Comments

Tree Planters' Notes

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Articles in all sections except the "Practical Tips" are peer-reviewed by two outside, anonymous referees. Articles in the "Practical Tips" section are reviewed by a tech transfer specialist and a scientist on the advisory board.

Cover: Columbia River Gorge (USDA Forest Service photograph by Jim Hughes)

Bringing *TPN's* Mission Into Focus

Tree Planters' Notes occupies a unique position in the forestry literature. We bring the latest research results and technology transfer from scientists and engineers working on seeds, seedlings, nurseries, and reforestation to the practitioners— the nursery managers, reforestation managers, tree farmers, and foresters. In a way, ***our position is our mission:*** we provide this crucial link between the finders of new information and the users of that new information.

We strive to publish articles that are not only full of useful information but also readable and interesting. We're working to find and commission articles on interesting new areas that relate to nurseries and reforestation, topics such as somatic embryogenesis, habitat restoration, and tropical species that you may not know that you need to know about until you read our article! We're also planning to commission articles on the scientific and technical bases of nursery operations, such as the physiology of fall acclimation in seedlings. These new articles will be called "Technical Background" and will join our other new sections— "Species Spotlight" and "Technical Updates." All of these articles plus the regular research reports will be peerreviewed and refereed. Please let us know about any ideas and suggestions for topics you are interested in. You may reach me by regular mail, e-mail, DG, and telephone. See Tom Landis' commentary following this one on another new section, entitled "Practical Tips," that we envision as an outlet for nursery managers, outplanters, etc.— all you practitioners out there— to write up your observations and ideas.

Another important service TPN provides to the fields of nurseries and reforestation is that we provide scientists who wish to reach our specific nursery audience a place to publish peer-reviewed, refereed research reports. There are other forestry journals that are peer-reviewed, etc., but they are all general forestry journals and aren't targeted to our specific audience. Scientists all need quality outlets for their papers, and we hope to provide scientists working in our specialties with the place to publish their papers. Also, we wish to encourage scientists to do more than just present their findings at a meeting and get them published in the meeting proceedings. Unfortunately, meeting proceedings do not always get included in bibliographic databases and important information may not be readily accessible.

Our New, Expanded Board of Advisory Editors

To carry out our mission at the interface of science and practice well, we must actively seek out and encourage publication of the latest information and ensure its quality and utility. To help us do so, we recently expanded our board of advisory editors, asking eight scientists, nursery folk, and State and Private Forestry (S&PF) administrators to join the board. We've

expanded the numbers of people who are not in the Forest Service; included on-the-ground nursery managers, more scientists, and representatives of two of the big nursery cooperatives; in addition, we've covered the United States better geographically. So here's the new team, and please welcome our new advisory editors (they've got asterisks after their names). Buttonhole them at a meeting with your needs and ideas!

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The Responsibility To Publish

In my travels, I have had the opportunity to visit many different nurseries and see a variety of unique and thoughtful innovations. Because forest tree nurseries practice such a small, highly specialized form of horticulture, nursery managers have had to adapt existing technologies and equipment to meet their needs. The cultural regimes of each nursery are also unique and reflect that nursery's particular physical, social, and economic environment.

As individuals, we each have a unique perspective that allows us to see different aspects of the same situation. These differences have stimulated creative ideas that lead to innovations. Each of us has a professional obligation to share this technical knowledge with others in our field. The best way to share information is to publish. I have to do a lot of technical writing in my job: the *Forest Nursery Notes,* articles for proceedings or journals, and books like the *Container Tree Nursery Manual* (five volumes done, two more to go). Initially I was intimidated, but soon I came to realize that I'd better learn how to express myself in writing if I was going to be effective. I came to understand that technical writing is the only efficient way to reach a large number of people. Because writing generates a permanent record of information, I found that I didn't have to continually explain the same technique or concept to different people.

There are other benefits of technical writing. Forcing yourself to put your ideas down on paper is a valuable mental exercise. Verbal ideas are generally "fuzzy" as well as extremely transient, and often have to be reevaluated after they are committed to paper (or computer screen). Because technical writing forces you to expose your ideas to criticism, it is the most honest form of communication.

Okay, assuming that I've convinced you to try technical writing, now let me explain why you should write for *Tree Planters' Notes*! If you are a nursery manager, you don't have the time or training to be a researcher and run experiments. But articles that reflect your direct experience or observation could be valuable. Perhaps you could report on one of the small operational trials that you've done. Almost every nursery has conducted field tests of a new pesticide or fertilizer, or slightly modified some cultural operation. These tests are rarely laid out in an experimental design and are therefore not suitable for rigid statistical analysis. Contrary to popular opinion, this does not make them worthless. Nursery people are also great tinkerers. There is always a way to make something better, and so *every* piece of nursery equipment has been modified to some degree. These modifications need to be documented and shared.

To encourage such "tech transfer" by nursery and reforestation practitioners, *Tree Planters' Notes is* inaugurating a new section called "Practical Tips," for publishing reports by on-the-ground nursery folk. These articles will be reviewed by one of the tech transfer specialists on the editorial board as well as by a scientist. If you feel uncomfortable about writing a technical article, find someone who can help. I feel that one of my technology transfer responsibilities is to identify promising new information and help get it into

print. My two counterparts in the USDA Forest Service's Northeastern Area (Ron Overton) and the Southeastern Area (Clark Lantz) would also be willing to help. Give us a call: Tom Landis (503) 326-6231, Ron Overton (612) 649-5241, and Clark Lantz (404) 347-3554.

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(Adapted from *Forest Nursery Notes*, January 1990)

Note: Our concept of this editorial space is that it should be a place to publish opinions and ideas relating to the reforestation profession. We invite you to submit ideas for commentaries. The views expressed here are solely those of the author(s) and do not necessarily reflect those of the ***Tree Planters' Notes*** editorial staff, the Forest Service, or the U.S. Department of Agriculture.

Field Testing a Modified Duster for Supplemental Mass Pollination in Douglas-fir Seed Orchards

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An agricultural Solex duster (Model 100) was modified for supplemental mass pollination (SMP) of Douglas-fir— Pseudotsuga menziesii *(Mirb.) Franco— seed orchard trees. In performance trials, isolated megastrobili (female cone flowers) were exposed briefly to the outside environment while the tractor-mounted duster blew pollen on the 7.6- to 10.7-m-tall clones. Average filled seed efficiency (FSE) obtained was 34 and 48% after 1 and 2 SMP's, respectively. This FSE difference was significant at* " *= 0.02. The numbers of filled seeds per cone, from cones averaging 57 round seeds, were 19 and 27 after 1 and 2 SMP's, respectively. When 2 SMP's were done, FSE values were generally similar from the ground to 8.5 m, which indicated that the duster uniformly distributed the pollen throughout the crown.* Tree Planters' Notes 46(4):118-125; 1995.

Wind-pollinated seed orchards of Douglas-fir– *Pseudotsuga menziesii* (Mirb.) Franco— usually fail to achieve high genetic efficiency or maximum seed production potential. Factors such as asynchronous flowering (Copes and Sniezko 1991, Erickson and Adams 1989), unequal fecundity among clones (Nakamura and Wheeler 1992b), inbreeding (Nakamura and Wheeler 1992a), and pollen contamination (Fast and others 1986) all negatively influence the quantity and quality of seed (Webber 1987). Friedman and Adams (1982) indicate that pollen contamination may be the most important factor limiting genetic efficiency of seed orchards. Pollen contamination levels in Douglas-fir orchards in British Columbia range from 44 to 89% (Fast and others 1986). In addition, young seed orchard trees often fail to produce enough pollen to adequately pollinate female megastrobili, thereby resulting in low seed production or, if the level of background pollen is high, excessive pollen contamination. One way orchard managers hope to reduce or eliminate the above problems is by using effective supplemental mass pollination (SMP) equipment and procedures.

Supplemental mass pollination is a technique in which viable pollen is broadcast over exposed female megastrobili when the megastrobili are receptive (El-Kassaby and others 1993). For SMP to be effective, pollen application must be properly timed to coincide with the optimum stage of megastrobili receptivity. Sufficient pollen must be applied to fill all available pollen chambers before they are filled with unwanted background pollen. Only pollen with excellent viability should be used. Procedures and equipment that can be used to apply pollen rapidly and uniformly over the entire crown are needed. For pollen application, it may be necessary to complete SMP's on several hundred medium to tall trees within a few hours. In addition, equipment and procedures that allow rapid and economical collection and long-term storage of large volumes of pollen (possibly several hundred liters for large orchards) must be available.

Developing and testing a satisfactory tractor-mounted pollen applicator was done through a joint research project between the USDA Forest Service's Pacific Northwest Research Station, Siuslaw National Forest, and Missoula Technology Development Center. Our primary objective was to modify a commercially available tractor-mounted dust applicator so it could be used effectively and economically to pollinate 7- to 11-m-tall conifers. Techniques and equipment had to do the following:

1. Not damage the pollen grains during application.
2. Distribute the pollen quickly and evenly over the exposed crown.
3. Accurately measure and dispense small amounts of pollen.

4.Be simple to operate so that one person could simultaneously drive the tractor and operate the pollen duster.
5.Be durable and reliable under field conditions so that equipment does not fail during critical pollina tion periods.

Equipment Selection and Modification

A Model 100 Solex duster was selected for modification and testing. The model had the following factory specifications: air flow, 82 m^3/min (2,900 ft^3/min); air velocity, 145 km/hr (90 miles/hr). Fifteen horsepower was required to power the equipment through a type I and II hitch attached to a 540-rev/min power takeoff outlet.

The factory duster was designed primarily for applying pesticide dusts to vineyards. It directed the airflow from the outlet parallel to the ground rather than vertically, which is needed for pollinating tall trees. To correct that problem, the airflow was directed 60º upward by inserting a flange between the fan housing and the exit baffles (figure 1). Further adjustment of the airflow was done by manually adjusting the three horizontal deflector plates in the outlet housing. The chemical hopper and metering system were replaced with a 2-liter pollen hopper and a more accurate pollen-metering system (figure 2). Metering of pollen was done at the base of the pollen hopper with a rotating rod that contained the metering chamber. A small electric motor-driven rod rotated one revolution each time the remote control was activated and released a prescribed volume of pollen into the airstream. Technical drawings of all equipment modifications can be obtained from the USDA Forest Service, Missoula Technology and Development Center, Building 1, Fort Missoula, Missoula, MT 59801.

Field-Testing Methods

Final field tests of the modified duster were made in April 1993 on 7.6- to 10.7-m-tall grafted trees near Monmouth, Oregon. To ensure that only the effect of pollen from SMP was measured, we covered branch tips bearing female megastrobili with kraft pollination bags before the reproductive buds opened. All male buds were removed from the isolated areas of branches of 5 clones (trees used as females). Twenty isolation bags were placed over megastrobili on each tree. All the bags were located on the same side of each tree so that every megastrobilus could be pollinated with a single pass of the duster. The isolation bags were positioned throughout the crown so the effectiveness of the duster to distribute pollen uniformly could be determined.

The 145-km/hr (90-mi/hr) airstream of the Solex duster was used immediately preceding each SMP to remove previously shed pollen grains from each tree. Immediately after that "cleaning," the 20 isolation bags were removed and SMP was done. Five to ten minutes per tree were required to complete the removal of the 20 SMP isolation bags, complete the SMP treatment, and position the isolation bags over the megastrobili. A total of 100 ml (62 g) of pollen was released into the airstream in 5 or 6 increments of 20 or 16.7 ml, respectively. Ground speed of the tractor during pollination ranged from 3.2 to 4.8 km/hr (2 to 3 miles/hr). Pollination of each half-crown took about 4 sec. Half of the isolation bags were given a second SMP treatment with an additional 100 ml of pollen 1 day (clones 185-17 and 167-8), 2 days (clones 195-16 and 129-10), or 3 days (clone 207-11) after the first SMP

Pollen used for SMP was collected in 1992 using vacuum techniques and equipment described by Copes and other (1991) and stored at -135 ºC. *In vitro* germination tests of pollens were done shortly before SMP Pollen germination averaged greater than 90% viability. Viability was determined by using the 10P10B *in vitro* solution [10%Brewbaker solution and 10% polyethylene glycol (mw 4000)] described by Webber and Bonnet-Masimbert 1993). The pollen was rehydrated before in vitro testing, but not before SMP.

Mature, unopened cones were collected from each clone in August 1993 when the cones began to turn brown. Five healthy cones were selected from each bag, and each cone was placed in a separate container so the seeds from individual cones could be evaluated. The average number of seeds per cone from each isolation bag was the experimental treatment for the test. All round seeds (RS) were extracted from each cone by tearing each cone apart by hand, counting the number of RS per cone (RSC), and making X ray images of all RS to identify the number of filled seeds per cone (FSC). Flat seeds were excluded from the total seed count of each cone and from the calculations of filled seed efficiency (FSE) because they were not viable at pollination. FSE was calculated by dividing the number of FSC by the number of RSC and multiplying the quotient by 100.

Transformation of data before analysis of variance (ANOVA) was not necessary when the most frost-damaged clone (167-8) was excluded from analysis. Analysis of variance of 1 and 2 SMP treatments revealed different linear slopes for each treatment, making covariance analysis inappropriate. Instead, regression procedures for each SMP were used to investigate the linear relation between FSE and megastrobili height aboveground (SAS 1993). Significance was judged achieved when $\alpha = 0.05$. Clone selection was random and type III mean squares were used.

