Effects of K-IBA on the Rooting of Stem Cuttings of 15 Taxa of Snowbells (*Styrax* spp.)[1]

Jason J. Griffin[2] and F. Todd Lasseigne[3]
Department of Horticulture, Forestry, and Recreation Resources, Kansas State University
John C. Pair Horticultural Center, 1901 East 95th Street South, Haysville, KS 67060

Abstract

Currently, information regarding asexual propagation of *Styrax* L (snowbells) is lacking in the literature. In this study, the influence of K-IBA treatment on root production was investigated on stem cuttings of 15 different taxa of *Styrax*. Softwood or semi-hardwood stem cuttings dipped in 0, 3000, or 8000 ppm (0, 0.3, or 0.8%) K-IBA were evaluated for percent rooting and root number per rooted cutting. Cuttings were allowed to root under intermittent mist in a perlite:peat (3:1 by vol) rooting substrate for 9 weeks. Percent rooting and the response to K-IBA varied by species and by cultivar within a species. All 15 taxa rooted to some extent. Auxin application improved percent rooting for *S. dasyanthus*, *S. japonicus* 'Carillon', *S. japonicus* 'Pink Chimes', *S. japonicus* 'Snowfall', and *S. tonkinensis*, and decreased percent rooting for *S. formosanus*, *S. japonicus* 'Issai', *S. japonicus* 'Pink Cascade', and *S. serrulatus* (clone B). Application of K-IBA increased the number of roots produced per rooted cutting on *S. calvescens*, *S. confusus*, *S. japonicus* 'Pink Chimes', *S. serrulatus* (clone A), and *S. tonkinensis*.

Index words: adventitious rooting, indolebutyric acid, plant propagation, storax, Styracaceae.

Species used in this study: *Styrax calvescens* Perkins (bald snowbell); *S. confusus* Hemsl. (Chinese snowbell); *S. dasyanthus* Perkins (silvery snowbell); *S. formosanus* Matsum. (Formosan snowbell); *S. japonicus* Sieb. & Zucc. (Japanese snowbell) (cultivars, 'Carillon', 'Crystal', 'Emerald Pagoda', 'Issai', 'Pink Cascade', 'Pink Chimes', 'Rubra Pendula', and 'Snowfall'); *S. serrulatus* Roxb. (sawtooth snowbell); *S. tonkinensis* (Pierre) Craib ex Hartwich. (Tonkin snowbell).

Significance to the Nursery Industry

The snowbells (*Styrax* L.) are a group of flowering shrubs and trees distributed throughout the warm-temperate regions of the northern hemisphere. There are approximately 120 species (5), of which only *Styrax japonicus* Sieb. & Zucc. (Japanese snowbell) and its cultivars are currently of commercial significance. This beautiful, small flowering tree is becoming increasingly popular with landscapers and homeowners alike. Increased awareness of more obscure taxa may lead to their eventual production and cultivation. Additionally, successful propagation will increase the availability of genetic material to plant breeders attempting to improve upon present selections. Asexual propagation of desirable plant material is necessary for production and selection of superior clones. Results herein demonstrate that propagation of a diverse assemblage of *Styrax* is possible by stem cuttings. However, species, and cultivars within a species, do not respond similarly to auxin treatment. Percent rooting of many taxa was improved when cuttings were treated with 3000 or 8000 ppm (0.3 or 0.8%) of the potassium salt of indolebutyric acid (K-IBA). However, for some taxa rooting was unaffected by K-IBA treatment; whereas in others, rooting was negatively affected by K-IBA.

Introduction

Although the Internet, trade magazines, and general gardening publications abound with recommendations regarding asexual propagation of *Styrax* by stem cuttings, these sources focus almost exclusively on *S. japonicus* and its cultivars. To date, few controlled studies have investigated cutting propagation of the snowbells, *S. japonicus* or other species. In general, most propagators consider *S. japonicus* easy to root by applying 1000 to 3000 ppm (0.1 to 0.3%) IBA to softwood cuttings, with little difficulty encountered subsequently in over-wintering these cuttings (4, 14). However, specific cultivar recommendations have not been reported. Stem cuttings of *S. japonicus* 'Emerald Pagoda' are known to be difficult to root, and even more difficult to over-winter (authors' personal experience, 2). Another popular cultivar, *S. japonicus* 'Pink Chimes' roots readily; however, over-wintering may be difficult (9). Propagation guidelines for other species of *Styrax* are lacking.

