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Phytochrome Control of Growth Cessation and Initiation of Cold Acclimation in Selected Woody Plants[1]

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B. J. WILLIAMS, JR.,[2,3] N. E. PELLETT, AND R. M. KLEIN
Department of Plant and Soil Science and Department of Botany, University of Vermont, Burlington, Vermont 05401

ABSTRACT

Short day enhancement of cold acclimation in twigs of *Cornus* (red-osier dogwood), *Weigela*, and *Pyracantha* (firethorn) was studied using dark interruptions with red or with red-far red radiation. Hardiness was estimated by freezing stem tissues to preselected temperatures and evaluating injury electrolytically. Dark-period interruptions with red radiation suppressed cold acclimation in *Cornus* and *Weigela*. When red light was followed by far red light, suppression was relieved. No radiation control of acclimation was found with *Pyracantha*. The short day enhancement of cold acclimation in *Cornus* and *Weigela* appears to be phytochrome-mediated.

The ability of woody plants to survive freezing temperatures depends on their acclimation during the prefreezing period (1). Cold acclimation is influenced by several environmental factors including light and temperature (12), with autumnal photoperiods serving in some plants to initiate the yet unknown alterations leading to acclimation (21). Initiation of cold acclimation may be most rapid at higher temperatures (5), with full acclimation induced by subsequent freezing temperatures (18).

Existence of a radiation-mediated step has raised the question of whether light is required solely for photosynthesis or is involved more directly in initiating acclimation. In nonwoody plants like alfalfa (2), wheat (16), and cabbage (11) acclimation appears best under long days. This suggests a photosynthetic role for the light. Citrus (22) and some conifers (19, 23) became more winter hardy under short day conditions. In *Cornus* (7), *Viburnum*, and *Acer* (9, 10), long day photoperiods suppressed acclimation. The reports (6, 8–10, 17) that a radiation-induced signal was formed in and translocated from leaves, that there was increasing acclimation as a function of the number of short day cycles, and that interrupting long night periods with white light reversed acclimation suggest possible participation of phytochrome in short day-induced initiation of cold acclimation.

This study was based on the assumption that there may be some relationship between phytochrome-controlled short day processes and initiation of cold acclimation. Radiation used for night break interruptions should have minimal effect on accumulation of photosynthate. Reversal by far red radiation of a red light-induced suppression of acclimation is evidence of participation of phytochrome.

MATERIALS AND METHODS

Three woody shrubs were selected for this study. *Cornus stolonifera* Michx. (Dickenson, North Dakota clone [5]) is very cold hardy with fully hardened stem tissues surviving freezing in liquid nitrogen. *Weigela florida* (Sieb. and Zucc.) A. DC. cv Bristol Ruby, and *Pyracantha coccinea* Roem. cv Kasan are less hardy compared with *Cornus*. The three species show different photoperiodic responses: *Cornus* and *Weigela* being markedly photoperiodic with respect to vegetative growth (3, 15) and *Pyracantha* being vegetatively unresponsive (15). *Cornus* and *Weigela* are deciduous and *Pyracantha* is evergreen.

Rooted softwood cuttings propagated from greenhouse-grown plants were planted in 1-quart plastic pots containing a soil mix of loamy sand-Perlite-sphagnum peat moss (1:1:1) supplemented with 4.8 kg/m^3 of dolomitic lime and 3.1 kg/m^3 of 20% superphosphate. Plants were fertilized at each watering with a 20:20:20 complete liquid fertilizer alternating every 3 weeks with 15:10:30 soluble fertilizer, both at the rate of 100 ppm N. Until used for the study, plants were grown in a greenhouse (about 21 C–15 C). Winter photoperiods were extended to 16 hr with incandescent lamps. *Cornus* was grown from October 1970 for 8 weeks, Weigela from December 1970 for 10 weeks, and *Pyracantha* from January 1971 for 12 weeks.

