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

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

BY

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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 Nalborcyzk, 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 synonomous. 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_3 and C_4 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_3 and C_4 plants showed the same pattern of ultrastructure changes to chloroplast thykaloids when exposed to moderate light intensities and chilling temperatures. These changes included reduction in the size of starch grains, contraction of the thykaloid 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

by subsequent changes in moisture levels (Chen et al 1977).

Although water-stressed dogwood plants do harden faster, dogwood will acclimate at low temperatures even in wet soils. Under these conditions of excessive moisture availability, acclimating plants will actively simulate conditions of water stress by accelerating their rate of water loss (Parsons 1978).

Soil moisture content had no significant effect on the winter survival of winter wheat (Pomeroy and Andrews 1978). Winter injury occurring under ice cover may be due instead to anaerobic conditions and the accumulation of toxic products such as ethanol and lactate (Pomeroy and Andrews 1978).

Low water levels, light and low non-freezing temperatures were the three environmental factors necessary for hardening cabbage (Cox and Levitt 1976). Fast droughting was most effective in inducing acclimation, whereas slow droughting was detrimental to it, possibly due to harmful effects on the photosynthetic machinery (Cox and Levitt 1976).

Drought hardening of the chilling sensitive species Phaseolus vulgaris (bean) at 25C and 40% RH for four days was as effective in preventing chilling injury as exposing the plants to 12C and 85% RH for the same time period. The primary factor inducing chilling hardiness was concluded to be water stress, not low temperature, and the primary cause of chilling injury was leaf dehydration due to stomatal opening at a time when root permeability to water is low (Wilson 1976).

The importance of plant water status to cold hardiness is related to the nature of freezing injury, which is thought to be similar to desiccation or dehydration injuries (Mazur 1972). Cold hardiness is apparently

due to an increased ability to tolerate the dehydration stresses induced by extracellular ice formation or to an ability to avoid these stresses by means of increased cell sap concentration and hence less dehydration (Levitt 1972). Although some researchers have supported the idea of an avoidance mechanism (Johansen and Krull 1970) and claim that it is applicable for some moderately hardy plants (Burke et al 1974), current research seems to refute the hypothesis that hardy plants contain higher levels of bound water (Burke et al 1974). Evidence for a tolerance mechanism is mounting. The quantity of unfreezable water, on a dry weight basis and as determined by nuclear magnetic resonance techniques, was not closely related to the degree of cold acclimation in cereals. However, the fraction of unfreezable water tolerated by non-acclimated winter cereals was much less than for acclimated plants (Gusta et al 1975).

This mechanism of tolerance to desiccation stress did not seem to exist in acclimated spring wheat, which had a similar tolerance to the fraction of unfreezable water in its tissue as did the non-acclimated winter cereals (Burke et al 1975).

To test Levitt's hypothesis that frost hardiness can be attributed to the cell's ability to tolerate desiccation stress induced by extracellular ice formation, the effects of various rates of freezing on the metabolism of a drought tolerant moss were studied. As expected, slow freezing did not inflict irreversible damage on this desiccation tolerant plant, whereas fast freezing did, presumably due to intracellular ice formation (Malek and Bewley 1978).

Not only the absolute amount of water in plant tissue, but also the rate of water movement and water permeability of the tissue are important

in cold acclimation (McKenzie et al 1974; Levitt 1972). The water permeability of dogwood cortex cells increased sharply during the initial phase of hardening induced by short photoperiods and accompanied by an increase in hardiness from -3 to -12C. No further increase in permeability occurred in the second phase of hardening to -65C. An increase in permeability is crucial to freezing tolerance since lethal intracellular ice formation occurs when water cannot move out of the cell quickly enough to extracellular ice nuclei. Intracellular ice formation may still occur during slow freezing if membrane permeability greatly restricts water movement out of the cell (McKenzie et al 1974).

Although water permeability increased in willow tissue with increasing hardiness until January, it is retained until May when much hardiness has been lost and is thus not the sole factor in freezing tolerance (Sakai 1965).

The role of membrane permeability in the avoidance of intracellular ice formation has been questioned as a result of recent studies of winter wheat tissue using nuclear magnetic resonance. Changes in the permeability of the plasmalemma were detected, but the authors concluded that these changes were not causally related to cold acclimation but are simply low temperature effects (Stout et al 1977, 1978; Chen et al 1978).

Desiccation stress due to extracellular ice formation is not the sole cause of freezing injury in plants; other direct low temperature effects have been reported (Burke et al 1974). However, most of the major hypotheses of freezing injury, including Levitt's sulfhydryl-disulphide theory (1962), Heber and Santarius' protein water shell theory (1964), Meryman's salting-out theory (1956) and Weiser's vital water theory (1970)

assign a major role to dehydration in freezing injury (Burke et al 1974).

It appears that plants can harden under a range of environmental moisture conditions, although water stress hastens the rate of acclimation. Hardy plants possess mechanisms for inducing the tolerance to desiccation stress necessary to survive the severe intracellular dehydration accompanying extracellular ice formation. However, even when ice formation is not the cause of injury, as in chilling sensitive plants, drought tolerance is important to low temperature acclimation (Wilson 1976). And although low moisture levels are beneficial to acclimation in the fall, adequate moisture levels during the previous growing season are equally important to the development of hardiness and the prevention of winter injury in perennials, presumably due to the plant's growth requirements (Alden and Hermann 1971).

Temperature

Low temperature influences the physiological and biochemical changes accompanying cold acclimation more than any other environmental factor (Alden and Hermann 1971) and is the primary inductive stimulus in the hardening process (Gusta and Fowler 1976). The effect of temperature on hardening must be divided into the differential effects of specific low temperature levels. As Levitt (1972) has noted, a distinction must be made between chilling and freezing temperatures, even though the effects of exposure to these temperatures may be similar in some plants (Guinn 1971).

In plants capable of cold acclimation, exposure to low but above freezing temperatures (chilling) induces considerable frost hardiness, given favourable environmental conditions, in a variety of species (Alden

and Hermann 1971; Garber and Steponkus 1976; Cox and Levitt 1976). However, decreasing temperature alone was not effective in hardening dogwood (Van Huystee et al 1967). Others claim that, given sufficient time, woody plants will acclimate under low temperatures even if other factors, such as photoperiod, are unfavourable (Irving and Lanphear 1967; Mooney et al 1978). On the other hand, decreasing temperatures may not be necessary to cold acclimation if photoperiodic conditions are favourable (Van Huystee et al 1967). Under natural conditions, however, decreasing temperatures are a primary environmental stimulus to cold acclimation.

In woody plants, low temperatures have differing effects during different phases of acclimation. During the initial photoperiodically induced phase involving translocatable promoters, low temperatures can inhibit acclimation (Fuchigami et al 1971a, 1971b). During this phase, a warm preconditioning period is required for the fulfillment of the plant's photosynthetic needs. In contrast, the second phase is induced by low temperatures (Fuchigami et al 1971a, 1971b). A third phase requiring prolonged exposure to freezing temperatures has been proposed, though not adequately explicated, for woody species (Tumanov 1967).

The chilling sensitive species, bean, can develop tolerance under low moisture conditions regardless of temperature levels, suggesting that low temperature is not essential for developing chilling resistance (Wilson 1976).

Duration of exposure to specific temperature levels affects the acclimation process. Perennial species require sufficient time at low temperatures to convert polysaccharides such as starch to cryoprotective substances, whereas winter annuals which do not store starch require sufficient

time to accumulate protective substances by photosynthesizing at low temperatures (Alden and Hermann 1971).

The destructive effects of low temperature on the photosynthetic apparatus of sensitive plants is also related to duration of exposure, and can be reversed if plants are restored to warm temperatures after a short exposure to chilling temperatures (Taylor and Rowley 1971).

Rate of cooling is also critical. A slow cooling rate is important to the avoidance of winter injury since the plant must be able to export its water at a rate fast enough to avoid intracellular ice formation (McKenzie et al 1974). The gradually decreasing temperatures encountered in the fall are thus most conducive to developing maximum hardiness (Alden and Hermann 1971).

The nature of freezing processes and resistance in plants is complex. Generally, frost hardy plants avoid intracellular ice formation, which is invariably lethal, and tolerate extracellular ice formation. As long as these plants are not exposed to sudden temperature drops, they are able to export their water to extracellular ice nuclei (Burke et al 1974).

Various survival mechanisms have been suggested. Some structures, such as seeds, become dehydrated and so avoid freezing injury altogether. Plants such as citrus species with high solute concentrations can avoid freezing injury by means of freezing point depression, although this is effective for only a few degrees of frost. Still other plants are capable of 'supercooling', wherein ice formation is avoided due to a lack of nucleating substances for ice initiation. While some plants supercool only a few degrees, others, in particular flower bud tissue and the living bark of moderately hardy trees such as apple, are capable of 'deep supercooling'

to -20C to -45C. Very hardy woody species do not supercool, but survive by tolerating the extreme desiccation stress induced by extracellular ice formation. Such species can survive with a very low fraction of bound or unfreezable water, and when properly acclimated can survive temperatures as low as -196C (Burke et al 1974).

Summary

Cold acclimation is induced in nature by a complex of environmental factors which interact to elicit a sequence of biochemical and physiological changes within the plant. Each one of these factors can be investigated individually, but their interaction should always be borne in mind (Alden and Hermann 1971).

Effect of Cold Acclimation on Growth

Overall Growth Response

Environmental factors which inhibit growth, including low temperature, shortening photoperiods, low soil moisture and low nitrogen enhance cold hardiness. Woody plants achieve maximum hardiness when growth ceases in the fall and deharden rapidly when growth resumes the following spring (Levitt 1972). Due to this observed inverse relationship between growth rate and acclimation in woody species, growth cessation is generally considered a prerequisite for cold hardening (Alden and Hermann 1971; Fuchigami et al 1971b).

Contradictions to this inverse relationship have been reported. A direct relation was found between growth rate of cabbage and its ability to harden (Cox and Levitt 1969). Rather than being antagonistic

to acclimation, growth was essential and little acclimation occurred in leaves exposed to hardening temperatures after growth had ceased. All actively growing leaves showed an ability to harden which directly paralleled their growth rates at time of exposure. Maximum hardening occurred in leaves which were at or near their maximum growth rates when acclimation began. Although the decrease in growth rate which occurs at the low hardening temperatures may be necessary for acclimation, growth cessation *per se* is not in cabbage (Cox and Levitt 1969).

Both RNA and protein synthesis increase during the fall in non-growing woody perennials and accompany cold acclimation in black locust (Siminovitch *et al* 1968) and dogwood (Li and Weiser 1967). Although these increases are preferentially described as 'augmentation' or accumulation rather than growth (Levitt 1972), it is clear that acclimation is an active metabolic process and that growth is not necessarily antagonistic to it. It may be useful to distinguish between growth as the initiation and enlargement of new organs (leaves, stem tissue etc.) plus the concommitant increase in surface area, and the 'invisible' growth accompanying increases in stored reserves (Wooledge 1969). In woody tissue, acclimation seems incompatible with the first type of growth while requiring the second (Alden and Hermann 1971), whereas in cabbage, an annual species, acclimation parallels both types of growth (Cox and Levitt 1969).

An hypothesis has been proposed to accomodate the seemingly contradictory data. Net protein synthesis at low temperature is taken to be the *sine qua non* of cold acclimation; if it occurs, the plant will harden whether it is growing or not. According to this hypothesis, growing plants such as cabbage and other species which lack a dormant period in their

growth cycle, are capable of hardening only if growth and active protein synthesis are not halted at hardening temperatures. Woody perennials, which do have a period of dormancy, can acclimate only if they can develop an active protein synthesis which is uncoupled from growth at low temperatures (Cox and Levitt 1969).

Neither the growth capacity nor efficiency of winter wheat cultivars differed significantly from spring wheat cultivars under hardening (4/2C) and non-hardening conditions. All cultivars exhibited an inverse relation between growth and hardiness, but this relation was not a causal one, since neither the growth coefficient under hardening nor non-hardening nor the transfer from one to the other condition was correlated with the degree of hardiness achieved (MacDowall 1974). Further attempts to correlate growth and acclimation require determination of the 'specific' or 'intrinsic' growth coefficient through measurement of dry weight exclusive of stored reserves. In contrast, the growth coefficients used in these experiments are determined largely by the increased storage of carbohydrate at low temperature. If growth is redefined in the way suggested, it may then be possible to establish a more meaningful relation to cold hardiness (MacDowell 1974).

The capacity of dogwood tissue to acclimate is dependant not only on growth rate but also phase of growth. Effective acclimation is possible only after the surge of growth promoting substances has subsided, i.e. one to two months after resumption of spring growth (Van Huystee et al 1967). Since the initiation of plant tissue involves a response to IAA and other growth promoters (Bidwell 1974), the capacity of acclimated cells to absorb and respond to IAA was investigated. Acclimation did not alter response to IAA, but hardened tissue breaks down IAA more rapidly than non-hardened

tissue (Sirois and MacDowell 1977).

Maintenance of the hardy state also depends on growth processes. Both Rideau and Cappelle, two cultivars of winter wheat, harden rapidly but the decline in hardiness during the winter is much more rapid in Cappelle. This rapid dehardening is due to Cappelle's higher metabolic rate and subsequent rapid depletion of energy reserves (Andrews et al 1974; Pomeroy et al 1975).

In chilling sensitive annual species, susceptibility to low temperature injury is also dependant on growth phase. Soybeans (Bramlage et al 1978) and cotton (Clay et al 1976; Clay 1977) are extremely vulnerable during germination and early seedling growth. The time period of maximum chilling sensitivity in cotton corresponds to the period of maximum DNA synthesis (Clay 1977).

Effects of Acclimation on Respiration and Photosynthesis

Low temperature effects on growth are determined in large part by effects on major growth processes, including photosynthesis and respiration. Several clarifying points are necessary. Firstly, the instantaneous effects of low temperature on photosynthetic and respiratory processes per se, e.g. enzyme activity, membrane permeability and substrate concentration should be distinguished from the long term effects of preconditioning or acclimating temperatures on the subsequent photosynthetic and respiratory performance of plants (Rook 1969; Leopold and Kriedemann 1975). In this study, the second type of effect is more pertinent. Secondly, many of the studies cited deal with preconditioning and measuring temperatures which are considerably higher than those used in my experiments. Although these studies are not relevant to a discussion of freezing tolerance, they do have a

bearing on general mechanisms of adaptation to temperature. Thirdly, many of the species used are warm climate plants, including C_4 species. Comparatively little work has been done with cool climate C_3 species in this area, and so I have not limited my discussion to temperate climate species.

Photosynthesis is generally considered to be the plant process most sensitive to environmental factors, especially light intensity and temperature (Bjorkman and Holmgren 1963). Species vary considerably in their photosynthetic temperature optimum. Some of these differences are innate and reflect differences in the photosynthetic apparatus; for example, most C_4 plants have higher optima than C_3 plants irrespective of growing conditions, possibly due to reduced or non-existent photorespiratory activity (Bidwell 1974; Leopold and Kriedemann 1975). In a study of the effect of temperatures ranging from 10C to 34C on the net assimilation rate (NAR) of rape, sunflower and corn, the temperature optima varied from a low of 20C for rape to a high of 30C for corn. At a light intensity of 3000 f.c., the NAR of rape varied only 10% between 12C and 30C (W. Wilson 1966). Similarily, most barley cultivars have a photosynthetic temperature optimum at 16-20C, although photosynthetic rates of some cultivars drop off much less than others as temperatures are lowered (Omrod 1968). Temperature optima of net photosynthesis in another temperate climate species, spring wheat, was 15C, whereas the temperature optima for translocation was 25C. At temperatures above 15C, increases in photorespiration override increases in photosynthesis (MacDowell 1973).

Inhibition of photosynthesis at temperatures below the optimum is thought to be caused by factors other than a direct temperature effect on the enzymatic dark reactions. Several factors may be involved, for example

1)mass action effect of accumulated assimilates; 2)end-product inhibition of photosynthetic reactions; 3)physical obstruction in the cytoplasm (W. Wilson 1966). Enhancement of respiration induced by accumulated assimilates would minimize this low temperature depression of photosynthesis.

Differences in temperature optima can be attributed to environmental factors as well as genetic. Temperature optima for net photosynthesis amongst plants of the same species can vary with growing conditions or pretreatment. The effect of varying day/night temperatures (18/10C, 24/16C and 29/21C) on CO_2 assimilation rates was studied in barley, peas and rape. For barley and peas, temperature optima did vary with growing regime; cold grown plants reached their maximum photosynthetic rate at a significantly lower temperature than warm grown plants. The CO_2 assimilation rate of rape, in contrast, was largely unaffected by temperature regardless of growing regime, indicating that this species possesses less plasticity of environmental response (Herath 1973).

Carbon dioxide assimilation rates of Arctic species not only have lower optima than the same species grown in warmer regions, but also have lower Q_{10} values, indicating a greater tolerance of temperature changes. Also, the respiration rate of the Arctic grown plants was higher at all temperatures tested (Wager 1941). Similarily, the photosynthetic rate of cold adapted cereals is not greatly affected by measuring temperature (Anderson 1944).

Net photosynthesis at low temperatures is determined by respiration as well as gross photosynthesis, and acclimation can have differential effects on these two processes, as indicated by both field and controlled

studies. Woody perennials show marked seasonal trends in their rates of photosynthesis and respiration. Gas exchange measurements were taken with an infra red analyzer at 25C and 4000 f.c. throughout the year on loblolly and white pine seedlings. Although the pattern of changes was not identical for the two species, both had significant seasonal variations in photosynthesis per unit fascicle length in addition to differences due to surface area. It was concluded that these changes were in the rate of photosynthesis per chlorophyll unit and that the loss in chlorophyll activity may be due to a reversible disorganization of the chloroplast. Changes in resistance to CO_2 uptake via changes in the mesophyll cells or the stomata were also considered possible causal factors in seasonal variations (Davis et al 1963).

Respiration also showed strong seasonal variations per unit fascicle length, but these variations were not identical to those observed for net photosynthesis. Respiration rates increased sharply in the spring, decreased to a minimum in September, but increased again in winter. This winter increase was thought to be due to an increase in soluble carbohydrate at cool temperatures and was not considered adaptive (Davis et al 1963). It should be noted that the gas exchange trends observed for these evergreen species, i.e. a decline in photosynthetic rates in the winter coupled with enhanced respiration, is related to the onset of winter dormancy. An acclimation process enabling the plant to continue growth at low temperatures has not occurred.

The effect of differing hardening regimes (20/15C and 10/5C) on the subsequent rates of photosynthesis and dark respiration in tall fescue leaves was studied at 10, 15, 20 and 25C. Net photosynthesis per unit leaf area differed little in plants from either regime, and was not

significantly affected by measuring temperature, possibly due to the low light intensities used (1000-1200 f.c.). On the other hand, photosynthesis per unit dry weight was greater in the warm grown leaves. These leaves had a higher optimum temperature and a shorter life. Respiration rates of the cool grown plants were significantly higher at all measuring temperatures; at 10C, for example, their respiration rates were two to three times higher. These respiratory increases were not considered adaptive but were attributed to higher levels of soluble carbohydrate. The lowering of the temperature optimum for net photosynthesis observed in the cool grown plants was also not considered to be due to adaptive changes in the photosynthetic apparatus, but as simply the effect of the increased respiration (Wooledge and Jewiss 1969).

Field studies of three cool semi-desert species showed no significant shifts in respiration rate, whereas net photosynthesis had a pronounced temperature optimum which was subject to considerable seasonal variation in Agropyron and Artemisia but not in Gutierrezia (Depuit and Caldwell 1975). The seasonal pattern in net photosynthesis of Artemisia depended on several factors, including plant water potential, leaf temperature, irradiation level and state of phenological development. No discernible circadian rhythm was apparent (Depuit and Caldwell 1973).

Climatic races of Mimulus cardinalis showed marked differences in photosynthesis at different temperatures and light intensities. Race 1, from a mild climate, had the highest overall photosynthetic activity from 0-49C. Races 5 and 6, from a region with cold winters and a much shorter growing season, show a much sharper drop in photosynthesis at high and low temperatures, i.e. a greater Q_{10} value (Milner and Heesey 1964).

Although these results seem discouraging in relation to the development of cultivars for cooler climates which have a wide range of photosynthetic efficiency over a range of temperatures, it should be remembered that this drop off in photosynthesis of the cool grown plants is probably related to the onset of dormancy as a prelude to developing winter hardiness. Arctic species which do not become dormant at these temperatures do not exhibit a similarily sharp decrease in photosynthesis and in fact have a smaller Q_{10} value than when grown in milder climates (Wager 1941).

All races of Mimulus had a photosynthetic temperature optimum of 30C, which contradicts results from other species indicating shifts in the optimum with differing environmental conditions (Milner and Heesey 1964). The authors attribute the differences in photosynthetic capacity over the temperature range tested to the natural selection of divergent genetic strains during evolution. It is generally agreed that plants do possess genetic adaptations which allow them to adjust to specific environmental conditions (Bjorkman and Holmgren 1963), although the limits of adaptability vary for each species.

Pronounced and characteristic variations in the temperature dependance of net photosynthesis per unit dry weight of two perennial desert species, Prunus armeniaca and Hammada scoparia were found during measurements under a variety of moisture conditions. No general correlation was discovered between these changes in temperature dependance, the daily photosynthetic maximum under field conditions, and the maximum photosynthetic capacity under experimental conditions. These seasonal variations were also not correlated with changes in respiratory activity, which showed only

slight seasonal variation. Under experimental conditions, acclimation of photosynthesis to different temperature regimes was very rapid for both species, occurring within 24 hours (Lange et al 1974).

Lange considers several reasons for the shift of the temperature dependance curve: 1) morphological and anatomical changes in the photosynthetic organs induced by water stress. These changes alter subsequent photosynthetic response. This cause is dismissed as unlikely since although Hammada plants grown under dry conditions do show anatomical changes not apparent in irrigated plants, both show the same shifts in temperature dependance.

2) changes in the temperature response of the stomata. Such changes have been suggested as a cause of shifts in the temperature dependance curves of Xanthium (Drake and Salisbury 1972), Prunus and Hammada (Schulze et al 1973). However, Lange (1974) dismisses this factor, since the temperature optimum and the CO_2 compensation point shifted in parallel throughout the season, making an important role for stomata unlikely.

3) seasonal changes in biochemical and/or biophysical properties of the photosynthetic and/or respiratory metabolism. For the species studied, seasonal shifts in respiration are not significant and so it is assumed that changes to the photosynthetic apparatus are involved, though these were not considered specifically (Lange et al 1974).

A variety of mechanisms may be involved in different species; for example, pronounced changes in the Hill activity of isolated chloroplasts of Oxyria were observed after temperature pretreatment (Billings et al 1971).

A conditioning effect of temperature on net photosynthesis in C_3 plants could be due to changes in photorespiratory activity (Lange et al

1974). A decrease in oxygen inhibition of net photosynthesis in Encelia was observed as the plants adjusted to warm temperatures after a cool pretreatment (Mooney and Harrison 1970). Plants conditioned to warm temperatures may be able to change their photosynthetic behaviour towards those of C_4 plants. Modifications in the temperature dependance of net photosynthesis may be based on shifts in the CO_2 fixation pattern (Lange et al 1974). These modifications are of significance only in the case of warm temperature acclimation, since photorespiration is negligible at low temperatures (Bidwell 1975).

In some cases the shift in temperature dependance of the net photosynthesis curve is accompanied by changes in the plant's absolute photosynthetic capacity, resulting in an inferior overall performance. Coastal ecotypes of Atriplex hymenelytra, a C_4 winter active perennial, grown at 23C have a photosynthetic rate at 40C which is 60% of maximum, which occurs at 30C. When the same clone is grown at 43C there is an upward shift of the temperature optimum such that the rate at 40C is 100% of maximum. However, the absolute rate at 40C declined to 1/2 the rate of the cool grown plants at 40C, indicating injury at the high temperature regime rather than photosynthetic acclimation (Pearcy et al 1974; Pearcy 1976, 1977). These results deal with high temperature regimes, but similar examples can be given regarding low temperature acclimation of C_4 plants. When Tidestromia plants from Death Valley are grown at 40C the optimum temperature is 43C. When grown at 16C the optimum shifted downward considerably to 28C, but the absolute maximum rate declined to less than 1/3 of the maximum for the warm grown plants. These results indicate an inability to respond to low temperatures rather than a true acclimation process (Bjorkman et al 1975). A shift in the

temperature dependance of net photosynthesis is therefore not sufficient evidence of acclimation to either low or high temperature (Mooney et al 1978).