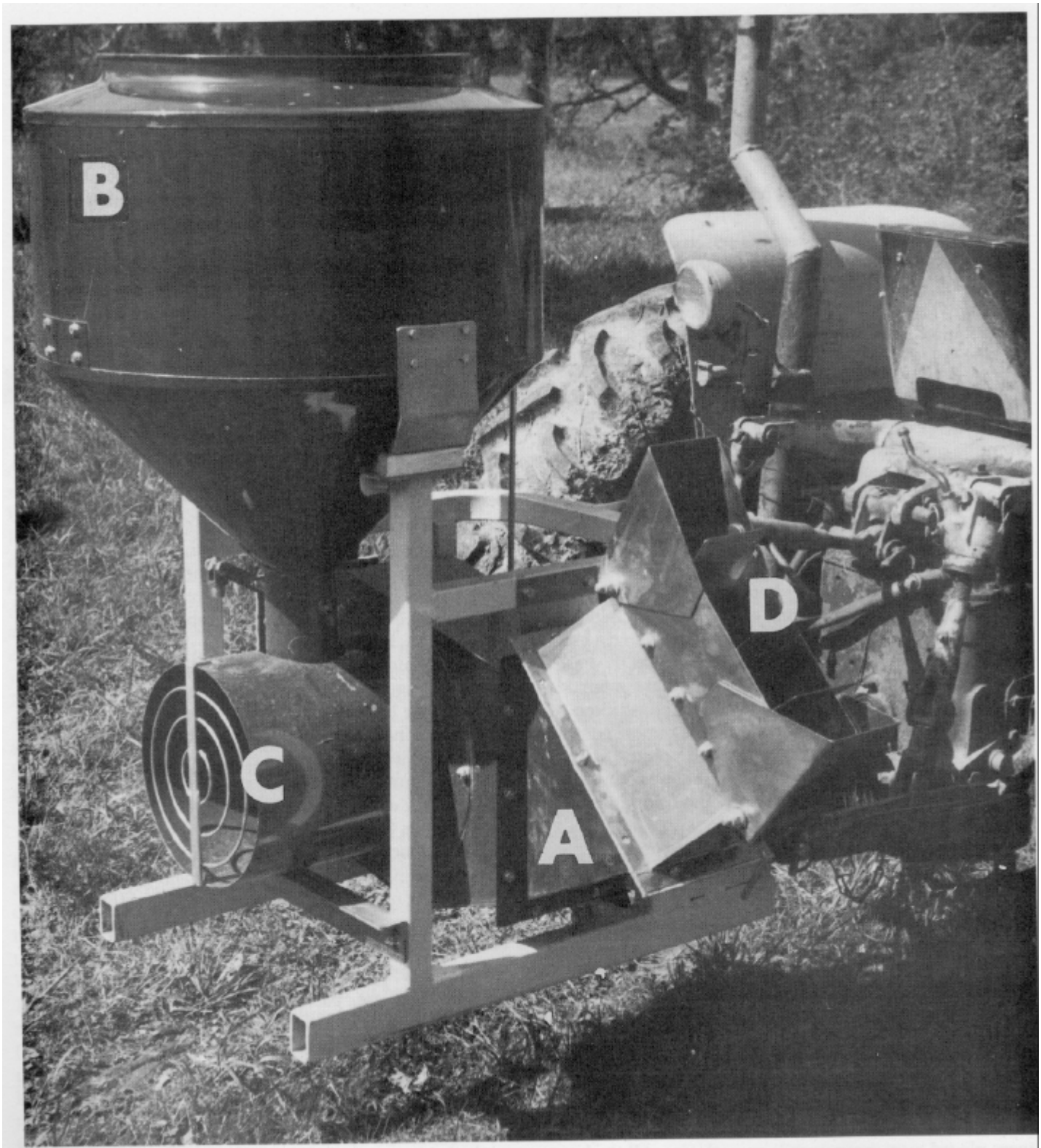

Figure 1—*The factory duster with a flange added to elevate outlet airflow to 60°. A = elevation flange, B = chemical hopper, C = air intake, D = adjustable baffle plate in air outlet.*

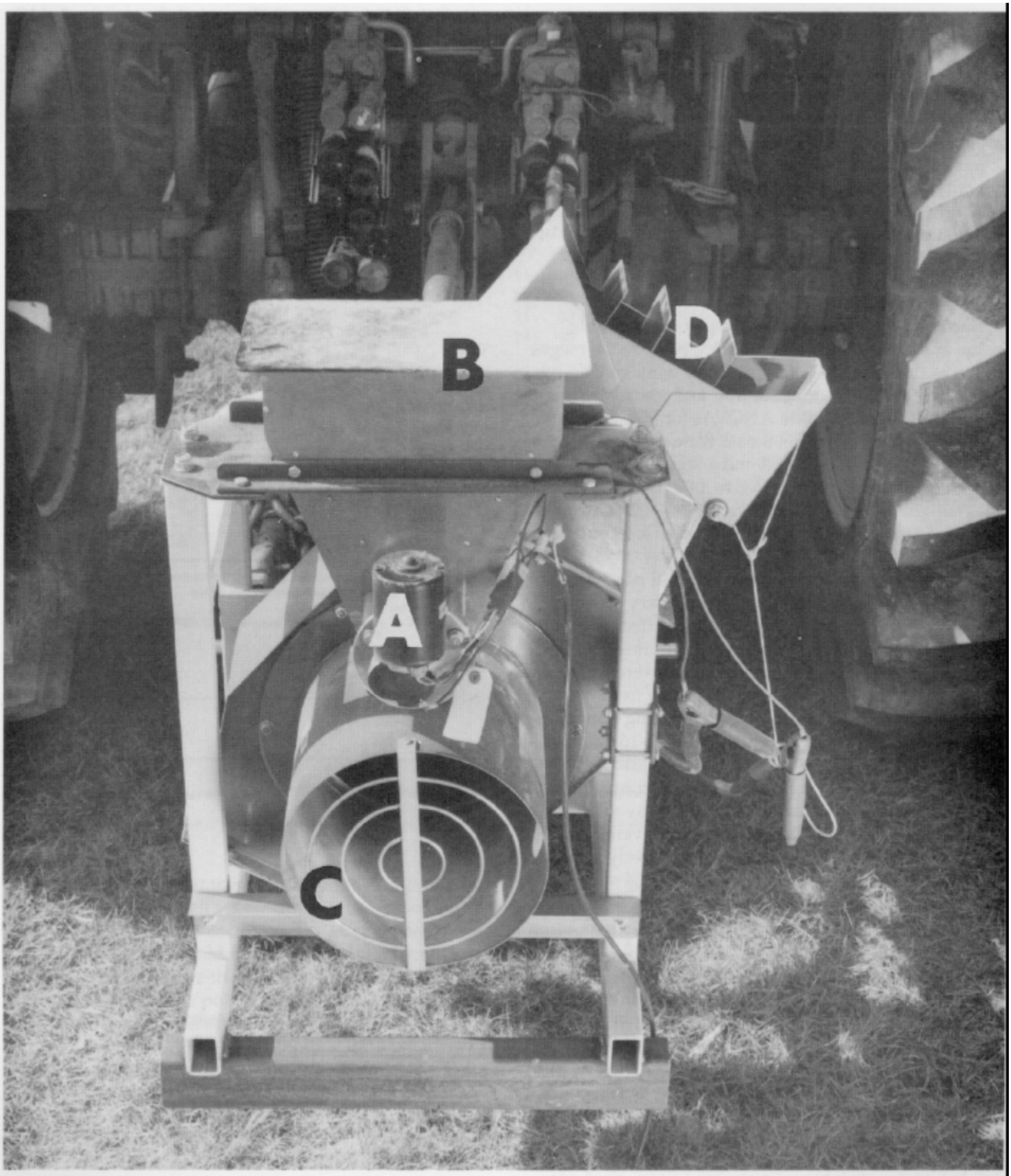

Figure 2—*Duster modified for SMP. **A** = electric motor for pollen metering, **B** = pollen hopper, **C** = air intake, **D** = adjustable baffle plate in air outlet.*

Results and Discussion

A damaging -5.5 °C (22 °F) frost occurred the night before the first SMP treatments on April 12, 1993. Damage from earlier frosts may have contributed to a reduction in the number of round seeds per cone and in lower FSE of both SMP and control pollinated cones. Severity of damage, as evidenced by variation in RS and FSE, differed among clones (tables 1 and 2). The RSC for the clones tested averaged about 60 (range, 50 to 65) of the 80 potential fertile ovule positions (Copes 1985). Results suggest there was a wide range in susceptibility of megastrobili to frost damage.

Seed data from clone 167-8 were of questionable value in evaluating duster efficiency because the megastrobili of that clone had been severely damaged, as evidenced by the low number of RSC and unusually low fecundity. Clonal effects in ANOVAs for FSE were not significant when clone 167-8 data were excluded. A single SNIP resulted in 34% FSE and a second SMP made 1, 2, or 3 days later improved FSE to 48% (table 1). This 14% FSE difference was significant (" = 0.02). Our present study supports the additive effect of a second SMP on FSE reported by El-Kassaby and others (1993).

Test results indicate the maximum FSE possible under the 1993 test conditions. Our primary objective was simply to determine the maximum effectiveness of the modified machine. To obtain that information, the masking effects of contaminating pollen were reduced or eliminated by keeping the megastrobili isolated except when the SMP treatments were being done.

Maximum FSE occurred with clone 195-16 (tables 1 and 2). The 56% FSE for clone 195-16 megastrobili approached the lower range achieved by conventional control-pollinations when megastrobili were saturated with pollen (Copes 1985). The study FSE averages of 34 and 48% FSE from 1 and 2 SMP's, respectively were greater than the 29% FSE average found in 4 wind-pollinated coastal Douglas-fir orchards (McAuley 1990). The average of 27 FSC (table 1) after 2 SMP's was larger than the 21 FSC reported in 4 orchards (McAuley 1990) and was comparable to 27 to 31 FSC from SMP and windpollinated cones from another orchard (El-Kassaby and others 1993). Two SNIP treatments in the present study produced clonal averages that ranged from 21 to 33 FSC (table 1).

Uniformity of FSE values among bags within clones after 2 SMP's indicated the duster uniformly delivered pollen as high as 8.5 m (table 2). A significant negative linear relation (" = 0.02) for average FSE and megastrobili height (bag height) existed after 1 SMP. That relation was not evident after 2 SMP's (table 2). On a clonal basis, significant negative-linear relations between FSE and isolation-bag height were detected for clones 207-11 and 185-17 after 1 SMP and for clone 185-17 after 2 SMP's. The general relation of FSE and number of SMPs is shown in figure 3 for data subjected to a 70% spline-smoothing procedure.

Table 1- *Results from 1 or 2 supplemental mass pollinations (SMP) applications (100 ml of pollen/application) on number of round (RSC) and filled seeds per cone (FSC) and filled seed efficien cy (FSE)**

	1 SMP				2 SMP's			
Clone	Mean bag height (m)	RSC (no.)	FSC (no,)	Mean FSE (%)	Mean bag height (m)	RSC (no.)	FSC (no,)	FSE (%)
207-11	6.4	72	16	22	6.4	72	33	47
195-16	7.6	58	24	42	7.3	56	31	56
129-10	6.7	53	19	37	5.8	50	24	50
185-17	7.3	46	17	36	7.3	51	21	42
167-8†	5.8	42	04	10	5.8	40	07	17
Mean	6.7	54	16	29	6.4	54	23	42
Mean without	7.0	57	19	34‡	6.7	57	27	48‡

* Filled seed efficiency was calculated by dividing the number of filled seeds by the number of round seeds per cone and multiplying the quotient by 100.

† Most Beverly frost damaged clone.

‡ Average FSE difference of cones that received 1 or 2 SMP's was significant (P=0.02).

