Research Reports:

Effects of Chemical Defoliation and Bare Root Storage on Carbohydrate Levels and Spring Growth in *Euonymus alata*[1]

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Abstract

For 2 seasons, levels of starch, sucrose and galactitol were measured in roots of *Euonymus alata* (Thunb.) Sieb. following fall spray treatments with a mixture of endothall [7-oxabicyclo- (2,2,1) heptane-2, 3-dicarboxylic acid] and ethephon (2-chloroethyl phosphonic acid) and overwintering in the field or in a common storage facility. The spray-storage treatment decreased starch levels by 80% and 43% during winter months of the first and second years, respectively. Dry weight of new shoot growth was reduced 66% as a result of the spray-storage treatment during the first year, but there was no reduction of dry weight relative to controls during the second year. During both years, galactitol levels in the roots were not affected by either treatment.

Index words: *Euonymus*, polyols, galactitol, growth regulator, bare root storage.

Introduction

In western New York state, nursery stock is commonly harvested in late fall and is stored as bare root, dormant plants in unrefrigerated storage structures. This practice allows grading and shipping during winter and early spring. As a result of fall harvesting, defoliation is often necessary to meet digging schedules.

Ethephon has been used experimentally as a defoliant for deciduous nursery stock (9) and its effectiveness has been improved by the addition of surfactants (10), salts (1) and herbicides (16). A mixture of ethephon and the herbicide endothall is a promising combination for defoliation of nursery crops (16). However, chemical defoliation may affect levels of energy reserves which may lead to "sleepy buds" or other physiological problems.

Winter storage conditions can also affect the physiological status of nursery stock since the relatively high temperature in some storage facilities (5 to 15 °C, 41 to 50 °F) can increase metabolic activity of dormant plants (17), resulting in depletion of energy reserves. Depletion

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of carbohydrates during winter storage has been reported in apple roots (14), Douglas-fir seedlings (15), and Jeffrey pine seedlings (7). In these studies, levels of either starch and sucrose or total nonstructural carbohydrates were determined.

Starch and sucrose are the most common and abundant reserve compounds in plants (8), but many horticultural crops contain other carbohydrates that are often present in high concentrations. Galactitol is a six carbon sugar alcohol that is present in all species of *Euonymus* (13). The route of galactitol metabolism is presently unknown, but it has been suggested that sugar alcohols may function as energy reserve compounds (2). Carbohydrates that function as energy reserves typically decrease in concentration as they are utilized in seasonal growth (8).

The purpose of this study was twofold: 1) to examine the effects of chemical defoliation and commercial storage practices on carbohydrate levels (including galactitol) and on subsequent spring growth in *E. alata*, and 2) to determine the extent of galactitol mobilization in *E. alata* following dormancy.

Materials and Methods

Plant material. Approximately 200 three-year-old plants of *E. alata* grown outdoors at a commercial nursery in western New York were sprayed to runoff with a mixture of endothall (774 ppm ai) and ethephon (1080 ppm ai) with an air blast sprayer on October 5, 1983 and October 5, 1984. A similar number of control plants were not sprayed. During the first year of the study, only sprayed plants were machine harvested (with a 'U blade') and were placed in storage on October 19, 1983. Six weeks after harvest and at roughly 6 week intervals thereafter, 3 sprayed plants were taken from storage and 3 nonsprayed plants were dug from the field for carbohydrate analysis. During the second year of the study, 100 sprayed and 100 nonsprayed plants were harvested and placed in storage on October 19, 1984. An additional 100 sprayed and 100 nonsprayed plants were left in the field. One day after harvest and at 6 week intervals thereafter, 3 sprayed and 3 nonsprayed plants were randomly selected from the storage facility and from the field. Samples were separated into shoots and roots (and leaves on the first sampling date) and were packed in dry ice at the collection site. Samples were later freeze-dried, ground in a Wiley mill to pass a #20 mesh screen and stored over anhydrous $CaCl_2$ at -60 °C (-76 °F).

Storage conditions. The storage facility was a 12.2 x 36.6 m (40 x 120 ft) barn with a 5.5 m (18 ft) ceiling and a cement floor. The walls were insulated with 19 mm (3/4 in) styrafoam and the building was heated, when necessary, by a propane burner thermostatically set to switch on at 0 °C (32 °F) and off at 1.7 °C (35 °F). When the indoor temperature exceeded 4.4 °C (40 °F) due to the heat of respiration from stored plants, cool air entered the building through a vertical rolling door which was the major source of ventilation during the winter. During the spring months, two 45.7 cm (18 in) ceiling vents were opened manually in addition to the rolling door. No fans were used for ventilation. The grower maintained relative humidity between 60% and 80% by periodically spraying the floor and ceiling with water. Bare root plants were stacked into 1.2 x 1.5 x 2.1 m (4 x 5 x 7 ft) framed pallets which in turn were stacked along the walls of the building. Plants used in these experiments were confined to one pallet (sprayed) during the first year and two pallets (sprayed and nonsprayed) during the second year.

