The Effect of Growth Regulators on Growth and Overwinter Survival of Rooted Cuttings

Anna Perkins and Nina Bassuk
Department of Floriculture and Ornamental Horticulture, Cornell University, Ithaca, New York 14853

INTRODUCTION

Successful cutting propagation of many woody plant taxa is limited by poor overwinter survival in the first propagation year (Smalley and Dirr, 1986). Plants which fail to survive the winter after rooting are often characterized as having had budbreak inhibition or bud dormancy following rooting. Application of the auxin rooting hormone, indole-3-butyric acid (IBA), to the cutting base has been shown to inhibit budbreak (Hartmann et al., 1990). Sun and Bassuk found that although IBA increased percent rooting and root number in *Malus* MM 106, it delayed and reduced axillary budbreak in rooted cuttings.

It has been hypothesized that growth inhibition in rooted cuttings results in insufficient carbohydrate reserves for sustaining the cuttings during winter and early spring, and for inducing insufficient cold hardiness (Smalley and Dirr, 1986). Smalley and Dirr found that cuttings of *Acer rubrum* 'October Glory' which broke bud had significantly more carbohydrates than cuttings which did not grow. Nevertheless, many cuttings that did not grow overwintered successfully. They concluded sufficient carbohydrate reserves may be more important than achieving budbreak to insure overwinter survival (Smalley et al., 1987).

As there is ample evidence that hormones are involved in the onset and breaking of dormancy (Hartmann et al., 1990), studies have also addressed the effectiveness of exogenously applied hormones in overcoming budbreak inhibition and poor overwinter survival. Loach and Whalley successfully promoted budbreak in *Berberis thunbergii* with application of gibberellic acid, GA_3, where extended photoperiod and supplemental CO_2 failed to promote budbreak; however, the breaks were irregular and "etiolated" in appearance (Loach and Whalley, 1975). Maynard and Bassuk found that $GA_{4/7}$ was more effective than GA_3 in stimulating shoot growth of *Stewartia pseudocamellia* rooted cuttings (Bassuk, 1992).

Thidiazuron (N-phenyl-N' -1,2,3-thidiazol-5-ylurea) (TDZ), a plant bioregulator with cytokinin-like properties, has also been used to break bud dormancy and release lateral buds in apple (19).

The objective of this research was to assess growth and overwinter survival of rooted cuttings after application of several post-rooting treatments designed to achieve as much growth as possible in the propagation year. *Acer rubrum* 'October Glory', *A. rubrum* 'Red Sunset', *Hamamelis vernalis, H. virginiana*, and *Stewartia pseudocamellia* were selected for the study because of their difficulty in overwintering after rooting (Dirr and Heuser, 1987; Smalley and Dirr, 1986).

MATERIALS AND METHODS

Plant Materials. Softwood shoots were taken from mature stock plants of *A. rubrum* 'October Glory', *A. rubrum* 'Red Sunset', *S. pseudocamellia*, *H. vernalis*, and *H. virginiana* over the period of May 27 to June 8, 1992 by the method described by Maynard and Bassuk (1985, 1987) except that IBA was not applied to the Velcro bands. All cuttings were treated with a 10-sec quick dip of 5000 ppm IBA 50% aqueous ethanol and rooted under mist. After 7 to 9 weeks, cuttings were transplanted to 4-in. (10.2 cm.) pots in a soil: peat: perlite medium (1 : 2 : 1 by volume) and then placed in a greenhouse with 21/15C (70/60F) day/night temperatures and 16-h photoperiods. Plants were fertilized weekly with Peter's 20N-10P-20K at a rate of 200 ppm up to October 25, 1992.

Post-Rooting Treatments. Three weeks after potting up, all plants were assessed for bud break and then randomly assigned to one of the five following plant growth regulator (PGR) treatments:

■ STS:	Foliar spray application of 1% silver thiosulfate (STS) prepared according to the method of Reid et al. (Perkins, 1994)
■ STS GA:	Foliar spray application of STS treatment followed 10 days later by a foliar spray application of gibberellin, $GA_{4/7}$ 250 ppm (ProVide, Abbott Laboratories, North Chicago, Illinois) .
■ TDZ:	Foliar spray application of thidiazuron 50 ppm (Nor-Am Chemicals)
■ TDZ GA:	Foliar spray application of thidiazuron followed 10 days later by a foliar spray application of $GA_{4/7}$ at 250 ppm.
■ H_2O:	Foliar spray of water

Each foliar spray contained 1 ml liter^{-1} Tween 20 surfactant. All plants were sprayed until run-off occurred.