All experiments were conducted in a Sherer Model CEL 4608 chest-type growth chamber partitioned into four equal sections. Chamber illumination was provided by S96T120 CW/VHO lamps plus 30 w incandescent reflector lamps providing a radiant flux (400–800 nm) of 0.6 w/cm^2. Lights and chamber were separated by sliding Thermopane doors. Temperatures were monitored with a YSI Telethermometer Model 43 Tz: temperature variations among chamber compartments did not exceed ±2 C. Red radiation was provided by 3 R-40 red reflector flood incandescent lamps filtered through 2.5 cm of $CuSO_4 \cdot 5H_2O$ (26 g/l) plus a single sheet of Cinemoid No. 5 (orange) plastic. The red-far red ratio was 17/1; radiant flux at plant level was 13×10^3 μw/cm^2 (Fig. 1). Far red radiation was provided by R-40 red reflector flood lamps filtered through 2.5 cm of $Fe(NH_4)_2(SO_4) \cdot 6H_2O$ (105 g/l) plus a 3 mm thick Rohm and Haas "Black" plastic filter. Radiant flux at plant level was 400×10^3 μw/cm^2 (Fig. 1). The filter systems were positioned under the sliding glass doors of the growth chamber.

After the period of greenhouse growth, test plants were

[1] Contribution 265 from the Vermont Agricultural Experiment Station.

[2] Present address: Essex Agricultural and Technical Institute, Hathorne, Mass. 01937.

[3] Taken in part from dissertation submitted by B. J. Williams to the University of Vermont in partial fulfillment of the requirements for the M.S. degree.

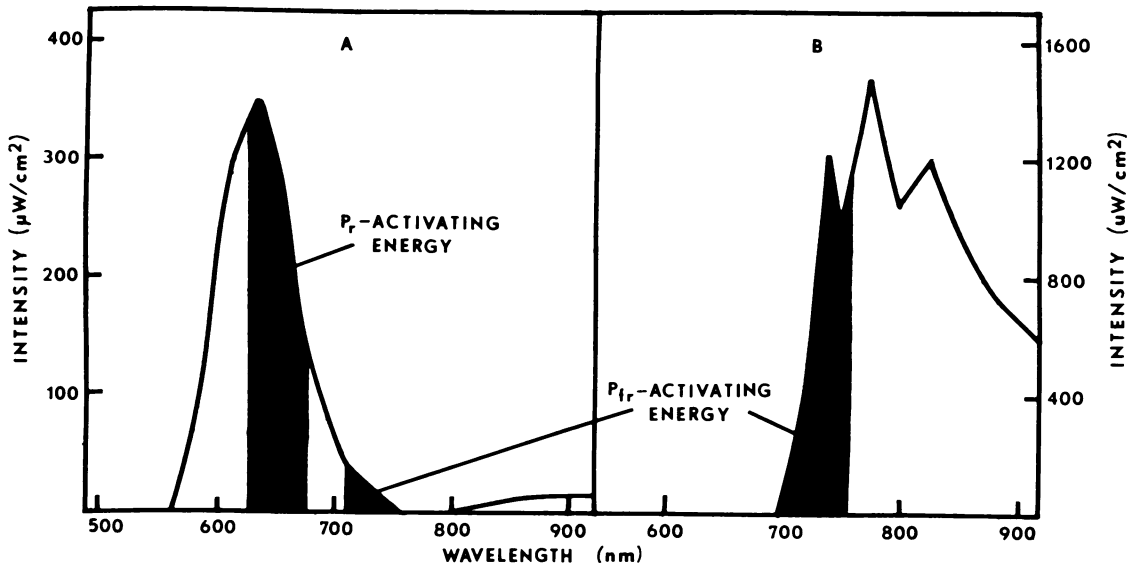

FIG. 1. Spectral energy distribution curves of radiations used for night interruptions. A: Red night interruption; B: Far red night interruption.

placed in the chamber. Each compartment received a different light regime: (*a*) long day of 16 hr; (*b*) short day of 8 hr; (*c*) short day plus 15 min night break of red radiation (11.7 joules/cm^2) presented at midpoint of the dark period; (*d*) short day plus red radiation immediately followed by 15 min far red radiation (36 joules/cm^2). For *Cornus* and *Weigela,* the temperature in all compartments was 20 C for the first 3 weeks followed by a week at 15 C and a final week at 10 C. *Pyracantha* was exposed to 20 C for 3 weeks, a week at 15 C, a week at 10 C, and a final week at 5 C.

Bud dormancy in *Cornus* was determined at the end of the 5-week test period. Axillary buds were scored as dormant, buds broken with aborted shoots, or growing. Buds were excised, dried at 70 C for 48 hr, and weighed.

