Ice tolerance and metabolite accumulation of herbage crops in Iceland and impact of climate change

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ABSTRACT

Ice tolerance of meadow plants and winter cereals was tested in field and also in artificial ice encasement experiments at -2°C in the laboratory. In general the ice tolerance was on the order of: Grass species > clover species > brassicae species, alpine plant and winter cereal species. Within the grasses the ice tolerance of Bering hairgrass and timothy was higher than in other grass species tested, such as cocksfoot and perennial ryegrass. White clover has significantly higher tolerance than red clover, and winter rye is slightly more tolerant than winter wheat and winter barley. In timothy carbohydrates were metabolised during ice encasement, resulting in carbohydrate decrease and accumulation of metabolites. Accumulation of metabolites in grasses, clover and winter cereals during ice encasement at -2°C was also measured in laboratory tests. All plant species tested accumulated CO_2 , ethanol and lactate. Timothy and winter wheat also accumulated malate. On a dry weight basis grasses accumulated metabolites to higher concentrations than winter cereals, and in general the more ice tolerant winter rye accumulated metabolites to higher concentrations than winter wheat. Bering hairgrass accumulated CO_2 to higher concentrations than timothy, indicating a faster metabolism. The winter temperature in Northern Iceland has increased substantially during recent decades as a result of climate change. Winter damage caused by ice cover is therefore almost eliminated from the agricultural lowland areas. However, there are indications that higher winter temperatures may increase ice damage in alpine areas and harm snow-loving snowbed plants that previously have been protected by a snow layer and therefore have low ice tolerance.

Keywords: Anaerobic respiration, climate change, ice damage, Iceland, ice tolerance, metabolite accumulation

YFIRLIT

Svellþol nytjaplantna, uppsöfnun öndunarefna undir svellum á Íslandi og áhrif væntanlegra loftslagsbreytinga

Svellþol túnjurta og vetrarkorns og nokkurra fleiri tegunda var metið í vallartilraunum og einnig í vinnustofutilraunum við -2°C. Í heild reyndist svellþolsröðin þessi: Grastegundir > smárategundir> krossblómategundir, fjallaplanta, vetrarkorn. Innan grastegunda voru beringspuntur og vallarfoxgras svellþolnari en aðrar grastegundir eins og axhnoðapuntur og vallarrýgresi. Hvítsmári var marktækt þolnari en rauðsmári, og vetrarrúgur heldur þolnari en vetrarhveiti og vetrarbygg. Orkuforði vallarfoxgrass brotnar niður undir svelli við -2°C og öndunarefni safnast fyrir. Allar tegundir sem mældar voru mynduðu CO_2 , etanól og mjólkursýru, og vallarfoxgras og vetrarhveiti mynda einnig eplasýru. Grös safna öndunarefnum í meiri styrkleika en vetrarkorn og í heild er uppsöfnun meiri í vetrarrúgi en vetrarhveiti og beringspuntur safnar CO_2 í meiri styrkleika en vallarfoxgras, til merkis um örari efnaskipti. Vetrarhiti á norðanverðu Íslandi hefur hækkað talsvert á síðari árum í tengslum við loftslagsbreytingar. Svellkal hefur því nær alveg horfið af ræktunarsvæðum á láglendi, en búast má við að svellkal geti í framtíðinni ógnað sumum snjódældaplöntum sem hafa lágt svellþol og hafa hingað til verið verndaðar af snjóalögum.

INTRODUCTION

Perennial plants are subject to many kinds of different winter stresses such as frost, frost heave, drought, flood and attack by snow moulds. These stresses may damage or kill perennial plants. In addition, longer or shorter periods of ice cover on the ground can be lethal to plants that do not protrude through the ice cover. Ice encasement damage has in certain areas in the last century occasionally been conspicuous on herbage crops such as grasses and winter cereals. This happens in alpine and arctic areas such as Northern Scandinavia, Iceland, Southwest Greenland, Eastern Canada and Northern Japan (Andersen 1963, Edey 1973, Friðriksson 1954, Hakamata et al. 1978). In some of these areas, i.e. Iceland, this kind of damage has periodically caused great yield losses and economic problems for farmers in the last century (Friðriksson 1954, Bergþórsson 2003, Gudleifsson 2009).

