

# Pollen Equipment for Seed Orchards

Tony Jasumback

*Project leader, USDA Forest Service, Missoula Technology and Development Center, Missoula, MT*

*A cyclone dust collector that uses centrifugal force to separate fine solid matter from the air has successfully collected pollen in Forest Service seed orchards. Preliminary field tests have shown that it can collect pollen in the quantity needed with minimal damage to the pollen. With 4 workers, the cyclone dust collector can be used to collect up to 6 liters of pollen per hour under optimal conditions. A tractor-mounted air duster modified for use as a pollen applicator is being evaluated. Tree Planters' Notes 42(4):4-5; 1991.*

Over 25 years ago, the Forest Service began establishing a network of seed orchards of genetically superior trees. Now that these trees are in the cone bearing stage, the problem of protecting the genetic quality of the seed is of prime importance. About 40% of the seed now produced in seed orchards can be the result of fertilization by pollen blown into the orchard from outside sources. This "outside" pollen threatens the decades of work accomplished by tree breeders in upgrading seed quality. For this reason, equipment and methods to control orchard pollination are essential to Forest Service managers.

In 1989, the National Forest Regeneration Committee directed the Missoula Technology and Development Center (MTDC) to investigate ways of developing orchard pollen collection and application equipment. This work is being done in conjunction with Don Copes of the Pacific Northwest Station in Corvallis, Oregon, and Floyd Bridgewater of the Southeast Station at Raleigh, North Carolina.

## Pollen Collection

Methods of collecting and disseminating pollen have traditionally involved cutting male flowers (catkins), drying them, and then shaking the pollen grains from the flowers. This pollen is then applied with hand-held applicators to single or small clusters of female flowers. The female strobili are then covered with bags to exclude windborne pollen.

Orchard managers needed a method of collecting a large supply of pollen and an efficient means of applying that pollen to the female flowers on target trees in a very short period of time. Geneticists call

this "supplemental mass pollination." It allows them to protect the genetic quality of orchard seed by minimizing the effects of non-orchard pollen and also increases orchard productivity by assuring adequate pollen supplies at the peak of female flower receptivity.

Various mass pollen collection techniques have been tried. In one example, the lower portion of a single tree was enclosed in a canvas-covered catch frame, then the tree was shaken to dislodge pollen. In other cases air was blown through the tree and the pollen collected on the far side of the enclosure. With these methods the actual removal of the pollen from the capturing fabric still posed problems. Setting up and dismantling the barriers around each individual tree proved time-consuming and labor intensive. Vacuum equipment with collector bags or similar separation methods also proved ineffective. The fine pollen grains quickly form a thick blanket layer on the inside surface of the bags and block the air passage.

MTDC found the solution in a cyclone dust collector that uses centrifugal force to separate fine solid matter from the air (figure 1). The collector is ideally suited for pollen work. A Model 20SN31P Cyclone Dust Collector manufactured by the Aget Manufacturing Company of Adrian, Michigan, was modified for field operations with an 8-horsepower gasoline engine as its power source. This unit was mounted in the bed of a pickup truck and configured with



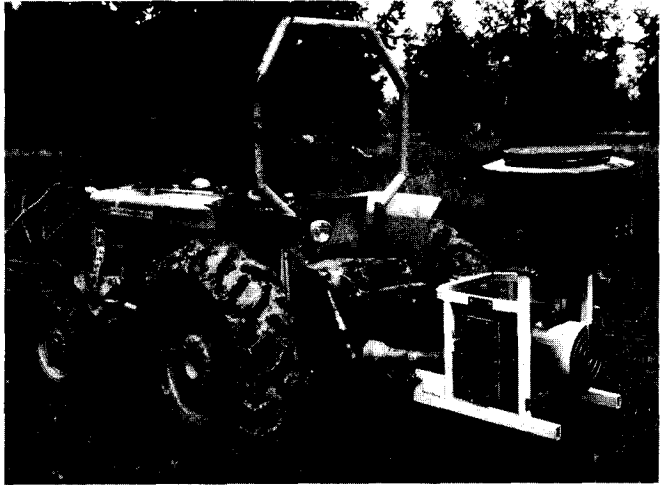
**Figure 1—Pollen collector.**

four collection hoses. These hoses were fitted with 10-cm by 35-cm (4-inch by 14-inch) vacuum heads and extension poles that allow an operator to reach approximately 7.6 m (25 feet) into a tree's crown. With this equipment, 4 workers can collect 6 liters of pollen per hour. This pollen can be easily cleaned and used immediately, or air-dried and placed in a freezer for long-term storage.

The cyclone collector operates very effectively with Douglas-fir pollen. However, species of pine, such as sugar, white, and lodgepole, have pollen grains with different aerodynamic characteristics that require minor modifications to the collector. Work is continuing in an effort to adapt the collection equipment to loblolly pine. Additional tests will be necessary to determine the effectiveness of the cyclone dust collector to various other tree species.

### Pollen Application

This spring, a tractor-mounted air duster was tested as a supplemental mass pollen applicator (figure 2). This duster utilized a squirrel-cage type blower with hopper and feed mechanism and operated from the tractor's power take-off system. It was originally designed as a vineyard duster for pesticide and fertilizer application. MTDC modified the feed system and blower outlet so that the pollen expelled from the machine could be directed high into the crown of orchard trees. Evaluation of this year's treatment will determine the effectiveness of this method of supplemental mass pollination.



**Figure 2—Pollen applicator.**

In FY 1992, MTDC will continue to work with both the Pacific Northwest and the Southeastern Experiment Stations to perfect the cyclone collector and the orchard blower applicator. Drawings and test results will be published.

Questions may be directed to either:

Tony Jasumback, project leader  
(406) 329-3922; FTS 585-3922

or

Debbie O'Rourke, project engineer  
(406) 329-3920; FTS 585-3920.

