Horticultural Applications of *Agrobacterium rhizogenes* ("Hairy-Root"): Enhanced Rooting of Difficult-to-Root Woody Plants

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INTRODUCTION

These studies tested the influence of *Agrobacterium rhizogenes* ("hairy root") dip-treatments on rooting of stem cuttings of difficult-to-root woody ornamentals. Prior work has shown that treatment of some woody plants with *A. rhizogenes* ("hairy root") resulted in root proliferation, as well as improved growth and performance in field and controlled environments (Han et al., 1993, Stomp, 1995; Strobel et al., 1988; Strobel and Nachmias 1985).

Rooting of woody plant cuttings by treatment with *A. rhizogenes* may make it possible to more profitably propagate difficult-to-root woody plants which are now grafted, or which are now expensive to produce from cuttings because of low rooting percentages. Woody plants propagated from stem cuttings induced to root with *A.rhizogenes* ("hairy root") may also show improved transplant recovery (Strobel et al., 1988). Positive results could lead to increased profitability and simplified production for growers of difficult-to-root woody ornamentals by enhancing rooting of stem cuttings. This work could therefore also positively affect consumer access and retail marketability of these plants.

Possible utility for *Agrobacterium*-treated, "extra-rooty" plants, in diverse arenas, has been discussed by Stomp, et al., 1993. However, little information exists regarding any potential uses for "extra-rooty" plants in horticultural applications—like improvement of vegetative propagation. We, therefore, screened a small number of commercially significant woody ornamentals for their susceptibility to *A. rhizogenes*, including botanical relatives of plants that have already shown enhanced rooting following treatment with *A. rhizogenes*.

Robinia pseudoacacia (Han et al., 1993) and *Prunus amygdalus* (Strobel and Nachmias, 1985) were reported to be successfully affected by *A. rhizogenes*. *Cercis* is a botanical relative of *Robinia*. *Prunus sargentii* and *P.* 'Kwanzan' are botanical relatives of *P. amygdalus* and therefore *C. canadensis, C. chinensis, P. sargentii,* and *P.* 'Kwanzan' were included in this study. In addition, two difficult- or slow-to-root conifers were included in a second study: *Abies fraseri* and *Cephalotaxus sinensis*. *Thuja plicata* was also included with the two conifers as an easy-to-root comparison species.

MATERIALS AND METHODS

Two propagation trials were conducted comparing percent rooting, number of roots greater than 1 cm, and length of longest root in stem cuttings of numerous difficult-to-root woody ornamental taxa when treated with standard rooting compounds and with two strains of wild-type ('hairy root') *A. rhizogenes*. The 'hairy-root' dip treatment was used in an analogous manner to that of an additional rooting hormone in the trials.

All trials were conducted in 1996 and 1997 using propagation facilities and plants from the living collections of the Arnold Arboretum of Harvard University, Jamaica Plain, Massachusetts. Stomp prepared and shipped to Tripp wild-type, bacterial cultures and the solutions necessary for dipping cuttings. Work with wild type *A. rhizogenes* strains is exempt from regulations governing genetically engineered plants (wild type strains are easily controlled with routine sanitary horticultural procedures such as cleaning all surfaces and implements with bleach solution).

In June 1996, Tripp collected and treated softwood stem cuttings of *Alnus hirsuta, A. maximowiczii, C. canadensis, C. chinensis, P. cyclamina, P.* 'Okame', *P. sargentii*, and *P.* 'Kwanzan'. Cuttings were treated with one of the following treatments: water, 8000 ppm KIBA (potassium salt of indole-butyric acid), *A. rhizogenes* strain A-4, *A. rhizogenes* strain R-1600, or combined treatments of *A. rhizogenes* strains A-4 or R-1600 with 8000 ppm KIBA. KIBA solution was prepared by dissolving KIBA salt in tap water at room temperature.

