Diseases Associated With Containerized Seedling Soil Mixes

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Although most peat-vermiculite mixes are relatively pathogen free, disease organisms can be introduced into containers on seed and plant debris or in irrigation water. Major root pathogens of containerized conifers include species of Fusarium, Pythium, *and* Phytophthora. Fusarium *spp. are the most common and produce several different signs of disease, including stunting, chlorosis, and needle tip dieback, and well as killing some seedlings. Composted tree bark included in the medium will suppress many pathogenic fungi.*

Production of containerized conifer seedlings by northern Rocky Mountain nurseries is increasing, and as it does so, associated disease problems have become more important. The most serious diseases of containerized seedlings are foliage and stem blights (24). Foliage pathogens spread rapidly, and environmental conditions within greenhouses are often ideal for infection and buildup of pathogens (2, 25).

Damping-off and root diseases may also be important in container operations. Most root pathogens are probably introduced either on contaminated seed or from infected plant debris within or adjacent to greenhouses (1, 9, 14, 17). In general, most container soil mixes are relatively pathogen free (25). However, some growers have used soil mixes containing sufficient pathogen populations to cause disease.

Most soil mixes for containerized conifers contain vermiculite or perlite incorporated with sphagnum peat. This type of mix is usually well drained and acidic, two factors that help reduce diseases (11, 20). Peat-vermiculite mixes are also lightweight, uniform in composition, relatively inexpensive, and readily available; they have high water-holding capacity and their acidic nature is conducive to growing conifers (20). Soil mixes with a pH of 4.5 to 6.0 are best for proper growth of seedlings and reduced incidence of disease (16).

Diseases

Major groups of pathogens associated with root diseases of containerized seedlings are species of *Fusarium* and water molds such as *Pythium* and *Phytophthora* (18). Although water molds may be seedborne (10), they are more often introduced into container nurseries through contaminated irrigation water (17). These fungi cause disease on very young seedlings and are favored by poorly drained soil mixes and prolonged wet conditions within greenhouses.

Root diseases associated with *Fusarium* are usually more common than those associated with *Pythium* or *Phytophthora*. These fungi may colonize seeds (7, 9, 19), either causing damping-off shortly after seedlings emerge or killing older seedlings. Several species of *Fusarium* are important causes of root disease of containerized conifers. These include *F. oxysporum* Schlecht. (7, 9), *F. solani* (Mart.) Sacc (7), and *F. moniliforme* Sheld. (17). These pathogens may cause chlorosis (11), stunting (22, 25), and needle tip dieback (8), as well as seedling mortality. *Fusarium* often produces spores on structures called sporodochia at the base of infected seedlings (22). These spores may spread to nearby seedlings and cause infection during watering (19). *Fusarium* may occur within peat-vermiculite mixes (8), but disease development is usually restricted if the mix is acidic (pH less than 6.0).

Disease Control

Root diseases in containerized conifer nurseries are usually sporadic, cause little damage, and do not require specific control measures. However, if disease levels are high, several procedures can help reduce losses.

Seeds should be as free of pathogens as possible. Seeds collected directly from the tree are usually less contaminated than seeds collected from the ground or squirrel caches (24). Seeds can easily be treated before sowing

to remove surface-contaminating fungi. A continuous tap water rinse for 48 hours is usually effective in removing most seedcoat fungi (7, 20). Seeds can also be treated with hydrogen peroxide or fungicides, although some effects on germination may occur (7, 24, 25).

Greenhouses should be kept clean to reduce damage from all diseases. Plant debris should be removed periodically, and benches and walls sterilized between crops (25). Diseased seedlings should be removed as soon as they are discovered (11). A noncontaminated water supply is also important (9, 17).

Soil mixes suspected of containing high populations of pathogens should be treated to reduce or eliminate these pathogens. Chemicals used to sterilize soil mixes include formaldehyde, chloropicrin, methyl bromide, and metham (Vapam) (20). The most widely used system of soil mix sterilization is heating with steam to about 82 °C (180 °F) for 30 minutes (2, 4). This will kill most harmful bacteria, fungi, nematodes, insects, and weed seeds. *Fusarium* species are killed at even lower temperatures (57 °C or 135 °F) (4). The treated soil mix should be placed in containers and handled as little as possible to reduce chances for reinfestation by pathogens (2).

Fungicides applied after root disease symptoms appear may not always be effective (24). Fungicides added to soil mixes may retard seedling growth (25). If fungicides are to be used, they should be applied as a drench immediately after sowing (19). Benomyl may control *Fusarium* and ETMT (Truban) may control *Pythium*. However, because of their uncertain effectiveness, fungicides should only be used when other control measures fail.

Another approach to controlling root diseases of containerized plants is to use composted tree bark in the soil mix. Composted bark has replaced peat in soil mixes for several ornamental species grown in containers (5). One of the major advantages of composted tree bark is that it suppresses several important plant pathogens, including *Phytophthora* (6, 22, 23), *Fusarium* (21), and *Rhizoctonia* (12, 13).

Composting is a process of partially decomposing conifer or hardwood bark to produce a more absorptive, uniform material. The process includes a thermophilic phase, during which high temperatures (40 to 80 °C) kill most organisms, and a stabilization phase, during which the rate of decomposition decreases, temperatures decline, and microorganisms, some of which are antagonistic to plant pathogens, recolonize the compost (5). Most growers use a 4:1 (v/v) mixture of bark and peat as the organic component of the soil mix. This ratio results in almost complete suppression of root diseases without the need for sterilizing the soil mix or applying fungicide.

There are three major mechanisms of root pathogen suppression from composted bark. Bark particles are generally coarser than peat, resulting in improved aeration, which is less conducive to disease occurrence (5). Composted bark supports high levels of antagonistic organisms, whereas peat does not (13). Also, water extracts from composted bark have fungicidal properties (6, 12). Composted tree bark has been used effectively in soil mixes to control plant pathogens in several ornamental plant industries. This approach should also be considered in containerized conifer seedling operations.

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Cylindrocladium crotalariae Kills Container-Grown Northern Red Oak (*Quercus rubra* L.)

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The fungus Cylindrocladium crotalariae (Calonectria crotalariae) *was associated with root-diseased container-grown northern red oak (*Quercus rubra *L.) seedlings. Inoculation trials confirmed the pathogenicity of the fungus. This is the first reported occurrence of* C. crotalariae *root rot of northern red oak. Control measures are discussed.*

Northern red oak seedlings are grown at the USDA Forest Service Resistance Screening Center (Asheville, NC) for the purpose of producing basidiospore inoculum of the fusiform rust fungus (*Cronartium quercuum* f. sp. *fusiforme*) to infect slash (*Pinus elliottii* Engelm.) and loblolly (*P. taeda* L.) pines. The acorns are collected, heat-treated to destroy insect pests, dusted with captan (50 WP), and stored in plastic bags at 0.5 to 3.5 °C for no less than 3 months and up to 3 years. Acorns are germinated in a vermiculite medium in a mist bed. Single acorns are then planted in 4- by 4-inch pots filled with a steam-treated mixture of loamy field soil, sand, and peat moss (3:2:1, v/v mix). Pots are watered every 3 to 4 days by individual drip tubes connected to a common supply pipe. Normally, 90 percent of acorns germinate.

The seedlings are incubated in a greenhouse on raised benches at 21 °C and 24 hours of daylight-artificial light for 6 weeks, when leaves are inoculated for eventual basidiospore production. After the leaves are harvested, the seedlings are removed and the potting medium is saved to grow future crops. The soil is reused 4 to 5 times before being discarded. Pots are washed with 50 percent aqueous sodium hypochlorite (Clorox) and thoroughly rinsed with clean water before reuse. These methods have been successfully used since 1974.

In December 1981, a 5-week-old seedling lot had many ungerminated acorns; seedlings showing foliage dwarfing, marginal necrosis, or wilt; and dead seedlings (fig. 1). Only 25 percent of the seedlings had developed normally. The roots of diseased seedlings were sparse, with black and necrotic tap roots. Radicles of ungerminated acorns were completely rotted just below groundline, and parts of the acorn were discolored. White fungus mycelium was occasionally present. In a few cases, perithecia of a *Calonectria* sp. were observed on affected acorns.

Fungus isolations from symptomatic roots and acorns were made on Czapek's medium with 100 parts per million streptomycin sulfate added (CZK agar).

