

The Environmental Control of Cold Acclimation in Apple¹

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ABSTRACT

The role of photoperiod and temperature in the cold acclimation of living Haralson apple (*Pyrus malus* L.) bark was studied in the autumn under field conditions in Minnesota. Whole trees, or different parts of the same tree, were exposed to either natural conditions, artificially lengthened days, or artificially warmed nights, or they were subjected to manual leaf removal. The results indicate that acclimation occurs in two stages which are induced by short days and frost (or low temperature), respectively. Leaves were stimulated by short days to produce translocatable substance(s) which promoted cold acclimation of the living bark. Leaves of plants grown under long days were the source of a translocatable substance(s) which inhibited acclimation. The second stage of hardiness, induced by frost (or low temperature), did not involve translocatable factors.

Inductive short days could overcome the effect of high temperatures, and low temperatures could overcome the effect of noninductive long days in promoting the first stage of acclimation to -30°C . Frost was necessary for maximum hardiness to -55°C . Plants grown in a greenhouse, in the autumn, under long days and high temperatures acclimated slightly in spite of the noninductive conditions. Short days and frost (or low temperatures) appeared to regulate different and independent endogenous acclimation processes.

Cold acclimation is necessary for the survival of perennial plants over large areas of the earth's surface. In these studies the roles of photoperiod and temperature in controlling the cold acclimation of the living bark of apple trees were examined under field conditions in Minnesota.

Low temperatures (1, 2, 5, 7, 9, 10, 16–18) and, in fewer instances, short days (2, 3, 5, 7, 8, 17) have been reported to promote the cold acclimation of temperate zone woody species. However, relatively little is known about the sites of perception, the interactions, or the nature of the response that each elicits. Low temperature alone induced hardiness in *Acer negundo*, *Weigela florida*, and *Viburnum plicatum tomentosum* (5, 6). With one exception, *Viburnum* plants grown under short days became harder than those grown under long days; manual or frost defoliation of plants grown under long days promoted hardiness; and, the hardiness inhibitor (hormone?) induced by long days was translocatable (6).

Van Huystee *et al.* (18) proposed a two-step acclimation process for *Cornus stolonifera*. Initiation of the first stage of hardiness

(to -18°C) was ascribed to short days, and the second stage (to below -196°C) to frost.

Photoperiodic induction was found to be a necessary prerequisite to the frost-induced stage. In studies of the same species, Hurst *et al.* (3) observed that even limited amounts of foliage (one pair of leaves) effectively promoted the cold acclimation of bark. In English ivy vines, Steponkus and Lanphear (14) found that the light-induced phase of acclimation involved translocatable hardiness promoters.

These results imply that substances are produced in the leaves which inhibit (6) or promote the acclimation of overwintering tissues (3), or do both. This study was designed to clarify the relationship of the photoperiod- and temperature-induced hardiness responses in apple, and to determine whether either involves translocatable hardiness-promoting substances.

MATERIALS AND GENERAL METHODS

Nine studies were conducted on Haralson apple trees (*Pyrus malus* L.) over a 2-year period. Two of the studies characterized the natural sequence of cold acclimation in mature orchard trees in the autumn of 1967 and 1968. The other seven studies were conducted on 3-year old trees which were potted in 3-gallon containers in April of 1967 and pruned to two leaders. In the autumn of 1967 and again in 1968, three experiments were conducted on these plants in the field where they were subjected to treatments involving natural or modified photoperiod, natural abscission or artificial leaf removal, and natural or modified temperatures. In 1968 a greenhouse experiment and a growth chamber experiment were conducted on similar plants to supplement the field photoperiod and temperature studies, respectively.

The following features were common to all experiments. Bark hardiness was determined periodically by subjecting excised 1-year-old branches to a controlled freezing stress as previously described (2). At each sampling date branches from three replicates were cooled (10°C/hr) in vacuum flasks in a freezer. The sample flasks were removed at 5°C intervals and slowly warmed. After 5 days of incubation the sample branches were dissected and rated visually for injury. The cambium, phloem, and cortex cells in the living bark become soft, brown, and water-soaked if injury has occurred. Uninjured tissues remain firm and turgid, retain their normal color, and have the ability to generate callus cells from cut surfaces (12). The seasonal pattern of cold acclimation was plotted as killing temperature. This is the highest temperature at which samples were killed at each sampling date.