As a group, the snowbells are flowering shrubs or small trees. Flowering occurs in late spring with solitary, bell shaped white (rarely pale pink) flowers produced beneath the foliage on terminal racemes. Flower color, abundance, fragrance, and size are species or cultivar dependent. Although clonal selections have been made, most have been based on growth habit and flower color (3). Current breeding programs are expanding on those characteristics as well as improving stress tolerance to expand the range in which snowbells can be grown.

To increase snowbell tolerance to environmental stresses such as drought, heat, or freezing temperatures, plant breeders may need to incorporate traits found in species other than *S. japonicus*. Currently, horticultural information is lacking on other species of *Styrax* (10, 13). One of the first steps in evaluating the diverse species and cultivars of *Styrax* for their potential in plant improvement programs is to ascertain their potential for clonal propagation.

Previous research has shown that hardwood cuttings of *S. japonicus* root poorly whereas softwood cuttings taken in May and treated with IBA, indoleacetic acid (IAA), or naphthaleneacetic acid (NAA) root easily (8). Best results were obtained for cuttings treated with a liquid formulation

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[2]Assistant Professor.

[3]Executive Director, Paul J. Ciener Botanical Garden, Post Office Box 1069, Kernersville, NC 27285.

of IBA at 1000 ppm (0.1%). A much higher IBA concentration of 7000 ppm (0.7%) was needed to obtain maximum rooting of softwood cuttings taken in July of *S. japonicus* (7). These discrepancies in auxin concentrations may suggest that growth stage, provenance, and stock plant genotype influence optimal auxin concentration. Another study demonstrated that *S. americanus* Lam. (American snowbell) rooted in high percentages (80 to 90%) when cuttings were taken from April through July and treated with 2500 or 5000 ppm (0.25 or 0.5%) IBA (1). There has been some research reported for micropropagation of *Styrax* (6, 11, 12). However, rooting of stem cuttings remains the primary method of asexual propagation.

The objective of this research was to determine the K-IBA concentration that resulted in the highest rooting percentage and the greatest number of roots produced on stem cuttings of different taxa of *Styrax*. Results herein discuss the rooting potential of 15 different taxa of *Styrax*. In all, the rooting response of eight cultivars of *S. japonicus*, two accessions of *S. serrulatus* Roxb., and five other species rarely seen outside arboreta (Table 1) are presented in this report.

Material and Methods

Stem cuttings of all taxa of *Styrax* (Table 1) were collected throughout the canopy of plants growing at the JC Raulston Arboretum, (North Carolina State University, Raleigh) on July 26, 2004. All stock plants had been growing in the ground less than 15 years and are considered physiologically mature as they had flowered previously. Plant material was placed in plastic bags, and then shipped, overnight, to the John C. Pair Horticultural Center, (Kansas State University, Wichita), where it was held overnight at 5.5C (42F). The only exception was for *S. formosanus* Matsum., of which cuttings were collected from plants growing in 3.8 liter containers (1 gal) at the John C. Pair Horticultural Center on July 28, 2004. Due to the variable growth patterns of species with such a wide geographic origin, not all taxa were in the same physiological state at the single cutting collection site. Some taxa were actively growing and thus considered softwood cuttings, whereas other taxa had set a firm terminal bud and were therefore considered semi-hardwood cuttings. Two taxa did not fit either description and so were considered to be in a transitional state and are so noted in Table 1.

Table 1. Taxa of *Styrax* investigated for rooting potential, and growth stage at the time of auxin treatment.

Species or cultivar	Growth stage
S. calvescens	Semi-hardwood
S. confusus (syn. *S. philadelphoides*)	Semi-hardwood
S. dasyanthus	Semi-hardwood
S. formosanus	Softwood
S. japonicus 'Carillon'	Semi-hardwood
S. japonicus 'Crystal'	Semi-hardwood
S. japonicus 'Emerald Pagoda'	Semi-hardwood
S. japonicus 'Issai'	Transitional[z]
S. japonicus 'Pink Cascade'	Softwood
S. japonicus 'Pink Chimes'	Semi-hardwood
S. japonicus 'Rubra Pendula'	Semi-hardwood
S. japonicus 'Snowfall'	Semi-hardwood
S. serrulatus (clone A)	Semi-hardwood
S. serrulatus (clone B)	Transitional
S. tonkinensis	Softwood

[z]Transitional = cuttings did not fit clearly into softwood or semi-hardwood classification. Shoot extension had ceased, newest leaves had not fully matured, terminal bud not yet visable.

