Improved Vegetative Propagation of Scouler Willow

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Demand has exceeded supply for conservation plantings of Scouler willow (Salix scouleriana *Barratt ex Hook.). To test possible ways to improve propagation, we treated 8- to 10-cm-long (3.2- to 4.8-in-long) hardwood cuttings with 0.0, 0.1, 0.3, 0.8, and 1.6% indole-3-butyric acid (IBA), and 5- and 10-cm-long (2- to 4-in-long) softwood cuttings, with 0.0 and 0.3% IBA. Best rooting (73% and 87%) occurred after treatment with 0.3% IBA in the hardwood and 10-cm long softwood cuttings, respectively. Microshoots were tested with the antibiotic cefotaxime and calcium gluconate to control bacterial contamination and shoot-tip necrosis. Microshoots, with or without naphthaleneacetic acid (NAA), rooted up to 92% both* in *and* ex vitro *without NAA. Similar micropropagation options may improve production of other difficult to propagate willows.* Tree Planters' Notes 46(2):58-63; 1995.

Many revegetation projects plant native willows to rehabilitate damaged habitat. Scouler willow *(Salix scouleriana* Barratt ex Hook.), an upland (non-riparian) willow of western North America, is useful for stabilizing steep erodible banks on drier sites above river courses. Scouler willow, however, does not root as readily as other willow species used for riparian revegetation (Platt and others 1987).

Willow cuttings develop adventitious roots from either the entire buried stem or from a restricted region at the base of the cutting, but species that produce basal roots are generally more difficult to propagate (Chmelar 1974). Only 4.5% of Scouler willow hardwood cuttings developed basal roots (Densmore and Zasada 1978). However, 78% of hardwood cuttings that were wounded and then treated with a powder containing 0.8% indole-3-butyric acid (IBA) achieved rooting success (Holloway and Zasada 1979). Alternative powder formulations, however, were not evaluated. Softwood (greenwood) cuttings rooted at a rate of 64% after similar treatment, but only about 50% of the rooted cuttings survived transplanting and only 38% of the surviving transplants survived 1 year.

Fog humidification and micropropagation methods have improved the propagation of many difficult-to-propagate species (Hartmann and others 1990). Survival of micropropagated *Salix schwerinii* (E. Wolf) was higher than that of conventional rooted cuttings (Gupta and others 1991).

Because demand has exceeded supply for conservation plantings of Scouler willow and propagation of softwood cuttings has been limited using conventional methods, we used fog humidification and micropropagation technology to develop improved vegetative propagation of the species. We evaluated rooting success and greenhouse survival of hardwood and softwood cuttings propagated under fog humidification and microshoots propagated *in vitro.* We established an optimal level of IBA powder treatment for hardwood cuttings, improved the rooting rate of softwood cuttings, and developed efficient *in vitro* multiplication and rooting procedures.

Materials and Methods

We conducted propagation studies with three types of cutting material:

1. Hardwood— dormant stems collected from the wild
2. Softwood— new shoots of the rooted hardwood cuttings
3. Microshoots— new shoot tips of rooted hardwood and softwood

Hardwood and softwood cuttings were macropropagated in a greenhouse, and microshoots were micropropagated in a laboratory.

Macropropagation techniques. For hardwood propagation material, 1-m-long (3.3- ft-long) dormant whips were harvested in April from several hundred genotypes from the Krassel Ranger District of the Payette National Forest in central Idaho and from Clearwater and Latah Counties of northern Idaho. These whips were stored for 3 weeks at 2ºC before propagation. Hardwood cuttings— 8- to-12-cm-long (3.2- to 4.8- in-long) stem segments— were then prepared according to the methods described by McCluskey and others (1983). For softwood (green-

wood) propagation materials, cuttings of partially lignified shoot tips were collected in mid-summer from container-grown rooted hardwood cuttings propagated the previous year.