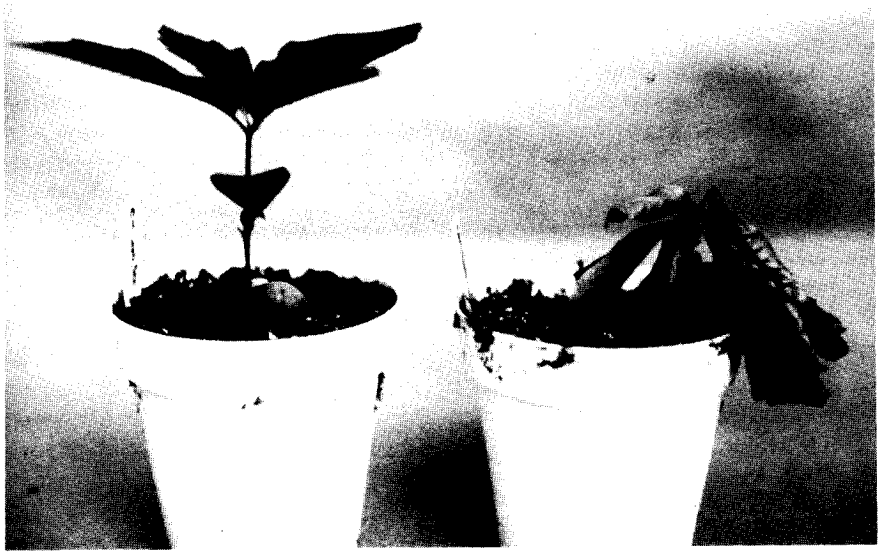

Figure 1—*Healthy and diseased 5-week-old northern red oak seedlings. Root system of diseased tree was sparse, discolored, and decayed.*

Additionally, alfalfa baiting (1), a rapid, qualititative method for detecting *Cylindrocladium* spp. from soil, was attempted on samples from the field-soil supply pile and from soil in pots with killed seedlings.

Cylindrocladium crotalariae was isolated from root systems, acorns, and killed alfalfa sprouts from baited potting medium. This fungus is the asexual stage of *Calonectria crotalariae*. Alfalfa baits from the field-soil supply pile did not yield any *Cylindrocladium* isolates. Inspection of stored acorns showed them to be free of any decay or degradation.

Cylindrocladium spp. are some of the most damaging root disease fungi found in bareroot hardwood tree nurseries. Yellow-poplar *(Liriodendron tulipifera* L.) (4), black walnut (*Juglans nigra* L.) (3), sweetgum *(Liquid-ambar styraciflua* L.) (4), and cherrybark oak (*Quercus falcata* var. *pagodifolia* Ell.) (5) have been severely damaged. Microsclerotia are formed in soil and infected root tissue and are difficult to control completely, even with methyl bromide-chloropicrin fumigants (MC 33) (1). Root diseases caused by *Cylindrociadium* spp. have been infrequently found in forest stands (2, 7).

Because *C. crotalariae* was associated with the symptoms and was not known to damage northern red oak, we evaluated its pathogenicity.

Methods

The inoculum used for pathogenicity trials was prepared by inoculating single-spored isolates of *C. crotalariae* into 2-quart glass jars filled with autoclaved oat seeds. Jars were incubated until the oats were completely colonized and microsclerotia developed (about 6 weeks).

Two inoculation methods were used. The first involved planting germinated acorns into the potting medium routinely used at the Resistance Screening Center but infested with colonized oats at high and low rates (1:10 and 1:50, oats potting medium, respectively). In the second method, washed root systems of 1-month-old seedlings were dipped in water suspensions of microsclerotia. Suspensions were obtained by grinding and repeated decanting of colonized oats until only microsclerotia and water remained. Three dilutions were prepared representing high-, medium-, and low-inoculum doses. Ten root systems were dipped in the inoculum and washed, and the rinsate was collected. The inoculum dose per seedling root system was determined by dilution-plating the rinsate on CZK medium (30, 12, and 8 propagules per root system for high, medium, and low doses, respectively). Seedlings planted in potting medium with sterilized oats and seedlings given root dips into the washings of ground sterilized oats served as controls.

Inoculations were repeated with a 1:1 mix of screened peat moss/vermiculite to determine if potting medium influenced disease.

All control and inoculated seedlings were fertilized at establishment and every 4 weeks thereafter with a soluble fertilizer (15N-30P-15K) in water until the soil was thoroughly wetted. Pots were irrigated by hand as needed. Seedlings were incubated for 10 weeks in a greenhouse under natural light between 12 and 30 °C. The media incorporation trial was terminated after the incubation period, and all seedlings were removed from their pots. Roots were evaluated for disease symptoms and cultured on CZK agar after surface sterilization (2 minutes in 10 percent aqueous sodium hypochlorite). Seedlings inoculated by root dip were removed immediately after they died and were similarly cultured.

Results

Some northern red oak seedlings were killed in all inoculation trials regardless of inoculation method, potting medium, or inoculum rate (table 1). The principal symptom for the medium incorporation method was damping-off (fig. 2)--only 6 of the 33 total killed seedlings initiated shoot growth before

Table 1—*Percentage of container grown northern red oak seedlings with symptoms of root disease after two root inoculation methods with* Cylindrocladium crotalariae *(incubation period = 10 weeks).*

Inoculum rate	Percentage diseased seedlings[a]					
	Inoculum in medium			Root dip inoculation		
	Soil	Mix	Average	Soil	Mix	Average
Low	66	83	75	0	33	17
Medium	NA	NA	NA	33	42	38
High	83	42	63	33	33	33
Average	75	63	69	22	36	29

[a] Symptoms include damping-off, dwarfed foliage, marginal leaf necrosis, and wilting. NA = not applicable.

Figure 2—*Medium-incorporated inoculation method. Controls at left and damped-off inoculated at right.*

death. Trees that died after initiating growth showed top symptoms that included dwarfed foliage, marginal leaf necrosis, and wilting. Roots of the damped-off seedlings were well decayed and often consisted of only a stub protruding from the germinated acorn. Root systems of seedlings without top symptoms at the end of the evaluations were well developed and fibrous, regardless of treatment.

Seedlings in the root dip method began dying 13 days after inoculation. Roots of killed and symptomatic seedlings showed varying degrees of necrosis, a water-soaked appearance in the laterals, and black discoloration in the woody part of the tap root.

One control seedling in the artificial medium/oat incorporation (high rate) treatment never completed germination. Two control seedlings in the root dip treatment showed marginal leaf necrosis and leaf dwarfing, but roots of these trees were asymptomatic.

Cylindrocladium crotalariae was reisolated from 43 percent of all symptomatic seedlings in the inoculation trials, but a higher percentage of the successful isolations were obtained from the root dip method than from the medium incorporation method (57 and 33 percent, respectively). Only saprophytic fungi were associated with the ungerminated control.

Control measures and discussion

When the outbreak was detected, all oaks were removed from the greenhouse and discarded, as was all previously used soil. Tools, pots, greenhouse benches and floors, and the drip tube watering system were thoroughly washed with 50 percent aqueous sodium hypochlorite and rinsed with clean water. A new crop of oaks was planted with the operational potting medium (steam-treated field soil, sand, and peat moss in 3:2:1, v/v mix). This crop grew normally for 2 to 3 weeks but then developed symptoms of root disease. Fruiting of *Calonectria crotalariae* (sexual stage of *Cylindrocladium crotalariae*) was observed on seedling roots and acorn tissue. After repeating the hypochlorite cleaning, oaks were planted in artificial medium (1:1, peat moss/vermiculite). Several subsequent oak crops have been grown in artificial medium without recurrence of the root disease. We suspect that field soil used in the potting medium mix was infested with the root disease organism.

Control recommendations based on our experience and the known action of *Cylindrocladium* spp. in bareroot hardwood nurseries (1, 3) can be suggested.

- ?? Avoid using field soil as a growing medium.
- ?? If field soil must be used, fumigate with methyl bromide-chloropicrin fumigants (MC 33 preferred).
- ?? Attempt to use commercially formulated or other "defined" potting media. Consider treating this material to reduce or eliminate potential pests as added insurance.
- ?? Avoid reusing potting medium from containers where seedlings have died; pests that may have had a role in the mortality could still be present in the medium.
- ?? Do not rely on steam treatment for total control of soilborne disease organisms.
- ?? Frequent reconnaissance of plant beds can aid in detecting problems before they limit production. Timely and aggressive pest control and culling of damaged stock, when necessary, will minimize losses.

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The Effects of White Spruce Stunting on Seedling Growth

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*Stunting of white spruce (*Picea glauca *(Moench) Voss) seedlings in northern Lake States tree nurseries continues to be a common occurrence. In this evaluation the cull percentage was seven times greater in stunted 3+0 white spruce stock than in nonstunted stock. Stunting of white spruce did not adversely affect seedling growth after outplanting.*

Stunting of white spruce and red pine (*Pious resinosa* Ait.) seedlings in Lake States tree nurseries is a common phenomenon. This condition is characterized by arrested seedling growth midway through the first growing season, with foliage that becomes purple in late summer. The following spring, stunted seedlings resume growth and the foliage turns green. Berbee et al. (1) examined several possible causes for this condition, including mycorrhizal deficiency and fungal infection but found no relationship between stunting and those factors.

