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Trees have been planted in the United States for more than 200 years. It has been said there are records of oak plantings dating back to the 1740s. In Alaska around 1805, the Russians planted a few spruce seedlings on Unalaska Island. Many hectares of plantations were established in Massachusetts during the 1840s. At the Biltmore Estate in North Carolina, there are a few pine plantations that are over 100 years old. The following is a short review of how things have changed over the past century and how, in respect to documenting the extent of plantations (stands established by planting or direct seeding), we still have far to go.

### **Early Plantations**

In 1925, there were about 154,600 hectares of "acceptable" plantations in the United States. Most of the plantations (75 percent) were in the North with 16 percent in the West, and less than 9 percent in the South. Three Lake States had the largest amount of plantations (47,350 hectares), followed by the Middle Atlantic States (27,730 hectares) and New England (16,190 hectares). Most of the seedlings planted before 1926 originated from Federal nurseries. Many early attempts failed due to a lack of experience in proper planting techniques. Some of the low success rates are probably explained by seedlings that were too small, shallow planting holes, and a lack of weed control. Some of these mistakes are still being repeated today.

### 1926-1952

About 1.97 million hectares of plantations were established during this 26-year period. From 1935-1942 there was a sharp increase in planting due to efforts of the Civilian Conservation Corps. Tree planting was still greatest in the North (49 percent), followed by the South (41 percent), and the West (10 percent). The Lake States were still the leader with about 515,600 hectares, followed again by the Middle Atlantic States (286,900 hectares). Success rates for tree planting were generally highest in the Pacific Northwest (90 percent) and in the South (85 percent), but were lowest in California (31 percent) and in the Southern Rocky Mountains (55 percent). By 1952, most seedlings were produced at State nurseries (70 percent) with Federal, commercial, and industry producing 16 percent, 12 percent, and 2 percent, respectively.

### 1953-1974

Large areas of farmland were converted to plantations from 1956-1961 due to the Soil Bank Program. This effort was responsible for the planting of 768,900 hectares on mostly "worn out" farmland. During years of high demand, many nurseries were producing seedlings at full capacity with no land in cover-crops. For example, the Morgan Nursery in Georgia produced more than 94 million seedlings in 1959. Tree planting was now greatest in the South (61 percent), followed by the North (20 percent) and the West (19 percent). The Southeastern States became the leader with about 4.59 million hectares, followed by the Pacific Northwest (1.69 million hectares) and the South Atlantic States (1.63 million hectares). In 1965, seedlings were produced at State (62 percent), industry (16 percent), Federal (14 percent), and commercial nurseries (8 percent). In total, about 12.46 million hectares of plantations were established during this 21year period.

### 1975-1997

During this 22-year period, large areas of farmland were converted to plantations due to the Conservation Reserve Program. This effort was responsible for the planting of more than 1 million hectares on erodible farmland. At the peak in the winter of 1987-88, about 2.3 billion seedlings were planted on 1.36 million hectares. In total, about 23.22 million hectares of plantations were established during this 22-year period. Tree planting in the South was 66 percent of the total, followed by the West (19 percent), and the North (15 percent). The Southeastern States remained the leader with about 9.12 million hectares, followed by the Western Gulf States (3.82 million hectares) and the Pacific Northwest (3.29 million hectares). By the end of this period, tree planting in the North had dropped to only 4 percent of the total (quite a turnaround from eight decades before when it was 75 percent). Many of the Northern States now rely mainly on natural regeneration. By 1997, seedlings were produced at industry nurseries (53 percent), with commercial, State, and Federal nurseries producing 23, 21, and 3 percent, respectively. Again, quite a major shift when compared to 1920.

### **Estimates of Plantations**

Accurate estimates for tree plantations in the United States are difficult to obtain. As a result, one international consultant had to guess that half of the plantations in the United States were on public land (13 percent would be much closer). In the past, some estimates were obtained simply by adding up the total for all previous planting. By 1952, this estimate was about 2 million hectares. Forty years later the estimate was 13 million hectares (72 percent in the South). By 1997, there were about 14.5 million hectares in the South, 1.7 million hectares in the North, and 5.5 million hectares in the West. The total, 22 million hectares, amounts to approximately 2.5 percent of the total land area (compare this to 26 percent for pasture land).

### **Dreams for the Future**

I have a dream of the future where I could pull a reference off of a shelf in 2010 and could find estimates for the number of hectares of plantations by State, year, species, and ownership. This reference would also report: (1) the

amount of plantations harvested and replanted; (2) the amount harvested but not replanted; (3) the amount of new afforestation; and (4) the amount of plantations lost due to fire, pests, agriculture, and development. Ideally, this reference would not classify old or direct-seeded plantations as "natural" stands, would not lump species together (as though they were planted together), and would not classify failed pine plantations as "oak-hickory" plantations. It would also not classify intensively managed naturally regenerated stands as plantations (as does the Forest Stewardship Council) or classify plantations without intensive management as "semi-natural" stands (as does the Food and Agriculture Organization). I know it is wishful thinking, but I would like to see plantation data presented in such a way that informs rather than confuses the reader.

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### Methyl Bromide Use in Forest Tree Nurseries What Happens After January 1, 2005?

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In 1987, the United States signed on to the Montreal Protocol to protect stratospheric ozone by limiting the production and consumption of ozone-depleting chemicals. The phase-out was set to be completed by January 1, 2005. In 1990, Congress enacted several amendments to the Clean Air Act regarding stratospheric ozone protection. Among other things, the act required that the United States maintain consistency with the requirements of the Montreal Protocol.

Both agreements included provisions for exemptions. In the case of the Montreal Protocol, an exemption allowing use of methyl bromide (MB) beyond January 1, 2005, could be granted by the Parties of the Protocol, if a lack of technical or economic feasibility could be demonstrated. This exemption is called the Critical Use Exemption (CUE).

Also included in the Montreal Protocol was a provision for another exemption called the Quarantine and Preshipment (QPS) exemption. Individual nations were permitted to exempt specific activities from the phase-out, if they could demonstrate that quarantine pests were being treated. In the January 2, 2003, Federal Register, the U.S.
Environmental Protection Agency (EPA) issued regulations

specifying the types of activities eligible for this exemption (January 2, 2003, Federal Register 68 FR 02-32986).

In 2002 and again in 2003, organizations and cooperatives representing forest tree nurseries submitted CUE applications requesting a quantity of methyl bromide for use in nurseries after the phase-out in 2005. Early in 2003, the U.S. Delegation to the Protocol filed a Critical Use Nomination (CUN) with the Methyl Bromide Technical Options Committee (MBTOC) of the Parties. It was denied due to insufficient information. A revised nomination was ultimately approved by MBTOC and by the Technical and Economic Assessment Panel (TEAP) in October 2003, but needed a vote of the parties.

Interestingly, it was believed that forest tree nurseries might qualify under QPS exemption, and, in fact, the nominated quantity was equal to the amount requested, minus any amount requested for new growth beyond 2001, less 50 percent for QPS exemption! The EPA said, although at the time undocumented, that the amount of MB used to treat forest tree seedlings destined for interstate shipment qualified under the QPS exemption. This decision was later documented in a letter from EPA Administrator Mike Leavitt to Senator Mike Crapo on January 20, 2004. In his letter, Mr. Leavitt states .... "we were able to review relevant state regulations related to interstate movement of forest tree seedlings. Our review allowed us to conclude quickly and generically that the focus of these rules was to ensure that no seedlings should be brought into the relevant state unless the seedlings were treated. Accordingly, we were able to state with assurance that interstate related uses, which amounted to approximately 50 percent of the consumption of MeBr used in the U.S. to treat forest tree seedlings, could be exempted under the protocol." As you can see, they `assumed' that seedlings in this category represented 50 percent of the total production and adjusted the nomination accordingly.

The good news is that if you ship 50 percent of your stock interstate, you can use MB to protect them under QPS exemption. The bad news is, that if you do not ship nursery stock interstate, you cannot purchase MB to protect those trees under the QPS exemption. The MB used to protect those seedlings must be allocated under the CUE allocation system, which has yet to be finalized in EPA regulations. To make matters worse, because EPA overestimated the amount of seedlings shipped interstate and, thereby, reduced the amount requested in the CUN, this combination could make MB in short supply for tree seedlings destined for intrastate shipments to customers.

The bottom line is that the U.S. Delegation nominated 195.5 metric tons for use by forest tree nurseries in 2005. A decision on the U.S. allocation could not be made at the annual meeting of the Parties of the Protocol in November 2003. At a subsequent meeting called the First Extraordinary Meeting of the Parties held in Montreal in March 2004, the U.S. nomination was approved.

So where are we? Currently, the EPA is working on an allocation process that will decide what the specific allocations will be. The EPA is also considering a QPS exemption for MB used to grow seedlings for intrastate shipment.

While the international process and rulemaking actions move forward, House Bill 3403 was introduced in October 2003 to modify certain provisions regarding MB. Specifically, the bill authorizes production of MB in the same amount requested by the United States under the CUE process of the Montreal Protocol, even if the parties to the protocol do not approve the entire amount. The status of this bill is that it has been referred to the Subcommittee on Energy and Air Quality.

What you should be able to count on at this point is the amount of MB needed to grow stock requiring interstate shipment to customers (QPS exemption). The remainder depends on whether you or someone representing your organization filed for a CUE. If a CUE was not filed, my understanding is that MB will not be available for your use in 2005. If a CUE was filed, expect half of what you submitted, since the EPA assumed you got the other half based on a OPS exemption for interstate shipment. Who knows, by the time 2005 rolls around, perhaps we will have in hand a QPS exemption for intrastate shipment. Word has it that the U.S. Delegation will make a supplemental request to the parties for additional MB that can be used by the forest seedling sector in 2005, reflecting a correction in the amount of MB that had been originally subtracted from the CUN when the EPA incorrectly assumed 50 percent of the seedlings grown were shipped interstate and, therefore, exempted under QPS exemption. We should have a clearer view of this outcome following the 24th Open Ended Working Group (OEWG) meeting of the parties in Geneva, Switzerland, July 2004.

So, what about 2006 and beyond? An interim evaluation of the 2004 CUNs was published by the party's Technology and Economic Assessment Panel (TEAP) in June 2004. The quantity that was nominated for use by the forest seedling sector in 2006 (157.7 metric tons) received a favorable recommendation by MBTOC. The TEAP will now review and present their recommendations to the parties at the 24th OEWG meeting in Geneva, July 2004. There is no question in my mind that the amount of MB approved for CUE by the parties will steadily decline to zero, and that the future of the QPS exemption is uncertain. What it says to me is that if you need to use a fumigation treatment and haven't experimented with some of the options, it is probably time to do so.

In the Pacific Northwest, we are still struggling to find an alternative that is on par with MB. In our situation, methyl isothiocynate agents (MIT) such as Basamid and metam sodium do not consistently reduce pest populations, and some trials have shown significant reductions in harvestable yield. Another limitation is that MIT should not be used in the Pacific Northwest in the spring due to wet, low soil temperature constraints. Often nurseries need to fumigate in the spring immediately after the current crop has been packed (March), and when planting the next crop begins (April). During this 30-day time period, which often occurs during periods of wet weather, there is insufficient time to complete soil preparation and fumigation activities. Delays in planting to accommodate the dissipation of Basamid, metam sodium, or even higher rates of chloropicrin will not leave sufficient time for adequate seedling growth. Even with late summer application to fields in fallow using products such as metam sodium, the current method of application is inadequate both in terms of distribution of material and prevention of offgassing, which has raised safety and liability concerns.

However, we continue to experiment and work with Telone (C-35), idomethane, and metam sodium in combination with chloropicrin, chloropicrin alone, and continue to seek herbicide solutions for the weed control shortcomings of Telone. The work with idomethane, the only spring option we have, has shown promise, but the treatment is cost prohibitive at this time. Improvements in the application technology of metam sodium by injecting at two depths followed by chloropicrin shanked to depth and then tarped has been a big improvement. However, there are still significant safety and liability concerns that need to be addressed. Chloropicrin alone used in a late summer fallow situation works well for disease control, but we lack sufficient weed control capability, especially for yellow nutsedge. We are making progress, but there is still much to do, and insufficient resources to do it in a timely manner.

It is hard to know what the future will bring, but the one thing that seems sure is that MB is going away.

### A Test of the Validity of Screening Poplar Clones for Long-Term Canker Disease Damage by Responses to Inoculation with *Septoria Musiva*

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### Summary

Septoria musiva (S. musiva) causes a stem canker disease that severely damages susceptible hybrid poplars in Eastern North America. An earlier field trial demonstrated the potential for short-term responses of poplar stems to inoculation with S. musiva to be predictive of long-term canker disease damage. In the summer of 2000, additional poplar clones primarily selected by a forest industry cooperator on the basis of growth potential (plus the resistant and susceptible standard clones used in the similar field trial in 1998) were inoculated in a test of the validity of the screening procedures. Trees were inoculated during their first season of growth by removing the fourth or fifth fully expanded leaf and placing an agar plug colonized by an aggressive isolate of S. musiva over the resulting wound. Four months after inoculation, incidence of cankers, canker length, and percentage of stem circumference affected (girdle) were recorded. Although incidence of cankers was slightly lower on the two standard clones than was observed in the earlier field trial, analyses of canker length and girdle data from both years showed that these clones responded similarly in the two trials. In the current trial, the 15 clones varied greatly in canker incidence (17-96 percent), mean canker length (6-55 mm, 0.24-2.17 in), and mean girdle (10-91 percent). Logistic regression analysis was used to compare these inoculation responses with canker disease damage categories assigned on the basis of subsequently obtained information from longer-term field studies. Incidence, canker length, and girdle data were all informative, allowing correct prediction of assigned canker disease damage categories, respectively, for 11, 13, or 12 of the 15 clones. In addition, when using these inoculation response data the probabilities of placement of clones that had been assigned to the high canker disease damage category (based on longer-term field studies) into the low category

were extremely low. Thus, it appears unlikely that clones that would be severely damaged by canker disease in a commercial rotation of 8-10 years or longer would not be detected in screening using these procedures. In addition to providing information about the likelihood of canker disease damage to clones of commercial interest in the Northcentral United States, these results validate previous work indicating the potential benefits of screening juvenile poplar clones for responses to inoculation with *S. musiva* before extensive field trials and release to growers.

### Introduction

The fungal pathogen *Mycosphaerella populorum* (anamorph = *Septoria musiva*) causes leafspot and canker diseases that affect poplar species and hybrids in the eastern United States and Canada (Bier 1939; Lo and others 1995; Ostry and McNabb 1985; Ostry and others 1989; Strobl and Fraser 1989; Waterman 1954). Significant damage can result from cankers on the branches and main stems of susceptible poplar clones. Cankers can cause stem defects that reduce economic value, may kill portions distal to girdling cankers, lead to stem breakage, and result in death of highly susceptible trees. In this region, Septoria canker has been a major barrier to the success of intensively managed poplar plantations as sources of fuel, fiber, and lumber.

Poplar clones are reported to differ greatly in responses to inoculation with *S. musiva* or in the amount of damage resulting from cankers that sometimes have been attributed to *S. musiva* (Bier 1939; Farmer and others 1991; Filer and others 1991; Hansen and others 1994, Lo and others 1995; Maxwell and others 1997; Mottet and others 1991; Netzer and others 2002; Newcombe and Ostry 2001; Ostry and McNabb 1985; Strobl and Fraser 1989; Waterman 1954; Zalasky 1978). Observations of Bier (1939) and Waterman (1954) indicate an apparent relationship between responses of some clones to inoculation and disease in the field. Weiland and others (2003) compared the incidence and severity of cankers 4 months after inoculation of young poplar trees in the field. Clones had been selected to represent a range in canker disease damage already observed in longer-term field studies. Incidence of cankers resulting from inoculation of these 27 clones varied from 28-98 percent, mean canker length 10-53 mm (0.39-2.09 in), and mean percentage of stem circumference affected (girdle) from 14-94 percent. Results of logistic regression analyses indicated that these responses to inoculation with S. musiva were predictive of canker disease damage that had been observed in the longer-term studies. Thus, the potential benefit of screening poplar clones was demonstrated.

Following our previous study (Weiland and others 2003), we were asked to characterize responses to inoculation of an additional group of poplar clones of commercial interest in the Northcentral United States, primarily selected by a forest industry cooperator on the basis of high potential productivity. At that time, little or no long-term performance data was available for the majority of these clones. Since then, however, field observations have allowed us to categorize these clones according to longerterm canker disease damage. Therefore, we were interested in whether analyses of these data would validate our methods (i.e., whether the range of responses of juvenile poplar clones to inoculation with S. *musiva* in this second, independent test could be indicative of canker disease damage in the longer-term field trials). Resistant and susceptible standard clones used in our previous study and 13 additional clones were included. The responses of the standard clones were compared to those obtained previously, and the predictive potentials of response data from all 15 clones were compared.

### **Materials and Methods**

### Clonal selection, propagation, and establishment.

Clones DN34 and NC11505 were considered resistant and susceptible standards, respectively, based on responses to inoculation in a previous study (table 1) (Weiland and others 2003) and ratings of canker disease damage reported in the literature (table 2). Clone MWH13 was selected as

**Table 1.** Resistant (DN34) and susceptible (NC11505) poplar clone standards and responses to inoculation(a) with Septoria musiva in 1998 and 2000 field experiments.

Clone	Year	Inc		Canker length <sup>c</sup>						Girdle (%) <sup>c</sup>				
		_				mm			in					
		%	(n)	(n) 1	mean	range	SE	mean	range	SE	(n)	mean	range	SE
DN34	2000	17	(42)	(7)	6	4-9	0.69	0.24	0 16-0 35	0.03	(7)	10	10-10	0.00
	1998	35	(40)	(14)	11	5-17	0.99	0.43	0.20-0.67	0.04	(14)	17	10-20	1.25
NC11505	2000	80	(44)	(35)	55	34-74	1.59	2.17	1.34-2.91	0.06	(35)	91	70-100	1.70
	1998	98	(40)	(26) <sup>d</sup>	53	43-68	1.01	2.09	1.69-2.68	0.04	(39)	94	70-100	1.49

a. Trees were inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of medium bearing mycelium on the resulting wound. Responses were evaluated approximately 4 months later.

b. Chi-squared tests (for each clone separately) supported the conclusion that canker incidence was not independent of the year (for DN34, p=0.06, for NC11505, p=0.01).

c. Analyses of variance indicated effects of clone on canker length and on girdle (values of p < 0.01), but not effects of the year or clone by the year interactions on canker length or on girdle (values of p > 0.10). Analyses for percentage data were performed after applying the arcsine of the square root transformation to the proportions.

d. Due to an error, data for calculation of mean length were obtained for only 26 individuals of this clone in 1998.

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Table 2.	Poplar clo	ones, parentage,	assigned car	ıker disease	? damage	categories,	references,	and respon	nses to
inoculatio	on(a) with	Septporia musi	va <i>in a 2000</i>	field exper	iment (in	order of in	creasing set	verity of gi	irdle).

