The Influence of Sugars on Growth and Cold Acclimation of Excised Stems of Red-osier Dogwood

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Abstract. Excised Cornus stolonifera Michx. stems cultured axenically in a liquid medium were acclimated to cold when subjected to short photoperiods and low temperatures. Foliate explants acclimated effectively and defoliated ones did not when they were cultured on White's medium which contained 0.083 M sucrose. Several other concentrations of sugars (0, 0.01, 0.1, and 0.5 M glucose and 0, 0.01, 0.05, and 0.1 M sucrose) did not enhance cold acclimation of foliated explants. The exogenously supplied sugars reduced stem growth, promoted leaf abscission, and enhanced the development of typical autumnal red coloration in leaves and stems. The highest sugar concentration (0.5 M glucose) caused death of the explants.

While a minimal level of photosynthate (sugar) is almost certainly required for the active metabolic phases of cold acclimation in hardy woody species, our studies provided no evidence that sugars bear a direct causal relationship to cold acclimation.

The possible role of sugars in the cold acclimation of plants has been the subject of research for many years (9, 16, 19, 22). That sugars may have a direct role in cold acclimation is suggested by several kinds of indirect evidence: 1) In the fall there is a conversion of starch to sugars in woody plant tissues which are capable of cold acclimation (10, 11, 12, 13, 14, 15). In winter cereals, there is an autumn build up of sugars arising directly from photosynthesis rather than from conversion of starch to sugar (7). 2) When coleoptiles of winter cereals (21, 22), cabbage leaf discs (8, 15), or gardenia cuttings (16) are incubated in sugar solutions for short periods, the lethal temp of such tissues drops. Also, the freezing resistance of isolated spinach chloroplasts increases when sugars are added to the suspension media (3, 4). This observation prompted Heber and Santarius to hypothesize (2, 4) that sugars protect sensitive proteins from freezing damage by forming a hydrogen-bonded sugar shell around protein molecules. 3) Sugar molecules can interact chemically in vitro with biological macro-molecules (21). Such interactions in vivo may possibly increase freezing stress resistance. 4) The leaves of short day induced woody plants are known to be the source of a translocatable hardiness promoting factor or factors (6). Previous studies suggest that one factor could be sugar (19).

Researchers who are not convinced that sugars play a direct role in the natural acclimation of hardy plants suggest that: 1) the increase in sugars during acclimation may be coincidental rather than causal. The low temp-induced conversion of starch to sugar occurs in many plant tissues (Irish potato tubers) which are incapable of cold acclimation. Sugar cane is very high in sugars, but it is quite sensitive to low temp (8). 2) Frequently there is a poor time correlation between sugar increase and cold acclimation even in hardy woody plants (7, 16). Simitnovitch suggested that the increase in hardiness in black locust is more likely to be related to the decrease in starch than to the increase in sugars (17). 3) Sugar concentration successfully lowered the killing point of leaf discs (15) and isolated organelles (3) are much higher than endogenous sugar levels. These “protective” concn cause plasmolysis and possibly increased freezing resistance by some indirect means. 4) Acclimation in hardy woody species is believed by some researchers to differ from acclimation in herbaceous plants (1). Results with information from isolated organelles, leaf discs, or cuttings from tender or semi-hardy plants (15, 16) should not be extrapolated to woody plants because cells of the 2 types of plants may not react to exogenously supplied sugars in the same way.

There is no evidence to indicate that exogenously supplied sugars can enhance the cold acclimation of hardy species. Two factors have made this relationship difficult to test. First, acclimation is a relatively slow process so some continuous system for feeding sugar to plant cells over prolonged periods is required. Second, an aseptic bioassay is required to insure that any effects are due to sugar and not to products of microbial degradation.

Tissue culture would provide the long-term aseptic feeding required. Preliminary studies, however, revealed that callus cultures of red-osier dogwood do not cold acclimate effectively. McCown failed to acclimate callus of hardy Dianthus species. Aseptic culture of whole plants or explants is also unsatisfactory because of the difficulty in surface sterilizing roots and buds of mature plants, the juvenility of seedlings grown from surface sterilized seeds, and the unnatural microenvironment in a culture flask.

A satisfactory solution was provided by the development of a simple technique for culturing excised mature stems in liquid media. Although the cultured explants lack roots, previous studies indicated that roots have little effect on cold acclimation of stem tissues in Cornus stolonifera. For example, root temp and girdling the stem just above the root system did not influence the development of hardiness in the stems.

