lifting. These were used for laboratory freezing tests and were not subjected to oscilloscope readings. A third set of five seedlings per group was potted and subjected to both freezing and square-wave analysis in the laboratory.

The freeze test was made at —4° C for 3 hours, 2 to 3 weeks after potting. After 6 weeks in a greenhouse under growing conditions, seedlings were classified as survivors if they had not been killed or as severely damaged if more than half the foliage was brown and the terminal bud injured.

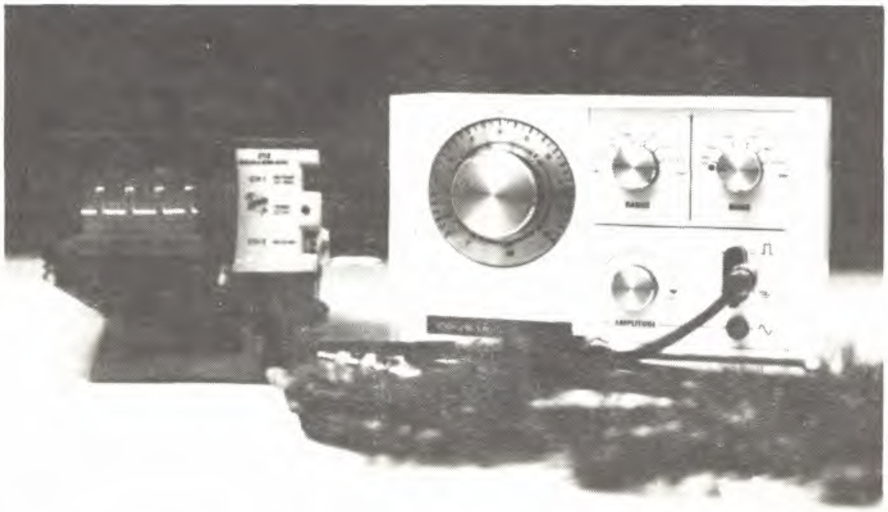

Figure 3—*Portable oscilloscope/square-wave apparatus used for square-wave observations.*

Results and Discussion

Test results (table 1) show no similarities between the percentages of seedlings classified as dormant by square-wave trace form and the percentages surviving freezing tests. For example, the highest percentage of seedlings classified dormant and the lowest percentage of seedlings surviving the freezing tests were from laboratory observations made in November on stock from both nurseries. Also, the lowest percentage of dormant seedlings and the highest percentage of survivors were from October observations of Phipps stock. Similarly, there were no correlations between percentage of seedlings dormant as determined by square-wave form and percentage undamaged by freezing tests in the 1977 tests (4).

We would expect that as dormancy and cold hardiness increase from September through November, wave-form indications of dormancy would also increase. A slight increase in the percentage of dormant seedlings from September to November occurred only in laboratory observations of Humboldt stock.

We would also expect that as cold hardiness of seedlings increases from September through November, severe damage or mortality following freeze tests would decrease. This did not occur. In all cases, there were fewer survivors in November than in September. Careful review of handling and testing procedures did not reveal any explanation for this result. Whatever the reason,

Table 1—*Comparison of dormant seedlings, as determined by square-wave form, and seedling survivors following freezing tests of Douglas-fir seedlings from the Humboldt and D. L. Phipps nurseries in 1978 tests of cold hardiness*

	September		October		November	
Nursery	Dormant	Survivors	Dormant	Survivors	Dormant	Survivors
			%			
Humbold						
Nursery observations	7	99	0	96	0	92
Laboratory observations	1	95	5	97	36	87
D. L. Phipps						
Nursery observations	3	87	1	96	3	82
Laboratory observations	4	85	0	94	32	79

square-wave readings did not give a good prediction of response to freezing.

The percentage of seedlings severely damaged or dead was greater in those subjected to freezing tests than in unfrozen seedlings placed under similar growing conditions. Higher mortality in frozen seedlings than in unfrozen seedlings indicates that mortality in the frozen seedlings was related to the treatment and thus to lack of cold hardiness.

The two sets of oscilloscope trace observations, one immediately following lifting at the nursery and one shortly before freezing tests, were carefully made to minimize factors that might influence test results, such as the time between lifting and freezing tests and the use of two separate samples or the same sample for observations of both square-wave trace and freeze damage. Using two separate samples for dormancy readings and freeze tests did not give results different from those made on the same seedlings.