The experiments were designed and statistically analyzed as follows. (a) Mature tree and controlled environment chamber studies were completely randomized designs with three replicates. (b) Each divided plant study consisted of a series of parallel plots since structural barriers limited randomization. (c) Data were analyzed via analysis of variance, and treatment comparisons were made via Treloar's statistic *K* (15).

METHODS AND RESULTS

Observations on Mature Trees. During the autumn of 1967 and 1968 the bark hardiness of mature trees growing under commer-

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cial orchard conditions was determined at weekly intervals from mid-September to early December. Phenological changes and daily maximum-minimum temperatures were recorded.

Figure 1 shows the seasonal pattern of cold acclimation in mature orchard trees in 1967 and 1968. Acclimation occurred in two stages. The first step, to -25 C , was initiated at the same solar time each year. The second step, to -50 C , was initiated at the time of the first killing frost in both years. The designation killing frost is used in the popular sense. In this, and subsequent figures, it refers to a frost which killed tomato and cucurbit foliage and does not imply that apple leaves were injured.

These results agree with the description of the two-stage acclimation process in *Cornus stolonifera* (18). Although the mature trees bore a light crop in 1967 and a heavy crop in 1968, the acclimation patterns and the ultimate levels of hardiness were very similar.

In the killing temperature curves plotted in Figure 1, and all subsequent figures, there were no differences among replicates. As such, each point on the plot is both the mean and the range of the killing temperature and all differences are statistically significant.

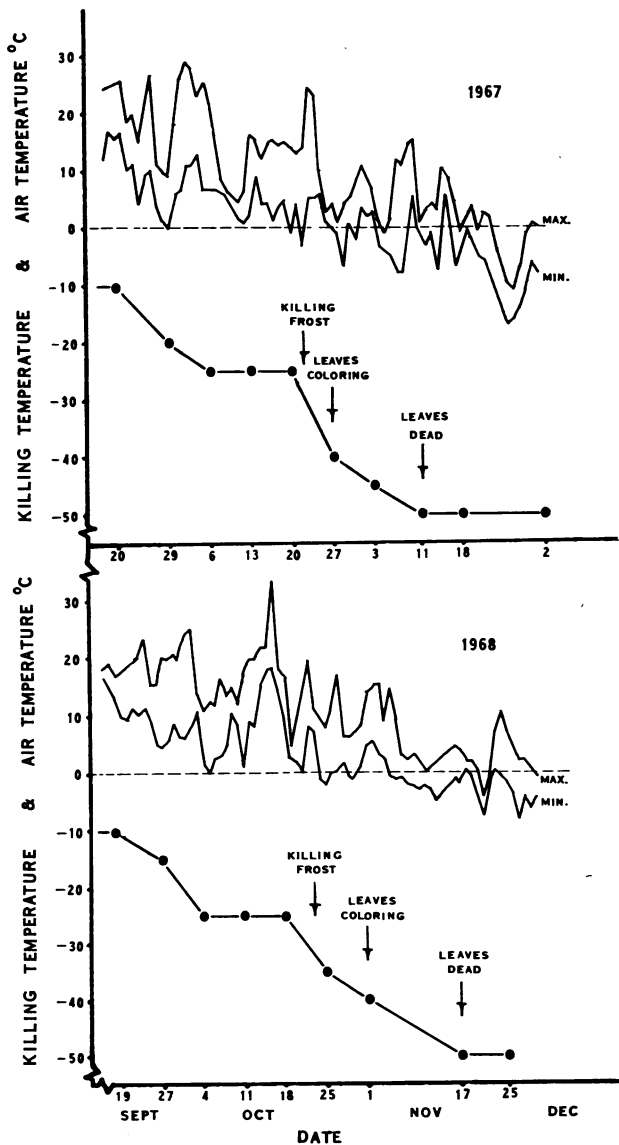


FIG. 1. Air temperature and seasonal hardiness patterns of mature apple trees in the autumn of 1967 and 1968.