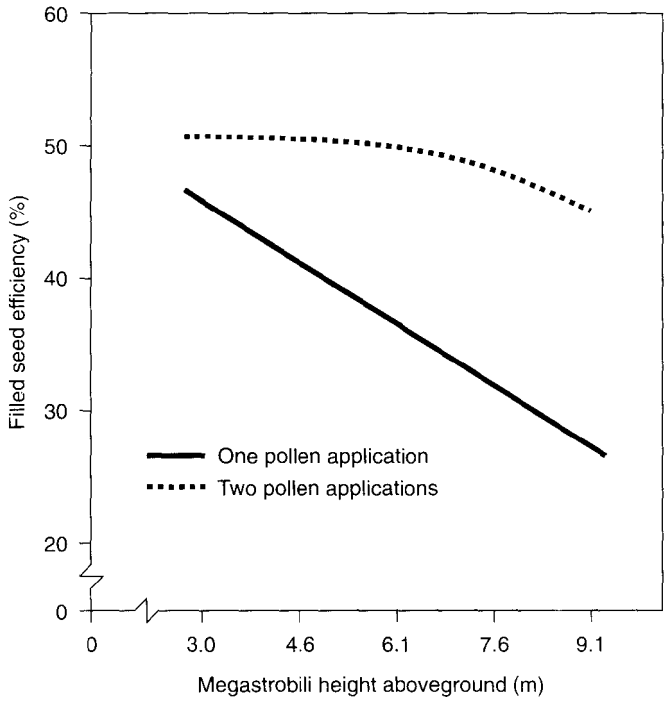

Figure 3— *The relation between filled seed efficiency (FSE) and height of megastrobili aboveground after a 70% spline-smoothing procedure.*

Table 2-*Filled seed efficiencies (FSE) after 1 or 2 SMP applications and number of isolation bags located between 2.7 and 9.7 m aboveground*

	FSE*										Average FSE/no. of bags			
													All clones	
Bag ht.	207-11		195-16		129-10		185-17		167-8†		All clones		exc. 167-8	
(m)	1	2	1	2	1	2	1	2	1	2	1	2	1	2
9.7			39								39/1		39/1	
9.4	12	30		45	18						15/2	38/2	15/2	38/2
9.1			39	50				19	05		22/2	35/2	39/1	35/2
8.8			34		26	54	21			09	27/3	32/2	27/3	54/1
8.5			23		47		30	36			33/3	36/1	33/3	36/1
8.2	11	54					31	33			22/2	44/2	22/2	44/2
7.9			46	62			24	49		19	35/2	43/3	35/2	56/2
7.6	19			62		64					19/1	63/2	19/1	63/2
7.3		50			32		34	48			33/2	49/2	33/2	49/2
7.0			65				37		09		34/3		51 /2	
6.7	21	49	55	56				50			38/1	52/3	9/1	52/3
6.4	08		22	50	24		53			19	27/4	35/2	27/4	50/1
6.1	29	56	41			61		50			35/2	56/3	35/2	56/3
5.8	38	60				32					38/1	46/2	38/1	
5.5	25				48				06	07	26/3	07/1	37/2	
5.2						51	61	50	13		37/2	51 /2	61 / 1	51 /2
4.9										15		15/1		
4.6	26	38		69		33				19	26/1	40/4	26/1	47/3
4.3			56							29	56/1	29/1	56/1	
4.0					54					19	54/1	19/1	54/1	
3.6									17		17/1			
3.3	28	36							11		20/2	36/1	28/1	
3.0														
2.7					48	53					48/1	53/1	48/1	53/1
0.0														
Mean	22	47	42	56	37	50	36	42	10	1	29	42	34	48
P>t ‡	.05	.95	.21	.09	.06	.45	.00	.02	.10	.6	NS	NS	02	.43

NS = no statistical test was done.

* FSE was calculated by dividing the number of filled seeds per cone by the number of round seeds per cone and multiplying the quotient by 100.

†Most frost-damaged clone.

‡ Probability of obtaining a larger value of t by chance for the negative linear relation between FSE and height of megastrobili aboveground.

The airstream outlet was large enough and properly oriented to pollinate all visible areas of the crowns as the duster moved past each tree. The effectiveness of the duster above 9.4 m could not be determined because of insufficient bags above that height. The machine appeared to move the pollen highest in the trees when the ground speed was slowest. Fast movement of the tractor and duster may create a horizontal wind-speed vector that reduces vertical wind speed, thereby decreasing the effective dusting height.

Variation in FSE and RSC among megastrobili located within isolation bags was detected. Such variation may result from the megastrobili receiving different amounts of SMP due to wind drift, poor megastrobili location in the crown in relation to the position of the duster, or simply because some megastrobili may not be receptive when SMP is done. Two SMP applications removed much of the among-bag variation in FSE (table 2). Individual megastrobili of a clone do not all become receptive the same day (Owens and Simpson 1982). During warm springs in western Oregon, it is common for 80 or 90% of the megastrobili on individual trees to open within 3 days of the opening of the first megastrobilus bud (personal observation). Similarly, individual trees in British Columbia complete shedding pollen some years in 3 to 5 days (Ebell and Schmidt 1964). Cool, wet weather occurring after opening of the earliest megastrobilus buds may delay opening of unopened buds by a week or more. Combinations of cool, wet weather prior to bud opening and warm, dry weather at the start of megastrobili bud opening often result in a short breeding seasons (Copes and Sniezko 1991,

Fashler and El-Kassaby 1987). Conditions that promote rapid and uniform megastrobilus opening enhance SMP success and lower costs by reducing the number of pollinations needed.

The second SMP applications were done 1, 2, or 3 days after the first SMP's for clones 185-17 and 167-8, 195-16 and 129-10, and 207-11, respectively. An average of 6 to 8% increase in FSE occurred for each day the second SMP was delayed (table 2). The statistical significance of this could not be tested due to lack of replication. Increased FSE may have occurred because additional megastrobili had become receptive following the first SMP. Owens and Simpson (1982) and Webber (1987) both report that Douglas-fir megastrobili remain highly receptive for 6 days following bud opening. El-Kassaby and others (1993) report FSE increases after 2 SMP applications, but with little increase from additional SMP

Effective SMP procedures must ensure that receptive megastrobili are pollinated before they receive significant amounts of contaminating pollen. Unwanted pollen fills pollen chambers and physically prevents late-arriving SMP pollens from being taken into the pollen chambers (Webber and Yeh 1987). In Douglas-fir, an average of 3 to 4 pollen grains/pollen chamber is common (Owens and others 1991). The significant within-clone variation in megastrobili development (Owens and Simpson 1982) will necessitate several SMP's on each tree. Timing of applications is important, as Webber and Yeh (1987) indicate that pollen arriving 6 hours after earlier pollination is less likely to be taken into the pollen chambers.

We suggest an operational SMP program in which pollen is applied to each receptive tree in early morning of the day following first megastrobilus bud opening. The first SMP should be followed with a second early morning SMP on day 2 or 3, and possibly with a third SMP on days 3, 4, or 5. Pollinating in early morning is important because little pollen is usually shed during the night or early morning hours when the relative humidity is high and the wind speed is low. Warm, dry and cool, wet weather can increase or decrease the effective length of the pollination period, respectively (Copes and Sniezko 1991). The actual SMP schedule each year must be based upon floral phenology of the clones in that particular year.

The "first come, first in" pollination mechanism in Douglas-fir (Webber and Yeh 1987) appears to lend itself to SMP manipulation. Technology exists for rapid and cost-efficient vacuum collection and long-term storage of the quantities of Douglas-fir pollen needed for the SMP of hundreds of large trees (Copes 1987, Copes and others 1991, Webber 1987). Our modification of the factory duster was necessary so that the machine could accurately and uniformly dispense precise volumes of viable pollen. Pollinations can be accomplished rapidly and efficiently with just 1 tractor operator.

Operational SMP will never succeed in excluding all unwanted pollen, but we believe that a meaningful reduction will occur if proper SMP procedures and equipment are used. We hope for additional gains in the future as we obtain more accurate knowledge of amount of pollen needed, the number of SMP's, and the optimum timing of SMP applications.

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Mexican Conifers' Response to Fertilizer Type Indicates Difference Between Value and Cost

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Mexican forest nurseries produce most seedlings in polyethylene bags containing forest soil. Fertilization practices often are imprecise and use an expensive, slow-release formulation. The objective of this study was to evaluate alternative fertilizer practices using two Mexican conifers: Pinus douglasiana *Mart. and* P. pseudostrobus *Lindl. Seedlings were fertilized with Osmocote™; Peter's Conifer Grow™; and Picomodulus™, a slow-release formulation common to many nurseries in Mexico. The controls were seedlings that were not fertilized.* Pinus pseudostrobus *responded to all fertilizers equally. There was no difference in seedling diameter, dry weight or root to shoot ratio.* Pinus douglasiana, *a species with a seedling grass stage, responded best to Osmocote and Picomodulus. However, of the three fertilizer types, only fertilization during irrigation (that is, "fertigation") with Peter's Conifer Grow resulted in seedling nitrogen contents greater than 2%. Seedlings responded to nitrogen fertilization at least 300 days after seeding, indicating that nursery managers can compensate for inadequate fertilization by instituting a fertilization program at almost any time. With little difference in response, managers should use the most cost-effective fertilization method.* Tree Planters' Notes 46(4):126-129; 1995.

Fertilization is an integral component of nursery production, and nitrogen is the most important nutrient for maximum benefit (Fisher and Mexal 1984). Switzer and Nelson (1963) found that loblolly pine *(Pinus taeda* L.) seedlings required about 120 mg of nitrogen for maximum growth and yield. Furthermore, fertilization effects last well beyond the nursery phase. Increased seedling size and nutritional status increase seedling survival and growth. Van den Driessche (1982) found survival of Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) was best when seedling nitrogen content was *2%.* Furthermore, Autry (1972) showed that the residual, fertilizer-induced size differences in seedlings resulted in size differences 16 years after outplanting. South and others (1988) found that nursery effects lasted 30 years after outplanting. Thus it is conceivable that nursery fertilizer responses could last throughout a plantation's life.

Although fertilization is important biologically, it is almost insignificant economically. Fertilizer accounts for only 0.03% of container seedling production cost (Landis and others 1995). Thus, the long-term benefits of a well-planned fertilization program can be attained at practically no cost.

Much of the published information on fertilizer response of timber species is based on nurseries in the United States and Canada There is little published information about fertilizer response of timber species native to Mexico. Most seedlings grown for reforestation in Mexico are grown in plastic bags with native forest soil as the growing medium, and many nurseries rely on the inherent fertility of these soils. Consequently, fertilizer use in Mexican nurseries ranges from none to using expensive soluble or slow-release fertilizers (table 1). There is little indication that commercial, agriculturalgrade fertilizers are used in nursery production. The wide range in fertilizer use across nurseries results in a wide range in subsequent seedling size and quality. The objective of this study was to evaluate the response of two Mexican timber species to different fertilization types.

The species selected— *Pinus douglasiana* Mart. and *P. pseudostrobus* Lindl.— are important timber species in central and southern Mexico (Perry *1991). Pinus douglasiana* is found primarily in the states of Guerrero, Jalisco, and Michoacan, between 1,500 and 2,500 m. *Pinus pseudostrobus* is found further east in the states of Hildago, Michoacan, Mexico, Puebla, and Tlaxcala. It grows between 1,600 and 3,200 m. Both species can attain heights of 35 to 40 m. *Pinus douglasiana* has a "grass stage" as a seedling.

Table 1— *Unit price and cost per kilogram of nitrogen (N) of different fertilizers in Mexican nurseries*

Fertilizer	Composition (NPK)	Type	Approximate cost N$/kg	Approximate cost N$/kgN
Urea	45:0:0	Granular	1.90	4.22
Ammonium sulfate	21:0:0	Granular	1.90	9.05
Peter's Conifer Grow™	20:7:19	Soluble	14.89	8.67
Bayfolan™	24:0:0	Soluble	26.40	110.00
GrowGreen™	20:0:0	Soluble	26.40	132.00
Osmocote™	14:14:14	Slow release	16.76	119.71
Picomodulus™	25:12:7	Slow release	204.60	818.40

Cost is in Mexican pesos at an exchange rate of N$6.10 to US$1.00 on May 1995.

Materials and Methods

Seeds of *Pinus pseudostrobus* and *P. douglasiana* were sown (2 seeds/container) on April 7, 1994, into RL Containers (164 ml) containing a bark–scoria–sand mixture (2:1:1). There were four fertilizer treatments:

1. Control (no supplemental fertilization)

2. Peter's Conifer Grow™ (20:7:19 + micronutrients), applied at 100 ppm N with every irrigation (that is, "fertigation")

3. Osmocote™ (14:14:14), incoportated into the medium at 4 kg/m^3

4. Picomodulus™ (25:12:7), a slow-release formulation manufactured in Mexico, applied at 1 tablet (350 mg)/container

Seedlings were irrigated as needed. Containers were thinned to 1 seedling in May and fertigated (treatment 2) from May through August. Height, diameter, and root and shoot dry weight were measured on 25 seedlings/treatment on August 30, 1994. Shoots were combined and analyzed for nutrient concentration at Grace-Sierra Technical Services Laboratories. The original study design consisted of 3 replications of 49 containers each in a randomized block.

Beginning September 1994, a subset of *Pinus pseudostrobus* seedlings from the control group (treatment 1) were fertigated with 100 ppm N for 6 months. At 320 days, the remaining control trees were fertilized for an additional 115 days. Height was measured at each date to determine the seedlings' ability to recover from poor fertilization.

Results

After thinning, many seedlings succumbed to damping off during June. Survival was poorest for seedlings receiving Osmocote (treatment 3) (table 2). Survival of *Pinus douglasiana* with Osmocote was only 32%. The other incorporated fertilizer, Picomodulus (treatment 4), did not increase mortality of either species. Consequently, the three replications were combined into one block for further evaluation.

As expected, no fertilization (treatment 1) resulted in stunted seedlings (table 3) like those seen in some nurseries in Mexico. This may indicate that fertilization is inadequate at these nurseries, especially if forest soil without supplemental nutrients is used. There was little difference in seedling morphology among fertilization treatments. Seedlings of *Pinus pseudostrobus* were shorter when fertilized with Picomodulus, and seedlings of *P. douglasiana* had larger diameters and root dry weights when fertilized with Osmocote or Picomodulus. Seedlings fertilized with Picomodulus had altered root morphology, apparently caused by the plant growth regulators present in the formulation. Lateral roots in the upper 25% of the rootball had bifurcated short roots resembling mycorrhizal roots. However, microscopic examination indicated a lack of fungal hyphae or mantle characteristic of ectomycorrhizal structures.

Morphologically, seedlings from all the fertilizer treatments were acceptable. However, only fertigated seedlings (treatment 2) had adequate levels of nitrogen (target = 2% N). Other treatments were considered deficient in nitrogen (table 3). The phosphorus and potassium levels were not different among the 4 fertilizer treatments.