In December 1996, Tripp collected and treated hardwood stem cuttings of *A. fraseri, C. sinensis,* and *T. plicata,* with 95% ethanol:tap water (1 : 5, v/v) as a control, 5000 ppm NAA (naphthalene acetic acid), 8000 ppm KIBA, strains A-4 or R-1600 of *A. rhizogenes*, or combined treatments of *A. rhizogenes* strains A-4 or R-1600 with 8000 ppm KIBA, or with 5000 ppm NAA. KIBA and NAA solutions were prepared by dissolving the hormone powder in a solution of 95% ethanol and tap water (1 : 5, v/v).

All trials were conducted using a randomized complete block design with two replications of 10 cuttings each per treatment and plant (20 cuttings per treatment per plant combination for 960 total softwoods and 540 total hardwoods). Cuttings were collected, treated, and installed on the same day by replication. Cuttings were trimmed to about 5 inches long and all foliage was removed from the lower 2 inches of each cutting before treatment application. Control, NAA, and KIBA treatments were applied as 8-sec quick-dips. *Agrobacterium* treatments were applied by dragging the cutting bases through the appropriate culture plates. Treatments combining both standard hormone and *Agrobacterium* treatments were done by applying the quick-dip treatment first, allowing cuttings to air dry for 1 min, and then dragging the cutting base through the culture plate. Cuttings were rooted in 4 inch × 12 inch × 6 inch deep plastic rooting trays filled with a pre-moistened medium of peat and perlite (1 : 2, v/v).

Cuttings were maintained in the Arnold Arboretum Dana Greenhouses, Jamaica Plain, Massachusetts under intermittent mist for 6 sec every 6 min for softwoods, or 6 sec every 15 min for hardwoods (with periodic reductions in mist frequency in response to prolonged cloud cover). Bottom heat was applied at 65F to the conifer cuttings only for the duration of that trial. Mist was applied for 10 h day^{-1} to softwood cuttings and for 7 h day^{-1} to conifer cuttings. Greenhouse temperatures were maintained at 80F maximum day and 55F minimum night for the softwood cuttings, and at 55F maximum day and 40F minimum night for the conifer cuttings.

All cuttings were harvested after 12 weeks in both trials. Root fresh weights, length of longest root, and number of roots greater than 1 cm were recorded for all individual cuttings in all treatments. Percent rooting per replication for each plant and treatment were calculated from these data. Cuttings were counted as rooted if they showed at least 2 roots $\geq$ 1 cm. All data were analyzed for statistically significant treatment effects using the General Linear Means procedure for analysis of variance as performed by Statistical Analysis Software (SAS, SAS Institute, Inc., Cary, North Carolina). Analysis of variance was performed on arcsin transformations of percentage data but for clarity actual percentages were presented in Tables 2 and 3. Treatments and plant taxa treated for both the softwood and conifer trials are summarized below in Table 1.

Table 1. Summary of treatments and plants used in two trials testing rooting response of ornamental woody plant cuttings treated with standard rooting compounds and exposure to two strains of wild type *Agrobacterium rhizogenes* ("hairy root" bacteria). The softwood trial was conducted for 12 weeks in summer 1996 and the conifer trial was conducted for 12 weeks in winter 1996-97. *Agro.*=*Agrobacterium rhizogenes.*

Softwoods		Conifers	
Treatments	Plants	Treatments	Plants
Control	*Alnus hirsuta*	Control	*Abies fraseri*
8000 ppm KIBA	*A. maximowiczii*	8000 ppm KIBA	*Cephalotaxus sinensis*
Agro. strain A-4	*Cercis canadensis*	5000 ppm NAA	*Thuja plicata*
Agro. strain R-1600	*C. chinensis*	*Agro.* strain A-4	
KIBA + *Agro.*A-4	*Prunus cyclamina*	*Agro.* strain R-1600	
KIBA + *Agro.*R-1600	*P.* 'Kwanzan'	KIBA + *Agro.* A-4	
	P. 'Okame'	KIBA + *Agro.* R-1600	
	P. sargentii	NAA + *Agro.* A-4	
		NAA + *Agro.* R-1600	

RESULTS AND DISCUSSION

Results for overall percent rooting and rooting characteristics for all plants are presented in Tables 2 through 5. *Agrobacterium* treatments had varying effects on percent rooting and rooting characteristics depending on the plant and the *Agrobacterium* strain. In general, treatments combining KIBA with *Agrobacterium* significantly enhanced percent rooting and rooting characteristics for some of the difficult-to-root plants but not others.