Clone (synonyms)	Parentage <sup>b</sup>	Assigned <sup>e</sup> damage category	References <sup>d</sup>	Incidence	2	m	Cank	er le	ength	in		G	irdle (*	%)
				% (n)	(n) <sup>e</sup>	mean	range	SE	mean	range	SE	mean	range	SE
DN34 (NC5326, Eugenei)	dxn	Low	2,3,4,6,7,8	17 (42)	(7)	6	4-9	0.69	024	0.16-0.35	0.03	10	10-10	0.00
I-476	dxn	Low	2,3,6,7	23 (13)	(3)	10	7-14	2.11	039	028-055	0.08	10	10-10	0.00
NE264	dxn	Low	36,89	24 (34)	(8)	7	4-12	1.04	028	0.16-0.47	0.04	11	10-20	125
145/51	dxn	Low	2,3,6,7,8	54(41)	(10)	12	4-31	154	0.47	0.16-1.22	0.06	16	10-40	2.04
DN2	dxn	Intermediate	13,6	58 (40)	(23)	16	5-68	2.79	0.63	020-2.68	0.11	17	10-50	2.01
DN170	dxn	Low	3,6	70 (10)	(7)	19	9-31	3.16	0.75	035-122	0.12	19	10-30	2.61
NC14105	dxm	Intermediate	OA	67 (42)	(28)	13	4-20	0.90	051	0.16-0.79	0.04	21	10-70	2.67
NC14106	dxm	Low	OA	67 (36)	(24)	15	7-38	1.77	059	028-150	0.07	29	10-70	3.61
113.64	dxm	High	OA	74 (31)	(23)	19	8-42	1.59	0.75	031-1.65	0.06	30	10-60	3.02
NC14103	dxm	High	OA	95 (38)	(36)	20	10-35	0.93	0.79	0 <i>39-13</i> 8	0.04	45	10-90	2.99
MWH14	dxm	High	OA	68 (40)	(27)	26	4-48	237	1.02	0.16-1.89	0.09	50	10-80	394
MWH13	dxm	High	OA	90(31)	(28)	25	12-55	1.70	0.98	0.47-2.17	0.07	53	20-80	2.85
313.55	dxm	High	OA	87 (39)	(34)	30	10-48	137	1.18	039-1.89	0.05	56	30-50	2.49
25	dxm	High	OA	96 (28)	(27)	43	32-63	150	1.69	126-2.48	0.06	67	40-90	2.20
NC11505 (Kingston, NE388)	mxt	High	45,8	80 (44)	(35)	55	34-74	1.59	2.17	134-291	0.06	91	70-100	1.70

a. Trees were inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of medium bearing mycelium on the resulting wound. Responses were evaluated approximately 4 months later.

b. Letters refer to Populus species as follows: d=deltoides, m=maximowiczii, n=nigra, t=trichocarpa.

c. Canker disease damage categories were assigned based on information from past field trials, as referenced in the next column.

d. Numbers refer to references except OA, which refers to observations by the authors of the present study of trees in clonal field trials located at Arlington, WI (unpublished): 1=Boysen and Strobl 1991; 2=Hansen and others 1983; 3=Hansen and others 1994; 4=Lo and others 1995; 5=Long and others 1986; 6=Netzer and others 2002; 7=Ostry and McNabb 1985; 8=Ostry and others 1989; 9=Schreiner 1972.

e. Number of cankers, for both canker length and girdle.

a second likely susceptible clone, having been observed by the authors of the current study to exhibit high incidence and severity of naturally occurring cankers. Twelve test clones of commercial interest in the Northcentral United States were included at the request of a forest industry cooperator, based primarily on field observations suggesting high-potential productivity.

Dormant cuttings were planted in spring 2000 on a spacing of approximately 0.6 m x 2.4 m (2 ft x 8 ft) in a completely randomized design into each of two plots at the University of Wisconsin-Madison West Madison Agricultural Research Station. Soil at the site is welldrained to moderately well-drained Plano silt loam that previously had been planted in alfalfa, but was used to grow poplars during previous 2 years. The second plot was planted 1 week after the first plot. Trees were mulched with 0.91 m x 0.91 m (3 ft x 3 ft) squares of perforated black plastic (Vispore tree mats, Treessentials Co., Mendota Heights, MN) and each plot was surrounded by a 2-row border of clone DN34. Plots were sprayed immediately after planting with the preemergent herbicides imazaquin and pendimethalin. Subsequent maintenance included both mechanical and chemical (glyphosate) management of competing vegetation and pruning of the trees to maintain a single leader.

**Inoculation.** Inoculum was produced from a single conidial isolate of *S. musiva* (isolate 92-49A, DAOM 229444) obtained from a hybrid poplar leaf lesion. This isolate has induced cankers after inoculation of poplar stems in previous studies (Maxwell and others 1997; Stanosz and Stanosz 2002; Weiland and others 2003). Conidia stored frozen in sterile water were streaked in three equally spaced lines on the surface of malt extract agar (MEA) in Petri dishes and allowed to grow for approximately 2 weeks at 21°C (70°F) under continuous fluorescent light. Plugs of inoculum 5 mm (0.20 in) in diameter were then cut from colony margins.

From 4 to 22 trees per clone in each plot (depending on availability of cuttings, survival, and size) were inoculated. Dates of inoculation were 24-25 July and 31 July - 1 August 2000, respectively, for plots 1 and 2. Each stem was inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of inoculum on the resulting wound with the mycelium side toward the stem. The inoculum plug was held in place by wrapping the plug and stem with Parafilm (American National Can, Menasha, WI), but the piece of plastic foam used to hold the plug in place on the stem in previous studies (Maxwell and others 1997; Stanosz and Stanosz 2002; Weiland and others 2003) was omitted. Additionally, up to three trees of each clone per plot were used as controls by applying a sterile MEA plug onto the fresh wound. Parafilm was removed 2 weeks after inoculation and a small spot of latex paint was applied above each inoculated and control leaf scar to aid in finding the inoculation site during harvest. The appearance of cankers that developed in response to inoculation was noted at intervals during the growing season.

Canker evaluation. Responses of clones were evaluated following harvest of plots 1 and 2 during the week of 19 November and 26 November 2000, respectively. A segment of each stem 30 cm (12 in) long, centered on the inoculation point, was collected and stored for up to 4 weeks in a plastic bag at  $5^{\circ}$ C (41°F) until the presence or absence of a canker on each segment was recorded. When a canker was present, the outer bark was carefully peeled away, working from healthy bark toward the inoculation point to reveal the canker margin. The length of the canker, as indicated by darkly discolored and/or necrotic tissue, was measured to the nearest 1 mm (0.04 in) along the stem axis. The percentage of the stem circumference affected by the canker, as indicated by darkly discolored and/or necrotic tissue (hereafter referred to as girdle), was visually estimated to the nearest 10 percent.

Canker damage category assignment. Based on information about incidence and severity of cankers in longerterm studies described in the literature or from observations made in field trials located at Arlington, WI, by the authors of the current study, each clone was placed into one of three canker disease damage categories (low, intermediate, and high) (table 2). For example, the resistant standard clone DN34 (assigned to our low damage category) previously received canker disease ratings of 1.2 and 0.2 (for harsh and good sites, respectively, in the Northcentral United States) on a 0 (low) to 3 (high) scale (Hansen and others 1994). In addition, Lo and others (1995) assigned DN34 canker disease ratings of 0.1 and 0.4 (at ages 3 and 9 years, respectively, in plots in New York). In contrast, the susceptible standard clone NC 11505 (assigned to our high damage category) received a canker disease rating of 2.2 on a scale of 0 to 3 at age 3 years in the same New York plots, and was no longer present at 9 years (Lo and others 1995). Field trials at Arlington, WI, were established in 1995 and 1997, and canker disease symptoms have been noted in these plots

starting the year after establishment through 2003 (unpublished data of second author).

Statistical analyses. Comparison of DN34 and NC11505 responses in 1998 and 2000. Responses of clones DN34 and NC 11505 were compared with data obtained for these clones during a similar study in the same location in 1998 by Weiland and others (2003). For each of these two clones and years, incidence data were analyzed using the Chi-square test of independence (Sokal and Rohlf 1995) to test whether canker incidence (i.e., each stem cankered or not cankered) was independent of plot. For each of these two clones and years, severity data (canker length and girdle) also were analyzed to test for an effect of plot. Girdle data were converted into proportions (percentage of stem circumference girdled/100) and transformed by the arcsine of the square root before analyses. Because there was not strong evidence of effects (values of p>0.05 for all tests), data from the two plots in each year were pooled for further analyses and presentation. Pooled incidence data were analyzed using the Chisquare test to test whether canker incidence was independent of the year (1998 or 2000). Pooled severity data were also analyzed using analysis of variance to test for effects of clone, year, and their interaction. These analyses were performed using Minitab Statistical Software release 14 (Minitab Inc., State College, PA).

Comparison of responses of all clones in 2000. Data collected for all clones were analyzed to compare responses to inoculation in 2000. Incidence data, regardless of clone, were analyzed using the Chi-square test to test whether canker incidence was independent of plot. Because there was not strong evidence for effect of plot on canker incidence (p=0.158), data were pooled for presentation. Severity data (canker length and girdle) were analyzed using analysis of variance to test for effects of clone, plot, and their interaction. Girdle data were converted into proportions (percentage of stem circumference girdled/100) and transformed by the arcsine of the square root before analyses. There was not strong evidence for effect of plot or clone by plot interaction on canker length, or effect of clone by plot interaction on girdle. Although there was evidence for a plot effect on girdle (p=0.02), the difference in mean girdle was only slightly greater in plot 1 than in plot 2 (47 percent vs. 40 percent), and this difference might be explained by the later inoculation of trees in plot 2. Therefore, data from the two plots were pooled for further analysis and presentation. Pooled length data and pooled girdle data were analyzed using analysis of variance to test

for effect of clone. Girdle data were transformed as described above. In addition, the relationships among incidence, mean canker length, and mean girdle for each clone were examined by calculating Pearson correlation coefficients. These analyses also were performed using Minitab.

Prediction of assigned categories using responses to inoculation. Using procedures employed in our similar, previous study (Weiland and others 2003), collected data (canker incidence, length, and girdle) also were analyzed as potential explanatory variables to determine whether they were predictive of the assigned long-term canker disease damage categories (low, intermediate, high). These categories are viewed as ordered categorical data requiring specific methods of analysis. Thus, methods for multinomial models with ordinal responses were used (Agresti 1996). Such models are an extension of standard (binary) logistic regression to the case with three or more ordered categories. A key underlying assumption of these models is that of proportional odds; this assumption effectively means that there is a common slope (in the logistic regression model) separating each pair of categories. A p-value of less than 0.05 would indicate a failure of the proportional odds assumption (i.e., the collected data do not support the ordinal logistic model). To assess the performance of the multinomial models (given that the proportional odds assumption was met), we calculated "prediction accuracy" as the proportion of the cases (poplar clones) that were successfully predicted by the particular model. A proportion close to 1 (e.g., 12/15) suggests high model prediction accuracy. The effects of the predictor variables (incidence, length, girdle) are quantified by a p-value (calculated by maximum likelihood) where each p-value indicates the impact of a given variable after all other terms have been accounted for. A p-value less than 0.05 indicates statistical significance.

In the implementation of model fitting to our data, means of incidence, length, and girdle for each clone were each used as predictors in logistic regression where the "outcome" variable was the disease damage category. All three predictors, each possible pair of predictors, and each predictor separately were tested for each of the two experiments. Logistic regression analyses were performed using SAS version 8 (SAS Institute, Cary, NC).

### Results

**Canker characteristics.** Cankers that developed on inoculated trees closely resembled cankers produced in

response to inoculation in our previous study (Weiland and others 2003), and those attributed to natural infection of poplars by *S. musiva* in the field. No control trees developed cankers.

Comparison of DN34 and NC11505 responses in 1998 and 2000. Chi-squared tests (for each clone separately) supported the conclusion that canker incidence was not independent of the year (for DN34, p=0.06; for NC 11505, p=0.01) (table 1). For NC11505, 98 and 80 percent of the inoculated trees developed cankers in 1998 and 2000, respectively. For DN34, 35 and 17 percent of the inoculated trees developed cankers in 1998 and 2000, respectively. Analysis of variance indicated effects of clone (values of p<0.001) on both canker length and girdle, but not effects of the year or interaction (values of p>0.05) on either. Mean canker lengths and girdle in each of these 2 years were approximately 5 to 10 times greater for the susceptible standard NC 11505 than for the resistant standard DN34.

### Comparison of responses of all clones in 2000.

*Canker incidence.* Incidence of cankers varied greatly among clones (table 2). Clones with relatively low incidence (<25 percent) were DN34, I-476, and NE264. Incidence was less than or equal to 90 percent for clones NC14103, MWH13, and 25, and was 80 percent for clone NC 11505. Incidence by clone was positively correlated to mean canker length (r=0.69, p=0.005) and mean girdle (r=0.74, p=0.002).

*Canker length.* Analysis of variance indicated an effect of clone on mean canker length (p<0.001) (table 2). Mean canker length was relatively short (<10 mm, 0.39 in) for clones DN34, I-476, and NE264. Mean canker length was greatest (55 mm, 2.17 in) for clone NC 11505. Mean canker length by clone was positively correlated to mean girdle (r=0.96, p<0.001).

*Canker girdle*. Analysis of variance indicated an effect of clone on mean canker girdle (p<0.001) (table 2). Mean canker girdle was relatively small (10 percent) for clones DN34 and 1-476. Mean canker girdle was greater than or equal to 50 percent for clones MWH14, MWH13, and 25, and was 91 percent for clone NC 11505.

*Prediction of assigned canker categories using data for responses to inoculation.* Results of logistic regression analyses indicated how well canker incidence, length, or girdle data (or their combinations) from the field experiment predicted the assigned long-term canker disease damage categories (low, intermediate, or high). For example, for the model using the mean canker girdle data as the sole predictor, clone DN34 had a much greater probability of placement in the low damage category (probability = (0.96) than in either the intermediate (probability = 0.04) or high (probability = 0.01) damage categories (table 3). In contrast, for the same model clone NC 11505 had a probability of 1.00 of placement in the high damage category. For this model, the damage category with the highest probability matched the assigned damage category for 12 of 15 clones (tables 3 and 4). Further, for four of the six clones that had been assigned to the low damage category (i.e., literature reports or our observations indicated low canker disease damage in the field), the probability of placement in the low damage category based on the field experiment girdle data alone was greater than or equal to 0.79 (table 3). All seven of the clones that had been assigned the high damage category (i.e., literature reports or our observations indicated high canker disease damage in the field), the probability of placement in the low damage category based on the canker girdle data alone was less than or equal to 0.11 (table 3). For this model, the corresponding proportional odds assumption p-value was sufficiently high (p=0.1313) to satisfy the assumptions for this model and the maximum likelihood estimate p-value was low (p=0.0326), indicating the significant contribution of canker girdle to the model after all other terms were accounted for (table 4). Other models (using other variables singly or in combination) performed similarly to the model using girdle alone, satisfying model assumptions and accurately predicting the longer-term field performance of most clones. The two to four clones that were incorrectly predicted using the various models were limited to DN2, DN170, NC14105, and NC14106, each of which had moderate responses to inoculation (table 2). All other clones, which had either lesser or greater responses to inoculation, were never incorrectly predicted. Finally, when the model using all predictors (incidence, length, and girdle) was used, errors in prediction were never by more than one category. That is, clones assigned to the high canker disease damage category based on observations from longer-term field trials were never predicted to be in the low category based on inoculation responses, and vice versa.

### Discussion

In addition to providing information indicating a wide range in the responses of clones of commercial interest in **Table 3.** Poplar clones, assigned canker disease damage categories, and probabilities of placement of respective clones in those categories as indicated by logistic regression analysis using percentage of stem circumference girdled by cankers resulting from inoculation(a) with Septoria musiva in a 2000 field experiment (in order of their appearance in table 2).

Clone	Assigned damage category <sup>b</sup>	Probability of placement in category					
		Low	Intermediate	High			
DN34	Low	0.96	0.04	0.01°			
I-476	Low	0.96	0.04	0.01			
NE264	Low	0.86	0.11	0.02			
I45/51	Low	0.79	0.17	0.04			
DN2	Intermediate	0.71	0.23	0.06			
DN170	Low	0.65	0.28	0.07			
NC14105	Intermediate	0.52	0.36	0.12			
NC14106	Low	0.13	0.37	0.49			
113.64	High	0.11	0.35	0.54			
NC14103	High	0.01	0.03	0.96			
MWH14	High	0.00	0.01	0.99			
MWH13	High	0.00	0.01	0.99			
313.55	High	0.00	0.00	1.00			
25	High	0.00	0.00	1.0			
NC11505	High	0.00	0.00	1.00			

a.Trees were inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of medium bearing mycelium over the resulting wound. Responses were evaluated approximately 4 months later.

b. Canker disease damage categories were assigned based on information from longer-term field trials (see table 2).

c. Probabilities for a clone may total more or less than 1 due to rounding.

Table 4. Res	ults of logisti	c regression a	ialyses oj	f data from	responses	s of 15	poplar o	clones a	of $3$	canker	disease
damage cates	gories to inoci	ulation(a) with	Septori	ia musiva	in a 2000	field ex	perime	nt.			

Predictor (s)	Proportion	Proportional odds	Maximum likelihood estimate p-value					
	matched <sup>b</sup>	assumption p-value <sup>c</sup>	Incidence	Length	Girdle			
Incidence, length, girdle	12/15	0.3095	0.6975	0.5768	0.4458			
Incidence, length	12/15	0.1227	0.3530	0.2328				
Incidence, girdle	12/15	0.1659	0.5501		0.2163			
Length, girdle	11/15	0.2814		0.4373	0.2245			
Incidence	11/15	0.1320	0.0559					
Length	13/15	0.1388		0.0348				
Girdle	12/15	0.1313			0.0326			

a. Trees were inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of medium bearing mycelium over the resulting wound. Responses were evaluated approximately 4 months later.

b. Proportion of the 15 clones tested for which the canker disease damage category predicted by responses to inoculation matched the damage category assigned based on information from longer term field trials (see table 2).

c. A value of less than 0.05 indicates a failure of the proportional odds assumption (i.e., collected data do not support the ordinal logistic model).

d. A value of less than 0.05 indicates statistical significance (impact of a given variable after all other terms have been accounted for).

the Northcentral United States, our results support the validity of screening poplars for long-term canker disease damage by inoculation with S. musiva. Although the inoculation method used is artificial and could allow S. musiva to bypass mechanisms of resistance that might operate in naturally infected stems, processes that either facilitate or limit canker initiation and expansion appear to operate under the conditions of these tests. The wide range in responses among the tested clones to inoculation with S. musiva is consistent with results reported in our previous research (Weiland and others 2003). In both 1998 and 2000, clones exhibited a continuum of responses from very few and small cankers to very many and large cankers. Thus, rather than being qualitative (i.e., clones are either damaged severely or not damaged at all), responses to inoculation with S. musiva using these methods appear to be quantitative.

In spite of trees of each clone being genetically identical, there also was considerable variation in responses of different trees of the same clone. This tree-to-tree variation has important implications for application of these screening procedures to test new plant material. Instead of relatively few trees, many trees per clone needed to be inoculated to produce incidence data and reliable means for canker length and girdle. This variation also indicates that pure chance and environmental factors also may have great influence on development of individual cankers on individual trees.

The similarity of responses of DN34 and NC11505 to inoculation in two different years, however, supports their inclusion as standards. In each year these clones exhibited relatively low and high frequencies of cankers, respectively, although canker incidence for each was somewhat lower in 2000 than in 1998. Omission of plastic foam that had been used to hold the plug in place on the stem in previous studies (Maxwell and others 1997; Stanosz and Stanosz 2002; Weiland and others 2003) may have resulted in reduced infection efficiency in 2000. Variation in other conditions, such as weather, also might have affected canker incidence. But for cankers that did develop on these two clones in either year, however, mean canker length and mean canker girdle were very similar. Thus, regardless of the year, these two clones represented the approximate extremes in the ranges of response to inoculation.

As seen in our earlier test, overall predictability of longterm canker disease damage from responses to inoculation with *S. musiva* was high. All responses (incidence, canker length, and girdle) can contribute to accuracy of prediction, and allow detection of clones most likely and least likely to be severely damaged. Clones that are intermediate in response might be continued in a breeding or selection program based on desirability of other characteristics of the clone and the degree of defect that can be tolerated (e.g., when used for pulpwood as opposed to lumber). Incorporation of screening for long-term canker disease damage from responses to inoculation with *S. musiva* will help to ensure that limited resources for further field testing in poplar clone development programs are used efficiently.

### Conclusions

Stems of poplar clones of commercial interest in the Northcentral United States that were inoculated with the canker pathogen *S. musiva* during their first season of growth varied greatly in resulting canker incidence, canker length, and percentage of stem circumference affected. The responses of standard resistant and susceptible clones were consistent with previous results. Logistic regression analyses indicated that responses of the 15 clones generally were predictive of long-term canker disease damage categories assigned from information in the literature or observations of the authors of trees in longer-term trials. Screening poplar clones for responses to inoculation with *S. musiva* allows detection of clones most likely and least likely to be severely damaged by canker disease in commercial rotations.

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- Zalasky, H. 1978. Stem and leaf spot infections caused by Septoria musiva and S. populicola on poplar seedlings. Phytoprotection. 59: 43-50.Table 1.
  Resistant (DN34) and susceptible (NC11505) poplar clone standards and responses to inoculations with Septoria musiva in 1998 and 2000 field experiments.

