Changes in Growth Regulator and Carbohydrate Levels in Roots and Shoot Tips of *Cornus sericea* during Cold Storage and Emergence from Dormancy

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Abstract. *Cornus sericea* L. rooted cuttings were held in cold storage for 60 days and then transferred to a growth chamber in hydroponic culture. Roots and shoot tips were sampled during storage and through resumption of vegetative growth. Samples were analyzed for abscisic acid (ABA), indole-3-acetic acid (IAA), zeatin, zeatin riboside, glucose, fructose, sucrose, and starch. Budbreak was associated with increasing levels of the cytokinins and IAA, and decreasing levels of sucrose and starch in the shoot tips. Regeneration of new roots was preceded by an increase in the cytokinins and IAA, and a decrease in ABA in roots. Root sucrose increased nearly two times 1 week after budbreak and starch content generally decreased throughout the experiment. The results agree, in general, with previous reports indicating decreasing levels of ABA and increasing levels of cytokinins to be associated with root regeneration and budbreak. They also indicate that, of the four carbohydrates studied, sucrose levels changed most dramatically during the root regeneration and budbreak processes.

Specific actions and roles of endogenous growth regulators in budbreak, shoot elongation, and root regeneration of woody plants are unclear due to potential interactions among compounds and inadequate methods for analysis. An understanding of root and shoot growth mechanisms may aid development of improved cultural strategies.

Little work has been done on the roles of endogenous growth regulators in spring root regeneration. The majority of plant growth regulator studies utilized only bud or shoot tissue. Of the few reports on root growth regulator levels, most did not simultaneously examine root and shoot tissue. In addition, many previous studies have relied on imprecise analysis methods such as bioassay (Bachelard and Wightman, 1974; Carlson and Larson, 1977; Taylor and Dumbroff, 1975) or have examined only one or two growth regulators at a time (Domanski and Kozlowski, 1968; Dumas and Zaerr, 1988; Sundberg et al., 1987; Taylor and Dumbroff, 1975). Because several studies have provided evidence for an interaction among growth regulators and carbohydrates during root initiation and growth (Butcher and Street, 1960; Haissig, 1982; Wareing and Saunders, 1971), there is a need for more comprehensive studies of carbohydrates, growth regulators, and their roles in the onset of root and shoot growth. The following report represents a detailed study on several endogenous factors associated with spring regrowth in woody plants.

Materials and Methods

Bare-root *Cornus sericea* rooted cuttings (30 to 45 cm tall) were obtained from a local nursery, packed in moist sphagnum moss,

and placed in a dark cooler at 4°C. The plants were removed from storage after 60 days and placed in a growth chamber in hydroponic culture [50% strength Hoagland's solution (Hoagland and Arnon, 1950), pH 6.5, changed weekly, constant aeration]. Plants were grown under a 15-h photoperiod at 600 mol·m⁻²·sec⁻¹ photosynthetic photon flux density (PPFD) at the canopy level, with a combination of cool-white (Sylvania 72t12/CW/VHO; GTE, Versailles, Kent.) fluorescent and incandescent lamps, at a day and night temperature of 24°C. Root and shoot tissue from each of eight plants was harvested as plants were placed in cold storage, following 30 and 60 days of storage, and 1, 4, 8, and 13 days after the plants were transferred to the growth chamber. The shoot system of each plant consisted of two main branches that were similar in length and development, both originating from the main shoot at the same node. One shoot terminal (1 g, ≈2.5 cm, including the terminal bud) was harvested for growth regulator analysis and the other for carbohydrates. One-gram samples of terminal portions of the root systems, to a maximum root diameter of 2 mm and including both new and previous growth, were harvested for the growth regulator and carbohydrate analyses. The experiment was a completely randomized design with eight replications. Data were analyzed using the PROC GLM procedure of SAS with mean separation by LSD, (SAS Institute, 1985).

Extraction of the hormones and preparative high-performance liquid chromatography (HPLC) were as described by Kling et al. (1987). Filtration of the crude plant extract was followed by a two-column preparative HPLC procedure. The first column was a PRP-1 (10 µm particle diameter, 10 cm × 10 mm; Hamilton, Reno, Nev.) from which a fraction was diverted to a Nucleosil C₁₈ column (7 µm particle diameter, 15 cm × 10 mm; Machery-Nagel, Duren, Federal Republic of Germany). This protocol produced three fractions, one containing indole-3-acetic acid (IAA), one containing abscisic acid (ABA), and the third containing both cytokinins.