Askren and Hermann (1) used ratios of voltage measurements on the oscilloscope/square-wave apparatus as a more quantitative way to detect dormancy and survival potential of Douglas-fir seedlings. Although their observations demonstrated a seasonal trend in square-wave trace form, they concluded that the technique offered little promise in predicting survival potential. In studies of the relation of electrical impedance to vegetative maturity and dormancy in red-osier dogwood, Parmelee (6) ruled out use of the oscilloscope/square-wave technique because of difficulties she encountered with interpretation and reliability of the square-wave trace.

Conclusion

Because of the lack of similarities between the percentages of dormant seedlings (as determined by the oscilloscope/square-wave apparatus) and survivors of freezing in this study, it appears that the oscilloscope/square-wave technique (3) is not useful in measuring cold hardiness of Douglas-fir nursery stock.

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Pocket Gophers Girdle Large True Firs in Northeastern California

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Serious damage to trees by pocket gophers is thought to be limited to those less than 5 inches (12.7 cm) in diameter at breast height (d.b.h.). This article reports the first known instance of high mortality of large true firs—up to 36.9 inches (93.7 cm) d.b.h.--caused by pocket gophers and evidence of heavy and repeated feeding on major roots of mature trees.

Pocket gophers *(Thomomys* spp.) can be a serious problem in forest environments, especially at higher elevations in the Western United States, where they are frequently a major cause of mortality in young plantations *(1)*. Gophers feed heavily on trees when their preferred foods—various roots, tubers, bulbs, and herbage—are not available, usually in winter. Serious damage by gophers generally is limited to below ground portions of trees, except where the winter snowpack allows the rodents to travel aboveground through tunnels dug in the snow. Damage then is deep gnawing of branches and stems *(2)*. Although large trees suffer a low level of root loss, serious damage by pocket gophers is generally believed to be limited to trees less than 5 inches (12.7 cm) in diameter at breast height (d.b.h.) *(3).*

This article reports the first known instance of mortality in large (up to 36.9 inches (93.7 cm) d.b.h.) true fir *(Abies spp.)* resulting from pocket gophers.

Situation

Damage to trees was discovered surrounding and within a 4-acre (1.7 ha) upland meadow adjacent to the southwestern side of a 23-year-old, 19-acre (7.7 ha) clearcut strip, 330 feet (101 m) wide and 2,470 feet (753 m) long. The clearcut and meadow are located at a 6,100-foot (1,859 m) elevation on the USDA Forest Service's Swain Mountain Experimental Forest in northeastern California. A young-to-mature, virgin stand of California red fir *(A. magnifica* A. Murr.) and California white fir *(A. concolor* var. *lowania* [cord.] Lemm.) surrounds the meadow on three sides. The meadow contains a rich variety of grasses and forbs dominated by lupine *(Lupinus* spp.). The clearcut contains a mixture of grasses; woody brush; and young red fir, white fir, and Sierra lodgepole pine *(Pinus contorta* var. *murrayana* [Grey. & Balf.] Engelm.). The meadow, originally about 3.5 acres (1.4 ha), expanded slightly into the clearcut and now includes some residual trees, submerchantable at the time of harvest. Meadow boundaries are delineated sharply, and vegetation changes to forest or clearcut are abrupt. Abundant gopher burrows, fan-shaped mounds, earthen plugs, and winter casts (dirt cores through the snow) from tunnels provided ample evidence of gopher activity in the meadow.

Observations

In 1978, two small (less than 6 inches (15.2 cm) d.b.h.) residual trees died. A few small trees died in each of the next 2 years. In 1981, several apparently healthy, large trees suddenly faded and died. Preliminary excavations with a shovel indicated the cause of death to be girdling, probably by gophers.

In September 1981, a sample of trees, including apparently healthy, fading, and dead trees was excavated. All trees except one had suffered some loss of living tissue from root crowns and major roots (table 1). At least four trees had been girdled for more than 90 percent of their circumference, but still appeared green and healthy in September, indicating that feeding was recent. Damage was limited to the zone from just below the forest floor where the thick, furrowed bark of the stem turned into the thin, succulent bark of the roots, to the depth where roots penetrated a layer of large stones. Callus tissue on major roots of several large trees clearly indicated that these feeding attacks had occurred for many years (fig. 1). The number of trees killed and the area affected expanded in 1982.

Several apparently healthy, if slow-growing, residual trees in the clearcut blew over during winter

1981-82. Little was left of structural roots except a strip of living tissue along the top of otherwise heavily decayed exposed wood.

Although no pocket gophers were observed or trapped, physical evidence available indicated that they had caused the damage. An extensive, typical, pocket-gopher burrow system throughout the loose soil above a layer of large stones; burrows immediately adjacent to recently gnawed tree roots; one burrow plugged with fresh bark strips; and marks typical of gopher teeth on the stripped tree roots were observed.

