Plant Physiol. (1989) 91, 1063–1068
0032-0889/89/91/1063/06/$01.00/0

Received for publication January 26, 1989
and in revised form July 1, 1989

Metabolic Acclimation to Hypoxia in Winter Cereals[1]

Low Temperature Flooding Increases Adenylates and Survival in Ice Encasement

Christopher J. Andrews* and M. Keith Pomeroy

Plant Research Centre, Agriculture Canada, Ottawa K1A OC6, Ontario, Canada

ABSTRACT

Cold hardened seedlings of winter wheat (*Triticum aestivum* L. em Thell) show an hypoxic hardening response: an exposure to low temperature flooding increases the tolerance of plants to a subsequent ice encasement exposure. Seedlings of winter barley (*Hordeum vulgare* L.) do not show such a response in similar experimental conditions. During ice encasement, there are general declines in adenylate energy charge (AEC), total adenylates and ATP:ADP ratios in the crown tissues of two winter wheat cultivars, and a winter barley, but rates of decline are faster in the barley. When the ice period is preceded by low temperature flooding of the whole plant, levels of the adenylate components are raised significantly in the wheats, and to a lesser extent in the barley. The survival of plants in ice preceded by flooding is related to the increased initial level of adenylates at the onset of the ice encasement stress, and the maintenance of higher levels of adenylates and ATP in the early stages of ice encasement as a result of accelerated rates of glycolysis. Higher survival of both winter wheat and barley plants during ice encasement in the light is also associated with significantly higher levels of AEC and adenylates in the early stages of ice encasement.

Rosette-type overwintering plants in northern temperate areas may be subject to low temperature flooding or snowmelt, which on freezing can give rise to ice encasement of the plants (19). This condition is normally severely damaging due to the restrictions to gaseous exchange of the plants, and the accumulation of anaerobic metabolites which interfere with cellular metabolism and function (4). Some of the effects of low temperature flooding on plants are similar to those of ice encasement but are slower to develop or less severe because of the greater gaseous exchange rates in flooded than in ice encased plants.

In field conditions, low temperature flooding frequently precedes ice encasement. Rakitina (22) reported that with flooding at 2°C and ice encasement at −5°C, flooding reduced the tolerance of winter wheats to subsequent ice. In conditions in eastern Canada, ice normally forms just below the freezing point, and we found that flooding at 2°C promoted the subsequent survival of winter wheat, but not winter barley plants in ice at −1°C (5). The flooding effect could be simulated by exposing the plants to a nitrogen atmosphere (6). This is a metabolic acclimation, in which the hypoxic stress of flooding increases tolerance to the subsequent more severely hypoxic or anoxic stress of ice encasement. Ice encased plants which were previously flooded accumulate higher levels of ethanol and have greater alcohol dehydrogenase (Adh)[2] activity, and it was proposed that they enter the ice encasement stress period with an accelerated rate of glycolysis (6). A similar metabolic acclimation at warm temperatures has recently been described in root tips of *Zea mays* (23), with increased levels of ethanol production and adenylates associated with the hypoxic treatment.

We have previously reported that the levels of adenylates in enzymatically isolated cells of winter wheat are not closely related to the decline in survival of those cells in ice encasement (18) and similarly, that carbohydrate energy reserves of plant crowns do not limit survival in ice (14, 17). In this paper we show the declining levels of adenylates in relation to survival of crown tissue in ice encasement in dark and the effects of a low temperature flooding (hypoxic) pretreatment on levels of adenylates in plants of different survival potential. We use for comparison the energy status of plants during ice encasement in light, which promotes their survival but is not an acclimation response.

MATERIALS AND METHODS

Cold hardened plants of winter barley *Hordeum vulgare* L. cv Dover, and winter wheats *Triticum aestivum* L. em Thell cv Fredrick (soft, white) and cv Norstar (hard, red) were used in this study. Seeds were germinated and plants grown as described previously (3) for 5 d at 20°C/15°C and 7 weeks at 2°C/0°C at a light intensity of 250 $\mu Em^{-1} \cdot sec^{-1}$.

Stress Treatments

Flats of plants were flooded in plastic tubs filled with tap water and the leaves trimmed to allow total submersion. Nonflooded plants in tubs without water were similarly trimmed and both series of plants were maintained at cold hardening temperatures. For ice encasement in dark, plants were washed free of soil, roots removed, crown trimmed to 1 cm length, rapidly weighed in groups of 5 and transferred to 10 mL water at 0°C in 50 mL plastic beakers. After freezing of this layer at −2°C a further 15 ml of water was added and the plants were maintained at −1°C for the required ice period.