Cold hardiness of *Cornus* and *Weigela* was evaluated after 5 weeks and after both 3 and 6 weeks for *Pyracantha.* Internodal segments, 1.3 cm long by about 4.8 mm in diameter, were taken from *Cornus* and *Weigela* stems three or four internodes below the youngest leaf pairs. Ten segments were excised from each of six plants per light treatment, and two segments were randomly assigned to each of five test tubes for exposure to a series of freezing temperatures. For *Pyracantha,* 7.5 mm long segments were cut from internodes 10 to 30 cm from the stem apex. It was difficult to obtain uniform samples because *Pyracantha* internodes are short and variable. Fifteen segments were excised from each of six plants per light treatment and three segments randomly assigned to each of five test tubes for exposure to series of freezing temperatures.

All tissue samples were rinsed with tap water and blotted on paper toweling before being placed in test tubes. The tubes were plugged with polyurethane foam and placed at 5 C in a freezing chest equipped with a circulating 95% ethanol bath and a temperature programmer.

Bath temperatures were monitored at 1-min intervals with a copper constantan thermocouple positioned centrally in the bath. Tissue temperatures of *Cornus* and *Weigela* were monitored with a thermocouple inserted longitudinally into the pith of a segment in a test tube located in the center of the bath. Because of their small diameter, tissue temperatures were not monitored in *Pyracantha;* it was assumed that they followed the same pattern as did *Cornus* and *Weigela.* After 1 hr, tubes were removed from the 5 C bath and were stored in a holding chest at 5 C to serve as unfrozen controls. The bath temperature was lowered in 1.5 C decrements over 5-min intervals and held constant at each level for 10 to 12 min, a rate of temperature reduction of 6 C/hr. The supercooling exotherm occurred at −5.5 C, heat of crystallization dissipated in 20 min, and tissue temperatures remained approximately equal to bath temperature throughout the freezing sequence. Tissues were held 10 to 12 min at selected test temperatures before being transferred to the holding chest where they remained for about 12 hr at 5 C for thawing.

To evaluate cold acclimation, 5 ml of glass-distilled water was added to each tube containing thawed tissue segments. The tissues were held at 5 C for an additional 24 hr to allow diffusion of electrolytes from injured cells. Conductivity readings were taken with a Beckman RC 1631 conductivity bridge unit after samples were equilibrated at 21 C for 30 min. All samples were then autoclaved at 129 C for 15 min and allowed to equilibrate again for 24 hr at 5 C before conductivity measurements were repeated. Cold injury was expressed as an index of injury (I_t) which converts the percentage electrolyte release to a scale wherein the unfrozen control samples are rated at zero and the autoclaved samples are rated at 100 (4):

$$I_t = 100\ (L_t - L_o)/(L_k - L_o)$$

where I_t = index of injury resulting from exposure to temperature (t), L_o = specific conductance of electrolytes leached from unfrozen control samples, L_t = specific conductance of electrolytes leached from sample frozen at temperature (t), L_k = specific conductance of electrolytes leached from sample frozen at temperature (t) and then autoclaved.

This method assumes that the level of low temperature injury to cell membrane systems can be determined by measuring the leached electrolytes. Although it does not differentiate between cells showing complete, partial, or no freezing-induced loss of membrane integrity, we assume (without rigid proof) that a consequence of cold acclimation is the ability to maintain membrane integrity. Unpublished studies in our laboratories have shown a general correlation between the I_t and observed cold injury to plants.

Indices of injury were compared by an analysis of variance and the significance of differences between means was tested using Duncan's new multiple range test.

