Similarities Between the Control of Flower Initiation and Cold Acclimation in Plants[1]

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It has been necessary for perennial plants to develop systems for sensing and reacting to environmental parameters. Their survival and perpetuation is determined, in large measure, by the timing of vital processes in relation to favorable and unfavorable cycles of environment. While the regulation of flowering by environmental stimuli has been extensively researched little is known about the control of cold acclimation. Recent studies show striking similarities between the control mechanisms for flowering and cold acclimation in temperate zone plants.

Growth: Vegetative growth is inversely related to both flowering and cold acclimation. It is axiomatic that a meristem cannot be both reproductive and vegetative at the same time. Beyond this, vegetative growth is commonly retarded at the time of flowering, and determinate plants flower only when vegetative meristems have ceased to grow. Though cold acclimation of tissues is a less localized response, actively growing woody plants invariably do not acclimate.

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When climatic races of red-osier dogwood were collected throughout North America and grown at St. Paul, Minnesota, northern and high mountain races stopped growing and became frost resistant in late summer (8). Southern and coastal races continued to grow and failed to cold acclimate before sustaining damage from early autumn frosts. However, all races were capable of surviving -196°C by mid-winter.

Environmental stimuli: Of the various factors which regulate plant responses, day-length is the most predictable and its measurement via the phytochrome system provides plants with a rather absolute annual calendar. In regions where the environment follows a consistant annual pattern, photoperiod can effectively regulate the timing of vital processes. Where the environment is less predictable, adapted plants must respond to additional stimuli which can override or modify photoperiodic control.

Cyclic seasonal temperature changes are reasonably consistent and temperature regulation of flowering (vernalization and other processes such as the breaking of physiological dormancy) are well known.

Photoperiod and temperature also appear to be the primary factors regulating cold acclimation in woody perennial plants. Van Huystee et al. (10) and Irving and Lanphear (4,5,6) have shown that a combination of long nights and low temperatures induces maximum cold hardiness in the living bark of *Cornus stolonifera, Acer negundo, Viburnum plicatum tomentosum,* and *Weigela florida.* In *Cornus* (10) cold hardening proceeds in two rather distinct steps; the first induced by short days, and the second by freezing temperatures. The checks and balances inherent in multiple factor regulation has distinct advantages for regulating cold acclimation where a few days can mean the difference between life and death.

Inhibitors. The absence of flowering at non-inductive photoperiods is caused not only by a lack of flowering promoter, but as Wellensick (11) and Thompson and Guttridge (9) have shown, by the presence of a flowering inhibitor.

Hardiness inhibitors are also induced by photoperiod. Irving and Lanphear (5) have shown that the leaves of plants grown on short nights inhibit cold acclimation. Removal of leaves promoted cold acclimation. They concluded that leaves inhibit cold acclimation, and that frost, by killing leaves, removed the inhibition and permitted acclimation of the overwintering tissues.

Promoters. Current research on

Table 1. The effect of leaf removal and photoperiod on the cold acclimation of Haralson apple bark. Hardiness expressed as the highest test temperature (5° intervals) at which bark was killed.

Sampling Date (1968)	Bark killing temperature °C *			
	Long night regime		Short night regime	
	Leaves present	Manually defoliated (9/2/68)	Leaves present	Manually defoliated (9/2/68)
9/13	-10	-10	-10	-10
9/20	-15	-10	-10	-10
9/27	-25	-10	-15	-20
10/4	-30	-15	-15	-25
10/11	-30	-20	-20	-30

*The killing temperatures were the same for all three replicates of each treatment at each date.

Table 2. The effect of photoperiod on the cold acclimation of Haralson apple bark. Plants with dichotomous branching were exposed to either long nights, short nights, or split with an opaque light barrier so that one branch was exposed to long nights (branch a) and the other to short nights (branch b). (Hardiness expressed as in Table 1.)

Sampling date (1968)	Bark killing temperature °C *			
	Whole plant	Split plants (branch a)	Split plants (branch b)	Whole plant
	Long night regime		Short night regime	
9/13	-10	-10	-10	-10
9/20	-15	-10	-10	-10
9/27	-25	-20	-20	-15
10/4	-30	-25	-25	-15
10/11	-30	-30	-30	-20

*The killing temperatures were the same for all three replicates of each treatment at each date.

three-year old 'Haralson' apple trees shows that leaves also promote cold acclimation at short photoperiods. Trees were grown on natural short days (long nights) in the autumn or on short nights produced by interrupting the middle of the dark period with three hours of 150-foot candle incandescent light. Half of the plants at each photoperiod were hand defoliated on September 2, 1968. At four weekly intervals during the autumn the killing temperature of the living bark was determined by exposing excised twigs to a series of successively lower temperatures at 5° intervals (10).