[1] Plant Research Centre Contribution No. 1199.

[2] Abbreviations: Adh, alcohol dehydrogenase; AEC, adenylate energy charge.

For ice encasement in light, roots were removed from the plants and leaves were trimmed to 4 cm (to retain green laminae) and weighed. After thawing of the ice, crowns were trimmed to 1 cm under water, the remnant leaves were weighed and the approximate fresh weight of crown tissue determined by subtraction. For survival determinations in these experiments, crown segments with 1 cm roots were iced as described above, thawed, and transferred to moist vermiculite. Survival was determined after 2 weeks regrowth as described previously (3).

Determination of Adenylates

There are inherent difficulties in the sampling of tissues within ice blocks. Standard procedures, such as extraction with cold diethyl ether (21) are unsuitable. A number of procedures were tested (see "Results") but routinely, ice blocks were thawed in air at room temperature with frequent gentle agitation such that the temperature of the tissues did not increase above 1°C. After release from the ice, tissues were transferred to 2.5 ml of chilled 2.5% TCA in 2 mM EDTA in a mortar, ground, rinsed with a further 2.5 ml of 2.5% TCA with EDTA, and held in ice for 30 min. After centrifugation, 100 μL of the supernatant was diluted to 2.5 mL with 0.1 M Tris acetate (pH 7.75) with 2 mM EDTA and was analyzed for the three adenylates by a modification (18) of the method of Pradet (20). In parallel determinations on the same samples, ATP was measured directly by luminescence in the luciferin/luciferase system; ADP was measured as ATP after conversion with pyruvate kinase and PEP, and AMP was measured as ATP after conversion with adenylate kinase. Luminescence was recorded on an LKB 1251 luminometer and the response rate in each sample was recorded from successive additions of 10 μL of 1 μM ATP. Energy charge was calculated by the method of Atkinson (8), and total solubilized protein was determined by the procedure of Peterson (15).

RESULTS

Determination of metabolic events in winter cereal tissue surrounded by ice is complicated by the requirement to minimize potential changes occurring in tissues during thawing of the ice. A number of thawing procedures prior to extraction were evaluated to obtain measurements of adenylates after thawing of the ice, which would satisfactorily estimate relative levels of adenylates and adenylate energy charge of plants within the ice. Thawing of the ice with gentle agitation at room temperature, with care to maintain thaw water temperature as low as possible (1°C) was chosen as generally the most acceptable (Table I). Addition of high TCA concentration to ice before thaw in air decreased the yield of total adenylates, while thawing in the presence of nitrogen caused no change in comparison to thawing in air. The various treatments induced no major changes in AEC, but ATP:ADP ratios were higher in treatments thawed in the absence of TCA. Lyophilization of the ice, or removal in boiling buffer lowered both AEC and total adenylates (data not shown). The chosen system is similar to, though faster than the thawing process in natural conditions and yielded consistent values throughout.

Thawing of the ice blocks takes about 1.5 h. During an approximate simulation of this process by a 1.5 h exposure to flooding at 0°C, a substantial decrease in total adenylates occurs in all three cultivars, but it is not accompanied by any significant change in AEC (Table II). Extension of this flooding period to 3 h does not further decrease the level of total adenylates suggesting that the initial rapid decline in adenylates is due to a flooding shock (9, 27).

During ice encasement after the flooding shock, there is a general decline in AEC in all three cultivars (Fig. 1). The decline is more rapid and slightly greater in the barley, which is more rapidly killed by the stress. Total adenylates decline throughout the ice encasement period, but more rapidly in the first few days of ice (Fig. 1). Dover barley demonstrates more rapid and greater loss of adenylates than the winter wheats, and decreases to very low levels in the later stages of ice encasement when most of the plant population fails to regrow.