Both softwood and hardwood cuttings were sized with a cut made directly below a node at a 45º angle, after which the stems were soaked for 30 sec in a fungicidal dip of 1 g/1 benomyl before setting in a 1:1:1 (v/v/v) mixture of peat, perlite, and vermiculite. The propagation trays were placed on a rooting bench under 88 to 92% relative humidity, natural photoperiod, and 70%o shade. Shoot tips and lower leaves of softwood cuttings were removed, leaving the uppermost leaves intact. Cuttings were not fertilized. Rooted cuttings were transplanted to polystyrene block (315A) containers (with one-hundred sixteen 75-ml-capacity cells) and received twice-weekly nutrient applications of 20:20:20 N/P/K at rates increasing from 50 to 200 ppm nitrogen over several weeks.

Determining optimal IBA levels for hardwood cuttings. To assess IBA's effect on rooting success, on May 1, 1992, cuttings were assigned to four replicates, 40 cuttings per replicate treatment, of 0.0, 0.1, 0.3, 0.8, and 1.6% IBA, arranged in a randomized complete block design. Two months after initiation, cuttings with roots longer than 2 mm (0.08 in) were counted. Both live and dead rooted cuttings were tallied after 6 months. Shoot extension and the number of plagiotropic leaders were recorded after terminal budset. A total of 45 plagiotropic rooted cuttings were transplanted to a polystyrene block (615A) container (with forty-five 340-mlcapacity cells) and retained for observation during 1993.

Evaluating short cutting length in softwood cuttings. The optimal IBA level for hardwood cuttings was chosen to test the rooting response of softwood cuttings. Because shoot availability for softwood cuttings was limited, the effect of short cutting length on rooting was evaluated. On July 29, 1992, softwood cuttings were assigned to four replicates, 50 cuttings per replicate treatment, of short stems (5 cm, or 2 in) and long stems (10 cm, or 4 in) with and without a basal powder dip of 0.3%o IBA, arranged in a 2 x 2 factorial randomized complete block design.

Rooting success was tallied monthly for 3 months. The number of roots longer than 2 mm per rooted cutting and length of the longest root per rooted cutting were recorded after 1 month. A random sample of 35 cuttings were measured for leader lengths and overall survival levels after 3 months' growth.

Micropropagation techniques. *Initiation and incubation.* Microshoots were unlignified shoot tips collected from new growth on potted plants in the greenhouse. Leaves were removed and stem tips were surface sterilized in a 20%o solution of laundry bleach for 20 min and rinsed three times in sterile, distilled, deionized water. Explants were cut into 2-node stem segments and placed on Murashige and Skoog medium (MSM) (Murashige and Skoog 1962). Within a few weeks, new leaves were turning yellow, white, and brown and then falling off. Explants were moved to woody plant medium (WPM) (Lloyd and McCown 1980) containing 0.1 mg/1 of the cytokinin benzyladenine (BA). New green leaves appeared and axillary buds broke, forming multiple shoots per explant. These and all subsequent cultures were maintained under cool-white fluorescent lights (40 W), which produced approximately 20 µmol/m^2/sec PAR on the leaf surfaces. Diurnal temperatures ranged from 22 to 27 °C.

To increase the number of microshoots available for experiments, axillary shoots were excised monthly from the cultures and transferred to fresh WPM + 0.1 mg/1 BA until sufficient numbers of uniform healthy microshoot tips were produced. Microshoots formed an average of 4.9 ± 0.4 new shoots each month.

Bacterial contamination. Many microshoots had an internal bacterial infection that spread and multiplied on the agar medium, sometimes overwhelming the microshoot. To control these bacteria, microshoots were given the antibiotic cefotaxime in WPM at 0, 200, and 300 mg/1 (100 replicates per treatment), arranged in a completely randomized design.

Shoot necrosis. Many shoot tips in the maintenance cultures turned black, possibly because of a calcium deficiency (Sha and others 1985). A total of 800 microshoots were assigned to treatment groups that were placed in WPM with or without calcium gluconate under high light (50 µmol/ m^2/sec) or low light (18 µmol/m^2/sec), arranged in a 2 x 2 factorial completely randomized design. The number of microshoots with black shoot tips was recorded after 1 month.

