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Short Communication

A Translocatable Cold Hardiness Promoter[1]

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Hardy woody species which cold acclimate late or slowly are often injured by early autumn frosts. Adapted hardy species characteristically stop growing and acclimate to some extent prior to autumn frosts. In nature this first stage of acclimation appears to be induced by shortening photoperiods in a number of woody species (4, 23, 24).

Because growth cessation is associated with cold acclimation in woody plants, a considerable amount of work has been done to determine whether growth retarding chemicals can induce cold acclimation in the absence of inductive photoperiods. The growth retardants tested have been found to have only slight and often inconsistent effects on hardiness (7, 8, 13, 15).

Such findings have stimulated interest in elucidating the effective endogenous system(s) which induce acclimation in hardy species (7, 8). Several researchers have recently reported that short-day-induced leaves are the source of translocatable hardiness promoting factor(s) (4, 5, 10). The existence of such a factor(s) is of practical interest because it (they) may prove to be a relatively simple compound(s) which could be applied exogenously to induce acclimation in the absence of inductive environmental stimuli. There is, as yet, no evidence to indicate whether or not the promoting factor(s) is genotype specific.

A considerable amount of research has been done on the environmental control of cold acclimation (1, 4, 10, 24), the natural patterns of acclimation (4, 22), and the metabolic changes associated with acclimation (14) in red-osier dogwood (*Cornus stolonifera* Michx.). In nature, acclimation usually proceeds in two distinct stages which appear to be induced by short days and low temperatures (4, 21, 22), respectively. Climatic races collected from various sites in North America were found to differ greatly in the timing and rate of cold acclimation when they were grown in a uniform environment field plot in Minnesota (18). In the field at St. Paul, Minnesota (45° North latitude), a clone from Dickinson, North Dakota (47° North latitude), was found to begin cold acclimating as much as 8 weeks earlier in the autumn than a clone from Seattle, Washington (47° North latitude) (20). Both races eventually acclimate to below −196 C, but the Seattle race is often injured severely by the first frost in the autumn at St. Paul.

The influence of gibberellic acid and abscisic acid on cold acclimation has been studied in the hardy tree, *Acer negundo* (8). Based on these results, Irving has suggested that abscisic acid may be the hardiness promoting factor (6).

This study was undertaken to answer four questions: Is a hardiness promoting factor(s) produced in the leaves of red-osier dogwood exposed to inductive short days? Is the hardiness factor(s) translocated from the leaves to overwintering living tissues? Can abscisic acid, gibberellic acid, or combinations of these compounds elicit an acclimating response similar to the endogenous promoter(s)? Is the hardiness factor(s) genotype specific or can the promoting factor(s) from one genotype induce acclimation in another?

METHODS AND RESULTS

Plants of two divergent climatic races of red-osier dogwood (*Cornus stolonifera* Michx.) native to Dickinson, North Dakota, and Seattle, Washington (18), were grown for 8 to 10 weeks under long days (15-hr photoperiods) and good cultural conditions in a warm (25 C day/18 C night) greenhouse and were moved either to the field or to controlled environment chambers for study. In grafting studies, rooted cuttings of the two races were side-grafted when they were about 8 inches in height. The grafted plants were pruned to one leader of each climatic race and grown for 4 to 6 weeks under the conditions previously described before they were moved to the field or greenhouse for study.

Cold resistance was measured periodically by freezing stem sections under controlled conditions using a technique similar to that previously described (3, 23). Uniform internode sections were slowly rewarmed to room temperature after controlled freezing and incubated for 7 days in a humid chamber at room temperature. Samples were then visually rated for injury. Samples with firm green bark tissues (red epidermis) and no internal discoloration were considered to be uninjured. Such samples produced callus from the cut surfaces after 20 to 30 days of incubation. Freezing tests were run on triplicate samples of each treatment at each test temperature. In almost all cases the three samples at each temperature were either all dead or alive. Hardiness is expressed as the lowest survival temperature.

Four experiments were conducted. Experiment 1 was a preliminary study designed to determine whether 4 weeks of pretreatment with ABA or GA_3 separately or in combination could enhance the subsequent cold acclimation of red-osier dogwood. The treatments, experimental design, and methods of treatment were similar to those used by Irving in studies on *Acer negundo* (6, 8).

Height measurements were made at weekly intervals from the beginning of the month-long foliar feeding period until growth stopped. Hardiness was evaluated after plants had been exposed for 4 weeks to a 12-hr photoperiod and a day/night temperature regime of 20 C/15 C plus an additional 8 weeks at 15 C/5 C. In this and the subsequent growth chamber experiments, light was supplied by a mixture of cool-white fluorescent and in-

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candescent bulbs. The intensity provided by the light source at a distance of 76 cm (pot height) was 8.2×10^4 ergs/cm^2 sec at 15 C as measured by a YSI Kettering Model 65 radiometer.

