BOTANICAL BRIEFING

How Do Plants Survive Ice?

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Plant species have had to adapt to freezing and the presence of ice in many climatic zones. Annual plants avoid ice by seed dispersal but, for biennials and perennials to survive they must cope with ice in various forms. Most plants that are regularly exposed to ice during their life cycles have acquired a dormant or quiescent winter period, when they are more tolerant to freezing temperatures. This Botanical Briefing explores some associations between plants and ice, with an emphasis on processes in plants that alleviate stress imposed by ice cover. Examples are taken from winter cereals which must reach an equilibrium both with ice and with freezing temperatures for survival and economic productivity.   © 1996 Annals of Botany Company

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ICE WITHIN THE PLANT

The formation of ice within a plant is potentially detrimental to its survival. The rigid ice lattice structure extends with decreasing temperature and may penetrate cell walls and cell membranes to an extent that is irreparable by normal cell processes. It is generally accepted that intracellular ice formation is lethal (Guy, 1990). Most tropical plants are unable to protect themselves against such events, but most species of temperate plants can acclimate to cold, so that ice forms only outside the cells as the temperature drops. The initial formation of extracellular ice is under the control of ice nucleators (Brush, Griffith and Mlynarz, 1994) and antifreeze proteins (Hon *et al.*, 1994). Extracellular ice is relatively benign, but acts as a nucleation site for water vapour drawn out of the cell, thereby desiccating the cell. As a consequence, cell volume decreases, but the cell wall remains appressed to the plasma membrane, and ice generally forms between cell wall junctions. It is in this stable state that most cold stressed tissues overwinter at sub-zero temperatures. A hydrophyllic protein with the potential of conferring desiccation tolerance has recently been found in the vascular transition zone of the wheat crown, which is critical tissue for winter survival (Houde *et al.*, 1995). However, ice-driven desiccation can continue to the point of irrevocable cellular damage. The formation of ice within plants and mechanisms of freezing tolerance have been discussed in a number of recent reviews (Guy, 1990; Steponkus, Uemura and Webb, 1993).

ICE IN THE PLANT ENVIRONMENT

The special property of water by which it has a lower density at 0 than 4 °C dictates that ice first forms on open water surfaces. This allows water-adapted plant and animal life to survive winter underneath the ice, albeit at a much reduced metabolic rate. While many aquatic plants enter a dormant state under an ice layer, others continue to gain biomass. Boylen and Sheldon (1976) recorded a light intensity of 4300 lx 5 m under an ice layer. This light level was considerably above saturation level for photosynthesis of several species. In midwinter, rates of photosynthesis of *Potamogeton robbinsii* and eight other submergent macro-phytes were 10–20 % of those in summer, and overall carbon gain was in the same proportion.

Similar comparisons were made by Spencer and Wetzel (1993), who found specific adaptations to the sub-ice environment. These were a decrease in net photosynthesis, a decreased temperature optimum for photosynthesis and an increase in dark respiration. Some of these characteristics are similar to those of shade plants. These acclimations of the submersed species serve functions other than winter survival, and are considered to confer a competitive advantage. Continued active metabolism allows the accumulation of phosphorus in winter when lake-water concentrations are high. The phosphorus is then utilized in summer growth when the ambient supply is severely depleted (Spencer and Wetzel, 1993).

Ice retards gaseous exchange between atmosphere and water and lowers oxygen content of lake water (Wetzel, 1983). In lakes, this is not normally a critical factor because of the large body of water in relation to ice and the small amount of biomass. Furthermore, some of that biomass is consuming CO_2 and producing O_2 through photosynthesis. Thus, for submersed species adapted to the aquatic environment, the ice only impinges on the plant system by causing a reduction in light intensity and is not itself stressful.