Photoperiod Experiments. On August 20, 1967, a divided plant experiment was begun in the field. Trees were exposed to natural outdoor temperatures and either (a) natural short days (SD); (b) long days produced by lengthening the photoperiod to 18 hr with 150 ft-c of incandescent light (LD); or (c) divided with a black polyethylene light barrier so that one leader was under each of the preceding photoperiod regimes (LD-SD). In the LD-SD treatment, pots were placed on either side of the light barrier for comparison of position effects. From mid-September until early December, the hardiness of branches from each of the treatments was evaluated weekly.

In 1968 a similar field experiment was conducted. The photoperiod treatments were begun on July 10, and the LD treatment was produced by a 3-hr night interruption with 150 ft-c incandescent light. Also, a replica of the 1968 field photoperiod study was conducted concurrently in a greenhouse where the minimum temperature was 12.5 C . Hardiness was determined bimonthly.

Figure 2 shows the results of the divided plant photoperiod experiments conducted in 1967 and 1968. The cold acclimation patterns for the 2 years were similar. Whole plants and branches of divided plants all ultimately acclimated to -55 C in both years, and treatments altered the rates at which plants acclimated. In both years plants in the SD treatment acclimated first, followed

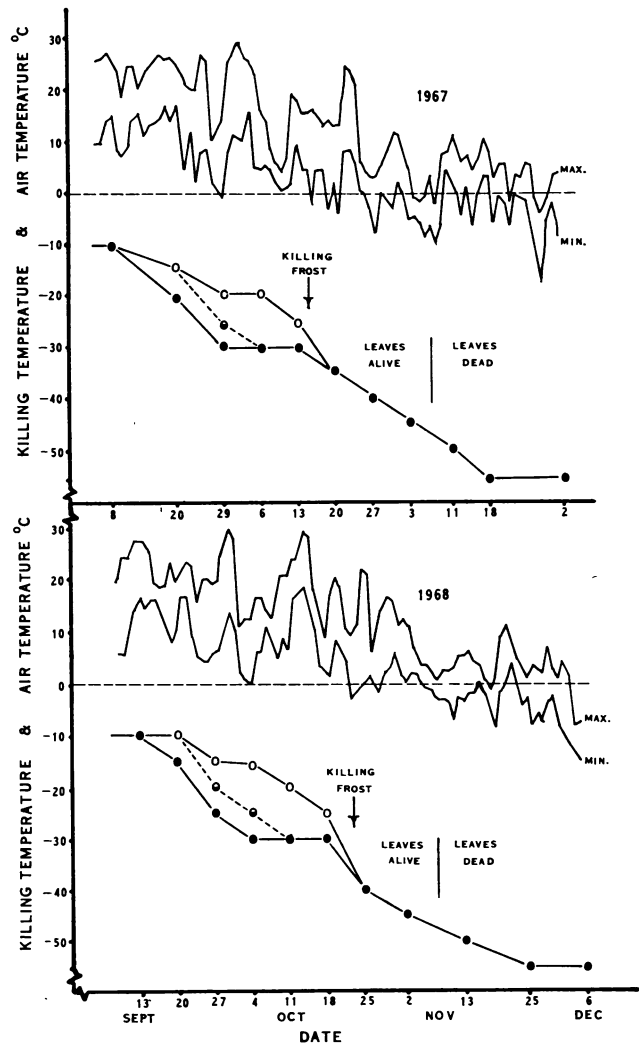


FIG. 2. Air temperatures and the seasonal hardiness patterns of young apple trees grown at different photoperiods in the field during the autumn of 1967 and 1968. ○: Long day treatment (LD); ●: short day treatment (SD); ●: LD-SD divided plant treatment.

by both the SD and LD branches of divided plants, and then plants in the LD treatment. Divided plants with the pots on either the LD or SD side of the light barrier behaved the same.

The fact that the plants under LD did eventually acclimate regardless of the noninductive photoperiod indicates that short days facilitate, but are not a necessary prerequisite for, cold acclimation in apple. This is true of several other woody species (5, 6) as well. The hardiness of both the SD and LD branches in the divided plant treatment was the same at all sampling dates, and the hardiness level was intermediate between that of plants in the SD and LD treatments. Such intermediate hardiness has also been reported in the case of divided *Viburnum* plants, but the hardiness of the LD and SD branches was not always the same (5).