## New Planting Tools

Dick Hallman

*Project leader, USDA Forest Service, Missoula Technology and Development Center, Missoula, MT*

*A power-driven auger and a hammer-action hand planter make reforestation tasks more efficient and economical.*  
Tree Planters' Notes 42(4):7-8; 1991.

### Planting Auger

The cone-shaped power-driven auger is designed to produce a planting hole configured more closely to the shape of a seedling's root system and it is designed to penetrate rocky soils. It has proved to be more effective in planting seedlings than currently used straight-shank augers. The auger is 76 cm (30 inches) long, has a bit length of 58 cm (23 inches), and weighs 3.4 kg (7½ pounds), which is comparable to commercial augers. The cone-shaped auger costs about \$200. Design drawings are available from MTDC.



**Figure 1**—Power-driven auger that produces a cone-shaped hole.

### Hammer-Action Hand Planter

The hammer-action hand planter is designed to plant seedlings in rocky soil. Although commercial hand planting tools perform well in ideal soil conditions, they are extremely tiring to operate because the operator continuously absorbs the shock generated when the tools strike rocks. The hammer-action hand planter absorbs the shock, while producing a suitable planting hole.

MTDC engineers converted two types of commonly used commercial planting tools, the wedge and the dibble, to a hammer-action shaft. T-handle and double-D handle options were fitted to the shaft to make gripping the tool easier.



**Figure 2**—Hammer-action hand planter with a double-D handle.

**Hammer-action wedge:** Creates holes for bareroot stock that are 30 cm (12 inches) or deeper. The blade is 7.6 cm (3 inches) wide by 27.3 cm (10<sup>3</sup>/<sub>4</sub> inches) long. Blade thickness at the top tapers to a thin wedge.

DD-handle

Length: 1.2 m (48<sup>5</sup>/<sub>8</sub> inches)

Weight: 9.4 kg (20 pounds, 10 ounces)

T-handle

Length: 1.1 m (44<sup>5</sup>/<sub>8</sub> inches)

Weight: 8.1 kg (17 pounds, 15 ounces)

**Hammer-action dibble:** Designed for planting containerized stock in super tubes. The dibble is 22.8 cm long (9 inches) with a 4.5-cm (1<sup>3</sup>/<sub>4</sub>-inch) diameter, tapering to 3.2 cm (1<sup>1</sup>/<sub>4</sub> inches) at the tip.

DD-handle

Length: 1.2 m (46<sup>3</sup>/<sub>4</sub> inches)

Weight: 9.7 kg (21 pounds, 8 ounces)

T-handle

Length: 1.1 m (42<sup>3</sup>/<sub>4</sub> inches)

Weight: 8.5 kg (18 pounds, 10 ounces)

The improved hammer-action planter costs about \$150. Design drawings are available from MTDC.

A project report, "New Resource Tools and Equipment," describes the development effort in detail (Pub. No. 8824-3806-MTDC). For more information, contact:

Dick Hallman, project leader

Missoula Technology and Development Center

Bldg. 1, Fort Missoula

Missoula, MT 59801

(406) 329-3946; FTS 585-3946

# Bareroot Seedling Inventory: Estimation of Optimal Sample Size

Carl Mize and Dave Hansen

Associate professor and forestry student, Iowa State University, Ames, IA

*Many State nurseries operate on an "inventory, sell, lift, and ship" schedule. One drawback of this schedule is that nurseries often end up with seedlings that cannot be sold, which results in reduced income. A fairly simple technique that allows nursery managers to estimate the number of plots needing to be measured during the inventory phase in order to achieve maximum profits is presented. The technique is discussed and an example is presented. Data supplied by seven State nurseries show consistent biases in estimated total number of salable seedlings. Tree Planters' Notes 42(4):9-13; 1991.*

In recent years, rising costs, budget cuts, and competition from privately owned nurseries have made operational efficiency increasingly important for government forest nurseries. Because a significant part of the production of most nurseries is bareroot seedlings, managers need to look closely at this aspect of their operation to maximize profitability.

An important phase of the production process is estimating the number of seedlings that can be sold. We have studied how some nurseries estimate the number of salable seedlings and are presenting our findings in this paper. For nurseries selling seedlings based only on an estimate of the number of seedlings and not an actual count, we are also presenting a relatively simple technique to calculate how many plots need to be measured to estimate the number of seedlings.

## Current Practices

In 1984, a questionnaire was sent to 56 State nurseries throughout the United States to determine existing practices and problems dealing with the inventory of bareroot seedlings. Thirty-one nurseries responded, and although management styles and customer bases varied widely, some generalizations could be made.

Most nurseries operated on an "inventory, sell, lift, and ship" schedule. The basic procedure is as follows:

- ?? Take an inventory to estimate the number of salable seedlings in the fall
- ?? Sell seedlings during the winter
- ?? Lift, package, and ship them in the spring.

The problem with this procedure is the uncertainty associated with selling seedlings based on an inventory estimate; the actual number of salable seedlings is not known until packaging is finished.

Nurseries inventoried each species and seedlot to obtain an estimate of the total number of salable seedlings. These inventories usually involved counting the number of seedlings meeting the nursery's standards in a number of 15-cm (6-inch) or 30-cm (12-inch)-wide plots (called frames) randomly or systematically spread throughout the seedbeds containing each species or seedlot. The number of plots measured varied widely among nurseries, but 20 plots were used fairly commonly, unless there were many beds, in which case more plots were used.

Because of normal seedling mortality and damage occurring during the lifting process, the number of seedlings that would be salable after lifting and packaging was expected to be less than the number estimated from the inventory. Moreover, because even if there was no mortality or damage, the estimate is not an actual count. Managers knew there was a 50-50 chance that the actual number would be less than the estimate. Because managers wanted to avoid overselling seedlings (which would require them to inform customers that they could not meet their commitment), they used a variety of techniques to adjust the estimated total. The most common technique was to reduce the estimated total by a percentage to arrive at a number that the manager felt could be sold safely.