THE PLANT WITHIN ICE

Unlike aquatic plants, the metabolic processes of terrestrial plants are immediately affected by flooding. Gaseous exchange slows down because of the lower concentrations of the relevant gases in water than in air (Wetzel, 1983). If the water should subsequently freeze, the rate of exchange of respiratory gases becomes extremely low, frequently with damaging effects. This is the condition of ice encasement (Fig. 1). It may develop in sub-continental and northern maritime climates, in areas of high winter precipitation where significant freeze/thaw cycles occur. Ice encasement occurs for many months annually in the arctic, and is associated with extreme anoxia tolerance in many high arctic species (Crawford, Chapman and Hodge, 1994). It is most frequently of economic concern in overwintering cereal and forage crops. A review of the effects of ice encasement on winter wheat was made by Andrews and Pomeroy (1991) and a detailed review of ice encasement research world-wide has been made by Gudleifsson and Larsen (1993).

Formation of ice over plants

Plants become encased in ice when snow layers partially thaw, the water percolates downward and refreezes (Fig. 2).

Fig. 1. Ice encasement at the Central Experimental Farm Ottawa. A, In early spring, over a seed plot of timothy grass cv. 'Bounty', following a winter of less than average snowcover. B, In January over a plot of winter wheat, before further snowfall. The shadow is from a solar panel and data-logger monitoring soil temperatures.

Rainfall may also contribute to the increase of the ice layer and compaction of the snow layer. This state will continue with further natural compaction until the next major thaw, which unless it is accompanied by run-off or soil drainage will further solidify the ice layer.

Solid ice is nearly impervious to respiratory gases (Rakitina, 1965) and, consequently, has the potential to induce severe hypoxia and anoxia within the plants (see below). But, conditions of this severity are not always realized. In the field, ice can develop in an irregular, granulated form which can allow low levels of aeration to the plants resulting in an alleviation of the ice encasement stress. Further, aeration may occur through the frozen soil to increase the chances of plant survival. Unsaturated frozen soil contains air filled pores (Cary and Mayland, 1972) which allow the movement of gases to and from the plant from unfrozen layers of the soil profile. Winter wheat has survived under 15 cm of ice, where the soil moisture in late winter was moderate, ranging from 20–30% (v/v). Thus, ice may form over the surface without penetration into the soil. Bolduc (Agriculture Canada, Ste. Foy, Quebec, pers. comm.) estimated in the field that 40% soil moisture is a threshold level to cause damage to winter wheat in ice encasement. This has been confirmed by controlled environment experiments (Pomeroy and Andrews, 1983). Even in midwinter an air space may occur between soil and the ice layer allowing complete survival of plants. This happens when the ice layer forms from standing water over unfrozen soil that continues to drain.

Ice-encased plants may also be aerated through the standing stubble of a previous crop. This is a 'snorkel' effect (Freyman, 1969) allowing internal movement of oxygen through hollow stems. The effect is also seen when the iced plant itself protrudes through the ice layer (Andrews and Pomeroy, 1975). This condition provides sufficient aeration to ensure survival.