Agriculture in Iceland mainly consists of animal husbandry based on hay production from permanent hayfields. Ice encasement damage in hayfields in Iceland is related to temperature. Surprisingly, hay yield in Iceland in the last century (1900-2006) was more closely correlated to winter temperature (December-May, r = 0.57***) than to summer temperature (June-August, r = 0.28***); the lower the winter temperature the lower the hay yield (Gudleifsson 2009). The impact of winter temperature on hay yield is not ascribed directly to low temperatures or to frost damage but rather to the formation of ice cover which often forms in cold winters with shorter or longer thawing periods in early or mid-winter. The intensity of ice damage in hayfields in Iceland is related to conditions of formation of ice (amount of melt water) and to the thickness and endurance of the ice cover (R= 0.70***) (Gudleifsson 2009).

Ice crusts may form on fields at different times during autumn, winter or spring. Most frequently, it occurs in early or mid-winter when the snow melts and thaw water accumulates on the soil surface, especially in depressions in the landscape (Gudleifsson 1975, Andersen 1976). At the end of the thaw period, meltwater refreezes to form an ice layer, which is highly impermeable to gases (Hemmingsen 1959). In some cases an ice layer can completely cover flat fields or depressions in the landscape for a longer or shorter period (Gudleifsson & Larsen 1992). The longer the ice stays the more extensive the damage becomes (Gudleifsson 1975). These weather conditions are frequently experienced in northern maritime areas with high winter precipitation and unstable winter temperatures fluctuating around zero (Gudleifsson & Larsen 1992).

Perennial plants respirate throughout the winter, although metabolism is slow at the low temperatures in the soil surface area (Gudleifsson 2009). Under normal aerobic conditions they metabolise carbohydrates to carbon dioxide and water. Plants encased in ice are exposed to either partial or complete oxygen deprivation. As a result, their normal pathways of respiration are restricted and pathways of anaerobic respiration predominate. Ice tolerance and metabolism of winter cereals and grasses encased in ice have been measured and the main products are CO_2 , ethanol and organic acids such as lactate and malate (Andrews & Pomeroy 1983, 1989, Pomeroy & Andrews 1986, Gudleifsson 1997). In winter wheat this also results in a decrease in the production of metabolic energy (Pomeroy & Andrews 1986), and plants are killed in larger or smaller areas.

It has been demonstrated that ice encasement damage does not occur as a result of oxygen deficiency itself but as a result of the injurious action of metabolites (Freyman & Brink 1967). The accumulation and toxicity of these main metabolites has been measured in ice-encased winter wheat and studies indicate a more significant role of CO_2 in the ice encasement damage than of ethanol and lactate (Andrews & Pomeroy 1979). In order to reach toxic levels the time of ice encasement is crucial; the longer the encasement period the higher becomes the concentration of metabolites, which results in more damage (Gudleifsson 1975). The cell membranes are considered the primary site of damage, resulting in cell and plant killing (Andrews & Pomeroy 1979, 1991).

In Iceland ice damage has been rather common on herbage crops because the winter climate is unstable and ground cover frequently changes from snow to compact ice (Gudleifsson 1989). The predicted climate warming in Iceland is expected to be greater in winter than in summer (ACIA 2004, Björnsson et al. 2008). This will in the future result in a decreased risk of ice cover formation in the lowlands in Iceland where agriculture is practiced. The climate change may therefore in the future decrease or eliminate ice damage on crops in those areas where ice damage has dominated. Additionally, the increasing winter temperatures may stimulate cultivation of less ice tolerant crop species such as winter cereals and perennial Brassica species. Ice damage, on the other hand, may appear at higher altitudes and latitudes where stable snow cover has previously protected alpine plants (Gudleifsson 2009).

Herbage plants differ substantially in ice encasement tolerance, grasses being more tolerant than winter cereals (Andrews & Gudleifsson 1983, Gudleifsson & Larsen 1992). The aim of this study was to verify the tolerance of different plant species and to determine the kind and amount of metabolites accumulated by different crops during ice encasement. The impact of climate change on the occurrence and consequences of ice damage is also evaluated.

MATERIALS AND METHODS

Ice tolerance tests in the field

The survival of seedlings of some meadow plant and winter cereal species was tested in field tests at the Möðruvellir Experimental Farm (65°46,239’N, 18°15,080’W, 15 m above sea level). Meadow plants were tested in four tests (1992-1994) and winter cereals in two tests (1991-1992), except for winter oat, which was only tested once (Table 1). The number of replicates in each experiment was two or three. In all these years plants in the experimental plots were damaged or killed by ice cover during the preceding winter. Survival was registered visually as % survival in the spring, 21-28 May each year.

In 2006 the field survival of six grass species was evaluated in the spring in one field experiment with two replicates close to the Möðruvellir Experimental Farm. Conditions are shown in Table 2.

Table 1. Plant material used in field experiments, 1991-1994.