Two species of gopher possibly could have been responsible, although the 6,200-foot (1,890 m) elevation strongly implies that the mountain pocket gopher *(Thomomys monticola)* was the damaging agent. The other possi-

Table 1—*Damage by pocket gophers to large true firs on edges of meadow and old clearcut, Swain Mountain Experimental Forest, Calif.*

Species	Diameter at breast height	Amount of tree base excavated	Portion of phloem missing in excavated area	Color of foliage[1]
	In	%		
Red fir	36.9	50	50	Green
Red fir	28.7	50	100	Brown
Red fir	25.4	100	100	Green
White fir	24.7	100	70+	Green
Red fir	21.7	100	90	Green
Red fir	21.2	100	95+	Green
Red fir	19.4	50	100	Brown
Red fir	19.0	30	100	Brown
Red fir	17.5	100	100	Green
Red fir	16.2	30	100	Brown
White fir	16.2	30	85	Green
Red fir	16.0	100	None	Green
Red fir	15.7	50	100	Green
Red fir	15.4	50	100	Brown
Lodgepole pine	15.4	50	45	Green
Red fir	15.2	100	5	Green
Red fir	11.8	50	100	Green
White fir	7.7	100	100	Brown
White fir	6.2	50	100	Brown
Red fir	5.8	50	100	Green

[1] At time of excavation in September 1981.

Figure 1—*Bark and phloem tissue have been eaten by pocket gophers in the base of this large (36.9 inches (93.7 cm) d.b.h.) red fir. The edge of the newly gnawed callus tissue that has partially healed, shown at the right, indicates a previous episode of feeding. (Photo by D. F. Roy)*

bility, Botta's pocket gopher *(Thomomys bottae),* usually is found only below a 5,000-foot (1,524 m) elevation (4).

Conclusions

The apparently isolated occurrence of gopher damage to trees between 5.8 and 36.9 inches (14.7 and 93.7 cm) d.b.h. on Swain Mountain Experimental Forest does not necessarily indicate serious problems elsewhere, but it demonstrates that pocket gophers can cause costly local damage to crop trees far beyond the regeneration stage. Removal of living root tissue over large areas of major structural roots may lead to massive infection by pathogenic and saprophytic fungi in exposed heartwood. Repeated feeding on tissue of major, first-order roots may also lead to significant growth losses on large trees.

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Root Deterioration of Black Walnut Seedlings During Overwinter Storage in Wisconsin

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Black walnut seedlings suffered root damage resulting from subfreezing soil temperatures during overwinter storage in nursery- and heeling-in beds. This problem can be avoided either by mulching beds in the fall or by winter storage of bareroot seedlings in shipping bags at 3° C.

In recent years, the demand for quality black walnut (*Juglans nigra* L.) has resulted in an increase in walnut plantings throughout the Midwestern United States. To ensure proper density and uniformity, new plantations are established with 1-year-old (1-0) nursery transplants, rather than by direct seeding. In Wisconsin alone, 150,000 to 300,000 black walnut seedlings are distributed annually.

One of the limiting factors in the production of 1-0 walnut seedlings is losses from root rot. Root rots may be a problem both in the seedbed during the growing season and in overwinter storage of the trees. Seedling root rot caused by *Cylindrocladium* spp. is a serious problem in nurseries in the Southeastern United States *(1, 6)*, while *Phytophthora citricola* Sawada is considered to be the major root pathogen of walnut seedlings in Indiana *(3, 5)*. So far, in Wisconsin, seedling root rots caused by these fungi, though present, have not produced extensive damage *(7)*.

In contrast, root deterioration of black walnut seedlings during overwinter storage has resulted in heavy mortality in Wisconsin in some years. Previous studies in other States indicate that both environmental *(8)* and biotic agents *(3, 4)* may be involved in storage losses. The purpose of this study was to evaluate several methods of overwinter storage and to identify factors contributing to seedling root deterioration during storage.

Materials and Methods

One-year-old black walnut seedlings, raised at the Wilson State Forest Nursery, Boscobel, Wis., were used in all studies. Seedlings were grown from open-pollinated nuts collected in southern Wisconsin and northern Iowa. Fall-lifted seedlings were undercut approximately 20 centimeters below the soil surface the first week in November of each year. Undercut seedlings were mechanically lifted, hand-graded, and bundled into groups of 20 or 25 trees. Those trees that had a taproot of less than 20 centimeters in length or a root system showing discoloration were culled before bundling. Spring-lifted seedlings were undercut and lifted the first or second week in April.