In contrast to previous studies on *Acer negundo* (6, 8), ABA appeared to have little if any influence on the first stage of cold acclimation in red-osier dogwood. Control plants were hardy to −14 C and those treated with 10^{-4} M ABA were hardy to −16 C. GA reduced hardiness (−8 C). ABA and GA treatments both promoted growth (0.05 level of significance, Duncan's multiple range test) (19). The apparent ABA enhancement of growth suggests that little intact ABA actually got into the plants under the conditions of this trial. Whereas the results are far from conclusive, they show that ABA, which enhanced acclimation in *Acer* (6), did not do so in *Cornus* under similar experimental conditions.

Experiment 2 was designed to determine whether the two clones differed appreciably in their ability to acclimate under controlled conditions and whether the scion of one climatic race influenced the hardiness of the other in graft combination. The cold resistance of branches of the Dickinson and Seattle clones was evaluated at weekly intervals on grafted and ungrafted plants subjected to short photoperiods and low temperatures in a controlled environment chamber. Plants were subjected to a preconditioning treatment consisting of a 12-hr photoperiod and a day/night temperature regime of 20 C/15 C for a period of 4 weeks. A 12-hr photoperiod was chosen because, based on previous field studies (18), it was estimated to be inductive for cold acclimation in the Dickinson clone but not for the one from Seattle. Following this preconditioning treatment, plants were exposed to day/night temperatures of 15 C and 5 C, respectively, and cold hardiness was determined at weekly intervals on individual branches of the two clones from grafted and ungrafted plants.

In this grafting experiment where no branches were defoliated, the hardiness of branches of the Seattle clone increased in hardiness from −8 C to about −14 C while the Dickinson clone increased from −8 C to about −36 C. This was true whether plants were grafted or not and there seemed to be no graft interactions.

Experiment 3 was similar to experiment 2 except that plants were permitted to acclimate naturally in the field. There were relatively minor differences in hardiness between the two clones. The maximal difference was observed on October 24, 1969, when Seattle plants were 8 C less hardy than those from Dickinson. The small differences in hardiness precluded meaningful evaluation of scion-scion interactions.

Experiment 4 was conducted to determine whether leaves influenced the effect of one scion clone on the acclimation of the other. Ungrafted and grafted plants of the Dickinson and Seattle clones, with or without the foliage removed at the beginning of the experiment, were acclimated under the same environmental regimes used in experiment 2. There were 12 plants per treatment.

After 9 weeks of acclimation in the growth chamber, hardiness was tested on 14 kinds of plants or parts of plants as shown in Figure 1.

Defoliation altered the cold resistance of grafted and ungrafted plants and branches (Fig. 1). As in previous experiments, the Dickinson clone became hardier (about 18 C) than the Seattle clone. Leaves were necessary for acclimation. All plants which were completely defoliated failed to develop hardiness (see treatments 3, 6, 10, and 14 in Fig. 1). They survived only to −4 C and were beginning to die-back from the tips at the time the experiment was terminated.

When one of the two branches of an ungrafted plant was defoliated, both the foliated and defoliated branches acclimated to the same extent (see treatments 2 and 5). This indicates that the hardiness promoting factor(s) produced in the leaves are translocated from a foliated to a defoliated branch. This is similar to the observation of translocatable hardiness factors in split plant studies when one branch of a plant is exposed to inductive photoperiods while another branch is not (3, 4, 10). The partially defoliated ungrafted plants acclimated as effectively (compare treatments 4 and 5) or almost as effectively (compare treatments 1 and 2) as the foliated ungrafted plants.

The defoliated Dickinson or Seattle branches acclimated somewhat more effectively when they were grown on their own roots. In several other treatments (compare 7 and 11, 8 and 12) the genotype of the root-stock appeared to influence the acclimation of branches.

When the Dickinson branches of grafted plants were defoliated they did not acclimate as effectively as they did if Dickinson leaves were present (see treatments 9 and 13). This indicates that Seattle leaves were not too effective in inducing hardiness.

When the Seattle branches of grafted plants were defoliated, they acclimated more effectively than they did in any other treatment (see treatments 8 and 12). This shows that the leaves of one genotype can induce and actually enhance the acclimation of branches of another genotype (compare treatments 8 and 12 with treatment 5). Such interactions were apparent only when one of the scion clones was defoliated (compare treatments 8, 9, 12, and 13 with treatments 7 and 11).

