Comments

Tree Planters' Notes

is published quarterly by the State and Private Forestry Staff, Forest Service, U.S. Department of Agriculture, Washington, DC 20250. The Secretary of Agriculture has determined that the publication of this periodical is necessary in the transaction of public business required by law of this Department.

Editor-in-chief: Robert Mangold
Managing editor: Rebecca Nisley
Advisory editors: Robert Karrfalt, Thomas Landis,
Clark Lantz, and Ronald Overton

Individual authors are responsible for the technical accuracy of the material mentioned in *Tree Planters' Notes.* The mention of commercial products in this publication is solely for the information of the reader and endorsement is not intended by the Forest Service or the U.S. Department of Agriculture.

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended. Caution: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish and other wildlife-if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

Subscriptions (\$5 per year domestic, \$6.50 per year foreign) are available from the **New Orders**, **Superintendent of Documents**, **PO Box 371954**, **Pittsburgh**, **PA 15250-7954**. A subscription blank is available on the back cover.

Cover: Colorado aspen (photograph by R.E. Grossman, USDA Forest Service, Collbran, Colorado).

International Nursery and Reforestation Exchanges

I have recently returned from an exciting trip to central Siberia that was part of a Forest Service scientific exchange with a series of institutes of the Russian Academy of Science. Russia, as you know, is a huge country, with forest resources roughly 3 times the size of the United States. The Russians have a nursery industry that produces about 800 million seedlings per year-roughly half of our production. Their nursery technology is developing but could use substantial infusions of capital and expertise to optimize effectiveness.

Russia is just one of several countries that have made overtures to the USDA Forest Service for technical and financial assistance in nursery production and reforestation efforts. Some of these other countries include Mexico, Armenia, Uganda, and India. In fact, there seems to be a steady stream of foreign travelers coming to the U.S. looking for assistance. The Rio Conference on the Environment held in 1992 (UNCED) and other factors have pushed tree planting for restoration of degraded ecosystems and reforestation in general to the forefront of many governments' agendas. Representatives are coming to the United States because we have a solid reputation of having state-of-the-art nursery and reforestation technology.

This need for assistance offers many of us in the profession a terrific opportunity to help other nations in their nursery efforts, to see other cultures, and make international contacts. Going to Siberia was a rare treat for me. I know that some of you also have been active travelers and know firsthand about the rewards of helping others learn more about nursery and reforestation techniques and technology.

You may ask, "What do you do on these trips?" Well, of course, a trip can be quite varied, but typically it can involve assessing the local situation-what species are they growing, what should they be growing, etc. Then, it can be a matter of helping to determine what is the appropriate technology to use for that particular situation. (It makes no sense to recommend a technology that is outside the financial grasp of a country.) There is very often also the matter of training the local professionals on the latest state-of-the-art technology (and of course learning from them tricks to bring home to your nursery). Incidentally, we are going to be doing an extensive nursery training course in Mexico in 1994 for an entire year.

If you want to become involved in international nursery work, the International Forestry Deputy Area of the USDA Forest Service maintains a computerized skills roster to store information about individuals who have experience in or who are interested in becoming involved in international forestry work. The roster application gathers information about the applicant's areas) of expertise, overseas experience, language skills, and education, in addition to a variety of other questions. When a request by an agency or institution is made to the Forest Service for short-term technical assistance, a computerized search is made of the roster to try and match a person's skills and qualifications with the technical assistance request.

If you are interested in international forestry, with particular emphasis on nursery and reforestation technology, we want to hear from you-regardless of whether you work for public or private organizations. Call me-at (202) 205-1379-and I will send you an application form for the skills roster. Filling out the application takes only a few moments. After your application has been processed, you will then be in the database and could be considered for an international mission. Travel costs and per diem are usually covered by either the Forest Service, the receiving country, or another entity. Usually salaries are not paid, as we expect your home unit to pay for salaries with the thought that international travel is a great training device. On some occasions, however, a consultant's fee is provided. In the meantime, a good thing to do is to obtain your personal passport, as this will facilitate getting a visa later on.

I think each of us benefits from seeing other countries, for both cultural and technical enrichment. Often, we can apply and learn much about what we see in other countries to our own situation. So, I encourage you to take part in this expanding world of international nursery and reforestation exchange.

Robert Mangold

Editor-in-chief Cooperative Forestry State and Private Forestry USDA Forest Service Washington, DC

Greenhouses Heated With Waste Oil A True Story

James D. Schwartz and Marla Schwartz

Owner-operators, North Woods Nursery, Inc., Elk River, Idaho

An alternative fuel source-used crankcase oil-has provided heat for three seasons at a forest tree nursery in northern Idaho. This environmentally safe system has proved economically sound as well, with fuel savings paying for the cost of the equipment in 2 1/2 years. Tree Planters' Notes 44(4): 146-148;1993

Waste oil has been used as an effective fuel for heating greenhouses where reforestation seedlings are produced. The EPA has stated that an environmentally safe way to dispose of used crankcase oil is to burn it in a certified burner. The heat energy released during that combustion can provide sufficient energy to heat greenhouses. A burner box inside a large tank of water can produce, store, and transfer many thousands of British thermal units of heat (BTU's) to extraction radiators. This system has been operated for three production seasons in a fairly extreme northern climate and has proved itself to be environmentally safe and economically sound. This same system can be converted to burn other fuels.

This description of our waste-oil burner system for heating our greenhouse does not present data of a scientific nature. Rather, the evidence that our heating system works is purely anecdotal, in other words it does work for us and works very well in our setting. The measure of BTU output, the insulation factor of the various greenhouses, the quality of fuel being burned, and many other factors would and could vary to change the overall efficiency of this system. As in other aspects of greenhouse production, the solution should fit the problem, but the problem is different in each unique setting. We hope that this solution to our particular problem will provide assurance that such a basic idea can be modified to provide an efficient and environmentally sound source of heat energy.

Geographic Considerations

North Woods Nursery, Inc., is located in a forested area of northern Idaho that can best be described as remote and isolated. At almost 47/ N latitude, Elk River is located in a snow belt with cold, snowy win-

ters. First snow falls in late October and remains on the ground until mid-April. Greenhouses at North Woods Nursery are in operation from March to mid December. It is more than 20 miles (32.2 km) to a natural gas pipeline. Propane is available from suppliers over 50 miles (80.5 km) away but it is expensive and requires bulky storage tanks that need costly maintenance calls.

History of Conversion to Nontraditional Fuels

In 1989, we decided to switch from expensive propane to a wood-burning system. We found a stove system that seemed to meet our needs in Turbo Burn of Spokane, Washington, and had it installed during the summer. We operated it through January of 1990 using slab wood as the only fuel. The system was successful in maintaining temperatures above freezing in five 108- x 30-foot (32.9- x 9.1-m) double-poly houses, approximately 16,200 square feet (1,506.6 m²), when outside temperatures fell to -40 / F (-40 / C). Wood proved to be adequate as a fuel source, but the labor required to feed the fire was the primary motivation in the decision to convert to a waste-oil burner for the spring crop of 1990. We have used waste oil as the heat fuel source for our greenhouses since that time.

How the System Works

The system consists of a burner box located inside a large metal tank that holds 1,500 gallons (5,670 liters) of water. A fire inside the box heats the water to nearly boiling. This is not a pressurized system, so water temperatures do not exceed 212 /F (100 /C). A network of pipes connects the hot water tank to radiators located in the greenhouses. Pumps controlled by thermostats circulate the water through this system. At the radiators, large fan jets (left over from the old propane burners) pull air through the radiators and conduct the warmed air under the growing benches in poly tubes. The cooled water is pumped back to the storage tank where it is reheated (figure 1).



Figure 1—Interior of barn showing burner box, oil drum, and tubing that transports hot air to 6 greenhouses.

How Well It Works

In planning the system, we paid close attention to anticipating and avoiding problems before they occurred. This proactive philosophy has probably saved hours of headaches. Insulating all areas where heat could be lost has made the system fairly efficient. The building that houses the burner and water storage tank has 18-inch-thick walls. Roof and walls are both filled with "blown in" insulation material (figure 2).

The pipes that connect the storage tank to the greenhouses and back are buried 2 feet (.6 m) underground and housed in 12 inches (.3 m) of rigid foam insulation. The loop of pipe that connects the storage tank to the radiator and back is a continuous polybutyl pipe with all its fittings above ground. The oil tanks are housed adjacent to the burner in insulated rooms,

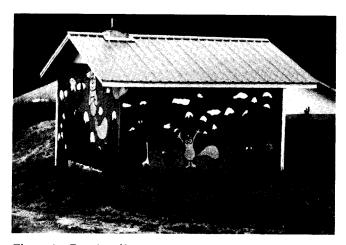


Figure 2—Exterior of barn.

to help keep the oil warm enough to flow efficiently and add more protection from cold temperatures to the burner room.

The first radiators that we installed in the system were used truck radiators. Although they proved adequate, we have since replaced them with more efficient heat exchange units that have four copper coil; and a much larger exposed area to extract the heat.

Handling, Safety, and Environmental Considerations of the Fuel

At this time, the only EPA-recognized way to dispose of waste oil is to incinerate it in a certified burner. The source of fuel for our burner has been used crankcase oil from farms and logging companies in our area. The fuel arrives at the nursery in 50-gallon (189.3-liter) drums and is pumped into the larger storage tanks adjacent to the burner. The ground immediately adjacent to the building is lined with a vinyl sheet in the case a serious spill needs to be removed. The rest of the area is lawn grass. The grass and soil organisms can successfully breakdown minor spills. The residue left after combustion is essentially inert, and its quantity and composition depend on the first use of the oil. We save this residue in a coffee can and take it to the nearby landfill once year on their household toxic chemical disposal day.

General Maintenance

We added a special anticorrosion chemical to the water storage tank as recommended by the dealer and covered exposed pipes and pumps with heat tapes f very cold temperatures. To prevent freezing damage, we remove and store the radiators when they are no in use in the winter.

Side Benefits

The storage tank is also fitted with a coil of copper pipe through which fresh water circulates. The resulting hot water at 170 /F (76.7 /C) is used to sterilize the trays.

Payback and Tax Benefits

At this time we are exploring the tax benefits and special loan opportunities that are available for individuals and businesses that are pioneering alternate energy technologies. We recovered the cost of the burner, pipe, and labor to install this entire system in $2\frac{1}{2}$ years by saving on the cost of propane fuel.

Should You Convert to This System?

The primary motivation to convert to this system could well be economic, but the environmental benefits are also great. Safe disposal of a large quality of waste oil can have a very positive impact on the environment. Some states do monitor burner sites and ask that operators test their fuel source on a regular basis. This minor requirement does not overshadow the major benefits of such a system.

Summary

Heating greenhouses with waste oil as a fuel appears to be a feasible solution to two problems: first, finding an inexpensive fuel source that is readily available, and second, using an environmentally safe and approved method to dispose of a potentially harmful product. The technology to convert to this system is available and new higher output burners and more efficient equipment are on the horizon.

Effects of a DCPA/Naropamide Herbicide Tank Mix on Germinants of Seven Hardwood Species in Tree Nursery Beds

J. D. Porterfield, J. D. Odell, and G. R. Huffman

Forest nursery specialist, senior forest nursery specialist, and nursery superintendent Oklahoma Department of Agriculture, Forestry Services, Forest Regeneration Center, Washington, Oklahoma

Two pre-emergence herbicides-DCPA (Dacthal®) and napropamide (Devrinol®)-were applied as a tank mix of 4.76 kg (10.5 pounds) and .45 kg (1 pound) active ingredient per acre, respectively, to nursery beds of seedlings of 7 different hardwood species-hackberry (Celtis occidentalis L.), persimmon (Diospyros virginiana L.), Russian olive (Elaeagnus angustifolia L.), euonymus (Euonymus bungeanus Maxim.), American plum (Prunus americana Marsh.), sand plum (P angustifolia Marsh.), and chokecherry (P virginiana L.)-at the time of germination but before seedling emergence. With the exception of American plum, germination counts and observations of seedling growth and condition revealed no damage from these herbicides on these hardwood species. Spring applications of these herbicides as a tank mix have several advantages over separate fall applications (just after sowing), including fewer applications of DCPA and napropamide needed to achieve adequate weed control, reduced loss of herbicide due to leaching and decomposition, reduced time spent spraying, and an increased window for application that achieves adequate control of weeds. Good weed control comparable to that achieved with separate applications of DCPA and napropamide was obtained with the tank mix. Tree Planters' Notes 44(4): 149-153; 1993.