Little is known about the influence of exogenous sugars on any plant processes. Vegis (23) found sucrose to induce physiological dormancy in Hydrocharis morsus ranae Linn. in
continuous darkness.

Our objective was to observe the influence of exogenously supplied sugars on growth and development, and specifically, to determine if sugars enhance cold acclimation.

Materials and Methods

Three-node terminal cuttings of the shrub, red-osier dogwood (Cornus stolonifera Michx.), about 20 cm long were collected from stock plants of a clone native to Dickinson, North Dakota. Live bark of this hardy clone resists -196°C in mid-winter. The stock plants were grown in a warm greenhouse on a 16-hr photoperiod. Cuttings were made with sterile scalpels, and the basal 5 cm of each cutting was immediately submerged in a surface sterilant (0.5% sodium hypochlorite). After 15 minutes the basal portion of each cutting was inserted into a sterile culture vial through a tight fitting opening in a rubber serum stopper. Insertion was facilitated with sterile silicone lubricant. Autoclaved nutrient solution (modified White's medium, pH 5.5 to 5.6) (24) and filtered air were supplied to each culture vial via centralized supply systems. Details of this axenic hydroponic culture technique have been described.

The explant cultures were transferred to a controlled environment chamber illuminated by a mixture of cool-white fluorescent and incandescent bulbs. The light intensity at a distance of 75 cm from the medium surface was approximately 8.2 x 10^4 ergs/cm^2 sec at 15°C, as measured with a YSI Kettering Model 65 Radiometer. The photoperiod and thermoperiod were maintained at 8 hr throughout all studies, and the day-night temp regimes were 20° to 15°C for the first 1 to 2½ weeks followed by 15° to 5° for the balance of each experiment. The duration of the experiments as shown in Tables 1 and 2 and Figure 1 was the total time that explants were exposed to short days in the growth chamber.

At the conclusion of the feeding period the explants were removed from the vials, cut into 2 cm internodal segments, and subjected to a controlled freezing test. Internodal segments were frozen in dewar flasks at a rate of 10° per hr. Flasks were removed from the freezer at 2° intervals, the internodal segments were rewarmed and incubated in a humid chamber at room temp. After 7 days the samples were scored for survival. Any segments not showing discoloration and breakdown of cells in the cambium or bark were considered alive. Previous tests had shown that such samples rated alive are capable of forming callus if incubated for 20 to 30 days.

All feeding and freezing tests were run in triplicate, and there was perfect agreement in the survival scores for the three replicates from each treatment at each test temp. Hardiness was determined as the lowest survival temp. The growth data were averages of all stem length measurements in each treatment. Differences in final growth between treatments were evaluated by Duncan's multiple range test (18).

Three experiments were conducted. The first was to determine if cultured explants could be induced to cold acclimate under environmental conditions which induce acclimation in intact plants. Leaves were removed from half of the explants. Explants were exposed to 8-hr photoperiods and a

### Table 1. Cold resistance of foliated and defoliated Cornus stolonifera explants grown at an 8-hr photoperiod and low temp for 6 and 8 weeks in White's medium containing 0.083 M sucrose.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of the feeding experiment (weeks)</th>
<th>Lowest survival temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With leaves</td>
<td>6</td>
<td>-16</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>-20</td>
</tr>
<tr>
<td>Without leaves</td>
<td>6</td>
<td>-4</td>
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<tr>
<td></td>
<td>8</td>
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</table>

2Defoliated explants were dead after 8 weeks.

2Hardiness determinations were made after 12 1/2 weeks of glucose treatment and after 9 weeks of sucrose treatment.

2Exploans cultured in medium containing 0.5 M glucose were dead after 12 1/2 weeks.

20° to 15° day-night temp for 2 1/2 weeks followed by an additional 3 1/2 or 5 1/2 weeks at a temp regime of 15° to 5°. The culture solution was White's medium, which contained 3% sucrose (0.083 M). Freezing tests were performed after explants were cultured a total of 6 weeks and 8 weeks.

The second and third experiments tested the effects of several concn of glucose and sucrose respectively in White's medium on growth and cold acclimation of foliate explants.