During the winter of 1980-81, fall-lifted trees were stored either in heeling-in beds or in heavy paper shipping bags. The heeling-in method consisted of placing the bundled, graded seedlings in trenches, 30 to 45 centimeters deep, dug in the nurserybed. The roots were covered with soil and the seedlings stored in this manner until they were lifted in the spring. Five bundles of 20 seedlings each were placed in a heeling-in bed and then mulched to a depth of 40 to 60 centimeters with marsh hay. Five other bundles were placed in a heeling-in bed that was not mulched. Other fall-lifted, bundled trees were placed in shipping bags and stored overwinter in a large walk-in cooler at 3° C. The relative humidity inside the bags remained above 90 percent throughout the storage period.

Several root treatments were applied to bundled, bareroot seedlings before bagging. Bundled seedlings were root-dipped for 1 minute in a fungicide suspension of benomyl (Benlate 50% WP at 6 g active ingredient/l) or captafol (Difolatan 4F at 5 g active ingredient/l). Another set of untreated bundles was placed into bags containing wetted marsh hay. Bundled seedlings not treated or root-dipped in water only served as controls. Each of the five treatments was replicated in five bags, with 20 seedlings per bag.

In 1981-82, the same treatments were used except that each treatment was replicated four times, with four bundles of 25 seedlings in each bag. Also, another treatment was added. The roots of four bundles were packed with wetted marsh hay, then covered with corrugated paper. The seedling tops remained exposed during storage in the cooler.

When the bags were opened in the spring, each bag was rated on a 1-to-3 scale for the amount of root and top mold present. Those bags having little or no superficial mold buildup on the bundled seedlings were graded as 1.0; those having a moderate amount of molding were graded as 2.0; and those having heavy mold on both roots and tops were graded as 3.0.

Further studies were conducted in 1981-82 to determine the effect of mulching and storage method on the incidence of root rot. Three adjacent plots (13 m^2) were established in a portion of the walnut nurserybed in late October. Trenches were dug between the rows of seedlings, and 16 bundles (25 trees/bundle) of fall-lifted seedlings were heeled-in per plot. One plot was then mulched to a depth of 40 to 60 centimeters with marsh hay. The second plot received no mulch. A sturdy polyethylene tent, supported by a wooden framework, was built over the third plot. The sides of the tent were open: that there was no impairment of air movement through the plot. The purpose of the tent was to prevent snow deposits, thereby simulating conditions in the bed during winters without snow cover.

Soil temperatures in each plot were monitored by thermistors buried 13 centimeters beneath the soil surface. Minimum and maximum temperatures for 5-day intervals from November 28, 1981, through April 3, 1982, were recorded with a CR 21 data logger (Campbell Scientific, Logan, Utah).

Fall-lifted, bundled seedlings were also stored in bags at 3° C or at —2° C. In the case of frozen storage, bags were held 2 months at 3°C before placement at —2° C. Four bags, each containing four bundles of 25 trees, were placed at each storage temperature.

All trees were examined the first week in April. The incidence of root rot and overall appearance of the trees after storage were recorded. For some treatments, seedlings were outplanted in the nursery. Seedling survival was recorded in August 1982.

Results

During the winter of 1980-81, there was no accumulation of snow on the nurserybeds. Seedlings that were left over winter in the beds and then lifted in April had extensive root rot. Surveys of the seedbed showed that 60 percent of the seedlings had complete taproot mortality, and many of the remaining trees had varying degrees of root deterioration. When pulled from the ground, injured roots were rubbery and discolored. The cortex of rotted roots was water-soaked and light brown to black. The discoloration never extended beyond the root collar region. This was in sharp contrast to the pearl-white cortex of healthy roots. Isolations were made from the rotted tissue, but no fungi or bacteria were isolated consistently. Because seedling mortality in the nursery was so high and the identification of healthy trees was so uncertain, the entire stock of over 150,000 black walnut seedlings was condemned.

Fall-lifted seedlings placed in heeling-in beds without mulch also had a high percentage (75 percent) of root rot and a low survival rate (14 percent) after outplanting (table 1). Mulching of the heeling-in beds reduced root rot incidence to 7 percent. Seedlings that were bagged and stored at 3° C had almost no root rot. Root treatments before bagging had no influence on the incidence of root rot. However, one problem associated with bag storage was the epiphytic colonization of roots and stems by fungi during the winter. When the bags were opened in the spring, we noted a sparse, unsightly covering of mycelium on the roots, but roots were still sound and the superficial mycelium did not affect seedling survival after outplanting. Both the benomyl and captafol root-dip treatments prevented molding on the roots and reduced molding of the stems.