Most managers indicated that the adjustments that they made were enough to ensure that they seldom ran out of seedlings. An unfortunate result of their success was that they almost always ended up with confirmed sales for fewer seedlings than the actual number of salable seedlings. Sometimes they could sell the extra seedlings, but other times they could not and had to destroy them, which reduced their income.

## Another Method

Most of the adjustments that nursery managers made to their inventory estimates might be best described as educated guesses. None of the managers indicated that their adjustments were based on statistical techniques. We have developed a technique for adjusting the inventory total based on the level of confidence the manager wishes to have in the estimate. The technique should improve profitability for nurseries that cannot sell their extra seedlings, can be easily defended, and can be easily adapted to changing circumstances.

The technique is developed by starting with the formula for a **one-sided confidence interval**. For this paper, we define a one-sided confidence interval as a value (number of seedlings) for which the probability that the actual number of salable seedlings is greater than the value can be specified. For example, if an inventory for a species was made, a one-sided 95% confidence interval for the total was calculated, and a value of 150,000 was obtained, then 95 times out of 100, in situations like this, there would be at least 150,000 seedlings. In such a situation, only about 5% of the time will the nursery end up with less than 150,000 seedlings. Managers who wish to be more or less sure can increase or decrease the confidence level accordingly.

The formula for the one-sided confidence interval is as follows: number of salable seedlings =  $T - t \cdot S_T$ , (1) where  $T$  = estimated number of salable seedlings from an inventory.

$t$  = value from a t-table that depends on the sample size used to estimate  $T$  and the desired likelihood of not having enough seedlings to fill orders. (A t-value of 1.729 would be used for a one-sided 95% confidence interval for a sample of 20 frames, which has 19 degrees of freedom. The degrees of freedom is the sample size minus 1. ).

$S_T$  = standard error of the total derived from the sample used to estimate  $T$ . The formula for  $S_T$  is square root of:  $[(N^2 \cdot S^2)/n]$  where  $S^2$  (the variance of the number of seedlings per plot) is calculated from the inventory,  $N$  is the population size (the length of the beds planted to the species or seedlot divided by the width of the plot), and  $n$  is the sample size (number of plots). The finite correction

factor  $[(N-n)/N]$  is not used because the sample size,  $n$ , is usually small compared with  $N$ .

Both  $t$  and  $S_T$  are affected by the size of the sample used to estimate  $T$ , that is, the number of plots measured. As sample size increases,  $t$  and  $S_T$  decrease, and the value of the one-sided confidence interval becomes close to the actual population value. But measuring many plots is expensive. The challenge is to identify the sample size that represents an optimal balance between increased precision and increased cost of inventory.

Formula 2 shows a simple marginal return model that can be used to predict net income. It shows that net income ( $I$ ) (not considering production costs, which are fixed and independent of the number of seedlings sold) is a function of  $t$  (the t-value); the value of individual seedlings ( $V$ ); the standard deviation of the total ( $S_T$ ); and the cost of measuring each sample plot ( $C$ ).

$$I = (T - t \cdot S_T) \cdot V - n \cdot C \quad (2)$$

By taking the derivative of formula 2, with respect to  $n$  (first substituting the formula used to calculate  $S_T$ ), setting it equal to zero, and rearranging the terms, the optimum sample size is estimated by formula 3. The value of  $n$  must be solved in a stepwise manner (explained in the example below) because the value of  $t$  depends upon the value of  $n$ .

$$n = \left[ \frac{t \cdot V \cdot S \cdot N}{2 \cdot C} \right]^{2/3} \quad (3)$$

To show how to solve for  $n$ , we will work an example using the following values, which are representative of the values provided in the questionnaires:

$V$	=	\$0.10 (10 cents per seedling)
$S$	=	19 (standard deviation of number of seedlings per plot)
$N$	=	800 (length of bed divided by plot width)
$C$	=	\$1.50 (\$1.50 to sample a frame)

Aside from these values, we need a value of  $t$  to estimate  $n$ . But the value of  $t$  depends upon  $n$  and how confident the nursery manager wants to be of the nursery's not running out of seedlings. Because we do not know, we can just guess that  $n$  might be equal to 20. (Note: the initial guess is not very important, it will not affect the final answer.) Moreover, let's assume the manager wants to be 95% confident that the nursery will not run out of seedlings, so we will start with a t-value of 1.729, which is for a

95% one-sided confidence interval with 19 degrees of freedom. By using formula 3 and the values just described, we estimate that  $n$  equals 92. But that value was calculated using a  $t$ -value for a sample size of 20. For a sample size of about 92, the  $t$ -value would be smaller. Therefore, then is between 20 and 92. So we will guess that  $n = 80$ , which would have a  $t$ -value of 1.667. Using 1.667,  $n = 89$ , which would have almost the same  $t$ -value as 80. So the sample size that should be taken to maximize profit is 89. For practical purposes,  $n$  can be estimated usually within two or three steps. A person moderately familiar with using spread sheets should be able to develop a simple spread sheet that can estimate sample size.

An examination of a  $t$ -table shows that  $t$ -values change very slightly for sample sizes ( $n$ ) above 30. Often, an inventory will require at least 30 plots. Therefore, a good initial estimate of the sample size would be achieved by just inserting the  $t$  value for infinite degrees of freedom (the one at the bottom of each column) in the equation and calculating  $n$ . Iterations are not really necessary if the estimate is 30 or more.