### Hot Air Cleaning of Styrofoam Containers in Forest Nurseries

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### Abstract

Fungal pathogens tend to accumulate within styrofoam containers that are reused to produce successive crops of container-grown seedlings. Most nurseries treat reused containers by immersing them in hot water for varying time periods. The efficacy of radio frequency waves (RFWs) and hot, dry air (82.2°C for 10, 20, and 60 minutes) to reduce levels of selected groups of potentially pathogenic fungi within styrofoam containers was evaluated. RFWs and hot air were effective only on prewetted containers heated under high humidity. Fungi were readily killed on container surfaces when a thin film of water was present on containers prior to treatment. Dry containers were not adequately sanitized. Fusarium proliferatum was the most commonly encountered potentially pathogenic fungus isolated from containers. Eight other species of Fusarium and two species of Cylindrocarpon were also isolated from containers. Common fungal saprophytes on containers included Trichoderma and Penicillium spp. Although wet RFW treatment was as effective as hot water immersion, such treatments may be much more expensive due to the high equipment costs. Wetting containers and exposure to dry, hot air is an effective alternative to hot water immersion for styroblock sanitization.

### Introduction

A variety of containers are used in forest seedling nurseries. Several popular types of containers are made of styrofoam. During their effective life, these containers are typically reused several times to produce multiple seedling crops. However, they require sufficient sterilization prior to reuse because they can harbor potentially pathogenic organisms that may cause important diseases on new seedling crops (James et al. 1988; Peterson 1990, 1991; Sturrock and Dennis 1988). Potential pathogens reside on residual organic matter and within the inner cell walls of styrofoam containers (James 1987, 1989a, 1992; James and Woollen 1989; James et al. 1988). They may also colonize residual roots from the previous seedling crop that remain attached to containers after seedling extraction (James et al. 1988; Peterson 1990; Sturrock and Dennis 1988).

Several approaches to cleaning styrofoam containers have been investigated. Chemical sterilants, such as sodium hypochlorite (bleach) (James and Sears 1990) and sodium metabisulfite (Dumroese et al. 1993), have produced varying results. Problems with worker exposure to and disposal of toxic chemicals limit their desirability (Dumroese et al. 1993). Because of these disadvantages, many nursery growers have sought alternative, cost-effective techniques for container cleaning. Steam treatment has often been used. However, steam treatments may not adequately reduce potentially pathogenic organisms (James 1987, 1990; James et al. 1988). Therefore, immersion in hot water for varying lengths of time has been evaluated (James 1992; James and Woollen 1989; Peterson 1990, 1991; Sturrock and Dennis 1988), In general, exposure of styrofoam containers to 60-70°C for about 120 seconds kills most residual pathogens (Dumroese and James 2004).

Hot water immersion of large numbers of containers is time-consuming and expensive due to the high energy costs required to maintain sufficient water temperatures for efficacious treatments (Peterson 1990; Sturrock and Dennis 1988). Recently, the U.S. Department of Agriculture (USDA) Forest Service Missoula Technology and Development Center began investigating possible alternative methods for container treatment. Their goal was to evaluate other methods that might be more time and cost effective.

One alternative method was to use radio frequency wave (RFW) ovens to raise styrofoam temperatures sufficiently to kill potential pathogens. Industrial RFW wave ovens are

used for baking, curing, and drying many different types of foods and materials. RFW ovens operate at an electrical frequency of 10-100 MHz. Heating is accomplished by subjecting the material to be heated to an alternating electrical field that makes the molecules inside the material rotate and move laterally millions of times per second in an attempt to align with the changing electric field. This generates heat within the material in a manner similar to friction. The ovens can be incorporated into a conveyor system to mechanize the operation to minimize handling.

Another method was to use hot, dry air to sterilize containers within specially-fabricated ovens. This method eliminates the need to constantly maintain hi <sup>g</sup>h water temperatures for water immersion treatments.

Evaluations were conducted to determine efficacy of RFW and hot air treatments on reducing populations of selected fungi colonizing styrofoam containers from a commercial forest seedling nursery. The goal was to determine if such treatments could kill potentially pathogenic fungi and thus render containers relatively safe for reuse from a disease potential standpoint. Some results have been previously reported (James and Trent 2001, 2002).

### **Materials and Methods**

#### **RFW Treatments**

Ten styrofoam containers used to grow several crops of conifer seedlin<sup>g</sup>s were tested. The containers varied in size and manufacturer. A random-number generator was used to select cells to be sampled; 24 cells were sampled per container (the same cells-designated by row and column-were sampled in each container). Each selected cell was sampled for fungal colonization prior to treatment. Sampling for fungi was restricted to the bottom of cells adjacent to the drainage hole where the highest populations of contaminating fungi, includin<sup>g</sup> potential pathogens, tend to congregate (Dumroese et al. 1995; James 1987, 1989b). Two pieces of styrofoam approximately 2 x 5 mm in size were aseptically extracted from each sampled cell and placed on an agar medium selective for Fusarium and closely-related fungi (Komada 1975). Plates were incubated for 7-10 days at about 24°C under diurnal cycles of cool, fluorescent light. Emer <sup>g</sup>ing fungi were identified to genus and selected isolates were transferred to potato dextrose agar and carnation leaf agar (Fisher et al. 1982) for species identification. Fusarium

After preliminary sampling, containers were treated with RFW heatin<sup>g</sup> in a laboratory test oven (PSC, Inc., Cleveland. OH). The oven operated at 40kW at a frequency of 18MHz and contained a parallel plate electrode system with variable electrode heights; the plate voltage was 12kV. The 10 containers were divided into 2 groups of 5 containers each. Five of the containers were "dry" treated; the other 5 containers were "wet" treated. These latter containers were initially immersed in cold water for a brief period of time, shaken to remove excess water, and placed in the RFW oven. Electrode heights were either 19.1 or 25.4 cm above containers that were exposed to the RFW field for 2 minutes. Blocks were then removed and their cell surface temperatures measured with an infrared (IR) sensor. Final temperatures varied somewhat among containers, but averaged 33.5°C (range 26.7-48.0°C).

After treatment, containers were again sampled for fungal colonization using the same presampling cells. Two pieces of styrofoam per cell were again sampled as described above. Statistical comparisons of fungal colonization (number of sampled styrofoam pieces colonized by particular fungi) between pre- and post-treatment were made for the "dry" and "wet" treated containers. Comparisons were made using the nonparametric test of Kruskal-Wallis (Ott 1984).

#### **Hot Air Treatments**

Six additional styrofoam containers used to grow several crops of seedlings at an Idaho nursery were tested. Each container was cut into thirds; within each third, 12 cells were randomly sampled for selected fungi. The same cells were sampled before and after treatment. Cell sampling and associated fungal identification was conducted as outlined above.

Five of the containers were exposed to hot, dry air in a small oven. Each third was exposed to 82.2°C for 10, 20, and 60 minutes, respectively. The sixth container was first wetted with tap water, shaken to remove excess water, and then exposed to dry heat; each third was exposed to the same temperature–time regime as the other five containers. After treatment, containers were again sampled for

presence of fungi. Results for nonwetted containers were collated and average colonization means for particular fungal groups before and after treatment were compared statistically using the Kruskel-Wallis Test (Ott 1984). The same statistical tests were used to evaluate treatment effects on fungal colonization for the wetted styrofoam container.

### **Results and Discussion**

Effects of wet and dry RFW treatments on colonization of styrofoam containers are summarized in table 1. Basically, styrofoam containers had to be wetted prior to treatment for RFWs to significantly reduce level of *Fusarium* and *Cylindrocarpon* (the only potentially pathogenic fungi assayed) colonization. Levels of *Trichoderma* spp., which are saprophytic and sometimes potentially antagonistic toward pathogens, such as *Fusarium* and *Cylindrocarpon* (Papavizas 1985), were also significantly reduced when containers were wetted prior to treatment. Wetting blocks prior to treatment also resulted in essentially sterilizing major portions of sampled containers. In contrast, if blocks were not wetted prior to treatment, RFWs did not significantly reduce level of potential pathogen (*Fusarium* and *Cylindrocarpon*) or saprophyte (*Trichoderma* and *Penicillium*) colonization (table 1).

Effects of treating styrofoam containers with hot, dry air for 10, 20, and 60 minutes on colonization by selected fungi are summarized in table 2. Although statistical differences varied, prewetting containers greatly improved efficacy of hot air treatments. This was particularly evident by the much larger number of sampled cells without any detectable fungal colonization after being wetted and exposed to hot air. The temperature to which containers were exposed (82.2°C) was at the upper limit possible because containers became disfigured and unusable at higher temperatures.

We found that air heated with either RFWs or in a standard oven did not adequately penetrate containers to kill resident microorganisms unless containers are wet prior to treatment. Since immersion of blocks into hot water is also usually efficacious (James 1992; James and Woollen 1989; Peterson 1990; Sturrock and Dennis 1988), apparently the water conducts heat to surfaces of cells where microorganisms reside.

<b>Fungus</b> <sup>1</sup>	Percent colonization <sup>2</sup>							
	Wet con	tainers <sup>3</sup>	Dry cont	tainers				
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment				
Fusarium	54a	3b	72a	71a				
Cylindrocarpon	2a	0Ъ	1a	1a				
Trichoderma	35a	5a	34a	30a				
Penicillium	4a	3a	15a	13a				
Other fungi	37a	29a	14b	39a				
No fungi	0b	60a	0a	Oa				

Table 1. Effects of RFW treatments on colonization of styrofoam containers by selected fungi.

<sup>1</sup> For each fungus, average values comparing pre- and post-treatments followed by the same letter (across columns) are not significantly different (P=0.05) using the Kruskal-Wallis test.

<sup>2</sup>Twenty-four cells sampled per container; the same cells were sampled before (pre) and after (post) treatment.

<sup>3</sup> Container surfaces wetted prior to RFW treatments.

<sup>4</sup> Unidentified fungi isolated from styrofoam pieces.

<sup>5</sup> No fungal growth from sampled styrofoam pieces.

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Table 2. Effects of hot air treatments for varying time periods on colonization of styrofoam containers by selected fungi.

Fungus		Percent colonization-											
		10 minutes			20 minutes				60 minutes				
	E	Dry	1	Wet <sup>3</sup>	I	Dry	V	Vet <sup>3</sup>	E	Dry	W	/et <sup>3</sup>	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
Fusarium	11a	19a	17a	0b	7a	8a	4a	4a	7a	7a	12a	Ob	
Cylindrocarpon	2a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	
Trichoderma	28a	36a	8a	4a	42a	46a	4a	0a	37a	33a	4a	0a	
Penicillium	22a	19a	33a	4b	28a	13a	37a	0b	27a	17a	29a	0b	
Other fungi	66a	65a	75a	0b	55a	52a	83a	12b	62a	51a	79a	8b	
No fungi	0a	0a	0a	87b	0a	0a	0a	87b	0a	136	0a	92b	

' For each fungus, average values comparing pre- and post-treatments followed by the same letter (across columns) are not significantly different (P=0.05) using the Kruskal-Wallis test.

<sup>2</sup> Twenty-four cells sampled per container; the same cells were sampled before (pre) and after (post) treatment.

<sup>1</sup> Container surfaces wetted prior to hot air treatments.

4 Unidentified fungi isolated from styrofoam pieces.

5 No fungal growth from sampled styrofoam pieces.

Several Fusarium spp. colonized styrofoam containers (table 3). One of the most common Fusarium species encountered was F. proliferatum (Matsushima) Nirenberg. This species is commonly associated with root diseases of container-grown conifer seedlings (James et al. 1995) and can be an aggressive pathogen (James et al. 1995, 1997). Another common species was F. sporotrichioides Sherb., which may or may not be pathogenic on conifer seedlings (James and Perez 1999). Other Fusarium species isolated from styrofoam containers included F. oxysporum Schlecht., F. avenaceum (Fr.) Sacc., F. acuminatum Ell & Ev., F. sambucinum Fuckel, F. equiseti (Corda) Sacc., F. culmorum (W.G. Smith) Sacc., and F. subglutinans (Wollenw. & Reinking) Nelson, Toussoun & Marasas. Several of these Fusarium species are potential pathogens on conifer seedlings, whereas others are probably saprophytic (James et al. 1991).

Two Cylindrocarpon species were isolated from either styrofoam containers: C. destructans (Zins.) Scholten and C. tenue Bugn. Both species were encountered at much lower frequencies than Fusarium species (tables 1 and 2). Cylindrocarpon destructans may be an important

1994).

containers tested with	hot air treatments.
Fusarium Species	Percent of Fusarium species

 Table 3. Fusarium species colonizing styroblock

pathogen of conifers (Beyer-Ericson et al. 1991; Dahm and Strezelczyk 1987; James et al. 1994), whereas C.

tenue is usually saprophytic (Booth 1966; James et al.

	RFW treatment	Hot air treatment
F. proliferatum	87	25
F. sporotrichioides	8	14
F. acuminatum	1	31
F. oxysporum	3	10
F. solani	0	4
F. avenaceum	1	6
F. equiseti	0	8
F. culmorum	1	0
F. subglutinans	1	0

Use of RFWs on wet containers effectively removed potentially pathogenic fungi from reused styrofoam containers at lower temperatures than required for hot water immersion. Although not all potentially pathogenic *Fusarium* propagules were killed by the wet RFW treatment, sufficient inoculum was reduced from used containers to greatly limit disease potential in future seedling crops (James et al. 1988). Apparently, RFWs heated the thin water film on containers to sufficient temperatures to kill fungal propagules. It is possible that exposure to RFWs for a shorter time period might be just as effective as the 2 minute exposure evaluated in this test. Because the dry treatments were ineffective, there was no indication in our tests that the RFWs themselves were toxic to pathogen propagules

The major disadvantage of wet RFW treatments is the cost of equipment. The oven and conveyor system required is much more expensive than existing hot water immersion tank systems. However, lower energy costs required for the RFW system as compared to hot water immersion may help offset the high initial equipment costs. In any event, our results indicated that wet RFW treatments may provide a suitable alternative to standard hot water immersion for cleaning reused styrofoam containers.

Only a thin film of water was necessary to conduct hot, dry air to where undesirable organisms reside. Heating large amounts of water is unnecessary and more costly than heating equal volumes of air. Therefore, heating air can replace heating large volumes of water to obtain similar efficacy in sanitizing containers, resulting in lower energy costs. However, systems must be designed to reduce heat loss when replacing containers within ovens and container surfaces must be wetted prior to heat exposure.

One question not adequately addressed is the effects of either hot air or hot water immersion treatments on longevity and useful life span of styrofoam containers. One treatment may be more damaging to containers than the other, resulting in another important "cost" of treatment.

Our work indicated that hot air may be as effective as hot water immersion treatments for sanitizing styroblock containers if the containers are wetted prior to treatment and heated under humid conditions.

### Acknowedgements

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# Determination of Soil Hydraulic Conductivity in Nurseries and Plantations

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### Introduction

Water management is one of the most important aspects of the nursery growing technology. At the same time, studies of soil water balance in forest stands require accurate description of the soil water transport, which is dependent on the physical characteristics of soils and influences water availability. A plethora of soil water movement models, mostly from agriculture, that could be adapted to nursery crops are now available for the estimation of the soil water balance of crops with various physical characteristics and irrigated and fertilized or not (De Jong and Hayhoe 1984, De Jong and Bootsma 1996). There are several benefits of these models; the most important being:

- Avoidance of hydric stress during the growing season,
- Improvement of irrigation activity (only as much water as necessary),
- Avoidance of fertilizers waste (prevention of situations when fertilizers are washed due to excessive irrigation), and
- Prevention of groundwater contamination.

The soil's hydraulic conductivity, K, measures the speed with which water progresses downwards into the soil. It influences the time residence of water in soils and determines to a large extent its usefulness for seedlings and trees. There are several empirical methods for the measuring of hydraulic conductivity (Dorsey et al. 1990).

One of these methods uses a relatively new device, Guelph Permeameter, largely adopted in agricultural practice, especially for irrigation and drainage design. This simple device should also be adopted in forest nurseries, where the permeability changes often due to soil manipulations, especially peat moss additions, of various qualities and in different proportions. This light, relatively simple and robust field instrument, manufactured by Soil Moisture Company in California, USA, uses a new embodiment of the Mariotte principle. In use, it needs only water and can be manned by only one operator. It is available as a kit with a series of attachments, making it fully operational within a few minutes. In this paper, we attempt to give a brief review of the method, a description of the device, and an illustration of its ecological usefulness.

### The Hydraulic Conductivity

Ontario has a great site diversity; from desertic, bare landscapes to forested wetlands. The 800 to 900 mm of annual precipitation in southern Ontario, with about an equal amount supplied each month of the year, results in very different water availability conditions due to soil's permeability. Soils given this amount of moisture can be well or poorly drained, depending upon how fast the water will move downward.

Simply speaking, hydraulic conductivity, K, is the measure of the ease with which water moves gravitationally through the soil. Generally, the higher the K value of a certain soil layer, the greater the flow rate. Since in a forest nursery irrigation and drainage are very important activities, which depend critically on the rate of water flow through the soil, measurement of the soil K is an essential component in the design and functioning of these utilities. At the same time, the additions of peat moss improve the soil water-related properties. However, the decomposition of peat moss causes a slight increase of K during a growing season and the soil has to be reamended with peat moss after a few years.

In the past 50 to 70 years, a number of field methods have been developed to measure the soil K. These include ring and cylinder infiltrometer methods, the air-entry permeameter method, the double tube method, and various well methods. All of these techniques, described in detail in irrigation and drainage handbooks published by the American Society of Agronomy or by the American Society of Agricultural Engineers, have been used with varying degrees of success and suffer from various theoretic and practical limitations. The limitations include low accuracy, complex and unreliable equipment, large time and water requirements per measurement and the need for at least two skilled operators.

The development of an "in-hole," permeameter has removed many of the practical limitations of one well method, known as the constant-head well permeameter or the dry auger hole method. This is based on the observed fact that around of a nucleus of wetting, after a short while, a sphere of wetting develops in the soil. Initially, in the soil, the absorption of water is fast, filling quickly the empty pores. After the sphere has developed, the absorption stabilizes to a constant rate. In this sphere, soil is at its maximum capacity for water. The standard conditions for the K measurement begin when this constant rate of absorption stabilizes. Hence, it will need more water and more time to a dry soil and less water/time to a soil close to field capacity, at the time of measurement. The formation of the sphere of influence depends critically on the texture. In sands, the sphere is small and forms quickly, while in clays it is much larger and may take more than a day to form. The K-value measured is in fact the conductivity when the soil is saturated with moisture, Ksat.

This new permeameter, made of plexiglas, is inexpensive, simple, and can be operated by one person after very little training—described in instructions that accompany the device. Usually, only small quantities of water, about 0.5 to 2 litres, are required per measurement, and in most cases, in typical nursery conditions, measurements can be made within 5 to 30 minutes.

In spite of these practical advances, which provide considerable advantages over many of the other techniques, the constant-head well permeameter method still has not seen widespread use in forestry.

### The Permeameter

The constant well permeameter method estimates Ks. by measuring the steady-state rate of waterflow out of a shallow well in which a constant depth of water, H, is maintained. Its principle is illustrated in figure 1. The well is constructed using a 1 1/2-inch soil auger to give a well radius, a, of approximately 4 cm. It uses a plastic storage tank and a float-valve system to maintain the constant depth of water in the well. The air-inlet maintains sufficient vacuum above the water in the permeameter such that water flows out of the permeameter, in the well, at a rate sufficient to keep the level of water in the well at the base of the air-inlet tube. With this system, the rate of flow of water out the well is obtained readily by measuring the rate of fall of the water level in the permeameter vs. time. The water **in** the well can, theoretically, be kept at any height, although 10 or 20 cm appear to be the most convenient.

The K-value of the soil has traditionally been calculated using the relationship —

$$K_{sat} = \frac{C \times Q}{2 \times \pi \times H^2} (1)$$

where

Q = Steady flow rate out of the permeameter and, therefore, out of the well (volume of water per unit time).

H = Depth of water in the well (length). C = Proportionality constant equal to 2 for H/a = 10 and equal to 1.3 for H/a = 5.