A 2-week period of low temperature flooding before ice encasement increases survival of wheat plants during subsequent ice exposure, in comparison with nonflooded plants. This is the effect of the low temperature hypoxic hardening, which occurs in the wheat, but not the barley (Fig. 2; refs. 4 and 5). Before ice encasement, there is a decrease in relative levels of ATP due to flooding, measurable as slight decreases in AEC, or as larger decreases in the ATP:ADP ratios (Table III). Subsequently, the overall decline in adenylate levels during ice encasement is influenced by previous flooding: small increases in AEC and larger increases in the ATP:ADP ratios due to flooding occur at all stages of ice encasement in the winter wheats and the barley. The ATP:ADP ratios are maintained or increased in the first 4 d of ice in the previously flooded wheats, in contrast to the nonflooded wheats, or the barley with either pretreatment. Total adenylates decline generally throughout ice encasement, but levels in the two wheat cultivars are about 40% higher in those plants that were previously flooded than in those that were not (Fig. 2). The effect of the flooding is more severe on the barley and reduces the content of total adenylates below that of the nonflooded control before the ice treatment. Nevertheless, during ice exposure the loss of adenylates is faster and to lower levels in those plants not previously flooded. After 2 d of ice, ATP levels in the previously flooded plants are almost twice those in the nonflooded plants, while the other adenylates show much smaller differences (Table IV). The AEC is also increased at this stage by the flooding treatment.

The effects of flooding are emphasized by interpolations among the data of Figure 2, which give the levels of ATP and total adenylate at the 50% killpoint due to the treatments influencing each of the three cultivars (Table V). In each case, when 50% of the plants fail to regrow, there is a higher level of ATP and of adenylates in plants previously flooded than in those that are not. Levels in the two wheats with corresponding treatments are similar. Higher levels of adenylates due to flooding are associated with a longer period to the 50% kill point in the wheats, indicating an adaptative, or promotive role. In the barley, the higher adenylate levels are associated with a considerably shorter period to the 50% kill point,

Table I. *Changes in AEC, ATP:ADP Ratios, and Total Adenylates Associated with Different Conditions of Thawing of Ice*

Crowns of cold hardened Frederick winter wheat seedlings without ice, or ice-encased for 4 d at −1°C.

	AEC	$\frac{ATP}{ADP}$	Total Adenylates	
			nmol g fresh wt	*nmol/mg protein*
Noniced	0.91	5.91	400 ± 22.2[a]	14.7 ± 1.24
Iced, thaw in air with TCA[b]	0.75	0.75	106 ± 3.6	4.7 ± 0.34
Iced, thaw in air without TCA	0.78	1.49	131 ± 7.8	5.5 ± 0.42
Iced, thaw in N_2 with TCA	0.75	1.08	126 ± 3.1	5.2 ± 0.09
Iced, thaw in N_2 without TCA	0.75	1.59	129 ± 5.5	5.5 ± 0.10

[a] Mean of four replications ± SE. [b] TCA added to ice surface to give a final concentration of 2.5% after thaw.

Table II. *AEC and Total Adenylates in Crowns of Cold Hardened Winter Cereal Plants With and Without a Low Temperature Flooding Shock*

	Flooding	AEC	Total Adenylates	
	h		*nmol/g fresh wt*	*nmol/mg protein*
Dover	0	0.83	334 ± 15[a]	15.4 ± 1.0
	1.5	0.81	184 ± 6	9.5 ± 0.5
	3.0	0.83	177 ± 10	9.1 ± 0.3
Fredrick	0	0.81	345 ± 37	14.8 ± 1.8
	1.5	0.80	214 ± 3	9.0 ± 0.1
	3.0	0.81	236 ± 8	10.4 ± 0.3
Norstar	0	0.74	343 ± 6	13.2 ± 0.3
	1.5	0.74	218 ± 4	9.2 ± 0.1
	3.0	0.81	219 ± 1	9.5 ± 0.3

[a] Mean of four replications ± SE.

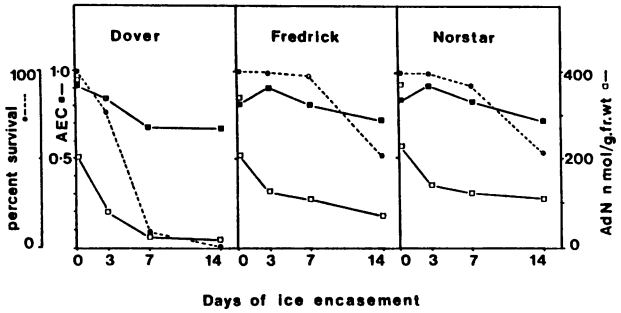

Figure 1. Changes in AEC, total adenylates (AdN) and survival of crowns of cold hardened winter wheat and barley during ice encasement at −1°C. Upper values of AdN on the vertical axis are from untreated plants; lower values are exposed to 1.5 h flooding at 0°C.

indicating the existence of a damaging component of the mechanisms leading to adenylate accumulation.

