

THE EFFECT OF COLD ACCLIMATION  
ON GAS EXCHANGE AND GROWTH  
OF SPRING RAPE

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Isobel Waters

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ISOBEL WATERS

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## ABSTRACT

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The effect of cold acclimation on gas exchange and growth of spring rape.

Major Professor; Dr. Lucien J. LaCroix.

The problem investigated in this thesis was the growth response of spring rape as affected by a cold acclimation regime. The gas exchange rates of hardened and non-hardened rape seedlings were measured at 10C and 20C using an infra red analyzer. Growth as indicated by four parameters (leaf area, plant height, fresh weight, and dry weight) was measured over a 10 day period at both 10C and 20C.

Cold acclimation for a 3 week period significantly increased rates of dark respiration at both 10C and 20C as compared to the non-hardened controls. A 6 week acclimation period also increased respiration at both temperatures, but the rate at 20C was intermediate between that obtained for the non-hardened plants and those hardened for 3 weeks.

Net photosynthetic rates of the plants hardened for 3 weeks were significantly higher than the non-hardened plants at 10C but identical at 20C. Plants hardened for 6 weeks had photosynthetic rates that were significantly higher than the non-hardened controls at 10C, but significantly lower at 20C.

Cold acclimation was observed to have a general stimulatory effect on subsequent growth rates at both 10C and 20C, as measured by increases in growth parameters. The cold hardened plants displayed greater rates

of increase in fresh weight, dry weight, and leaf area at both temperatures. Rates of increase in plant height was the only parameter that was not significantly affected by cold acclimation, although the hardened plants did attain lower plateau values than the non-hardened controls.

Comparison of the fresh and dry weight values showed that the hardened plants gradually increased their water content after removal from the cold acclimation pretreatment chamber, as well as increasing their leaf area relative to dry weight increase. These changes are indicative of morphological changes to the plant during cold hardening.

## INTRODUCTION

Response of economic crops to a low temperature pretreatment or cold acclimation is of vital importance to the agricultural economy of Western Canada. Because of the short growing season, plants must be able to not only withstand occasional spring frosts, but also grow efficiently under the sub-optimal temperature conditions encountered early in the season.

Past studies of the effect of cold hardening on the supercooling point of seedling spring rape tissue showed that cold acclimation during germination not only increased the frost resistance of the germinating tissue, but that these effects were retained throughout the total growth of the plant (Stout 1972). These findings raised questions about the effect of cold acclimation on growth rates of hardened tissue, as measured by increases in general growth parameters such as leaf area, plant height, dry weight, and fresh weight, and by specific growth processes such as dark respiration, net photosynthesis, and photorespiration. If acclimation had permanent effects on the frost resistance of rape tissue, does it also have permanent effects on the growth performance of the plant?

Preliminary gas exchange studies indicated that cold hardening did have stimulatory effects on net photosynthesis of hardened plants, but had no effect on respiratory activity (LaCroix and Nalborczyk, unpublished data).

The objective of this study was to continue these investigations into the growth responses of cold acclimated rape seedlings.

## LITERATURE REVIEW

### Introductory Comments

Plant response to low temperature has been reviewed frequently. Levitt (1956, 1972) has compiled the most general overview of the subject, surveying the possible mechanisms whereby plants are thought to a) avoid or b) tolerate low temperature stress. He also examined freezing and chilling injury in plants.

Other reviewers have focussed on more specific areas. Parker's 1963 review discussed the importance of low temperature acclimation to the ecological distribution of woody species. Olien (1967) and Mazur (1969) examined the physical and biochemical events associated with freezing in plants; the former dealt largely with herbaceous species. Weiser (1970) concentrated on freezing processes in woody species. Most recently, Burke et al (1974) investigated freezing injury and plant resistance. Alden and Hermann (1971) stressed the role of environmental factors other than low temperature in cold acclimation and species distribution. They also stressed the biochemical changes in plant cells associated with acclimation, including a discussion of changes in cell membranes and their phospholipid components. This topic is discussed in greater detail by Lyons (1973) in his review of chilling stress and injury.

Despite these numerous investigations of the cold acclimation mechanism in plants, no clear picture has emerged. In part this is due to

the complexity of environmental and internal factors involved in acclimation, factors which are difficult to unravel (Alden and Hermann 1971). As Mazur (1969) pointed out, researchers have dealt with this complexity by searching for a single biochemical alteration, or a single sequence, which will explain how all plants acclimate to low temperature. However, no such single, coherent explanation has been put forward which can account for the many and sometimes contradictory findings reported in the literature.