The relation between optimum sample size and projected net income (forgetting about fixed costs and assuming that only the number of seedlings estimated by the one-sided confidence limit are sold) can be quantified by adjusting formula 1 to include the cost of sampling and the value of the seedlings. The resulting formula is as follows:

$$\text{Income} = (T - t^*S_T) * V - (n * C) \tag{4}$$

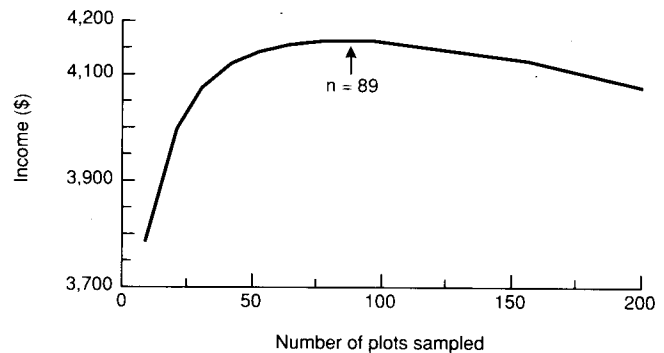
Formula 4 was used to examine the relation between income and the number of plots sampled, the variability of the number of seedlings per plot, and the cost of measuring a plot. Figure 1 illustrates the relation between income and sample size (number of plots measured), using the values previously listed, the appropriate  $t$  value, and calculating the  $S_T$  for each sample size.

If the nursery had sampled 89 plots (figure 1), the income, using formula 4, would have been \$4,137. If the nursery were to use 20, which is commonly done, the income would be \$4,000, or \$137 less than the optimum. These calculations assume that only the number of seedlings estimated by the one-sided confidence interval are sold and do not consider production costs.

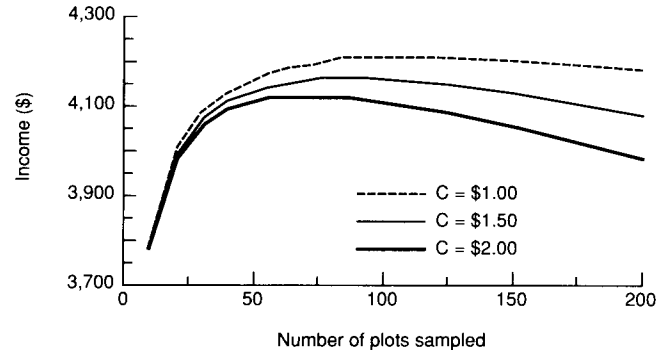
Much variability exists between nurseries and species and even between beds. Changing the values of the terms used in formula 4, however, does not alter the basic relation between sample size and

income. For example, figures 2 and 3 illustrate the effect of different standard deviations ( $S$ ) and sampling costs ( $C$ ) for the example just worked out.

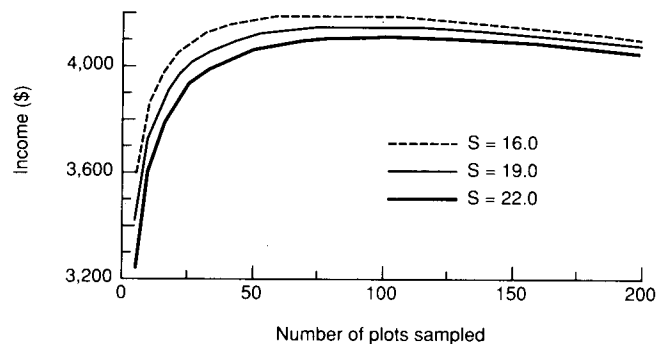
As seen in figures 2 and 3, the optimal sample size increases as the cost per plot decreases and the variability increases. The curves are fairly flat on top, which means that if the actual number of measured plots is "close" to the number of plots estimated by formula 2, income should be about the same. Income is reduced less by taking too many samples than by taking too few.



**Figure 1—Income versus sample size for hypothetical nursery bed.**



**Figure 2—Effect of different costs of measuring a plot on income.**



**Figure 3—Effect of different standard deviations on income.**

The value of seedlings is readily available to the nursery manager. The acceptable percentage of unfilled orders (tolerance level) will require managers to decide how often they want to contact purchasers and inform them that there are no seedlings available. Estimating the cost per plot will require an estimate of the time required to measure a plot and travel to another. (Note: some managers estimated that it cost nothing to do an individual plot. But assuming that it takes 2 workers, each making \$9 per hour, 5 minutes to count a plot and travel to another plot, each plot will mean a cost of \$1.50, so a cost of nothing is unrealistic.) The values of the standard deviation (S) can be taken from previous inventories if records are kept, or they can be calculated from samples of the seedbed being inventoried. (Note: if nurseries kept records of S for different species for some years, fairly consistent values may be observed.)

To make it easier to estimate the optimum sample size and to give nursery managers an indication of the sample sizes needed, we used equation 3 to develop a table of optimum sample sizes (table 1). Because equation 3 has five unknowns (t, V, S, N, and C), we decided to assume that V = \$0.12 per plant, C = \$1.50 per plot, and the likelihood used is 95%. This left two terms to vary, S and N. Values of S and N were chosen to span most of the range that nursery managers will likely encounter.

**Table 1—Optimum sample size for a range of standard deviations (5 to 30 SD) and bed lengths\***

Bed length	No. of seedlings					
	5 SD	10 SD	15 SD	20 SD	25 SD	30 SD
400	45	71	92	112	130	147
800	71	120	147	178	206	233
2,000	130	206	270	327	380	429
4,000	206	327	429	520	603	681
10,000	380	603	790	957	1,111	1,254
20,000	603	957	1,254	1,519	1,763	1,991
50,000	1,111	1,763	2,310	2,799	3,247	3,667
75,000	1,455	2,310	3,037	3,667	4,255	4,805
100,000	1,763	2,799	3,667	4,442	5,155	5,821

\*Using equation 3 and  $t = 1.645$ ,  $V = \$0.12$ , and  $C = \$1.50$ .