Table 1—*Effect of storage method and root treatments on root rot incidence, superficial molding, and survival of black walnut seedlings after outplanting*

Storage method[1]	Treatment	1980-81 Root rot[2]	1980-81 Survival[3]	1981-82 Root rot	1981-82 Mold index[4]
		%	%	%	
Bagged, 3° C	No-dip	2	98	0	2.5
Bagged, 3° C	Water dip	0	96	1	2.5
Bagged, 3° C	Benomyl dip	0	100	0	1.0
Bagged, 3° C	Captafol dip	1	97	0	1.0
Bagged, 3° C	Root bundle	—[5]	—	0	1.5
Heeling-in	Straw mulch	7	87	—	—
Heeling-in	No mulch	75	14	—	—

[1] Seedlings were fall-lifted, bundled, and stored either in paper shipping bags in coolers at 3° C or placed in heeling-in beds in the nursery.
[2] Mean percentage of root rot for five replicates (20 seedlings/replicate) in 1980-81 and four replicates (100 seedlings/replicate) in 1981-82.
[3] Mean percentage of survival of seedlings after outplanting for five replicates.
[4] Superficial molding of roots and stems rated on 1-3 scale, where 1.0 = slight or no molding of the roots in each bag, 2.0 = approximately 50 percent of the seedlings showing some mold, and 3.0 = heavy molding of all seedlings.
[5] — = not available.

Root treatments of bagged seedlings in 1981-82 gave results similar to those in 1980-81 (table 1). Almost no root rot was found in any treatment; however, the molding of bagged seedlings was heavy in the no-dip and water-dip treatments. Molding was prevented by root-dip treatments with benomyl or captafol.

In the winter of 1981-82, the entire nursery was covered with snow from the first week in January to the second week in March. Snow depths ranged from 22 to 38 centimeters. Spring-lifted seedlings that had been mulched with marsh hay or had snow cover survived the winter in excellent condition (table 2). Samples taken throughout the seedbed showed less than 1 percent of the seedlings had discolored roots. These appeared to be carry-over infections of *P. citricola* during the growing season. Root rot was not found in those heeling-in plots that had been covered by snow or hay and snow.

Soil temperatures in both the mulched and nonmulched plot with snow cover were similar (fig. 1). Soil temperatures dropped to the freezing point in early December. In the nonmulched plot, minimum temperatures dropped to —4.5°C in mid-December, but then increased as snow cover accumulated on the plot. Soil temperatures remained constant and near 0° C in both the mulched and nonmulched plot throughout much of the winter.

In contrast, soil temperatures in the bare-ground plot had great temperature fluctuations throughout the winter (fig. 1). Soil temperatures dropped to —14°C in Feb-

Table 2—*Effect of storage method on the incidence of root rot during overwinter storage in 1981-82*

Storage method	Treatment	Root rot	Survival	Stem necrosis
		%	%	%
Bagged	3° C	0[1]	—[2]	5.6
Bagged	−2° C	0	98.0	1.6
Heeling-in	Mulch + snow	0	—	5.0
Heeling-in	Snow only	0	98.0	1.4
Heeling-in	Bare ground	74.4	31.2	4.2
Spring-lift	Mulch + snow	0	—	5.3
Spring-lift	Snow only	0	—	4.6
Spring-lift	Bare ground	59.6	—	1.2

[1] Percentages are averages from four replicates containing 100 seedlings per replicate.
[2] — = not available.