The theory represented by equation (1) neglects the influence of gravity and estimates the values of C by approximate analytical means. The inclusion of gravity and estimation of the C-values by more accurate numerical procedures have resulted in the relationship:

$$K_{sst} = \frac{C \times Q}{2 \times \pi \times H^2 \times [1 + \frac{C}{2} \times (\frac{a}{H})^2]}$$
(2)

Where: C=3.3 for H/a=10, and C=2.2 for H/a=5. Equation (2) results in a 77-percent increase in the estimate of K over equation (1), when H/a=5 and a 68-percent increase when H/a=10 (Elrick and Reynolds 1985). These increases effectively eliminate the previously observed underestimation of Ksat relative to the values obtained using other methods.

For the field, the principle shown in figure 1 has been modified to the more useable form in figure 2. The sliding seal in the reservoir cap allows the operator to change the depth of water in the well, H, easily by adjusting the height of the air-inlet tube. The interchangeable reservoir tube makes it possible to use one permeameter for several soil types, since sandy soils generally require a larger reservoir than those with high clay content. The removable cap and shutoff valve make it easier to fill and start





- air inlet tube
- removable cap
- sliding air tight seal
- draw string for shut off valve
- rubber band for clamping draw -
- scale
- reservoir tube (interchangeable)
- threaded coupling
- outlet tube
- 10. tripod assembly
- steady water level in well
- 13. ball type shut off valve 14. funnel - shaped port
  - 15. permeameter tip

16. rubber stopper

Figure 1. The head of the in-hole Mariotte-type pereameter.

the permeameter. The tip of the permeameter, which consists of a perforated section of outlet tube, filled with coarse sand, serves to reduce erosion of the sides of the well while it is being filled to the steady-water level. Finally, by extending the length of the outlet tube, vertical profiles of Kan of more than 1-meter depth can be obtained readily in deep soils.

Usually, by choice, the soils of forest nurseries are sandy, noncrust forming, and easy to work. Normally they are conditioned with minced peat moss to improve their water time residence and reduce the percolation. The time and volume of water required to complete a Ksat measurement were found to depend on the soil moisture content at the beginning of the procedure. On sandy soils at moisture content close to field capacity, a K. measurement requires less than 5 minutes and only 0.3 to 0.5 liters of water. Conversely, at the other extreme, dry, sandy soils, required measurement times of about 15 to 30 minutes and up to 2 or even more liters of water. Measurement times can be reduced by prewetting the well and surrounding soil to increase the initial moisture content. This might be important for the clay soils, where times of about 20 to 60 minutes and 0.5 to 1.0 liters of water are required.

Three different methods for measuring K were compared on a structureless, Fox loamy sand soil at the Cambridge Agricultural Research Station; namely, the

Figure 2. The constant-head well pereameter in position.

constant head well permeameter, the air entry permeameter, and soil cores. The first two procedures are field methods and the third is a laboratory method. Statistical analysis revealed that the measurements with the constant well and the air entry devices were not significantly different, but that the soil core measurements were significantly lower (Elrick and Reynolds 1986). These differences are, however, not large enough to be of practical importance. For highly structured soils, such as well-aggregated clay loam<sup>y</sup>, a much larger variation in K,at amount was found among the methods. This is believed to reflect the differing degrees to which the methods respond to soil macropores such as cracks, worm holes, and root channels. The Guelph permeameter was also independently tested with good results (Gallichand et al. 1990; Kanwar et al. 1989), and is now in use on large scale in Ontario's agriculture.

In soils containing a significant amount of clay, both the soil probe and the soil auger tend to smear the walls of the well as it is being dug. Since all the water must flow out of the wall and bottom of the well, it is critical that this region not be partially sealed by smearing as this would result in an underestimation of the K., value. There can also be a gradual sealing of the inside surfaces of the well in soils high in silt and clay which also results in an underestimation of the Ksat value.

### Applications in Nurseries and Plantations

On the base of Ks., theoretical equations allow the calculation of some very important and useful characteristics:

- Relation between the soil suction (or hydraulic head) and soil moisture and
- Relation between hydraulic conductivity and soil moisture.

These relations are determined from a simple physical model described by Campbell (1974). This model links the soil matric head and the volumetric water content through a power function of saturated water content. On this base, it is possible to determine the suction head of the soil, at every level where soil moisture is measured, and implicitly, the stress level under which the roots operate at that depth. This is then expressed in terms of suction, cm or MPa, that the seedling or the tree have to exert in order to snatch water from the soil. In figure 3, we presented the suction heads developed in by "typical" sand, loam, and clay, having a certain Ks., corresponding to typical sandy, loam, and clay soils (soils 1, 6, and 11 from Clapp and Hornberger, 1978), respectively. It is easily noticeable that the same soil moisture content results in very different suction heads in soil of various textures. Thus, if we have in soil a suction head of 15,000 cm (equivalent to wilting point), we notice that this suction is achieved at less than 5-percent soil moisture in sand, 12 percent in loam and 22 percent in clay.

The second relation, conductivity versus soil moisture, allows to know the speed with which water percolates through soil. In a fine textured soil, with a low Ks., this



Figure 3. Suction head vs. soil moisture for sand, loam, and clay.

time is very long. Conversely, water passes quickly through a coarse soil, where the particles of soil matrix don't develop large attraction forces, and Ksa, is large. This is the typical situation of the washed sands, where water is available only for a short interval after a rain event. The water-related properties of such soils will improve only through lowering of Ks.. In figure 4, using the same soils, a comparison is given amongst the soil hydraulic conductivities at various levels of soil moisture. It can be observed that, in sand, water is very slowly mobile under 12 percent of soil moisture. Its mobility increases gradually up to 40 percent, when it is completely mobile (at maximum soil capacity). Here, the values of K allows also to calculate the amount of percolation vs. time.

In real life, the lowering of Ksat is achieved in nurseries through peat moss addition—which inflates soil mixture and retains water; while in a forest stand it is the result of the evolution under vegetation from unstructured material to a soil—adding continuously small amounts of humus that lower the Ks. in time.

At the next level of sophistication, when operational irrigation policies will be expressed by models, a computer will control the irrigation activity, triggerin<sup>g</sup> the re-supply of a certain area when a certain level of stress, in respect to the wilting point, has been attained. In a forest stand, the knowledge of local hydraulic conductivity may be an important factor in selecting the species to be planted. Thus, on sites with high Ksat , implying that frequent alternatives of soil water suction occur, the most appropriate species to plant would be, for instance, red pine, European larch or oaks, due to their proven ability to grow in these conditions, while the sites with lower Ks. would



Figure 4. Hydraulic conductivity vs. soil moisture for sand, loam, and clay.

in a forest stand in which both the soil moisture and the diameter growth are periodically recorded, for instance in spacing/density treatments, it is possible to represent, in retrospect, the stress evolution, suggesting optimal times for silvicultural interventions.

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### Sowing Depth, Media, and Seed Size Interact To Influence Emergence of Three Pine Species<sup>1</sup>

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### Summary

Seedling emergence in nursery production is commonly affected by many factors. The effect of sowing depth and growing media on emergence of pine species with different sized seed—Pinus greggii (P. greggii), Pinus brutia var. eldarica (P. brutia), and Pinus cembroides (P. cembroides)-was studied in a greenhouse experiment. Both emergence rate and percentage were reduced significantly at greater depths for all species. The optimum sowing depth for seeds of P. brutia (92-percent emergence), and P. cembroides (82-percent emergence) was at once or twice the thickness of the seed while the smallest seeded species, P. greggii (87-percent emergence) germinated best at one times the thickness of the seed. Agricultural soil, used in polybag production in many countries, reduced emergence of all species in comparison to horticultural mixture. Nursery managers in Mexico can improve seedling emergence and performance by using the correct sowing depth and selecting a growing media with good drainage to ensure high emergence.

### Introduction

In Mexico, as well as in many developing countries, forest nursery production using polybags is a common practice. In this seedling production system, seeds are sown into aboveground seedbeds called "almacigos." Two or 3 weeks after germination, the seedlings are generally transplanted ("pricked-out") into polybags containing native soil. This practice often deforms the tap root, which may hamper survival in the nursery and establishment success following outplanting. Furthermore, the transplanting process is time consuming. The rule of thumb is about 1,000 transplants/person/day. Thus, large nurseries require a large labor force, and the sowing season can extend several weeks or even months. The growing media used to fill the polybags varies from that used for the "almacigos." Usually "almacigos" are prepared with a mixture of forest soil and sand in a 1:1 proportion (Camacho 1995) in order to facilitate the emergence of the seedlings. However, polybags are usually filled only with native forest soil (Mexal 1996, Sánchez 1995), which occasionally may be mixed with sand in a 3:1 (soil:sand) proportion (Patiño-Valera and Marín-Chávez 1993). The reason for using this fertile soil is many forest nursery producers do not fertilize the seedlings after transplanting. Furthermore, some practitioners believe the seedling should be grown in a medium similar to outplanting conditions.

Besides growing media, there are other factors that affect seed germination and seedling emergence. Some of these, such as soil temperature, affect germination percentage (Vozzo 1983) while others, like sowing depth, are critical for seedling emergence (Minore 1985). Sowing depth varies depending on the species and seed size, and many nursery managers in Mexico usually sow pine species at an average depth of twice the thickness of the seed (Camacho 1995).

Each species has specific sowing depth requirements based on the type of seed and the environmental conditions (Agboola 1996, McWilliam *et al.* 1998). Sometimes the sowing depth can vary for the same species depending upon if the seedlings are going to be grown in a greenhouse or directly in the field (Roath 1998, Rowan 1980, Shipman 1963) because of the weather conditions. Another factor that affects seedling emergence is the growing media used for the germination process (Devaranavadagi and Sajjan 1997). Soil mixtures like soil/sand are preferred instead of single materials for the germination process (Bahuguna 1996) because they usually have better physical and chemical characteristics that increase germination percentage in comparison to single materials. Furthermore, sometimes when an adequate mixture is used as a growing media, the seeds can be sown deeper without negative effects on the seedlings (Minore 1985).

The objectives of this study were to compare the influence of sowing depth on seedling emergence and to determine optimum sowing depth for seeds of three different sized species in media used in container and traditional polybag production.

### **Materials and Methods**

The experiment was carried out in a greenhouse at Fabian Garcia Science Center at New Mexico State University. Plastic trays (15 cm deep) were filled with a commercial growing media, Metromix 360, or with a Glendale clay loam soil taken from under a 27-year old *P. brutia* forest stand.

Seeds of *P. greggii* Engelm., *P. brutia* var. *eldarica* Medw., and *P. cembroides* Zucc. were used in this study. The seeds of *P. greggii* were collected in 1995 and *P. cembroides* in 1993 in central Mexico. The seeds of *P. brutia* were collected in Las Cruces, NM, in 1997. Ten seeds of each species were measured to obtain the average thickness. The seeds were soaked in water for 24 hours and the floaters were removed before sowing. All species were sown at three depths (1-times, 2-times, and 4-times seed thickness) in the two growing media. The seeds were sown at depths of 4, 8, and 16 mm for *P. greggii* (diameter=3.5 mm (sd=0.25)); 5, 10, and 20 mm for *P. brutia* (diameter=4.9 mm (sd=0.84)); and 8, 16 and 32 mm for *P. cembroides* (diameter=7.6 mm (sd=0.57)) in both growing media.

The experimental design was a completely randomized design. All factorial combinations of sowing depth and growing media for each species formed the treatments. Each treatment combination was replicated four times. The experimental unit was 50 seeds for each species. Light irrigation was provided by hand as needed after sowing. Emergence was recorded daily for 4 weeks.

### Results

The mean differences in percentage and rate of seedling emergence were statistically significant (P<0.01) for all the species. *Pinus brutia* and *P. cembroides* seedlings emerged significantly earlier than *P. greggii* seedlings at all sowing depths (table 1). *Pinus brutia*, a freshly collected seed source, had the best emergence percentage overall (figure 1). **Table 1.** *Percentage and rate of emergence (E-50) at three sowing depths for three pine species sown in Metromix.* 

Species	Sowing depth	Emergence	E-50
	( <b>mm</b> )	(percent)	(days)
P. greggii	4	87 a	30 a
	8	49 b	37 b*
	16	27 с	—
P. brutia	5	92 a	21 a
	10	88 a	26 b
	20	71 b	30 c
P. cembroides	8	82 a	20 a
	16	63 a	30 b
	32	40 b	31 b**

Note: Values within a species followed by the same letter are not significantly different (p<0.05).

\* Only two replications achieved 50-percent emergence.

\*\* Only one replication achieved 50-percent emergence.

**Effect of sowing depth.** For *P. greggii*, a small seeded species, the emergence percentage decreased with depth of sowing from 87 percent at 4 mm to 27 percent at 16 mm. Furthermore, the rate of emergence also decreased with sowing depth. The shallow sowing depth (4 mm) achieved 50-percent emergence 30 days after sowing, but it failed to reach 50-percent emergence at the deepest sowing (table 1).

There were no significant differences in emergence percentage between 5- and 10-mm depths for *P. brutia*, but further comparisons among all depths showed that 5 and 10 mm were better than 20-mm sowing depth (figure 1). However, the rate of emergence was significantly better in the case of the shallow depth (5 mm) where 50 percent of emergence was achieved at 21 days from sowing compared to 26 days for the 10-mm depth and 30 days for deepest sowing (table 1).

The results for *P. cembroides* were similar to *P. brutia*. For depths of 8 and 16 mm, emergence percentages were not significantly different but both were significantly better than 32mm. However, the shallow depth (8 mm) achieved



**Figure 1.** *Emergence of three pine species at three depths. Within a species, correlation coefficients are significantly different* (P=0.05).

50-percent emergence at 20 days after sowing in comparison to 30 days for 16 mm, and 31 days for 32 mm.

**Effect of growing media.** The use of soil as a medium to germinate the seeds resulted in poorer emergence of all species. In the case of *P. greggii* and *P. cembroides*, emergence percentage was under 20 percent for all depths. Furthermore, seedling emergence in the deepest sowing was near 0 percent for both species. These two species were excluded from further analysis.

The results with P. brutia followed a similar pattern when using soil compared to the horticultural mixture. Shallow seeding always was better than deep seeding (figure 2). Data from the comparison of both growing media indicated that Metromix 360 was significantly better than agricultural soil for emergence (figure 2). This mixture had adequate seedling emergence for depths of 5 (92 percent) and 10 mm (88 percent). In contrast, when using agricultural soil, emergence percentage was low for all sowing depths. Shallow treatment (5 mm) had 55-percent seedling emergence, which was significantly better than 10 mm (26 percent), and 20 mm with only 12 percent (figure 2).

### Discussion

Both sowing depth and media used for germination affected emergence rate and percentage. The influence of increasing the sowing depth more than twice the thickness of the seeds unfavorably affected percentage and rate of seedling emergence for all species tested. The greater the sowing depth, the fewer seedlings emerge and the greater the number of days to emergence. The results of this experiment are consistent with previous studies with other species (Minore 1985). The sowing depth at 1-times the thickness of the seed might be better for greenhouse conditions. However, in exposed conditions like most nurseries in Mexico, the traditional rule of sowing pine species at depths of twice the thickness of the seed might be a better option. On the other hand, this rule should not be applied indiscriminately for all the species and growing conditions because, as Minore (1985) stated, sowing depth affects the emergence of each species depending on seed size and the media used for germination. In this experiment, we found that the emergence for *P. brutia* and *P.* cembroides was statistically similar for sowing depths of 1-times or 2-times the thickness of seed. However, sowing



**Figure 2.** *Emergence of* P. brutia *at three depths in two media. Within a media type, columns followed by the same letter are not significantly different (P=.05).* 

at twice the thickness reduced the speed and rate of emergence for *P. greggii*, a small seeded species. However, these results might be confounded by the age of the seed, which were collected in 1995. If the seed were not stored properly, a common practice in Mexico where freezer storage is scarce, reduced vigor might have interacted with sowing depth to result in an extraordinary reduction in emergence.

The low seedling emergence associated with the soil used in this study may have been caused by the formation of a thin superficial crust that impeded emergence of the seedlings. This problem could be avoided by using a coarser soil as the growing medium. However, many nurseries in Mexico prefer a finer-textured soil because of improved water-holding capacity. In the case of *P. greggii* and *P. cembroides*, this situation was more serious possibly due the low vigor of the seeds because those seeds were stored for at least 1 year at room temperature. These storage conditions can reduce the vigor and germination capacity of the seeds (Donald and Jacobs 1990; Krugman and Jenkinson 1974). On the other hand, *P. brutia* seeds were collected shortly before sowing and had vigorous germination. When trying to apply the results obtained in this experiment, it should be taken into account that the controlled environmental conditions in the greenhouse where the experiment was carried out differ from normal conditions in a typical nursery in Mexico, where the plants are exposed to adverse environmental conditions. As Rowan (1980) pointed out, in nursery conditions a more significant number of seeds can be washed out at shallow depths in comparison to greenhouse conditions. So even if there is no significant difference between two depths a deeper depth might be preferred to avoid losses by exposure of the seed to adverse factors. On the other hand, shallow sowing coupled with an organic mulch (pine needles) could provide the same protection (Rowan 1980).

The use of soil as a growing medium is a common practice in forest nurseries in Mexico but almost always is associated with the practice of transplanting from "almacigos." This rational is borne out by the results of this experiment because transplanting would reduce the risk of poor establishment. However, using a well-drained, porous media may permit direct sowing in the containers and achieve acceptable seedling emergence. Certainly direct seeding of polybags similar to other container systems should be the goal. One nursery in the state of Mexico recently converted from "almacigos" to direct seeding of *Cupressus lusitania* with good success. After seeding two seeds/polybag, the nursery had to thin the excess seedlings, but root deformation was eliminated, and thinning costs were considerably less than transplanting costs (Alvarez, pers. comm.)

The possibility to increase the practice of direct seeding into polybags in Mexican nurseries should be associated with an improvement in the quality of the seed collection and storage conditions. Direct seeding is only recommended when there is no restriction in the amount of seed to be used and the quality of the seed is good. In addition, the storage conditions used in many nurseries in Mexico need to be changed from room storage at ambient temperature to cold storage in order to avoid a decrease in the vigor of the seed.

The type of growing medium used for polybag production may be more difficult to change from the use of forest soil. However, there are other local materials like agricultural wastes (bagasse, coir, coffee residue), pine bark, and sand that can be used to prepare a good mixture. This, in combination with a good fertilization program can support the practice of direct seeding into polybags to eliminate the use of "almacigos" and transplant labor.

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### Effect of Weeds on the Survival and Growth of Scots Pine Seedlings

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### Abstract

Weeds are one of the most serious problems in growing high-quality seeding material in the open ground in forest nurseries. About 100 weed species were found in the fields of 11 forest nurseries in Republic of Karelia (Russia). The species can be formally divided into three groups according to morphological characteristics and damaging capacity: (1) annual and biennial herbs, (2) perennial weeds with the bulk of the underground organs in the upper soil layer, and (3) perennial weeds with deeplying root system. The effects of weeds on the seedlings can be divided into direct (physical damage) and indirect through redistribution of nutrients and water, as well as through stimulation or inhibition of microorganism development in the soil.

Reforestation activities in Republic of Karelia still widely employ coniferous seedlings grown in open-air forest nurseries. This paper offers data on the effect of weeds on the performance of 1- and 2-year-old pine seedlings. Because the nurseries in question are located on poor sandy podzols, the following information may be useful in the recultivation of sand quarries by sowing or planting of pine.

Forest nurseries in Karelia host the total of about 100 weed species (Kuznetsova 1972 and Kryshen 1990). Standing out of the general number are several typical species most adapted to the conditions in forest nurseries: *Cirsium arvense (L.) Scop., Fallopia convolvulus (L.) A. Love, Polygonum lapathifolium L., Vicia cracca L., Trifolium repens L., Chenopodium album L., Elytrigia repens (L.) Nevski., Spergula arvensis L., Achillea millefolium L., Viola arvensis Murr., Chamomilla suaveolens (Pursh.) Rybd., Equisetum arvensis L., Rumex acetosella L.* All these species are widespread in Karelian nursery fields, the incontestable dominants being *Spergula arvensis* and *Elytrigia repens*, replaced in moister sites by *Equisetum arvensis.* 

### **Methods**

Percent cover of weeds was determined with a 1-by-0.25-m frame. The size (Vasilevich 1969 and Smirnov and Smirnova 1976) and shape of the frame are convenient as the width of the sowing row is normally 1 m, and weeds are absent or heavily damaged in tractor tracks in strips between the rows. The frame orientation in the sample was across the row to make the seedling counts more convenient (6 stretches, 25 cm each). The frames were established at given distances from each other following the path planned in advance. So that the total number of plots in each field was not less than 15, the number of samples depended on the species diversity, homogeneity of the ground cover, and size of the area. The most typical field sections were chosen on the basis of geobotanical description with regard to the species composition and weed abundance-weeds were mown down to determine the aboveground mass and soil was sampled to measure the root biomass. Soil samples were taken from 5 points of a 0.25-m<sup>2</sup> plot with a 5.7-cm<sup>2</sup> corer (Stankov 1951). Roots were rinsed on sieves with a mesh diameter of 0.25 mm, and then separated from the soil under a binocular microscope and using an electrically charged glass stick (Stankov 1951 and Taranovskaya 1957).