The divided plant study in the greenhouse during the fall of 1968 (Fig. 3) was conducted to examine the acclimation of plants in the absence of low temperature. SD, LD, and divided plants all acclimated to some extent (-15 to -25 C). Both branches of divided plants were again equal in hardiness and intermediate between the SD and LD treatments. Hardiness was initiated at the same time in the warm greenhouse as in the parallel field study even in the LD treatment. SD and divided plants hardened to -25 C in the absence of inductive temperatures.

In summary, it appears that SD, *per se*, can induce hardiness to approximately the level of the first hardiness plateau; that, given time, low temperatures can fully harden plants grown on noninductive photoperiods; and that plants can acclimate to some extent in the autumn in spite of noninductive photoperiod and temperature.

Leaf Removal Experiment. In 1968, a leaf removal experiment was run concurrently in the field with the photoperiod experi-

ment. On September 2 all leaves were manually removed from trees grown under natural SD and under artificial LD. The influence of leaf removal on hardiness was tested weekly from mid-September to early December.

Leaves have been said to inhibit (6) and promote (3) the hardiness of overwintering stems. The results of the leaf removal experiment (Fig. 4) and the photoperiod experiments indicated that both concepts have merit. SD plants with leaves acclimated first, followed by defoliated LD plants, LD plants with leaves, and defoliated SD plants, in that order.

Temperature Experiments. On August 20, 1967, a divided plant experiment was begun in the field. Trees were exposed to natural SD and either (a) naturally occurring temperatures; (b) naturally occurring temperatures except that the minimum temperature was never allowed to drop below 4.5 C; or (c) divided so that one leader was in each of the preceding temperature regimes. The modified temperature treatments were achieved via a clear plastic, double-walled frame structure through which warm air was circulated from an adjacent greenhouse by a thermostatically controlled fan when the outside temperature reached 5 C. In the divided plant treatment, pots were placed on either side of the temperature barrier for comparison of position effects. When the outside temperature was above 5 C during the daylight hours, the structure was opened to permit good air exchange and prevent warming. The parallel study was begun on August 5, 1968. In both years, leaves were manually removed in the modified temperature treatments on the same date that leaves were killed by frost in the other treatment.

The ability of plants to acclimate in response to low temperature under long day conditions was tested in a controlled environment chamber. Plants which had been growing in a greenhouse under long days since July 10 were transferred to a chamber programmed to provide a 15-hr photoperiod and a 12-hr thermo-period. The day-night temperatures were decreased stepwise (see Fig. 6) during the 35 days of the experiment, and the hardiness was determined weekly.

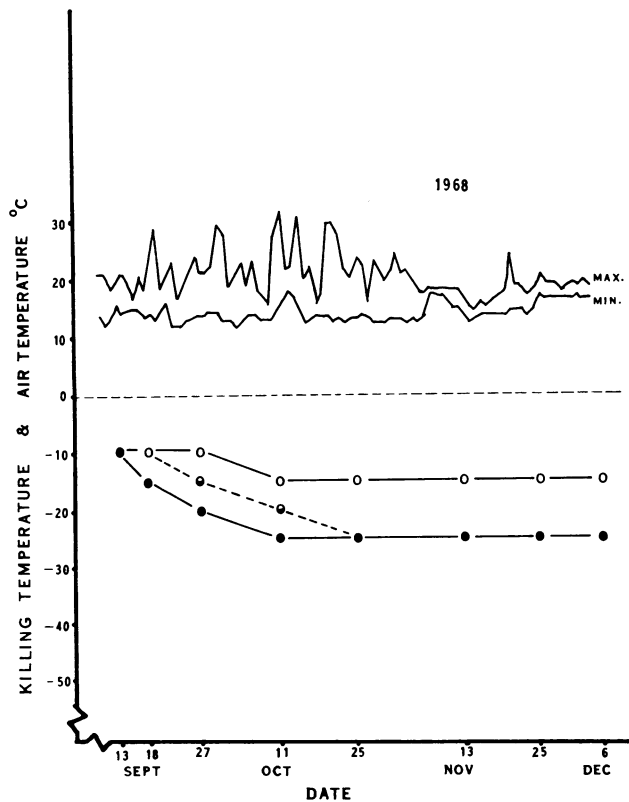


FIG. 3. Air temperature and the hardiness of young apple trees grown in a warm greenhouse at different photoperiods during the autumn of 1968. \circ — \circ : LD treatment; \bullet — \bullet : SD treatment; \ominus — \ominus : LD-SD divided plant treatment.