In vitro *rooting.* Healthy shoot tips were assigned to two treatments (90 replicates per treatment) of WPM + 0.1 mg/1 of the rooting hormone naphthaleneacetic acid (NAA) or WPM + 0 hormone (controls) in a completely randomized design. The microshoots were cut to 1.5 cm (0.6 in) and their bases were inserted 0.5 cm into the gel media. Rooting success was tallied weekly for 4 weeks, and the number of roots longer than 2 mm (0.08 in) and length of the longest root per rooted cutting were recorded after 1 month.

Ex vitro *rooting.* Shoot tips were inserted 0.5 cm into a 2:1:1 (v/v/v) sterile mixture of perlite, peat, and vermiculite in a germination tray. Half of the

microshoots (26) were dipped in Rootone® (0.2% NAA + 4.04% thiram) for 5 sec and the other half were dipped in water. The microshoots were placed in a fog humidification chamber and covered with a 20% shade cloth for several days. Rooting success was recorded weekly for 3 weeks.

Data analysis. Summaries of continuous and count data were expressed as the sample mean ± standard error. Counts of rooting success, contamination, and shoot tip necrosis underwent categorical loglinear maximum likelihood analysis of variance (PROC CATMOD, SAS Institute, Cary, NC, USA). Treatment comparisons were made by single degree of freedom contrasts at " =0 .05.

Results

Macropropagation. *Hardzvood cuttings.* Hardwood cuttings treated with 0.3% IBA had the highest average rooting rate (table 1), 17% more than untreated cuttings (P = 0.0006) and 24%, higher than cuttings in the 1.6% IBA treatment (P = 0.0002). By 6 months, however, the number of rooted cuttings declined by over 10%, with mortality associated with dieback and stem canker symptoms typical of *Cytospora* spp. (Westcott 1971).

After 3 months' growth, new shoots had extended an average of 18.3 ± 1.0 cm (7.2 ± 0.4 in). Initially, 73% of the rooted cuttings grew plagiotropically. In 1993, the transplanted plagiotropic plants developed an orthotropic form typical of Scouler willow. Furthermore, shoots released from below the plug surface often became detached from the cutting by natural growth and produced a less vigorous root system (figure 1), possibly because of the loss of their carbohydrate source.

Softwood cuttings. Adventitious roots appeared within 10 days and 85% of the rooting occurred within 4 weeks of setting the cuttings (figure 2). No further rooting was observed after 8 weeks. Of the 541 cuttings