A number of experiments were staged to study vegetative propagation of *Elytrigia repens*. In the first experiment, quackgrass rootstocks were dug out and cut into sections. The sections were weighted, their length measured, and the number of nodes counted. A total of 28 rootstock sections were replanted. Precisely a year after the rootstocks were planted, part of them was excavated to be measured and weighted.

Various methods can be employed to study relationships between plants. Part of the methods is based on the comparison of various parameters in plants growing separately, surrounded by plants of the same species or plants belonging to other species. The parameters compared are biomass, percent cover, height (Gaudet and Keddy 1988, Goldberg 1987, Schoener 1983), productivity of photosynthesis, and other physiological processes (Bazzaz and Garbut 1988; Wray, Strain, 1987). The principal technique under field conditions is to place a plant into a community and monitor its development. This way competitive hierarchy is revealed (Miller and Werner 1987), which enables researcher to check whether the absence of a species from the community is a result of competition (Goldberg 1987). We used this approach to the study of relationships between plants to investigate the effects of weeds on Scots pine growth. Elytrigia repens and Cirsium setosum plants were planted in 1-m<sup>2</sup> plots. Spergula arvensis was not sown specifically, as it was abundant in the nursery soil. Pure one-species communities were established by removing all plants of other species from the plots, and removing all weeds from the control. The following year Pinus sylvestris seeds were sown. Sown in each plot were 600 seeds (6 rows of 100 seeds). Thirty plots were established for each variant. The surviving pine seedlings were counted twice. In 2 years, all seedlings were excavated, measured and weighted. In addition, the number of weed shoots was counted in each plot. At the termination of the experiment all weeds were dug out, air dried, and the above- and below-ground parts were weighted separately.

Observations were performed to study the effect of *Elytrigia repens* on 1-year-old pine seedlings: points where quackgrass rootstock crossed the seeding row were chosen, each centimeter all seedlings were excavated at various distances from the point. The number of seedlings in 1 cm of the row was counted, the masses of the aboveand below-ground parts of each seedling were measured. Counts were made in 13 points in two directions.

The distance from the weeds to the closest surviving pine seedling was measured. Isolated weeds were chosen in fields of 1-year-old seedlings so that the stem base was in the sown row. These two conditions do not often happen to appear together, therefore impact zones were analyzed in just 17 cases for *Elytrigia repens*, 22 for *Spergula arvensis*, 11 for *Achillea millefolium*, 16 for *Senecio vulgaris*, 18 for *Viola arvensis*, 8 for *Rumex acetosella*, 9 for *Equisetum arvense*, and 4 for *Chamomilla suaveolens*.

Weeds for the study of the qualitative and quantitative composition of mycoflora were sampled from plots where only the species of interest grew. Microbiological analysis of the soil root layer was carried out following M.A. Litvinov (1969) technique by S.N. Kiviniemi, researcher of the Forest Research Institute.

### **Results and discussion**

Geobotanical description of the nursery fields of the Kondopoga integrated forestry enterprise revealed a connection between the number of Scots pine seedlings and the species composition of weeds (tables 1 and 4).

**Table 1.** Survival of 1-year-old Scots pine seedlings in relation to the species composition of weeds.

Species	No of seedlings in 1 linear m of the row	No. of observations		
All observations	75.4±4.60*	266		
No weeds	102.3±24.10	15		
Spergula arvensis	112.2±12.10	42		
Elytrigia repens	59.1±8.77	23		
Equisetum arvense	43.7±12.74	34		

Note: Equal number of pine seeds was sown in all plots. In the study of a species effect on the survival of pine seedlings, we took into account observations where the percent cover of the species in question was no less than 10 percent, given that no other perennials were present and the percent cover of other annual species was lower than 10 percent.

\* This and other tables show the arithmetic mean  $\pm$  single standard error.

Analysis proved that *Fusarium* fungi was the main reason for the death of some first-year seedlings in seeded Scots pine plantations. Along with other microorganisms, phytopathogenic fungi are constantly present in the plant root zone. The species composition of microorganisms depends on their competitive ability and the composition of active substances excreted by the plants (Krasilnikov 1955, Bilaj 1977, Mirchink 1988, Kiviniemi, and Kryshen 1994, Durynina et al. 1998, Westover et al. 1997). The surface of weed roots is inhabited by *saprotrophic* fungi and facultative parasites. The latter are represented mainly by root rot pathogens, which include i. a. some *Fusarium* species, as well as fungi of genera *Pythium, Phytophtora,* and *Rizoctonia* (Krasilnikov 1955).

Microbiological analysis of the soil root layer in plots occupied by different weeds demonstrated that *Elytrigia* 

repens and Equisetum arvense enhance the development of Fusarium, whereas the rhizosphere of Spergula arvensis shows the accumulation of fungi of the genus Trichoderma (table 2) antagonistic to soil pathogens (Shubin and Kiviniemi 1986), which explains a certain positive effect of Spergula arvensis on the survival of pine seedlings (table 1).

The distance from the weeds growing right in the sowing row to the nearest surviving seedling was measured in the fields with 1-year-old Scots pine seedlings. Despite the small size of their above-ground part, the greatest effect on the seedlings in their first year is produced by rhizomatous plants (table 3).

In addition to the species composition of weeds, the survival of the second-year pine seedlings depends also on their percent cover (table 4). The assumed reason for the death of the seedlings is the lack of nutrients and water, as well as possible mechanical damage.

Tables 5, 6, and 7 show the results of experiments on the effects of weeds on Scots pine seedlings in pure onespecies weed communities artificially established a year prior to pine seeding. The aim of the experiments was to assess the damaging capacity of the weeds. Upon the completion of the experiment, all plants growing in a plot were excavated simultaneously with pine seedlings. By this time, all *Spergula arvensis* plants had died out, therefore, *Spergula arvensis* above- and below-ground biomass

 Table 2. Fungi content in the soil weed root layer.

**Table 3.** Distance from weeds to the nearest survivingseedling.

Species	Weed leaf area, cm <sup>2</sup>	Distance to the nearest surviving seedling, cm
Achillea millefolium	321.8	2.8±0.65
Elytrigia repens	29.0	2.7±0.47
Equisetum arvense	123.6	5.2±0.82
Rumex acetosella	249.1	2.1±0.61
Senecio vulgaris	724.0	2.2±0.71
Spergula arvensis	192.6	1.4±0.34
Viola arvensis	690.6	1.3±0.45

was estimated indirectly, by means of the percent cover, which was around 50 percent in all plots. The purity of one-species communities was maintained by means of hand weeding, which could not stop the invasion of rootstocks from outside the plot. All these factors prevent the comparison of the effect of the studied weeds on pine seedlings. Although leaving the possibility of a general evaluation of the weeds' damaging capacity, the experiment demonstrated that the most expressed effect of the weeds is the reduction in the seedlings biomass. This result is corroborated by data on the effect of *Rumex* 

Variant	Sampling site	No. of micr	No. of microorganisms in 1 g of dry soil, 1000 ind.				
		Fuzarium	Trichoderma	Penicillium			
Control	soil	sparse*	3.6	24.0			
Spergula arvensis	soil	sparse	-	19.2			
	rhizosphere	-	6.0	4.8			
Rumex acetosella	soil	-	3.6	9.6			
	rhizosphere	sparse	2.4	2.2			
Equisetum arvense	soil	-	-	15.6			
	rhizosphere	3.6	-	6.0			
Elytrigia repens	soil	1.2	4.8	13.2			
	rhizosphere	3.6	-	3.6			

\* Not over 1,000 germs in 1 gram of absolutely dry soil.

Table 4.	Correlation	between the	survival of	<sup>e</sup> Scots pine	e seedlings	and the	percent	cover o	of weeds in	plantations	s of
different	age.										

Species	Correlation of	coefficient	Correlation ratio square		
	1-year-old	2-year-old	1-year-old	2-year-old	
Achillea millefolium	-0.05	-0.18	0.02	0.16	
Elytrigia repens	-0.07	-0.12	0.03	0.12	
Equisetum arvense	-0.15	<u>-0.48</u>	0.08	<u>0.33</u>	
Rumex acetosella	0.08	-0.11	0.06	<u>0.34</u>	
Spergula arvensis	0.17	-	0.08	-	
Total cover	-0.09	<u>-0.54</u>	0.10	<u>0.44</u>	
No. of observations	264	102			

Note: Underlined numbers are reliable values at P=0.05.

**Table 5.** Effect of weeds on the survival of Scots pine seedlings.

Variant	Number of 1-year-	old seedlings	Number of 2-year-old seedlings				
	Percent of the no. of seeds sown	Percent of the control	Percent of the no. of seeds sown	Percent of the control	Percent of the no. of 1st year seedlings		
Control	82.0	100.0	81.8	100.0	99.9		
Spergul arvensis	62.2	76.0	59.0	72.1	94.8		
Cirsium setosum	54.3	66.2	49.2	60.1	90.6		
Elytrigia repens	61.8	75.4	51.8	63.3	83.7		

*acetosella* on Scots pine seedlings (table 8). In this case, pine seedling height was observed to grow with the growth of *Rumex acetosella* biomass, which happens in dense pine plantations (Igaunis 1974). In the author's opinion, the similar response of pine seedlings in these two cases indicates that the reason is competition for light. In addition, weeds, which consume water and nutrients, reduce the supply of the resources to seedlings. Competition for nutrients is an essential factor in forest nurseries in Karelia, most of which lie on poor soils.

If the distribution of nutrients in a community is assessed by the biomass produced by plants, and the distribution of the biomass across horizons is taken into account, the negligible effect produced on pine seedling performance by *Cirsium setosum* can be explained by its deep-lying root system. The weed, therefore, does not compete with seedlings for nutrients and water. At a depth of 20 cm, *Cirsium setosum* root mass is not over 10 g/m<sup>2</sup>. The species does not cause heavy shading either, while the number of aboveground shoots is rarely more than 20 in  $m^2$ , and their total mass does not exceed 200 g in  $m^2$ .

The effect of *Spergula arvensis* on the growth of pine seedlings is more pronounced as the species is noted for the strong development of the above-ground part (the cover often reaches 100 percent). Its root system, though not very powerful, lies in the upper soil layer, just as the bulk of pine seedling roots. *Spergula arvensis* completes its development cycle in the period between two weeding events, developing a biomass of 500 g per m<sup>2</sup>. Plantations are hand-weeded two to three times during the growing period, so the total mass reaches 1,000 and more gram in square meters, whereas the increment of total pine seedling biomass is not over 150 g in m<sup>2</sup> in the first year and no more than 400 g in the second year.

Variant Seedling height		ıg height	Root le	ngth	Bioma	Biomass of air-dried 2-year-old seedlings					
	cm	% of control	cm	% of control	Above p	-Ground art	Roots		Total		
					g	% of control	g	% of control	g	% of control	
Control	4.6± 0.14	100	23.8± 0.30	100	0.30± 0.14	100	0.12± 0.005	100	0.42± 0.017	100	
Spergula arvensis	3.0± 0.11	65	21.8± 0.35	92	0.19± 0.012	63	0.10± 0.005	83	0.28± 0.015	67	
Cirsium setosum	4.0± 0.16	88	21.3± 0.37	89	0.30± 0.033	100	0.11± 0.006	92	0.41± 0.042	98	
Elytrigia repens	2.8± 0.08	62	21.7± 0.34	91 0.017	0.13±	43	0.06± 0.003	50	0.19± 0.009	45	

**Table 6.** Effect of weeds on the growth of Scots pine seedlings.

**Table 7.** Effect of weeds on the growth of 1-year-old Scots pine seedlings.

Variant	Bioma	Biomass of 100 absolutely dry Scots pine seedlings							
	Above-ground part		F	Roots	Total				
	g	% of control	g	% of control	g	% of control			
Control	5.12	100	1.89	100	7.02	100			
Spergula arvensis	2.45	48	0.94	50	3.39	48			
Cirsium setosum	3.66	71	1.45	77	5.10	73			
Elytrigia repens	2.25	44	1.03	54	3.28	47			

Table 8. Effect of Rumex acetosella on the growth of Scots pine seedlings in the Segezha forestry enterprise nursery.

Rumex acetosella	<i>mex acetosella</i> Seedling height -dried above- ound biomass		<b>Biomass of air-dried seedlings</b>					
air-dried above- ground biomass			Above-	Above-ground		Roots		Total
			part					
g/m <sup>2</sup>	cm	% of control	g	% of control	g	% of control	g	% of control
0	5.7± 0.17	100	0.13± 0.0150	100	0.04± 0.001	100	0.17± 0.016±	100
115	7.2± 0.019	127	0.11± 0.012	85	0.03± 0.001	75	0.14± 0.013	82
287	5.9± 0.26	104	0.06± 0.009	46	0.01± 0.001	25	0.07± 0.010	41

*Elytrigia repens* produces the heaviest effect on the performance of Scots pine seedlings. The percent cover may reach 80-100 percent, and above-ground biomass of up to 500 g in m<sup>2</sup>. The principal reason for the powerful effect of *Elytrigia repens* on pine growth is the well-developed rootstock system lying in the upper soil layer, which also contains a significant part of active pine roots. The biomass of quackgrass rootstocks at a depth of 20 cm may reach 4,000 g in m<sup>2</sup>.

The study of the relationship between pine seedling survival and performance, and the distance from the growing quackgrass rootstock showed the rootstock impact zone to cover 3-4 cm (figure 1). On the other hand, substances excreted by *Elytrigia repens* roots were not found to affect the germination of pine seeds. The growth of the seedlings could not be observed because of abundant mould growth in variants with extracts from quackgrass roots.

### Conclusion

Survival of Scots pine seedlings in the first year of cultivation depends on the species composition of weeds. The greatest threat are rhizomatous *Elytrigia repens* and *Equisetum arvense*, which enhance the development of pathogenic fungi of the genus *Fusarium* resulting in the seedling damping-off.

Survival of pine seedlings in the second year of cultivation depends both on the species composition and percent cover of weeds. The major reason for seedling death is the lack of nutrients. The heaviest damage is produced by rhizomatous plants, particularly *Elytrigia repens* and *Equisetum arvense*.

The major manifestation of the adverse effect of weeds on Scots pine seedling growth is reduction in the seedlings' biomass.

All weeds may be formally joined into three groups with regard to morphological characteristics and damaging capacity in respect of pine seedlings: (1) annual, biennial grasses, (2) perennial grasses with deeply lying root system, and (3) perennial grasses with the bulk of the belowground organs in the upper soil layer. The latter are the most harmful for the growth and survival of both 1- and 2-year-old pine seedlings.

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### An Inexpensive Rhizotron Design for Two-Dimensional, Horizontal Root Growth Measurements

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### Abstract

We designed, constructed, and tested an observational system that supports two-dimensional, horizontal root growth measurements over time without disturbing aboveground plant growth and without the need for destructive sampling of roots. Our rhizotrons allow for (1) studying relatively greater numbers of plants at any given time than is now possible under traditional technologies in a crop development context, (2) observing the horizontal orientation of root systems, which ultimately supports the study of competition among crop trees and between crop trees and invasive weed species, and (3) acquiring novel rooting data that can be input to a plant growth model. Our system is primarily constructed using common materials such as plexiglass and aluminum channel at approximately \$375.00 per unit.

### Introduction

Aboveground growth of many trees has been studied extensively (Gower and others 1993; Wu and Stettler 1994; Orlovic and others 1998). In contrast, study of root growth has been limited because of the difficulty of acquiring meaningful data, the cost associated with excavation, the logistics associated with accessibility, and limited efficiency of sampling procedures (Carlson 1965; Lauenroth and Whitman 1971; Friend and others 1991). Knowledge of the growth and spatial orientation of roots can be valuable to better assess plant responses to a wide variety of factors, including but not limited to genetic effects, fertilization, animal browsing, herbicide application, and climatic conditions. Joint knowledge of aboveground and belowground growth could lead to development of technologies supporting increased plant yields and greater overall health of the plant community.

We have developed a new method of studying root growth and development over time without disturbing aboveground plant productivity (Kokko and others 1993; Stoermer 1996; Kaspar and Ewing 1997). One of the major problems often associated with rooting research is the collection of fine roots from the planting medium, which is tedious and may result in underestimates because of loss of fine tissue (Bohm 1979; Heilman and others 1994). Another problem is the inability to view a large area in which the roots are contained, while leaving the plant undisturbed in order to observe growth responses over extended time periods (Newman 1966; Yorke 1968).

As a result of the aforementioned problems, existing systems for studying belowground root growth failed to meet our needs. Our objectives were to (1) observe relatively greater numbers of plants at any given time than is now possible under traditional technologies in a crop development context; (2) observe root systems from a two-dimensional, horizontal orientation so that we can ultimately study competition among crop trees and between crop trees and invasive weed species; and (3) acquire novel rooting data that can be input to a plant growth model (i.e., measurements of root geometry over time). The following sections provide detailed plans and steps describing construction of our rhizotron, along with a summary of the kind of data that can be collected while using the rhizotron.

### **Rhizotron Construction**

Refer to figure 1 for a photograph of operational rhizotrons.

**Materials/Equipment.** The rhizotrons cost approximately \$375.00 each, with 0.25-in- (0.64-cm-) thick plexiglass accounting for the majority of the total cost (approximately \$159.00/sheet). Our rhizotrons supported six equally divided compartments within a 4- x 8-ft (1.219- x 2.438-m) sheet of plexiglass; however, the rhizotrons are versatile enough to accommodate other spacing systems, based on specific experimental objectives. In addition, we



**Figure 1**. Operational rhizotrons used for twodimensional, horizontal root growth measurements. Observations of the root systems are taken on the underside of the rhizotrons by removing the tarps.

built a rhizotron framework consisting of a 2- x 4-in (5.08- x 10.16-cm) outer frame with a 1- x 4-in (2.54- x 10.16-cm) inner frame to support the weight of the rhizotron, which was approximately 80 lb (36.287 kg) when complete. The rhizotron framework may be optional if your greenhouse table can support the weight load and still allow working room under the rhizotrons to view root development. A support framework constructed with 1.25-in (3.175-cm) galvanized steel pipe held the rhizotron and its framework above the ground, while leaving adequate space under the rhizotron to view root growth. Table 1 provides a list of equipment and materials needed to build the rhizotron, rhizotron framework, and support framework, according to our spacing system.

Rhizotron Assembly. Figure 2 illustrates the rhizotron.

Step 1. Remove the protective covering of paper from one of the plexiglass sheets (a). Use a marker to equally divide a full sheet of plexiglass into six rectangular compartments. The measurements should be 24 in (60.96 cm) wide and 32 in (81.28 cm) long. Once the lines have been drawn, connect opposite points making an (x) in each of the boxes. The (x) determines the point at which to drill the hole that will receive the terminal adapter and nut (b). This piece of plexiglass is the top section of your rhizotron (A<sub>1</sub>). *Note*: the 24- x 32-in (60.96- x 81.28-cm) grid system worked best for our studies and is optional depending on your specific needs. *Step 2.* After marking the glass, compare your hole-saw kit to the size of the terminal adapters you have purchased. The threads of the terminal adapter should fit just inside the hole-saw bit with little room to spare. Proceed to drill holes through the plexiglass at the marked center of the (x). Depending on the terminal adapters you purchased, you may have to grind, sand, or cut the thread length down on the terminal adapters so they will allow room for the roots to emerge against the bottom piece of plexiglass and spread horizontally through the viewing window when the nut is threaded to the underside. *Note:* when drilling holes in the glass, be sure to lay the glass on a flat wooden surface in order to prevent fracturing the glass and dulling the hole-saw bit.

*Step 3.* After finishing steps 1 and 2, secure the 0.5-in (1.27-cm) aluminum channel (c) to the outside edge of the plexiglass by drilling pilot holes through the plexiglass and the aluminum framework slightly smaller than the self-tapping screws (d).

