Propagation of Loblolly, Slash, and Longleaf Pine from Needle Fascicles

C. Dana Nelson, Zeng Linghai, and James M. Hamaker

Research geneticist, USDA Forest Service, Southern Forest Experiment Station, Gulfport, Mississippi
Guangdong Forestry Research Institute, Guangzhou, Guangdong, China
Biological laboratory technician, USDA Forest Service, Southern Forest Experiment Station, Gulfport, Mississippi

*A method of vegetatively propagating pine from needle fascicles, previously reported in China, was tested for its potential use in propagating loblolly (*Pinus taeda *L.), slash (*P. elliottii *var.* elliottii *Engelm.), and longleaf (*P. palustris *Mill.) pine. The method consists of a 16- to 20-hour auxin treatment, a 30- to 60-day rooting stage, and a 60- to 120-day shooting stage. All stages are completed under greenhouse conditions, the first two in water solutions and the third in potting soil. The results of two experiments are reported here. In experiment 1, four auxins were tested with loblolly and slash pine on two collection dates. Overall, slash pine rooted better than loblolly, but loblolly shooted better. Indole-3-acetic (IAA) and indole-3-butyric (IBA) acids were effective at promoting roots and shoots with both species, while a-naphthaleneacetic (NAA) and indole-3-propionic (IPA) acids were effective at promoting roots with slash pine only. Several treatment combinations produced regeneration rates above 25%. In experiment 2, needle fascicles from loblolly, slash, and longleaf pine stock plants of varying ages (1 to 10 years) were cultured. Rooting declined sharply between ages 1, 2, and 3 for loblolly and slash, with slash rooting better than loblolly and longleaf. Slash and longleaf rooted fascicles produced more shoots than loblolly, although relatively few shoots were formed in this experiment. It appears that this vegetative propagation method has potential for the rapid increase of selected loblolly, slash, and longleaf pine seedlings, and it may have special significance for longleaf pine, since the "grass stage" of this species severely limits the production of standard stem cuttings.* Tree Planter's Notes 43(3):67-71: 1992.

Vegetative propagation of pine seedlings is an important tool in both the production and experimental components of pine genetics and breeding programs. Mass vegetative propagation of selected families is a useful adjunct to improvement programs based on recurrent and non-recurrent selection. Experimentally, statistical efficiency is increased when treatments are applied to clones instead of families, due to the absence of genetic variance within the clones.

Several factors, known and unknown, limit the success of propagation systems for the various species of pine. One of the most problematic factors is the inability to produce large numbers of viable explants in a short time. Several tissue culture techniques hold promise for solving this problem; however, they are costly and require specialized facilities and labor. An alternative method employs the short shoots (that is, needle fascicles or fascicles) of pine as explants in a non-sterile greenhouse propagation system. Because individual pine seedlings produce 30 to 100 needle fascicles in their first year of growth, a successful fascicle culture technique may provide a practical solution to this problem.

The literature contains many reports of needle fascicle culture experiments (Toda 1948, Mergan and Simpson 1964, Rudolph and Neinstaedt 1964, Hare 1965, Wells and Reines 1965, Kummerow 1966, Libby and Conkle 1966, Larson and Dingle 1969, MacDonald and Hoff 1969, Sargento and Barker 1978, Andrews 1980, Struve and Blazich 1984). Most indicate acceptable rooting rates (greater than 50%), though with little or no shoot initiation or elongation. In general, the higher rooting rates have been associated with lower shooting rates, thus, regeneration rates have been very low. Wang and Wei (1988), Wang, Wang, and Chao (1984), and JFRI (1981) summarized needle fascicle culture studies of slash pine conducted in China. Rooting results for 1- and 2-year-old slash pine were consistently high (46 to 95%) and relatively rapid (4 to 5 weeks). Shoot production on rooted fascicles also fell in this range (50 to 80%) at 10 weeks, resulting in 25 to 75% regeneration rates in 14 *to* 15 *weeks.* In terms of regeneration rate and quantity, these results compare favorably with standard stem cutting propagation methods.

To our knowledge, the method developed in China is different than any reported in the U.S. lit-

erature. The primary difference is that in China the rooting phase is completed in a water-based medium, instead of a solid medium (such as peat, perlite, sand, etc.). To evaluate the water-based method, we conducted several preliminary experiments with loblolly, slash, and longleaf pine. Here we report the results of two experiments designed to test the effects and interactions of species, auxins, and ages of stock plants on root and shoot initiation.

