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Short Communication

Interactions of Low Temperature, Water Stress, and Short Days in the Induction of Stem Frost Hardiness in Red Osier Dogwood[1]

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ABSTRACT

The induction of stem frost hardiness by low temperature, water stress, short days, and their combinations in 2- and 4-month-old growing dogwoods (*Cornus stolonifera*) were investigated. When plants were subjected to more than one factor, the increased hardiness was the sum of the effects of the individual factors involved. No interactions among these factors on hardiness were observed during a 3-week treatment. Results indicate that low temperature, water stress, and short days initially trigger independent frost-hardening mechanisms. Plant ages significantly influenced the change in low temperature-induced frost hardiness, but not the water stress or short day-induced frost hardiness.

Frost hardiness in woody perennials can be induced by either low temperature (5–7), water stress (15), short days (4, 7), or their combinations (4, 6, 7). In red osier dogwoods: (*a*) short days alone induced frost hardiness to some extent (10, 13); (*b*) water stress also increased frost hardiness under either long day or short day regimes (2, 3); and (*c*) low temperature alone did not induce hardiness very effectively in long day regimes but was effective after growth cessation in short days (9, 13). There has been relatively little study on interactions of low temperature and water stress, or of low temperature, water stress, and short days in the induction of stem frost hardiness in red osier dogwoods. Therefore, a study was carried out and results are reported here.

MATERIALS AND METHODS

Plant Culture. Red osier dogwood plants, *Cornus stolonifera* Michx., were propagated by tip cuttings from the same genetic clones. Plants were then transplanted in 15-cm pots with a 3:2:2 mixture of soil, sand, and peat. Plants were maintained in a greenhouse under an approximately 16-hr-long day and 20/15 C day/night temperatures. Three weeks after transplanting, plants were trimmed to two leaders/pot to provide uniform experimental materials. After 2 months of growth, half of the plants were transferred to controlled environment chambers for treatments (trial I). The other half remained in the greenhouse for an additional 2 months of growth and were then transferred to growth chambers for treatments (trial II).

Experimental Design and Treatments. In each trial, 72 uniform plants were selected for a 2^3 factorial experiment in a completely randomized design. This included (*a*) two photoperiods—16-hr LD[2] and 8-hr SD; (*b*) two controlled water supplies—plants were watered once daily either to the point of saturation or with 30 ml of water/pot (WS); and (*c*) two temperatures—20/15 C day/night warm temperature and 5/5 C day/night LT. Each pot constituted an experimental unit and each treatment was replicated three times. Through the combining of LT, WS, and SD, eight treatments including a control were imposed. Prior to treatments, all plants were watered to field capacity and each pot was wrapped with a plastic bag to eliminate evaporation during the experiment (3). Hardiness determinations were carried out after 0, 7, 14, and 21 days of treatment. The status of soil moisture content and stem water potential under 30 ml/pot·day WS was reported elsewhere (2).

Evaluation of Frost Hardiness. Frost hardiness was determined by visual observations (8, 11) and was expressed as the minimum survival temperature (the lowest temperature at which stem cortical tissues still remained alive).

RESULTS AND DISCUSSION

During a 3-week treatment, control plants maintained a consistent level of −4 C frost hardiness in both trials, while LT increased hardiness to −15.5 and −25.5 C, WS to −12.3 and −12.0 C, and SD to −6.7 and −7.0 C in the 2- and 4-month-old plants, respectively. Figure 1 illustrates increases of stem frost hardiness induced by LT, WS, SD, and their combinations. As shown, the factor of LT played a predominant role in terms of increasing hardiness in the growing plants as compared with WS and SD. This is not surprising because it has been shown that plants exposed to LT during long days can acclimate to the greatest hardiness level and that the exposure of plants to SD at relatively high temperature results in less hardiness (10, 14).