PHYSIOLOGICAL EFFECTS OF ICE ENCASEMENT

Total ice encasement, even at mild sub-freezing temperatures, kills non-cold acclimated (non-hardened) cereal seedlings within 1 or 2 d. Cold acclimated plants survive the conditions much longer, according to species. Barley is killed in less than 1 week, wheat in about 2 weeks while a number of pasture grasses can survive many weeks (Gudleifsson, Andrews and Bjornsson, 1986). In the cereals, ice exposure significantly reduces the subsequent tolerance to freezing stress (Andrews and Pomeroy, 1975), which may be critical in the likely event of late winter or early spring freezes. Also, the cooler the temperature of ice encasement the more quickly the plants die, and even freezing after 1 h of total ice encasement reduces the LD_{50} temperature tolerance by about 6 °C. Whereas severe low temperature can accompany ice encasement in the field, normally the temperature at crown depth in ice is about -1 °C because of snow insulation. This is the temperature that has been used for most experimental work. There is no detectable ice within a wheat plant at -1 °C (Andrews and Pomeroy, 1975; Pukacki and McKersie, 1990).
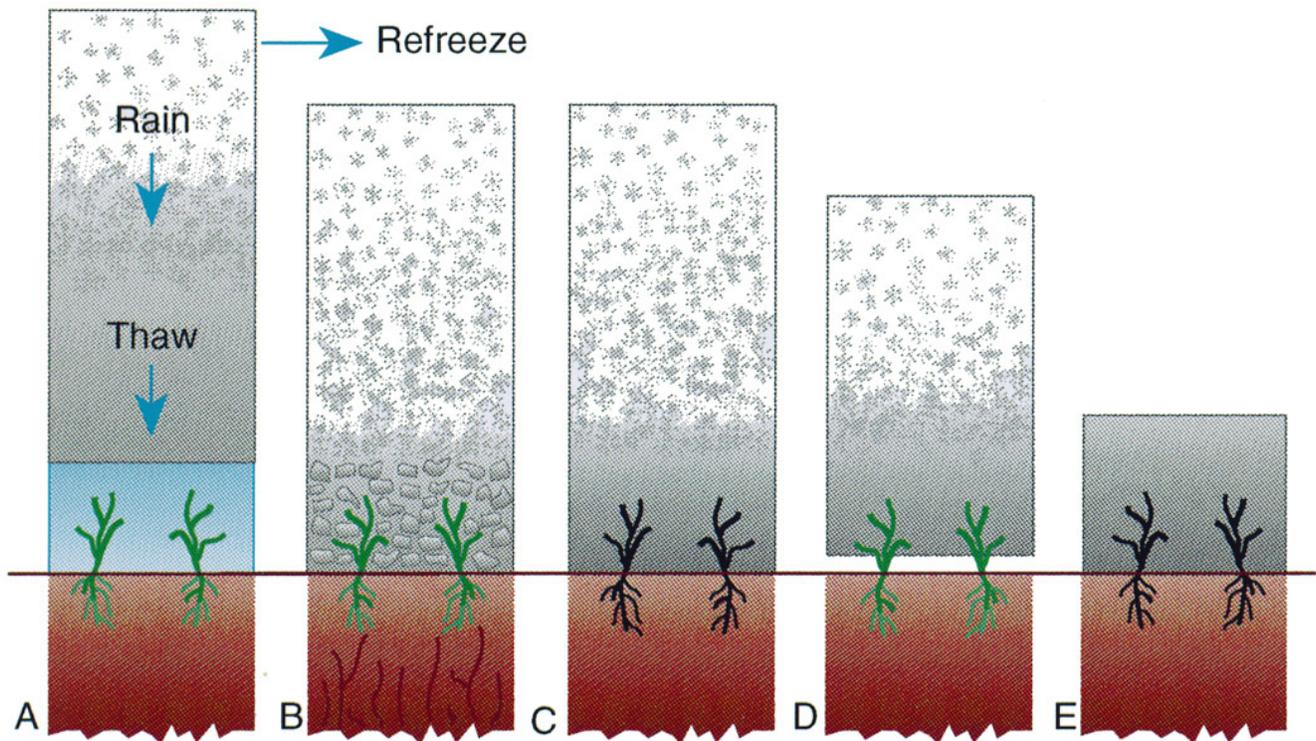
FIG. 2. A schematic diagram of possible consequences of midwinter thaw or rain on snowpack characteristics in relation to damage to overwintering cereals. A, Meltwater percolates through snow to accumulate at the soil surface where it refreezes. B, If granulated or porous, ice and/or soil layers can allow aeration of the plants. C, Solid ice and saturated frozen soil induce severe hypoxia. D, An air layer at the soil surface. E, Solid ice with loss of insulating snow cover. Plants in (B) and (D) are likely to survive, those in (C) and (E) probably will not.