A strong, positive *Agrobacterium*-related response for increased percent rooting, enhanced rooting characteristics, or both (compared to controls and traditional hormone treatments), was observed in the difficult-to-root plants *A. fraseri, C. sinensis, A. hirsuta, C. chinensis,* and *P. sargentii.* The more readily rooted plants *P.* 'Kwanzan', *P.* 'Okame', and *T. plicata* showed little or no *Agrobacterium*-related response for enhancement of percent rooting or rooting characteristics. No roots were formed on any *A. maximowiczii, C. canadensis,* or *P. cyclamina* cuttings (although there was significant callus formation on some treatments) therefore no results were reported for these taxa.

There was no apparent relationship between enhancement of percent rooting and rooting characteristics in taxa that responded to *Agrobacterium* treatments compared to traditional hormone and control treatments. In some plants both percent rooting and rooting characteristics were enhanced by *Agrobacterium* treatments relative to traditional hormone and control treatments (*Abies, Cephalotaxus*). In some plants, percent rooting only was enhanced by *Agrobacterium* treatments relative to traditional hormone and control treatments (*C. chinensis*). In some plants, rooting characteristics only were enhanced by *Agrobacterium* treatments relative to traditional hormone and control treatments (*A. hirsuta, P. sargentii*).

Softwoods.

Effect of Treatments on Mean Percent Rooting of Softwoods (see Table 2). There were no effects of treatments on mean percent rooting of *A. hirsuta, P.* 'Okame' or *P. sargentii*. For *P.* 'Kwanzan', all KIBA treatments showed significantly greater percent rooting compared to all other treatments. KIBA alone and combined with either *Agrobacterium* strain showed 100% rooting, while controls and *Agrobacterium* strains alone showed significantly lower rooting at only 55% to 65%. For *C. chinensis*, KIBA combined with *Agrobacterium* A-4 gave significantly increased percent rooting compared to all other treatments (100%). *Agrobacterium* A-4 alone gave intermediate results (90%) and all other treatments were significantly lower ($\leq$ 80%).

Table 2. Mean percent rooting of softwood stem cuttings of five different ornamental trees following treatment of cuttings with one of five rooting promoters or control after 12 weeks of standard intermittent mist treatment in a medium of perlite and peat medium (2 : 1, v/v). *Agro.*=*Agrobacterium rhizogenes.*

	Percent rooting of five ornamental trees[x]				
Treatment	*Alnus hirsuta*	*Cercis chinensis*	*Prunus* 'Kwanzan'	*Prunus* 'Okame'	*Prunus sargentii*
Control	30	75[a]	65[a]	85	45
8000 ppm KIBA	15	80[a]	100[b]	100	70
Agro. A-4	65	90[ab]	55[a]	65	45
Agro. R-1600	35	70[a]	65[a]	80	55
KIBA + *Agro.* A-4	45	100[b]	100[b]	90	75
KIBA + *Agro.* R-1600	5	50[a]	100[b]	90	85
	NS	*	**	NS	NS

NS=No statistically significant differences among column means.
*Statistically significant differences at $p \leq 0.05$ among column means.
**Statistically significant differences at $p \leq 0.01$ among column means.
[a,b,c] Column means with different letter designations are significantly different as per indicated p value.
[x]Statistical analysis was performed on arcsin transformations of percentages. Actual percentages are presented here for clarity. Statistically significant differences indicated apply to transformed data.

Effect of Treatments on Rooting Characteristics of Softwoods (see Table 3). Treatments had no significant effects on rooting characteristics of *C. chinensis* or *P.* 'Okame'. For *P.* 'Kwanzan', KIBA treatments alone, or in combination with either *Agrobacterium* strain, significantly improved all rooting characteristics (length of longest root, root number, root fresh weight) compared to all other treatments, but the addition of *Agrobacterium* did not enhance results compared to KIBA alone. For *P. sargentii*, KIBA treatments alone, or in combination with either *Agrobacterium* strain, significantly improved rooting characteristics compared to all other treatments. The combination of KIBA with *Agrobacterium* R-1600 increased mean root fresh weight only compared to all other treatments, however, the combination of KIBA with *Agrobacterium* A-4 increased mean root number and mean root fresh weight compared to all other treatments. *Prunus sargentii* cuttings showed enhanced rooting characteristics when treated with KIBA combined with *Agrobacterium* A-4. These root systems were more highly branched and more fully developed than those of other treatments. *Agrobacterium* strain treatments alone, in contrast, had no effect on rooting characteristics.