In 1977, stunting affected from 13 to 30 percent of the total production of white spruce and red pine in five nurseries in Michigan, Wisconsin, and Minnesota (1). A later evaluation (2) indicated that 28 percent of the white spruce at the Eveleth Tree Nursery, Eveleth, MN, were stunted in 1979. I have observed similar levels of white spruce and red pine stunting in many of the region's northern nurseries and also that the percentage of stunted stock varies from year to year in any one nursery.

Croghan and LaMadeleine (2) found that the average height of stunted white spruce seedlings was 22 percent less than the height of nonstunted seedlings at the end of 3 years in the nursery. However, this was not a true indication of the comparative size of the seedlings because the nursery beds had been top pruned. The stunted seedlings had attained 78 percent of the top pruned height, not the actual height. It was not known if the growth of stunted seedlings continued to lag behind nonstunted seedlings after outplanting. If it did, the dollar losses associated with white spruce stunting were greater than those attributed to culling seedlings alone. This evaluation was designed to assess losses of white spruce in the nursery due to stunting and to determine if the growth differential persists after planting.

Methods

Nursery. In September 1980, seven plots of stunted and seven plots of nonstunted 1+0 white spruce seedlings were marked in the Eveleth nursery. In April 1982, these seedlings were hand-lifted as 3+0 stock.

Nursery personnel used the specifications developed by USDA Forest Service, region 9 for class B white spruce planting stock (13 to 23 centimeters shoot height and 3 to 8 millimeters stem caliper) to place seedlings in one of the following grading categories: 1) stunted culled, 2) stunted accepted, 3) nonstunted culled, and 4) nonstunted accepted. Height and root collar caliper measurements were taken for all seedlings. Student's t-test was used to test for differences between the stunted and nonstunted groups.

Outplanting. Sixty-two seedlings were randomly selected from each of two grading categories (2 and 4, above) and outplanted on the Superior National Forest. The nonstunted seedlings were planted in a single row at approximately 2.4-meter (8-foot) intervals. A parallel row of stunted seedlings planted at the same spacing was installed 2.4 meters from the nonstunted row. Height and stem caliper of the outplanted seedlings were recorded at the time of planting. At the end of the second growing season (October 1983), shoot growth, stem caliper, mortality, and pest damage were recorded. Based on the assumption that planting in separate rows had no effect on seedling growth, Student's t-test was used to test for differences between the stunted and nonstunted groups.

Results

Nursery. Mean percent cull in the stunted plots was significantly greater than in the nonstunted plots (table 1). Height and caliper were significantly greater in nonstunted seedlings than in stunted seedlings for both accepted and culled categories.

Table 1—*Cull, shoot height, and stem caliper for stunted and nonstunted 3+0 white spruce at the Eveleth Nursery (April 1982)*

	Stunted	Non-stunted
Number accepted [a]	90	202
Number culled	121	16
Mean percent plot cull [b]	57	7*
Mean seedling height (cm)		
Accepted	20.23	26.37*
Culled	12.41	15.38*
Mean stem caliper (mm)		
Accepted	2.63	3.78*
Culled	2.27	2.56

* Significantly different from paired value at P = 0.05 (Student's *t*-test).
a Accepted = seedling met USDA Forest Service region 9 planting stock specification.
b Mean of 7 stunted seedling plots and 7 nonstunted seedling plots.

The mean height of seedlings from stunted plots was only 62 percent of the height of seedlings from nonstunted plots. However, it should be noted that the beds were top-pruned during the second nursery growing season. The corresponding comparison of mean stem caliper shows that the stunted group was only 66 percent as large in caliper as the nonstunted seedling group.

Outplanting. There were significant differences for initial and final mean shoot height for the outplanted stunted and nonstunted white spruce (table 2). Similarly, initial mean stem calipers for the stunted and nonstunted seedlings were significantly different. However, by the end of the second growing season no statistically significant caliper difference was found. Similarly, no differences in shoot and caliper growth or growth rate (growth/initial size) were observed.

Twice as many of the outplanted stunted seedlings died (10 percent) as did nonstunted seedlings (5 percent). Mortality was due to dessication and did not result from insect or disease activity.

Discussion

The cull rate in 3+0 stunted seedlings was 57 percent whereas the cull rate in nonstunted seedlings was 7 percent, a difference of 50 percent. This value is almost twice the amount observed in an earlier evaluation (2). Although the exact amount

Table 2—*Shoot height and stem caliper for 62 stunted accepted and 62 nonstunted accepted 3+0 white spruce stock on the Virginia Ranger District, Superior National Forest, Minnesota, at outplanting (April 1982) and after 2 growing seasons (October 1983).* [a]

Measurement	Stunted	Nonstunted
Total seedling height (cm)		
April 1982	20.1	24.32*
October 1983	29.30	34.61*
Shoot growth since outplanting (cm)	9.50	10.04
Shoot growth rate [b] (cm)	.52	.45
Total stem caliper (mm)		
April 1982	2.77	3.74*
October 1983	4.19	5.13
Caliper growth since outplanting (mm)	1.46	1.39
Caliper growth rate (mm)	.59	.43

* Significantly different from paired value at P = 05 (Student's *t*-test).
a Accepted = seedling met USDA Forest Service region 9 planting stock specifications.
b Growth rate = total 1982 and 1983 growth/initial size.

of 1+0 stock stunted in 1979 is not known, based on my past observations, the nursery probably had from 10 to 35 percent white spruce stunting. If we use the 10 percent figure, the cull due to stunting for the nursery's entire 3+p white spruce crop for 1982 would be 5 percent. In 1982 the nursery shipped 2.1 million 3+0 white spruce seedlings. With a 10-percent stunting rate, 105,000 seedlings would be lost; with a 35-percent rate, 367,500 seedlings would be lost.

Comparisons of the four seedling classes showed that the stunted seedlings remained smaller than the nonstunted seedlings for both shoot height and stem caliper throughout the third nursery growing season.

At planting and at the end of two growing seasons, stunted stock had significantly smaller mean shoot heights than did nonstunted stock. However, the initial difference in mean caliper disappeared by the end of the second growing season. Although the final mean shoot height of the stunted group was smaller by 5.13 centimeters, this difference should not increase over time, as there were no significant differences between the stunted and nonstunted groups for shoot growth or shoot growth rate. Clearly, a 5-centimeter difference would not be of consequence at rotation age.

Excellent survival rates were observed for both the stunted and nonstunted groups of seedlings, 90 and 95 percent, respectively.

Conclusions

Although stunted 3+0 white spruce stock is smaller than nonstunted stock, the stunted seedlings that pass the region 9 specifications grow at rates comparable to those of nonstunted seedlings when planted out. Based on these results, it appears that economic losses due to stunting of white spruce are limited to the nursery. The need to understand the phenomenon of stunting in the nursery continues, however.

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Variation in Rooting Ability Among Selected Clones of Eastern Cottonwood (*Populus deltoides* Bartr. ex Marsh) in Southern Louisiana

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*Rooting of eastern cottonwood (*Populus deltoides *Bartr. ex Marsh) clones selected along the lower Mississippi River was highly variable. Early results indicated that some of these clones rooted as well as or better than some superior check clones obtained from the Texas Forest Service and the USDA Forest Service.*

Eastern cottonwood is a fast-growing, commercially valuable, genetically diverse, and easily propagated poplar species that is planted extensively in the Mississippi River Valley (3,5,6) (fig. 1). Most planters have been using genetically superior clonal stock that was developed through intensive selection and testing procedures by personnel of the USDA Forest Service at the Southern Hardwoods Laboratory at Stoneville, MS (1,4,7,8). Several of these select clones, planted near Baton Rouge, LA, failed to perform satisfactorily and showed even poorer growth than cuttings collected locally from young trees.

A study was initiated at the Louisiana State University Agricultural Center to identify and test selected cottonwood clones that would be adapted for superior growth in the lower Mississippi Valley. Superior native cottonwoods have not been selected in this area, and locally selected material should perform

Figure 1—*Five-year-old eastern cottonwood* (Populus deltoides) *plantation at Stoneville, MS.*

better than clones originating from more northerly latitudes.

Rooting ability can be used as a criterion for the early evaluation of clonal tests (3). Reported here are the results of a rooting study done after the initial selection of parent cottonwood trees along the lower Mississippi River. Rooting of the locally selected clones (LSU clones) was compared to superior check clones obtained from the Texas Forest Service and genetically improved (blue-tag) clones from the Southern Hardwoods Laboratory.

