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Effects of Auxin Concentration and Medium Temperature on Four Woody Plant Taxa Propagated by Stem Cuttings using Recirculating Subirrigation in a Growth Chamber[1]

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Abstract

Four woody plant taxa ('Sparkleberry' holly, 'Mariesii' viburnum, 'Shasta' viburnum, and Red Sunset® maple) were propagated from softwood stem cuttings in a recirculating subirrigation propagation system to evaluate the effect on adventitious root formation of three auxin (Dip-n-Grow®) concentrations [0 (water), 20:1, or 10:1] and three medium temperatures [20C (68F), 23C (74F), or 26C (79F)]. All taxa showed a positive rooting response to auxin treatment. Compared to cuttings treated with water, root number of all taxa doubled when treated with the highest auxin concentration (10:1 Dip-n-Grow® dilution). Length of the longest root of rooted cuttings increased with auxin treatment of two taxa. Medium temperature also affected rooting of all taxa. The intermediate temperature evaluated, 23C (74F), was optimal for all rooting parameters on cuttings of 'Mariesii' viburnum, and for root length and percentage rooting on cuttings of 'Shasta' viburnum and Red Sunset® maple. A propagation medium temperature of 26C (79F) resulted in the greatest root number on cuttings of 'Shasta' viburnum, while 20C (68F) was optimal for root number on cuttings of Red Sunset® maple. Root numbers on cuttings of 'Mariesii' viburnum were reduced at 20C (68F) and 26C (79F), which appeared to be sub- and supra-optimal temperatures, respectively, for root initiation. All rooting parameters on cuttings of 'Sparkleberry' holly increased with propagation medium temperature. Temperature-auxin interactions were apparent for root number and length on cuttings of 'Mariesii' and 'Shasta' viburnum. The recirculating subirrigation system was useful for evaluating the basal temperature and auxin dose interactions and was effective for rooting softwood stem cuttings without intermittent mist irrigation.

Index words: indole-3-butyric acid, 1-napthaleneacetic acid, rooting environment, propagation medium temperature.

Species used in this study: red maple (*Acer rubrum* L. 'Franksred'); Sparkleberry holly (*Ilex* 'Sparkleberry'); Japanese snowball viburnum (*Viburnum plicatum* Thunb. var. *tomentosum* (Thunb.) Rehd. 'Mariesii'); Japanese snowball viburnum (*Viburnum plicatum* Thunb. var. *tomentosum* (Thunb.) Rehd. 'Shasta').

Significance to the Nursery Industry

Plant propagators use auxin and bottom heat, separately or in combination, to increase rooting percentages of stem cuttings, enhance root quality, and reduce propagation time. Previous studies on the effect of medium temperature, auxin treatment and their interaction on root formation of cuttings have been contradictory because these factors can be sub- or supra-optimal to the rooting process and their effect may be species-dependent. Use of recirculating subirrigation propagation systems as a research tool allows insight into factors affecting root formation while maintaining a constant medium moisture content and reducing the environmental variability inherent in the use of overhead mist irrigation. In this study a recirculating subirrigation propagation system was used to evaluate the effects of three propagation medium temperatures and three auxin concentrations on the rooting of four woody plant taxa. 'Sparkleberry' holly, 'Mariesii' viburnum, and 'Shasta' viburnum yielded the greatest rooting percentage, mean root numbers, and mean length of longest root when treated with a 10:1 aqueous dilution of Dip-n-Grow®, or rooted at a medium temperature of 23C (74F). Rooting percentage, mean root number, and mean length of longest root of Red Sunset® maple were optimum when propagules were treated with 10:1 Dip-n-Grow® or rooted at a lower medium temperature [20C (68F)]. Recirculating subirrigation systems are promising tools for plant propagation because of improved product quality and reduced production costs, and as a means of addressing increased government environmental regulation. Recirculating subirrigation may also be used to enhance our understanding of the optimum environment for propagating many different plant species and to increase the overall success of rooting stem cuttings.