Ten weeks after the rooted cuttings were potted up, long day treatments ceased and all plants were exposed to short days and fluctuating, cool temperatures 10/4.4C (50/40F) day/night to insure that they hardened-off and dormancy was established.

Carbohydrate Analysis. Once dormant, (characterized by fall color and leaf drop) rooted cuttings were measured for total new shoot growth. Next, a sample of three plants was taken in each of three growth categories (no budbreak; budbreak yet no growth, budbreak and growth) for every growth accelerator treatment group. Samples were analyzed for total non structural carbohydrates according to da Silveira, Teles and Stull (1978) of the roots and shoots expressed as a percent of the dry weight (% TNC).

Winter Storage. On December 21, 1992, rooted cuttings from all treatments were divided into two winter storage locations. Half the plants were located in a constant 3C (37.4F) cooler. Half the plants were stored in outdoor glass covered cold frames with fluctuating and freezing temperatures. On March 21, 1993, plants were removed from cold storage to the greenhouse and assessed for survival.

Statistical Analysis. The experiment was a completely randomized complete factorial design. Data was analyzed by logistic regression and general linear model.

RESULTS AND DISCUSSION

Plant Growth. Growth in all species except'October Glory' red maple was significantly increased when GA was applied as a follow-up treatment. STS and TDZ showed little effect on their own, with TDZ occasionally causing mild growth inhibition (Table 1).

Table 1. Comparison of amount of shoot growth (cm) as affected by plant growth regulator treatment.

	Growth regulator				
Species	Water	STS	STS-GA[1]	TDZ	TDZ-GA
Stewartia pseudocamellia	3.5	6.0	11.0[2]	4.5	9.0[2]
Acer rubrum 'Red Sunset'	8.5	9.0	15.0	5.0	8.5
Hamamelis vernalis	14.5	15.0	17.0	11.0	15.0
H. virginiana	7.0	11.0	13.0	5.0	7.5

[1] STS-GA = STS treatment followed by $GA_{4/7}$ 10 days later; TDZ-GA = TDZ treatment followed by $GA_{4/7}$ 10 days later

[Z] All treatments with GA follow-up (STS-GA and TDZ-GA) vs. no GA follow-up were significant at .02 or better level.

Carbohydrates. There were no significant differences in the total nonstructural carbohydrates, expressed as percent dry weight, in the root tissue of *S. pseudocamellia* for cuttings which grew vs. cuttings which did not grow (Figure 1). *Hamamelis vernalis* and *H. virginiana* both showed significantly higher percent total nonstructural carbohydrates in the root tissue of cuttings which grew than the root tissue of cuttings which did not grow, and *A. rubrum* 'October Glory' cuttings demonstrated a similar trend. *Acer rubrum* 'Red Sunset' cuttings were not analyzed for % TNC. The % TNC in the root tissue of *S. pseudocamellia* cuttings that did not grow were greater than the % TNC of the root tissue of the other species by at least 93%. Analysis of % TNC in the whole plant tissue (roots and shoots combined) was similar to the analysis of % TNC in root tissue alone (Perkins, 1994).

Survival Results. Winter storage in a 3C (37.4F) cooler resulted in significantly higher percentage of survival for every species than winter storage in the cold frame with fluctuating and freezing temperatures (Fig. 2). 'October Glory' was least affected by the fluctuating conditions; percent overwinter survival was only 7% lower than the cooler stored cuttings. *Hamamelis virginiana* and *S. pseudocamellia* were most harmed by cold frame conditions. One *Stewartia* cutting survived out of 395, and only 14% of the *H. virginiana* cuttings survived in the cold frame. *Hamamelis virginiana* was the only species to exhibit poor survival in the cooler (58%).

***Acer rubrum* 'October Glory'.** Overall survival was high for *A. rubrum* 'October Glory' cuttings. Increased shoot growth significantly increased the survival rate of *A. rubrum* 'October Glory' (Tables 2, 3) and was more critical to survival for cuttings exposed to fluctuating temperatures.