Materials and Methods

Standard protocol. The propagation method is a modification of that described by Wang and Wei (1988) and Wang, Wang, and Chao (1984). One-year-old needle fascicles were carefully removed from the stock plants. For 1-year-old plants, fascicles located at or just below the median point of the main stem were used. The fascicles were bundled together by tree, and placed in moist, cool sand until all collections for the day were made. Following collection, the bundles were thoroughly rinsed in cold running tap water. The basal 2-mm (approximately) of tissue was then trimmed from each fascicle with a razor. The fascicles were rebundled (consistent with the particular experimental design) and stood upright in a shallow glass pan containing an auxin solution (75 to 150 ppm auxin, pH 5.5). Following 16 to 20 hours in auxin, the bundles were removed, thoroughly rinsed under running tap water and returned to a glass pan containing a nutrient solution (60 ppm H_3B0_3, 40 ppm NH_4N0_3, and 20 ppm thiamine-HCI, adjusted to pH 5.5). Water naturally buffered to pH 6.0 (in these experiments, water from Palmer Creek in Harrison County, Mississippi) was used in both the auxin and nutrient solutions. We have found that both natural and RO (reverse osmosis) water are superior to tap water (unpublished data). The nutrient solution was replaced and the bundles were rinsed one time each week. The level of the nutrient solution (5 to 7 mm) was increased to compensate for evapotranspiration losses as needed (usually 2 or 3 times each week). When roots had emerged and grown approximately 5 to 10 mm, the rooted fascicles were transplanted into a potting mix of forest soil, peat, and vermiculite (2:3:1). All culturing phases-auxin treatment, rooting, and shooting-were completed in a temperature (21 to 29 °C) and relative humidity (75 to 85%) controlled greenhouse under natural photoperiod.

Experiment 1. Seeds from a bulk collection of Livingston Parish (Louisiana) loblolly pine trees (75 total) and an open-pollinated collection of a slash pine clone were sown in the Harrison Experimental Forest (HEF) nursery in April 1990. About 60 vigorous seedlings from each species were selected and transplanted to a separate nursery bed in June 1990. These transplants were spaced approximately 30 x 30 cm. During the last week in August the distal ends of each shoot were cut off in an effort to stimulate fascicle bud and needle development.

Needle fascicles were collected on November 29, 1990 (date 1, N = 540 fascicles) and February 12, 1991 (date 2, N = 900 fascicles) from 30 to 40 trees of each species. The fascicles of each species were grouped at random into bundles of 15. Three bundles on date 1 and 5 bundles on date 2 of each species were treated with 1 of 12 auxin treatments (treatment combinations of IBA, IAA, IPA, and NAA each at 75, 100, and 150 ppm). The treatments were completed during 16 hours, on the day following collection, in a temperature and humidity controlled greenhouse. Following the auxin treatment, the bundles were transferred to the standard nutrient solution (see standard protocol) in the same greenhouse. Nutrient solutions were replaced weekly and the fascicles were inspected for rooting. Fascicles with root(s) approximately 5 mm long were scored as rooted, removed from the bundles and planted into potting media. The transplanted fascicles were further observed and scored for spontaneous shoot initiation (shoot length > 10 mm).

Experiment 2. Loblolly**,** slash, and longleaf pine stock plants aged 1, 2, 3, 5, 7, and 10 years from seed were located in nursery beds and various field plantings on the HEF. The 1-year-old loblolly and slash explants came from the same material used in experiment 1, while the 1-year-old longleaf explants came from potted wind-pollinated seedlings of several local trees. These seedlings had been grown outdoors, under shadecloth (approximately 33% shade).

Needle fascicles were collected on January 15, 1991, from 3 to 14 (mostly 7) stock plants in each species-age combination. Seven to 15 fascicles were collected from each tree and grouped into 3 to 7 bundles of 15 or 30 fascicles per species-age combination. The bundles were treated in 100 ppm IBA for 16 hours and then cultured according to the standard protocol. Rooting and spontaneous shoot initiation were scored as in experiment 1.

Data Analysis. Two variables were statistically analyzed--rooting in each experiment and shooting in experiment 1-date 1, only. Rooting was scored as the proportion of needle fascicles rooted (less than 90 days), and shooting was scored as the proportion of the rooted fascicles that produced a shoot (less than 210 days).