For *A. hirsuta*, combined treatment with KIBA and *Agrobacterium* A-4 significantly enhanced all rooting characteristics. These cuttings had more highly branched and fully developed root systems. Treatment with KIBA alone increased mean root fresh weight but did not effect mean length of longest root or mean root number. There were no other treatment effects.

Summary of Effects of Treatments on Softwoods (see Tables 2 and 3). Use of *Agrobacterium* combined with KIBA produced significantly enhanced results in some difficult-to-root softwoods in this trial (*A. hirsuta, C. chinensis, P. sargentii*). Use of *Agrobacterium* alone, however, had no effect on percent rooting or rooting characteristics of softwoods tested. There were significant treatment effects on mean percent rooting of *C. chinensis* and *P.* 'Kwanzan'. There were no treatment effects on mean percent rooting in *A. hirsuta, P.* 'Okame', or *P. sargentii*. Any KIBA treatment (alone or combined with either *Agrobacterium* strain) increased mean percent rooting of *P.* 'Kwanzan'. Use of *Agrobacterium* A-4 combined with KIBA, however, significantly enhanced percent rooting in *C. chinensis* compared with the control and traditional KIBA treatments. These results show promise for further study and application to *Cercis* cutting propagation. There were significant treatment effects on rooting characteristics of *A. hirsuta, P. sargentii,* and *P.* 'Kwanzan'. Potassium indolebutyric acid combined with *Agrobacterium* A-4 enhanced all rooting characteristics of *A. hirsuta* such that cuttings had more highly branched and fully developed root systems compared to those of control and traditional hormone treatments. KIBA alone enhanced rooting characteristics of *Prunus sargentii* but root fresh weight and root number were further increased when KIBA was combined with *Agrobacterium* A-4, and root fresh weight was also further increased when KIBA was combined with *Agrobacterium* R-1600. Any KIBA treatment enhanced all rooting characteristics of *P.* 'Kwanzan'. There were no treatment effects on rooting characteristics of *P.* 'Okame' or *C. chinensis*.

Conifers.

Effect of Treatments on Mean Percent Rooting of Conifers (see Table 4). For *A. fraseri*, KIBA combined with either strain of *Agrobacterium* significantly increased percent rooting compared with all other treatments. *Agrobacterium* A-4 alone gave intermediate results and there were no differences between all other treatments. For *C. sinensis*, KIBA combined with either strain of *Agrobacterium* significantly increased percent rooting compared with all other treatments. KIBA alone and either *Agrobacterium* strain alone all gave intermediate results and there were no differ-

Table 3. Mean length of longest root (cm) (LN), mean number of roots ≥ 1 cm long (NM), and mean fresh weight of roots (g) (FW), per cutting, for softwood stem cuttings of 5 different ornamental trees following treatment of cuttings with one of 5 rooting promoters or control after 12 weeks of standard intermittent mist treatment in a medium of perlite and peat (2:1, v/v). *Agro.*=*Agrobacterium rhizogenes*.

