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Response of winter barley to low temperature flooding

P. RICHARD HETHERINGTON
and
WILLIAM S. STEWART *)

Department of Agricultural & Environmental Science, The University, Newcastle upon Tyne, NEl 7RU, U.K.

SUMMARY

When eight winter barley cultivars (*Hordeum vulgare* L.) were flooded at low temperature (6°C days, 2°C nights), a range of responses were found. Sensitivity to low temperature flooding was associated with gradual leaf wilting, whilst more tolerant cultivars produced only leaf chlorosis after longer durations of flooding. When a 'sensitive' (Maris Otter) and 'tolerant' cultivar were examined in more detail, flooding the sensitive cultivar produced an enhanced leakage of cell electrolytes (indicative of a loss of membrane semipermeability), firstly in the roots, then in the leaves. Wilting, electrolyte leakage and turgor loss in leaf tissue of the sensitive cultivar was associated with ethanol accumulation. Moreover, application of exogenous ethanol to control tissue (to produce similar endogenous levels to those found in flooded tissue) could reproduce flooding symptoms. This occurred in both cultivars, suggesting that ethanol avoidance may be important in preventing flooding damage to leaves of the tolerant cultivar. Seedling roots appeared to be more flooding sensitive than adventitious roots, and cortical cell collapse in the latter was not associated with flooding tolerance in the two cultivars examined.

Key words: flooding, low temperature flooding, winter barley.

YFIRLIT

Áhrif lághitaflóða á vetrarbygg

Átta afbrigði vetrarbyggs (*Hordeum vulgare* L.) voru umflotin vatni við lágt hitastig (6°C að degi og 2°C að nóttu) og komu þá fram allmörg einkenni á plöntunum. Blöð næmari afbrigðanna sölnuðu smám saman en blöð þolnari afbrigðanna gulnuðu fyrst eftir lengri flóð. Næmt afbrigði (Maris Otter) og þolið afbrigði (Athene) voru rannsökuð nánar og kom í ljós hjá næma afbrigðinu aukinn leki á rafvökva úr frumunum sem bendir til skemmdar á hálfgegndræpi frumuhimnunnar, og gerðist þetta fyrst í rótum en síðar á blöðum. Etanól safnaðist fyrir í blaðvefjum næma afbrigðisins samhliða sölnun, rafvökvaleka og vökvaspennutapi. Auk þess komu flóðskemmdir fram á plöntum sem voru settar í etanól, þannig að etanólmagn vefjanna varð svipað og við flóð. Þetta gerðist í báðum afbrigðum og bendir það til þess að þolna afbrigðið komist hjá flóðskemmdum á blöðum með því að forðast etanólið. Frærætur vetrarbyggsins virðast viðkvæmari fyrir flóðskemmdum en hjárætur og hrun barkarfruma í hjárótum tengdist ekki flóðþoli afbrigðanna tveggja.

*) To whom correspondence should be addressed.

INTRODUCTION

Winter cereals growing in temperate areas can often experience periodic flooding from autumn through to spring. In the United Kingdom this is seldom severe enough to cause mortality, but if prolonged, outdoor flooding of either winter wheat or winter oats substantially reduces grain yields (Belford, 1981; Cannell *et al*., 1985). In the Nordic countries such as Finland, however, flooding damage is considered to be frequently more severe and is a probable component of winterkill (Ravantti and Miettinen, 1989). For example, flooding during winter may predispose plants to other overwintering stresses such as freezing (Andrews and Pomeroy, 1981; Gao *et al*., 1983). Unlike freezing, relatively little attention has been given to factors influencing flooding tolerance in winter cereals. Of the studies that have been performed, most, if not all, have been at temperatures much higher than those experienced by overwintering plants. Therefore, as temperature is one factor known to modify the response of plants to flooding (Trought and Drew, 1982), more information is needed about plant response to low temperature flooding.