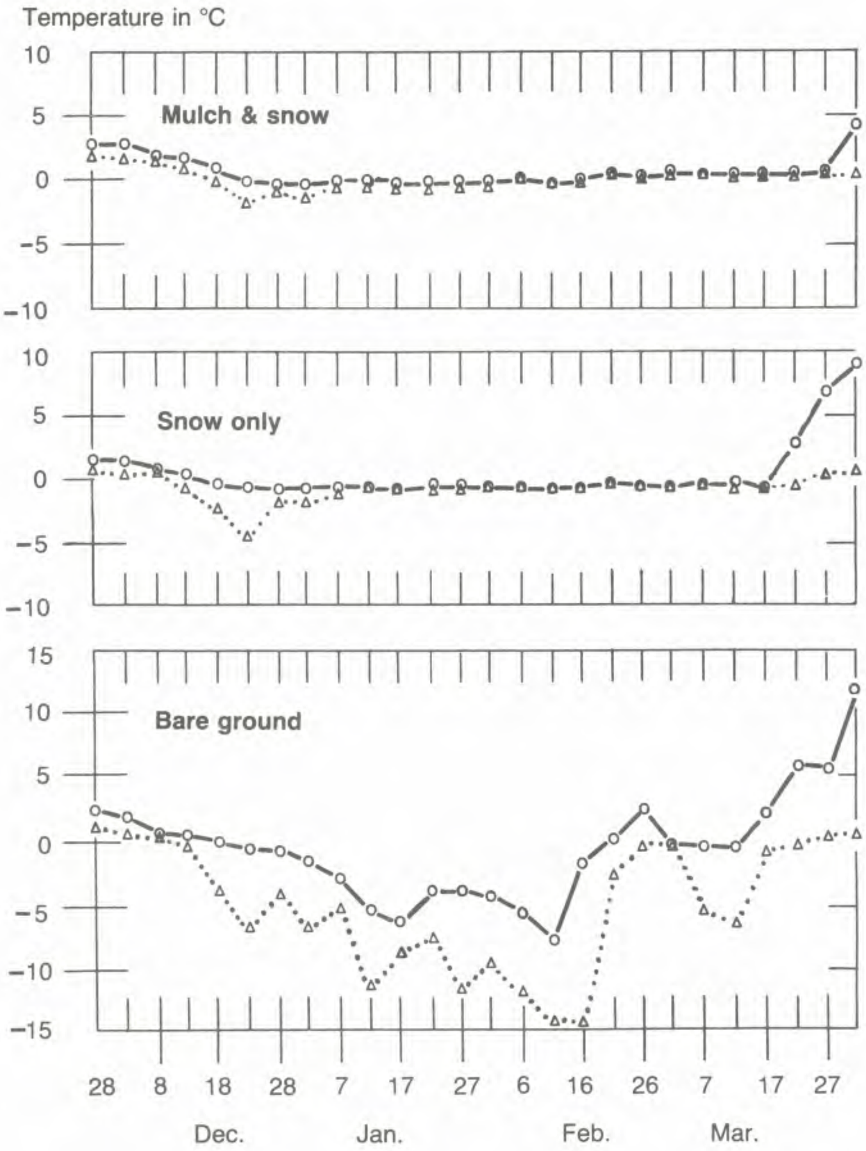

Figure 1—*Five-day minimum and maximum soil temperatures for nurserybed plots having mulch plus snow cover, snow cover only, or bare ground during the winter of 1981-82. Soil temperatures were taken at a depth of 13 centimeters.*

ruary. Trees overwintered in this plot had a high incidence of root rot (table 2). An average of 74 percent of the fall-lifted seedlings in the bare-ground heeling-in bed suffered root mortality.

Seedlings that were bagged and stored at 3°C or —2°C had no root rot (table 2). It was noted that, in all treatments, some seedlings had discoloration of the taproot where it had been cut upon lifting. This discoloration did not extend above 1 or 2 centimeters from the base of the root.

In the 1981-82 experiment, 3.5 percent of all seedlings had discoloration of the stem. These seedlings were found in all storage treatments, indicating that the damage occurred before fall lifting. No fungi or bacteria were isolated consistently from the discolored areas.

Discussion

In past years, overwinter storage of black walnut seedlings in nurserybeds or heeling-in beds resulted in extensive root rot. Our results indicate that root deterioration during overwinter storage in Wisconsin is caused principally by cold temperature damage to the roots. In both years, root damage was confined to those seedlings stored outdoors in nonmulched plots. Soil temperatures at a depth of 13 centimeters in the nonmulched areas often dropped below —10°C during January and February.

Young *(8)* found that bareroot walnut seedlings, exposed to —12°C for 30 minutes, developed root rot during overwinter storage. He also found a high incidence of root rot in both mulched and nonmulched heeling-in beds in sandy soil in an Iowa nursery. This is similar to the results we obtained in Wisconsin. It is still not known whether the roots are injured by low temperatures or by fluctuations in temperatures in the nurserybed, which may result in freezing and thawing of root tissue during storage.

Our results suggest alternative methods for storage of black walnut seedlings during the winter months. Root rot can be avoided by mulching the beds in the fall to a depth of 20 centimeters with marsh hay. Other mulches also would be suitable. Spring-lifting of mulched beds and immediate distribution of seedlings are recommended where cold storage facilities are not available. Because Wisconsin is located along the northern limits of the geographic range of blackwalnut, some loss in nursery stock should be expected. Trees that are susceptible to early frost or are not winter-hardy will be killed. We believe that the stem discoloration found in 1981-82 probably was caused by an early frost in the fall, since a biotic agent was not isolated and the damage was found in all treatments. However, Green *(2)* has reported a discoloration of walnut stems caused by *Phomopsis elaeagni* and has suggested that this pathogen maybe responsible for top damage previously ascribed to frost injury. Further studies and isolations should be made to confirm the cause of tip dieback in Wisconsin.