Given the numerous areas of cold hardiness research, no attempt will be made to cover all aspects in this review. Firstly, although I will be concerned primarily with the effects of freezing temperature on plant growth, since exposure to such temperatures is a crucial part of the hardening regime, I will not be concerned with injury sustained by exposure to extremely cold temperatures. Plant adaptations to avoid such injury, for example supercooling, will be mentioned only in passing. Instead, I will discuss wherever possible the ability of plants to maintain normal metabolic functioning under less than optimal environmental temperatures. For this reason, the effects of chilling temperatures, i.e. temperatures which are low but above freezing (Levitt 1966) will also be discussed where appropriate. Secondly, I will include as many findings as possible from work done with annual species, since these will be most applicable to my study. However, since the great majority of cold hardiness work has been conducted with perennial species, this will form the bulk of the material cited.

In this thesis, cold acclimation will refer to the process whereby plants exposed to appropriate environmental conditions are able to protect themselves from the damaging effects of low temperature. The older term, cold hardening, will be considered synonymous. These terms should be

differentiated from cold adaptation, which refers to the inherited traits which determine the plant's ability to undergo cold acclimation and endure subsequent cold stress (Stout 1972).

### Environmental Factors in Cold Acclimation

#### Light

The effect of light on cold acclimation has been investigated from two perspectives (Alden and Hermann 1971). Light is thought to play a photosynthetic role in acclimation by enabling the plant to accumulate energy reserves and/or specific cryoprotective substances. A variety of crops, including winter wheat, cabbage and alfalfa hardened more effectively at 0C when exposed to light (Dexter 1933).

Supplementary light during acclimation increases resistance to winter injury in winter wheat. Moreover, the degree of acclimation in winter wheat declines sharply five weeks after transfer to the dark, indicating that the photosynthetic reserves necessary for cold acclimation have been exhausted (Andrews 1960).

Russian workers have also concluded that normal gas exchange, including substantial photosynthetic rates, is crucial for developing cold hardiness in winter wheat (Rakitina 1967). Sucrose uptake can replace light during low temperature treatment of winter annuals, suggesting that photosynthesis is necessary for accumulation of carbohydrate since these species do not store starch (Tumanov and Trunova 1963). Not only the intensity of light but also its spectrum is critical to acclimation in winter annuals; effective wavelengths correspond to the absorption maxima of the photosynthetic pigments (Voblikova 1963).



Accumulation of photosynthate is also necessary for acclimation in cabbage, and resistance to cold in this annual species depends on the ability to photosynthesize at low temperatures (Kohn and Levitt 1965; Cox and Levitt 1969, 1976).

The effect of light on acclimating winter annuals depends in part on the physiological age of the material and the acclimation temperature. Continuous light is most effective in enhancing hardiness at sub-freezing temperatures, whereas darkness favours hardiness at chilling temperatures (Panchenko 1963). These findings contradict other work done with winter annuals, and may suggest an alternate role for light (Alden and Hermann 1971).

Although light enhances cold acclimation in the perennial broad-leaved evergreen, English ivy (Hedera helix), it is not necessary (Steponkus and Lanphear 1968; Steponkus 1971). It was concluded that the light-enhanced production and accumulation of sugars is only one step in acclimation, as indicated by the kinetics of acclimation observed during alternating periods of light and dark. Incubation with 50mM sucrose solution can replace the light requirement (Steponkus and Lanphear 1967, 1968).

Steponkus (1971) explains these findings by explaining acclimation as a two-step process involving a) light-stimulated production of a translocatable hardiness promoter, possibly sucrose, and b) a dark reaction requiring low temperatures which enables the plant to respond to sucrose. These two steps are thought to proceed independantly, with the second more likely to be limiting (Steponkus and Lanphear 1966, 1967, 1968). However, although English ivy can be acclimated in the dark, light-grown plants preferentially use the accumulated photosynthate rather than the reserves.

Dark-grown plants can hydrolyze starch, but ultimately they require light to replenish reserves (Steponkus and Lanphear 1968).

In woody species, the photosynthetic role of light is not necessarily fulfilled during the acclimation period as it seems to be for the winter annuals, but rather during the previous growing season before the onset of dormancy (Alden and Hermann 1971). A preconditioning period when plants are exposed to both warm temperatures and short days enhances cold acclimation in dogwood by accumulating photosynthetic reserves (Fuchigami et al 1971b).