Methods

Forty-nine mature parent trees (ortets) were selected, because of their phenotypes, from along the Mississippi River between Cat Island (near St. Francisville, LA) and New Orleans. The trees were selected in early fall in 1981 for high leaf retention, straightness, and average or above-average height and diameter growth.

Primary ramets (cuttings) were collected from the selected trees from mid-February to early April 1982. Ten to twenty terminal cuttings 12 to 20 millimeters in diameter and 28 to 30 centimeters long were collected from the crowns. The cuttings were soaked in water overnight, and then in 100 parts per million rooting hormone (indole butyric acid) for 24 hours. Each cutting was planted in a 20-centimeter-tall clear polyethylene bag 10 centimeters in diameter that contained 1:1:1 sand/peat/vermiculite rooting medium. The planted bags were placed on heating pads in a greenhouse. Four to six weeks after planting, all rooted cuttings were transplanted to large pots 25 centimeters in diameter by 50 centimeters tall. The selected clones from the Texas Forest Service and the Southern Hardwoods Laboratory were included and treated the same as the LSU clones.

All propagules were watered, fertilized with 20N-20P-20K soluble fertilizer, and sprayed periodically with insecticide. Some were cut back twice during the summer of 1982 to multiply and expand the clonal material. The propagules resprouted each time.

In late February 1983, 10 to 46 secondary cuttings were taken from the primary ramets of each surviving clone. Nineteen LSU clones survived to this stage. Four clones (ST244 from Stoneville and S7C4, 57C20, and KEN8 from Texas) were used as check clones. A total of 798 cuttings 20 centimeters long were taken over a 3-day period. All cuttings were dipped in fungicide (benomyl), soaked in 500 parts per million rooting hormone (indole acetic acid) for 2 hours, and planted in clear polyethylene bags containing a 1:1 perlite/vermiculite rooting medium. The bags were placed on heating pads in a greenhouse, watered daily with deionized water, and sprayed weekly with fungicide (captan). Each cutting was checked for roots every 7 days. An overhead intermittent mist system was turned on after several weeks, when many cuttings had sprouted and leafed out before rooting.

Results and Discussion

Mean clonal rooting for the primary ramets was 47.5 percent and ranged from 0 to 100 percent (table 1). Rooting for twelve LSU clones was 80 percent or higher, and rooting for two clones (LSU-23 and LSU-35) was 100 percent. There were significant differences in rooting among clones (table 1). Rooting for the LSU clones (47.5 percent) was similar to that obtained by Farmer (2) (52.3 percent) for dormant cuttings of mature cottonwood collected in February and March. The check clones all had 100 percent rooting.

Rooting of the primary ramets was probably limited by the age of the cutting material. Maisenhelder (5) pointed out that cuttings taken from mature trees root and survive poorly. However, if the mature cutting material is rejuvenated, or repeatedly propagated over several generations, it will eventually return to a more easily rooted juvenile state. Rooting was lowest for ortets over 25 years old ($r = -0.34$). Maisenhelder (5) and Allen and McComb (1956)

reported that rooting ability usually decreases with increasing ortet age.

Mean rooting for the secondary ramets was 67.4 percent for the 19 surviving clones and ranged from 33.3 to 100 percent (table 2). Clones LSU-32, LSU-39, LSU-50, and LSU-14 all had over 90 percent rooting. Although there were significant differences among clones, a grouping of clones could not be established because of the high variability in the number of cuttings per clone. Mean rooting of the four check clones (81.4 percent) was not significantly different from the mean of the LSU clones (67.4 percent). These results verify that some of the LSU clones rooted as well as or better than the superior check clones (table 2).

The secondary ramets of the LSU clones were not in a fully rejuvenated state, so that the age of the cutting material probably still limited rooting. The secondary ramets generally had higher rooting percentages than did the primary ramets. Rooting for the primary ramets of the check clones was higher than for the secondary ramets. The primary ramets of the check clones were taken from young nursery-grown stock, so that the cutting material was at an optimum rooting age. The secondary ramets were grown in containers, which may have affected rooting success.

Table 1—*Ranking of clones by rooting percent of primary ramets taken from selected eastern cottonwood trees*

Clone	Ortet age* (yr)	Number of cuttings rooted/total	Percent rooted†
LSU-23	20	10/10	100a
LSU-35	20	10/10	100a
LSU-19	20	9/10	90a,b
LSU-27	14	18/20	90a,b
LSU-28	16	9/10	90a,b
LSU-13	13	17/20	85a,b
LSU-46	21	17/20	85a,b
LSU-08	12	8/10	80a,b
LSU-14	16	8/10	80a,b
LSU-15	17	8/10	80a,b
LSU-38	21	16/20	80a,b
LSU-42	16	8/10	80a,b
LSU-32	16	15/20	75a,b
LSU-25	28	7/10	70a,b
LSU-29	22	7/10	70a,b
LSU-01	19	6/10	60b
LSU-09	9	6/10	60b
LSU-37	20	6/10	60b
LSU-39	18	6/10	60b
LSU-40	23	12/20	60b
LSU-47	20	6/10	60b
LSU-06	20	5/10	50b
LSU-20	28	5/10	50b
LSU-31	20	5/10	50b
LSU-33	10	5/10	50b
LSU-36	21	5/10	50b
LSU-41	13	5/10	50b
LSU-43	21	5/10	50b
LSU-49	ND	5/10	50b
LSU-48	21	4/10	40
LSU-30	22	3/10	30
LSU-44	22	3/10	30
LSU-51	ND	3/10	30
LSU-07	16	5/20	25
LSU-24	19	5/20	25
LSU-50	15	5/20	25
LSU-05	18	2/10	20
LSU-34	23	2/10	20

Continued on next page

Table 1—*Continued*

Clone	Ortet age* (yr)	Number of cuttings rooted/total	Percent rooted
LSU-45	21	2/10	20
LSU-03	ND	1/10	10
LSU-10	30	1/10	10
LSU-16	16	1/10	10
LSU-21	21	1/10	10
LSU-26	28	1/10	10
LSU-11	27	0/10	0
LSU-12	21	0/10	0
LSU-17	20	0/10	0
LSU-18	25	0/10	0
LSU-22	17	0/20	0
Mean			47.5
Check clones‡			
ST67		20/20	100
ST74		20/20	100
ST92		20/20	100
ST109		15/15	100
ST244		15/15	100
S07C01		10/10	100
S07C02		4/4	100
S07C04		10/10	100
S07C08		10/10	100
S07C13		10/10	100
S07C15		10/10	100
S07C20		10/10	100
S13C15		10/10	100
S13C20		10/10	100
KEN08		10/10	100
Mean			100

* ND = Age could not be determined.

† Clones with 50-percent rooting or higher followed by the same letter do not differ in rooting from each other. ($P = 0.05$).

‡ Clones with ST preceding the number were obtained from the Southern Hardwoods Laboratory, Stoneville, MS; the other check clones were obtained from the Texas Forest Service.

Conclusions

Rooting ability among the primary ramets and the secondary ramets was highly variable. Rooting of the primary ramets appeared to be limited mainly by the age of the cutting material. Statistically significant differences for rooting were evident among clones for both the primary and secondary ramets, indicating that useful variation for selection was present. Significant differences for rooting means were not evident between the secondary ramets of the LSU clones combined and the four superior check clones, indicating that some LSU clones performed as well as the superior check clones.

Finally, long-term conclusions should not be drawn from this study because the results are based on first-year data. The ranking of the top clones (the top five in terms of rooting percent were LSU-19, LSU-14, LSU-13, LSU-32, and LSU-39) could change with time as different clones having consistently high rooting emerge and perform better in the long run. All clones should therefore be tested and observed for several more years so that more definite trends for rooting and survival of each clone can be established.

Table 2—*Ranking of clones by rooting percent of secondary ramets of eastern cottonwood*

Clone*	Number of cuttings	Percent rooted
LSU-39	11/11	100.0
S07C04	19/19	100.0
KEN08	24/25	96.0
LSU-14	23/25	92.0
LSU-32	33/36	91.7
LSU-50	9/10	90.0
LSU-19	15/17	88.2
LSU-13	28/34	82.4
S07C20	36/46	78.3
LSU-06	12/17	70.6
LSU-29	13/19	68.4
LSU-07	8/12	66.7
LSU-42	14/21	66.7
LSU-27	16/25	64.0
LSU-46	19/30	63.3
LSU-37	7/12	58.3
LSU-41	6/11	54.6
LSU-09	12/23	52.2
ST244	19/37	51.4
LSU-28	8/16	50.0
LSU-25	9/20	45.0
LSU-15	11/25	44.0
LSU-35	5/15	33.3

* Check clone ST244 was obtained from the Southern Hardwoods Laboratory, Stoneville, MS. S07C04, S07C20, and KEN08 were obtained from the Texas Forest Service.