Maximum photosynthetic and leaf respiratory rates of field grown Larrea divartica, a desert shrub, showed no appreciable seasonal shifts. However, there were pronounced shifts in the temperature dependance of net photosynthesis. The optimum shifted from near 20C in January to 32C in September. Stomatal conductance was little affected by measuring temperature and remained constantly high throughout the season, suggesting that stomata play no role in these shifts. No similar shift was observed in the temperature dependance of quantum yield, nor in leaf nitrogen content in per cent of dry weight. No positive correlation was found between rates of light saturated photosynthesis or dark respiration and nitrogen content or specific leaf weight, when all are expressed on a leaf area basis. It was concluded that the seasonal shift in temperature optimum was due to adaptive changes in intrinsic photosynthetic characteristics rather than changes in respiration (Mooney et al 1978).

Similar studies conducted in the lab holding all other environmental conditions constant showed a similar shift in temperature dependance, indicating that temperature alone is sufficient to alter the temperature dependance of net photosynthesis. Two distinct effects of temperature acclimation on net photosynthesis were distinguished though not described; growth at low temperature increased photosynthetic capacity, whereas growth at high temperature increased the thermal stability of the photosynthetic apparatus (Mooney et al 1978).

The possibility that growing Larrea plants under different temperatures influences the inhibitory effect of 21% O_2 on net photosynthesis,

i.e. affects net photosynthesis indirectly through photorespiration, was also investigated. However, results discredited this theory since there were no significant differences in the relative affinites to CO_2 and O_2 of ribulose-1, 5 diphosphate carboxylase/oxygenase nor in other possibly limiting aspects of photorespiratory metabolism (Mooney et al 1978).

The observed increase in photosynthetic capacity of plants adapted to low temperature may be due to an increased amount of one or several rate-limiting enzymes. Increases in the quantity of RuDP carboxylase have been reported for some species (Bjorkman and Pearcy 1971). However, for Larrea plants grown at low temperature, increased photosynthetic capacity is not attributable to such quantitative changes in enzymes, nor to increased chlorophyll content (Mooney et al 1978). Alternatively, qualitative changes resulting in increased catalytic activity at low temperatures may be involved, although what these might be in Larrea has not been discovered (Mooney et al 1978). Alterations in enzyme activity have been suggested as responsible for increased photosynthesis at low temperatures for acclimated specimens of Lolium (Wilson and Cooper 1969). The authors determined that preconditioning effects could not be attributed to mesophyll cell size or thickness, nor to stomatal characteristics, although which particular enzymes are affected and how is not known. Of a number of enzymes investigated in cotton, a chilling sensitive species, a correlation between enzyme activity and temperature dependant photosynthetic response after temperature pretreatment was found for carbonic anhydrase only. Changes in the activity of this enzyme could alter CO_2 availability at the carboxylation site (Downton and Slayter 1972).

Influence of three growing regimes (15/10C, 24/19C, and 33/29C) on

the photosynthetic and respiratory rates of Pinus raidata seedlings was investigated by gas analysis. Measurements were taken at 9, 16, 24 and 30C under a light intensity of 3000 f.c. Rates of acclimation to differing regimes were also studied by taking measurements daily after transfer. Net photosynthesis was expressed as mm^3 CO_2 absorbed per plant per minute. Rates of net photosynthesis were markedly lower at 30C for the 15/10C plants and at 9C for the plants from the two warmer regimes. The temperature optimum was 16C for both the 15/10C and 24/19C plants, and 24C for the 33/29C plants (Rook 1969). A shift of four to seven degrees C also occurs in the photosynthetic temperature optimum of Douglas fir as the result of preconditioning (Sorenson 1964). In Pinus, gross photosynthesis shows a slightly different trend due to the estimated effects of respiration in the light, which was presumed to be equivalent to dark respiration (Rook 1969).

Changes in respiratory activity of acclimated Pinus seedlings were even more marked. Cold grown plants had respiration rates twice that of the warm grown plants (Rook 1969). These results again concur with those obtained for Douglas fir, where increases of 100% were reported in cold grown plants (Sorenson 1964). These respiratory increases are attributed to high soluble carbohydrate levels and are considered a wasteful consumption of reserves which is not adaptively linked to growth. Moreover, translocation rates are presumed to be more inhibited than photosynthesis, and this factor, combined with a reduced sink size due to growth inhibition, has the ultimate effect of severe inhibition of photosynthesis at low temperatures. The photosynthetic apparatus can adapt somewhat, but respiration rates remain high and represent a continual drain on the

plant's energy reserves. The observed changes in gas exchange rates of Pinus seedlings occur very rapidly and are complete several days after transfer (Rook 1969).

Adaptiveness of these Changes in Gas Exchange Rates of Acclimated Plants

In general, then, alterations to the temperature optimum of net photosynthesis and a concommitant increase in respiration have been cited as a frequent response to temperature stress (Pisek and Kemnitzer 1968; Bauer et al 1969), although shifts in respiratory activity have not been observed for all species. Whether or not these changes are adaptive is not always clear. It is evident that low temperature acclimation requires the efficient use of available energy, which results from a high photosynthetic capacity as well as a properly balanced respiratory activity (Bjorkman 1966). Bjorkman (1966) distinguishes two causes of increased respiration during low temperature acclimation: 1) increased respiration due to exposure during active growth to low temperatures which inhibit growth and lead to an accumulation of substrates no longer being used in growth processes. This is the cause most often cited for increased respiration and is not considered adaptive.

2) consistently higher respiration rates observed in Arctic species due to increased activity and possibly increased amounts of respiratory enzymes. These changes are an integral part of acclimation and result from the augmentation of protein in the mitochondria of acclimated plants (Siminovitch et al 1967). Photosynthesis at low temperatures must replace this chemical energy used in respiration, and the rate of photosynthesis possible determines the maximum respiration rate beneficial to the plant (Bjorkman 1966). In

this context, respiratory increases are adaptive.

Although it is possible to acclimate many species to a particular set of environmental conditions, some researchers doubt whether it is possible to acclimate a plant to respond favourably to a range of environmental conditions. Tradeoffs seem to exist such that optimization for one environmental condition leads to less effective performance in another. Hence the capacity to tolerate or function effectively over a range of temperatures may necessitate sub-optimal efficiency at any one temperature. (Berry 1975).

It is not clear why these tradeoffs should exist. For example, the high photosynthetic capacity of Atriplex at low temperatures is due to massive amounts of enzymes which catalyze steps normally limiting to photosynthesis at low temperatures (Bjorkman and Pearcy 1971). These adaptive changes prove an unexplained liability to the photosynthetic efficiency of these plants at high temperatures (Bjorkman et al 1973). Acclimation seems geared to the production of specialized ecotypes rather than plants adapted to a wide range of environmental conditions. Although the latter capacity is more desirable for agronomic applications, the former seems more successful in nature (Berry 1975).

Inhibitory Effects of Cool Night Temperatures on C_4 Plants

A C_4 plant, Panicum, was grown under three temperature regimes: 30/30C, 33/20C and 30/10C. As night temperatures were lowered, the plants took progressively longer to reach maximal photosynthetic rates the following day. This inhibitory effect was closely correlated ($r=0.90$) to the stomatal resistance to CO_2, which was significantly greater at cool night

temperatures (Ku et al 1978). These studies question the hypothesis that there is a negative feedback inhibition of photosynthesis by the accumulation of soluble assimilates in the leaf. Although there is a slight correlation between photosynthetic rates and carbohydrate concentration, it is not as marked as that between photosynthetic rates and stomatal resistance to CO_2 transfer. Also, activities of RuDP and PEP carboxylase were similar in plants from all regimes (Ku et al 1978).

The sluggishness observed in stomata of prechilled leaves which results in delayed opening and inhibition of photosynthesis may be mediated by increases in ABA content. Chilling induces an increase in ABA in young leaves, which in turn sensitizes stomata to CO_2 (Raschke et al 1976). Alternatively, delays in stomatal opening may be due to photosynthetic inhibition at low temperatures, which results in increased CO_2 concentrations in intracellular spaces and substomatal cavities. These increases inhibit stomatal opening in spruce and pine seedlings (Christersson 1974).

The photosynthetic rate of Sorghum, a C_4 grass which is more chilling sensitive than Panicum , is greatly reduced by exposure of the shoots to cool night temperatures ranging from 5-15C. This inhibition is attributed to similar stomatal effects and is associated with the development of temporary water deficits (Pasternak and Wilson 1972). In other C_4 plants, low night temperatures interfere with starch hydrolysis and translocation from the chloroplast due to temperature sensitive hydrolytic enzymes (Hilliard and West 1970; Lush and Evans 1974; Hilliard 1975). These authors have suggested that subsequent growth reductions caused by low night temperatures are due to carbohydrate accumulation rather than changes in stomatal resistance.

Just as warm night temperatures are critical for maintaining the photosynthetic efficiency of many C_4 plants, cool night temperatures are beneficial to numerous other species (Leopold and Kriedemann 1974). Lowering night temperatures enhances root growth, yield, earliness of flowering and fruit development of many temperate species (Went 1945, 1957, 1959). These stimulating effects on growth are thought to be due to a systematic plant response involving hormones rather than the direct stimulation of a particular organ (Nielsen and Humphreys 1966). The lack of a day/night differential, as opposed to specific day/night regimes, is detrimental to the growth of many species including peas (Highkin and Lang 1966).

Other Deleterious Effects of Low Temperatures on Plant Growth Processes

Some cultivars of corn are sensitive to photobleaching at temperatures as high as 16C (Teeri and Stowe 1976). Of all the environmental factors tested, daily minimum July temperatures have the strongest correlation to the relative abundance of C_4 grasses in regional flora (Teeri and Stowe 1976). Besides the avoidance of low temperature injury, this requirement of C_4 plants for warm day and night temperatures is due to the higher translocation rates characteristic of C_4 species (Teeri and Stowe 1976).

Low temperature inhibition of photosynthesis in C_4 plants may also be due to the inhibition of both chlorophyll synthesis and chloroplast development in the 2-12C range, as well as the already mentioned stomatal effects and the possibly deleterious effects of carbohydrate accumulation. No single metabolic or physical factor controls low temperature stress response; the changes which occur are determined by the kind and duration of stress as

well as the plant's genetic constitution (Teeri 1977).

The Role of Membranes in Cold Acclimation

General Comments

Because of their important role in the physiology of the plant cell, membranes are critical to cold acclimation. Membranes perform two important functions which are affected by acclimation: 1)structural role as a semi-permeable barrier controlling movement of substances in and out of the cell and its membrane bound organelles. Membrane structural properties are vital for maintaining the cell's osmotic balance and must be preserved to avoid freezing injury. Membranes are the primary site of freezing injury in plants (Levitt 1972); 2)biochemical role as the site to which many photosynthetic and respiratory enzymes are bound, particularly those involved in electron transport. The integrity of the mitochondrial and chloroplast membranes must be maintained if these systems are to function effectively at low temperatures. Loss of these functional membrane properties causes chilling injury (Lyons 1973).

In this section I will examine some of the alterations to plant membranes which occur during acclimation and which allow plants to preserve both of these important membrane functions, as well as some of the freezing and/or chilling injuries which result when they are impaired. Similar alterations may protect plant membranes from both types of low temperature stress, but each will be considered separately.

Acclimation of Membranes to Freezing Temperatures

Cellular membranes have been proposed as the major site of freezing

injury since membrane damage invariably accompanies freezing damage and is in fact the basis of all major methods used to evaluate damage (Stout et al 1978). Cold acclimation enables membranes to survive freezing temperatures by inducing changes in membrane quantity, composition, environment and function (Stout et al 1978). For example, the amount of membrane phosopholipid increases during acclimation of black locust (Siminovitch et al 1968) and poplar (Yoshida and Sakai 1973, 1974; Yoshida 1976). There is a direct relationship between amount of membrane phospholipid and environmental temperature. At 0 C, levels of two main phospholipids, phosphatidylcholine and phosphotidylethanolamine increased sharply, accompanied by a sharp drop in triglycerides (Yoshida and Sakai 1973). These increases parallel the seasonal development of hardiness in poplar and are due to augmentation of membrane systems, specifically an infolding of the plasma membrane and a proliferation of small vesicles (Yoshida 1976). Species incapable of acclimation undergo rapid degradation of membrane phospholipids (Yoshida and Sakai 1974).

Functional alterations to membranes during cold acclimation have received the most attention, and have been attributed largely to compositional changes (Levitt 1972). Membrane permeability to water was thought to increase as a result of these changes, allowing the plant to avoid intracellular ice formation (Levitt 1972).

As a result of subsequent experiments this theory has been questioned (Stout et al 1978; McKenzie et al 1974). Early indications that freezing and desiccation resistance in young bean roots could be induced by decenylsuccinic acid, a compound which increases membrane permeability and makes it less temperature dependant (Kuiper 1964) have since been refuted (Weiser 1970). It seems, then, that increases in membrane permeability to water

at low temperature do not represent a functional change which is causally related to acclimation. In fact, excessive increases in membrane permeability, particularly to ions, are usually an indication of freezing injury, which causes a rapid disorganization of the plasma membrane and subsequent release of electrolytes into the intercellular spaces (Alden and Hermann 1971; Nobel 1974). Chilling injury in cotton (Powell 1969), corn (Creencia 1971) and cucumber (Wright and Simon 1973) is due to a similar loss of membrane semi-permeability. Therefore, although increases in water permeability may not be critical to acclimation, maintenance of the cell's semi-permeability is essential for avoiding the osmotic shrinkage which is a major factor in freezing injury (Towill and Mazur 1977) and for maintaining cell compartmentalization (Alden and Hermann 1971).

The second and possibly more critical alteration in membrane function during acclimation involves protection of the phosphorylating system. However, most of the emphasis has been on freezing damage to this function, with less attention given to how acclimation prevents such damage. Freezing inactivates phosphorylation in isolated spinach chloroplasts (Heber 1967; Williams 1970). Heber (1967) cites two possible causes of inactivation: 1)denaturation of cold-labile enzymes; 2) physical alterations to membrane structure resulting in the uncoupling of electron transport from phosphorylation. Although ATPase was inactivated by freezing and could be protected by sucrose, the major effects were on membrane structure. Freezing inactivated proton uptake and the light dependant shrinkage of choloroplast lamellae by inducing conformational changes in the membrane which prevent the formation of the proton gradient necessary for phosphorylation, as explained by the chemiosmotic theory. Uncoupling and subsequent freezing injury occurs because of

leakage resulting from freezing induced permeability changes (Heber 1967). Exactly how freezing inactivates proton uptake and results in uncoupling is not clear, but it is known that sucrose can protect membranes from these non-adaptive conformational changes (Heber 1967).

High concentrations of electrolytes resulting from desiccation stresses associated with extracellular ice formation may be the causal factor which inactivates membranes by irreversibly uncoupling phosphorylation from electron transport (Santarius 1971). Sugars, sugar alcohols, soluble proteins, salts or organic acids can protect membranes; moreover, the relative amount of these cryoprotectants can enhance or retard their protective properties (Santarius 1971). These results suggest a non-specific colligative action, although others suggest a specific cryoprotective role for sucrose (Garber and Steponkus 1976) or a low molecular weight protein (Heber 1970).

Without the protection of sucrose, isolated poplar chloroplasts are unable to form a light dependant proton gradient (Garber and Steponkus 1976). The concentration of sucrose neeeded for protection is inversely related to the prevailing temperature. Reasons for this loss of membrane functional properties are multiple and include 1)vesicularization, which results in an unavoidable release of plastocyanin; 2)release of CF_1, which can be prevented by sucrose; 3)disruption of thylakoid membrane semi-ermeability, also preventable by sucrose. Freezing, then, involves multiple stresses to membranes which result in a loss of activity (Garber and Steponkus 1976). In these experiments, uncoupling was reversible, and membrane activity could be restored.

In contrast to these results with chloroplast membranes, mitochondria

in both hardened and non-hardened rye cells were relatively insensitive to extracellular freezing stress and retained their functional capacity (Singh et al 1977). It was concluded that alterations to mitochondrial membranes and the associated respiratory apparatus could not acccount for differences in cold hardiness.

The issue of differential effects of acclimation on different plant membranes has not been resolved (Stout et al 1978). Alterations to the plasma membrane of English ivy have been observed during acclimation, but it is not known whether these alterations are related to the degree of cold hardiness (Wiest and Steponkus 1977). Other workers have stressed the importance of alterations to the plasma membrane as well as organelle membranes (Chen et al 1978). Proliferation of another membrane system, the endoplasmic reticulum, has been observed during ice encasement of winter wheat, but whether these changes are protective or destructive is not known (Pomeroy and Andrews 1978).

Freezing injury, then, is not necessarily due to complete membrane disruption but rather to biochemical alterations which interfere with membrane function (Chen et al 1978). The adaptive changes which occur during acclimation and protect membranes from damage may be strictly environmental, involving sucrose and other cryoprotectants, but more likely also involve compositional changes to the membranes themselves which are not fully understood (Levitt 1972; Stout et al 1978).

Effect of Chilling Temperatures on Membranes

Chilling injury is also due to a loss of membrane function, but whereas freezing temperatures uncouple electron transport from phosphorylation

in frost sensitive plants, chilling temperatures are thought to disrupt membrane function by increasing the activation energies of membrane bound enzymes (Lyons 1972, 1973). However, which compositional features in the membrane cause these abrupt increases in activation energies in chilling sensitive plants, and which compositional alterations prevent these increases during acclimation, is a subject of considerable debate.

One side of this debate, associated mainly with Lyons and Raison, and reviewed several time by them (Lyons 1972, 1973; Raison 1973), can be summarized as follows. The inability of chilling sensitive plants to maintain physiological activity at chilling temperatures is correlated with an increase in activation energy of the respiratory enzymes (Lyons and Raison 1970; Raison et al 1971; Raison 1973, 1977; Peoples et al 1978), as well as an increase in the activation energy of the photoreduction of $NADP^+$ (Shneyour et al 1972; Raison 1973). These increases are in turn correlated with a temperature induced phase change in the membrane lipids (Raison et al 1971; Shneyour et al 1972; Raison 1973; Lyons 1972, 1973). These phase changes have been detected by a change in the mobility of spin labels, and occur at the same temperature as the changes in activation energies (Murata et al 1975; Raison et al 1971). In physical terms, this phase change is described as a sharp transition of the membrane phospholipids from a liquid crystalline to a 'cogel' state which in turn induces conformational changes in the active centre of the enzyme (Raison 1973). Similar changes in activation energy or in the phase state of the membrane lipids were not found in chill resistant plants (Lyons and Raison 1970; Raison et al 1971; Shneyour et al 1972; Murata et al 1975). Chilling resistant plants retain greater membrane flexibility and activity at low

temperatures due to a higher proportion of unsaturated fatty acids in their membrane lipids (Lyons et al 1964; Friedman 1977).

The central tenet of this theory is that the observed alterations in enzyme kinetics are not intrinsic properties of the enzymes at low temperatures, but are due rather to the association of the enzyme with the mitochondrial membranes. Enzymes extracted from the mitochondrial membranes of rat liver and sweet potato, including succinic oxidase, succinic dehydrogenase and cytochrome c oxidase, have a uniform activation energy (E_a) over the temperature range from 1 to 36C. The breaks observed in the E_a of these same enzymes when still bound to the membrane are thought to be caused by a phase change in the lipid component of the membrane, which in turn causes configurational changes in the enzymes (Raison et al 1971; Raison 1973).

These effects are not limited to the kinetics of respiratory enzymes. C_4 plants exhibit sharp drops in photosynthesis at temperatures below 12C, purportedly due to changes in the activation energies of membrane bound enzymes involved in electron transport, notably the photoreduction of $NADP^+$ (Raison 1973). These increases in E_a can be as high as three fold, but unlike temperature responses in mitochondria, are not related to the relative proportion of saturated and unsaturated fatty acids in the chloroplast membranes. However, the high proportion of linolenic acid present would suggest a phase transition should occur well below 10C, indicating that factors other than fatty acid unsaturation may determine phase changes in chloroplast lipids (Raison 1973). It seems that in both cases membrane lipids are the locus of chilling temperature sensitivity, but it is not clear whether changes in total lipid or specific lipids associated with

enzyme sites on the membrane are more important (Raison 1973).

Chloroplasts from tomato, a chilling sensitive C_3 plant, showed a similar break in E_a at 12C, whereas none was apparent in chloroplasts from chilling resistant pea and lettuce. This temperature effect was reversible and localized in photosystem 1 (Shneyour et al 1972). However, not all of the reactions involved in photosynthetic electron transfer undergo increases in E_a, as was the case in respiratory electron transfer; only the terminal electron transfer reaction is affected. Since the increase in E_a is not an intrinsic property of the enzyme, it was concluded that this terminal enzyme is located in a distinct region of the chloroplast membrane apart from the other enzymes and that this region alone is affected by phase changes in the membrane lipids. The ultimate results of these changes are a decrease in ATP levels and injurious metabolic imbalance (Shneyour et al 1972). Electron transfer in spinach chloroplasts is also inhibited at low temperatures by physical changes in the chloroplast membranes (Yamamoto and Nishimura 1976).

Chilling resistant alfalfa cultivars exhibited only moderate reductions in photosynthesis at 10C and contained a significantly higher percentage of unsaturated fatty acids in their chloroplast membranes, as well as a greater double bond index than chilling sensitive cultivars, which exhibited severe reductions in photosynthesis at 10C. These reductions were negatively correlated ($r=-0.90$) with the double bond index of the chloroplast membrane (Peoples et al 1978). In contrast to aforementioned studies (Raison 1973), these results suggest that photosynthetic reductions at chilling temperatures are related to the fatty acid composition of the membranes in the chloroplast due to the increased flexibility associated with a higher proportion of unsaturated fatty acids (Peoples

et al 1978).

It is not clear whether the main effect of chilling temperatures on the respiratory apparatus is on oxidation or phosphorylation. In sweet potato tissue stored at OC, a sharp decrease in respiration was observed after ten days, indicating chilling injury. Oxidative activity dropped off sharply, but the P/O ratio remained relatively constant, suggesting that the primary effect is on the oxidative system (Minamikawa et al 1961).

Others claim that the major effect is on phosphorylation. The primary effect of chilling in cotton is as inhibition of membrane associated phosphorylation, leading to a depletion of ATP (Creencia 1971; Stewart 1969). THis inhibitory effect is reversible in cotton, and ATP levels are restored if plants are removed from chilling conditions. Inhibition is attributed to the relative inflexibility of membranes in chilling sensitive plants (Stewart 1969).

Enzyme effects may be involved as well. In unhardened cotton plants, hydrolytic phosphatase activity increases with chilling. Acclimation prevents this increase as well as inducing structural changes in the membranes which also help to preserve phosphorylating ability (McStewart and Guinn 1971). Resistance to chilling damage in germinating cotton is correlated to a higher degree of fatty acid unsaturation in the membranes. The function of these compositional changes is believed to be the avoidance of phase changes which decrease membrane flexibility and lead to a progressive inactivation of membrane systems, including phosphorylation (Clay et al 1976). Decreases in DNA activity during exposure to 2C are also attributed to similar membrane effects (Clay et al 1976).

Other examples which support Lyons' general theory of chilling injury

and resistance are numerous in both plant and animal species. Mitochondrial membranes from warm blooded animal tissue is incapable of swelling at low temperatures, whereas membranes isolated from cold blooded animals retain this ability. A correlation was observed between this ability to swell and the fatty acid unsaturation of the mitochondrial membrane. Cold blooded animals had a higher degree of unsaturation, permitting greater flexibility and hence the continuation of metabolic functions at low temperatures (Richardson and Tappel 1962). Warm blooded animals show similar increases in activation energies at low temperatures as seen in chilling sensitive plants; these increases are attributed to changes in the phase state of the membrane (Raison 1973).

Chilling sensitivity in soybeans also seems to derive from two related factors: 1) changes in membrane molecular organization and 2) increases in E_a of membrane bound enzymes as a result of phase changes (Duke et al 1977; Bramlage et al 1978). These phase changes also result in membrane leakage and extensive disruption due to a loss of compartmentalization (Bramlage et al 1978). Further examples supporting this theory can be found in Lyons' review articles (1973, 1974).

Despite the large body of experimental work which supports this theory, increasing numbers of researchers have disputed it in whole or in part. A temperature induced phase change was observed in the mitochondrial membranes of apple, but the temperature at which it occurred did not correlate with susceptibility to low temperature injury. Metabolic changes other than phase changes in the lipids, such as the accumulation of toxic products like ethanol, may be the primary cause of injury. Some apple cultivars can compensate for these metabolic imbalances which occur at low temperatures

and so prevent injury (McGlasson and Raison 1973).