Pinus pseudostrobus seedlings that had not been fertilized for 150 days from seeding (F_2) responded immediately to fertilization (figure 1). Furthermore, seedlings fertilized for the first time 324 days after seeding (F_3)

Table 2—*Percentage of containers with live seedlings 2 months after seeding for replications 2 and 3 (replication 1 was not surveyed)*

Species & treatment	% Survival	SD
Pinus pseudostrobus		
Control	83	13
Peter's™	88	4
Osmocote™	64	3
Picomodulus™	73	6
Pinus douglasiana		
Control	47	0
Peter's™	55	2
Osmocote™	32	9
Picomodulus™	73	4

Table 3- *Seedling morphology and nutrient content after 145 days under different nutrition treatments*

Species & treatment	Height (cm)	Diameter (m m)	Dry weight Shoot (g)	Root (g)	R/S	Nitrogen (%)	Phosphorus (%)	Potassium (%)
P. pseudostrobus								
Control	2.8 a	0.92 a	0.14 a	0.12 a	.90 a	0.50	0.21	1.37
Conifer Grow™	20.7 c	1.71 b	0.59 b	0.29 b	.50 b	2.19	0.27	1.12
Osmocote™	20.3 c	1.84 b	0.60 b	0.29 b	50 b	1.62	0.20	0.99
Picomodulus™	15.7 b	1.69 b	0.56 b	0.33 b	.61 b	0.86	0.16	0.77
*P. douglasiana**								
Control	-	1.46 a	0.16 a	0.14 a	.77 a	0.44	0.20	1.05
Conifer Grow™	-	1.90 b	0.52 b	0.18 b	.35 c	2.01	0.28	1.51
Osmocote™	-	2.03 bc	0.57 b	0.22 bc	.40 bc	1.55	0.26	1.45
Picomodulus™	-	2.18 c	0.51 b	0.24 c	.48 b	1.06	0.24	1.26

Values followed by the same letter are not significantly different (P=.05).

*Seedling with grass stage.

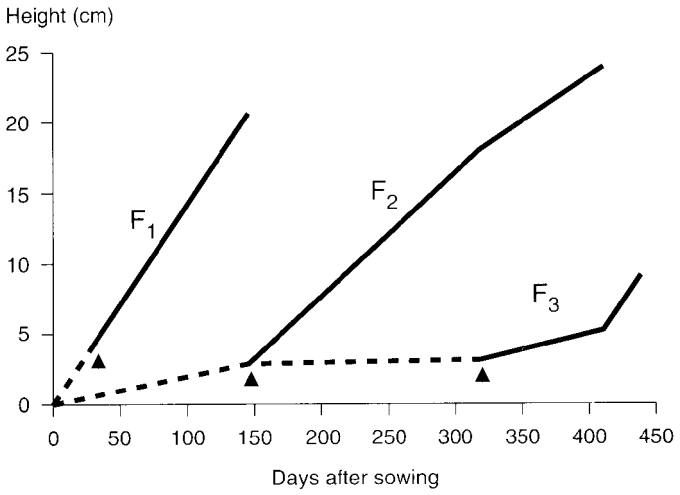

Figure 1— *Height growth of* Pinus pseudostrobus *seedlings in response to fertilization. The initiation of fertilization is indicated by an arrow.*

also responded to fertilization, although the growth rate appeared to be slower. Seedlings fertilized from seeding grew at 1.4 mm/day. Seedlings fertilized after 150 days grew only 0.8 mm/day during the fertilization period, and seedlings fertilized after 324 days grew only 0.5 mm/day during fertilization. Although seedlings maintain the ability to respond to fertilization, the level of response is greatest if fertilization begins shortly after emergence.

Implications

There was little difference in the biological response of these two species to different types of fertilization. 1n fact, both species responded similarly in spite of different growth habits. However, the cost of these fertilizers vary considerably (table 1). Obviously, there is no fertilizer cost associated with a lack of fertilizer, but a different price is paid in poor seedling growth. Granular fertilizers are the least expensive (<N$10/kg N). The commercial fertilizers used in this study range in price from N$100/kg N for Peter's Conifer Grow, to N$122/kg N for Osmocote and more than N$800/kg N for Picomodulus. With no biological difference in response, there is no need to use limited financial resources on a fertilizer costing nearly 7 times more than more cost-effective alternatives. The actual cost per seedling for the Picomodulus fertilizer is even higher because of the tablet's size. The Picomodulus costs about N$0.07/ seedling compared to about N$0.002 for other slow-release or soluble fertilizers. Without a proven benefit, nursery managers should use cost-effective fertilizers, and conduct fertilizer trials periodically to ensure optimum growth rates. Fertilization should begin shortly after emergence, within 1 month of sowing, to maximize seedling growth response.

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Effect of Seed Size on Seedling Growth of a Shade-Tolerant Tropical Tree (*Hymanea stilbocarpa* Haynes)

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The effect of seed size (weight and length) on seedling growth o f a tropical species adapted to shaded environments was tested. Seeds of a climax tree species characteristic of the Atlantic Forest (Hymanea stilbocarpa *Haynes) were sized by weight (heavy, medium, or light) and length (long, mean, or small). Seeds were scarified and sown under 3 light regimes: (1) full sun-an open forest gap (100% full solar radiation flux at noon), (2) partial shade— a closing forest gap (80% full radiation at noon), and (3) deep shade— under a forest canopy (40% full radiation at noon). Twelve weeks after the development of primary leaves, the seedlings were measured for total height and root collar diameter.* Hymanea *seedlings grew best under shaded conditions despite seed category. Seed weight did not significantly influence seedling height or root collar diameter under either shading treatment. Seed length influenced seedling height and root collar diameter when seedlings were grown under either shade treatment. In deep shade, seeds with longer-than-average axes yielded seedlings 24.0 mm taller and 5.5 mm thicker at the root collar than seedlings from short seeds.* Tree Planters' Notes 46(4): 130-133; 1995.

Hymanea stilbocarpa Haynes occurs through Brazil, Argentina, and Paraguay on sites between 40 and 900 m above sea level, with annual precipitation of 1,000 mm and annual mean temperature of 19 to 28 ºC. It is a 10-to 15-m-tall tree with a dbh of 40 to 80 cm and a dense, large rounded crown (figure 1). Leaves are composite and alternate, each with 2 shiny and thick leaflets (figure 2). Inflorescences are hermaphroditic, white or creamlike, with 14 terminal flowers producing long and brown fruits bearing 2 to 8 seeds (figure 2).

One possible adaptation for ensuring successful seedling establishment is the possession of a large (long and heavy) seed that provides an ample reserve of nutrients during the period immediately after germination. This reserve allows the seedling to achieve critical size and capture external resources in competition with other plants. Within a population, a range of seed sizes is produced. Several studies have demonstrated that the seedlings derived from larger seeds consistently maintain a size advantage over the seedlings from smaller seeds— for the species *Lupinus texensis* (Schaal 1980), *Mirabilis hirsuta* (Weis 1982), and *Raphanus raphanistrum* (Stanton 1984)whereas several other species had the opposite or no effects.

Figure 1— *Full-grown* Hymanea stilbocarpa *Haynes.*

Figure 2— *Close-up of ripe seeds of* Hymanea stilbocarpa *Haynes.*

The importance of seed weight for seedling establishment in the shade is indicated by experiments carried out by Grime and Jeffrey (1965) on saplings of 9 North American tree species. The species used included light-seeded trees characteristic of open woodland and heavy-seeded trees of dense forests. The seedlings were grown in artificial shade conditions by surrounding each with a blackened cylinder that provided a gradient of light to dark. After 12 weeks, mortality was found to be inversely proportional to the weight of a seed's food reserve. More seedlings from light seeds died than did those from heavy seeds.

If the possession of large seeds is an adaptation for establishment in shade, it can be expected that large seeds store more carbon than small seeds because of the need to compensate for reduced carbon assimilation in the early stages of seedling growth. However, seed weight, by itself, cannot always be taken as an indication of shade tolerance. Augspurger (1984) working with light requirements of 18 forest tree species in Central America found that survival in shade was not correlated with seed weight but was related to the successional status of the species. Late-stage successional trees tended to have seedlings that were more shade-tolerant despite their seed weight.

The objective of this study was to test if seed size, as quantified by fresh weight or by long axis, influenced seedling growth under different radiation flux regimes— a distinct characteristic of late successional stages.

Materials and Methods

Fruits of *Hymanea stilbocarpa* Haynes were collected from 5 trees at the Universidade Federal Rural do Rio de Janeiro (UFRRJ) campus, Itaguai (22º 34' S and 42º 19' W), Rio de Janeiro, Brazil. Seeds were manually extracted, individually weighed, and measured at their long axis, then scarified in hot water for 30 minutes.

The seed lot was divided in two sublots: in the first, seeds were classified as heavy, medium, or light, based on their fresh weight (table 1); in the other, seeds were classified as long, medium, or small, based on their length (table 2). The cut-off point was the mean (for either seed weight or seed length) ± 1 standard deviation of the mean. Seeds were sown in plastic boxes measuring 40 X 30 X 10 cm filled with sterilized river sand; 9 boxes were used for each seed category. Each box received 28 seeds randomly chosen within a size class planted in an evenly spaced grid of 4 rows and 7 columns.

After germination indoors, 3 boxes for each seed category were placed under one of the following conditions, all within the UFRRJ campus:

Table 1-*Classification of seeds of* Hymanea stilbocarpa *Haynes based on seed weight*

Seed weight class	Mean wt. (mg)	SD (mg)
Light	439.4	50.3
Medium	537.7	28.2
Heavy	627.8	39.9

Table 2-*Classification of seeds of* Hymanea stilbocarpa *Haynes based on seed length*

Seed length class	Mean length (mm)	SD (mm)
Small	24.03	1.41
Medium	26.35	10.2
Long	28.82	0.81

1. Full sun— large forest gap (100% of full solar radiation flux at noon)
2. Partial shade— partial canopy opening (80% of full solar radiation flux at noon)
3. Deep shade— a secondary forest canopy (40% of full solar radiation flux at noon)

Percentage radiation was measured by a radiometer. Extra seeds were germinated to replace those that did not germinate. After germination, the boxes received water as necessary as well as 500 ml of nutrient solution (10-10-10 NPK plus micronutrients) every other week. Manual weeding was performed accordingly. Seedlings were harvested 12 weeks after the development of primary leaves. Data from the inner 20 seedlings in each box were used for statistics. Analyses of variance (ANOVA), using a randomized design, were conducted to determine the effect of seed fresh weight (SFW) and seed length (SL) on total aboveground length and root collar diameter.

Results

There was 100% germination and no seedlings died during the experiment. As expected with a shade-tolerant tree species, the data analysis (table 3) revealed that seedlings under either shade treatment attained more height ($P < 0.05$) growth (figure 3); mean seedling height, pooled across seed weight and seed length, under full sun after 12 weeks was 231 mm whereas those under the shade treatments were 243 and 254 mm for partial and deep shade, respectively. On the other hand, secondary growth (root collar diameter) did not differ among seedlings submitted to different light regimes (figure 4).

Table 3— *Significant level for seedling height and seedling root collar diamenter due to shading and interactions of shade with seed weight and seed length*

Source of variation	DF	Significance level: Seedling height	Significance level: Seedling root collar diameter
Shade	2	0.0037 **	0.4245 ns
Seed weight x shade	4	0.8984 ns	0.7066 ns
Seed length x shade	4	0.0266 **	0.0242 **

ns = not significant, ** = significant at P < 0.05.