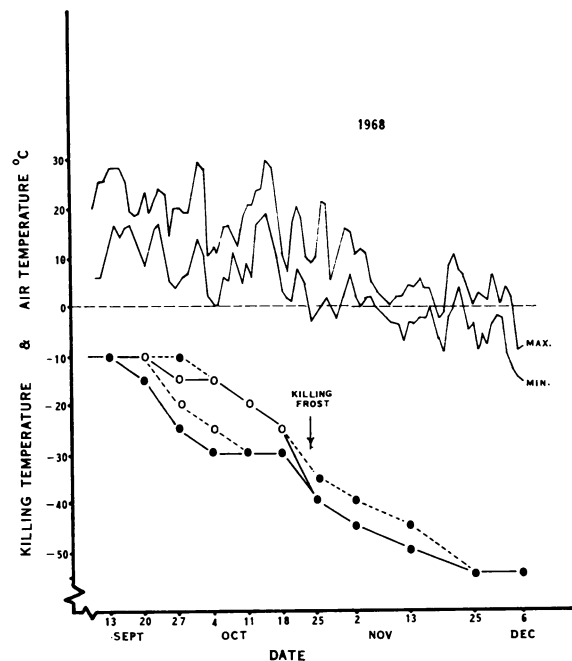


FIG. 4. Air temperature and the seasonal hardiness patterns of young apple trees grown at different photoperiods in the field during the autumn of 1968 with and without manual leaf removal. \circ — \circ : LD plants with leaves; \circ — \circ : LD plants with leaves removed; \bullet — \bullet : SD plants with leaves; \bullet — \bullet : SD plants with leaves removed.

Figure 5 shows the results of the temperature experiments conducted in the field in 1967 and 1968. As in the case of the photoperiod experiments, the data from the 2 years were very similar. Plants or branches of divided plants which were exposed to natural outdoor temperatures ultimately acclimated to -55°C . Plants and branches never cooled below 4.5°C failed to acclimate beyond -30°C , even though leaves were removed at the same time that leaves were killed in the natural temperature treatments. The hardiness plateau of the first stage of hardiness, at -25 to -30°C , was the same as previously observed (Figs. 1, 2, and 3). In contrast to the photoperiod studies, the low temperature induction of hardiness did not involve translocatable factors. Divided plants were not intermediate in hardiness, and each branch on divided plants became as hardy as whole plants exposed to the same temperatures.

The study conducted under controlled conditions, in a growth chamber, provided further evidence that low temperature could effectively induce acclimation in the absence of inductive SD. Figure 6 shows that the plants acclimated to -25°C before exposure to frost; to -30 or -35°C before the leaves were killed; and ultimately to -45°C , even though the photoperiod was maintained at 15 hr.