### Biased Estimates

For the system on sample size estimation to function, the estimate of the total, T, developed from the sample must be a good (unbiased) estimate. From the data provided by 7 nurseries, 6 nurseries showed a consistent tendency to underestimate their actual seedling populations. The seventh nursery tended to

overestimate the actual number of seedlings. Thus, it seems likely that most nurseries produce biased estimates of the number of salable seedlings. For a nursery to decide whether or not the estimates of the total are biased requires records from previous inventories. An easy check would be to examine the ratio of estimated to actual number of seedlings and look for a tendency to over or under estimate the actual number of salable seedlings. If there is a tendency, the sampling procedure and the criteria for deciding whether a seedling is salable or not need to be reviewed.

### Using Stratified Sampling

In many nurseries, the density of seedlings in beds seeded to a species can be quite variable. An important consequence of the variability is that the variance,  $S^2$ , of the number of seedlings per sample plot will be higher than that in uniform beds. As a result, for a given number of plots, the one-sided confidence interval for the total number of seedlings in the variable beds will be lower than that for the uniform beds. One way of dealing with this is to use stratified sampling because it results in narrower confidence intervals for overall totals than do samples from a simple sample. If the manager can separate beds or portions of beds into different groups (strata) by density, then stratified sampling can be used effectively. A good reference on the use of stratified sampling is Freese (1962).

### Summary and Conclusions

The optimum sample size for an inventory in a nursery bed is estimated by the following equation:

$$n = \frac{[t \cdot V \cdot S \cdot N]^{2/3}}{[2 \cdot C]}$$

where t is from a t-table, V is the value of an individual seedling, S is the standard deviation of the number of seedlings in a sample plot, N is the length of the bed(s) divided by the width of the sample plot, and C is the cost of measuring an individual sample plot.

Effective management of bareroot seedling stock can enhance the profitability of any nursery operation. Use of appropriate inventory procedures, including economically optimal sample sizes, is important to maximize effectiveness. Although time, personnel, and other considerations may influence the nursery manager to use less than the calculated optimal sample size, the technique discussed in this



paper will provide the manager with additional information on the trade-offs of using various sample sizes.

#### Literature Cited

Freese, F. 1962. Elementary forest sampling. USDA Agriculture Handbook No. 232. 89 pp. [Reprinted by Oregon State University Book Stores, Corvallis, OR]

#### Acknowledgments

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The authors thank the nurseries that returned their questionnaires and, particularly, those that sent inventory data. They also thank Paul Hinz, Statistics Department, Iowa State University, and Jerry Grebasch and Roger Jacob, Iowa Department of Natural Resources State Forest Nursery for their comments and suggestions concerning the preparation of this article.

# Growth and Colonization of Western Redcedar by Vesicular-Arbuscular Mycorrhizae in Fumigated and Nonfumigated Nursery Beds

S. M. Berch, E. Deom, and T. Willingdon

Assistant professor and research assistant, Department of Soil Science, University of British Columbia, Vancouver, BC, and manager, Surrey Nursery, British Columbia Ministry of Forests, Surrey, BC

Western redcedar (*Thuja plicata* Donn ex D. Don) seedlings were grown in a bareroot nursery bed that had been fumigated with methyl bromide. Seedlings grown in fumigated beds were stunted and had purple foliage. Microscopic examination showed that roots from these seedlings were poorly colonized by mycorrhizae, and only by fine vesicular-arbuscular mycorrhizae. In contrast, roots from seedlings grown in non-fumigated beds had larger shoots and green foliage and were highly colonized by both fine and coarse vesicular-arbuscular mycorrhizae. *Tree Planters' Notes* 42(4):14-16; 1991.

Species of cypress (Cupressaceae) and yew (Taxodiaceae) that make up significant parts of the forest landscapes of northwestern North America are dependent on vesicular-arbuscular mycorrhizae (VAM) for good growth. These fungi are mutualistic root colonizers that take carbon (C) and energy from the host plant in exchange for soil nutrients, notably phosphorus (P), and water gathered by the soil hyphae. Many other kinds of mycorrhizae exist (Harley and Smith 1983), but forest nursery crops in North America only form either ectomycorrhizae or endomycorrhizae, which are also called VAM. Most pines form ectomycorrhizae with the characteristic mantle of fungal hyphae surrounding the fine feeder roots and the *Hartig net* of hyphae surrounding cortical cells. VAM are characterized by fungal storage organs called *vesicles* and minutely branched intracellular hyphae known as *arbuscules*. VAM come in two types: the more common coarse VAM's and the fine VAM's, which are more common in stressed environments, as discussed later.

Kough et al. (1985) inoculated incense-cedar (*Libocedrus decurrens* (Torn.)), redwood (*Sequoia sempervirens* (D. Don) Endl.), giant sequoia (*Sequoiadendron giganteum* (Lindl.)), and western redcedar (*Thuja plicata* Donn ex D. Don.) with three different

VAM. Positive growth responses of up to 20 times the nonmycorrhizal controls occurred under conditions of limited soil phosphorus. Incense-cedar, redwood, and giant sequoia seedlings in northern California nursery beds are routinely inoculated with *Glomus* sp. (Adams et al. 1990), as experience has shown that the absence of VAM after soil fumigation leads to phosphorus deficiency and poor growth.

When western redcedars in fumigated transplant beds at the British Columbia Ministry of Forest's Surrey Nursery began to show signs of phosphorus deficiency, a deficiency of mycorrhizal colonization was suspected. Many studies have demonstrated improved P status of VAM-inoculated plants (see Harley and Smith 1983). The objective of this study was to determine whether fumigation decreased VAM colonization.

## Materials and Methods

Bareroot cedar nursery beds were fumigated in May 1986 with the typical rate of 390.18 kg/ha of methyl bromide to control strawberry root weevils (*Otiorhynchus ovatus* (L.)). In July 1987, 4-month-old containerized western redcedar seedlings from the same nursery were randomly planted in fumigated and adjacent nonfumigated beds. By May 1988, plants in the fumigated beds had purple foliage and smaller shoots than plants from nonfumigated beds.