Plant species		Cultivar	Country of origin	Number of experiments
Timothy	*Phleum pratense* L.	Adda	Iceland	4
Red clover	*Trifolium pratense* L.	Bjursele	Sweden	4
Perennial ryegrass	*Lolium perenne* L.	Svea	Sweden	4
Winter rye	*Secale cereale* L.	Jussi	Finland	2
Winter wheat	*Triticum aestivum* L.	Norstar	Canada	2
Winter barley	*Hordeum vulgare* L.	WB 185-25	Canada	2
Winter oat	*Avena sativa* L.	Peniarth	UK	1

Table 2. Grass species tested in field test, 2006.

Plant species		Cultivar	Country of origin
Timothy	*Phleum pratense* L.	Engmo	Norway
Meadow fescue	*Festuca pratensis* Huds.	Salten	Norway
Cocksfoot	*Dactylis glomerata* L.	Hattfjelldal	Norway
Perennial ryegrass	*Lolium perenne* L.	Svea	Sweden
Meadow foxtail	*Alopecurus pratensis* L.	Seida	Norway
Smooth bromegrass	*Bromus inermis* Leyss.	Leif	Norway

Ice tolerance tests in the laboratory

Ice tolerance of meadow plants and winter cereals was tested several times (1993-1995) in artificial ice encasement experiments at -2°C (Table 3). Juvenile plants (vegetative seedlings) sown the previous summer were used in these experiments, grasses sown in June, winter cereals sown in July-August. Mature (generative) plants of meadow crop species were also tested. Before freeze-up of the soil in the autumn, turfs from field sown plants were placed in plastic trays. Trays were kept outdoors at the Möðruvellir Experimental Farm for hardening under natural conditions. After hardening, plants were taken to the laboratory for treatment on different dates in November - March each winter, roots washed clean of soil under cold tap water, and top and roots trimmed to about 3 cm each. Groups of five or ten plants were placed in 200 ml plastic beakers containing cold tap water. The beakers were then placed in a modified domestic freezer (Voisey & Moulton 1968) and frozen to -2°C to form solid ice cubes. Beakers of ice-encased plants were collected from the freezer for thawing, daily for winter cereals and weekly for grasses. Survival was evaluated by transplanting plants from four beakers (replicates) of thawed plants into peat soil for growth at 15°C and a day length of 12 hours. Surviving plants were counted 3 weeks later and % survival calculated. Ice tolerance of plants was calculated from the regression line and expressed as LD_{50} , i.e. the number of days in ice that killed 50% of the plant population.

From 16-20 October 2009 plant turfs of sev-

Table 3. Conditions in laboratory tests of survival and metabolite accumulation of juveniles in ice encasement (1993-1995). Mature plants of Bering hairgrass (2059 mg DM plant^{-1}) and timothy (4523 mg DM plant^{-1}) were also tested.

Plant species		Cultivar	Country of origin	Number of experiments	Number of plants in beaker	Dry matter mg plant^{-1}
Bering hairgrass	*Deschapsia caespitosa* Hult.	Nordcoast	USA	5	5.0	36
Timothy	*Phleum pratense* L.	Adda Snorre	Iceland Nordic	6	5.2	142
Red clover	*Trifolium pratense* L.	Bjursele 2n Torunn 4n	Sweden Norway	3	3.0	401
Winter rye	*Secale cereale* L.	Jussi Reetta	Finland Finland	5	7.3	233
Winter wheat	*Triticum aestivum* L.	Norstar Skjaldar Stava	Canada Norway Sweden	6	9.0	370
Winter barley	*Hordeum vulgare L.*	Dover WB158-25 Borwina	Canada Canada Germany	5	8.8	274

eral crop species (Table 4) were taken from three field experiments at the Möðruvellir Experimental Farm. The turfs were put in plastic trays and left outdoors for natural hardening until 18 January when they were taken into the laboratory for ice tolerance measurements. The plants consisted of three grass species (sown 2 July 2009), two clover species (sown 26 May 2008), two winter cereal species and two Brassica species (sown 21 July 2009), as well as one species of alpine plant (*Omalotheca supina*) taken from the highland (Vaðlaheiði, 500 m.a.s.l.). On 19 January plants were washed clean of soil, as described earlier, and a variable number of plants encased in ice in 200 ml (grasses and winter cereals) or 500 ml (clovers and Brassica) plastic beakers at -2°C for different lengths of time. Beakers, three replicates, were withdrawn from the freezer for thawing, weekly for grasses and daily for other plant species. The plant roots were then wrapped in wet filter paper and plants placed in plastic beakers for regeneration at 15°C and a 12 hour day length. The survival rate of single plants was evaluated after 10 days on a scale of 0-100% survival and the LD_{50} was calculated from the regression line.