Indole-3-acetic acid was further purified using an Ultrasphere ODS column (5 µm particle diameter, 25 cm × 4.6 mm Altech, Deerfield, Ill.) using ion-pairing protocol with tetrabutyl ammonium hydrogen sulfate (TBA) as the ion-pair reagent (Kling et al.,

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1987). Quantitation of IAA was with fluorescence detection (FS 950; Kratos, Westwood, N.J.). Absciscic acid from the preparative fraction was methylated with diazomethane, and the fraction was dried and redissolved in ethylacetate, and subjected to gas-liquid chromatography employing a 0.3 mm $\times$ 30 m fused silica capillary column (model SE-30; J & W Scientific, Rancho Cordova, Calif.) (Hein et al., 1984). The zeatin and zeatin riboside fraction was further purified by selective elution from a 4-ml column of polyvinylpolypyrrolidone (PVPP) (GAF Corp., New York)(Cappiello and Kling, 1990). The resulting fraction was separated on a Vyadac 401TP SCX column (5 μ m particle diameter, 4.6 $\times$ 150 mm, Alltech Assoc. Deerfield, Ill.) using sodium acetate buffer solvents. Quantitation was by ultraviolet (UV) absorbance at 254 nm. Purity and identity of all the plant hormones was verified by gas chromatography-mass spectroscopy.

Analysis of sugars was according to Juvik and LaBonte (1988). Trimethylsilyl derivatives of the sugars were separated on a 12.5-m cross-linked methyl silicone capillary column (model Ultra-1; Hewlett-Packard, Avondale, Pa., 0.1 mm i.d.) with a gas chromatograph (model 5790; Hewlett-Packard), with detection by flame-ionization.

Starch was extracted from the pellet remaining after the sugar extraction. The starch extraction procedure was similar to that for sugars, except the solvent was 10% dimethylsulfoxide (DMSO) and the extraction time was 2 h per extraction (Dickinson et al., 1983). Quantitation of starch was accomplished with the phenol sulfuric acid test (Hodge and Hofreiter, 1962).

Results

Only statistically significant results are presented in the Results and Discussion sections. In the shoot tips, all four growth regulators remained at relatively constant levels throughout the cold storage period except ABA, which increased between days 30 and 60 of storage (Fig. 1). From the time that the plants were transferred to the growth chamber until the end of the experiment, all four growth regulators increased on each date with three exceptions; the levels of IAA and ABA decreased during the first day in the growth chamber, and the level of ABA remained unchanged over the last two sampling dates (Fig. 1). Levels of IAA, zeatin, and ABA

increased at least four times and zeatin riboside increased almost three times by the end of the experiment as compared to levels found in cold storage.

The levels of growth regulators in roots remained relatively constant during cold storage (Fig. 2). The levels of zeatin and zeatin riboside increased three to four times from the time the plants were moved into the growth chamber until the end of the experiment. The level of IAA decreased during the first day that the plants were in the growth chamber followed by small to moderate increases until day 73. Root ABA levels decreased steadily from 97 ng·g⁻¹ at the end of cold storage to 64 ng·g⁻¹ at the end of the experiment.

Glucose and fructose in shoot tips increased slightly during storage, decreased during the first day in the growth chamber, and

Fig. 2. Levels of ABA, IAA, zeatin, and zeatin riboside in roots of *Cornus sericea* during cold storage and through the resumption of vegetative growth in the growth chamber. Day 0 = before cold storage; days 30 to 60 in cold storage; days 61 to 73 in growth chamber. Budbreak occurred at day 63 and root regeneration occurred at day 67.

Fig. 1. ABA, IAA, zeatin, and zeatin riboside in shoot tips of *Cornus sericea* during cold storage and through resumption of vegetative growth in the growth chamber. Day 0 = before cold storage; days 30 to 60 in cold storage; days 61 to 73 in growth chamber. Budbreak occurred at day 63 and root regeneration occurred at day 67.

Fig. 3. Levels of glucose, fructose, sucrose, and starch in shoot tips of *Cornus sericea* during cold storage and through the resumption of vegetative growth in the growth chamber. Day 0 = before cold storage; days 30 to 60 in cold storage; days 61 to 73 in growth chamber. Budbreak occurred at day 63 and root regeneration occurred at day 67.

Fig. 4. Levels of glucose, fructose, sucrose, and starch in roots of *Cornus sericea* during cold storage and through the resumption of vegetative growth in the growth chamber. Day 0 = before cold storage; days 30 and 60 in cold storage; days 61 to 73 in growth chamber. Budbreak occurred at day 63 and root regeneration occurred at day 67.

then returned to levels previously found during storage (Fig. 3). Shoot sucrose levels increased during cold storage while starch decreased. When the plants were moved into the growth chamber, the level of sucrose in shoot tips decreased on every sampling date except the last. Starch levels decreased after 1 day in the growth chamber and increased on the next sampling date and then decreased to the end of the experiment.

Root sucrose levels increased during cold storage while starch levels decreased (Fig. 4). When the plants were moved to the growth chamber, the level of sucrose remained fairly constant for the first 6 days and then rose sharply through the end of the experiment. The level of starch in roots increased slightly during the first day in the growth chamber and then decreased for the remainder of the experiment. Root fructose and glucose levels remained relatively unchanged throughout the experiment.