Survival is increased when plants are illuminated at low levels during the ice encasement period and, after the ice has melted, the plants have greater freezing tolerance than plants frozen in the dark (Andrews, 1988). There is evidence that this promotion of survival by light is a result of oxygen generation by photosynthesis. Rakitina (1970) reported that a gaseous mixture aspirated from ice blocks containing wheat seedlings at $-5\,^{\circ}C$ contained 3% oxygen and up to $44\%\ CO_2$ (v/v). Higher O_2 levels and lower CO_2 levels were found in leaves than in crowns. Similarly, polarograph measurements of oxygen concentrations in wheat leaf segments at $-1\,^{\circ}C$ have shown that substantial O_2 is produced in the light, but is rapidly reabsorbed (utilized) on return to dark (Andrews, 1988). Non-photosynthetic crowns did not influence O_2 levels in light or dark. Thus, while direct evidence is lacking, there is circumstantial evidence that cereal crowns in ice, at least in the light, are hypoxic rather than anoxic, and may experience diurnal cycles of hypoxia and anoxia.

METABOLIC CONSEQUENCES OF ICE ENCASEMENT

When encased in ice, cereal seedlings produce ethanol, CO_2 and lactic acid. They consume approximately stoichiometric amounts of carbohydrate (Andrews, 1988), with an increased proportion of free sugars (Pomeroy and Andrews, 1983). The observed ratio is approximately one mole of carbohydrate consumed to four moles of (ethanol plus CO_2)

produced, which is close to the familiar equation of anaerobic metabolism. The carbohydrate loss is not significant for plant survival (McKersie *et al.*, 1982; Pomeroy and Andrews, 1983), but the concentrations of ethanol and CO_2, with lactic acid are sufficiently high to cause cellular disruption and death of the plants (Andrews and Pomeroy, 1979).

Carbon dioxide is the most toxic of the products of anaerobic respiration, and is likely to be the major contributor to cytoplasmic acidosis which is recognized to be a significant cause of cellular damage during anoxia at ambient $(20–25\,^{\circ}C)$ temperatures (Roberts *et al.*, 1982, 1984; Fox, McCallan and Ratcliffe, 1995). When wheat plants are iced in light, they not only survive longer, as discussed above, but they consume less carbohydrate, accumulate more CO_2, but considerably less ethanol than they do in the dark. These observations have been interpreted in the following way. The decrease in ethanol accumulation is a response to increased oxygen levels generated by photosynthesis, which also benefits the tissue with increases in ATP by photophosphorylation. The increase in CO_2 is also associated with this photosynthetic O_2; mitochondria which are present and potentially active during ice encasement (Andrews and Pomeroy, 1977) generate CO_2 from the TCA cycle and additional ATP from oxidative electron transport. A proportion of this CO_2 is recycled in photosynthesis, thus reducing its toxic concentration. The net effect of light on survival is positive. Thus, it is deduced that the higher O_2 level and increased

ATP supply is more significant to cellular maintenance than is the contribution to cell acidification caused by CO_2.

Calcium increases the survival in ice encasement of isolated winter wheat mesophyll cells (Pomeroy and Andrews, 1985). This is accompanied by a protection by calcium of the capacity of the cells to take up ^{86}Rb. The calcium was thought to stabilize the plasma membrane and its ATPase ion pumps. More recently, Subbaiah, Zhang and Sachs (1994) have been able to antagonize the activity of intracellular pools of calcium by Ruthenium Red, and proposed that calcium is an anoxic-signal transducer in maize root cells. In considering this response at low temperature, it is interesting that calcium has been reported to act as a cold-signal transducer in alfalfa cell cultures (Monroy, Sarhan and Dhinsda, 1993).