Introduction

A majority of plant propagation research has focused on the atmospheric environment of the propagation area and on the use of auxin treatment to promote rooting. Since the 1950s, overhead intermittent mist irrigation has been the conventional means of maintaining cutting turgidity during rooting. Subirrigation, another method to reduce water stress for successful rooting of cuttings, was first introduced in 1946 as a constant water table method (18) and again in 1956 as a 'hydroponic' method (14). Subirrigation propagation systems provide water from a water table to the base of stem cuttings by capillary action through the propagation medium. One of the first reports of subirrigation propagation of woody stem cuttings in the United States compared the rooting of cuttings of several lilac cultivars in subirrigation and under intermittent mist (16). Boland and Hanger (3), using components of hydroponics and nutrient film techniques, developed a recirculating subirrigation system that used heated water

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to solve problems of algae growth, stagnant water, and providing basal heat associated with earlier subirrigation systems. Further improvements in subirrigation propagation systems have come from studies examining the chemical properties of the propagation medium. Increased rooting percentages of *Rhododendron* L., *Acer* L. and *Daphne* L. stem cuttings have been obtained, respectively, by decreasing the propagation medium pH (10), adding mineral nutrients (20) or auxin (19) to the subirrigation water reservoir.

Indole-3-butyric acid (IBA) and 1-napthaleneacetic acid (NAA), the plant growth regulators used most widely in nursery and propagation commerce, promote adventitious root formation, increase rooting percentage, rate of root initiation, rooting uniformity, and number of roots when applied at close to optimal concentrations, which in turn varies with plant taxon and edaphic factors (2). Liquid formulations are the preferred means of applying auxins in the nursery industry because of the uniformity of results and ease of use (2). Rooting percentage, roots per rooted cutting, length of longest root, and the extent of basal necrosis on stem cuttings of various taxa of *Ilex* L. have been shown to change dramatically in response to optimal, sub- and supra-optimal auxin concentrations (6).

Propagation medium temperature also affects rooting success. As with auxin application, plant propagators can attempt to optimize root initiation, emergence, and elongation through adjustments in root-zone temperature during the propagation of stem cuttings. Dykeman (7) demonstrated that the optimal propagation medium temperature differed for root initiation and root elongation, and that these temperature optima also varied among species. For example, while more roots formed and roots emerged faster at propagation medium temperatures of ≈ 30C (86F), elongation and overall root mass were optimal at temperatures ≤ 25C (77F). Carpenter (5) reported that medium temperatures ≤ 22C (72F) yielded better quality roots, which were whiter, thicker, and less branched than the weak, filamentous roots formed in a medium maintained at 34C (93F).

The effect of propagation medium temperature on adventitious root initiation and root development is a net result of air temperature, air movement, irradiance, and heat provided to the base of the stem cutting. Drawing from various reports examining environmental effects on the rooting of woody cuttings, it may be generalized that optima for adventitious root formation include a photosynthetic photon flux (*PPF*) ≤ 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR (15), a stem base temperature of ≈ 22C (72F) (9), a minimum greenhouse air temperature of 10C (50F) (1) and a 10C (18F) differential between air and the stem base temperatures (13). These optima correspond to the greatest metabolic rate of the cutting and a balance of respiration and photosynthesis that maintains the carbohydrate supply needed for initiation and growth of adventitious roots.

The objective of this research was to evaluate the interaction of auxin concentration and propagation medium temperature in a controlled edaphic and atmospheric environment that met previously reported criteria for propagation of stem cuttings. The formation and development of adventitious roots on softwood cuttings of four woody plant taxa was evaluated in a recirculating subirrigation system without intermittent mist. The experiment was conducted in a controlled environment growth chamber that maintained an optimal, yet realistic atmospheric environment during evaluation of adventitious rooting at different root-zone temperatures and auxin concentrations.