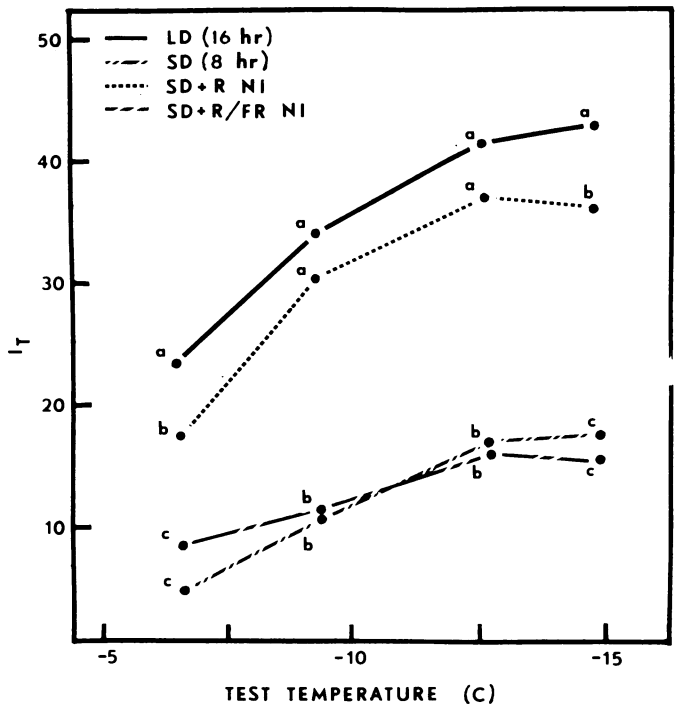

FIG. 2. Indices of injury (I_t) of stem segments of *Cornus stolonifera*, cold acclimated for 5 weeks under four light regimes and frozen to four test temperatures. Each point is the mean of six samples, one each from six plants. Means with the same letter for a test temperature are not significantly different at the 0.01 probability level. LD: long day; SD: short day; R: red; FR: far red; NI: night interruption.

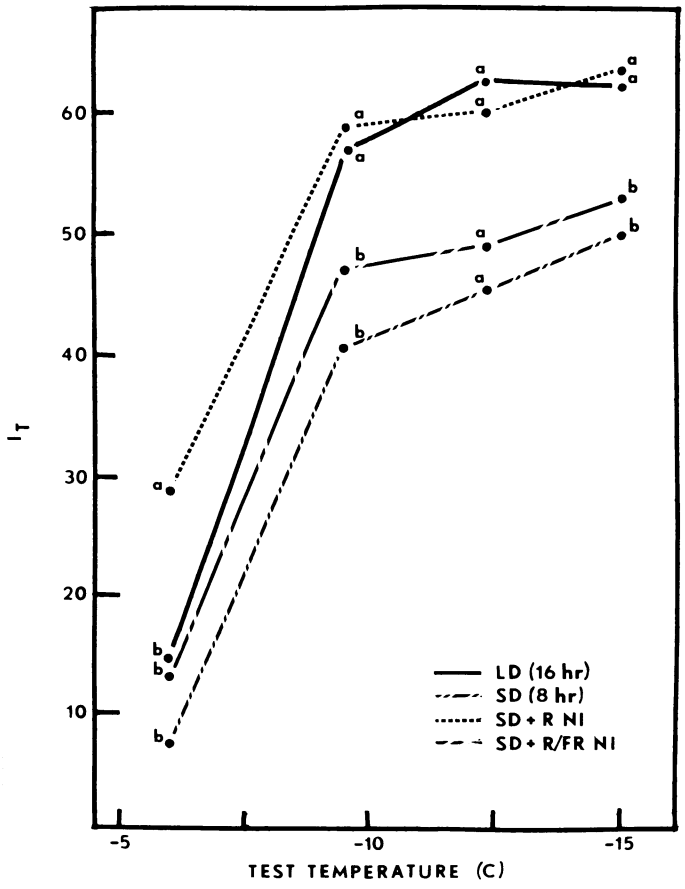

FIG. 3. Indices of injury (I_t) of stem segments of *Weigela florida*, cold acclimated for 5 weeks under four light regimes and frozen to four test temperatures. Each point is the mean of six samples, one each from six plants. Means with the same letter for a test temperature are not significantly different at the 0.01 probability level.

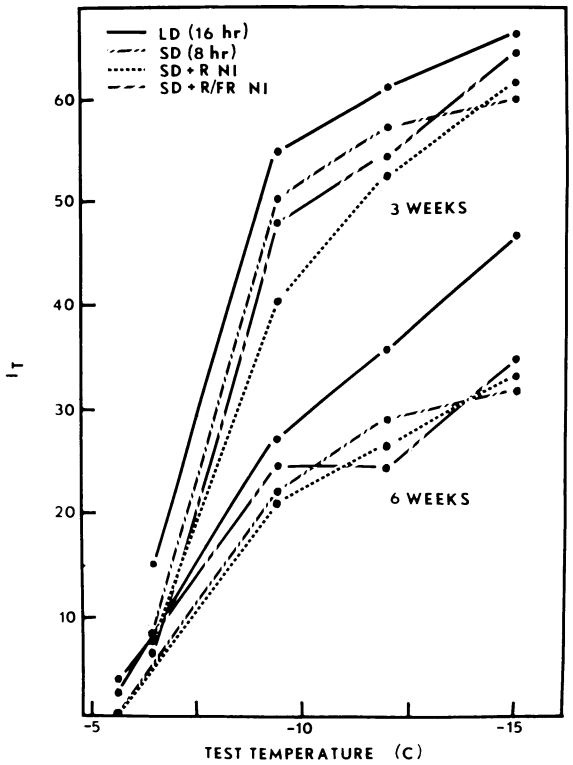

FIG. 4. Indices of injury (I_t) of stem segments of *Pyracantha coccinea* cv. Kasan cold acclimated under four light regimes for 3 and 6 weeks and frozen to four test temperatures. Each point is the mean of four samples, one each from four plants. Means with the same letter for a test temperature are not significantly different at the 0.05 probability level. Means without letters were not significantly different at any given temperature at the 0.05 probability level.