The development of new post- and pre-emergence selective herbicides for agricultural use has refined the control of weeds in agronomic crops. Many of these selective herbicides can be used in forest tree nurseries. Weed control at the Oklahoma Department of Agriculture's Forest Regeneration Center in Washington, Oklahoma, has been a perpetual battle requiring considerable investments of time and labor.

Pre-emergence herbicides provide selective control of weeds before they germinate and compete with desirable plants for water, nutrients, light, and space. Dacthal® (DCPA or dimethyl tetrachloroterephthalate) and Devrinol® (napropamide or 2-("-naphthoxy)-N,N-diethylpropionamide)) are pre-emergence herbicides commonly used in the control of annual broadleaf and grass weed species throughout the United States (Weed Science Society of America 1989). In forest nur-

series, DCPA and napropamide are used extensively in the Northeast and West, but to a much lesser degree in the South (Abrahamson L.P., personal communication).

At Oklahoma's Forest Regeneration Center, a tank mix of DCPA/napropamide was applied soon after sowing to Russian olive (Elaeagnus angustifolia L.) in the fall of 1988, and to American plum (Prunus americana Marsh.) and sand plum (P. angustifolia Marsh.) in the fall of 1989 without phytotoxic damage. The tank mix consisted of DCPA at 4.76 kg (10.5 pounds) of active ingredient (AI) per acre and napropamide at .45 kg (1 pound) AI per acre. Application of these chemicals as a tank mix versus separate application reduces the time needed for spraying. Also the label recommends application of napropamide at a rate of 1.8 kg (4 pounds) AI per acre, but the previous studies detailed above have shown that .45 kg (1 pound) AI per acre is adequate in a tank mix with DCPA. This lower rate would reduce the risk of crop damage by napropamide (a problem in prior studies), and possibly allow another application of napropamide later in the growing season, if necessary, without a build-up of herbicide residue.

Fall-applied herbicides are often leached from the soil by winter and early spring rains, broken down by microorganisms, and/or absorbed by organic matter. However, very few weeds usually emerge during the fall or winter, whether or not pre-emergence herbicide is present. It would be desirable if these herbicides could be applied in the spring just before seedling emergence or just before significant numbers of weeds emerge. Spring application would make more preemergence herbicide available when it is needed most so that maximum use is made of the active life (DCPA and napropamide are effective for about 3 months) of the chemical in the soil. Less chemical would be needed for spring applications compared to fall applications, because spring applications would provide weed control for up to 3 months into the growing season whereas fall applications would barely provide

weed control beyond spring weed and crop emergence and would require another application to achieve the same degree of weed control. Labor and weather constraints often limit the time when herbicides can be applied, and spring applications would allow more latitude in terms of when and possibly how often they may be applied. In the past, it was believed that fall applications on a fall-sown crop were necessary to avoid phytotoxic damage and provide adequate weed control, but no studies had been conducted to test alternatives to this idea.

The present study was conducted to determine if a tank mixture of DCPA/napropamide causes any phytotoxic effects on seven different fall-sown hardwood species when applied in the spring at the time of gerurination, but before seedling emergence.

Materials and Methods

All seven hardwood species in this experiment were sown in early November 1990 in 1.2-m-wide (4-footwide) by approximately 198-m-long (650-foot-long) nursery beds. The nursery beds were located on a loam soil with a pH of 6.2. Organic matter content of the soil in these nursery beds at the Forest Regeneration Center was 2%. At the time of the herbicide application in March 1991, most of the hardwood species had begun to germinate. The American plum and sand plum radicals had begun to extend, but the epicotyl had not broken the surface of the ground. Chokecherry (P. virginiana L.), euonymus (Euonymus bungeanus Maxim.), and hackberry (Celtis occidentalis L.) radicals were extending, and for some seedlings the epicotyl had reached the surface of the ground. The persimmon (Diospyros virginiana L.) had no radicals extending. Russian olive had radicals extending with many epicotyls above ground.

Five unpaired treatment sample plots were randourly established (measuring 1.83 m (6 feet) long x 1.22 m (4 feet) wide) for each species in the 1990 fallsown beds. The treatment plots were sprayed with a tank mix of DCPA at 4.76 kg (10.5 pounds) and napropamide at .45 kg (1 pound) active ingredient per acre on March 9, 1991. At the time of application, the weather was sunny, with a temperature of 10 /C (50 /F), and the wind was north at 8 km/hr (5 miles per hour). Each plot was divided in half. Within each half plot (.9 m or 3 feet), half of the drill rows for a given species were chosen at random and marked. These rows were used for destructive germination counts whereas unmarked drill rows were used to assess the condition of the seedlings over time. Each new germinant was gently removed to facilitate countsampling also eliminated the need for counting each new germinant more than once on future dates of assessment. All plots were mapped. Five control sample plots were established, mapped, and evaluated in the same manner as the treatments but were ran dourly established in relation to the treatment plots and were not sprayed with the herbicide mix.

At varying intervals from March 23 until the final tallies were made, destructive counts were made by gently pulling, examining, and counting the number of newly germinated seedlings within each marked drill row in each half plot. American plum and Russian olive had finished germinating on April 26. Sand plum germinated until April 17. Hackberry, euonymus, and chokecherry germinated through May 1, and persimmon germinated until May 30. All species were examined for any new germination on May 30. Rows not sacrificed for germination counts were used for observations of developmental stages and the general health of the seedlings. Mean germination for both the treatment and control (five samples each) of each species for each date, and for the total cumulative germination were computed, and 95% confidence intervals were developed for each mean. Each sample (depending upon species) was the mean of 12 to 30 subsamples from the drill rows within each plot. A two-tailed t-test for unpaired plots was performed on the treatment and control means for final cumulative germination per .3 m of bed length (i.e., bedfoot) for each species. Significance of the differences between treatment and control means (5 samples for each mean) was assessed using the t-test method of Freese (1967).

Results

There was considerable overlap in the 95% confideuce intervals for treatment and control means for number of new germinants for all species on any date of assessment and for total germination (table 1). Overall, for total cumulative germination, only the control and treatment means for sand plum showed a significant difference, with the herbicide mix treatment having higher germination than the control for sand plum. It appears that this herbicide mix, when applied in a tank mix at the given rate, will not adversely affect germinating seedlings of chokecherry, euonymus, American plum, sand plum, hackberry, Russian olive, or persimmon. Observations of the seedlings' health and growth indicated no differences between plots treated with DCPA/napropamide and the control. Later, during the middle of the 1991 growing season, it was noticed that the stems of American

Table 1-Mean number of seedling germinants per .3 m of bed length (bed foot) for treatments and controls of fall-sown hardwood species after spring pregermination treatment with an herbicide tank mix

				Dates of asse	essment (1991)			
Species	Treatment	3/23	4/6	4/17	4/26	5/1	5/30	total no. germinants
	C					0 1		
chokecherry	C	2 ± 4	1 ± 3	1 ± 2	0 ± 1	0 ± 1	0	5 ± 6
	T	3 ± 4	2 ± 3	1 ± 2	0 ± 2	0 ± 2	0	6 ± 7
euonymus	C	4 ± 6	2 ± 4	0 ± 1	0 ± 0	0 ± 0	0	6 ± 5
	T	5±8	1 ± 3	0 ± 1	0 ± 0	0 ± 0	0	6 ± 9
American plum	C	1 ± 2	20 ± 17	6 ± 6	0 ± 1	0	0	27 ± 16
	T	1 ± 2	16 ± 8	5 ± 6	1 ± 5	0	0	23 ± 7
sand plum	C	22 ± 28	20 ± 21	4 ± 17	0	0	0	45 ± 12*
	T	27 ± 26	27 ± 26	3 ± 9	0	0	0	56 ± 16*
hackberry	C	0 ± 2	30 ± 11	7 ± 10	0 ± 1	0 ± 0	0	38 ± 6
	T	0 ± 1	22 ± 19	6 ± 8	0± 1	0 ± 1	0	29 ± 22
Russian olive	C	24 ± 26	3 ± 6	0 ± 1	0 ± 1	0	0	27 ± 38
	T	31 ± 26	4 ± 11	0± 1	0± 1	0	0	35 ± 40
persimmon	C	0 ± 0	0 ± 1	3 ± 10	12 ± 40	13 ± 38	25 ± 77	53 ± 198
	T	0 ± 0	0 ± 0	0 ± 0	7 ± 21	13± 27	31 ± 73	54 ± 162

The herbicide mixture is DCPA (Dachtal®) at 4.76 kg (10.5 pounds) AI per acre and napropamide (Devrinol®) at 4.5 kg (1 pound) AI per acre. C = control, T = treatment. 95 % confidence intervals are computed as the mean ± standard

the roots appeared to be unaffected (Weatherford, G., personal communication).

Discussion

Seedlings tend to be most sensitive to herbicides like DCPA and napropamide during or just after gerurination. They cause root stubbing, malformation, and restricted growth. Usually there is no foliar activity. In studies by Abrahamson (1987 and 1988), both DCPA and napropamide were separately applied to euonymus and Russian olive just after seeds were sown (post-seeding) and at 4 to 5 weeks after seedling emergence (post-germination). There were no phytotoxic effects to the seedlings. In other studies by Abrahamson (1988), DCPA applied post-germination was shown to have some phytotoxic effects on American plum, chokecherry, and hackberry. However, napropamide applied post-germination to hackberry caused no noticeable phytotoxic effect. The effect of these preemergence herbicides on seedlings at different times greatly depends upon the species and the particular chemical applied. Of the species examined in the present study, Abrahamson (1988) has only investigated the effects of DCPA and napropamide separately upon euonymus and Russian olive post-seeding, but not in a tank mix or applied close to when seedlings germinate as in this study.

Although not formally examined in this study, use of a DCPA/napropamide tank mix at the rate used in this study has historically provided good weed control of many grasses and broadleaved weeds at the Forest Regeneration Center, and good weed control was

napropamide were mixed in this study and in previous studies to replace the tank mix of bifenox (Modown®) and napropamide formerly used. Modown is no longer manufactured, and DCPA will help control weeds not controlled by napropamide and vice versa (table 2). In addition to the further species controlled by each chemical in the mix, it is possible that a synergistic effect of the mix may control even more weeds than those listed.

In the present study, the 95% confidence intervals overlapped considerably for the treatment and control means for germination for each species on each date of assessment and overall. Large confidence intervals probably indicated a lack of precision in the experiment and the need for more subsamples to account for the environmental variation and reveal any further significant differences if they existed. However, 95% confidence intervals do not indicate the significance of differences between means as t-tests do, and it is posible for 95% confidence intervals to overlap considerably between two means and for two-tailed t-tests to show the difference between those means to be significant at "= .05 (Patton, D., personal communication). Nevertheless, it is interesting that the sand plum treated with the mixture had significantly higher over all germination than the control because napropamide has been shown to have beneficial plant growth regulating effects on other plants (Weed Science Society of American 1989). A study comparing the effect of higher differing rates of the mixture on sand plum would be interesting to ascertain if this pre-emergence mix does indeed enhance germination. This study should also be repeated with American plum to deter-

^{*}Treatment and control values for total no. of sand plum germinants were statistically significant using a two-tailed t-test at "= .05

Table 2-Weed control by DCPA and napropamide

Common name	Scientific name
Controlled by DCPA but not by napropamie	de
burning nettle	Urtica wrens L.
copperleaf	Acalypha spp.
deadnettle	Lamium spp.
dodder	Cuscuta spp.
[European] field pansy	Viola arvensis Murray
Florida purslane	Richardia scabra L.
groundcherry	Physalis spp.
knotweed	Polygonum spp.
lovegrass	Eragrostis spp.
nightshade	Solanum spp.
spurge	Euphorbia spp.

Controlled by napropamide but not by DCPA

brome	Bromus spp.
cheatgrass	B. secalinus L.
Anna Channa	B. tectorum L.
ripgut brome	B. rigida Roth
[wooly] cupgrass	Eriochola villosa (Thunb.)
	Kunth
fall panicum	Panicum dichotomiflorum
	Michaux
filaree	Erodium spp.
[common] groundsel	Senecio vulgaris L.
knotweed	Polygonum spp.
little mallow	Malva parviflora L.
mallow	several genera have plants called mallow: <i>Malva</i> ,
	Malvella, Urena, Abutilon,

panicum (panicgrass) Panicum spp. Matricaria matricarioides pinapple weed (Less.) Porter Lactuca serriola L prickly lettuce soft chess Bromus hordeaceus L. sowthistle Sonchus spp. sprangletop Lentochloa spp ryegrass Lolium spp. Ambrosia spp. ragweed wild barley Hordeum spp. wild oats Avena spp.