Material and Methods

Propagation experiments were conducted in a 15 cu m storage cooler retrofitted as a controlled-environment growth chamber. Three 400-watt (General Electric Co., Louisville, KY) high-pressure sodium (HPS) lamps were spaced 0.6 m (1.9 ft) on center and suspended 1 m (3.3 ft) above the propagation canopy to provide light. Nine recirculating subirrigation units were arranged in a block three chambers wide by three chambers deep. The recirculating subirrigation units (described fully in 17) were constructed using modified Rubbermaid® storage boxes (Rubbermaid Inc., Wooster, OH) as propagation trays, filled with degassed horticultural perlite (Whittemore Co., Inc., Lawrence, MA), and 125-liter (33 gal) Sterilite™ totes as water reservoirs (Sterilite Corp., Townsend, MA). The water reservoirs contained ≈ 90 liters (24 gal) of de-ionized water circulated at 3 liters/min (0.8 gal/min) and heated to the desired medium temperature with 100 watt Rena Cal™ aquarium heaters (Aquarium Pharmaceuticals, Inc., Chalfont, PA). In the propagation trays, a water table of approximately 8 cm (3.1 in) was maintained with a 44.5 cm (17.5 in) length of perforated schedule 80 polyvinyl chloride (PVC) pipe covered with a 1 mm (0.04 in) mesh screen (17).

A CR21X Micrologger (Campbell Scientific Inc., Logan, UT) connected to a CS500 temperature and relative humidity (RH) probe, 105T thermocouple probes (Campbell Scientific Inc.) and a LI-190SA quantum sensor (LI-COR, Inc., Lincoln, NE) recorded air temperature and relative humidity, propagation medium and water reservoir temperatures, or photosynthetically active radiation (*PAR*) ($\mu mol \cdot s^{-1} \cdot m^{-2}$), respectively, at 15 min intervals for the duration of the experiment.

One-hundred-thirty-five 10-cm (4 in) leafy sub-terminal stem cuttings of *Acer rubrum* L. 'Franksred' (Red Sunset® maple), *Ilex* 'Sparkleberry' (*I. serrata* (Thunb.) x *I. verticillata* (L.) A. Gray; 'Sparkleberry' holly), *Viburnum plicatum* Thunb. var. *tomentosum* (Thunb.) Rehd. 'Shasta' ('Shasta' viburnum), and two hundred seventy 10 cm (4 in) sub-terminal leafy stem cuttings of *Viburnum plicatum* Thunb. var. *tomentosum* (Thunb.) Rehd. 'Mariesii' ('Mariesii' viburnum) were collected from the grounds of the University of Rhode Island, misted, placed in polyethylene bags and stored in a 4C (39F) cooler for 24 hrs before being inserted into the propagation medium. All cuttings, except those of 'Mariesii' viburnum, were inserted on July 12, 2000, and rooted for 33 days. Cuttings of 'Mariesii' viburnum were inserted on August 29, 2000, and rooted for 17 days. Leaves were stripped from the lower portion of each cutting, leaving two or three leaves. All cuttings were given a 2 cm (0.8 in) heavy wound on one side of the stem base that exposed the cambium. Cuttings were dipped for ≈ 3 sec to a depth of 2.5 cm (1 in) in water (0 ppm), or a 20:1 (500 ppm IBA, 250 ppm NAA) or 10:1 (1,000 ppm IBA, 500 ppm NAA) dilution ratio of deionized water:Dip-N-Grow® concentrate (Dip-N-Grow, Inc., Astoria-Pacific, Inc., Clackamas, OR). Each replication of 'Shasta' viburnum, Red Sunset® maple and 'Sparkleberry' holly contained five cuttings, while each replication of 'Mariesii' viburnum comprised 10 cuttings. Cuttings were inserted ≈ 4 cm (1.6 in) deep in recirculating subirrigation propagation trays maintained at one of the three desired me-

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Table 1. **Significance of mean Dip-n-Grow® (DNG) ratio, medium temperature and their interaction on the rooting response (rooting percentage, number of roots, length of longest root) of four species rooted in a growth chamber maintained at an air temperature of 13C (55F) with a day length of 11 hours at 300 μmol·m^{-2}·s^{-1} PAR.**