A generalized theory of flooding tolerance has been proposed, whereby, as a consequence of hypoxic conditions around the roots, alcohol dehydrogenase activity (ADH) was induced in flooding-sensitive plants, leading to the accumulation of ethanol to deleterious levels. Flooding-tolerant plants, on the other hand, showed no accumulation of ethanol due to maintenance of low levels of ADH activity (Crawford and McManmon, 1968). However, the importance of ethanol accumulation in relation to flooding injury has since been questioned (Jackson *et al*., 1982). In barley, the basis of flooding tolerance appears unclear with Fagerstedt (1984) finding flooding sensitivity to be related to increased ADH activity, but Wignarajah *et al*. (1976) showing higher levels of ADH activity to be associated with flooding-tolerant cultivars.

Other adaptations to flooding have been reported including the formation of air-spaces ("aerenchyma") in the roots of tolerant plants, which act as an oxygen reservoir, thus alleviating hypoxia. Aerenchyma has been found in several species including wheat and barley (Erdmann *et al*., 1986; Larsen *et al*., 1986). Bearing in mind the above considerations, we examined the response of a range of winter barley cultivars to low temperature flooding.

MATERIALS AND METHODS

Growth conditions, flooding treatment and cultivar screening

Seeds were germinated on moist filter paper in sterile crystallizing dishes for 5 days in the dark at room temperature. Germinated seedlings were planted in small trays (20×15×5 cm) containing John Innes No. 2 potting compost, 20 seedlings per tray. The seedlings were watered as required and grown for the next 6 weeks at 6°C day, 2°C night temperatures, with a 12 hour photoperiod of 270 µmol m^{-2} s^{-1} (PAR), and 50% relative humidity. After this period, plants had three fully expanded leaves. Trays of plants were then allowed to continue growth under freely-drained conditions with water given as required (controls), or flooded to l cm below the soil surface (level checked daily). Plant measurements were made after varying durations of treatment specified later. Eight cultivars (Table l) consisting of 5 two-row and 3 six-row types were evaluated in a preliminary screening for flooding tolerance. After 25 days of treatment the following parameters were recorded for flooded and control plants: dry weight of shoots and roots (seedling and adventitious), number of live leaves on the main shoot, and number of shoots produced per plant. For a single cultivar, comparisons between control and flooded treatments were made using Student's T-test.

Table 1. Mean values of some characteristics of plants of eight winter barley cultivars after 25 days of flooding at l2 hour, 6°C day/2°C night.
1. tafla. Meðalgildi nokkurra eiginleika átta afbrigða vetrarbyggs eftir 25 daga flóð við 12 klst. á dag, 6°C að degi og 2°C á nóttu.

Cultivar	Treatment	Plant dry wt. mg	Shoots plant^{-1}	Live leaves Main shoot^{-1}	Shoot: root ratio[a)]	Seedling roots: adventitious roots ratio[a)]	Flood tolerance index[b)]
Distichon group							
Sonja	Control	316	2.8	3.3	3.3	4.2	0.11
	Flooded	300^{NS}	3.0^{NS}	0.4***	5.3**	1.8***	
Fenella	Control	257	3.4	3.6	5.8	1.7	0.26
	Flooded	282^{NS}	2.8^{NS}	0.9***	4.8^{NS}	1.1^{NS}	
Maris Otter	Control	356	3.9	3.6	2.8	2.4	0.31
	Flooded	241*	2.7*	1.1***	3.4^{NS}	1.0***	
Tipper	Control	280	1.7	2.8	3.0	2.3	0.56
	Flooded	283^{NS}	1.5^{NS}	1.6***	3.6^{NS}	0.8***	
Igri	Control	312	2.8	3.3	2.7	2.1	0.68
	Flooded	300^{NS}	3.1^{NS}	2.2***	3.7**	0.8***	
Hexastichon group							
Pirate	Control	243	2.3	2.2	3.4	3.2	0.39
	Flooded	238^{NS}	2.5^{NS}	0.8***	4.2**	0.9***	
Gerbel	Control	448	4.5	3.1	3.2	2.5	0.48
	Flooded	436^{NS}	3.9^{NS}	1.5***	4.6***	0.5***	
Athene	Control	359	2.6	3.0	2.9	2.4	0.77
	Flooded	385^{NS}	3.0^{NS}	2.3**	4.2***	0.8***	

a) Calculated on a dry weight basis.
b) Calculated as: Live leaf no. (main shoot) FLOODED/Live leaf no. (main shoot) CONTROL.
NS=Not significantly different from the freely drained control.
*, **, ***=Significantly different from the freely drained control at the 0.05, 0.0l and 0.00l levels of P, respectively, n=10.