We believe that the best method of storage in Wisconsin is fall-lifting and storage of seedlings indoors at a constant temperature. Moreover, fall-lifting and cold storage may be more convenient for nursery managers, enabling them to avoid the spring rush in lifting other tree species. Bareroot seedlings can be stored in sealed shipping bags at 3°C throughout the winter with little or no root rot. Seedlings dc; develop epiphytic molding during storage in bags, but it does not appear to affect survival. Both the benomyl and captafol dips prevented molding of the roots. Green and Plourde *(4)* previously reported reduction in molding by the use of captafol. While molding did not affect survival, it was unsightly and could reduce the marketability of bagged seedlings. Top molding could also be prevented by wrapping the roots in wetted straw and corrugated paper, but leaving the tops exposed.

Results from frozen storage experiments at —2° C are promising. Seedlings stored below freezing had no root rot or superficial mold. Survival after outplanting was also very high. Frozen storage might eliminate the need for fungicide dips to prevent molding. Care should be taken to avoid fluctuating temperatures. Previous experiments *(7)* have indicated that cold storage at —5°C results in a decrease in survival over seedlings stored at 3°C.

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Stratification and Germination of Arkansas Oak Acorns

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Results indicate there are approximately 390 Arkansas oak acorns per pound. Germination is best when acorns are stratified for 120 days.

Arkansas oak (*Quercus arkansana* Sarg.) is a medium-sized tree reaching 70 feet in height and 2 feet in diameter. Its distribution is limited largely to the sandy soils and hills of southwestern Arkansas, southeastern Alabama, northern Florida, and southwestern Georgia. The leaves, which are remotely three-lobed, are similar to those of blackjack oak (*Quercus marilandica* Muench.). The bark closely resembles that of water oak (*Quercus nigra* L.), being smooth when young, but becoming rough with scaly ridges when older *(1)*. This tree has been suggested as a hybrid between black jack oak and water oak *(5)*, but it is now considered a separate species *(2)*.

Oaks are commonly classified into two groups: the white oak group and the red oak group. With a few exceptions, the members of the white oak group produce acorns that do not require stratification. Acorns of the red oak group may require from 30 to 120 days of cold stratification for optimal germination.

Arkansas oak belongs to the red oak group, and its acorns require an overwintering period before germination. Little information is available concerning the cold stratification requirements and acorn yields of Arkansas oak. This information is needed by nursery personnel for efficient seed handling and growing of seedlings. This study was conducted to determine the number of acorns per pound and to determine the cold stratification requirements of acorns from Arkansas oak.

Materials and Methods

Approximately 5 pounds of acorns were collected in Nevada County, Ark., in October 1982. All acorns were washed, and those obviously defective were discarded. Clean acorns were weighed into five 1-pound samples. The number of acorns per pound was determined for each of the five samples.

Next, acorns were randomly divided into five subgroups of 150 each and soaked overnight in water. Each of the five subgroups was cold-stratified for either 0, 30, 60, 90, or 120 days in moist sand at 2° C. Stratified acorns were planted approximately 1 centimeter deep in number 8 styroblocks containing a 1:1 mixture of peat moss and vermiculite, and they were watered three times weekly. Germination was tallied when any part of the epicotyl was visible above the planting medium. Germination counts were recorded daily for 45 days.

All treatments were replicated three times with 50 acorns planted per treatment. Data were analyzed according to the techniques of McLemore and Czabator *(3)* utilizing an analysis of variance and Duncan's multiple range test (4).

Results and Discussion

Acorns in each of five 1-pound samples were counted. The average number of acorns per pound was 390 with a range of 387 to 393 acorns per pound.

The acorns receiving 0, 30, and 60 days of cold stratification did not show epicotyl development after 45 days (fig. 1). They did, however, show radicle development of 6, 90, and 98 percent, respectively. Epicotyl development was evident in the 90- and 1 20-day treatments (table 1). Mean germinations were 92.6 and 92.0 percent for the 90- and 1 20-day treatments, respectively. Peak germination of seeds stratified 120 days occurred an average of 3 days earlier than that of seeds receiving the 90-day treatment. Results from an analysis of variance of germination values were statistically significant at the 5-percent probability level. A Duncan's multiple range test indicated that germination of acorns stratified 120 days is significantly greater than that of acorns stratified 90 days and that germi-