DISCUSSION

While the answers to the four questions this study was designed to examine are not wholly conclusive they are informative: (*a*) A hardiness promoting factor is apparently produced in the leaves of red-osier dogwood exposed to short days as has been found in other species (4, 10). (*b*) The hardiness promoter is translocated from the leaves to the bark of defoliated branches on the same plant. (*c*) ABA and GA_3 separately and in combination did not induce or enhance acclimation under the conditions of this experiment. (*d*) The translocatable hardiness promoter is not genotype specific at least between diverse climatic races of red-osier dogwood, *e.g.*, a foliated branch of the Dickinson clone enhanced the acclimation of a defoliated branch of the Seattle clone.

The implication of the results of the grafting and defoliation study (experiment 4) is that the hardiness promoting factor(s) is not genotypically specific. It was interesting that branches of grafted plants acclimated independently and in a manner characteristic of the genotype when they were foliated. It was not until the leaves were removed from branches of one or the other genotype that the influence of the foliated branch was expressed. Researchers who are attempting to induce acclimation with growth retardant sprays may find it useful to defoliate treated plants shortly after treatment.

The grafting experiments in this study offer a basis for evaluating the concept of hardiness promoters and inhibitors since the 12-hr photoperiod was essentially a long day to the Seattle clone and a short day to the Dickinson clone in terms of acclimation induction. Previous research on other woody plants has indicated that the long day leaf is a source of translocated hardiness inhibitors just as the short day leaf is a source of hardiness promoters (4, 10). The results with partially defoliated grafted plants in this study were consistent with the inhibitor promoter concept (4), in that Seattle leaves inhibited the hardiness of Dickinson branches and Dickinson leaves promoted the acclimation of Seattle branches.

There appear to be several inter-related endogenous ingredients necessary for efficient acclimation in hardy woody species. In simplest terms they can be described as: (*a*) growth cessation (22), (*b*) substrate for synthetic processes (3, 5 and Fig. 1), (*c*) a high proportion of hardiness promoter to hardiness inhibitor,

FIG. 1. The frost resistance of grafted and ungrafted Seattle, Washington, and Dickinson, North Dakota, clones of *Cornus stolonifera* after 8 weeks of acclimation at a 12-hr photoperiod and a 15 C/5 C day/night temperature regime. Plants were defoliated as indicated at the beginning of a 4 week preconditioning period (12-hr photoperiod, 20 C/15 C day/night temperature regime) which preceded the acclimation cycle. Sketches identify the genotype and the parts of the plant which were defoliated.

and (*d*) an efficient metabolic machinery for translating the three preceding ingredients into the biophysical or physiological changes responsible for resistance.

If a plant is not short day induced (low hardiness promoter-inhibitor ratio) it acclimates less effectively (23). Removing leaves from plants exposed to short days inhibits acclimation while removing leaves from plants exposed to long days enhances acclimation (4). A genotype with an efficient acclimating machinery (Dickinson clone), or perhaps a lower promoter-inhibitor threshold, becomes hardier in a given period of time than a less efficient genotype (Seattle clone) when the common source of promoters and inhibitors is the foliage of the latter (treatments 9 and 13, Fig. 1). The converse is also true (treatments 8 and 12).

While the data seem to fit this interpretation, there are still a number of questions. Are growth cessation and the hardiness promoter and inhibitor ingredients really separate phenomena or does the hardiness promoter enhance acclimation by merely stopping growth? Is it possible that the hardiness promoter is a growth inhibitor and that the hardiness inhibitor is a growth promoter? This possibility is supported by the observation that low temperatures, which stop growth, can induce maximal hardiness eventually under noninductive photoperiods (9). Although ABA did not enhance acclimation in this study, it also did not inhibit growth, and it is doubtful that very much intact ABA was taken up by the plants.

It has been suggested that ABA and GA_3 may be the endogenous hardiness promoter and inhibitor, respectively (8). If this were true, one would expect ABA treatments to elicit a more marked response under long day conditions than under short days where growth cessation would be photoperiodically induced in both the control and treated plants. In *Acer*, ABA had similar effects under long days and short days (6). Another bothersome piece of information is the observation that low temperatures inhibit the first stage of acclimation in dogwood (3). This and the numerous metabolic changes associated with acclimation (2, 11, 12, 14, 16) tempt one to conclude that the hardiness promoter(s) plays a more active role in the process than just stopping growth.

Another question is whether sugars function merely to provide an energy substrate for active acclimation processes or whether they are directly and causally involved in resistance to freezing as several workers suggest (17, 20, 21). In red-osier dogwood (3) and other hardy woody plants it has not been possible to demonstrate that exogenously supplied sugars have any direct effect on cold resistance.