It is a common observation that ice encased plants appear healthy for a short period after thawing of the ice, but develop symptoms of damage within 1–2 d. Tanino and McKersie (1985) showed that the crown of winter wheat remained alive after 8 d of ice encasement, and that damage (electrolyte loss, lack of tetrazolium staining, mitotic spindles) was not evident until 1–3 d later. Further work showed that this deterioration was due to a peroxidation of lipids. During ice encasement itself only a minor decrease in unsaturation of lipid fractions was found (Hetherington, Broughton and McKersie, 1988). Upon thawing of the ice, there was a major decrease in unsaturation and an increase in free fatty acids (Hetherington, McKersie and Borochov, 1987). This indicated a breakdown of lipid bilayers as a result of free radical reactions at the polar head groups of the phospholipids. Post-anoxic injury of this kind has been described by VanToai and Bolles (1991), Albrecht and Wiedenroth (1994) and others. There is evidence that in some flood tolerant species, superoxide dismutase proteins are induced during flooding, which have the capacity to protect tissues from peroxidation after removal of the anoxic stress (Monk, Fagerstedt and Crawford, 1987).

ACCLIMATION TO ICE ENCASEMENT

Acclimation by prior flooding

Cold acclimation increases the tolerance of plants to freezing as well as to ice encasement stress. The survival of such cold acclimated wheat plants in ice encasement is *further* increased if they are first exposed to flooding at low temperature. This is itself an acclimation response (Andrews and Pomeroy, 1981, 1983, 1989), which is in addition to, and separate from cold acclimation. Investigation of this interaction began as a simulation of combined flooding and icing stresses that might occur in the field, and as an attempt to duplicate earlier work of Rakitina (1977). The acclimative interaction was found (Andrews and Pomeroy, 1981) when an ice encasement temperature of −1 °C was employed, rather than −5 °C used by Rakitina as typical of regional conditions in Russia.

The acclimative response is not specific to low temperature flooding but can be duplicated by an atmosphere of nitrogen gas, and by the application of 10^{-5} M ABA, a response also noted by Hwang and VanToai (1991) and VanToai *et al.*
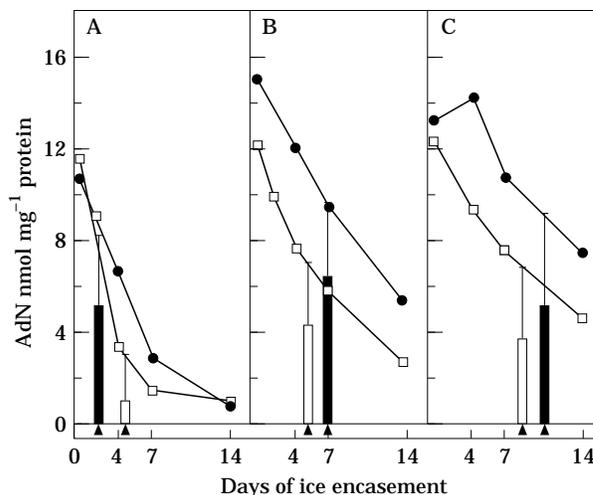
FIG. 3. Declining levels of adenylates in cereal crowns during ice encasement. Acclimation by prior low temperature flooding takes place in wheat, but not barley. Arrows indicate the time required in ice encasement to inflict 50 % fatality, and the bars show the ATP content at that time. These amounts differ between the acclimated and non-acclimated treatments. A, Dover barley; B, Frederick wheat; C, Norstar wheat. ●, Flood; □, non-flood.

(1995) at warm temperatures. Low temperature flooding itself produces ethanol in winter wheat (Pomeroy and Andrews, 1979, McKersie *et al.*, 1982), but because of rapid diffusion from the plant, it is considered to be of little significance to the overall survival of flooded plants (Jackson, Herman and Goodenough, 1982). However, plants enter ice encasement with an increased ability to produce ethanol, indicating an increased rate of glycolysis. Previously flooded plants accumulate more ethanol in ice, and show higher activity of alcohol dehydrogenase (ADH) than those not previously flooded (Andrews and Pomeroy, 1983). They also accumulate slightly more CO_2 and less malate. The effect of flood-induced ethanol itself is not a means of protection, as even low concentrations (2–3 mg g^{-1} f. wt) cause reductions in both ice encasement and freezing tolerance. Plants flooded at low temperature accumulate low levels of lactate, and continue to do so for a short period when ice encased, but there is no additional lactate produced under ice by acclimated plants (Andrews and Hope, 1994). There is no evidence to indicate that the formation of aerenchyma as found in flooded wheat roots at warm temperature (Jackson and Drew, 1984) is a factor in the acclimation response.