***Acer rubrum* 'Red Sunset'**. Increased shoot growth was not critical for survival of *A. rubrum* 'Red Sunset' cuttings in either winter storage location (Tables 2, 3). Survival rates were high for cuttings stored in the 3C cooler, but the cuttings were not sufficiently cold hardy to survive well in the fluctuating and freezing cold frame environment.

Hamamelis vernalis. Similarly, increased shoot growth significantly increased survival rates of *H. vernalis* for cuttings in both winter storage locations. Without shoot growth, survival rates were very poor, 38% in the 3C cooler and 14% in fluctuating temperatures (Tables 2, 3). Overall, *H. vernalis* cuttings exhibited high survival rates in the 3C cooler (Table 2).

Hamamelis virginiana. Shoot growth significantly increased survival of *H. virginiana* cuttings in both winter storage locations (Tables 2, 3). However, there was a great disparity between cutting survival rates in the 3C cooler and cutting survival rates in fluctuating temperatures. Cuttings in the 3C cooler which grew between 10 and 20 cm had 96% survival. Those that didn't grow had only 37% survival. Shoot growth for cuttings exposed to fluctuating temperatures only increased survival rates from 5% to 21%.

Stewartia pseudocamellia. Shoot growth was not critical for survival of *S. pseudocamellia* cuttings (Table 2, 3)—97% survived in the cooler regardless of growth, and 1% survived in the cold frame regardless of growth.

Table 2. Percent overwinter survival as affected by amount of shoot growth in 3C storage.

Species	Amount of shoot growth 0 cm (%)	 1 - 10 cm (%)	 11 - 20 cm (%)	 21 - 30 cm (%)
Acer rubrum 'October Glory'	84 a	98 b	--	--
	(87)†	(285)	(0)	(0)
A. rubrum 'Red Sunset'	86 NS	98 NS	100 NS	--
	(28)	(40)	(23)	(0)
Hamamelis vernalis	38 a	83 b	93 b	96 b
	(29)	(120)	(294)	(113)
H. virginiana	37 a	59 b	96 c	--
	(139)	(147)	(69)	(0)
Stewartia pseudocamellia	93 NS	100 NS	--	--
	(440)	(50)	(0)	(0)

Within species, numbers in rows followed by different letters are significantly different at the .05 level . Differences tested by logistic regression.

† Numbers in parentheses represent the sample size (n).

Was Growth Important for Survival? *Acer rubrum* 'October Glory', *H. vernalis* and *H. virginiana* cuttings all had higher overwinter survival rates for cuttings which grew *Acer rubrum* 'Red Sunset' showed a similar trend, but *S. pseudocamellia* did not exhibit increased survival with growth. Several studies support the finding that growth improves overwinter survival. In a study by Loach and Whalley, extended photoperiod and CO_2 enrichment promoted growth and increased overwinter survival in *Betula pendula* and *Berberis thunbergii* (Loach and Whalley, 1975). Drew et al. used extended photoperiod to induce budbreak and growth of *Quercus* cuttings and found that 100% of cuttings which grew survived (Drew et al., 1993). Goodman and Stimart also found that growth improved survival when *A. palmatum* and *Cornus florida* cuttings were fertilized with nitrogen, but growth was not necessary for survival when nitrogen fertilizer was withheld (Goodman and Stimart, 1987). All species in this study received 20N-10P-20K fertilizer, at a rate of 200 ppm, once a week for 4 weeks. Nitrogen fertilization did not limit the survival of cuttings that did not break bud because, depending on winter storage environment, or response to plant growth regulators, fertilized cuttings which did not break bud survived the winter in high percentages for all species except *H. virginiana*.

Table 3. Percent overwinter survival as affected by amount of shoot growth in fluctuating cold frame.