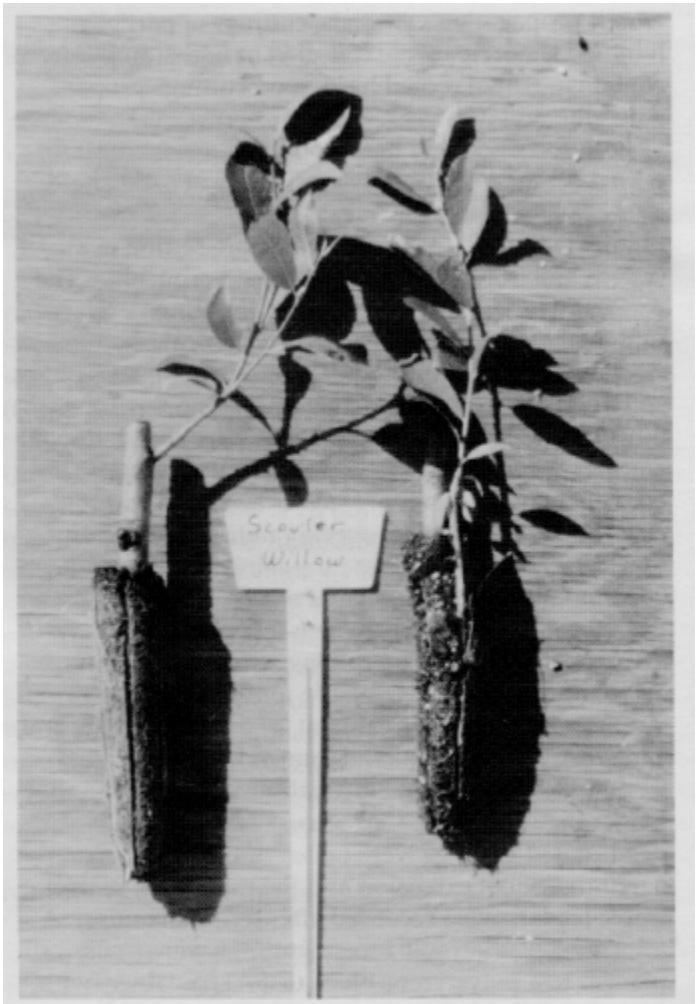

Figure 1—*Hardwood rooted cuttings of Scouler willow 3 months after rooting. Note the well-developed root system on the left. On the right, the shoot is detached from the hardwood stem and has fewer roots.*

Table 1-*Influencc of IBA on the rooting of hardwood cuttings 8 weeks after treatment with alternative powder formulation (n = 160)*

	0.0% IBA	0.15% IBA	0.3% IBA	0.8% IBA	1.6% IBA
Rooting success (%)	56 a	64 abc	73 c	63 ab	49 a
Survival (%)	48 a	55 ab	62 b	41 a	43 a

Within rows, treatment means with the same letter are similar as determined by contrasts of maximum likelihood estimates, "= 0.05. Maximum likelihood ANOVA of treatment effect yielded X^2= 28.56 (P= 0.0001).

rooted (67.6%), 9.2% died on the rooting bench with stem canker symptoms.

Both longer cutting length and auxin treatment resulted in enhanced rooting success (P < 0.0001). An average of 41% more of the 10-cm-long (4-in-long) cuttings treated with 0.3% IBA rooted than the 5-cm-long (2-in-long) cuttings lacking IBA treatment (table 2). The longer softwood cuttings produced slightly more roots per rooted cutting but the maximum root length remained constant. Cuttings developed overall averages of 3.4 roots per rooted cutting and maximum root length of 4.8 cm (1.9 in). After 3 months' growth, new shoots extended an average of 20.3 ± 1.3 cm (8.1 ± 0.5 in) with generally well-developed root systems.

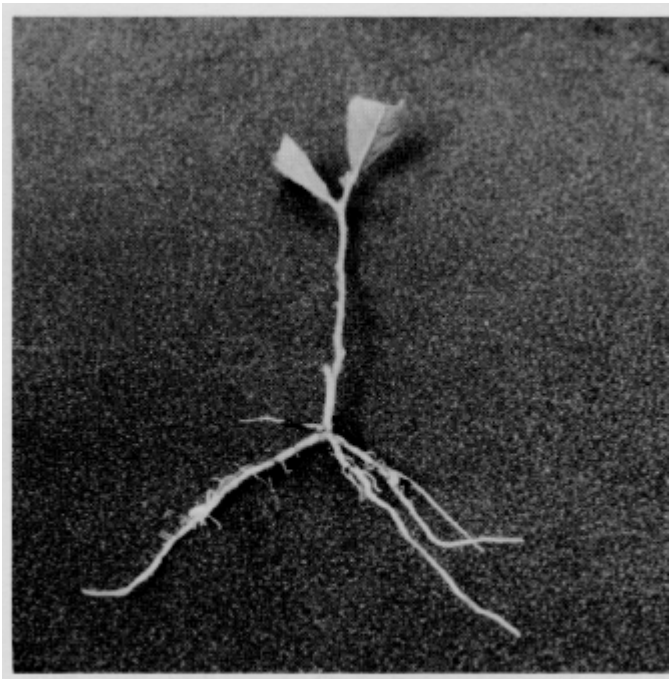

Figure 2—*A softwood rooted cutting of Scouler willow 3 weeks after setting. The shoot tip was removed and the upper leaves cropped to reduce transpiration.*