Root morphology and anatomy

After 25 days of treatment, control and flooded plants were removed from the growing medium, and the number of primary roots and their length were recorded for each root system. Numbers of lateral roots initiated from the primary seedling roots were also counted. Anatomical studies were conducted on the median adventitious root (by length). Transverse sections were cut using a clean razor blade and stained in l% aqeous toludine blue for l min. A camera lucida was fitted to the microscope, and root sections were drawn on paper allowing the relative area of tissues and collapsed cells to be determined gravimetrically.

Leaf diffusive resistance and water relations

These measurements were made at the midpoint of the second leaf (adaxial surface) using an automatic diffusion porometer (Delta-T Devices, Cambridge). Daily values were taken between 5 and 6 hours into the light period. Water relations were measured by thermocouple psychrometry. Leaf discs (6 mm diameter) were excised from the midpoint of the second leaf lamina, sealed in C-52 sample chambers and allowed to equilibrate at constant temperature. Measurements of water potential were made in combined psychrometric and dew-point mode using an HR-33T dew-point microvoltmeter (Wescor

Inc., Utah, USA). For solute potential, discs were placed in small capped vials, frozen in liquid nitrogen, then thawed, and measured in combined mode as above.

Electrolyte leakage

Single excised laminae, or an entire root system carefully washed in distilled water and blot-dried were placed in glass tubes (2×30 cm), and immersed in 25 ml double-distilled water per tube. After storage of tubes in darkness at 5°C for 24 hours, the conductivity of the bathing solution was measured at 15°C (CDM 3 conductivity meter, cell type CDC 314, Radiometer, Copenhagen). Tubes were sealed and autoclaved at 1.05 kg cm^{-2} for 30 min and the conductivity of the bathing solution again measured at 15°C. The conductivity of the double-distilled water was subtracted from both readings, and the conductivity of the bathing solution before autoclaving was calculated as a percentage of that after autoclaving. This is referred to as '% leakage'. All % leakage data were given the angular transformation before statistical analysis, after which mean and SE values were transformed back to % leakage for presentation.

Ethanol measurements

Extraction of ethanol from roots, leaf laminae or crowns was by the method of Bertani *et al*. (1980), and ethanol concentration was determined spectrophotometrically (Bergmeyer, 1974).

Treatment with ethanol

Shoots from control plants (grown as described prior to treatment) were exposed to ethanol in the range 0 to 50 µM by excising them under the test solution, and placing in test tubes in the 6°C day/2°C night environment. Only the lowest part of the shoot was submerged in the test solution. With this method only leaf sheaths were in actual contact with the test solution, and any effects on the visible leaf laminae would be caused by uptake into the laminae via the sheaths. Observations of symptoms, and measurements of ethanol, water relations and electrolyte leakage were made as described previously.

RESULTS

Screening of cultivars

Visual symptoms appeared on susceptible cultivars after 12 days flooding, and consisted of small pin-head sized necrotic flecks on the oldest (first) leaf laminae of cvs Maris Otter, Sonja and Fenella. Over the next 4 days this lamina wilted and died, and the process was repeated on the second leaf, with wilting starting at the tip and no chlorosis being evident. The remaining cultivars showed different flooding symptoms which appeared one week later, their progression being dependant upon cultivar. These symptoms were chlorosis of the older leaves leading to premature senescence, with no obvious wilting symptoms at all. In all cases, flooding caused no mortality, with plants still producing new leaves, and in some cases poorly developed tillers (Table 1).