In August 1988, 10 plants each were randomly collected from one fumigated and one nonfumigated nursery bed, wrapped in plastic and shipped to the University of British Columbia for analysis. Height and root collar diameter were determined on fresh shoots, which were then oven-dried at 70 °C for 48 h and weighed. All roots under 2 mm diameter were fixed in 50% aqueous formalin/acetic acid/ ethanol (90:5:5), then later cleared and stained by a

modified method of Phillips and Hayman (1970).

Colonization of these fine roots by VAM was examined under the dissecting microscope at magnifications of 8 to 25 x and categorized into six classes: 0 = none; 1 = very low (1 to 5% of total fine root length colonized); 2 = low (6 to 15%); 3 = medium (16 to 30%); 4 = high (31 to 50%), and 5 = very high (>50%).

Colonization by coarse VAM was determined under the dissecting microscope at 8 to 25 x, based on the presence of stained hyphae and arbuscules in roots and large vesicles. Stained hyphae in roots with arbuscules but no large vesicles were suspected of being fine VAM. To confirm presence of fine VAM (which are too small to see without high magnification) and the absence of coarse VAM and other types of root-inhabiting fungi, a minimum of five 2-cm-long mycorrhizal root segments per plant were mounted in lactic acid on slides and examined at 400 x. We differentiated the two VAM based on the following criteria: fine VAM species have hyphae 2 to 5  $\mu\text{m}$  wide and vesicles up to 5 to 10  $\mu\text{m}$  long; coarse VAM species form hyphae 5 to 10  $\mu\text{m}$  wide and vesicles up to 100  $\mu\text{m}$  long.

Growth data were checked for normality and heterogeneity and analyzed statistically using the Student's *t*-test at  $P < 0.05$  (Zar 1984) for differences between means of shoot diameter, height, and dry weight. Mycorrhizal colonization data were not normally distributed and so were analyzed using the  $\chi^2$  test for nonparametric data at  $P < 0.05$  (Siegel 1956).

## Results and Discussion

Shoot diameter and shoot dry weight were significantly lower in seedlings planted in fumigated than in nonfumigated beds (table 1). Shoot height was lower, though not significantly so, in the fumigated treatment and might have been even more so if 3 seedlings in the nonfumigated plot had not had their leaders cut to control growth.

Mycorrhizal colonization of plants in the nonfumigated bed was significantly higher than in the

fumigated bed ( $\chi^2 = 81.6$ ). Only fine VAM were present in roots from the fumigated bed, whereas both fine and coarse VAM were present in 6 of the 10 plants from the nonfumigated bed. From this study, we do not know if the decreased plant growth resulted from changes in VAM colonization due to fumigation or from the lack of other beneficial soil organisms that were also killed. Residual soil toxicity from fumigation is not considered a problem in this case as a full year had passed between fumigation and planting. However, replacement of VAM fungi in fumigated bareroot nurseries has corrected similar poor growth of incense-cedar, giant sequoia, and redwood (Adams et al. 1990), and we believe that the same would be true with bareroot western redcedar.

Twenty-seven months after fumigation and 13 months after transplanting, almost all of the plants from the fumigated bed were mycorrhizal, though not always to the same extent as the seedlings from the nonfumigated bed (table 1). Based on other studies of containerized nurseries in British Columbia, we are confident that the plants were virtually nonmycorrhizal at transplanting even though we did not examine these plants. It appears that plants going into the nonfumigated bed were colonized shortly after transplanting, whereas those going into the fumigated bed were either not colonized until mycorrhizal propagules were reintroduced into the soil or colonized very slowly by a small number of surviving propagules. Of particular interest, however, is the apparent difference in colonization behavior of the fine and coarse VAM. If both VAM had been eradicated by the fumigation, then we can deduce, based on these data, that the fine VAM is a better recolonizer than the coarse VAM. On the other hand, it is possible that some propagules of the fine VAM survived fumigation.

We have found that fine VAM often dominate western redcedar roots under stressful or disturbed conditions (Berch et al. in prep.). Containerized seedlings from the MacMillan Bloedel Nursery at Nanaimo raised in peat-vermiculite mix were essentially nonmycorrhizal, but for the occasional plant

**Table 1—Mean shoot growth and root colonization of western redcedar in fumigated and nonfumigated beds at Surrey Nursery (N = 10)**

	Diameter (mm)	Height (cm)	Dry weight (g)	Mean root colonization class*	Type of endophyte
Fumigated	9.43 ( $\pm$ 2.10) a	62.7 ( $\pm$ 10.1) a	22.7 ( $\pm$ 10.4) a	3.3 a	F
Nonfumigated	12.42 ( $\pm$ 2.57) b	67.6 ( $\pm$ 6.60) a	43.1 ( $\pm$ 16.9) b	5.0 b	F or F + C

Numbers within columns with the same letter in common differ significantly ( $P < .05$ ). Root colonization is expressed as mean colonization class, with 0 = none, 1 = 1 to 5%, 2 = 6 to 15%, 3 = 16 to 30%, 4 = 31 to 50%, 5 = 50%. Type of endophytes: F = fine only; F + C = fine and coarse. Values are  $\pm$  1 standard deviation.

\*  $\chi^2$  Test of heterogeneity = 81.6 (critical value,  $P < 0.05 = 18.31$ ).

with low levels of the fine VAM. After outplanting on sites pretreated with different slash-burn intensities, the majority of these young plants on the severely burned site were colonized by fine VAM alone or by both VAM. On the lower intensity burn site, the coarse VAM was dominant, which suggests that the fine VAM is better adapted to stressful conditions. Further substantiation of this comes from a pot bioassay in which soils were dried, ground, and used as inocula to determine propagule density; in this test only fine VAM colonized the test plants (Mike Curran, Ministry of Forests, Nelson, BC, personal communication). This implies that fine endophyte propagules survive the rigors of drying and grinding better than coarse endophytes. Parke et al. (1983) also reported that western redcedar formed only fine VAM after inoculation with fresh, sieved forest litter or mineral soil.