Discussion

The methods of analysis used allowed for the determination of four growth regulators or four carbohydrates from the same sample. As a result, the data should present a more reliable picture of changes in the levels of these growth substances than past reports. This study also evaluated these hormones and carbohydrates in plants grown under well-defined and controlled environmental and nutritional conditions. Determination of the changes in levels of these substances from uniform plants will give a much clearer picture of the timing and magnitude of those changes than has been demonstrated in the past.

The data from shoot tips of *Cornus sericea* in the present study agree with that presented by Wood (1983) from a study of pecan bud tissue. Both studies showed rapidly increasing levels of cytokinins at the time of budbreak, decreases in the level of IAA to a minimum 3 days before budbreak followed by sharp rises, and decreases in ABA before budbreak. The rise in cytokinins before budbreak and root growth is similar to that reported by Taylor and Dumbroff (1975) in *Acer saccharum*. The continuing increase in cytokinin levels following budbreak in roots and shoots is similar to results reported by Dumas and Zaerr (1988) for xylem exudate in *Pseudotsuga menziesii* and Hewett and Wareing (1973) in buds of *Populus × robusta*. Englebrecht (1971) reported only a slight

increase in cytokinins during shoot expansion of *Populus tremula* and *Acer platanoides*, whereas Domanski and Kozlowski (1968) and Young (1989) reported a decrease in cytokinins after budbreak. Abscissic acid and other inhibitors have also been shown to decrease in bud and stem tissue of several species during the time leading up to budbreak (Alvim et al., 1976 and Taylor and Dumbroff, 1975). Increases in levels of free ABA following budbreak are reported both in this study and by Wood (1983).

There were several differences between the trends in growth regulator levels in pecan (Wood 1983) and *Cornus sericea*. In *Cornus*, the levels of both cytokinins increased throughout the entire course of the experiment except for a decrease in zeatin on the last sampling date. In the study by Wood, bud cytokinin content peaked 15 days before budbreak, dropped to a minimum 3 days before budbreak, and rose rapidly at budbreak. In pecan, the level of IAA in buds peaked at the time of budbreak, while in *Cornus sericea* the level of IAA continued to increase during the time of active shoot elongation. Alden (1971) also reported increasing IAA activity as shoots of *Pinus sylvestris* developed, with the highest levels exhibited in June.

Results from the present study and those of others provide evidence of a common phenomenon in expansion of bud and shoot growth, such as decreases in ABA and increases in cytokinins before budbreak. However, the timing and continuance of the increases for the cytokinins in relation to bud expansion and growth appears to differ with species. There were strong similarities between roots and shoots for changes in the levels of IAA, zeatin, and zeatin-riboside in this study. Only ABA showed marked differences between roots and shoots. The significance of the differences in ABA levels in roots following budbreak reported here and by Wood (1983) is unknown.

A comparison of the absolute levels of the growth substances in tissues of the species discussed above might provide additional insight into potential common systems of regulation. Now, however, these comparisons are not possible because of methodologies used. Most studies have used bioassay systems for determination of levels of growth-regulator-like activity. The few studies that have used strict physiochemical methods of analysis have done so for xylem exudate rather than for macerated buds and shoot tips as in this study.

The changes in carbohydrate levels reported in this study are markedly different from those reported in apple by Young (1989). In apple, glucose, fructose, and sucrose levels followed similar trends as plants progressed from rest, through budbreak and into vegetative growth. The combined level of these three carbohydrates decreased markedly before budbreak and then increased as shoot growth proceeded. In *Cornus sericea*, glucose and fructose levels remained essentially unchanged throughout the experiment in both roots and shoots; however, sucrose levels changed significantly. Sucrose levels in shoots of *Cornus sericea* rose throughout the cold storage period and then, as reported for apple, the level decreased significantly just before budbreak.

The significant decrease in root starch and increase in root sucrose may indicate mobilization of stored carbohydrates for export to expanding shoots. Hansen (1971) demonstrated in apple that stored root carbohydrates were mobilized and exported to support new shoot growth.

This work represents a detailed study on a variety of endogenous factors associated with spring regrowth of roots and shoots in woody plants. This is in contrast to previous studies which related their work only to budbreak without consideration of root growth. Spring root regrowth is very important to the horticultural industries which rely on rapid regrowth of root systems following transplanting.

The methods of analysis used in this study allowed for the determination of four growth-regulating substances or four carbohydrates from the same tissue sample, unlike much previous work. As a result, the data present a more reliable picture of magnitude and timing of changes in the levels of these growth substances than past reports, many of which were based upon bioassay systems of separate extractions. This study represents an improvement in the efforts to understand how both carbohydrates and plant growth regulators act to control development in plants subjected to cold storage—an increasingly common technology in the horticulture industries. Because of the small number of studies conducted on the relationship of woody plant growth substances and root regrowth, these data provide an increased understanding of these processes.

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