Most of the controversy has centred around the importance of fatty acid unsaturation to chilling sensitivity and resistance. During hardening of bean, a chilling sensitive species, the percentage of fatty acid unsaturation did increase, preventing water loss and electrolyte leakage by lowering the temperature of the phase changes in the membrane lipids (Wilson 1974). However, comparison of chilling resistant and sensitive species shows that chilling at 5C reduces the percentage of linolenic acid and the total weight of fatty acids in sensitive species but has no similar effect on resistant species such as barley. Acclimation, then, may reduce the degree of chilling injury in susceptible species by slowing down detrimental processes such as the drop in percentage of linolenic acid rather than by increasing unsaturation levels as such (Wilson and Crawford 1974). The temperature of the phase change seems to be determined by a more complex set of factors than fatty acid unsaturation, including the possible role of sterols (Wilson and Crawford 1974; Miller et al 1974).

Determination of the role of fatty acid composition in chill hardening requires precise analytical techniques. Changes in the fatty acid composition of specific lipids may occur during hardening and not be detected by studies conducted on the total lipid fraction. For example, hardening may prevent chilling injury by increasing the degree of unsaturation of phospholipids which are only a small component of the membrane in terms of total fatty acid composition (Wilson and Crawford 1974). The physiological age of the tissue is also important. Degree of unsaturation in membrane lipids increases only in young bean leaves, stressing the importance of using juvenile tissue to study acclimation. Fruit tissue is often incapable of

acclimation due to advanced age (Wilson and Crawfor 1974). Moreover, changes in fatty acid composition for plants exposed to freezing temperatures cannot be extrapolated to chilling temperatures, since different changes may occur (Wilson and Crawford 1974). These represent just a few of the complicating factors which make it difficult to evaluate the multitude of data regarding the role of unsaturation in acclimation (Alden and Hermann 1971).

Seedlings of winter wheat grown at 2C did have higher phospholipid content, a marked increase in linolenic acid, enhanced membrane synthesis and a greater degree of fatty acid unsaturation as compared to the 24C controls (De la Roche et al 1972). However, only one cultivar was used, making it impossible to determine if these changes were simply low temperature effects or directly related to acclimation.

Subsequent experiments using four cultivars of contrasting hardiness showed that similar changes occurred in all cases and that differing proportions of linolenic acid were not correlated with hardiness. Changes in fatty acid unsaturation are therefore not a primary factor in the cold hardiness of winter wheat (De la Roche et al 1975). Observed increases in fatty acid unsaturation at low temperatures have been attributed to an increase in O_2 in solution, and are strictly low temperature responses (Harris and James 1969).

Similar results have been obtained by other workers using cultivars of contrasting hardiness. Not only levels of unsaturation but also the functional properties of mitochondria are similar and unrelated to hardiness when samples from all cultivars are grown in the dark at acclimating temperatures (Pomeroy 1974). Pomeroy (1974) has also questioned the importance of

lipid phase changes to mitochondrial functioning at low temperatures. Discontinuities were observed in the Arrhenius plots of respiratory activity versus temperature for wheat and rye seedlings grown at both 2C and 24C, indicating phase changes in the membranes. However, no correlation was observed between the cold hardiness of the cultivars and the temperature of the phase changes. Respiratory control and efficiency of phosphorylation were also not affected by acclimation (Pomeroy and Andrews 1975).

These results demonstrate the absence of a growth temperature effect on whole cell respiration or on oxidation rates of isolated mitochondria, since no correlation was observed between respiratory rates, efficiency of phosphorylation, respiratory control, transition temperature and the degree of hardiness of the seedlings. Changes in respiratory parameters, it was concluded, are not directly related to acclimation either to freezing or chilling temperatures in winter cereals. Furthermore, phase change temperatures cannot be attributed solely to the degree of fatty acid unsaturation in winter wheat, since although unsaturation does increase during growth at 2C, transition temperatures are similar for both 2C and 24C seedlings. Other factors must be involved in the abrupt phase changes observed at low temperatures (Pomeroy and Andrews 1975).

Another chilling resistant plant, barley, also exhibits abrupt changes in E_a of its membrane bound enzymes, which according to Lyons' theory are not supposed to occur in resistant plants. Both respiratory and photosynthetic enzymes are affected, and these changes in enzyme kinetics are correlated neither with degree of chilling sensitivity nor degree of fatty acid unsaturation (Nolan and Smillie 1977). Temperature induced changes in Hill activity observed at 9C coincided with changes in the fluidity of

thykaloid membranes as detected by electron spin resonance. However, rather than being related to chilling injury, these changes seem to be a part of the control mechanism for the regulation of chloroplast development and photosynthesis at low temperatures (Nolan and Smillie 1976). Structural changes to chloroplast membranes during acclimation are thus not necessarily related causally to cold hardiness (Garber and Steponkus 1976).

Phase changes have also been reported in other chilling resistant species. Growing winter wheat at 2C lowered the temperature of the phase change when compared to the 24C control. This drop was attributed to increased unsaturation levels, which decreased the ordered packing of the hydrophobic portions of the membrane. By lowering the temperature of the phase change, acclimation maintained the structural properties necessary for high levels of respiratory activity at low temperatures (Miller et al 1974).

Such contradictory findings on the occurrence and importance of phase changes and alterations in enzyme kinetics may be due to errors arising from the detection of these phase changes. Variation with temperature of the substrate-binding affinity of a membrane bound respiratory enzyme can strongly influence its Arrhenius plot, altering the measured E_a values and the phase change temperature. Moreover, if enzyme activity is measured at a single fixed substrate concentration, artifactual breaks can occur. Thus, serious errors in the interpretation of Arrhenius plots can arise if temperature variations in substrate binding affinity are not considered. These errors may contribute to the confusion surrounding the relation of membrane properties to function at low temperatures (Silvius et al 1978). The interpretation of ESR data and the construction of

Arrhenius plots from this data has also been criticized on mathematical grounds as being inaccurate and misleading (Cannon 1975).

Another matter of debate is the importance of mitochondrial swelling and contraction at low temperatures, as related to membrane flexibility. Mitochondria from unhardened wheat and rye seedlings show a spontaneous or energy independant swelling and an energy dependant contraction, whereas cold grown plants are incapable of contraction (Pomeroy 1974). This inability to contract is due to an impairment of electron transport which prevents the establishment of the proton gradient required for the energy dependant contraction (Pomeroy 1974).

In contrast, chilling sensitive tissue, such as tomato, is incapable of the initial spontaneous swelling, whereas mitochondria from pea and turnip show a striking ability to swell. However, mitochondria from bean, also a chilling sensitive species, retained their swelling ability as well, making generalizations impossible (Lyons 1964). Although swelling and contracting behaviour of plant tissue is not clearly understood, it is believed to be an important indicator of membrane flexibility (Lyons 1964). Recently the importance of membrane flexibility has been questioned. No correlation was found between cold hardiness and the extensibility of mitochondrial membranes of wheat and rye (Pomeroy 1976).

No clear cut conclusions can be stated about the alterations in membrane quantity, composition, environment, or function which are important to acclimation against freezing and/or chilling temperatures. The controversies surrounding this issue highlight the difficulty of establishing a causal relationship between acclimation and the changes which occur in the plant during growth at low temperatures (Pomeroy and Andrews 1976).

Changes in Important Classes of Plant Compounds During Acclimation

General Comments

Acclimation is an active process involving complex metabolic events within the plant. In order to understand these events, researchers have catalogued the changes in amounts and types of important compounds during acclimation. More specifically, changes in proteins, amino acids, carbohydrates, lipids and nucleic acids have been reported for a wide variety of species (Levitt 1956, 1972; Parker 1963; Alden and Hermann 1971). It is not possible for me to review these findings, nor would it be profitable. As Weiser (1970) points out, it is unlikely that the results of descriptive studies of metabolic changes will explain acclimation, since these results can be interpreted to support almost any and all hypotheses which have been proposed. However, I will report some of the more important changes discussed in the literature.

To indicate the extent of metabolic alterations during acclimation, changes were reported in all of the following compounds during cold hardening of dogwood tissue: total protein, specific proteins, lipid unsaturation, translocatable hardiness promoting and inhibiting factors, starch, sugars, nonvolatile organic acids, free and bound amino acids, organic and inorganic phosphorus, total RNA, tRNA, rRNA and DNA (Weiser 1970). Moreover, different biochemical trends are apparent during different phases of acclimation in dogwood. RNA and protein levels increase during the initial phase but subsequently decrease, as do sugars (Chen 1977). Some of the trends reported are obviously related; for example, the drop in starch is related to the rise in sugar levels. Similarily, a rise in

total protein is related to the observed drop in free amino acids (Li et al 1966).

Carbohydrates

Perhaps the most widely recognized event accompanying the onset of hardening is the increase in sugar levels, due either to photosynthate accumulation or starch-sugar interconversions (Levitt 1956, 1972). Levitt (1956) lists more than 50 references to such conversions, and he includes many more in his later review (1972). However, the possible conversion of these sugars to other sugar complexes and the actual role of sugars during acclimation is not fully understood (Parker 1963; Alden and Hermann 1971). Moreover, a correlation has not been established to cold resistance in all species studied, leading some to conclude that starch-sugar conversions are not crucial during hardening (Siminovitch et al 1968).

Such seemingly contradictory results about the relation of sugars to frost hardiness may be explained by the fact that sugar levels, though important, are not limiting to acclimation. Their role is not merely an osmotic effect acheived through accumulation, and hence it is not surprising that although total sugar content and hardiness increase during acclimation of English ivy, there is no direct parallel (Steponkus and Lanphear 1968).

Sugars are believed to be important in acclimation to chilling as well as freezing temperatures. Sugar levels increased during exposure to chilling temperatures in cotton, and are believed to protect proteins from dehydration stress, although such increases did not ensure hardiness. Starch levels also increased during acclimation of cotton, while levels of

RNA, protein and lipid soluble phosphate decreased (Guinn 1971).

Sugars are thought to be important cryoprotectants in plants. Different explanations have been given for their protective role. In dogwood, the effect of sugars is thought to be either a non-specific osmotic or colligative effect which permits the cell to avoid freezing, or an unknown metabolic effect which allows the cell to tolerate freezing stress (Chen et al 1977). Sucrose can protect ATPase from inactivation at freezing temperatures as well as preserving proton uptake and the osmotic properties of the chloroplast thylakoids (Heber 1967). Protection by sugars against freezing induced inactivation of electron transport and phosphorylation is thought to be due to a non-specific stabilization of membrane structure through alteration of their water binding properties (Santarius 1973).

Other possible roles of sucrose include: 1)retardation of ice crystal growth and hence prevention of sudden water loss from membrane proteins during freezing; 2)replaces water of hydration on membrane proteins; 3)increases water holding power of the protoplasm; 4)inhibits growth and so prevents bud break during warm periods; 5)energy source (Alden and Hermann 1971). Therefore, although the action of sugars is still in dispute, they are thought to protect membranes from the physical damage caused by freezing temperatures and to preserve membrane biochemical functions as well.

Various factors can affect the concentration of sugars needed. A low sugar concentration is needed to protect electron transport relative to that required to protect phosphorylation, indicating that the latter is more frost sensitive (Santarius 1973). Concentration required to protect

thylakoids varies with temperature, higher concentrations being required to preserve semi-permeability at lower temperatures (Garber and Steponkus 1976). Stage of physiological development and tissue age also affect the protective action of sugars, since tubers can increase in sugar concentration at low temperatures with little increase in cold hardiness (Sakai 1967).

Whether the protective action of sucrose is specific is not resolved. Other soluble sugars have been linked to hardiness, including raffinose, which increased four-fold during acclimation of dogwood (Li et al 1966). Raffinose is more effective than sucrose in stabilizing membrane structure at low temperature (Santarius 1973). Other sugars mentioned in the literature include glucose, fructose, stachyose, and melibiose (Alden and Hermann 1971). Different evaluations have been made of their protective capacity in different species. Earlier workers stressed the role of pentosans, but their importance has since been questioned (Alden and Hermann 1971). Cell wall polysaccharides may also influence freezing resistance either directly as cryoprotectants or indirectly through effects on cell wall properties or by interfering with ice crystal growth (Olien 1967; Alden and Hermann 1971).

Proteins and Amino Acids

Levels of water soluble proteins have also been correlated with cold hardiness (Levitt 1956, 1972). To cite one important example, the water soluble protein content of black locust increased during acclimation in the fall and decreased in the spring. This strong correlation suggested that soluble proteins are more important than carbohydrates in acclimation

since no correlation was found between hardiness and levels of soluble carbohydrate or starch (Siminovitch and Briggs 1953).

Some claim that the increase in protein is due to an increase in protein synthesis, while others claim that it is due to a breakdown of more complex proteins. Alternatively, both processes may be involved (Alden and Hermann 1971). Soluble proteins are thought to protect membranes from freezing damage in a non-specific fashion (Santarius 1973). Heber (1959) has postulated that a specific low molecular weight protein is involved, either by protecting the vital properties of protoplasm during freezing stress or by protecting sensitive membrane sites.

The importance of proteins to acclimation has also been questioned. Protein levels in English ivy increased gradually from July to April but showed no sharp increase paralleling an increase in hardiness. Protein levels did not drop off with the loss of hardiness in the spring (Parker 1962). Soluble proteins increase during hardening of alfalfa, purportedly due to a preferential increase in specific enzymes. However, differences between protein levels of alfalfa cultivars do not differentiate hardening capacity (Gerloff et al 1967). Similarily inconsistent results with various species may be due to the extractants used, since pH increases during acclimation and affects the extraction process. Suitable buffers must be used to avoid misrepresentative results (Faw 1976).

Total amino acid content of water soluble proteins in crowns of three cultivars of winter wheat followed seasonal variations in cold hardiness. Levels of five specific amino acids (alanine, arginine, aspartic acid, glutamic acid and histidine) were also correlated to hardiness (Pauli and Zech 1964). Other studies indicate an increase in amounts of

all amino acids in winter wheat (Alden and Hermann 1971). No differences were found in the amino acid composition of winter wheat cultivars of contrasting hardiness (Toman and Mitchell 1968). Similar experiments on amino acid levels in acclimating dogwood tissue also failed to produce conclusive results, although it was shown that some amino acids decreased, some increased, and others remained constant (Li et al 1965). Generally, amino acids are thought to be important because of the proteins synthesized from them, though a specific role has been suggested for proline (Alden and Hermann 1971).

Besides the cryoprotective role of proteins, specific enzyme proteins may also be important. Differences in the amounts and activities of a number of enzymes, including invertase, nitrate reductase, and various oxidases have been reported, with some indication that different forms of an enzyme, or isoenzymes, may dominate under different temperature conditions (Alden and Hermann 1971).

Nucleic Acids

Alterations in nucleic acid metabolism occur during acclimation, presumably because of the synthesis of substances important in the development of hardiness. Exogenous applications of purines and pyrimadines enhanced hardiness in alfalfa and were correlated with increases in water soluble and TCA precipitable protein, nucleic acids, and pH. Although these applications were not always effective, they can increase winter survival by 100% by inducing changes in the metabolism of non-hardy plants similar to those which occur in hardy plants under low temperatures and short photoperiods (Jung et al 1967). Ribosome number increased during

hardening of black locust. Ribosome structure was altered as well, possibly due to an altered cell environment at low temperature, or due to the production of hardiness-related proteins (Bixby 1976). Cold resistance may be related to high guanine and cystosine content in RNA (Jung et al 1967; Shih and Jung 1968).

Although increases in RNA are commonly reported, there is less consensus concerning changes in DNA levels during acclimation (Alden and Hermann 1971).

Lipids

Because of the important role played by membranes in acclimation, major changes occur in the lipid metabolism of the acclimating plant. A direct correlation exists between changes in total phospholipid and cold hardiness in poplar. These increases in total as well as specific phospholipids, namely phosphatidylcholine and phosphatidylethanolamine, are also correlated to a drop in triglycerides due to interconversions (Yoshida and Sakai 1973, 1974; Yoshida 1976). Similar trends in phospholipids have been reported for alfalfa (Kuiper 1970). However, this stimulation of phospholipid biosynthesis may be a low temperature effect only and not a prerequisite for hardening (Willemot 1975).

Acclimation affects the levels of other lipid compounds as well, notably sterols. In wheat shoots stored at 0 C, sitoserol, stigmosterol and campesterol decreased while cholesterol levels remained constant. Sterol levels in root tissue decreased as well, but after one to two weeks they increased to even higher levels than at the outset, indicating that sterol synthesis in root tissue is better able to acclimate to low

temperatures. The importance of these alterations in sterol levels is not known (Davis 1972).

Not only the amounts of specific lipids formed during acclimation but also their composition has been linked to cold hardiness. In chilling sensitive tissue, acclimation specifically repressed the initial low temperature stimulation of oleic acid desaturation to linoleic acid without affecting the stimulation of palmitic acid elongation and stearic acid desaturation (Grenier et al 1975). The specificity of these effects suggests that lipid changes are not strictly a low temperature response, but involve complex control at the enzymic level. Increased unsaturation is believed to enhance membrane flexibility and function at low temperature, and also increase dehydration tolerance (Grenier et al 1975). Inhibiting linolenic acid synthesis in cotton inhibited the onset of hardiness in newly developing tissue, purportedly because increasing levels of linolenic acid are necessary to preserve membrane fluidity in chilling sensitive species (St.John and Christiansen 1976; Lyons 1973).

Many more examples could be given of changes in the levels of lipids and other compounds during acclimation. However, because of the difficulty of incorporating the large number of often contradictory findings into a comprehensive picture of the sequence of changes occurring in the acclimating plant, only a brief summary has been attempted.

Concluding Remarks

The assimilation of the preceding wealth of information regarding cold acclimation in plants into a consistent and coherent mechanism of cold adaptation in plants is a formidable task. Most of the general

theories attempt to explain the acclimation mechanism in woody perennials. One such theory is given in Weiser's 1970 paper, in which he incorporates observed changes in water relations, hormonal balance, and metabolic activity. However, since cold acclimation in a herbaceous species is the subject of this thesis, no further elaboration of these theories will be attempted. Studies of cold acclimation in woody tissue are pertinent only if similar changes occur in herbaceous species, but, as the literature indicates, there is no guarantee that identical mechanisms will be operating. Therefore, as a concluding section, I will summarize one of the few theories of cold acclimation which have been developed with specific reference to an herbaceous species. As this theory deals specifically with winter rape (Kacperska-Palacz 1978), it is especially pertinent to this thesis.

Kacperska-Palacz (1978) describes cold acclimation in winter rape as a three stage process, induced primarily by low temperatures. Light is also an important inductive factor in the initial stage of acclimation, although the photosynthetic role appears to be more important than the photoperiodic role in the case of herbaceous species. However, temperature is the most critical environmental factor since each stage of acclimation is related to a specific temperature level: 1) Temperatures in the 2-5C range increase frost tolerance by only a few degrees, but this initial stage is nevertheless very important because it is related to growth cessation and an accompanying inhibition of cell expansion. In turn, this decreased cell expansion results in decreased tissue hydration and indicates important metabolic shifts. 2) This stage is initiated by exposure to subfreezing temperatures in the 0 to -3C range and results in the .....

development of maximum hardiness. 3) This stage may be coincident with the second, and its importance is not fully understood.

Step-by-step cold acclimation as a response to decreasing temperature levels is similar to the pattern observed in woody tissue (Levitt 1972). However, the temperature levels required for maximum acclimation of herbaceous species are higher, and there is no observed response to prolonged exposure to extremely low temperatures as is reported for woody species (Kacperska-Palacz 1978).

Kacperska-Palacz (1978) stresses that the induction of dormancy, a phenomenom which is considered by many to be crucial to acclimation in woody species (Alden and Hermann 1971), is not involved in cold acclimation of winter rape. She claims that herbaceous species retain their growth capability throughout the winter despite the fact that environmental factors have brought about a cessation of growth. This growth cessation, however, is critical to the first stage of acclimation and is not merely a low temperature effect since it results in important metabolic shifts accompanying acclimation.

The metabolic alterations observed in winter rape during the initial stage of acclimation include starch hydrolysis, accumulation of reducing sugars, accumulation of water soluble proteins, as well as extensive lipid and phospholipid transformations. Preferential synthesis of some metabolites occurs during this stage as well as an enhancement of some hydrolytic activities. The driving force behind these metabolic shifts appears to be alterations to the hormonal balance induced by growth cessation. Specifically, the ABA/GA ratio is of most importance. Changes in this ratio modify protein synthesis and/or activity and may also directly affect the

properties of cellular membranes, for example the ABA induced increase in water permeability. Again, the role postulated for hormones is similar to their suggested role in the acclimation of woody species (Alden and Hermann 1971). Acclimation may also alter the properties of the hormone receptors in herbaceous species, influencing the response of the tissue and directing cell metabolism into specific cold-affected pathways (Kacperska-Palacz 1978).

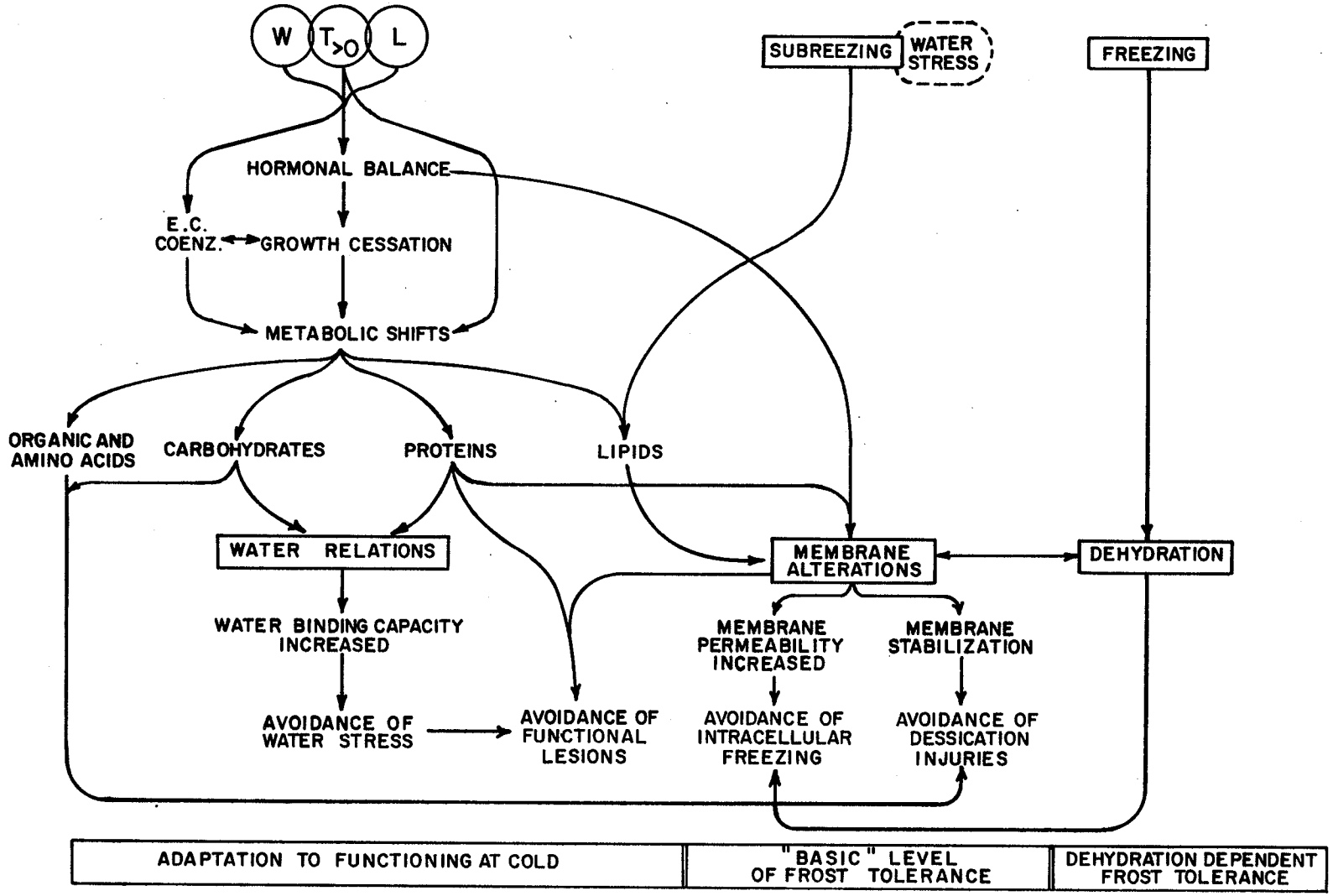
A second driving force behind the important metabolic shifts is the energy relations of the plant during acclimation. Not only the photosynthetic but also the respiratory system may contribute to increased energy availability during low temperature treatment. Changes in the energy charge of the plant cells will in turn produce shifts in important metabolic pathways (Kacperska-Palacz 1978).