When cultivars were assessed 25 days after flooding, a significant reduction in live leaf number on the main shoot, and in many cases an increase in the shoot:root ratio were observed (Table 1). Also, within the root system, flooding often reduced the ratio of seedling to adventitious root biomass. In Maris Otter, flooding also reduced plant dry weight and shoot numbers. An index of flooding tolerance based upon the live leaf number of the main shoot is also given. With this index a range of flooding tolerance is shown for the cultivars tested, with values ranging between 0.11 and 0.77. From these preliminary tests, two cultivars were selected for further study viz. – Maris Otter (flooding sensitive) and Athene (flooding tolerant).

Electrolyte leakage, water relations and leaf resistance

In Athene, up to 16 days flooding caused no

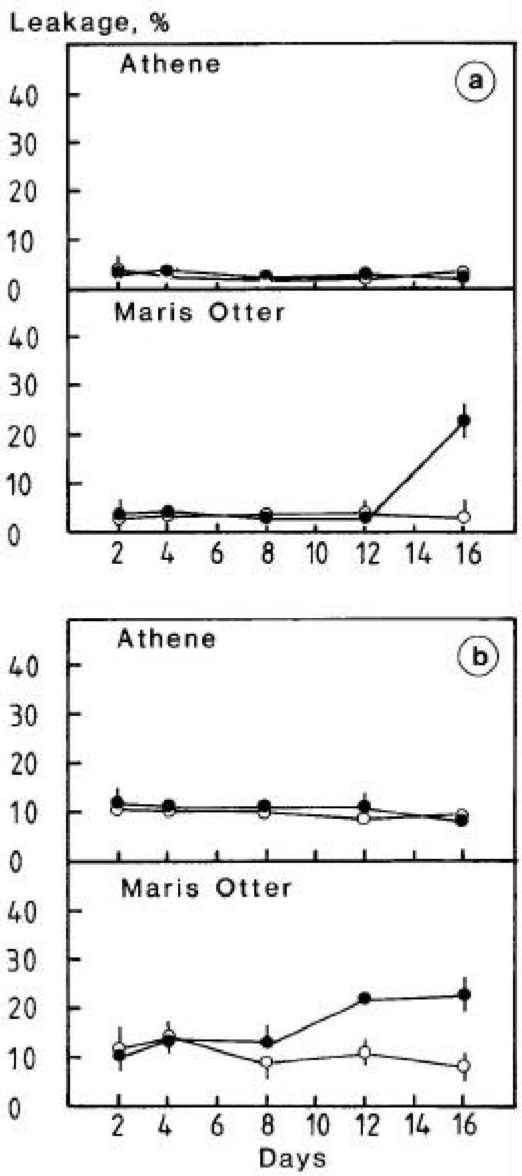


Figure 1. Leakage of electrolytes from (a) the second leaf, and (b) roots for two winter barley cultivars with (●) or without (○) flooding over 16 days. Mean values ± SE (shown where symbol size is exceeded), n=5.

1. mynd. Rafvökvaleki frá öðru blaði (a) og rótum (b) tveggja afbrigða af vetrarbyggi, með (●) eða án (○) flóða í 16 daga. Meðaltöl ± staðalskekkja (sýnd þar sem hún er stærri en táknið), n=5.