Althaea, Abelmoschus, and

Hibiscus

Controlled by both chemicals

annual blu	uegrass	Poa annua L.
barnyard (grass	Echinochloa crus-galli (L.) P Beauv. var. crus-galli
carpetwee	ed	Mollugo verticillata L.
chickweed	Ė	Stellaria spp. or Cerastium spp.
crabgrass	;	Digitaria spp.
foxtail		Setaria spp. or Alopecurus spp.
goosegras	ss	Eleusine indica (L.) Gaertner
Johnson g	grass (fom seed)	Sorghum halepense (L.) Pers
lamb's-qu	arters	Chenopodium album L.
pigweed		Amaranthus spp.
purslane		Portulaca spp.
sandbur		Cenchrus spp.
witchgras	S	Panicum capillare L.

ture, and if so at what rate(s), or if it is due to an interaction of the herbicides with another environmental factors) (e.g., fertilizer). A study examining the phytotoxicity and weed control of separate applications of DCPA and napropamide upon these species versus a tank mix would also be helpful in understanding the results of this study. Despite the limitations of this research, the application of DCPA/napropamide as a pre-emergence tank mix at the time of germination shows promise for weed control in nursery beds of all species tested in this study.

The results of this 1-year study must be accepted with caution because the treatments have not been repeated over time or under different temperature, mositure, wind, soil texture or other environmental conditions (Owston and Abrahamson 1984). Nevertheless, the results are encouraging, and the experiment will be repeated at the Forest Regeneration Center. Further studies could include such measurements as height, stem caliper and/or root weight to help detect possible phytotoxic effects. Also, the phytotoxic safety limit of this herbicide mixture could be determined by applying higher (2 or 4 x) doses for a species (Owston and Abrahamson 1984).

In this study, the DCPA component of the mix was applied near the label's maximum recommended level of active ingredient per acre while the napropamide part was applied at much less than the recommended rate. Neither chemical's label prohibits nor gives recommendations for mixing these chemicals. In the past, this tank mix has been applied as a part of ongoing research conducted by Dr. Larry Abrahamson of the State University of New York at Syracuse in cooperation with the Oklahoma Department of Agriculture's Forest Regeneration Center. The present study was conducted with Dr. Abrahamson's advice and consent, and all applicators were licensed by the Oklahoma Department of Agriculture with demonstration and research category pesticide applicator licenses.

Fermenta Plant Company advises application of Dacthal® W-75 separately (not as a tank mix) to nursery stock at the rate of 14 to 16 pounds (6.3 to 7.2 kg) in 50 to 100 gallons (190 to 380 1) of water per acre (.4 ha). This would be 10.5 to 12 pounds (4.7 to 5.4 kg) of active ingredient per acre. Weed control at this rate should last 3 months or more. For DCPA (dimethyl tetrachloroterephthalate), the active ingredient of Dacthal, the average half life is 60 to 100 days in most soil types, with no leaching. This chemical is absorbed by organic matter, and microorganisms are the primary factor in the disappearance of the chemical. No loss occurs from photodecomposition and/or volatilization. DCPA is not absorbed by foliage or

translocated in the plant (Weed Science Society of America 1989).

For Devrinol® 50-WP, the Stauffer Chemical Company recommends separate surface application to fruit and nut crops at the rate of 8 pounds (3.6 kg) per broadcast acre (.4 ha). This would be 4 pounds (1.8 kg) of the active ingredient, napropamide, per acre for Devrinol. Napropamide has a half life of 8 to 12 weeks in loam soils when incorporated at 70 to 90 /F (21 to 32 /C). This chemical is quite resistant to leaching and is slowly broken down by microorganisms; very little is lost to volatilization from the soil surface. Also, napropamide is only absorbed somewhat by foliage but is rapidly taken up by roots, trapslocated, and metabolized (Weed Science Society of America 1989). Fortunately both DCPA and napropamide are fairly stable, nonreactive, and nonflamma ble chemicals that carry only d caution label. Given the characteristics Of the active ingredients of Dacthal® and Devrinol®, their label recommendations, and the

site arid weather conditions of the study area, one would expect that other southern nurseries might also have success using these chemicals at rates similar to those used in this study.

Conclusions

Preliminary results indicate that an application of DCPA (Dacthal)/napropamide (Devrinol) as a tank mix at 4.76 kg (10.5 pounds) and .45 kg (1 pound) of active

ingredient per acre, respectively, just before seed gerurination will not damage young seedling germinants of sand plum, chokecherry, euonymus, hackberry, persimmon, or Russian olive, but will provide good weed control for these species. With the exception of

American plum, this application had no adverse effect upon the number of seedling germinants or the growth of the seedlings over time. The germination of sand plum appears to be enhanced by this herbicide application. Further studies of these herbicides on these and other hardwood species should be conducted and the phytotoxicity of this herbicide mixture determined for each species. The results, if positive, would indicate that the DCPA/napropamide mixture could be applied in the spring just before seedling emergence of a fall sown hardwood crop at the Forest Regeneration Center. However, other nurseries must do their own studies to determine the safety and effectiveness of these chemicals on each species they grow. Differing soil and weather conditions often alter the weed control and phytotoxicity of these chemicals, and the phytotoxicity of these chemicals appear to be species specific.

Literature Cited

Abrahamson LP 1987. Forest tree nursery studies at the Oklahoma Forest Regeneration Center. In: Proceedings, Annual meeting of the Intermountain Forest Nursery Association; 1987 August 10-14; Oklahoma City, OK: 49-57.

Abrahamson LP 1988. Forest tree nursery herbicide studies in the Great Plains and the northern United States-1978 to 1987. In: Proceedings, Forestry Herbicides in the Northeast; 1988 March 15-16; New Brunswick, NJ: 17-53.

Freese F 1967 Elementary statistical methods for foresters. Agric. Handbk. 317. Washington, DC: USDA Forest Service: 24-26.

Owston PW, Abrahamson LP 1984. Weed management in forest nurseries. In: Duryea ML, Landis TD, eds. Forest nursery manual: Production of bareroot seedlings. The Hague: Martinus Nijhoff/Dr. W Junk Publishers for Oregon State University Forest Research Laboratory: 193-202.

Page BG, Thomson WT. 1992. The insecticide, herbicide, fungicide quick guide. Fresno, CA: Thomson Publications: 81, 94.

Reed CF, Hughes RO. 1970. Selected weeds of the United States.

Agric. Hndbk. 366. Washington, DC: USDA Agricultural

Research Service. 463 p.

Terrell EE, Hill SR, Wiersema, Rice WE. 1986. A checklist of names for 3,000 vascular plants of economic importance. Agric. Hndbk. 505. Washington, DC: USDA Agricultural Research Ser vise. 241 p.

Weed Science Society of America. 1989. Herbicide handbook, 6th ed. Champaign, IL: Weed Science Society of America: 144-146, 337-339.

Economic Analysis of Two-Spotted Spider Mite Management on Greenhouse-Grown Poplars

Vincent A. Smith, David B. Orr, and Elwood R. Hart

Senior research technician, Department of Entomology, University of Missouri, Columbia, Missouri; assistant professor of entomology, Department of Entomology, Michigan State University, East Lansing, Michigan; and professor of entomology, Department of Entomology, Iowa State University, Ames, Iowa

The two-spotted spider mite- Tetranychus uriticae Kochis a perennial pest problem on greenhouse-grown poplar (Populus spp.). Although weekly applications of miticide effectively managed this pest, other issues such as managemeat costs and worker safety prompted an effort to test another approach. An integrated pest management (IPM) program for spider mite suppression was developed using pest monitoring, release of predatory mites-Phytoseiulus persimilis Athias-Henriot and Amblyseius californicus (McGregor)-and spot-treating with a miticide when neces sary. One year after implementation of this IPM program, mite populations have been suppressed to acceptable levels, pest management costs have been reduced by 81%, and concerns regarding miticide exposure by personnel sharing the greenhouse facilities have been eliminated. Tree Planters' Notes 44(4): 154-156; 1993

At Iowa State University in Ames, research is being conducted by the Department of Entomology on the insect-plant interaction of the cottonwood leaf beetle (*Chrysomela scripta* F) and poplar (*Populus* spp.) selections. The laboratory colony of beetles requires a continual food source. Selected tree clones are grown in a greenhouse.

The two-spotted spider mite (*Tetranychus uriticae* Koch) is a common pest in greenhouse environments. This mite has been a perennial problem associated with the cultivation of *Populus* in our greenhouses but is not a problem in field plantings. In the past we have managed this pest effectively with weekly applications of a miticide, usually dienochlor (Pentac®) or propargite (Ornamite®). However, economic considerations, environmental concerns raised by non-project personnel sharing the greenhouse space, and the need to use pesticide-free plant materials as insect colony food prompted a reconsideration of our program.

We decided to attempt implementation of an rotegrated pest management (IPM) program. The IPM strategy utilizes more than one pest control method to increase the performance of a pest management program so that it is both economically and ecologically sound (Pedigo 1989). One common tactic employed in greenhouse IPM programs is the release of natural enemies (predators, parasitoids, and diseases) of pests (Hussey and Scopes 1985, Parrella 1990). In this paper, we describe how we used predatory mites- *Phytoseiulus persimilis* Athia-Henriot and *Ambyseius californicus* (McGregor) for the suppression of the two-spotted spider mite *Tetranychus urticae* on *Populus*, and the benefits that accrued from using IPM.

Methods

We implemented our IPM program in two completely enclosed greenhouse bays. Each bay was 6.15 m (20 feet) long and 3.69 m (12 feet) wide, with a total floor area of 22.3 m 2 (240 square feet). About 70 trees are grown to a height of 1 m (39.37 inches) in each bay. Populations of spider mites reached extremely damaging levels as evidenced by the wide spread chlorosis and the webbing covering the leaves. On March 28, 1991, prior to the beginning of the program, dienochlor (Pentac® Aqua Flow) was applied at label rate to suppress T. urticae populations ($\frac{1}{2}$ tea spoon AI per gallon). This was done to provide a more favorable predator-to-prey ratio and increase the probability that the released predators might manage pest populations (Hussey and Scopes 1985, Weinzerl and Henn 1991).

It has been reported that for proper control of the two-spotted spider mite, 10 to 50 predators were needed per plant (Hussey and Scopes 1985, Weinerl and Henn 1991). Because there are no published recommended release rates for Populus spp., we estimated what our release needs would be, based on pest infestation and tree size. On April 6, 1991, 1,000 of each of two predator mites----*Phytoseiulus persimilis*-Athias-Henriot and *Amblyseius californicus* (McGregor)-were released on 150 trees (Weinzierl and Henn 1991), a ratio of 13.3 mites per tree (Pest Management Supply, Amherst, MA). Because of high temperatures in the greenhouse (> 32.2 °C), the predators died, making this release a failure. When the

temperature was more moderate (< 26.5 /C), the same number of mites was released on April 11, 1991. An additional 1,000 *A.californicus* were released .on September 26, 1991, to increase the numbers of mites and provide added control of the pest during the hottest part of the summer. Trees were monitored daily for symptoms of pest mite population increases. Leaves were sampled randomly every week to determine the continued presence of predators. No additional labor costs were added to the program as both these surveys were conducted while watering the trees.

Results and Discussion

Suppression of spider mite. Before we began the IPM program, we had to use a weekly application of a miticide to ensure that our trees had low levels of damage. Whenever we reduced the frequency of application to a biweekly schedule in an attempt to reduce pesticide use and cost, spider mite populations consistently reached outbreak levels and the majority of leaves on our trees were heavily damaged. This damage compromised the quality and quantity of the food supply for our insect colony and reduced growth of the trees.

Over the 1-year period since we began the IPM program, predators have provided good levels of spider mite suppression. Only four additional spot applications of dienochlor (1/4 teaspoon AI in 1/2 gallon water) were required to suppress minor outbreaks on some of the trees.

The qualitative measures of effectiveness of this IPM program were (1) the lack of damaged, chlorotic leaves and mite webbing, (2) the lack of a need to spray miticide to ensure food quality for the insect colony, and (3) the continued presence of predator populations on the trees throughout the entire year period, as evident on weekly randomly sampled leaves.