Treatment	Rooting response	'Mariesii' Viburnum	ω^2[y]	'Sparkleberry' Holly	ω^2[y]	Red Sunset® Maple	ω^2[y]	'Shasta' Viburnum	ω^2[y]
DNG ratio	percentage	NS	—[x]	**	—	•••	—	*	—
	number	***	0.415	***	0.291	**	0.170	***	0.521
	length (mm)	***	0.245	*	0.105	*	0.395	**	0.183
Medium temperature (C)	percentage	NS	—	***	—	NS	—	*	—
	number	***	0.231	*	0.100	***	0.365	***	0.281
	length (mm)	***	0.740	*	0.094	***	0.235	***	0.542
DNG ratio × medium temp. (C)	percentage	NS	—	NS	—	NS	—	*	—
	number	NS	0.000	NS	0.017	NS	0.111	***	0.292
	length (mm)	**	0.107	NS	0.000	NS	0.000	***	0.254

[z]'Mariesii' viburnum rooted in 17 days, all other taxa rooted in 33 days.

[y]ω^2 is a relative measure of magnitude for the variance of the treatment and error (Keren and Lewis, 1979).

[x]ω^2 was not used to measure relative magnitude of rooting percent.

NS, *, **, ***. Not significant, or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

dia temperatures. Cuttings were overhead irrigated immediately after insertion. De-ionized water was added, as needed, directly to the 125-liter Sterilite™ Totes to maintain the initial reservoir volume.

The experimental design was completely randomized with a 3 × 3 factorial array (3 propagation medium temperatures × 3 auxin concentrations) for each of the four taxa. At the end of the study, rooting percentage, root number, and length of longest root of rooted cuttings were recorded. A rooted cutting had at least one root ≥ 1 mm (0.04 in) in length. Main effects and interactions were analyzed using SPSS™ 11.0 (SPSS Science, Chicago, IL). Means were separated, when appropriate, using Tukey's honestly significant difference (HSD) post hoc test. Rooting percentage was evaluated separately from root number and length of longest root. All analyses were tested at $P < 0.05$. Partial omega squares ($\hat{\omega}^2$) were calculated to measure the amount of variance explained by main effects or interactions associated with root number and length of the longest root of rooted cuttings. This measure of relative treatment magnitude explains the variance associated with the main effect relative to the overall error variance, independent of other treatment variables (12).

Results and Discussion

Environmental conditions inside the growth chamber included an average air temperature of 12.6 ± 0.2C (54.7 ± 0.5F), a day length of 11 hrs at a *PPF* of 300 μmol·m^{-2}·s^{-1} (*PAR*), and a mean RH of 71 ± 14% (range 48–89%). Subirrigation units were set to maintain media temperatures of 20C (68F), 23C (74F), or 26C (79F), each ± 2C (3.6F). In general, the greatest rooting percentage, root number, and length of the longest root of rooted cuttings occurred when cuttings were treated with auxin and rooted at the intermediate temperature of 23C (74F). Auxin concentration and medium temperature significantly affected mean root number and mean length of the longest root of all four taxa. An interaction of auxin and temperature was observed for root length of 'Mariesii' viburnum and for all three rooting parameters of 'Shasta' viburnum (Table 1). As a main effect variable,

Table 2. **Mean rooting percentage, root number, and length of the longest root of rooted cuttings of four taxa treated with water or 20:1, or 10:1 Dip-n-Grow® (DNG) and medium temperatures of 20C (68F), 23C (74F), or 26C (79F). Key: % = rooting percentage, No. = number of roots per rooted cutting, LRL = longest root (mm) per rooted cutting.**