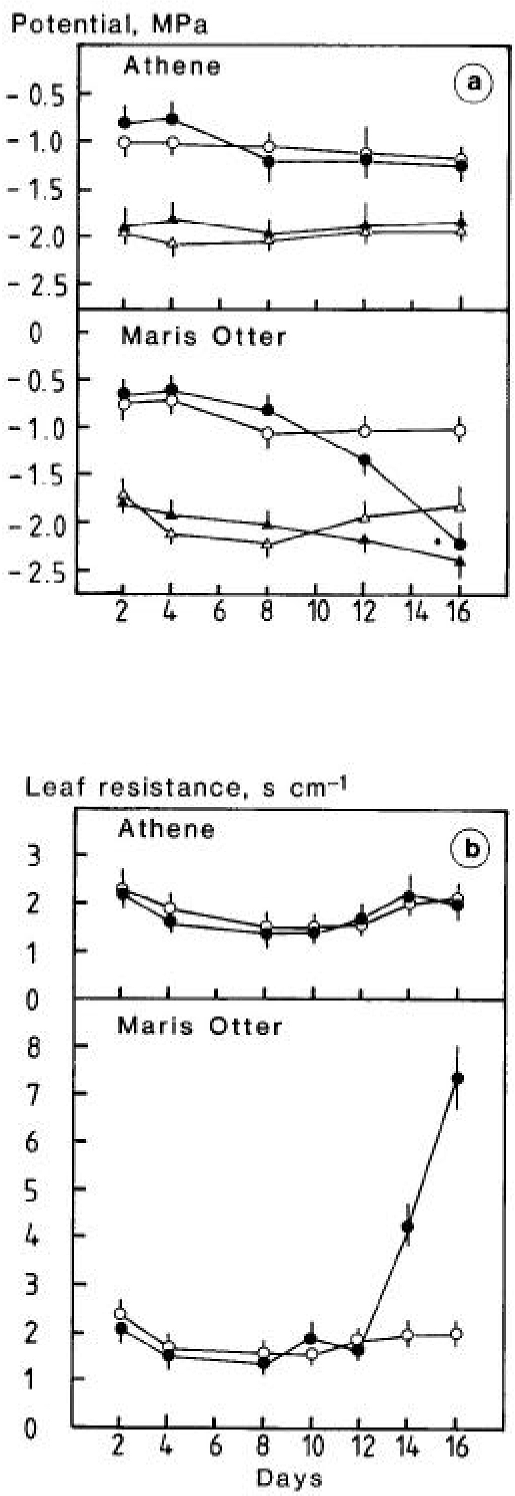
detectable damage to either roots or the second leaf as assessed by electrolyte leakage (Figure 1). However, for Maris Otter there was an increase in leakage from laminae after 16 days flooding, and from roots after 12 days flooding.

For Athene, flooding did not substantially affect leaf turgor, with values for the laminae ranging from 0.7 to 1.0 MPa. However, in Maris Otter a marked decline in leaf water potential was not paralleled by solute potential and the result was a turgor loss over this period from normal levels of around 0.8 MPa, to 0.15 MPa (Figure 2a). This loss of leaf turgor in Maris Otter was associated with a simultaneous increase in adaxial resistance (Figure 2b) presumably due to stomatal closure.

Ethanol measurements

The ethanol content of leaf laminae, roots and crowns was measured when symptoms first began to appear in the flooding sensitive cultivar Maris Otter (Table 2). Flooding both cultivars for 12 days resulted in the production of larger quantities of ethanol than those found in the controls, but the distribution of ethanol within the plants differed. In Athene, ethanol accumulated in the crown, whereas in Maris Otter the additional ethanol was found in the leaf laminae. The flooding sensitive cultivar accumulated higher ethanol levels during flooding than the flooding tolerant cultivar. In neither cultivar was there an increased accumulation of ethanol in roots because of flooding.

In order to examine the possibility that leaf wilting and electrolyte leakage result from ethanol accumulation, shoots of control plants at the third leaf stage were incubated in a range of ethanol solutions to see if such symptoms could be reproduced; ethanol being introduced through the base of the shoot. After 6 days incubation in 10 µM ethanol, similar symptoms were found in the laminae of both cultivars. Shoots incubated in distilled water produced no visual symp-



toms, the laminae remaining turgid, showing no leakage of electrolytes or presence of ethanol in detectable amounts (Table 3). However, in shoots incubated with 10 µM ethanol all three leaves showed chlorosis at the tip and severe wilting (turgor loss) which was quantified by water relations measurements. Such leaves of both cultivars were found to contain measurable amounts of ethanol and showed enhanced leakage of cell electrolytes (Table 3).

Root system

More detailed studies revealed different root morphologies in the two selected cultivars, with Athene initiating more seedling and ad-ventitious roots (Table 4). Low temperature flooding for 25 days reduced the lengths of seedling roots but not adventitious roots, this reduction being greatest in Maris Otter. Also in this cultivar, flooding reduced the number of laterals initiated by the primary seedling root.