The critical adaptations which occur during the second stage involve the avoidance of intracellular freezing and the tolerance of the concommitant desiccation stress. These adaptations allow the development of maximum frost tolerance in herbaceous plants, as they do in the case of woody species. However, the nature of these adaptations is not known, although the phospholipid fraction appears to be critical. These changes facilitate water movement across membranes and result in the production of specific protective compounds designed to protect cell constituents against desiccation stress. It appears that the degree of lipid unsaturation is not causally involved in these changes, but alterations in phospholipid content and the proportion of phosphatidycholine and phosphatidylethanolamine appear to be important. The driving force behind this second stage is also not known. Kacperska-Palacz (1978) suggests that

phospholipid transformations affecting membrane rapid alteration may be the major inductive factor but the evidence is not conclusive.

The adaptations which occur during this second stage are the ultimate result of the cold acclimation process in herbaceous species since they permit maximum frost tolerance. Similar adaptations have been reported for woody species, although the degree of frost tolerance achieved can be substantially greater (Alden and Hermann 1971).

Kacperska-Palacz's cold acclimation mechanism for winter rape is summarized in Figure 1. Although some aspects are disputable and others are not fully developed, this mechanism provides a useful framework for evaluating the ever increasing data regarding acclimation in both herbaceous and woody species.



From: A. Kacperska-Palacz: Mechanism of cold acclimation in herbaceous plants, in Plant cold hardiness and freezing stress, ed. P.H. Li and A. Sakai, New York, Academic Press (1978), p.119.

MATERIALS AND METHODS

Preparation of Material

Planting Procedure

The plant material used for all experiments was rapeseed, Brassica napus, cv. Target. The planting mixture used was a 2:1:1 mixture of loam, peat, and sand, fertilized with 16-20-0 and 11-48-0 at the rate of one teaspoon of each per two shovelfuls of loam.

Seeds were planted in 4 inch plastic pots at the rate of ten per pot. A thin layer of finely sifted sand and loam was then placed over the seeds. Initial watering was done by hand using a solution of No-damp fungicide of the recommended strength. Subsequent waterings were done on a regular basis by greenhouse staff, except for those plants in the cold hardening chamber which were watered less frequently and by hand.

All plants were germinated at 20C under an 11 hour day. Emergence occurred in six to nine days, at which time the seedlings were thinned to one per pot.

Non-hardening Conditions

The non-hardened or control plants were maintained under these growing conditions (20C and 11 hour day) and a photon flux density of approximately 280 microeinsteins m^{-2} sec^{-1} for the gas analysis experiments, and 400 microeinsteins m^{-2} sec^{-1} for the growth analysis

experiments, as measured by a Li-Cor Quantum/Radiometer/Photometer model Li-185A. All lights used were Sylvania Gro-lux wide spectrum fluorescent.

Cold Hardening Conditions

The cold hardening regime used in these experiments is identical to that used by a previous worker (Rossnagel, unpublished data). Temperatures ranged from a low of -1C to a high of 15C over an eleven hour day. In the gas analysis experiments, photon flux density was maintained at approximately 280 microeinsteins m^{-2} sec^{-1} by leaving only 2/3 of the lights on. This was done in order to duplicate light conditions in the non-hardening Coldstream growth chamber. For the growth analysis experiments, identical Econaire growth chambers were used, allowing a photon flux density of approximately 400 microeinsteins m^{-2} sec^{-1}.

Hardening Procedure

Gas analysis experiments. Plantings of 20 pots were made weekly to provide a continual supply of plant material. Plants to be hardened were transferred after reaching the two true leaf stage to the hardening chamber where they were hardened for three weeks. At this time, the plants had progressed to the three leaf stage, which was the state of morphological development chosen for gas analysis measurements. This choice was partly arbitrary and partly influenced by the size of the plant chamber used during gas analysis and the ease of insertion. Non-hardened plants at the same developmental stage were also selected for measurement.

To investigate the effects of a longer hardening period, some plants were transferred at the one leaf stage for six weeks of hardening. At

this time they had progressed to the three leaf stage.

Growth analysis experiments. A modified hardening procedure was followed during the growth analyis experiments. An identical planting procedure was followed, except that mass rather than weekly plantings were made. The plants which were to be hardened were planted ten days before the non-hardemed plants, in order to allow for the slower rate of growth in the cold hardening chamber.

Transfer to the hardening chamber was done shortly after germination when the plants were still at the cotyledon stage; this occurred two weeks after planting. These plants were then hardened for three weeks. This change in procedure was intended to ensure that all plants, hardened and non-hardened would be at the same developmental stage at the onset of the growth analysis experiments. This had not been a problem in the gas analysis experiments; since only one or two plants were measured daily, selections of plants of comparable developmental stage could easily be made from the continual supply of plant material. Due to the mass planting necessary for growth analysis, precautions had to be taken to ensure developmental comparibility between the hardened and non-hardened material. However, during the second set of growth analysis experiments, the lights were inadvertently left on for two full 24 hour periods due to an electrical error which I failed to notice until the following Monday. Continuous light for this period set back the non-hardened plants and resulted in a disparity in developmental stage and size at the outset of the experiments.

Estimation of Physiological Development

In both the gas analysis and grwoth analysis experiments, an estimation of physiological development was made on the basis of morphological development. For example, in the gas analysis experiments, plants were chosen at the three leaf stage for measurement even those these plants were quite different in chronological age. This procedure was necessary because of the different growth rates of the plants in the hardening and non-hardening chambers. However, this estimation of physiological development poses theoretical problems.

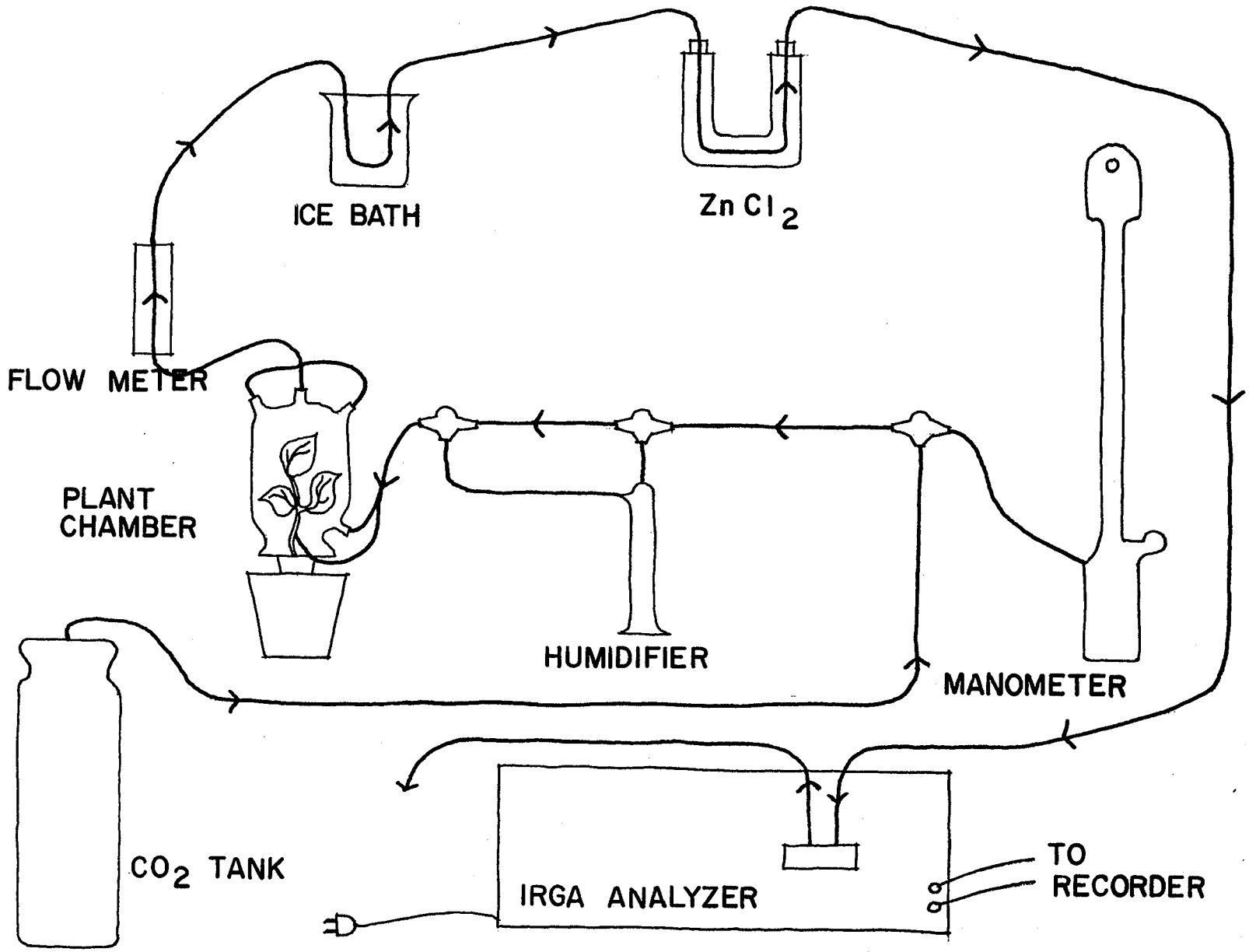
The major growth which plants undergo during acclimation does not involve the initiation of new organs such as leaves, but rather involves metabolic alteration reflected in qualitative rather than quantitative changes in morphology (Wooledge 1969). Therefore the hardened plants may appear to be at the same physiological stage as their non-hardened counterparts because they have the same number of leaves, but they may be considerably more advanced in less apparent but nonetheless important respects which may affect subsequent growth.

As problematic as this estimation of physiological development may be, it was the only feasible basis for comparison which could be devised for these experiments.

Experimental Measurements

Gas Analysis

Gas analysis measurements were made using a Beckman infrared gas analyzer model 215 attached to a gas system outlined in figure 2. The gas tanks were purchased from Linde. For the daily calibration, a tank


ICE BATH
$ZnCl_2$
FLOW METER
PLANT CHAMBER
HUMIDIFIER
MANOMETER
CO_2 TANK
IRGA ANALYZER
TO RECORDER

of pure nitrogen (N_2) was used, as well as a tank of precise CO_2 concentration (350 ppm). For experimental purposes the tanks used were:

1) 397 ppm CO_2; balance air

2) 386 ppm CO_2; 1% O_2; balance N_2

The CO_2 concentrations of these tanks were determined after calibration of the machine. The first tank was used for determining rates of net photosynthesis and mitochondrial respiration, the second for estimating photorespiration by an indirect means, that is removing the O_2 inhibition.

As figure 2 shows, gases from these tanks were channelled through a system of Tygon tubing of 3/8 inch diameter through a glass humidifier before entering the plant chamber. This circular glass chamber had three inlets at the bottom, allowing the gas mixture to circulate evenly around the enclosed plant, and two outlets at the top. After leaving the chamber, the gas mixture was then dehumidified by passing through two ice-baths and finally a tube containing a powerful desiccant, $ZnCl_2$. Finally the gas mixture passed into the anlayzer, which is attached to a calibrated 100 mv. Honeywell recorder. After the CO_2 concentration of the air leaving the plant chamber was recorded, the gas mixture was vented to the atmosphere.

Rate of gas flow in the system was measured by a Gilmont flow meter attached to the system. Throughout the experiments, flow rates were kept at approximately 50 liters/hour, which was the maximum rate possible without disturbing the water seal on the plant chamber.

Temperature in the plant chamber was measured by a copper-constantan thermocouple, which was calibrated at regular intervals and attached to a 1.0 mv. Honeywell recorder to provide continuous temperature measurements.

To avoid excessive heating by the lights, the thermocouple was shielded by an aluminum foil cap.

Ambient temperature in the growth chamber was regulated manually by the growth chamber controls. Due to the heating effect of the lights, it was necessary to keep ambient temperature several degrees below the temperature desired in the plant chamber. For example, in the 10C measurements, the growth chamber was set at 5-8C.

A wet bulb-dry bulb psychrometer attached to a 1.0 mv. Honeywell recorder monitored humidity levels in the plant chamber, which varied from 90-98% R.H.

For gas exchange measurements, the test plant was inserted into the plant chamber and fastened with a rubber stopper which contained a hole for the stem. The edges of this rubber stopper were then secured into the earth in the plant pot, with water added to form a seal. This seal was the weak point in the gas system; if flow rates were not allowed to exceed 50 liters/hour, this water seal was effective in preventing leaks. Any leaks which did occur could be detected by a bobbing of the flow meter.

The following sequence of gas exchange measurements was practised:

1) 20C in dark - tank 1 (balance air)
2) 20C in light - tank 1
3) 20C in light - tank 2 (balance 1% O_2)
4) 10C in light - tank 2
5) 10C in light - tank 1
6) 10C in dark - tank 1

This sequence was chosen to minimize shock effects on the plants due to changes in temperature and/or light conditions. Sufficient time was allowed the plant to equilibrate before any readings were taken on the Beckman 215.

Gas exchange rates were calculated on the basis of leaf area (dm^2), as measured by a leaf area meter (Lambda Instruments, model LI-3000). This choice was made partly because of the ease of determination, but also because I was more interested in the rates of gas exchange relative to the type of pretreatment, hardened or non-hardened, rather than the absolute values. Specifically, my concern was the performance of the hardened plants at 10C relative to the performance of the non-hardened controls, and relative to the performance of the hardened plants at 20C.

If absolute values were desired, a basis other than leaf area might be more desirable (Sestak et al 1971). The authors discuss the merits of various alternatives and suggest that for low temperature studies the amount of carboxylating enzyme would be the best choice since this factor is most likely limiting at low temperatures.

Sample Gas Exchange Calculation

Since the Beckman analyzer provides readings in terms of ppm CO_2, it was first necessary to convert these to mg CO_2/liter, using conversion tables found in Sestak et al (1971). These tables relate ppm CO_2 to mg CO_2/liter at different temperatures.

The final concentration of CO_2 is then subtracted from the initial CO_2 concentration of the tank, again converted to mg CO_2/liter, to give the change in CO_2 concentration as a result of photosynthesis and/or

respiration. Then, using the value for leaf area obtained from the leaf area meter, the rate of CO_2 exchange can then be determined:

$$\frac{\text{change in } [CO_2] \quad X \quad \text{flow rate}}{\text{leaf area}}$$

$$= \frac{\text{mg } CO_2/\text{liter} \quad X \text{ liter/hour}}{dm^2}$$

$$= \text{mg } CO_2/\text{hour}/dm^2$$

Growth Analysis Experiments

After the hardened plants had been in the hardening chamber for three weeks, ten plants were randomly selected from both the hardening and non-hardening chambers. The values of leaf area, plant height, fresh and dry weight were recorded for these plants and used as base values for determining the changes in these parameters over time as a result of hardening and temperature.

One-half of the remaining hardened plants and one-half of the remaining non-hardened plants were then selected at random and placed in a growth chamber held at 10C and an 11 hour day. The remianing plants, again both hardened and non-hardened, were placed in a second growth chamber held at 20C and an 11 hour day.

Ten plants from each group (hardened 10C; hardened 20C; non-hardened 20C; non-hardened 20C) were then selected at random every second day for the following sequence of growth analysis measurements:

1) number of true leaves

2) plant height (as measured from the soil level)

3) leaf area (as measured by a leaf area meter)

4) fresh weight (as measured by a Fisher Scientific Gram-atic Balance)

5) dry weight (as measured by a Fisher Scientific Gram-atic Balance)

Plants were dried in brown paper bags under well-ventilated greenhouse conditions for two weeks prior to the dry weight measurements.

In the first set of growth analysis experiments, plants were recorded every 2 days for a total of 8 test dates. In the second set, only 6 test dates were used to avoid the complication of floral tissue development.

Methodological Problems

Measuring Temperatures

The use of only two temperatures, 10C and 20C, caused difficulties in interpretation of data. With only two temperatures it is impossible to construct meaningful line graphs, although it is of course still possible to compare results at the two temperatures.

The particular temperatures used are less than ideal. Since this is an experiment investigating the effects of cold hardiness, it would be preferable to use a temperature lower than 10C. It was my original intention to use 5C, but, due to the heating effect of the lights, it would have been necessary to keep the growth chamber below freezing in order to keep the plant chamber at 5C. The chamber used for the gas analysis measurements was incapable of being maintained below freezing. A water filter would have provided a more satisfactory solution.

The same temperatures were chosen for the growth analysis experiments in order to be consistent with those used for the earlier gas

analysis experiments. Although a third temperature would have been desirable here as well, the amount of growth space this would have required was prohibitive.

There is perhaps some justification for the choice of 10C since this temperature is quite characteristic of temperatures encountered during the early spring growing conditions.

Light Intensity

Light intensities used for the gas analysis experiments may have affected measurements taken at 20C. At this temperature, light intensity is probably limiting net photosynthesis. Measurements taken at this temperature are probably lower than they would be at the same temperature under higher flux densities. This assumption is based on the observation that rape has a very high light saturation point for a C_3 species (Rossnagel, unpublished data).

At 10C, on the other hand, the enzymatic dark reactions are more likely limiting photosynthesis. Hence the gas exchange measurements taken at this temperature are probably more indicative of what they would have been in the field.

The net result of the fact that light intensity is limiting to photosynthesis at 20C but not at 10C is that the range of photosynthetic rates recorded at these two temperatures is considerably smaller than it would be in the field, resulting in a smaller Q_{10} value.

Studies which cite very small Q_{10} values for photosynthesis in rape may be due to similar problems arising from limiting photon flux densities (Herath 1973).

In the growth analysis experiments, photon flux densities may be limiting growth at 20C by means of the limiting effect on net photosynthesis.

Statistical Analysis

Gas Analysis Data

The gas exchange data was analyzed as a completely randomized design. Corrections were made for the fact that there were an unequal number of observations per treatment, i.e. six non-hardened, five hardened three weeks, and seven hardened six weeks.

Calculations of sum squares, mean squares, F values and standard deviations were performed on a Corvus calculator, and then checked on a Hewlett Packard model 10 calculator.

Growth Analysis Data

The growth analysis data was analyzed as a factorial experiment using a completely randomized design.

Calculations of sum squares, mean squares, F values and standard deviations were made on a Hewlett Packard model 10 calculator.

RESULTS AND DISCUSSION

Gross Morphology of Acclimated and Non-Acclimated Plants

Seedlings of Target rape exhibited characteristic changes in gross morphology when subjected to cold acclimation.

When seedlings at the one or two true leaf were transferred to the cold hardening chamber, they experienced an initial period of shock. In several hours these plants were already showing signs of wilting and in twenty-four hours they resembled plants undergoing severe moisture stress even though soil moisture levels were kept uniformly high. In several days the plants showed some signs of recovery; leaf and stem tissue slowly regained turgor and the stems began to straighten.

However, there was still considerable variation in the appearance of the plants during this period depending on the time of day they were examined. In the morning, after exposure to the coldest period of the hardening regime, the plants were again bent over and highly stressed. Later in the day, when temperatures climbed to 15C, the plants regained a normal appearance. By one week after transfer the seedlings appeared to be fully recovered, in that they no longer exhibited signs of stress at any time in the hardening cycle.

Growth did not resume in these plants until a second week had passed in the hardening chamber, and then only at a very slow rate when compared to the non-acclimated plants. The major changes observed during this third and final week of acclimation were qualitative in nature, involving changes

in their morphology. Quantitative increases in the amount of tissue were limited.

In contrast, when plants were transferred when still in the cotyledonary stage for use in the growth analysis experiments, the seedlings did not exhibit such severe outward indications of shock. They showed no apparent signs of water stress. Moreover, although all visible signs of growth were again arrested upon transfer to the hardening chamber, the seedlings resumed growth after one rather than two weeks, and at a faster rate. Less differences in growth rate in terms of amount of new leaf tissue were apparent between hardened and non-hardened material when transfer was made at the cotyledon stage than when transfer was made at the one to two leaf stage. This observation supports the belief that the more juvenile the tissue, the better able it is to acclimate effectively and resume normal quantitative growth rates at low temperatures.

Despite these differences in quantitative growth rates, all of the seedlings exposed to the acclimating regime exhibited the same qualitative changes in gross morphology regardless of the time of transfer. These changes can be summarized as follows: 1) development of a rosette growth appearance. The stem internodes of the acclimated plants were much shorter than those of the non-acclimated tissue. 2) thicker and less fragile stems. 3) smaller, thicker leaves, with a distinctive rubbery appearance. 4) darker green colouration in all plant tissue.

Even after transfer to non-acclimating conditions, the acclimated plants retained these morphological alterations, although gradually their tissue did become less rubbery and leaf size increased.

Evaluation of Shock Effects During Gas Exchange Measurements

Besides the shock effects observed upon transfer of the seedlings to the acclimating regime, shock effects might be anticipated after transfer of the plant from its preconditioning temperature regime, either acclimating or non-acclimating, to the measuring temperature. This factor is of particular importance in the gas analysis experiments when readings are made within a short interval after transfer, and might possibly lead to misleading results.

In this experiment, such shock effects are believed to be negligible since neither of the measuring temperatures used, 10C or 20C, was extreme and hence neither was considered likely to result in severe setbacks to the plants.

To test this assumption, a preliminary experiment was conducted to investigate the possibility and the severity of such shock effects occurring. The net photosynthetic rate of a non-acclimated plant was recorded after immediate transfer to 8.2C, after two hours, and after four hours. The lowest possible temperature which could be maintained was used in order to maximize the likelihood of shock effects. The rates obtained were 7.3 mg. CO_2/plant/hour at 0 time; 7.1 mg. CO_2/plant/hour after two hours; 7.0 mg. CO_2/plant/hour after four hours.

It was concluded from these results that shock effects were negligible. However, after transfer to either measuring temperature, the plants were allowed to acclimate for at least thirty minutes prior to measurement. Also, a sequence of measurement was adopted which minimized shock effects on the plants.

Effect of Cold Hardening on Gas Exchange

Respiration

Cold acclimation causes marked changes in the respiratory rates of the hardened plants as compared to the non-hardened controls, as seen in table 1.

TABLE 1. Mean rate of dark respiration of hardened and non-hardened rape plants expressed as mg $CO_2/dm^2/hr$.

Temperature	Pretreatment		
	Non-hardened	Hardened 3 weeks	Hardened 6 weeks
10C	1.1 ± 0.1	2.8 ± 1.0	2.8 ± 0.7
20C	2.6 ± 0.9	6.8 ± 2.3	5.3 ± 1.0

A detailed analysis of variance for dark respiration rates is found in the Appendix (table 11). For discussion purposes, I will refer to a summary of the F values for respiration as well as other gas exchange parameters (table 2).

TABLE 2. Summary of F values of temperature and hardening effects on gas exchange rates in rape seedlings.

Source of variation	F value			
	Respiration	Net photo-synthesis	Photoresp-iration	P.S./ Resp.
Temperature	64.6327**	0.7290$^{n.s.}$	3.8972$^{n.s.}$	25.7240**
Hardening	29.0488**	3.2189$^{n.s.}$	3.1747$^{n.s.}$	18.7812**
Temp. X Hard.	4.1940*	11.9555**	1.5181$^{n.s.}$	0.3306^{ns}

As table 2 indicates, hardening regime and temperature both had a significant effect on dark (mitochondrial) respiration. However, when tested against the temperature X hardening interaction, neither of these main effects are significant (table 3).

TABLE 3. Main effects of temperature and hardening on respiration tested against the TxH interaction.

Source of variation	Degrees of freedom	Mean square	F value
Temperature	1	60.5803	$15.4105^{n.s.}$
Hardening	2	27.2274	$6.9262^{n.s.}$
Temp.X Hard.	2	3.9311	

This finding is surprising, since dark respiration is enzymatically controlled and hence highly affected by temperature. However, failure to show significance for the main effects is largely attributable to the small number of degrees of freedom for temperature (1) and hardening regime (2). Sample variation was also a factor.