Exposure of plants to light during ice encasement increases survival of plants of all three cultivars (Table VI; ref. 1). Substantial decreases in AEC occur after 2 d in ice in the dark, before plants show any visible damage from ice stress, whereas AEC is little affected by 2 d of ice in the light. Total adenylates similarly decrease by 40 to 50% in dark during the first 2 d of ice encasement, but the losses are considerably less

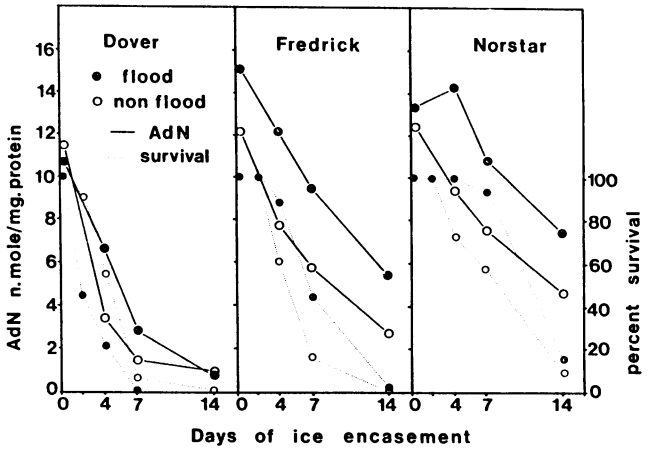

Figure 2. Changes in total adenylates (AdN) and survival of crowns of cold hardened winter wheat and barley during ice encasement with and without a previous 2-week exposure to low temperature flooding.

in light. Decreases in adenylates and AEC continue in ice, and at 7 d of exposure the levels are higher in light than in dark by 10 to 40%. At this stage, higher total adenylates are associated with higher survival levels, and there are lower adenylate levels in the more sensitive Dover barley. If crowns alone without leaf laminae are ice encased in this system, no differences are observed in the effects of light and dark on AEC or total adenylates (data not shown).

DISCUSSION

The increased survival of winter wheat in ice encasement preceded by low temperature flooding has been previously reported as a promotive response analogous to cold hardening, or as an hypoxic hardening response (5, 6). It has been proposed to be due to an increased rate of glycolysis (6) based on an increased level of ethanol production and Adh activity during ice encasement when the plants were previously exposed to a flooding period. The current work supports this proposal and demonstrates higher levels of adenylates throughout ice encasement after a previous exposure to flooding. The higher levels are apparent before any change in the survival of the tissue is observed, and hence are considered to

Table III. *AEC and ATP:ADP Ratios in Crowns of Cold Hardened Winter Cereal Seedlings during Ice Encasement With and Without Previous Flooding*

Values are means of three experiments, each of three or four replicates.

	Ice Encasement (days)							
	0		4		7		14	
	AEC	$\frac{ATP}{ADP}$	AEC	$\frac{ATP}{ADP}$	AEC	$\frac{ATP}{ADP}$	AEC	$\frac{ATP}{ADP}$
Dover								
Nonflood	0.87	4.94	0.58	0.44	0.56	0.38	0.54	0.21
Flood	0.85	2.34	0.72	0.98	0.62	0.47	0.55	0.24
Fredrick								
Nonflood	0.91	7.12	0.76	1.84	0.74	1.25	0.75	0.71
Flood	0.87	4.81	0.83	4.23	0.77	1.87	0.76	0.95
Norstar								
Nonflood	0.85	3.71	0.77	1.76	0.75	1.64	0.73	0.59
Flood	0.79	2.67	0.84	5.32	0.79	2.95	0.76	1.12

Table IV. *Content of Individual Adenylates in Crowns of Cold Hardened Winter Cereals after 2 d of Ice Encasement at −1°C*