Economic benefits. The key to success for an IPM program is vigilant monitoring of the plants for symptoms of insect activity or damage. In our project, personnel needed no additional training as they are entomology or pest management students (figure 1). However, other organizations that implement an IPM program will probably need to train their personnel to scout for pest problems, preferably while performing other tasks such as watering. This will ensure that outbreaks are stopped before they become damaging.

Because of the rapid reproduction rate of the pest, miticide had to be applied weekly to ensure healthy trees. In addition to the cost of the miticide, there are many extra costs associated with weekly spraying:



Figure 1 -Entomology student monitoring greenhouse-grown poplars for pest and predator activity.

Someone from the project had to spray the miticide, which took about 2.5 hours each week to mix and apply. At a base pay of \$5.00/hr for student labor, spraying costs \$650.00/year in wages. The sprayers need to wear protective clothing (table 1). The filters for protective masks (changed monthly, at \$4.39/pair) cost \$52.68 annually, gloves (replaced monthly at \$1.21/ pair) cost \$14.52, and coveralls (replaced every other week at \$4.70 each) cost \$122.20 annually. (These are 1991 prices obtained from the Iowa State University's Central Stores Catalog.)

Table 1 -Economic comparison between two methods for management of the twospotted spider mite (Tetranychus urticae) on greenhouse-grown Populus, Ames, Iowa (March 1991-March 1992)

	Integrated	Pesticide
	pest	application†
	management*	
Predator release 1	\$ 37.20	\$ 0.00
Predator release 2	37.20	0.00
Predator release 3	13.00	0.00
Pesticide‡	0.90	15.60
Labor	20.00	650.00
Protective coveralls	9.40	122.20
Filters	0.00	52.68
Gloves	0.00	14.52
Total	\$117.70	\$855.00
Estimated savings	\$737.30	

^{*}Three releases of predator mites (Phytoseiulus persimilus and Amblyseius californicus) plus 1 initial and 4 spot applications per year of dienochlor (Pentac®), ¼ tsp. AI, ½ gallon solution per application.

[†]Weekly application of dienochlor (Pentac®) ½ teaspoon AI per gallon of water, 2 gallon solution per application

[‡]Pentac® Aqua Flow price is \$57.50 per quart. Actual cost of annual applications, based on amount of pesticide applied in IPM program (one initial application and four spot program.treatments), and weekly miticide applications in non-IPM

Conclusion

The use of an IPM program involving the release of two species of predators was determined by us to be effective in controlling two-spotted spider mite in our

greenhouse environment. Although this was not a quantitative, controlled experiment, it does illustrate the practical applications of an IPM program in a greenhouse environment. The purpose of this program was to reduce our pesticide use while maintain-

ing healthy food trees for our insect colony. We reached this goal 1 year after we began this program, on March 28, 1991, and as an added benefit we saved \$737.30 in wages and protective clothing. This, along with freeing time for other purposes and alleviating concerns by non-project personnel, is seen as a positive attribute for the IPM program. We believe that this program has made a substantial contribution to our project and will have many long-term benefits.

Literature Cited

Hussey NL, Scopes N, eds. 1985. Biological pest control: The greenhouse experience. Ithaca, NY: Cornell University Press. 240 p.

Pedigo LP 1989. Entomology and pest management. New York: Macmillan. 646 p.

Parrella ML. 1990. Biological pest control in ornamentals: Status and perspectives. SROP/WPBS Bull. 13/5: 161-168.

Weinzierl R, Henn T 1991. Alternative in insect management: Biological and biorational approaches. North Central Reg. Ext. Publ. 401. Urbana, IL: University of Illinois Cooperative Extension Serrice. 73 p.

Acknowledgment

This research was carried out at the Department of Entomology, Iowa State University, Ames, Iowa.

Genotyping of Löngleaf Pine Ramets After Hurricane Hugo by Using DNA and Isozyme Markers

K. D. Jermstad, P A. Guge, E. R. Carroll, S. T Friedman, and D. B. Neale

Biologist, USDA Forest Service, Pacific Southwest Research Station, Institute of Forest Genetics, Placerville, California; biological laboratory technician and laboratory director, USDA Forest Service National Forest Genetic Electrophoresis Laboratory, Camino, California; research coordinator, USDA Forest Service, Resources Program and Assessment Staff, Washington, DC; and research plant molecular geneticist, USDA Forest Service, Pacific Southwest Research Station, Institute of Forest Genetics, Placerville, California

Isozyme and restriction fragment length polymorphism (RFLP) markers were used to determine the genetic identities of 12 longleaf pine (Pinus palustrus Mill.) ramets whose identities came into question after Hurricane Hugo. Isozyme assays were performed for 12 enzyme systems representing 15 loci. Variation at 6 loci revealed unique identities for 6 ramets. Four RFLP probes showed that 9 of the 12 ramets were genetically unique. This study confirms the power of isozyme analysis for determining genetic identity of seed orchard clones and, in addition, demonstrates the increased power of genetic discrimination offered by RFLP analysis. Tree Planters' Notes 44(4):157-160;1993.

In September 1989, Hurricane Hugo swept through the Atlantic seaboard, causing extensive damage to forests, seed orchards, and clone banks. The USDA Francis Marion Seed Orchard near Charleston, South Carolina, presented the National Forest Genetic Electrophoresis Laboratory (NFGEL) in Camino, California, with the challenge of determining the identities of 12 longleaf pine (*Pinus palustris* Mill.) ramets from a clone bank that could be used to replenish the longleaf pine clonal orchard that was damaged by the hurricane. It was unclear whether the 12 ramets were ramets from one clone, individual ramets from each of 12 clones, or something in between. It was known, however, that the clone bank ramets were not included in the damaged clonal orchard. NFGEL asked the molecular genetics laboratory at the Institute of Forest Genetics (IFG) in Placerville, California, to genotype the 12 unlabelled ramets using the restriction fragment length polymorphism (RFLP) technique to test the efficiency of DNA technology for solving practical genetic identity questions. For comparative purposes, isozyme assays were conducted by NFGEL on needle tissues from the same 12 ramets.

Isozyme genetic markers have been used extensively over the past decade for numerous applications in forest tree improvement, including paternity analysis, varietal identification, seed lot certification, and verification of controlled crosses (Adams 1981, 1983; Adams et al. 1988, Cheliak et al. 1987; Miller et al. 1989). Isozyme assays are relatively inexpensive and technically easy to perform on large samples and are thus the preferred marker in cases where they provide adequate discrimination.

DNA technology has developed rapidly in human forensic science in recent years. This technology is now being used for applications in agricultural science and forestry. The recent development of DNA marker provides a new tool to tree breeders that promises to increase the power of genetic discrimination (Nybom and Schaal 1990, Rogstad et al. 1988, Kreike et al. 1991, Neale and Williams 1991, Friedman and Neale 1993, Wagner 1992). Assays of DNA markers are more costly and time consuming to apply than isozyme assays but have several important advantages: (1) there are potentially a large number of DNA markers, (2) DNA assays can be performed on most tissue types, and (3) DNA markers are less affected by environmental variation. Restriction fragment length polymorphisms are simple Mendelian genetic markers that result from various types of mutations and rearrange ments of the DNA. These alterations are detected when the DNA is cleaved with restriction enzymes and the fragments of varying lengths are separated by electrophoresis in an agarose gel. The fragmented DNA is transferred to a nylon membrane and incubated in a solution containing a radioactively labeled DNA probe. Because of "hybridization" between the probe and filter-bound DNA, the location of the probe-specific fragments can be visualized using x-ray film (figure 1) (Neale and Williams 1991).

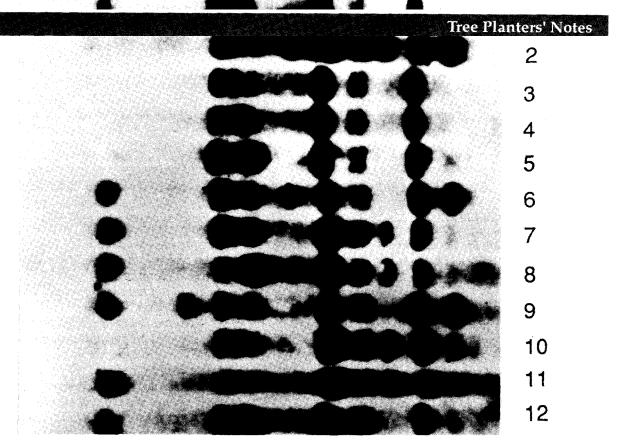


Figure 1—DNAs of 12 longleaf pine clone bank ramets of unknown genetic identities were cleaved with HindIII and hybridized with the loblolly pine RFLP probe pPtIFG669.

Materials and Methods

158

Needle tissue from 12 longleaf pine clone-bank ramets was sent by the USDA Forest Service's Francis Marion Seed Orchard in Charleston, South Carolina. The genetic relationship among the ramets was unknown.

RFLPs. *DNA isolation and blot preparation*. Needle tissue was ground to a coarse powder in liquid nitrogen and stored at - 70 /C. Total genomic DNA was extracted from 10 g dry-weight tissue using methods described by Devey et al. (1991). Two 10-μg samples of DNA from each ramet were digested with 50 units of either restriction enzyme *Hind*III or *Bam*HI and fractionated on 0.8% agarose gels submerged in 1 x (TAE) buffer. DNA was transferred to Zetaprobe GT nylon membrane (BioRad) using methods described by Reed and Mann (1985).

DNA probes and southern hybridizations. Four probes (pPtIFG: 669, 1628, 660, 1963) were selected from a loblolly pine cDNA library because they reveal poly-

morphism in loblolly pine and were known to hybridize with other *Pinus* species. Southern hybridizations were conducted using methods described by Devey et al. (1991).

Isozymes. Isozyme analyses were performed at NFGEL for 12 enzyme systems (DIA, GOT, GLY, SKD, ME, PGI, LAP, UGPP, PGM, IDH, MDH, GPGD) coded by 15 loci. Assays were performed on protein extracts of fresh needle tissues following standard methods (Adams et al. 1990, Conkle et al. 1982). Six loci (Skd, Pgi-2, Ugpp, Pgm-1, Mdh, 6-Pgd) were polymorphic among the 12 ramets, and allozyme genotypes at these loci were determined for each ramet.

Results

RFLP's. The RFLP genotypes of the 12 ramets are shown in table 1. Based on their multilocus genotypes, ramets 1, 2, 6, 7, 8, 9, 10, 11, and 12 had unique genetic identities. Probe pPtIFG669 revealed unique DNA band patterns among ramets 2, 6, 9, 10, and 11

Table 1-RFLP band pattern types in 12 longleaf pine clone-ban	nk ramets treated with 6 probe enzymes
---	--

	669	1628	1628	660	660	1963	1963	Multi-locus
Ramet	HindIII	BamHI	HindIII	BamHI .	HindIII	BamHI	HindIII	genotype
1	1	ND	1	1	1	1	1	Α
2	2	2	2	2	2	2	2	В
3	3	3	3	3	3	3	3	С
4	3	3	3	3	3	3	3	С
5	3	3	3	3	3	3	3	С
6	4	4	4	4	4	4	4	D
7	5	5	5	5	5	5	5	E
8	5	5	5	5	5	10	10	F
9	6	6	6	6	6	6	6	G
10	7	7	7	7	7	7	7	Н
11	8	8	8	8	8	8	8	I
12	1	1	1	1	9	9	9	J

ND= no data. Numbers are assigned to unique RFLP band pattern genotypes for each probe-enzyme combination. Letters are assigned to each multi-locus RFLP band pattern genotype.

when hybridized to DNA's restricted with HindIII (figure 1). The remaining 7 ramets were clustered into 3 distinct groups: ramets 1 and 12; ramets 3, 4, and 5; and ramets 7 and 8. Southern hybridization with probe pPtIFG1628 gave the same results as probe pPtIFG669. Probe pPtIFG660 revealed variation between ramets 1 and 12 when cleaved with HindIII. Lastly, probe pPtIFG1963 gave unique RFLP band patterns for ramets 7 and 8. Repeat hybridizations were conducted for confirmation of results. Ramets 3, 4, and 5 consistently revealed identical RFLP patterns.

Isozymes. Isozyme genotypes of the 12 ramets at the six polymorphic loci are presented in table 2. On the basis of their multilocus genotypes, ramets 1, 6, 7, 9, 10, and 12 had unique genetic identities. The remaining 6 ramets were clustered into two distinct groups: ramets 2, 3, 4, and 5; and ramets 8 and 11.