Table 2-*Effects of cutting length and .3% IBA treatment on the average proportion of softwood cuttings rooted, roots produced per rooted cutting, and maximum root length*

Length (cm)	% IBA	% Rooting (n = 200)	Ave no. roots per cutting	Ave length of longest root (cm)
5	0.0	46 a	2.8 ± 0.3	6.7 ± 1.4
5	0.3	68 b	2.9 ± 0.5	4.7 ± 0.9
10	0.0	65b	4.3±0.7	5.5±1.0
10	0.3	87c	4.5±0.5	5.5±0.9

Treatment means for percent rooting with the same letter are similar as determined by contrasts of maximum likelihood estimates, " = 0.05. The additive model (with factoral interaction p = 0.3390) produced a likelihood ratio $X^2 = 12.81$ ($P = 0.2343$).

Micropropagation. *Bacterial contamination.* After 1 month from the beginning of the experiment, we found that, as cefotaxime concentrations increased from 0 to 200 to 300 mg/1, bacterial contamination decreased significantly from 100 to 46 to 32%, respectively ($P < 0.05$). Just as important, we found that growth and development of microshoots was not inhibited by the antibiotic. Control microshoots produced an average of 6.8 new shoots a month compared to 8.9 and 8.0 new shoots produced by microshoots treated with 200 and 300 mg/1 cefotaxime, respectively.

Shoot necrosis. After 1 month, the combination of high light intensity (50 µmol/m^2/ sec) and addition of calcium gluconate to the culture medium completely inhibited the formation of black tips. The next best treatment, high light without calcium, produced significantly more dead tips than the high light with calcium ($P = 0.02$) (3% vs 0%, respectively). The low light (18 µmol/m^2/sec with calcium and low light without calcium treatments increased the number of dead tips (14% and 58%, respectively) as compared to the high light treatments, although the low light with calcium showed significantly less necrosis than without the calcium ($P < 0.0001$).

In vitro *rooting.* Microshoots rooted vigorously during the first 2 weeks (figure 3), the shortest time of any rooting method in our study, with only a few microshoots rooting in the third and fourth weeks. Control microshoots (0 NAA) rooted at 87%, which was not significantly different from the 92% rooting of NAA-treated microshoots, but the addition of NAA to the medium significantly increased the number of roots formed on each plantlet as compared with the control ($P < 0.01$) (table 3). Roots formed on control microshoots were significantly longer, though, than those roots initiated on NAA-treated cultures ($P < 0.01$) (table 3). All transplanted plantlets survived.

Ex vitro *rooting.* After 3 weeks, all rooting had taken place. Control microshoots rooted at 92.3% as compared to microshoots treated with Rootone® (57.7%). Thirty-eight percent of the microshoots treated with

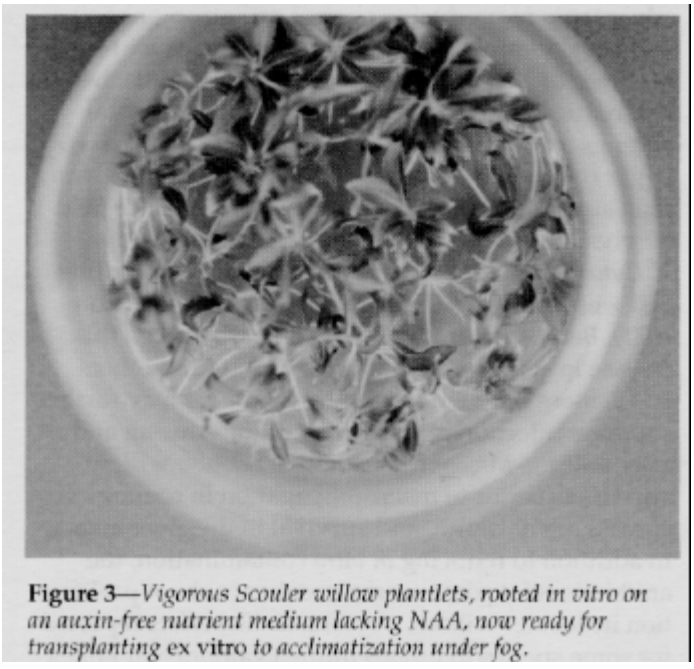

Figure 3—*Vigorous Scouler willow plantlets, rooted in vitro on an auxin-free nutrient medium lacking NAA, now ready for transplanting* ex vitro *to acclimatization under fog.*