Microscopic examination of adventitious root sections, revealed flooding to have caused the appearance of large areas of collapsed cells in the cortex of Maris Otter, but not Athene, 4 cm from the root tip. Such cell collapse substantially reduced the amount of root cross-sectional area due to cortical tissue (Table 4).

DISCUSSION

On flooding barley at a minimum temperature of 15°C Larsen *et al*. (1986) found leaf symptoms of wilting and chlorosis to appear in 5 to 6 days. In the present study when

Figure 2. Water (○,●) and solute (+, % potentials (a), and adaxial resistance (b) of the second leaf for two winter barley cultivars with (closed symbols) or without (open symbols) flooding over 16 days. Mean values ± SE, n=3 or 4.

2. mynd. Spenna (a) og áslægt viðnám (b) vatns (○,●) og uppleystra efna (+, % á öðru blaði tveggja afbrigða af vetrarbyggi, með (fyllt tákn) eða án (ófyllt tákn) flóða í 16 daga. Meðaltal ± staðalskekkja, n=3 eða 4.

Table 2. Ethanol content (µmol g^{-1} fresh wt.) of crowns, roots and leaf laminae in two winter barley cultivars, with (F) and without (C) a flooding treatment of 12 days. Mean values (SE), n=5.
2. tafla. Etanól (µmól/g votvigt) í rótarhálsi, rótum og blöðum í tveimur afbrigðum af vetrarbyggi, með (F) og án (C) flóða í 12 daga.

	Maris Otter		Athene	
	F	C	F	C
Roots	0.57 (0.03)	0.56 (0.10)	0.18 (0.08)	0.15 (0.07)
Crowns	0.39 (0.06)	0.50 (0.10)	0.67 (0.12)	0.12 (0.02)
Laminae	0.87 (0.23)	a)	a)	a)

a) Not detectable.

plants were flooded at 6°C day and 2°C night temperatures, such symptoms took over twice as long to appear. This delay in appearance of flooding symptoms at lower temperatures is also found in winter wheat (Trought and Drew, 1982). In addition to the flooding protocol used, the nature and magnitude of symptoms appear to be cultivar dependant, with wilting being associated with flooding-sensitive cultivars such as Maris Otter, and leaf chlorosis occurring later in more tolerant cultivars like Athene, with no observed wilting. Other cultivars displayed symptoms intermediate to these. Chlorosis caused by flooding of barley can be alleviated by application of nitrate or cytokinins to the leaves (Drew *et al*., 1979), however little is known about the cause and development of wilting, which was associated with greater sensitivity to flooding.

Electrolyte leakage measurements in the sensitive Maris Otter, indicate a loss of semipermeability in the root plasmalemma to precede any similar effect in the leaves. Also dry matter measurements show in many cases roots to be more flooding-sensitive than shoots, and seedling roots to be more sensitive than adventitious roots. In the flooding-sensitive cultivar used here, seedling roots had reduced lengths, and a reduction in the initiation of lateral roots. However, for the two cultivars tested in this study, flooding-tolerance was not associated with the production of aerenchyma in adventitious roots. In fact, to the contrary, cortical cell collapse was found in the sensitive cultivar. However, we did not establish whether such collapsed cells resulted in increased tissue air spaces. Several of these morphological changes have been reported in wheat seedlings following

Table 3. Ethanol content (µmol g^{-1} fresh wt.), turgor (MPa) and electrolyte leakage (%) in leaves of winter barley after incubation of shoots for 6 days at 6°C day/2°C night with 10 µM ethanol (E). Turgor was calculated as the difference between water and solute potentials of samples. Controls (C) were incubated in distilled water for the same time period. Mean values (SE), n=3.
3. tafla. Etanól (µmól/g votvigt), vökvaspenna og rafvökvaleki í blöðum vetrarkorns eftir 6 daga við 6°C að degi og 2°C að nóttu við 10 µM af etanóli (E). Vökvaspenna var reiknuð sem mismunur spennu vatns og uppleystra efna í sýnunum. Viðmiðun (C) stóð sama tíma í eimuðu vatni.