Light also affects cold acclimation through a photoperiodically induced stimulus. Short photoperiods enhance cold acclimation in alfalfa, red clover and sweet clover (Alden and Hermann 1971). In cabbage, short photoperiods are most effective in inducing the accumulation of reserves (Cox and Levitt 1976). However, in contrast to the findings of Russian workers with winter wheat, Paulsen (1968) found that cold hardiness develops more effectively under long days. He suggested that his results may have been due to low light intensities.

The initial phase of hardening in dogwood (Cornus stolonifera) involves a photoperiodic response (Van Huystee 1965; Van Huystee et al 1967; Fuchigami et al 1971b). However, a decreasing photoperiod alone was not sufficient. The most effective acclimation treatment under artificial conditions was a rest period induced by gradually decreasing photoperiod, followed by a low temperature treatment (Van Huystee et al 1967). Short photoperiods followed by low temperature was also effective in hardening other woody species, including Acer, Viburnum and Weigela (Irving and Lanphear 1967).

Dogwood leaves exposed to short days produced a translocatable hardiness promoting factor, whereas long day conditions resulted in the production of a hardiness inhibiting factor (Fuchigami et al, 1971a). The function of the photoperiodic stimulus to cold hardiness may be simply to cease growth and induce dormancy in woody species (Tumanov et al 1964, 1965; Garber and Steponkus 1976). However, specimens of Viburnum, Acer and Weigela which remain in a non-growing state without bud dormancy after hardening in the dark at 5C developed the same degree of hardiness under a six hour photoperiod as dormant plants hardened under similar conditions. Furthermore, the gain in frost hardiness obtained after two weeks of short photoperiods is reversed by two weeks of long photoperiods (Irving and Lanphear 1967). They concluded that cold acclimation is induced by a photoperiodic stimulus in much the same way that dormancy is induced, but that dormancy is not a prerequisite for acclimation. In addition, application of growth retardant chemicals has only slight and inconsistent effects on acclimation (Irving and Lanphear 1968; Irving 1969).

The photoperiodic response is thought to be mediated through the phytochrome system (Alden and Hermann 1971; McKenzie et al 1974). Removal of leaves from dogwood and interruption of the dark period with a twenty minute light period interfered with acclimation (Hurst et al 1967). The nature of the hardiness promoting factor induced by short days and mediated by phytochrome interconversions is not known, although it is non-genotype specific in dogwood (Fuchigami et al 1971a). The factor is translocated from the leaves to overwintering bark tissue; speculations that it is abscisic acid (Irving 1969) have not been confirmed with dogwood. Abscisic acid or gibberellic acid ( $GA_3$ ) alone or in combination did not induce or

enhance acclimation (Fuchigami et al 1971a).

Researchers are unsure whether this unknown factor acts simply to retard growth or whether it has a more direct role (Fuchigami et al 1971a). Steponkus (1971) suggested that sucrose may be the hardiness promoting factor, since this compound inhibits growth and has been found to increase in hardening plant tissue. Others claim that sucrose is merely a substrate necessary for effective acclimation and thus plays an indirect role only. According to this theory, growing plants may be unable to harden fully because they are low in these substrates (Fuchigami et al 1971a, 1971b).

Under long days, acclimation is inhibited until the leaves are removed, indicating that the hardiness inhibiting factor is also a translocatable hormone-like substance (Irving 1967). Speculations that this factor may be gibberellic acid have not been verified for dogwood (Fuchigami et al 1971a), but it seems likely that the relative proportion of inhibitor to promoter is important in cold acclimation. However, hardening can be induced with an unfavourable photoperiod in some woody species, providing that acclimating temperatures are kept sufficiently low to lead to leaf loss and growth cessation (Irving 1967).

Short days enhance cold acclimation in conjunction with a reduced water supply (Chen et al 1975. 1977). Under conditions of low water and short days, hardiness increased rapidly, whereas under normal water and short day, hardiness increased gradually but was accompanied by similar physiological changes in protein, nucleic acids and carbohydrates. However inductive photoperiods do seem necessary since under long day no change in hardiness was observed despite water stress. Ultimately, short day conditions simulate water stress conditions in the hardening plant through

alterations in stomatal and root resistance (Chen et al 1977).