The temperature X hardening interaction is of greater interest since it illustrates changes in temperature response of the respiratory apparatus as a result of the cold hardening treatment. As figure 3 shows, Q_{10} values are little altered by hardening; relative increases are similar over the temperature range tested for hardened (3 and 6 weeks) and non-hardened plants. The temperature response curve for the plants hardened for 3 weeks is steeper than the other two, but the most notable trend is the upward shift in the temperature response curve as a result of hardening. Rates

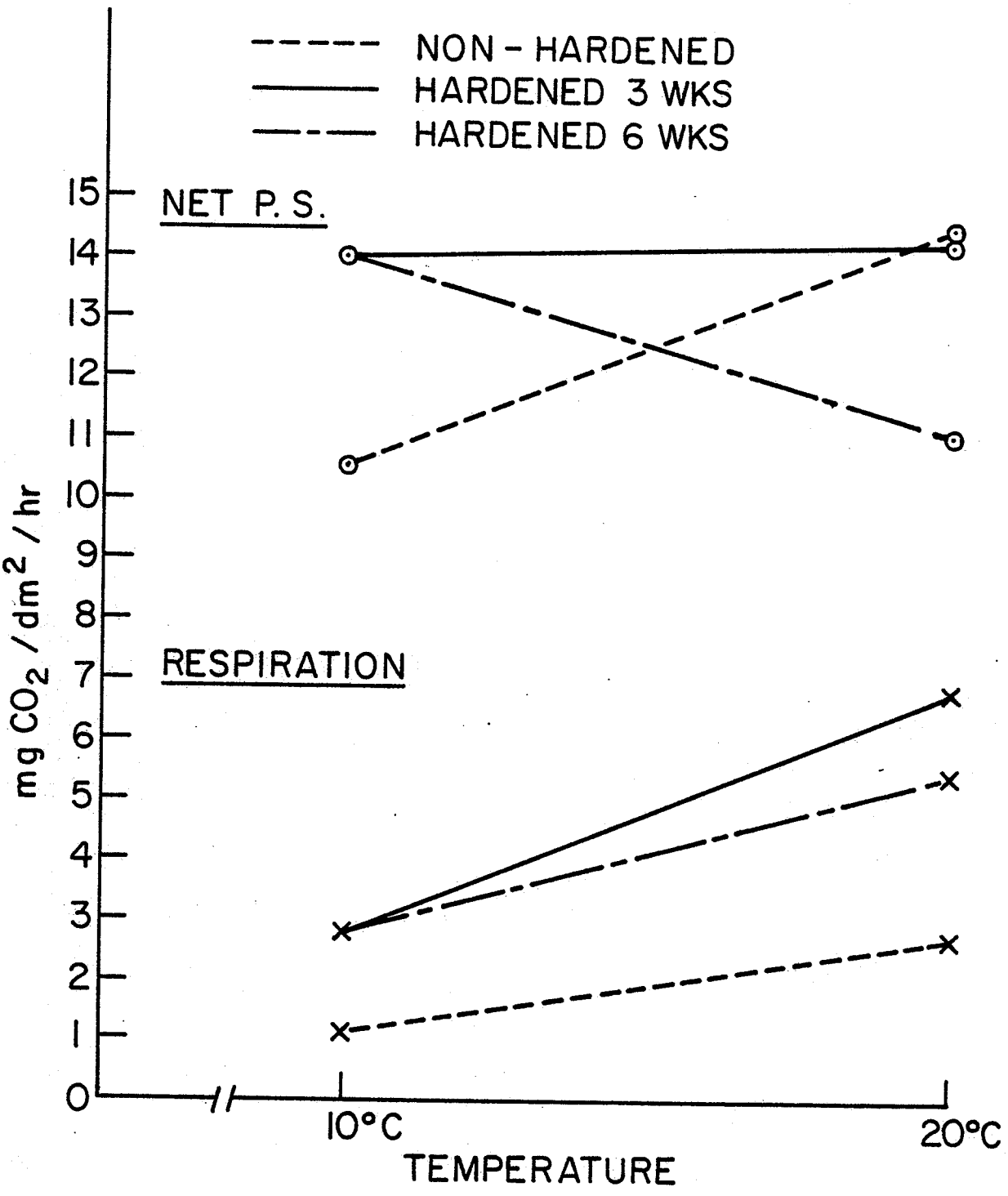


FIGURE 3. Effect of temperature and cold hardening on mean rates of net photosynthesis and dark respiration in rape.

of dark respiration are higher at both temperatures for both sets of hardened plants when compared to the non-hardened controls. This upward shift is in agreement with other findings in the literature indicating enhanced respiration (Pisek and Kemnitzer 1968; Rook 1969; Wooledge and Jewiss 1969), and may be due to one of two factors (Bjorkman 1966).

The first of these two causes, and the one which is most commonly held, is an increase in the level of respiratory substrates in the cold hardened plants as a result of the inhibition of growth observed during the hardening treatment. The resulting accumulation of substrate would not only stimulate respiration, but would at the same time inhibit subsequent photosynthesis via feed-back inhibition and hence inhibit growth even further. According to this explanation, enhanced respiratory rates represent a wasteful or non-productive consumption of stored reserves.

If this explanation is correct, exposure to more favourable temperature conditions would bring about a reduction in respiration, since growth would resume and alleviate the feed-back inhibition process. Alterations to the respiration rate during acclimation would therefore be a short term effect only, not involving complex changes to the respiratory apparatus of the acclimated plant. Unfortunately, it was not determined in these experiments whether and for how long the hardened plants retained their enhanced respiratory rates. However, earlier experiments involving testing of rape plants which had been growing in non-hardening conditions subsequent to transfer from the hardening chamber indicated no significant effect of cold hardening on respiration at 5, 10, and 20C (La Croix and Nalborcyzk, unpublished data). These findings support this first explanation of enhanced respiration in hardened plants

by indicating that this enhancement is a short term effect only.

The second cause cited for enhanced respiratory rates involves alterations to the respiratory apparatus of the plant during cold acclimation. Cold hardening may increase the number of mitochondria, increase the amount or catalytic activity of respiratory enzymes and/or alter membrane properties. Such effects would facilitate respiration at low temperature and hence enable the plant to continue growth (Bjorkman 1966). Any further account of these quantitative and/or qualitative changes would be purely speculative, but if they do occur, the enhanced respiratory rates which they result in would be a productive use of plant reserves in that it would be tied to the continuation of growth at low temperatures. This explanation has been suggested to account for the adaptive increases in respiration of Arctic plants (Bjorkman 1966). Moreover, such changes would more than likely be long-term due to the alterations to the mitochondrial apparatus rather than merely to substrate levels.

No conclusive statements are possible about the adaptiveness of the enhanced respiration rates observed in this experiment. If they were adaptive, they would presumably be accompanied by a resumption of growth. It is true that after the initial adjustment period, the cold hardened plants did resume visible growth by initiating new leaf and stem tissue, as well as the qualitative changes to the morphology of their tissue. Continuation of growth did not appear to be antagonistic to acclimation in rape as it does in the case of many woody species (Alden and Hermann 1971), although growth resumed only after the changes accompanying cold hardening appeared complete, i.e. one to two weeks after transfer

into the hardening chamber. Resumption of growth after this period of acclimation did not seem to be accompanied by a loss of hardiness.

Prolonged exposure to hardening conditions had no effect on the respiratory rates of the plants at 10C but inhibited respiration at 20C. The respiratory rates of the plants hardened for 3 weeks were significantly higher than those hardened for 6 weeks, although both were much higher than the rate recorded for the non-hardened plants (table 1 and figure 3). A possible explanation for these results is that the plants hardened for 6 weeks have had a longer period of growth at low temperature after the initial 1-2 week adjustment period. During this period after the resumption of growth, photosynthetic products would be used for growth processes, relieving the feed-back inhibition of photosynthesis and reducing the stimulative effect of increased substrate levels on respiration.

In summary, cold hardening enhances the rate of dark (mitochondrial) respiration at both 10C and 20C, although there is insufficient evidence to determine whether this enhancement is advantageous to the plant or merely a wasteful consumption of reserves. Visual observation of the renewed growth rate of the rape plants after their adjustment period in the hardening chamber lends support to the theory that the resumption of growth is not antagonistic to cold hardiness in this herbaceous species. Accordingly, alterations to the respiratory apparatus may be related to this adjustment of the plant to growth under low temperatures.

Net Photosynthesis

Cold hardening affects photosynthetic processes in rape, as seen in table 4.

TABLE 4. Mean rate of net photosynthesis of hardened and non-hardened rape plants expressed as mg $CO_2/dm^2/hr$.

Temperature	Pretreatment		
	Non-hardened	Hardened 3 weeks	Hardened 6 weeks
10C	10.5 ± 3.1	14.1 ± 2.6	13.9 ± 3.8
20C	14.4 ± 3.5	14.2 ± 4.6	11.0 ± 2.0

A detailed analysis of variance for net photosynthesis is found in the Appendix (table 12). A summary of the F values for photosynthesis as well as other gas exchange rates is found in table 2; in contrast to dark respiration, only the temperature X hardening interaction and not the main effects of temperature and hardening was significant.

The magnitude of this temperature X hardening interaction is indicated numerically in table 4 and graphically in Figure 3. Rates of net photosynthesis were graphed together with the rates of dark respiration in order to illustrate the comparative effects of hardening on these two parameters. Both the plants hardened for three weeks and the non-hardened controls had virtually identical rates of net photosynthesis at 20C, and both groups were significantly higher than the mean rate recorded for the plants hardened for six weeks. At 10C, in contrast, the non-hardened plants were considerably inhibited in their photosynthetic performance

whereas the plants hardened for three weeks had a rate that was only slightly reduced from their performance at 20C. The plants hardened for 6 weeks, on the other hand, had photosynthetic rates that were considerably higher than at 20C.

From these results it is tempting to conclude that the hardened plants have obtained their optimal photosynthetic capacity at 10C as a result of acclimation and that raising the temperature to 20C has no further stimulatory effect on photosynthesis. These changes in the photosynthetic adaptations of the acclimated plant might be due to changes in enzyme catalytic activity and/or amount, or to some other alteration to the photosynthetic apparatus (possibly alterations in membrane properties) as yet unconfirmed. The ultimate effect of these changes, however, is a levelling of the photosynthetic response curve and a corresponding drop in the Q_{10} value for this gas exchange parameter. Net photosynthesis would appear to be much less temperature dependant in the plants hardened for three weeks.

Moreover, these changes do seem to be adaptive to the plant, for not only is the rate of photosynthesis at 10C greatly enhanced when compared to the non-hardened controls, but also there is no concommitant inhibition of photosynthesis at 20C. Photosynthetic performance has indeed been levelled off by cold hardening over the temperature range tested, but at a comparatively high level and one which is virtually equal to the performance of the non-hardened plants at 20C. These findings contradict other indications in the literature that acclimation to a wider range of environmental temperatures may necessarily involve a decrease in optimal performance (Berry 1973).

However, several reservations about this optomistic interpretation of the data must be made. First, due the recognized limitation of using only two measuring temperatures, it is difficult to determine even roughly the overall photosynthetic trends, for example maximum, minimum, and so on. Thus it is impossible to say whether the hardened plants have in fact attained their maximum capacity, though it is possible to conclude that their photosynthetic performance is less temperature dependant over the temperature range investigated and at prevailing levels of radiation.

Secondly, the observed decrease in Q_{10} value may be due in part to the radiation levels used. Radiation is likely to be a limiting factor for the hardened plants at 20C, resulting in a misleading levelling off at the 10C level of photosynthetic performance. It is possible that the photosynthetic rates at 20C might have been higher for the hardened plants at higher levels of radiation, and that the entire response curve might have been shifted upward by cold hardening in a fashion more similar to that observed for dark respiration (Figure 3). Thus the decrease in Q_{10} observed for the hardened plants may be artifactual, though it is not possible to confirm or dismiss this possibility given available data.

Thirdly, this apparent enhancement of net photosynthesis at low temperatures as a result of acclimation might be offset by a similar enhancement of dark respiration rates during the night period, making overall increases in photosynthetic accumulation to be used for growth purposes unlikely. The metabolic changes accompanying acclimation would, if such were the case, not represent an adaptive change to the plant which would allow continued growth at low temperatures. Given the high respiratory rates recorded for the hardened plants, this possibility

must be considered.

Lastly, it is impossible to determine from this data how long-term these effects of cold hardening on net photosynthesis are in rape. In order to determine this important point, delayed investigations of gas exchange rates would have to be conducted. Such investigations would also provide a better indication of the nature of the metabolic changes occurring in the plant during acclimation which result in the observed alterations in gas exchange. If they were in fact long-term in nature, the alterations to the photosynthetic apparatus would then more likely involve physical changes rather than merely due to substrate concentrations, for example.

It was noted in the discussion of dark respiration that enhanced mitochondrial activity at low temperature is often attributed to the inhibition of photosynthesis at these temperatures (Bjorkman 1966). In these experiments, however, the plants hardened for three weeks not only had significantly higher rates of respiration at 10C than the non-hardened controls, but also significantly higher rates of net photosynthesis. This was also true for the plants hardened for six weeks.

The differential response of the plants hardened for the longer period (six as opposed to three weeks) requires further comment. Like the plants hardened for three weeks, they also have enhanced rates of net photosynthesis at 10C as compared to the non-hardened controls. In contrast, their rates of net photosynthesis are greatly inhibited at 20C. Over the temperature range tested their Q_{10} value is much closer to that of the non-hardened controls, but in the reverse direction (Figure 3). Their photosynthetic performance is much more temperature dependant than the

plants hardened for three weeks.

It is possible that the prolonged acclimation period leads to further alterations to the metabolic apparatus such that the plant again becomes more specialized, but to a lower environmental temperature than the non-hardened plants. The optimum temperature for net photosynthesis would thus be shifted downwards, indicating that the plants have lost the capacity to perform optimally over an expanded temperature range, a capacity which was retained in the plants hardened for the shorter period. This interpretation would be in keeping with findings reported in the literature of an overall downward shift in the temperature response curve for net photosynthesis as a result of low temperature acclimation (Stestak 1971) as well as the observation that plants do become specialized to environmental temperature conditions (Berry 1973).

Photorespiration

An attempt was made to estimate the effects of cold hardening on photorespiration as well as dark respiration (table 5).

TABLE 5. Mean rate of photorespiration of hardened and non-hardened rape plants expressed as mg $CO_2/dm^2/hr$.

Temperature	Pretreatment		
	Non-hardened	Hardened 3 weeks	Hardened 6 weeks
10C	0.4 ± 9.0	1.5 ± 1.5	3.3 ± 1.4
20C	2.8 ± 1.0	2.8 ± 5.6	3.3 ± 3.8

The indirect method employed did not prove satisfactory, leading to such a high coefficient of variability (75%) that the results were not reliable. A detailed analysis of variance for photorespiration is found in the Appendix (table 13), but an examination of table 2 shows that because of the huge sample variation it is impossible to show significance for either the main effects or the temperature X hardening interaction. Although all the results are extremely variable, the most erratic values as indicated by the standard deviations were recorded for the non-hardened plants at 10C, followed by the hardened plants (three weeks) at 20C (table 5). These results may be interpreted as a greater sensitivity of the non-hardened plants' photorespiratory apparatus at the lower temperature, although an explanation for this effect is not apparent.

Although it is not possible to draw any statistically sound conclusions from this data, it is possible to state that the results indicate that cold hardening has less effect on photorespiratory rates at 20C than at 10C (table 5).

No explanation is immediately apparent for the extreme variability of the results obtained by using a low O_2 gas mixture. As a consequence, no conclusions could be drawn about the effects of cold acclimation on gross photosynthesis.

Net Photosynthesis/Dark Respiration

Because the plant's overall growth performance is a result not only of its rate of accumulation determined by photosynthesis but also its rate of breakdown achieved through respiration, the ratio of net

photosynthesis/dark respiration was studied in order to get some general indications of the plant's gas exchange efficiency. The mean ratios are given in table 6.

TABLE 6. Mean ratio of net photosynthesis/dark respiration of hardened and non-hardened rape plants.

Temperature	Pretreatment		
	Non-hardened	Hardened 3 weeks	Hardened 6 weeks
10C	10.3 ± 4.5	5.5 ± 3.8	5.1 ± 6.3
20C	6.0 ± 3.6	2.1 ± 0.5	2.1 ± 0.4

A detailed analysis of variance for this ratio is found in the Appendix (table 14), but the summary of the F values given in table 2 shows that although the main effects of temperature and hardening were significant, the more important temperature X hardening interaction was not. Like the photorespiratory results, there is considerable sample variation as indicated by the standard deviations from the mean (table 6). Generally greater variability was recorded in the results at 10C, with the lowest variability being for both groups of hardened plants at 20C. However, this variability in the ratios is entirely due to the variability recorded for each of the two gas exchange parameters involved in the ratio.

Due to the failure to show significance, the data in table 6 was not graphed, but the general trend is towards much more efficient gas exchange at 10C as compared to 20C, as indicated by the higher ratios. Photosynthetic rates did not increase sharply enough at the higher temperature to

compensate for the increases in respiration. This trend is similar for all plants studied irregardless of temperature pretreatment, but the cold-hardened plants had lower ratio values at both temperatures and are apparently less efficient.

Several reservations are in order concerning this conclusion. First, radiation levels used may again be causing a serious misrepresentation of the performance of the hardened plants. If light were a severely limiting factor on photosynthesis at 20C for the hardened plants, then this would greatly reduce the ratio obtained since respiration would not be similarily limited. The hardened plants would, as a result, appear much less efficient than they in fact are.

Secondly, conclusions about the relative efficiency of the plants at either temperature are problematic without an indication of the longevity of the cold hardening effects on gas exchange. For example, the effects on respiration may be short-term, due to the accumulation of photosynthate not used in growth or not yet translocated to another part of the plant for storage. On the other hamd, the effect on photosynthesis may be long-term, due to alterations in membrane properties or some other integral part of the photosynthetic apparatus. Such a differential longevity of cold hardening effects would lead to a badly distorted ratio, and hence a distorted indication of the relative efficiency of the plant.

Thirdly, this ratio is determined with the dark respiration figures. However, it is not reasonable to assume that dark respiration rates will be identical to rates of photorespiration. Due to the extreme variability of the photorespiratory results, they could not be used in the

ratios, even though theoretically this would have been more appropriate.

Lastly, the calculation of a ratio value results in an undesirably high coefficient of variability (approximately 40%).

Conclusion

A significant temperature X hardening interaction was observed for both net photosynthesis and dark respiration (Figure 3). However, it was not possible to determine whether these effects of cold acclimation on subsequent gas exchange rates were adaptive in nature, that is linked to an increased growth capacity at low temperatures, or non-adaptive. In the case of dark respiration, it seems likely that the enhanced rates do involve a wasteful consumption of the plant's reserves, although alternative explanations are possible. It is more difficult to dismiss the changes in the temperature response curve of net photosynthesis as non-adaptive changes. They may be indicative of important metabolic alterations in the plant which acclimate it to lower environmental temperatures. Because of limitations inherent in experimental method, it was not possible to draw stronger conclusions regarding the effects of cold acclimation on gas exchange. The longevity of the effects, as well as the effects on photorespiration, are still undetermined.

Effect of Cold Hardening on Growth Parameters

General Comments

The previous study of gas exchange rates in hardened and non-hardened rape plants was intended to study the effect of cold acclimation on these important growth processes. By studying gross parameters of growth, on

the other hand, it should be possible to see how these individual growth processes interact to prevent or enhance growth at low temperatures.

Data was collected for five growth parameters or dependant variables over a ten day period: increase in fresh weight, dry weight, leaf area, plant height, and leaf number. Mean increases in each of these parameters are given in individual tables within each section, along with graphical representations. Detailed analyses of variance are found in the Appendix (tables 15-18), while a summary of the F values for all parameters is found in table 7 in order to facilitate comparisons within parameters.

TABLE 7. Summary of F values obtained in growth analysis of hardened and non-hardened rape plants.

Source of variation	Growth Parameter			
	Fresh weight increase	Dry weight increase	Leaf area increase	Plant height increase
Temperature	228.2983**	69.4175**	422.9300**	297.5769**
Hardening	308.0688**	251.6143**	164.3012**	.0.6959$^{n.s.}$
Date	171.2131**	189.6067**	188.2534**	106.3974**
TxH	21.3868**	0.8072$^{n.s.}$	26.4142**	0.6098$^{n.s.}$
TxD	17.7327**	12.5194**	31.8104**	5.3434**
HxD	8.6687**	8.1929**	4.0632**	4.1679**
TxHxD	0.7655 $^{n.s.}$	1.2229$^{n.s.}$	0.9132$^{n.s.}$	1.1443$^{n.s.}$

Although there are strong similarities in the trends for these variables, each will be considered separately. The only exception will be fresh and dry weight, which are considered together because of their obvious relation to one another.

The data for increase in leaf number was not analyzed because it was felt that little could be learned from it. Counting the number of leaves is of limited value as a growth parameter since there is no possible way of estimating how much of the leaf has developed. Instead, this parameter was included as a tool to provide a rough indication of the physiological stage of the plant.

Growth analysis data obtained in an earlier set of experiments is summarized in the Appendix (table 19). Because of the undesirable variability of the results, this data was not included in the Results section but was used instead as a trial experiment.

Fresh and dry weight increase

Table 8 summarizes the trends observed for these two important growth parameters in rape, as measured on five sampling dates over ten days.

TABLE 8. Mean increase in fresh and dry weight expressed in grams of hardened and non-hardened rape plants.

	Fresh weight increase		Dry weight increase	
	10C	20C	10C	20C
Hardened				
Date 1	0.704 ± 0.109	1.281 ± 0.347	0.090 ± 0.002	0.111 ± 0.002
Date 2	1.689 ± 0.256	2.974 ± 0.583	0.211 ± 0.003	0.207 ± 0.004
Date 3	2.477 ± 0.358	4.370 ± 1.386	0.307 ± 0.007	0.368 ± 0.013
Date 4	2.918 ± 0.331	6.265 ± 2.799	0.370 ± 0.005	0.553 ± 0.022
Date 5	4.070 ± 1.002	7.092 ± 1.385	0.500 ± 0.014	0.749 ± 0.017
Non-hardened				
Date 1	0.257 ± 0.048	0.298 ± 0.021	0.030 ± 0.001	0.023 ± 0.000
Date 2	0.412 ± 0.061	1.081 ± 0.162	0.049 ± 0.001	0.089 ± 0.001
Date 3	0.837 ± 0.069	1.842 ± 0.132	0.110 ± 0.001	0.189 ± 0.002
Date 4	1.331 ± 0.032	3.201 ± 0.449	0.156 ± 0.000	0.307 ± 0.005
Date 5	2.390 ± 0.446	4.182 ± 0.534	0.296 ± 0.009	0.448 ± 0.015

The main effects of temperature, date and hardening are all highly significant for both fresh and dry weight increase (table 7). The large main effect of temperature is expected, since exposure to the warmer temperature conditions is bound to result in much larger increases in fresh and dry matter accumulation than observed at 10C. Indeed, this is true for all growth parameters studied, which all had highly significant main effects of temperature. Similarily, the highly significant main effect of sampling date on fresh weight, dry weight, leaf area and plant height (table 7) is predictable and requires no further explanation.

The significant main effect due to hardening is more noteworthy. The hardened plants undergo larger increases in both fresh and dry weight at both 10C and 20C (figures 4 and 5; table 8), suggesting that they are capable of more vigorous growth as a result of the acclimation treatment. Thus, while low temperature acclimation was observed to bring about a cessation of growth after the rape seedlings were first transferred to the cold hardening chamber, the plants did resume growth after this initial period. Moreover, cold acclimation was by no means antagonistic to growth after the plants were returned to more favourable temperatures. Both measuring temperatures used, 10C and 20C, were higher than the mean temperature experienced during the hardening regime.