The data in Table 1 shows that leaves promoted the cold acclimation of plants grown on long nights and inhibited cold acclimation of those grown on short nights. On October 4th the bark of foliated plants on long nights was killed at -30°, while the bark of defoliated long-night plants was killed at -15°. Killing temperatures in the short night treatments were -15° and -25° respectively. The promotion of hardiness by leaves under the short-day conditions has also been reported for dogwood where leaf removal or complete shading of leaves inhibited cold acclimation (3).

Translocated responses. The photoperiodic flowering-stimulus can be translocated across a graft union from an induced to a non-induced branch (1). In apple trees the stimulus to cold acclimation induced by photoperiod is also translocated. Three lots of apple trees were trained to double leaders and grown 1) entirely under long nights, or 2) short nights (as previously described), or 3) split with a light barrier so that one branch was under each of the two photoperiod regimes.

The data in Table 2 shows that the hardiness was the same for both branches of split-plants at each sampling date. The hardiness of split plants was generally intermediate between plants from the short night or long night treatments. For example, on 9/17/68, the bark of short-night plants was killed at -25°, long night plants at -15°, and both branches of the split-plants at -20°. These data suggest that there is translocation of both the hardiness

Table 3. The effect of temperature below 4.5°C on the cold acclimation of Haralson apple bark. Plants with dichotomous branching habits were exposed to either natural field temperatures, or natural temperatures which were never allowed to go below 4.5°, or split with a temperature barrier so one branch received each of the preceding treatments. (Hardiness expressed as in Table 1.) (The first frost occurred on 10/14/67 and field temperatures dropped below 4.5° on 34 days or nights between 9/20/67 and 12/2/67.)

Sampling date (1967)	Bark killing temperature °C *			
	Whole plant	Split plants (branch a)	Split plants (branch b)	Whole plant
	Natural field temperatures		Natural field temperatures but never below 4.5°.	
9/20	-20	-20	-20	-20
9/29	-30	-30	-25	-25
10/6	-30	-30	-25	-25
10/13	-30	-30	-30	-30
10/20	-35	-35	-30	-30
10/27	-40	-40	-30	-30
11/3	-45	-45	-30	-30
11/11	-50	-50	-30	-30
11/18	-55	-55	-30	-30
12/2	-55	-55	-30	-30

*The killing temperatures were the same for all three replicates of each treatment at each date.

promoter (from long night foliage) and the hardiness inhibitor (from short night foliage) to the overwintering bark. Plants grown on short nights did cold acclimate slowly in spite of exposure to non-inductive photoperiods.

Low temperature induction. The promotion of flowering by low temperature (vernalization) is a non-translocated response. The one possible exception to this (Hyocyamus (7)) has been questioned (2). Frost is necessary for maximun cold hardening of *Cornus stolonifera* bark (3). Table 3 presents the results of a split-plant field study on young apple trees where temperature was the only variable. Ten plants were exposed to natural outdoor temperatures in the autumn of 1967, ten others to the same conditions except that the minimum temperature was never allowed to drop below 4.5°, and ten plants were divided with a double walled layer of clear plastic so that one branch was exposed to each of the preceding temperature regimes. The temperature was held above 4.5° by the thermostatically regulated circulation of warm air through the enclosed polyethylene structure when the outdoor temperatures reached 5°. Air temperature was recorded continually, and the structure was open except when marginal temperatures were anticipated.

The temperature that a plant or plant part was exposed to determined its hardiness. On 11/18/67, for example, the killing temperature of bark was -55° for whole plants and branches of split plants which were subjected to field temperatures. Whole plants and branches of split plants not cooled below 4.5° were killed at -30°. These results show that temperatures below 4.5° promoted cold acclimation and that the low temperature-promotion of cold acclimation is a non-translocatable response.