Kough et al. (1985) reported that growth response to VAM inoculation in pots of incense-cedar, redwood, giant sequoia, and western redcedar generally decreased with seedling age up to about 11 months, which may reflect the depressing effects of small rooting volume. Our cedars were approximately 4 months old when transplanted and 17 months old when harvested, yet they still showed growth differences that may be due to colonization. This may reflect the difference in growth potential of plants in pots and plants in a bareroot nursery.

### Recommendations

This study suggests that VAM fungi are important to the growth of bareroot western redcedar. Ideally, our observations should be verified in a study designed specifically for that purpose in which nonmycorrhizal redcedar seedlings would be planted into prefumigated soils and some would be inoculated with pure VAM inoculum free of other microorganisms that might affect plant growth. It would also be possible to examine the differences between fine and coarse vesicular-arbuscular mycorrhizae in terms of propagule survival of treatments such as soil fumigation and slash burning. This could be achieved in closed chambers, where airborne pro-

pagules are eliminated, if cedars were germinated in treated and untreated soils and allowed to form mycorrhizae with the VAM that survived treatment. Given the improved growth of redwoods at the Ben Lomond State Forest Nursery after inoculation with a VAM (Adams et al. 1990) and the fact that western redcedar in pots grows best when mycorrhizal (Kough et al. 1985, Parke et al. 1983), we can hypothesize that it grows best in bareroot nurseries when mycorrhizal. Soil transfer or application of commercial inoculum will result in the recolonization of sterilized seed beds. Because of their ability to improve nutrient status of seedlings, including western redcedar (Kough et al. 1985), it would be interesting to determine whether VAM could substitute wholly or in part for P fertilizer application in bareroot nurseries.

### Acknowledgments

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# Carry-Over of Loblolly Pine Seeds on Cutover Forest Sites

James P. Barnett and John M. McGilvray

*Principal silviculturist and biological technician, USDA Forest Service  
Southern Forest Experiment Station, Pineville, LA*

*The role of carry-over seed in the natural regeneration of loblolly pine (*Pinus taeda* L.) was investigated in central Louisiana. Three lots of loblolly pine seeds were sown on two forest sites (dry and moist) and at two seasons (fall and spring). Observations indicated that all viable seeds germinated by April. No viable ungerminated seeds were found to remain on the forest floor after the first growing season. These data suggest that no significant amounts of loblolly pine regeneration occurs from seeds carried over to the following year. Tree Planters' Notes 42(4):17-18; 1991.*

The long-term survival of seeds on the ground or buried in the soil has been reported for many tree species (Baldwin 1942). However, little information is available for southern conifers. There are persistent but unsubstantiated reports of loblolly pine (*Pinus taeda* L.) seed carry-over from one year to the next. The number and viability of loblolly pine seeds remaining ungerminated at the end of the first year after natural seed fall or sowing is unknown. Wahlenberg (1960) reported that very few loblolly pine seeds remain viable on the forest floor through the second winter after seed fall. However, it has also been reported that under certain extreme conditions, such as different seasons and soil types, some seed may remain ungerminated for lengthy periods (Little and Somes 1959).

The results of this study further clarify the question of longevity of loblolly pine seeds. Specifically, the number and viability of loblolly pine seeds that lie ungerminated for extended periods were evaluated. The data are of particular interest given the resurgence of interest in natural regeneration (Barnett and Baker 1991) and the unclear role of southern pine seed carry-over in stand establishment.

## Methods

Two sites on the Palustris Experimental Forest were used in this study. The sites were cutover areas with no trees and only light grass competition. The soil on the dry site was a well-drained sandy loam

(Ruston soil series) and on the moister site, an imperfectly drained silty loam (Beauregard soil series).

Lots 1 and 2 were fresh seeds collected locally in central Louisiana, and lot 3 had been collected in Polk County, Texas, and stored for 10 years. Empty seeds were removed by water flotation before sowing. Seeds were sown on November 9, 1971, and February 11, 1972. The spring-sown seeds were stratified for 30 days; the fall-sown seeds were not. Standard laboratory germination tests were conducted to determine seed quality before and after field exposure (AOSA 1980).

For each site-season combination, short-term (1-year) germination rates were determined by counting germinants from an 1,000-seed sample. Long-term viability (greater than 1 year from sowing) was determined from a 3,000-seed sample. Each seedlot of 48,000 seeds was divided into 24 seedlots-12 with 1,000 (field germination tests) and 12 with 3,000 (long-term germination tests) seeds. Each group of 12 sublots provided seeds for the two sites, two seasons of sowing, and three replications. Plots were arrayed according to a randomized split-plot design for each phase of the study. Seeds from each subplot were sown on mineral soil in spots 1 by 1.5 feet in size and were protected from predators with screen-wire baskets.

The number of sound seeds remaining after 1 year was determined by counting the germinated seed about twice weekly in the early spring during peak germination, and at lesser intervals at other times. Seedlings were removed when counted. For the longer term study, field plots were sampled in later December (more than 1 year after sowing), by lifting the top half inch of soil from each 3,000 seed plot and sifting to obtain ungerminated seeds. By sowing such a large number of seeds, we tried to ensure that enough would be available to test germination of ungerminated seeds after various periods in the field. The design of the study provided for statistical evaluation by analysis of variance; however, insufficient quantities of seed remained after the first spring to quantify long-term (> 1 year) carry-over.