Step 4. Mark and cut two of the 0.5-in (1.27-cm) aluminum channel pieces that will divide your rhizotron into thirds and connect them to the plexiglass with self tapping screws. These pieces should be 47 in (119.38 cm) long. Use the same procedure as in step 3.

*Step 5*. Connect the terminal adapters to the top piece of plexiglass making sure the threads are facing the center of the unit. This will be the side the aluminum channel has been attached to.

*Step 6.* Place the rhizotron on the working surface with the aluminum channel facing up. After removing the protective covering of paper from another full sheet of plexiglass, screw the new sheet of plexiglass to the aluminum channel framework. This should be done in the same fashion as in steps 3 and 4, and will create the bottom of the rhizotron.

Step 7. After the rhizotron is screwed together, for ease of measuring and estimating the root lengths, drill 0.125in (0.318-cm) holes halfway through the bottom piece of glass every 3.94 in (10 cm). By drilling holes on the angle, or from the center of the planting hole, root length can be measured digitally and estimated when photographing growth due to the pre-established distance that will show up in your digital photograph. In order to establish these holes, draw a grid system as in step 1 so that one can work from the center of the (x) outward. This will increase the accuracy of measurements.



- Notes: 1. Drill pilot holes and use self-tapping screws to secure plexiglass to aluminum channel.
  - Use eye screws to secure dark-colored tarps to the rhizotron framework to eliminate light penetration into the underside of the rhizotron.
  - 3. Secure the rhizotron to its framework using wood screws, and place the rhizotron and its framework on the support framework.

**Figure 2**. Sketch of an inexpensive rhizotron design, including the rhizotron, its framework, and a support framework. Observations of the root systems are taken on the underside of the rhizotrons. Lowercase letter designations in parentheses correspond to those in the text and table 1.

**Table 1.** *List of equipment and materials for construction of an inexpensive rhizotron, along with a rhizotron framework and support framework, used for two-dimensional, horizontal root growth measurements. The designations correspond to those given in the text and figure 2.* 

#### Equipment

Mitre saw, reciprocating saw with blade for cutting metal, screw gun, wrenches, drill bits, hole saw kit, marker

#### Materials

System component	Designation	Quantity	Description of part(s)		
A. Rhizotron	а	2 sheets	Plexiglass [4 ft x 8 ft x 0.25 in (1.219 m x 2.438 m x 0.64 cm)]		
	b	6 pieces	Terminal adapter with nut [1.5-in (3.81-cm) diameter]		
	с	4 pieces	Aluminum channel [8 ft x 0.5 in (2.44 m x 1.27 cm)]		
	d	1 box	Self-tapping screws		
	e	48 in (121.92 cm)	Polyvinyl chloride pipe (PVC) [1.5-in (3.81-cm) diameter]		
B. Rhizotron framework	f	3 pieces	Lumber [2 in x 4 in x 8 ft (5.08 cm x 10.16 cm x 2.44 m)]		
	g	3 pieces	Lumber [1 in x 4 in x 8 ft (2.54 cm x 10.16 cm x 2.44 m)]		
	h	10 pieces	90-degree angle		
	i	1 box	Wood screws		
C. Support framework	j	9 pieces	Galvanized steel pipe [8 ft x 1.25 in (2.438 m x 3.175 cm)		
	k	18 pieces	Pipe adapter [1.25 x 1.25 in (3.175 x 3.175 cm)]		
	1	18 pieces	Bolt with nut [3 x 0.25 in (7.62 x 0.635 cm)]		
D. Filling and planting	m	3 tarps	Dark-colored tarp [4 x 8 ft (1.219 x 2.438 m)]		
	n	1 box	Eye screws		
			Planting medium		
			Planting stock		

*Step 8.* After the two pieces of plexiglass are fastened to the aluminum channel framework, flip the rhizotron over and cut the polyvinyl chloride pipe (PVC) (e) to the desired length based on experimental objectives and plant material used. We cut our PVC to 8 in (20.32 cm) to accommodate the planting medium and offer vertical support for our 8 in (20.32 cm) cuttings. Insert the PVC into the receiving end of the terminal adapters to complete the rhizotron. By cutting the PVC to 8 in (20.32 cm), the bottom of the cutting will be approximately 0.5 in (1.27 cm) from the bottom piece of plexiglass and allow early root development to be viewed soon after planting. *Note*: for easy cleanup and storage do not glue the PVC to the terminal adapters.

**Rhizotron Framework Assembly.** Figure 2 illustrates the rhizotron framework.

*Step 1*. Cut two 45-in (114.3-cm) pieces from one piece of the 2- x 4-in (5.08- x 10.16-cm) lumber (f). This will be

attached to the full 8-ft (2.44-m)-long pieces of the 2- x 4in (5.08- x 10.16-cm) lumber by screwing through the 8 ft (2.44 m) length and into the shorter pieces of wood creating the outside of the rhizotron framework.

Step 2. Cut one piece of the 1- x 4-in (2.54- x 10.16-cm) lumber (g) to 93 in (236.22 cm) and attach it to the center of the 2- x 4-in (5.08- x 10.16-cm) framework by using two of the 90 degree angles (h) and wood screws (i).

Step 3. Cut the remaining pieces of 1 - x 4 - in (2.54 - x 10.16 - cm) lumber to 22.125 in (56.197 cm) and install with 90 degree angles and screws. You will end up with four pieces that will fit between the outside framework and the dividing piece of 1 - x 4 - in (2.54 - x 10.16 - cm) that was installed in step 2. Be sure to space these four pieces directly under the aluminum framework previously installed in the rhizotron to allow full view of your root-viewing window from below.

**Support Framework Assembly.** Figure 2 illustrates the support framework.

*Step 1.* Using a reciprocating saw, cut six pieces of the 1.25-in (3.175-cm) galvanized steel pipe (j) into 4-ft (1.219-m) sections. Divide each 4-ft (1.219-m) section in half by drawing a line with a marker at 2 ft (0.61 m) from either end.

Step 2. Take a section from step 1 to be your horizontal support. Connect the end of the horizontal support at the 2-ft (0.61-cm) line on another of the sections from step 1 using a 1.25- x 1.25-in (3.175- x 3.175-cm) pipe adapter (k). Insert a 3-in long x 0.25-in diameter (7.62- x 0.635- cm) bolt (l) into the adapter and tighten the nut. Repeat this process with another 4-ft (1.219 m) section of steel pipe on the other end of the horizontal support, forming an "H" shape.

*Step 3*. Connect a third 4-ft (1.219-m) section to the horizontal support at the 2-ft (0.61-cm) line of each section using an adapter and nut as in step 2.

*Step 4*. Repeat steps 2 and 3 for the remaining 4-ft (1.219-m) sections. *Note*: you should end up with three "vertical units."

Step 5. Draw a line at 32 in (81.28 cm) from one end of each of the remaining 8-ft (2.438-m) sections of steel pipe. Connect each end of the 8-ft (2.438-m) section to one end of two vertical units using an adapter and nut as in step 2. Similarly, connect the third vertical unit to the 8-ft (2.438m) section at the 32-in (81.28-cm) line. Repeat the process with the remaining 8-ft (2.438-m) sections of steel pipe. *Note*: do not center the third vertical unit on the 8-ft (2.438-m) sections because the support framework will impede clear view of the middle root-viewing windows.

Securing the System Components. Secure the rhizotron to its framework using wood screws. Place the rhizotron and its framework on the support framework, with the weight of the rhizotron and its framework keeping them in place on the support framework.

### Filling and Planting.

Step 1. Our planting medium consisted of one part peat to three parts mason sand. To fill the rhizotron, remove the upper cover and fill, then reapply after leveling the medium in the bottom section of the rhizotron. Our soil mixture allows for easy root removal and viewing of fine roots while photographing the root system. Also, this mixture offers no confusion between peat and root material and still aids in maintaining some moisture if irrigation systems fail during extended leave.

Step 2. After the rhizotron is filled, wrap the sides with 4- x 8-ft (1.219- x 2.438-m) dark-colored tarps (m) to eliminate the potential of light penetration to the underside of the rhizotron (figure 1). In order to do this, attach eye-screws (n) to the 2- x 4-in (5.08- x 10.16-cm) rhizotron framework in line with the grommet openings and allowing the tarps to come into contact with the floor.

*Step 3*. Before planting any material, test all irrigation systems to ensure proper operation and water volume needed to support the plant material. These volumes are subject to change as plant material matures. *Note*: drainage may be necessary.

*Step 4*. Plant the cuttings (or other planting stock) in the soil of the PVC (figure 3). *Note*: the size of the PVC depends on the planting stock used.

### **Rhizotron Data Collection**

We developed our rhizotron because existing systems for studying root growth failed to meet our research needs. Our system supports two-dimensional, horizontal root growth measurements over time without disturbing aboveground plant growth and without the need for destructive sampling of roots. Our third objective in the development of the rhizotron was to acquire novel rooting data that can be input to a plant growth model. The uniqueness of the data that can be acquired with this system is a result of the potential to view an entire root system as it develops and comes into contact with root systems of neighboring plants. Therefore, one can map the growth of the roots continuously during development to better understand how root systems respond to contact with obstructions, moisture and temperature gradients, and competition from other plants.

Rooting parameters that can be observed with our system include but are not limited to length and number of primary, secondary, and tertiary roots, as well as parameters associated with the spatial orientation of the roots (root geometry) (figure 4). For example, one can measure the angle of secondary/tertiary root branching over time to observe how roots occupy spaces in the soil. Points of differentiation of lower-order roots from higher-order roots also can be studied. The distance between secondary roots or between secondary roots and the root tip is an example of root geometry data that can be very useful in the development of a computer-generated rooting model. Continual observation of the root system also can lead to the identi-



**Figure 3.** *Populus cutting, 8 in (20.32 cm) long, in a PVC planting tube with a soil medium of three parts sand to one part peat. The photograph was taken 29 days after planting.* 



**Figure 4**. Root system of a Populus cutting 46 days after planting. Measurements can be taken from primary roots (A), secondary roots (B), and tertiary roots (C), with examples of root geometry parameters such as angle of secondary/tertiary root branching (D) and distance between secondary roots and the root tip (E). The bottom of the cutting is located 0.5 in (1.27 cm) above the dashed, white circle (F). Photographs such as this can be taken throughout development of the root system without destructive sampling.

fication and selection of specific genotypes adapted to variable soil conditions. For example, the development of a root system mostly composed of long, thick primary roots compared with one having more secondary and tertiary roots may depend on the genotype.

Our rhizotron can be used for more than learning about the structure and development of root systems. For example, the rhizotron can be used to study the effects of simulated browse on the rooting of cuttings or seedlings. In addition, the rhizotron can be used in phytoremediation treatability studies testing if specific genotypes will develop roots in contaminated soil before the genotypes are used for *in situ* trials. The aforementioned use of the rhizotron in competition studies also supports its potential for broad-scale application. We believe, with a balanced combination of scientific and creative ingenuity, our inexpensive rhizotron design can be modified to assist almost anyone interested in learning about plant root systems.

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# Factors Influencing the Quality of Nursery Seedlings of *Pinus Pseudostrobus* Lindl.

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### Abstract

This study presents the results of cultivating seedlings of *Pinus pseudostrobus* Lindl. in nursery conditions for 1 year. Germination and survival were tested in five container types and three substrates. A "mixture" (50-percent peat moss, 25-percent agrolite and 25-percent vermiculite) and the "pine bark" substrates provided better germination than the "forest soil." Survival was better (89 percent) in 100-cm<sup>3</sup> (6-in<sup>3</sup>) tubular containers. Seedlings growth was proportional to the capacity of the containers. Most robust seedlings were obtained in 400-cm<sup>3</sup> (24-in<sup>3</sup>) plastic bags. However, given the expected economical and environmental benefits, we recommend *Pinus pseudostrobus* be grown in 160-cm<sup>3</sup> (10-in<sup>3</sup>) tubular containers with pine bark substrate.

### Introduction

With the objective of increasing the forest cover of Mexico, the National Reforestation Program, PRONARE (SEMARNAP 1998) was created in 1989. Since that time, the production of conifer seedlings has greatly increased due to the introduction of technically improved production systems. However, the indiscriminate adoption of methodologies used in advanced countries for the production of seedlings has not always yielded satisfactory results due to different ecological conditions prevailing in several Mexican regions (Cetina et al. 1999; Tienda 2000).

In rural reforestations, it is common to observe low survival rates as a consequence of deficient quality of the seedlings used (Dominguez and Navar 2000). Johnson and Cline (1991) pointed out that high-quality seedlings could survive a prolonged environmental stress and present a vigorous growth after plantation.

This study is aimed at contributing to the design of a technological package for the production of high-quality nursery seedlings of *Pinus pseudostrobus* Lindl. (*P. pseudostrobus*), using adequate substrates and containers to allow better results in the regional reforestation tasks in Nuevo Leon, Mexico.

### Materials and Methods

**Experimental site**. The nursery of the Faculty of Forestry Sciences, Universidad Autónoma de Nuevo León (UANL) is located in the county of Iturbide, N. L., at 24° 43' N, 99° 53' W and 1,600 meters (5,249 ft) above sea level. According to Garcia (1973), the regional weather is hot—dry with rainy summer. The average annual precipitation is 629.1 mm (2.5 in.), and the average annual temperature 18° C (64.4° F). The thermal oscillation varies from minus 10° to plus 35° C (14° to 95° F).

**Experimental material.** The experiment was conducted in October 1999 in the nursery of the Faculty. We used three substrates: (1) composted pine bark "MASVI"; (2) a mixture of peat moss, perlite, and vermiculite in proportions of 50 percent, 25 percent, and 25 percent, respectively; and (3) a control represented by natural forest soil. The seeds were placed in four container types: (1) polyurethane planting trays with 108 cavities of 100 cm<sup>3</sup> (6 in<sup>3</sup>) each, (2) tubular containers apb<sup>®</sup> of 100 cm<sup>3</sup> (6 in<sup>3</sup>), (3) tubular containers apb<sup>®</sup> of 160 cm<sup>3</sup> (10 in<sup>3</sup>), (4) polystyrene bags of 400 cm<sup>3</sup> (24 in<sup>3</sup>), used as controls. Four container types with two substrates plus a control (bags with forest soil) resulted in nine treatments. There were 12 experimental units per treatment, making a completely randomized design. The treatments were:

- T1 = 100-cm<sup>3</sup> (6-in<sup>3</sup>) polyurethane planting trays filled with bark.
- T2 = 160-cm<sup>3</sup> (10-in<sup>3</sup>) tubular containers filled with mixed substrate.
- T3 = 100-cm<sup>3</sup> (6-in<sup>3</sup>) round tubular containers filled with bark.
- T4 = 160-cm<sup>3</sup> (10-in<sup>3</sup>) tubular containers filled with bark.
- T5 = 400-cm<sup>3</sup> (24-in<sup>3</sup>) plastic bags filled with natural forest soil.
- T6 = 100-cm<sup>3</sup> (6-in<sup>3</sup>) squared tubular containers filled with bark.
- T7 = 100-cm<sup>3</sup> (6-in<sup>3</sup>) round tubular containers filled with mixed substrate.
- T8 = 100-cm<sup>3</sup> (6-in<sup>3</sup>) squared tubular containers filled with mixed substrate.
- T9 = 100-cm<sup>3</sup> (6-in<sup>3</sup>) polyurethane planting trays filled with mixed substrate.

Those treatments are conventionally used in several nurseries in northern Mexico, and the experiment was intended to test their adequate applicability for the production of seedlings.

Statistical evaluation and measures. We made weekly observations beginning with the date of seeding, recording the emergence of the seedlings (percent), the temperature, and the precipitation. We also analyzed substrates to determine their more important physical and chemical characteristics. We determined the responses of the seedlings to the substrates and the container types on the velocity of the germination and the development of the seedling heights through variance analyses and confidence intervals.

After a 12-month period of cultivation in the nursery, we randomly selected 15 seedlings per treatment and performed a biomass analysis. For each seedling, we recorded diameter, stem, and root length; number of secondary roots; and weight (g) produced in the aerial and radicular portions. These parameters were considered as indicators of the quality of the seedling. We evaluated the registered variables through variance analysis and a means comparison with the Tukey test, following conventional statistical methods.

### **Results and Discussion**

Germination evolution of seedlings for all the treatments is shown in figure 1.

During the first 20 days of observation, the mixed substrate yielded the highest percentage of germinated seeds (55 percent), while the bark and natural forest soil substrates yielded 28 percent and 16 percent, respectively. The last two values are statistically different from the first one (P=0.05). The highest germination rate observed in the mixture substrate was probably due to the high content of organic matter (14 percent) and to the proportion of organic and inorganic materials (50:50) used in this study.

An acid substrate (pH between 5.0 and 6.0) with an organic matter content around 14 percent fosters germination (Landis et al., 1990). The substrates used in this study did not simultaneously present these two conditions. The mixture and the forest soil were alkaline, while the bark substrate was acidic. Organic matter content was higher in the substrates mixture and forest soil.

The alkalinity and the lower percentage of organic matter in the forest soil, probably affected the germination rate and efficiency. At the end of the study, the germination levels in the substrates mixture (90 percent) and bark (89 percent) were higher compared with the percentage in the forest soil (78 percent). Their final values, however, were not significantly different ( $P^n=0.05$ ). All of the these values can be considered highince they are superior to those reported by Prieto and Trujillo (1999) for *Pinus* engelmannii and Prieto et al. (1999) for *Pinus cooperi*, using the substrates mixture and bark respectively in each species. The survival rate of the seedlings grown in all the different treatments is shown in figure 2.



**Figure 1**: Germination rate of the seedlings of P. pseudostrobus in three substrate types. Grown in the nursery of the Faculty of Forestry, FCF/UANL.





Two periods can be observed with a decrease in the survival rate in almost all of the treatments. Mortality was probably caused by the low registered temperatures (-2°C) and also by damping-off negatively affecting all seedlings. At the end of the 12-month observation period, the best survival rate (89 percent) was shown by the seedlings grown in trays filled with the mixture (T9) and the poorest (71 percent) was registered in the tubular 100-cm<sup>3</sup> (6-in<sup>3</sup>) containers with the mixture (T7). The variance analysis indicates significant differences (P=0.05) in this parameter for the different treatments.

Better survival rates were observed in the shorter seedlings, which were grown in the lower capacity 100cm<sup>3</sup> containers (T1 and T9). The benevolent conditions in the nursery allowed the observation of acceptable survival percentages in all treatments. However, the quality of these seedlings was lesser than that of those observed under other parameters (table 1).

The precarious quality of seedlings, together with the poor soil conditions of the planting sites in this region (heat and drought) suggest that these seedlings should not be planted here since they would be very susceptible to drying out (Domínguez and Návar 2000).

In table 1, the morphological parameters of 12-month nursery seedlings are shown. The results of the variance analysis indicate that there are significant differences in all of the registered parameters (P=0.05). In general, it was possible to observe that the containers capacity influenced

the size of the seedlings and, therefore, also the biomass production. This can be observed in the treatments with greater containers (T2 and T4 with 160 cm<sup>3</sup>, and T5 with 400 cm<sup>3</sup>).

Those results are coincident with the ones reported by Aguirre (2000) for seedlings of P. pseudostrobus of similar age grown in containers of the same capacity and with substrates similar to those used in the current study. It is interesting to note that the length of the root system observed in the seedlings grown in the containers previously described, is greater than the root length recorded in the seedlings of the rest of the treatments. Similar results were reported by Aguirre (2000) for P. pseudostrobus in containers of the same capacity and are also included in the rank mentioned by Montoya and Cámara (1996) for seedlings intended to be established in dry sites, since these can absorb moisture from greater soil depths. Montoya and Cámara (1996) presented similar observations for seedlings that were to be established in dry sites, in which seedlings were ranked for moisture absorption at greater depths.

The number of secondary roots also increases the moisture absorption capacity of the seedling. In this experiment, seedlings grown in larger volume containers had more secondary roots. These characteristics of the radical systems are important, taking into account northeastern Mexico's irregular precipitation.

The average basal diameter of the stems at the end of the nursery phase is shown in figure 3. The variance analysis indicates that there are significant differences (P=0.05) between the average diameters of the seedlings of different treatments. The best results were observed with the seedlings cultivated in the larger capacity containers (T2, T4, and T5), in which seedling diameters were greater than those observed in the other treatments.