	Rooting characteristics for each of 5 ornamental trees														
	Alnus hirsuta			*Cercis chinensis*			*Prunus* 'Kwanzan'			*Prunus* 'Okame'			*Prunus sargentii*		
Treatment	LN	NM	FW	LN	NM	FW	LN	NM	FW	LN	NM	FW	LN	NM	FW
Control	7.60[a]	34.6[a]	0.52[a]	10.8	20.8	0.41	12.3[a]	43.2[a]	1.96[a]	19.6	79.4	0.62	11.3[a]	22.3[a]	1.13[a]
8000ppm KIBA	4.70[a]	43.2[a]	0.83[b]	10.9	25.1	0.37	20.9[b]	108.8[b]	4.29[c]	21.8	101.4	0.77	19.8[b]	81.5[b]	2.14[b]
Agro. A-4	5.60[a]	15.1[a]	0.32[a]	11.3	30.6	0.41	9.81[a]	26.8[a]	1.63[a]	14.8	60.8	0.54	8.42[a]	21.6[a]	0.86[a]
Agro. R-1600	7.52[a]	24.1[a]	0.42[a]	11.6	21.2	0.36	12.7[a]	39.5[a]	1.78[a]	13.5	50.5	0.43	11.4[a]	29.2[a]	0.98[a]
KIBA+ *Agro*A-4	11.1[b]	102.2[b]	1.99[c]	10.7	22.7	0.42	20.4[b]	94.5[b]	3.31[b]	16.3	82.9	0.60	26.8[b]	146.0[c]	3.56[c]
KIBA + *Agro.* R-1600	12.1[y]	44.0[y]	1.09[y]	9.54	21.5	0.48	19.5[b]	97.9[b]	3.19[b]	17.7	77.2	0.69	25.1[b]	102.8[b]	3.18[c]
	*	**	**	NS	NS	NS	**	**	**	NS	NS	NS	**	**	**

NS=No statistically significant differences among column means.
*Statistically significant differences at $p \leq 0.05$ among column means.
**Statistically significant differences at $p \leq 0.01$ among column means.
[a,b,c] Column means with different letter designations are significantly different as per indicated p value.
[y]These data were included for observational comparison only and were not included in any statistical analyses because they are the means of only 2 surviving cuttings in this treatment group.

ences between all other treatments. Using combined treatments of *Agrobacterium* with IBA significantly increased percent rooting in both of the difficult-to-root conifers compared to traditional hormone treatments. There were no significant treatment effects on percent rooting of *T. plicata* which roots readily under most conditions and treatments.

Table 4. Mean percent rooting of hardwood stem cuttings of three different ornamental conifers following treatment of cuttings with one of eight rooting promoters or control after 12 weeks of standard intermittent mist treatment in a medium of perlite and peat (2 : 1, v/v). *Agro.=Agrobacterium rhizogenes.*

	Percent Rooting of Three Ornamental Conifers[x]		
Treatment	*Abies fraseri*	*Cephalotaxus sinensis*	*Thuja plicata*
Control	5[a]	30[a]	75
8000 ppm KIBA	20[a]	60[ab]	90
5000 ppm NAA	10[a]	20[a]	75
Agro. A-4	30[b]	60[ab]	95
Agro. R-1600	10[a]	65[bc]	75
KIBA + *Agro.* A-4	70[c]	100[c]	80
KIBA + *Agro.* R-1600	60[bc]	95[c]	95
NAA + *Agro.* A-4	10[a]	45[a]	90
NAA + *Agro.* R-1600	15[a]	30[a]	55
	*	*	NS

NS=No statistically significant differences among column means.
*Statistically significant differences at $p \leq 0.05$ among column means.
**Statistically significant differences at $p \leq 0.01$ among column means.
[a,b,c]Column means with different letter designations are significantly different as per indicated p value.
[x]Statistical analysis was performed on arcsin transformations of percentages. Actual percentages are presented here for clarity. Statistically significant differences indicated apply to transformed data.

Effect of Treatments on Rooting Characteristics of Conifers (see Table 5). For *A. fraseri*, combined treatments of KIBA with either strain of *Agrobacterium* significantly increased all rooting characteristics in comparison to all other treatments. Root systems from these treatments showed strongly enhanced branching and development. For *C. sinensis*, combined treatments of either *Agrobacterium* strain with KIBA significantly increased all rooting characteristics compared to all other treatments. Root systems from these treatments showed enhanced branching and development. Either strain of *Agrobacterium* alone, KIBA alone or NAA in combination with either strain of *Agrobacterium* gave intermediate results compared to controls and NAA alone. For *T. plicata* there were no treatment effects on root number. KIBA combined with *Agrobacterium* R-1600 increased the length of longest root compared to all other treatments and all NAA treatments reduced length of longest root compared to the control. All treatments using KIBA, NAA or *Agrobacterium* A-4