The hardening X temperature interaction (HxT) is particularly important since, as table 7 shows, it was significant for fresh weight increase but not for dry weight increase. Figure 6 depicts the HxT interaction for all the growth parameters studied. The enhanced growth capacity of the hardened plants is apparent, but the hardened plants

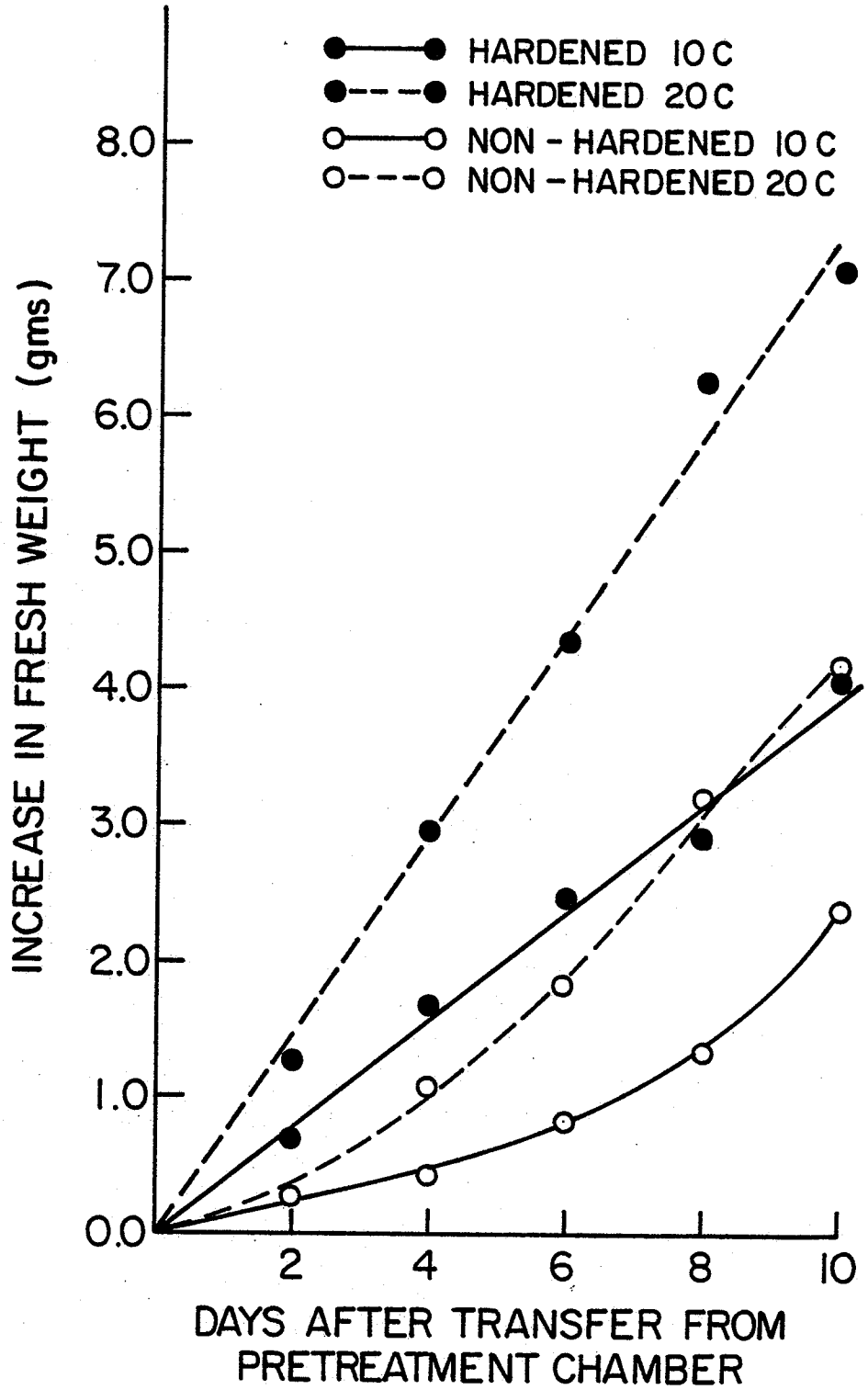


FIGURE 4. Effect of temperature and cold hardening on mean rates of fresh weight increase in rape.

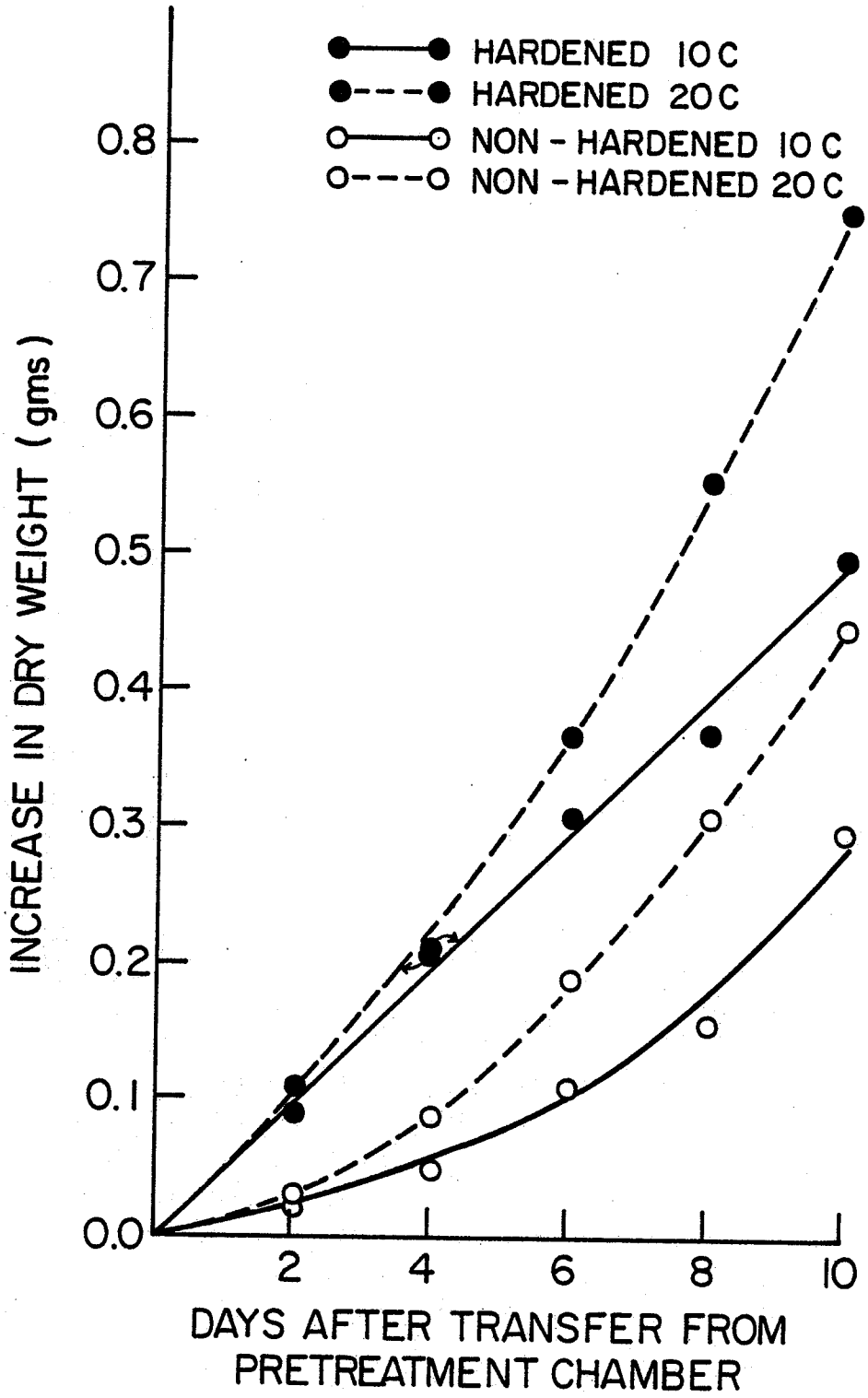


FIGURE 5. Effect of temperature and cold hardening on mean rates of dry matter increase in rape.

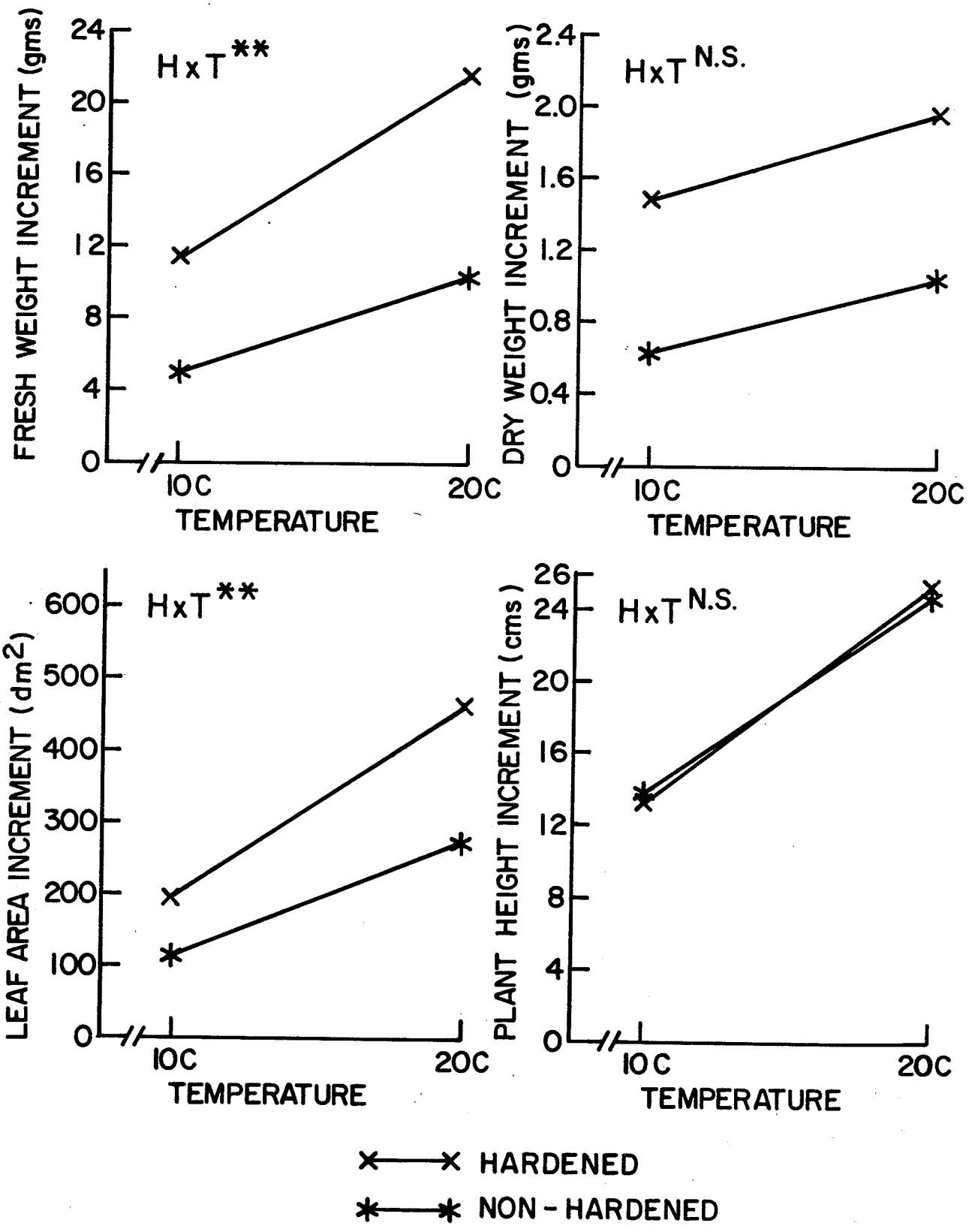


FIGURE 6. Hardening x temperature interaction for four growth parameters in rape.

undergo a proportionately greater increase in fresh weight over the temperature range from 10C to 20C than the non-hardened plants. This difference is reflected in the greater slope of the HxT interaction line for the hardened plants in figure 6, signifying a higher Q_{10} value for fresh weight increase. For dry weight increase, this interaction is non-significant, and the temperature response lines of the hardened and non-hardened plants are very nearly parallel to one another.

The reason for this discrepancy between the two related parameters is not fully clear. It may be due to the fact than the enhanced fresh weight increase of the hardened plants at 20C is due to an increase in the relative water content of the tissue. A comparison of the mean increases for fresh and dry weight (table 8) indicates the hardened plants do contain proportionately less water initially than the non-hardened controls, but that when these hardened plants are transferred to the 20C conditions the water content of their tissue increases to a level more like that of the non-hardened controls. These changes in water content might explain why the fresh weights of the hardened plants would increase proportionately more at 20C than their dry weights. This hypothesis is also supported by gross morphological observations, since after prolonged exposure to warm conditions the hardened plants gradually lose the rubbery appearance associated with low water content and become more tender in appearance, indicating a higher proportion of water in their tissue.

The hardening X date (HxD) interaction is significant for both fresh and dry weight increase (table 7). Figure 7 summarizes the HxD interactions for all the parameters; the curves for fresh and dry weight are

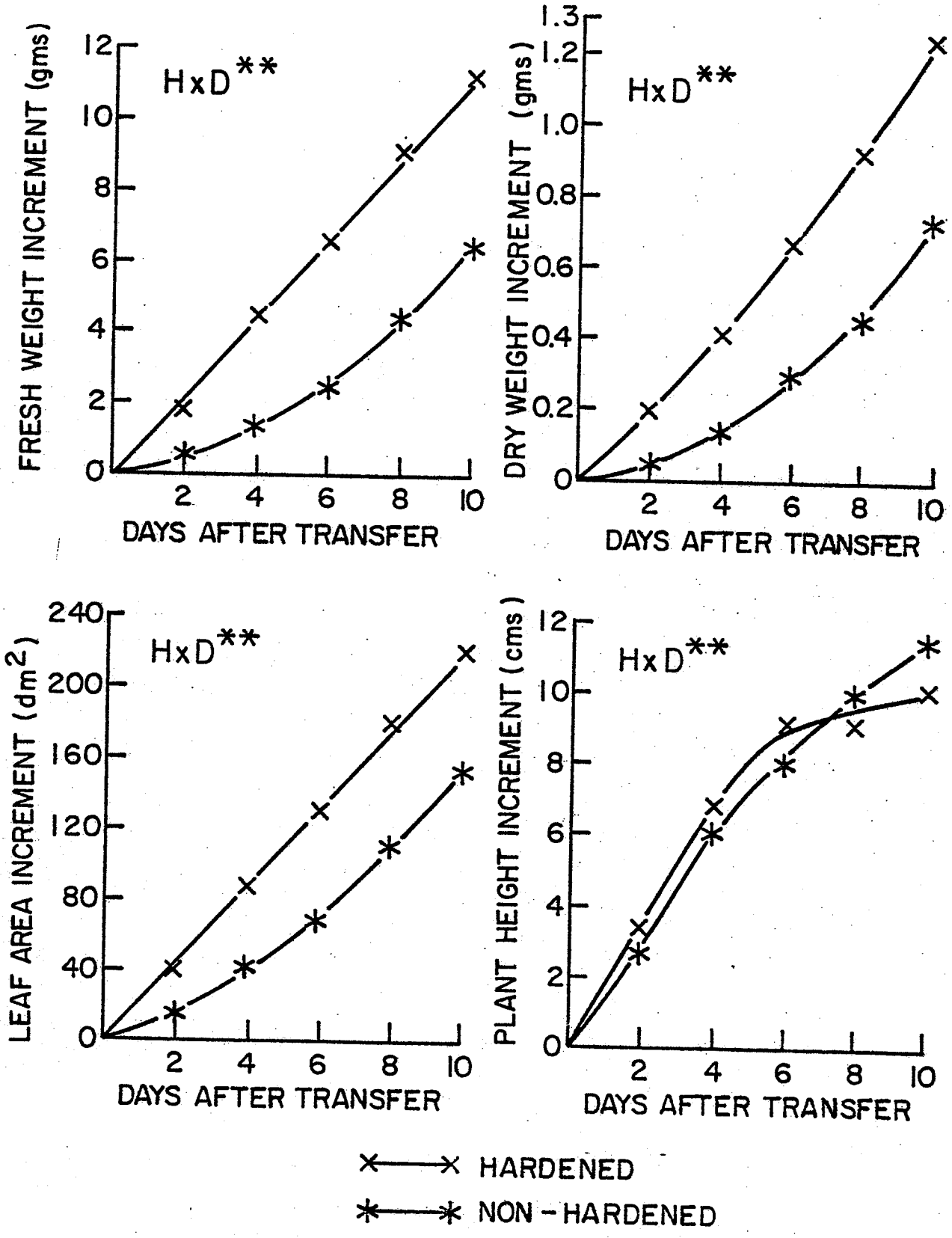


FIGURE 7. Hardening x date interaction for four growth parameters in rape.

very similar. For both parameters, the hardened plants show enhanced vigour and a more highly linear response over time. The curve for the non-hardened plants is more exponential in outline, due to an initial lag phase presumably caused by the period of adjustment required when the plants are transferred to the 10C chamber. After this initial set-back, analogous to an acclimation period, the non-hardened plants show rates of increase in both parameters which are similar to the non-hardened plants.

The temperature X date interaction is significant for both parameters (table 7). It is not pertinent to this discussion since it is not due to any effect of acclimation.

The overall trends in fresh and dry weight accumulation as affected by cold hardening are shown in figures 4 and 5. In both cases, the hardened plants have a more linear or constant response curve, whereas the non-hardened plants undergo an initial lag period at 10C. There is no levelling off in either growth parameter after the ten day period for either the hardened or non-hardened plants.

Leaf area

Table 9 summarizes the mean increases in leaf area as measured over the ten day period. Both the main effects and the interactions were all highly significant for this parameter, as seen in table 7. Only the triple interaction was non-significant.

TABLE 9. Mean increase in leaf area expressed as dm^2 of hardened and non-hardened rape plants.

	10C	20C
Hardened		
Date 1	12.41 ± 5.83	28.64 ± 7.86
Date 2	27.17 ± 7.74	61.08 ± 13.90
Date 3	43.19 ± 8.90	90.34 ± 21.67
Date 4	50.74 ± 11.56	130.54 ± 34.27
Date 5	66.28 ± 17.91	152.58 ± 26.83
Non-hardened		
Date 1	8.00 ± 5.13	9.49 ± 3.77
Date 2	11.43 ± 5.93	32.22 ± 4.55
Date 3	19.70 ± 5.87	49.99 ± 8.82
Date 4	31.02 ± 3.56	83.32 ± 17.54
Date 5	50.94 ± 12.37	104.16 ± 14.85

The main effect of temperature on leaf area increase was exceedingly large. This result was expected since leaf area was observed to be one plant characteristic which is highly sensitive to environmental conditions. When grown under cool (acclimating) conditions, rape developed leaves of noticeably smaller proportions than under warm conditions.

The main effect of date is also highly significant, and not at all surprising. The rape plants used were still in the juvenile period of their growth cycle, and hence are expected to undergo rapid expansion of photosynthetic surface area over time.

Hardening also has a highly significant effect on leaf area increase. The reason postulated for this effect is similar to that offerred for the enhanced growth capacity observed for the hardened plants in the case of fresh and dry weight accumulation. Acclimation seems to prime the plant

for optimal performance upon transfer from the cold hardening chamber to warmer conditions. Although their growth rate is visibly inhibited during acclimation, they quickly adapt when exposed to more favourable conditions and perform with apparent efficiency.

As indicated in table 7, the hardening x temperature interaction is highly significant, and indicates that the hardened plants undergo a proportionately greater increase in leaf area over the temperature range from 10C to 20C than the non-hardened controls. A comparison of the HxT interactions for all growth parameters (figure 6) shows that they are virtually identical for fresh weight and leaf area increase. Moreover, a similar explanation may apply. Just as the hardened plants when grown at 20C gradually attain a water level in their tissue more similar to the non-hardened controls, so also the hardened plants grown at 20C gradually change their morphological appearance to that of the non-hardened plants in terms of developing leaves of larger surface area. These changes in morphology would explain the proportionately greater increases in both fresh weight and leaf area for the cold hardened plants grown at 20C as opposed to 10C. At the lower temperature the hardened plants retain the morphology developed during the hardening regime.

The hardening X date interaction is also similar to that observed for fresh and dry weight (figure 7), except that the non-hardened curve is more nearly linear.

Figure 8 illustrates the overall response of this growth parameter to cold acclimation. The response of the hardened plants is more linear over time than the non-hardened plants, which display a characteristic exponential response. This difference is most apparent when the performance

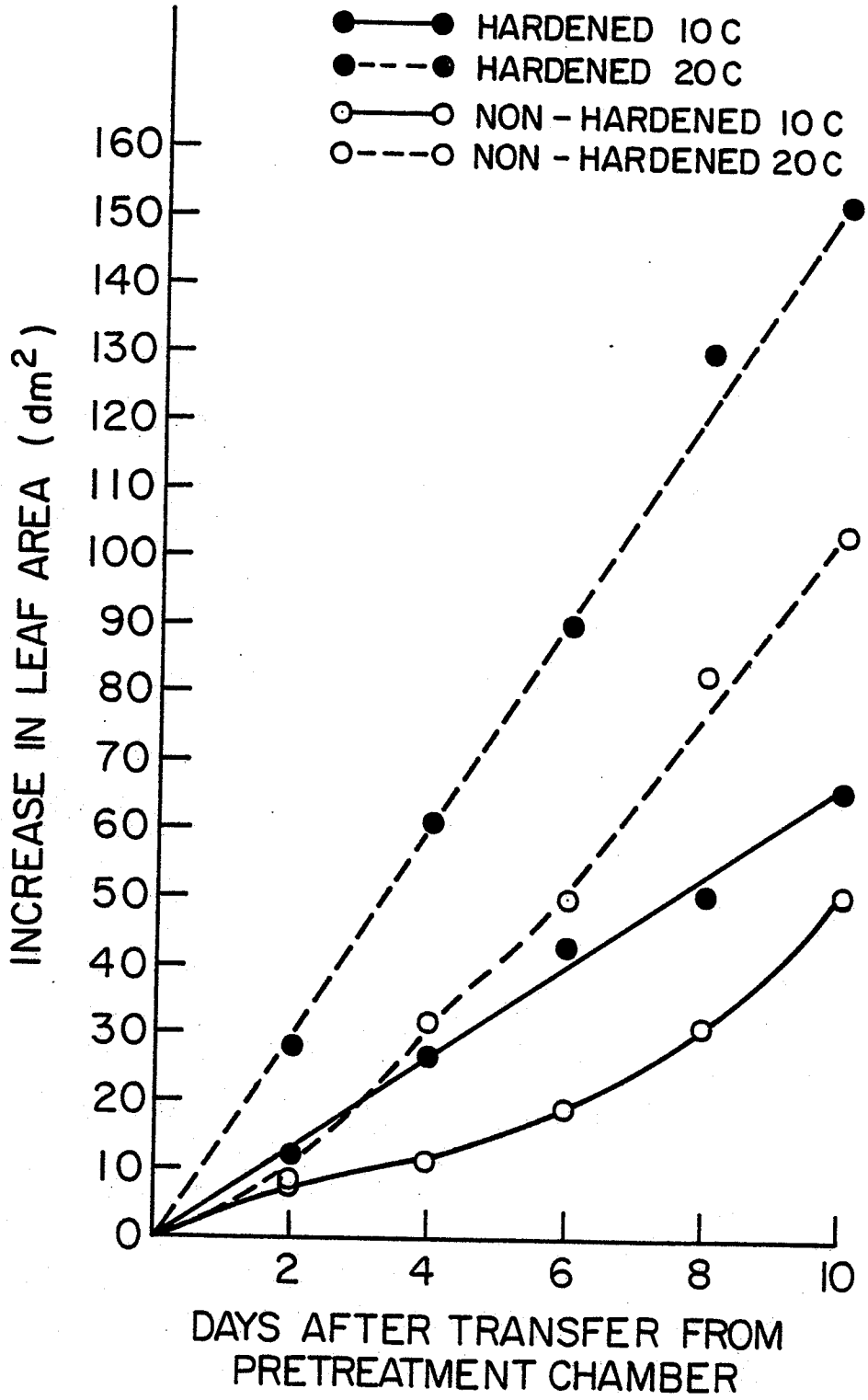


FIGURE 8. Effect of temperature and hardening on mean rates of leaf area increase in rape.

of the hardened and the non-hardened plants at 10C is compared. At this low temperature the non-hardened plants undergo a lag period of approximately four days, during which they experience virtually no increase in leaf area. After this adjustment period, the non-hardened plants resume healthy increases in leaf area. If a temperature lower than 10C had been used, the lag period would presumably have been longer since it took the hardened plants between one and two weeks before they resumed growth after transfer to the hardening regime, which included temperatures as low as -1C. Because the hardened plants are acclimated to temperatures much lower than 10C, they show no such lag in leaf area increase but instead have reasonably constant increments in leaf area over the ten day period, resulting in their linear response curve.

As was the case for dry and fresh weight, there is no levelling off in leaf area increase for either the hardened or non-hardened plants.

Comparing the leaf area results with that for fresh and dry weight, the most notable difference is the greater sensitivity of this parameter to temperature differences. This difference is apparent not only in the F values for the temperature effect (table 7), but also in the graphical presentations of the data (figures 4, 5, and 8).

Plant height

Plant height increase showed some important differences from the other growth parameters studied. Table 10 summarizes the response of this parameter to cold acclimation.

TABLE 10. Mean increase in plant height expressed in cms of hardened and non-hardened rape plants.

	10C	20C
Hardened		
Date 1	1.0 ± 0.6	2.4 ± 0.9
Date 2	2.0 ± 0.7	4.9 ± 0.9
Date 3	3.2 ± 0.8	6.0 ± 0.9
Date 4	3.1 ± 0.8	6.0 ± 1.5
Date 5	4.1 ± 0.8	5.9 ± 1.2
Non-hardened		
Date 1	0.9 ± 0.6	2.0 ± 1.0
Date 2	2.2 ± 0.8	3.9 ± 1.5
Date 3	2.8 ± 0.9	5.3 ± 0.9
Date 4	3.3 ± 1.0	6.7 ± 1.1
Date 5	4.8 ± 0.9	7.0 ± 0.8

Plant height is the only growth parameter studied which did not show a significant main effect due to hardening (table 7). Prior acclimation treatment had no significant effect on the subsequent increase in plant height; this is most clearly seen in figure 9. It should be remembered, in contrast, that during the acclimation period itself plant height is very noticeably affected by acclimation, resulting in the rosette appearance of the hardened plants.