Analyses of variance (SAS proc GLM, Type III) were computed for each of the three data sets. All effects were considered fixed. Pairwise t-tests ($a < .05$) were used for treatment mean comparisons when differences were suggested ($a < .10$) by the F-tests. In experiment 1-date 1, the data were inadvertently pooled (during collection) across replicate bundles within the treatment combinations, thus reducing the model to two factors and their interaction. Since the range in auxin concentration was small (75 to 150 ppm), we chose to analyze the effects of species and auxin, allowing the levels of concentration to serve as replications. The experiment 1-date 2 data were analyzed with the same model, with 15 replications (that is, 5 replicate bundles X 3 concentrations = 15) of 15 fascicles per treatment combination. In experiment 2, a two factor-plus-interaction model was used, with 3 to 7 replications of 15 or 30 fascicles per treatment combination. Because no fascicles from 10-year-old trees rooted, the number of levels of age was reduced to five (1, 2, 3, 5, and 7 years).

Results

Experiment 1. The percentages of needle fascicles rooting, shooting, and regenerating (that is, rooting X shooting) are presented in table 1. For both species, fascicles from date 1 responded better than did those from date 2. Analysis of variance and F-test results for dates 1 and 2 are presented in table 2.

Date 1. Differences were significant between species and auxins for rooting and between auxins for shooting (table 2). The species X auxin interaction was not significant for rooting or shooting. Slash pine was significantly more responsive to root initiation than was loblolly pine. In contrast, a higher percentage of rooted loblolly pine fascicles produced shoots than did slash pine, although this difference was not significant. IBA and IAA were significantly more effective at promoting roots and shoots than were IPA and NAA. When treated with either IBA or IAA, both species showed similar regeneration percentages, however by different

Table 1—*Summary of rooting (R), shooting (S), and regenerating (P) data for 2 dates of fascicle collection in experiment 1*

Auxin (ppm/mM)	Date 1 (Nov. 29, 1990)						Date 2 (Feb. 12, 1991)					
	Slash			Loblolly			Slash			Loblolly		
	R%	S%	P%	R%	S%	P%	R%	S%	P%	R%	S%	P%
Overall	63	19	12	23	40	9	36	3	1	17	8	1
IAA overall	70	28	19	39	53	21	20	7	1	38	6	2
75/.43	42	53	22	58	58	34	20	0	0	32	8	3
100/.57	100	18	18	38	65	25	35	4	1	65	6	4
150/.86	69	16	11	22	20	4	7	40	3	17	0	0
IBA overall	87	22	19	41	51	21	56	2	1	28	10	3
75/.37	100	27	27	47	38	18	84	0	0	24	11	3
100/.49	91	20	18	18	50	9	35	4	1	44	6	3
150/.74	69	19	13	58	62	36	51	3	1	16	17	3
IPA overall	53	20	10	7	0	0	47	3	1	3	17	1
75/.40	60	22	13	4	0	0	20	0	0	4	0	0
100/.53	36	13	5	13	0	0	63	2	1	3	0	0
150/.79	62	21	13	4	0	0	57	5	3	3	50	2
NAA overall	43	5	2	5	57	3	22	0	0	0	0	0
75/.40	38	0	0	4	0	0	0	0	0	0	0	0
100/.54	69	0	0	12	80	10	27	0	0	0	0	0
150/.81	22	30	7	0	0	0	39	0	0	0	0	0

Notes: R% is percentage of fascicles rooting in 90 days, S% is percentage of rooted fascicles producing a shoot in 210 days, and P% is the product of R% and S%, that is, the percentage of fascicles regenerating whole plants in 210 days. Numbers of needle fascicles treated per species-auxin-concentration treatment combination were 45 for date 1 and 75 for date 2. Levels of auxin concentration were treated as replications in all analyses. Date 1: least significant difference (LSD, $\alpha < .05$) between species for rooting = 16%, LSD's between auxins for rooting = 22% and for shooting = 26% Date 2: LSD's between auxins for slash rooting = 32% and for loblolly rooting = 19%.

Table 2—*Analysis of variance for rooting and shooting data in experiment 1*

	Date 1			Date 2	
Source	df	Roots MS	Shoots MS	df	Roots MS
Species	1	.954*	.074	1	1.083*
Auxin	3	.217*	.117	3	0.501*
Species × Auxin	3	.007	.063	3	0.513*
Error	16	.034	.045	112	0.054

*Significant at the .01 level of probability.
Notes: Species were loblolly and slash pine. Auxins were IAA, IBA, IPA, and NAA at concentrations of 75, 100, and 150 ppm. MS = mean square, df = degrees of freedom.

means-slash with higher rooting and lower shooting, and loblolly with lower rooting and higher shooting.