Cutting material was trimmed to 8 to 13 cm (3 to 5 in) terminal shoots, with care taken to ensure the proximal portion of the cutting was of current season's growth. Leaves were stripped from the lower half of the cuttings and the basal 1 cm (0.4 in) of each cutting was dipped for 5 sec in 0, 3000 (0.3%), or 8000 ppm (0.8%) K-IBA dissolved in distilled water. The treated cuttings were allowed to air dry for 5 min prior to insertion into flats 9 cm (3.5 in) deep containing a rooting substrate of perlite:peat (3:1 by vol). The flats were placed on a raised greenhouse bench under natural photoperiod and intermittent mist operating for 5 sec every 7 min during daylight hours at the John C. Pair Horticultural Center. Greenhouse temperatures were set at days/nights of 28/24C (82/75F). For each taxon, 75 individual cuttings were assigned to the three treatments and arranged in a randomized complete block design with five blocks, and five cuttings per treatment per block.

At 9 weeks, cuttings were harvested and data recorded. Data included percent rooting and number of roots ≥ 1 mm (0.04 in) in length on rooted cuttings. A cutting was considered rooted if it had one adventitious root ≥ 1 mm (0.04 in) in length. Data were subjected to analysis of variance and where appropriate, means were separated by least significant difference (LSD). Due to a lack of cutting material, only three auxin treatments were utilized. Therefore, a means separation was employed rather than regression analysis. Means separation was not utilized to detect K-IBA effects across taxa, only within a taxon. Therefore, no statistical comparisons are made between taxa in a softwood growth stage and taxa in a semi-hardwood growth stage.

Results and Discussion

Percent rooting or root number were affected by K-IBA application in 12 of the 15 taxa examined (Table 2). Application of K-IBA affected percent rooting of nine taxa. Of these nine taxa, percent rooting was increased by auxin application in five: *S. dasyanthus* Perkins, *S. japonicus* 'Carillon', *S. japonicus* 'Pink Chimes', *S. japonicus* 'Snowfall', and *S. tonkinensis* (Pierre) Craib ex Hartwich. Percent rooting increased two-fold for *S. japonicus* 'Carillon', *S. japonicus* 'Pink Chimes' and *S. tonkinensis* when treated with 3000 ppm (0.3%) K-IBA. These results agree with previous reports that suggested *S. japonicus* softwood cuttings could be rooted and that rooting percentage was influenced by IBA concentration (7, 8). Nontreated cuttings of *S. dasyanthus* and *S. japonicus* 'Snowfall' failed to root. However, 3000 ppm (0.3%) K-IBA improved rooting in these two taxa to 36 and 25%, respectively, with a further increase in rooting when cuttings were treated with 8000 ppm (0.8%) K-IBA. Interestingly, *Styrax japonicus* 'Snowfall' was the only taxa in which application of 8000 ppm (0.8%) K-IBA improved rooting over the 3000 ppm (0.3%) treatment. Four taxa were affected negatively by auxin application: *S. formosanus*, *S. japonicus* 'Issai', *S. japonicus* 'Pink Cascade', and *S. serrulatus* (clone B). In most of these instances, 3000 ppm (0.3%) K-IBA did not improve rooting over the control (0 ppm), while 8000 ppm (0.8%) significantly reduced rooting. Percent rooting of the remaining taxa was unaffected by K-IBA treatment.

Root number per rooted cutting was also affected by K-IBA concentration (Table 2). Of the 15 taxa examined, auxin

Table 2. Percent rooting and number of roots per rooted cutting of various taxa of *Styrax* as affected by K-IBA treatment.