The absence of a significant effect due to hardening is surprising since for all other growth parameters studied the hardened plants had much greater rates of increase at both 10C and 20C than did the non-hardened controls. However, plant height is perhaps more valuable as an indicator of morphological type than it is of plant vigour. Increase in dry weight and leaf area are generally more reliable indicators of the

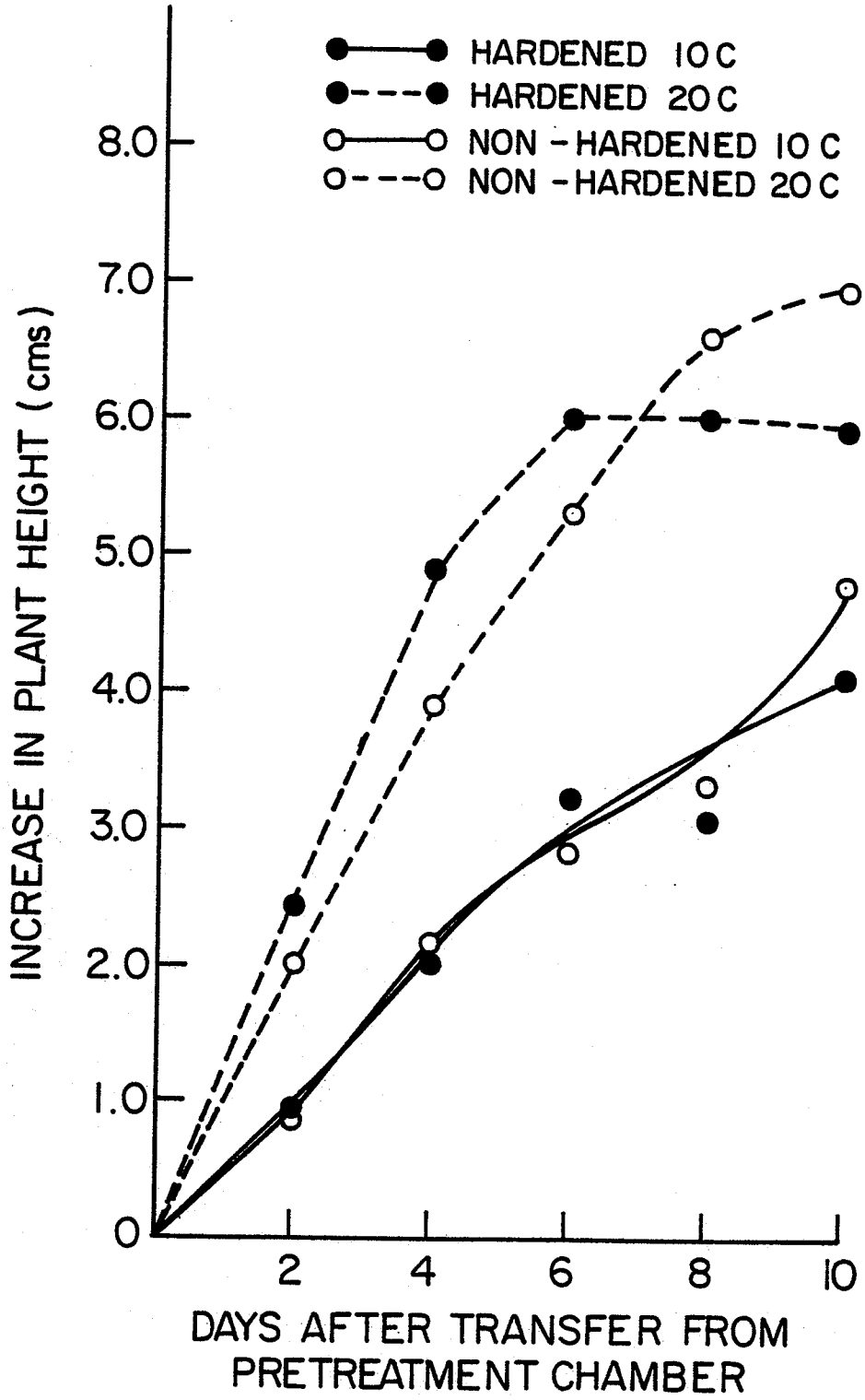


FIGURE 9. Effect of temperature and hardening on mean rates of plant height increase in rape.

plant's growth performance, and hence the failure to show a significant main effect due to hardening need not be disturbing. What these results do show is that the dwarfing effect of acclimation on plant height is remarkably short-lived once the plant is removed from acclimating conditions.

The other main effects, temperature and date are highly significant and require little comment. The explanation of these effects is similar to that put forward for the other growth parameters.

As is the case for dry weight, there is no significant hardening X temperature interaction (figure 6). Both hardened and non-hardened plants show identical responses in terms of plant height increase over the temperature range from 10C to 20C.

There is, however, a significant hardening X date interaction (figure 7). Hardened plants show a much more distinct plateau in plant height than do the non-hardened controls. This levelling off is particularly apparent in the hardened plants grown at 20C, which show little further increase in plant height after six days, whereas the non-hardened plants continue to show substantial increases (figure 9). Plant height is the only parameter studied which did show an apparent levelling off during the ten day measuring period. Consequently, the overall response of the hardened plants is not linear as it was for leaf area and dry weight increase.

The establishment of a plateau is presumably due to the fact that the plant has reached its optimum height for the prevailing light and temperature conditions. From this point on, the plant will continue to grow in terms of leaf area and fresh and dry weight, but will not increase significantly in height. No predictions are possible about the actual value

of this height plateau, but it is possible to say that the hardened plants grown at 20C seem to be levelling off at a lower value than the non-hardened plants. It would seem, then, that although acclimation does not affect the rate of plant height increase, hardened plants do appear to level off at a lower ultimate height.

The complicating factor of floral initiation should be mentioned at this point. In the earlier growth analysis experiment, which took place over a longer time period, floral initiation was observed after 10 to 14 days. If flowering was induced by prevailing light conditions, then this would be largely responsible for the levelling off in plant height observed.

Relationships between growth parameters

Fresh versus dry weight.

Bar graphs plotting increases in both fresh and dry weight over time for hardened and non-hardened rape seedlings show important differences in temperature response.

For the hardened plants grown at 10C, dry weight increases at a faster rate than does fresh weight, as indicated by the slope of the bars. This means that the hardened plants are gradually increasing their dry matter content (or, alternatively, lowering their moisture content) when grown at 10C (Figure 10).

When grown at 20C, the hardened plants show similar rates of increase for both parameters, indicating that they do not significantly alter their dry matter content when transferred to the warmer conditions, at least

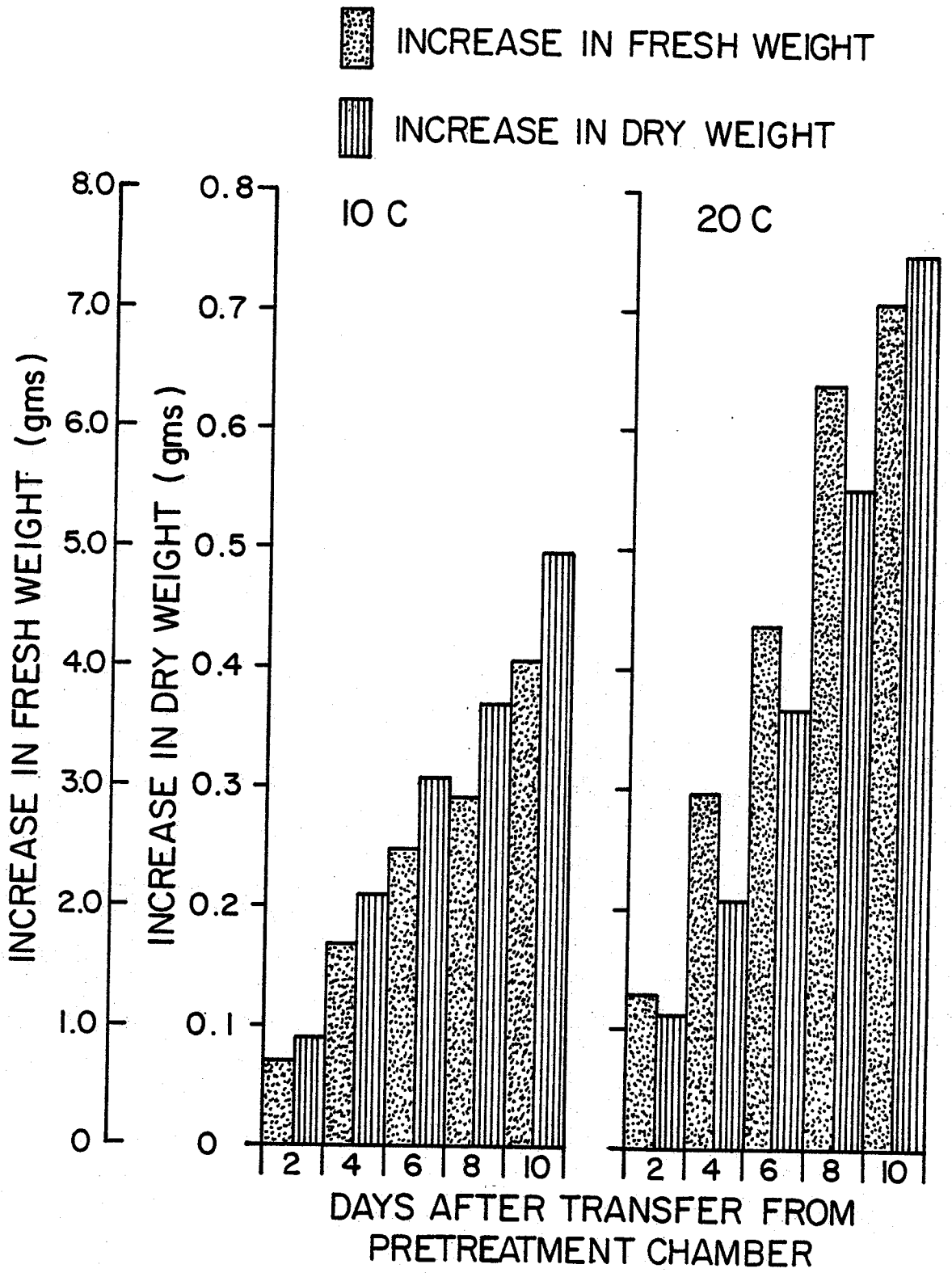


FIGURE 10. Mean increase in fresh weight versus dry weight for cold hardened rape plants.

during the time period studied (Figure 10).

Non-hardened plants grown at 10C have virtually identical rates of increase for both dry and fresh weight, as indicated by the slope of the bar graphs (Figure 11). This means that they have not begun lowering their moisture content in response to the lower temperature. Temperatures lower than 10C would probably be required for such a response to occur.

The non-hardened plants grown at 20C also show no significant differences in their rates of dry and/or fresh weight increase (Figure 11).

Besides the large quanitative differences in dry and fresh weight increase for hardened and non-hardened plants, the only notable difference in rate of increase, then, is that observed in the case of the hardened plants grown at 10C.

Leaf area versus dry weight.

When the hardened plants are grown at 10C, the rate of increase in dry weight over time far outstrips the rate of increase in leaf area, indicating that the hardened plants are continuing their morphological trends towards smaller photosynthetic surface area (Figure 12). This trends implies an improved efficiency of photosynthetic performance as well, since the high rates of dry matter increase are supported by a relatively small leaf area.

At 20C, on the other hand, the hardened plants show no significant differences in rates of dry weight or leaf area increase (Figure 12).

The non-hardened plants grown at 10C also have similar rates of

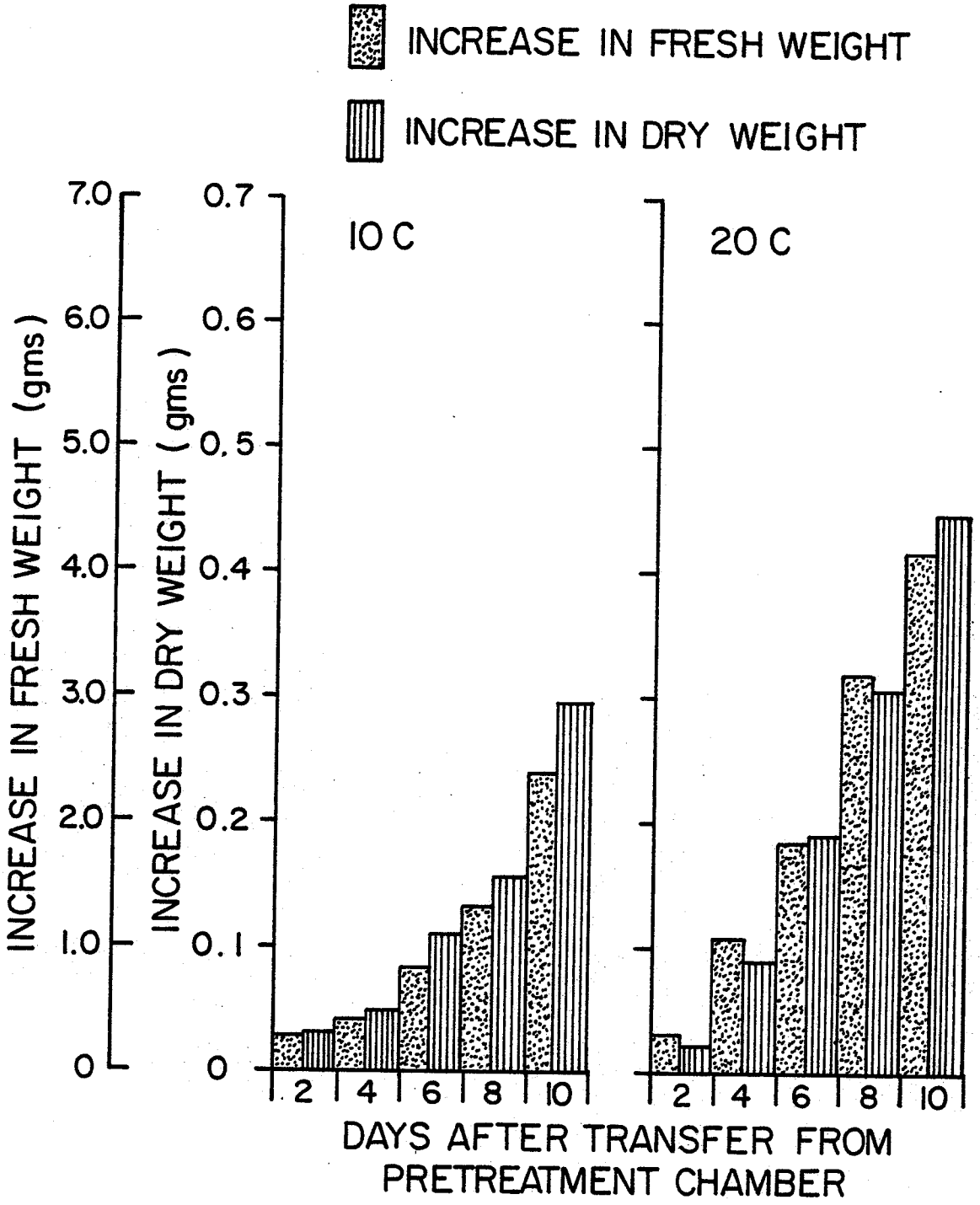


FIGURE 11. Mean increase in fresh weight versus dry weight for non-hardened rape plants.

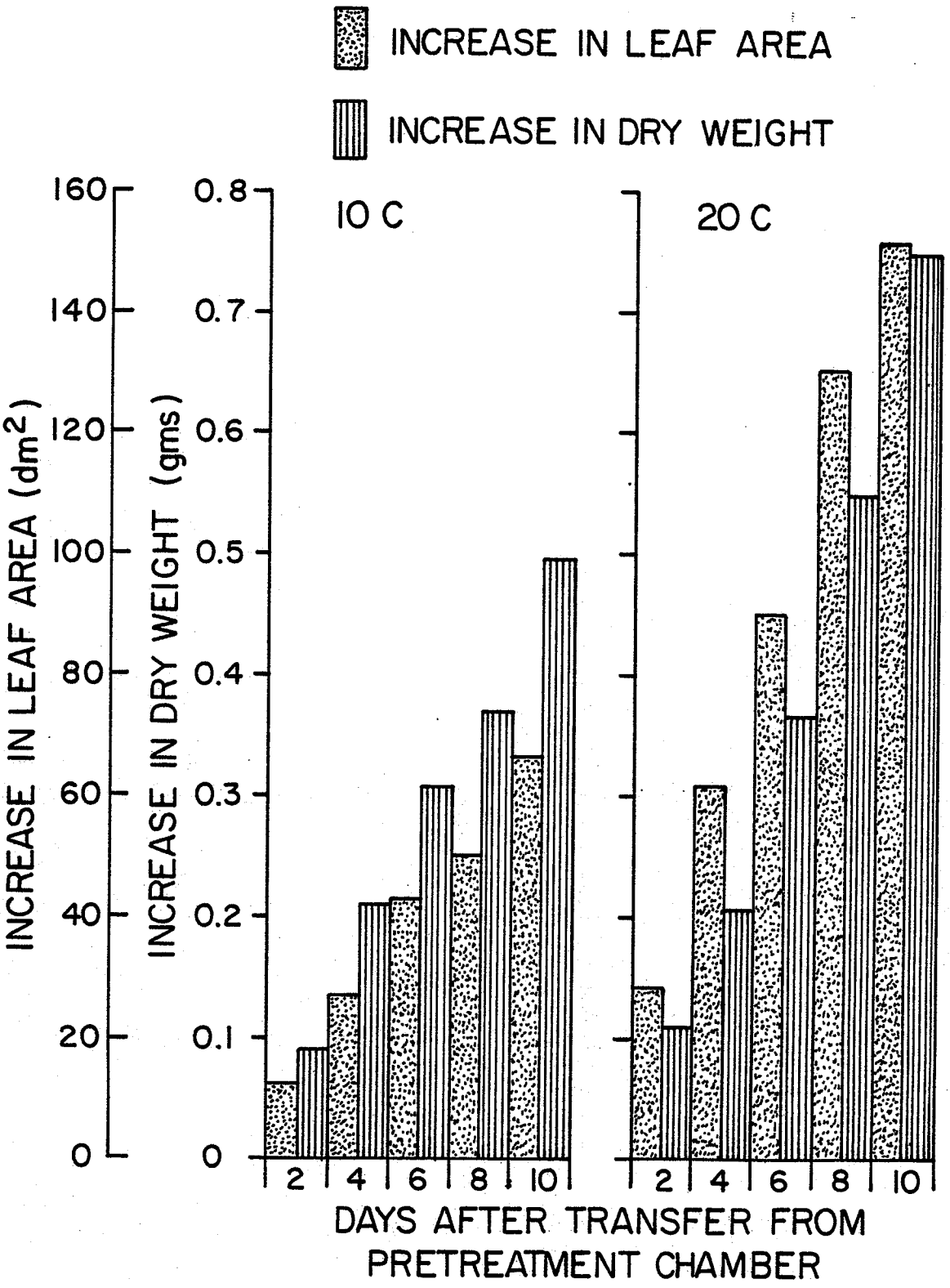


FIGURE 12. Mean increase in leaf area versus dry weight for cold hardened rape plants.

dry weight and leaf area increase, as indicated by the slope of the bar graphs (Figure 13). Again, the 10C temperature is not low enough to induce the alterations in morphological type associated with the acclimation process.

At 20C, differences in rates of increase are again not great for the non-hardened plants, although leaf area is increasing at a slighter higher rate than dry weight, indicating the trend at warmer temperatures towards plants of larger surface area (Figure 13).

Plant height versus dry weight.

When the hardened plants are grown at 10C, rates of increase for plant height and dry weight are similar until the third sampling date, at which point the rate of plant height increase drops off sharply (Figure 14). This is due to the levelling off observed for the hardened plants.

At 20C, a similar plateau effect is observed, although after much greater quantitative increases in plant height (Figure 14).

No plateau in plant height is observed for the non-hardened plants when grown at 10C, indicating an important difference in morphological type from the hardened plants (Figure 15). The rate of increase in plant height is also greater than the rate of increase in dry weight when the non-hardened plants are grown at 20C as well.

These results suggest that though the non-hardened plants are not as capable of as substantial rates of dry matter increase at either temperature as are the hardened plants, their growth in terms of plant

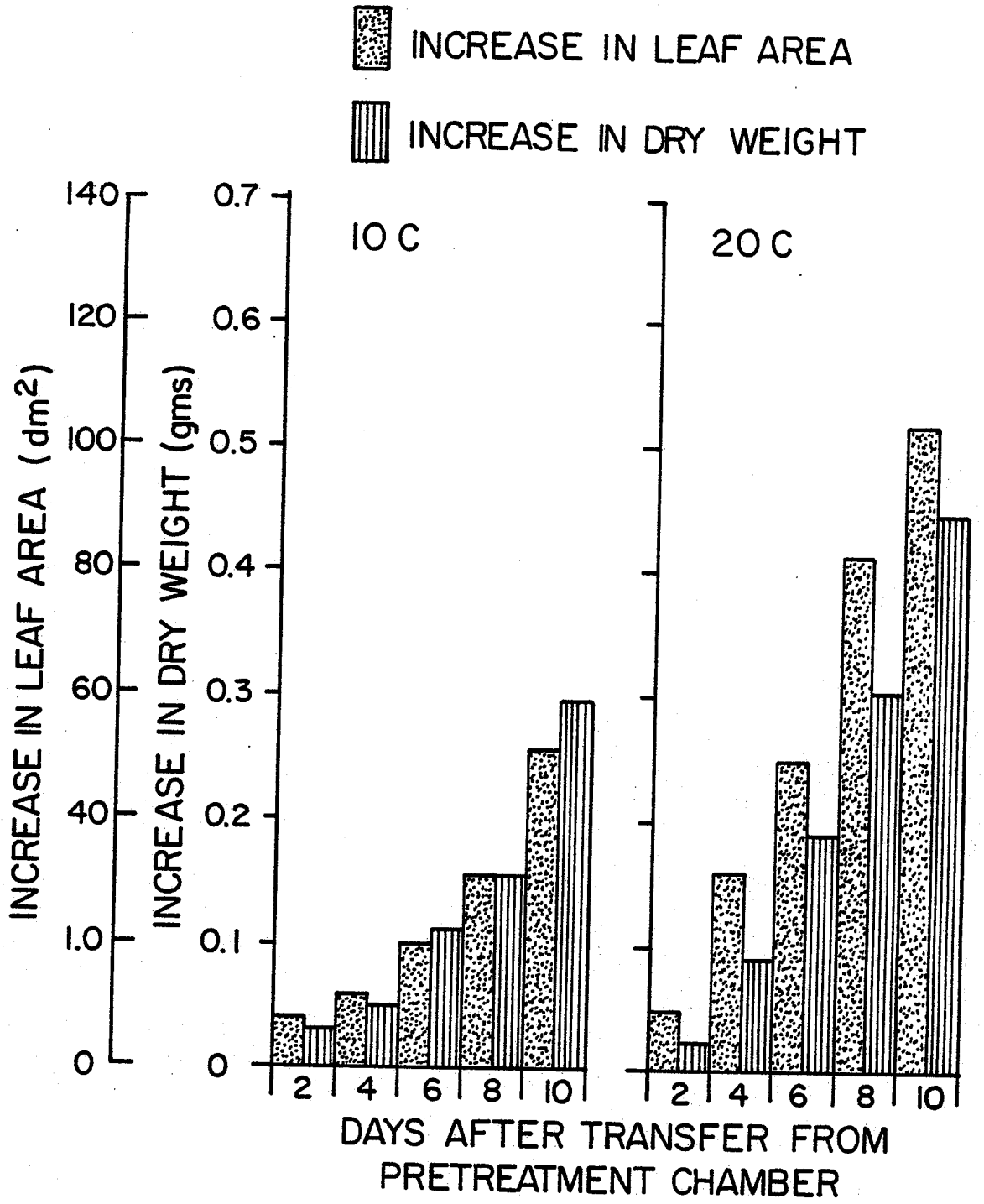


FIGURE 13. Mean increase in leaf area versus dry weight for non-hardened rape plants.