Metabolite accumulation during ice encasement

Carbohydrates were determined in 1997 in timothy plants which had been ice encased for different lengths of time. At weekly intervals (up to 9 weeks, 64 days) three beakers (replicates) were withdrawn from the freezer. Beakers were thawed and the dry weight of the plants determined and the samples then boiled in distilled water for 1 hour, the liquid filtered on Watman paper no. 1 and analysed in HPLC with the same setup and conditions as described below for analysing organic metabolites.

In the laboratory tests for ice tolerance (Table 3) plants from beakers not used for tolerance tests (at least 2 replicates) were taken on each sampling date for analysis of metabolites. Ice blocks in beakers were thawed and plants were ground in a mortar in the thaw water, the liquid volume measured and plant residue dried in an oven at 70°C for dry matter determination. Ethanol was quantified enzymatically in the liquid with a UW test kit (Boehringer Mannheim) at 340 nm using a Vitratron universal photometer. Liquid samples for analysis of organic acids were filtered through 0.45 µm HA membrane filters before high performance liquid chromatography (HPLC Hewlett Packard 1050 series) with a solvent flow

Table 4. Plant species used in ice tolerance measurement, 2009-2010. Three beakers (replicates) were withdrawn from ice on each date.

Plant species		Cultivar	Country of origin	Number of plants in beaker	mg DM $plant^{-1}$
Timothy	*Phleum pratense* L.	Snorre	Nordic	6	31
Meadow fescue	*Festuca pratensis* L.	Salten	Norway	5	25
Perennial ryegrass	*Lolium perenne* L.	Birger	Sweden	5	42
Cocksfoot	*Dactylis glomerata* L.	Laban	Sweden	5	29
White clover	*Trifolium repens* L.	Norstar	Norway	5	59
Red clover	*Trifolium pratense* L.	Bjursele	Sweden	5	148
Winter rape	*Brassica napus* L. *var. oleifera subvar. biennis*	Goya	Sweden	3	104
Stubble turnip	*Brassica rapa* L. *var oleifera subvar. biennis*	Largo	Sweden	3	119
Alpine arctic cudweed	*Omalotheca supina* (L.) DC.	Wild ecotype (Vaðlaheiði)	Iceland	10	13
Winter rye	*Secale cereale* L. *var biennis*	Reetta	Finland	4	93
Winter wheat	*Triticum aestivum* L. *var biennis*	Stava	Sweden	10	32

rate of 0.35 µl min^{-1} and the UV detector set at 210 nm. The analytical column was an Animex HPX-87H (Bio-Rad) ion exclusion column, 300-8.7 mm stabilized at 50°C. The mobile phase consisted of 3% isopropyl alcohol, 1% 0.8 N H_2SO_4 and 96% distilled water. The separation of some metabolites was incomplete and as a check L-lactic acid, D-lactic acid, formic acid and malic acid were sometimes quantified enzymatically with a UV test kit as described for ethanol. CO_2 was analysed with a carbon dioxide ion selective electrode (Orion, model 95-02, see Orion Research 1982). During ice melting the CO_2 was retained in solution by addition of 1M NaOH or 1M citric acid and acidified to pH 4.8-5.2 during analysis. In all cases two or more replicates were analysed. In most cases metabolite accumulation was analysed, both in juvenile plants (seedlings) and mature grass plants.

Impact of climate warming on occurrence of ice damage

The climate change in Iceland from the ninth century settlement until today is based on temperature data from Bergþórsson (1969) with additions from Jónsson (2007) and Moberg et al. (2005) and information from the Icelandic Meteorological Office (IMO). Climatic data for Akureyri are from the IMO.

Calculations

In the field the two or three experiments were considered replicates. In laboratory experiments beakers were considered parallels and experiments replicates. Survival of plants in field tests is given in % survival while in laboratory experiments it is expressed as LD_{50} calculated from the 50% point on the regression line, where 50% plant survival was expected. The differences between plant species in ice tolerance was calculated by one way Anova analysis. The regression lines and R^2 were also calculated for carbohydrate breakdown and metabolite accumulation under ice, the means for all experiments with the same plant species.

RESULTS

Ice tolerance in field tests

Field tests of winter cereals indicated that winter rye was slightly more tolerant than winter wheat, winter barley and winter oat (Table 5). Field tests showed that timothy was a relatively ice tolerant meadow plant species, while perennial ryegrass and red clover were not. It should be pointed out that meadow plants and winter cereal species were not tested the same years and therefore the exact survival rates of these two plant groups are not comparable. The high ice tolerance of timothy was also verified in one field test from 2006 where timothy was compared to five other grass species.