Spring growth experiment. On March 25, 1984 and March 28, 1985, 4 single plant replicates were removed from the field and from the storage facility and were planted at the Cornell test garden in Ithaca, New York. Budbreak was first observed on April 29, 1984 in the first study and April 15, 1985 in the second study. The oldest leaves were approximately half expanded on May 19, 1984 and May 5, 1985 when the new shoots were removed by hand, freeze-dried, weighed, and stored for carbohydrate analysis. The second flushes of shoot growth were treated similarly, but were harvested on June 12, 1984 and June 5, 1985.

Neutral sugar analysis. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO 63178). Tissue samples (50 mg) were extracted in test tubes (13 x 100 mm) in methanol:chloroform:water (12:5:3) following the method of Haissig and Dickson (6). The residue was saved for starch analysis. The aqueous phase was bubbled with nitrogen for 1 hour to remove methanol. The remaining aqueous phase was passed through a mixed bed ion exchange-PVP column and analyzed by high pressure liquid chromatography (HPLC) as described by Boersig and Negm (3).

Starch analysis. After solvent extraction, the residues from the 50 mg samples were oven-dried at 37 °C (98.6 °F) and were incubated for 48 hours at 55 °C (131 °F) in 3 ml *Rhizopus* amyloglucosidase solution (50 units/tube in 0.1 M acetate buffer pH 4.5). Each sample was assayed for glucose by the glucose oxidase method modified from Goldstein and Lampen (5). After vortexing, a 25 *u*l aliquot was mixed with 3 ml of a solution containing the following components: 10 parts enzyme solution (type II glucose oxidase from *Aspergillus niger* + type I peroxidase from horseradish, 16 units/ml each in 0.1 M Na-phosphate buffer pH 6.0) plus 5 parts 0-dianisidine (6 mg/ml in H_2O) plus 85 parts 45% glycerol (v/v). This reaction mixture was incubated at 30 °C (86 °F) for 30 min. The reaction was then stopped by the addition of 0.5 ml concentrated HC1. Tubes were vortexed and absorbance was read at 540 nm using a Perkin-Elmer 552 spectrophotometer. Glucose standards from 5 to 75 *u*g were used.

Water potentials. Water potentials of shoots and roots were measured at each sampling during the 1983-1984 season with a pressure bomb (Soil Moisture Testing Equipment, Santa Barbara, CA 93105). Temperature and relative humidity were measured in the storage facility during the 1983-1984 season with a steady state diffusive resistance porometer (Licor, Inc., Lincoln, NB 68504). Outdoor air temperatures were obtained from the National Weather Service Station at Colden, New York.

Statistical analysis. Carbohydrate data for the 1983-84 season were analyzed by a two way analysis of variance (AOV) comparing effects of time and sprayed-

storage. Carbohydrate data from the 1984-85 season were analyzed by a three way AOV comparing effects of time, storage and spraying. Least significant differences were determined when the appropriate interaction effects were significant. The SAS statistical package was used for all analyses.

Results and Discussion

Effectiveness of defoliant. Ethephon and endothall at the concentrations used in this study proved effective in hastening leaf abscission of *E. alata.* Two weeks after spraying, the dry weights of foliage remaining on sprayed plants and controls were 1.5 g (s.e. = 0.4 g) and 11.6 g (s.e. = 1.7 g), respectively. These findings are in agreement with previously reported results (16).

Storage environment. Temperatures in the storage building were relatively constant compared to air and soil temperatures outdoors (Table 1). Temperatures during the winter months were generally warmer indoors than outdoors. Relative humidity indoors ranged from 61% to 71% (data not shown). This level of moisture was sufficient to maintain plants in a reasonably hydrated state. Shoot water potentials of plants stored indoors ranged from -2.0 bars to -5.6 bars, essentially the same as field plants (data not shown).

Carbohydrates and spring growth. Carbohydrate concentrations in *E. alata* roots were 3 to 6 times greater than in shoots (Fig. 1). Likewise, the greatest fluctuations in carbohydrate pool size occurred in roots. During the first year of the study, the combination of chemical defoliation and storage through April decreased the starch and sucrose contents in the roots by 90% and 70%, respectively. Starch concentration in nonsprayed field plants remained high until May when budbreak occurred. As leaves expanded, starch concentration in roots of control plants fell to a level near that of treated plants. Dry weight of the new shoot growth was 66% less from indoor-sprayed plants than from outdoor-nonsprayed plants (Table 2). Since the leaves were acting as net sinks when this new growth was removed (11), the dry weight of the tissue was largely dependent on stored reserves.