RESULTS AND DISCUSSION

Cornus acclimated more under short day (SD) than under long day (LD) conditions; mean I_t values for SD plants were significantly lower than those for LD plants at all test temperatures (Fig. 2). The SD response was significantly repressed by the red radiation supplied as a 15-min night break. The repressive effects of the red light night break were relieved by subsequently presented far red radiation. I_t values for plants under SD conditions without night break irradiation were not significantly different from those of plants that had received the red-far red sequence. *Weigela* responded to red (R) and to red-far red (R/FR) treatments in the same manner as did *Cornus* (Fig. 3). I_t values for *Weigela* were significantly greater than those for equivalent *Cornus* tissues, suggesting that *Weigela* may be slower in cold acclimation, less dependent on photoperiodic induction, or genetically or physiologically incapable of reaching the level of acclimation achieved by *Cornus*. Van Huystee *et al.* (20) noted that the capacity for acclimation may be a function of vegetative vigor of a plant. It is possible that the lower I_t values obtained with *Cornus*, as compared with those of *Weigela*, reflect differences in the level of vegetative growth activity of these two plants at the time of the study.

Pyracantha, in contrast, was much less responsive to photo-

Table I. *Influence of 5 Weeks of Light Treatment on Dormancy of Axillary Buds of Cornus stolonifera*

Data represent means of six plants except where noted. Plants were treated for 3 weeks at 20 C, followed by 1 week each at 15 C and 10 C.

Light Regime	Percentage of Buds			Mean Dry Wt of Axillary Buds and Shoots
	Dormant	Aborted	Growing	
				g
Short day (8:16)	69.2 ± 11.4	30.8 ± 11.4	0	0.0689 ± 0.099
Long day (16:8)	33.9 ± 5.6	61.5 ± 3.1	4.6 ± 4.5	0.5001 ± 0.030[1]
Short day + red NI[3]	47.7 ± 19.5	52.3 ± 19.5	0	0.2126 ± 0.053[2]
Short day + red/far red NI	79.2 ± 9.6	20.7 ± 9.7	0	0.0407 ± 0.026[1]

[1] Mean of five plants.
[2] Mean of four plants.
[3] NI: night interruption.

period; quantitative differences among treatments were not significant at the 0.05 probability level (Fig. 4). *Pyracantha*, day-neutral for vegetative growth (15), appears to be day-neutral for initiation of cold acclimation.

Cornus plants under LD and SD + red light treatment had a high percentage of buds that developed before growth had ceased (Table I). This probably occurred during the first 3 weeks of treatment when the temperature was 20 C. Short day and SD + R/FR treatments appeared to repress both bud break and subsequent growth of axillary buds, with the mean dry weight of axillary buds and shoots of both LD and SD + R plants being significantly higher than that of either SD or SD + R/FR plants. Stem coloration, characteristic of winter twigs of *Cornus stolonifera*, was pronounced in SD and in SD + R/FR; leaf senescence and abscission were more advanced under these treatments. Observations on vegetative characteristics are consistent with the concept of a phytochrome-mediated short day response.

Weigela plants under LD and SD + R treatment schedules were still producing terminal shoot growth and had developing leaves after 5 weeks of light treatment. Plants under SD and SD + R/FR schedules ceased vegetative growth; the youngest pair of partly expanded leaves was terminated by a small pair of abnormal leaves. Although not as convincing as the observations on *Cornus*, the pattern of response was similar.

Weiser (21) postulated that short days induce only partial hardiness and serve as the first stage of cold acclimation. He suggested that the short day signal leads to cessation of growth and to the activation of metabolic changes that facilitate the alterations induced by freezing temperatures that result in full cold hardiness. The reports (13, 14) that growth retardants paralleled increase in frost resistance, as well as the data given here, tend to support these contentions.

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