Rooting variable	'Mariesii' Viburnum[z]			'Sparkleberry' Holly			Red Sunset® Maple			'Shasta' Viburnum		
	%	RN	LRL	%	RN	LRL	%	RN	LRL	%	RN	LRL
DNG ratio[y]												
0 (water)	88.9[w]	27c[x]	15b	48.9b	12b	8a	31.1b	4b	62b	91.1[v]	32[v]	58[v]
20:1	87.8	48b	19a	68.9ab	10b	10a	51.1b	9a	143a	100.0	63	66
10:1	91.1	58a	22a	80.0a	23a	13a	80.0a	10a	132a	100.0	61	70
Medium temp (C)												
20	81.1[w]	36b	8c	37.8b	7b	5b	53.3[w]	15a	134a	100.0	40	53
23	100.0	55a	27a	80.0a	17a	12a	60.0	7b	156a	100.0	54	81
26	86.7	39b	18b	80.0a	18a	12a	48.9	4b	67b	91.1	64	60

[z]'Mariesii' viburnum rooted in 17 days, all other taxa rooted in 33 days.

[y]DNG ratios are of de-ionized water:stock Dip-N-Grow® (1.0% IBA, 0.5% NAA)

[x]Means followed by different letters were separated with Tukey's HSD after analysis of variance ($P \leq 0.05$).

[w]Not significant ($P \geq 0.05$).

[v]No post hoc test performed because of disordinal nature of significant interaction.

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Fig. 1. Mean and standard error of length of longest root of rooted cuttings of 'Mariesii' viburnum. Cuttings were treated with 0 (water), 20:1, and 10:1 Dip-n-Grow® (DNG) and rooted at propagation medium temperatures of 20C (68F), 23C(74F), or 26C (79F) for 17 days.

auxin concentration accounted for 29%, 42%, and 52% of the variance associated with mean root number for 'Sparkleberry' holly, 'Mariesii' viburnum, and 'Shasta' viburnum, respectively. For the same taxa the variance of root length associated with auxin concentration was less. Propagation medium temperature was more strongly associated with the variance of longest root length than of root number for rooted cuttings of both viburnum taxa, but was equivalent for cuttings of 'Sparkleberry' holly (Table 1).

At the highest auxin concentration, root number of rooted cuttings of all taxa increased 2-fold over the water control (Table 2). Root number and length of the longest root of 'Mariesii' viburnum were significantly greater on cuttings rooted at a propagation medium temperature of 23C (74F) as compared to higher or lower medium temperatures. Rooting percentage and root number of 'Sparkleberry' holly approximately doubled when rooted at 23C or 26C (74F or 79F) or treated with 10:1 ratio of Dip-n-Grow®. Mean longest root length on cuttings of 'Sparkleberry' holly was not significantly affected by auxin concentration, but more than doubled when cuttings were rooted at 23C or 26C (74F or 79F). Auxin at the highest concentration applied significantly increased rooting percentage of Red Sunset® maple, while either auxin concentration doubled root number. Lower medium temperatures favored both root number and longest root length of rooted cutting of Red Sunset® maple (Table 2).

The response of the mean longest root length of rooted cuttings of 'Mariesii' viburnum to auxin treatment varied in response to basal temperatures of 23C (74F) ($F = 23.5$, $P < 0.05$) or 26C (79F) ($F = 24.6$, $P < 0.05$). As illustrated in Fig. 1, medium temperatures of 20C (68F) and 26C (79F) were sub- and supra-optimal, respectively, for root elongation. The length of the longest roots [33 ± 7 mm (1.3 ± 0.3 in)] occurred when cuttings were treated with a 10:1 ratio of Dip-n-Grow® and rooted at 23C (74F). Similarly, root number of rooted cuttings of 'Shasta' viburnum responded to auxin treatment differently at 23C (74F) ($F = 23.9$, $P < 0.05$) and 26C (79F) ($F = 42.8$, $P < 0.05$). Cuttings at a medium temperature of 26C (79F) and treated with a 10:1 ratio of Dip-n-Grow® yielded many more roots (Tukey's HSD, $p \leq 0.05$) than the water control. Root elongation on cuttings of 'Shasta' viburnum rooted at 23C (74F) was uniformly high and not affected by auxin application, while root length on cuttings rooted at 26C (79F) without auxin was suppressed (Fig. 2).