Ice tolerance in laboratory experiments

The survival rates of juveniles and mature plants of meadow species tested in three laboratory experiments did not differ signifi-

Table 5. Survival in three field test series at Möðruvellir Experimental Farm expressed as % survival. Meadow plant species tested in one field test (2006) and in three tests (1993-1994). Winter cereal species tested in two tests (1991-1992). Plant species in the same column marked with different letters were significantly different.

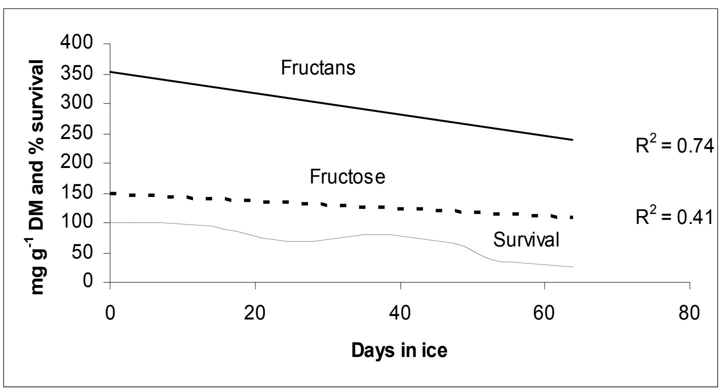
Species	% survival		
	2006	1993-1994	1991-1992
Timothy	80 a	78 a	
Meadow fescue	32 ab		
Cocksfoot	42 ab		
Perennial ryegrass	14 b	20 b	
Meadow foxtail	5 b		
Smooth bromegrass	0 c*		
Red clover		22 b	
Winter rye			90 a
Winter wheat			72 a
Winter barley			41 a
Winter oat			0 b*
p-value	0.011	0.002	0.092

* Not included in statistical calculations

Table 6. Ice tolerance of plant species tested in three laboratory series. Plant species in the same column marked with different letters were significantly different.

	Ice tolerance, days (LD_{50})		
Crop species	**2010**	**1993-1995**	**1993-1995**
Bering hairgrass		53 a	40a
Timothy	34 a		26b
Tall fescue	33 a		
Perennial ryegrass	26 a		
Cocksfoot	23 a		
White clover	25 a		
Red clover	11 b	11*	
Stubble turnip	9 b		
Winter rape	7 b		
Alpine arctic Cudweed	7 b		
Winter rye	2 b	7*	
Winter wheat	2 b		7c
Winter barley		2 b	2c
p-value	< 0.001	0.030	<0.001

*not included in statistical calculatior



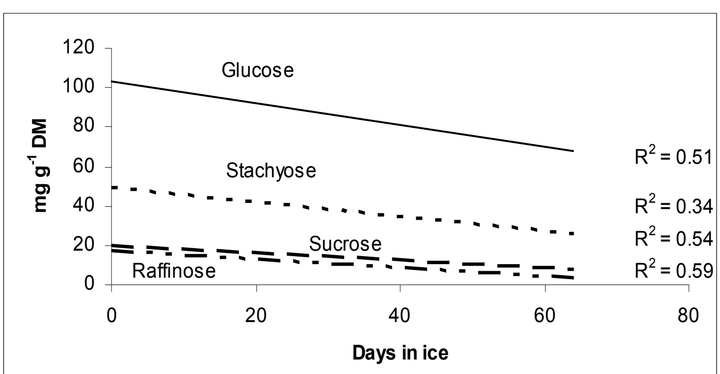


Figure 1. Carbohydrate content in timothy plants encased in ice at -2°C up to 64 days. R^2 describes how well the regression line fits the data.

cantly. The survival results are therefore a mean of juveniles and mature meadow plants. Meadow plants tested are significantly more ice tolerant than winter cereals (Table 6) and hairgrass was more ice tolerant than timothy. Red clover was significantly less ice tolerant than grasses and white clover. These results are all in good accordance with the results from the field tests.

In these tests, the grass species were much more ice tolerant than the winter cereals. Brassica species and the alpine plant tested also had low ice tolerance (Table 6).

Metabolite accumulation during ice encasement

The content of all carbohydrates measured in timothy decreased in prolonged ice encasement. Fructans and fructose dominated, then glucose and stachyose and sucrose, while sucrose and raffinose had the lowest concentration (Figure 1). The survival of these timothy plants is also illustrated with a curved line and the calculated LD_{50} in this experiment was 51 days.