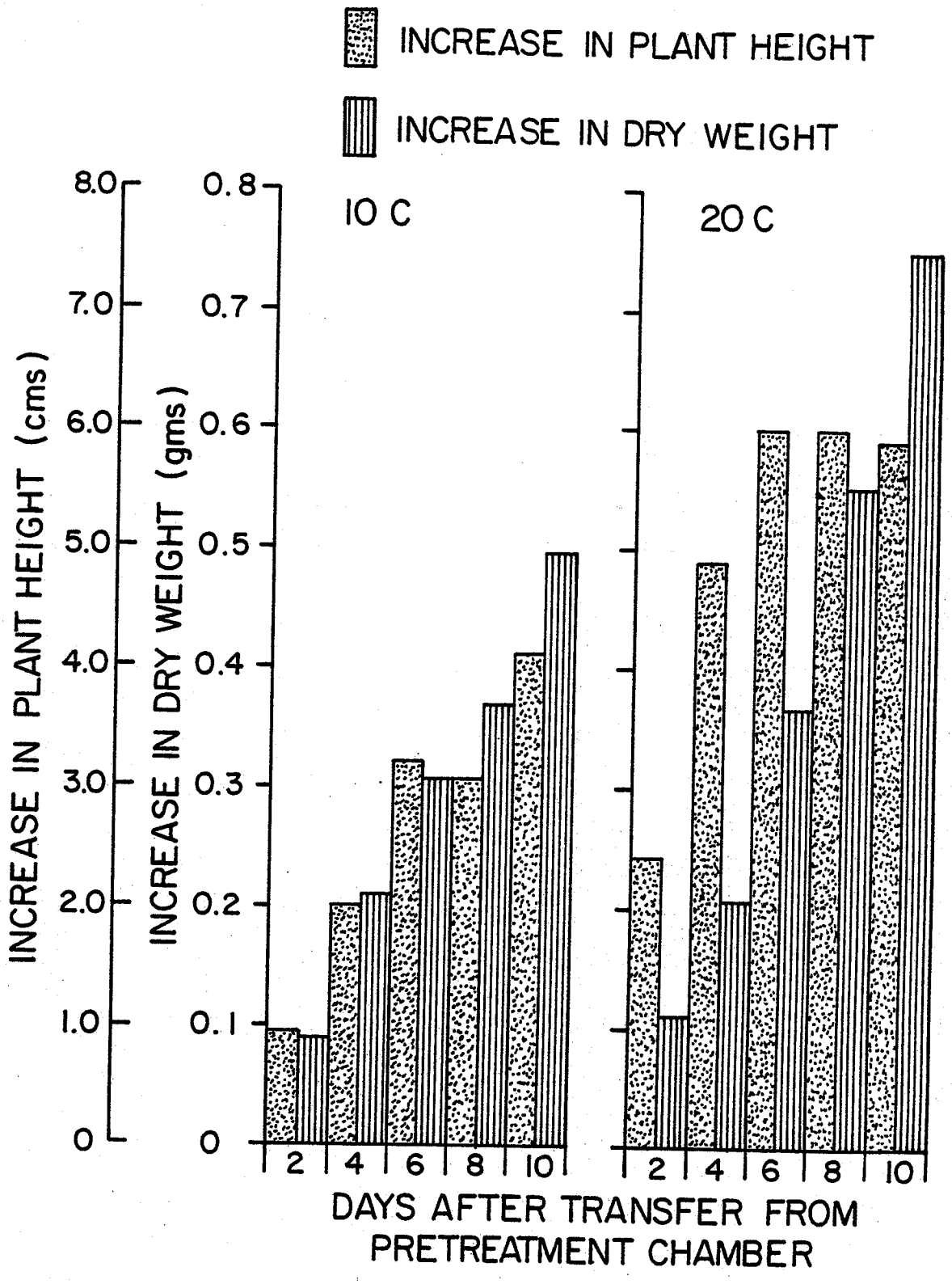


FIGURE 14. Mean increase in plant height versus dry weight for cold hardened rape plants.

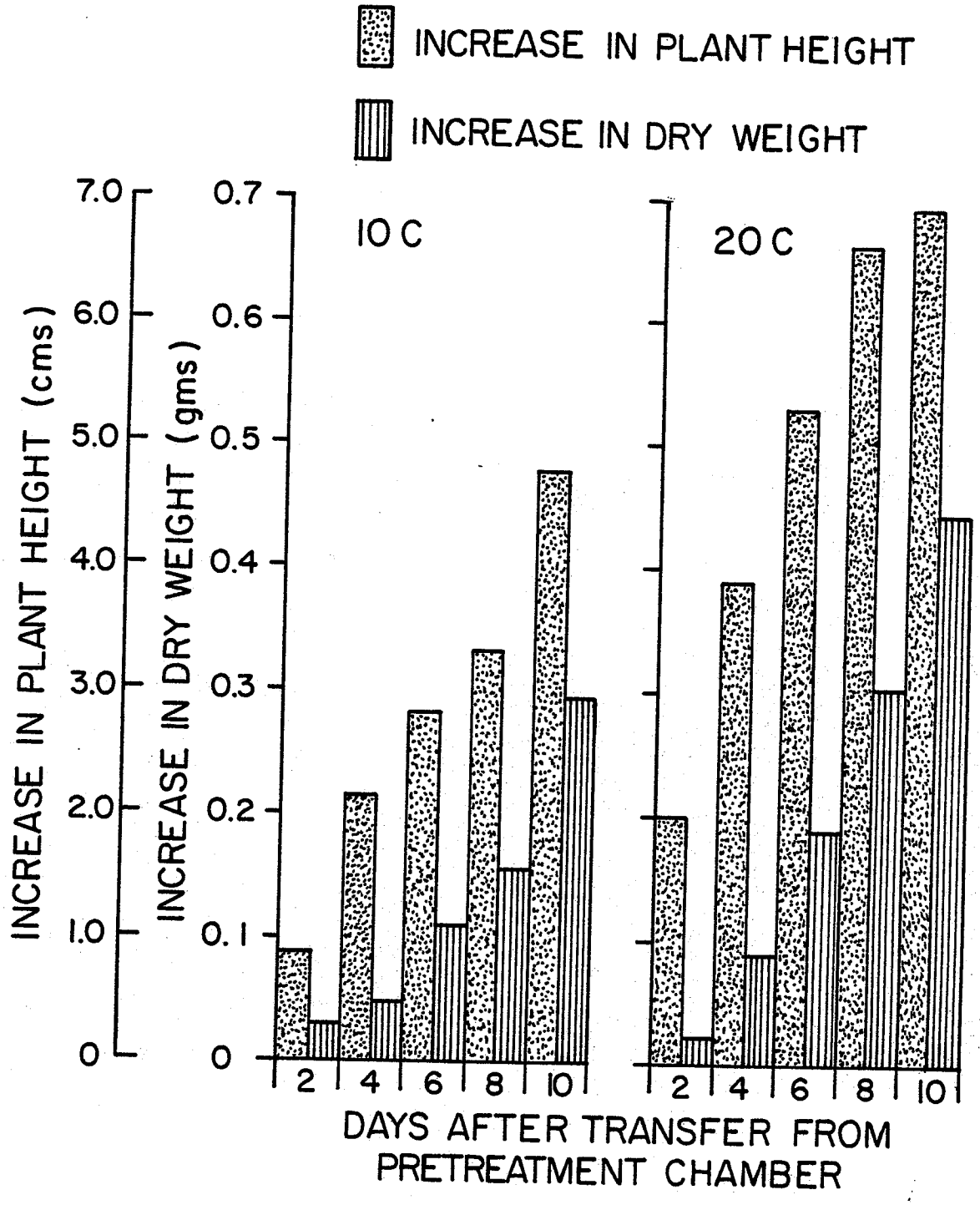


FIGURE 15. Mean increase in plant height versus dry weight for non-hardened rape plants.

height is much less inhibited. The growth pattern initiated under non-hardening conditions has been continued even when plants are transferred to the 10C chamber.

These characteristically large increases in plant height relative to dry weight seem to be indicative of the non-hardened state, since hardened plants are more dwarfed in appearance. Increase in plant height, therefore, is not a reliable indicator of the growth efficiency or the acclimation of the plant, since the cold hardened plants had much higher rates of dry matter increase, as well as much lower rates of plant height increase when compared to the non-hardened plants.

In general, then, this examination of the relative rates of increase in the various parameters shows that whereas quantitative increases differ greatly between the hardened and non-hardened plants, rates of increase are much more constant. The important differences show up in the hardened plants grown at 10C, where both the rates of increase in leaf area and fresh weight decrease relative to the increase in dry weight, indicating the continued development of a plant of smaller surface area and lower moisture content. The only ntoable exceptions observed were in the rates of plant height increase, which indicated differences in morphological type between the hardened and non-hardened plants, rather than differences in growth efficiency.

Relationship of growth analysis results to gas analysis results

It is difficult to draw strong conelusions about the relationship of the gas exchange results to the overall growth performance of the plant since many factors are involved in the plant's growth rate besides its rates of photosynthesis and respiration. However, it is possible to determine whether or not the two sets of results support each other or are contradictory.

The gas exchange experiments indicated that cold acclimation results in enhanced rates of net photosynthesis at 10C. This would suggest a greater growth capacity of the hardened plants at cooler temperatures as compared to the non-hardened controls. At 20C, on the other hand, the hardened plants displayed no such advantage in terms of photosynthetic capacity. At both temperatures enhanced respiration rates would seem to detract severely from the growth capacity of the hardened plants. In terms of their overall growth efficiency, as indicated by the ratio of net photosynthesis/dark respiration, the hardened plants appeared to be at a disadvantage, certainly at 20C where they have similar photosynthetic rates but much greater respiration rates than the non-hardened plants.

These conclusions make the important assumption that the enhanced respiratory rates will continue during further growth at both 10C and 20C. However, if one assumes instead that they result from an excess of photosynthate accumulated during acclimation, it is reasonable to conclude instead that the enhanced rates of respiration are a short term effect

that will be alleviated once the plant has reached a new metabolic balance under prevailing environmental temperatures. The hardened plant, then, will undergo a period of enhanced respiratory rates upon transfer to warmer temperatures during which it burns up excess photosynthate that has not been transported to storage sites due to reduced translocation rates at low temperatures. This process amounts to a wasteful consumption of fuel, but once it is accomplished, the feedback inhibition on photosynthesis will be alleviated and the plant will be able to resume a more efficient photosynthetic performance. The temporary nature of these respiratory increases of the hardened plants, plus their increased photosynthetic capacity at 10C (which is presumed to be more long-term in nature than the respiratory effects) means that over time the hardened plants will indeed be capable of greater growth rates than the non-hardened controls, at least at 10C.

The growth analysis results support this second interpretation of the gas analysis data. For all growth parameters studied apart from plant height, the hardened plants exhibited much greater rates of increase. Moreover, this advantage is not only apparent at 10C as one might expect, but at 20C as well. Cold acclimation seems to prime the plant for a more vigorous growth performance over a range of environmental temperatures. That this does not occur in the case of plant height is due to this parameter's importance as an indicator of morphological type rather than growth performance.

The hardened plants retain this advantage over the non-hardened

controls throughout the ten day measuring period, indicating that it is relatively long-term in nature rather than an immediate effect. This finding also supports the conclusions from the gas analysis data that the effects of cold acclimation on photosynthesis are long-term, presumably involving changes to the photosynthetic apparatus, whereas the effects of hardening on respiration are short-term, involving a temporary excess of substrate which leads to a wasteful consumption of fuel not tied to growth.

Results discussed in light of issues arising in the literature

Several issues arising in the literature deserve comment insofar as they relate directly to findings reported in this thesis.

One of the main issues concerning cold acclimation is whether cessation of growth is a necessary prerequisite (Alden and Hermann 1971; Levitt 1972). In the case of spring rape, growth cessation was not necessary for effective acclimation. In fact, during the second growth analysis experiments when plants were transferred to acclimating conditions shortly after germination, the period of shock was only one week in duration. After this period, the plants resumed growth under the low temperature conditions in the acclimating chamber. In contrast, when the plants were transferred to the acclimating chamber at the 1-2 true leaf stage, as they were for the gas analysis experiments, the period of adjustment was noticeably longer, about two weeks. Only after this period did growth resume. From these observations it seems safe to conclude that in the case of an annual species such as spring

rape which does not have a dormant period in its life cycle, actively growing tissue is capable of effective acclimation. This would concur with Levitt's (1965, 1974) findings with another annual species, cabbage.

Not only is growth cessation not necessary for acclimation in spring rape, but acclimation appears to enhance growth rates after plants are removed from the acclimating chamber and returned to more favourable temperature conditions. As seen in the growth analysis experiments, this invigorating effect of cold acclimation on subsequent growth rates in retained by the plant for the duration of the ten day measuring period. The cause of this enhancement effect is not clear, although the gas analysis results suggest that it involves adaptive changes to the photosynthetic apparatus.

A second issue which is unresolved in the literature is whether the changes accompanying acclimation are merely low temperature effects not causally related to enhanced cold hardiness or an integral part of the acclimation process. This issue arises from the difficulty of establishing causal relationships, as opposed to correlations, between observed phenomena, be they biochemical or biophysical alterations in the plant, and alterations in the degree of cold hardiness. The role of unsaturation in fatty acids, changes in protein levels and carbohydrate transformations are prime examples of phenomena which may or may not be directly involved in cold acclimation. In these experiments, no attempt was made to look at correlations between these phenomena and cold hardiness

but the same issue can be raised about alterations to gas exchange rates. Enhanced respiratory rates as a result of low temperature acclimation are probably not an integral part of the cold acclimation process in rape, though they may be in Arctic species (Bjorkman 1966), but are more likely a low temperature effect caused by the accumulation of unused photosynthate at low temperature brought on by the observed slow-down in growth processes, including translocation.

A third issue is the degree to which plants are capable of cold acclimation, that is their degree of plasticity of response to environmental temperature. This characteristic of adaptability is presumably genetic in origin, and it has been reported for rape that temperature optima do not vary greatly with environmental temperature (Herath 1973). Based on my results, I would dispute that claim, since the plants did show alteration in gas exchange rates related to preconditioning temperatures, and that these alterations were reflected ultimately in alterations in growth capacity, as measured by a number of parameters. Limiting light intensities might partly explain why other workers have not observed alterations in temperature optima for photosynthesis in rape.

The importance of environmental conditions during the cold acclimation period should be emphasized. The long discussion on the effects of environmental factors on acclimation was included to stress the role of light and water, as well as temperature, on cold hardening. Many cold acclimation experiments involve an acclimation period of total darkness, in part for ease of mitochondrial extraction. However, in view of the

critical role of light — both its intensity, quality (spectrum) and duration — results obtained after such an artificial acclimation period are questionable. It was felt than the acclimation period which most closely resembles natural conditions would be desirable. For this reason, a regime involving an 11 hour day and a continuous fluctuation of temperatures from a low of -1 to a high of 15C was chosen.

Photoperiod is less critical to acclimation of an annual species such as spring rape since there is no dormancy requirement for cold acclimation in this species. However, photoperiod may have a role apart from inducing dormancy, in particular the stimulation of hardiness promoting substances (Alden and Hermann 1971). In this experiment an 11 hour day was chosen to avoid the complication of floral initiation, although in the preliminary growth experiments this occurred towards the end of the measuring period despite this precaution. An alternate photoperiod would have been preferable.

SUMMARY AND CONCLUSIONS

1. Cold acclimation significantly increased dark respiration rates at both 10C and 20C as compared to non-hardened controls.

2. Rates of net photosynthesis were identical at 20C for both non-hardened plants and those hardened 3 weeks, and significantly above rates for plants hardened 6 weeks.

3. At 10C rates of net photosynthesis were significantly lower for the non-hardened plants than for either group of hardened plants.

4. Ratios of net photosynthesis/dark respiration were significantly higher at 10C than at 20C for both hardened and non-hardened plants.

5. Cold hardened plants had significantly higher rates of increase in fresh weight, dry weight and leaf area as compared to the non-hardened controls at both 10C and 20C.

6. Non-hardened plants had an initial lag period when transferred to 10C, after which they resume growth as measured by basic growth parameters, but at a lesser rate than the hardened plants.

7. Cold acclimation had no significant effect on increase in plant height. However, hardened plants levelled off at a lower height than did the non-hardened controls.

8. Hardened plants had decreased rates of fresh weight increase as compared to dry weight increase when grown at 10C.

9. Hardened plants had decreased rates of leaf area increase as compared to dry weight increase when grown at 10C.

The gas exchange findings indicate that cold acclimation enhances respiration and makes the temperature response curve for net photosynthesis less temperature dependant. However, these conclusions are tentative since the longevity of these effects was not determined. Respiration may be enhanced by excess substrate, indicating a short-term non-adaptive stimulus, whereas changes in photosynthetic rates may result from alterations to the apparatus and hence may be more long-term. Future work would have to settle this issue before any more definite conclusions could be drawn. Such studies should also include a greater range of measuring temperatures, as well as higher radiation levels. An alternate method of estimating photorespiration would also be advisable.

The growth analysis results appear to support this tentative conclusion that cold acclimation enhances the overall growth capacity of spring rape as indicated for all parameters studied except plant height. Alterations to the ratios of leaf area and fresh weight to dry weight indicate that growth at low temperatures results in decreased water content and leaves of smaller surface area. These effects appear to be low temperature rather than cold acclimation effects since they are evident for both the hardened and non-hardened plants. Future growth analysis work would also benefit from the use of more than two measuring temperatures and a higher radiation level.

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APPENDIX

TABLE 11. Analysis of variance in dark respiration rates of hardened and non-hardened rape plants.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Temperature	1	60.5803	60.5803	64.6327**
Hardening	2	54.4547	27.2274	29.0488**
Temperature x Hardening	2	7.8621	3.9311	4.1940*
Error	30	28.1193	0.9373	
Total	35	151.0164	c.v. = 28.02%	

TABLE 12. Analysis of variance in rates of net photosynthesis of hardened and non-hardened rape plants.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Temperature	1	0.2336	0.2336	0.7290n.s.
Hardening	2	20.6274	10.3137	3.2189n.s.
Temperature x Hardening	2	76.6131	38.3066	11.9555**
Error	30	96.1223	3.2041	
Total	35	193.5964	c.v. = 13.86%	

TABLE 13. Analysis of variance in rates of photorespiration of hardened and non-hardened rape plants.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Temperature	1	12.7212	12.7212	$3.8972^{n.s.}$
Hardening	2	20.7255	10.3628	$3.1747^{n.s.}$
Temperature x Hardening	2	9.9110	4.9555	$1.5181^{n.s.}$
Error	30	97.9279	3.2642	
Total	35	141.2856		c.v. = 74.93%

TABLE 14. Analysis of variance in ratio of net photosynthesis/dark respiration of hardened and non-hardened rape plants.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Temperature	1	109.9002	109.9002	25.7420**
Hardening	2	160.3651	80.1826	18.7812**
Temperature x Hardening	2	2.8228	1.4114	$0.3306^{n.s.}$
Error	30	128.0794	4.2693	
Total	35	401.1675		c.v. = 39.93%

TABLE 15. Analysis of variance in increase in dry weight of hardened and non-hardened rape plants.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Temperature	1	0.4300	0.4300	69.4175**
Hardening	1	1.5586	1.5586	251.6143**
Date	4	4.6980	1.1745	189.6067**
Hardening x Temperature	1	0.0050	0.0050	$0.8072^{n.s.}$
Hardening x Date	4	0.2030	0.0508	8.1929**
Temperature x Date	4	0.3102	0.0776	12.5194**
Hardening x Temperature x Date	4	0.0303	0.0076	$1.2229^{n.s.}$
Error	180	1.1115	0.0062	
Total	199	8.3501		c.v. = 30.51%

TABLE 16. Analysis of variance in increase in fresh weight of hardened and non-hardened rape plants.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Temperature	1	120.1762	120.1762	228.2983**
Hardening	1	162.1674	162.1674	308.0688**
Date	4	360.5062	90.1266	171.2131**
Temperature x Hardening	1	11.2580	11.2580	21.3868**
Hardening x Date	4	18.2527	4.5632	8.6687**
Temperature x Date	4	37.3379	9.3345	17.7327**
Hardening x Temperature x Date	4	1.6118	0.4030	$0.7655^{n.s.}$
Error	180	94.7562	0.5264	
Total	199	806.0664		c.v. = 30.91%

TABLE 17. Analysis of variance in increase in leaf area of hardened and non-hardened rape plants.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Temperature	1	88831.59	88831.59	422.93**
Hardening	1	34509.63	34509.63	164.30**
Date	4	158161.37	39540.34	188.25**
Temperature x Hardening	1	5547.04	5547.04	26.41**
Hardening x Date	4	3407.82	851.96	4.06**
Temperature x Date	4	26727.51	6681.88	31.81**
Hardening x Temperature x Date	4	767.58	191.90	$0.91^{n.s.}$
Error	180	37807.11	210.04	
Total	199	355759.65		c.v. = 27.26%

TABLE 18. Analysis of variance in increase in plant height of hardened and non-hardened rape plants.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Temperature	1	258.3265	258.3265	297.5769**
Hardening	1	0.0041	0.6041	0.6959$^{n.s.}$
Date	4	369.4543	92.3636	106.3974**
Hardening x Temperature	1	0.5294	0.5294	0.6098$^{n.s.}$
Hardening x Date	4	14.4727	3.6182	4.1679**
Temperature x Date	4	18.5543	4.6386	5.3434**
Hardening x Temperature x Date	4	3.9737	0.9934	1.1443$^{n.s.}$
Error	180	156.2646	0.8681	
Total	199	821.5796		c.v. = 22.39%

TABLE 19. Mean increases in four growth parameters in rape: preliminary experiment.

	Fresh weight increase (gms)		Dry weight increase (gms)	
	10C	20C	10C	20C
Hardened				
Date 1	0.10 ± 0.20	0.22 ± 0.20	0.01 ± 0.02	0.03 ± 0.03
Date 2	0.14 ± 0.23	0.74 ± 0.60	0.01 ± 0.02	0.06 ± 0.05
Date 3	0.35 ± 0.38	2.00 ± 1.04	0.04 ± 0.05	0.16 ± 0.08
Date 4	0.70 ± 0.56	3.29 ± 2.48	0.09 ± 0.07	0.26 ± 0.22
Date 5	1.33 ± 0.75	6.54 ± 3.17	0.14 ± 0.09	0.50 ± 0.26
Date 6	1.48 ± 1.15	9.18 ± 3.95	0.18 ± 0.14	0.85 ± 0.41
Date 7	1.86 ± 0.72	12.37 ± 4.71	0.21 ± 0.08	1.22 ± 0.52
Non-hardened				
Date 1	-0.61 ± 0.86	-0.42 ± 1.00	-0.05 ± 0.07	-0.03 ± 0.96
Date 2	-0.09 ± 1.38	1.25 ± 2.49	0.02 ± 0.14	0.14 ± 0.26
Date 3	-0.05 ± 1.53	2.40 ± 2.57	0.03 ± 0.16	0.25 ± 0.29
Date 4	0.36 ± 2.15	1.75 ± 1.78	0.09 ± 0.24	0.20 ± 0.19
Date 5	1.18 ± 2.76	5.14 ± 5.01	0.18 ± 0.32	0.49 ± 0.49
Date 6	1.98 ± 3.23	6.01 ± 3.65	0.31 ± 0.39	0.77 ± 0.55
Date 7	2.02 ± 3.24	9.07 ± 4.96	0.31 ± 0.44	1.14 ± 0.71

	Leaf area increase (dm^2)		Plant height increase (cms)	
	10C	20C	10C	20C
Hardened				
Date 1	2.96 ± 4.82	5.99 ± 7.16	1.7 ± 1.7	2.3 ± 2.1
Date 2	3.83 ± 5.65	17.58 ± 13.04	1.2 ± 1.3	5.1 ± 2.7
Date 3	8.55 ± 8.36	48.15 ± 23.32	3.5 ± 1.8	8.7 ± 1.9
Date 4	16.12 ± 12.06	66.14 ± 46.45	3.3 ± 2.0	9.5 ± 3.3
Date 5	29.02 ± 15.49	131.89 ± 67.26	5.1 ± 1.5	12.4 ± 1.8
Date 6	31.86 ± 21.96	192.97 ± 77.70	5.2 ± 2.1	13.0 ± 4.5
Date 7	36.15 ± 13.56	257.65 ± 87.81	5.6 ± 1.3	14.3 ± 3.9
Non-hardened				
Date 1	-11.76 ± 22.97	-7.62 ± 23.73	-2.0 ± 2.8	-1.7 ± 2.9
Date 2	-1.29 ± 31.97	27.58 ± 55.34	-1.7 ± 0.8	1.9 ± 7.6
Date 3	-1.68 ± 33.14	55.97 ± 55.91	-1.2 ± 3.6	3.4 ± 2.7
Date 4	1.68 ± 42.00	45.08 ± 40.32	-1.1 ± 3.8	1.9 ± 2.7
Date 5	22.84 ± 55.75	114.94 ± 152.01	-0.7 ± 3.7	4.4 ± 4.5
Date 6	34.71 ± 63.75	133.53 ± 71.00	-0.5 ± 4.2	5.8 ± 2.4
Date 7	38.90 ± 60.54	191.91 ± 101.05	1.1 ± 3.2	6.3 ± 2.5