	ATP	ADP	AMP	Total	AEC	$\frac{ATP}{ADP}$
	nmol/mg protein					
Dover						
Nonflood	2.19 ± 0.16[a]	1.90 ± 0.18	0.18 ± 0.08	4.27 ± 0.26	0.73	1.15
Flood	3.80 ± 0.25	2.23 ± 0.14	0.18 ± 0.08	6.22 ± 0.31	0.79	1.70
Fredrick						
Nonflood	3.67 ± 0.35	2.08 ± 0.11	0.23 ± 0.14	5.98 ± 0.44	0.78	1.76
Flood	6.21 ± 0.23	2.32 ± 0.21	0.05 ± 0.02	8.58 ± 0.16	0.86	2.67
Norstar						
Nonflood	2.68 ± 0.12	2.01 ± 0.15	0.12 ± 0.09	4.81 ± 0.02	0.76	1.33
Flood	5.43 ± 0.02	2.05 ± 0.21	0.38 ± 0.14	7.86 ± 0.25	0.82	2.65

[a] Mean ± SE of eight replicates (two experiments each of four repetitions).

Table V. *Total Adenylates and ATP in the Crowns of Cold Hardened Winter Cereal Plants at the LD_{50} (days) of Ice Encasement*

	LD_{50}	Total Adenylates	ATP
	d in ice	*nmol/mg protein*	
Dover			
Nonflood	4.2	3.1	0.9
Flood	2.6	8.7	4.9
Fredrick			
Nonflood	4.8	7.5	4.4
Flood	6.9	9.7	6.7
Norstar			
Nonflood	8.7	7.3	3.8
Flood	10.6	9.6	4.9

be a major contributory factor to the greater level of tolerance to ice encasement induced as a response to the earlier flooding. Proof of a direct causal relationship between raised adenylate levels and higher survival in ice is not possible from these studies, but further correlative evidence is provided by the enhanced survival of plants in light-exposed ice. In this situation also, raised adenylate levels are observed prior to any differential survival occurring as a result of light, as compared to dark ice encasement.

A similar hypoxic acclimation has been reported recently from corn seedlings at warm temperatures (23), where exposure to low oxygen partial pressure increased survival of root tips in a subsequent fully anoxic treatment. Moreover, declines in ATP and total adenylates were markedly slower in anoxia preceded by hypoxia than in anoxia preceded by an enhanced oxygenation treatment. Hypoxia also induced a substantial increase in Adh activity leading to increased levels of ethanol production during subsequent anoxia. These results are similar to those reported here, and earlier (5, 6) but over a shorter time period associated with the higher temperature.

It is an interesting contrast that winter barley does not show similar metabolic acclimation at low temperature as the wheat; flooding of barley for 2 weeks induces greater plant death in subsequent ice encasement. Whereas there is a higher level of adenylate during ice encasement of previously flooded plants, a contributory factor to the lower survival is likely to be the lower level of adenylate at the beginning of the ice period after the 2 weeks of flooding. The barley plants appear healthy at this stage, but their reduced adenylate content may be caused by their greater sensitivity at low temperature to CO_2 (6), to ethanol (1), and to anaerobiosis in general (26) in comparison to wheat. Further, a greater level of ultrastructural

Table VI. *Changes in AEC and Total Adenylates in Crowns of Cold Hardened Winter Cereal Plants in Ice Encasement at −1 °C in Light or Dark*

	Ice Period	Survival	AEC	Total Adenylates	
	d	%		*nmol/g fresh wt*	*nmol/mg protein*
Dover	0	100	0.86	275 ± 8[a]	12.9 ± 0.8
Dark	2	100	0.66	135 ± 12	4.7 ± 0.4
Light	2	100	0.80	260 ± 52	8.0 ± 0.9
Dark	7	0	0.63	32 ± 4	3.4 ± 0.8
Light	7	10	0.65	57 ± 7	3.9 ± 0.9
Fredrick	0	100	0.89	328 ± 25	11.2 ± 0.7
Dark	2	100	0.68	194 ± 11	5.5 ± 0.2
Light	2	100	0.84	330 ± 31	8.7 ± 1.0
Dark	7	6	0.64	119 ± 4	5.5 ± 0.4
Light	7	83	0.73	186 ± 17	6.3 ± 0.4
Norstar	0	100	0.81	326 ± 14	10.7 ± 0.8
Dark	2	100	0.70	178 ± 14	4.5 ± 0.2
Light	2	100	0.79	259 ± 37	6.2 ± 0.4
Dark	7	36	0.64	126 ± 11	5.2 ± 0.5
Light	7	94	0.77	140 ± 9	5.9 ± 0.1

[a] Values are means of two experiments, each of three replicates ± SE.

damage occurs in crowns of barley than in those of wheat after several weeks of flooding at 2°C (16). Nevertheless, despite the initial lower level of adenylate, a metabolic response to the flooding does occur because in ice the level of adenylate drops much more slowly in the preflooded plants than in those not flooded. In the barley, in contrast to the wheat, this slower adenylate decline is not reflected in higher survival in ice encasement.