The effect of short days on the water relations of hardening dogwood plants has been well documented (Parsons 1978). An eight hour day produced dramatic changes in transpiration rate, stomatal resistance and root conductivity in hardening dogwood as compared to control plants grown under long days at the same low (15/5C) temperatures. Six days after transfer to short days, stomatal resistance decreased significantly below that of the long day plants, and transpiration rates increased 20-30%. The net result was an acceleration of water loss in the acclimating plants under short days in the first 30-40 days of treatment. During the last two weeks, the stomata of the short day plants closed completely due to changes in leaf water potential, and transpiration rates dropped rapidly. Root conductivity was significantly lower in the short day plants (Parsons 1978). These results suggest that the reduced water content observed in dogwood and other woody species during hardening (Fuchigami et al 1971b; Chen et al 1975, 1977) is due to the relative increase in the rate of water loss due to increased stomatal opening over the reduced rate of water uptake due to reduced root conductivity (McKenzie et al 1974). Thus, although the mechanisms by which these processes are affected by short days is unclear, the evidence suggests that inductive photoperiods initiate a sequence of changes in the plant during acclimation, the result of which is a reduced water content and subsequent stomatal closure (Parsons 1978).

Steponkus and Lanphear (1968) concluded that the effect of light on acclimation is two-fold, involving a complex interaction of photosynthetic and photoperiodic effects. They suggest that in English ivy a phytochrome system is activated which in turn, through hardiness promoters and inhibitors

influences the plant's metabolic processes, including its photosynthetic apparatus. Photosynthetic products such as sucrose are important indirectly by providing necessary substrates, and may have a direct effect as well. This view of the importance of light in acclimation is thought to apply to other perennial species as well (Irving 1968; Fuchigami et al 1971b). The interaction of light effects with other environmental parameters has also been examined (McKenzie et al 1974; Parsons 1978).

Light can also have detrimental effects on chilling sensitive plants. Exposure to high light intensities during cold temperature treatment can lead to chloroplast damage (Slack et al 1974). When chilling-sensitive plants are exposed to low temperatures, light causes a temperature-dependant destruction of the photosynthetic apparatus. For such plants, low light intensity is protective rather than limiting to acclimation (Taylor and Rowley 1971; Taylor and Craig 1971). Visible light at 10C causes progressive and permanent damage to the photosynthetic capacity of leaves of chilling-sensitive C<sub>3</sub> and C<sub>4</sub> plants. Extent of damage increases with intensity and length of exposure to 10C (Taylor and Rowley 1971).

Attempts to acclimate sorghum to chilling temperatures showed that leaves formed during the acclimation period had significantly lower chlorophyll content, which appeared to protect the plants somewhat from light damage at chilling temperatures (Taylor and Craig 1971). Chilling sensitive C<sub>3</sub> and C<sub>4</sub> plants showed the same pattern of ultrastructure changes to chloroplast thylakoids when exposed to moderate light intensities and chilling temperatures. These changes included reduction in the size of starch grains, contraction of the thylakoid intraspace, and swelling of the stroma (Taylor and Craig 1971).

### Nutrients

Cold acclimation is an active process and as such requires adequate amounts of the essential plant nutrients. Although I will not attempt a detailed account of the effects of various nutrients on acclimation, it is important to stress their role as an environmental factor influencing acclimation.

Although adequate soil nitrogen is essential to acclimation, high levels may delay the onset of hardening and increase winter injury in perennial species, presumably by prolonging growth (Alden and Hermann 1971). Excessive amounts of phosphorus can also be detrimental to cold acclimation. High in vivo levels of inorganic phosphate predisposes dogwood plants to winter injury, and total inorganic phosphorus levels decrease as degree of hardiness increases. Total organic phosphate, in contrast, increases with hardening, suggesting that organic phosphorus compounds enhance hardening by means other than acting as an energy source (Li et al 1966). Alden and Hermann (1971) cite other experiments indicating that additional phosphate can enhance hardening.

### Water

Soil moisture availability also affects cold acclimation. The onset of hardiness in dogwood was hastened by low soil water; plants grown under normal water conditions and identical photoperiod hardened at a significantly slower rate than the water-stressed plants (Chen et al 1977). Once hardiness developed, after seven days of treatment, increased water availability had no further effect on the degree of hardiness, indicating that the increase in hardiness induced by water stress is rapid and not reversible