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Irrigation Effective in Increasing Fruitfulness of a Shortleaf Pine (*Pinus echinata* Mill.) Seed Orchard

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*Irrigation with a drip system significantly increased flowering and cone survival in a shortleaf pine (*Pinus echinata *Mill.) seed orchard in Arkansas. Irrigation was especially effective during the severe drought of 1980.*

Various irrigation systems are operational in some southern pine seed orchards. Considerable optimism has been expressed about their benefits, but sound experimental data are scarce. Spring droughts, common in the South, could very well be deleterious to both growth and seed production. In a recent study of a loblolly pine seed orchard (3), irrigation greatly increased female flower and cone production. On the other hand, Jett (4) found that irrigation inhibited female flowering. Abundant moisture may favor vegetative growth at the expense of reproductive growth. Thus, mild moisture stress at the time female strobili are initiated (probably late summer) may enhance strobili production. One well-controlled experiment indicated that midsummer to late summer drought, with abundant moisture at all other times, was beneficial to cone production in loblolly pine (2). Others report that abundant early summer rain, followed by a relatively dry late summer, resulted in good female flower crops in loblolly pine the following spring (6). In the same study, the opposite rainfall pattern, i.e., a dry early summer followed by a wet late summer, appeared to enhance male flowering.

The objective of the present study is to determine effects of the timing of irrigation on production of male and female strobili, cones, and seed in a shortleaf pine seed orchard. Year-to-year variation in male and female strobili will also be examined.

Materials and Methods

The study was established in a USDA Forest Service shortleaf pine seed orchard in central Arkansas. The orchard, near the western extremity of the southern pine region, where rainfall becomes a limiting factor, consists of 13,000 ramets of 50 clones. The majority of the ramets were planted in 1968 with 15- by 30-foot spacing. The clones were arranged sequentially with each adjacent row offset by five clones (fig. 1). The well-drained soils in the orchard are primarily Goldston shaley silt loams or Herndon gravelly silt loams. A drip system of irrigation seemed advantageous (5) for experimental work, for it allowed irrigation of individual trees with very little carryover to adjacent trees. Ten clones, adjacent or nearly adjacent within the rows, were chosen from those having a large number of ramets planted in 1968. Trees used in the study averaged 5 inches in d.b.h. and 20 feet in height at the beginning of the study.

A split-plot/completely random design was used. The main plots were the group of clones within a row, which were irrigated as a unit (fig. 1); subplots were ramets of a given clone within the main plots. The four treatments consisted of 1) full, irrigated entire growing season (when needed) (April 1 to October 2); 2) early, irrigated when needed before July 1; 3) late, irrigated when needed after July 1; and 4) none, nonirrigated control.

The 5 replications included 20 main plots and 200 trees. In the spring of 1980, polyvinyl chloride (PVC) pipe was laid on the ground along the rows next to the trees. Four emitters were installed in the pipe under each irrigated tree (fig. 1) to distribute the water evenly under the crown. The plots were arranged so that adjacent rows were not used, to prevent carryover effects from adjacent plots. Water was applied for 1 night (15 hours) any week in which less than 1 inch of rain fell. According to measurements of emitter output and area wetted, this approximated applying 1 inch of rain within the area beneath the crown of the trees.

[1] The author is grateful to James McConnell, Robert Kitchens, Jim McMahon, and Charles Studyvin, USDA Forest Service, Southern Region Tree Improvement personnel, for their assistance during the course of this study.

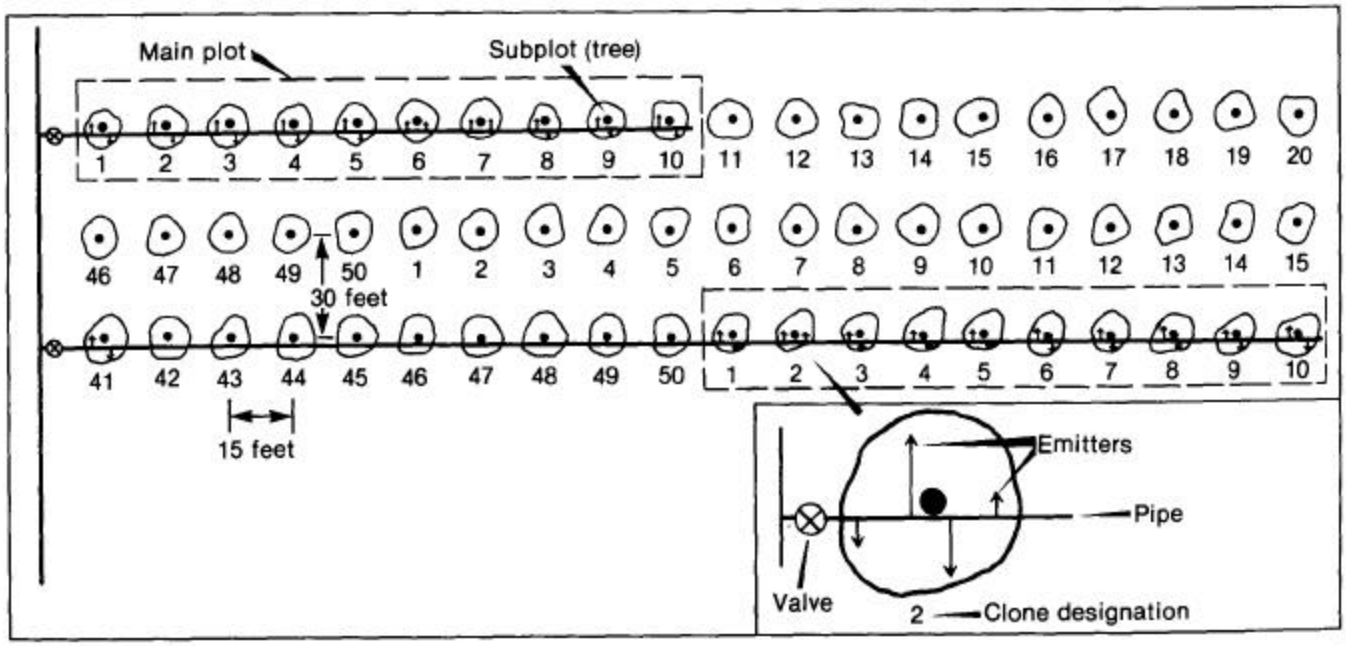

Figure 1—*Diagram of the orchard showing clone arrangement, experimental plot, and irrigation system layout.*

Female strobili and male strobili clusters were counted in the spring of 1979, 1981, 1982, and 1983. Cones were counted in the fall of 1980 and 1981; at the same time a 5-cone sample was taken from each ramet for seed-yield information.

Analysis of variance appropriate for the split-plot/completely random design was used to test the significance of treatment, clone and treatment by clone effects at P = 0.05. Duncan's multiple range test was also used to separate treatment means.

Results and Discussion

Year-to-Year Clonal Variation.

Both male and female flowering varied considerably over the length of the experiment (fig. 2). Severe drought in the summer of 1980 in Arkansas (8) undoubtedly accounts for the poor flowering in 1981. Male and female flowering in 1982 was four times as great as in 1981 but dropped off slightly in 1983.

There was a strong year-by-clone interaction for both male and female flowering (fig. 3). Although, in general, good-flowering clones perform well

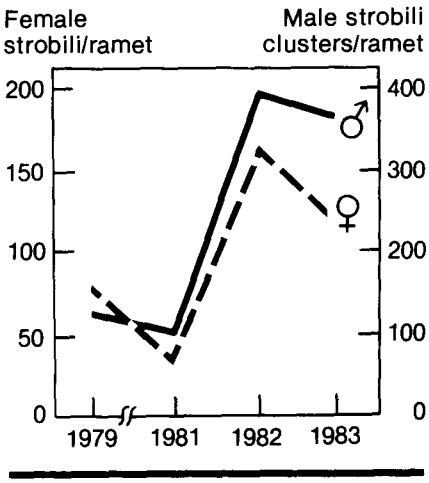

Figure 2—*Variation in male and female flowering in the orchard for each year during the experiment. Based on nonirrigated controls.*