## Results and Discussion

The results of this study indicate that loblolly pine seed carry-over on the forest floor is essentially non-existent after the first spring. An initial germination test of all seedlots conducted in the laboratory indicated over 90% of seeds were viable before field sowing. Cumulative field viability after sowing ranged from 75 to 92%, with statistically significant ( $P = 0.05$ ) differences due to seedlots (table 1) but not the time of sowing and site. Seeds from the stored seeds (lot 3) sowed in the spring germinated less relative to the other lots on the dry site.

Overall field germination was high, averaging 85% across the sites (table 1). Germination was essentially complete in April after both fall (November) and

spring (February) sowing. No ungerminated seeds were present for the periodic summer and fall sampling from the 3,000-seed plots, although there were many seedcoats from germinated seeds. There was no additional germination on the 1,000-seed plots during summer or fall. These results indicate that long-term survival of seeds is not a typical feature in the life history of loblolly native to central Louisiana. These findings are similar to those of Little and Somes (1959) from Maryland, which showed that few loblolly pine seeds from natural seed fall remain viable through a second winter. Based on these results, it seems highly unlikely that natural regeneration from seeds remaining on the forest floor for more than a year contributes significantly to reproduction of loblolly pine.

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**Table 1—Percent germination for three seedlots sown in the field germination test on two dates and two sites.**

Seedlot	% Germination for fall sowing		% Germination for spring sowing		Overall average
	Dry site	Moist site	Dry site	Moist site	
1	86 a	89 a	90 a	92 a	89
2	84 a	84 a	83 a	88 a	85
3	89 a	86 a	75 b	81 b	82
Avg.	86	86	82	87	85

Germination results through April 1972 (2 and 5 months after sowing). Germination values within columns followed by the same letter are not statistically different at the 0.05 level.

## Seedling Box Lifter

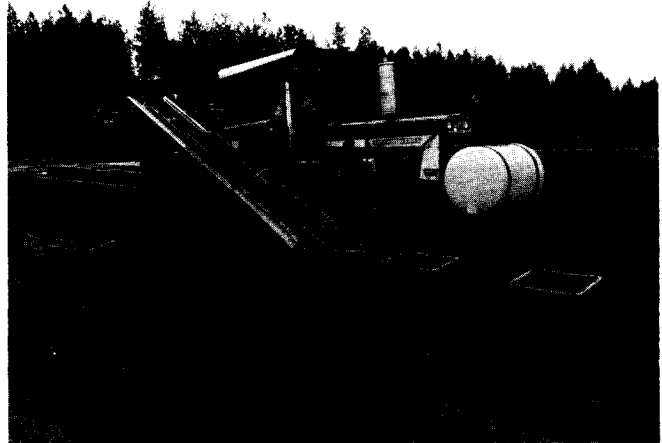
Dick Karsky

*Project leader, USDA Forest Service, Missoula Technology and Development Center, Missoula, MT*

*A seedling box lifter, mounted on the side of a farm tractor, is designed for use in bareroot nurseries, to lift and deliver boxes from the ground to a transport trailer, thus minimizing manual box handling and moving boxes from the field to the packing shed quickly. Manual loading of seedling boxes onto transport trailers is labor intensive and has the potential of causing back injuries in field workers. Tree Planters' Notes 42(4):6; 1991.*

A 1984 survey of Forest Service nursery managers indicated that an improved method of lifting seedling boxes from the ground to the transport trailers was a high priority. The Missoula Technology and Development Center (MTDC) initially used a hay bale loading device to determine what features would be required of the new machine. It had to be capable of loading different sized boxes and elevating them to a trailer at a level of approximately 1.2 m (4 feet) above the trailer floor. From there, personnel can handle and stack the boxes. A prototype box pickup was designed and built by MTDC engineers. Initial tests were conducted at the Forest Service's Coeur d'Alene, Lucky Peak, and J. Herbert Stone Nurseries.

The seedling box lifter is mounted along either side of a farm tractor and attached to the tractor's 3-point hitch. The tractor also tows the transport trailer (figure 1). A frame mounted onto the side of the tractor with a lift cylinder attached raises and lowers the front of the lifting machine. The first part of the box lifter is a pickup unit that grabs the box and places it on an elevator chain. This elevator chain lifts the box to a height .9 to 1.2 m (3 to 4 feet) above the trailer floor and delivers it to an inclined gravity conveyor, which then moves the box to the front center of the trailer and provides temporary storage of up to three seedling boxes. Stackers or box handlers then move the previously lifted boxes to the appropriate position on the trailer. A hydraulic motor driven by the tractor's hydraulic system provides power for the lifting mechanism. The speed of the lifting mechanism can be changed by adjusting an hydraulic flow valve. The side-mounted mechanism allows boxes to be picked up in only one direction of travel. The machine can be installed on either



**Figure 1—***The seedling box lifter in use.*

side of the tractor, but it is not easily switched from side to side. The tractor moves at a speed of about 1.6 km (1 mile) per hour.

A corrugated belt/chain assembly can be adjusted to pick up boxes from 35 to 48 cm (14 to 19 inches) wide. It can typically deliver 10 to 12 boxes per minute to the trailer. Boxes containing the lifted seedlings should be aligned in a row to allow minimal maneuvering of the tractor. However, the tapered entry of the pickup mechanism reduces the need for precise alignment.

The seedling box lifter does an excellent job of picking up both plastic and corrugated seedling boxes and elevating them to personnel on a trailer. From there, the boxes must be off-loaded from the seedling box lifter. Thus, additional work is needed to develop a complete seedling handling system that integrates all aspects of the seedling harvesting process.

Drawings are available for the side-mounted box pickup from MTDC. For information on the seedling box lifter (drawing No. MTDC-850) contact:

Dick Karsky, project leader  
Missoula Technology and Development Center  
Bldg. 1, Fort Missoula  
Missoula, MT 59801  
(406) 329-3921; FTS 585-3921