For many plants flowering and cold acclimation are a) dependent on growth cessation; b) controlled by photoperiod and/or temperature; c) inhibited by factors produced in foliage at non-inductive photoperiods; and d) promoted by factors produced in the foliage at inductive photoperiods. e) The promotion of both processes by photoperiod is translocatable, f) while the low temperature response is localized in the tissues exposed to the stimulus.

The high degree of similarity between the control of flowering and cold acclimation suggests that traditional techniques for studying flowering could be used advantageously to elucidate the control of other vital processes such as cold acclimation in temperate zone species.

The timing of growth cessation and cold acclimation is particularly critical for plants growing in areas with short growing seasons and subject to sudden severe frosts. For plants in such regions, a multifactor environmental control system would appear to provide the high degree if physiological flexibility required to permit maximum growth while insuring survival.

Literature Cited

1. Vajlachjan, M. Ch. 1937. Concerning the hormonal nature of plant development processes. *Acad. of Sci. U.S.S.R.* 16:227-230.
2. Hillman, W. S. 1962. *The Physiology of Flowering.* Holt, Rinehart, and Winston. New York, N. Y. 164p.
3. Hurst, C., T. C. Hall and C. J. Weiser. 1967. Reception of light stimulus for cold acclimation in *Cornus stolonifera* Michx. *Hort. Science* 2:164-166.
4. Irving, R. M. and R. O. Lanphear. 1967. Environmental control of cold hardiness in woody plants. *Plant Physiol.* 42:1191-1196.
5. Irving, R. M. and R. O. Lanphear. 1957. The long day leaf as a source of cold hardiness inhibitors. *Plant Physiol.* 42:1384-1388
6. Irving, R. M. and R. O. Lanphear. 1968. Regulation of cold hardiness in *Acer negundo. Plant Physiol.* 43:9-13.
7. Lang, A. 1952. Physiology of flowering. *Ann. Rev. Plant Physiol.* 3:265-306.
8. Smithberg, Margaret and C. J. Weiser, 1968. Patterns of variation among climatic races of red-osier dogwood. *Ecology* 49 (3): 495-505.
9. Thompson, P. A. and C. G. Guttridge. 1960. The role of leaves as inhibitors of flower production in strawberry. *Ann. Bot.* 24:482-490.
10. 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.
11. Wellensick, S. J. 1962. The control of flowering. *Nethlands J. Agri. Sci.* L): 390-398.

A Rapid Method for Obtaining Leaf Samples for Electron Microscopy

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This report, describes a rapid and convenient method to obtain leaf disc samples for electron microscopy. It was developed in connection with bio-chemical-ultrastructural studies of herbicidal effects on protein synthesis. It was important to speed up the sampling time and to obtain uniformly sized samples.

The method requires inexpensive tools, namely a Luer-lok multifit interchangeable syringe, a 16 gauge needle and a petri dish (Fig. 1). The needle is approximately 1 mm inside diameter. The tip was machined to serve as a punch, much the same as a cork borer. The needle tip can be satisfactorily machined on a fine grain emery wheel. A few drops of cold fixing solution are placed in the petri dish. The leaf material to be sampled is floated on the fixative. For convenience, the needle is removed from the syringe and used to punch out the leaf discs. The leaf discs are retained within the needle, together with traces of the fixative. When a sufficient number of samples has been taken, the needle is reinserted in the cylinder previously filled with the cold fixative. Leaf discs and fixative are then injected into collecting vials.

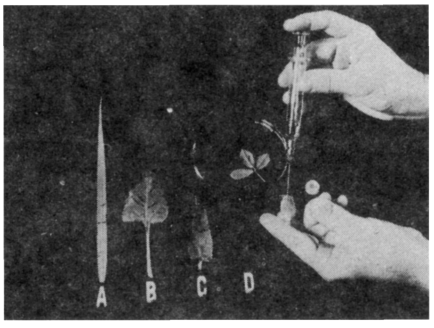

Fig. 1. Leaves of (A) *Bromus inermis,* (B) *Beta vulgaris,* (C) *Kalanchoe blossfeldiana,* and (D) *Medicago sativa.*

This method has been used on both thin. and succulent dicotyledonous leaves and on grass leaves (Fig. 1). It reduced the sampling time about 65% as compared to hand sectioning. The average time required to take 10 leaf discs was 21.9 seconds in our studies. Injury to the tissue was reduced considerably, and all sections were of uniform size.

This method has further application in obtaining uniform leaf discs between veins for respiration and photosynthesis studies involving herbicides.