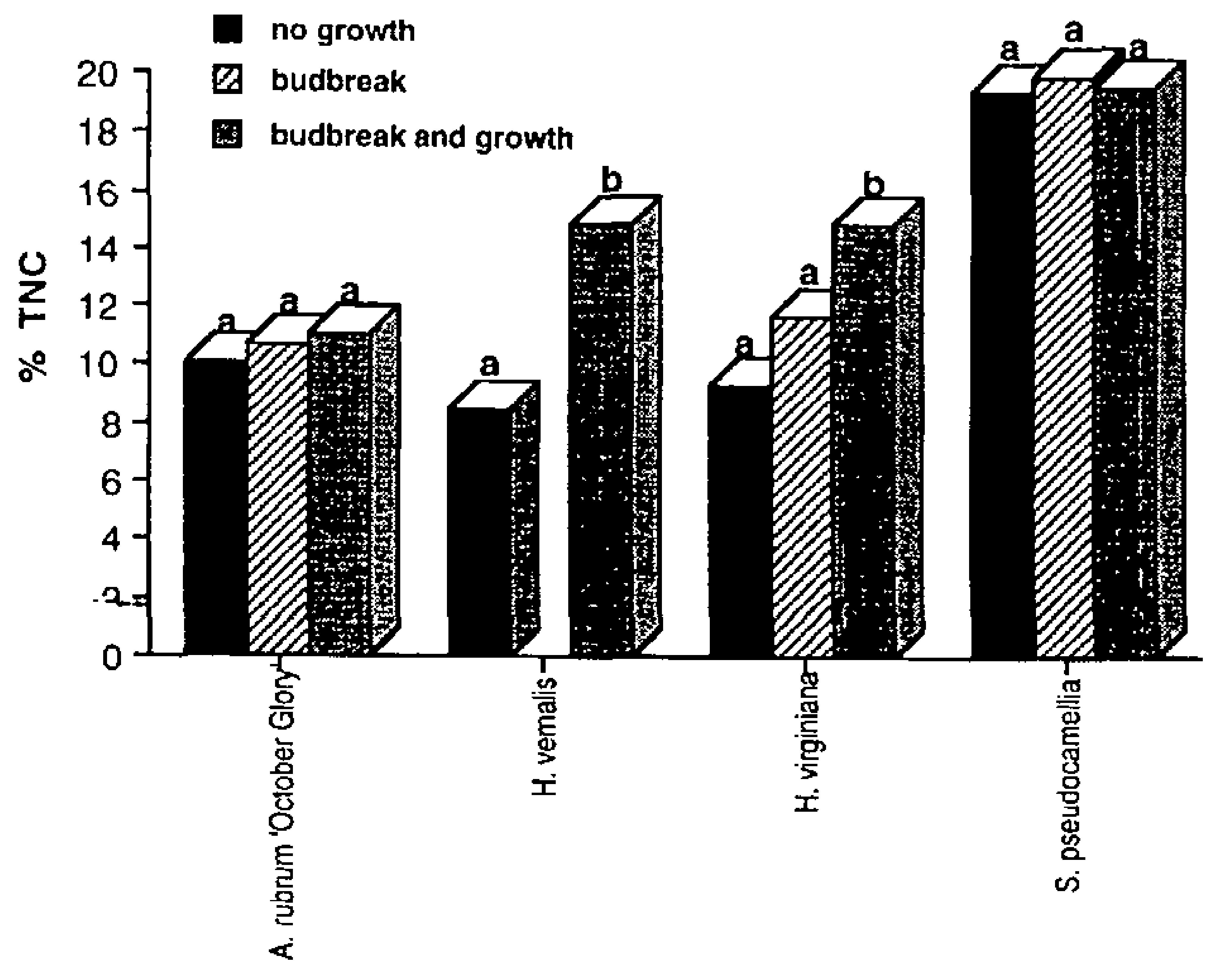
	Amount of Shoot Growth			
Species	0 cm %	1 - 10 cm %	11 - 20 cm %	21 - 30 cm %
Acer rubrum 'October Glory'	71 a	94 b	--	--
	(155)†	(486)	(0)	(0)
A. rubrum 'Red Sunset'	52 NS	55 NS	--	--
	(23)	(71)	(0)	(0)
Hamamelis vernalis	14 a	67 b	--	--
	(37)	(433)	(0)	(0)
H. virginiana	5 a	21 b	--	--
	(108)	(169)	(0)	(0)
Stewartia pseudocamellia	1 NS	0 NS	--	--
	(343)	(50)	(0)	(0)

Within species, numbers in rows followed by different letters are significantly different at the .05 level. Differences tested by logistic regression.

† Numbers in parentheses represent the sample size (n).

Did Growth Result in Increased Carbohydrate Reserves? Both *H. vernalis* and *H. virginiana* support the hypothesis that increased growth after rooting results in increased carbohydrate reserves, and increased carbohydrate reserves are necessary for insuring winter survival. *Acer rubrum* 'October Glory' exhibited a similar trend, although increases in carbohydrates were not statistically significant. Carbohydrates were not anlayzed for *A. rubrum* 'Red Sunset' cuttings. *Stewartia pseudocamellia* cuttings did not have higher carbohydrates in cuttings which grew. For *S. pseudocamellia* cuttings which did not grow, carbohydrate reserves comprised at least 93% more of the dry weight of a cutting than any other species tested. This indicated that carbohydrate reserves, after rooting, were not critically low for *S. pseudocamellia* cuttings.