The relative concentrations of accumulated metabolites were much higher in juveniles than in mature plants, as had been established before (Gudleifsson 1997). The metabolite concentrations in grasses presented here are only from

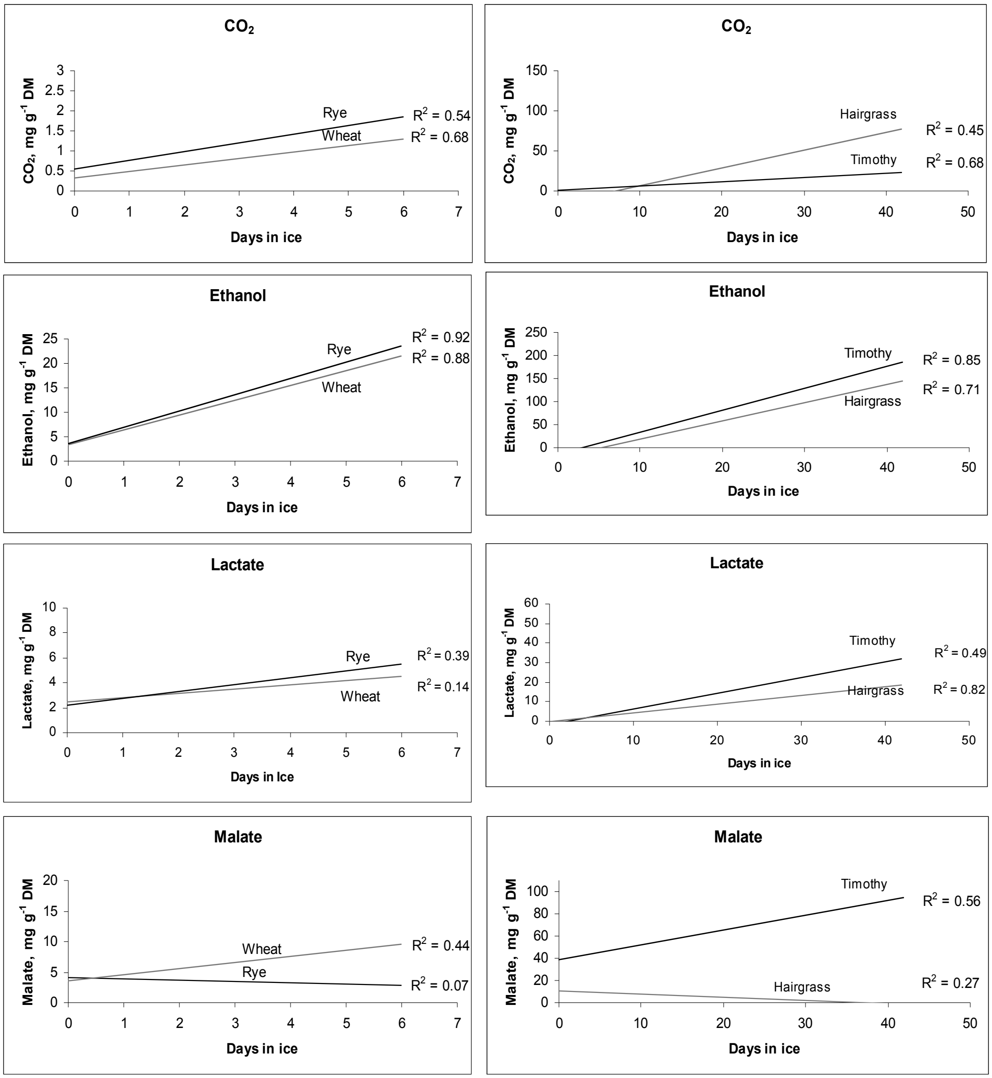


Figure 2. Accumulation of four metabolites in juveniles of four crop species, winter cereals to left, meadow plants to right.

juveniles. The use of juveniles makes the comparison between cereals and grasses more justified, as the winter cereals were of course only juveniles. The metabolite accumulation was tested in the laboratory on the same six plant species as were tested for ice tolerance in 1993-1995 (the two last columns in Table 6). Only four of the six species are illustrated in Figure 2, two grass species and two winter cereal species. All plant species tested accumulated carbon dioxide, ethanol and lactate, but only wheat and timothy, accumulated malate (Figure 2). On a dry weight basis grasses accumulated most metabolites to higher concentrations than winter cereals. In winter cereals the winter rye accumulates three out of four metabolites to higher concentrations than winter wheat; only malate is higher in winter wheat. Hairgrass accumulates CO_2 to higher concentrations than timothy, but timothy accumulates ethanol, lactate and malate to higher concentrations. Winter barley accumulated CO_2 and ethanol to similar concentrations as the other winter cereals and red clover accumulated CO_2 , ethanol and lactate to low concentrations compared to the grass species (data not shown). Besides the four metabolites presented in Figure 2, the following compounds were also measured: citrate, pyruvate, shikimate, fumarate, succinate, malonate and acetate. None of these increased systematically during ice encasement of these six plant species and all of them were in low concentrations. Citrate reached the highest concentrations, 5-15 mg g^{-1} DM.