Discussion

This study reaffirms the power of isozymes for genetic analysis in tree populations but also demonstrates the added discrimination that can be achieved through the use of molecular markers, such as RFLP's. Isozyme analysis was able to resolve the identities of 6 of the 12 ramets; the other 6 ramets were classified into 2 groups of 2 and 4 ramets each. RFLP analysis, however, resolved identities for 9 of the 12 ramets; ramets 3, 4, and 5 had identical RFLP patterns for all probes. On the basis of the results of isozyme and RFLP analysis we conclude that ramets 1, 2, 6, 7, 8, 9, 10, 11, and 12 are genetically unique. Ramets 3, 4, and 5 appear to be from the same clone, but further analysis may reveal unique genetic identities.

Table 2-Isozyme genotypes of 12 longleaf pine clone-bank ramets

1 doic	2 isozymie genotypes o	1 12 longicui pine	cione bank ramets				
				Isozyme locus			Multi-locus
Ramet	Skd	Pgi-2	Ugpp	Pgm-1	Mdh	6-Pgd	genotype
1	22	11	11	12	11	11	A
2	11	11	12	11	11	12	В
3	11	11	12	11	11	12	C
4	11	11	12	11	11	12	C
5	11	11	12	11	11	12	C
6	12	12	12	11	11	11	D
7	11	11	11	11	12	12	E
8	11	11	11	11	11	12	F
9	11	12	12	11	11	11	G
10	14	11	11	11	11	12	Н
11	11	11	11	11	11	12	I
12	11	11	11	12	11	11	J

Numbers refer to diploid allozyme genotypes at each locus.

Information obtained from this genetic study enabled the USDA Forest Service's Francis Marion Seed Orchard to make practical management decisions

regarding genetic resources. Clonal material from the genetically differentiated longleaf pine ramets was included in the restoration of the clonal orchard that had been damaged by Hurricane Hugo. In addition, scions from the differentiated longleaf pine ramets

were grafted into a new clone bank, and open-pollinated seed has been collected for future use.

Summary

This study demonstrates the high level of genetic

discrimination that can be achieved using just a small number of RFLP probes and restriction enzymes. The genetics laboratory at IFG is attempting to identify additional DNA probes that reveal substantial levels of

genetic variability in the tree populations. If such diagnostic probes can be identified, then a cost-effective method for genotyping in tree populations could be devised. The Francis Marion Seed Orchard has demonstrated a useful application of information

obtained from analysis with RFLP markers for the purpose of genetic resource management.

Literature Cited

Adams WT. 1981. Applying isozyme analyses in tree-breeding programs. In: Conkle MT, tech. coord. Proceedings, Symposium on Isozymes of North American Forest Trees and Forest Insects; 1979 July 27; Berkeley, CA. Gen. Tech. Rep. PSW-48. Berkeley, CA:

USDA Forest Service, Pacific Southwest Forest and Range Experiment Station: 60-64.

Adams WT 1983. Application of isozyme in tree breeding. In: Tanksley SD, Orton TJ, eds. Isozymes in plant genetics and breeding. Amsterdam: Elsevier: 381-400. Adams WT, Neale DB, Loopstra CA. 1988. Verifying controlled crosses in conifer tree improvement programs. Silvae Genetica 37:147-152

- Adams WT, Neale DB, Doerksen AH, Smith DB. 1990. Inheritance and linkage of isozyme variants from seed and vegetative bud tissues in coastal Douglas-fir [Pseudotsuga menziesii var. menziesii (Mirb.) Franco]. Silvae Genetica 39:3-4.
- Conkle MT, Hodgskiss PD, Nunnally L, Hunter, SC. 1982. Starch gel electrophoresis in conifer seeds: A laboratory manual. Gen. Tech. Rep. 64. Berkeley, CA: USDA Forest Service, Pacific South west Forest and Range Experiment Station.
- Cheliak WM, Yeh FCH, Pitel JA. 1987. Use of electrophoresis in tree improvement programs. Forestry Chronicle (April): 89-96.
- Devey ME, Jermstad KD, Tauer CG, Neale DB. 1991. Inheritance of RFLP loci in a loblolly pine three-generation pedigree. Theoretical and Applied Genetics 83:238-242.
- Friedman ST, Neale DB. 1993. Biochemical and molecular genetic markers. In: Advances in pollen management. Agric. Handb. 698. Washington, DC: U.S. Department of Agriculture.
- Kreike J, Burg K, Zechmeister M, Haider T, Glossl J. 1991. DNA fingerprint and RFLP analysis as tools to study genetic diversity in populations of fir, spruce and oak. In: Muller-Starch G, Ziehe M. Proceedings, E-C Workshop on Genetic Variation of Forest Tree Populations in Europe; 1990 October 9-11; Gdttingen, Germany. Frankfurt am Main: Sauerlander's Verlag.
- Miller RG, Conkle MT, Friedman ST 1989. The Forest Service laboratory for genetic analysis of trees. Tree Planters' Notes 40:25-29.
- Neale DB, Williams, CG. 1991. Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. Canadian Journal of Forestry Research 21:545-553.
- Nybom H, Schall BA. 1990. DNA "fingerprints" applied to paternity analysis in apples (*Malus x domestica*). Theoretical and Applied Genetics 79:763-768.
- Reed KC, Mann DA. 1985. Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Research 13:7207-7221.
- Rogstad SH, Patton JC II, Schaal BA. 1988. M13 repeat probe detects DNA minisatellite-like sequences in gymnosperms and angiosperms. Proceedings of the National Academy of Science

USA 85:9176-9178.

Wagner DB. 1992. Nuclear, chloroplast, and mitochondrial DNA polymorphisms as biochemical markers in population genetic analyses of forest trees. New Forests 6:373-390.

Sodium Metabisulfite Reduces Fungal Inoculum in Containers Used for Conifer Nursery Crops

R. Kasten Dumroese, Robert L. James, and David L. Wenny

Research associate, Forest Research Nursery, Department of Forest Resources, University of Idaho, Moscow, Idaho; plant pathologist, USDA Forest Service, Northern Region Timber, Cooperative Forestry, and Pest Management, Coeur d'Alene, Idaho; and professor/manager, Forest Research Nursery, Department of Forest Resources, University of Idaho

Containers from eight nurseries in the northern Rocky Mountains were treated with a 5% (wlv) solution of sodium metabisulfite. In general, after being used for at least one crop, Styroblock containers were more contaminated than pine cells before and after treatment. Treatment significantly reduced levels of both Fusarium and other groups of fungi. Seedling disease levels were usually reduced in crops grown in treated containers; Douglas-fir (Pseudotsuga menziesii var. glauca [Beissn.] Franco) seedling growth was otherwise unaffected by the treatment. Tree Planters' Notes 44(4): 161-165;1993.

Fusarium root disease is a serious problem in the production of container-grown seedlings, especially Douglas-fir (Pseudotsuga menziesii var. glauca (Beissn.) Franco) (James 1986). Losses to disease vary with seed source and individual nursery, ranging from a few percent to over 70%. Control of this disease is best realized through integrated pest management (James and others 1990). Reducing exposure to Fusarium inoculum is paramount to reducing infection and subsequent disease (James and others 1991). Inoculum has been associated with seeds, growing media, containers, and weeds (James and others 1987, James and others 1991). After several crops, Styrofoam®-and to a lesser degree, hard plastic containers-develop cracks and holes in which root pieces and organic matter collect. Seedling roots often penetrate the inner walls of Styroblock containers and remain embedded when the seedlings are extracted. This residue, especially at the bottom of cells, harbors Fusarium inoculum (James and others 1988a, James and Woollen 1989) and is often the largest inoculum source for new crops of seedlings (James and others 1988b).

Sodium metabisulfite (Na₂S₂O₅) is used as an antifermenting agent in beer and winemaking and as a preservative for fruits and vegetables (Sturrock and Dennis 1988). When mixed with water, sodium metabisulfite (SMBS) releases sulfur dioxide (SO₂), which is toxic to many fungi (Hibben 1966). Therefore, treatment with this chemical may reduce inoculum of pathogenic fungi within growing containers.

Our main objective in this study was to determine whether SMBS could effectively reduce levels of inoculum of potential root-pathogenic fungi without adversely affecting seedling growth.

Materials and Methods

Uncleaned containers, previously used to grow conifer seedlings, were provided by eight forest seedling nurseries in the northern Rocky Mountains. Four nurseries each provided eight trays of Ray Leach Pine Cells® (200 cells per tray) while the other four nurseries each provided eight Styroblock 4's (160 cells per block). Before treatment, all containers were assayed for Fusarium, Cylindrocarpon, Trichoderma, and other fungi by aseptically removing two pieces of container, each about 10 mm long, 5 mm wide, and 1 mm thick. from near the bottom drainage hole of 10 randomly selected cells per container. Pieces were placed on a selective medium for Fusarium and closely related fungi (Komada 1975) and incubated under cool fluorescent light at 22 to 24 /C for 7 to 10 days. Selected isolates of Fusarium were single-spored onto both carnation leaf (Fisher and others 1982) and potato dextrose agar for identification using the taxonomic methods of Nelson and others (1983).

Four pine cell trays and four Styroblocks were completely immersed for 30 sec in a 5% (w/v) solution of SMBS and allowed to dry uncovered because the growers who requested this work were interested in using this chemical as a dip, without the additional expense of tarps and tarping. Once dry, the containers were again assayed using the above procedure; the same cells previously sampled were once again evaluated for the presence or absence of fungi.

To test effects of container treatment with SMBS on seedling growth, two seedlots of Douglas-fir from northern Idaho (8002 and Bovill) were surface sterlized in a solution of 2 parts laundry bleach (5.25% sodium hypochlorite) with 3 parts water for 10 min (Wenny and Dumroese 1987), rinsed in running tap water for 48 h, and stratified for 28 days at

3 /C. After stratification, the seeds were rinsed for 24 h in running tap water and sown into treated and untreated containers filled with a 50:50 peat-vermiculite growing medium commonly used by container nurseries in the northern Rocky Mountains. Each treated and untreated container had half of its cells sown with the Bovill seed source and the other half with 8002. Containers were placed on tables in a randomized block design. Seedlings were grown under the regime of Wenny and Dumroese (1992). One month after planting, seedlings were fertilized and irrigated twice weekly with Peter's Conifer Starter® at 42 ppm N for 4 weeks, followed by twice weekly applications of Peter's Conifer Grower® at 120 ppm N alternated with liquid calcium ammonium nitrate at 81 ppm N for 6 weeks. Seedlings were then fertilized only when irrigation was necessary and received Peter's Conifer Finisher® at 24 ppm N alternated with liquid calcium ammonium nitrate at 161 ppm N until seedlings were extracted for cold storage.

Seedling emergence (based on germination of handsown seed within individual container cells) was calculated after germination was deemed complete. Postemergence damping-off and root disease were periodically monitored throughout the growth cycle. Diseased seedlings were collected and isolations made from their roots to identify associated fungi. Thirty randomly selected seedlings from each treatment were measured for height and root collar diameter (caliper) at the end of the growth cycle, about 7 months after sowing. These same seedlings were assayed for root colonization by potentially pathogenic fungi. Isolations were made from randomly selected, surface-sterilized root tips onto Komada's medium and incubated as described above. Selected *Fusarium* isolates from roots were identified. Biomass was determined on a sub sample of 15 seedlings after drying for 24 h at 60 /C.

Cell colonization before and after treatment was compared using the non-parametric test of Kruskal Wallis (Ott 1984). Means were converted to percentages after the analysis of variance. Diseased and non-diseased seedlings and seedling morphological characteristics were statistically tested with a one-way analysis of variance (Snedecor and Cochran 1980) and means separated using Tukey's honestly significant different test at P = 0.05.