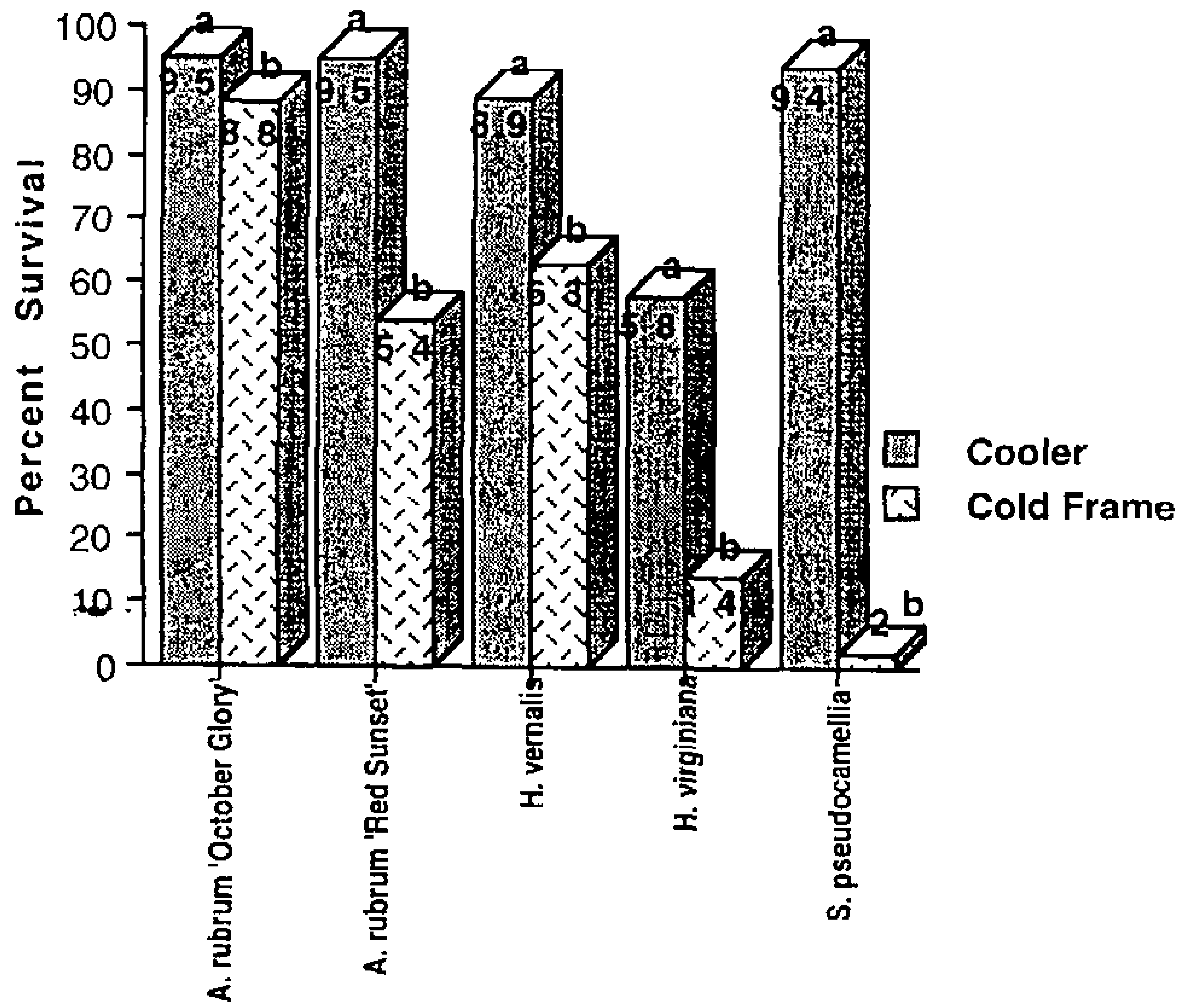
Smalley et al. reported similar results with *A. rubrum* 'October Glory' (Smalley et al., 1987). In their study, *A. rubrum* 'October Glory' cuttings which did not break bud had high survival rates, but had carbohydrate levels similar to *A. rubrum* 'October Glory' cuttings that did break bud. This result indicated that carbohydrates were not necessarily low in cuttings that did not break bud.