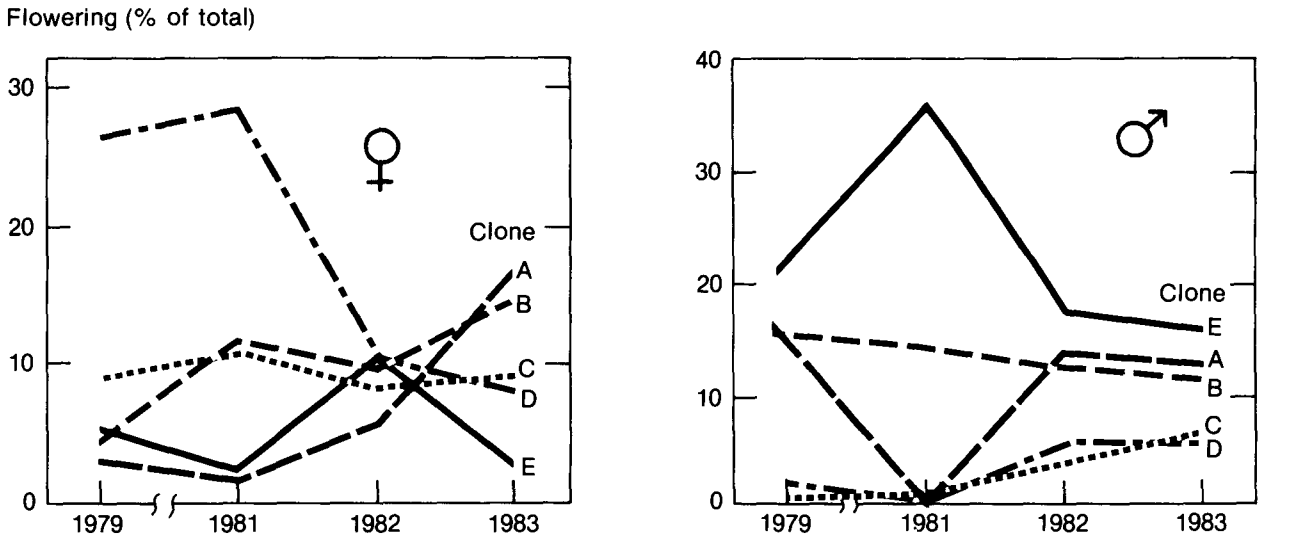

Figure 3—*Clonal variation in male and female flowering over the course of the study of 5 of the 10 experimental clones. Based on nonirrigated controls.*

every year, it is evident in figure 3 that the considerable change in rank for both male and female flowering is similar to that which has been observed in loblolly pines (7). Year-to-year variation in flowering results in genetic variation in the seed produced each year, even in seeds collected separately by clone.

Irrigation effects. *Female flowering*. In 1981, the first spring after the irrigation system was used, late-season and full-season irrigation nearly doubled the female flower crop (fig. 4) over that of both controls and over that of the early-season irrigation treatment. However, the system was only used twice before July 1, in late June, and only a very limited amount of water was applied. Some problems with clogged filters reduced water flow in the first 2 months of the use of the system. In July, August, and September of 1980, the system was used nearly every week in treatments 1 and 3 because of the extended drought.

In 1982, after the second year of irrigation, treatment effects followed the pattern that was expected: i.e., early-season irrigation was best, late-season irrigation was not effective, and full-season irrigation was intermediate (fig. 4). Rainfall was nearly average in 1981, the previous year, although the system was used six times before July 1 and eight times after July 1.

In 1983, after the third year of irrigation, the irrigated treatments had more female strobili that did the controls, but irrigation regimes did not differ significantly (fig. 4) among themselves. Carryover effects from the previous 2 years' treatment may have affected 1983 results. Mean diameter growth from 1980 through 1983 is in the same order as flowering for 1983: the control averaged 6.8 centimeters, those irrigated early averaged 7.1 centimeters, those irrigated late averaged 7.2 centimeters, and those irrigated the whole season averaged 7.9 centimeters of growth.

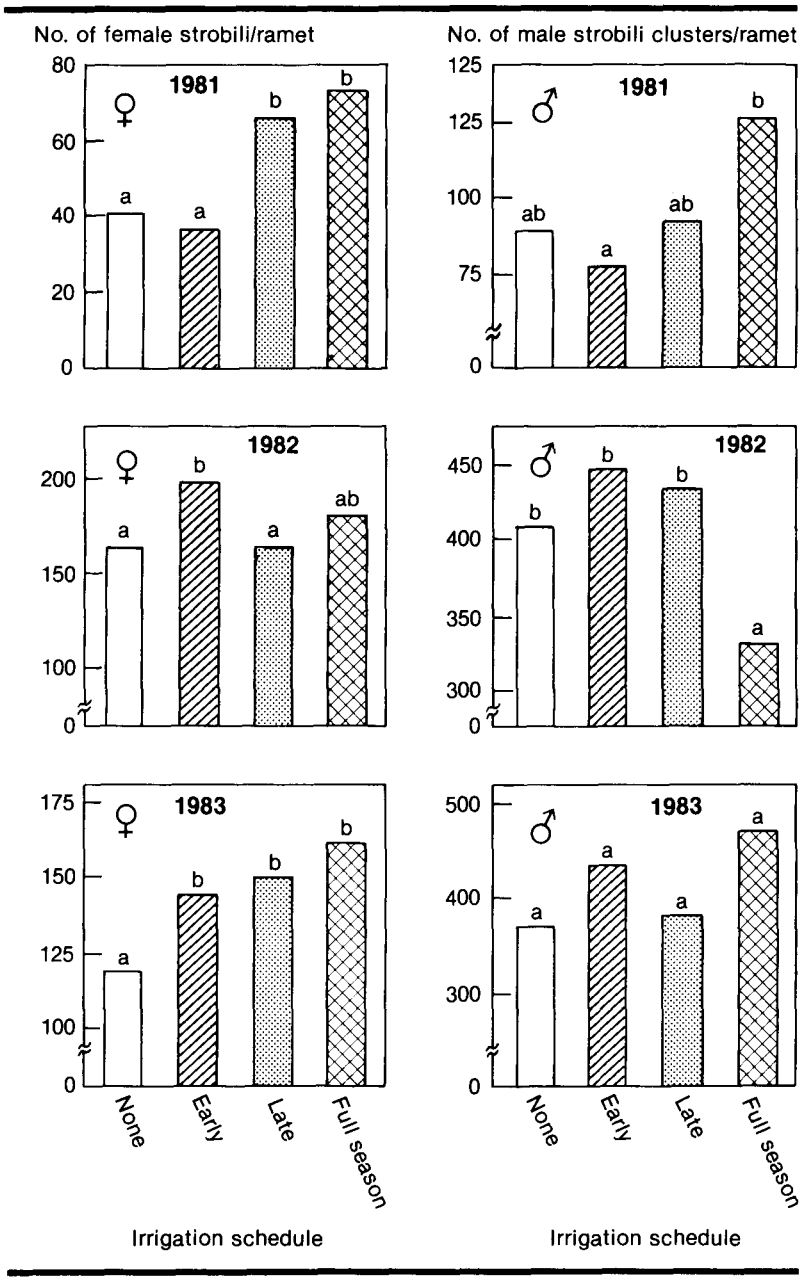

Figure 4—*Effects of irrigation on male and female flowering in the orchard for 1981-83. Bars topped by the same letter do not differ significantly (P = 0.05) according to Duncan's multiple range test.*

Male flowering. Significant differences occurred among irrigation treatments in male flowering in 1981 and 1982, but the pattern of variation is difficult to explain. In 1981, the only clear difference appeared between full-season irrigation, which averaged 125 male strobili clusters per ramet, and the early irrigation, which averaged only 75 per ramet (fig. 4). The control and late-season irrigation treatments were intermediate, averaging around 85 male strobili clusters per ramet. In 1982, the pattern of variation seemed the reverse of the 1981 pattern. The control and early- and late-season irrigation treatments averaged around 430 male strobili clusters per ramet, whereas the full-season irrigation averaged only 330 clusters per ramet. Treatment differences were not significant in 1983.

Barnes and Bengtson (1), not finding a significant effect of irrigation on male flowering of slash pines, did find a significant irrigation-by-clone interaction in male flowering. Clone-by-irrigation interactions were not significant for any trait in this study, but the effects of irrigation on male flowering are clouded.

Cone and seed yields. Seed yield per cone was unaffected by irrigation in either 1981 or 1982. Conelet survival was affected by irrigation in 1979-80. Survival was only 21 percent in the controls, lower than the 46-percent average for the 3 irrigated treat-

ments. Differences among irrigation regimes were not significant. Since irrigation was not applied until June of 1980, the treatments must have enhanced cone survival in the second growing season. Further, because all three irrigation regimes enhanced survival, a period around July 1 may be critical to survival. Second-year cones are growing rapidly at this time, and they reach full size by the end of July.

Cone survival for 1981-82 did not vary significantly by treatment. Rainfall was near average for both years, so apparently conelet survival is affected only in severe droughts such as the one that occurred in 1980.

Conclusions

Irrigation can apparently be effective in increasing female flowering in a shortleaf pine seed orchard. In a severe drought, irrigation not only increases female flowering but also enhances conelet survival. It does not appear that male flowering can be increased reliably by irrigation, although both male and female flowering would eventually be enhanced in subsequent years because irrigation increases the size and vigor of the ramets.