![Fig. 1. The influence of glucose and sucrose concn on stem elongation of Cornus stolonifera explants grown at an 8-hr photoperiod in White's medium. Day-night temp regimes were 15°C-5°C during preconditioning and 20°-15° after preconditioning.](chart)

This technique was shown to increase cold resistance of herbaceous and semi-hardy plant tissues (15, 16, 21, 22). The glucose concn were 0, 0.01, 0.1, and 0.5 M. Sucrose concn were 0, 0.01, 0.05, and 0.1 M. Explants in the glucose experiments were exposed to a 20° to -15°C day-night temp regime for 2½ weeks and then to a 15° to -5°C regime for 10 weeks. Those treated with sucrose were exposed to a 20° to -15°C regime for 1 week and then to 15° to -5° for 8 additional weeks. Growth measurements (stem length) were made until elongation ceased. Controlled freezing tests were made after a total of 12 1/2 weeks of feeding in the glucose experiment and after a total of 9 weeks in the sucrose experiment. Visual changes in explants were noted during the course of the studies.

The total concn of hydrolyzable reducing sugar soluble in 80% ethanal of 10 samples, each, of bark and leaves of control, 0.1 M glucose, and 0.1 M sucrose-treated explants was determined with a Technicon Auto-Analyzer (3).

Results

When explants were cultured in a medium containing 0.083 M sucrose, those with leaves became acclimated to -15°C in 6 weeks and to -20° in 8 weeks. The leaves and stems of these explants developed red autumn coloration, and leaves began to abscise during the sixth week of treatment. In contrast, the defoliated explants had not acclimated in 6 weeks and were dead after 8 weeks. These explants continued to produce new leaves (which were removed) and stems reddened slightly at the base before death occurred. The level and rate of acclimation of the foliate explants were similar to those observed in studies of intact plants subjected to similar photoperiod and temp regimes.

In the second and third experiments, the glucose and sucrose treatments induced changes normally associated with the onset of natural cold acclimation in intact plants (i.e. growth reduction, stem reddening, and leaf senescence and abscission), but the treatments did not enhance cold acclimation.

Both glucose and sucrose reduced the rate of growth and total growth. The reduction was greater as the glucose or sucrose concn was increased (Fig. 1). Growth cessation did not appear to be affected by the sugars since explants in all treatments stopped growing at about the same time.

The typical autumnal red coloration of leaves and stems and leaf abscission were hastened with increasing concn of glucose and sucrose up to 0.1 M, and the explants were healthy until the experiments were terminated.

At the highest sugar concn (0.5 M glucose), explants wilted and within 1 week the leaves were flaccid, puckered, and mottled. Leaf veins became chlorotic while some areas between veins remained green. Red color development and leaf abscission were inhibited, and explants at this concn of glucose showed severe symptoms of stress before they died during the eighth week of treatment.

Glucose and sucrose treatments did not enhance the cold acclimation of dogwood stems at any concn studied. The hardness of explants treated with sugars was either identical to or less than that of the untreated controls (Table 2).

The total hydrolyzable reducing sugar concn of the leaves and bark of the 0.1 M glucose-treated explants were 46 and 57% higher than the controls, while the 0.1 M sucrose treated explants were 139 and 23% greater than the controls. Thus, the explants were able to take up the sugars provided in the nutrient media.

Discussion

Incubation of semi-hardy plant tissues and organelles in sugar solutions is known to increase their frost resistance (4, 16, 20, 21). Sugars have also been used as cryoprotective agents for the freeze preservation of animal cells (11). In contrast there is no evidence in the literature to show that sugars directly enhance the cold acclimation of hardy woody species. This lack of evidence could be due to the difficulties encountered in effectively feeding exogenous sugars to woody plants for sustained periods of time, or to the lack of any real causal relationship between sugars and cold resistance in hardy woody species.

The excised dogwood stems cultured hydroponically in our study grew successfully for long periods of time and were induced to cold acclimate like intact plants in response to a short photoperiod and low temp treatment. Leaves were necessary for acclimation and sucrose did not induce cold acclimation of bark tissues in the absence of leaves. Glucose and sucrose promoted typical autumn phenomena such as leaf senescence and abscission, reduced growth, and red coloration of stems and leaves. Neither glucose nor sucrose enhanced cold acclimation.

These findings indicate that sugars per se do not directly cause cold acclimation of hardy woody species such as Cornus stolonifera. The concn of sugars which have effective short-term cryoprotective properties on semi-hardy plant tissues and organelles usually range from 0.5 M to 1.5 M. Glucose at a concn of 0.5 M caused severe stress symptoms and ultimately death of Cornus stolonifera explants. Extrapolation of conclusions derived from the results of short-term incubation studies with non-biological concn of sugars to natural cold acclimation in plants is probably unwarranted.