As for the influence of seed size upon growth, seed weight did not bear any statistical influence on seedling development among radiation treatments. Seed length, on the contrary, had positive effects on the growth of seedlings under deep shade. Long and medium seeds produced seedlings taller than those grown from short seeds (figure 5). Mean root collar diameter from seedlings grown from long seeds was statistically ($P < 0.05$) different from those grown from short seeds (figure 6). Correlation coefficient between seed weight and seed length was 0.486 ($P > 0.05$). Interpretation of the results agreed with the interpretations of Augspurger

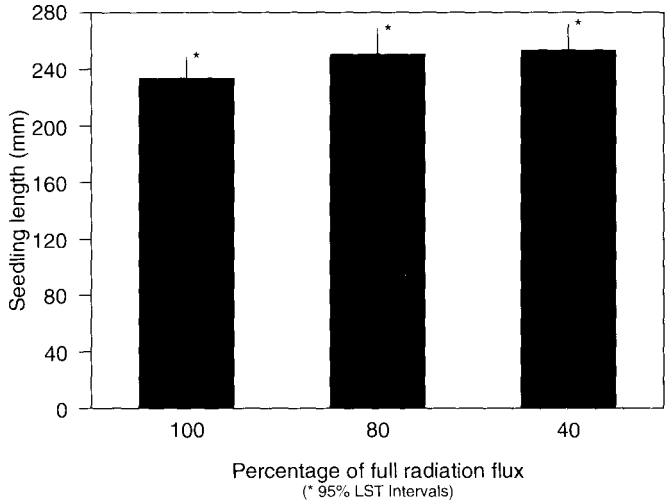

Figure 3— *Effect of radiation flux regimes on seedling length at 12 weeks after development of primary leaves pooled across seed weights and lengths (means and 95% LSD).*

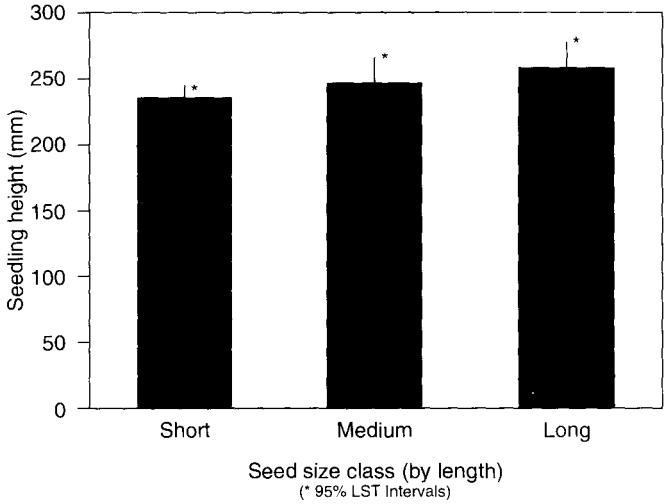

Figure 5— *Influence of seed size class on height of seedlings grown under full shade (40% of full solar radiation flux at noon) (means and 95% LSD).*

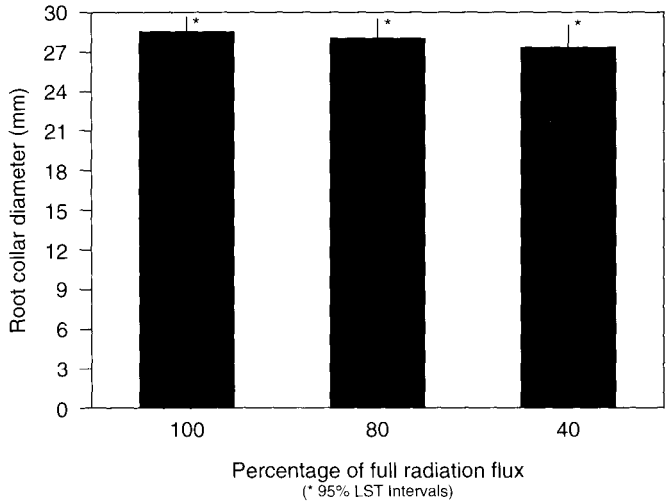

Figure 4— *Effect of radiation flux regimes on seedling root collar diameter at 12 weeks after development of primary leaves pooled across seed weights and lengths (means and 95% LSD).*

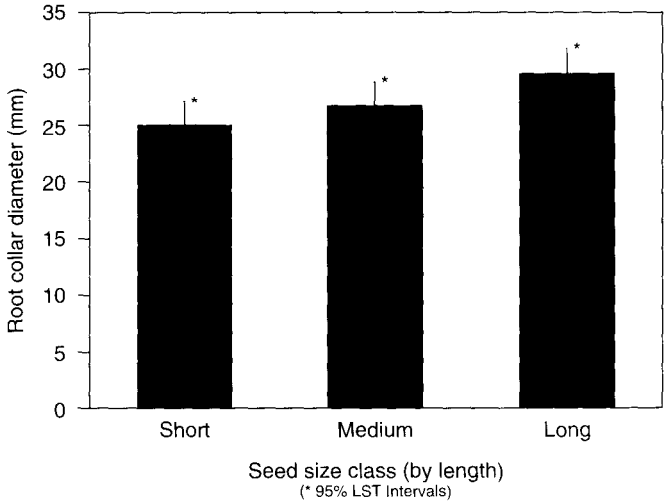

Figure 6— *Influence of seed size class on root collar diameter of seedlings grown under full shade (40% of full solar radiation flux at noon) (means and 95% LSD).*

(1984) and Fenner (1983), for whom seed weight, in itself, is not an indicator of adaptation to shade conditions. With *Hymenae,* seed length had more influence on seedling size attained 12 weeks after development of definitive leaves than seed weight. It is hypothesized (Malavasi and Malavasi 1992) that there may exist a direct relationship between seed length and embryo length for species of shaded environments. Further studies with X-ray techniques to measure embryo length of intact seeds would be appropriate.

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Germination of Carolina Silverbell Seed

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Seeds o f Carolina silverbell (Halesia tetraptera *Ellis var.* tetraptera) *from 4 seed lots were subjected to 7 pretreatment/stratification regimes. Germination rate and percentage differed significantly among seed lots, ranging from 0 to 80% at the end of 65 weeks. It appeared that seed lots varied in their requirements for cold stratification. An 8-hour sulfuric acid soak, followed by a warm-cold-warm stratification cycle significantly increased the rate and cumulative germination at the end of the 65 weeks for 3 of the 4 seed lots examined.*
Tree Planters' Notes 46(4):134-137; 1995.

Carolina silverbell *(Halesia tetraptera* Ellis var. *tetraptera,* formerly *H. carolina* L.) is a native understory tree that provides a source of food for wildlife (Bonner and Mignery 1974). It also has potential for increased usage as an ornamental in the landscape. The species shows a high degree of pest tolerance and cold hardiness (Dirr 1990). Found in riparian and wooded areas, the species has a native range extending from Florida to West Virginia to eastern Oklahoma. The cultivated range is broader— USDA hardiness zones 5-8 (USDA 1990)— with limited possibilities in zone 4 (Dirr 1990).

Carolina silverbell can be propagated by seed or asexually by cuttings or micropropagation (Dirr and Heuser 1987). Seed propagation of this native tree would allow the nursery industry to preserve genetic diversity for reforestation and landscape uses. Seed propagation is usually done by dewinging the fruit, a 4-winged, reddish-brown, corky drupe with a 4-celled ellipsoid stone ovary (Bonner and Mignery 1974), and sowing the stone (seed).

Seed propagation has been reported to require either complex temperature regimes or a 2-year natural cycle for maximum uniform germination. Seed requires warm moist stratification, followed by a cold moist stratification period for germination (Bonner and Mignery 1974, Dirr and Heuser 1987, Giersbach and Barton 1932). Moist stratification at 13.3 °C (56 °F) for 60 to 120 days, followed by 60 to 90 days at 1 to 5 °C (33 to 41 °F) was effective in one study (Bonner and Mignery 1974); however, a second cold stratification period was required in another study (Dirr and Heuser 1987).

Deno (1993) reported that Barton and Crocker (1948) increased germination with sulfuric acid but did not give details of the treatment. Cutting both tips of fresh ly ripened fruits may also increase rate of germination (Dr. W. Witte. Personal communication. Orono: University of Maine); however, Bir (1987) was not able to overcome fruit dormancy by cutting a notch in the seed coat.

The reported discrepancies suggests that other factors, such as seed source, may affect germination requirements. Seeds lots have been reported to differ in their degree of dormancy (Young and Young 1992).

The objectives of this study were to (1) examine fruit pretreatments and stratification procedures to determine if accelerated uniform germination could be improved over the standard procedure of no pretreatment followed by warm-cold-warm stratification and (2) examine the role of different seed lots in optimizing germination.

Materials and Methods

In November 1992, mature fruits were obtained from 4 wild-collected sources (table 1). These collections were part of a larger provenance trial being conducted and were chosen because they had large numbers of fruit. They are not meant to represent the geographic range from which they were sampled.

Immediately following collection, fruits were stored in sealed plastic bags and placed in a refrigerator (4 °C). Wings were removed from each fruit by hand in February/March 1993. Fruits from each of the 4 seed lots were mixed and divided into 7 groups of 100, or 7 equal quantities for seed lots with less than 700 fruit. The number of fruit per subgroup for each seed lot was West Virginia, 100; Tennessee, 33; Georgia, 100; and North Carolina, 98. Fifty surplus fruit from the West

Table 1-*Carolina silverbell seed lots examined for germination*

Location	No. of trees	Collector
Kanawha Co., West Virginia	12	Dr. Edward Garvey U.S. National Arboretum
Macon Co., Tennessee	1	Dr. Scott Schlarbaum University of Tennessee
Polk Co., North Carolina	1	Dr. Scott Schlarbaum University of Tennessee
Richmond Co., Georgia	6	Mr. George Barrett

Virginia lot and 100 from the Georgia lot were removed from cold storage in the spring of 1995 and examined with a cut test to determine the percentage of sound seed.

The 7 subgroups were subjected to four pretreatments and three stratification cycles (table 2). Insufficient fruit did not allow for all 12 possible combinations. The fruit pretreatments included (1) nontreated, (2) tip cutting, (3) hot water, and (4) sulfuric acid. For the second pretreatment, the pedicel end of each fruit was excised with pruners carefully, so that the embryo was not cut into. The process was time consuming because embryo chamber location varied with fruit size. The purpose of this treatment was to provide an easy imbibition point for moisture and an "exit" point for embryos. For the third pretreatment, hot water (exact temperature unknown, >85 ºC) was poured over the fruit and left to soak for 24 hours. For the fourth pretreatment, fruits were soaked for 8 hours in concentrated sulfuric acid, followed by a 21-hour soak in water to thoroughly rinse the fruits. The water was changed after 16 hours and the fruits were rubbed to remove any of the outer deteriorated fruit/stone. The 8-hour acid soak was selected after a preliminary trial with a local seed source indicated that embryos were not damaged in that time (visual observation only).

On March 11 and 12, 1993, after pretreatments were performed, fruits for each of the seven treatments were sown in plastic seed flats (24 x 58 x 9 cm) with drainage holes. The medium consisted of half milled sphagnum and half Q-roc #4 sand. Flats were filled with 8 cm of medium and fruits planted approximately 2 cm deep.

To reduce the length of the study, stratification cycles lasted for approximately 12 weeks. These are shorter than natural cycles, but a 2 to 2 ½-year natural time period was simulated in 65 weeks. If germination occurred while a tray was undergoing cold stratification it was taken into the greenhouse the week germination began. This reduced the cold stratification for some flats (noted in table 2). Three stratification regimes were examined: no cold stratification, warm-cold-warm-cold-warm (W-C-W-C-W), and cold-warm-cold-warm-warm (C-W-C-W-W). Cold stratification was accomplished by placing the flats in a dark walk-in refrigerator (4 ºC). During warm periods, flats were placed in a greenhouse where temperatures were maintained between 24 and 35 ºC. Seed flats were kept moist, but not saturated.

The flats were examined weekly for shoot emergence, that is, germination. Cumulative germination, the total number of germinants divided by the number of fruit sown, was determined for each seed lot by treatment combination at the end of each stratification cycle.

Differences in germination were tested using a linear model with the SAS CATMOD procedure (SAS 1988). The first model examined the effect of seed lot, treatment, and their interaction (independent variables) on germination at the end of each cycle (dependent variable). This procedure is similar to linear regression/ ANOVA for continuous dependent variables, except that the dependent variable is categorical instead of continuous. The categorical model allowed for the testing of the treatment-by-seed lot interaction term; this would not have been possible using normal linear regression.

Table *2-Cumulative germination percentage by treatment at 14 to 65 weeks after sowing averaged over 3 seed lots of Halesia carolina (NC,GA, and WV); dates represent the end of each stratification cycle*

Pretreatment	Stratification[z]	Percent germination				
		Week 14 (6/18/93)	Week 26 (9/17/93)	Week 39 (12/10/93)	Week 51 (3/11/94)	Week 65 (6/17/94)
Untreated	W-C-W-C-W	1	2[y]	32	35[x]	54
Cut tip[w]	W-C-W-C-W	3	3	41	44[v]	52
Hot H20[u]	W-C-W-C-W	3	4[t]	32	41[s]	53
Acid[r]	W-C-W-C-W	15	17[y]	72	73[q]	88
Untreated	C-W-C-W-W	0	5	6	43	51
Cut tip	C-W-C-W-W	0	4	4	29	45
Cut tip	W-W-W-W-W	6	17	25	34	47

z Refers to warm and cold periods, each lasting about 12 weeks.
y North Carolina and Georgia lots moved to greenhouse 2 weeks early because of active germination; cumulative germination reflects 24 weeks' cumulative germir.ation.
x North Carolina lot moved to greenhouse 7 weeks early because of active germination.
w The pedicel tip of seed was cut, with care taken to avoid the embryos.
v Georgia lot moved 6 weeks early and North Carolina lot moved 4 weeks early to green house because of active germination.
u Twenty-four-hour water soak pretreatment.
t West Virginia and Georgia lots moved to greenhouse 6 weeks early because of active germination.
s Georgia lot moved to greenhouse 7 weeks early because of active germination.
r Eight-hour soak in concentrated sulfuric acid, followed by a 24-hr rinse/soak in water.
q North Carolina lot moved to greenhouse 3 weeks early because of active germination.

Chi-squares (χ^2) are produced for each dependent variable and used to test the significance level. For a more detailed explanation see Grizzle and others (1969) and SAS (1988). A second model tested for differences in seed lot germination patterns over time. Cumulative germination was the dependent variable and the independent variables were: week, treatment, lot, treatment-by-lot, week-by-lot, and week-by-treatment. Week (time) was modelled as a categorical variable and represented the end of each cycle. This was done because the cold stratification periods resulted in uneven germination patterns, and a complex function would be required to model time as a continuous variable.

Results and Discussion

Seed lots differed significantly in cumulative germination at the end of each cycle. Ignoring the Tennessee seed lot, which did not germinate in these accelerated treatments (7% germination was found in the accompanying provenance study), significance levels (α) for seed lots ranged from 0.034 to 0.000 (table 3). An examination of 6 fruits from the Tennessee lot halfway through the study found that half had embryos. The cut tests of 50 and 100 fruits showed the West Virginia seed lot had 82% filled fruit (1.18 embryos/fruit) and the Georgia lot had 86% filled fruit (1.09 embryos/fruit). These statistically nonsignificant (α=0.27) differences in filled fruit did not reflect the substantial and statistically significant germination differences (43% germination for West Virginia, 80% germination for Georgia). Rankings of total germination by lot remained constant throughout the study; however, the rate of germination differed among lots as indicated by a significant lot-by-week interaction in the second model (χ^2=84.21, df=8, α=0.000). The Georgia and North Carolina seed lots germinated more quickly than the other two lots (table 4).