When plants were exposed to either WS, SD, or a WS/SD combination, the 2- and 4-month-old plants developed similar hardiness levels (Fig. 1, solid lines). Age did not influence the changes of WS- and SD-induced hardiness. When the LT factor was involved, the 4-month-old plants had a net hardiness increase of 21.5 C as compared to 11.5 C in the 2-month-old plants after a 3-week LT treatment. The initiation of cold acclimation in dogwood is closely associated with growth cessation (10), and the capacity for cold acclimation is also dependent on the growth phase (13). Growth cessation, however, was not observed in these studies because apical growth and new leaf expansion occurred during the treatment period. Growth activity in the 4-month-old plants might have already slowed down at the beginning of trial

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[2] Abbreviations: LD: long day; SD: short day; WS: water stress; LT: low temperature.

II even though they had not yet reached the stage of growth cessation.

Analyses of variance were used to test the effects of each factor (LT, WS, SD) and their interactions on survival temperatures. Sources of variance and their significance on frost survival are shown in Table I. Temperature, water supply, and day length treatments all showed a significant (99% level) effect on frost survival, but there were no significant interactions among them.

TABLE I. *Significance of Effects and Interactions on Survival Temperatures of Red-osier Dogwood Stem Sections After Three-Week treatments.*

Source	df	Significance Trial I	Trial II
Blocks	2	NS	NS
Photoperiod (A)	1	**	**
Water supply (B)	1	**	**
Temperature (C)	1	**	**
A x B	1	NS	NS
A x C	1	NS	NS
B x C	1	NS	NS
A x B x C	1	NS	NS
Error	14		
Total	23		

**: Significant at the 99% confidence level.
NS: Not significant.

Analysis indicates that the total frost hardiness increase should be the sum of the individual effects from all factors involved in cold acclimation.

By subtracting the minimum survival temperature of controls (−4.0 C) from the minimum survival temperatures of each of the other seven treatments, the net frost hardiness increases were obtained; these are listed in Table II for both trials. It is surprising that hardiness increases which resulted from any combinations of LT, WS, and SD are the sums of the individual effects. The observed results coincide with the variance analysis (Table I). If the effects of LT, WS, and SD on the minimum survival temperatures are really additive, then the observed increases of hardiness should be in agreement with the values resulting from any of the possible calculations. Such agreements, as a matter of fact, are illustrated in columns 3 and 4 for trial I and columns 6 and 7 for trial II in Table II.

It has been suggested that SD may be involved in the production of a translocatable hardiness promoter because leaf removal can delay the SD-induced cold hardiness in stem tissues (4). There is also abundant evidence indicating that day length has a substantial influence on the change in plant hormonal balance which in turn affects growth. Leaf removal, on the other hand, had little effect on LT-induced cold hardiness (6). LT may affect the availability

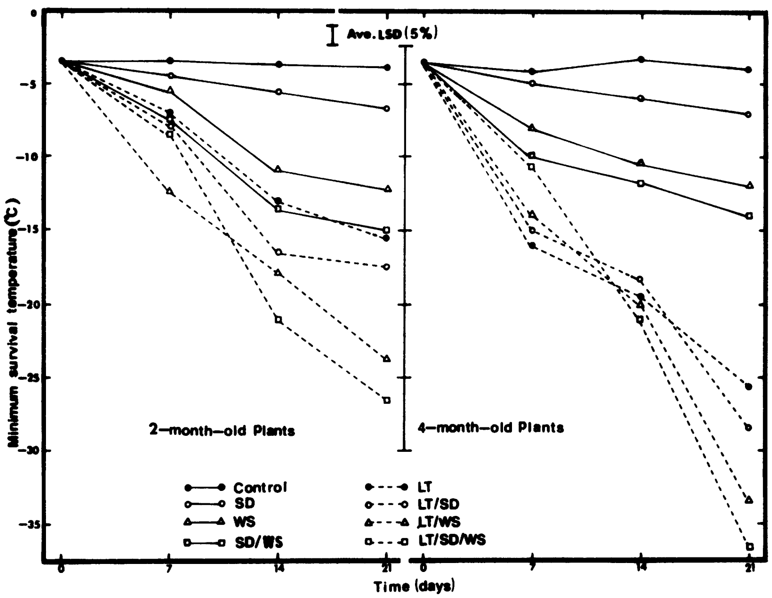

FIG. 1. Increases of frost hardiness in stem sections of growing red osier dogwoods under controlled environments. Control: long day/warm temperature (16 hr light, 20/15 C day/night temperature); SD: short day (8 hr light); WS: water stress (30 ml of H_2O/pot·day); LT: low temperature (5/5 C day/night).