Date 2. All sources of variation in rooting were significant (table 2). Slash and IBA produced the highest rooting responses, although the significant species x auxin interaction made interpretation of the main effects difficult. The effect of auxins was analyzed separately for each species (not shown). For slash pine, IBA and IPA were significantly ($a < .05$) more effective for rooting, while, for loblolly, IAA and IBA were significantly more effective. Shooting rates were not statistically analyzed, but the summarized data showed trends similar to date 1 (table 1). Rooted loblolly fascicles produced a higher percentage of shoots than did those of slash, and IBA was the more effective auxin.

Experiment 2. The percentages of needle fascicles rooting, shooting, and regenerating are presented in table 3. The analysis of variance and F-tests revealed that all sources of variation were significant (table 4). The significant species x age interaction was primarily due to the sustained rooting rate of needle fascicles taken from 2- and 3-year-old longleaf pine stock plants (table 3). It is interesting to note that the 2-year-old longleaf stocks produced higher shooting rates than the land 2-year-old loblolly and slash stocks, although the number of shoots in this experiment was low (34 shoots on 283 rooted fascicles).

Discussion

Our results demonstrate that water-based needle fascicle culture is feasible for propagating loblolly, slash, and longleaf pine seedlings. However, much additional research and development will be required to refine the protocol for practical use in pine genetics and breeding programs. Under the conditions of our experiments, IBA and IAA (75 to 150 ppm) were the superior auxins for root and subsequent shoot initiation and elongation. That IBA was equal or superior to the other auxins is consistent with data from previous loblolly and slash pine rooting experiments (Grigsby 1962, Hare 1974). In addition to the reported observations, we noted that IBA and IAA induced a faster rooting response compared to IPA and NAA, as well as healthier appearing roots in both loblolly and slash pine.

Several treatment combinations in experiment 1date 1 produced greater than 25% regeneration rates. Most of the failure to root (and therefore regenerate) in all treatment combinations appeared to

Table 3—*Summary of rooting (R), shooting (S), and regenerating (P) data for experiment 2*

Species	Age of stock plants (yrs)	No. of stock plants sampled	No. fascicles cultured	R%	S%	P%
Slash pine	1	13	195	72	16	12
	2	6	90	26	0	0
	3	7	105	7	0	0
	5	7	105	7	0	0
	7	7	105	3	0	0
Loblolly pine	1	14	105	15	6	1
	2	7	105	1	0	0
	3	7	105	1	0	0
	5	7	105	0	0	0
	7	7	105	1	0	0
Longleaf pine	1	3	45	31	0	0
	2	7	105	30	28	9
	3	4	75	49	1	0
	5	7	105	0	0	0
	7	7	105	0	0	0

R%, S%, and P% same as in table 1.

Table 4—*Analysis of variance results for rooting data in experiment 2.*

Source	df	MS
Species	2	.387*
Age	4	.418*
Species × age	8	.173*
Error	82	.014

*Significant at the .01 level of probability.
Notes: Species were loblolly, slash, and longleaf pine. Ages were 1, 2, 3, 5, and 7 years from seed. MS = mean square, df = degrees of freedom.

be associated with fungal contamination of the fascicles. The contamination increased with time, so that treatments producing slower rooting responses were more affected. However, from our observations, we concluded that the contamination did not bias treatment comparisons, and that a reduction in contamination would improve the rooting responses of all treatments. It also appears that extreme care in raising the stock plants is necessary for successful fascicle propagation. In agreement with Isikawa and Kusaka (1959) and Zeng (unpublished data), we observed that mature, slightly swollen needle fascicles from vigorous stock plants responded best in culture. Physical manipulations of the stock plants, such as hedging, strangulating (Koh, Menzies, and Hong 1990), or girdling, appear to be the best methods of promoting viable fascicles.

We are uncertain as to any apparent influence of collection date upon root and shoot initiation differences in experiment 1. One hypothesis is that physiological changes detrimental to root and shoot initiation occurred in the stock plants between the collection dates (29 November to 12 February = 78 days). This seems reasonable in light of many time-of-year rooting experiments in pine species, which show seasonal variation in root initiation (Reines and Bamping 1964, Boeijink and Broekhuizen 1974, Struve and Blazich 1984). Thus, our November collection may have occurred during a physiologically optimal rooting and shooting opportunity. Future experiments will need to further address this factor to indicate proper timing for needle fascicle collection.