Treatment effects were examined using only the 3 seed lots that germinated (Georgia, North Carolina, and West Virginia). The acid-treated group had the highest germination percentage of any treatment group at the end of every cycle, with one exception (table 2). After 2 cycles (26 weeks), fruit with cut tips that had not undergone cold stratification had the same germination (statistically) as acid-treated fruit. After 3 cycles (39 weeks), the acid-treated fruit had almost twice the germination of the next best treatment group. The tip cutting and hot water pretreatments did not statistically increase germination on any date as compared to the standard warm–cold–warm stratification with no pretreatment.

After 65 weeks, total germination ranged from 45 to 88%. Except for the acid-treated group, germination was between 45 and 54%. A χ-square test indicated that these treatments were statistically equal (α=0.22). The acid treatment produced significantly more germination than any other treatment.

The 2 treatment groups that started out with cold stratification had minimal germination after their first warm period (table 2). This agrees with other studies that have shown the need for an initial warm stratification period before cold stratification (Bonner and Mignery 1974, Giersbach and Barton 1932).

Table 3—*"Analysis of variance" table examining the effect of the 7 accelerated treatments, 3 seed lots, and their interaction on germination at the end of each stratification cycle. The chi-square (χ^2) and significance level (α) are shown for the end of each cycle.*

		Week 14		Week 26		Week 39		Week 51		Week 65	
Lot	DF	χ^2	α	χ^2	α	χ^2	α	χ^2	α	χ^2	α
Intercept	1	278.9	0.000	315.9	0.000	139.9	0.000	41.3	0.000	38.4	0.000
Treatment	6	18.0	0.006	27.0	0.000	225.4	0.000	143.9	0.000	115.3	0.000
Lot	2	6.8	0.034	24.6	0.000	94.1	0.000	323.1	0.000	167.7	0.000
Treatment × lot	12	12.5	0.408	0.3	0.594	26.2	0.010	57.0	0.000	110.3	0.000
Residual	0										

Table 4 - *Cumulative germination percentage of 4 Carolina silverbell seed lots at 14 to 65 weeks from sowing averaged over 7 treatments. Dates represent the end of each stratification cycle*

	Percent germination				
Seed Lot	Week 14 (6/18/93)	Week 26 (9/17/93)	Week 39 (12/17/93)	Week 51 (3/11/94)	Week 65 (6/17/94)
West Virginia	0	0	15	20	43
Tennessee	0	0	0	0	0
Georgia	7	12	49	71	80
North Carolina	6	10	27	36	44

The tip cutting pretreatment was an unsuitable substitute for an initial warm stratification period. At the end of the second stratification cycle, the group receiving the tip- cutting pretreatment with no cold stratification had better germination than the treatment groups that began started with cold stratification. However this temporary improvement appeared to be a result of not having a cold period to slow germination, because by the end of the fourth cycle it had lost its superiority. When comparing within stratification regimes, the tip-cutting treatment group never had significantly more germination than the untreated groups. This is in agree ment with Bir (1987).

Treatment effects differed by seed lot; the treatment-lot interaction was significant at the end of the last 3 stratification cycles (table 3). The Tennessee seed lot did not germinate with any accelerated treatment (table 4). The acid-treated Georgia and North Carolina lots began to germinate without any cold stratification in the first cycle, whereas the West Virginia lot appeared to require a cold stratification period (figure 1). It is possible that the acid pretreatment overcame the need for a cold stratification period for the Georgia and North Carolina seed lots. Unfortunately it was impossible to tell whether these 2 lots would have continued to germinate without a cold stratification period because the study did not include an acid pretreatment without a cold stratification period. Examination of the non-pretreated W-C-W-C-W germination showed that the Georgia lot needed only one cold stratification period to achieve most of its germination potential, whereas the North Carolina and West Virginia lots benefited greatly from a second cold stratification (figure 1).

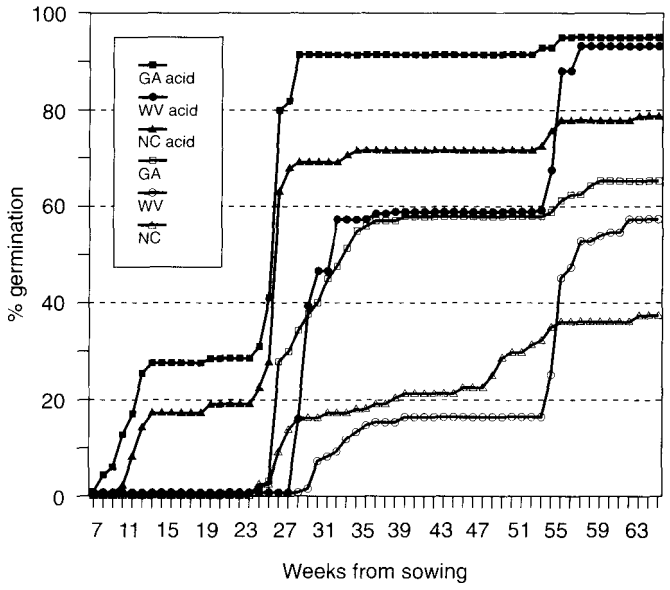

Figure 1— *Cumulative germination of 3 seed lots comparing no pretreatment and an 8-hour acid soak. Stratification cycle is weeks 1–4, warm; weeks 15–26, cold; weeks 27–39, warm; weeks 40–51, cold; and weeks 52–65, warm*

Conclusions

To achieve adequate germination a reliable source of seed must be used. Germination at 65 weeks for the four seed lots examined in this study ranged from 0 to 83%. The 8-hour soak in concentrated sulfuric acid increased germination rate and total germination for 3 of 4 seed lots. An acid soak before beginning a warm-cold-warm stratification cycle should increase overall germination and germination speed.

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Effect of Seed Condition, Stratification, and Germination Temperature on the Laboratory Germination of Loblolly Pine Seed

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Stratified and nonstratified loblolly pine (Pinus taeda *L.*) *seed of dormant, nondormant, and weak lots were germinated at 6 temperatures for 28 days. Germination percentages and germination values were evaluated. Constant 22 or 25 ºC is best for laboratory germination. An alternating 20-30 ºC and 30-20 ºC sometimes acted as a mean temperature, a low temperature and a heat sum equivalent depending on the seed condition.* Tree Planters' Notes 46(4):139142; 1995.

Several experiments have been conducted to identify the optimum temperature for germinating loblolly pine *(Pinus taeda* L.) seed. Belcher and Jones (1966) reported that a constant 22 /C and an alternating 20-30 ºC provided equal germination on unstratified seed with light, but that 22 ºC provided faster germination. Dunlap and Barnett (1982) found that 13-22 ºC provided even faster germination. McLemore (1966) found optimum germination of loblolly seed in the dark to occur between 17.5 and 27.5 ºC. Barnett (1979) found a peak between 19 and 24 ºC with stratified loblolly seed in container culture. Bonner (1984) found 30-20 ºC to provide the fastest germination on a thermogradient table. His data also suggested that 22 ºC may be too low for optimum laboratory germination of loblolly pine. Dunlap and Barnett (1983) achieved 50% faster germination of loblolly with 35-22 ºC and reported seed source to effect speed of germination.

None of the published data considered the effect of seed condition. Loblolly pine appears in 3 conditions: (1) dormant seed, which will provide greater germination following stratification; (2) nondormant seed, which will provide the same germination either with or without stratification; and (3) weak seed, which has lower germination after stratification. The nondormant lots can also differ in germination rate, so careful selection is required for investigations. This study evaluated germination percent and germination value over a range of temperatures for both stratified and unstratified seed of each of the 3 condition classes.

Materials and Methods

Four lots of each of the three seed condition classes were selected from test samples received at the USDA Forest Service's National Tree Seed Laboratory in 1984. All nondormant lots evaluated had the same rate of germination to minimize confounding. Twelve 100-seed sublots from each of the 12 seed lots were planted on crepe cellulose paper in clear plastic boxes. Six sublots from each seed lot were stratified for 5 weeks at 3 to 5 ºC. One stratified and one unstratified sublot from each lot were germinated at each of 6 temperatures: 20, 22, 25, 30, 20-30, and 30-20 ºC.

Temperatures were controlled within ± 1 ºC. Eight hours of light from cool white flourescent lamps was provided to all tests during one 24-hour period. The 20-30 ºC cycle was 16 hours at 20 ºC and 8 hours at 30 ºC with light. The 30-20 ºC was 16 hours at 30 ºC and 8 hours at 20 ºC with light.

Germination was counted each Monday, Wednesday, and Friday. A seed was considered germinated when the hypocotyl was at least 1 cm long. This allowed for the detection of some abnormalities. Seedlings from one lot were saved on the ninth-day count for evaluation of temperature effects on seedling development. The root length, hypocotyl length, hypocotyl diameter, and hypocotyl color were evaluated. Germination was closed at 28 days.

Germination percentages were adjusted for empty seed and transformed to arc sine for analysis. Germination percent at 28 days and germination value (Czabator 1962) were analyzed by a split-plot ANOV Treatment means were evaluated with the Duncan's multiple range test at the 1% level.

Results and Discussion

Approximately 40% of the variation could not be accounted for in this study. Variation between seed lots (replication) was not significant and accounted for only 4% of the variation. Seed condition and the interaction of seed condition with stratification accounted for 18 and 10%, respectively, in both germination and germination value. Temperature and stratification effects accounted respectively for 12 and 19% of the variation in germination percent whereas for germination value they accounted for none and 29% of the variation.

Temperature had no significant effect on root length (table 1) or hypocotyl diameter. Hypocotyl color darkened as the prevailing temperature decreased. Seed germinated at 20 ºC produced seedlings that developed slowly and, therefore, were reduced in size. At 20-30 ºC, seedlings elongated rapidly and were significantly taller than at all other temperatures.

Week seed produced more than half of all abnormal germinants (table 2). The number of abnormal seedlings was reduced 29% by stratification. The number of abnormals was not significantly different among replications.

The seed lots fell into the three seed conditions that the service test data indicated that they should (figure 1). Stratification improved the total germination for dormant lots, had no significant effect with the nondormant lots, and decreased the total germination on the weak lots. Therefore, the assumption that the study was conducted on seed in 3 physiological conditions is probably correct.

Germination at 22 ºC was significantly higher than at 30, 20-30, and 30-20 ºC, and equal to that at 20 and 25 ºC (figure 2). Dormant lots germinated best at the lower

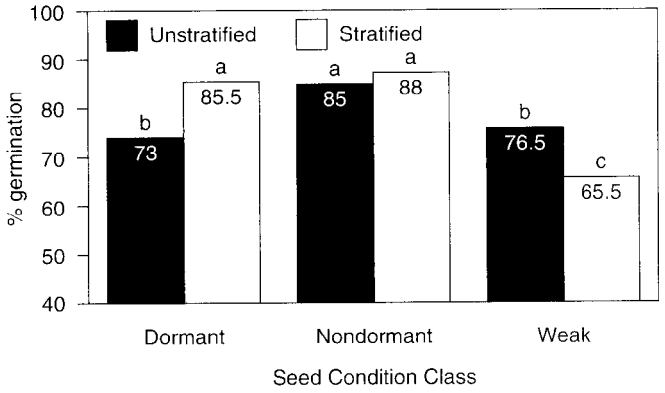

Figure 1—*Mean germination of unstratified and stratified seed of each seed condition class. Bars with different letters are significantly different at the 1% level of probablility.*

Table 1-*Summary of data on seedlings sampled at ninth day from each temperature with a single seed lot*

Temperature (/C)	Top length (cm)	Root length (cm)	Hypocotyl	
			Thickness (cm)	Color
20	1.6 ± 0.2	1.8 ± 0.5	0.12	Red black
22	3.6 ± 0.3	2.2 ± 0.3	0.12	Med. red
25	3.0 ± 0.5	1.8 t 0.5	0.12	Med. red
30	2.6 ± 0.2	1.5 ± 0.9	0.12	Pale red
20-30	4.4 ± 0.2	1.6 ± 0.4	0.12	Dark red
30-20	3.7 ± 0.4	1.4 ± 0.1	0.12	Dark red

Values are mean ± 1 standard deviation.

Table 2-*Summary of abnormal seedling counts for the 3 seed conditions at each germination temperature*

	No. of abnormal seedlings												
	20 /C		22 /C		25 /C		30 /C		20-30 /C		30-20 /C		
Seed condition	N	S	N	S	N	S	N	S	N	S	N	S	Sum
Dormant	4	1	6	1	0	4	2	3	0	3	1	3	28 b
Nondormant	2	1	4	1	4	1	1	0	0	3	0	2	19 c
Weak	1	2	10	3	6	2	2	3	7	3	12	8	59 a
Totals	7	4	20	5	10	7	5	6	7	9	13	13	
Sum for each temp.	11 c		25 a		17 b		11 c		16 b		26 a		

Values for sums in rows or columns with different letters are significant at the 1 % level of probability.