TABLE II. *The Observed and Calculated Net Increases of Stem Frost Hardiness in Red-osier Dogwood Plants After Three-Week Treatments of LT, WS, SD or any Combinations of these Factors.*

Treatments	Trial I (2-month-old plants) Minimum survival temp. (C)	Observed net hardiness[a] increase (C)	Calculated hardiness increase (C)	Trial II (4-month-old plants) Minimum survival temp. (C)	Observed net hardiness increase (C)	Calculated hardiness increase (C)
Control	-4.0			-4.0		
SD	-6.7	2.7	2.8[b]	-7.0	3.0	2.8
WS	-12.3	8.3	8.2[c]	-12.0	8.0	7.8
LT	-15.5	11.5	11.6[d]	-25.5	21.5	22.0
SD/WS	-15.0	11.0	11.0[e]	-14.0	10.0	11.0
LT/SD	-18.5	14.5	14.2[f]	-28.3	24.3	24.5
LT/WS	-23.7	19.7	19.8[g]	-33.5	29.5	29.5
LT/WS/SD	-26.5	22.5	22.6[h]	-36.5	32.5	32.5

[a]Net hardiness increase = the absolute value of the minimum survival temperature of treatment minus the minimum survival temperature of control.
[b]Average of: SD/WS-WS, LT/SD-LT, LT/WS/SD-LT/WS, LT/WS/SD-LT-WS.
[c]Average of: SD/WS-SD, LT/WS-LT, LT/WS/SD-LT/SD, LT/WS/SD-LT-SD.
[d]Average of: LT/SD-SD, LT/WS-WS, LT/WS/SD-SD/WS, LT/WS/SD-SD-WS.
[e]Average of: SD+WS, (LT/WS-LT) + (LT/SD-LT), LT/WS/SD-LT.
[f]Average of: LT+SD, (LT/WS-WS) + (SD/WS-WS), LT/WS-SD-WS.
[g]Average of: WS+LT, (LT/WS-LT) + (LT/SD-SD), LT/WS/SD-SD.
[h]Average of: LT+WS+SD, WS/SD+LT, LT/SD+WS, LT/WS+SD.

of energy in controlling the shift of metabolic events. It is reasonable to say that SD- and LT-induced cold hardiness is probably the result of different physiological routes, at least at the initial stages. Undoubtedly, WS will affect the tissue water content level and its properties (2). Such a change could well determine the amount of extracellular ice formation reducing, perhaps, mechanical stress. It is also known that WS can increase the tolerance of freeze-induced dehydration (2). Judging from the results in this report, we concluded that LT, WS, and SD initially trigger independent mechanisms in inducing frost hardiness, although WS and SD accomplish the same physiological end(s) in terms of biochemical changes (1).

Siminovitch *et al.* (12) suggested that total frost hardiness results from independent physiological events whose effects are additive. Results reported in this communication support their suggestion. In conclusion, the total frost hardiness in dogwood plants which resulted from 3-week LT, WS and SD treatments could be expressed by an equation:

$$H_t = C + I_{\mathrm{LT}} + I_{\mathrm{WS}} + I_{\mathrm{SD}}$$

where H_t is the total degree of frost hardiness; C is the minimum survival temperature degree during active growth (summer levels); and I_{LT}, I_{WS}, and I_{SD} are the induced net increases of frost hardiness individually resulting from LT, WS, and SD.

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