In the second study, root starch in all treatments declined sharply between November and January and continued to decline gradually until May (Fig. 2). Despite this early decrease, starch concentration of indoor-sprayed plants was significantly lower than field-nonsprayed plants (at the 5% significance level) from November through April.

Table 1. Indoor and outdoor median air temperatures on 10 sampling dates.

	Indoor temp (°C)	Median Outdoor temp (°C)
10/20/83	3.5	3.9
12/10/83	3.4	-2.3
02/03/84	3.5	-2.0
03/17/84	6.0	0.3
05/07/84	6.5	11.1
10/19/84	4.5	11.4
11/27/84	4.3	6.4
01/04/85	3.5	-6.6
02/18/85	3.4	-3.8
05/16/85	6.8	18.3

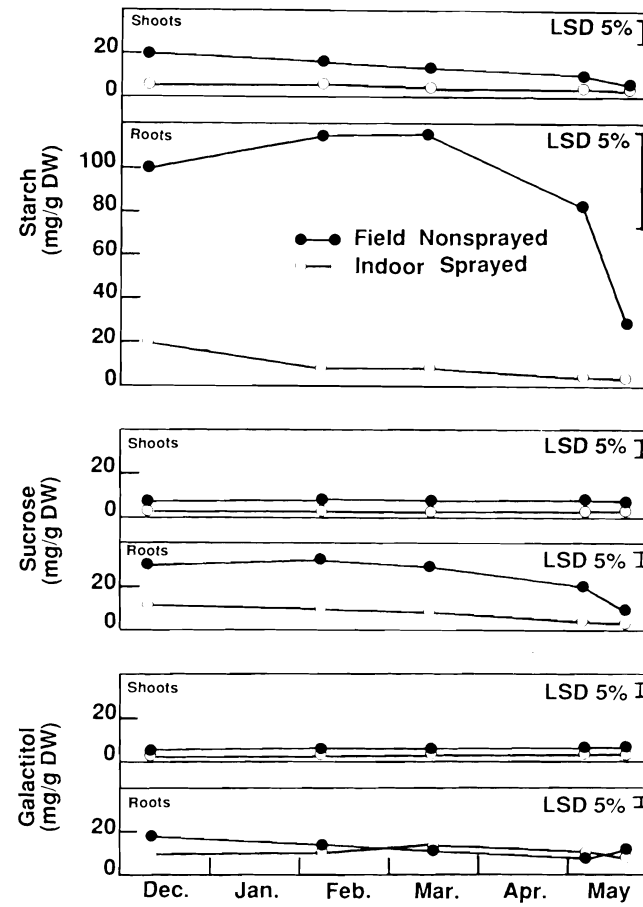

Fig. 1. Effect of ethephon-endothall and storage on carbohydrate levels in overwintering shoots and roots of *Euonymus alata* during 1983-84. Each point represents the mean of 3 replications.

Sucrose levels were only slightly affected by the treatments during the second storage season. There were, likewise, only minor differences (statistically insignificant) between treatments in the dry weights of new shoots during the second season (Table 2).

The early decrease in starch content of control plants during the second season coincided with an unusually warm December. The average daily maximum temperature in December 1984 was 5 °C (41 °F), compared to -2.2 °C (28 °F) in December 1983. Differences in winter temperatures between the two years may have also influenced dates of budbreak (April 29 in 1984, compared to april 15 in 1985).

Effect of spraying. During the second year of the study, an attempt was made to distinguish between the effects of the defoliant and the effects of the storage environment. The effect of spraying on starch levels in field plants was significant only on the November and February sampling dates (Fig. 2). Among indoor-stored plants, the spray treatment had a significant effect only on the October sampling date.

Role of galactitol. The differences between galactitol levels in field and stored plants or sprayed and non-

Table 2. Dry weights of new shoot growth of *Euonymus alata* treated with or without ethephon-endothall and bare root storage.

	Dry Wt[z] (g)			
	Field		Indoor	
	Nonsprayed	Sprayed	Nonsprayed	Sprayed
1983-84				
1st Flush	22.2 ±4.9	——	——	7.4 ±1.9
2nd Flush	8.6 ±3.7	——	——	1.7 ±0.3
1984-85				
1st Flush	11.5 ±2.0	13.8 ±1.3	13.6 ±2.3	14.9 ±2.9
2nd Flush	5.8 ±1.0	6.7 ±0.5	5.0 ±1.1	7.0 ±0.9

[z]Each value (± 1 SE) represents the mean of 4 replications.