The main effect of auxin concentration explained a greater proportion of the variance associated with root number than with root length (Table 1). The opposite trend was seen for

Fig. 2. Mean and standard error of (A) root number and (B) length of longest root of rooted cuttings of 'Shasta' viburnum. Cuttings were treated with 0 (water), 20:1, and 10:1 Dip-n-Grow® (DNG) and rooted at propagation medium temperatures of 20C (68F), 23C(74F), or 26C (79F) for 33 days.

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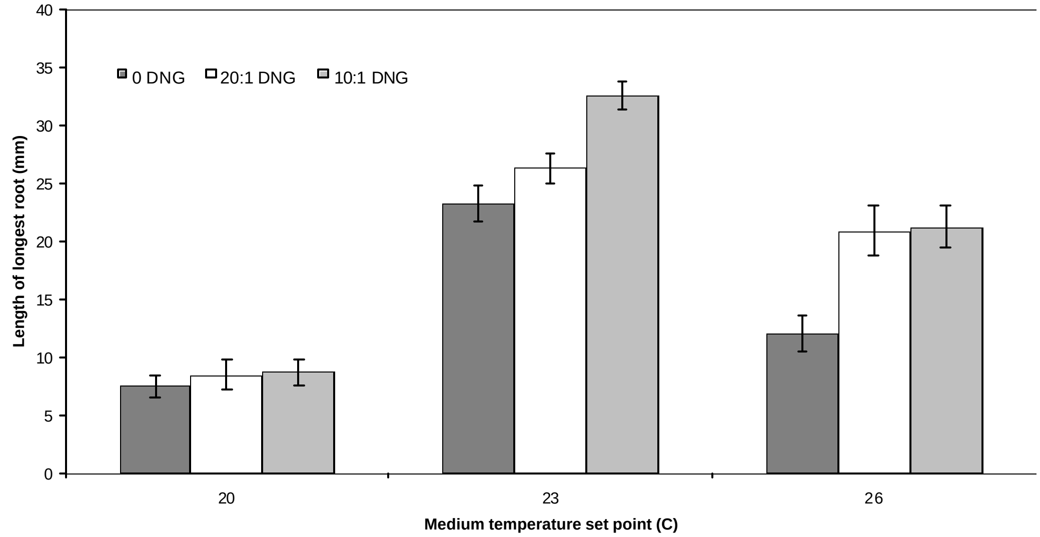

Fig. 1. Mean and standard error of length of longest root of rooted cuttings of 'Mariesii' viburnum. Cuttings were treated with 0 (water), 20:1, and 10:1 Dip-n-Grow®(DNG) and rooted at propagation medium temperatures of 20C (68F), 23C(74F), or 26C (79F) for 17 days.