In dogwood, and several other woody species (6), it appears that leaves are the source of a translocatable factor(s) which promotes the hardiness of overwintering stem tissues. It is unlikely that this factor is endogenous sugar. Identification of this factor(s) is important because of a potential for artificially inducing acclimation in woody species subject to winter injury.

Literature Cited

13. ________ 1959. Seasonal variations in sugars of conifers with some observations on cold resistance. Forest Sci. 5:56-63.
Fluoride Induced Necrosis of *Cordyline terminalis* Kunth ‘Baby Doll’ as Influenced by Medium and pH

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**Abstract.** Although grown by foliage growers for its colorful red leaves, *Cordyline terminalis* Kunth ‘Baby Doll’ often develops foliar necrosis during propagation. Propagating media had a pronounced influence on amount of necrosis, but soluble salts, pH, and cation exchange capacities of the media offered few clues for the variation. Necrosis was related to soluble soil fluoride. Addition of superphosphate containing 1.6% fluorine (F) increased leaf tissue F and tip necrosis, and both were reduced when media pH increased with liming amendments.

Foliage plants with good keeping quality and bright colors are highly prized for use in dishgarden combinations to provide a focal point. ‘Baby Doll’ grows upright, roots readily, and possesses attractive maroon leaves with light rose margins. Although available to the nursery trade for years, ‘Baby Doll’ was not grown in large quantity because of foliar necrosis which appears during propagation. The necrosis was thought to be pathological in nature, but no active pathogen was isolated (7). Although F induced toxicity from soil solutions was reported to be rare (9), improbable (8), and relatively unimportant (3), Conover and Poole (2) showed that uptake of soluble F from irrigation water through the cut stem during propagation caused necrosis of ‘Baby Doll’, and levels of 0.25 ppm F or more in the irrigation water could reduce the sales value of this crop.

There are several reviews concerning effects of atmospheric fluoride on plants (3, 8, 9), but reports of F toxicity due to solution uptake is limited (5, 12). Several workers have studied methods of alleviating toxicity due to atmospheric fluoride. Woltz (10) and Allmendinger et al. (1) reported that alkaline dusts reduced toxicity. Woltz et al. (11) also found that low soil acidity enhanced F toxicity. We determined effects of various media and amendments on necrosis of ‘Baby Doll’ during propagation.

**Materials and Methods**

Six-inch, necrosis-free tip cuttings of ‘Baby Doll’ were placed in various media (Table 1) under 3000 ft-c in a glass greenhouse and misted with tap water containing .25 ppm F 15 seconds every 30 minutes from 8 AM to 6 PM. Cuttings were placed in the media during the fall of 1970, and number of leaves showing necrosis were counted 3 weeks later. There were 3 replications in randomized block design with 5 cuttings per replication. Propagating media were analyzed for cation exchange capacity (C.E.C.) as determined by the ammonium acetate method (4); soluble salts by mixing soil and water (1:2) on a volume basis, filtering, and multiplying solution readings from an RD-15 Solubridge Soil Tester by 14, pH by readings of the filtered 1:2 solution on an Ionalyzer, Model 404 and F by stirring into 50 ml of water a 10-g sample of dried media. The sample was allowed to stand for 30 min with occasional stirring, filtered through Whatman 41, and analyzed for F content utilizing a Techicon Auto Analyzer.

Cuttings, 1 per pot, were placed in 4-inch pots containing either Turface, a calcined clay or German peat amended with or without 10 lb/yd³ superphosphate which contained approximately 1.6% F on April 1, 1971. Treatments were replicated 10 times in a completely random design. Plants were rated April 22, from 1 (no necrosis) to 10 (complete necrosis) (Fig. 1). Leaf tissue was collected at that time for F analyses according to the procedure developed by Mandl et al. (6).

Figure 1. Toxicity ratings used to evaluate necrosis of *C. terminalis* ‘Baby Doll’.

August 6, 1971 tip cuttings of ‘Baby Doll’ were placed in amended German peat (Table 3) to determine the effect of superphosphate and 2 liming materials, Ca(OH)₂, and dolomite, on development of necrosis. Toxicity ratings were taken August 17. This experiment was repeated in 1972 and determinations...

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2Fluoride analyses were obtained through the courtesy of C. D. Leonard and H. B. Graves, Agricultural Research and Education Center, Lake Alfred, Florida.