Results and Discussion

Immersing containers in SMBS significantly increased the percentage of clean cells (those not colonized by fungi) in both Styroblock and plastic containers. Styroblock containers had fewer clean cells before and after treatment when compared to pine cells (table 1). However, Styroblocks also had the highest levels of *Trichoderma*, a known *Fusarium* antagonist (Papavizas 1985), before and after the treatment. Percentage of cells colonized by *Fusarium* spp. was significantly decreased by the treatment for both con tainer types. However, the inoculum level remaining in containers was higher than that reported by Sturrock and Dennis (1988). Their lower levels of inoculum may have been a function of enclosing the containers after treatment. Peterson (1990), using a 5% solution

Table 1 -Percentage of cells in each container type colonized with Fusarium, Cylindrocarpon, Trichoderma, and other fungi before and after immersion in sodium metabisulfite (5%)

Container type and	Fusarium	Cylindrocarpon	Trichoderma	Other fungi	Clean cells*
treatment	(%)	(%)	(%)	(%)	(%)
Ray Leach Pine Cells		•	• • •	, ,	
Before	17.9 a	4.0 a	34 a	67 a	39 a
After	7.5 b	0.0 a	31 a	29 b	68 b
Styroblocks					
Before	41.2 a	13.0 a	55 a	35 a	2 a
After	20.8 b	15.0 a	56 a	32 a	35 b
All containers					
Before	29.8 a	8.7 a	45 a	51 a	20 a
After	14.2 b	7.5 a	43 a	30 b	51 b
Pine Cells vs. Styroblocks					
Pine Cells before	17.9 a	4.0 a	34 a	67 a	39 a
Styroblocks before	41.2 b	13.0 b	55 b	35 b	2 b
Pine Cells after	7.5 a	0.0 a	31 a	29 a	68 a
Styroblocks after	20.8 b	15.0 b	56 b	32 a	35 b

Means were converted to percentages after being compared with the Kruskal-Wallis test. For each container type, values in each column followed by the same letter are not significantly different at P #0.05.

^{*}Cells without fungal or bacterial growth.

of SMBS and a 10-sec dip, effectively removed *Fusar*ium spp. and other fungi by promptly enclosing containers with plastic. Containment of treated containers probably allows for a longer and more concentrated fumigation effect. The most frequently isolated species of *Fusarium* from containers was *F. proliferatum* (Matsushima) Nirenberg. Other isolated species included *F. oxysporum* Schlechtend.:Fr., *F. acuminatum* Ellis & Everh., *F. sporotrichioides* Sherb., and *F. tricinctum* (Corda) Sacc.

The percentage of cells colonized by other fungi was also significantly decreased by treatment with SMBS, indicating that the chemical was toxic to several different groups of fungi. Levels of *Trichoderma* and *Cylindrocarpon* on containers were unaffected. Species of *Trichoderma* were unidentified but most isolates of *Cylindrocarpon* were C. *destructans* (Zinssmeister) Scholten.

Table 2 shows the variation between inoculum levels in containers from different nurseries and subsequent inoculum reduction by the treatment. There was extensive disparity in container cleanliness among the nurseries sampled. Much of this disparity reflected container age-i.e., those containers that were older and had been used to grow several seedling crops (evident because of wear and deterioration of the cells) were colonized with all types of fungi at much higher levels than newer containers reused fewer times.

Seedling emergence was unaffected by treatment (data not shown), and levels of post-emergence disease were very low for both treatments (table 3). Significant reductions in number of diseased seedlings were observed in the Douglas-fir crops grown in SMBS-treated pine cells. Similar trends were noticed in Styroblock containers, with P=0.12. Although the number of diseased seedlings were similar in treated and control containers, initial container inoculum was significantly reduced. When data from all containers were pooled, the number of diseased seedlings were significantly reduced in SMBS-treated containers.

Table 3-Effect of treating containers with sodium metabisulfite (5%) on the number of post-emergent diseased Douglas-fir seedlings throughout the growth circle

Container type and	Diseased seedlings		
treatment	n	%	
Ray Leach Pine Cells	10 a	1.2	
Treated	16 b	2.0	
Untreated			
Styroblocks	24 a	3.8	
Treated			
Untreated	32 a	5.0	
All Containers	34 a	2.4	
Treated	48 b	3.3	
Untrooted a			

Because of low disease levels within each tray or block, data from each treatment/container-type combination were pooled prior to analysis. For each container type, values in each column followed by the same letter are not significantly different in P# 0.05.

Table 2 -Percentage of cells colonized with Fusarium, Cylindrocarpon, and Trichoderma spp. in containers from 8 nurseries before and after treatment with sodium metabisulfite (5%)

Container type and		Fusarium	Cylindrocarpon	Trichoderma	Clean cells*
nursery Treatment		(%)	(%)	(%)	(%)
Ray Leach Pine Cells					
1 Before		0 a	5 a	55 a	60 a
	After	0 a	0 a	40 a	70 a
2 Before		8 a	2 a	3 a	43 a
	After	0 a	0 a	3 a	97 b
3 Before		0 a	7 a	23 a	52 a
	After	O a	O a	10 a	97 b
4 Before		64 a	3 a	55 a	0 a
	After	30 b	0 a	70 a	7 b
Styroblocks					
5 Before		77 a	2 a	55 a	0 a
	After	33 b	10 a	80 b	10 b
6 Before		13 a	5 a	35 a	3 a
	After	3a	3a	17a	87b
7 Before		3 a	40 a	88 a	3 a
	After	7 a	40 a	73 a	20 b
8 Before		73 a	7 a	42 a	0 a
	After	40 b	7 a	53 a	23 b

Means were converted to percentages after being compared with the Kruskal-Wallis test. For each nursery, values in each column followed by the same letter are not significantly different at P # 0.05.

^{*}Cells without fungal or bacterial growth.

Root colonization of non-diseased seedlings by selected fungi is summarized in table 4. Significant differences between seedlings grown in SMBS-treated containers and those grown in untreated containers were not apparent for root colonization by Fusarium, Cylindrocarpon, and Trichoderma spp. Most sampled seedlings were colonized by Fusarium spp., whereas about one-third were colonized by Cylindrocarpon spp. and nearly one-half by Trichoderma spp. The percent age of infected seedlings was probably influenced and confounded by inoculum from other sources than the containers, including seed and airborne additions. For both treatments and seedlots, about 50% of the root tips sampled were infected with Fusarium (data not shown). The most common Fusarium and Cylindrocar pon species colonizing roots were the same recovered from containers: F. proli feratum and C. destructans. Fusarium proliferatum was also the most commonly iso lated fungus from diseased seedlings. We believe this species is capable of extreme virulence on Douglas-fir seedlings (unpublished data) and may also commonly colonize seedling roots without eliciting disease symp toms. It is readily adapted to greenhouse environ ments and is a common colonizer of roots of many different conifer species. Unlike several other species of Fusarium, E proliferatum does not produce resistant chlamydospores or sclerotia (Nelson and others 1983), which aid in carrying the fungus between crops of susceptible seedlings. However, it (along with most Fusarium species) is able to remain viable for extended periods on both Styroblock and pine cell containers, as verified by this and other investigations (James and others 1988a, Sturrock and Dennis 1988).

Table 4-Effect of container treatment with sodium metabisulfite (5%) on percentage of non-diseased seedlings with Fusarium, Cylindrocarpon, or Trichoderma spp. colonizing their root systems

Fusarium	Cylindrocarpon	Trichoderma
Treatment (%)	(%)	(%)
Treated 95 a	37 a	40 a
Untreated 92 a	35 a	42 a

Percentages compiled for both pine cell and Styroblock containers at all nurseries. Values in each column followed by the same letter are not significantly different at P # 0.05.

Seedling height, caliper, and ovendry weight were unaffected by SMBS treatment (table 5). However, seedlings from the Bovill seedlot in SMBS-treated containers were significantly taller than those in untreated containers. Apparently, residual SMBS on the containers was non-phytotoxic to Douglas-fir seedlings. This confirms work by Peterson (1990), who did not find phytotoxicity on sensitive lettuce plants in treated

Table 5-Effects of container treatment with sodium metabisulfite (5%) on mean height, caliper, and ovendry weight at lifting of Douglas-fir seedlings

Seedlot and treatment	Height	Caliper	Ovendry weight	
	(cm)	(mm)	(g)	
Bovill				
Treated	18.3 a	2.25 a	1.33 a	
Untreated	17.1 b	2.16 a	1.25 a	
8002				
Treated	16.6 a	2.12 a	1.26 a	
Untreated	16.4 a	2.18 a	1.22 a	
Seedlots combined				
Treated	17.5 a	2.19 a	1.30 a	
Untreated	16.7 a	2.17 a	1.23 a	
For each condict values in each	a column followed by t	ha cama lattar ara r	at algorificantly	

For each seedlot, values in each column followed by the same letter are not significantly different at P # 0.05.

Management Implications

Our results indicate that SMBS was effective in reducing levels of several different groups of fungi, including potential pathogens in the genus Fusarium, on both pine cells and Styroblock containers. However, Fusarium propagules were not completely eliminated from the containers, indicating that this treatment is less effective in removing inoculum than immersing containers in hot water (James and Woollen 1989). Residual levels of pathogenic fungi following SMBS treatment were sufficient to cause some disease and seedling root infection during the crop cycle. However, effects on seedling growth as measured by height, caliper, and biomass production were usually undiscernible. We found that Douglas-fir seedlings with low levels (10 to 40%) of Fusarium infection on their roots grow and survive as well as uninfected seedlings after outplanting on a forest site (Dumroese and others 1993). Other concentrations and submersion times may improve the efficacy of SMBS treatments.

The major disadvantages of using SMBS commercially in container nurseries involve potential health risks to workers and environmental concerns involved in disposal after use. Workers should avoid skin exposure to SMBS by using chemical goggles and rubber boots, gloves, and aprons. Even with such protective clothing, some workers may show hypersensitivity to the sulfites produced in SMBS solutions. Because of the extreme corrosiveness of SMBS solution, it should only be used in stainless steel, plastic, or fiberglass tanks. Disposal problems can be reduced by adjusting the pH of the solution to neutral, separating any insoluble liquids or solids for hazardous-waste disposal, and flushing the aqueous solution down the drain with plenty of fresh water (Peterson 1991).

We conclude that SMBS is effective in reducing levels of potentially pathogenic fungi on Styroblock and plastic containers used in conifer seedling nurseries.

Treatment with SMBS may especially be desirable if equipment is unavailable for immersing large numbers of containers in hot water solutions. Tarping treated containers immediately after dipping may enhance the fumigation effect. Whichever system is used, it is

important that containers used to grow several crops of seedlings are sterlized between crops.

Acknowledgments

We thank the nurseries that participated in this research: Bitterroot Native Growers, Hamilton, Montana; Champion International, Plains, Montana; North Woods Nursery, Inc., Elk River, Idaho; Plum Creek Timber Company, Pablo, Montana; Raintree Nursery, Libby, Montana; University of Idaho Forest Research Nursery, Moscow, Idaho; USDA Forest Service, Coeur d'Alene Nursery, Coeur d'Alene, Idaho; and Western Forest Systems, Inc., Lewiston, Idaho.

Literature Cited

- Dumroese RK, James RL, Wenny DL. 1993. Fusarium root infection of container-grown Douglas-fir: Effect on survival and growth of outplanted seedlings and persistence of the pathogen. New Forests 7:143-149.
- Fisher NL, Burgess LW, Toussoun TA, Nelson PE. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151-153.
- Hibben CR. 1966. Sensitivity of fungal spores to sulfur dioxide and ozone. Phytopathology 56:880-881.
- James RL. 1986. Diseases of conifer seedlings caused by seedborne Fusarium species. In: Shearer RC, ed. Proceedings, Conifer Tree Seed in the Inland Mountain West Symposium; August 5-6, 1985; Missoula, MT. Gen. Tech. Rep. INT-203. Ogden, UT: USDA Forest Service Intermountain Research Station: 267-271.
- James RL, Woollen RL. 1989. An evaluation of the efficacy of hot water-chemical treatments to clean Styroblock containers-Champion Timberlands Nursery, Plains, Montana. Rep. 89-5. Missoula, MT: USDA Forest Service, Timber, Cooperative Forestry and Pest Management Northern Region.
- James RL, Dumroese RK, Wenny DL, Myers JF, Gilligan CJ. 1987.
 Epidemiology of Fusarium on containerized Douglas-fir seedlings.
 Seed and seedling infection, symptom production, and disease progression. Rep. 87-13. Missoula, MT: USDA Forest Service Northern Region.
- James RL, Dumroese RK, Wenny DL. 1988a. Occurrence and persistence of Fusarium within Styroblock and Ray Leach containers.
 In: Landis TD, tech. coord. Proceedings, Combined Meeting of the Western Forest Nursery Associations; August 8-11, 1988; Vernon, BC. Gen. Tech. Rep. 167. Ft. Collins, CO: USDA Forest Service Rocky Mountain Forest and Range Experiment Station: 145