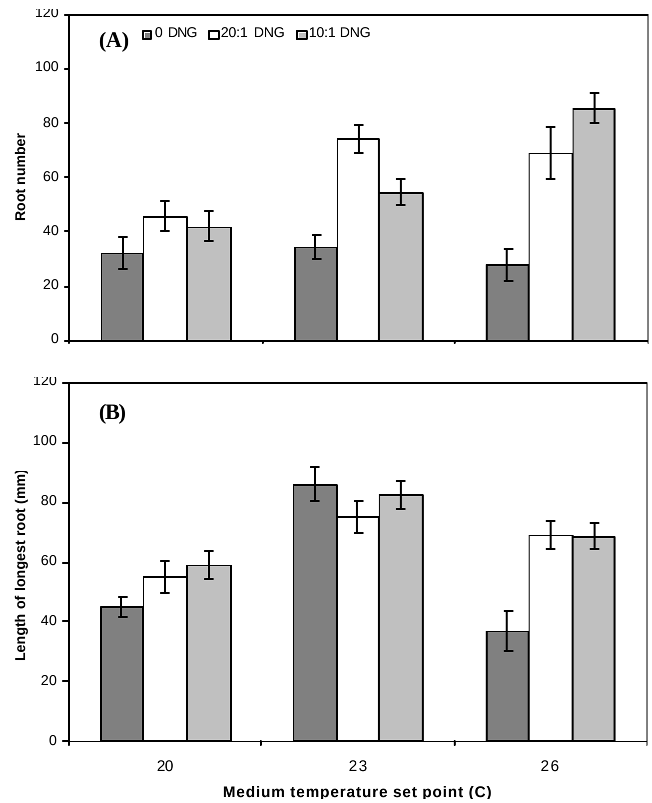

Fig. 2. Mean and standard error of (A) root number and (B) length of longest root of rooted cuttings of 'Shasta' viburnum. Cuttings were treated with 0 (water), 20:1, and 10:1 Dip-n-Grow® (DNG) and rooted at propagation medium temperatures of 20C (68F), 23C(74F), or 26C (79F) for 33 days.

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the main effect of medium temperature. Auxin is generally considered a stimulus for cell dedifferentiation and division resulting in root initiation, a temperature-regulated process that is coupled to the rate of mitotic cell divisions, with an optimum ≤ 30C (86F) reported for *Chrysanthemum* L. spp. (not specified) (7). Burholt and Van't Hof (4), using *Helianthus annuus* L. *'Russian Mammoth'*, reported that root elongation, which results from cell differentiation and elongation, also is a temperature-regulated process with an optimum of ≈ 25C (77F). The optimum temperatures for adventitious root formation and root elongation reported above are guidelines from previous research only; plant provenance and genotype must also be considered when considering optimum medium temperature.

The concept that auxin and temperature interact to affect root number, and that root elongation is a more sensitive indicator of non-optimal medium temperature, is confirmed in this study by the response of cuttings of both viburnum taxons. In the absence of auxin, cuttings of 'Shasta' viburnum formed longer roots on cuttings rooted at a propagation medium temperature of 23C (74F) as compared to cuttings treated with auxin and rooted at 20C (68F) or 26C (79F), but were not responsive to auxin treatment (Fig. 2). The main effects reported in Table 2 confirm these results, where mean root number of 'Shasta' viburnum was greatest at 26C (79F), but peak root elongation occurred at 23C (74F). Conversely, both root number and longest root length on cuttings of 'Mariesii' viburnum were greater at 23C (74F). As well, root length on cuttings of 'Mariesii' viburnum was more responsive to auxin treatment at the optimal propagation medium temperature of 23C (74F) (Fig. 1). This might represent a confounding artifact of longest root length as a rooting parameter in that earlier root initiation would allow more time for root elongation (11). Ellyard and Ollerenshaw (8) reported that IBA concentrations > 4,000 ppm are detrimental to rooting of *Grevillea johnsonii* McGillivray at propagation medium temperatures ≤ 23C (74F). If we had evaluated the cutting base and developing roots throughout the present experiment we might have found that roots formed at higher propagation medium temperatures emerged earlier, but then stop elongating after exposure to supra-optimal temperatures, as reported by Dykeman (7).

This research demonstrates that recirculating subirrigation propagation systems may be used to root a variety of cuttings in a controlled environment, while allowing insight into the effects of a range of factors on adventitious root formation. Degassed perlite was a suitable medium for this method of propagation because it retains high soil moisture content (17) while allowing the maintenance of desired medium temperatures. Further studies are needed to evaluate recirculating subirrigation as a practical method of cutting propagation. In this study it appeared that both optimum root number and length could be obtained by rooting cuttings at one medium temperature. The more rapid rate of root emergence under higher propagation medium temperatures might offer more time for roots to elongate and reach acceptable quality, if the same temperatures are not detrimental to subsequent root growth. Studies that would sample cuttings throughout an experiment could increase our understanding of propagation medium temperature and auxin interaction effects on root initiation versus elongation, and allow the propagator to decrease the time needed for propagation while achieving higher root quality.

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