The initial level of adenylates at the start of the ice period is considered to be the significant contributory factor in subsequent plant survival in ice. Increased availability of substrate ATP contributes to plasma membrane activity (10, 13, 24, 25) thereby maintaining cellular ion balances and turgor. It also allows enhanced turnover and repair of cellular synthetic systems. Continued accelerated operation of the glycolytic sequence yields the increased levels of adenylate with the potential additional accumulation of NADH. Increases in the activity of Adh (6) and other dehydrogenases allow the regeneration of NAD and the maintenance of redox balance. These processes may all contribute to an increased survival rate of the plant tissues in ice, but the final reductive steps of the process enabling the regeneration of NAD also involve the production of anaerobic metabolites of known toxicity (4), which increase as a result of the flooding pretreatment (6). Recent evidence indicates that plasma membrane function is inhibited by accumulated anaerobic metabolites, particularly the CO_2 related ionic species (7). The declining level of adenylates in iced tissues reported here, and in iced cells (18) is only approximately correlative, and there is no direct evidence that cellular damage is induced by the decline of adenylates below a critical threshold value. A similar conclusion was drawn earlier from the declines in carbohydrate energy reserves during ice encasement which also are not closely related to decreases in survival (14, 17).

The survival of cereal plants during ice encasement is promoted by exposure to light (2) as well as the preflooding treatment. The promotive effect of light is not a sequential acclimation process but it also is mediated by enhanced levels of adenylates in the early stages of ice encasement before any change in survival of tissues can be observed. The higher relative levels of adenylates in plant crowns in ice in the light are accompanied by oxygen evolution (2) indicating at least the partial functioning of photosynthesis. Whether the increased adenylate level is due to photophosphorylation, or to oxidative phosphorylation in the presence of low levels of photosynthetically generated oxygen, or to both of these factors is not yet known. After longer periods in ice, similar or smaller differences in adenylates exist between the flooded and nonflooded treatments, associated with substantial differences in survival. Present evidence supports the concept that eventual death of plants in ice in light, as in the dark, is due to the impact of anaerobic accumulations on membrane function (2). Increases in free fatty acids, phospholipid breakdown, and membrane lipid peroxidation during and after ice encasement have been recently described (11, 12). The immediate stimulus of these changes is not known, but it may be associated with deleterious anaerobic accumulations. The changes would be expected to influence membrane function.

The significance of the presently described hypoxic acclimation response in practical agriculture or in the ecological distribution of species is difficult to assess. Frequently in winter conditions an ice encasement period is preceded by flooding of varying duration, which could serve to increase tolerance in ice. Certainly, variation in tolerance to ice in field conditions is encountered which could be due to previous flooding, but other factors such as light penetration of snow layers (2) and soil porosity (17) also are directly involved in the control of survival. The observation that winter barley does not show the same response as wheat, at least in the conditions tested, is very likely to be associated with the limited survival of barley under high stress conditions, despite its relatively high cold tolerance (5). Standard winter barley cultivars have limited flooding (4) and ice tolerance, and as described here and elsewhere (5, 6) they have no, or limited, adaptive response between the two stresses. As low temperature flooding and icing are frequent winter conditions in northern parts of the cereal growing areas, it follows that barley is more limited in its distribution than winter wheat.

ACKNOWLEDGMENT

We thank Pat Bonn for excellent technical assistance.

LITERATURE CITED

1. **Andrews CJ** (1977) The accumulation of ethanol in ice encased winter cereals. Crop Sci **17:** 157–161
2. **Andrews CJ** (1988) The increase in survival of winter cereal seedlings due to light exposure during ice encasement. Can J Bot **66:** 409–413
3. **Andrews CJ, Pomeroy MK** (1975) Survival and cold hardiness of winter wheats during partial and total ice immersion. Crop Sci **15:** 561–566
4. **Andrews CJ, Pomeroy MK** (1979) Toxicity of anaerobic metabolites accumulating in winter wheat seedlings during ice encasement. Plant Physiol **64:** 120–125