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Acid Scarification Requirements of Kentucky Coffeetree (*Gymnocladus dioicus* (L.) K. Koch) Seeds From Southcentral Minnesota

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*Kentucky coffeetree (*Gymnocladus dioicus *(L.) K. Koch) is reported to have an impermeable seed coat that requires 3 hours of acid scarification for germination. In this study, acid scarification did not improve the germination of seeds from southcentral Minnesota.*

Kentucky coffeetree is a woody legume indigenous to the United States. Its range covers Missouri, Illinois, and Indiana and extends into adjacent states. Isolated populations are also found in Minnesota, Tennessee, West Virginia, Pennsylvania, and New York (2). Kentucky coffeetree is reported to have an impermeable seed coat that requires scarification for germination to occur (3). The published scarification recommendations for Kentucky coffeetree are all from the central part of its range. Isolated populations may produce seeds with different scarification requirements.

Methods

Pods were collected from trees in Blue Earth County, MN, during February 1984. The pods were opened immediately and the seeds removed. Eighty seeds were soaked in water for 24 hours and then divided into four scarification treatments. Twenty seeds each were soaked for 0, 1, 2, or 3 hours in concentrated sulfuric acid. After the treatment the seeds were rinsed in water, air dried for 24 hours, and then planted in vermiculite. The seeds were placed in a greenhouse in which day temperatures were maintained at 21 °C, with a 3 °C drop at night.

Results and Discussion

Seeds of Kentucky coffeetree from southcentral Minnesota did not significantly benefit from acid scarification (table 1). This result does not agree with two other studies, which found that germination did not reach 80 percent unless the seeds had been treated for 2 hours in acid (1, 4). The difference in scarification requirements may be due to the origin of the seed trees. The other two studies were conducted with seeds collected from trees in central Illinois and Ohio, whereas ours were from an isolated population in southcentral Minnesota. Kentucky coffeetree growers in other regions may wish to test the acid scarification requirements of local seeds before following the standard recommendations.

Table 1—*Percentage of germination for Kentucky coffeetree seeds scarified in concentrated sulfuric acid.*

Scarification time period (hours)	N	Percentage germination
0	20	80
1	20	80
2	20	85
3	20	75

No significant difference at the P = 0.05 (Duncan's multiple range test).

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Effects of Substrate Moisture on Germination of Scotch Pine (*Pinus sylvestris* L.) Seed From Several Sources

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*Scotch pine (*Pinus sylvestris *L.) seed from 15 sources were germinated on five different levels of substrate moisture (5, 10, 15, 20, and 25 percent). A substrate moisture level of 10 percent or less was less desirable with crepe cellulose paper than were higher levels. Substrate moisture levels of 15 to 25 percent produced acceptable results.*

Scotch pine seeds from many sources are imported for Christmas tree farms. Previous research showed that Scotch pine seed is sensitive to high substrate moisture during germination. Scotch pine has a very broad natural range, from latitude 40°N to 70°N and longitude 5°W to 140°E. Previous work did not determine if seeds from differing environmental conditions such as those derived by latitude, longitude, and elevation would demonstrate variations in substrate moisture tolerance. This study was undertaken to examine the tolerance to substrate moisture with seeds from the western portion of the range.

Methods and Materials

Seeds from each of 15 sources (table 1) were germinated at 5 different substrate moisture levels: 5, 10, 15, 20, and 25 percent of the water-holding capacity of the substrate (a double layer of crepe cellulose paper). Four germination dishes of 100 seeds each were prepared for each treatment. The seeds were germinated at 22 °C with 3 hours of light and 16 hours of darkness. Tests were terminated at 28 days.

Table 1—*Sources of Scotch pine seed used in this study*

Lot no.	Source	Elevation[a] (m)	Latitude	Longitude
1.	Nfruskovskoe Forest, Orlovsk Oblast, USSR	Unk.	53.0°N	35.0°E
2.	Neliskovitskoe Forest, Kiev Oblast, USSR	Unk.	50.0°N	30.0°E
3.	Novousmansky Forest, Woronesh Oblast, USSR	Unk.	52.0°N	
4.	Vintila Voda Forest District, Romania	800-900	45.5°N	26.3°E
5.	Pirscov Forest District, Romania	950	45.5°N	26.3°E
6.	Nehovia Forest District, Romania	1100-1350	45.7°N	26.3°E
7.	Kamon Seed Orchard, Sarvar, Hungary	Unk.	47.2°N	16.6°E
8.	Kamon Seed Orchard, Sarvar, Hungary	Unk.	47.2°N	16.6°E
9.	Kranichfeld, German Democratic Republic	370	50.5°N	11.1°E
10.	Landskrona, Sweden	300	65.7°N	20.0°E
11.	Landskrona, Sweden	200	65.7°N	20.0°E
12.	Jamtland, Sweden	450	62.3°N	14.5°E
13.	Jamtland, Sweden	350	62.3°N	14.5°E
14.	Jamtland, Sweden	400	62.3°N	14.5°E
15.	Jamtland, Sweden	500	62.3°N	14.5°E

[a]Unk. = unknown.

Percent water-holding capacity has proven to be an accurate measure of substrate moisture. Each piece of crepe cellulose paper was weighed before placement in the dish and the appropriate weight (volume) of water (+ 0.5 gram) was spread evenly over the surface with a calibrated sprayer. The dishes were covered and set overnight to allow the water to spread uniformly throughout the medium. The 100 percent water-holding capacity was determined by saturating the medium with a measured amount of water, allowing it to drain for 1 minute, and then measuring the drained water.

Each lot of 100 seeds was radiographed before placement on the medium, and the full seed percent was determined from the radiograph. Germination was evaluated every 7 days.

Germination percent, days to reach 50 and 90 percent of total germination, percentage abnormal germination, percentage of moldy seeds, and vigor evaluations by Czabator's method (2) were subjected to analyses of variance. Significant differences were examined with Duncan's multiple range test. Correlation coefficients were calculated between latitude, longitude, elevation, and the test measurements.

Results and Discussion

The 5 percent moisture level was so dry that the effects overshadowed all other effects in the analysis. Therefore the data were reanalyzed without the 5-percent data (table 2). Seed mold increased slightly as the substrate moisture increased.

Seed source significantly affected all measurements except percentage of moldy seed and percentage of abnormal seedlings. Correlation coefficients were calculated from the components of the seed sources (latitude, longitude, and elevation) and the germination variables. Only source elevation proved significant (table 3).

Germination decreased as source elevation increased, but seeds from higher elevations germinated faster. This finding might be expected though, because Scotch pine seed development has been reported to be delayed with decreasing mean temperatures (3). Slower seed development may result in less dormancy and thereby less delay in germination.

The sources were also summarized by germination percentage with those above 80 percent in one group (strong) and those below 80 percent in another group (weak) (fig. 1). Although the differences between means at moisture levels above 5 percent are not significantly different, the impact of moisture levels is more noticeable in weak seeds than in strong seeds. Weak seeds appeared to be more moisture specific. This may be related to the specific needs of deteriorating material and competition for food reserves.

In terms of seed testing, there appears to be no interaction between substrate moisture and seed source for Scotch pine. With crepe cellulose paper, a substrate moisture of 15 to 25 percent of the water-holding capacity should produce acceptable results for all of these sources. This means a standard seed test can be used to determine viability of all sources of Scotch pine examined in this study.

Table 2—*Summary of germination measurement means*

Measurement	10% Moisture	15% Moisture	20% Moisture	25% Moisture
Moldy seed (%)	11.4 b	13.9 a	13.4 ab	14.9 a
Germination of filled seed (%)	74.9 b	77.8 a	76.9 ab	76.3 ab
Days to 90% of total	10.4 a	10.3 a	10.0 ab	9.7 b
Days to 50% of total	5.6 a	5.4 ab	5.3 ab	5.2 b
Abnormal germination (%)	4.9 a	3.3 b	3.5 b	3.9 ab
Vigor, Czabator's method	22.2 b	24.6 a	24.6 a	24.5 a

Means within each measurement not followed by the same letter are significantly different at P = 0.01.

Table 3—*Coefficient of determination* (r^2) *of seed source and substrate moisture level as related to laboratory measurements*

Evaluation	Mold	Germination	Speed to 90%	Speed to 50%	Abnormal	Vigor by Czabator
Substrate moisture	0.87*	0.51	0.64	0.60	0.56	0.57
Elevation	0.17	0.65*	0.85*	0.68*	0.01	0.82*
Latitude	0.01	0.20	0.65	0.28	0.13	0.13
Longitude	0.13	0.08	0.42	0.41	0.18	0.04

* Significant at P = 0.01.