The response of the 1- to 3-year-old longleaf pine to this propagation system is intriguing. Longleaf pine seedlings, unlike loblolly and slash pine, remain in a grass stage of development (that is, stemless) for 2 to several years following germination. The non-declining rooting response of 1-, 2-, and 3-year-old longleaf pine may be associated with the grass stage of development, since these stock plants were in the grass stage, while the older longleaf stocks were not. It will be interesting to learn whether or not the grass stage maintains an aging tree in a juvenile condition. We conclude that a water-based needle fascicle culture technique has potential for the rapid vegetative increase of southern pine seedlings, especially longleaf seedlings, since the grass stage of this species severely limits the production of standard stem cuttings.

Literature Cited

Andrews, D.S. 1980. Rooting western white pine, *Pinus monticola* Doug]., needle fascicles and branch cuttings. Res. Note INT-291. Ogden, UT: USDA Forest Service. 11p.

Boeijink, D.E.; van Broekhuizen, J.T.M. 1974. Rooting of cuttings of *Pinus sylvestris* under mist. New Zealand Journal of Forestry Science 4:127-132.

Grigsby, H.C. 1962. Propagation of loblolly pine by cuttings. International Plant Propagators Society Proceedings 11:33-35.

Hare, R.C. 1965. Breaking and rooting fascicle buds in southern pines. Journal of Forestry 63(5):544-546.

Hare, R.C. 1974. Chemical and environmental treatments promoting rooting of pine cuttings. Canadian Journal of Forest Research 4:101-106.

Isikawa, H.; Kusaka, M. 1959. The vegetative propagation of cuttings of *Pinus* species. I. vegetative propagation of Japanese black pine using leaf-bundles. Bull. No. 166. Meguro, Japan: Government Forest Experiment Station: 59-64.

JERI. 1981. Needle fascicle culture of slash pine. Jinzhou Forest Research Institute 1980-81:13-15.

Koh, D.S.; Menzies, M.I.; Hong, S.O. 1990. Strangulation pretreatment effect on the development and rooting of fascicle cuttings of *Pinus radiata*. New Zealand Journal of Forestry Science 202(2):129-137.

Kummerow, J. 1966. Vegetative propagation of *Pinus radiata* by means of needle fascicles. Forest Science 12(4):391-398.

Larson, F.E.; Dingle, R.W. 1969. Vegetative propagation of lodgepole pine (*Pinus contorta* Dougl.) from needle fascicles. Forest Science 15(1):64-65.

Libby, W.J.; Conkle, M.T. 1966. Effects of auxin treatment, tree age, tree vigor, and cold storage on rooting young Monterey pine. Forest Science 12(4):484-502.

MacDonald, G.I.; Hoff, R.J. 1969. Effect of rooting mediums and hormone application on rooting of western white pine needle fascicles. Res. Note INT-101. Ogden, UT: USDA Forest Service. 6p.

Mergan, F.; Simpson, B.A. 1964. Asexual propagation of *Pinus* by rooting needle fascicles. Silvae Genetica 13(5):133-139.

Reines, M.; Bamping, J.H. 1964. Rooting of needle bundles. Journal of Forestry 62:181-182.

Rudolph, T.D.; Neinstaedt, H. 1964. Rooting, shoot development, and flowering of jack pine needle fascicles. Silvae Genetica 13(4):118-123.

Sargento, J.O.; Barker, J.E. 1978. Vegetative propagation of *Pinus caribaea* var. *hondurensis* Morelet and *Pinus oocarpa* Schneide by means of needle fascicles. Pterocarpus 4(1):5261.

Struve, D.K.; Blazich, F.A. 1984. Pre-severance environmental condition influence rooting response of eastern white pine needle fascicles. Forest Science 30(2):343-354.

Toda, R. 1948. Rooting responses of leaf bundle cuttings of pine. For Bull. 36. Tokyo: Tokyo University: 49-53.

Wang, D.Z.; Wang, J.Y.; Chao, J. 1984. Experiments on needle fascicle culture in slash pine. Funan Forestry Science and Technology 1:13-15.

Wang, Y.S.; Wei, Z. 1988. Summary of studies on needle fascicle culture of *Pinus massoniana* and *Pinus elliottii*. Forestry Science and Technology of Guangdong Province No. 1:29-30

Wells, D.W.; Reines, J.M. 1965. Vegetative propagation of needle bundles of pine. Res. Paper 26. Macon, GA: Georgia Forestry Research Council. 8p.