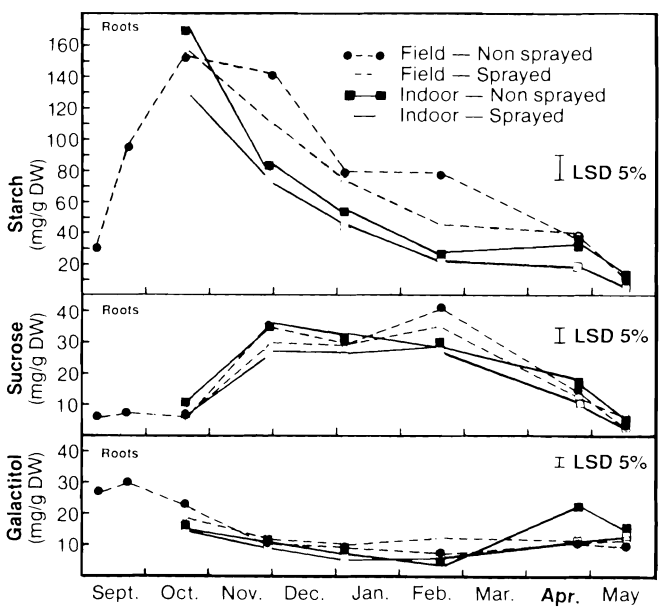

Fig. 2. Effect of ethephon-endothall and storage on carbohydrate levels in overwintering roots of *Euonymus alata* during 1984-85. Each point represents the mean of 3 replications.

sprayed plants were small during both storage seasons (Figs. 1 & 2). In addition, when new shoot growth occurred in the spring, galactitol levels in roots and shoots did not decline. These results suggest that either the galactitol pool was not utilized in new shoot growth or, if it was utilized, it may have been replenished at the expense of starch or sucrose. In either case, galactitol was not the ultimate source of carbon from the perennating organs. Galactitol was, however, the predominant nonstructural carbohydrate in the new expanding shoots (Fig. 3). It is not known whether galactitol was formed in the leaves as an early product of photosynthesis or was translocated from the roots and woody shoots following its rapid synthesis in those tissues. The most likely substrate for galactitol synthesis is galactose which is readily reduced by an NADPH-dependent aldose reductase in the leaves of *E. fortunei* and *E. japonica* (12). In the present study, galactose was not detected in the roots or woody shoots but was present in young expanding shoots at a level of 10.9 mg/g dw. Galactose does not normally occur at such high a concentration in most plants and is in fact toxic to many species (4). The presence of galactose suggests that the leaves are the most likely site for galactitol synthesis, but this has yet to be demonstrated conclusively.

Significance to the Nursery Industry

The chemical defoliant ethephon-endothall, and bare root storage in the described facility, both significantly decreased the level of starch in roots of *E. alata*. Dry weight of new shoot growth from plants that were sprayed and stored indoors was significantly less than that from non-sprayed plants that overwintered in the field during the 1983-1984 season. No significant difference in dry weight resulted from these treatments during the 1984-1985 season, possibly because starch levels in control plants declined in midwinter. Under the conditions of this study, starch depletion resulting from ethephon-endothall treatment was less significant than that resulting from storage. However, all treatments resulted in plants of acceptable appearance before hand

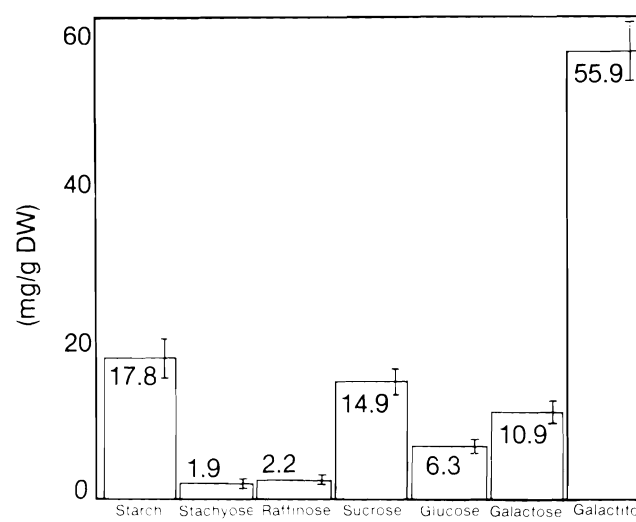

Fig. 3. Concentrations of various carbohydrates in expanding shoots of *E. alata*. Vertical bars represent ± 1 SE.

defoliation. Based on the results of this study, ethephon-endothal appears to be a safe and effective defoliant for *E. alata*.

(*Ed note:* This paper reports the results of research only, and does not imply registration of a pesticide under amended FIFRA. Before using any of the products mentioned in this research paper, be certain of their registration by appropriate state and/or federal authorities.)

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