Based on our present state of knowledge on the control of cold acclimation in woody plants, it would seem worthwhile to

continue attempts to elucidate the nature of the endogenous promoting factor(s).

LITERATURE CITED

1. CHANDLER, W. H. 1954. Cold resistance in horticultural plants: A review. Proc. Amer. Soc. Hort. Sci. 64: 552–572.
2. CRAKER, L. E., L. V. GUSTA, AND C. J. WEISER. 1969. Soluble proteins and cold hardiness of two woody species. Can. J. Plant Sci. 49: 279–286.
3. FUCHIGAMI, L. H. 1970. The environmental, physiological and chemical control of cold acclimation in *Cornus stolonifera* Michx. Ph.D. thesis. University of Minnesota, St. Paul.
4. HOWELL, G. S. AND C. J. WEISER. 1970. The environmental control of cold acclimation in apple. Plant Physiol. 45: 390–394.
5. HURST, C., T. C. HALL, AND C. J. WEISER. 1967. Reception of the light stimulus for cold acclimation in *Cornus stolonifera* Michx. Hort. Sci. 2: 164–166.
6. IRVING, R. M. 1969. Characterization and role of an endogenous inhibitor in the induction of cold hardiness in *Acer negundo*. Plant Physiol. 44: 801–805.
7. IRVING, R. M. 1969. Influence of growth retardants on development and loss of hardiness in *Acer negundo*. Proc. Amer. Soc. Hort. Sci. 94: 419–421.
8. IRVING, R. M. AND F. O. LANPHEAR. 1968. Regulation of cold hardiness in *Acer negundo*. Plant Physiol. 43: 9–13.
9. IRVING, R. M. AND F. O. LANPHEAR. 1967. Environmental control of cold hardiness in woody plants. Plant Physiol. 42: 1191–1196.
10. IRVING, R. M. AND F. O. LANPHEAR. 1967. The long day leaf as a source of cold hardiness inhibitors. Plant Physiol. 42: 1384–1388.
11. JUNG, G. A., S. C. SHINK, AND D. C. SHELTON. 1967. Influence of purines and pyrimidines on cold hardiness in plants. III. Associated changes in soluble proteins and nucleic acid content and tissue pH. Plant Physiol. 42: 1653–1657.
12. LI, P. H., C. J. WEISER, AND R. VAN HUYSTEE. 1966. The relation of cold resistance to the status of phosphorus and certain metabolites in red-osier dogwood (*Cornus stolonifera*, Michx.). Plant Cell Physiol. 7: 475–484.
13. MARTH, P. C. 1965. Increased frost resistance by application of plant growth chemicals. J. Agr. Food Chem. 13: 331–333.
14. MAZUR, P. 1969. Freezing injury in plants. Annu. Rev. Plant Physiol. 20: 419–448.
15. MODLIBOWSKA, I. 1965. Effects of (2-chloroethyl) trimethylammonium chloride and gibberellic acid on growth, fruit bud formation and frost resistance in one-year old pear trees. Nature 208: 503–504.
16. PARKER, J. 1963. Cold resistance in woody plants. Bot. Rev. 29: 124–201.
17. SAKAI, A. 1962. Studies on the frost hardiness of woody plants. I. The causal relation between sugar content and frost hardiness. Low Temp. Sci. 11: 1–40.
18. SMITHBERG, M. H. 1964. Patterns of variation among some climatic races of red-osier dogwood. Master's thesis. University of Minnesota, St. Paul.
19. STEEL, R. G. D. AND J. H. TORRIE. 1960. Principles and Procedures of Statistics. McGraw Hill, New York.
20. STEPONKUS, P. L. 1969. Protein-sugar interactions during cold acclimation. Abstract C-60. 6th Annual Meeting of the Society for Cryobiology.
21. TUMANOV, I. I. 1967. The frost-hardening process of plants. *In*: A. S. Troshin, ed., The Cell and Environmental Temperature. Pergamon Press, New York. pp. 6–14.
22. TUMANOV, I. I. AND T. I. TRUNOVA. 1963. The first phase of frost-hardening in the dark of winter plants on sugar solutions. Plant Physiol. U.S.S.R. 10: 176–188.
23. VAN HUYSTEE, R. B., C. J. WEISER, AND P. H. LI. 1967. Cold acclimation in *Cornus stolonifera* under natural and controlled photoperiod and temperature. Bot. Gaz. 128: 200–205.
24. WEISER, C. J. 1966. Postulated relationships between rest period, dormancy, cold acclimation and survival potentials in woody plants. Proc. XVII Inter. Hort. Congr. 1–99.