Ice encased wheat plants which have been previously flooded have higher levels of ATP and energy charge than those not flooded (Andrews and Pomeroy, 1989) (Fig. 3). The plants lose adenylates during the ice period, but the loss does not correspond precisely to the decline in survival. At the LD$_{50}$ point in ice, there is still more measurable ATP and total adenylate in the acclimated than the non-acclimated plants (Fig. 3). Similar levels of adenylates would be expected if survival was dependent on ATP supply only. Recent observations from hypoxically acclimated maize root tips (Xia, Saglio and Roberts, 1995) may be significant to an explanation of energy relationships in the

ice acclimation response. In maize roots, reduction of adenylates by artificial means (fluoride, mannose) does not appreciably affect the survival rate of acclimated tips. Correspondingly, lower ATP levels brought about by the inhibitors do not reduce the ability of the cells to regulate cytoplasmic pH. Following from these observations, energy levels are not central to the survival of tissues in anoxia. This counteracts a long-held dogma of anoxia survival, and warrants continued investigation. It is tempting to apply these conclusions to the acclimation of plants in ice, but the nature of the materials, the time spans as well as the temperatures are all very different, and a separate analysis is required.

Further evidence for the basis of the acclimation response in ice was sought from enzyme activities. No promotion by flooding of the activity of ATP-dependent phosphofructokinase (PFK), PPi-dependent phosphofructo-phosphotransferase (PFP) or pyruvate kinase (PK) was found (Andrews, 1996). This was in agreement with the work of others on systems at higher temperatures (Bailey-Serres, Kloeckner-Gruissem and Freeling, 1988). However, activity of two enzymes of the fermentation pathway was found to increase with time of flooding at low temperature. Alcohol dehydrogenase and pyruvate decarboxylase (PDC) activity both increased in proportion, but PDC was one tenth the activity of ADH (Andrews, 1996). The activity of both enzymes was maintained at approximately the same flood-determined level through 1–2 weeks of ice encasement. The low activity of PDC in this system makes it a candidate for a regulatory site of alcoholic fermentation, which has been proposed by others working at warmer temperatures (Morrell, Greenway and Davies, 1990; Waters *et al.*, 1991). This has led to a study of a transgenic tobacco with a bacterial PDC (Bucher, Brändle and Kuhlemeier, 1994). In anoxia this produces greatly increased ($\times 20$) levels of ethanol, but also a major accumulation of acetaldehyde, resulting from a breakdown in coordination with ADH activity.

We have evidence that the acclimation effect of prior low temperature flooding on ice tolerance is based on substantial changes in protein synthesis (Hope, Andrews and Seguin, unpubl. res.). Incorporation of ^{35}S-methionine into newly synthesized polypeptides of wheat crowns was reduced relative to the cold hardened controls by flooding, and reduced to a greater extent by ice encasement. However when ice encasement was preceded by flooding, the level of incorporation was similar to that of the control. While the level of many pre-existing polypeptides was increased, label was also found to be incorporated in 15 new ones, not present in the other treatments. The nature of these polypeptides remains unknown, but it is assumed that the number includes new forms of glycolytic and fermentation enzyme proteins, and that some are associated with the increased survival of the acclimated plants.

In addition to the accelerated metabolism resulting from the acclimation process described here, it is possible that there is a protection against post-anoxic injury reported by Hetherington *et al.* (1987). We have been unable to protect ice encased plants from damage by applying ascorbate during thaw, or before ice, as has been reported from an

analagous situation (Crawford and Wollenweber-Ratzer, 1992). Preliminary data have shown that flooding at low temperature does however promote the activity of superoxide dismutase, but not catalase in wheat crowns (Andrews and Hodges, unpubl. res.).