- James RL, Gilligan CJ, Reedy V 1988b. Evaluation of root diseases of containerized conifer seedlings at the Champion Timberlands Nursery, Plains, Montana. Rep. 88-7. Missoula, MT: USDA Forest Service, Timber, Cooperative Forestry and Pest Management Northern Region.
- James RL, Dumroese RK, Wenny DL. 1990. Approaches to integrated pest management of *Fusarium* root disease in container grown seedlings. In: Rose R, Campbell SJ, Landis TD, eds. Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations; 1990 August 13-17; Roseburg, Oregon. Gen. Tech. Rep. 200. Ft. Collins, CO: USDA Forest Service Rocky Mountain Forest and Range Experiment Station: 240-246
- James RL, Dumroese RK, Wenny DL. 1991. Fusarium disease of conifer seedlings. In: Sutherland JR, Glover SG, eds. Proceedings, First Meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). 1990 August 23-30; Victoria, BC. Info. Rep. BC-X-331. Victoria, BC: Forestry Canada, Pacific Forestry Centre: 181-190.
- Komada H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research 8:114-125.
- Nelson PE, Toussoun TA, Marasas, WFO. 1983. *Fusarium* species: An illustrated manual for identification. University Park, PA: Pennsylvania State University Press. 193 p.
- Ott L. 1984. An introduction to statistical methods and data analysis, 2d ed. Boston: Duxbury Press. 676 p.
- Papavizas GC. 1985. Trichoderma and Gliocladium: Biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-54
- Peterson M. 1990. Sanitation of Styroblocks to control algae and seedling root rot fungi. FRDA Rep. 140. Victoria, BC: Forestry Canada and the British Columbia Ministry of Forests.
- Peterson M. 1991. Guidelines for the sanitation of nursery seedling containers. FRDA Rep. 140. Suppl. Victoria, BC: Forestry Canada and the British Columbia Ministry of Forests.
- Snedecor GW, Cochran WG. 1980. Statistical methods, 7th ed. Ames, IA: Iowa State University Press.
- Sturrock RN, Dennis JJ. 1988. Styroblock sanitization: Results of laboratory assays from trials at several British Columbia nurseries. In: Landis TD, tech. coord. Proceedings, Combined Meeting of the Western Forest Nursery Associations; 1988 August 8-11; Vernon, BC. Gen. Tech. Rep. 167. Ft. Collins, CO: USDA Forest Service Rocky Mountain Forest and Range Experiment Station: 149-154
- Wenny DL, Dumroese RK. 1987. Germination of conifer seeds surface sterilized with bleach. Tree Planters' Notes 38(3):18-21.
- Wenny *DL*, Dumroese RK. 1992. A growing regime for container grown Douglas-fir seedlings. Idaho For. Wildl. Range Exp. Bull. 49. Moscow, ID: University of Idaho.

Survival and Growth of Trees and Shrubs on Different Lignite Minesoils in Louisiana

James D. Haywood, Allan E. Tiarks, and James P Barnett

Silviculturist, principal soil scientist, and project leader, USDA Forest Service Southern Forest Experiment Station, Pineville, Louisiana

In 1980, an experimental opencast lignite mine was developed to compare redistributed A horizon with three minesoil mixtures as growth media for woody plants. The three minesoil mixtures contained different amounts and types of overburden materials, and normal reclamation practices were followed. Loblolly pine (Pinus taeda L.), sawtooth oak (Quercus acutissima Carruthers), yaupon (Ilex vomitoria Ait.), Amur honeysuckle (Lonicera maackii Maxim.), water oak (Q. nigra L.), white oak (Q. alba L.), longleaf pine (P palustris Mill.), and Osage-orange (Maclura pomifera (Raf.) Schneid.) were planted in each reclaimed soil. Survival and growth of all eight species were good on all soils. Therefore, replacement of the A horizon is not always necessary to satisfactorily revegetate lignite minesoils. Tree Planters' Notes 44(4): 166-171;1993.

Under the 1977 Surface Mining and Reclamation Act (PL. 95-87), the reclamation of lands stripped for lignite involves replacing the overburden (the material overlying a useful mineral deposit) to return the land to approximately its original contour, restoring the land to its original productivity, and reestablishing its original plant cover (Torbert and Burger 1990). Rapid establishment of ground cover is important for protecting newly contoured slopes from erosion, and woody species used for reclamation must tolerate this ground cover and be adapted to growing in the reclaimed soil (Torbert and others 1985).

Capping the overburden with the A horizon (the topsoil) aids in rapid establishment of ground cover and benefits tree growth (McGinnies and Nicholas 1983). However, there are two drawbacks to using the A horizon. Separately stockpiling A horizon material is an added expense, and the A horizon may be too shallow and difficult to recover or have such poor physical properties that its recovery is not practical or desirable.

Therefore, the objective of our research was to determine if A horizon restoration was necessary on lignite mine lands in Louisiana, or if establishing selected tree and shrub species on several overburden mixtures would be sufficient. To study this objective, an experimental opencast lignite mine was operated, closed, and reclaimed in northwestern Louisiana in 1980.

Methods

Study site. The study site is in DeSoto Parish, Louisiana. Before mining, the soils were in the Woodtell (fine, montmorillonitic, thermic Vertic Hapludalfs) and Metcalf (fine-silty, siliceous, thermic Aquic Glossudalfs) series. These are moderately well to somewhat poorly drained, very slowly permeable soils formed in gently sloping to nearly level Coastal Plain uplands. These soils are best suited to growing forest crops but may be converted to pasture. The typical native vegetation is loblolly pine (*Pinus taeda* L.), shortleaf pine (*P echinata* Mill.), southern red oak (*Q. falcata* Michx.), and post oak (*Q. stellata* Wangenh.). The site is nearly level.

Stockpiles. During mining in 1980, soil material was extracted from different depths, segregated, and stored in seven stockpiles (SP1 through SP7) (figure 1):

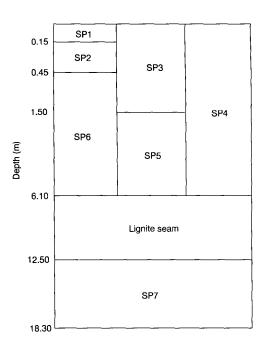


Figure 1-Depths from which the different stockpiles (SP1 through SP7) were extracted. The depths given in the figure are not to scale, and the lignite seam may have varied in thickness.

- SP1 A horizon -the upper 0.15 m (0 to 0.5 feet) of the soil profile. Texture is sandy loam, available water-holding capacity is 0.07 kg/kg of soil (0.07 pounds per pound of soil), and total sulfur content is 0.3 g/kg of soil (0.005 ounces per pound of soil).
- SP2 *B horizon* -the 0.15- to 0.45-m (0.5- to 1.5-foot) layer of the soil profile. Texture is clay, available water-holding capacity is 0.11 kg/kg of soil (0.11 pounds per pound of soil), and total sulfur content is 0.2 g/kg of soil (0.003 ounces per pound of soil).
- SP3 A, B, and C horizons -the upper 1.5 m (0 to 5 feet) of the soil profile containing 10% A horizon by volume, and the remainder is B and C horizon material. Texture is clay, available water holding capacity is 0.11 kg/kg of soil (0.11 pounds per pound of soil), and total sulfur content is 0.1 g/kg of soil (0.002 ounces per pound of soil).
- SP4 0 to 6.1 m overburden -the upper 6.1 m (0 to 20 feet) of the soil profile containing 2.5% o A horizon material by volume. Texture is clay loam, available water-holding capacity is 0. 07 kg/kg of soil (0.07 pounds per pound of soil), and total sulfur content is 0.2 g/kg of soil (0.003 ounces per pounds of soil).
- SP5 Clay loam subsoil overburden -the 1.5- to 6.1-m (5 to 20-foot) layer of the soil profile. Texture is clay loam, available water-holding capacity is 0.06 kg/kg of soil (0.06 pounds per pound of soil), and total sulfur content is 0.5 g/kg of soil (0.008 ounces per pound of soil).
- SP6 Sandy clay loam overburden -the 0.45- to 6.1-m (1.5-to 20-foot) depth in the soil profile. Texture is sandy clay loam, available water-holding capacity is 0.07 kg/kg of soil (0.07 pounds per pound of soil), and total sulfur content is 0.2 g/kg of soil (0.003 ounces per pound of soil).
- SP7 Deep loamy sand -the layer was at a depth of 12.5 to 18.3 m (41 to 60 feet), which was below the lignite. Texture is loamy sand, available water-holding capacity is 0.05 kg/kg of soil (0.05 pounds per pound of soil), and total sulfur content is 0.2 g/kg of soil (0.003 ounces per pound of soil).

Soil samples from each stockpile were analyzed at the Louisiana State University Soil Analysis Laboratory, Baton Rouge, LA. Based on these chemical analyses, ammonium nitrate and triple superphosphate were applied to all stockpiles at the minimum rates recommended by the laboratory. Rates were different for each stockpile, reflecting the different nutritional status of each overburden mixture. However, the rates per unit volume of overburden were not determined. Muriate of potash was also added to the SP7 stockpile. Each stockpile was seeded with Pensacola bahiagrass (*Paspalum notatum* Fluegge) and common Bermuda grass (*Cynodon dactylon* (L.) Pers.) and mulched with straw.

Plot installation, fertilization, and plantings. The mine was closed in winter 1980, and the pit was backfilled to within 3.05 m (10.0 feet) of the final, leveled surface. The pit area was divided longitudinally into four parallel east-west strips, each 13.7 m (45.0 feet) in width and 88.4 m (290 feet) in length (figure 2).

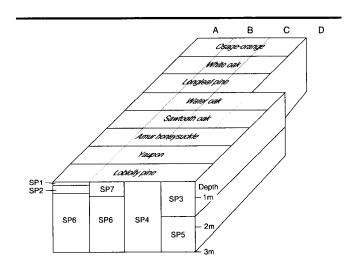


Figure 2-Placement of stockpiled minesoils (SP1 through SP7) in strips (A through D) and species rankings.

The northernmost strip (A) was backfilled with 2.6 m (8.5 feet) of SP6 overburden, overlaid with 0.3 m (1.0 foot) of the SP2 material, and surfaced with 0.15 m (0.5 foot) of SP1 material (figure 2). Strip B consisted of 2.45 m (8.0 feet) of SP6 overburden covered by 0.6 m (2.0 feet) of the SP7 material. Strip C was entirely filled with SP4 overburden. Strip D consisted of 1.5 m (5.0 feet) of SP5 overburden covered by 1.5 m (5.0 feet) of SP3 material. Strip D was 13.0 m (43.0 feet) short on the east end because it contained the access ramp for backfilling.

Therefore, the stockpiles in strip A were returned to the pit in the same order in which they were extracted, resulting in the restoration of the A horizon (figures 1 and 2). In strip B, the loamy sand from beneath the lignite seam replaced the A and B horizons. Strip C was a mixture of all soil horizons from a depth of 0 to 6.1 m, and this mixture contained 2.5% A-horizon material. Strip D was a coarse attempt to restore the original soil structure by returning to the pit a

mixture of the upper 1.5 m of soil material (an aggregate of the A, B, and C horizons) overlying a mixture of the soil material found at a 1.5 to 6.1 m depth.

In 1981, lime and fertilizer were applied uniformly along each strip, but at a different rate for each strip (Burton and Tiarks 1986). A native grass, carpetgrass (*Axonopis affinis* Chase), was seeded over the entire test pit, but common Bermuda grass and Pensacola bahiagrass quickly reestablished and formed a vigorous stand, overtaking the carpetgrass.

Woody species were hand-planted in pure stands at a 0.9- by 0.9-m (3- by 3-foot) spacing (11,960 stems/ha or 4,840 stems/acre). The planting rows ran perpendicular to the backfilled strips (A through D), and each species-strip combination formed a plot (figure 2). Therefore, species were not randomized or replicated. The planting scheme resulted in 24 rows of 15 plants (360 plants/plot) of loblolly pine or sawtooth oak (Quercus acutissima Carruthers), and 8 rows of 15 plants (120 plants/plot) of yaupon (*Ilex vomitoria* Ait.), Amur honeysuckle (Lonicera maackii Maxim.), water oak (Q. nigra L.), longleaf pine (P palustris Mill.), white oak (Q. alba L.), or Osage-orange (Madura pomifera (Raf.) Schneid.) per species-strip combination. The backfilling ramp precluded the plots at the end of strip D.

Not all species were planted in the same year because of poor seedling quality, delays in obtaining planting stock, poor success at stand establishment, and species replacement (Burton and Tiarks 1986). However, between 1981 and 1986, the following species were successfully established in terms of survival and early growth: loblolly pine and sawtooth oak in February 1981; water oak, Amur honeysuckle, and yaupon in January 1982; white oak in January 1983; longleaf pine in May 1985; and Osage-orange in October 1986 (figure 2). Plantings were made with 1 + 0bareroot seedlings, except for yaupon, longleaf pine, and Osage-orange. The yaupon seedlings were grown in peat pots by a commercial nursery. The longleaf pine and Osage-orange seedlings were grown in containers by USDA Forest Service personnel.