Table 5. Mean length of longest root (cm) (LN), mean number of roots ≥ 1 cm long (NM), and mean fresh weight of roots (g) (FW), per cutting, for hardwood stem cuttings of three different ornamental conifers following treatment of cuttings with one of eight rooting promoters or control after 12 weeks of standard intermittent mist treatment in a medium of perlite and peat (2 : 1, v/v). *Agro.*=*Agrobacterium rhizogenes.*

	Rooting characteristics for each of three ornamental conifers								
	Abies fraseri			*Cephalotaxus sinensis*			*Thuja plicata*		
Treatment	LN	NM	FW	LN	NM	FW	LN	NM	FW
Control	0.86^{a}	1.45^{a}	0.15^{a}	1.98^{a}	3.40^{a}	0.24^{a}	7.81^{bc}	13.0	0.55^{a}
8000 ppm KIBA	2.50^{a}	1.70^{a}	0.36^{a}	5.37^{b}	13.9^{ab}	0.77^{ab}	7.42^{bc}	30.8	1.15^{b}
5000 ppm NAA	2.13^{a}	4.01^{a}	0.49^{a}	1.29^{a}	3.02^{a}	0.23^{a}	5.41^{ab}	14.7	0.69^{ab}
Agro. A-4	2.65^{a}	4.19^{a}	0.40^{a}	7.41^{b}	22.8^{b}	0.95^{b}	10.2^{bc}	37.1	1.24^{b}
Agro. R-1600	0.95^{a}	1.45^{a}	0.07^{a}	10.0^{b}	32.2^{b}	1.56^{b}	6.52^{b}	9.84	0.48^{a}
KIBA+*Agro.*A-4	10.0^{b}	31.2^{c}	1.97^{b}	14.5^{c}	60.0^{c}	2.69^{c}	8.02^{bc}	46.5	1.27^{b}
KIBA+*Agro.*R-1600	8.56^{b}	16.3^{b}	1.33^{b}	15.9^{c}	58.1^{c}	2.19^{c}	11.6^{c}	35.0	1.29^{b}
NAA+*Agro.*A-4	2.00^{a}	1.99^{a}	0.28^{a}	3.97^{ab}	8.59^{a}	0.60^{ab}	5.00^{ab}	24.0	1.21^{b}
NAA+*Agro.*R-1600	3.05^{a}	2.24^{a}	0.29^{a}	3.52^{ab}	11.2^{ab}	0.64^{ab}	1.84^{a}	7.36	0.32^{a}
	**	**	**	**	**	**	*	NS	*

NS=No statistically significant differences among column means.
*Statistically significant differences at $p \leq 0.05$ among column means.
**Statistically significant differences at $p \leq 0.01$ among column means.
[a,b,c] Column means with different letter designations are significantly different as per indicated p value.

increased root fresh weight compared to the control, but did not differ in other respects, while all treatments using *Agrobacterium* R-1600 reduced root fresh weight compared to all other treatments.

Summary of Effects of Treatments on Conifers (see Tables 4 and 5). Use of *Agrobacterium* combined with IBA significantly enhanced results in the difficult-to-root conifers (*A. fraseri, C. sinensis*) but not the readily rooted conifer (*T. plicata*). Use of *Agrobacterium* alone enhanced some results in comparison to controls, but not in comparison to traditional hormone treatments. *Agrobacterium* combined with KIBA strongly increased percent rooting and enhanced rooting characteristics in both of the difficult-to-root conifers compared to traditional hormone treatments and controls, but did not result in a strong response in the readily rooted conifer. These results show promise for further study and application to *Abies* and *Cephalotaxus* cutting propagation.

CONCLUSIONS

Use of *A. rhizogenes* wild type strains in combination with KIBA as a rooting promoter shows good promise for some difficult-to-root woody ornamentals — particularly conifers in these trials. There were large differences in response among the taxa tested. Significantly more work clearly needs to be done to elucidate which species and cultivars could benefit from such treatment.

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