METABOLIC AND MOLECULAR BASIS OF HYPOXIC ACCLIMATION

Recent work on hypoxic acclimation of tissues at ambient temperatures has supplied information relevant to an understanding of ice encasement tolerance. Saglio, Drew and Pradet (1988) reported an increase in anoxia tolerance in maize root tips following a short 2–4% O_2 exposure in comparison with root tips transferred directly from air. In the hypoxically-acclimated tips, ATP and total adenylate levels were greater in anoxia. Activity of ADH and ethanol production were also increased (Johnson, Cobb and Drew, 1989). A molecular basis of acclimation was indicated by a greater induction of ADH mRNA transcripts and activity at 4% O_2 than in anoxia, and both level of transcript and enzyme activity were maintained longer in the hypoxic than in the anoxic condition (Andrews *et al.*, 1993). The hypoxic pretreatment allowed further induction of ADH transcripts and activity in anoxia, but to a greater extent in tips from young than older seedings. As there was no difference in the root survival in anoxia of seedlings of these two ages, the significance of ADH in this system was questioned (Andrews *et al.*, 1994*a*).

Analysis over time of transcript levels in maize seedling segments showed that mRNA of two fermentation enzymes (PDC and ADH2) increased in hypoxia much more than that of the glycolytic enzymes enolase and aldolase. In anoxia, transcripts of the fermentation enzymes increased more rapidly when hypoxically acclimated than did transcripts of glycolytic enzymes. All transcripts declined to uninduced levels by 48 h, while enzyme activities did not, due to different post-translational events (Andrews *et al.*, 1994*b*). The changing levels of enzyme activity described in this work in maize have many similarities to the changes in PDC and ADH activity during the acclimation of wheat to ice encasement. The associated increases in transcript levels and enzyme activities in maize support the view that polypeptide synthesis in wheat during and following the acclimation treatment includes new and augmented glycolytic and fermentation enzyme proteins.

A lower level of lactic acid in tissues has been proposed as a reason for increased survival of acclimated maize root tips (Xia and Saglio, 1992). Acclimated tips produce less lactate when anoxic, but are also able to move it out of the tissues to the medium faster than non-acclimated tips. This is an active efflux that can be inhibited by cycloheximide, but the nature of the putative transporter protein is not known. The effects of acclimation on lactate efflux are observed in the first few hours of anoxia. No evidence of such a process has been seen in wheat acclimated to ice encasement. It is unlikely that an acclimation based on the efflux of toxic compounds would be effective in ice encasement where diffusion from the plant is severely restricted. In any event, such changes in lactate content *per se* are thought to make

only a small contribution to cytoplasmic acidosis (Menegus *et al.*, 1991; Fox *et al.* 1995), and other processes result in changes to acidification. One of these processes appears to divert carbon during hypoxia from lactate to alanine with a reduction in acidosis and an associated enhancement of survival in anoxia (Xia and Roberts, 1994). A similar diversion may occur in wheat crowns during acclimation to ice encasement. During low temperature flooding there is an increase in the activity of alanine aminotransferase, and an accumulation of alanine (Andrews and Bonn unpubl. res.) which may be associated with reduced acidosis and contribute to enhanced survival.

HIGH ICE TOLERANCE OF FORAGE GRASSES

A number of the forage grass species have an especially high tolerance to ice encasement (Gudleifsson *et al.*, 1986). This tolerance is of interest to northern wheat breeders as a potential source of improvement in winter survival of the crop. Gudleifsson (1994) has shown that during ice encasement of timothy grass (*Phleum pratense*) ethanol accumulates to relatively low levels, and there are increases in the levels of a number of organic acids. *Deschampsia berengensis* (berings hairgrass) is an arctic grass with high ice tolerance (Gudleifsson *et al.*, 1986) as well as extreme anoxia tolerance when measured at 10 °C (Crawford and Braendle, 1996). Comparative studies between winter wheat, *D. beringensis* and timothy have revealed major differences iSn levels of metabolic end products in ice (Andrews, 1997). There is also a much smaller acclimation response by flooding to ice encasement in these forage species. Ethanol, CO_2 and lactate in the grasses accumulate in ice to substantially lower levels than in wheat (Fig. 4), and the activity of PDC is much reduced. The acclimation process reduces ethanol and CO_2 accumulation in the grasses, but promotes it in wheat as described above. Clearly, a different