	Rooting (%)				Root no./rooted cutting			
	K-IBA concn. (ppm)				K-IBA concn. (ppm)			
Species or cultivar	0	3000	8000	Sig	0	3000	8000	Sig
S. calvescens	28	36	32	NS[z]	3.0b	9.5b	21.0a	**
S. confusus	28	44	32	NS	3.1b	14.1a	12.0a	**
S. dasyanthus	0b	36a	44a	**		3.1	5.2	NS
S. formosanus	84a	80a	48b	*	7.6	14.2	5.9	NS
S. japonicus 'Carillon'	30b	75a	45ab	*	1.7	3.9	5.0	NS
S. japonicus 'Crystal'	12	28	16	NS	10.8	7.3	9.7	NS
S. japonicus 'Emerald Pagoda'	0	4	16	NS		1.0	2.5	NS
S. japonicus 'Issai'	60a	68a	24b	*	7.7b	23.0a	17.9ab	*$P = 0.09$
S. japonicus 'Pink Cascade'	48a	24ab	8b	*	3.9	1.5	1.5	NS
S. japonicus 'Pink Chimes'	48b	92a	76ab	*	5.4b	19.3b	38.8a	**
S. japonicus 'Rubra Pendula'	68	56	64	NS	2.8	4.9	3.6	NS
S. japonicus 'Snowfall'	0b	25b	75a	**		6.5	8.0	NS
S. serrulatus (clone A)	72	84	88	NS	13b	38.2a	37.4a	**
S. serrulatus (clone B)	68a	36b	16b	**	13.9b	39.3a	27.5ab	*$P = 0.08$
S. tonkinensis	32b	68a	36ab	*	2.0b	6.3a	4.0b	**

[z]Effect of K-IBA treatment on percent rooting or root number is nonsignificant (NS), or significant at $P \leq 0.05$ (*) or $P \leq 0.01$ (**). Means within a row and followed by the same letter are not significantly different based on a protected LSD ($P \leq 0.05$) (n = 25). If significance is relaxed to $P \leq 0.1$, root number of *S. japonicus* 'Issai' and *S. serrulatus* (clone B) become significant.

application increased the number of roots produced on a rooted cutting for five of the taxa: *Styrax calvescens* Perkins, *S. confusus* Hemsl., *S. japonicus* 'Pink Chimes', *S. serrulatus* (clone A), and *S. tonkinensis*. Of these five taxa, 8000 ppm (0.8%) K-IBA negatively affected root production in *S. tonkinensis* only. The other four taxa either exhibited no improvement or a substantial increase in root production at the higher auxin concentration. If the level of significance is relaxed to $P \leq 0.1$, root number on two additional taxa was influenced by K-IBA application. In both instances, root number of *S. japonicus* 'Issai' and *S. serrulatus* (clone B) increased with K-IBA at 3000 ppm (0.3%) followed by a slight decrease at 8000 ppm (0.8%). Overall, when root production was improved by auxin application, numerous roots were produced (14 to 40 roots per rooted cutting). Taxa where K-IBA application failed to improve root production generally produced few roots overall (1 to 14 roots per rooted cutting).

Although these results may be considered a preliminary report on adventitious rooting of the taxa investigated, in nearly all instances these data represent the first, experimentally-based, replicated values reported for these taxa. Once sufficient stock plant material is acquired, further studies examining multiple cutting collection dates, additional auxin concentrations, and various auxin formulations can be incorporated utilizing a greater quantity of cuttings, thereby increasing statistical precision. Our results were obtained utilizing a growth stage that often is suggested as optimal for adventitious rooting of *Styrax* (4). With the knowledge that only one cutting collection date was available for most taxa, the decision to target a transitional growth stage from softwood to semi-hardwood was a logical compromise. As is frequently a challenge when studying rare and unusual taxa, a limited supply of available stock plants results in a low number of experimental units. In the current study, a total of 25 cuttings per treatment were available. While this number of cuttings was sufficient to detect large treatment effects, additional cuttings for future experiments may increase the precision of the data and elucidate additional treatment effects.

These results suggest that clonal propagation of *Styrax* is possible although there is great variability in rooting potential between species, and between clones and cultivars within a species. In general, cultivars of *S. japonicus* rooted moderately well, which had been previously reported (3). *Styrax japonicus* 'Pink Chimes' rooted easily, and rooted cuttings began growing in the rooting bench. However, *S. japonicus* 'Emerald Pagoda' rooted poorly, as has been noted previously for this cultivar. The two accessions of *S. serrulatus* demonstrated a very different response to auxin application, with 'clone A' showing no response to auxin and 'clone B' showing a decrease in rooting in response to increasing K-IBA. Overall, each of the 15 taxa produced some rooted cuttings, although some taxa were clearly more difficult to root by stem cuttings than others. However, personal experience has shown that over-wintering newly rooted cuttings of *Styrax* can be difficult, and this subject requires further investigation to ensure successful clonal propagation of these beautiful small landscape trees. Future studies may explore multiple cutting dates and auxin formulations. For the taxa that are particularly recalcitrant, grafting onto seedling understock or tissue culture methods may be explored.

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