Impact of climate change

At the settlement of Iceland, around the year 900 AC, the temperature seems to have been fairly high, making barley cultivation possible (Figure 3). In the 13th century temperatures in the northern hemisphere started to decrease and this decline continued until the 18th century (Bergþórsson 1969, 2003, Jónsson 2007, Moberg et al. 2005). Iceland also experienced a drop in temperatures in that period, creating substantial problems for the inhabitants (Bergþórsson 2003). No barley was cultivated, grasses were frequently killed, and the population declined (Friðriksson 1954). Temperature increased again in the last century (Gudleifsson 2009) so that nowadays the annual temperature in Iceland is similar to what it was at the time of the settlement (Figure 3) and barley has now been cultivated again since the 1980s.

Climate change is expected to increase winter temperatures more than the summer temperatures (ACIA 2004, Björnsson et al. 2004). The increase in winter temperatures is already very conspicuous in Iceland (Figure 4). Winter temperatures during the last decade have been higher than ever in the last century and most probably higher than in the last 800 years. Thus the impact of climate warming is easily measurable in Iceland. Now, as winter temperatures have increased as a result of climate change, ice damage on cultivated crops has almost disappeared in many places where it dominated or has moved to new areas. This is visualised in the numbers on the top of each column in Figure 4, indicating the number of years with substantial ice damage in each decade.

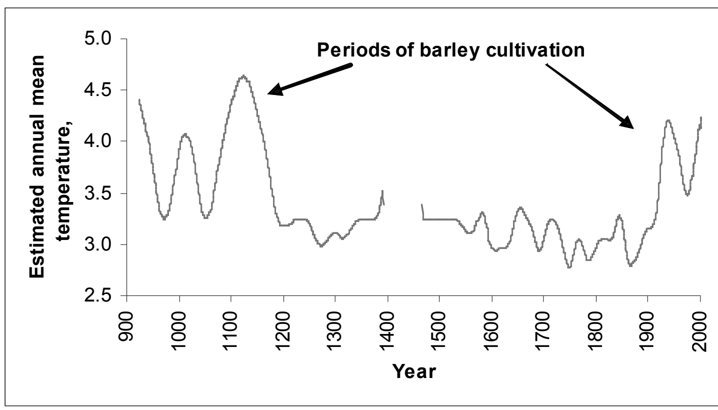


Figure 3. Estimated annual temperature in Iceland since settlement around 900 to 2008. Data from Bergþórsson (1969, 2003) with additional data from IMO.

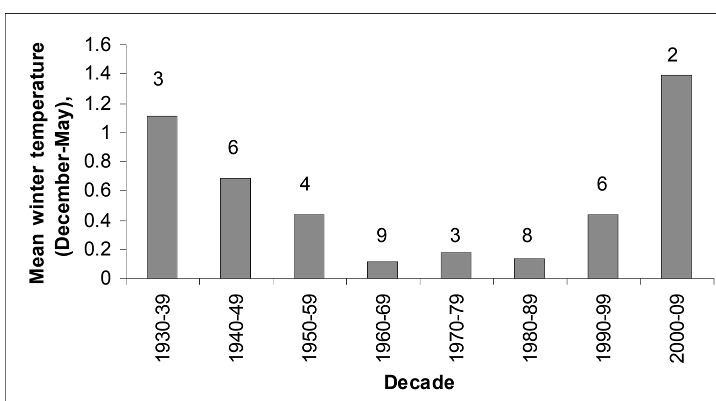


Figure 4. Mean winter temperature (December-May) in Akureyri, Northern Iceland, 1930-2009. Figures on top of columns indicate number of years with ice damage on hayfields in each decade. Climatic data from IMO.

DISCUSSION

Field tests of ice tolerance are very unreliable as the stress intensity depends mainly on winter climate and small topographical differences (Gudleifsson & Larsen 1992). Therefore the variability in survival is very high (Table 5). This underlines the importance of more reliable laboratory tests to measure ice tolerance. Laboratory tests for ice tolerance were first developed in Canada (Andrews 1977).