	Maris Otter		Athene	
	E	C	E	C
Ethanol content	0.86 (0.03)	a)	0.55 (0.01)	a)
Turgor	0.28 (0.12)	1.40 (0.20)	0.37 (0.13)	1.08 (0.08)
Leakage[b)]	42 (3.0)	4 (0.8)	49 (6.9)	6 (0.9)

a) Not detectable.
b) Measurements made on second leaf lamina.

Table 4. Some morphological and anatomical characteristics of the root system in two winter barley cultivars, with (F) and without (C) a 25 days flooding treatment. Mean values (SE), n=l0.
4. tafla. Form- og líffæralegir eiginleikar rótarkerfisins í tveimur afbrigðum vetrarbyggs, með (F) og án (C) flóða í 25 daga. Meðalgildi (staðalskekkja), n=10.

	Maris Otter		Athene	
	F	C	F	C
Seedling roots				
No. primary roots	4.8 (1.1)	4.2 (1.0)	7.2 (0.4)	6.4 (0.8)
Mean length, mm	77 (7)	l24 (16)	84 (6)	l10 (10)
No. lateral roots/cm primary root	l.4 (0.2)	2.4 (0.3)	2.7 (0.2)	2.5 (0.2)
Adventitious roots				
No. primary roots	4.6 (0.2)	2.6 (0.4)	6.8 (0.2)	5.0 (0.7)
Mean length, mm	69 (7)	62 (13)	66 (6)	66 (6)
% root cross[a)]	5l (3.2)	73 (1.8)	6l (1.6)	62 (1.7)
% of cortex cross[b)]	25 (1.7)	0.4 (0.4)	2.4 (1.1)	0.8 (0.7)

a) Sectional area due to intact cells, at 4 cm from tip, n=5.
b) Sectional area due to collapsed cells, at 4 cm from tip, n=5.

root anaerobiosis (Wiedenroth and Erdmann, 1985).

Leaf symptoms appeared soon after damage to the roots was detected, with loss of membrane permeability, turgor and an associated increase in leaf resistance occurring presumably due to stomatal closure. This response is at variance to that found on flooding pea (*Pisum sativum* L.) where stomatal closure was not controlled by the turgor of the leaf tissue as a whole (Zhang and Davies, 1986). Nevertheless, any of the above measurements, which are relatively easy to perform, could be useful in evaluating flooding tolerance in both the field and controlled environments.

At the onset of wilting, leaves of the flooding-sensitive cultivar had accumulated substantial amounts of ethanol suggesting it to have been transported from more hypoxic regions of the plant such as the roots, and possibly the basal region of the crown. Previous workers have found a movement of ethanol from the root system to the leaves via the xylem during flooding (Fulton and Erickson, 1964). Furthermore, symptoms of turgor loss and electrolyte leakage in laminae could be reproduced when shoots were incubated with a 10 µM ethanol solution for 6 days, after which time tissue ethanol levels were similar to those found in the sensitive cultivar after 12 days flooding. Interestingly, damage symptoms were also found in leaf laminae of the tolerant cultivar after exogenous ethanol treatment, indicating avoidance of ethanol accumulation in the leaf laminae to be important in preventing damage.

It is unclear as to any possible role for ethanol in causing damage, such as electrolyte leakage in the root system of the flooding-sensitive cultivar. After 12 days flooding, endogenous root ethanol levels were similar to the controls. However, at this stage root membranes were already becoming leaky to electrolytes, and ethanol may have already been leached from the tissue.

In conclusion, it appears that the response of winter cereals to flooding varies depending upon the exact nature of the flooding treatment and the cultivar used. Sensitivity to flooding is shown in the root system first, as changes in membrane permeability (electrolyte leakage). Leaf symptoms associated with flooding-sensitivity are also related to a loss of membrane semipermeability (wilting, turgor loss, electrolyte leakage and stomatal closure), and a toxic role for ethanol in this situation can not be discounted. Ethanol together with carbon dioxide is considered to be toxic in relation to mortality caused by another overwintering anoxic stress, namely ice encasement (Andrews and Pomeroy, 1979).

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