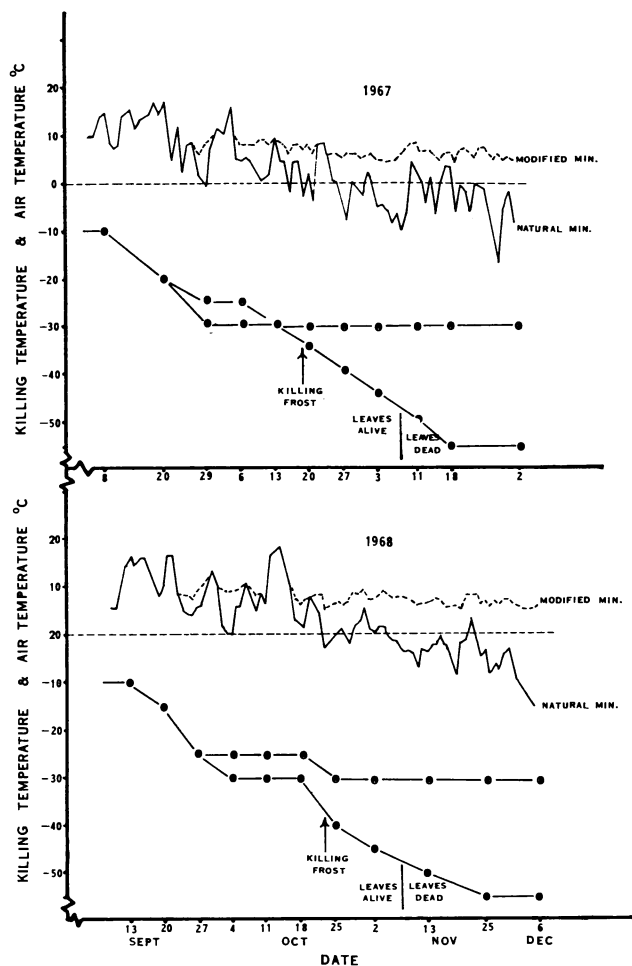


FIG. 5. Minimum air temperatures and the seasonal hardiness patterns of young apple trees grown at different temperature regimes in the field during the autumn of 1967 and 1968. ●: Plants and branches of divided plants in treatments at natural temperatures; ○: = plants and branches of plants in treatment at modified minimum (4.5°C) temperatures.

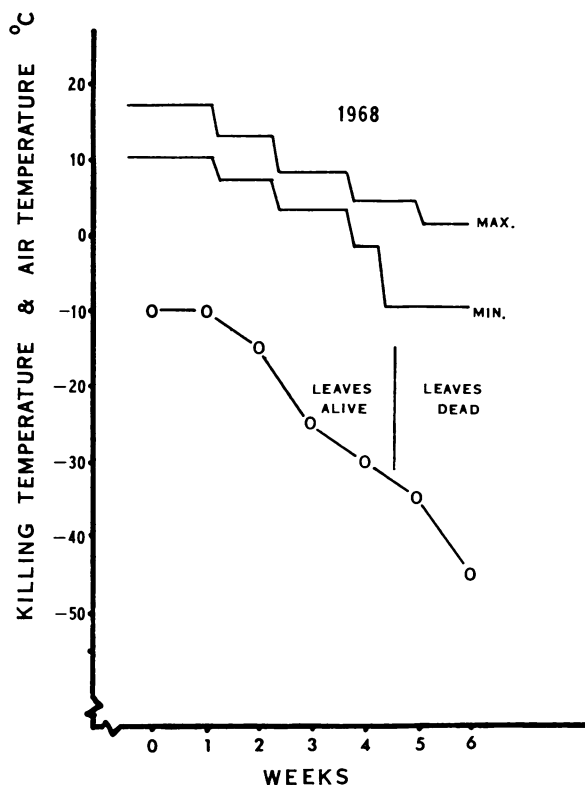


FIG. 6. Day and night temperature regimes and the hardiness of young apple trees grown at a 15-hr photoperiod in a controlled environment chamber.

DISCUSSION

Cold acclimation of apple trees appears to be controlled by several environmental factors. Plant responses to various combinations of these factors can provide a high degree of physiological flexibility which enables plants to acclimate and resist low temperature stress.

The observations for 2 years on mature trees (Fig. 1) and the divided plant studies of photoperiod (Fig. 2) and temperature (Fig. 4), in the fields, indicate that hardiness normally occurs in two stages in Haralson apple. This is consistent with observations on other species (10-12, 18).

In apple, the first stage of acclimation to -25 or -30°C can be induced by short days and probably is in nature. Mature trees started to acclimate at the same solar time each fall (Fig. 1) as did SD plants in a warm greenhouse (Fig. 3). The SD plants in the greenhouse acclimated to the level of the first hardiness plateau (-25°C) in the absence of low temperature (Fig. 3).