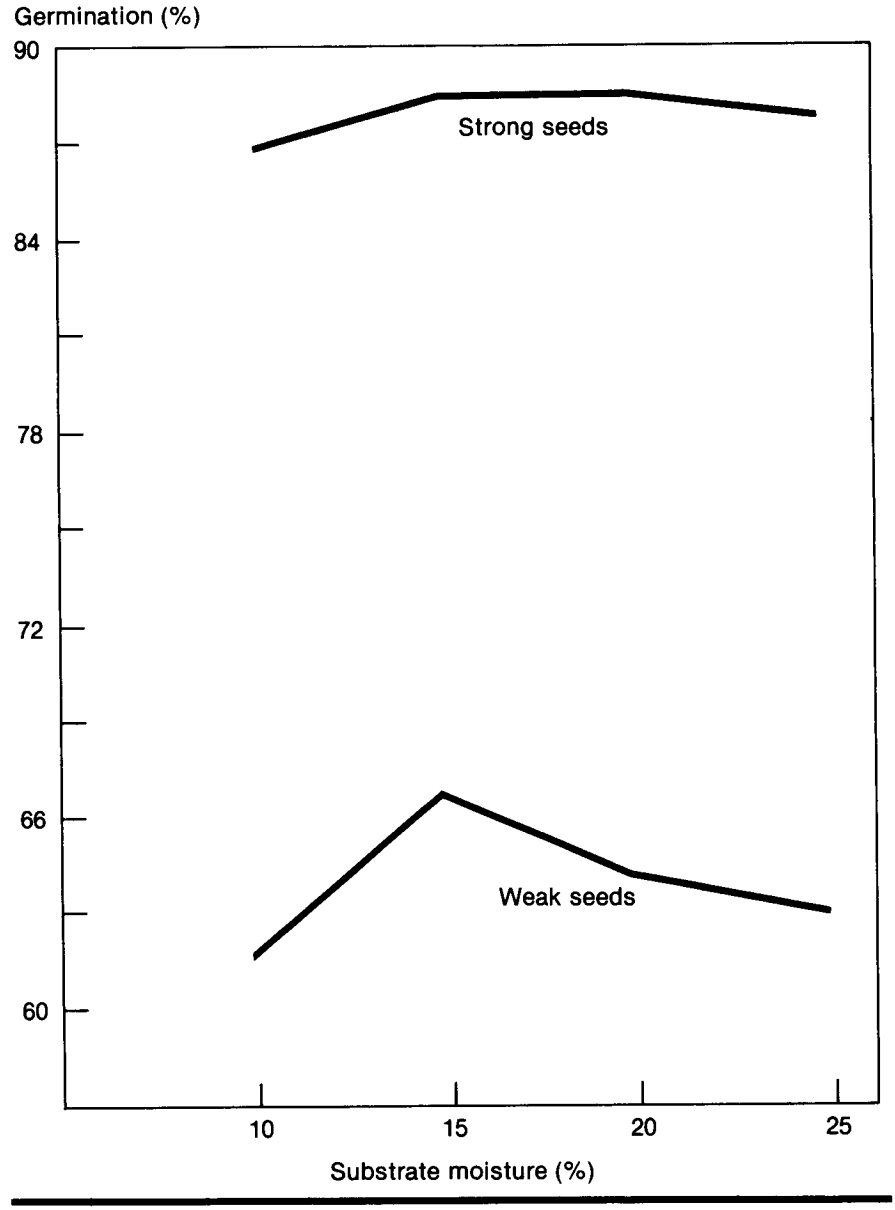

Figure 1—*Mean germination of strong and weak seed lots on five substrate moisture levels.*

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Reliability of Height and Diameter Remeasurements on Red Pine *(Pinus resinosa* Ait.) Seedlings

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A study was conducted to determine the error associated with remeasurement of diameter and heights of red pine (Pinus resinosa *Ait.) seedlings by the same and different observers. There were no significant differences between initial measurements and remeasurements of seedling height, either by the same or different observers. A major source of error in seedling diameter measurements is correct placement of the calipers on the stem. By marking the measurement point with an indelible felt-tip pen, the remeasurement error between two observers was significantly reduced.*

Many studies involving the effects of various treatments on seedling survival and growth use repeated measurements of height and diameter (1,4). After establishing a starter fertilizer study involving repeated annual height and diameter measurements of nearly 1,000 seedlings, we became concerned about the reliability of the measurements. The study would certainly not be effective if treatment differences between seedling heights and diameters were masked by variability associated with the measuring process. This variability would also be increased if different individuals were engaged in the measuring process.

One way of reducing measurement errors of seedling root collar diameters is to permanently mark the seedling at the point of measurement, because placement of the calipers on the stem can be a major source of error. Consistent orientation of the calipers is fairly simple, requires no extra time, and also reduces error. The act of marking the stem is, however, quite time-consuming, and a question arose as to whether or not the time investment was worthwhile. The objectives of this study were to determine if significant differences exist a) between repeated diameter measurements of unmarked and marked seedling stems, using the same observer and different observers and b) between repeated seedling height measurements using the same and different observers.

Methods

In May 1984, 25 freshly planted 3+0 red pine seedlings were isolated to serve as a representative sample. From previous work we determined that height and diameter measurements on a sample of 25 seedlings would allow us to estimate within ± 5 to 10 percent of the population mean, using the 95-percent level of probability (3).

On each seedling the root collar diameter or the diameter at the soil surface (if the root collar was not visible) was measured to the nearest 0.02 millimeter using a set of calipers. The measurements were made by the two independent observers, first on unmarked seedlings, then on seedlings with the measurement point marked with a red ring using an indelible felt-tipped pen. All measurements were repeated. Seedling heights were also measured by the same observers. The duplicated height measurements were recorded to the nearest centimeter.

Statistical analyses were conducted using paired t-tests at 5 percent probability (2). For both diameter and height, two statistical tests were calculated. For diameter, the first test was for a difference between repeated measurements by the same observer on unmarked seedlings and on marked seedlings. The second test was for a difference between repeated measurements by observers 1 and 2 on the unmarked seedlings and then on the marked seedlings. The tests for height involved differences between repeated measurements by the same observer and repeated measurements by different observers. For testing purposes the following null hypotheses were established:

Diameter

(1) H_o: D1 = D2

where D1 = mean of differences between repeated measurements of unmarked seedlings (observer 1); and

D2 = mean of differences between repeated measurements of marked seedlings (observer 1).

(2) H_o: D3 = D4
where D3 = mean of differences between measurements of unmarked seedlings by observers 1 and 2 and

D4 = mean of differences between measurements of marked seedlings by observers 1 and 2.

Height

(1) H_o: H1 = H2
where H1 = mean height of seedlings as first measured by observer 1 and

H2 = mean height of seedlings measured by observer 1 in second run.

(2) H_o: H1 = H3
where H3 = mean height of seedlings as measured by observer 2.

Table 1—*Results of paired t-tests for diameter and height measurements*

Null hypothesis	Alternate hypothesis	Means	Calculated test statistics	Result of test
H_o: D1 = D2	H_a: D1 ≠ D2	D1 = 0.18 mm D2 = 0.13 mm	1.532	Accept H_o
H_o: D3 = D4	H_a: D3 ≠ D4	D3 = 0.29 mm D4 = 0.14 mm	2.955	Fail to accept H_o
H_o: H1 = H2	H_a: H1 ≠ H2	H1 = 20.16 cm H2 = 20.24 cm	0.811	Accept H_o
H_o: H1 = H3	H_a: H1 ≠ H3	H3 = 20.36 cm	1.879	Accept H_o

Results and Discussion

The results of the paired t-tests and the means used in the tests are presented in table 1. The tests show that when the same observer is making diameter measurements, there is no benefit in marking the point of measurement on the seedling stem. Although the mean difference between marked and unmarked stems was lower (0.13 compared to 0.18 millimeter), the reduction was not significant at P = 0.05. When a different observer is making the measurements, however, marking the measurement point significantly reduced the error. The mean difference between measurements made by observers 1 and 2 without marking was 0.29 millimeter, and with marking it was 0.14 millimeter.

Measurements of seedling height have very little error associated with them. Repeated measurements by the same observer and measurements by different observers were not significantly different, indicating that height growth measurements are probably the most reliable for assessing treatment differences.

Conclusion

In studies in which measurements of seedling heights and diameters are used to assess treatment effects, marking the diameter measurement point on the seedling stem can significantly reduce remeasurement error if a different observer is making the remeasurement. An indelible felt-tipped pen ring on the stem will remain visible for about a year in Wisconsin and should be reestablished at each annual measurement. If the same observer is making the diameter measurement, with careful caliper alignment the marking ring may not be necessary.

Seedling height measurements can be made with greater ease and lack of remeasurement error, especially with different observers. Repeated measurements of height to obtain growth rates would provide the variable with the least error.

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