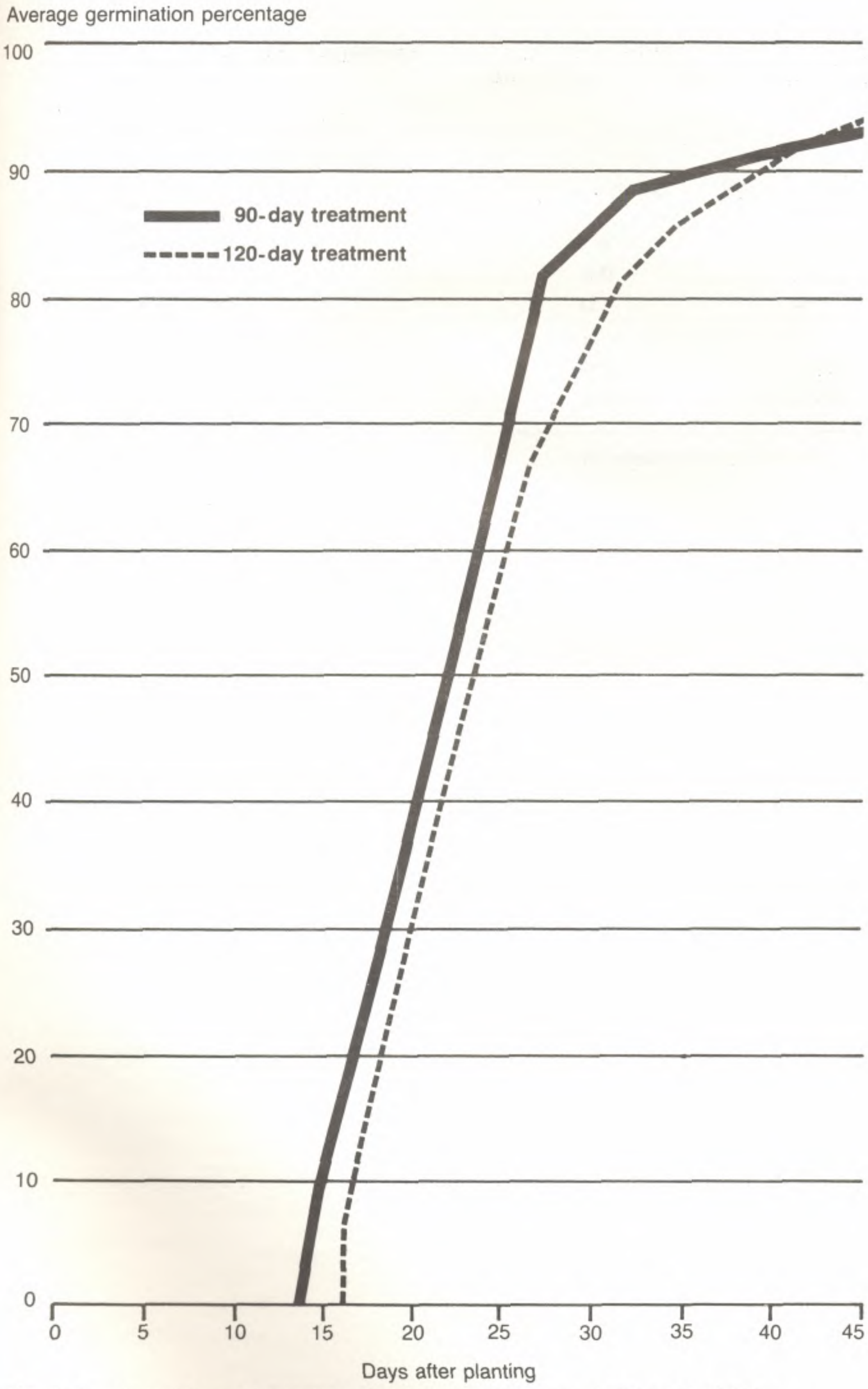

Figure 1—*Cumulative germination of Arkansas oak acorns stratified at 2° C for different periods.*

nation of acorns stratified 120 or 90 days is greater than that in shorter stratification periods.

Conclusion

Nursery personnel can expect Arkansas oak acorns stratified for 120 days to germinate significantly faster than those stratified for shorter lengths of time.

Table 1—*Duncan's multiple range test of germination values, day of germination peak, and percentage of germination for acorns stratified up to 120 days*

Stratification period	Germination value	Day of germination peak	Germination
Days			%
0	0.0c[1]	0.0	0.0
30	.0c	.0	.0
60	.0c	.0	.0
90	6.0b	33.3	92.6
120	7.9a	30.3	92.0

[1] Values followed by the same letter are not significantly different at the 5-percent probability level.

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