The short day induction of hardiness involves translocatable factors which promote hardiness (3, 5). This was apparent in the photoperiod studies of acclimation in divided plants. Both LD and SD branches acclimated more effectively than branches of plants totally under LD (Fig. 2). The hardiness-promoting factor could be a hormone, perhaps a growth inhibitor, since growth cessation is closely associated with acclimation. Treatment with growth retardants can enhance acclimation to some extent in other woody species (4, 7).

Although growth cessation appears to be a prerequisite to acclimation in woody plants, this does not imply that plants must be physiologically dormant. Van Huystee *et al.* (18) and Irving and Lanphear (5) have demonstrated that woody species acclimate substantially at a stage of development when they are capable of resuming growth under favorable environmental conditions.

As in other woody species (6), long days inhibited acclimation in apple, and the inhibition also resulted from translocatable factors. Divided plant studies showed that LD and SD branches acclimated less effectively than branches of plants totally under SD (Fig. 2).

If leaves are the source of these hardiness promoters and inhibitors, removal of LD leaves should enhance hardiness and removal of SD leaves should inhibit hardiness. This, in fact, was the case (Fig. 4). However, stem factors are apparently involved also because LD stems acclimated sooner than SD stems when both were manually defoliated (Fig. 4). Long day leaves are known to be the source of hardiness inhibitors in other woody species (4, 6). These results suggest that the first stage of hardiness in apple may be determined by a balance of promoters and inhibitors, and that the interaction of promoters and inhibitors is quantitative.

While the short day response can account for the first stage of acclimation, other factors are probably involved. Plants in a greenhouse stopped growing in the autumn and acclimated, to some extent, in spite of noninductive long days and high temperatures (Fig. 3). This rhythmic type of acclimation may be associated with endogenous growth cycles, or perhaps it is triggered by seasonal changes in environmental stimuli other than short days or low temperature (seasonal changes in light quality?). Acclimation of this type may be significant in nature during late summer and early autumn when unseasonable frosts can occur.

There is good circumstantial evidence that frost is the stimulus which triggers the second stage of acclimation in Haralson apple and other species (11, 12, 18). Plants did not acclimate beyond the level of the first plateau (-25 to -30 C) in the absence of frost (Figs. 1, 2, 3, 4, 5, and 6); and the second stage of acclimation was always initiated when plants were exposed to frost (Figs. 1, 2, 4, 5, and 6). Acclimation induced by frost under LD conditions began in the fall before leaves senesced or abscised. Frost apparently did not induce hardiness by merely killing leaves and removing the source of the hardiness inhibitor, as has been suggested (6).

The frost induction of cold acclimation, in contrast to SD induction, does not involve translocatable factors. The localized nature of this response is illustrated by the divided plant study (Fig. 6) where the minimum temperature was controlled. Branches of divided plants or whole plants exposed to frost become fully hardy (-55 C), while branches or whole plants not exposed to frost did not acclimate beyond -30 C. Because the inductive temperatures are low, it is tempting to speculate that the second stage of acclimation may be a physical rather than a metabolic phenomenon. This conclusion is unwarranted, however, because there may be enzymes induced by low temperature, and there are often warm days in the autumn when night frosts are common.

Acclimation was most rapid when plants with leaves were exposed to SD followed by frost (Figs. 2, 4, and 5). However, plants

with or without leaves (Fig. 4) could acclimate when they were exposed to only one of these inductive treatments. SD induced the first stage of acclimation (to -30 C) in the absence of frost (Fig. 5), or in the absence of temperatures below 12.5 C (Fig. 3).

Frost (low temperature), on the other hand, fully induced acclimation (to -55 C) under LD conditions in the field (Fig. 2), and to -45 C in the growth chamber (Fig. 6). Some acclimation occurred when both the photoperiod and temperature were non-inductive (Fig. 3). The ability of plants to acclimate in response to several environmental or endogenous stimuli improves adaptability and enhances survival potential.

From a practical point of view, the most interesting feature of cold acclimation in Haralson apple is the translocatable factor that promotes hardiness. If this short day-induced promoter proves to be a relatively nonspecific hormone, it may ultimately be possible to trigger hardiness in plants which are commonly injured by early frosts although they possess the inherent capacity to acclimate (13). This may apply to cultivated woody species grown at latitudes far removed from their sites of origin.

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