Table 3- *Summary of germination and difference between paired tests for the 3 seed conditions at each germination temperature*

Seed condition	Germination percent											
	20 /C		22 /C		25 /C		30 /		20-30 /C		30-20 /C	
	N	S	N	S	N	S	N	S	N	S	N	S
Dormant	85	97	87	94	85	92	47	76	7 8	76	52	68
Nondormant	93	92	94	93	92	90	63	81	91	86	71	85
Weak	74	71	88	69	81	58	60	51	79	59	69	68
	Difference between paired tests											
Dormant	+12		+7		+7		+29		-2		+16	
Nondormant	-1		-1		-2		+18		-5		+14	
Weak	-3		-19		-23		-9		-20		-1	

temperatures of 20, 22, and 25 °C (table 3). Stratification promoted germination of dormant lots at all temperatures except 20-30 °C. Stratification did not promote the germination of nondormant lots at 20, 22, 25 and 20-30 °C but did promote germination at 30-20 and 30 °C. Weak lots gave substantially lower germination with stratification at all temperatures except 20 and 30-20°C. Results at these last two temperatures were similar to the nondormant seed class. The depression of germination with weak lots increased with increasing constant temperature from 20 to 25 °C, and 20-30 °C provided results similar to the mean equivalent of 25 °C.

Loblolly pine is adapted to the moderate temperatures of early spring in the southern United States and, therefore, might be expected to germinate better at moderate temperatures. With increasing constant temperatures, physiological processes are increased until at some point weak seeds are unable to synchronize their biochemical processes correctly and die instead of germinating (Gulliver and Heydecker 1973). This is a likely explanation for the decreasing germination of weak seed lots at higher constant temperatures that was observed in this study.

The germination temperature used can also change the seed condition class assigned to the seed lot. The weak seed lots perform in this study as nondormant when germinated at 20 and 30-20 °C, the nondormant seed perform as very dormant when germinated at 30 or 30-20 °C, and the dormant seed as nondormant at 20-30°C.

Stratification increased the rate of germination for dormant and nondormant lots while weak lots showed no benefit from stratification and germinated as slowly as the unstratified dormant lots (figure 3). Significantly greater germination values were obtained at 22 and 25 °C than at 30 or 30-20 °C, but not at 20-30 °C nor 20 °C (figure 4).

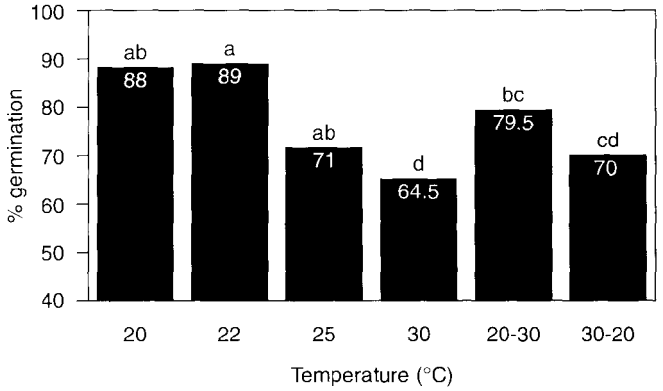

Figure 2—*Mean germination for each germination temperature. Bars with different letters are significantly different at the 1% level of probability.*

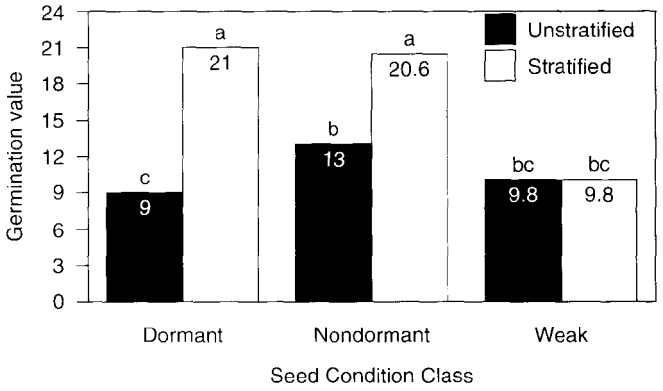

Figure 3— *Mean germination value of unstratified and stratified seed of each seed condition class. Bars with different letters are significantly different at the 1% level of probability.*

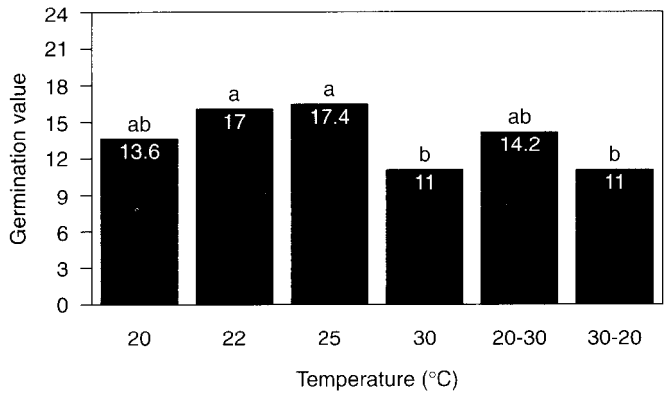

Figure 4—*Mean germination value for each temperature used in the study. Bars with different letters are significantly different at the 1% level of probability.*

Germination values increased with stratification of nondormant lots and the effect increased with increasing constant temperature until 25 °C. When the seed was not stratified, germination value was significantly lower at 30 and 30-20 °C than at 22, 25, and 20-30 °C .

The results of the weak seed were less uniform than the results of the other seed condition classes. At 20, 30, and 30-20 °C the stratified results exceeded the nonstratified. The highest germination values were obtained with nonstratified seed at 22 °C and stratified seed at 30-20 °C. The alternating 20-30 °C results were those expected of its constant heat sum counterpart of 23 °C, but the 30-20 °C did not respond as would be predicted for a constant 27 °C.

Temperature had less effect on determining seed condition class by germination value than it did by germination percent (table 4). The dormant seed lots showed a positive increase at all temperatures when stratified. The increase was less pronounced at the alternating temperatures. Therefore, the classification of a seed lot as dormant was affected by the germination temperature. The nondormant seed lots gave a large positive increase from stratification at 30 and at 30-20 °C, which would classify the lots as dormant. Although the weak seed gave positive and negative responses to stratification, the germination values are all low which is typical of weak seed.

These results do not support Bonner's findings (1984) that loblolly pine seed germinates twice as fast at 30-20 °C as they do at 20 °C, nor that 25 and 30 °C provide the same rate of germination (figure 4). These findings do support the earlier findings of Belcher and Jones (1966) that 22 °C is a reasonable germination temperature for loblolly pine. This study was originally designed with a dark treatment, but that was eliminated from the analysis because all the results in the dark were so much less than in the light. Thus, light is recommended for laboratory germination of loblolly pine seed.

Container nursery managers have sometimes the option to closely control germination temperatures and could use these data to manipulate seed lots. For example, germination of weak seed lots could be maximized by keeping temperatures low, or dormant lots could in a emergency be more successfully germinated without stratification at the alternating temperature of 20-30 °C.

Conclusions

The optimum temperature may be taken to be that temperature at which the highest percentage of germination is attained in the shortest time. The data presented suggest the following optimum temperatures:

Table 4— *Summary of germination value and the difference between paired tests for the 3 seed conditions at each germination temperature*

	Germination percent											
	20 °C		22 °C		25 °C		30 °		20–30 °C		30–20 °C	
Seed condition	N	S	N	S	N	S	N	S	N	S	N	S
Dormant	11.27	24.31	11.23	27.43	13.43	27.08	4.28	17.72	11.75	15.08	4.22	10.22
Nondormant	12.06	17.41	16.45	22.07	16.53	24.65	8.59	21.04	16.73	21.04	9.61	19.98
Weak	7.42	8.81	13.86	10.50	11.35	9.43	6.84	7.75	11.40	9.25	8.32	13.47
	Difference between paired tests											
Dormant	+13.04		+16.20		+13.65		+13.44		-3.33		+6.00	
Nondormant	+5.35		+5.62		+8.12		+12.45		+4.31		+10.37	
Weak	+1.39		-3.36		-1.92		+0.91		-2.15		5.15	

Summary of germination value and the difference between paired tests for the 3 seed conditions at each germination temperature.

Dormant lots— 22 °C or 25 °C
Nondormant lots— 22 °C, 25 °C, or 20-30 °C
Weak lots— 22 °C, 25 °C, or 20-30 °C

Because the condition of a seed lot is not known until after the test is completed, the poorer results of very dormant seed at 20-30 °C suggest that loblolly seed should be germinated at 22 °C or 25 °C in laboratory tests.

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Test of the Float Method of Assessing Northern Red Oak Acorn Condition

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The float method of *assessing the condition* of *acorns and other tree seeds is widely used, yet its efficacy has not been rigorously studied. In this study, 18,334 northern red oak* (Quercus rubra *L.) acorns collected from 2 sites in West Virginia were tested using the float method and then dissected to assess true condition. Immersion in water was found to be a reliable means of identifying insect-infested, diseased, and otherwise damaged northern red oak acorns. It was most effective in identifying undeveloped or aborted acorns and those infested by the stony cell gall wasp* (Callirhytis fructosa *Weld.), fly larvae (Drosophilidae, Psychodidae, and/or Anthomyiidae), and lepidopterous larvae-the filbertworm* (Melissopus latiferreanus *Walsingham) and acorn moth* (Valentinia glandulella *Riley). Use* of *the float method was also found to result in the unnecessary rejection* of *variable numbers* of *apparently sound acorns. On average, half of the 4,257 sound acorns used in this study failed the test. Based on these results, it is recommended that large collections of acorns from many sources be made to compensate for genetic differences and desiccation due to microsite conditions. Alternately, acorns may be soaked in water to raise moisture content before testing.* Tree Planters' Notes 46(4):143-147; 1995.

The float method of assessing acorn condition has been widely recommended as a fast, inexpensive, and nondestructive means of differentiating between sound and insect-infested or otherwise damaged acorns (Korstian 1927, Schopmeyer 1974, Stockton and Morgan 1979, Bonner and Vozzo 1987). Batches of acorns are placed in water; those that sink to the bottom are deemed viable whereas those that float are assumed to be insect-infested, aborted, malformed, or diseased. Although true viability of the embryo cannot be assessed using the float method, it may be used to differentiate between sound acorns and those that have a reduced probability of germination.

This method of sorting acorns has been used in most studies of oak *(Quercus)* seedling establishment and is widely used by planters and nursery managers. However, the reliability of the method has not been rigorously studied. Therefore, it is unknown how many damaged acorns remain undetected and are futilely planted out nor how many sound acorns are inadvertently rejected after failing to sink. In addition, damage due to certain agents, such as infestation by specific insects, may tend to be detected whereas damage caused by other agents may remain concealed. Therefore, it is possible that the reliability of the method may vary as the species composition of acorn-infesting insects and the incidence of disease fluctuate.

This study was conducted in October and November 1993 to determine the reliability of the float method in assessing the condition of northern red oak *(Quercus rubra* L.) acorns. The primary objective of the study was to determine what percentage of northern red oak acorns damaged by several different agents could be expected to pass the test (sink) and, therefore, be falsely assumed sound. Also of interest was the percentage of sound acorns that could be expected to fail the test (float) and be unnecessarily discarded.

Methods

Study site. This study was conducted on the West Virginia University (WVU) Forest located in north-central West Virginia along the westernmost range of the Allegheny Mountains. This 7,600-acre experimental forest is part of the Coopers Rock State Forest, which straddles Interstate 68 in Monongalia and Preston Counties (figure 1).