strategy of ice tolerance has developed in these grasses than in wheat. Glycolytic rates are slow, resulting in low levels of potentially toxic products. In particular, smaller amounts of CO_2 would lessen cytoplasmic acidosis (Roberts *et al.*, 1982, 1984). Xia and Roberts (1994) have shown in maize roots that hypoxic acclimation stabilizes cytoplasmic pH sufficiently to preclude further decrease on transfer to anoxia. It is possible that the metabolism of the grasses has achieved this stability without a requirement of acclimation.

CONCLUSIONS

The presence of ice in the environment of plants is potentially lethal. Tolerance is dependent upon the restriction of ice formation within the plant to non-damaging locations. However, the effect of ice encasement is frequently lethal to overwintering rosette plants. Nevertheless, plants may survive because of leaks in the surrounding layers, either as channels in granular ice or as pores in the frozen soil, which allow gaseous exchange in the plants. Similarly, survival is increased by light exposure and the O_2 generated by the resulting photosynthesis. An acclimation process simulating early winter flooding, in addition to that provided by cold acclimation, also increases survival of winter wheat plants in ice. This hypoxic acclimation accelerates glycolysis and generates more adenylate and a higher energy charge. This in turn increases proton pumping and supports repair mechanisms in stressed cells. Current evidence indicates that accumulated CO_2 is the most toxic of the metabolites accumulating in ice-encased plants. Carbon dioxide is confined within the plants, not freely diffusing from them as it can from plants in many hypoxic and anoxic situations. From comparisons of the effects of anoxia on plants at ambient temperature, the accumulation of CO_2 in ice is deduced to contribute markedly to acidification of the cytoplasm, the major cause of anoxic damage. Survival of wheat plants in ice encasement appears to be dependent upon a balance between energy generation and potentially damaging CO_2. The highly ice-tolerant grasses accumulate very little CO_2. Further comparative studies of wheat and the grass species should reveal fundamental and potentially exploitable differences in their ice tolerance mechanisms.
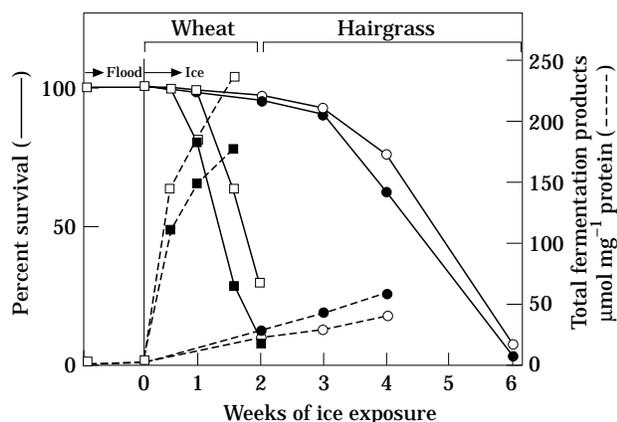
FIG. 4. Relationships of survival in ice encasement and accumulation of fermentation end products (total of ethanol, CO_2 and lactate) in wheat and berings hairgrass (*Deschampsia beringensis*). The hairgrass accumulates markedly less fermentation product than the wheat, and in contrast to the wheat, accumulates less product following acclimation by prior flooding. ■, NFL wheat; □, FL wheat; ●, NFL hairgrass; ○, FL hairgrass.

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