Table 3-*The effect* of *NAA on rooting of Scouler willow microshoots after 3 weeks of exposure to 0.1 mg/l NAA*

Treatment (mg/I NAA)	% Rooting (n = 90)	Ave no. roots per plantlet	Ave length of longest root (cm)
0.0	87 a	2.36 a	2.53 a
0.1	92a	3.41 b	2.17b

For percent rooting, treatment means with the same letter are similar as determined by contrast of maximum likelihood estimates. "= 0.05. For average no. of roots or length of longest root, means with the same letter are similar as determined by the Cochran and Cox approximation

Rootone rotted whereas only 8%, of the control microshoots blackened.

Discussion

Best rooting successes suggest that Scouler willow softwood cuttings can root at least as well or better (87%) than hardwood cuttings (73%). This contrasts with a reversed trend previously reported by Holloway and Zasada (1979, who found that softwood cuttings (64%) rooted less than hardwood cuttings (78%). The higher softwood rooting rate attained in our study may have been enhanced by using fog humidification rather than conventional misting. Our optimal hardwood treatment of 0.3% IBA produced a similar result to the 0.8% IBA treatment of Holloway and Zasada (1979), but the rooting decline with 0.8 and 1.6% IBA suggests that hardwood cuttings require only moderate 1BA concentrations to enhance rooting. Cuttings rooted from softwood have the additional advantages of producing normal orthotropic shoots and fully occupying the container cell. Because the presence of a large stem in a plug, whether attached or not, reduces potential root volume, both softwood cuttings and micropropagated plantlets can produce a larger root volume than hardwood shoots for a given plug size, which in turn could possibly enhance field survival.

Cytospora infections are exacerbated by wounding and wet conditions (Filip and others 1992, Westcott 1971). Because benomyl treatment has reduced this disease in some hardwood trees (Spotts and others 1990), more frequent fungicidal treatment may further suppress infection in softwood cuttings during and after rooting. Micropropagation of Scouler willow appears superior to macropropagation in rooting success, plant health, and survival in the greenhouse. In addition to reducing *in vitro* contamination, the antibiotic cefotaxime seems to enhance shoot proliferation in Scouler willow, a cytokinin-like effect reported for some species in tissue culture (Valobra and James 1990). Increased light reduces shoot-tip necrosis, and calcium added to the culture medium provides further benefit, particularly in low light conditions. Calcium supplements have reduced shoot-tip necrosis in other species (De Block 1990, Sha and others 1985). In contrast with the apparent exogenous auxin requirement for optimal rooting of macrocuttings, auxins seem unnecessary to promote rooting of microshoots. Auxin treatment also may inhibit *ex vitro* root formation by increasing microshoot mortality.

Because results achieved were similar for the many genotypes collected from widely separated areas of central and northern Idaho, the propagation protocols are likely to be broadly useful for propagating Scouler willow.

Conclusions

This propagation study of Scouler showed that the rooting rate of softwood cuttings could be improved and developed an efficient method to micropropagate the species. These promising alternative options to hardwood propagation could increase the flexibility of producing stock for conservation plantings. With plentiful, disease-free cutting material available, propagating softwood cuttings could provide more stock plants with a larger root mass than rooted hardwood cuttings. The option to micropropagate Scouler willow can produce microshoots that root faster and survive at higher rates in the greenhouse than either softwood or hardwood cuttings, minimizes stem disease, bulks up propagation material rapidly when cutting material is in short supply, and allows year-round production. The protocols developed here should improve nursery production of Scouler willow. A similar approach of applying softwood and micropropagation options to other upland, nonriparian willows may improve production of other species now considered difficult to propagate.

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