Two study sites were chosen. The first site was located in the Lick Run watershed of the WVU Forest, where cove hardwood stands were selected on a northeast-facing slope. This study area was characterized by very high site indices (81 to 97) for northern red oak *(Quercus rubra* L.*),* an abundance of mature yellow-poplar *(Liriodendron tulipifera* L.) and northern red oak, and a moderate ground cover of herbaceous and woody vegetation. The SAF cover type was yellow-poplar-white

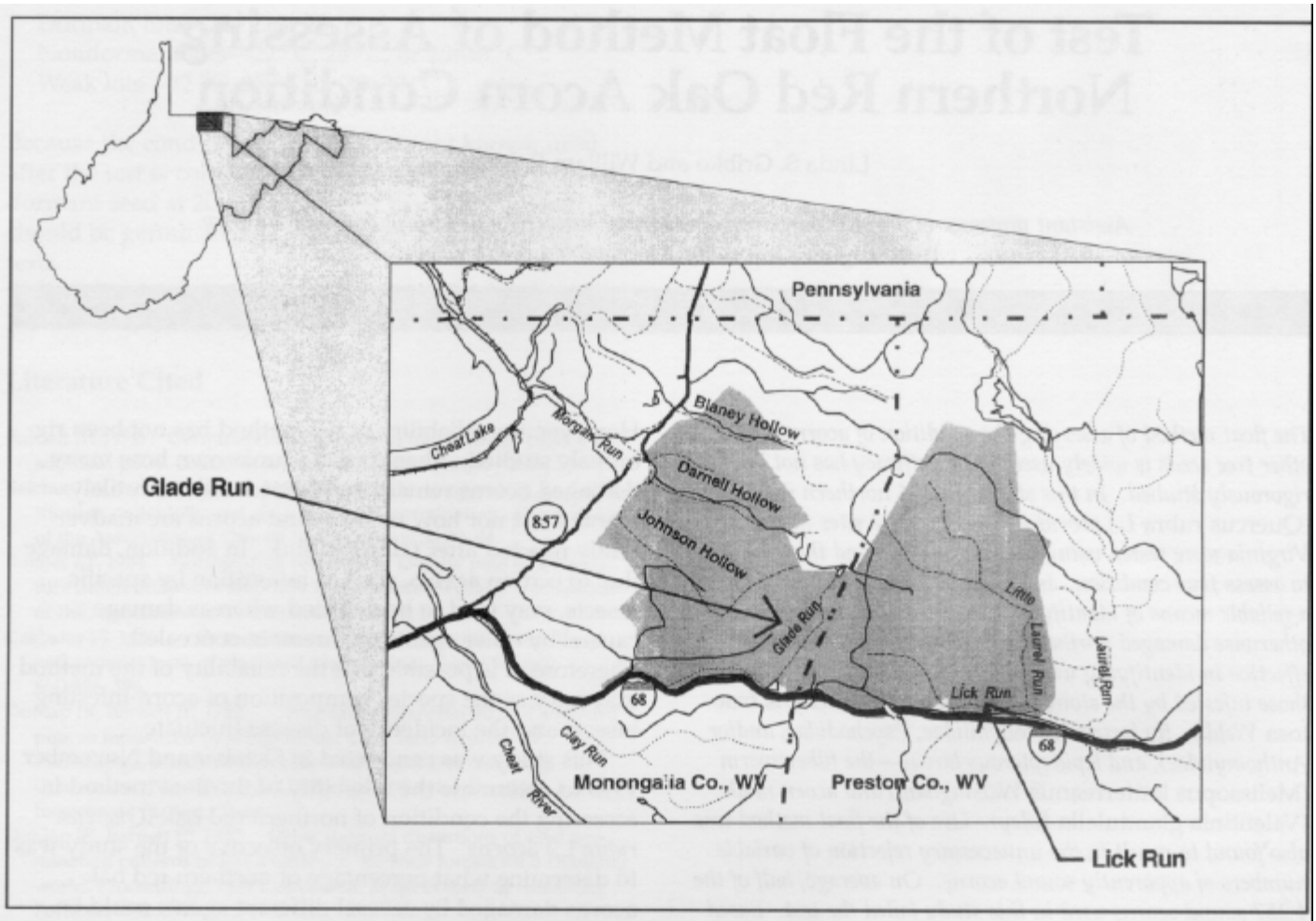

Figure 1—*Location of the West Virginia University Forest (shaded area).*

oak-northern red oak (Eyre 1980). This site was located on a mid-slope position with an average slope of 12% (McNeel 1993). The soils were Dekalb stony sandy loams (Baur 1959). This study area will henceforth be referred to as the Lick Run site.

The second site was located in the Glade Run watershed of the WVU Forest. The stands selected in this area were located on a drier northwest-facing slope and were characterized by lower site indices (68 to 73) for northern red oak, a relative absence of yellow-poplar, a significant component of mature black cherry *(Prunus serotina* Ehrh.), and sparse ground cover. The SAF cover type on this site was also yellow-poplar-white oak-northern red oak (Eyre 1980). This site was located on a higher mid-slope position with an average slope of 17% (McNeel 1993). Soils were Dekalb stony sandy loams; however, the A horizons were thinner and contained more stone than those on the Lick Run site. This study area will henceforth be referred to as the Glade Run site.

Acorn collection. Ten 0.5-acre (0.2 ha) square plots were established in the study area; 6 on the Lick Run site and 4 on the Glade Run site. Each plot was divided into quarters and a mature (> 12 inches dbh), mast-producing, dominant or codominant northern red oak was located within each quarter. In mid-October 1993, all acorns, regardless of apparent condition, were manually collected from the ground under the crown of each of the 40 study trees. Acorns collected under the 4 study trees on each plot were then combined to provide a representative sample of acorns produced on each plot.

The acorns were tested 3 times over a 2-week period using the float method (Korstian 1927, Schopmeyer 1974, Stockton and Morgan 1979). All acorns were submersed in water immediately after collection. Those that either floated on the surface or remained suspended in the water column were classified floaters; those that sank to the bottom were considered sinkers. The floaters were removed for dissection and the sinkers were covered and refrigerated for 1 week to allow fur-

ther development of any larvae contained within. These acorns were then immersed in water a second time. As was done previously, all acorns that floated were separated for dissection and all that sank were held for 1 additional week, after which the final floatation test was conducted.

Dissection of acorns. Of the 19,774 northern red oak acorns collected, 14,775 floated in water (or failed the test) and 4,999 sunk (passed the test). One thousand, four hundred and forty (1,440) randomly selected sinkers were used in an unrelated germination study and so were not available for dissection. This left 3,559 sinkers and the 14,775 floaters to be dissected with anvil shears and classified based on condition. Each acorn was cut in quarters and given one of the following classifications (Dorsey and others 1962):

1. undamaged
2. damaged by acorn weevil larvae (*Curculio* spp.)
3. containing galls of the stony cell gall wasp *(Callirhytis fructosa* Weld.)
4. infested with fly maggots (presumably Drosophilidae, Psychodidae, and/or Anthomyiidae)
5. damaged by caterpillars— the filbertworm *(Melissopus latiferreanus* Walshingham) or the acorn moth *(Valentinia glandulella* Riley)
6. undeveloped with shriveled cotyledons
7. diseased
8. infested with gnat maggots (presumably Cecidomyiidae or Mycetophilidae)
9. aborted

In cases where infestation by more than one insect species or type was apparent, an individual acorn was tallied in more than one category. For example, an acorn infested with fly maggots but containing weevil exit holes was noted as having been infested by both weevils and fly maggots. Damage was classified based on photographs published by Dorsey and others (1962).

Statistical analysis. Eight of the classes included sufficient observations to allow statistical analysis. The acorns were kept segregated by plot so that analysis of variance comparisons could be made between the mean percentages of sinkers and floaters within the 8 condition classes. Because of the large differences in sample sizes among the plots, all means and associated statistics were weighted by sample size. Analyses of variance were conducted using these weighted statistics.

Results

Overall, the float method appears to be a reliable method of culling damaged or insect-infested northern red oak acorns from collections. An average 91.6% (SE_M = 7.7%) of the acorns damaged by any agent floated in water and correctly failed the test (table 1). The technique was particularly good at identifying undeveloped (μ_f = 99.7%, SE_M= 0.2%) or aborted acorns (μ_f = 99.8%, SE_M = 0.2%) and those infested by gall wasps (μ_f = 99.0%, SE_M = 0.7%) or dipterous larvae (μ_f = 99.5%, SE_M= 0.3%) (table 1). Nearly 100% of the acorns damaged by these agents failed the test, regardless of plot. On average, the test was also effective in distinguishing acorns infested by lepidopterous larvae, although there was more variability in efficacy among plots with small sample sizes (μ_f = 99.4%, SE_M = 1.0%). The test was least effective and least consistent in the identification of diseased (μ_f = 89.8%, SE_M= 3.9%) or weevil-infested (μ_f = 88.4%, SE_M = 3.3%) acorns; however, these success rates appear high enough for most applications. Analyses of variance were all highly significant within these condition classes (P = 0.0001). Individual Fvalues will not be reported here.

The float method was neither reliable nor consistent in the discrimination of sound northern red oak acorns. On average, only 56.0% (SEM = 9.1%) of the apparently sound acorns sank and, therefore, passed the test (table 2). Success rates on individual plots ranged from 4.9%0 (n = 451) to 92.8% (n = 741), and there was no significant difference between percentages of sound acorns that floated or sank (F = 0.78, P = 0.3896).

Discussion

The float method is based on the presupposition that damaged acorns contain more air space than do sound acorns; therefore, damaged acorns should be more apt to float in water. This generally proves to be the case when acorns are infested by gall wasps, fly larvae, or lepidopterous larvae. The presence of wasp galls in particular changes the entire structure of the cotyledons; the resulting ligneous mass of galls is lightweight and often surrounded by air space rather than healthy hydrated plant tissue. Infestation by the filbertworm can cause rapid desiccation due to the caterpillar's habit of chewing an entrance hole through the acorn shell. In addition, this species feeds rapidly and replaces the cotyledon with light, fibrous frass that is often held together with strands of silk. Fly larvae and larvae of the acorn moth often infest acorns vacated by weevil larvae. The combined feeding of the weevils and the secondary invaders converts much of the acorn contents from moist cotyledon to light, dry frass. In addition, the large exit hole(s) excavated in the acorn shell by the departing weevil larvae provide a significant route of desiccation.

Undeveloped and aborted acorns also tend to consistently contain more air space than do sound acorns.

Table 1- *Percentages* of *acorns that correctly failed the float test by condition; mean percentages and standard errors are weighted by plot-level sample size*

Condition	Total no.	Mean sample size	Standard error (SE_M)	Mean percentage failed	Standard error (SE_M)
Weeviled	8395	839.5	18.1	88.4	3.3
Diseased	1832	183.2	30.0	89.8	3.9
Dipterous larvae	1295	129.5	13.7	99.5	0.3
Undeveloped	1182	118.2	12.8	99.7	0.2
Aborted	627	62.7	10.4	99.8	0.2
Wasp galls	406	40.6	11.1	99.0	0,7
Lepidopterous larvae	332	33.2	7.8	99.4	1.0
Total	14077			91.6	7.7

Table 2-*Percentages of sound acorns that passed the float test by plot; mean percentages and standard errors are weighted by sample size*

Site & plot	No. of sound acorns	Percentage passed
Lick Run		
1	806	39.8
2	451	4.9
3	208	42.8
4	499	46.5
5	741	92.8
6	843	70.3
Glade Run		
7	72	11.1
8	139	16.5
9	106	47.2
10	392	90.8
Total	4257	
Mean	426	56.0
SE_M	93	9.1
Confidence interval		38.2 - 73.8

Generally, acorns in this condition have very small or shriveled cotyledons and appear to be "dried up" (Dorsey and others 1962). These acorns are often severely desiccated and can be picked from the sample visually based on their dull appearance or the presence of a cap.

The fact that the float method failed to perform consistently in the identification of weeviled or diseased acorns is not particularly surprising. Initially, weeviled acorns exhibit very little evidence of desiccation and the narrow tunnels created in the cotyledons during feeding are densely packed with heavy moist frass. Acorns in this condition could still be heavy enough to sink in water and would, therefore, falsely pass the test. As the larvae develop and continue to feed, the tunnels become much more substantial and the frass produced by the weevils becomes larger and looser. Heavily weeviled acorns, therefore, tend to float even in the absence of exit holes. Once weevils begin to emerge, acorn contents dry rapidly; acorns with exit holes seldom sink and falsely pass the test.

Substantial numbers of diseased acorns may also sink in water, especially if the cotyledons are fully infected. In this study, many of the apparently diseased acorns were very wet internally and, consequently, dense and heavy. Conversely, acorns that apparently succumbed to other bacteria or fungi developed large air spaces as the cotyledons pulled away from the shell. These were more apt to float and, therefore, correctly failed the test.

Overall, however, the float method appears to be a reasonably reliable means of removing damaged acorns from a collection. This is particularly true when used in combination with visual checks of acorn condition. For example, many diseased acorns that pass the test are dark and discolored, many undeveloped acorns retain their caps, and oviposition scars are often visible on the shells of weevil-infested acorns. In fact, the float method should be used as a supplement to these more obvious indicators of condition.

The efficacy of the test in identifying sound acorns varied greatly among the plots (table 2). This probably reflects genetic variability and differential site conditions. Structurally, many sound acorns that floated contained more open space either between the two cotyledons or between the cotyledons and the shell. This condition did not appear to affect viability, but it did cause the acorns to float. In addition, the size of the acorns varied widely from tree to tree and from plot to plot. Although no record of acorn size was made in this study, this variable may have had some affect on the outcome of the test.

In the absence of structural differences, drier microsites on some of the plots may have resulted in the slight desiccation of certain samples of acorns, allowing them to float in water. This may be evidenced by the fact that 92.8% (n=741) of the sound acorns collected from a very moist site in the Lick Run watershed sank whereas only 4.9% (n=451) from a drier site in the same watershed sank.

Regardless of the cause, use of the float method as a means of ascertaining acorn condition can be expected to result in the rejection of, on average, approximately half of the sound acorns in a collection. In the absence of a more reliable test, it may be prudent to collect many more acorns than might otherwise be needed for germination and to collect acorns from many sites and sources to avoid local anomalies in acorn structure and hydration. In addition, Bonner and Vozzo (1987) suggest that acorns collected from the ground should be soaked in water for 16 to 24 hours before testing. They note that this extra step is particularly critical when conditions are extremely dry at collection. Although not tested in the present study; use of this technique could be expected to significantly reduce the number of sound acorns that fail the test.

Summary

The float method of assessing acorn condition was found to be a reliable means of identifying insect-infested, diseased, and otherwise damaged northern red oak acorns. It was most effective in identifying undeveloped or aborted acorns and those infested by gall wasps, fly larvae, and lepidopterous larvae. Satisfactory, although more variable, results were obtained from acorns that were diseased or infested with *Curculio* spp. weevil larvae.

Use of the float method was also found to result in the unnecessary rejection of variable numbers of apparently sound acorns. On average, half of the apparently sound acorns used in this study failed the test. Based on these results, it is recommended that large collections of acorns from many sources be made to compensate for genetic differences and desiccation due to microsite conditions and that acorns be soaked for 16 to 24 hours before testing to increase moisture content.

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