All plots were irrigated frequently in 1981 and 1982. All the planted trees and shrubs were quickly overtopped by herbaceous plants, and frequent mowing was necessary.

Foliage samples were collected from the loblolly pine, sawtooth oak, and water oak plots in August 1983. Nitrogen (N), phosphorus (P), and potassium (K) concentrations were determined on a Kjeldahl digest by ammonium probe, by colorimetry, and by atomic absorption, respectively (Burton and Tiarks 1986). Nitrogen was applied as ammonium nitrate to all plots at 56 kg/ha (50 pounds per acre) in May 1984

and 28 kg/ha (25 pounds per acre) in May 1985. Foliage samples were collected from loblolly pine, sawtooth oak, and water oak again in August 1992 to determine N, P, and K concentrations.

In April 1984, loblolly pine and sawtooth oak numbers were reduced (by thinning) from 360 plants/plot to 40 loblolly pines/plot or 180 sawtooth oaks/plot (from 11,960 plants/ha to 1,329 pine and 5,980 oak/ha or from 4,840 plants/acre to 539 pine and 2,420 oak/acre). Individual plants among the other established species were too small to warrant thinning in 1984.

Tree and shrub measurements. Each plot (or species-strip combination) was inventoried periodically through March 1990. Plot results were based on the center 32 loblolly pines, 99 sawtooth oaks, and 66 plants for each of the other six species. The other plants were in the unmeasured border area of each plot. Because of the differences in planting dates, the most recent measurements were taken after nine growing seasons for loblolly pine and sawtooth oak; eight growing seasons for yaupon, Amur honey suckle, and water oak; seven growing seasons for white oak; five growing seasons for longleaf pine; and three growing seasons for Osage-orange. Survival was determined, total height was measured, and percent age of crown cover was estimated for each species strip combination. Diameter at breast height (dbh) was measured on the loblolly pines, and groundline diameter was measured on all other species. Statistical analysis was not performed because there was no replication; all comparisons and interpretations are subjective.

Results and Discussion

Tree and shrub survival and growth. In terms of height and diameter growth, loblolly pines were the largest trees on all four strips (A through D). First year survival of loblolly pine ranged from 63 to 90% across the four strips, probably because of the initial irrigation. No mortality occurred in the second and third growing seasons. Few of the remaining loblolly pines died after thinning to a stocking of 1,329 trees/ ha (538 trees/acre) at age 3, and among the remaining trees, survival averaged 98% across the four strips at age 9 (table 1). Loblolly pine trees were no larger on strip A than on strip D. Nine-year-old loblolly pine height and quadratic mean dbh averaged 9.0 m (29.6 feet) and 15.4 cm (6.0 inches), respectively, across the four strips. Plot canopies were not closed at age 9 be cause of the earlier thinning treatment.

Longleaf pine was planted 4 years after loblolly pine. Because longleaf pine has an extended "grass" stage and its plots were not irrigated, the longleaf Fall 1993 . 169

Table 1-Percent survival, heights, diameters, and crown cover by species, date, and overburden mixture*

Lignite minesoil strips Species and variables Α В С D Growing season Loblolly pine 9th Survival (%) 96 100 100 97 9.1 9.0 9.3 8.7 Height (m) Diameter (cm) 16.3 14.5 14.3 16.3 Crown cover (%) 45 45 45 45 9th Sawtooth oak Survival (%) 95 93 94 93 Height (m) 5.1 1.7 2.2 4.5 Diameter (cm) 6.8 3.4 3.8 6.2 100 90 100 100 Crown cover (%) Water Oak 8th Survival (%) 67 88 85 85 Height (m) 3.2 3.1 3.0 4.2 5.9 Diameter (cm) 4.4 3.9 3.8 Crown cover (%) 80 90 80 100 Amur honeysuckle 8th 92 27 89 Survival (%) 94 Height (m) 2.1 0.4 1.0 1.4 ND Diameter (cm) ND ND ND Crown cover (%) 100 5 60 80 8th Survival (%) 79 79 77 80 Height (m) 1.9 0.8 1.0 1.1 Diameter (cm) 3.0 1.6 1.6 2.1 Crown cover (%) 40 90 30 60 White oak 7th 91 NΡ Survival (%) 70 56 2.1 ΝP Height (m) 2.1 1.6 Diameter (cm) NP 3.9 2.9 3.8 80 70 90 NP Crown cover (%) Longleaf pine 5th NP Survival (%) 47 64 94 0.9 NΡ 1.3 Height (m) 1.6 Diameter (cm) ND ND ND NΡ Crown cover (%) ND ND ND NP 3rd Osage-orange Survival (%) 92 89 100 NP Height (m) 0.6 0.4 NP 1.0 NΡ Diameter (cm) ND ND ND

seedlings averaged only 1.3 m (4.3 feet) tall after 5 growing seasons, and trees on strip A were the shortest (table 1). However, height growth was comparable to or better than that of the three oak species at the same age (data not shown). Longleaf pine survival averaged 68% across the three strips (A through C), which is better than the average survival of 60% for 1-year-old planted longleaf pine seedlings in Louisiana (State of Louisiana 1993).

Sawtooth oak was the largest hardwood species through three growing seasons. Few of the remaining sawtooth oaks died after thinning to a stocking of 5,980 trees/ha (2,420 trees/acre) at age 3, and among

the remaining trees, survival averaged 94% across the four strips at age 9 (table 1). After nine growing seasons, the trees on strips A and D were taller and had greater quadratic mean groundline diameters than those on strips B and C. Tree height and diameter averaged 3.4 m (11.1 feet) and 5.1 cm (2.0 inches), respectively. The tree crowns covered covered 98% of the four strip surfaces after 9 years.

Water oak was not initially as large as sawtooth oak. However, after eight growing seasons, water oaks were as large as the older sawtooth oaks, with water oaks having an average total height of 3.4 m (11.2 feet) across the four strips (table 1). The water oak plots

were not thinned, which influenced groundline diameter growth comparisons with sawtooth oak. Survival averaged 81%, and crown cover averaged 88% across the four strips. Water oak was largest on strip D after eight growing seasons.

Height and diameter growth of white oak after seven growing seasons was as good as those of either sawtooth oak or water oak at the same age, although the white oak plots were not irrigated. After seven growing seasons, survival, total height, quadratic mean groundline diameter, and crown cover averaged 72%, 1.9 m (6.2 feet), 3.5 cm (1.4 inches), and 80%, respectively, across the three strips (table 1). The trees on strip A were similar in size to those on strip C.

Amur honeysuckle and yaupon survival averaged 76 and 79%, respectively, after eight growing seasons (table 1). Average height and crown cover of both shrub species were greater on strips A and D than on strips B and C.

Osage-orange was planted last and had completed only three growing seasons by 1990. Survival averaged 94%, and the seedlings were 0.7 m (2.3 feet) tall across the three strips at age 3 (table 1, figure 2).

Nutrient contents of foliage and soil properties. Foliage sampled in August 1983 from the three fastest growing species showed that trees on strip A had the highest concentration of N (table 2). However, N was applied to all strips in both 1984 and 1985. By August 1992, foliar N concentrations in strip A for loblolly pine, sawtooth oak, and water oak were 10.0, 14.1, and 14.0 g/kg, respectively. For the other three strips, foliar N concentrations of loblolly pine, sawtooth oak, and water oak averaged 10.1, 15.5, and 13.4 g/kg, re-

spectively. The nitrogen levels in loblolly pine foliage indicated deficiency (Wells and Allen 1985), which is not surprising because the N-supplying capacity of new minesoil frequently is less than that of older, long-vegetated minesoil or of undisturbed soil with similar N concentrations (Hons and Hossner 1980, Reeder and Berg 1977).

The foliar concentrations of P and K were not consistently affected by the kind of lignite minesoil (table 2). However, the P concentration in loblolly pine foliage from both the 1983 and 1992 samples were above growth-limiting levels (Wells and Allen 1985). Thus, the differences in P are not affecting growth. Potasium concentrations in loblolly pine foliage were less in 1992 than in 1983, but concentrations in sawtooth oak foliage were greater in 1992 than in 1983.

Conclusions

Plant survival and growth were generally satisfactory on all four kinds of minesoil, and therefore, replacement of the A horizon is not always necessary when correct reclamation practices are followed. The two pine and three oak species studied here are important forest tree, and all five species became readily established and grew well on these lignite minesoils, although established common Bermuda grass and Pensacola bahiagrass are effective competitors of planted tree seedlings (Barnett and Tiarks 1987, Fisher and Adrian 1981). In fact, loblolly pine and water oak grew best when a mixture of the A, B, and C horizons was used as the surface soil (strip D). Reclamation should continue to be successful as long

Nutrionto (a/ka)

Table 2 -Nutrient concentrations of foliage samples collected from selected species in August 1983 (before nitrogen amendment in May 1984 and 1985) and in August 1992

Species and lignite minesoil strip	Nutrients (g/kg)						
	August 1983			August 1992			
	N	Р	K	N	P	K	
Loblolly pine							
A	13.1	1.5	8.4	10.0	1.2	5.0	
В	9.5	1.3	8.4	9.9	1.7	7.0	
С	10.5	1.4	9.0	10.0	1.5	5.4	
D	11.4	1.3	7.6	10.4	2.1	5.3	
Sawtooth oak							
A	14.1	1.4	5.3	14.1	1.3	6.3	
В	9.7	1.7	6.2	15.2	1.5	8.4	
С	10.0	1.1	3.7	16.5	1.7	8.0	
D	10.8	1.2	4.5	14.9	1.8	7.4	
Water oak							
A	12.4	1.0	6.1	14.0	1.0	6.4	
В	10.8	0.9	6.7	13.4	1.2	5.1	
С	11.1	0.9	4.6	14.4	1.2	6.7	
D	11.7	0.9	5.4	12.4	0.9	5.7	

Fall 1993 171

as these lignite minesoils provide acceptable chemical and physical soil properties and an adequate amount

of nutrition is supplied through fertilization.

Literature Cited

- Barnett JP, Tiarks AE. 1987. Reforesting disturbed sites in the South with pine species. In: Fourth biennial symposium on surface mining and reclamation on the Great Plains and fourth annual meeting of the American Society for Surface Mining and Reclamation, 1987 March 17-19, Billings, MT Bozeman, MT: American Society for Surface Mining and Reclamation: H-5-1 to H-5-7
- Burton JD, Tiarks AE. 1986. Available nutrients and early growth of woody plants vary with overburden material in lignite minesoils of Louisiana. In: Proceedings, National Meeting of the American Society for Surface Mining and Reclamation, 1986 March 17-20, Jackson, MS. Princeton, WV: American Society for Surface Mining and Reclamation: 79-85.
- Fisher RF, Adrian F. 1981. Bahiagrass impairs slash pine seedling growth. Tree Planters' Notes 32(2):19-21.

- Hons FM, Hossner LIZ. 1980. Soil nitrogen relationships in spoil material generated by the surface mining of lignite coal. Soil Science 129(4):222-228.
- McGinnies WJ, Nicholas PJ. 1983. Effects of topsoil depths and species selection on reclamation of coal-mine spoils. In: Smith JA, Hays VW, eds. Proceedings of the 14th International Grasslands Congress. 1981; Lexington, KY Lexington, KY: Westview Press, 352, 356.
- Reeder, JD, Berg WA. 1977. Nitrogen mineralization and nitrification in a Cretaceous shale and coal mine spoils. Soil Science Society of America journal 41(5):922-927
- State of Louisiana, Department of Agriculture and Forestry, Office of Forestry. 1993. Pine plantation survival report-1992. Baton Rouge, LA. 1. p.
- Torbert JL, Burger JA. 1990. Tree survival and growth on graded and ungraded minesoil. Tree Planters' Notes 41(2):3-5.
- Torbert JL, Jr, Burger JA, Lien JN, Schoenholtz SH. 1985. Results of a tree species trial on a recontoured surface mine in southwest ern Virginia. Southern Journal of Applied Forestry 9(3):150-153.
- Wells C, Allen L. 1985. A loblolly pine management guide-when and where to apply fertilizer. Gen. Tech. Rep. SE-36. Asheville, NC: USDA Forest Service, Southeastern Forest Experiment Station. 23 p.