	Sample Size Analyzed for Each Species			
Amt. of Growth	A. rubrum 'October Glory' (n)	H. vernalis (n)	H. virginiana (n)	S. pseudocamellia (n)
No growth	9	1	14	8
Budbreak (< 1 cm growth)	9	0	7	14
Budbreak and Growth (> 1 cm growth)	15	15	15	5

Figure 1. Changes in %TNC with growth. Growth effect tested by general linear model, P=.05. Within species, columns with same letters are not different at the P=.05 level. See tables for sample sizes and mean separation by orthogonal contrasts.

Did Plant Growth Regulators Increase Growth? Post rooting plant growth regulators did not increase the growth of *A. rubrum* 'October Glory' cuttings, STS and TDZ did not increase growth of *H. vernalis*, but application of $GA_{4/7}$ to the leaves and buds after rooting and after application of STS or TDZ increased growth, as it did for *A. rubrum* 'Red Sunset', *H. virginiana*, and *S. pseudocamellia*. This result indicated



SPECIES	Winter Storage Location 3° C. COOLER (*n*)	 COLD FRAME (*n*)
A. rubrum 'October Glory'	388	283
A. rubrum 'Red Sunset'	96	94
H. vernalis	556	470
H. virginiana	367	277
S. pseudocamellia	490	395

Figure 2. Comparison of overwinter survival in two winter storage facilities. Winter storage location effect tested by Logistic Regression, P=.05. Within species, columns with different letters are signficantly different at the P=.05 level. See table for sample sizes.

that $GA_{4/7}$ alone should be investigated as growth enhancing treatment. McConnell and Herman reported increased growth of softwood cuttings with GA_3 treatments, but not overwinter survival (McConnell and Herman, 1980), and Loach and Whalley reported increased, but irregular and weak, growth with GA_3 treatments on *Betula pendula* and *Berberis thunbergii* cuttings (Loach and Whalley, 1975). In this study, growth induced by $GA_{4/7}$ was from the apical bud and neither irregular nor weak.

Despite the fact that growth was not necessary for insuring survival of *S. pseudocamellia*, added growth in the first year may be desired simply because it would give *S. pseudocamellia* cuttings, generally characterized by one flush of growth in a season, a head start on development.

Although TDZ did not effectively increase growth for any species in this study, a general statement about the response of cuttings to post rooting foliar spray application of TDZ should be noted. TDZ was selected as a plant growth regulator because Wang et al. reported that TDZ effectively increased lateral budbreak and bud development in *Malus domestica* (Wang et al., 1986). In this study, TDZ did break bud dormancy, but did not increase the amount of growth for any species. Overall, TDZ treated cuttings had weaker extension shoots, and did not have a central leader.

Did Cold Hardiness Affect Survival? In the case of *A. rubrum* 'October Glory', *A. rubrum* 'Red Sunset', and *S. pseudocamellia*, cold hardiness was the most critical factor for survival. All three plants had extremely high survival (95%, 95%, and 94%, respectively) when stored in the 3C cooler regardless of growth, and significantly lower survival (88%, 54%, and 2%, respectively) in the cold frame regardless of growth. Until it is determined exactly what winter conditions are intolerable for rooted cuttings, mild winter storage conditions are essential. Research is needed to determine rooted cutting cold tolerance for all the species.

Lastly, when treatments successfully increased cutting growth, hardening off may require a longer period of time than cuttings with less growth or no growth. In this study, STS GA treatment effectively increased growth of *A. rubrum* 'October Glory', but had very low survival (Perkins, 1994). This poor survival, despite growth, is likely due to insufficient cold acclimation.

CONCLUSIONS

What Conclusions can be Drawn Concerning Growth and Overwinter Survival? Not all plants that exhibit budbreak inhibition and poor overwinter survival fit the hypothesis that rooted cuttings are carbohydrate depleted and must break bud and grow to survive the first winter. Some cuttings are not carbohydrate depleted by rooting and therefore not carbohydrate enhanced by budbreak and growth. These cuttings survive without growth if their cold hardiness capacity is not exceeded.

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