The diameter is also considered to be an indicator of the quality of the seedling, and it is related to the growth of the radical system (Prieto et al. 1999); therefore, its dimension can influence the behavior of the seedling in the planting site.

Mexal and Landis (1990) pointed out that it is possible to ensure a survival rate superior to 80 percent in the field if the seedlings leave the nursery with stem diameters between 5 and 6 mm. The same authors indicate that the Table 1. Treatment effects on morphological participation of P. pseudostrobus seedlings at 12 months age in the forestry nursery of the Faculty of Forestry, FCF/UANL.

Treatments	Dry weight biomass (gr)			Diameter	Length (cm)		Average	
	Tallo	Raíz	Total	( <b>mm</b> )	Tallo	Raíz	secondary roots .	
100-cm <sup>3</sup> tray with bark (T1)	0.8	0.4	1.2c	1.2	6.3	11.2d	14d	
160-cm <sup>3</sup> tubular container with mixture (T2)	3.3	2.6	5.9a	1.7	10.5	20.1b	20c	
100-cm <sup>3</sup> round tubular container with bark (T3)	2.5	1.8	4.3b	1.5	9.1	11.4	20c	
160-cm <sup>3</sup> tubular container with bark (T4)	3.7	2.3	6.0a	1.7	11.4	18.7c	26b	
400-cm <sup>3</sup> plastic bag with forest soil (T5)	4.5	2.1	6.6a	1.8	14.1	26.4a	30a	
100-cm <sup>3</sup> square tubular container with bark (T6)	2.6	2.0	4.6b	1.5	9.01	13.9d	24b	
100-cm <sup>3</sup> round tubular container with mixture (T7)	2.1	2.0	4.1b	1.5	8.6	10.3e	25b	
100-cm <sup>3</sup> tubular container with mixture (T8)	2.0	2.0	4.0b	1.4	8.1	12.8d	21c	
100-cm <sup>3</sup> tray with mixture (T9)	2.0	1.3	3.3c	1.7	9.0	9.4e	17d	

survival rate is increased from 5 to 7 percent for each millimeter increase in the diameter of the seedling.

• The diameter differs among individuals of the same species and also among the species themselves. So for example, Rodriguez (1993) and Aguirre (2000) reported larger dimensions for seedlings of the same species than the dimensions reached by the seedlings in this study. Prieto et al. (1999) also mention larger diameters for *Pinus engelmannii* and *Pinus arizonica* grown in conventional systems, in contrast with seedlings grown in other containers and with other substrates.

The development in height of the nursery seedlings is shown in figure 4. In the first 5-month observation period, the increment in height is barely perceptible in all treatments. Probably low temperatures prevented the growth of the seedlings. However, once the average temperature reached values above 12 °C, a generalized increase in the height was observed in all of the treatments. Variance analyses performed for height showed significant differences (P=0.05) among treatments beginning with the 7<sup>th</sup> observation (8 March 2000). From this date until the last observation, four groups of treatments can be distinguished whose heights are not statistically different (P=0.05).



Figure 3: Average diameter and confidence intervals for seedlings of P. pseudostrobus at the end of the nursery phase, reached at the age of 12 months in the nursery of FCF/UANL.



**Figure 4**: Growth in height of seedlings of P. pseudostrobus cultivated in five different containers and three different substrates in the nursery of the School Forest of the FCF/UANL.

The first group, with height of 5.6 cm (2.2 in), is made up of the seedlings from the treatment 100-cm<sup>3</sup> (6-in<sup>3</sup>) tray with bark (T1). The seedlings from the 100-cm<sup>3</sup> (6-in<sup>3</sup>) tubular container with mixture (T6) and 100-cm<sup>3</sup> (6-in<sup>3</sup>) planting trays with mixture (T7) treatments constituted the second group with the heights reaching approximately 8.0 cm (3.1 in) at the end of the study. The third group, with height of 10.1 cm (4 in), was conformed by the plants from the 100-cm<sup>3</sup> (6-in<sup>3</sup>) tubular container with bark (T3) treatment. The seedlings of the 160-cm<sup>3</sup> (10-in<sup>3</sup>) tubular container with mixture (T2) and 160-cm<sup>3</sup> tubular (10-in<sup>3</sup>) containers with bark (T4) treatments reached about 11.0 cm (4.3 in.), and they were the second tallest seedlings.

The last group was composed of the treatment bags with forest soil (T5), which produced the tallest seedlings, reaching 13.6 cm (5.3 in) at the end of the study. The height of the seedlings in this last treatment can be considered acceptable, and it is very similar to that reported by Aguirre (2000) for the same species in identical growth conditions.

Dominguez and Navar (2000) pointed out that the height of the seedlings is strongly influenced by the capacity of the containers and that it is a function of the growth rate of the species. This coincides with the results obtained in the current study, where we observed taller seedlings in the larger volume containers.

Regarding Mexican pines, Prieto et al., (1999) report lesser heights for *Pinus engelmanni* and *Pinus arizonica* and greater heights for *Pinus durangensis* of the same age and grown in conventional systems, in comparison with the heights reported for *P. pseudostrobus* in the present study.

#### Tree Planter's Notes, Vol. 51, No. 1, 2005 Conclusions and Recommendations

On the basis of the results of the present study, we conclude that the substrate mixture and bark substrate are the best media to accelerate the seed germination, a factor that may shorten nursery production cycles. The observed high germination percentages and the statistical similarity registered in the tested substrates allow us to recommend the utilization of composted pine bark. This could reduce production costs because this substrate is less expensive than the components conventionally used for planting substrate mixtures (perlite, agrolite, peat, etc.). In addition, the utilization of sawmill waste could contribute to increase income to logging communities in the region.

Seedlings survival in the nursery phase was not show to be dependent of any specific treatment. However, it was possible to confirm that the nonwoody stems of the seedlings grown in smaller containers were more susceptible to drying out.

In regard to dimensions and biomass production the superiority of seedlings cultivated in larger capacity containers was evident. Among them, the seedlings grown in the conventional system of bags with forest soil (T5) and secondly the seedlings grown in tubular containers of 160 cm<sup>3</sup> (10-in<sup>3</sup>) were outstanding, given their strength. The low cost and easier management of the seedlings in the nursery using these containers and substrates makes them our recommended choice. However, it is important to point out that the utilization of larger tubular containers (more than 250 cm<sup>3</sup> (15 in<sup>3</sup>)) and the application of agrochemical products will produce better quality seedlings, increasing the success probabilities of rural plantations in this region.

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### A Review of the "Pull-Up" and "Leave-Down" Methods of Planting Loblolly Pine

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Two schools of thought exist regarding the planting of bare-root seedlings. One school favors the "pull-up" method where the seedling is pulled-up 3 to 10 cm after placing the roots in the planting hole. Although this action purportedly straightens the taproot, data are lacking to show this extra step actually improves field performance. Pulling the seedling up usually results in a root-collar 5 cm or less below the groundline (which could increase mortality on some sites). The "leave-down" school advocates making a deep planting hole, placing the roots near the bottom of the hole, and no "pull-up." The "leavedown" technique results in planting the root-collar 3 to 10 *cm deeper than the "pull-up" technique. Those from the* "leave-down" school say that shallow holes kill seedlings; bent roots do not. Planting guidelines should be rewritten to: (1) emphasize the "proper" depth of planting to increase seedling survival; (2) de-emphasize intuitive beliefs that taproots and lateral roots must be oriented downward after planting; (3) not recommend unnecessary refinements in planting technique; (4) explain the advantages of machine planting; (5) explain the species, site, planting depth interaction for survival; and (6) cite references to support recommendations.

A high percentage of planted seedlings in the South (40 to 80 percent) can be classified as having deformed roots (Schultz 1973, Hay and Woods 1974a, Mexal and Burton 1978, Senior and Hassan 1983, Harrington and others 1989, Gatch and others 1999). However, just because a planted loblolly pine (*Pinus taeda* L.) seedling has a bent taproot or compressed lateral roots does not mean its performance will be less than seedlings that originate from direct seeding. In fact, on four sites in Arkansas (Harrington and others 1989), 32 percent of the trees originating from seed had bent taproots (likely due to rocks and compact soil layers). Therefore, bends in the taproot can be "natural" as well as "human-made." Even so, some claim that planting methods that result in J-roots or

L-roots (table 1) will kill seedlings and that utmost care should be exercised during planting to ensure the taproot is straight. Since planting the root-collar 15 cm below the surface (in a 25-cm hole) will bend the taproot, several planting guides indicate the "proper" planting depth is so the root-collar is slightly below the groundline.

Typically, survival and average diameter growth of transplanted 1+0 loblolly seedlings are greater than for trees in direct seeded fields (this may not hold for other species and stocktypes; Halter and others 1993). This partly explains why tree planting in slits (with flattened roots) is more common in the South than direct sowing. However, planting method can make a significant difference in survival (Muller 1983, Xydias 1983, Rowan 1987, Shriver and others 1990, Paterson 1993, Harrington and Howell 1998). For example, operational planting techniques can lower survival by 10 percent or more (Rowan 1987, Shriver and others 1990, Harrington and Howell 1998). The practice of stripping roots just before planting can reduce new root growth (South and Stumpff 1990) and lower survival (Marx and Hatchell 1986). On some sites, planting the roots just 8 cm deeper than the depth used by operational crews can increase survival by 15 percentage points (Blake and South 1991). In most years, machine planting provides better survival than hand planting (McNab and Brendemuehl 1983, Barber 1995, South and Mitchell 1999), probably because depth of planting is typically greater, the frequency of loose planting is lower, there is less root exposure, and there is less root pruning and root stripping by tree planters. Moderate root pruning can reduce seedling survival by 4 to 19 percent (Mexal and South 1991). When poor planting techniques are used, the productivity of stands will be decreased (Mullin 1974, Rowan 1987, Paterson 1993).

Since planting technique affects survival, it is imperative that supervisors of tree planters know which techniques **Table 1.** Definitions of various root shapes at time oftransplanting.

Code	Orientation
I-root	A taproot pointed straight down (0-20°)
D-root	1 cm or more of the taproot pointed down at an angle (21°-69°)
L-root	1 cm or more of the taproot pointed horizon- tally $(70^{\circ} - 110^{\circ})$
J-root	Less than half of the taproot in a J-shape pointed up (>110°)
N-root	Two bends in the taproot with the tip pointed down
P-root	A loop in the taproot with the tip pointed down
U-root	Half or more of the taproot pointed up (>110°)
Ψ-root	A taproot pointed straight down (0-10°) but with two or more first-order lateral roots pointed up (>110°)

In addition to the letter code, a number code can be added to provide more information on the root-collar diameter, planting depth, rooting depth, and taproot length. For example, seedling with an L-root and a code of (L:5:3:13:15) has a 5-mm root-collar with the root-collar 3 cm below the surface, it has a root depth of 13 cm, and the taproot is 15 cm long. A U-root with a 6-mm root-collar diameter (U:6:8:15:16) would the root-collar 8 cm below the groundline, the roots are up to 15 cm below ground, and the taproot is 16 cm long. A N-root with a 4-mm root-collar diameter (N:4:0:7:18) would have the root-collar at groundline, the roots would only extend to 7 cm below the surface, and the bent taproot (if extended) would measure 18 cm long. An I-root with a 5-mm root-collar diameter (I:5:-1:18:15) would have the root-collar 1 cm above the groundline, the lateral roots would extend to 18 cm below the surface, and the taproot is 15 cm long.

actually improves survival. If supervisors provide the wrong information to tree planters, they might encourage pruning of taproots and lateral roots. There is no doubt that root pruned seedlings are easier to plant and it is easier to get the roots straight in the hole without folding (Dierauf 1982). Although root pruning can make tree planting easier and can increase the percentage of straight taproots, it does not improve field performance. In fact, pruning roots after lifting often reduces seedling survival (table 2). Despite this information, some recommend tree planters root prune seedlings when taproots are longer than 18 cm. Dierauf (1982) believes that root pruning to a length not less than 13 cm is a good practice. In one study, 25-cm taproots **Table 2.** Effect of root pruning after lifting on survival (percent) of pine seedlings (Wakeley 1954, Dierauf and Garner 1978, Dierauf 1984b, Dierauf 1992, Harrington and Howell 1998).

Year	Species	No prune	Pruned	Heavily pruned
1954	Slash	77	36	6
1954	Slash	40	24	1
1954	Longleaf	81	80	42
1954	Longleaf	61	39	11
1978	Loblolly	95	94	90
1978	Loblolly	95	95	87
1978	Loblolly	93	90	92
1978	Loblolly	93	85	87
1978	Loblolly	100	93	82
1984	Loblolly	94	98	_
1984	Loblolly	90	93	_
1984	Loblolly	90	91	_
1992	Loblolly	82	80	_
1992	Loblolly	100	95	_
1992	Loblolly	97	95	_
1998	Loblolly	80	76	74

were pruned to a length of 17 cm and lateral roots were pruned to a 5 cm length (Wilder-Ayers and Toliver 1987). At the end of the first growing season, the mean survival of these root-pruned seedlings was 39 percent.

In my opinion, most tree planting guides for loblolly pine exaggerate the dangers of both root deformation and deep planting (planting seedlings with the root-collar 7 to 18 cm below the groundline). Some planting guides emphasize the need for tree planters to prune long lateral roots and taproots in order to facilitate "proper" planting. These guides should be rewritten to stress the important aspects of planting and eliminate the unimportant.

This paper reviews the J-rooting and L-rooting studies that have been conducted with bare-root pines in the Southern United States. It does not cover root-strangulation occasionally caused by growing seedlings in containers or when twisting bare-root seedlings during planting (Ursic 1963). It reviews data mainly from the compression method of planting where root systems are compressed into a vertical plane (also know as slit planting).

### **Two Schools of Thought**

Two schools of thought exist regarding the planting of loblolly and slash pine (Pinus elliottii Englem.) seedlings. The difference in planting recommendations between these schools are illustrated in figure 1. The older-school favors the "pull-up" technique, where the seedling is placed deep into the planting hole and then pulled up 3 to 10 cm in order to straighten out the roots. Some from this school recommend pulling up the seedling so the root-collar is about 1 to 6 cm below the soil surface. This action purportedly improves field performance by straightening out the roots. Several planting guides recommend this technique when hand planting (Wakeley 1954, Balmer and Williston 1974, Anonymous 1981, Moorehead 1988, Anonymous 1989, Carlson and Miller 1990, Trewin 2004) or machine planting (Anonymous 1998). We do not know if pulling the seedling up 3 cm is really enough to straighten out the roots or if this technique makes any difference in survival or growth of loblolly pine. To avoid  $\Psi$ -roots, some members of this school recommend pruning of long fibrous roots by tree planters (Moorhead 1988, Anonymous 1989). Some recommend pruning roots with a sharp knife, machete, axe, or hatchet when taproots exceed 18 cm in length. This school prefers straight taproots to planting bent roots 3 to 10 cm deeper. Some claim the "correct" planting depth is to have the root-collar 1 to 6 cm below the groundline (Martin et al. 1953, Wakeley 1954, Balmer and Williston 1974, Anonymous 1981, Anonymous 1989, Fancher et al. 1989, Carlson and Miller 1990). Others recommend making planting holes that are 20 to 25 cm deep (Dierauf 1982) or less (Martin et al. 1953).

The other school recommends the "leave-down" technique. As a result, the roots are generally planted 3 to 10 cm deeper than when the "pull-up" method is used. The "leave-down" technique favors leaving the roots bent at the bottom of the planting hole over attempts to straighten taproots and laterals by pulling the root-collar closer to the soil surface. Due to an increase in probability of success, members of this school prefer machine planting to hand planting. (Average planting hole depth for machine planting is about 30 cm, and the root-collar is typically about 15 cm below the soil surface; this sometimes results in a high percentage of L-roots.) Planting on agricultural lands using machines that make slits that are only 15 to 20 cm deep will likely result in many L-roots (Gatch and others 1999). On sites where hand-planting is required, leaders in this school recommend making a wide (15 to 20 cm) and

deep (27 to 34 cm) planting hole. The roots are placed at the bottom of the hole and there they remain. As a result, the root-collar ends up at least 5 to 10 cm deeper than usually recommended by the "pull-up" school. For many sites, the "correct" planting depth will result in the rootcollar 15 cm below ground and the bottom of the roots will be 25 to 34 cm deep (VanderShaaf and South 2003). They allow J-roots, L-roots, and  $\Psi$ -roots but prohibit shallow planting holes (less than 25 cm deep), as well as pruning or stripping of roots by tree planters. However, due to a three-way interaction between species, site, and planting depth, members of this school do not recommend the same planting depth for all pine species or for all sites. Deep planting on poorly drained sites (where the water table is near the soil surface) can decrease survival of loblolly pine (Switzer 1960). Therefore, the "correct" planting depth varies with site.

Because less time is required to make a narrow, shallow hole, hand planters prefer recommendations made by those who favor pruning roots (Dierauf 1982, Anonymous 1989). Making a deeper planting hole by hand increases planting costs. This is one reason those from the "leavedown" school favor machine planting. On many sites, the cost of machine planting is similar or less than that for hand planting (Straka et al. 1992).



**Figure 1.** A comparison of hand planting recommendations from members of the "pull-up" school (Anonymous 1981) with hand planting recommendations from members of the "leave-down" school.

### Definitions

Tree planting terminology can sometimes be confusing. For example, some planting guides say the seedling should not be planted deeper than the length of the dibble bar. Some from the "pull-up" school say the correct depth of planting should be 3 to 6 cm "below" the root-collar (Carlson and Miller 1990). Others define a seedling as being planted "deep" when the root-collar is just 3 cm below the soil surface (Brissette and Barnett 1989, Jones and Alm 1989). The "recommended" planting depth in Virginia is 3 to 5 cm deeper than the "normal depth" (Dierauf 1984a). To improve the terminology of root classifications, I propose a new code system to define root shape, root-collar diameter, planting depth, rooting depth, and taproot length (table 1). In addition, I offer the following definitions:

- Root depth = Distance between groundline and deepest point of the roots after planting.
- Planting depth = Distance between the root-collar and the groundline (negative values indicate the root-collar is aboveground).
- Correct planting depth = Depth where survival and early growth are reduced when planting the root-collar deeper or shallower.
- Shallow planting = Depth where survival is increased when planting the root-collar deeper.
- Excessively deep planting = Depth where survival or growth would be increased if the root-collar was planted closer to the groundline.

Shallow planting hole = Hole less than 20 cm deep. Deep planting hole = Hole greater than 25 cm deep.

## History of Transplanting Recommendations

The debate about proper planting techniques has been going on for more than a century. For example, Jarchow (1893) recommended planting the root-collar a little above ground and he could not comprehend how Hough (1882) could recommend "setting the seedlings deeper than they stood before." Jarchow said the "experts in this matter agree in accepting the reverse to be true." Likewise, those in the "pull-up" school today might not comprehend how those in the "leave-down" school could allow seedlings to be planted deep with J- and L-roots. Debates on proper planting techniques will likely continue since data from empirical studies contradicts intuition. But even Jarchow (1893) realized that pine seedlings were different. He said that on very poor soil, the "seedling should be buried so deep that only its top shows above the soil." So apparently, over a century ago he realized there existed a three-way interaction between species, site, and planting depth. Many planting guides today do not mention this interaction and make recommendations as though the correct planting depth is the same for all species and for all sites.

According to Cheyney (1927), "The directions for the planting of a tree have become more or less stereotyped and have been copied for so many years that it is practically impossible now to say on what the directions are based...." The same can be said today. Some of the current recommendations regarding the "correct" planting depth can be traced back to 19th century Europe. For example, Toumey (1916) admitted that there was little information on deep planting in the United States (page 385) but said "... the investigations of many European foresters clearly prove that poor results are likely to follow the setting of plants too deep in the soil." He said that "many investigators have recorded the bad effects from the deep planting of Picea abies (L.) Karst. The older the plants, the more disastrous the results." However, he did say that 1-year seedlings of Pinus sylvestris L. (in Prussia) can be safely set considerably deeper than their original position in the seedbed. Even though he said planting with the root-collar well below the soil surface will often enable trees to survive summer drought, he warned this would not be desirable even on dry sites "because of its effect upon later root development." Almost a century later, there is still a fear that deep planting of loblolly pine is undesirable because something bad might happen before the stand is 50 years old. This fear is not supported by published studies (Hunter and Maki 1980) but has been passed down by word-of-mouth from forester to forester. Zon (1951) stated that one of forestry's early mistakes was the "Uncritical, almost slavish following of European patterns." For example, many loblolly pine planting guides today still have a figure to illustrate that planting the root-collar 10 cm (or more) below the soil surface is "incorrect" planting.