5. **Andrews CJ, Pomeroy MK** (1981) The effect of flooding pretreatment in cold hardiness and survival of winter cereals in ice encasement. Can J Plant Sci **61:** 507–513
6. **Andrews CJ, Pomeroy MK** (1983) The influence of flooding pretreatment on metabolic changes in winter cereal seedlings during ice encasement. Can J Bot **61:** 142–147
7. **Andrews CJ, Pomeroy MK** (1989) Ice encasement injury at the cellular and membrane level. Icel Agric Sci (in press)
8. **Atkinson DE** (1969) Regulation of enzyme function. Annu Rev Microbiol **23:** 47–68
9. **Gronewald JW, Hanson JB** (1982) Adenine nucleotide content of corn roots as affected by injury and subsequent washing. Plant Physiol **69:** 1252–1256
10. **Hellegren J, Widell S, Lundborg T, Kylin A** (1983) Frost hardiness in *Pinus sylvestris*: the involvement of a K^+ stimulated Mg^{++} dependent ATPase from purified plasma membranes of pine. Physiol Plant **58:** 7–12
11. **Hetherington PR, Broughton HL, Mckersie D** (1988) Ice encasement injury to microsomal membranes isolated from winter wheat crowns II. changes in membrane lipids during ice encasement. Plant Physiol **86:** 740–743
12. **Hetherington PR, Mckersie BD, Borochov A** (1987) Ice encasement injury to microsomal membranes from winter wheat crowns I. Comparison of membrane properties after lethal ice encasement and during a postthaw period. Plant Physiol **85:** 1068–1072
13. **Jian L-C, Sun L-H, Dong H-Z** (1982) Adaptive changes in ATPase activity in the cells of winter wheat seedlings during cold hardening. Plant Physiol **70:** 127–131
14. **McKersie BD, McDermott BM, Hunt LA, Poysa V** (1982) Changes in carbohydrate levels during ice encasement and flooding of winter cereals. Can J Bot **60:** 1822–1826
15. **Peterson GL** (1977) A simplification of the protein assay method of Lowry *et al* which is more generally applicable. Anal Biochem **83:** 346–356
16. **Pomeroy MK, Andrews CJ** (1979) Metabolic and ultrastructural changes associated with flooding at low temperature in winter wheat and barley. Plant Physiol **64:** 635–639
17. **Pomeroy MK, Andrews CJ** (1983) Responses of winter cereals to various low temperature stresses. *In* DD Randall, DG Blevins, RL Larson, BJ Rapp, eds, Current Topics in Plant Biochemistry and Physiology 2. University of Missouri, Columbia, pp 96–100
18. **Pomeroy MK, Andrews CJ** (1986) Changes in adenine nucleotides and energy charge in isolated winter wheat cells during low temperature stress. Plant Physiol **81:** 361–366
19. **Pomeroy MK, Andrews CJ** (1989) Low temperature injury in winter cereals. *In* PH Li, ed, Low Temperature Stress Physiology in Crops. CRC Press, Boca Raton, FL, pp 107–122
20. **Pradet A** (1967) Etude des adenosine 5′ mono, di, et triphosphates. 1. Dosage enzymatique. Physiol Veg **5:** 209–221
21. **Pradet A, Raymond P** (1983) Adenine nucleotide ratios and adenylate energy charge in energy metabolism. Annu Rev Plant Physiol **34:** 199–224
22. **Rakitina ZG** (1977) Effect of a surrounding ice crust on winter wheat plants as a function of their flooding before freezing. Sov Plant Physiol **254:** 317–324
23. **Saglio PH, Drew MC, Pradet A** (1988) Metabolic acclimation to anoxia induced by low (2–4 Pa partial pressure) oxygen pretreatment (hypoxia) in root tips of Zea mays. Plant Physiol **86:** 61–66
24. **Sobczyk EA, Marszalek A, Kacperska A** (1985) ATP involvement in plant tissue response to low temperature. Physiol Plant **63:** 399–405
25. **Spanswick RM** (1981) Electrogenic ion pumps. Annu Rev Plant Physiol **32:** 267–289
26. **Wignarajah K, Greenway H, John CD** (1976) Effects of waterlogging on growth and activity of alcohol dehydrogenase in barley and rice. New Phytol **77:** 585–592
27. **Zocchi G, Hanson JB** (1982) Calcium flux into corn roots as a result of cold shock. Plant Physiol **70:** 318–319