The correlation between field tests and laboratory tests for winter tolerance was studied by Pulli et al. (1996). In winter cereals freezing tolerance fitted better to the field survival than ice or snow mould tolerance. On the other hand, in grasses, especially timothy, ice tolerance was best related to field survival. This indicates that winter tolerance breeding of winter cereals should be based on freezing tests whereas on grasses it should concentrate on ice tests.

In laboratory experiments of plant tolerance the stress intensity has to be regulated to allow partial survival. If all plants or no plants are killed, minimal information on plant tolerance is obtained. The LD_{50} figures presented here from the laboratory experiments are not comparable to the number of ice days that plants can tolerate in nature. The stress is much stronger in the laboratory where the plants are completely encased in ice without any soil air. In nature the soil may always contain some air bubbles and the encasement is often incomplete. The rule of thumb in Iceland is that meadow grasses can survive up to 90 days of natural ice encasement, legumes about 20-30 days and winter cereals only 7-14 days (Gudleifsson 2009).

The field tests presented here indicate the order of ice tolerance within the two groups of plants tested, meadow plants and winter cereals. Within the meadow plants the decreasing tolerance is from timothy, red clover to perennial ryegrass (Table 5). Within the winter cereal species it is from winter rye, winter wheat, winter barley to winter oat. These field results from 1991-1994 (Table 5) do not allow comparison between the two plant groups as they were not tested the same winter and the stress may have been different from one winter to another.

The laboratory tests (Table 6) confirm these species differences and that meadow plants are in general more ice tolerant than winter cereals (Gudleifsson & Larsen 1992). This difference has been measured before (Andrews & Gudleifsson 1983). The high tolerance of Bering hairgrass and timothy and low tolerance of cocksfoot has been measured before (Gudleifsson et al. 1986, Andrews 1997), as well as the relatively high tolerance of winter rye compared to winter barley (Andrews et al 1986). The laboratory tests presented here only separated plant species with relatively great differences in ice tolerance but did not discriminate between species with small differences. The ranking is similar to that revealed in the field tests.

The carbohydrate reserves accumulated during summer and hardening in autumn are used for respiration for cellular maintenance in the anoxic conditions under ice. All six of the measured carbohydrate fractions decreased in timothy plants up to 60 days in ice (Figure 1). Fructans and fructose were accumulated to the highest levels during hardening. Barley was also tested (data not shown), but surprisingly the content of all carbohydrates did not decrease but rather increased slightly with prolonged encasement. This might be explained by the low ice tolerance of barley, with an LD_{50} of 2 days (Table 6). Therefore the plant cells were mostly dead during the six days of testing. The dry matter of the plants might decrease, as cell content, other than carbohydrates, may leak out. The apparent increase in dry matter content may then be an artefact of the lower dry matter. Since clovers store carbohydrates mainly as starch, their metabolism might differ from that of grass species. In these tests they produced less CO_2, ethanol and lactate than the grasses (data not shown).

During ice encasement metabolites are accumulated, mainly as a consequence of anaerobic respiration. The results in Figure 2 confirm the accumulation of CO_2 , ethanol and lactate, as has been established before in winter cereals (Pomeroy & Andrews 1983) and timothy (Gudleifsson 1997). In general all the tested crop species accumulated these three metabolites. On the other hand accumulation of malate was only confirmed in two crop species, winter wheat and timothy. There was no indication that winter rye, winter barley, Bering hairgrass and red clover accumulated malate. There are indications (Figure 2) that the more ice tolerant plants accumulate metabolites to higher concentrations than plants with low tolerance, as has been shown by McKersie et al (1982). This may be a consequence of greater ice tolerance, rather than a cause.

The increasing winter temperatures and climate change in Iceland have almost eliminated winter damage to crops (Figure 4). On the other hand this may result in increased ice stress on alpine plants where snow cover has previously isolated plants from low temperatures and ice stress. In warmer temperatures ice stress may occur for example on snow-loving plants previously growing on snow beds. The ice tolerance of these plants is not well known, but alpine arctic cudweed was used in the laboratory tests to represent them. As seen in Table 6 it had very low ice tolerance, an LD_{50} of 7 days, similar to Brassica species, which might indicate that these alpine plants could be harmed in a future warmer climate with more ice formation in alpine areas. On the other hand this alpine species has a rather high freezing tolerance (Gudleifsson 2009).

The decreased hardening of plants due to warmer winter temperatures may also be a threat to overwintering crops. One of the predictions of climate change is increased variability of weather events (ACIA 2004). Thus, warm early winter conditions in the future could combine with severe low temperature events to damage traditional crops, or new crops developed to exploit generally warmer winter temperatures. A rapid and stable hardening response will then be important for the success of such new plant materials.

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