### **Three Types of Recommendations**

Regardless of the century, tree planting recommendations can be placed into three types: (1) recommendations based on intuition, (2) recommendations based on observations, and (3) recommendations based on experiments designed to test a hypothesis. Little confidence should be placed on guidelines that rely on 19th century intuition. Likewise, guidelines that cite results from empirical experiments deserve more confidence than recommendations based solely on survey data.

**Intuition.** Recommendations based on intuition indicate that root distortion will: (1) kill seedlings, (2) reduce the seedling's ability to uptake water, (3) increase the susceptibility of disease, (4) slow growth of any surviving seedlings, and (5) cause seedlings to blow over. Some planting guides (Stephen 1928, Martin and others 1953) warn that J-rooting will kill seedlings but these guides cite no data or references. When data do not support intuition, the accuracy of these guesses can be questioned. For example, intuitive recommendations that longleaf pine (*Pinus palustris* Mill.) be planted with the root-collar above ground were made before 1940. However, after conducting an empirical test, Wakeley (1954) stated that this practice should be abandoned.

The "pull-up" method of tree planting is an "intuitive" recommendation. This technique (possibly started by Floyd Cossitt about 1939) can be found in several planting guides. As far as I know, there are no data to show this method of planting improves survival, growth, or field performance of loblolly pine. Perhaps some researcher in the future will decide to test this "intuitive" method of tree planting. Seiler and others (1990) anticipate that this method of tree planting increases seedling mortality. My intuition says if the root-collar is "pulled-up" to the groundline, it could also increase the probability of toppling. In New Zealand, the method has been modified so that after pulling up, the root-collar remains 15 cm below the groundline (Trewin 2004). This modification was made to improve the wind-firmness of the seedling.

Root pruning after lifting is another example of an "intuitive" recommendation. Instead of improving the survival of loblolly pine (by planting a smaller I-root), root pruning can reduce root growth potential and seedling survival. Even so, some planting guidelines recommend that tree planters prune long roots before planting (Moorhead 1988, Anonymous 1989). Sometimes 44 percent of the roots are removed so the pruned roots will match the planting hole (Wilder-Ayers and Toliver 1987).

**Observations.** Operationally planted seedlings are sometimes excavated 1 or more years after planting and the root shape is reported. These observational reports do not involve an experiment laid out in a randomized complete block design. As a result, analyses often involve simple correlations or sometimes multiple regressions. After root systems are examined, a subjective root score is given to reflect the degree of distortion. In some cases, recommendations regarding the negative effects of root distortion are made without excavation of any direct seeded seedlings.

In a few cases, planted seedlings are compared with wildlings (Little and Somes 1964, Harrington and others 1989). These studies are useful for identifying the frequency of root abnormality of trees with "natural" root systems. Usually, differences in both location and genetics exist between the "natural" seedlings and the planted stock. Therefore, any observed differences are often confounded with site, genotype, and sometimes there is a 2year difference in seedling age. However, such studies can illustrate what we think of as "abnormal" can occur to some extent in nature.

**Empirical trials.** The scientific method involves: (1) identifying a problem, (2) researching the known literature, (3) formulating a hypothesis, (4) deciding on a procedure to test the hypothesis, (5) collecting data and conducting a proper analysis to test the hypothesis, (6) deriving a conclusion, (7) publishing the results, and (8) reevaluating the hypothesis. Observational studies are good for formulating a hypothesis but planned experiments must be conducted in order to test a hypothesis. Carefully designed experiments (designed to minimize confounding) may require years before adequate growth data are obtained (which may explain why some researchers only report observational data). However, there are several examples of empirical studies in the literature (e.g., Deleporte 1982, Haase and others 1993). Results from these trials are more reliable than those where root form at planting is not known. However, not all empirical studies are designed properly (e.g., Cheyney 1927).

### J-rooting per se does not kill seedlings: shallow planting kills seedlings

Some tree planting guides state that root deformation will kill seedlings (Stephen 1928, Martin and others 1953). However, for both loblolly pine and slash pine, there is no proof to show this is true. Not only do most Jrooting trials show no significant effect on survival (table 3), almost all these trials confound root depth with treatment. Therefore, the real cause of mortality in such trials could simply be due to shallow planting. Apparently, the idea that L-rooting can kill seedlings might have originated from a misinterpretation of a photo in a book by Toumey (1916). He shows two L-rooted seedlings: one alive and one dead (figure 2). Apparently some authors of tree planting guides assumed the tree died because of the L-root. But the photo clearly shows the deeper planted L-root seedling in good condition. The cause of mortality was a shallow planting hole.

Brissette and Barnett (1989) established an empirical study where both root depth and J-roots were tested. Roots were pruned to a length of 15 cm and were placed into shallow holes (8 cm to 18 cm deep). A close examination of their data suggests that root depth (not J-rooting) was the primary factor affecting survival (figure 3). In fact, when planting in a very shallow hole (13-cm root depth), J-roots had 18 to 27 percent greater survival than I-roots.



**Figure 2.** Yellow pine killed from crowding its roots into a shallow planting hole (A). Yellow pine in the same plantation in good condition and tap-root re-established (B). L-root planted in a deeper hole.



**Figure 3.** The effect root depth, water stress, and root form on the survival of loblolly pine seedlings 12 weeks after planting in shallow holes (8 to 18 cm deep) in a greenhouse (adapted from Brissette and Barnett 1989).

Extrapolating the equations in figure 3 suggest that 90percent survival could have been obtained if rooting depth was 22 to 28 cm. However, the researchers planted no roots this deep.

A new OST planting bar (Council Tool Co., Lake Waccamaw, North Carolina 28450) can be used to make a 25-cm-deep hole and a Whitfield planting bar (R.A. Whitfield Manufacturing Co., P.O. Box 188, Mableton, Georgia 30126) can help make a 34-cm-deep hole. Ursic (1963) and Bilan (1987) planted trees deep using a 45-cm bar. Malac (1965) recommends using a dibble with a 30to 35-cm blade when planting Grade 1 seedlings but his recommendation is rarely followed. Trewin (2004) recommends the tree planter make a 30 cm deep hole. In contrast, one planting guide recommends that planting holes be 15 cm to 20 cm deep (Martin and others 1953). Therefore, when planting pruned roots in holes only 8 to 19 cm deep, tree planters should expect some mortality (even under well-watered conditions in a greenhouse).

I agree with those who say a shallow planting hole is the main reason for increased mortality and not root deformation per se. Toumey (1916) states that "One of the most frequent defects in planting arises from crowding trees with large roots into shallow holes." Wakeley (1954) concluded that U-rooting "usually has a negligible effect on initial survival." He said that setting depth probably reduces survival more often and more seriously than any and all other errors in planting depth combined. After evaluating the performance of many operational plantings throughout the South, Xydias and others (1983) stated "Probably root deformation, per se, has no effect on survival. A too shallow planting slit results in root deformation, but the real cause of mortality is shallow planting." Seiler and others (1990) said "instructing planters to avoid J-roots by pulling back up on the seedlings when they are planted in the bottom of the planting hole may do more harm than good since the end result could be shallower root placement."

Twenty studies that compared I-roots with bent roots of southern pines are listed in table 3. On average, survival of bent roots was about 0.6 percent less than I-roots. However, in all cases, bent roots had less root depth than I-roots. Therefore, confounding exists between root depth and root form.

### Effect of Planting Depth on Survival

Wakeley (1954) conducted several planting depth studies and found that planting the root-collar of longleaf pine seedlings 1.3 cm above the groundline reduced the survival by 25 to 29 percent. In contrast, planting the seedlings with root-collars 1.3 cm below the groundline increased the survival 7 or 8 percent. Similar results were reported by Smith (1954).

Planting loblolly pine or slash pine with root-collars 5 to 28 cm below the groundline (on drained sites) tends to increase outplanting survival (table 4). On average, the increase is about 4 percentage points. Unfortunately, several studies during the 1950s and 1960s dealt with cull

seedlings. Although the data are limited, there appears to be a site-by-planting-depth interaction for loblolly pine. Deep planting is not recommended on poorly drained sites (Switzer 1960).

Although data by Koshi (1960) suggest a detrimental effect of deep planting loblolly pine during a wet year, he made a math error. Apparently, he reported percentages as survival data instead of mortality data. Overall survival for four loblolly pine seedling grades was 67 percent (not 33 percent).

Sutton (1969) reviewed the research on planting and stated that "deep planting has been damned by many... as a common cause of plantation failure..." However, he said that the evidence indicated that deep planting is beneficial on many sites. Data supplied by others (Blake and South 1991; VanderShaaf and South 2003) support Sutton's conclusion.

# Effect of Bent Roots on Short-Term Growth

According to Toumey (1916), Möller (1910) conducted a series of experiments with *Pinus sylvestris* on sandy soil in Prussia and concluded "that it does not matter apparently whether roots are bent to one side, tied together, or crowded into the planting hole. He found that if roots were not permitted to dry out, the above manner of treatment was not likely to kill the trees or even appreciably to check their growth." Toumey (1916) concluded that unnecessary refinements in the planting technique should be avoided.

Ursic (1963) excavated 13 seedlings that had been planted with U-roots. Examinations showed that roots had either elongated and turned to grow downward or that new roots had developed along the U-root (figure 4). Ursic indicated that the dangers attributed to U-roots "have been exaggerated."

Hay and Woods (1974a) excavated 348 saplings and found a positive correlation between root deformation and size of loblolly pine seedlings 4 to 6 years after planting. On one site, seedlings with the most root deformation were more than twice as heavy as seedlings with I-roots. However, this apparent correlation may be simply due to more root deformation when planting seedlings with larger roots. Seedlings with larger roots and a larger root-collar diameter at time of planting tend to grow more than seedlings with small roots (South 1993).

**Table 3.** Effect of root distortion on outplanting survival (percent) of bare-root pines in the Southern United States (Wakeley 1954, Ursic 1963, Little 1973, Hay and Woods 1974b, Hunter and Maki 1980, Woods 1980, Dierauf 1992a, Harrington and Howell 1998). In no case was a statistically significant difference reported.

Year	Species	Straight roo	ots Bent-roots	Root form	Difference
1954	Longleaf	86	86	U	0
1954	Longleaf	42	42	U	0
1954	Longleaf	82	88	U	+6
1954	Slash	62	69	U	+7
1954	Slash	71	56	U	-15
1954	Slash	96	94	U	-2
1963	Loblolly	87	75	U	-12
1963	Loblolly	89?	89?	U	?
1963	Loblolly	94?	94?	U	?
1973	Loblolly	89	86	L+J	-3
1973	Loblolly	60	67	L+J	+7
1974	Loblolly	90	90	J	0
1980	Loblolly	89	91	Curl	+2
1980	Loblolly	70	78	L	+8
1980	Loblolly	55	51	L	-4
1992	Loblolly	80 ***	82	$\Psi$	+2
1992	Loblolly	95 ***	100	$\Psi$	+5
1992	Loblolly	95 ***	97	$\Psi$	+2
1998	Loblolly	87 *	80 **	J	-7
1998	Loblolly	76 ***	80 **	J	+4

\* Planted with shovel—roots not pruned.

\*\* Planted with hoedad—roots not pruned.

\*\*\* Roots pruned.

Mexal and Burton (1978) excavated 100 seedlings 2 to 4 years after planting. As one might expect, they found a positive relationship between initial seedling size and early growth on all four sites but found no correlation between taproot deformation and height growth. However, on one site, they found a positive relationship between taproot deformation and volume growth ( $r^2=0.10$ ). On a bedded site, they found a positive relationship between planting depth and height ( $r^2=0.14$ ).

Mexal and others (1978) excavated trees from 30 stands across the South. Five trees were excavated per plot (for a total of 150 excavated trees). A strong positive relationship (r<sup>2</sup>=0.14; n=30) was reported between the number of seedlings per plot with good roots and seedling height. Average height (4 to 9 years after planting) was 20 cm taller for plots with four "good" roots compared to plots with just three "good" roots. A root system was judged to be "poor" if it had less than six lateral roots, had a deformed taproot, or was encircled by lateral roots. Although some trees had missing taproots (or twisted laterals resulted in strangulation), it is possible that tree height in this study was correlated with the number of large seedlings (those containing six or more first-order lateral roots at time of planting). **Table 4.** Effect of planting depth on survival (percent) of southern pine seedlings (Slocum 1951, Smith 1954, Wakeley 1954, Malac and Johnson 1957, Slocum and Maki 1956, Switzer 1960, Shoulders 1962, McGee and Hatcher 1963; Swearingen 1963, Ursic 1963, Donald 1970, Dierauf 1984a, Bilan 1987, Blake and South 1991). Where reported, numbers in parentheses indicate the distance in centimeters between the root-collar and the soil surface.

Year	Species	Root-collar near surface	Deeper	Difference
1954	Longleaf	73 (0)	83 (1.3)	+10
1954	Longleaf	74 (0)	90 (1.3)	+16
1954	Longleaf	68 (0)	76 (1.3)	+8
1954	Slash	83 (0)	83 (5)	0
1954	Slash	92 (0)	95 (5)	+3
1957	Slash	40	61	+21
1963	Slash	80 (0)	90 (15)	+10
1963	Slash	80 (0)	95 (28)	+15
1963	Slash	86	89	+3
1963	Slash	71	70	-1
1951	Loblolly	97 (0)	97 (5.7)	0
1956	Loblolly	97	97	0
1956	Loblolly	97	91	-6
1956	Loblolly	99 (2)	94 (9.3)	-5
1956	Loblolly	85 (1.5)	98 (5.5)	+13
1960*	Loblolly	59	66	+7
1963	Loblolly	87	76	-11
1970	Loblolly	72 (0)	82 (6)	+10
1984	Loblolly	79 (2.5)	86 (7.5)	+7
1984	Loblolly	84 (2.5)	86 (7.5)	+2
1984	Loblolly	84 (2.5)	90 (7.5)	+6
1987	Loblolly	90 (0)	87	-3
1991	Loblolly	70 (4.8)	85 (11.9)	+15
1991	Loblolly	69 (1.3)	84 (9.4)	+15
		Poorly drained soil		
1960	Loblolly	90	73	-17
1960	Loblolly	90	32	-58

\* Data from Koshi (1960) assumes he made an error and reported data as survival instead of mortality.

Harrington and others (1987) excavated 192 loblolly pine seedlings (ages varied from 3 to 9 years old). Half of the 16 plots were from natural or artificial seedling. Distance between sites within each of the eight pairs was less than 15 km. Although planted trees exhibited more root deformation, there was no difference in growth (i.e., past 3 years height growth) between planted and seeded trees. However, on four plots in Arkansas, they found a total of three planted trees with L- or J-roots (root class #2) that grew 58 cm during the year prior to excavation while 14 trees with single taproots averaged 70 cm of height growth (a difference of 12 cm). Likewise, in the Gulf Coastal Plain, they found a 24-cm difference in growth between I-roots (22 trees: 127-cm-height growth)

and J-roots (7 trees: 103-cm-height growth). Although the trees may not have been the same age, they concluded that root system deformation and orientation are factors in the long-term performance of loblolly pine plantations.

Seiler and others (1990) found no difference in thirdyear height growth between J-roots and I-roots. Likewise, Dierauf (1992) found no difference in height growth between I-roots and  $\Psi$ -roots. On an agricultural site, Harrington and Gatch (1999) found better height growth for J-roots than for I-roots.



**Figure 4.** New growth of U-roots of loblolly pine often turn downward. This seedling was excavated from a sandy textured soil in April of the third growing season (from Ursic 1963).

# Effect of Bent Roots on Long-Term Growth

An argument against bent taproots planted deeply is that something bad might happen to the stand after it reaches an age of 20 or 30 years. Stated another way, deep planting and the associated root deformation might be bad even if we cannot prove it to be so today. Indeed, reports from Europe suggest this might have occurred with pine and spruce in Germany and Austria (Toumey 1916). Since scientists cannot prove a null hypothesis, followers of the "leave-down" school cannot prove that something bad will not happen in the future. They can only say that in three stands, nothing bad happened for 10 years (Harms 1969) and in another stand nothing bad happened for 24 years (Hunter and Maki 1980).

### Effect of Bent Roots on Toppling

"Toppling" occurs when high winds blow over young (1 to 6 year-old) seedlings. Toppling is almost nonexistent for slow-growing wildlings (Burdett and others 1986). Toppling of fast growing pines is a problem in some windy countries such as South Africa and New Zealand (Mason 1985, Zwolinski and others 1993). For Pinus radiata, researchers believe that bent roots will give poor anchorage to the seedling and it will result in toppling at a later date (Maclaren 1993). However, even in areas with hurricanes, toppling of bare-root loblolly pine is rare in the United States. Infrequent toppling has occurred when planting bare-root stock on good sites between the ages of 3 and 5 (Klawitter 1969, Hunter and Maki 1980; Harrington and others 1989), especially when the foliage is loaded with ice or snow (Dierauf 1982). Older bare-root loblolly pine trees tend to snap as opposed to lean (Fredericksen and others 1993). However, some guess that that if shallow planted seedlings are so cramped that the root systems defy classification by form, high winds might cause toppling of bare-root loblolly pine (Gruschow 1959). In the Southern United States, I have observed a few cases of toppling of both container-grown stock and bare-root stock.

Slit planting might affect toppling more than J-rooting. For example, Schultz (1973) excavated five slash pine seedlings that had blown over by a high wind. Although all five had deformed taproots, he concluded the primary reason for toppling was compression of the lateral root system as a result of slit planting (there was only one or no lateral roots on the windward side of the tree). After excavating 163 trees, he concluded that root deformation did not appear to be detrimental to tree growth.

My intuition suggests that toppling might be negatively related to planting depth. The "ball-and-socket" effect that precedes toppling might be reduced when the stem above the root-collar is supported by 15 to 18 cm of firm soil. Instead of preventing toppling, the "pull-up" method of tree planting might result in more toppling than planting loblolly pine seedlings deep. If toppling becomes a problem in the South, this would be an interesting hypothesis to test.

### Effect of Bent Roots on Sinuosity

For pines, sinuosity of the stem (also known as speedwobble) is related to genetics and growth rate. Slow growing provenances of loblolly pine have less sinuosity than fast growing provenances (Anonymous 1993). The heritability for bole sinuosity can range from 0.2 to 0.35 for loblolly pine and 0.2 to 0.55 for *Pinus radiata* D. Don (Bail and Pederick 1989, Anonymous 1993). If the bole is sinuous, the branches will also be sinuous (genetic correlation = 0.93 or greater). In Australia, sinuosity occurs on soils with high fertility (Birk 1991, Turvey and others 1993).

Crooked stems can result from toppling. Some pines that have a 50° lean at age 2 will recover and only have a 5° lean at age 6 (Harris 1977). As seedlings gradually recover, compression wood forms on the underside of the lean. Although this enables the seedlings to recover, some of the seedlings develop a crook in the stem (Dierauf 1982, Harris 1977).

If shallow planting results in toppling, this can cause sinuosity. Harrington and others (1999) excavated 144 trees and observed stem sinuosity on trees with and without straight taproots. However, the amount of sinuosity on trees with bent taproots was about twice as great as trees with straight taproots. If seedlings are machine-planted with a lean (Klawitter 1969) or have a lean after handplanting (Gleason 1981), this might also result in the formation of compression wood and butt sweep. In fact, the frequency of stem curvature was the same for J-rooted and I-rooted seedlings but the frequency increased when seedlings were planted at an angle (Murphy and Harrington 2004). Examination of empirical trials (e.g., Harrington and Howell 1998) will confirm or fail to confirm the hypothesis that L-roots cause sinuosity.

### Conclusion

For bare-root loblolly pine or slash pine, shallow planting regardless of taproot form can kill seedlings. Therefore, a loblolly pine seedling that has a bent taproot (J:5:15:25:15) but is planted deeply (on a drained soil) will have a higher probability of survival than a shallow planted seedling (I:5:0:15:15) with a straight taproot. Research needs to be conducted to determine if planting seedlings deep will reduce the frequency of toppling and subsequent butt-sweep.

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